

THE PONTIFICAL  
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Scripta Varia

103

# The Challenges of Sciences

*A Tribute to the  
Memory of Carlos Chagas*



VATICAN CITY  
2002

Working Group  
23-24 February 2001

**THE CHALLENGES OF SCIENCES  
A TRIBUTE TO THE MEMORY OF CARLOS CHAGAS**

*Address:*  
THE PONTIFICAL ACADEMY OF SCIENCES  
CASINA PIO IV, 00120 VATICAN CITY

THE CHALLENGES  
OF SCIENCES  
A TRIBUTE TO THE MEMORY  
OF CARLOS CHAGAS

23-24 February 2001



EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

MMII

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VATICAN CITY



*John Paul II*





The Pontifical Academy of Sciences  
Casina Pio IV





The Participants of the Symposium in Memory of Carlos Chagas 23-24 February 2001

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THE PONTIFICAL ACADEMY OF SCIENCES

Symposium on:

THE CHALLENGES OF SCIENCES  
A TRIBUTE TO THE MEMORY OF CARLOS CHAGAS

(23-24 February 2001)

Programme

*Friday, 23 February 2001*

Chairman: N. Cabibbo

- 9:00 – 9:15     *Welcome*  
(N. Cabibbo)
- 9:15 – 9:25     *General Introduction*  
(M. Sánchez Sorondo)
- 9:25 – 9:30     *Greetings*  
(M. Sela)
- 9:30 – 9:40     *A Tale by Carlos Chagas Filho*  
(A.M. Chagas)
- 9:40 – 10:10   *The Main Lines of Multi-disciplinary Research Implanted by  
Carlos Chagas Filho at the Institute of Biophysics*  
(D. de Almeida)
- 10:20 – 10:50   *The Contribution of Cell Biology for a Better  
Understanding of Chagas' Disease*  
W. de Souza

11:00- 11:30 Coffee-Break

Chairman: W. Arber

11:30- 12:00 *Science and Solidarity*  
(P. Germain)

12:10 – 12:40 *L'homme de science et la société actuelle*  
(A. Blanc-Lapierre)

12:50 – 14:30 Lunch

Chairman: A. Rich

14:30 –15:10 *The Meaning of Biological Diversity: What Should We Do?*  
(P.H. Raven)

15:10 - 15:50 *Genetic Modification of Plants*  
(M.F. Singer)

15:50 – 16:30 Coffee-Break

16:30 – 17:10 *Molecular Evolution: Comparison of Natural  
and Engineered Genetic Variations*  
(W. Arber)

*Saturday, 24 February 2001*

Chairperson: M.F. Singer

9:00 – 9:30 *Chance and Necessity in the Origin and Evolution of Life*  
(C. de Duve)

9:40 – 10:10 *Challenges and Achievements in Infectious  
and Autoimmune Diseases*  
(M. Sela)

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10:20 – 10:50 *Chagas' Leadership in Science and in the Problem of Values*  
(R. Levi-Montalcini)

11:00 – 11:30 Photograph of Participants and Coffee-Break

Chairman: C. Pavan

11:30 – 12:00 *How to Find Better Drugs for the Treatment of AIDS*  
(P.A.J. Janssen)

12:10 – 12:40 *Protein Aggregates in the Brain:  
Cause of Neurodegeneration and Dementias*  
(M.F. Perutz)

12:50 – 14:50 Lunch

Chairman: P.H. Raven

15:00 – 15:30 *A Role for Left-Handed Double Stranded  
Nucleic Acid Helices in a Right-Handed World*  
(A. Rich)

15:40 – 16:10 *Society, Genetics, and the Future of Our Species*  
(C. Pavan)

16:20 – 16:50 Coffee-Break

16:50 – 17:30 General Discussion

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## GENERAL INTRODUCTION

I would like to join the President in extending to you my best greetings and a very warm welcome to the Pontifical Academy of Sciences. It would be superfluous to observe how important science is for contemporary man. Indeed, it is evident that today man lives within conditions powerfully shaped by science and technology, as is well borne out by the recent discoveries relating to man's genetic structure and by the mapping of the genome. Therefore, without entering into an analysis of the questions, issues and problems which science raises for contemporary man (which will be examined and discussed by the various distinguished speakers during this symposium), I would like once again to observe that science cannot but be at the service of man. This is something which is very easily understood: as science is a product of man, it must not be turned against its creator but must increasingly act to humanise man and all men. There can be no doubt that President Chagas strove during his long presidency of this Pontifical Academy of Sciences to ensure that this institution was not only a centre of research but also a centre of action for the cause of man and above all for the cause of developing countries, an approach, one can well understand, encouraged by the Pontiffs that Professor Chagas so devotedly served - Pope Paul VI and John Paul II.

I am very happy that the subject addressed by this symposium - 'the challenges of sciences' - will be discussed and examined in great detail and with great expertise by the distinguished speakers who have agreed to take part. Some of these speakers, who come from Brazil, such as Professor de Almeida and Professor de Souza, will tell us about the special and specific contribution to scientific advance made by Carlos Chagas. As a citizen of Argentina, I am more than familiar with the malady named after the father of this former President of our Academy - 'Chagas' Disease' - which is common in the Pampas. And thus I am more than aware of how much the Chagas family and the Chagas Institute have done for the health and well-being of our peoples of South America. Professor Germain will then talk

about 'science and solidarity', a subject of great topical relevance, and Professor Blanc-Lapierre will give a paper on 'the man of science and contemporary society'. Of no lesser importance will be the contributions made by Professor Raven on 'biological diversity', and by Professor Singer on 'the genetic modification of plants'. Of great topical relevance will also be the papers given by Professor de Duve and Professor Sela on 'chance and necessity in the origin and evolution of life' and 'the challenges and achievements in infectious and autoimmune diseases'. Our symposium will then hear most instructive papers on the subjects of neurobiology, AIDS, and neurodegeneration, by Professor Levi-Montalcini, Professor Janssen, and Professor Perutz respectively, and will finish with contributions by Professor Rich on nucleic acid helices and by Professor Pavan on society, genetics and the future of our species.

Because of the importance of the subjects addressed, the prestige and eminence of the speakers, and the geographical provenance of those taking part in this symposium, we can well hope that this scholarly encounter will constitute a valuable contribution to scientific investigation at the service of man. Indeed, this symposium well expresses two of the principal aims of the Pontifical Academy of Sciences – the achievement of inter-disciplinary discussion (through the participation of scholars from different fields of study) and the promotion of inter-regional interchange (through the presence of experts from different geographical areas of the world). Finally, I would like to thank the organisers of this symposium, Professor Sela, Professor Pavan, and Margarita Chagas, for all their efforts and expertise in ensuring the success of this commemorative symposium, and express my gratitude to all the distinguished participants who, in accepting our invitation, have at times had to come from very distant lands.

MARCELO SÁNCHEZ SORONDO,  
Bishop-Chancellor of the Pontifical Academy of Sciences

## SPEECH OF GREETING

We are gathered here today to commemorate Carlos Chagas Filho, a great scientist, a great humanist and a towering moral figure. In honor of his memory this Symposium was organized, covering – on the one hand – various scientific disciplines, and – on the other hand – moral and philosophical problems either caused by science or which can be helped by science. Thus, the expression ‘challenges of sciences’ includes not only what we as scientists usually consider the latest results of the progress of science, but also the challenges provoked by science, in short – as is so well said in french ‘les defits de la science’.

To me personally Carlos Chagas Filho was a great friend whom I had the privilege and pleasure of meeting in Israel in October 1963, and as a result of this visit he invited me to give a course in 1964 at his institute of biophysics in Rio de Janeiro. After that we met often, in Rio, Paris, Boston and – first and foremost – here in the Pontifical Academy of Sciences. We attended together many of the splendid study weeks, and I remember especially the study week we organized jointly on the role of non-specific immunity in the cure and prevention of cancer.

Carlos Chagas was enormously interested in all areas of science, but especially in those aspects which were of crucial importance to the progress of humanity. At the same time he was totally committed to the development of science in Brazil, and I am sure he would be exceedingly proud of the Brazilian achievement of sequencing the complete genome of the bacterium *Xylella fastidiosa*.

The great achievement of Carlos Chagas Filho during his sixteen years at the helm of our Academy will never be forgotten. I would like to express my gratitude to the speakers at this memorial Symposium who have so enthusiastically accepted our invitation. I wish all a most successful and intellectually stimulating symposium.

MICHAEL SELA

## INTRODUCTION

This tale by Carlos Chagas was sent to his family shortly after his death in February 2000. It was written in 1984 for a young daughter of one of his colleagues of the Brazilian Academy. The child, whose name is Taciana, probably reminded him of his own only direct niece, Tatiana, as he wrote in the tale. His daughter Ana Margarida Chagas Bovet translated it into English, French and Italian for the Symposium “Challenges of Science” held on his behalf at the Pontifical Academy of Sciences one year after his death.

The scientists and philosophers mentioned in the tale are referred to with fictitious names. The real names can be found in the notes.

ANA M. CHAGAS

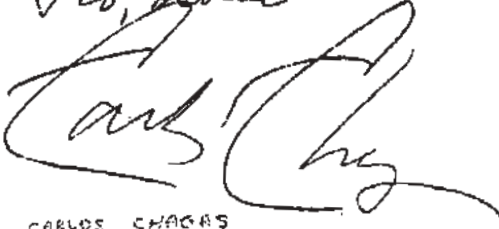








Este Tarciano é o filho de Antônio. Ele lhe dá a "confidência"  
 e a palavra para sua Omelete e assim, em três, pois  
 o Sabedão he de favorecer entre as "omelas" e o  
 passar, substituindo ~~o~~ <sup>no substituído</sup> a flor a de orvalho,  
 no mês a omel, as cascadas a cantar e a.  
 baya-film a trieta-lo.

Rio, Janeiro 1984  

  
 CARLOS CHAGAS

## A TALE: THE TRANSLATION <sup>1</sup>

I don't know, Taciana, (the only niece I have, her parents changed the c into a t, so her name is written Tatiana), whether you are two, four, six or ten years old. It doesn't matter, for an old friend of mine, who by the way I didn't know, always used to tell me: "In each woman's heart there is a bit of a child" (in French, which was her mother tongue, it sounds much more beautiful),<sup>2</sup> so, even if you are 18 or 20, I shall tell you a story which I would have told to my first greatgrand-daughter (that I still don't have). I imagine that you – who have the beauty of the mother and the intelligence of the father – as Bandeira<sup>3</sup> would have said – will like this story.

It starts like all stories: "Once upon a time there was a very powerful man, very rich, and with names that may change from a region to another (Let's call him Lord, with capital L) I think the city in which he lived most of the year – for during the rest of the time he lived with the stars, the galaxies, the planets – was in Africa – could it be Donogo Tonga? – I don't think so. It was in an extraordinary oasis, in the middle of the Sahara. People who recall many ancient things say that one day he called his older son – perhaps his favourite one – who was called Knossos and told him: Take this shell, my son, and go out into the world. Everything you see and you will not know what it is, the shell will explain it to you. It will make you understand the mysteries that surround you.

Knossos departed. During his long way, the shell explained to him everything that he saw: what it was, and how was it formed, how it lived; birds, plants, wild animals, stones, everything. After a long walk, Knossos arrived at Alexopolis,<sup>4</sup> during a wonderful night, and the magical shell

<sup>1</sup> This manuscript was found by the family in May 2000 after the death of Carlos Chagas Filho, which took place on 16 February of the same year. The notes are not a part of the original text.

<sup>2</sup> Marceline Desbordes – Valmore (1786 – 1859)

<sup>3</sup> Manuel Bandeira (1886 – 1968)

<sup>4</sup> Alexandria

explained to him heaven, the stars and the planets. Knossos was enthusiastic and entered by chance into a big room full of books, where some old men were trying to explain heaven. They were very surprised with what Knossos told them, and many of their knowledge gaps were fulfilled. After some weeks, Knossos continued his trip. After many vicissitudes which escape our plot, Knossos met, near a place called Teparnon,<sup>5</sup> a person who attracted him immediately. It was a kind of teacher who gave lessons dialoguing with students – not lecturing but debating – (just think, Taciana how different it is today).

The friendship between Knossos and Aristogildo<sup>6</sup> was immediate. Knossos told him everything he knew and even lent him his shell for some years while returning to a well deserved rest at his father's home. I should have written Father. The teachings of the pair Knossos-Aristogildo started to spread immediately across the world, but were much criticized: they disturbed what the world considered the truth and, moreover, they were transformed by a group of imitators who were protected by important people called Chatterastics.

When Knossos knew about that, he returned. He searched and searched, and finally found who could arrange all things. It was a monk, Thomas, the son of the Count d'Axino,<sup>7</sup> to whom Knossos revealed everything. Thomas accepted but because Knossos didn't bring the shell, the only thing he could do was to publish books, one after the other. But, because the books were hand written, they had a limited circulation. They were not best-sellers. It didn't help much.

Therefore Knossos came back and remained a long while with an italianese called Liopardo<sup>8</sup> and later with an Englishman with whom he left the shell for one week. The Englishman was called Gil, and more precisely Gil Gilson,<sup>9</sup> who benefited from what he had learned and rediscovered the magnets. Then Knossos being tired, decided to retire, also because another Britton, Chico Bascone,<sup>10</sup> told him that he would have soon published a book in which he would summarize everything Knossos had learned. Before returning home, however, Knossos decided to visit Italy. He started

<sup>5</sup> Partenon

<sup>6</sup> Aristotle (384 – 322 b.c.)

<sup>7</sup> Aquinas (1225 – 1264)

<sup>8</sup> Leonardo da Vinci (1452 – 1519)

<sup>9</sup> William Gilbert (1544 – 1603)

<sup>10</sup> Sir Francis Bacon (1561 – 1626)

from Venice, what a marvel! He passed by Verona, when they told him that here took place one of the most beautiful love stories that had ever been. Then he went to Ferrara from where he left in a hurry because that was a land of many “paramours”; he passed through Assisi, where he cried because of the emotions he felt. Then he passed through Florence, that he loved very much, but which was disturbed by the sermons of an extremely radical priest,<sup>11</sup> and had to pass by Pisa, where he stayed for hours in order to understand if the tower would fall or not.

He was so tired that he decided to rest in the first open house he encountered. There he found a group of young people who were listening to, drinking I should say, the words of a man, whose eyes seemed like illuminated from within, and who was very nice. The latter was right away friendly with him, and housed him. They became friends and, when he left the house of Gasparelo Gaspareli,<sup>12</sup> because this was the name of the host, Knossos left him the shell. Gasparelo Gaspareli did as it had been done before and saw the sky in a different way, for the shell had taught him how telescopes were made, and he discovered so much that, from then on the knowledge that the shell provided men was multiplied, also because other men started to discover how shells were done.

Nelson, or better, Israel Nelson,<sup>13</sup> in Albion, Kapler,<sup>14</sup> in Austria, even got to think that every thing had been discovered. Hugolino<sup>15</sup> in Holland, and others, and with new shells, new knowledges were made. Remi Desoutez,<sup>16</sup> a Frenchman who once lived in the Hague, saw that there was such a confusion that he tried, through his “Method” to use the shells, to bring some order in their utilization. But there was no way to stop it: shells after shells, first in Europe, then in America were discovered daily, and new shells, ever more complex, were invented.

Meanwhile, Knossos was quietly resting in his palace, waiting for the Lord, convinced that he had really fulfilled his mission. But two days after the arrival of the Lord, the palace was shattered by a tremendous explosion. The Lord said to Knossos: This is, my son, the result of the multiplication of the shells. So I have acted in a wrong way, my Lord! said Knossos. No, I

<sup>11</sup> Girolamo Savonarola (1452 – 1498)

<sup>12</sup> Galileo Galilei (1564 – 1642)

<sup>13</sup> Isaac Newton (1642 – 1727)

<sup>14</sup> Johannes Keplero (1571 – 1630)

<sup>15</sup> Christiaan Huygens (1629 – 1696)

<sup>16</sup> Rene' Descartes (1596 – 1659)

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knew this was going to happen, the Lord answered, and this is why I sent, without your knowing it, my twins, Ethos and Eros, because I knew that the uncontrolled development of the shells would bring, and perhaps will still bring about, the destruction of plants, animals, houses. But more seriously still, is the fact that neighbours will not speak to one another. This is why I asked Ethos and Eros to follow you. Ethos spoke much but few people heard him, but a day will come when he will be heard. Eros, even if he taught many silly things after the shell invented the TV, left a deep mark in everyone. This is why, when people who heard Ethos will get closer to those who have heard Eros, everything will calm down.

Also because I, who am not a fool, have made someone be born in Galilee, someone who, in spite of having appeared on earth in a stable, brings a message of love which will overcome the bad use of the shells men do with the shells they have invented.

This is, Taciana, the end of the story. It will give you the necessary peace and the poetry that a child and a girl must have, because wisdom will prevail over the “shells”, and birds will continue to fulfill us with tenderness, rivers to run, waterfalls to sing, and humming-birds to imitate you.

Rio, February 1984

CARLOS CHAGAS FILHO





## SCIENTIFIC PAPERS



THE MAIN LINES OF MULTIDISCIPLINARY RESEARCH  
IMPLANTED AT THE INSTITUTE OF BIOPHYSICS BY  
CARLOS CHAGAS FILHO

DARCY F. DE ALMEIDA

To introduce the question of the implantation of science at the University of Rio de Janeiro I chose to offer you some not very well-known aspects of the routes followed by Chagas to face such challenge. For that purpose, I shall bring evidence from several sources, besides my own recollections from countless talks we had throughout the last fifty years of his life. I have arbitrarily established two phases to tell the story: (1) The starting point: a revolution from within; (2) A brief look at the first 25 years of the Institute of Biophysics.

*1. The starting point: a revolution from within*

A brief description of the academic and institutional life at the University of Rio de Janeiro in the 30s would seem quite fit as a background against which the initial actions take place. The characteristics of the XIX century and first two decades of the XX century still prevailed. The University existed exclusively as an assembly of professional charter-delivering schools. Internally, interchanges between the various chairs were not noticeable. Practically isolated from each other, every chair was regarded as personal property of its respective Professor, whose appointment depended on personal and/or political interests rather than on academic merits. Even though the title of University Professor carried with it a social prestige very useful for the private practice, the duties were strictly limited to magisterial classes, the only source of contact between the professor and his students. There was plenty of time left for the faculty to earn additional salaries, from alternative public and/or private activities,

to make up for their small wages from the University. In short, a thing such as a professional scientist was undreamed of (Schwartzman, 1979). Therefore the implantation of scientific investigation at the University of Rio Medical School was indeed a challenge, and not a trivial one.

Being trained at the Oswaldo Cruz Institute, Chagas had made up his mind rather early in his career. He was 21 years old in 1931, when he graduated in Medicine. Admitted as assistant professor to the Department of Pathology, he was seemingly oriented towards the study of tropical diseases. But a conference at the Oswaldo Cruz Institute made an extraordinary impression on him. It was given by Fauré-Frémiet, professor of Comparative Embriogenesis at the Collège de France from 1928 to 1954. The subject was the “kinetics of development”, and the French professor showed that biological phenomena could not only be subjected to a mathematical treatment, but interpreted as well under the light of physical and chemical events. Chagas was, to use his own words, “*dazzled*” (Chagas Filho, 2000) by this approach and there and then decided to dedicate himself to it.

His father did not seem much disturbed when informed of that transmutation; only he recommended, before it was accomplished, that Chagas should spend a season at the hospital in Lassance, the small country town where the Chagas disease had been discovered. The intentions behind this paternal advice become clear when one hears from Chagas Filho that in Lassance he received the finishing touches of his medical education. There he acted not only as a physician, but also as pharmacist, confessor and, in general, a public authority. “*It was there that I learned really to know people (...) dealing with the patients, the simplicity and sincerity of those people feelings were of the utmost value to me*” (Chagas Filho, 2000). He was by then sure that the sciences were essential to help solving the problems posed by human diseases.

Back in Rio, events precipitated in rapid succession. While following complementary University courses on chemistry, physics and mathematics, in Manguinhos Chagas went through training in bacteriology and physiology; most importantly, he improved his physical chemistry knowledge under Carneiro Felipe. He was transferred in 1934 to the Department of Biological Physics, chaired by Prof. Rodrigues Pereira. Two years later, due to the untimely death of the department head, Chagas was appointed to the vacant chair, after the usual academic competition. Much earlier than he might have thought, since he was then 27 years old, the opportunity came for Chagas to start on the route to make his dream come

true. In the opening address to the Medical School class, not surprisingly dedicated to Carneiro Felipe, he insisted once more on the importance of the physical and physical-chemical interpretation of biological phenomena (Chagas Filho, 1938).

Aware of the importance of modern research techniques, and intent on extending his own scientific experience, in 1938 he worked in Paris, with René and Sabine Wurmser, at the laboratory of Physical-Chemical Biology and with Denise and Alfred Fessard, at the laboratory of electrophysiology of the Institut Marey. In England, he was received by A.V. Hill, at the University College Department of Biophysics, in London, and by lord Adrian, at the University of Cambridge Laboratory of Physiology.

Upon his return, in the address to the medical students, on August 8, 1938, he expressed his convictions: "*The introduction of new physical and physical-chemical techniques has propitiated the upsurge of a series of investigations that open new horizons for medicine and often modify generally accepted concepts*" (Chagas, 1940).

Meanwhile, a new Constitution had been proclaimed in 1937 by the Vargas government, which suppressed the often used double appointment in the public service. On account of the small salaries paid by the University, as mentioned before, the whole faculty of the Biological Physics chair left, with a single exception. Chagas looked at multiple quarters for help from idealistic people like himself. Tito E. Leme Lopes, his friend and colleague throughout the Medical School years, was the first to come. There followed two assistants, J. Moura Gonçalves and J.B. Veiga Salles, from the Department of Biochemistry in Minas Gerais, indicated by Baeta Viana, who had trained what was at the time perhaps the best group of biochemists in the country. Others, like M. Frota Moreira and H. Martins Ferreira, were locally recruited, from the University of Rio. This is a proper occasion to pay a tribute of praise to Guilherme Guinle, patron of the Institute of Biophysics, for the support that the Laboratory and later the Institute received from him. During the difficult initial years he was the main provider of funds for the experimental work and for the salaries of most of the staff.

A number of strategic innovations which might be called *patterns of development* were adopted by Chagas to achieve the implantation of scientific research in the University. An example of this kind is found as early as 1938, when he created the "Laboratory of Biophysics", defined as "*the research section of the Biological Physics chair (...) to make the experimental method accessible to the medical students and to make biologists and*

*technicians acquainted with the new physical methods used in the study of vital processes*" (Chagas Filho, 1942). Without any formality at all, Chagas institutes an activity which, in spite of being physically superposed on the traditional chair, possesses an identity of its own. That was a typical gambit played by Chagas, the first one in a series of creative innovations which strategically avoided conflicts with ingrained bureaucratic procedures. It is quite possible that the simple substitution of the designation of *laboratory* for *chair* would have faced, at that time, a strong opposition. Chagas had started what I chose to call *a revolution from within*.

In fact, to describe the Laboratory of Biophysics, Chagas cautiously states that *"its organization is dependent on the scientific activity there developed, and tries to answer the questions posed by problems arising in the course of experimental work"* (Chagas Filho, 1942). This rather vague declaration is quite in line with his usual saying, later on, that *"Biophysics is everything we do in the lab"*. Altogether they were about half a dozen local people, plus Sabine and René Wurmser, who had been taken in by Chagas as war refugees. Possibly there were no facilities for a more comfortable arrangement, such as independent individual laboratories.

However, it would appear as possible that, beyond the initial limitations, Chagas might also have found some inspiration in Bernal. Indeed, analysing the role of flexibility on the strategy of scientific advance, one reads from Bernal: *"Nothing could be more fatal to science than rigid adherence to a plan laid down beforehand (...). Perhaps a five- or ten-year scheme for the whole of science and shorter schemes for individual sciences would be workable (...) and provision would have to be made for changes, as at any moment (...) new integrating discoveries might (...) demand a complete recasting of pre-existing schemes"* (Bernal, 1939). I submit that these thoughts and propositions were not unknown to Chagas since in 1946, on the occasion of the First Joint Meeting of Biological Societies, in São Paulo (Chagas Filho, 1947), he chose to quote, from that same source: *"(...) the biophysicists' task is only just beginning. All the new methods of examination of the structure and changes of matter – electron microscopes, X-ray analysis, ultra violet and polarizing microscopes, thermal, electric, and acoustic detectors – require to be put in the service of biology and used by men who understand the significance of their finds both physically and biologically"* (Bernal, 1939). The profusion of quotes in documents produced during the few years antedating the creation of the Institute of Biophysics strongly indicates that Bernal's ideas on science planning were contemplated by Chagas at that time.

Two main research lines were soon established. First, the choice of the *Electrophorus electricus* as a research model organism was considered as quite appropriate for “a multiplicity of research lines, besides (offering) the opportunity of a multidisciplinary investigation (of bioelectrogenesis), which is the surest way for scientific development” (Chagas Filho, 2000). The choice of a common research model for the Institute had a predominant role, particularly in the early years, in propitiating the set up of a closely united group. Everybody had an interest in everybody else’s work. That is probably the reason why the basic training of newly arrived students passed invariably through the study of bioelectrogenesis.

Second, in line with the familial scientific tradition, Chagas had planned to study the infection caused by the *Trypanosoma cruzi*. A laboratory was built for that purpose and acting upon his brother’s advice Chagas invited Hertha Meyer, a German refugee, then working in the Rockefeller Foundation-sponsored Laboratory of Yellow Fever, in Manguinhos, to take the lead. She used the tissue culture technique to investigate “the protozoan morphology, its evolution in the tissues and the cell changes it might cause” (H. Meyer, 1943).

The 1942 publication classifies the activities of the Laboratory of Biophysics in groups of studies. No mention is yet made of specific laboratories, which indicates that the Laboratory of Biophysics remained a common space for research within the chair of Biological Physics.

The same organizational picture is portrayed in Chagas’s “Comments on Biophysics”, published the following year (Chagas Filho, 1943). While dealing with the extremely close relationship between the basic sciences, he provides an outline of Biophysics through a brief review of some of the main advances in the field, based on their respective experimental approaches. Once more he quotes Bernal: “The great value of this approach as against that of either the older histologists or the biochemists is that with refinement of technique it is easier to approximate to the detailed study of the mechanism of an intact animal or plant” (Bernal, 1939).

## 2. A brief look at the first 25 years of the Institute of Biophysics

Profound changes in the existing organization were brought by the creation of the Institute on December 17, 1945. The first article of its bylaws reads: “the Institute aims at the investigation in Biophysics and the cooperation in the teaching of Biology, Chemical and Medical Physics, and in the development of cultural, scientific and technical activities of the University



of *Brazil*". The scientific investigation comes explicitly to the forefront, and teaching is regarded as a research-derived activity. This was, with no doubt, a history-making pattern of development for the Brazilian University. It is furthermore possible to identify in the text a suggestion of multidisciplinary activities, through the integration with the university's multiple areas of study.

The *First Memory on the Institute of Biophysics*, published in 1948, is a most revealing document for the early history of the Institute. It shows that four Research Divisions, with their specific equipments and heads, had been created. They were the Divisions of Biological Physical Chemistry (J. Moura Gonçalves), Medical Physics and Radiobiology (M. Frota Moreira), Cell Biophysics (A.M. Couceiro and H. Meyer), and Electrobiophysics (A.A.P. Leão). These were then the main lines of research implanted by Chagas at the Institute of Biophysics. The study of the electric activity of the brain cortex had been quite recently added, under the direction of A.A.P. Leão, the discoverer of the cortical spreading depression, back in Rio after obtaining in 1944 his Ph.D. from Harvard University.

The faculty as a whole included ten graduate researchers – seven of which figured already in the 1942 document – and every Division contained undergraduate students (graduate courses did not exist until the early sixties).

Last, but most importantly, every one of the Divisions heads were under contract as a peculiarly named, unheard-of class, the "technical specialist".

Each one of these characteristics contains a pattern of development and deserves a comment. First, a University Institute primarily devoted to research is officially recognized.

The second is the strategy, successfully tried at the University of São Paulo, of regularly inviting foreign scientists to visit the Institute, which prevailed from the very beginning. Sometimes the visitors stayed for a few weeks or months, during which time they would work assisted by a chosen undergraduate student. The visits were reciprocated during each side respective summer, for several semesters. A most important result, not always duly stressed, derived from such strategy. Often it originated new laboratories which, in due time, developed into Departments of the Institute. Examples illustrating this point, together with the respective foreign visitor and the student associated to him, are: Radiobiology, with R. Latarjet and L.R. Caldas; Neurobiology, with Denise and Alfred Fessard and C.E. Rocha-Miranda and E. Oswaldo-Cruz; Radioisotopes, with J.D. Cooper and the graduate student E. Penna Franca; Heart

Electrophysiology, with B. Hoffman and A.P. de Carvalho; Electron Microscopy and Cell Ultrastructure, with H. Meyer (already a permanent member) and W. de Souza.

Third, as for the position of technical specialist, created by the public service authorities under Chagas inspiration, its real purpose was to provide a more becoming salary for recognizably competent people recruited for research work. At hindsight, this seems to have been an inventive temporary device to maintain the senior researcher while the full time regime did not come into practice.

Scholars studying the history of the Institute compared the initial group to a “family” (Mariani, 1978; Schwartzman, 1982). Outside observers and several members of the Institute of Biophysics associated this solidarity to the idea of a “*esprit de corps*” (Schwartzman, 1979).

At the Institute’s 25th anniversary, its structure had taken a more conventional shape. There were four Departments, but it is still possible to trace their origin to the pre-1945 informal Divisions. The total count of laboratories went up to 26, in 1971. Today, scientific interests are considerably expanded: the Institute includes six Programs and a total of 39 laboratories.

Finally, I selected what I consider three landmarks of the work carried out at the Institute during its first 25 years. The first, in the early fifties, was the application of the modern microelectrode technique to the electroplate, which served to show the production of a sodium-dependent action potential on its innervated side (Keynes and Martins-Ferreira, 1953). Besides, Keynes visit in 1952 showed that this pattern of development could also function as a two-way road. According to Hogkin (1992), this work generated two by-products: one, the successful use of the electric organ as a source of material for the determination of the amino-acid sequence of the sodium channel and two, the development of Keynes’s interest in South America, which led to his writings on the voyage of the Beagle and his discovery of the drawings by Darwin’s artist Conrad Martens (Keynes, 1979; Hodgkin, 1992).

The second one has more to do with internal affairs of science administration, but it has been chosen because it was fundamental for the Institute survival, at the end of the sixties. E. Penna-Franca, then director of the Institute, obtained funding from a most unusual source – the Brazilian National Bank for Economic Development – thereby changing the face of biomedical sciences in the country. The contract provided the means of a thorough updating of the Institute’s equipment,

there included, most notably, a modern Unit of Electron Microscopy and the introduction of the PDP-12 (LINC) computer into the research work.

To describe the third, I take the words of Rita Levi-Montalcini, guest of the Institute between September 1952 and January 1953, on the history of the nerve growth factor discovery. *“The finding that (...) extraembryonic transplants elicited the same effects as intraembryonic grafts gave definite evidence for the diffusible nature of the tumor nerve growth-promoting factor. (...) I then thought of resorting to the tissue culture technique (...) Lack of facilities in this field at (...) Washington University prompted me to ask hospitality from Professor Carlos Chagas, director of the Biophysics Institute (...) There, a friend of mine, Hertha Meyer, had built and was director of a most efficient tissue culture unit. (...) The tumor had given a first hint of its existence in St. Louis but it was in Rio de Janeiro that it revealed itself, and it did so in a theatrical and grand way, as if spurred by the bright atmosphere of that explosive and exuberant manifestation of life that is the Carnival in Rio.”* (Levi-Montalcini, 1987).

It is difficult, perhaps impossible, to single out an efficient cause to explain why the implantation of research in the University was a successful project, but I would suggest that it took essentially a group of people who could individually repeat the saying many times uttered by Chagas: *“I have got the science under my skin”*.

# THE CONTRIBUTION OF CELL BIOLOGY FOR A BETTER UNDERSTANDING OF CHAGAS' DISEASE

WANDERLEY DE SOUZA

First I wish to thank the organizers of the symposium to invite me to be here today, and to congratulate the Pontifician Academy of Sciences for the initiative to organize this symposium as a tribute to the memory of its past President Carlos Chagas Filho. Since 1970, when I was 18 years old and started my scientific career at the Instituto de Biofisica Carlos Chagas Filho under the supervision of Hertha Meyer, I had the privilege to be in permanent contact and under the positive influence of Professor Carlos Chagas Filho. I still remember when I showed him the first micrographs where the sub-pellicular microtubules of the protozoan *Toxoplasma gondii* could be seen. He became so enthusiastic with the results and decided to present them in the French Academy of Sciences. The paper, which was my first one, was published in 1972 in the Comptes Rendues de la Academie de Sciences

**Table I**

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**TROPICAL PARASITIC DISEASES-WHO / 1999**

|                         |             |
|-------------------------|-------------|
| Infected people.....    | 500.000.000 |
| Dead per year .....     | 2.000.000   |
| Malaria.....            | 500.000.000 |
| Schistosomiasis.....    | 200.000.000 |
| Filariasis .....        | 120.000.000 |
| Amebiasis .....         | 16.000.000  |
| Leishmaniasis .....     | 15.500.000  |
| Chagas disease .....    | 14.000.000  |
| Sleepness disease ..... | 3.000.000   |

de Paris (1). Figure 1 (see page III) illustrates one important moment of my career when I was introduced in 1984 as Member of the Brazilian Academy of Sciences and received the diploma from the hands of Professor Carlos Chagas Filho, at that time President of the Pontifician Academy of Sciences.

In the last 30 years I have been involved in the study of parasitic diseases from a cell biology perspective. Tropical Parasitic Diseases still constitute one of the most important public health problem in the world affecting about 500 millions of people, according to information from the WHO (Table I).

Chagas Disease, caused by the protozoan *Trypanosoma cruzi* discovered in 1909 by Carlos Chagas, father of Carlos Chagas Filho, is the most important parasitic disease in large areas of South and Central America (Fig. 2, see page III), affecting about 14 million people, with about 100 million people remaining at risk.

Important health programs have been established in the last years aiming to control transmission of the parasites from the insects to man as well as from man to man during blood transfusion as exemplified in Figure 3 (see page IV) and Table II.

These data could led us to suggest complete elimination of new infections caused by *T. cruzi* in the next years. However, recent studies using isoenzyme and riboprinting analysis, rRNA promoter activity, sequence of mini-exon genes and microsatellite markers have provided clear evidences that *T. cruzi* is not a single species but it corresponds to two highly diver-

**Table II: Human Infection by *Trypanosoma cruzi* and reduction of incidence Southern Cone Initiative,**

| Country   | Age Group<br>(Years) | 1983-1999                             |                                       | Reduction of<br>Incidence<br>(%) |
|-----------|----------------------|---------------------------------------|---------------------------------------|----------------------------------|
|           |                      | Infection in<br>1983<br>(Rates x 100) | Infection in<br>1999<br>(Rates x 100) |                                  |
| Argentina | 18                   | 4.5                                   | 1.2                                   | 85.0                             |
| Brazil    | 7-14                 | 18.5                                  | 0.17                                  | 96.0                             |
| Bolivia   | 1-4                  | 33.9                                  | ND                                    | ND                               |
| Chile     | 0-10                 | 5.4                                   | 0.14                                  | 99.0                             |
| Paraguay  | 18                   | 9.3                                   | 3.9                                   | 60.0                             |
| Uruguay   | 6-12                 | 2.5                                   | 0.06                                  | 99.0                             |

gent genetic subgroups, designated as lineages 1 and 2 (2). Lineages 1 predominates in the domestic cycle while lineage 2 is mainly represented in the sylvatic cycle. Both are potentially pathogenic for man. It is important to point out that although at present there is no transmission of Chagas disease in places like the Rio de Janeiro state 100% of the small primitive monkeys known as gold lion tamarins and 84 % of the opossums found in a residual forest localized 80 km from the city of Rio de Janeiro are infected with *T. cruzi* (3). Therefore, it is important to intensify basic work on the biology of the parasite in order to develop new strategies of disease control.

From the cell biology point of view *T. cruzi* is a very interesting model to study processes such as reversible differentiation or cell transformation, mechanisms of cell invasion, etc. The central developmental stage is the trypomastigote form (Fig. 4). The basic structure of the protozoan, which is



Fig. 4 General aspect of the trypomastigote form of *T. cruzi* as seen with the high voltage electron microscope. K. Kinetoplast.

common to most of the members of the Trypanosomatidae family, is schematically shown in Figure 5. Some characteristic features deserve a few comments (4). The protozoan is an asymmetric and polarized cell where at least three surface domains exist: 1) the cell body, 2) the flagellum, and 3) the flagellar pocket, a specialized region formed due to an invagination of the plasma membrane lining the cell body, which is continuous with the flagellum. The flagellum establishes contact with the cell body through a special type of junction. The coupling of the flagellum with the cell body is so strong that when the flagellum moves the body also seems to move, giving the impression of the existence of an undulating membrane. At the base of the flagellum there is the kinetoplast, a structure which contains up to 30 % of the total cell DNA and formed by DNA arranged as cocatenated

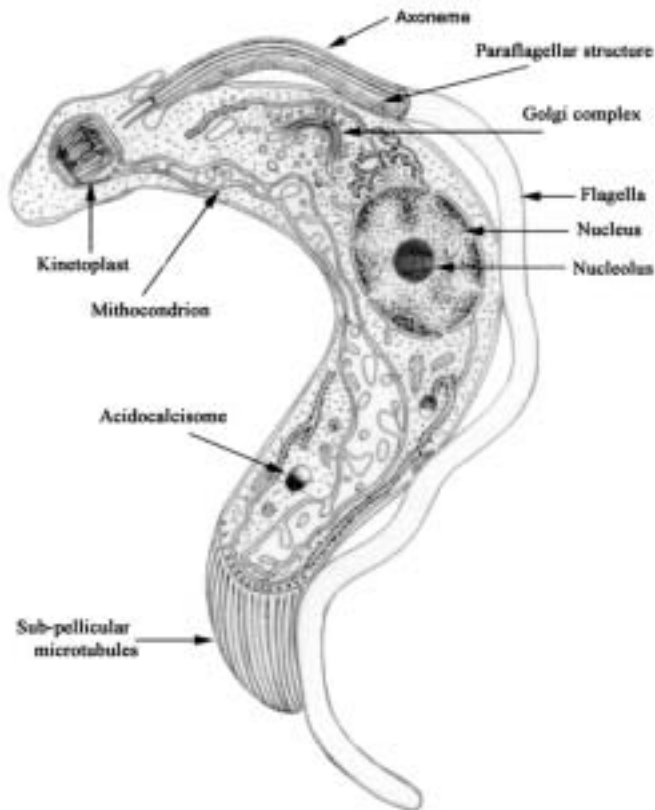


Fig. 5 Schematic view of the trypomastigote form of *T. cruzi*



Fig. 6 Deep-etching view of the association of sub-pellicular microtubules to each other (white arrow) and the plasma membrane (asterisk).

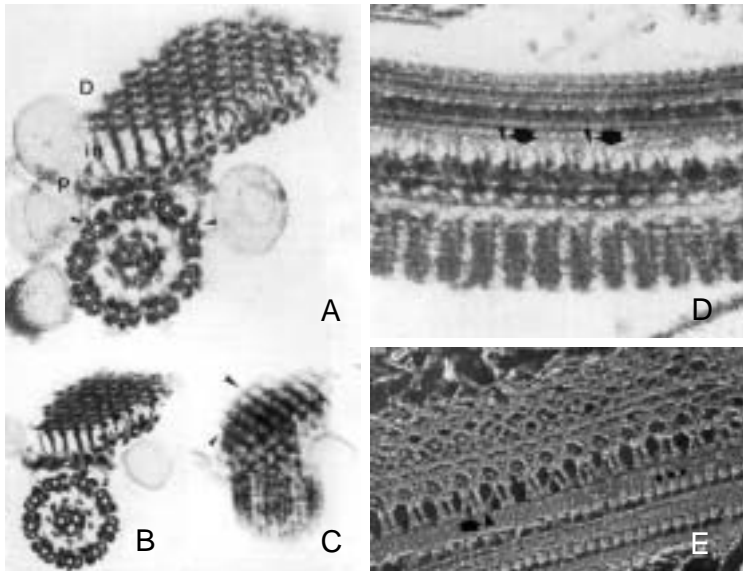


Fig. 7 General view of the flagellar axoneme and its association with a network of filaments which make the paraflagellar structure.



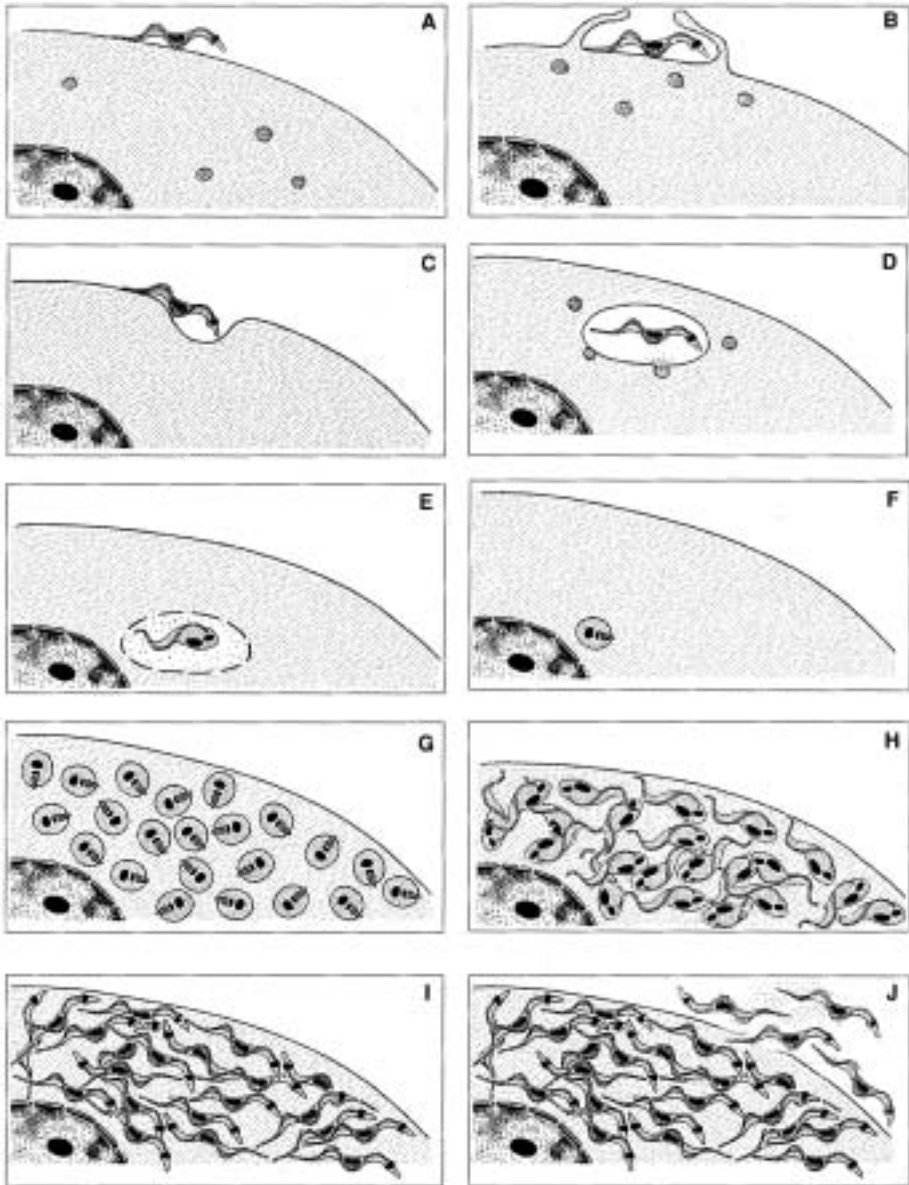


Fig. 9 Schematic view of the process of interaction of *T. cruzi* with host cells.

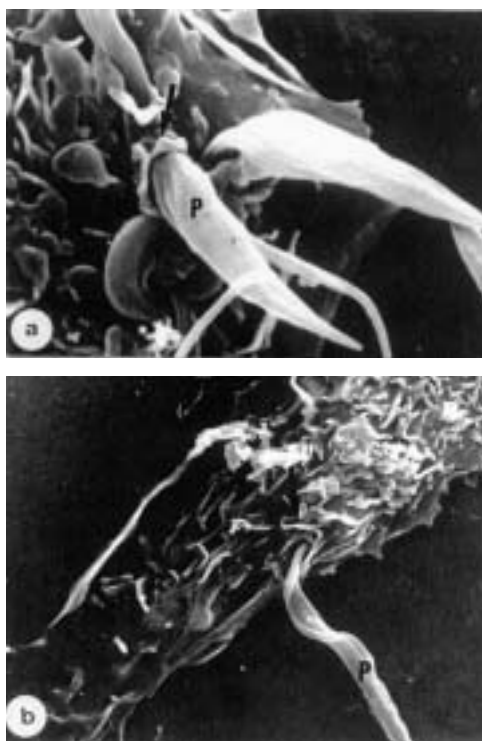


Fig. 10 Scanning electron microscopy of the process of interaction of *T. cruzi* with macrophages.

minicircles and maxicircles located within the single mitochondrion. Below the plasma membrane there is a layer of microtubules, known as sub-pellicular microtubules, connected to each other and to the plasma membrane via small bridges (Fig. 6). Two special organelles are observed in all trypanosomatids: (a) the glycosome, a special type of peroxisome, which contains the glycolytic enzymes, which has been characterized in detail by Fred Opperdoes and co-workers in Belgium (5), and (b) the acidocalcisome, which is an acidic organelle involved in the uptake of calcium, thus helping in the regulation of the intracellular concentration of this ion (Review in 6). The flagellum of the trypanosomatids exhibits, in addition to the axoneme, a complex array of filaments that form the paraxial structure (Fig. 7) whose function is still not completely defined. Recent studies implicate its role in the flagellar movement. The paraxial structure is formed by a complex

array of 25 and 70 nm thick filaments made of two major proteins of 69 and 80 kDa (7-8).

The life cycle of *Trypanosoma cruzi* starts with the ingestion of trypomastigote forms, found in the blood of the vertebrate host, during biting by insects of the Reduviidae family (Fig. 8, see page IV). Following blood meal the insect dramatically increase its size and weight. In the stomach the long trypomastigote forms transform into rounded, flagellated forms known as spheromastigotes (Reviews in 9-10). These forms migrate to the intestine and transform into short and long epimastigote forms. Subsequently, intense division of the epimastigote forms takes place in the intestine and later on they transform into infective trypomastigote forms, also known as metacyclic forms, which are released through the feces and urine of the insect during its bloodmeal in vertebrates, including the man. The parasites deposited on the



Fig. 11. Transmission electron microscopy of a thin section showing a trypomastigote form of *T. cruzi* within a parasitophorous vacuole whose membrane is in process of desintegration (arrows).

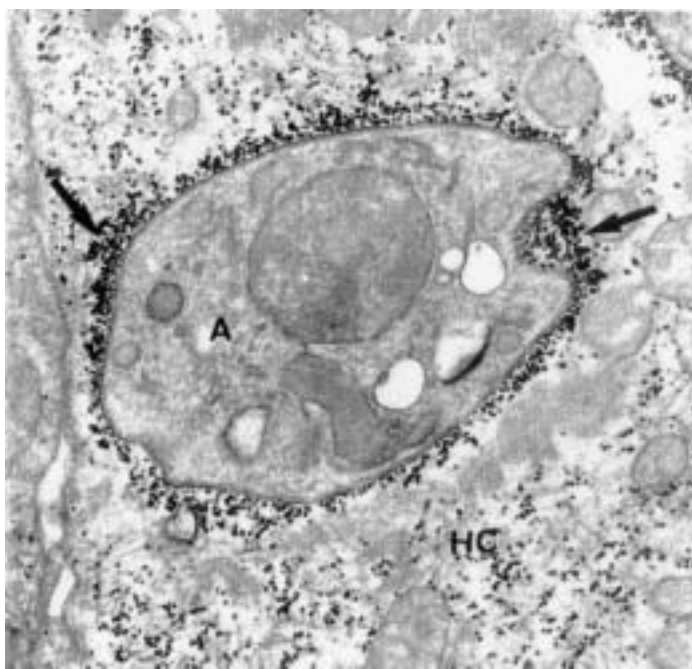


Fig. 12. Amastigote form of *T. cruzi* (A) in direct contact with components of the host cell cytoplasm (HC). The arrows indicate the presence of glycogen particles.

skin of the vertebrate host may have access to the host tissues if small skin lesions exist or are formed during insect biting, and then enter in contact with the surface of host cells (Fig. 9) such as macrophages, muscle cells, neurons and fibroblasts. Such contact involves a process of cell-to-cell recognition event. Studies carried out in the last years have shown that sialic acid-containing macromolecules found on the surface of the host cells are involved in the recognition process (11). In addition, a large number of parasite surface molecules play a fundamental role. Among them I would like to emphasize the importance of a family of proteins known as trans-sialidase which has a dual function since they simultaneously express neuraminidase and sialyl transferase activities (Reviews in 12-14). Using not yet clarified mechanisms these molecules may interfere with the level of sialylation of surface-exposed molecules found both on the parasite and on the host cell. Once attached to host cell surface the infective trypomastigote form is either ingested through

a typical phagocytic process (Fig. 10) or induces an endocytic activity of the host cell in a process that involves calcium release, protein phosphorylation, and lysosomal migration (Reviews in 15-17). Both processes correspond to an endocytic event with formation of a parasitophorous vacuole (Fig. 11). Next, the parasite changes its form to a rounded one (amastigote), with the concomitant disruption of the membrane lining the vacuole (Fig. 11) due to release of enzymes. Then the parasite enters in direct contact with the cytoplasmic structures of the host cell (Fig. 12) interacting with cytoskeletal elements such as microtubules and microfilaments (Fig. 13, see page V). After about 35 hours the amastigote forms start to divide, with a generation time of

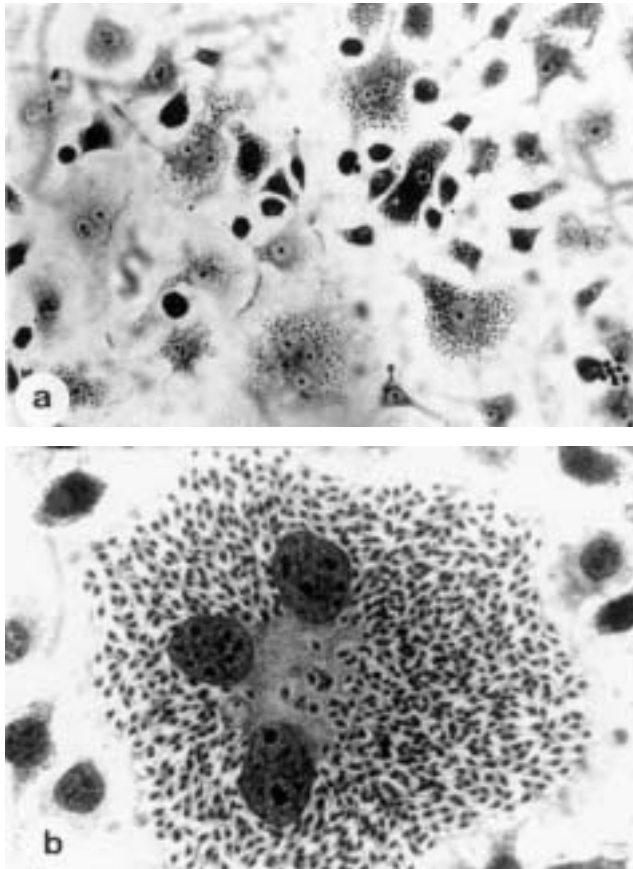


Fig. 14. Light microscopy of cells infected with *T. cruzi*.

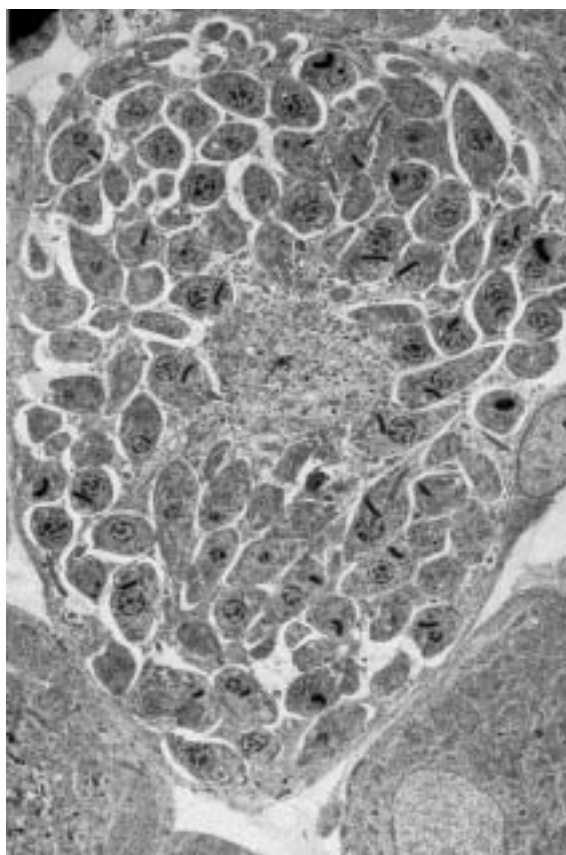


Fig. 15. General aspect of a neuron infected in vitro with *Trypanosoma cruzi*. Most of the cytoplasm of the cell is occupied by intracellular parasites. (Courtesy of H. Meyer.)

about 14 hours. After about 5 days the host cell is completely filled with amastigote forms (Figs. 14-15), which then starts a gradual process of transformation into trypomastigotes. Due to the intense movement of the intracellular parasites there is a rupture of the host cell and release of hundreds of trypomastigote forms into the intercellular space, from where they infect neighboring cells or reach the bloodstream and infect other tissues such as heart, skeletal muscle and the nervous system.

From the cell biology point of view *T. cruzi* is also very interesting. The analysis of the mechanisms used by the parasite to ingest macromolecules



Fig. 16. The section of an epimastigote form of *T. cruzi* showing the kinetoplast (K), two basal bodies(b), and the region of the attachment of the flagellum (F) to the cell body (arrows). An oblique section through the cystostome (C) is also seen.

from the medium and how it regulates the biosynthesis of sterols have provided information which is opening possibilities to develop new alternatives for the chemotherapy of Chagas disease. Biochemical studies have shown that *T. cruzi* is not able to synthesize cholesterol (18). However, it incorporates this important molecule as part of the LDL complex through a highly polarized endocytic process which takes place at the cystostome (19), a highly organized region of the protozoan surface (20) (Figs. 16-18). In the absence of LDL in the medium the parasites synthesize ergosterol which is subsequently incorporated into the cell membranes. This observation opened the possibility to use inhibitors of the ergosterol biosynthesis, largely employed against fungi, to kill *T. cruzi*. In collaboration with Julio



Fig. 17. Freeze-fracture view of the membrane lining the cell body (B), the flagellum (F) and the cystostome (C) of *T. cruzi*.

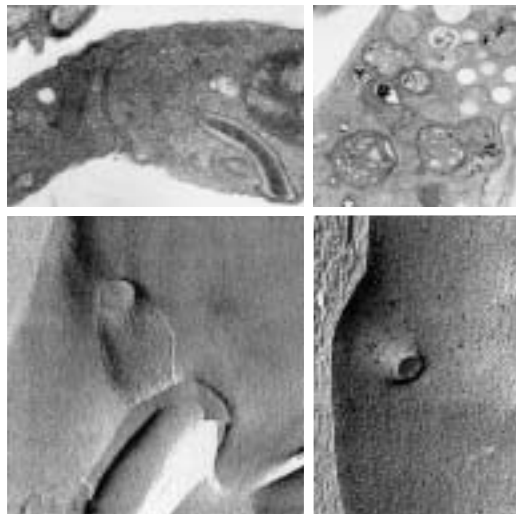


Fig. 18. Different views of the endocytic system of the epimastigote form of *T. cruzi*. A and B are thin sections of cells incubated in the presence of gold-labeled transferrin C and D show the cystostome.



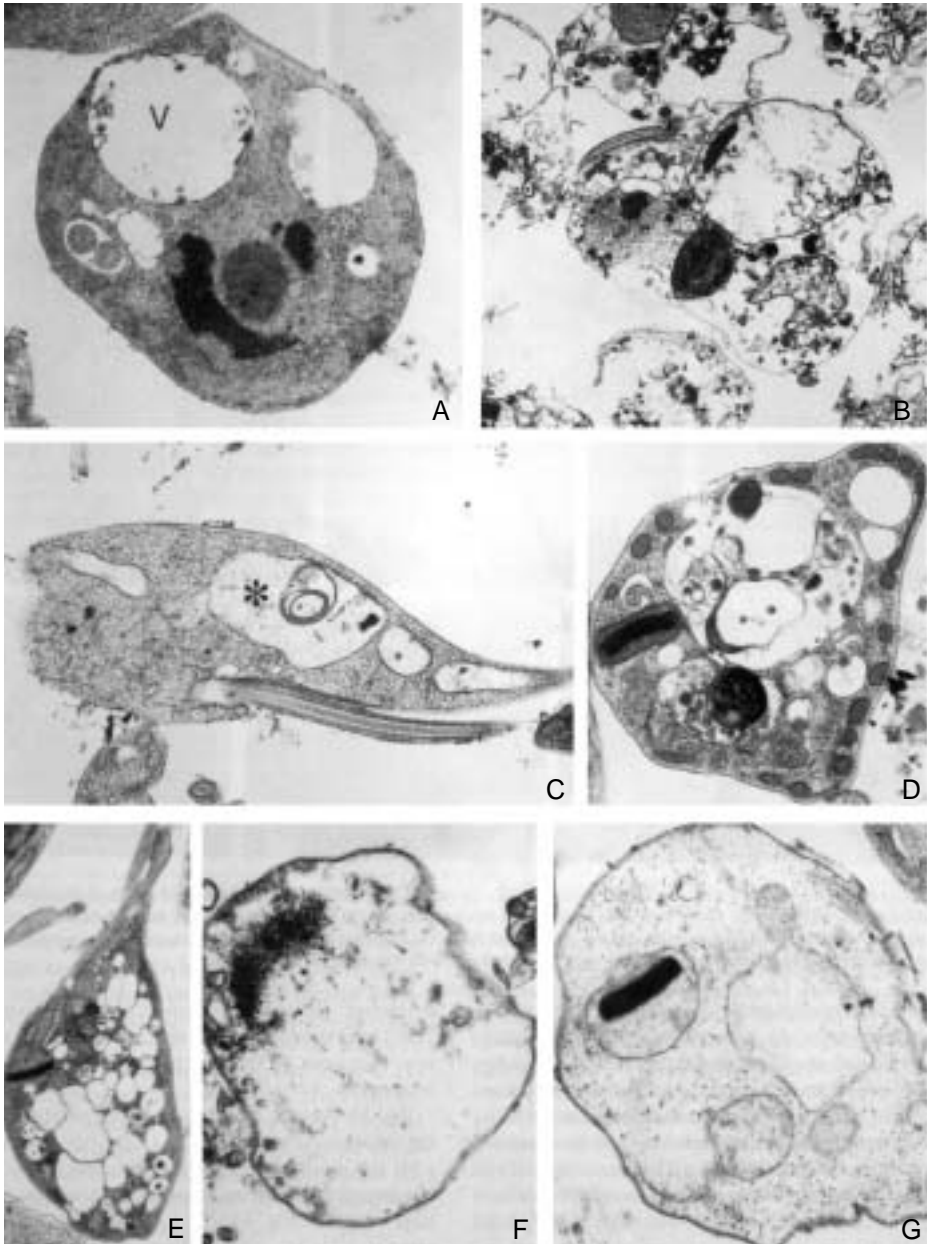


Fig. 19. Different views of *T. cruzi* incubated in the presence of an inhibitor of the biosynthesis of ergosterol. The parasite is completely desintegrated.

Urbina and colleagues in Venezuela we have tested several of such compounds (21-22). The results we have obtained confirmed this assumption and show that some drugs are highly active, efficiently killing the parasite (Fig. 19) and are now in clinical trials.

I would like to conclude saying that much need to be done on the study of basic aspects of the cell biology of *T. cruzi* in order to identify new parasite targets for more specific chemotherapy and for the development of a vaccine.

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# SCIENCES AND SOLIDARITY

PAUL GERMAIN

## 1. *The explosive expansion of the sciences*

It is not necessary to express at length the fantastic progress achieved by the scientific disciplines during the last century. All of them are today completely new, even those which already existed at its beginning. One may foresee that this expansion will continue and probably at an increasing speed. The twenty-first-century information sciences will allow communication of exchanges of knowledge and unprecedented performances in calculation. Within a few decades we should be able to build computers in quantity that will be a million times more powerful than the personal computers of today. These achievements will provide new methods of research in every scientific field and will give rise to extraordinary applications.

In physics, they will favor the possibility and the development of direct atomic-level manipulations. We will be able to conduct dissecting, manipulating and designing tasks with great success at dimensions which are today unavailable and even unbelievable. In particular, we will increase the field of nanotechnology and its numerous applications and build new devices for accomplishing new experiments. Chemistry will still increase its ability to realize new materials satisfying some prescribed properties for specific industrial use. In biology, we are cataloguing our own genes. We should be able to manipulate them to prevent and to cure many diseases and to reduce human suffering. Many unpredictable advances in molecular and cellular biology will allow a better understanding of the history of life and of the behavior of animals, plants, insects bacteria and so one. Sciences concerning the earth and the universe will benefit from the new achievements of launchers and satellites and will give us a new knowledge

of our world and of its history. Cosmology will be greatly enriched and will become a more well-founded scientific discipline. We are studying intelligence and consciousness. Maybe within the next century, we will be able to create astonishing artificial intelligence.

But what is more important for the daily life of people is the application of this scientific knowledge to what is called, for a few decades, new technology. It is the art of using scientific results and known techniques in order to build new objects or apparatus to be sold in the market. To be a good engineer, capable of developing a new technology requires a creative imagination, very broad knowledge, good judgement and a great capacity for predicting the chances of the success of the operation, which means its marketing. The work in technology requires a lot of research but of a kind quite different from the research in scientific disciplines. Time plays a different role: in technology the total time required for a realization including research, development, industrialization and sale must always be shorter, thanks to improvements and innovations. In science, the most important progress requires very often a long time of reflection and research which is not of crucial importance. In science the result is judged by peers and is appreciated by way of a good reputation. In technology, it is judged by the market and the appreciation is in the hands of the shareholders. Despite these essential differences between sciences and technology, for the public they are very close and the economists make no distinction and speak of technosciences. For them, the concept of science has no importance or it must be understood as a component of technoscience. Very often the word of science implies at the same time science and the technology which is generated by science

## *2. The great challenge for humanity*

It is quite obvious that these wonderful achievements as well as the powerful methods and tools which have been found and used, open fantastic possibilities. We discover that we are in position, in a very fundamental way, to reshape ourselves and our world. The new technologies provide us with great hopes, for example hopes of eliminating diseases and poverty. But also they come with grave challenges and great dangers. I borrow the following example from a paper by Bill Joy in a recent issue of the Bulletin of the American Academy of Arts and Sciences: "We struggled for much of the twentieth century with controlling our capacity for biological, chemical and nuclear weapons of mass destruction.

The great advantage we had with these technologies was that these often required large scale activities or very specialized knowledge and facilities that were not widely available. In the twenty-first century, the new technologies of great power are much more likely to be small, portable and capable of being used by small groups of individuals and inherently much more difficult to control”.

Here is the great challenge to be met by humanity in the coming years: the necessity to make decisions concerning the orientation of the program of scientific and technological activities which could have many consequences for the future of human beings and for the societies, often without knowing what it wants to achieve. The situation is quite new. People for a long time wanted to travel by air and that was the perspective or the orientation of various scientific and technical research which gave rise to balloons and to airplanes. The same may be said for the wish to have a powerful and easily usable form of energy for improving people’s work and life. However when one has to deal with scientific and technical decisions which would affect the human being himself, his own nature or his environment or the life of future generations, the situation is quite different. What kind of a human being do we want to produce? What is our dream? It is impossible to formulate an answer which would receive a quasi unanimous agreement. Even now, at the present time , it is difficult to define what makes the dignity of a human being, what makes his singularity among all the living beings.

All these problems arise namely in the history of humanity at a time when science and technology have reached the level which permits an action on man himself, but also at a time when, as noted by Bill Joy, our connection to the spiritual and sacred is relatively weak. In the West, the principal conceptions of man, religious or philosophical, may be considered as “humanism”, which means that, for them, man is a very special living being who has special privileges and rights and who is worthy of great consideration and of great respect. Today, the validity of humanism could be doubtful. Sure, man is endowed with a great power. But, for a biologist, the specificity of man as a living being is not at all obvious. The difference between the human genome and the genomes of apes or monkeys like chimpanzee, gorilla, bonobo, orang-outang is very small. As far as the behavior is concerned, the difference is not as great as was thought. Some monkeys may have feelings and ethical behavior. Some people think that the “animal rights” have to be taken into consideration. Others may be tempted to deal with human beings, especially with embryos

or with very old people, in a somewhat loose consideration, at least less than it was in the past.

We will have to determine the fate of our species. But the problem is that we don't know what we want to become. Another unexpected consequence of this situation, a paradoxical one, is the decline of the scientific ideal. In most of the advanced countries, in the West especially, the number of young people who want to become a scientist or an engineer is decreasing.

This great challenge would be the most difficult problem of humanity during the coming years. It will require a lot of thinking and many discussions about what we want to become. Like Bill Joy, I do not believe that science can tell us what we should become. Our choices should come from our spiritual, artistic and ethical values. To find the good one will require time. The academies can help bring together groups of people to discuss the shape of our future. Scientists involved in the most advanced and crucial fields will, of course, like to go very fast. In my opinion, we must avoid taking decisions in a hurry. One must try to make a step only when a great proportion of people is thinking that it may be done safely.

But an urgent task and a useful one for the scientific community would be to redefine, as clearly as possible, the foundation of its ideal and to give precise examples of actions that the scientists intend to do for the benefit of society; in other words, to justify the choice to become a scientist. It is worth proposing already initiatives which may improve the present situation. An example is given by Claude Allègre, a recent Minister of Education, Research and Technology in France. Noting the diminution of the number of young students in science and in engineering, he decided to create for them new courses in history and philosophy of sciences in many faculties of sciences. He thought that that would be a good way to convince them of the high cultural value of a scientific education. The purpose was a good one, even if one may think that it was not the best decision to be taken.

### *3. "Human solidarity" as a good foundation for the new scientific ideal*

The classical scientific ideal was to increase the knowledge on the universe and on the material and living world for the intellectual and moral benefit of "man" considered in an abstract way, independent of space and time. "Science has no frontier". In the present paper, it is proposed that this "man", the abstract man of the "enlightenment", be replaced by "human solidarity"; or if you want the "concrete man", which means all the people

of any nation or territory and all the future generations. The great difference is the following. In the classical conception, the scientists have to elaborate new results. Their application is done by engineers, technicians and companies which have to ensure their diffusion. In the new conception recommended in this paper, the scientists will have, as always, as first duty to increase knowledge, but they will also have the duty to see that it meets effectively the needs and the expectations of the world's inhabitants and, eventually, to participate in this action.

It would not be difficult to show the important consequences of this change of perspective. Generally speaking, it would imply partnership between scientists, and in particular between scientists belonging to the developed world and of the developing world. It is not necessary to comment on the benefit for Education which is for any country the best lever for improving its situation. As far as Research is concerned, it would mean, for instance, that more work would have to be done to cure tropical diseases or in biotechnology, for creating genetically modified organisms which could be used for overcoming the deficiencies due to parasites or to drought. As is now obvious, especially with the very recent report of IPCC, much investigation is required in order to limit the damage caused by the global warming.

The actions inspired by this new ideal would have a deep influence on the life of all countries, especially on the developing ones. A better understanding of what science is, what it can do and what it cannot do, would help to stimulate a good exercise of democracy which is necessary today for the decision-makers in order to take the best decision after good expert advice. A country which would be scientifically illiterate would not be able to take advantage of the new scientific and technical achievements. That is an unfortunate situation, not only for this country, but also for the whole world because it is a factor which would continue to increase the gap – or even the fracture- between the rich and the poor countries, a dangerous situation which has been worsening these past decades as shown by recent reports – see for instance the last paper of Pierre Papon, the Président of the *Observatoire des Sciences et des Techniques* in France.

This last observation shows that the ideal of human solidarity which is offered here to the scientific community is a factor antagonistic to the perspective of the technosciences. It tries to favor cooperation and not competition. The present economic world puts a strong accent on the free-market and globalization which is the main cause of the fracture already mentioned. Scientific solidarity would happily temper and balance this great and sometimes dangerous economic influence.

I want to conclude this paper by greeting the recent reinforcement of IAP – the Inter Academy Panel on international issues – and the creation of the IAC- Inter-Academy Council. Both seem to reveal an evolution in the direction recommended in this paper. It does not mean, nevertheless, that all the scientists are ready to adopt this position. The two first articles of the last issue of the “Bridge”, the publication of the National Academy of Engineering, are very enthusiastic about the expected progress, but, at least apparently, they don’t take account of public acceptability. On the contrary, the last reference is a plea for “a modern humanism”. Sooner or later “man” will be the crucial question of our future.

I am very grateful to have been invited to this wonderful symposium and to have the possibility to express my gratitude to Professor Carlos Chagas. Most of the ideas I have tried to express have been greatly influenced by many discussions and exchanges of view with our former President either in Rio or in Paris when he visited our Academy as one of its “Associés Etrangers” and of course in Rome in this marvellous Casina where he will never be forgotten.

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# L'HOMME DE SCIENCE ET LA SOCIÉTÉ ACTUELLE

ANDRÉ BLANC-LAPIERRE

Notre regretté président, notre ami, le professeur Carlos Chagas, qui a tant fait pour le rayonnement de cette Académie, a, naturellement, orienté beaucoup de travaux de celle-ci vers le thème "Science et Société". Le programme de ce symposium nous donne la chance de profiter de très intéressantes communications sur les dernières avancées dans un large éventail de domaines scientifiques, et, évidemment, sur ce que ces avancées rapportent ou laissent espérer à l'humanité. C'est essentiellement aux positions et aux missions, dans notre société en rapide évolution, des scientifiques eux-mêmes et de ceux qui appliquent les résultats de la science, que sont consacrées les considérations qui suivent.

Les progrès de la connaissance ont, certes, toujours à des degrés divers et avec des délais variables, influencé la vie de la société ; mais, avec le prodigieux développement actuel des applications, l'interaction de plus en plus serrée entre elles et la science, jointe à la fascinante rapidité des évolutions correspondantes et à la possibilité de mobiliser des moyens de communication et de diffusion quasi instantanés vers le monde entier, *la situation de l'homme de science dans la société a fortement changé* et, même, si j'ose m'exprimer ainsi, *la place, au sein d'un même individu, de l'homme de science par rapport au citoyen qu'il est, s'est notablement modifiée.*

Au regard de cette présentation – peut-être un peu pour la tester – permettez-moi de me reporter à mon propre passé... les hommes de mon âge aiment bien évoquer leur passé, qui est l'assise de leur expérience ! J'ai essentiellement travaillé en électronique, dans la théorie et la technique de la communication (avec une forte incursion dans le calcul des probabilités), et en physique des particules. Certes, j'ai mesuré toute l'importance de l'électro-

nique et des communications pour la société (la physique des particules relevant essentiellement de la recherche fondamentale), mais ce ne sont pas vraiment mes propres recherches, elles-mêmes, qui m'ont fait pleinement vivre l'évolution résumée ci-dessus, mais bien les possibilités qui m'ont été offertes de participer vigoureusement à l'action d'organismes ou d'institutions tels que, en France, le Comité Consultatif de la Recherche Scientifique et Technique, le Conseil Supérieur de la Sécurité et de l'Information Nucléaire, l'Académie des Sciences... et, hors de France, notamment l'Académie Pontificale des sciences, le Conseil de l'Université des Nations Unies...

Ce qui suit concernera, mais avec des pondérations variables, à la fois l'homme de science fondamentale et celui qui, au moins partiellement, oeuvre pour les applications. Je l'articulerai en deux parties:

1 - *La science et la société.*

2 - *L'homme de science et la société.*

## 1. LA SCIENCE ET LA SOCIÉTÉ

Le monde de la recherche et celui de la technique, de l'entreprise, ont longtemps suivi des voies parallèles, avec, certes, des passerelles, mais en maintenant une certaine distance, la démarche du premier visant à accroître la compréhension de la nature et celle du second ayant pour objectif essentiel la production de biens et de services. Mais, depuis un siècle, et avec une rapidité surprenante au cours des dernières décennies due en particulier à la mondialisation, ces deux mondes, tout en conservant leurs originalités de leurs vocations propres, se sont considérablement imbriqués et collaborent dans leur activité quotidienne. Il n'est plus d'entreprise, même de taille moyenne, sans recherche, et il n'y a guère plus d'organisme de recherche qui ne développe des projets communs avec le monde de l'entreprise. Je ne résiste pas au plaisir de citer ici un paragraphe emprunté au discours d'introduction prononcé par André Cauderon<sup>1</sup> à l'ouverture, le 3 mai 2000, d'une séance de l'Académie d'Agriculture de France consacrée au thème: "*Les biotechnologies chez les végétaux*".

"L'opinion ne peut qu'être impressionnée par cette conjonction entre deux mondes, la recherche et l'entreprise, également dynamiques : elle peut avoir l'impression d'une sorte d'hybridation, évidemment contre nature, qui donne naissance à un être fabuleux, un Dragon, puisque l'année 2000 est

<sup>1</sup> Membre de l'Académie des Sciences, Secrétaire Perpétuel honoraire de l'Académie d'Agriculture de France.

celle du Dragon. Cet être mal connu, compétent et puissant, tourné vers le changement, est impatient dans ses projets. Il tend à formuler seul les questions et à donner immédiatement la bonne réponse générale. L'accélération des changements fait que le public se sent de plus en plus dépossédé de son avenir. Comment faire équilibre à la force et à l'efficacité, mais aussi à la surdité et à l'arrogance éventuelle du Dragon? Comment ménager un espace de réflexion et de choix pour la société?"

Naturellement, l'imbrication science-applications est allée de pair avec l'établissement de liens étroits – tout aussi rapidement croissants – avec l'économie, les grands problèmes de la société: nourriture, santé, énergie, éducation..., tous problèmes qui rejoignent ceux de la paix, de l'éthique, de la vie spirituelle, religieuse... Mais là, l'homme de science n'est pas seulement engagé par sa compétence dans un domaine scientifique, mais bien en tant que *citoyen*, plus généralement en tant qu'*homme*. D'où le second thème.

## 2. L'HOMME DE SCIENCE ET LA SOCIÉTÉ

La communauté scientifique fait partie de la société en général. D'un point de vue logique, peut-être un peu trop strict, il me paraît raisonnable de développer ce thème conformément au plan suivant :

a) *Le scientifique en tant que tel: dans sa spécialité.*

a-1 L'éthique de la recherche scientifique.

a-2 L'expertise.

a-3 Les brevets.

b) *Le scientifique en dehors de sa spécialité mais en tant que participant à la vie de sa commune, de son pays, du monde ... Qu'est-ce qui découle, dans ses rapports avec l'ensemble de la société, du fait qu'il est scientifique?*

b-1 Une certaine rigueur, prudence professionnelle.

b-2 Un certain sens du questionnement sur les besoins de la société.

b-3 Le souci de la formation des jeunes, de l'information du public, du développement général de la culture dans le sens plein du terme.

a) *Le scientifique dans sa spécialité*

a.1 *L'éthique de la recherche scientifique (pour mémoire)*

Il n'y a pas lieu de s'appesantir ici sur ce point. Il est évident que toute recherche doit respecter des qualités de soins, de méthode, de précision et

de probité dans son accomplissement et dans l'énoncé de ses résultats, d'honnêteté vis-à-vis des autres chercheurs dans les publications, dans la répartition des mérites, ... La diffusion des résultats de la recherche fondamentale est de règle. Certes, une certaine confidentialité permettant de mener à bien l'exploitation d'une idée ou la poursuite dans le calme d'une série d'expériences, ou encore de protéger des applications, est normale, mais le but est d'accroître le patrimoine scientifique de l'humanité ce qui implique que l'on communique.

### a.2 *L'expertise*

Lorsqu'un décideur, un homme politique, fait appel à l'*expertise* d'un scientifique, d'un technicien ou, plus généralement, d'un groupe de scientifiques, de techniciens, ces derniers doivent éclairer les premiers sur l'état *actualisé* de la connaissance scientifique et technique relative aux problèmes considérés, éventuellement sur l'incertitude de cette connaissance, sur ce qui pourrait réduire cette incertitude... et faire abstraction de ce qui relève de leurs intérêts personnels, de leurs propres convictions philosophiques, politiques... L'expertise doit être pluridisciplinaire, contradictoire et indépendante.

Je pense que, s'il est absolument indispensable que les gouvernements, les ministères, aient leurs spécialistes, par contre, il ne me paraît pas bon qu'ils créent leurs propres "Comité d'expertise", ceci pour sauvegarder l'indépendance des avis exprimés. Je crois que les Académies ont, elles, un grand rôle à jouer en offrant leur cadre à de tels comités. En plus des compétences qu'elles possèdent en leur sein, elles fournissent un lieu de réflexion indépendante dans lequel les experts réunis peuvent s'exprimer de façon infiniment plus libre que dans une structure hiérarchique. Elles ont, par ailleurs, l'avantage de fournir un cadre adapté à la mise en oeuvre d'une expertise collective en général nécessaire dans l'étude des grands problèmes de société et dans les besoins en évaluation prospective qui sont les leurs.

### a.3 *Les brevets*

L'accroissement de la connaissance scientifique pure est, à juste titre, considéré comme un accroissement du patrimoine de l'humanité et, en principe du moins, ne donne pas lieu à prise de brevet. On ne brevète pas la loi d'Ohm, ni l'équivalence de la masse et de l'énergie. C'est dans le domaine des applications que les brevets interviennent. Il est normal de rémunérer les

bons inventeurs et les compagnies qui développent des applications découlant de leurs inventions. Il y a d'ailleurs là un effet de stimulation incontestable. Il faut cependant prendre garde à ce que ces rémunérations ne se transforment pas en rente de situation exagérée permettant la pratique de prix abusifs, ce qui, en fin de compte, bloquerait les progrès ultérieurs.

Il faut, aussi noter que les dispositions juridiques et financières qui régissent les brevets et les droits d'obtention ne sont vraiment justifiées que dans des échanges économiques équilibrés, entre pays ayant déjà atteint un certain niveau de développement. Qu'ont-elles à faire, s'il s'agit de réduire l'effet de serre qui concerne toute la planète, d'améliorer la situation des pays pauvres, de se préoccuper de leur alimentation... Une recherche délibérément orientée vers la solution de ces grands problèmes intéressant l'ensemble de l'humanité est nécessaire. Elle doit être, pour une large part, affranchie des impératifs financiers des compagnies privées, c'est-à-dire être, en grande partie, soutenue par des Fondations et des crédits publics. Un bon exemple est constitué par le "*riz doré*"<sup>2</sup> provenant de l'insertion dans le riz de sept nouveaux gènes codant pour des enzymes et des protéines qui permettent au riz de produire et de stocker du  $\beta$ -carotène (-> carence en vitamine A) et également d'accumuler, toujours dans les grains, du fer absorbé en excès. Ce projet, issu des résultats d'une étude de chercheurs de l'Institut de Technologie fédéral suisse à Zurich et de l'Université de Fribourg, a été financé par l'Union européenne et par la Fondation Rockefeller. Les résultats sont importants car, selon l'organisation mondiale de la santé, entre 140 et 250 millions d'enfants d'âge préscolaire présentent, dans le monde, des carences en vitamine A. La carence en fer, elle, est la carence la plus importante dans les régimes à base de riz ; elle affecte un nombre considérable de personnes.

Il faut noter que certaines inventions importantes, qui ont été largement – et depuis longtemps – utilisées dans le monde entier n'ont pas fait l'objet de brevets à l'origine. À titre d'exemples remarquables, je citerai la photographie (Niepce et Daguerre en 1839), l'accumulateur au plomb (Planté, 1859), l'utilisation des champs tournants dans les moteurs électriques asynchrones (Ferraris, 1888) ... Il faut cependant reconnaître que des brevets ont été pris par la suite par des chercheurs, autres que les inventeurs initiaux, qui ont perfectionné les techniques correspondantes.

<sup>2</sup> Les données concernant le riz doré sont extraites de la communication de B. Le Buanec à la séance du 3 mai 2000 de l'Académie d'Agriculture de France, consacrée au thème "Les biotechnologies chez les végétaux".

b) *Le scientifique en dehors de sa spécialité, mais en tant que participant à la vie de sa commune, de son pays ..., du monde. Qu'est-ce qui découle, dans ses rapports avec l'ensemble de la société, du fait qu'il est scientifique ?*

b.1 *Une certaine rigueur, prudence professionnelle*

Dans son travail, le scientifique acquiert une méthode, une rigueur qui, à condition d'être bien employées, restent précieuses, même face aux grands problèmes de société dans lesquels il faut aussi prendre en compte des aspects économiques, psychologiques, politiques, éthiques, spirituels...

Le scientifique est habitué à respecter les faits, à se reporter à l'expérience. Il a le souci de remettre en cause les modèles et les théories après le retour d'expérience; il est capable d'une appréhension raisonnée de la notion de risque. Il a le sens des constantes de temps alors que notre monde est de plus en plus fasciné par l'instantané.

Naturellement, toute cette expérience, cette formation acquise dans le domaine scientifique ne peuvent être mises à profit qu'avec la plus extrême prudence et beaucoup d'esprit de finesse surtout lorsqu'on sort de ce domaine.

b.2 *Un certain sens du questionnement sur les besoins de la société*

La curiosité est la base de la recherche. Il faut savoir se poser des questions. Il me semble que le scientifique doit s'efforcer de transposer cette formation au questionnement, avec prudence certes, dans des domaines extérieurs à sa recherche... dans des domaines qui le touchent comme citoyen... dans des domaines qui intéressent les besoins de l'humanité.

Je mentionne ici deux questions qui, dans cette perspective, ont, ces temps derniers, mobilisé ma réflexion.

1 – Lors de la dernière Assemblée Plénière de notre Académie, notre attention a été attirée sur l'importance des *plantes transgéniques* pour faire face aux problèmes de la *nourriture de l'humanité*,<sup>3</sup> *spécialement dans les pays en développement*. Je suis tout à fait incompetent sur le sujet, mais il me paraît important et, je suis allé interroger des confrères bien informés.

<sup>3</sup> J'ai été très intéressé à propos de ce sujet par le projet de texte d'une communication que P. Louisot doit présenter à la réunion Diétecom 2001, texte qu'il m'a aimablement confié.

Ne faut-il pas, par exemple, orienter systématiquement et plus énergiquement les recherches vers les biotechnologies qui assureront aux pays en développement la sécurité des productions : plantes résistantes au gel, cultivables sur des sols salés, ou pouvant se développer sur des sols très secs...?

2 – Marc Pèlerin<sup>4</sup> a évoqué devant le Conseil des Applications de l'Académie des Sciences (Paris),<sup>5</sup> le premier symposium international des ingénieurs (Hanovre – juin 2000) auquel participaient 3600 personnes dont 1000 étudiants et élèves.

“Lors de la séance de clôture, le Prof. Dr. Ing. U. Seiffert fit, en ouverture de son exposé, la remarque suivante qui met en exergue le problème majeur auquel les (actuellement) *jeunes* ingénieurs vont être confrontés:

*“Il y a actuellement 700 voitures pour 1000 habitants aux États-Unis, 7 pour 1000 habitants en Chine ; bien avant la fin du siècle, si la croissance maintient son rythme, il y aurait 700 voitures pour 1000 habitants en Chine (... et des situations analogues dans beaucoup d'autres pays). Ceci correspond à des marchés de centaines de millions de voitures à construire, voire plusieurs milliards si l'Inde, la Russie et d'autres pays 'démunis' suivent la même évolution. La planète peut-elle fournir les matériaux, l'énergie ... pour construire ces véhicules puis assurer leur fonctionnement?”*

N'est-ce pas le problème majeur auquel, dans un avenir très proche les ingénieurs vont être confrontés? Il ne s'agira pas de construire des voitures dont les vitres seront commandées par la parole, mais d'imaginer, puis de construire, des moyens de transport bien adaptés aux pays et compatibles avec les sources de matières premières, avec les ressources énergétiques, avec les impératifs de la préservation de l'environnement... Il s'agit probablement aussi de revoir nos politiques de croissance irréfléchie, nous, *les pays industrialisés, qui représentons 20 % de la population et consommons 80 % des ressources de la planète.*

“Personne, ajoutait Pèlerin, n'oserait, j'imagine, dire: il faut convaincre ces pays qu'ils ne peuvent espérer les 700/1000 car l'équilibre de la planète serait compromis! Est-ce sûr que personne ne le dira?

L'exemple pris par Seiffert est excellent, il résume en une phrase la place de l'éthique dans les activités humaines, surtout pour ceux qui ont des responsabilités qui peuvent – qui doivent – orienter les activités technologiques des pays dits développés”.

<sup>4</sup> Membre correspondant de l'Académie des Sciences

<sup>5</sup> Conseil qui est en voie de transformation en Académie des Technologies.

### b.3 *Formation des jeunes, information du public, développement général de la culture*

La communauté scientifique, les Académies des Sciences, des Technologies... doivent participer pleinement à la poursuite de ces objectifs.

#### a) *La formation des jeunes, soit directement, soit à travers l'élaboration des programmes d'études*

Je crois qu'il est essentiel dans le monde actuel, de développer l'enseignement des sciences expérimentales, de faciliter le contact direct avec le réel (les personnes, la matière...), de bien faire comprendre que *les modèles*, si utiles soient-ils, ne sont valables que dans le cadre de certaines hypothèses et de certaines approximations, et qu'il faut toujours revenir à leur validation par la réalité qui a pu changer.

L'enseignement doit s'efforcer de développer *l'esprit critique, l'aptitude à réfléchir*, qui permettent de prendre du recul, de trier et de faire le point dans le flot des informations, vérités ou contre vérités, qui déferlent.

#### b) *l'information du public*

*Il s'agit d'éclairer l'opinion publique* sur l'état précis et actualisé des connaissances scientifiques disponibles relatives aux grands projets en cours en distinguant scrupuleusement ce que la science peut dire et ce qu'elle ne peut pas dire et en indiquant quels types de recherches pourraient améliorer ces connaissances. Il s'agit de lutter contre la confusion souvent faite entre les *sciences* et les *pseudo-sciences*, contre l'existence, dans les médias, d'un certain dénigrement de la science et du progrès souvent présentés et perçus comme des menaces, contre l'attraction exercée sur les médias par le sensationnel au détriment d'une information correcte. Il faut lutter contre la *dés-information* du public produite par des *rumeurs* rapidement et habilement propagées basées sur des affirmations non vérifiées et souvent erronées. Ce contact avec le public, notamment à travers ceux qui influencent l'opinion: médias, associations, organisateurs de débats,... peut poser des problèmes aux scientifiques car il faut faire preuve de grande qualités pédagogiques pour trouver un langage commun, mais c'est là un effort indispensable.

#### c) *La culture scientifique fait partie intégrante de la culture générale*

Ce n'est pas perçu comme évident par tout le monde: à ce sujet, il me paraît important que les étudiants qui ne se destinent pas à des activités



proprement scientifiques (juristes, littéraires, artistes, hommes de médias, candidats à des postes d'administration,...) bénéficient au cours de leurs études, d'une formation suffisante relativement à la démarche scientifique et à ses méthodes, et, ceci, au titre même du développement de leur propre culture. Il ne s'agit pas de détailler, pour eux, tel ou tel chapitre de la science, mais de leur faire saisir l'essence de la démarche scientifique, de son évolution et de son insertion dans la connaissance générale. Je pense d'ailleurs que cet enseignement ne serait pas des plus simples et nécessiterait des efforts de formation des enseignants eux-mêmes.

### 3. EN MANIÈRE DE CONCLUSION: ORIENTER L'ACTIVITÉ HUMAINE VERS LE DÉVELOPPEMENT DE L'HOMME, LUI-MÊME, ET VERS CELUI DE L'HUMANITÉ

Ce titre peut paraître provoquant ou d'une grande naïveté. Je l'ai choisi comme on choisit une lumière pour assurer le chemin, un phare sur la mer...

Il est trivial de dire que les possibilités ouvertes par la science peuvent être utilisées pour le bien et pour le mal; ceci est vrai de tous types d'activités humaines. Les problèmes qui en découlent ne sont pas l'apanage du scientifique, mais concernent le citoyen, l'homme. Où est donc l'aiguillage qui orientera vers le bien ou vers le mal? Ou encore: *où sont les forces* qui font pencher la balance:

- vers le respect de l'homme ou vers son utilisation,
- vers la recherche d'un développement durable ou vers la satisfaction du court terme, la défense des droits acquis,
- vers le respect de l'environnement ou vers l'exploitation démesurée des ressources naturelles,
- vers le fait que les médias ont pour mission principale la formation et la culture bien avant la propagande et la publicité,
- vers le fait que l'argent est considéré comme un moyen facilitant les échanges et non comme un but, un facteur de puissance.

Pour ma part, je crois qu'on touche là à un profond problème de mentalité, à une vision éthique et morale de la société, au respect de l'homme et de la création, à l'amour du prochain, à la Foi en Dieu...

Comme pour tous les problèmes de mentalité, celui-ci demande conviction, patience et foi. Je crois qu'il est très important qu'avec tout le respect qui leur est dû, nos jeunes soient sensibilisés à ces importantes questions. Je suis sûr qu'elles ont été l'objet de grandes réflexions de notre ami Carlos Chagas.

## BIODIVERSITY AND THE HUMAN PROSPECT

PETER H. RAVEN

The world's biological diversity, or biodiversity, is a priceless gift to humanity, one on which we base our existence on Earth. Biodiversity feeds us, provides most of our medicines, protects our watersheds, and enriches our lives with beauty. For these reasons, I would like to state clearly from the beginning that I consider the loss of biodiversity caused by human beings to be a moral outrage, a completely unacceptable treatment of the planet that supports us, and in contradiction of all religious teachings. In addition, it is a disastrous strategy from the standpoint of human survival and human options in the future.

Human beings have grown and developed in a rich context of biological diversity, and the properties of our bodies and our minds reflect relationships that are millions of years old. In form and inspiration, our art, our music, and many of our deepest aesthetic and cultural inspirations reflect aspects of biological diversity. We feel nurtured when we are in a place where plants or animals are also present, and by the same token, often feel isolated when we are in a place where it is not. Plants make us feel comfortable, and most of the rooms, offices, and dwellings in which we live and work have plants, aquarium fishes, cats, dogs, or other animals around. We have a natural affinity for them, an affinity that is not surprising given our history, which only for a very short time has us living in villages, towns, and cities.

Given these relationships, and the increasingly rapid extinction of large numbers of organisms, one can wonder with our colleagues Paul Ehrlich and E.O. Wilson, whether man has a right to destroy such a large proportion of what, as far as we know, are our only living companions in the universe. It is for this fundamentally important reason that we must pay careful attention to the fate of biological diversity and our responsibility in

bringing about its loss. In addition, there are numerous practical reasons to be concerned with this loss, and I shall discuss these briefly.

From an individual point of view, biological diversity, and specifically plants, provides all of our food, either directly or indirectly (after being consumed by animals or other organisms), so we are for this reason alone utterly dependent on it. In addition, for the majority of people in the world, plants provide the sole or overwhelmingly predominant source of medicine. It is interesting to note, in connection with the use of plants as medicines by human beings, that higher primates, such as baboons and chimpanzees, also have been observed to use plants essentially as medicines, as dietary supplements in cases of nutritional deficiency or in cases of infestation by parasitic worms; and it is clear that among the more highly evolved vertebrates the use of medicines really is broader than the human race itself. No wonder, then, that we should have begun to find, and that most people in the world should still find, their major source of medicines in natural materials such as plants. The complex and highly diverse biological molecules that evolved in plants and in other organisms as a way of maintaining their status in the world's ecosystems by repelling potential herbivores or preventing diseases, have become important for us as medicines. Even in this age of combinatorial chemistry one cannot, in the final analysis, find enough basic molecules to work with, and by examining those that have evolved naturally one can still find useful starting points for further chemical discoveries. Indeed, the methods of microanalysis and microseparation now being developed promise many new opportunities for the use of natural products from plants and other organisms over the next decade or so.

Many other uses of biological materials can also be enumerated: for example, wood and other building materials; cloth, from such sources as cotton or linen (from flax plants); or biomass, for energy, either fossil (petroleum, natural gas, or coal) or living. Organisms also provide materials that form the basis of many diverse industrial processes, whether actively, as fermentation by yeast, or chemical processes stimulated by microfungi, or simply as starting points in the industrial production of many chemicals. What is not talked about quite as often as examples of this kind, however, is the application of modern biology to the diversity of organisms. Watson and Crick's postulate of the double-helical model of DNA came only in 1953, less than 50 years ago; the first transfer of a gene from one kind of organism to another unrelated one came in 1973 (Boyer and Cohen, about 28 years ago); the first experiments with genetically modified organisms, less than 20 years ago. Increasingly widespread use of the kinds of geneti-

cally modified plants, discussed by Maxine Singer (this symposium), really began in the early 1990s, and continues to increase rapidly. As we begin the 21st century and the new millennium, many of us share the opinion that this should be the “century of biology”; and what we mean by this slogan is that our understanding of biological processes, and particularly of sustainable biological systems, has been developed to the point at which we should be able to create sustainable systems, both at the level of the sustainable productivity of individual species productive and at the level of ecosystems. These new applications should enable us to achieve further progress without at the same time destroying even more of the Earth’s natural capacity to support us, a topic that I shall discuss subsequently.

Over the past few years, we have begun to elucidate the complete base sequences of the genomes of individual kinds of organisms, starting with viruses and bacteria, and continuing on to yeasts, *Drosophila*, nematodes, flowering plants (*Arabidopsis* and rice), and human beings. These remarkable achievements open up whole new vistas for human progress, since they enable us to understand more fully than has earlier been possible the relationships between different kinds of organisms and their genes in an evolutionary context. In the face of such developments, the loss of a major proportion of biological diversity just as we are beginning more fully to understand is poses real obstacles for future progress, both basic and applied. Questions can now be asked, for example, about why human beings may have only about 30,000 genes, but something on the order of 100,000 kinds of proteins. In addition, we immediately begin to ask how it can be that all placental mammals, taking basic sequences and gene function into consideration, have basically the same array of genes, but can still be as strikingly diverse as they are. It has long been known that the genes of human beings and chimpanzees are virtually identical, even in base sequence: what does this mean for interpreting the differences between them? At least a third of the genes of flowering plants are essentially like those of human beings and other placental mammals: what does this signify for the interpretation of GMOs and the nature of opportunities for their further development and use?

In fact, it is the evolutionary relationship between genes and families of genes during the 3.8 billion or more years that life has existed on Earth that will ultimately provide the most exciting and most informative analysis of life on Earth at a genetic level, and the best tools for applying that knowledge to human benefit. Such knowledge can be based only on a thorough appreciation of diversity; it is therefore exceedingly ignorant to allow a

major proportion of diversity to disappear before we have even begun to understand it. It is estimated that we have given names to only about 1.6 million species of plants, animals, fungi, and microorganisms, of the 10 million or more that exist – so most of the organisms that we are driving to extinction in such large numbers as we enter the 21st century have never been studied by anyone: their very existence is unknown. The great American conservationist Aldo Leopold said, in his collection of essays “A Sand County Almanac” (1949) that the first rule of intelligent tinkering is to save all the cogs and wheels. Collectively, however, we are discarding. That is precisely the opposite of what we are collectively doing, however: we are throwing away the cogs and wheels as we try to make better and better machines from the ones that are left!

In addition to the importance of individual organisms for human beings, we also need to consider their importance as components in biological communities and ecosystems. For example, organisms in nature mitigate the effects of soil erosion and protect watersheds. In New York City in the 1990s, for example, water quality was restored to approximately its original state by restoring the ecology of the watersheds in the Catskill Mountains from which the City’s water supply was derived, as for example by limiting the use of pesticides and building up the cover of shrubs and other vegetation, for about a third of the price (\$1.5 billion) that it would have cost to construct water purification plants to accomplish the same purpose. This kind of ecosystem restoration makes possible the continuation of ecosystem services, services that natural ecosystems provide free: other such services include providing homes for insects that pollinate crops and for birds that eat destructive insects, controlling local climates and precipitation – about half of the rainfall in the Amazon is associated with the forest cover – and in generally keeping ecosystems in balance, whether they be terrestrial, freshwater, marine inshore, or deep-sea ecosystems. The value of those ecosystem services is incalculable – we simply could not survive without them.

Four hundred human generations (approximately 10,000 years) ago, at a time when human beings were first discovering how to cultivate crops to produce food for themselves, the population of the entire whole world – all of the continents together – was a few million people – equivalent to the population of Rome at the present time. When human beings were hunter-gatherers, and before they began to live together in villages, towns, and cities, and to develop what we consider civilization, behaviors (whether genetic or learned) that were adaptive, useful, and constructive were favored. Since

those behaviors predominated over some two million years of human history, while people have lived together in communities for only about 10,000 years, it seems certain that we still have the original ones to a large extent, and that we probably have just begun the process of learning how to live together in such a way as to allow everyone to contribute what they can. In the world of the present, where nearly half of a total of more than 6 billion people live in cities of more than a million inhabitants, we must forge new rules for living in harmony with nature and with ourselves.

Over the past 10,000 years, human populations have grown slowly to probably several hundred million at the time of Christ, to probably half a billion in late medieval to early Renaissance times, to perhaps 800 million at the start of the Industrial Revolution in the middle of the 18th Century, and just about reached 1 billion at a time in the 1790s when the Reverend Thomas Malthus was predicting that human population growth would inevitably outstrip our ability to produce food, and that we therefore were in for mass starvation. What the Reverend Malthus did not take into his calculations was the invention of the steam engine and many other productive mechanical systems, which first depended on the massive use of fuels such as wood and subsequently on the millions of years of accumulation of fossil fuels of biological origin – petroleum, natural gas, and coal. The discovery of these fuels and their widespread utilization made it possible for human beings to find other ways to wring more productivity out of the Earth. But as the human population over that 200-year period has grown from a billion to over 6 billion people, radical changes have occurred in life on Earth, many of them during the last 50 years. In this period of runaway growth, it is striking to remember that the last billion people were added in the last 12 years, and a billion people in the 13 years before that. As recently as 1975, the global population was four billion; 50 percent were added to the global population in the last quarter century, and although both absolute growth and percentage growth have slowed down, the first decades of the next century are likely to see the addition of another 2 billion people – the first billion of them in not much more than the 12 years in which the last billion people were added, because the base is now so high that even though percentage growth and absolute numbers have gone down, the overall number is still increasing rapidly.

Population is by no means the only problem in the world, although we often tend to overemphasize it. The problem is one of population multiplied by affluence, the level of consumption, multiplied by the technology that is used – a factor that can be positive or negative. If one calculates that peo-

ple in affluent countries such as Europe and North America consume at about 30 times the rate of rural people in Brazil or Indonesia, about 30 times as much energy or any other substance to support their lives, then one can readily calculate that, multiplying the 200 million people added in Europe and the United States since the end of World War II by 30, one would have a total consumption equal to that of 6 billion rural Indonesians. That, of course, leads right into another very serious moral problem that many have pointed out: whether those of us who live in affluent countries have the right to consume so much of what the world has to offer; or whether we ought not (and I think the answer is clear) be thinking much more carefully about our common management of this planet. In any event, over the past 50 years, while the global population has grown from 2.5 billion to over 6 billion people, we have exhausted about a fifth of the world's topsoil, a similar amount of its agricultural lands, cut down about a third of its forests without replacing them, increased carbon dioxide – the primary human-produced greenhouse gas in the atmosphere – by about a sixth, and depleted the stratospheric ozone layer by about 7 or 8 percent, thus increasing the incidence of malignant skin cancer by about 20 percent in temperate latitudes. These changes are extraordinarily serious, and they clearly are going on to a degree that cannot be sustained.

This relationship was pointed out very clearly by participants at the 1992 Earth Summit in Rio de Janeiro, but very little has been done as a result. Nevertheless, as George Schaller, the great American conservationist, of the Wildlife Conservation Society in New York, put it, we cannot afford another century like the past one. We simply cannot afford it; because we, the human race, are living on our accumulated natural capital and not on the interest or natural productivity – just the same as a family would be living on a bank account that way, and it is a proposition that cannot continue. The world will find sustainability, because continued human activities lead to a condition that is sustainable worldwide; what we are talking about, therefore, is not whether we are going to survive or die. We are clearly going to survive. What we are discussing is the kind of a world in which we want our children and grandchildren and their grandchildren to live. This world will differ remarkably from place to place, depending on the efforts of individual people in different places. Some will be healthy and prosperous, and rich in biological diversity and natural beauty and productivity, and others will be impoverished, homogenized, desertified, and not places that we can consider fit for human beings. The choice is ours.

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Regardless of whether we have enough moral character to worry about the fate of people all over the world, where a quarter of our fellow human beings are living on less than \$1 a day and half of them are literally starving, we are nonetheless engaged in a collective management of our planet, a partnership that can work out for everyone's benefit only if we do begin in some way to form a kind of partnership. This partnership will, in my opinion and the opinion of many others who have thought about it, require substantial change in moral viewpoint. It will not come about simply by recitation of dreary facts about how different parts of the world are going downhill or how many people are starving. The change that it will take to make us address the world dilemma overall is basically a change that must occur within us. Along the way, however, paying attention to biological diversity is something that we can do to our very great advantage. In the spirit of Carlos Chagas, whose life we remember with great appreciation, let us dedicate ourselves to developing our common appreciation of the whole human race, and of the biological diversity that supports it in its great variability and magnificence throughout the world.



## GENETICALLY MODIFIED ORGANISMS

MAXINE F. SINGER

Some of us joined this Academy when Carlos Chagas was president. Upon meeting him for the first time, we knew that we were in the presence of a person of great vision, enormous integrity, and unquestioned dedication to science. His dedication to science had two foundations. One was his inherent fascination with the natural world. The second was his conviction that science could advance human welfare. Ever since last summer I have been thinking of how interested he would have been in the extraordinary scientific news coming from his beloved Brazil.

On July 13, 2000, the cover of Nature Magazine displayed an insect, a leaf hopper, sitting on an orange or grapefruit. Inside, was a paper reporting the entire genome sequence, more than 2 million 600 base pairs (2,679,305), of the bacterium *Xylella fastidiosa*.<sup>1</sup> The many authors were from 34 different laboratories and a bioinformatics center in the State of Sao Paulo, Brazil. For part of its life cycle, *Xylella fastidiosa* lives in the leaf hopper's gut. From there, it is delivered into the xylem of a plant by the feeding leaf hopper where it multiplies and causes chlorosis, the loss of chlorophyll by citrus trees. The tree produces useless fruit prematurely with consequent loss of the crop. Relatives of this bacterium cause diseases in coffee, nuts, fruits including grapes, and other important plants. *Xylella* is a major problem for Brazil which produces a third of the world's oranges and half of the world's orange juice concentrate. Remarkably, at least 83 *Xylella* genes are derived from bacteriophage genomes... the viruses that infect bacteria. Among these are genes associated with virulence in other bacteria infecting other plants. Thus, the bacteriophage have been the agents of gene transfer between species.

<sup>1</sup> A.J.G. Simpson *et al.*, *Nature*, 406, pp. 151-157, 2000.

The consortium of laboratories in Sao Paulo started this project with resources of \$13 million as a matter of deliberate policy by a state research agency (FAPESP). Handsome support is continuing for projects to sequence the genomes of additional pests of Brazilian agricultural plants. Laboratories in Sao Paulo are now well equipped both to pursue the sequencing and the biology experiments necessary to exploit this work for the benefit of the state and its people.

The investigators are reportedly working to identify all the genes involved in pathogenicity. Some suspects have already been noted. Once identified, knowledge of these genes can be used in several ways to help control *Xylella* infections and the loss of crops. It can facilitate breeding of new varieties of plants by traditional methods. It can also be used to direct genetic modifications by modern molecular gene transfer techniques. Presumably the first approach would be acceptable in Brazil. Ironically, the second method, which is likely to be faster and more precise, is not now possible.

The report of the *Xylella* genome sequence, the first public sequence of a free-living plant pathogen, made international celebrities of the scientists in Sao Paulo. The event stimulated the federal government of Brazil to provide more funds for research, nation-wide. But, as the Nature editorial lauding this great achievement said, the challenge to Brazil is to “persuade the Brazilian public that transgenic plants can play an important economic role and at the same time take firm steps to avoid untoward social and environmental consequences”.<sup>2</sup> At present, Brazil does not allow the planting of transgenic crops although the scientific director of FAPESP, José Fernando Perez, was a member of a panel set up by 7 National Academies which recently endorsed the use of genetically modified plants to help meet the food needs of the world’s poor.

*Xylella* was only identified in 1993 and its residence in the leaf hopper not until 1996. In less than a decade, the science moved so far that the genes responsible for pathogenicity are now being identified. The history of the last 50 years has been like this for biologists. Startling insights and new information have accumulated at a dizzying pace, constantly challenging us to change ideas and fundamental concepts. Like most scientists, Carlos Chagas had little trouble adjusting to such revolutionary changes.

Revolutionary change does not go down so easily outside the scientific community. Change is often resisted, sometimes because it’s discomfoting,

<sup>2</sup> *Nature*, 406, p. 109, 2000.

even scary, sometimes because it challenges philosophical or religious notions, sometimes because it conflicts with economic interests... although economic interests can also be a powerful catalyst for change.

Paul R. Ehrlich, the distinguished environmentalist, put it this way in a recent article.<sup>3</sup> “A major contemporary human problem, for instance, is that the rate of cultural evolution in science and technology has been extraordinarily high in contrast with the snail’s pace of change in the social attitudes and political institutions that might channel the uses of technology in more beneficial directions”.

On their part, scientists are often just as surprised and distressed at public reactions to new science as the public is to the science itself. In our exuberance over the new discoveries, scientists may not provide understandable explanations of what is going on, or listen carefully to the public concerns.

For much of the 19<sup>th</sup> century, a lot of what we now call biology was called “natural history”. Tramping around in the countryside looking for new species of beetles or fossils or plants was seen as a charming and harmless pursuit of the wealthy, leisured class. Then, around the middle of that century, three great discoveries signaled a new kind of biology. One was the formulation, by Schleiden, Schwann, and Virchow of the cell theory – the concept that all organisms are composed of one or more living cells. One was Mendel’s elaboration of the laws of inheritance. The third was Darwin’s concept of evolution and the origin of the species. By the end of the 20<sup>th</sup> century, these three paths had converged into one biology, a science that is extraordinarily sophisticated and productive if less charming and acceptable to some.

Mendel was, among other things, a plant breeder. This ancient skill, in modernized form, is as relevant today as it was in his time, even for making transgenic plants.

One of the most important facts recognized by Mendel was that any particular gene, for example the gene responsible for the color of a pea, could occur in different forms. Depending on the two forms present in an individual plant, the peas would be green or yellow. These different versions of genes are responsible for variation within a species... including the variation you see if you look around this room at all the different faces.

<sup>3</sup> P.R. Ehrlich, ‘The tangled skeins of nature and nurture in human evolution’, *Chronicle of Higher Education*, 22 September 2000. Online at <http://chronicle.com/free/v47/i04/04b00701.htm>.

Mendel had read Darwin a few years before 1866 when he reported the characteristics of inheritance in the peas he bred in his monastery garden. The 1863 edition of the German translation of Darwin's *The Origin of the Species*, with Mendel's notes in the margins, is still in the monastery library in Brno. But Darwin apparently never knew of Mendel. If he had he might have realized, as 20<sup>th</sup> century scientists did, that Mendel's work could explain the source of variations in nature upon which natural selection operates to produce new species. The idea of natural selection had its origins in the selection techniques practiced by plant and animal breeders and farmers for at least the last 10,000 years.

The earliest of plant and animal breeders made use of these variations, though they were ignorant of the underlying causes. They observed new, rare forms in fields and when the new property was advantageous, they bred it into their standard varieties. Wild potatoes, for example, have high levels of alkaloid toxins. At least 4000 years ago, Central Andean populations, probably around Lake Titicaca, began selecting and breeding potatoes, perhaps with alleles that reduce the poison. It's useful when thinking about the current debate over genetically modified plants to recall that when potatoes were brought to Europe in the middle of the 16<sup>th</sup> century, the French, suspicious of new foods, refused them and kept on refusing for 200 years while the rest of Europe enjoyed them. Poison was not the issue. The word in France was that potatoes caused leprosy. Similarly, tomatoes, another 16<sup>th</sup> century new world contribution to worldwide diets, suffered a similar fate. At first, only the Italians were bold enough to challenge the widespread notion that tomatoes were poisonous... as indeed some of its relatives and foliage are.<sup>4</sup>

Today, we know that genes are made of segments of DNA. Different forms of genes differ from one another in the sequence of the four DNA bases, or by lacking portions of the sequence, or by containing extra DNA sequences, or by changes not in the coding sequence itself, but in the surrounding DNA sequences that regulate the level at which the gene operates, or even whether it operates at all, under particular conditions. All of these naturally occurring changes in DNA structure can be mimicked by genetic engineering techniques.

A change in the regulation of gene activity... or what biologists call gene expression, underlies one of the five genetic differences between modern

<sup>4</sup> H. McGee, *On Food and Cooking*, MacMillan, New York, 1984.

maize and teosinte, a wild relative of the same species (*Zea mays*) that is indigenous to central Mexico. Very few wild plants are closely related to maize and none of them look much like the maize we know. Nevertheless, teosinte, is the likely ancestor of maize on the basis of archeological, anthropological, and biological considerations. The two plants interbreed efficiently. Still, for more than a century they were assigned to different species and genus. It's easy to understand why. Teosinte is a bushy plant, with many tassels, the organ that produces pollen, and also many seed bearing stalks. The stalks are about an inch or 2 long and have two rows of tiny seeds, each of which is covered in a very hard case. Unlike maize, these seed stalks have no green, encasing husk. The seeds eventually fall to the ground, sowing next year's plants and providing food for birds which also disperse the seeds. Early inhabitants of middle America likely made food of teosinte by grinding the seeds, or popping them. Sometime more than 4000 years ago, they began to domesticate maize-like variants. Maize could never have arisen by natural processes and cannot propagate itself without human intervention. The seeds (or kernels) are tightly attached to the cob and unable to disperse because of the husks. Starting about 5000 years ago, smart middle American plant breeders selected and grew teosinte variants because they were advantageous. One of these variants resulted in growth as a single, straight stalk rather than a bush-like teosinte. The change is not in the coding segment of the gene, but in the regulation of gene activity.<sup>5</sup> The wealth of the ancient middle American empires depended on breeding for unusual mutations in teosinte. Their achievement now feeds the world. Yet today, such modifications made by molecular techniques would be rejected by many.

Plant breeders before the era of genetic engineering even combined different species and still do, using special techniques. Grapefruit, for example, is the result of an 18<sup>th</sup> century breeder's crossing of oranges and pummelo. What neither breeders nor anyone else knew until recently, was that DNA sequences have been transferred between organisms since evolution began, as is apparent in the genome of *Xylella* and as we have recently learned from the sequence of the human genome. Thus, besides different alleles, organisms have evolved through the acquisition of foreign DNA sequences.

Altogether, our modern diets are composed almost entirely of genetically modified organisms. If the history was better understood, the

<sup>5</sup> J. Doebley, A. Stec, and L. Hubbard. 1997. Nature 386 485-488.

current public debates about genetically modified organisms, GMOs, might have greater clarity and depth. Few people understand the continuum that exists between ancient and modern methods. The general public is now at the mercy of the imprecise, even misleading statements made both by those who are opposed to the use of GMO's and those who are promoting them.

The new molecular techniques emerged about 30 years ago, when biologists developed the ability to make precise, conscious manipulations of genes through the techniques variously called recombinant DNA or DNA cloning. These techniques allow directed changes in DNA structure, changes that accomplish a predetermined purpose. These same capabilities have advanced basic biological understanding. The methods being used in plants are essentially the same as those being used to understand and develop treatments for a significant number of human diseases. All these methods generally involve changes in only a small number – from one to several thousands – of the billions of base pairs in the organism's genome.

Traditional interspecific breeding joins many genes from the two species. A lot of the subsequent breeding required over many years to achieve the desirable plant, involves breeding out those genes that were not wanted in the first place. The results are unpredictable and the successes are rare among the many failures to produce safe, hardy plants. There is a significant probability that undesirable traits – undesirable with respect to agriculture, the environment or food safety, will remain. In contrast, the new techniques permit introducing a single change in a single gene in a precise and verifiable manner. There is still a possibility that undesirable properties will occur, but the probability is much less.

What kinds of genes are introduced? Genes from varieties of the same species. Genes from related species. And genes from totally unrelated species, including from bacteria and animals. This is of course very different from what can be done with traditional breeding methods. And the apparent strangeness of the idea of using, for example, a fish gene to protect strawberry plants from frost, has attracted a great deal of discussion.

It is important then to consider exactly what is meant when we say we are putting a bacterial or a fish gene into a plant. The appropriate gene, a segment of DNA, is identified and isolated free of the rest of the DNA of the source organism by techniques known as cloning. Usually this means allowing a bacteria to reproduce the DNA segment. Once a bacteria is isolated that carries only the single, desired new gene, in addition to its own DNA, the bacterial population is expanded so that a sufficient amount of

the DNA segment can be isolated... say a few micrograms. Sometimes that DNA can be introduced directly into a plant. Often, however, it will be modified to make it more suitable for its new location. For example, DNA code words might be changed to permit more efficient gene activity in its new plant host. This entails multiple cycles of expansion in bacteria. Then it is introduced into a plant, sometimes by shooting it in and sometimes by having it carried in on the DNA from a special bacteria which in nature, transfers its own DNA into plants. The original gene may have come from a fish, but it's been around and about in many different bacterial cells before it is finally inserted in the plant's genome-and it has been altered. At that point, it is a pure, definite chemical structure, a piece of DNA. Is it still a fish gene? That is a philosophical question, not a scientific one.

Some recent polls suggest that the descriptions of GMOs in the media have lead a substantial number of people to believe that only modified organisms contain DNA. Of course, the DNA itself is not an issue. The issues center on the particular proteins that the new gene encodes.

As with all complicated problems, there is no simple yes or no answer to the question of whether genetically modified plants will be safe for human health and the environment and views are sure to differ. The issues are not different from those posed by new plant varieties produced by traditional breeding. The questions do not arise from the process used to produce the plants, but rather the nature of the modified plant.

Each type of modified plant needs to be assessed on its own in relation to its use and the environment in which it is to be grown. Several different classes of concerns should be evaluated. For example, for food, we are interested in the safety of the engineered plants for human and animal consumption. With all modified plants, environmental effects, both positive and negative, need to be addressed. In addressing these questions, hypotheses will be made about possible problems.

For each plant, we need to consider the probability of the reality of the hypothetical concerns. This is essential if intelligent decisions are to be made about how to use limited resources to perform experimental evaluations of possible harm. If a hypothetical problem proves to be real, we need to consider the plant in the light of expected benefits. Equally important, the alternatives to using a particular engineered plant need to be considered. For example, maize and cotton have been engineered to resist certain insect pests by introducing a bacterial gene for an insecticidal protein, so-called Bt cotton and maize. Maize and cotton have also been bred for insect resistance by traditional breeding methods using naturally

occurring insect-resistant plants as the source of resistance genes. Insects are also controlled by chemical spraying of fields. On balance, which method or combination of methods is safer for human use and for the environment as well as economically feasible?

For genetically engineered, or traditionally bred food plants, we need to ask whether the newly introduced changes yield a protein that is allergenic or toxic to humans or animals. Is the quantity of some toxic component found in the normal plant increased? If an antibiotic resistance marker gene was used for convenient manipulation when the DNA changes were made, we might be concerned if it compromised use of an important drug. But if the resistance gene is already ubiquitous and the antibiotic therapeutically useless, or the marker has only a very small possibility of being transferred into human pathogens, such a concern could be set aside. Demands for absolute assurance of the absence of any problem cannot be answered by science. Scientific data do not give absolute certainty. This of course is one of the challenges to making sensible public policy.

Five years ago, US farmers began planting Bt maize and cotton. The Bt gene yields a protein that is toxic to a major maize pest, the European maize borer and other pests that destroy cotton. The gene was copied from a bacteria called *Bacillus thuringensis*, or Bt for short. In summer of 1999, more than 30 percent of the maize and 48 percent of the cotton planted in the US was Bt – a total of 30 million acres. A lot of that maize gets fed to animals or goes into maize oil. Most people in the US have eaten those animals or that oil. There is no sign whatsoever that it is harmful to us or animals. The goal for these plants is to protect the 30-40% of potential food estimated to be lost to pests of various kinds, worldwide. Actually, organic farmers have used the bacteria themselves, by the ton, for more than 40 years, to control insect pests, so there was good reason to think that the Bt toxin would be harmless.

With respect to health, there are no indications of untoward effects from eating food from any of the currently harvested GM plants. Excepting for the problem of allergies, which all corporate and academic researchers and government regulators are aware of and attentive to, there are no obvious reasons to worry about the health effects of foods and fibers in the pipeline.

What about the balance between undesirable and desirable effects on the environment, including biodiversity, by insect-resistant GMO's? Crops of these plants require much less chemical insecticide than do unmodified crops. This means less pollution of air, water, and soil by noxious



chemicals, a decrease in the substantial number of pesticide poisonings in farm communities, and a cost saving to farmers. For cotton alone, between 1996 and 1998, there was, according to USDA, a reduction of more than 1 million gallons in chemical pesticide use.<sup>6</sup> Spraying chemicals indiscriminately eliminates all the insects in a field – billions of them, including species that are vital for pollination and biological control. Thus insect biodiversity can be positively affected by GMOs.

However, two years ago, two scientific reports showed that milkweed leaves dusted with heavy concentrations of Bt maize pollen are toxic to Monarch butterfly larvae in laboratory experiments.<sup>7,8</sup> This was not surprising because it was known that the Bt toxins are toxic to lepidoptera in general. Nevertheless, these findings garnered enormous public attention although the authors pointed out that it remained to be seen what happens under field conditions. Concern was amplified by the well known fact that there has been an unexplained drop of about 70% in the population size of Monarchs wintering in Mexico since 1996. Is there a relation between the use of Bt maize and the decline in Monarchs? Perhaps, but it is likely that the effect of Bt maize will be at most slight compared to the known effects of habitat destruction in Mexico and the use of chemical insecticides in both Mexico and the US. More recent experiments, some in the field, indicate that the lethal effect of Bt maize depends on the particular variety of Bt maize and the level of the Bt toxin produced as well as the amount of pollen that spreads, and how far.<sup>9</sup>

Wise policy making will need to take into account the relative effects on Monarch mortality of chemical insecticide, the spraying of tons of the *Bacillus thuringiensis* bacteria, and the use of genetically modified plants, as well as the crop yields, costs per acre, and the local conditions (such as the abundance of Monarchs, and the timing of larval feeding compared to pollen production).

Another environmental concern is that pest-resistant plants can spread, through seed dispersal or transfer of pollen to wild relatives of crop plants. This could lead to insect resistant weeds. A paper published in *Nature* this

<sup>6</sup> Biotech Knowledge Center. 1999. Reference No. 1653, Monsanto company, July 7 On line at <http://biotechknowledge.com/showlibsp.php3?uid=1653>.

<sup>7</sup> J.E. Losey, L.S. Rayor, and M.E. Carter. *Nature* (London) 399, p. 214, 1999.

<sup>8</sup> L.C. Hansen and J.H. Obrycki. 'Field deposition of Bt transgenic maize pollen: lethal effects on the monarch butterfly', *Oecologia*, 19 August 2000. Online at <http://link.springer.com/link/service/journals/00442/contents/tfirst.htm>

<sup>9</sup> D.S. Pimentel and P.H. Raven. 97 pp. 8198-8199, 2000.

month reported that several crop plants (e.g., rape, maize) made resistant to the herbicide glufosinate or containing Bt genes, did not survive beyond two years in natural habitats.<sup>10</sup>

Another concern is that the insect (and other) pests may develop resistance to the agent in the GMO. Such resistance is already a problem with respect to chemical insecticides and to the use of naturally occurring resistance genes introduced into crops by traditional plant breeding. It has also been observed in Hawaiian organic water cress as a consequence of heavy doses of the Bt bacteria, a situation unrelated to the use of GMOs.<sup>11</sup>

The development of resistance to all insecticides is a fact of life for farmers just as resistance to antibiotics is a medical problem. That's one reason farmers constantly look for new ways to control pests, including Bt maize and cotton.

Many of these environmental concerns can be mitigated by the nearby planting of unmodified crops. Since January of last year, farmers in the US have been required, by the EPA, to plant as much as 20 to 50 percent of their acreage in conventional maize to decrease the probability of these sorts of concerns.<sup>12</sup> There are ongoing discussions about whether this is sufficient, but the general principle is embedded in US regulatory structure.

These are complex matters. We should not be acting on 'hunches' or preliminary findings, or irrational concerns but on thoughtful, informed analysis. In the US, we do have mechanisms for such analysis and regulation. It is important that the regulatory process be monitored so that it is rigorous, open, transparent, and well-enforced so that the public can judge for itself whether or not its interests are being served.

Thus far I have been describing those aspects of the use of GMO's that can be addressed by science. There are other issues being raised for which science can provide at best limited useful information. For many, food is a personal and cultural issue, not a scientific one, as the stories about potatoes and tomatoes demonstrate. Surely we all need to recognize that it is reasonable for people to have choices about what they themselves eat. This is an argument for labeling foods, if scientifically valid and informative labels can be devised. However, this argument is sometimes carried to extremes. One example is the new 'golden rice', which has been

<sup>10</sup> M.J. Crawley, S.L. Brown, R.S. Hails, DD. Kon and M. Rees, *Nature*, 409, pp. 682-683, 2001.

<sup>11</sup> F. Gould. *Private communication*.

<sup>12</sup> Rick Weiss, *Washington Post*, p. A2, 16 January 2000.

engineered to produce significant amounts of beta-carotene, the precursor to vitamin A. The hope is that widespread use of golden rice can ameliorate the blindness suffered by many in Asia and Africa because of dietary deficiency in vitamin A. Yet, some argue that golden rice cannot succeed because it will not be palatable to people accustomed to white rice. That is a choice that affected populations will make for themselves. Personally, I find it hard to imagine that people anywhere would be willing to see their children go blind rather than change their habits.

One set of arguments in opposition to the use of GMO's is especially difficult for scientists. These are the arguments that derive from a sense that such plants are not 'natural'. What, after all, is natural? Certainly not our standard diets, derived as they are from millennia of careful, directed breeding. All species tend to alter the natural world starting with the earliest photosynthetic organisms that increased the meager 'natural' levels of oxygen in the air. Yes, surely we need to protect our planet and all the organisms that share it with us. We need to adopt from the new technologies, those elements that can help us do that. One conundrum illustrates for me the difficult choices that need to be made. Some experts believe that the older breeding methods have achieved just about as much as they can in terms of the productivity of current farm land and water. In many parts of the world, the response to this is to clear more and more forest and cultivate more and more land to feed increasing populations. Yet, most people agree that preserving forests is essential for the balance on the planet and the preservation of biological diversity. The new genetic engineering techniques appear to offer potential for improving the productivity of current agricultural land and water and saving forests. How do we choose?

Novelty engenders suspicion. In time, familiarity and particularly obvious utility, tend to dissipate those suspicions that were ill-founded to begin with. When benefits become clear, especially if they are directly to individuals and not just to farmers, the public view may become less suspicious. Twenty years ago a debate raged over the production of useful therapeutic agents through genetic engineering. Like the engineered plants today, people argued that the methods were dangerous, unnatural, even immoral. Today, few people object to human insulin, or growth hormone, materials that are made by recombinant DNA techniques.

There are other aspects of the vocal opposition to genetically modified (GM) plants that elude scientific consideration. One is antagonism to the practices of large agricultural industries. Another is that the com-

mercialization of the plants means they will be unavailable in developing countries, where they may be most important to alleviate starvation. This is a legitimate concern because an estimated 80 percent of the new plants have been developed by companies. We should surely strive to avoid injustices like those associated with the limited distribution of drugs to fight AIDs.

Yet another aspect of the opposition comes from the organic food industry which can hope to advance itself through this campaign. The industry lobbied hard to include, in the official U.S. definition of organic, the absence of GMOs, although organic farming techniques could benefit greatly from the use of certain GMOs. At least parts of the opposition to GMO's are also violent and disrespectful of law and private property. In Europe, the willful destruction of greenhouses, laboratories, and experimental fields has been going on for years and similar acts are now occurring in the U.S.

None of this is to say that the promoters of GMO's are blameless. Several large corporations invested heavily in the development and production of seeds of genetically modified plants. They have been aggressive in promoting the sale of these seeds. The concerned public is suspicious of their promotional emphasis on the value and harmlessness of the plants. Suspicion is fed by the fact that the corporations have not tried hard to develop a labelling system that will be informative and scientifically sound. Can the 6 billion people on earth now and increasing numbers in the future be adequately and economically fed without the investments and products of the large companies? Some think so. But even in the U.S., billions of dollars of food is lost to insect and nematode pests each year.

There is a moral imperative to feed and improve the health of all the world's people while preserving our planet. The public needs to decide whether to support the development of GMO's that can bring, on balance, real advantages to agriculture, health and the environment. With careful attention, we can avoid situations that result in harm and reap a good harvest.

# MOLECULAR EVOLUTION: COMPARISON OF NATURAL AND ENGINEERED GENETIC VARIATIONS

WERNER ARBER

## *Abstract*

Genetic engineering implies deliberate alterations of the genomic information of the considered organism. Such alterations may concern a local variation of the inherited nucleotide sequence, it can involve a structural rearrangement of genomic sequences, e.g. by bringing a given gene under a different expression control signal, and it can involve the addition of genetic information taken from another organism. These processes closely resemble the spontaneous generation of genetic variations, which represent the substrate for natural selection. A critical comparison of similarities and differences in the formation of genetic variations in genetic engineering and under natural conditions of biological evolution can represent an essential help in the evaluation of conjectural risks of genetic engineering.

## *Introduction*

Genetic engineering was developed in the 1970's as a novel research strategy on the basis of scientific knowledge that had mainly been acquired in microbial genetics. In the meantime this strategy has proved to be an extremely powerful tool both in basic genetic research and in biotechnological applications (Sambrook *et al.*, 1989). The strategy includes steps of combining genetic information of sometimes different origin and/or steps of site-directed mutagenesis. These kinds of designed genetic variations are compared here to naturally occurring genetic variations known to be the driving force of biological evolution (Arber, 2000). Such comparison is a welcome contribution to the evaluation of conjectural risks of gene technology.

### *Background knowledge*

Genetic information is contained in the linear sequence of nucleotides or base pairs in very long filamentous molecules of DNA. One can compare the genetic message with a written message in our common way of communication, by setting one nucleotide or base pair equal to one letter. In this comparison, the bacterial genome, i.e. the entire genetic information of a unicellular microorganism, corresponds to the content of a book. Depending on the kind of bacteria considered, the book can be more or less thick. Higher organisms have considerably larger genomes. The human genome, for example, corresponds to a library of roughly 1000 books.

The gene is the functional unit of the genetic information. Its size is quite variable from one gene to another. A gene may be as short as 100 nucleotides or as long as many thousand nucleotides, hence corresponding to a few lines or to one or a very few pages of the genetic library. The two essential parts of a gene are, on the one hand, the information serving for directing the synthesis of a gene product (usually a protein) and, on the other hand, the nucleotide sequences serving to control time and efficiency of gene expression, i.e. the synthesis of the gene product.

Following the definition generally used in molecular genetics, we define here as a mutation any alteration of the inherited nucleotide sequence. A mutation may affect a gene by changing either the reading frame serving in the biosynthesis of the gene product or else an expression control signal. In the former case the property of the gene product may become altered, while in the latter case the availability of the gene product may change.

### *Major components of gene technology*

Due to its large size, the genome is generally too big to serve directly in analytical studies of gene structure and function. Hence, specific DNA segments must be purified from the bulk of DNA and ideally highly amplified to have enough material available for further studies (Sambrook *et al.*, 1989). This can be accomplished as follows. Entire DNA is extracted from the organism under study. This DNA can be cleaved into specific fragments by using restriction enzyme preparations. If needed, the resulting DNA fragments can be specifically sorted out by electrophoretic separation. In a next step DNA fragments can be spliced into a so-called gene vector. This is often a viral DNA molecule or a bacterial plasmid. Both of these are able to replicate in an appropriate host cell. Therefore, if the product of *in vitro* recom-

bination between a vector and a DNA fragment is introduced into a host cell, this hybrid product will undergo replication, i.e. the passenger DNA becomes amplified together with its vector DNA. The reproduced DNA segment of interest can then easily be sorted out and used for further studies.

An efficient alternative method to amplify selected stretches of DNA is the polymerase chain reaction (PCR). This is carried out *in vitro* by using a DNA polymerase preparation. It requires as primers short oligomeric chains of DNA complementary to the two sites on the DNA between which the amplification will occur. Hence, for the PCR amplification short partial DNA sequences flanking the DNA to be replicated must already be known.

Highly amplified segments of DNA can serve for the determination of their nucleotide sequence by using appropriate chemical methods. If this process is carried out with a series of different DNA fragments prepared from a larger DNA molecule, the sequences of the fragments can be joined together like a puzzle. Upon reiteration, this process can eventually result in the availability of the sequence of an entire genome.

A next step in experimental investigations can be a search for open reading frames as potential parts of genes as well as for potential expression control signals for such genes. However, the presence of an open reading frame does not necessarily indicate the presence of a functional gene, nor could it generally reveal what kind of biological function the suspected gene would carry out.

In order to find answers to these questions, mutations are deliberately introduced into the DNA sequences under study. There is a number of particular strategies to do so (Sambrook *et al.*, 1989). For our present discussion it is sufficient to refer in general terms to the principles of such site-directed mutagenesis. By either *in vitro* or *in vivo* methodologies a given DNA sequence can be altered by introducing additional nucleotides, by provoking substitutions, by deleting inherited nucleotides, or by a combination of these strategies.

In any case, the consequences of such manipulations will then be investigated by comparative studies of the functional characteristics of the mutated genetic information and of the unaltered parental DNA. These studies are normally carried out in the concerned living organisms with appropriately altered genomes. Phenotypic differences point to an impairment or an improvement of particular biological functions by the introduced mutation. This can guide the researcher in the identification of the gene function under study.

*Research strategies of classical genetics and of molecular genetics differ*

Investigations of both classical genetics and modern molecular genetics are based on the availability of mutants. However, we must be aware that there are fundamental differences between these two approaches. These differences relate to the definition of a mutation and to the principle of the research approach.

In classical genetics, spontaneous or mutagen induced mutants are first identified by an altered phenotype, hence at the level of a biological function. Inheritance of an altered phenotype into the progeny indicates that an identified phenotypical alteration is due to a mutation on the genome. Genetic crosses between independent mutations serve to establish genetic maps. These are normally linear, but give no hint as to the chemical nature of the carrier of genetic information. Nevertheless, based on today's scientific knowledge, we can conclude that classical genetics starts from the biological function and ends up with a map of sites of mutations on the genome, i.e. on the DNA molecule.

In contrast, investigations in molecular genetics, which is sometimes also called "reverse genetics", start with a segment of DNA and they aim to identify the biological function encoded by that DNA. To do so, the DNA is isolated as we have already discussed, amplified and its nucleotide sequence is determined. The sequence is screened for potential open reading frames, expression control signals and other controlling elements. The inherited sequences are then deliberately altered at expected strategic sites. The altered sequences are compared with the unaltered parental sequences with regard to their effects on biological functions in the concerned organisms. Changes in the phenotype become manifest at that moment, and this can guide the researcher in the identification of the biological function encoded by the DNA sequence in question. Principally this strategy can be applied to any gene as well as to any living organism, although it will not always immediately lead to valid conclusions, depending on the possibility to identify the respective phenotypes.

While in the classical genetic approach the researcher cannot know the molecular nature of the mutation responsible for an altered phenotype, the investigator in reverse genetics designs himself the site-directed mutation and novel sequence combinations, and he can verify the result of his intervention by sequence analysis and other molecular genetic approaches. Any given genetic alteration introduced by genetic engineering into a genome may concern one to a few letters in the genomic library,



in other cases a few lines, a part of a page or at most a few pages. Some of such alterations are additions, others deletions and sometimes substitutions or reshufflings of sequences. In any case, the alterations generally affect a very small part of the genome. Remember that the genome of higher organisms represents a library of a large number of books. As we will discuss later, this strategy of genetic alteration in small steps is quite essential for the maintenance of viability. This has to do with natural selection.

*The impact of genetic engineering on biotechnology*

Efficient new impulses were given to biotechnology by gene technology as it was developed in the 1970's. As a matter of fact, genetic engineering led to strategic changes in biotechnological applications of scientific knowledge.

Classical biotechnology, including agricultural practicing, uses organisms as originally found in nature on the basis of some properties which were identified as useful for human applications. In more recent times breeding between related forms of organisms has served and still serves to obtain more productive and qualitatively more valid recombinants. Thereby, mutagens are sometimes used to increase the chance to find organisms with improved properties. In these investigations, systematic screening for phenotypes serves to identify rare derivatives responding to the expectation, while neither the relevant DNA sequence changes nor any other mutations resulting from the applied mutagenesis are usually identified.

In contrast, modern biotechnology relying on genetic engineering is scientifically much more straightforward and precise. First of all, biotechnological applications can profit of knowledge on specific biological functions as revealed by basic research in molecular genetics. Secondly, genetic engineering can help to improve a particular gene product, if relevant, by site-directed mutagenesis within the open reading frame of a particular gene. Similarly, by genetically altering an expression control signal, the yield of a gene product can be improved. In this latter case, this can, for example, imply the fusion of a reading frame with another, unrelated expression control signal. Finally, and most importantly for some biotechnological applications such as the biosynthesis of natural products for the use as medical drugs, the relevant genes can be introduced into the most appropriate organism for the synthesis of the gene product in question, rather than to use the natural host of the relevant gene.

*Conjectural risks of genetically modified organisms*

So far, strict natural laws which could enable the researcher to precisely predict the functional consequences of any given specific alteration in the genome sequences are not available. Gene technology and its application in biotechnology have thus to rely on experimental investigations of the outcome of any intervention in the genetic outfit of an organism. Predictions and expectations are often fulfilled, but with many exceptions. For example, a possible consequence of a genetic alteration is that the produced organism is not viable due to a major disturbance of the functional harmony causing a severe selective disadvantage. Most researchers working with genetic engineering have experienced this response of nature to one of their genetic constructs. Of more severe concern are those consequences of genetic alterations which might, for example, cause pathogenicity or more generally undesirable long-term consequences on the environment. To avoid such effects, guidelines to carry out the work with appropriate care and controls have been introduced at an early time in the 1970's. Still today, the scientific basis to give green light for some novel applications is sometimes relatively weak, particularly if an application involves the deliberate release of a genetically modified organism into the environment.

A better understanding of the process of biological evolution at the level of the biologically active molecules might be a welcome answer to these pending questions. As a matter of fact, any designed alteration of genetic information represents a reflected construction of a genetic variation. As we have seen, such variations can, in terms of the comparison already made, affect one or a few letters, a few lines, or one to several pages of added, deleted, substituted or scrambled genetic information in the context of the inherited genetic library. To which degree does this correspond to genetic alterations occurring spontaneously any time in genomes, upon the generation of natural genetic variations which form the substrate for natural selection in biological evolution?

*Bacterial genetics reveals the process of molecular evolution at work*

Bacteria are haploid unicellular organisms. They propagate by cell division and thus grow exponentially. As long as plenty of nutrition is available, growth is fast, typically with generation times in the order of 30 minutes. Occasionally, a cell may suffer a spontaneous mutation. Therefore, the propagating clone representing the progeny of a single cell contains an

increasing number of different genetic variants. In view of the haploid nature of the genetic information of bacteria, genetic variants express their phenotype rapidly. At any time the mixed population of parental and mutant cells is submitted to evolutionarily relevant natural selection. For these reasons bacteria are very well suited for population genetic and evolutionary studies. In particular, methodology now available in molecular genetics has made it possible to investigate the molecular nature of individual spontaneous mutants in bacterial plasmids, viruses and even in the chromosome.

On the basis of a wealth of genetic data made available in the literature it has become clear that a relatively large number of particular sources of spontaneous mutagenesis is at work in bacteria. It is possible, however, to group these sources into a few principal strategies followed by nature to ensure a steady, but slow influx of novel genetic variants into the microbial populations (Arber, 1995, 1999, 2000).

The three main strategies of genetic variation in bacteria are:

(1) *Small local sequence changes*, such as the substitution of one nucleotide by another, the deletion of one or a few nucleotides, the insertion of one or a few nucleotides, or a local scrambling of the inherited nucleotide sequences. The causes for such mutations are seen in replication infidelity, nucleotide instability and in the action of many known mutagens.

Genetically encoded repair systems keep the effects of this mutagenic strategy at low, tolerable frequencies. Such repair is of increasing importance for larger genome sizes. This has to do with the generally observed fact that spontaneous mutants are only rarely useful in terms of evolutionary adaptation and advance. More frequent are lethal mutations and variations providing selective disadvantage. Without repair activities this could lead to the eradication of organisms with large genomes. Of course, many DNA sequence alterations may remain without immediate effect on the organism's phenotype; they are neutral or silent.

(2) *Intragenomic DNA rearrangements*. These are mostly influenced by enzyme-mediated recombination processes, such as general (homologous) recombination between largely homologous sequences at different genomic locations, site-specific recombination (including DNA inversion) involving also deviations from the consensus crossover sites and last, but not least, transposition of mobile genetic elements.

(3) *DNA acquisition* based on the horizontal transfer of genetic information between different bacteria. The well understood processes of horizontal gene transfer are: transformation by free DNA molecules, conjuga-

tion mediated by conjugative plasmids, and bacteriophage-mediated transduction in which the virus serves as a vector for bacterial host genes.

These three strategies of genetic variation differ in the quality of their evolutionary contributions. The local sequence change, on which the evolution biologists base the molecular clock to measure evolutionary times of separation of related organisms, is a slow, stepwise process from which novel biological functions can result. However, this process gains in efficiency only when expressed functions start to represent a substrate for natural selection. Hence, local sequence changes must mainly contribute to the amelioration of available biological functions and it can also serve for a steady adaptation to changing living conditions.

DNA rearrangements can bring about novel combinations of available capacities, particularly by the fusion of functional genetic domains of different origin and by the combination of a given reading frame with another, previously unrelated expression control signal. DNA rearrangements thus contribute both to the improvement of existing gene functions and - by assembling different functional domains and sequence motifs - to the innovative generation of novel gene functions.

DNA acquisition is a quite efficient evolutionary strategy. It is a sharing in successful developments made by other organisms. The chance that a particular novel function will also exert its actions in another genetic context is often quite high.

As to the extent of genetic information involved in intragenomic DNA rearrangements and in DNA acquisition it is in general relatively small, typically involving between a part of a page and a few pages of the genetic library. One may assume that very exceptionally larger texts may become acquired without seriously disturbing the functional harmony of the recipient. Such events could perhaps account for cases of a sudden emergence of complex novel properties which cannot easily be explained otherwise. But in general, DNA acquisition as well as intragenomic DNA reshuffling proceed in small steps involving one or a very few genes or even only part of a gene such as a functional domain.

*Products of evolution genes together with nongenetic elements generate spontaneous genetic variations*

As was already mentioned, genetic variants are the substrate for natural selection and they represent the driving force of biological evolution. The theory of molecular evolution postulates that the products of specific

evolution genes are important actors in the steady supply of genetic variations (Arber, 1997, 2000). In the outline of the three distinct natural strategies of the generation of genetic variations we have repeatedly encountered gene products involved in spontaneous mutagenesis. Many of these products are inessential for the normal bacterial life cycle from one cell division to the next. This, and the fact that such genes are widely present in microorganisms, is consistent with the postulate that they primarily serve biological evolution and exert their activities at the level of populations.

One can distinguish between two major types of action of evolution gene products. One class englobes the enzymes which actively generate genetic variants. Examples for this class are transposases and various other types of DNA recombinases involved in DNA rearrangements. Interestingly, this kind of gene products acting as variation generators do not and cannot conform with what one usually expects from gene activities; they work inefficiently and non-reproducibly. The second class is formed by those gene products which modulate the frequencies of genetic variation to low levels that are tolerable for the long-term maintenance of a given strain or species. Examples for this kind of evolution genes are those belonging to DNA repair systems. A good example for evolution genes limiting the frequency of horizontal transfer of DNA and at the same time stimulating occasional DNA acquisition to occur in small steps are restriction enzymes.

A number of non-genetic factors contribute each in its specific way to the generation of genetic variants. These factors include the chemical instability and the structural flexibility of biologically active molecules, environmental mutagens and random encounters (Arber, 2000).

In brief, nature makes use of naturally given factors that contribute to the generation of genetic variations and in addition it has developed enzyme systems to provide a steady influx of novel genetic variants of different qualities serving as substrate for natural selection. This principle ensures the maintenance and renewal of a rich genetic diversity in the living world.

At present time, there is ample evidence from microbial genetics for the views expressed here on evolutionary strategies. It is likely that precisely these microbial capacities might have been instrumental together with symbiotic associations (Margulis, 1981) for the evolution of higher eukaryotic and multicellular organisms. We can thus expect that the further evolution of higher organisms also benefits from activities that are exerted by evolution genes and that contribute together with non-genetic factors to the steady, but not excessive generation of genetic variations. An increasing number of circumstantial evidences is in support of this view (Caporale, 1999).

### *Conclusions and Outlook*

The strategies used in genetic engineering for the production of genetic variations resemble quite closely the natural strategies of generating genetic variants. In both instances, three major strategies with qualitative differences can be distinguished, namely small local sequence changes, intragenomic sequence reshuffling and DNA acquisition involving horizontal gene transfer. Recombinant DNA technology was, as a matter of fact, developed on the example of naturally occurring horizontal gene transfer involving vector DNA molecules. Similarly, site-directed mutagenesis simulates the natural strategies of either local sequence change or DNA rearrangement. Similarities are also seen in the extent of sequences involved in these processes, either natural or designed in genetic engineering. One might thus argue that what is done by design in the laboratory must have occurred already sometimes in nature. However, this rapid conclusion is not strictly correct, certainly not for any very specific DNA sequences resulting either from a genetic manipulation or from the natural generation of a genetic variant. This can be seen in the following reflexions on the uniqueness of DNA sequences longer than about 80 base pairs.

We can estimate today's number of living cells on our planet to be roughly  $10^{30}$  (Arber, 1993). In the course of time this number may somewhat fluctuate but empty places due to cell death become rapidly reoccupied. Assume that this was so for the last three billion of years, which is  $10^{15}$  minutes. Let us also assume that during that long time period each genome would have explored 1000 novel sequences each minute. This corresponds to a mutation rate much higher than what is usually observed. Under these conditions the total number of already explored sequences would be  $10^{30} \times 10^{15} \times 10^3 = 10^{48}$  different sequences. With the four different nucleotides of DNA available,  $10^{48}$  corresponds to the number of different closed sequences that can be written with the length of only 80 base pairs. Therefore, novel DNA sequences longer than 80 base pairs have a fair chance to be universally unique and many possible sequences are likely to have never existed. This is surprising, since 80 bp is much shorter than the length of an average gene of about 1000 base pairs!

In view of these considerations it appears justified to maintain the introduced precautions and to carefully explore properties of novel genetic constructions before they are introduced into the environment, although the chance of undesirable effects is very low. Indeed, the same kind of uncertainty with regard to long-term effects must apply not only to

designed genetic variants but also to spontaneous mutations and, at least as far as local sequence changes and some DNA rearrangements are concerned, to mutagen induced genetic variants. In the past, these processes have only very rarely been noted to have caused undesirable effects such as increased pathogenicity of the resulting variant subclones.

In this context, it is relevant to note that up to now, not a single completely novel gene has been invented by genetic engineers. All functional DNA sequences so far involved in experimentation have been taken from natural, living organisms. It is plausible that at some future times entirely novel genes can be designed on the basis of by then increased scientific knowledge. Special care should be taken at that time to widely explore properties and effects of such biological functions before they become introduced into the environment.

To finish, let us briefly discuss a difference between natural biological evolution and evolutionary steps due to genetic engineering. As far as we know, nature has not developed any specific sensory organ that would enable an organism to identify any evolutionary need under particular living conditions and to react by preparing the appropriate mutations which could overcome novel selective disadvantages. Rather, nature follows the principle of producing more or less randomly enough different genetic variants to ensure evolutionary progress. In contrast, the genetic engineer reflects on possible developments which could serve his expectations. Although not all designed genetic modifications keep their promise, some biotechnological applications may guide a branch on the tree of biological evolution to grow into a specific direction.

This kind of reflexions would deserve to be deepened as well as extended into considerations on possible effects of various human activities on natural selection which largely influences the directions taken by biological evolution.

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# CONSTRAINTS ON THE ORIGIN AND EVOLUTION OF LIFE<sup>1</sup>

CHRISTIAN DE DUVE

As an introduction to my topic, let me quote from the 1970 bestseller, *Chance and Necessity*, by the late Jacques Monod. In this “essay on the natural philosophy of modern biology”, as the book is subtitled, the celebrated French biologist attempted, as many have done before and after him, to derive some sort of *Weltanschauung* from the science of his day. On the existence of intelligent life on Earth, Monod wrote the famous sentence: “The Universe was not pregnant with life; nor the biosphere with man”.<sup>2</sup> He did not mean this literally, of course, considering that the Universe did give birth to life and the biosphere did give birth to human beings. Birth without pregnancy would imply a miracle, which is certainly not what Monod had in mind. What he meant is that life arose through the combination of highly improbable circumstances, so improbable that life may have arisen only once in the whole history of the Universe and might well, but for a fantastic stroke of luck, never have arisen at all. Given the fact that life did arise, the probability that it would evolve into intelligent beings is once again, according to Monod, extremely low. In other words, we owe our existence to the succession of two extremely improbable events, a near-miracle squared, so to speak, a cosmic quirk. And Monod concludes: “Man knows at last that he is alone in the Universe’s unfeeling immensity, out of which he emerged only by chance”.<sup>3</sup> Beautiful poetry, but somewhat shaky science. I propose to explain briefly why I disagree with my late friend Jacques Monod.<sup>4</sup>

<sup>1</sup> Read 14 November 1997.

<sup>2</sup> Jacques Monod, *Chance and Necessity*, translated from the French by A. Wainhouse, New York: Knopf (1971), 145.

<sup>3</sup> *Ibid.*, 180.

<sup>4</sup> For more details see my *Vital Dust*, New York: BasicBooks (1995).

Let us look at the origin of life first. I will do so succinctly, because Monod's view is no longer shared by many scientists today. According to the most recent observations, we have two landmarks. On one hand, there is the evidence that living organisms, most probably primitive bacteria, were already present on Earth some 3.6 billion years ago, perhaps earlier. On the other, there are the many messages, relayed by radiation from outer space or provided directly by the analysis of comets and meteorites, indicating that the Universe is a hotbed of organic syntheses leading, among others, to amino acids and other typical building blocks of life. This "vital dust"<sup>5</sup> permeates the entire Universe and most likely represents the chemical seeds from which life arose. The problem of the origin of life is, how did these simple molecules interact and combine to give rise to the first primitive cells?

There are two possible approaches to this question: bottom-up and top-down. The paradigm of the bottom-up approach is Miller's historical experiment of 1953.<sup>6</sup> He simply attempted to re-create the chemical conditions he believed, on the strength of a hypothetical model developed by his mentor, Harold Urey, to have prevailed in the atmosphere in the early days of our planet. In no way was he trying to make amino acids. They just happened to be made, by processes that may be related to those now known, from the the analysis of meteorites, to operate on some celestial bodies. No other experiment in abiotic chemistry carried out since is so purely of the bottom-up kind. Workers have always had some substance or substances in mind in setting up their experimental conditions. To be true, they have chosen those conditions to be of the kind that might possibly have obtained on the archaic Earth, often, however, stretching this compatibility to, or even beyond, the limits of plausibility.

Ideally, the top-down approach starts from existing biochemical processes and tries to reconstruct the simpler ancestral mechanisms from which they could have been derived. It is often assumed that this approach is unlikely to be fruitful because the pathway from abiotic to biotic biogenesis must have involved so many changes that hardly any trace of the early chemistry can have been left in present-day biochemistry.<sup>7</sup> There are, however, reasons to believe that this may not be so,<sup>8</sup> in

<sup>5</sup> See note 4.

<sup>6</sup> S.L. Miller, "A Production of Amino Acids under Possible Primitive Earth Conditions", *Science*, vol. 117, pp. 528-29 (1953).

<sup>7</sup> Witness this quotation from *The Origins of Life* (Englewood Cliffs, NJ: Prentice Hall, 1973) by S.L. Miller and L.E. Orgel, two of the major experts in the field. Referring to the

which case origin-of-life research might benefit from a greater input by biochemists than it has enjoyed so far.<sup>9</sup> In the meantime, we can look at the problem in a more general way.

To me, the key word here is *chemistry*. Life is a chemical process, which relies entirely, including its all-important informational aspects, on the operation of specific molecules – proteins, nucleic acids, carbohydrates, lipids, and other typical components of living beings. Likewise, the origin of life was an essentially chemical process, or rather a long succession of intricately interwoven chemical processes. Now, chemistry deals with highly deterministic phenomena, which depend on the statistical behavior of huge numbers of molecules of different kinds and owe hardly anything to chance. Given a specific set of physical-chemical conditions, the same reactions always take place in the same manner. Applying this rule to the origin of life, I conclude that, given the conditions that prevailed on Earth four billion years ago – or wherever and whenever Earth life originated – the chemical processes that gave rise to life were bound to take place. Given the same conditions elsewhere, life would similarly arise there.

This view is reinforced by the fact that a very large number of steps must have been involved. Something as complex as a living cell cannot possibly have arisen in one shot, or even in a small number of steps. That would require a miracle. Now, if there are a great many steps, the probability of reaching the end of the chain within realistic confines of space and time soon approaches zero if the probability of each individual step is even moderately low.<sup>10</sup> My conclusion therefore, is, using Monod's terminology: the Universe *was* pregnant with life. In other words, we belong to a Universe of which life is a necessary component, not a freak manifestation. This view

possibility that "metabolic pathways parallel the corresponding prebiotic syntheses that occurred on the primitive earth", the authors write: "It is not difficult to show that this hypothesis cannot be correct in the majority of cases. Perhaps the strongest evidence comes from a direct comparison of known contemporary biosynthetic pathways with reasonable prebiotic pathways – in general, they do not correspond at all" (p. 185). But what is reasonable?

<sup>8</sup> See C. de Duve, "The RNA World: Before and After?" *Gene*, vol 135, pp. 29-31 (1993).

<sup>9</sup> My proposal of a "thioester world" is an example. Günter Wächtershäuser's "iron-sulfur world" is another. For details see my *Blueprint for a Cell* (Burlington NC: Neil Patterson Publishers, Carolina Biological Supply Company, 1991).

<sup>10</sup> To set an order of magnitude, a chain of 100 steps, each with a probability of 50%, has one chance in  $2^{100}$ , or  $10^{30}$ , of coming to successful completion. With 200 steps the chance is one in  $10^{60}$ . With an individual probability of 10%, the overall probability for a 100-step process is one in  $10^{100}$ .

implies that if, as a number of astronomers believe, many other Earth-like planets exist in our galaxy and elsewhere in the Universe, these planets are very likely to bear life, in a form not very different in its main chemical features from its form on Earth.<sup>11</sup> I won't expand further on this topic. I believe it to be accepted by a majority of scientists, certainly among biochemists.<sup>12</sup>

Let us now look at the second half of Monod's statement, namely that the biosphere was not pregnant with man or, more generally, with conscious, intelligent beings. Here, Monod is in much better company. The majority opinion among evolutionists today is that, given the enormous number of chance events that have traced the pathway from the common ancestral form of life to the human species, the probability of this outcome must be considered vanishingly small and its reproduction elsewhere extremely unlikely.<sup>13</sup> In the view of these experts, we are indeed alone, as Monod stated. There are,

<sup>11</sup> This point is implicit in the deterministic view of the origin of life that I defend.

<sup>12</sup> Examples are George Wald, who in 1963, alluded to the "dawning realization ... that life in fact is probably a universal phenomenon, bound to occur wherever in the universe conditions permit and sufficient time has elapsed" ("The Origin of Life", in Philip Handler, ed., *The Scientific Endeavor*, New York: Rockefeller University Press, 1963, pp. 113-34; see p. 120); Albert Lehninger, who in his classic textbook *Biochemistry* (New York: Worth Publishers, Inc., 1st edition, 1970, p. 771), visualized the origin of life as "the result of a long chain of single events, so that each stage in their evolution developed from the preceding one by only a very small change", adding "each single step in the evolution of the first cells must have had a reasonably high probability of happening in terms of the laws of physics and chemistry"; and Manfred Eigen, who expressed the same view even more forcefully in 1971, writing: "We may furthermore conclude that the evolution of life, if it is based on a derivable physical principle, must be considered an *inevitable* process (italics are his), despite its indeterminate course ... it is not only inevitable in principle but also sufficiently probable in a realistic span of time. It requires appropriate environmental conditions (which are not fulfilled everywhere) and their maintenance. These conditions have existed on earth and must still exist on many planets in the universe" ("Selforganization of Matter and the Evolution of Biological Macromolecules", *Naturwissenschaften*, vol. 58, pp. 465-523, 1971, see p. 519). Nothing has happened since these various lines were written to make them less relevant today.

<sup>13</sup> Following are quotations from three prominent American evolutionists who express their beliefs in no uncertain terms: "The assumption, so freely made by astronomers, physicists, and some biochemists, that once life gets started anywhere, humanoids will eventually and inevitably appear is plainly false" (George Gaylord Simpson, *This view of Life*, New York: Harcourt, Brace & World, 1963, p. 267); "An evolutionist is impressed by the incredible improbability of intelligent life ever to have evolved" (Ernst Mayr, *Toward a New Philosophy of Biology*, Cambridge, MA: Harvard University Press, 1988, p. 69); "Wind back the tape of life to the early days of the Burgess Shale, let it play again from an identical starting point, and the chance becomes vanishingly small that anything like human intelligence would grace the replay" (Stephen Jay Gould, *Wonderful Life*, New York: Norton, 1989, p. 14.

of course, a number of astrophysicists who believe otherwise and have succeeded in obtaining considerable support for their project of searching for extraterrestrial intelligence (SETI).<sup>14</sup> But few biologists agree with them.

The case for utter contingency is very strong. According to all we know or have reasons to suspect, every single fork in the tree of life results from the coincidence in time and site between two chance events: an accidental genetic change or rearrangement affecting a given individual in a population and a set of environmental circumstances allowing the mutant individual to survive and produce progeny.<sup>15</sup> Between the common ancestral form of all life on Earth and humankind, thousands, if not more, such coincidences must have taken place. The conclusion is thus inescapable that we owe our existence to the succession of a large number of chance events. Hence the view, held by Monod and by a majority of biologists today, that we are the products of an extremely improbable chain of circumstances

Now, I want to make it clear that I am in no way questioning the first statement. I am not advocating some kind of woolly, holistic, finalistic, anti-Darwinian theory of evolution. I fully subscribe to the neo-Darwinian view, as substantiated and specified by the findings of modern molecular biology. What I am questioning, however, is the inference from chance to improbability. One does not enforce the other. *Chance does not exclude inevitability*. All depends on how many opportunities there are for an event to take place, as compared to its probability of actually taking place. Whatever the odds, an event becomes virtually bound to occur if you give it a sufficient number of trials. A flipped coin has one chance in two of falling on its heads side. But flip it ten times, and the odds of its doing so at least once are 99.9 percent. At roulette, some 250 spins of the wheel are needed to reach the same probability of 99.9 percent for a given number to come out at least once. In a lottery, the probability of a seven-digit number coming out in a single drawing is one in ten million. But with ten million drawings, the probability becomes two in three. And with one hundred million drawings, the probability is 9,999.5 in 10,000, close to certainty.<sup>16</sup> This

<sup>14</sup> See: F. Deake and D. Sobel, *Is Anyone Out There?* (New York: Delacorte Press, 1992).

<sup>15</sup> As stated, this is true only of microorganisms multiplying by simple division. Things are more complicated in the case of sexual reproduction, but the principle of each evolutionary bifurcation depending on the coincidence between two chance events, one genetic and the other environmental, remains valid.

<sup>16</sup> These values are readily computed by considering the probability of the event *not* occurring. Let this probability be  $P$ , then the probability of the event actually taking place is:  $1 - P^n$ , in which  $n$  is the number of trials.

will not help you win in a lottery. But things are different in the evolutionary lottery, which is played with millions, often billions or more, individuals, following each other, generation after generation, over periods of up to several million years. Within such a framework, the probability of a given mutation occurring under conditions conducive to its being retained by natural selection appears as very much higher than is often affirmed on the strength of little more than some kind of gut feeling.

It must be stressed that the number of possible mutations is not unlimited. It may be large, but it is finite, strictly limited by the size and structure of genomes. It is often relatively small when compared with the total number of mutations that occur in a given population.<sup>17</sup> Contrary to a commonly held opinion, evolution rarely has to wait very long for chance to offer a mutation that will be beneficial in a given set of circumstances. More often than not, the mutations are there, waiting, so to speak, for an opportunity to prove useful or, if merely neutral or not overly harmful, to provide a viable alternative that will later be advantageously exploited.

What we witness of evolution in action supports this contention. See, for example, how in just a few decades, organisms have become resistant to the substances used to kill them – bacteria to antibiotics, malarial plasmodia to chloroquine, mosquitoes to DDT, and so on. Note that some of these substances do not even exist in nature. It is clear that the resistance mutations did not occur as a response to exposure to the drugs. This would imply some sort of intentionality, which is ruled out by molecular biology. The mutations were always present or happened regularly, and

<sup>17</sup> As a very rough yardstick for such an estimate, consider the following. The average spontaneous mutation rate, as determined experimentally in bacteria (roughly the error rate of DNA replication throughout the living world), is on the order of  $6 \times 10^{-10}$  per base per replication (See F. Hutchinson, "Mutagenesis" in: F.C. Reinhardt, ed.-in-chief, *Escherichia coli and Salmonella, Cellular and Molecular Biology*, 2<sup>nd</sup> Edition, Washington DC: ASM Press, 1996, Vol. 2, pp. 2218-35). On the other hand, the number of possible point mutations (replacement of one base by another) is 3 per base pair. This means that  $3/6 \times 10^{-10}$ , or  $5 \times 10^9$ , replications – the number accomplished in one cycle by some 5 mg of dividing bacteria or 50 g of dividing mammalian cells – suffice for the number of spontaneous mutations to equal the total number of possible point mutations. Needless to say, this does not mean that all possible mutations will take place in just this number of replications. Chance may have some occurring several times, others not at all. Furthermore, not all mutations have the same probability of taking place. Finally many other genetic changes can occur beside simple point mutations. But the main message is clear. Given the huge number of individuals participating in the evolutionary lottery, mutations in most cases are not rare events.

we provided them with an opportunity to flourish by putting the drugs in the environment. Also revealing is the fact that chance can easily be solicited to provide a desired mutation. In the early penicillin days, cultures of the drug-producing mold were exposed to X-rays in the hope that mutants producing larger amounts of penicillin might arise. The yield of the precious drug was multiplied more than twenty-fold by this chancy device. It is a well known fact among molecular biologists that almost any desired trait compatible with the cells' general organization can be elicited in a population of growing cells by sufficiently stringent selection conditions, once again illustrating the enormous potential of chance mutations. In fact, there is now evidence that natural selection has retained a mechanism whereby bacteria enhance the mutability of parts of their genome under stressful conditions, where survival may depend on some rapid genetic readjustment.<sup>18</sup>

Once we accept mutations as banal rather than improbable, we are led to the conclusion that it is the environment that plays a major role in shaping evolution by providing the conditions under which given genetic chances will be selected. Which brings us back to chance. It is important here to distinguish between what I call horizontal and vertical evolution. Horizontal evolution is the kind that leads to *diversity* without significant change in body plan. Some 750,000 species of insects are known and several millions may exist. But all are insects. Here is where contingency was given an almost free rein, with all sorts of different environments screening all sorts of different variant forms of the insect body plan, thereby opening a multitude of distinct pathways that led, through the vagaries of circumstances, to forms as different as beetles and dragonflies, bees and praying mantises, as well as those astonishing insects that look for all the world like the branch or leaf they sit on. Incidentally, this extravagant profusion of forms is itself proof of the richness of the mutational field.

Things are very different when it comes to vertical evolution, the kind that leads to increasing *complexity*. Here, chance enjoys much fewer degrees of freedom, with inner constraints playing an increasingly important role. There are not so many ways of moving, for example, from a fish to an amphibian or from a reptile to a mammal, especially if every intermediate stage in this long transformation is to be viable and able to pro-

<sup>18</sup> Recent discussions of this topic are to be found in articles by B.A. Bridges, In *Nature*, vol. 387, pp. 557-58 (1997) and by E.R. Moxon and D.S. Thaler, *ibid.*, pp. 659-62 (1997).

duce adequate progeny under the prevailing conditions. These constraints become all the more stringent the greater the complexity of the developmental program undergoing the changes. Certain directions are thus imposed on further evolution, in spite of the purely fortuitous character of the underlying events. For example, different groups of aquatic animals have evolved different ways of adapting to terrestrial life, each within the constraints imposed by the existing body plan. Also, in vertical evolution, selective advantages tend to be more fundamental and less linked to trivial environmental factors than in horizontal evolution.

It is impressive that, both in animal and in plant evolution, there is a consistent rise in reproductive efficiency, from random, aqueous fertilization and development to increasingly protected forms of embryogenesis. Vertical evolution has successively produced spores, seeds, and, finally, flowers and fruits, in the plant line. Its main innovations in the animal line have been copulation and then the amniotic egg, developing first outside the animal's body, and then inside, with the help first of a marsupial pouch and later of a placenta. These improvements have not prevented each intermediate stage from diversifying its perfectly viable reproductive mode horizontally. Contrary to what is often maintained by critics of the notion of evolutionary complexification, accepting vertical evolution, does not imply denying horizontal evolution. Both proceed simultaneously to shape the tree of life.

Particularly remarkable, in animal evolution, is the unswerving vertical drive – with horizontal evolution producing side branches all along the way, of course – in the direction of polyneural complexity. Starting some six hundred million years ago with a necklace of about a dozen neurons circling the body opening of some primitive jellyfish, the complexity of the nervous system has consistently increased, culminating, in just the last few million years, in the stupendous three-fold expansion of the cerebral cortex in the human line. No doubt the environment played an important role in molding the details of this pathway – the change from forest to savannah is often cited as a significant factor in human evolution – but the overriding factor, surely, is the fact that a more complex brain is an asset in almost any circumstance. Viewed in this context, the emergence of humankind, or at least of conscious, intelligent beings, appears as much less improbable than many maintain. Contrary to what Monod stated, the biosphere *was* pregnant with man.

It has become fashionable, almost politically correct, to deny any significance to the emergence of humankind. We are just one little twig on the



tree of life, on par with plague bacilli, amoebae, oak trees, puffballs, scorpions, koala bears, and the millions of other species of bacteria, protists, plants, fungi, and animals that now exist or have existed in the past. Some even claim that bacteria are superior to us, just because there are more of them or because they can do all kinds of things, such as synthesizing vitamins or thriving in boiling water or in drying brine, that we are unable to accomplish.<sup>19</sup> This is utter nonsense, of course. Bacteria have not invented the wheel, decorated the walls of the Lascaux caves, written the *Divine Comedy*, composed the *Well-tempered Clavier*, discovered relativity or natural selection, or drafted the Ten Commandments or the Bill of Rights. In fact, no living organism other than human beings has accomplished anything approaching such feats, which one must be either deranged or dishonest not to view as immensely important and significant.

The nonsense would be harmless were it not presented as incontrovertible, scientifically established truth, and gleefully relayed by a number of philosophers, social scientists, writers, and journalists who, for some strange reason, appear to take a perverse pleasure in denigrating the human condition. This appeal to science in support of human bashing is, to put it mildly, unwarranted. We may, in some way, appear as a mere twig in the rich canopy of the tree of life. But trim the tree of its canopy and you see that our little twig obviously occupies the top of a trunk that, while continually extending ever more varied branches horizontally, has simultaneously grown vertically, over almost four billion years, in the direction of increasing complexity. To deny this is to deny what to most of us is self-evident.

This, however, is no reason for bragging. Our position most likely is temporary. It was occupied three millions years ago by a young primate called Lucy, and six million years ago by the last common ancestor we share with chimpanzees. What form of life will occupy it in the future is anybody's guess. It could well, in fact, go far beyond anybody's capacity to guess. The astronomers tell us that the Earth will be able to sustain life for another five billion years before it becomes engulfed in the fiery expansion of the dying Sun. If the tree of life goes on growing vertically, it may reach

<sup>19</sup> See, for example, *What is Life?* by L. Margulis and D. Sagan (New York: Simon & Schuster, 1995), and *Full House*, by S.J. Gould (New York: Harmony Books, 1996), and my reviews of these two books in *Nature*, vol. 379, p. 409 (1996) and vol. 383, pp. 771-72 (1996). See also Gould's presentation to this Society, "Redrafting the Tree of Life", *Proc. Amer. Philos. Soc.* Vol 141, pp. 30-54 (1997).

more than twice its present height. Extrapolating what has happened until now, this opens the possibility of mental powers that are simply unimaginable to our feeble means. This development could happen through further growth of the human twig, but it does not have to. There is plenty of time for other twigs to bud and grow, eventually reaching a level much higher than the one we occupy, while the human twig withers.

What will happen depends to some extent on us, since we now have the power of decisively influencing the future of life and humankind on Earth. One can only hope that the generations to come will carry out this awesome responsibility with greater wisdom than humankind has done so far.

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## CHALLENGES AND ACHIEVEMENTS IN INFECTIOUS AND AUTOIMMUNE DISEASES

MICHAEL SELA

Pasteur coined the expression “vaccine” from “vacca”, to honor Jenner, who pioneered vaccination against smallpox by making use of cowpox. These days the notion of therapeutic vaccines has been extended to cancer, autoimmune diseases and recently a vaccine approach has been envisaged even for Alzheimer disease. With recent advances in isolating and defining tumor-specific antigens and their genes, there is little doubt that specific tumor vaccines will become a significant factor in the armamentarium against cancer. The central hypothesis behind active vaccination for cancer treatment is that tumor cells express unique antigens that are capable of inducing a specific response. A proposed vaccine would have to deliver this antigen to the immune system, which would recognize it as foreign and destroy any cell bearing this antigen. Many of the tumor associated antigens in humans, however, are non mutated self proteins. Overcoming the tolerance of the immune system toward them is crucial for their utilization as anti-tumor vaccines, and this could be achieved by employing tumor-specific peptides.

### *Cancer*

There is a vast literature concerning this subject (1) and only a few examples can be given as illustration. Most efforts in the field of tumor vaccination have been in the area of melanoma, above all, around the family of MAGE genes and the proteins or peptides derived from them (2, 3), as well as around MART antigens (4). In human studies, 39 tumor-bearing patients with metastatic melanoma were treated with three subcutaneous injections of the MAGE-3 A1 peptide at monthly intervals. Of the 25

patients who received the complete treatment, 7 displayed significant tumor regressions. Three regressions were complete and two of them led to a disease-free state which persisted for more than two years (Thierry Boon and his colleagues in Bruxelles).

Humanized monoclonal antibody against the ErbB2-HER2 receptor, under the trade name Herceptin, is now being used successfully – in conjunction with chemotherapeutic drugs – against breast cancer in those individuals who exhibit a high density of the receptor on their cancerous cells. We have been studying for the last 10 years, together with Professor Yossi Yarden, monoclonal antibodies of similar specificity (5). Most of them inhibit tumor growth in nude mice, but some accelerated tumor growth. Among the inhibitory anti-ErbB2 antibodies, some acted after internalization, whereas others prevented the heterodimerization of ErbB2 with other members of the ErbB family.

### *Specificity*

Specificity is one of the most important considerations in the development of vaccines against infectious diseases, and consequently, small structural antigenic variations may lead to drastic changes in the efficacy of a vaccine. It is of interest that in the case of the first vaccine ever developed, against smallpox, the vaccine consisted of the heterologous cowpox virus, *Vaccinia*, that was not identical to the disease-causing pathogen. Yet it was capable of inducing very effective protection against infection and eventually led to complete global eradication of this dreadful disease. However, in the case of most viruses, even small antigenic changes resulting from genetic variation, lead to change of specificity and interfere completely with the protective capacity of the vaccine. Thus, in the case of influenza for example, a new vaccine is required almost annually to fend off the newly emerging strains.

I would like now to extend this concept of specificity to cancer and to autoimmune diseases (6). I mentioned before the MAGE genes and the studies of Boon. Similarly, Steven Rosenberg had success with MART genes, also in melanoma.

### *Autoimmune diseases*

Whenever it is possible to identify the putative cause of the disease, it should be possible to find a close molecular analog which will combat the disease. In one case of an autoimmune disease, that of multiple sclerosis,

we have succeeded in developing a drug/vaccine which has by now been approved by the FDA in the United States, as well as seventeen other countries (6, 7).

This drug – or vaccine – as I prefer to call it – is a polymer composed of four kinds of amino acids, and prepared so as to resemble and cross-react immunologically with the main troublemaker of the myelin sheath of the brain, the myelin basic protein (MBP). This myelin basic protein can provoke an experimental disease – allergic encephalomyelitis, and our substance, denoted by us Copolymer 1, or Cop 1 – can suppress the onset of the disease, and in rhesus monkeys and baboons, we showed that it can heal the actual disease. As this is an experimental model disease for multiple sclerosis, we moved to clinical trials. The phase 2 clinical trial was most successful. This was followed by several more big trials, before the FDA approved the drug/vaccine for daily injections for the exacerbating-remitting type of multiple sclerosis. We have proved recently that it can be given efficiently by oral route (8), and a trial involving 1800 participants is going on now in 18 countries. Copolymer 1 does not seem to have any effect on any other autoimmune disease.

Copolymer 1 is a potent inducer of Th2 regulatory cells in both mice and humans. Highly reactive Cop 1 specific T-cell lines were established from both brains and spinal cords of Cop 1 treated mice. In contrast, no reactivity to the control antigen lysozyme could be obtained in lymphocytes isolated from the central nervous system (CNS) of mice injected with lysozyme. Adoptively transferred labeled Cop 1 specific suppressor cells were found in brain sections seven and ten days after their injection to the periphery, whereas lysozyme specific cells were absent in the CNS (9). Cop 1 induced Th2 cells cross the blood-brain barrier and accumulate in the CNS, where they can be stimulated in situ by MBP and thereby exert therapeutic effects in the diseased organ.

In the same spirit we have approached another autoimmune neurological disease: myasthenia gravis, in which the disease is caused by an immunological attack on the acetylcholine receptor of our nerve cells. We are already successful in preparing a specific drug/vaccine against myasthenia gravis by limited amino acid substitution in two myasthenogenic peptides from the  $\alpha$ -subunit of acetylcholine receptor. The analogs formed can heal the experimental myasthenia gravis in mice and rats provoked by injecting the complete acetylcholine receptor of the Torpedo electrical fish (10), and we hope to start clinical trials next year. In principle, in every autoimmune disease in which you can put your finger on

a potential candidate causing the disease, it should be possible to produce a close chemical relative that will suppress the disease.

### *Alzheimer's disease*

From recent reports it appears that even immunization against Alzheimer's disease becomes a cogent possibility (11-13). One hallmark of Alzheimer's is amyloid plaque, a protein deposit that builds up in brains of those with the disease. In mice genetically engineered to develop an Alzheimer's-like condition, immunization with b-amyloid (Ab), the protein fragment that forms the plaque, reversed or prevented plaque formation and neural damage. The finding raised the possibility that immunization with Ab may eventually be used as a treatment, or prophylactically, for Alzheimer's disease. Indeed, a phase I clinical trial has begun with 24 patients. Trials in mice of a possible vaccine for Alzheimer's disease have shown that it reduces the behavioural defects and the brain damage seen in the disease, and it actually prevents memory loss. As promising as these results are, a human vaccine remains a long way off.

### *Infectious diseases*

Coming back to infectious diseases, WHO recently reported that almost two million people die from tuberculosis every year, malaria kills over 1 million people per year, mostly in Africa, and newly-released figures show that AIDS killed an estimated 3 million people in 2000. Coming back to tuberculosis, it is estimated that between 2000 and 2020, nearly one billion people will be newly infected, 200 million people will get sick, and 35 million will die from tuberculosis, so we are badly in need of a successful vaccine.

### *Concluding remarks*

We understand well many immunological phenomena at the cellular and molecular level, but the increase in our knowledge also extends the definable 'unknown'. Today we wish to understand not only the role of antibodies and immune cells, the signals triggering them and the various soluble macromolecules which immunocytes spill out, but also the pathways the signals provoke, the nature of the movement of the cells and the extent to which this knowledge may help us to develop newer and better drugs and vaccines, and as the next generation struggles with these problems, they too

will learn that the more the sphere of knowledge grows, the larger becomes the surface of contact with the unknown.

For all these approaches to diseases, we must work together, as one world, globally. Globalization describes trends dramatically and relentlessly, increasing connections and communications among people, regardless of nationality and geography. But globalization without integration leads to a Babel Tower. So to improve health, and I mean first of all the developing world – we need both globalization and integration of our efforts. And this must be done with great speed, as standing still is the fastest way of moving backwards in a world that is changing at an ever more rapid pace.

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## CARLOS CHAGAS FILHO: SCIENTIST AND HUMANIST

RITA LEVI-MONTALCINI

After receiving a degree in medicine from the University of Turin in 1936, I attended the Institute of Anatomy and the clinic of neuro-psychiatry because I was attracted both by pure research and by the practice of the medical profession for which I felt I had special aptitudes. My uncertainty as to which career to choose was ended by the issuing of the racial proclamation of 14 July 1938 which deprived all non-Aryan citizens of the right to a university career and to pursue all the open professions. In the spring of 1939 I accepted the invitation of a neurological institute in Brussels and I moved to that city, where I stayed until December of the same year when the invasion of Belgium by German troops was considered imminent. Given that I did not want to be separated from my family I returned to Turin. Unable to attend the university institutes I decided to set up a small laboratory of experimental neuro-embryology in the bedroom of my home.

Giuseppe Levi, who like me had also returned from Belgium, to which he had emigrated at the beginning of the anti-Semitic campaign, asked if he could come and work with me and with joy I offered him the post of my first and sole assistant. These were the months before Italy entered the war at the side of Germany and when the daily newspapers were vomiting forth anti-Semitic slogans. The Fascist Children's Organisation and the Fascist Youth paraded in the streets of the city singing: 'If we need a bit of land we'll take England, if we like salmon we'll take Japan, then we'll have a ring-a-ring-a-roses, and we'll take the whole world'. This glorification of the omnipotence of Fascist Italy alternated with the declaration of hate and contempt for the members of the vile Jewish race who had to be exterminated so that they would not contaminate the members of the pure Aryan race. To the articles and the writing on the walls were added the posters which were put up all over the city. On 16 October my brother Gino, who

was already a famous architect despite his young age, came home proud of the honour which had been bestowed on him: "They've put me together with Einstein", he told us. In a poster, written down by Emanuele Arton in his diary, Gino's name appeared immediately above that of Einstein and other personalities held to be of 'Jewish race': 'They are Jews: from Verona, Moravia,... Levi-Montalcini, Einstein, Blum, La Pasionaria, Roosevelt... All the heads of the Freemasonry are Jews and all the abettors of the stock exchange. The most despicable cowards are Jews, as are those who make the people go hungry, the most unrepentant denigrators, the most perverse defeatists, the exploiters of men and women. The homosexuals are Jews, as are those who have never sweated, never worked, those who have always betrayed the homeland, those who wanted the sanctions. So do we want to finish all this once and for all? Not in the concentration camps but with flame-throwers against the walls. Long live the Duce! Long live Hitler! PS. We will also settle accounts with the accomplices of the Jews, the so-called honorary Jews.'

In an atmosphere which every day grew darker and more threatening, Levi and I work very hard in my minuscule laboratory intent on studying, from morning until night, the effects of ablation and the grafting of limbs on the development of the nervous systems of chicken embryos. The results of these studies were not accepted for publication by the Italian scientific journals because of the race laws and this was a stroke of fortune because it meant that they did not fall into oblivion. They were instead published by a famous Belgian scientific journal and by the Vatican scientific journal.

In 1943 the fall of Fascism and the declaration of the armistice by General Badoglio opened the doors to the Nazi hordes and there began the ferocious hunt, deportation and mass extermination of the small Jewish population which had been in Italy for many centuries. With my family I found refuge in Florence – the city was full of evacuees from the North and the South and it was not difficult to get lost amongst the other refugees by adopting a different name. Giuseppe Levi had also sought refuge in the same city. He arrived one day unexpectedly from the North and declared to our landlady that he was totally unaware of our real identity. He did this with his voice which had made legions of students tremble and introduced himself in the following way: 'Prof. Giuseppe Levi, Oh no, Prof. Giuseppe Lovisato. Call Rita for me'. He had the foresight, which was not to be expected in such a person who held every rule of safety in contempt, to call me by my name, given that he did not exactly remember what surname he had bestowed upon me. From that day onwards, however, our landlady had

some suspicions about our real identity and that of our many friends who came to visit us and declared that they were refugees from the South despite their unmistakable Piedmontese accents.

The winter and spring of 1944 went by with strong contacts with our friends who were partisans and active in the Partito d'Azione ('Party of Action'). We made false identity cards for those who had no papers and engaged in the revision, together with Levi, of his monumental work on histology. In the Party of Action, which had been founded by Carlo Rosselli, anti-Fascist intellectuals such as Ferruccio Parri, who played a role of primary importance in the activity of the Resistance, as well as other personalities (Bruno Zevi and Aldo Garosci, Riccardo Bauer etc.), were active members. Because of internal disputes, as described in the excellent volume 'L'orologio' ('The Watch') by Carlo Levi, the Party of Action broke up in 1947. Its prestigious president, Ferruccio Parri, joined the Republican Party at the side of Ugo La Malfa, and others joined the Socialist Party led by Nenni. The political field was characterised by mass parties whose broad organisational structures meant that they became the chief actors on the stage of national politics. The Communist Party was led by Palmiro Togliatti. The Right, hostile to the emerging new organisation of the country, which included Monarchist groups and those who looked back with nostalgia to the previous Fascist regime, established the Italian Social Movement which was led by Giorgio Almirante.

The liberation of Florence in August of that year marked the end of a long nightmare. Equipped with a real identity card I presented myself at the Anglo-American general headquarters and was employed as a medical doctor for the large number of people who had been evacuated from the Apennine regions of the Gothic line where the war was still very much in progress. This was my last and most painful experience as a medical doctor – the refugees arrived exhausted in army lorries which by day and night brought their human load to the barracks which had been turned into a lazaret. It was here that I was stationed.

In 1945 I actively took part in the campaign in favour of recognising the right of women to the vote.

With the end of the war in April 1945, Giuseppe Levi was given back his chair of anatomy in Turin and offered me the position of assistant. I held this position until 1946 when the famous scientist Viktor Hamburger, who had read my article which had been published in the *Archivie de Biologie*, invited me to go to St. Louis and join the Department of Zoology, of which he was the director, at the University of Washington. He wanted a clarifi-

cation of our different points of view on the mechanisms of action of the periphery tissues on the nervous centres used for their innervation. I left for the United States of America in September 1947. The invitation was for a few months and although I had intended not to stay there for long I remained in the Department for three decades, indeed until 1977.

In 1961 I wanted to meet my family relatives again and so I decided to go back at least for a few months to Italy, continuing at the same time with my scientific and teaching activity at Washington University. The choice of the city where I would continue my research on NGF fell on Rome thanks to the generous hospitality which was offered to me by the late Prof. Giovanni Battista Marini Bettòlo, the Director of the Department of Biochemistry at the Advanced Institute of Health.

Thus it was that I went back to that city which had exercised a great charm on me ever since the far off days of my childhood, when for the first time I was so struck by the Coliseum, by the Forum, and by the ruins of great imperial Rome.

On arriving in Rome I found the eternal city as I remembered it in those distant years. The vile traces of the invasion of the Nazi hordes had disappeared and there only remained the memory of their impiety in the short phrases written in blood in the walls of a sad building in Via Rasso and the horrors of the lugubrious Ardeatine mass graves. Having overcome the tragic and ignominious period of Nazi-Fascist domination, Rome had gone back to what it had always been, shining with light and pervaded by that natural Romanesque sense of humour immortalised by Gioacchino Belli and Trilussa. Rome in the 1960s was also animated by a feverish zeal of recovery in all sectors, ranging from the industrial to the cultural. This latter sector, fortunately and miraculously, had not been destroyed by the storm which had fallen on the city, invaded as it was by barbarians. The formidable energy and creativity of a small group of young scientists known to history as the 'boys of Via Panisperna' had kept this flame as alive and burning as it have ever been. During the centuries of the triumphs of the Roman empire and the centuries of the Renaissance, Rome had asserted itself for its extraordinary contributions to Western civilisation, these 'boys' were now to open up a new era with the discovery of nuclear fission, and this in a city which was besieged and hungry.

In 1969 the group that I directed moved from the Advanced Institute of Health to a location belonging to the National Research Council. In this Centre young researchers gathered around me. They were fascinated by the research of nerve growth factor which was then in top gear and were driven

by enthusiasm and the spirit of adventure which reigned in the laboratory, unconcerned by the fact that I could not guarantee them any certain future.

During the first part of the 1970s I took part in the activity of the women's liberation movement to support the liberalisation of abortion. The movement was led in Rome by the late Simonetta Tosi, who was then a young researcher who formed a part of the group of experimental biology which I directed. Simonetta campaigned very strongly in favour of the defence of the health of women and of the right, in cases of need, to engage in the interruption of a pregnancy. Her activity was marked by a courageous opposition to both institutional realities and groups who were hostile to any change.

During the last decades of the twentieth century in our country and in other highly industrialised countries a slow and gradual increase in the presence of women took place, not only in the social sphere but also in political and scientific sectors. In an article published in the volume 'The Road to Liberation' I wrote: 'Two X chromosomes have for millennia determined the destiny of hundreds of millions of women, in a way that is totally independent of their natural talents and inclinations. But the gates which blocked the road to liberation are today thrown open. This writer who in her youth found them bolted today with joy observes the long line of young women who proceed in great numbers along this road which was so rigidly closed to them in the past'.

In addition to scientific activity, which I am still engaged in, I dedicate the time available to me to social ethical questions and issues which today, at the dawn of the third millennium, I believe is of primary importance as much as ever before to address ourselves to.

In May 1999 at the time of the *honoris causa* degree conferred on me by the University of Trieste, I proposed the drawing up of a 'Magna Carta of Duties'. This project was received favourably by the Rector and the Academic Body. In December 1992 a first conference of scientists, from European and American universities, met at Trieste and drew up a draft of the 'Charter of Human Duties'.

This Charter, sponsored by the Italian government and approved by the United Nations, aspires to flank that on the rights of man of 1948 and others which have often and in different contexts been approved. It seeks to face up with the greatest urgency to the dangers which threaten the globe, the biosphere, and the survival of millions of species endangered by the action of man. As Ghandi expressed it: 'on the River Ganges of human rights there rise the Himalayas of human duties'.

It should thus be considered a moral duty of us all, both as human beings and even more as scientists and educators, to perform these duties, even at the cost of having to come up against opposing interests, dictated by the traditional spheres of influence connected with power. The International Council of Human Duties, of which I am the President, has made thousands of students of middle and high schools in Italy aware of the document of this Magna Carta.

In addition, in November 1993 at a meeting on the Charter of Human Duties in Trieste I proposed the idea of establishing the creation of an international network which would favour the exchange of information between existing women's groups and other which are being developed in all the regions of the planet. The plan of this organisation of women's solidarity, of which I am the Honorary President and which is called the Women's International Network, with the acronym WIN, Emergency and Solidarity, has today as its president the sociologist, Prof. Eleonora Barbieri Masini.

This initiative, which emerged in February 1995, gave rise to a meeting in the Campidoglio of Rome to women leaders from different parts of the world who described the situation which was to be encountered in their respective countries. At that time I observed that the art of war had been invented and administered exclusively by men and I expressed the hope that it was the more arduous task of the descendants of Eve to invent and administer peace.

The importance of the role that women can play is demonstrated by the results which have already been obtained, both at an individual level in small groups, without resorting to the support of the mass media, especially in developing countries. The solving of the questions and issues of different regions has required exceptional courage and organisational capacity because in some cases it has been in opposition to centuries-old dogmas.

In 1992 my sister Paola and I created a Foundation, giving to it the financial sources that we had available, in memory of our father whose career was that of an industrial engineer in Bari. The aim of this Foundation is today to help young African women from early childhood in basic education (literacy). The activity of this initiative flanks those which are already in existence and which are conscious of the fact that education is the only access key to a better future not only to mitigate the suffering of these tragically exploited populations but also because of the help that can be given to them depends the future of mankind. An African

proverb says: 'If we educate a boy we educate a person, if we educate a girl we educate a family, a nation'.

At the dawn of the third millennium, as an actor and a spectator of the twentieth century, I send to young people of both sex the message of knowing how to realise the potential that they are endowed with and to apply it within the most worthy human activities which are open to them.

## HOW TO FIND BETTER DRUGS FOR THE TREATMENT OF AIDS

PAUL A.J. JANSSEN

The Center for Molecular Design (CMD), which became operational in April 1996, was conceived as a think-tank. The objective of the CMD is to provide ideas for the synthesis of chemical compounds by means of molecular modeling and using crystal structures of proteins of therapeutic interest. The design team is multi-disciplinary and comprises biochemists, chemists, a physicist, mathematicians and a computer engineer. It relies heavily on the use of dedicated computers and modeling software.

Current targets for molecular design are HIV-reverse transcriptase, HIV-protease and Influenza-neuraminidase. Most of the effort of the CMD is presently concentrated on the design of nonnucleoside inhibitors of HIV-reverse transcriptase (NNRTI). The immediate goal of the CMD is to design compounds using a 'de novo' approach, i.e. from first principles using only the three-dimensional structure of a target protein and the rules of chemistry.

Molecular modeling at the CMD proceeds in three steps. First, a proposed ligand is 'docked' into the binding site of the target protein, using the ligand's minimal energy conformation. This operation can be regarded as a test for geometrical fit of a ligand. After successful docking, the change in free energy between the ligand in solution and the ligand-protein complex is determined. In reverse transcriptase, most of the interactions between the ligand and its environment are governed by the polar and lipophilic properties of the ligand. It is also possible to identify the specific amino acid residues of the binding site, which account for the largest part of the ligand-protein interaction. Finally, using computed binding energies and observed antiviral activities of known compounds, a prediction can be made for the activity of a newly designed compound.



The 'do novo' approach of the CMD makes use of docking, binding and prediction of activity in an automatic design process which mimics natural evolution and which is, therefore, referred to as 'genetic algorithm'. At the start of this process one has to define a tractable initial 'population' of chemical structures. Each of these is assigned a 'fitness' value, according to its predicted biological activity, using the molecular modeling procedure described above. 'Parent' structures are selected for 'breeding' according to their fitness, i.e. structures with high fitness stand a better chance of being selected than those with low fitness. Diversification of chemical structure in the 'offspring' is obtained by exchanging structural fragments from the parents, which is equivalent to 'cross-over' in natural reproduction. 'Child' structures can be diversified further by adding and removing fragments, which corresponds to 'mutation'. Finally, the fitness of the offspring is evaluated and the best-performing ones may replace structures in the population that are less fit. At this point one 'generation' is completed. After several generations, the process of variation and survival of the fittest will breed a population of chemically diverse structures with high-predicted activity.

Molecular modeling and 'de novo' design are by no means substitutes for chemical imagination and serendipity. Rather, the role of the chemist is strengthened by the need to evaluate a large quantity of computer-generated compounds with respect to the ease with which these can be synthesized, as well as their stability and solubility. The approach has led to the design of a novel class of highly active anti-HIV compounds (substituted dianilino pyrimidines or DAPY's) which are presently being evaluated in clinical trials.

## THE CHALLENGES OF SCIENCE

MAX PERUTZ

I thought I would just tell you about a great adventure which Carlos Chagas and myself shared. I got to know him a little better. I do not remember which year the Pope made an appeal against nuclear weapons to the Heads of the nuclear States, stimulated I think mainly by Ricky Vasco who had persuaded Chagas to propose this to the Pope, and the Pope enthusiastically endorsed it. So the Pope wrote letters to Reagan, Brezhnev and the Queen of England, and the idea was that delegations from the Academy would actually submit his appeal personally to the Heads of State.

To my surprise, Chagas asked me whether I would accompany him to deliver this to the Queen. Now, I felt a bit of a sort of Charlie Chaplin about this, a sort of immigrant to Britain of Jewish extraction acting as a messenger from the Pope to the Queen of England; it looked a bit funny. But anyway the next thing that happened was that the Foreign Office decided that it was a political message and that it should be delivered to the Prime Minister and not to the Queen. So Chagas and Hermann Bruck, another member of the Academy, and I, made an appointment with Margaret Thatcher at number 10 Downing Street. She received us in her drawing room dressed to the nines and with not a single hair out of place, looking very smart indeed, and Chagas delivered this message, a passionate message against nuclear weapons – it was really marvellous to see him in action – to which she replied with an impromptu well-reasoned statement that she would not accept any such move unless there already existed a reliable system to verify that nuclear disarmament really had taken place. However, she allowed us to deliver this message for the Queen and so Chagas gave the message to Margaret Thatcher, she read it and she said: “I shall decide, I shall tell the Queen what to reply”. With that we were accompanied out and of course were really amused by this outcome, although a little saddened

because we realised that we could not get any further. But as I said I admired Chagas' determination and his persuasiveness on this occasion. I think he would have melted anybody else's stony heart but he did not melt Margaret Thatcher's.

Now, to come to my story, in 1949 Linus Pauling and others published a sensational paper in *Science* entitled 'Sickle-cell Anaemia, a Molecular Disease'. Sickle-cell anaemia is a blood disease which affects black people and others living in malarial regions. It manifests itself by a reversible change. As we can see from the diagram, on the left is shown the normal shape of a red blood cell, a sort of saucer shape. On loss of oxygen the shape of the red cell changes to this sickle shape, which is why it is called sickle-cell anaemia. The venous circulation contains the sickle cells, the arterial contains the normal ones, and what Pauling showed in his paper was that the normal and sickle-cell haemoglobin differ by the electric charge which the haemoglobin molecule carries. The sickle-cell molecule carried too few electric charges. He thus examined the blood of people who had heterozygotes, who had inherited the disease from only one parent, and it contained two haemoglobins, one with the normal charge and one with the abnormal charge.

I managed to obtain some sickle-cell blood after that and Murdoch Mitchinson and I looked at it with a high-powered polarised microscope and found that this transition is accompanied by a crystallisation of the haemoglobin in the red cell. The haemoglobin is a solution; it crystallises; and it has the typical refringence of crystals of human blood, the oxyhaemoglobin. This observation showed me for the first time that protein aggregates are not tolerated by living cells and this was very important.

Shortly after that there was a young chemist in northern England, John Irovolsci, and Francis Crick and I needed to him to try and look into this chemically. He developed a very good new method of chromatography that he called fingerprinting, which involved spreading the haemoglobin. He digested the haemoglobin with an enzyme, with trypsin, and then spread out the peptides in one direction by their electric charge and in the other direction by their mobility, and found that the difference appears in those from peptides. The year after that he showed that the change in charge that Pauling and his colleagues had observed was due to the displacement of a single pair of amino acids among the more than 500 other pairs: the normal haemoglobin carries glutamic acid, which has a carboxylic group, which carries a negative charge, and in sickle-cell haemoglobin this is replaced by another amino acid, valine, which differs from the carboxyl

group in that two oxygen molecules are replaced by three hydrogens. Thus it was discovered that chemical exchange in a protein can give rise to a deadly disease. This discovery was also crucial because this was the first time that we actually realised what genetic mutation does.

We did not as yet know anything about the genetic code, but we realised that this had to be due to a change in the genetic code which replaces one amino acid in a protein by another. This posed in acute form the question of the genetic code, and really stimulated Crick and Brenner to work on the discovery that the triplet code was the three basis code for one amino acid. Since then I think literally thousands of other such amino acid replacements have been discovered as the causes of a variety of genetic diseases: most are caused by amino acid replacement, some are caused by extensions, and others by deletions, of amino acids.

However, in 1993 an entirely new and different form was found as a result of a tremendous cooperative effort, an unprecedented cooperative effort by about a hundred medical people and scientists to discover the gene for Huntington's disease, a terrible neurodegenerative disease, one of the worst diseases there is. It is dominantly inherited and leads first to uncontrolled movements, which is why it is also called Huntington's chorea, then to movement disturbances, then to progressive dementia, and finally to death. It is a late onset disease. It starts in middle age, and people are healthy and unsuspecting; they have children not knowing that they actually have the disease and will transmit it to an average half of their children. It is deadly and sinister. There was this tremendous international effort and in 1993 a paper appeared in *Cell* by 61 authors from six American and two British universities reporting the discovery of the gene. The gene was huge, it consisted of over 60 axons spread over thousands of kilobases and it coded for a protein of a single chain of almost 3,140 amino acid residues. But near one end, near the amino end, was an extraordinary feature, a repeat of a single amino acid, glutamine, as can be seen from the diagram.

The glutamine is a little different from glutamic acid, and as can be observed instead of there being two oxygens, one oxygen is replaced by nitrogen, and when this oxygen carries a negative charge the nitrogen carries a positive one. There is a dipole here: the oxygen carries a partial negative charge and the nitrogen a partial positive charge, and this is crucial for the mechanism of the disease. What they found was that in healthy people the length of this repeat varies anything from half a dozen to 37 glutamines, but in people with the disease the length is over forty, and that the longer the repeat, the earlier the disease sets in and the more

severe it is. There were some juvenile cases, but the repeat was over a hundred glutamines long.

I read this one night, coming home on the train and became very excited because a few weeks earlier my attention had been drawn to a repeat of glutamines in certain proteins of *Drosophila*, mainly transcription factors, and I wondered what they could possibly do, what the meaning of this was. What does a crystallographer do? He builds a model. So I built an atomic model of two glutamine chains and I found that they act as polar zippers, that is to say, as can be seen from the diagram, there are two chains: one protein chain on one side and another chain on the other, and I found that there were protein chains known to stick together by hydrogen bonds between their main chain CO and H groups. That was well known but I found when I built this model that actually these dipoles at the end of the glutamine can also form hydrogen bonds, so that this chain is held together not only by hydrogen bonds in the main chain, but by pairs of hydrogen bonds all along on the side chain. In the diagram can be seen in blue the bonds that that held together. And in fact I had a paper in press on polar zippers in proteins.

When I read this paper about Huntington's disease it seemed to me that this might be the clue to the molecular mechanism of the disease: that these longer repeats of glutamines might lead to aggregation of the protein, that it adheres at two appendices. How could I test this? I asked the chemist in our lab to make me a synthetic chain of polyglutamine which would have been in solution and I asked him to put two aspartates at one end of the chain and two lysines at the other end, and the optical properties of this showed that it does indeed form aggregates in solution with this sort of structure. When the aspartates at the end were discharged at neutral pH it actually formed little fibres and the fibres actually grew and gave the typical X-ray refraction picture of this sort of structure, and so I published this and sent it to *Nature* who rejected it as of no interest without sending it to referees. I then published it in the proceedings of the National Academy.

In the accompanying diagram the atomic model can be seen rather better than it can be seen in real life: there are hydrogen bonds in the main chain and H and CO groups, and there are the hydrogens between bonds in the side chains. The next diagram contains my suggestion in that paper that the extension of the glutamine repeat may cause the affected proteins to agglomerate and precipitate in neurons. Symptoms may set in when the precipitates have reached a critical size or have resulted in a critical number of neural blocks. I published this in 1994 but there was at

that time no evidence whatever that such aggregates existed. The immunostaining showed the protein to be in sort of little dots, isolated molecules in the cytoplasm, with no aggregates. But in August 1997 a paper by Bates and Davies appeared in *Cell* which really put an entirely new complexion on the disease.

Gillian Bates for the first time succeeded in reproducing the disease in mice, in an animal, and she did it by a piece of genetic engineering. She just used the first axon of the very large gene which expressed the first hundred or so amino acids, which included the glutamine repeat, and she made transgenic mice and one set of mice with a repeat of 20 glutamines, and another set of mice with a repeat of 150 glutamines. Among these the ones with 20 glutamines remained healthy and the ones with 150 developed the typical symptoms of Huntington's disease. This was a great success and her mice have since been used the world over for experiments on Huntington's disease.

In the same paper there was a report by Steven Davies, a lecturer at University College, London. Gillian Bates and Davies worked at the university hospital in London and Davies cut up and then examined sections of the brains of these mice under a electromicroscope. One morning he stormed into my room in Cambridge in a very excited state. He started telling me about his experiments even before he had shut the door and showed me a series of electromicrographs demonstrating the aggregates that I had predicted three years earlier, that is to say, he found that in the cell nucleoid of the neurons in these mice there were aggregates. There were clumps of protein which stained with antibodies against pectide and also with antibodies against ubiquitin and other proteins.

They published this discovery and in the same issue of *Cell* there was a paper by Henry Shwanken and his group at the Max-Planck Institute for Molecular Genetics in Berlin, who used Gillian Bates' axon to introduce it into colibacteria. They introduced it with a series of different lengths of glutamine repeats and found that with repeats shorter than 40 the protein remained soluble and with repeats longer than 40 glutamines it produced aggregates. The important fact in this discovery was that the length of glutamine repeat that produced aggregates in vitro was exactly the same as the length that produced this disease in the patients.

After this, Marian Di Figlia's group at the Harvard Medical School looked again at the sections of the post-mortem brains of Huntington's patients and found the same aggregates there. They had been overlooked before but since then very many groups have been found in patients. The

largest structures of neurons blocked by these aggregates were found in juvenile patients, and there is a distinct relationship: the fraction of neurons carrying these aggregates depends on the length of the repeat – the shorter the repeat, the rarer they are, and vice versa.

Since that time seven other neural degenerative diseases have been discovered caused by the extension of glutamine repeats in totally different proteins of different molecular weights. In some the glutamine repeat is near the amino end, and in others near the carboxyl end, and in others in the middle, but it does not seem to make any difference, and the striking thing is that in six of these diseases the length of repeat that produces the disease is the same as in Huntington's disease. Thus the rule is: with fewer than 37 repeats people are healthy, if they have more than 40 glutamines in the repeat they are likely to experience this neural degeneration.

I wondered what this meant and, thinking about the question, it seemed to me that there would be entropic reasons why longer repeats form ordered hydrogen bondage sheets, while shorter repeats would form random ones. The following diagram shows this. If the roots are free they attract water. The group depicted in it attracts the water molecules which attach themselves because they also carry partial electric charges. The water molecules become immobilised at the cost of entropy within the system. When they come together and form hydrogen bondage with each other, these water molecules become liberated and the entropy of the system increases. At the same time, when the chain is a random coil it has a large entropy, but when it forms a hydrogen bondage structure of this kind it loses rotational and translation entropy.

The chain becomes longer and more and more hydrogen bonds are formed. The loss of rotational and translational entropy becomes smaller and smaller until it becomes negligible, but the gain in entropy through the liberation of water molecules in that group remains constant, so eventually the gain in entropy wins and the two come together.

This is expressed in the next diagram. There are a number of glutamine repeats against the entropy changes in the system and there is a gain of entropy; there are liberated water molecules which remain constant; a loss of rotational and translational entropy which decreases; and eventually the cross over in between. Due to this entropic effect, a length of more than 40 would form a hydrogen bondage structure which would need the aggregation. Certainly I have not been able to test this idea because anything you make with long repeats is insoluble in the first place, and you cannot obtain any spectral information in solution.

The situation now is that many people have tried to reproduce the disease in other animals and cells. Nancy Bonini has reproduced it in Philadelphia, has produced it in flies, in *Drosophila*, and other people have introduced the Huntington gene into all kinds of different cell lines and done experiments on it. As a result readers become absolutely confused: reports of aggregates without cell death, neural cell death, and of cell death without aggregates. Whereas workers in England are convinced that it is the aggregates that cause the disease, in the United States it is now widely believed that the aggregates are what they call an epiphenomenon and that the true cause of the disease is still unknown and has yet to be sought.

I was disturbed about this controversy and was wondering what to do about it when I came across two interesting observations. Last July a paper appeared in *Nature* reporting the probability of neural death in twelve neural degenerative diseases, and they found that in Huntington's disease the probability of neural death remained constant with time. In other words, it behaved like a random event that was due to singeing heat rather like radioactivity, so that each neuron in Huntington's disease has a constant half-life. But they offered no interpretation for this result.

Last September a paper appeared in a new journal, *Nature Reviews*, by Guzella and McDonald of the Harvard Medical School. They plotted the age of onset of the disease against the lacks of glutamine repeats in all eight diseases due to extension of glutamine, and found an extraordinary thing: in every case the age of onset, or rather its inverse, was an exponential function of the length of glutamine repeat.

In the next diagram are to be found Steven Davies' aggregates and in the following one are Marian DiFiglia's aggregates in the human neuron. These aggregates form in the cell nucleoid and they are composed not of the entire Huntington's disease molecule but of a fraction of it. So the first step in the disease seems to me to be a proteolytic severance of the molecule and then a fragment in the nucleus about one tenth the size of the whole molecule, and the nucleus forms this granular of aggregates.

The next diagram shows this remarkable fact. In it you have a number of CAG glutamines and proteins against the age of onset of the disease, and the larger the number of CAG glutamines the faster the age of onset: hence these remarkable logarithmic curves.

These two observations made me wonder. I said to myself: surely they are telling me something? And if you look at the cell as a collection of large molecules, what sort of event could give rise to this phenomenon, to this radioactive life decay, and to this exponential dependence on the moment



of onset of the disease? And it struck me that there was only one phenomenon, and that was nucleation.

The classical system of nucleation is clouds; water droplets in a cloud. Supersaturate cloud water molecules come together and form aggregates, but as long as the aggregates are small the surface tension is very high and the weight of loss of water molecules from the aggregates exceeds the weight of aggregation. But at some moments in time the aggregate reaches a critical size where the rate of loss is less than the rate of aggregation, and then it forms into a drop, and this is a totally random event which is also obtained in crystallisation, which is another example, and in solutions of large molecules.

It struck me that this was probably due to the same thing, but I found that I did not really know enough about the theory of nucleation, so I got together with a physicist, Alan Wildon, who is a Professor of Material Science in Cambridge and works on the aggregation of synthetic polymers and is an expert on the nucleation of such polymers into aggregates. He taught me a great deal and together we produced a paper on this subject in which we showed that nucleation is exactly such a random event and that in high polymers the rate of nucleation is indeed an exponential function of the length of the polymer, exactly as you see in the curves in the diagram. This paper has been submitted to *Nature*. I hope that this will end the controversy, but I am not sure that it will because in the case of sickle-cell haemoglobin it took twenty years for the paper by Mitchinson and myself to be accepted, and I fear it may take just as long again. But I will no longer be here.

# FUNCTIONAL ASPECTS OF LEFT-HANDED NUCLEIC ACIDS

BERNARD A. BROWN II and ALEXANDER RICH

## *Introduction*

Both DNA and RNA form stable double helices held together by Watson-Crick base pairs. However, the presence of an added oxygen atom in the backbone of RNA results in a considerable change in the geometry of the molecule. The B-DNA molecule has both major and minor grooves, with the major groove fully accessible. On the other hand, A-RNA, because of the altered pucker of the ribose ring, forms a double helix in which the minor groove is quite accessible, but the major groove is constricted so that it is almost inaccessible to neighboring molecules. Right-handed B-DNA and A-RNA are the most stable forms of these duplexes. Both of these conformations can be transformed with the input of energy into left-handed double helical conformations, which are still held together by Watson-Crick base pairs, but with the backbones in an unusual *zig-zag* shape, hence the name "Z"-conformation. In the Z-conformation, the puckers of the furanose sugar rings in the polynucleotide chain alternate between that found in B-DNA (*C2' endo*) and that found in A-RNA (*C3' endo*). It is this alternation that produces the unusual shape of the phosphate backbone, the most striking feature of the Z-conformation.

Here we discuss some of the research on the left-handed Z-forms of both DNA and RNA double helices. Much more is known about Z-DNA than Z-RNA at the present time.

DNA can assume many shapes (Rich 1993). A dramatic change in shape is found when the familiar right-handed B-DNA double helix changes to the slightly thinner and elongated left-handed Z-DNA conformation (Figure 1) (Wang *et al.* 1979). This conformational change occurs most readily in seg-

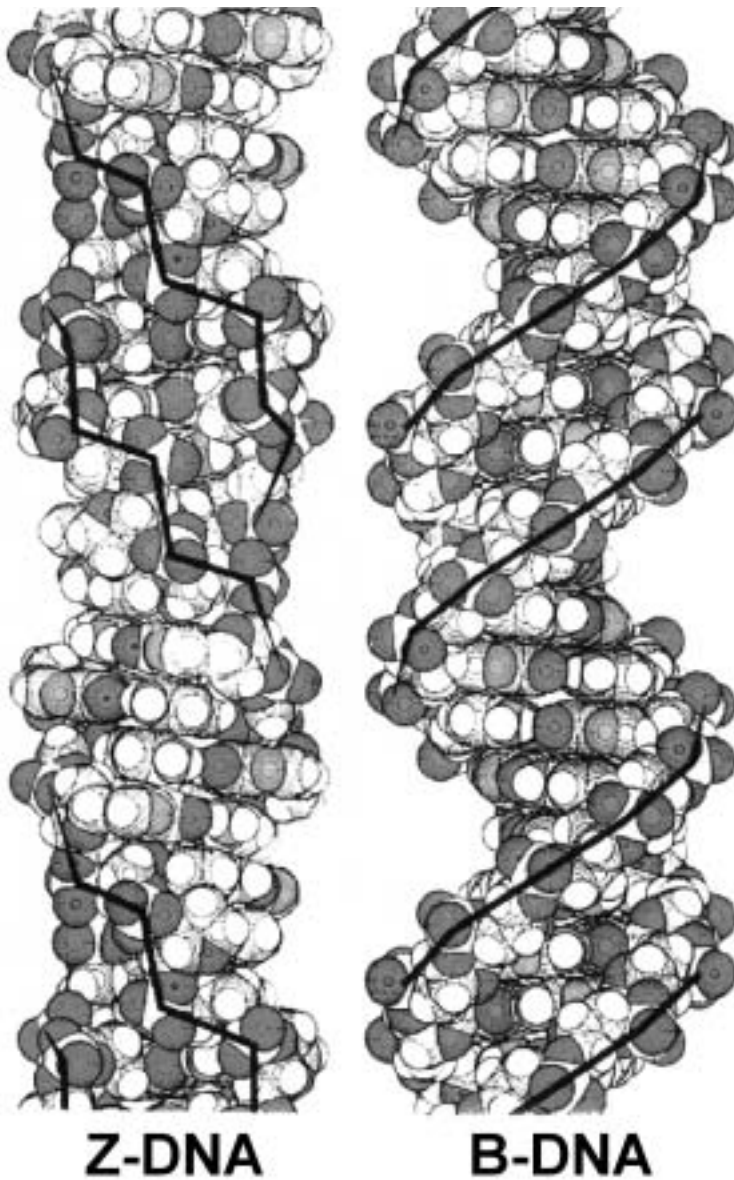


Figure 1. Overview of B- and Z-DNA helices (Wang *et al.* 1979; Wang *et al.* 1981). The “information-rich” residues that allow sequence-specific recognition of the major groove of B-DNA lie on the convex surface of left-handed Z-DNA helix. The two DNA strands of each duplex are highlighted by solid black lines. The “zig-zag” nature of the Z-DNA backbone is clearly seen. The sequence shown is  $(dC-dG)_n$ .

ments with specialized sequences, favored largely by alternations of purines and pyrimidines, especially alternating deoxycytidine and deoxyguanosine residues (Klysik *et al.* 1981; Haniford & Pulleyblank 1983; Peck *et al.* 1982). The alteration in ring pucker reflects differences in the stabilities of furanose sugar puckers for particular nucleosides.

### *Negative Supercoiling Stabilizes Z-DNA*

An alternative conformation was first suggested by optical studies of Pohl and Jovin showing that polymers of alternating deoxyguanosine and deoxycytidine residues,  $d(CG)_n$ , produced a nearly inverted circular dichroism spectrum in *ca.* 4 M salt solutions (Pohl & Jovin 1972). The physical reason for this finding remained a mystery until an atomic resolution crystallographic study of  $d(CG)_3$ , surprisingly revealed a left-handed double helix, which maintained Watson-Crick base pairing (Figure 1) (Wang *et al.* 1979). The Z-DNA helix is formed by a  $d(CG)$  dinucleotide repeat with the deoxycytidines in the familiar *anti* conformation while the deoxyguanosines are in the unusual *syn* form. In Z-DNA, there is a single narrow groove that corresponds to the minor groove of B-DNA; there is no major groove. Instead, the information-rich residues that allow sequence-specific recog-

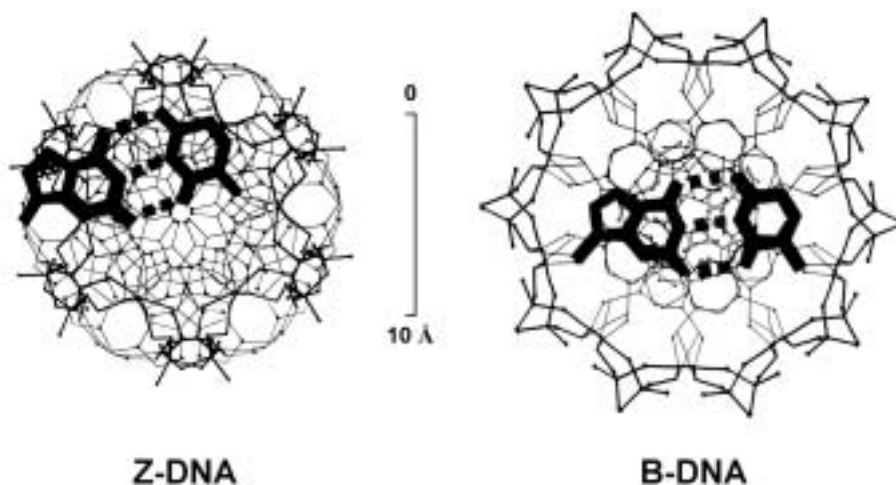


Figure 2. End views of Z-DNA and B-DNA. (Wang *et al.* 1979; Wang *et al.* 1981). End views of Z-DNA and B-DNA are shown in which a G-C base pair has been shaded. The guanine residues in B-DNA are located closer to the center of the molecule and the phosphates are on the outside, while in the thinner Z-DNA conformation, the base pair is displaced to one side, with the guanine C8 of the purine ring near the periphery of the helix.

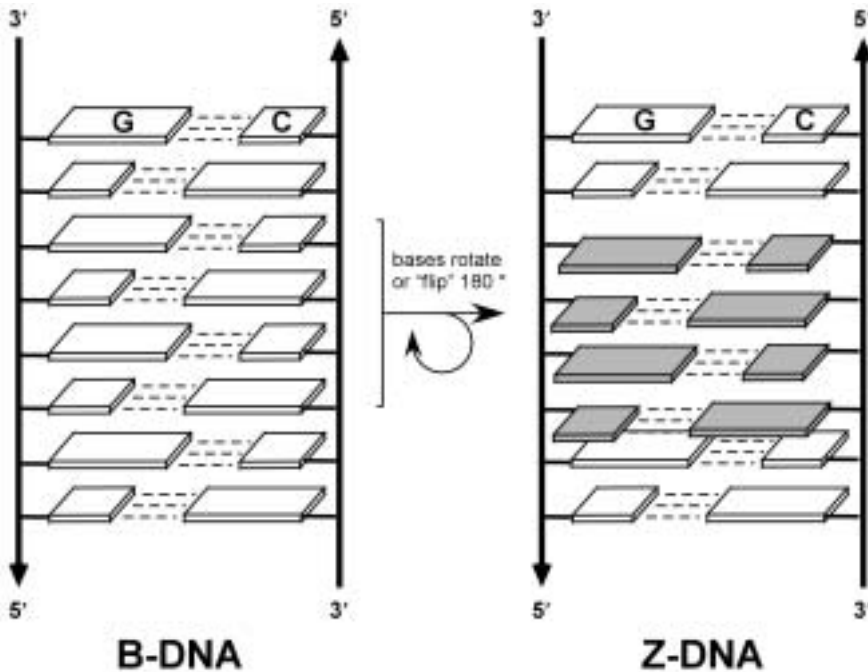


Figure 3. B-to-Z "Flipping" (Wang *et al.* 1979). This diagram illustrates the changes in topological relationship if a four-base pair segment of B-DNA were converted into Z-DNA. Base pairs are represented by flat boards; the base pairs in the Z-conformation are shaded. The conversion is associated with a rotation or "flipping" of the base pairs as indicated. Rotation of the guanine residues about the glycosidic bond yields deoxyguanosine in the *syn* conformation (with a C3' *endo* sugar pucker), as shown in Figure 4. In contrast, for deoxycytidine residues, both the cytidine base and deoxyribose are rotated, keeping cytosine in the *anti*-glycosidic orientation with a C2' *endo* sugar pucker.

nition of B-DNA lie exposed on the convex outer surface of Z-DNA. This is shown in an end view where in Z-DNA the base-pair is at the edge of the helix, especially the guanine base (Figure 2). The transition from B-DNA to Z-DNA involves "flipping" the base pairs upside down (Figure 3). During this process, deoxycytidine remains in the *anti* conformation because both the sugar and base flip over, while only the base of deoxyguanosine inverts, moving it into the *syn* conformation. In addition, the guanosine deoxyribose adopts the C3' *endo* sugar pucker, normally found in riboses of A-RNA, while the deoxycytidine deoxyribose remains in the normal C2' *endo* sugar

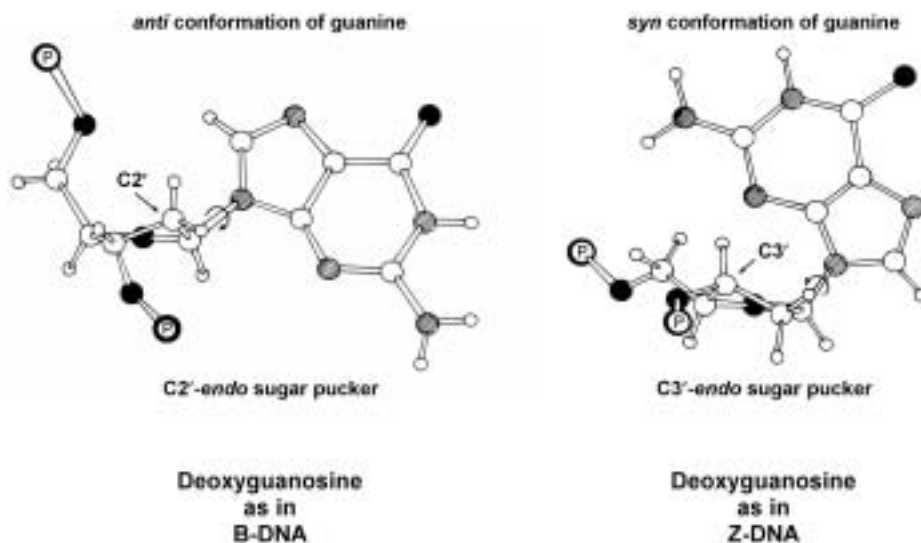


Figure 4. Conformation of deoxyguanosine in B- and Z-DNA (Wang *et al.* 1981). In both diagrams, the sugars are oriented so that the plane defined by C1'-O1'-C4' is horizontal. Atoms lying above this plane are in the *endo* conformation. In B-DNA all of the sugar puckers are C2' *endo*; in A-RNA all sugars are in the C3' *endo* conformation. In Z-DNA and Z-RNA the guanine sugars adopt the C3' *endo* conformation. However, in the Z-conformation the guanine bases are in the *syn*-orientation with respect to the glycosidic-bond. In contrast, only the *anti*-position is found in B-DNA and A-RNA. A curved arrow around the glycosyl carbon-nitrogen bond indicates the site of rotation.

pucker (Figure 4). These features consequently mold the phosphate backbone into a zig-zag path (see Figure 1). B-DNA can form Z-DNA under physiological salt conditions when deoxycytidine is C5-methylated or brominated (Behe & Felsenfeld 1981). The demonstration that Z-DNA formed under conditions of negative superhelical stress was notable as this brought the left-handed conformation within the realm of biology (Klysik *et al.* 1981; Peck *et al.* 1982; Singleton *et al.* 1982).

Z-DNA is a higher-energy conformation than B-DNA and will only form in plasmids when they are torsionally stressed, thus Z-DNA is stabilized by negative supercoiling. The energy necessary to stabilize Z-DNA can be determined by measuring the plasmid superhelical density at which Z-DNA formation occurs, and it is proportional to the square of the number of negative supercoils (Peck & Wang 1983; Ellison *et al.* 1985). Sequences other

than alternating purines and pyrimidines can also form Z-DNA. The ease with which this occurs depends on the sequence; d(CG) is best, d(TG/AC) is next, and a d(GGGC) repeat is better than d(TA)<sub>2</sub> (McLean *et al.* 1986; Ellison *et al.* 1986). In addition, formation of a B-Z DNA junction, which has a  $\Delta G$  of about 4 kcal/mol, is a significant energetic barrier to Z-DNA formation (Peck & Wang 1983). Based on many empirical findings, computer models have been developed to rank the Z-DNA-forming potential of naturally occurring sequences (Ho *et al.* 1986).

As pointed out by Liu and Wang (Liu & Wang 1987), negative supercoils arise behind a moving RNA polymerase as it ploughs through a DNA double helix. The torsional strain generated by passage of RNA polymerases then becomes a potent source of energy to stabilize Z-DNA. An analysis by Schroth *et al.* of 137 fully sequenced human genes demonstrated that sequences that could easily form Z-DNA were present in 98 and they were distributed non-randomly throughout the gene; sequences were 10 times more frequent in 5' than in 3' regions with the highest frequency near the transcription start site (Schroth *et al.* 1992). This finding supports the expectation that the energy necessary to form Z-DNA *in vivo* is generated by transcription.

#### *Detection of Z-DNA*

Z-DNA formation *in vivo* can be detected by chemical modification. Through use of either osmium tetroxide or potassium permanganate, plasmids containing a d(CG)<sub>n</sub> can be seen to form Z-DNA *in vivo* (Palecek *et al.* 1988). UV cross-linking of bacteria treated with psoralen dyes have confirmed these results and permitted precise quantitation of unrestrained supercoiling present within *Escherichia coli* (Zheng *et al.* 1991). A more indirect approach has used a construct in which an *EcoRI* restriction site is embedded within a Z-DNA-forming sequence (Jaworski *et al.* 1987). In the bacterial cell, this fragment can be methylated when it is in the B-DNA conformation, but is resistant to methylation while in the Z-DNA conformation. Susceptibility to methylation thus can be used as a measure of *in vivo* torsional strain. Results obtained with this system show that Z-DNA formation in *E. coli* occurs in the absence of external perturbation and is regulated by transcription, an effect that is enhanced by mutations inactivating topoisomerase I (Rahmouni & Wells 1989; Jaworski *et al.* 1991).

Krasilnikov and co-workers were able to quantitate the effects of negative supercoiling in *E. coli* by assessing the efficiency of cruciform forma-

tion at varying distances upstream of a promoter (Krasilnikov *et al.* 1999). Chemical probing assays showed cruciform formation decreased to one half by placing a promoter 800 bp upstream, and it could still be detected over 2 kb upstream. This was the first demonstration *in vivo* that supercoiling generated by transcription could change DNA structure at such great distances.

Detection of Z-DNA in eukaryotic systems is more complex, although a number of early observations clearly suggested its existence. Unlike B-DNA, Z-DNA is highly immunogenic, and polyclonal as well as monoclonal antibodies can be made that specifically recognize this conformation (Lafer *et al.* 1981). The first indication that Z-DNA exists in eukaryotic systems came from analyses of sera obtained from patients with autoimmune diseases such as systemic lupus erythematosus. These experiments showed that lupus patients produced antibodies which were highly specific for Z-DNA (Lafer *et al.* 1983). The blood concentrations increased during the exacerbations of the disease, together with antibodies to many other nuclear components.

Anti-Z-DNA antibodies raised in rabbits and goats have been used in staining fixed (Nordheim *et al.* 1981) and unfixed polytene chromosomes of *Drosophila melanogaster* (Lancillotti *et al.* 1987). These produced an unusual pattern with staining in the interband regions but not in the bands. Staining was especially intense in the puffs, which are associated with high levels of transcriptional activity (reviewed by Hill (Hill 1991)). Antibodies were also used in staining the ciliated protozoa *Stylonychia mytilus*, which has both a macronucleus and a micronucleus (Lipps *et al.* 1983). The micronucleus is used for genetic reproduction, but the macronucleus is the site of all transcriptional activity. In this case, the macronucleus stained exclusively, with no staining in the micronucleus, even though they both had the same DNA sequences. These findings suggested a link between transcriptional activity and the presence of Z-DNA.

There are a number of limitations in the analysis of Z-DNA in intact mammalian systems. No phenotype has ever been associated with the presence or absence of Z-DNA-forming sequences, thus limiting the use of genetic approaches. Furthermore, regulation of Z-DNA is likely to be very complex. Moving RNA polymerases can generate negative torsional strain. RNA polymerase I is known to work on some favorable Z-DNA-forming sequences in ribosomal RNA genes, but it is not known how the torsional strain in regions 5' to RNA polymerase II promoters is regulated. The effect of potential Z-DNA-forming sequences upstream in a promoter must be



interpreted carefully. Deletion or mutation of such regions, as in the case of the SV40 enhancer which has regions of alternating purine/pyrimidine repeats, may have many different consequences (Gruskin & Rich 1993).

Several experiments have been carried out using metabolically active, permeabilized mammalian nuclei, which were formed by embedding living cells in agarose microbeads (Jackson & Cook 1985). A low concentration of detergent is used to lyse the cytoplasmic membrane and permeabilize the nuclear membrane. The treated nuclei are transcriptionally active and replicate DNA at 85% of the rate observed in the intact cell (Jackson *et al.* 1988). The amount of Z-DNA present under these conditions was detected by diffusing biotinylated anti-Z-DNA monoclonal antibodies into the permeabilized nucleus and measuring the amount of radioactive streptavidin that would bind (Wittig *et al.* 1989). The amount of Z-DNA measured was independent of the antibody infused, over a 100-fold range in antibody concentration. Furthermore, the amount of Z-DNA depended on DNA negative torsional strain. It increased dramatically as transcription increased, but was largely unaffected by DNA replication (Wittig *et al.* 1991).

Individual genes can be assayed by cross-linking the antibody to DNA using a 10-ns exposure of a laser at 266 nm (Wittig *et al.* 1992). The release of DNA fragments with cross-linked antibody was carried out by diffusing in restriction endonucleases, an *in situ* DNA digest. Following isolation of biotin-labeled antibody-DNA complexes with streptavidin magnetobeads, free DNA restriction fragments were obtained by proteolysis. Thus it was possible to determine which regions of a gene forms Z-DNA. Using hybridization or PCR techniques, the *c-myc* oncogene was studied in mouse U937 cells (Wittig *et al.* 1992). Three transcription-dependent Z-DNA-forming segments were identified in the 5' region of the gene with two of them near promoters (Wolfl *et al.* 1995b). Retinoic acid, which induces the cells to differentiate into macrophages, was then used to down-regulate expression of *c-myc*. Loss of *c-myc* expression was accompanied a loss of Z-DNA over 15–20 min. in these regions. As a control, Z-DNA was detectable by PCR amplification with probes for actin genes under all conditions tested; actin is not down-regulated during differentiation.

Induction of Z-DNA was also measured in the corticotropin hormone-releasing gene in a primary liver cell line (Wolfl *et al.* 1996). Z-DNA formation increased when the gene was up-regulated and decreased when it was down-regulated. This finding suggests that physiological events are being measured in these systems. A major conclusion from these studies is that Z-DNA forms largely, if not exclusively, behind a moving RNA polymerase

and is stabilized by the negative supercoiling generated by DNA transcription. After the polymerase stops transcribing, topoisomerase is able to catch up and release torsional strain caused by negative supercoiling, and the Z-DNA reverts to the lower energy B-conformation.

It is possible that Z-DNA formation has a functional role without recognition of its shape by proteins. For example, the *E. coli* RNA polymerase does not transcribe through Z-DNA (Peck & Wang 1985). Thus, the formation of Z-DNA behind a moving polymerase may block a following RNA polymerase from re-initiating transcription from that region of the gene. This might ensure spatial separation between successive polymerases. In a mammalian system, RNA transcripts would then be physically and temporally separated from other transcripts, perhaps minimizing non-functional eukaryotic trans-splicing (Rich 1994).

Alternatively, formation of Z-DNA could facilitate recombination of homologous chromosomal domains by relieving topological strain that arises when intact duplexes are intertwined (Pohl 1967). The Z-DNA-forming sequence  $d(\text{CA/GT})_n$  has been shown to be recombinogenic in yeast (Trecó & Arnheim 1986) but is found to be less efficient than  $d(\text{CG})_n$  in human cells (Bullock *et al.* 1986; Wahls *et al.* 1990). Finally, Z-DNA formation could affect the placement of nucleosomes as well as the organization of chromosomal domains (Garner & Felsenfeld 1987).

#### *An Adenosine Deaminase (ADAR1) Recognizes Z-DNA*

A number of laboratories have searched for Z-DNA binding proteins. Early studies were unfruitful and caused widespread skepticism that Z-DNA would be associated with any biological function. Many of the positive results reported in these studies may have been due either to artifacts or misinterpretation of data (Wolfl *et al.* 1995a; Krishna *et al.* 1990; Rohner *et al.* 1990). However, absence of proof should not have been confused with absence of existence.

A protein which was found to specifically bind Z-DNA is the RNA editing enzyme double-stranded RNA adenosine deaminase. This enzyme deaminates adenine to create inosine. Inosine hydrogen bonds in a manner similar to guanine. In effect, the editing enzyme changes codons in mRNA by converting selected adenine residues to the functional equivalent of guanine. These enzymes are called adenosine deaminases acting on RNA (ADAR, formerly known as dsRAD or DRADA), and the enzyme that binds tightly to Z-DNA is ADAR1. The human ADAR1 protein is nearly 140 kDa

in size (1226 aa) and consists of three major domains. The C-terminal region contains the adenosine deaminase domain and the central region consists of three double-stranded RNA binding domains. These domains recognize double-stranded A-RNA and bind solely to that conformation in a sequence-independent manner. The N-terminal region consists of a bipartite Z-DNA binding domain, termed Zab, which has two homologous subdomains ( $Z\alpha$  and  $Z\beta$ ) that are separated by a tandem-repeated linker (Herbert *et al.* 1995; Schwartz *et al.* 1999a).

The  $Z\alpha$  domain, containing approximately 80 amino acids, has been cloned and studied independently in great detail.  $Z\alpha$  was found to bind to Z-DNA with a low nanomolar binding constant (Herbert *et al.* 1993; Herbert *et al.* 1995; Herbert *et al.* 1997). The interaction between  $Z\alpha$  and DNA can be measured in several ways. Upon incubating poly(dGdC)<sub>n</sub> with increasing amounts of  $Z\alpha$ , the circular dichroism changes from right-handed B-DNA to the left-handed Z form. In addition, the binding can be measured directly using surface plasmon resonance (BIAcore) or ultracentrifugation experiments (Herbert *et al.* 1998; Schade *et al.* 1999).

The ADAR enzymes exist as a small family. ADAR-2 contains an adenosine deaminase domain and a double-stranded RNA binding domain but does not have a Z-DNA binding domain (Melcher *et al.* 1996). These ADAR proteins are found in all metazoan tissues, suggesting that RNA editing is of great evolutionary significance (Bass 1993; Wagner & Nishikura 1988; Herbert 1996). The activities of these enzymes may be an important source of phenotypic variation as they have the potential to significantly alter the linear flow of information from DNA to RNA (Herbert 1996; Maas & Rich 2000). A number of substrates have been identified including the glutamate and serotonin receptors in the central nervous system, as well as the  $\alpha$ -2,6-sialyltransferase in the liver (Sommer *et al.* 1991; Lomeli *et al.* 1994; Burns *et al.* 1997; Ma *et al.* 1997; Maas & Rich 2000). In all examples the edited form of the protein, with changes in specific amino acids, results in the production of a modified function for the protein.

In the case of the glutamate receptor which is an ion channel, a glutamine (codon CAG) is edited to code for arginine (codon CGG) (Sommer *et al.* 1991). This is located in GluR-B, one of the subunits that make up the glutamate ion channel. The positively-charged arginine is found in the center of the ion channel, and its presence prevents the influx of calcium ions. This results in a rapid excitatory transmission, a change that is so beneficial to the organism that the GluR-B message is almost completely found in the edited form (Kask *et al.* 1998).

The serotonin receptor is a G-coupled protein; the edited form of the enzyme which interacts with the G protein is modified so that the coupling is somewhat weaker than the unedited form (Burns *et al.* 1997). This results in a modified serotonin receptor that produces a weaker signal. Both of these receptors are used in the central nervous system, thereby permitting a finer-tuned level of serotonin regulation. In the liver  $\alpha$ -2,6-sialyltransferase, the edited protein has a different secretory pathway, giving rise to a longer half life for the protein (Ma *et al.* 1997). Again, the unedited and the edited transcripts are both used in the organism, probably to gain greater control over the regulation of the sialyltransferase activity.

The crucial step in the editing process is the formation of a hairpin or fold-back structure in the pre-mRNA molecule, resulting in the formation of an RNA duplex (Higuchi *et al.* 1993). The RNA duplex is the binding site for the ADAR enzyme through its double-stranded RNA binding domains. This leads directly to deamination of an adenine residue somewhere in the duplex. The mechanism concerning the selection of the particular adenine are not well understood. An important point is that the

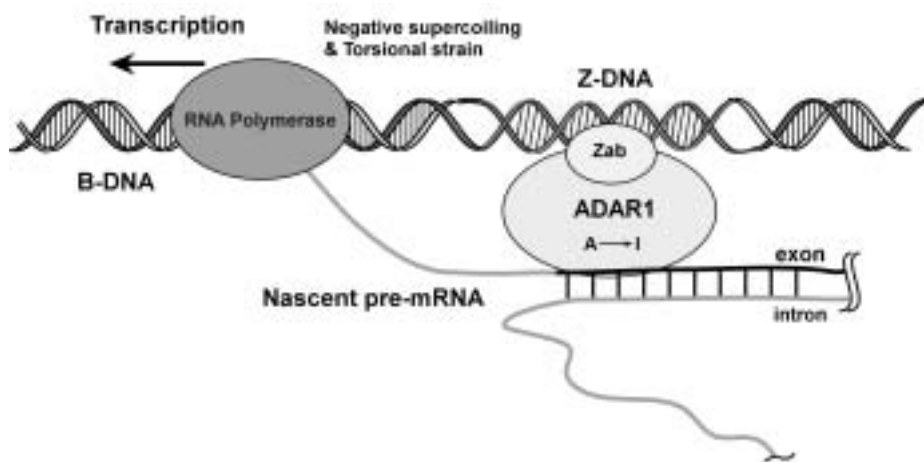


Figure 5. *In vivo*, Z-DNA is thought to be stabilized by the negative supercoiling generated by an RNA polymerase moving through a gene. Transcription also gives rise to regions of double-stranded RNA (dsRNA), formed when a nascent RNA transcript folds back on itself. The RNA editing enzyme, double-stranded RNA adenosine deaminase (ADAR1), has been shown to bind both Z-DNA and dsRNA with nanomolar affinity. Each nucleic acid is bound through a separate domain. This enzyme then catalyzes the hydrolytic deamination of an adenine within the dsRNA to form inosine, which is subsequently translated as guanine. Several editing sites may exist in a single pre-mRNA.

duplex RNA substrate is frequently formed by the pairing of an intron with an exon, and the exon is edited to change the amino acid codon. This has a number of interesting consequences. The control of the editing system rests in particular intronic sequences that are complementary to exonic sequences. In addition, it raises the question how does the enzyme manage to carry out all of its editing activity before the introns are removed by the splicing apparatus, which is known to be attached to the end of the nascent mRNA chain. This is where the postulated role of the Z-DNA binding domain becomes important.

The problem that the editing enzyme has is that of finding an actively transcribing gene in contrast to a gene that is not transcribing. Actively transcribing genes with their moving RNA polymerases generate the negative torsional strain upstream of the polymerase that transiently stabilizes Z-DNA while the polymerase is moving (Liu & Wang 1987). Hence, transcribing genes have Z-DNA in them, while non-transcribing genes do not. It is possible that the high-affinity Z-DNA binding domain at the N-terminus of ADAR1 localizes itself on the Z-DNA as a way of targeting a transcribing gene, as distinct from a non-transcribing one. In effect, it increases the local concentration of the ADAR1 editing enzyme in the vicinity of areas undergoing active transcription. The manner in which ADAR1 may bind to both Z-DNA and double-stranded RNA is shown in Figure 5.

#### *Z $\alpha$ -Z-DNA Crystal Structure*

By carrying out controlled proteolysis of the Zab domain of ADAR1, it was possible to isolate a stable Z $\alpha$  domain which could be over-expressed and purified in sufficient quantities for crystallographic studies (Schwartz *et al.* 1999a; Schwartz *et al.* 1999c). Schwartz and coworkers were able to co-crystallize Z $\alpha$  with a segment of Z-DNA and the structure of this complex has been solved at 2.1 Å resolution (Schwartz *et al.* 1999b). The structure that emerged from the complex was quite surprising and revealed the mechanism that nature uses for detecting Z-DNA. One Z $\alpha$  molecule binds to each strand of a Z-DNA duplex, but the two proteins do not interact with one another. The protein domain is folded in the form of a helix-turn-helix (HTH), a motif that is widely used in transcription factors for the recognition of specific B-DNA sequences. In the case of B-DNA-binding HTH proteins, there usually is a recognition helix that fits into the broad, deep major groove of B-DNA and contacts specific base pairs. However, in Z $\alpha$  a recognition helix is used, but it does not bind in a groove, and rather it runs along

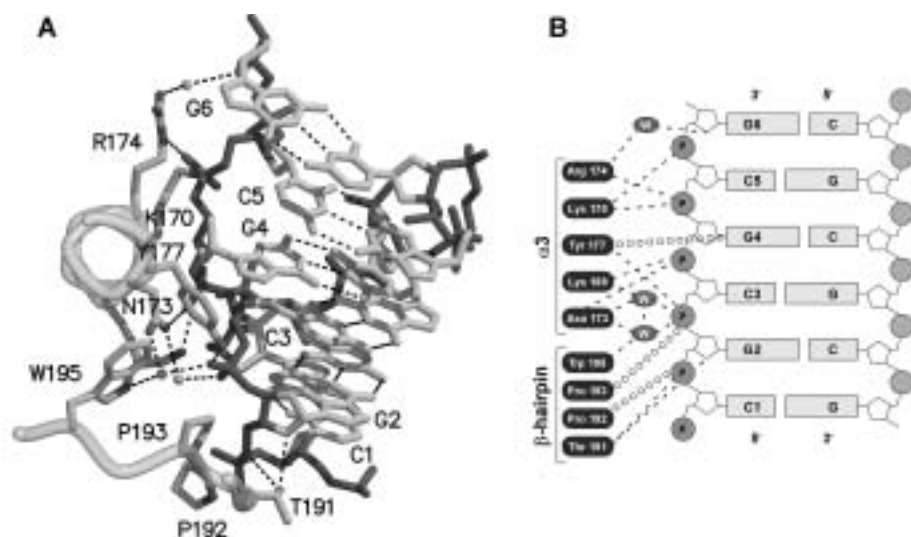


Figure 6. Recognition helix and specific interactions of the  $Z\alpha$ -Z-DNA Complex (Schwartz *et al.* 1999b). (A) A view down the recognition helix ( $\alpha 3$ ) axis shows the entire region of Z-DNA recognized by  $Z\alpha$ . Five consecutive backbone phosphates of the Z-DNA segment are contacted by an extensive hydrogen bonding network. Protein side chains in direct or water-mediated contact with the DNA are labeled. Water molecules are represented as green spheres. Tyrosine 177 is involved in the only base-specific contact seen in the complex and is within van der Waals contact of the exposed carbon 8 of the guanine base G4; this feature is characteristic of Z-DNA. (B) Schematic of the protein-DNA contacts. Dashed lines represent H-bonds, and open circles show van der Waals contacts.

the side of the Z-DNA double helix where, together with an adjacent  $\beta$ -sheet, it recognizes five adjacent phosphate residues in the zig-zag backbone using a complex of 11 different hydrogen bonds. This recognition is shown in Figure 6, together with a schematic diagram illustrating the interaction modes. Another striking aspect of the interaction is the complementarity both in shape and in electrostatic interactions between the protein and the Z-DNA (Figure 7, see page VI). The only base-specific interaction is made by a tyrosine residue (Y177) which is in van der Waals contact with the C8 residue of a guanine in the *syn* conformation. By referring to Figure 2, it can be seen that the C8 residue is on the exterior of the Z-helix. This interaction is a stabilizing edge-to-face contact. On the other side of the tyrosine residue, a tryptophan is in van der Waals contact with the tyrosine

in a second stabilizing edge-to-face interaction. The tyrosine residue in this conformation can interact with guanine in the *syn* conformation, or any other base in the *syn* conformation. Thus, the  $Z\alpha$  domain recognizes Z-DNA by its two most distinct features which differ from B-DNA, the zig-zag phosphate backbone and the *syn* conformation of a purine nucleotide.

The major difference between the interaction of  $Z\alpha$  and Z-DNA and the structurally similar helix-turn-helix domain recognizing B-DNA is that the recognition helix has a different "angle of attack". In the B-DNA interaction, the recognition helix is almost perpendicular to the axis of B-DNA, while in the Z-DNA interaction, the recognition helix is rotated so that it is more in line with the helix axis.

*The ADAR1  $Z\alpha$  domain also interacts with Z-RNA*

When an RNA virus such as measles infects a cell, the anti-viral interferon response leads to increased activity of interferon-inducible genes. This includes the ADAR1 gene, which is strongly up-regulated and produces the full-length protein including the  $Z\alpha$  domain (Patterson & Samuel 1995). In addition, the distribution of ADAR1 changes from primarily nuclear localization to both nuclear and cytoplasmic localization. The measles virus replicates in the cytoplasm (as do most RNA viruses), and late in infection it has been observed that the viral RNA has been subjected to hypermutation in which a significant fraction of adenines have been changed to guanines, and many uracil residues have been changed to cytosines (Cattaneo & Billeter 1992). Such mutations are the expected result of the action of ADAR1 on the viral RNA replication system, and may be an attempt on the part of the host cell to disable the virus. Hypermutation similar to that found in the measles virus has also been found in the RNA of vesicular stomatitis virus, respiratory syncytial virus and para-influenza virus 3 (Cattaneo 1994; Bass 1997).

RNA viruses generally utilize a double-stranded intermediate during some period of their life cycle (Jacobs & Langland 1996). Little is known about the conformation of this double-stranded RNA or the forces acting upon it during replication. However, the evidence of the interferon-inducible full-length variant of ADAR1 localized in the cytoplasm prompted us to investigate the possible interactions between  $Z\alpha$  and double-stranded viral RNA in the Z-conformation.

Z-RNA was discovered a few years after Z-DNA (Hall *et al.* 1984). Although the low energy forms of right-handed duplexes of B-DNA and A-

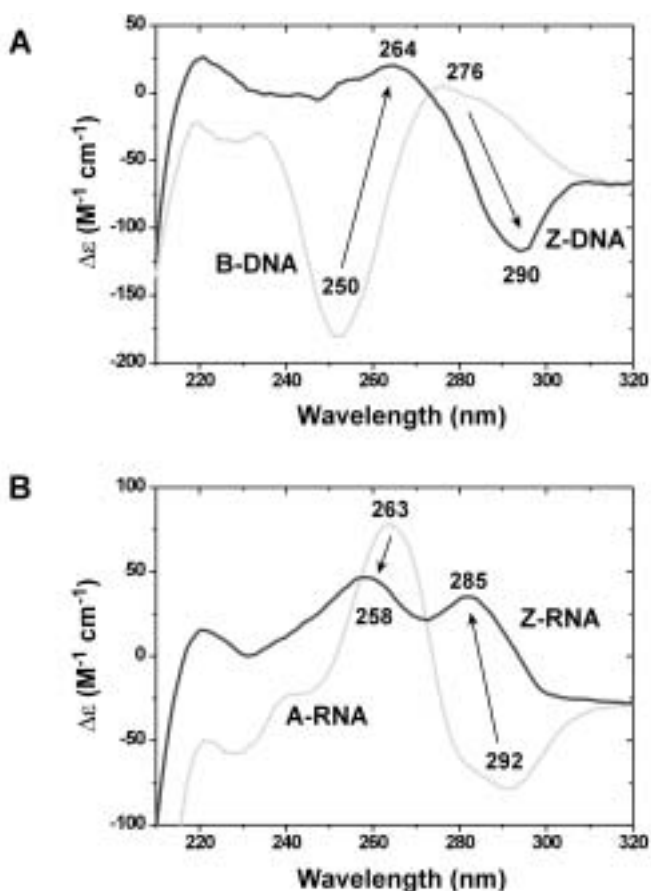


Figure 8. CD spectra of B, Z-DNA, and A, Z-RNA. Circular Dichroism is often used to observe the transition from the A-RNA, or B-DNA conformations to the Z-conformation. (A) CD spectra of the B- and Z- conformations of duplex  $d(CG)_6$ . B-DNA has a deep negative CD band at 250 nm and a broad positive ellipticity at 276 nm. Upon addition of NaCl to approximately 3.5 M, the spectrum nearly inverts to the Z-form with a positive ellipticity at 264 nm and a negative band at 290 nm. (B) CD Spectra of A- and Z-forms of duplex  $r(CG)_6$ . A-RNA has a distinctly different CD spectrum than B-DNA, characterized by a positive band at 263 nm and a broad negative ellipticity peak at 292 nm. Differences in base-stacking and the C3' *endo* sugar pucker both contribute to the differences compared to B-DNA. When  $NaClO_4$  is added to *ca.* 6.5 M, the spectrum changes dramatically with the negative peak at 292 becoming a positive band at 285, and the band at 263 shifting to 258 nm, with reduced intensity. These spectral changes are due to the structural transitions in the phosphodiester backbone, alterations in base-stacking, and change of the cytosine sugar pucker. Note that much higher salt concentrations are required to shift A-RNA to the Z-conformation, than those necessary for the corresponding B-to-Z transition.



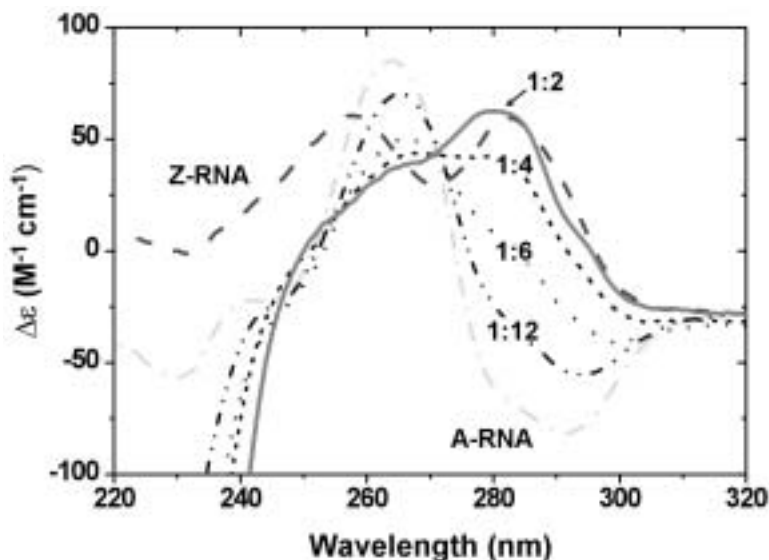


Figure 9. The Z-RNA conformation can be stabilized by  $Z\alpha$  as shown by circular dichroism (CD) spectroscopy (Brown *et al.* 2000). Spectra are shown for  $5\ \mu\text{M}$  of duplex  $r(\text{CG})_6$  in the A-form (---). All samples contained  $10\ \text{mM}\ \text{Na}_2\text{HPO}_4$  (pH 7),  $20\ \text{mM}\ \text{NaCl}$ ,  $0.5\ \text{mM}\ \text{EDTA}$ . In  $6.5\ \text{M}\ \text{NaClO}_4$ , a typical Z-RNA spectrum is seen (- - -). The A-RNA spectrum changes as  $Z\alpha$  is added ( $Z\alpha$  has no CD signal above  $250\ \text{nm}$ , but a strong negative ellipticity below  $250\ \text{nm}$ ). Spectra are shown for addition of  $5\ \mu\text{M}\ Z\alpha$  (-·-·-·), which is 1  $Z\alpha$ :12 bp;  $10\ \text{mM}\ Z\alpha$  (· · · ·) 1:6;  $15\ \text{mM}\ Z\alpha$  (- - - -) 1:4; and  $30\ \mu\text{M}\ Z\alpha$  (—) 1:2. Inversion of the CD bands around  $285\ \text{nm}$  and the decrease in signal at  $266\ \text{nm}$  are characteristic of the A-to-Z-RNA transition.

RNA are structurally very different, both adopt similar left-handed Z-conformations (Teng *et al.* 1989; Davis *et al.* 1990). The Z-DNA conformation is stabilized *in vitro* by high concentrations of salt and other agents that screen repulsion between electronegative phosphate residues, which are closer together in the Z-conformation. The transition from the right-handed A-form of duplex RNA to the left-handed Z-form is much less favorable than the B-to-Z-DNA transition; consequently, higher concentrations of chaotropic salts or low dielectric solvents combined with elevated temperatures are required to induce the transition *in vitro* (Tinoco *et al.* 1986; Klump & Jovin 1987). In order to shift from the right-handed A-RNA duplex, every other residue must change pucker from the C3'-endo into the C2'-endo conformation as both Z-DNA and Z-RNA alternate sugar puckers

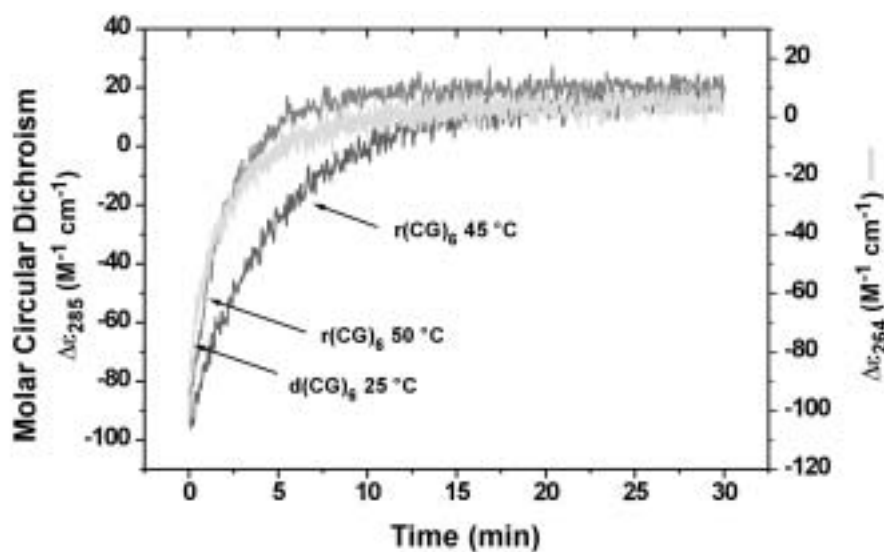


Figure 10. Temperature dependence of the B-to-Z-DNA and A-to-Z-RNA transitions (Brown *et al.* 2000). The rates of the A-to-Z-DNA transitions for the  $r(\text{CG})_6$  duplex at 45 and 50 °C (monitored at 285 nm) are comparable to the B-to-Z-RNA transition of  $d(\text{CG})_6$  (monitored at 264 nm) at 25 °C, demonstrating the higher energy requirements of the A-to-Z-RNA transition.

in the Z-conformation. The energy for changing the sugar pucker of a ribonucleotide is considerably greater than for a deoxyribonucleotide (Olson & Sussman 1982; Sanger 1984). This difference in energy accounts for the high concentrations of salts or increased temperatures which are necessary to stabilize poly  $r(\text{CG})_n$  in the Z-conformation.

It is easiest to observe the change from right-handed RNA duplex A-conformation to the Z-conformation by observing changes in the circular dichroism. Figure 8 illustrates the CD spectroscopic changes of a 12 base pair  $(\text{CG})_6$  duplex of DNA or RNA when they change from the right-handed duplex to the left-handed Z-conformation resulting from the addition of 3.5 M NaCl for the DNA and 6.5 M  $\text{NaClO}_4$  for the RNA. It can be seen that there are near inversions of the CD spectrum although the actual direction of the changes is different, depending if one starts with right-handed B-DNA or right-handed A-RNA. When similar experiments were carried out using  $r(\text{CG})_6$  and gradually increasing amounts of  $\text{Z}\alpha$ , the spectroscopic changes shown in Figure 9 were observed. This clearly indicated that the A-

RNA changed into the Z-conformation in the presence of Z $\alpha$  in a manner analogous to that which had been previously observed for DNA (Herbert *et al.* 1998; Schwartz *et al.* 1999a). The presence of Z-RNA in this complex was corroborated by Raman spectroscopic studies (Brown *et al.* 2000).

Analysis of the change of r(CG)<sub>6</sub> in the presence of Z $\alpha$  revealed that it took place at a slower rate than the conversion of d(CG)<sub>6</sub> to the Z-conformation in the presence of Z $\alpha$ . Figure 10 shows a scan of the change in the circular dichroism signal at fixed wavelength as a function of the rate of change from the A- and B-forms of RNA or DNA to the Z-conformation (Brown *et al.* 2000). The rate of conversion of the duplex r(CG)<sub>6</sub> at 50 °C is approximately equal to that of the analogous DNA duplex, d(CG)<sub>6</sub>, at 25 °C. This is a reflection of the activation energy required for the transition to the Z-conformation which is 24 kilocalories per mole for d(CG)<sub>6</sub>, compared to 38 kcal mole<sup>-1</sup> for r(CG)<sub>6</sub>. The increased activation energy for RNA is largely due to the energy required to change the sugar pucker of ribonucleotides compared to that required for deoxyribonucleotides.

It is not surprising that proteins which bind to B-DNA do not bind to A-RNA, and *vice versa*, since these right-handed duplexes differ significantly in shape; however, the left-handed Z-form duplexes are very similar. Z $\alpha$  may be the first nucleic acid binding domain that binds specifically to *both duplex DNA and RNA*. The role of this domain in the hypermutation of RNA viruses has yet to be explored. A great deal is known about the negative superhelicity generated by transcription of dsDNA, but little is known about negative torsional strain in replicating RNA molecules. This subject needs to be more fully explored in order to understand the possible participation of the Z $\alpha$  binding domain in the hypermutational activities of ADAR1 during infections by RNA viruses.

### *Conclusions*

Since the discovery of Z-DNA in 1979 and Z-RNA in 1984, many groups have worked to understand whether specific biological functions are associated with these unusual nucleic acid conformations. The discovery that the N-terminus of ADAR1 bound Z-DNA with high affinity and the subsequent efforts, including a detailed structural view of the interaction, have shed much light into this interesting system, but this is only a first step. Less is known about Z-RNA. Although a few studies attempted to identify this structure in cells (Zarling *et al.* 1987), research on Z-RNA has been dor-

mant for almost a decade. The recent finding which demonstrated that the Z $\alpha$  domain of ADAR1 could also bind Z-RNA has reinitiated interest, and raised more questions than it has answered.

The future for the Z-conformations of nucleic acid has many difficult questions which need to be addressed. Work is ongoing to identify biological activities that may be associated with these structures and the current abundance of genomic information has fueled efforts to seek additional proteins which may specifically interact with the Z-conformations and to identify sequences which have the propensity to form Z-DNA or Z-RNA. New developments from biochemical and structural studies of other organisms may provide insight into the problems of biological functions. There is little doubt, however, that some of the answers will be unexpected.

#### *Acknowledgements*

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## SOCIETY, SCIENCES, AND THE FUTURE OF OUR SPECIES

CRODOWALDO PAVAN

Among computer annotations made by my friend Julio Viegas (10 years of age), I found the following reflection: 'The Universe is very big, the Earth, the sky, the stars; and I don't have any idea of what we are doing here'. This doubt burdens a good part of humanity and has given rise to interesting answers even from great thinkers. Certainly it is an important thought that reflects among other things, the mystery that is the origin of the Universe and the existence in it of a thinking-being *Homo Sapiens*. We should not forget however that one should also consider, beside that transcendental reflection, the material part of the problem and the role of *Homo Sapiens with his* activities and consequences on the surface of the earth. *Homo Sapiens* has characteristics not found in any other species, but shares with them the capacity, among others, of reproducing and evolving. This allowed mortal individuals organized as species to compose the immense system of living beings which has survived for about 3.5 billion years without interruption, and will continue existing for a length of time.

Species have been extinguished by asteroid accidents, competition with other species or changes in their environment. On the other hand, new species are produced by the interaction of mutations and natural selection. This process in the more developed organisms which include *Homo Sapiens* always obey the same principle: species are relatively long-lived in spite of being formed by short-lived individuals.

In the past, cultural inheritance has created enormous amounts of aggressivity among different cultural groups, with consequences as disastrous as the destruction of entire civilizations. As a result of our increasing population, and its excess of activities, the very survival of the human

species is in jeopardy. Fortunately, after the dramatic crisis of the second world war, a general tendency toward cooperation among countries and races is developing. This creates the possibility of sustained ethical advance, but involves a tremendous responsibility.

We are not presenting any theory about 'the position of man in the Universe'. We only suggest that we take into account that man has a very active part and is immersed into the perpetual flow of life and, through the hazards of evolution, has conquered unique features. This enables man to overcome a great number of other species by creating technology and using it to transform the environment and modify other species according to its necessities and fantasies.

Unfortunately, this apparent success in life, is creating very undesirable situations that is causing serious concerns about the future of the species on earth. With the ability to think and foresee, man has the obligation of attending to the crucial problems confronting him. Some of these problems will be commented.

A. The shameful social, economic and cultural inequalities existing today inside each nation and in the human population as a whole.

Homo sapiens, as it was said above, is distinguished today from all the other existent species, by having beside a genetic inheritance, that also exists in the other species, its own cultural inheritance that is the main basis of his success in nature.

Intellectual capacity and its manifestation are the result of the sum of genetic heredity and cultural inheritance.

The latter is controllable by society, the former, even though understood in many details, has its millions of types distributed at random in the population. Privileged genotypes, good and bad occur by chance in the individuals of any human population.

Presently, cultural inheritance privileges preferentially people of some countries and also certain individuals, among others, inside each country. This is the result of the absurd social and economic inequalities prevalent in all parts of the world.

Actually those developed groups are immersed in the exhilaration of progress – progress and more progress without any consideration of the impact on civilization and the so called 'rich' (groups in populations) and self proclaimed 'privileged' are taking from nature all that they can with very few considerations for the consequences of what they are doing, not only for the society, but also in the destruction or pollution of the environment with bad consequence for the future generations.

Without a doubt, a great part of the human development and progress, is directly linked to the use of science and should be a very universal one. Unhappily, in some cases it is being used improperly and even in a nonethical way used by groups not only in the exploitation of the environment as well as in an immoral exploitation of fellow creatures less fortunate, of the same or of different races.

The profits obtained with patent of linked genes in the processes of production of food and medicines is acceptable when applied in the developed countries, but without a doubt it is immoral and unjust when applied, as it happens today, for underdeveloped countries.

Human food and medicines, two basic elements for the development of an individual are not only scarce but are expensive for a great part of the populations of the underdeveloped countries. This situation causes an enormous amount of deaths and, not less important, disabilities in the physical and mental development of that majority that doesn't get a decent survival level in the fight for the life.

The argument that if patents not be allowed for production of foods and of medicines, the great firms won't apply resources for researches in those areas with the consequences that they won't obtain very desirable progress for the species, is inconsistent and cynical.

The situation is such that in the current world organization, about 80% of the victuals and 80% of the medicines are consumed by approximately 20% of the world population, as represented by the developed countries. Are not the obtained profits of those 80% consumed by the privileged ones enough to cover the expenses of the researches to obtain the processes patented along with the due profits?

The problem is moral and of justice, because the application of those patents causes a situation which contributes to the malnutrition that affects billions of people in the underdeveloped countries and hundreds of millions of children die in several parts of the world from a combination of malnutrition and the impossibility of obtaining medicines by the members of poor populations.

Three are the basic and necessary conditions that a person can have a normal development and be capable to participate in the natural competition in the society, using their physical and mental resources to obtain what they deserve to have as a human being.

In a natural sequence the three basic conditions are: feeding, health and education. It is obvious that rare cases are found today of people that had in the childhood deficiencies of one or more of those basic conditions and

that still has achieved success in life. Those cases are rare and without a doubt he/she would still have larger success had they received the normal basic conditions.

Those conditions are basic and they should be supplied for all. However, in the current situation in the human populations the privileged ones are few that in childhood and youth receive them and many or the great majority receive them with different degrees of deficiencies and even a portion die for not receiving them at all.

Report of UN 1998 informs us: 'World consumption has expanded at an unprecedented pace over the 20th century, with private and public consumption expenditures reaching \$24 trillion in 1998, twice the level of 1975 and six times that of 1950. In 1900 real consumption expenditure was barely \$1,5 trillion'.

In another chapter of the same report we found: 'The poorest 20% of the world's people and more have been left out of the consumption explosion. Well over a billion people are deprived of basic consumption needs. Of the 4,4 billion people in developing countries, nearly three-fifths lack basic sanitation. Almost a third have no access to clean water. A quarter do not do adequate housing. A fifth have no access to modern health services. A fifth of children do not attend school to grade 5. About a fifth do not have enough dietary energy and protein. Micronutrient deficiencies are even more widespread. Worldwide, two billion people are anaemic, including 55 million in industrial countries. In developing countries only the privileged minority have motorized transport, telecommunications and modern energy'.

All these deficiencies are important and we should be ashamed to have them existing in such proportions in the human populations, but worse is that due to the lack of nutrition and health at the youth stage, we have the existence of billions of persons which, in their entire life, never reached the stage of normal human development.

If the firms that possess the patents of foods and medicines are not satisfied with the profits they are obtaining from the 80% or more of the market, that is the percentage of food and medicines consumed today by the developed countries, then we find that such an inhuman and egotistical attitude must be counterbalanced by removing from these 'little ethical groups' the privilege that is given them through patents which provide incredible profits and produce benefits for the rich while neglecting the poor, that represent an enormous portion of the human population.

It is of interest to evidence that the sub-human situation in which a

great percentage of the human population of the underdeveloped countries live today is not just a repugnant social excrescence that should be avoided but also represents important focuses of serious problems, mainly on public health that affect the global population, as we shall see further on.

By the initiative of the London Royal Society, the US Academy of Science and the adhesion of the Brazilian, the Chinese, the Indian, the Mexican and the Third World Academies of Science a proposition was made to facilitate food production to meet the needs of the poor farmers of the world. The project Transgenic Plants and World Agriculture is extraordinary in its purpose and in the way it should operate. It is not a project of charity, but of human cooperation following the chinese proverb:

‘If you want to solve the problem of the hunger, don’t give him fish, teach him how to fish’.

The basic beginning is, through agronomic researches, to produce organisms genetically modified and to cultivate a high production for several areas, supplying seeds to the farmers at low prices or even free, without any restrictions on the repeated use of the initial seeds.

It is without a doubt a project of high human interest and great humanitarian value which deserves the collaboration of all.

Unhappily, the improper use of the industrial property (patents) by multinationals as discussed above and later the excessive admonitions of possible negative consequences for the environment has resulted in the use of organisms genetically modified being incriminated by groups of people in several countries.

The criticisms in relation to the nonethical and inhuman use of the industrial patents against the poor farmers are justifiable, but the prohibition of the use of the genetically modified organisms because of the possible dangers to the environment or human health is absurd.

The transgenesis is an extraordinary technique with great potency in food production and great economical value and, when controlled, it is as safe or even more so than the conventional processes. In relation to the negative effects on public health and the environment, the transgenic is less offensive than the traditional approaches to the problem.

Another series of important negative circumstances created by human activities deals with changes in the biosphere, the earth environment on which humans depend for survival.

The continuous deterioration of the ecosystems is the consequence of the absurd and irrational processes of pollution of water, soil and air which are

eliminating so many different species of organisms. This has to be curtained or otherwise grave problems will descend upon the future generations.

As stated by Raven (1998), over the last 50 years, about 25 percent of the world's topsoil has been lost and at present we are losing about 26 billion tons of topsoil a year. Also 15 to 20 percent of the land under cultivation in 1950 has been lost. We have to emphasize, however, that even with this loss of topsoil the food produced has still been sufficient to feed the extra 3.5 billion people added to the population between 1950 and 1998. We should not be proud of this, since the loss of part of the topsoil system will produce dire consequences in the future.

It is also true that 2 billion, or one third, of the people in the world subsists on less than one dollar a day. They are living in a condition that the World Bank defines as extreme poverty, therefore they lack adequate nutrition.

The problem is of such importance that in May of 2000 an assembly of 63 National Academies of Sciences published a statement entitled 'Transition to Sustainability in the 21st century'. In the chapter on scientific achievements and future challenges, the following problems are discussed:

- a) Meeting the needs of a Large World Population, Reducing Hunger and Poverty and Preserving Human Wellbeing;
- b) Preserving and Maintaining the Environment and the Natural Resource Bases;
- c) Moving toward Sustainable Human Consumption Patterns.

The document follows with: what can and must be done by the Scientific and Technological Community and the Necessary Steps to transform the statements in Action.

This joint declaration of the 63 Academies constitute a paradigm for multiple coordinated actions, aimed at mitigating the terrible situation in which a great part of humanity lives, jeopardizing the happiness of future generations. It is mandatory that the leading countries in the world accept their responsibility and attend to this problem mobilizing resources and ingenuity, as if they were in times of war.

Another anguishing situation that necessitates the urgent support of the developed countries is the aids epidemic that is occurring principally in the subsaharan African countries.

The situation is extremely serious not only for the African countries but for the entire global population. Of the 36 million people stricken with aids that existed in the world in the year 2000, about 25 million of them live in subsaharan Africa and from the way that this epidemic is being treated in

these countries we may consider the situation as being the perfect biological laboratory for the improvement of the HIV against humans.

To this we must add the fact that the HIV presents various biological characteristics that make the problem even more serious.

'Genetics variability is the hall mark of HIVs. A major source of variation is the high error rate of the viral reverse transcriptase, which generates approximately one mutation per replication cycle' (Thomson, 2000). This without a doubt gives it a large adaptive capacity; the HIV inside the human body maintains itself in direct contact with the microorganisms responsible for practically all the infectious diseases of man. The very large number of people afflicted with AIDS are characterized by possessing in the organism a large quantity of HIV frequently associated with different infectious agents (microorganisms) of the other human diseases. This contact, direct and frequent, becomes extremely serious when we realize that the HIV also possesses a capacity to function as a transposon. As such it can be moved horizontally by means of other infectious agent such as herpes simplex. It can also acquire a piece of genetic material of one of the microorganisms accompanying the infection that would cause the newly infected undesirable complications.

The possibility that the HIV acquires by this process the capacity to be transmitted in the different form from one individual to another cannot be discounted.

To show that the problem is even more serious we must not forget the fact that the HIV is transported by their hosts from one end of the world to the other becoming a global pandemic.

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## TESTIMONIES

ENRICO DI ROVASENDA

During four four-term years as President of the Pontifical Academy of Sciences, which is composed of scientists of the highest level from all over the world, among whom several Nobel Prize winners, Chagas, within the vast horizon of his mind open to truth, considered the world, in the words of Henri Bergson, a “machine à faire des dieux”, a house open to transcendence, a light and shaded home of divine and human knowledge.

When Man’s house ran the risk of being universally ruined because of the use of nuclear energy for warfare, the Pontifical Academy of Sciences, through Chagas’s initiative, drew up four documents on the universal catastrophe of nuclear conflict. Pope John Paul II supported with his authority the documents of the Academy, which were presented by delegations of the Academy, accompanied by the Pontifical Nuncios, to the major world powers, from the USSR to the USA.

The Galileo case, which during previous centuries had caused a contrast between scientific and religious authorities, was the object of resolute attention by Chagas in his specific function as President of the Pontifical Academy of Sciences. Chagas invited the Nobel Prize winner, Paul Dirac, to review the most up-to-date scientific theory of relativity, and asked John Paul II to proclaim the authentic relationship that existed between science and faith, an initiative which was in the highest tradition of the Church’s teaching. Chagas’s initiative had the widest cultural and religious echo, and re-established in the scientific world a deeper confidence in the Church’s teaching.

Carlos Chagas, an authoritative guide of illuminated command, and an expert on the human soul, secured, wisely and mildly, the adhesion of his co-workers of all functions and levels.

Light of science and transcendence, wisdom of command and goodness of direction, are sculpted in the perpetual memory of Carlos Chagas.

RENATO DARDOZZI

Mi associo con tutto il cuore alla celebrazione dell'indimenticabile e non-dimenticato Presidente Carlos Chagas, nel dolore. Serie ragioni di salute mi impediscono di essere presente.

Mi rivolgo particolarmente a Donna Anna Sua consorte confidente e amata, alle magnifiche e amate Figliole: Mara da Gloria – Silvia Amelia – Margarita – e Cristina –, che Egli tanto amava e che sono anche nel mio cuore.

Don Carlos, uno dei Primi Padri Fondatori, con Gemelli, con Gianfranceschi, con Lemaître, con O'Connell, con Enrico di Rovasenda, ha avuto l'intuizione, la capacità e la forza, di interpretare e attuare il particolare ruolo che il Fondatore Pio XI, assegnò e assegnava specificamente all'Accademia. E cioè approfondire, in un secolo dinamico esigente e difficile, e promuovere autonomamente le basi della scienza, la scienza stessa con le sue applicazioni. E ciò a fianco e a supporto del pensiero sulla trascendenza metafisica; nella distinzione *non* nella separazione in una integrazione globale.

Egli (Don Carlos), saggio prudente ed acuto, propose sempre l'obiettivo del Fondatore (Pio XI) alla riflessione delle alte menti pensanti del mondo per raggiungere l'Unità del Sapere, perfetta e superiore nella Carità.

A questo grande Spirito, che vive in Dio ancora fra noi, innalzo sentimenti profondi di immensa riconoscenza, chiedendoGli di continuare a illuminarci con quella particolare attenzione e amore che abitualmente riservava a tutti noi.

La Consorte e le Sue Figliole anche da noi amate ci portino nella mente e nel cuore.

Sempre memore il dev.mo e aff.mo Renato Dardozzi.

## TABLES





Fig. 1.



Fig. 2.

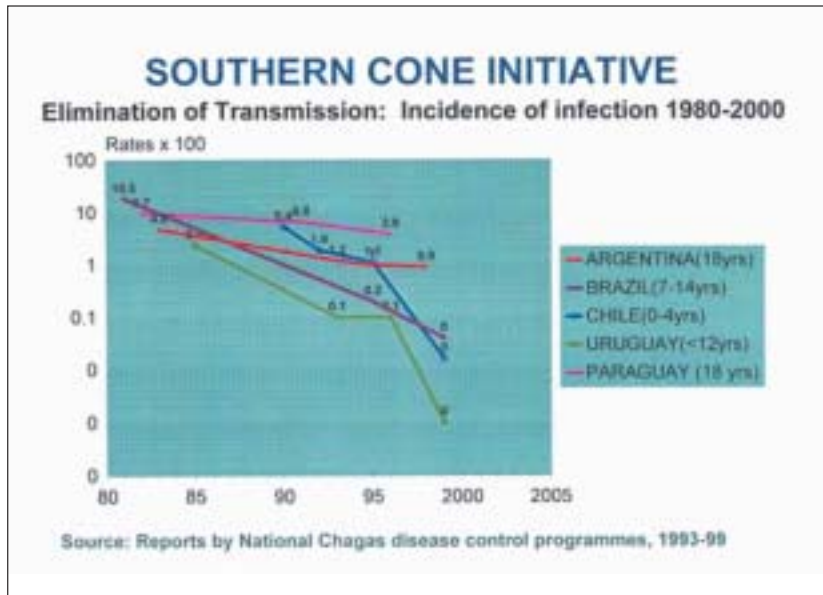
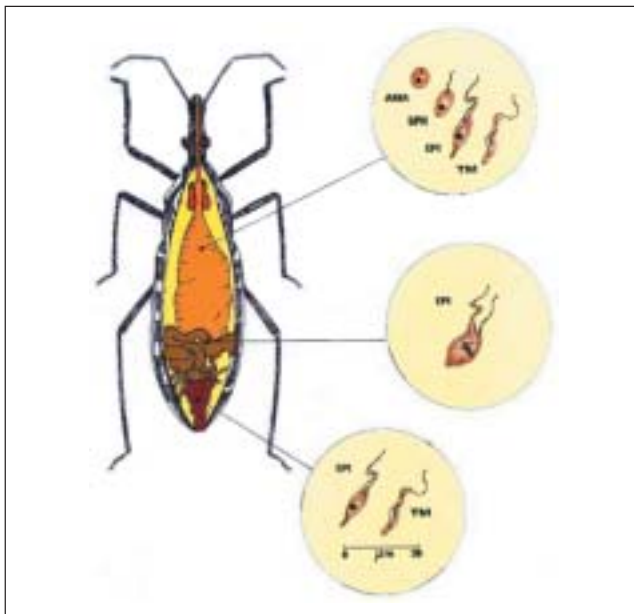


Fig. 3.

Fig. 8. Schematic view of the life cycle of *T. cruzi* in the insect vector.

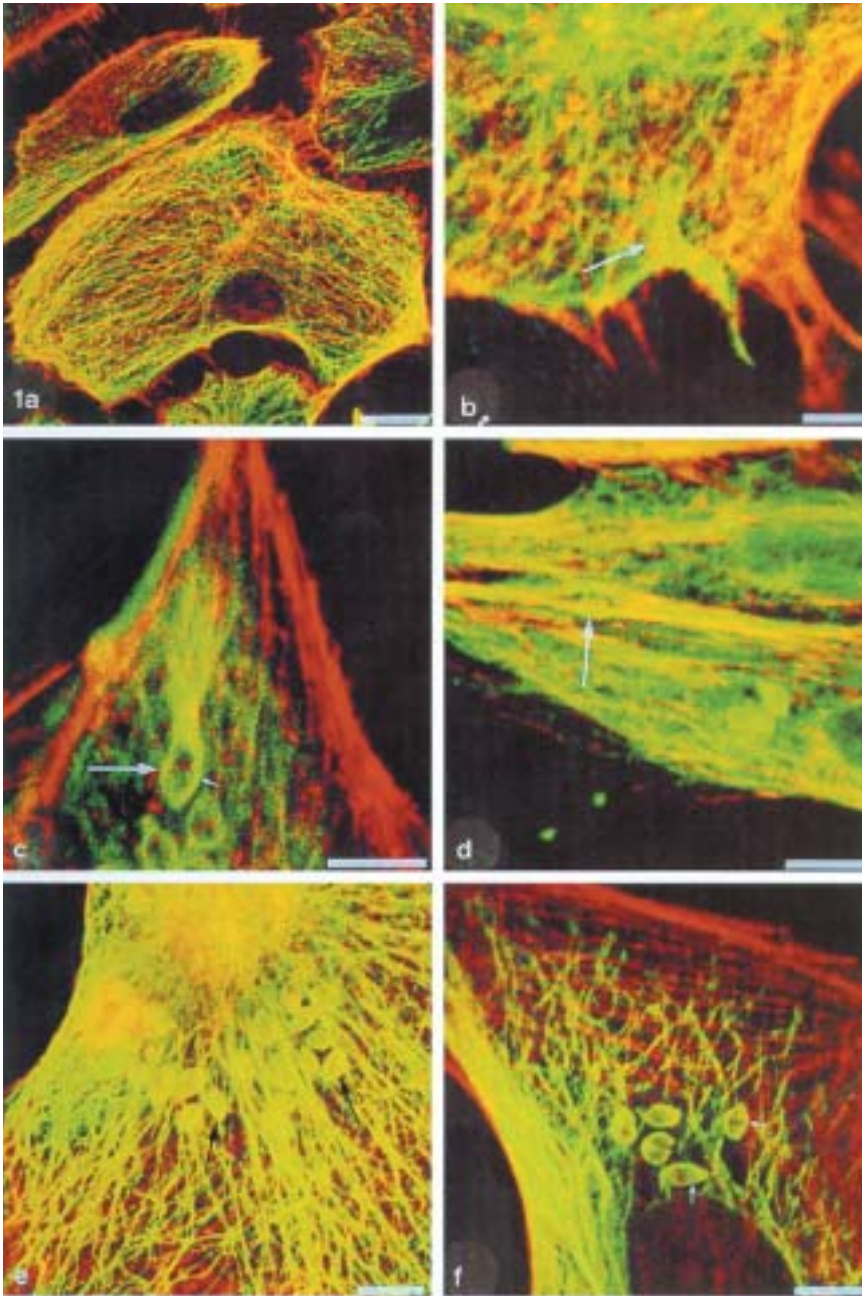


Fig. 13. Confocal laser scanning microscopy of cells infected with *T. cruzi*.



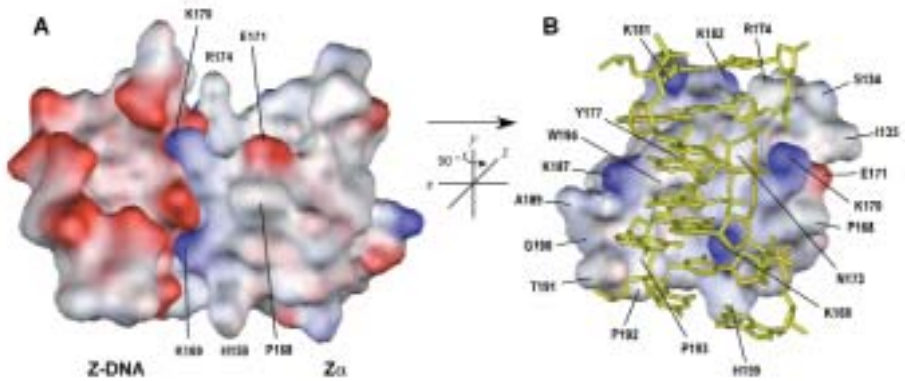


Figure 7. Electrostatic complementarity of the Z $\alpha$ -Z-DNA complex. (A) Residues 134 to 198 (numbering from human ADAR1) of one Z $\alpha$  protein and the 6 bp duplex d(TCGCGCG) are shown represented as solvent-exposed van der Waals surfaces with coloring indicating electrostatic potentials (red indicates negative potential, and blue represents positive potential). The complementarity in shape and electrostatic potential is striking. (B) The same complex as in A, rotated by ~90°, with the DNA duplex displayed as a stick-model allowing direct viewing of the Z $\alpha$  interaction surface. Amino acid residues on the surface are labeled.