PROGRESS IN BOTANY 66

Edited by

K. Esser
U. Lüttge
W. Beyschlag
J. Murata

Genetics
Physiology
Systematics
Ecology

Springer

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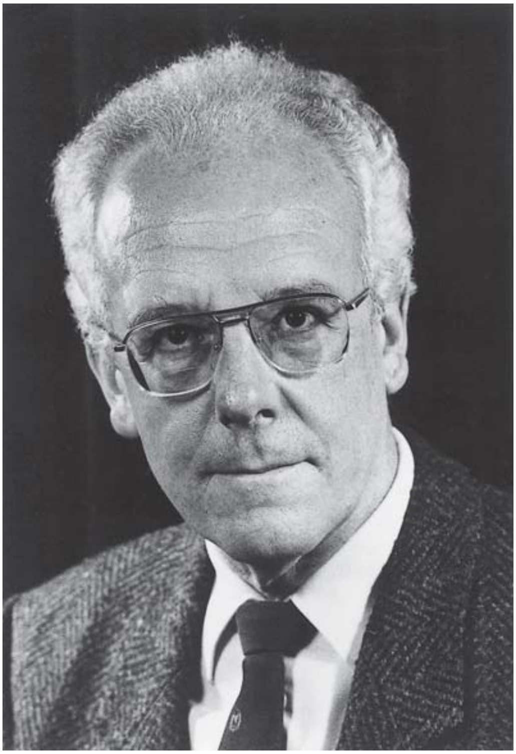

Curriculum vitae

1930 Hans Mohr was born on a farm in the Black Forst/Germany

1949 Graduation from a German Gymnasium under French supervision

1950 Undergraduate student at the University of Tübingen/Germany: Philosophy or science?

1953 Graduate student in biology with Professor Erwin Bünning

1955 Submission of a Ph. D. thesis to the Faculty of Sciences at Tübingen University

1956 Postdoctoral research fellow at the Plant Industry Station at Beltsville, Md., U.S.A.

1957 Married to Dr. Iba Kraut, brilliant biochemist and wonderful partner

1959 Habilitation at Tübingen University

1960 Professor of Biology, University of Freiburg; Visiting Professor, University of Massachusetts

1966 Member of the German Academy of Sciences (Leopoldina)

1982 Member of the Heidelberg Academy of Sciences and Humanities

1982 Honorary Editor of the International Journal of Plant Biology, Planta

1992 Director at the Governmental Insitute of Technolgoy Assessment in Stuttgart

1992 Member of the presidency of Leopoldina

1992 Member of the 'Innovationsbeirat' (State Government, Baden-Württemberg)

1995 Member of the 'Technologiebeirat' (Federal Government, Bonn)

1997 Retirement

2000 Honorary Member of the German Botanical Society

2002 Member of the University Council, University of Konstanz

Many honours, including honorary doctorates, the Cothenius medal in gold, the Max Born medal and the Bundesverdienstkreuz, 1st class

Author/Editor of 18 books and approximately 400 articles

A Life History Between Science and Philosophy

Hans Mohr

1 Philosophy or Science?

When I graduated from an old-fashioned German Gymnasium under French regime my interests were divided between science (physics) and philosophy, with an intuitive preference for the latter. However, by the end of my first semester at Tübingen University I realized that I needed a more solid education in science to study that branch of philosophy I was particularly interested in, namely epistemology. By that time I considered philosophy as primarily an epistemological subject.

Epistemology investigates the origin, nature, methods and limits of human knowledge. Since in the modern world the sciences had become the major source of positive knowledge, a deepened introduction to the basic sciences was obviously a prerequisite for any career in epistemology.

Moreover, I was dissatisfied with the prevailing German academic tradition of teaching philosophy as a history of philosophical thoughts. My deep interest in modern fields, such as modern logic and analytical philosophy, was hardly met. As far as the leading philosophical fashions of the time were concerned, neither Heidegger's existentialism or fundamental ontology nor the late Husserl's transcendental phenomenology attracted me.

Fortunately, some of the famous science professors in Tübingen had strong philosophical minds: Walter Kossel in physics, Max Hartmann in natural philosophy and Erwin Bünning in botany. Bünning, who by that time had established the concept of the physiological clock, had just published a superb treatise on *Theoretische Grundfragen der Physiologie* (Bünning 1949) which I studied with keen interest. Moreover, I enjoyed reading a well-thumbed copy of A.J. Ayer's brilliant book of 1936 *Language, Truth and Logic*. This ideally accessible, lucidly written work stabilized my plans to become a real scientist before considering any career in philosophy. Ayer, the genuine philosopher, had argued convincingly: "If you want a philosopher to be constructive..., then I think you've got to marry him to a scientist." At the end of *Language, Truth and Logic*, Ayer saw the future of

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philosophy only in its being the logic of science. In retrospect, I gratefully acknowledge that Bünning and Ayer put me on the right track.

Equipped with a stipend of the Studienstiftung des Deutschen Volkes, I studied physics and biology. At the graduate level, I preferred biology even though quantum physics and thermodynamics remained favorite topics. Fortunately Erwin Bünning accepted me as candidate for a doctorate in 1953. He was a great mentor and a fine person.

2 Early Steps in My Scientific Work

2.1 Towards Phytochrome

The action spectrum of a photobiological response represents, with certain assumptions, the absorbance spectrum of the effective absorbing substance (photoreceptor). Bünning wanted me to identify by means of action spectroscopy the photoreceptor involved in the germination of fern spores. After some preliminary studies I chose the spores of the common male fern, *Dryopteris filix-mas*, for the following reasons:

- These spores never germinate in complete darkness.
- The light requirement can be satisfied by a short light treatment of the fully imbibed spores. That is, germination can be 'induced' by light. If the spores are placed in darkness after the light treatment, complete germination takes place.
- In the Botanical Garden in Tübingen I detected a population of cloned *Dryopteris* sporophytes. They had been derived in the 1930s from a single rhizome by the late Prof. Lehmann, who was interested in the appearance of somatic mutations. From this clone I collected the most homogenous spore population you can imagine.
- The spore material could easily be germinated and inspected on a thin agar medium. I have only very rarely observed contaminations within the time span required for germination.

As far as my experimental equipment was concerned I was equally lucky: interference filters had just become available, and I, together with my friend G. Schoser, could construct an interference filter monochromator unit for photobiological purposes. This type of irradiation device has had decisive advantages compared with prism- or grating-equipped monochromators. Measurement of the photon flux of the monochromatic light beams posed a problem since photocells turned out to be a poor choice. However, Bünning provided the money to buy an expensive bolometer, and Kossel offered me an extremely sensitive thermopile which I could use over night

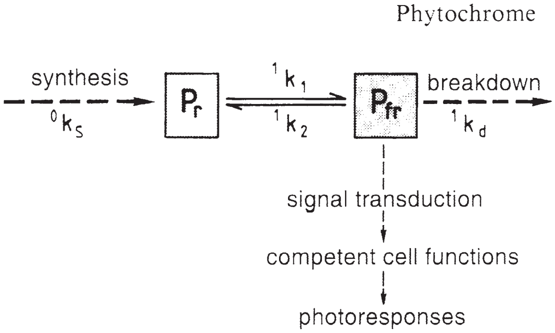

Fig. 1. Scheme of the phytochrome system. Explanation: phytochrome appears in two forms, P_r and P_{fr}. Without light only P_r, the physiologically inactive form, is made. Under the influence of light P_r changes into P_{fr}, the physiologically active form. The photoconversion P_r–P_{fr} is photoreversible; it follows first-order kinetics $^1k_1$, $^1k_2$ in both directions. The signal induced by light is passed on from P_{fr} (signal transduction) and received by cell functions competent for this signal, e.g. by promoter regions of competent genes. Specificity of photoresponses is determined by the spatial and temporal competence pattern for P_{fr}. Light (P_{fr}) has no influence on development of this competence. Phytochrome is a predominantly hydrophilic chromoprotein which is easily isolated from the cell. The absorption maximum of P_r is 665 nm in vitro, i.e. in the red (R); that of P_{fr} is 730 nm in the far-red (FR). (Mohr and Schopfer 1995)

(since it was needed in the Physics Department during the day). In fact, with the help of my girlfriend Iba (who fortunately agreed to marry me later), I could produce and measure with high precision all types of monochromatic beams which I needed for my work. The result was worth the effort: I was able to elaborate a very precise action spectrum, and we found the 'reversible red/far-red photoreactive system', which was later named 'phytochrome' (Mohr 1956).

In the meantime, however, the reversible red/far-red photoreactive system had been discovered and described by the Beltsville research group in studies on light-induced seed germination. A modern version of the phytochrome system is reproduced in Fig. 1.

Even though I was only second, I received the Research Prize of the University – the first time in my life I owned 2,000 Marks – and, more important in the long run, I was invited by H.A. Borthwick to join the Beltsville group as a postdoctoral research fellow.

2.2 The Beltsville Group

The discovery of the reversible red/far-red control of plant growth and development and the subsequent in vivo identification and isolation of the photoreceptor pigment phytochrome constitutes one of the great achievements in modern biology (Sage 1992). It was primarily a group of investigators at the Plant Industry Station, Beltsville, Maryland, USA, headed by the botanist H.A. Borthwick and the physical chemist S.B. Hendricks, who made the basic discoveries and developed a theoretical framework on which the progress in the field of (molecular) plant development has been largely based.

I joined the Beltsville group in mid-1956. The mode of cooperation at the Plant Industry Station opened my eyes to the benefits of teamwork, and the wisdom, humility and helpfulness of the two senior scientists was an unforgettable experience which has been a constant inspiration throughout my research career.

2.3 Photomorphogenesis

Photomorphogenesis (Mohr 1972) has remained the major theme of my scientific efforts after my return to Germany, following Bünning's advice to try for habilitation, by that time a prerequisite for an academic career in Germany. By 'photomorphogenesis', we designate the fact that light controls growth and differentiation (and therewith development) of a plant independently of photosynthesis. In order to grasp the full importance of this phenomenon we must recall that the specific development of any living system depends on its particular genetic information and on its environment. In the case of higher plants, the most important environmental factor is light. Of course, light does not carry any specific information with regard to plant development. Rather, light – operating via photoreceptor molecules – must be regarded as an elective factor which influences the manner in which those genes that are contained in the particular organism are being used. In this sense, the study of photomorphogenesis (Fig. 2) became central to a worldwide program to investigate the influence of the environment on the development of higher organisms, including man. I contributed to this latter aspect with a book chapter in *Freiburger Vorlesungen zur Biologie des Menschen* (Mohr 1979a).

3 My Academic Career – A Short Story

I stayed at Tübingen University only for a little while. In February 1960, some months before my 30th birthday, I was offered the traditional chair for botany at the University of Freiburg to succeed Professor Friedrich Oehlkers, an eminent cytogeneticist.

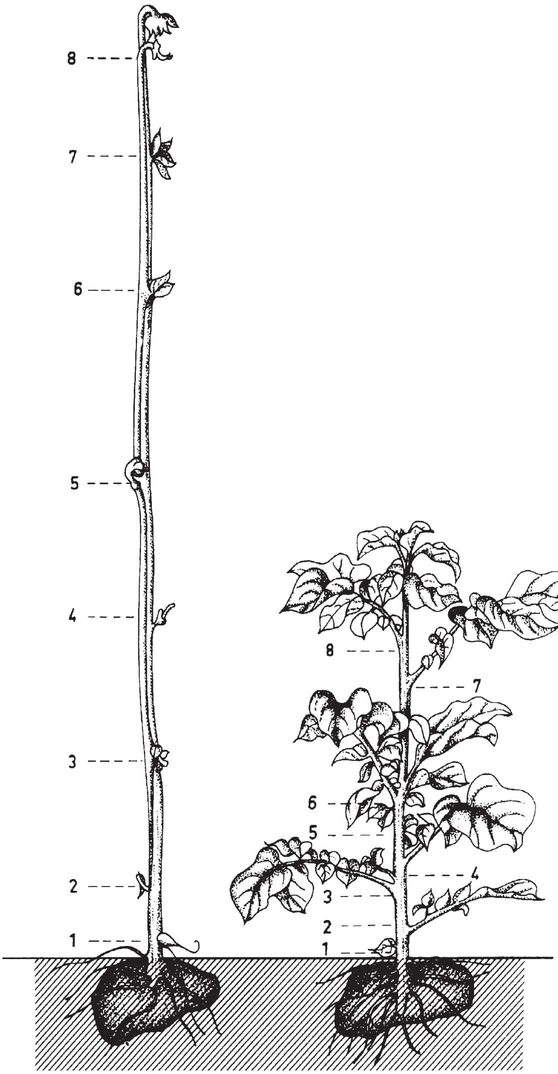

Fig. 2. Both potato plants (*Solanum tuberosum*) are genetically identical. *Left* An etiolated plant grown in darkness; *right* the normal light-grown plant. Scotomorphogenesis (etiolation) is characteristic of development of plants under light-deficient conditions; the alternative development in light is called photomorphogenesis. It is noteworthy that light affects the expression of patterns, the specification of which is light independent. In the present case, patterns of leaf arrangement (called phyllotactic patterns) are precisely the same in etiolated plants as in light-grown plants. However, development from leaf primordia to leaves takes place only in the light. Etiolation is an adaptive response of plants, because as long as the plant is grown in the dark, the limited supply of storage substances is predominantly invested in production (growth) of the shoot axis. This is the most likely way of ensuring that the plumule reaches the light before reserves are depleted. This scotomorphogenesis may be interpreted as a strategy for survival. Photomorphogenesis, on the other hand, is the suitable strategy for development in light (affluence strategy). (Mohr 1972)

To make the right choice was not easy, since I had planned firmly to return to the United States after habilitation, at least for a couple of years. Eventually, my wife and I decided to accept the position (Professor and Department Head) in Freiburg. An important factor at that time was that the administration agreed that I could spend at reasonable intervals a couple of months at American institutions to maintain close personal contacts with my colleagues in research and teaching.

Freiburg University turned out to be an excellent place. Once we had established a new faculty and moved into new buildings, the plant biology department was ready for top research and new kinds of teaching. In 1968, we were chosen by the Deutsche Forschungsgemeinschaft to become a center of excellence (SFB). This implied that we could count on sufficient support provided that we could meet the strict requirements in 3-year intervals.

During the political turmoils in the period 1968–1972, my wife and I reconsidered emigration to the USA. However, since 1972, I have had no doubts that Freiburg was the place where I wanted to work and to live. Only in 1991 did I accept an offer by the State Government of Baden-Württemberg to become a director at the newly established Institute for Technology Assessment in Stuttgart. This meant, in early 1992, the final departure from the laboratory and from regular academic teaching.

4 Some of my Research Topics in Freiburg

4.1 Photosensors in Photomorphogenesis

In order to react optimally to the light conditions in their environment, higher plants require various sensor pigments. Based on molecular physics, you can predict that phytochrome alone is not sufficient to measure all the relevant solar spectrum (290–800 nm) with the required accuracy. Today, it is known that three types of photosensors are involved in the process of photomorphogenesis in higher plants: phytochromes (> 520 nm; red/far-red), cryptochromes (340–520 nm; blue/UV-A) and the UV-B photosensor (290–350 nm).

We have tried over the years to understand at the physiological level the mode of coaction of the photosensors in bringing about photomorphogenesis, including control of gene expression. It can be seen from an early diagram (Fig. 3; Mohr 1987) how we felt the three photosensors worked together. It appeared in all cases that phytochrome (P_{fr}) is the effector proper whereas the blue/UV-A and the UV-B photoreceptors (together with phytochrome) determine the plant's responsiveness to P_{fr}. Even though in

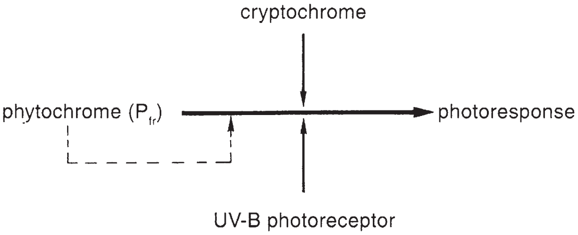

Fig. 3. Schematic of cooperation between phytochrome and the blue/UV-absorbing photosensors. Light absorption in cryptochrome and the UV-B photoreceptor determines the sensitivity of a photoresponse towards *P_{fr}. Dashed line* indicates that light absorbed by phytochrome can also increase the efficiency of P_{fr} or, expressed differently, it can increase the sensitivity of plants towards P_{fr}. (Mohr 1987)

the meantime the experimental approaches have become 'molecular' rather than 'physiological', the basic message contained in Fig. 3 has remained: in addition to the transduction chain triggered by P_{fr} and influencing responsive promoters by direct contact with transcription factors, "extensive cross-talk between signaling cascades downstream of multiple photoreceptors has become apparent..." (somewhat confusing, I admit, but this is the tribute of our field to becoming 'molecular') (Frankhauser and Staiger 2002).

4.2 Multiple Effects of Phytochrome

Obviously, P_{fr} has multiple effects at the organ level (Fig. 2). Multiple effects of P_{fr} can also be demonstrated at the cell and tissue level (Fig. 4). Subepidermal cells of mustard hypocotyls synthesize large amounts of anthocyanin under the influence of P_{fr}. Other cell layers of the axis do not form anthocyanin, even though they all react to P_{fr} with respect to their longitudinal growth. Some epidermal cells (trichoblasts) grow under the influence of P_{fr} into long hairs, but do not form anthocyanin, etc. The obvious multiple effect of P_{fr} at the level of tissues and cells can only be explained by the assumption that cells are differently competent for P_{fr} and that this pattern of competence exists before active P_{fr} is first formed. Epidermal trichoblasts of mustard hypocotyls (see Fig. 4) react differently to P_{fr}: on the one hand, they grow into long hairs, whilst on the other hand, their longitudinal growth is inhibited. Subepidermal cells show a corresponding pattern, i.e. one may say that they react positively by the synthesis of anthocyanin to P_{fr}, whilst they react negatively to P_{fr} with respect to their growth. We must inevitably assume specifically competent cell functions.

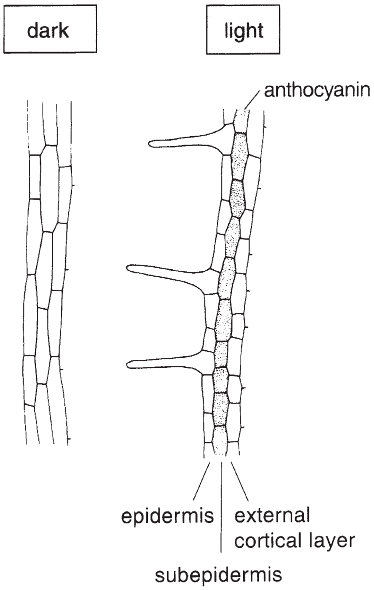

Fig. 4. Drawings representing the three outer cell layers of a mustard seedling hypocotyl in longitudinal section. *Left* Dark-grown seedling; *right* seedling kept for 24 h in light. (Mohr 1972)

4.3 Control of Gene Expression by Phytochrome

In 1966, we reported the induction of enzyme synthesis by phytochrome (Durst and Mohr 1966). Unexpectedly, the scientific community responded quite reluctantly. When I gave a seminar on the subject at Beltsville (October 1966) I could not convince colleagues that they should join us in investigating photomorphogenesis in terms of control of gene expression. Though readily accepted in Germany, the gene regulation hypothesis faced an icy reception in the USA. I received ironic (insulting?) comments throughout my lecture tour, even by some prominent colleagues who only a few years later enthusiastically joined the crowd once molecular physiology had become popular, and nobody any longer opposed the concept that phytochrome operates on development via control of gene expression. In principle, I was not very concerned because I knew that I was right, and I made precise plans while travelling in the USA to substantiate the gene regulation hypothesis after my return to the Freiburg laboratory. It worked!

A first step was to demonstrate that within the same tissue – we had chosen mustard seedling cotyledons where cell number and DNA content remained constant during the experimental period – phytochrome (P_{fr}) could simultaneously induce (Dittes et al. 1971) and repress (Oelze-Karow et al. 1970) enzyme synthesis while syntheses of some marker enzymes of the basic metabolism were not affected at all (Karow and Mohr 1967).

Unfortunately we were not able in the late 1960s to elucidate the nature of the signal transduction cascade from the cytosol to – what we assumed – competent promoters.

Our studies of nuclear-encoded chloroplast enzymes have finally demonstrated that phytochrome in fact regulates gene transcription (Schuster and Mohr 1990). However, we did not suggest that gene expression is controlled only at the transcriptional level. Rather I pointed out: "Full gene expression means the appearance of a final direct gene product – a protein – active at its physiological site of action... In principle, there are many steps between the initiation of transcription and the accumulation of the gene product at its functional location where gene expression could be regulated." As an example, in the case of nitrite reductase phytochrome produces the mRNA, whereas in order to make the enzyme out of the mRNA you need nitrate (Schuster and Mohr 1990). So you have a beautiful two-step control, transcriptional as well as post-transcriptional, which we could take apart.

4.4 Nitrate Assimilation and the 'Plastid Factor'

In 1982, I was elected member of the venerable (and well-endowed) Heidelberger Akademie der Wissenschaften. From 1986 onwards, the Academy financed a research unit in Freiburg to study the formation of the apparatus of nitrate/ammonium assimilation during the development of chloroplasts. The final goal of the research was to breed plants with an improved potential to assimilate nitrate (Mohr and Neininger 1994).

Since the research unit attracted a couple of excellent graduate students we could establish within a few years a consistent model for the formation of the apparatus of nitrate assimilation during the development of chloroplasts (Mohr 1990a). A fascinating result of this research may be mentioned briefly: the plastid factor (Oelmüller and Mohr 1986). Research from different angles (defect mutants, photo-oxidative damage of plastids) led to the conclusion that there is a plastid signal which acts as a transcription factor on nuclear genes. This signal (plastid factor) informs genes in the nucleus, which code for plastid proteins, that plastids are receptive to their protein products (Fig. 5). If the signal is missing, for example as a consequence of photo-oxidative damage to the plastids, transcription of nuclear genes coding for plastid proteins is blocked. In this case phytochrome is ineffective as an inducer of transcription. The plastid factor, the molecular nature of which is still unknown, is thus at a higher level of the regulation of transcription than is phytochrome (and nitrate) (Rajasekhar and Mohr 1986). The gene expression of typical cytosolic enzymes is not affected by

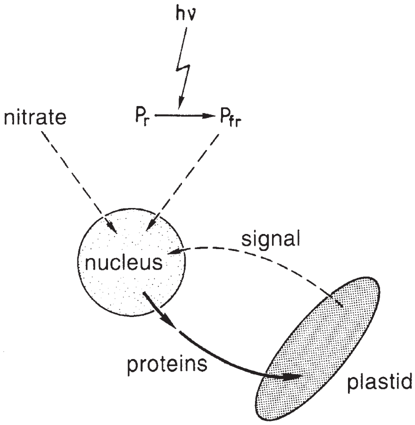

Fig. 5. Schematic showing the significance of plastid factor (*signal*) for expression of nuclear genes coding for plastid proteins. The plastid factor can be interpreted as an unspecific transcription factor without which neither light (via phytochrome) nor nitrate can become effective at the level of transcription. (Mohr and Schopfer 1995)

the lack of plastid factor. Under experimental conditions where, for example, SSU-mRNA and LHCII-apoprotein-mRNA disappear completely, synthesis of representative cytosolic, mitochondrial and glyoxysomal enzymes proceeds normally (Oelmüller and Mohr 1986).

5 A Textbook on Plant Physiology

In the 1960s a modern textbook of plant physiology was urgently needed to support teaching and to improve learning. Erwin Bünning persuaded me in the mid-1960s to publish my lectures on plant physiology which I had delivered in Freiburg and in part abroad. The original version of the textbook was written in German (Mohr 1969). Later, once the text was well established and Peter Schopfer had agreed to join me as an author, new editions as well as English (Mohr and Schopfer 1995) and Japanese translations followed.

To write a textbook is very different from preparing lecture notes! Expressed differently: to transform lecture notes into a coherent, consistent and balanced text requires an enormous concentration, in particular if the only time during the week left over for this task was from Friday night to Sunday afternoon. To achieve this goal you need stable health and a tolerant family. Fortunately, I had both.

The text was readily accepted by the students – and even by most of my colleagues. This was by no means a matter of course, since I left no doubt in my 'concepts of physiology' that physiology is not something like immature biochemistry but a science of its own, namely the science of organismic regulatory and control processes (Mohr 1988). Moreover, in my view, physiology is a quantitative (or exact) science. By analogy with physics the aim of physiology is to elaborate general statements (laws).

This was not my personal hybris! Physiologists have always wished to postulate at least some statements with the same authority and validity as our colleagues in physics. The recent successes in biochemistry and molecular biology have significantly reduced the self-confidence of physiologists and even, at times, produced a kind of neurosis, leading to the statement "that physiology has moved to the periphery of the problems." I am still convinced that my view of physiology as sketched above is right, but I am no more convinced that the present generation of physiologists can prevent what I called some years ago "the molecular collapse of quantitative physiology." Of course, I appreciate and enjoy the amazing discoveries made in molecular plant biology during the last 15 years, but the limits to reductionism may not be ignored (Mohr 1989). A very important challenge in the next decades will be constructing an interface between genomics and whole plant and animal physiology (Melvin 2003).

6 Steps in my Philosophical Thinking

I do not claim to be a professional philosopher of science. Rather, I consider myself a natural scientist with a profound interest in the nature of scientific thought and in the significance – including the cultural significance – of science. Since nobody can afford time to follow up every interesting idea, I had to select philosophical problems with a high probability of a pay-off for a practicing natural scientist. As a consequence, my 'philosophy', including my 'political philosophy', has remained closely connected with the progress of the sciences.

6.1 Structure and Significance of Science

The discoveries of science had a profound effect on man's philosophy, ethics and spiritual beliefs. I had planned for many years to organize my ideas on this subject matter. The opportunity of writing a treatise was made possible by a Visiting Professorship granted to me by the University of Massachusetts during the autumn term of 1975. The published text is based on a series of 15 lectures that I delivered at the University. I am still grateful to the students, and to my colleagues and friends at the University, for the cordial reception, continuous interest and constructive criticism. It was the positive response of my class and the fascinating intellectual climate at

Amherst, Maas., that encouraged me to revise the lectures for print (Mohr 1977a).

I dedicated the book to Erwin Bünning and to Walter Kossel. As I mentioned above, Bünning's book *Theoretische Grundfragen der Physiologie* was a major determinant in my decision to become a biologist (Bünning 1949). The late Walter Kossel introduced me to physics. He was not only a great physicist, but also a fascinating philosopher and an admirable personality. I had some bad feelings when I submitted the final text for print. I could only hope that the professional philosophers would forgive me if my treatise did not always respect the conventional division of labor between science and philosophy. I fully agreed with David Hull who had just criticized some noted scientists who tried their hands at 'philosophizing': "Just as scientists are entitled to established standards of competence for their undertakings, philosophers have a right to expect at least minimal competence in theirs" (Hull 1975). On the other hand, I felt that it was legitimate to base a reflection about 'structure and significance of science' primarily on the self-understanding of the practicing scientist. My deep-rooted respect for philosophy in toto and for epistemology in particular would hopefully prevent me from becoming chauvinistic in favor of the scientific world view.

The influential Anglo-Saxon schools in the philosophy of science have generally equated philosophy with epistemology, treating ethics as not properly part of academic philosophy. Since I was not obliged to any philosophical school, but looked at the problems from the point of view of a practicing scientist, I did not follow the tendency of excluding anything from consideration that might raise moral problems. Rather, I intended to emphasize this aspect. Moreover, I took the liberty of looking at some traditionally epistemological problems, such as empiricism and rationalism, from the point of view of scientific knowledge.

Another point was that by this time (1975) most philosophers of science, in particular within the dominant positivist school, took the Comtean view of physics as the paradigmatic science and of biology as a relatively immature and secondary study. Even as an enthusiastic biologist who was proud of his trade, I could not ignore this tendency since there is some truth in it.

While there is no principal difference between physics and biology, the general approach in both fields and the nature of physical and biological theories and laws obviously differ to a considerable extent. I often referred to physics rather than to biology not only for the sake of simplicity, clarity and brevity, but also for the reason that physics has a far wider scope than biology. Physics deals with the properties of all matter whereas biology is only concerned with living systems or with ecosystems in which living systems play the major part. All living systems are physical objects, but only a very small number of physical objects are considered to be living systems.

In the treatise I have often used the term 'responsibility'. This term implies, and I did emphasize this at the very beginning, that we are responsible for our acts. Indeed, I presume that moral responsibility is part of human nature, irrespective of the century-long discussion on determination, free will and moral responsibility. Determination to a scientist conveys the general proposition that every event has a cause. Whether this general proposition is true is a difficult question to decide, but it is certainly assumed to be true by most scientists. Otherwise science, in particular prediction, explanation and purposive action, would not be possible. On the other hand, we presume that we are responsible for our acts. It is implied as a matter of course that moral responsibility is an integral part of human nature. Indeed, we all believe that moral responsibility is real.

Since moral responsibility implies free will and self-determination (in the sense that we can create de novo determinants for our conduct and thus break causal continuity), the very serious and difficult question arises of whether moral responsibility (which implies free will) is compatible with our scientific knowledge, which plainly says that the concept of a breach in causal continuity is not acceptable. From the point of view of science, the reality of free will cannot be conceded. On the other hand, as human beings, we depend on the belief that at least some of our actions (called 'willed actions') are preceded by deliberation and choice and that our choice can be influenced by consideration of consequences.

Of course, I could not solve the paradoxes of free will (Stent 2002), but a thorough description of the paradoxes turned out to be a great advantage when I analysed – later in the book – the principle of causality and the structure of teleological action.

6.2 Epistemology and Evolution

Einstein once stated that for him the most unintelligible thing about the world is that it is intelligible. Why can we use the axioms and theorems of Euclidian geometry to reason about the physical world (Mohr 1977b)? Why is it legitimate to apply to a wheel (a physical object) the mathematical formula derived for a circle:

$$c = 2\pi r$$

As you all know, the use of diagrams is not essential to geometry. Geometrical reasoning per se is purely abstract. If diagrams are introduced it is only as an aid to our reason. In any case, a circle and a wheel are totally different things, but nevertheless the wheel obeys the formula obtained for the circle.

I have written here another formula, derived by Gauss, for a purely mathematical relationship between two variables:

$$y = \frac{1}{\sigma\sqrt{2\pi}} \cdot e^{-(x-\bar{x})^2/2\sigma^2}$$

Why is it possible to describe and treat the frequency distribution in biological populations with the help of this relationship, e.g. the frequency distribution of intelligence test scores in a human population? In brief: why is mathematics, a purely deductive system of axioms and theorems, applicable to nature? Galileo stated in 1623 that "nature is written in mathematical language" (and this phrase has been repeated and followed by scientists ever since), but he could not give any naturalistic explanation why this is so.

We may extend our question to the whole of logic (I consider mathematics to be part of logic). Why can we rely on syllogistic reasoning? It is probably that the principle of the syllogism was formulated not before but long after the usefulness and validity of syllogistic reasoning was discovered by man. Logic is the theory of deductive argument, not its source. Why does the real world obey logic? Wittgenstein (in his early phase) was very concerned about this question. As he puts it, our justification for holding that the world could not conceivably disobey the laws of logic is simply that we could not say of an illogical world how it would look. This is obviously not a good argument. Rather, it is a sign of perplexity and ignorance.

Or remember Ayer in *Language, Truth and Logic* (Ayer 1936): His second class of propositions were the formal propositions of mathematics and logic, and they were held to be tautologies. Ayer thought of them (as did Wittgenstein) "as being merely rearrangements of symbols which did not make any statement about the world" (Magee 1971). As Ayer pointed out:

The empirist does encounter difficulty...in connection with the truths of formal logic and mathematics. For whereas a scientific generalization is readily admitted to be fallible, the truths of mathematics and logic appear to be necessary and certain to everyone. But if empiricism is correct, no proposition which has a factual content can be necessary or certain. Accordingly, the empirist must deal with the truths of logic and mathematics in one of the two following ways: he must say either that they are not necessary truths, in which case he must account for the universal conviction that they are; or he must say that they have no factual content, and then he must explain how a proposition which is empty of all factual content can be true, useful, and surprising. If neither of these courses proves satisfactory, we shall be obliged to give way to rationalism. We shall be obliged to admit that there *is some truth about the world which we can know independently of experience; that there are some properties which we can ascribe to all objects, even though we cannot conceivably observe that all objects have them. And we shall have to accept it as a mysterious inexplicable fact that our thought has this power to reveal to us authoritatively the nature of objects which we have never observed. (Magee 1971)*

We know today that the fact Ayer is referring to is neither mysterious nor inexplicable. Ayer (and nearly all philosophers so far) did not take into account that experience has been accumulated and preserved as genetic information during biological evolution. From the point of view of the individual, this inherited foreknowledge about the structure of the world has the character of synthetic judgements a priori; from the point of view of evolution, however, the same statements must be regarded as synthetic judgements a posteriori, based on experience.

From the standpoint of the individual, a synthetic judgement about the world that we can know independently of experience is a synthetic judgement a priori in a strict sense. However, from the point of view of evolution, the same judgement is a synthetic judgement a posteriori; it is based on experience, namely on the experience of our phylogeny, which is preserved and stored in the genetic information, in the peculiar nucleotide sequence of the genetic DNA we have inherited from our parents. Kant's dictum that, although there can be no doubt that all our knowledge begins with experience, it does not follow that it all arises out of experience, can no more be maintained. The fact is that we combine in our individual life two kinds of experiences: the genetically inherited experience of our ancestors and the experience we have made in our personal life, including the experience transmitted to us by cultural tradition and social imitation. This is, in brief, the message of what has been called evolutionary epistemology (Vollmer 1975).

Having introduced this new branch of epistemology, I want to return to Ayer (and to Kant). Ayer (1936) writes in *Language, Truth and Logic*:

...the admission that there were some facts about the world which could be known independently of experience would be incompatible with our fundamental contention that a sentence says nothing unless it is empirically verifiable...the fundamental tenet of rationalism is that thought is an independent source of knowledge, and is moreover a more trustworthy source of knowledge than experience; indeed some rationalists have gone so far as to say that thought is the only source of knowledge. And the ground for this view is simply that the only necessary truths about the world which are known to us are known through thought and not through experience....

From the point of view of evolutionary epistemology, the venerable confrontation of empiricism and rationalism is a fictive problem. In reality, it does not exist, since synthetic judgements a priori are also based on experience. The rationalism vs. empiricism debate is a striking example of a philosophical discussion that scientific advance – in this case, the theory of biological evolution – has rendered pointless.

Let me close my apologetics of this new branch of philosophy with a personal remark: in my opinion, further progress in science and in epistemology will depend on the strong and steady, i.e., not only occasional, interaction of both fields. Epistemology must respect and consider seriously the actual, genuine knowledge elaborated by scientific disciplines. Science has proved that it is capable of producing genuine knowledge, although the theory of how knowledge can actually be obtained, epistemology, may not yet be satisfactory. On the other hand, those scientific disciplines, in particular those fields that have advanced far beyond the realm of common sense, such as classical quantum physics, elementary particle physics or molecular genetics and neurobiology, must consider epistemology as part of their endeavor. Fortunately, a considerable number of scientists have realized that epistemology is part of their trade. Professional philosophers, in particular in Germany and France, are more reluctant, following Wittgenstein at least in this regard, who firmly believed that the dominance of scientific thought since the Renaissance has been a disaster. Following the late Ayer, I do not see how one is to effect changes of this attitude except by changing the conceptual outlook among modern philosophers, and I do not think one can do this except in conjunction with scientific theories (Magee 1971).

Philosophy and science diverged in the nineteenth century, partly in consequence of the romantic movement and partly because science got too difficult, and now we must utilize the outlook that they are coming together again.

6.3 The Normative Code of Science (Mohr 1979b)

Scientists consider science as a systematic attempt of the human mind to obtain genuine knowledge. However, scientific knowledge in a true sense is 'public knowledge', i.e. knowledge shared by a scientific community. Repeatedly, I have investigated the normative prerequisites, the intrinsic values of science, which enable the scientific communities to obtain genuine objective knowledge. As a rule the intrinsic values of science are being shared by all members of a scientific community. This moral commitment is the reason why science became the prime cultural force of our age. Upon questioning, most scientists will tell you that they consider 'freedom to inquire into the nature of things' a great privilege. To what extent, however, is the individual scientist 'free'? In fact, he or she is not free at all. Rather, the activity of every scientist is subject to a strong social control. He/she is faced with a normative code which itself is based on the intrinsic values of science.

Why does the scientist accept this normative code as compulsory? Because scientists need and desire recognition by other scientists they cannot but conform to the goals and norms of the scientific community. The striving for professional recognition is in fact the major motivation for scientists to conform to the normative code.

The normative code is a heterogeneous complex. It consists of at least two parts: basic assumptions that are rigorously shared by the members of the scientific community and actual commandments. Since usually the assumptions as well as the actual commandments are not written up explicitly, the following lists are possibly not complete in the eyes of some scientist.

Among the basic assumptions, we may discriminate two sets:

1. There is a real world (negative version: the notion of solipsism is not acceptable); the real world is intelligible at least in part; formal logic (including mathematics) is valid, without any restriction, in the description of the real world; there is no break in causal continuity.
2. Freedom of thought and freedom of inquiry must indeed be guaranteed (this does not necessarily imply freedom in the choice of any particular goal; it implies, however, that the result of a scientific inquiry may not be influenced by factors extrinsic to science); genuine, objective knowledge is good, i.e. it is superior to ignorance under all circumstances (this implies that there is no code of forbidden knowledge). Complete freedom to publish cannot be assumed in reality. The industrial scientist as well as the scientist working on classified governmental projects must be aware of the possibility that he may not be permitted by his employer to publish the results of his research without delay.

A tentative list of the actual commandments includes:

- Be honest.
- Never manipulate data.
- Be precise.
- Be fair (e.g. with regard to priority of data and ideas).
- Be without bias (e.g. with regard to data and ideas of your rival).
- Do not make compromises, but try to solve a problem.

This list of fundamental commandments could be extended by more explicit formulations:

- Use words and symbols with explicitly defined meaning.
- Try to improve singular and general propositions with regard to inner perfection and degree of credibility.
- Make accurate predictions and indicate the range of error.
- Consider observational (experimental) data as the ultimate court of appeal.
- Be ready, any time, to modify or replace a theory in view of inner inconsistencies or experimental refutation.
- Always keep in mind that the members of the scientific community must depend on each other for reliability of material and intellectual methods, data, conclusions and theories.
- Regard simplicity as of high value. Do not create new constructs if unless absolutely necessary.

It is expected by the scientific community that the normative code is obeyed as a matter of course whenever a person works as a scientist. Misconduct and deliberate fraud are extremely rare in scientific practice, since every scientist is aware of the fact that penalties for misconduct are more severe in science than in any other profession.

6.4 Contributions to Economics

My amateur contributions to economics concerned the theory of evolutionary economics as compared to the biological theory of evolution (Mohr 1990b), and the concept of sustainable development. I published a carefully investigated book on the latter subject under the title *Qualitative Growth as a Survival Strategy* (English translation) (Mohr 1995). Even though I still consider it as my best book, it was not a success. My ideas and suggestions were indeed appreciated and even hailed by many readers, but the book had no detectable influence on political decisions or economic practice.

6.5 Science and Politics

Science as I understand it is a global adventure. It comprises all mankind. Science is less political than other issues (Mohr 2003). I preach to my students that science is a bridge for peace. Moreover, science is a moral adventure based on values and on mutual trust among the members of a scientific community.

The aim of science is reliable knowledge. Some call it 'truth'. Irrespective of semantics, it is agreed, that knowledge is a supreme value, the only means to overcome ignorance, superstition and poverty (Mohr 1999). Truth, reliable knowledge, can only be achieved if two functional values are obeyed: (1) the scientific method and (2) the ethics of science. Both the search for truth and ethical autonomy are alien to politics, which is necessarily ruled by doctrine and power, public opinions and compromise. Thus, science and politics are distinct and basically unrelated segments of social reality. However, science and politics are connected by two fluxes:

– Science needs alimentation. This implies that we must make the public aware of the value that basic science contributes to society.
– Politics needs knowledge. There is not the slightest chance to run a modern society without reliable knowledge.

Obviously, problems will arise: the individual scientist is expected to be a loyal member of the global scientific community and – at the same time – he or she is expected to be a loyal citizen of a politically defined community. Tensions and conflicts will arise whenever the two loyalties are not compatible.

When I wrote the present chapter, a story from the late 1960s came to mind. James Shapiro, who by that time belonged to the left wing liberals on the Harvard campus, declared his intention of renouncing his scientific career at the AAAS meetings in Boston in 1969. He had been part of the Harvard team that isolated the lac operon. Shapiro argued "that so long as men like Nixon and Agnew prescribe what is going to be done with scientific knowledge, we scientists should quit giving them the materials for their bad decisions." Shapiro could hardly be blamed as long as he spoke as an individual. However, could we fellow researchers still follow him when he tried to make his conviction compulsory for the scientific community? Could we be convinced that he had thought enough about the relationship between the scientific community, society and political power in different parts of the world? Or about the relationship between knowledge, wealth, security and power?

As for myself I reached the following conclusion: no scientist should be forced to do research he or she does not want to do for ethical reasons. If a scientist feels that society would inevitably and immediately use the knowledge originating from his/her research for purposes he/she considers to be evil, he/she must have the right to give up at any time. However, the scientist may leave no doubt that his/her decision is based on his/her personal value system, which is extrinsic to science.

This distinction is essential, since allegiance to any particular ideology may blind even a scientist. As an example, in the 1930s partisans of the

Marxist 'social relations of science' movement in England such as Bernal and Haldane openly used their high prestige as scientists to communicate and spread their ideological convictions even though the horrific excesses of Stalinism were already known. During the same period, some German physicists, among them two Nobel Prize laureates, created what they called German Physics, a direct support of Nazi ideology. Obviously, the outstanding analytical powers of the Nobelists had fallen sway under political prejudice.

6.5.1 First Case Study: Science and War

The utmost problem for the integrity of science is war among nations. Let us briefly consider the matter of war and law in view of the present tensions, in particular in view of biological weapons. A majority of the world's leading scientists stands behind the call – recently issued by John Polanyi – for replacement of war by international law. It is a call, an impressive document indeed, not to arms, but to disarm the sources of major tensions in the present world. Polanyi justifies the call: "I don't think politics can afford to discount the thinking of scientists in an age of science." Others such as Mario Molina from the Massachusetts Institute of Technology (MIT) are more sceptical about the political power of the scientific community: "Science alone, technology alone, is not sufficient to deal with these issues, we need strong commitments and signals from society that science and technology are put to good use." This, of course, is the crucial point: what does good use of knowledge mean in view of terror and warfare?

Let me briefly look back to 1940. World War II made science the most powerful political institution humankind has yet devised. Radar, pioneered in Britain, had the greatest effect on the war. "Radar won the war", US scientists used to say, "but the atomic bomb ended it." During World War II, science became subordinated to government to reach a superior goal, to win the war against Hitler and the Japanese generals.

However, the nuclear bombing of Hiroshima, which was intended to be and conceived of as an ethical act (to save the lives, both American and Japanese, which would be lost in a full-scale invasion), was later classified as unethical, even by brilliant thinkers such as the fabulous British physicist and Nobel Prize winner of 1948, Blackett, one of the inventors of airborne radar. His book of 1949 – *Fear, War and the Bomb* – has had a strong impact on the political scene as well as on the scientific community. Moreover, the apparent subordination of science to government in military matters became a major cause of public distrust of scientists. In any case, the use of the atomic bomb in World War II illustrates the obvious in the starkest terms, namely that even the most considered application of knowledge as well as moral calculus do not lead to unambiguous answers. In any case, Hitler and his allies as well as the Japanese generals no longer had a chance once science entered the war scene in England and America in 1940/1941. Moreover, the ultimate triumph of Western democracy over Marxist doctrine in the Cold War is to be attributed to superior science.

The excelling performance of Western science over German and Soviet science is to be attributed to freedom of thought and freedom from censorship. The catastrophic consequences of constraining freedom of inquiry for ideological reasons are well illustrated by the cases of Galileo – the loss of Italy's leading position in the rise of science during the Renaissance – and Lysenko – the rapid loss of genetic knowledge and competence and concomitantly of agricultural potential and productivity in the Soviet Union during the Lysenko–Stalin era. Millions of people died because an obsolete political doctrine prevented the use of scientific progress in Soviet agriculture and medicine.

6.5.2 Second Case Study: Robust Knowledge

This case study is based on my mixed experience as a science adviser to the government. Under the prevailing contract between science and society, science has been expected to follow sound scientific practice and to produce 'reliable' knowledge, provided merely that it communicates its progress to society. Some social scientists (Michael Gibbons, Helga Nowotny, Peter Scott) have argued that a fresh approach – virtually a complete rethinking of science's relationship with the rest of society – is needed (Nowotny et al. 2001). The argument is that we are currently witnessing a significant shift from 'reliable' to 'socially robust' knowledge. The latter characterization is intended to embrace the growing contextualization and socialization of knowledge. For knowledge to be 'socially robust' three aspects are decisive:

1. It is valid not only inside by also outside the laboratory.
2. This validity is achieved through involving an extended group of experts, including lay 'experts'.
3. Because 'society' has participated in its genesis, such knowledge is less likely to be contested than that which is merely 'reliable'.

This means, as my colleagues believe, that science can no longer be validated as reliable by conventional discipline-bound norms; while remaining robust, science must now be sensitive to a much wider range of social implications.

An example is the current debate surrounding genetically modified organisms (GMOs). Here, specialist peer groups have been challenged not only by parapolitical forces such as Greenpeace but also by ordinary consumers, for whom the research process is far from transparent and who are demanding that it be more so. To emphasize the decisive point again, knowledge of the health and ecological implications of GMOs may be 'reliable' in the conventional scientific sense; but it is not socially robust, and will not become so until the peer group is broadened to take into account the perspectives and concerns of a much wider section of society.

This novel type of scientific activity has been established in the USA and here in Germany under the term 'technology assessment'. I summarize my experience in this new discipline as follows (Mohr 1998): in the modern world at least some first-class experts must respond to issues and questions that are never merely scientific and technical, and must address audiences that never consist only of other experts. The limits of competence of the individual expert call for the involvement of a wide base of expertise that has to be carefully orchestrated if it is to speak in unison.

The suggestions of the advocates of 'robust knowledge' extend further, however. My colleagues from the social sciences demand that the process of knowledge *production* – not only knowledge application – become, what they call, transparent and participative. To quote Mike Gibbons (Gibbons 1999): "Under the prevailing contract, science was left to make discoveries and then make them available to society. The new contract will be based upon the joint production of knowledge by society and science."

Most natural scientists insist that the rigor of the scientific method/ethics and the robustness of the scientific practice may not be weakened. More than ever the modern world depends on 'reliable knowledge'. The advocates of socially 'robust knowledge' concede, of course, that "reliable and/or objective knowledge continues to provide the foundations on which our knowledge of the natural world depends" (Helga Nowotny) (Nowotny et al. 2001). However, the inherent problems have not been solved yet: to what extent does the engagement with extrascientific forces during the process of research undermine science's capacity to produce reliable knowledge? How can scientific quality be maintained?

While the proponents of 'robust knowledge' argue that there is no suggestion that scientific objectivity, consensibility and consensuality cannot be practiced in such a context, my (and others) practical experience in the field of technology assessment has been sobering. In fact, as far as I am aware, participation of lay 'experts' in the process of research has been a disaster – wherever it was tried, including my own efforts in the field of gene technology. Most citizens are neither willing nor prepared to accept this role. Moreover, most scientists are equally unprepared to assess the significance of their scientific results in the context of public opinion or policy guidance. The distinction between science and policy advice cannot simply be suspended by a strong dose of good will.

A particular confusion will be produced when well-intended but politically naive scientists enter the political fray as individuals or groups, as in the case of the Intergovernmental Panel on Climate Change: science as a whole becomes politicized and compromised. This is my major concern at present: how can we prevent the practice of science from being compromised? (Mohr 1996). A realistic strategy is to avoid any merging of science and politics and instead to strengthen the trust between science and the public, in particular between science and the political decision-makers.

What does 'trust' mean in this context? The politician must be sure that our factual statements are as 'reliable' as possible by any means. Scientists, on the other hand, must be sure that political practice is based on factual knowledge, Aristotelian logic and a coherent and consistent value system.

To emphasize again the decisive point: irrespective of the degree of 'public understanding of science', the modern world is totally dependent on *reliable* knowledge, on scientific truth. Scientific truth is the greatest treasure mankind has. It is the privilege and the obligation of the established institutions of science to foster and to preserve this knowledge and to guarantee the cultural boundary conditions for scientific truth and human welfare. This requires the autonomy of science *and* a positive coaction with the political institutions on the basis of mutual trust. In fact, our most urgent task is to restore the confidence of the public – including the political sector – in the trustworthiness of the natural sciences. The scientific enterprise – objective knowledge as the supreme good – must prove its integrity beyond any serious doubt or it will lose its status as the prime cultural force of our age (Mohr 1996).

7 Retrospect and on to the Future

Why did I write the present essay? What kind of message did I want to communicate to the inclined reader? Let me summarize my intentions by referring to a recent interview.

I was asked by a TV-master whether or not – under the present circumstances – I would try again to become a professor? My answer: Given my propensity I would certainly try anew to become a researcher but not necessarily strive for the position of a university professor. The politically overregulated German university of today is no longer the best place to pursue a scientific career.

Do you regret, the moderator continued his inquiry, that in the late 1950s you did not venture a career in the USA? My answer: Even though I liked and admired America, my wife and myself preferred in the end to live closer to the genuine European culture.

Next question: Do you feel that your long-lasting flirtation with Queen Philosophy has affected your scientific achievements in an adverse sense? My answer: Yes and no! Undoubtedly, I have neglected at times my scientific work (and my family!) in favor of urgent philosophical (and political) challenges. On the other hand, the fact that my scientific studies were embedded in a philosophical (or at least epistemological) framework made me sure of my scientific aims and fostered my purposeful behavior in the laboratory and in the lecture hall.

Towards the end of our conversation the moderator asked me four very distinct questions and requested a brief answer to each of them:

- Do you believe in the future of science? – Of course, I responded quickly, since mankind cannot survive without knowledge. Science is the only source of genuine, reliable knowledge (and consistent ethics). Moreover, like it or not, science has become the prime cultural force of our age, and in practice an ever-more pervasive way of life for all people on this planet. In brief: the future of man is linked irreversibly to the future of science.
- Will there be any future for philosophy? – The power of philosophical thinking, mainly an advanced form of hermeneutics, will become an integral force in the ripening processes of the great naturalistic theories. Critical realism will be the common epistemological basis. As A.J. Ayer, the ingenious philosopher, pointed out decades ago (Ayer 1936), philosophy will flourish as the 'logic' or metatheory of the modern sciences. On the other hand, traditional but largely outdated disciplines such as ontology, metaphysics, idealism or phenomenology in its varieties will simply disappear.
- Will your scientific discipline, plant physiology, disappear as well, in this case in favor of molecular biology? – As I have argued repeatedly (Mohr 1989), there are limits to reductionism in the sciences. I am indeed afraid of 'the molecular collapse of quantitative physiology'. An important challenge in the next decade will be constructing an interface between genomics and whole plant and animal physiology.
- Science and technology often encounter scepticism and wariness in academia as well as in the media and general public. How can the scientific community promote a much more positive view of science in society? – A traditional response of the scientific community to what it views as a lack of appreciation by the media and the general public has

been to mount public understanding campaigns to 'enlighten' the populace about specific issues in question. I have participated ex officio in quite a number of these well-aimed campaigns (Mohr 1998). The results have been sobering: simply trying to educate the public about specific science-based issues is not working; what is needed is a broadening of science education in general far beyond the present scope. Science in the modern world must definitely become an integral part of liberal education and cultivated life.

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Genetics

Recombination: RNA – A Powerful Tool for Recombination and Regulated Expression of Genes*

Dirk Müller and Ulf Stahl

1 Introduction

The natural frequency of any base mutating to another is about 10^{-7} to 10^{-8}. The process of screening for a special phenotype is extremely time-consuming when one just relies on this natural mutation. Thus, a broad spectrum of methods have been developed to speed up the process and to obtain mutated or recombinant organisms.

Random methods such as UV mutagenesis and chemical mutagenesis yield clones with one or more unknown mutations. Although these methods can provide a desired phenotype, e.g. a high producing strain, several additional and undesired mutations can counteract the improvement by slowing growth or introduce other unwanted abilities. These side effects can have severe consequences for the strains concerned, particularly as the whole metabolic spectrum can be thrown out of balance which then results in reduced yield of certain metabolites.

Targeted mutations can only produce the desired phenotype (e.g. homologous recombination, loss-of-function/gain-of-function, gene disruption, and gene replacement) and are thus used when one wants to avoid unwanted mutations. A major disadvantage of both random and targeted mutageneses is that it targets and/or recombines the DNA of the organism. The mutations introduced therefore cannot be reversed. The choice of targets in this case is limited to those which, after mutagenesis, are non-lethal for the organism.

Recombination techniques that do not target the DNA but rather the messenger RNA have become more and more reliable over recent years. Even genes that are essential for the organism can specifically be targeted when the remaining level of the messenger is sufficient to guarantee survival of the organism. The application of these RNA techniques enables scientists to analyse gene function, even in certain developmental phases.

* This chapter is dedicated to Prof. Dr. Karl Esser on the occasion of his 80th birthday

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RNA technologies are also a promising tool for the production of metabolites as cells can be grown to the desired growth phase. The use of the appropriate RNA technology can lead to product formation without necessarily having to shift to an inducing medium. Products that are growth inhibiting or, in the end, lethal for the cell, can even be synthesized when the fermentation process is broken down into separate growth and production phases.

The antisense technique which utilizes RNA molecules in an antisense orientation to the target RNA was the first RNA technique used for this purpose. The discovery of the catalytic properties of some kinds of RNAs called ‘ribozymes’ caused a sensation among researchers which was only topped by elucidation of RNAi mechanisms. Apart from being promising therapeutic agents, these RNA methods also give us insight into RNA chemistry and evolution.

This chapter provides an overview of the progress in RNA-based recombination methods and their application.

2 RNA Methods

Basically, RNA-based methods for recombination do not change the genetic identity of an organism as they do not attack the DNA, but rather the transcribed RNA. This has the advantage over mutating the target DNA, which can be lethal for the organism. In addition, it is a better system when the mutation takes effect at a particular stage of development. Some genes even have open reading frames on the complementary strand which can be affected when the target gene is silenced by classical means.

2.1 Antisense RNA

Antisense oligonucleotides bind specifically to a complementary mRNA. In order to be able to bind specifically to a given target RNA, the size of the molecule can range from anything as small as 12 to several hundred nucleotides; however, the probability for unspecific binding with additional undesired side effects increases with very small antisense molecules. After binding to the target site, antisense molecules lead to digestion by RNase H, impede splicing, or to translational arrest.

The size of antisense molecules determines the probability for specific or unspecific binding. Given an antisense molecule with a size of 8 nt, we can find this binding motive statistically every 65 kb. The human genome consists of about 2,900,000 kb which would result in nearly 45,000 possible binding sites for this small antisense molecule. In contrast, an antisense molecule of 20 nt would bind every 1,099,511,627 kb, thereby ensuring the required specificity.

Although the antisense approach is universal and specific, many antisense molecules show little or no antisense activity in practice. It is therefore crucial to find the optimal binding site for an efficient antisense molecule. RNA molecules fold into complex secondary structures which can exclude accessibility of some regions on the target RNA. Both a high number of external nucleotides and global flexibility of the antisense RNA can improve the formation of the RNA–RNA duplex. In practice, this dependency on unpredictable binding requires that whole libraries of antisense constructs need to be tested in order to obtain only a few functional antisense molecules. Despite the relatively simple theory behind antisense technology, the need for a large setup can make the antisense approach very expensive and time consuming with no guarantee of success.

Binding site selection could, in principle, be based on secondary structure prediction (Lehmann et al. 2000). However, as our knowledge of RNA folding, duplex stability, and the quality of computer programs for structure prediction is rather limited, a combined approach using computer prediction and experimental methods (either based on hybridization techniques or on ribonuclease H, i.e. RNase H activity) are most likely to succeed at constructing efficient antisense RNAs (Sczakiel 2000; Sohail and Southern 2000).

Various chemical modifications can be introduced to improve stability and affinity for the target. Common modifications of antisense molecules include the exchange of phosphodiester internucleotide linkages by phosphorothioate, polyamides (see Braasch and Corey 2002), replacement of the phosphate-sugar backbone with uncharged N-(2-aminoethyl)glycine (PNA, see Fig. 1; Doyle et al. 2001), replacement of the sugar backbone with locked nucleic acids (LNA; Wahlestedt et al. 2000), or addition of functional groups such as 2-methoxyethyl (Chen et al. 2001). Although chemical modification of RNA leads to increased stability, it must be balanced with effects on activity and potential toxicity.

The application of RNA antisense molecules is always accompanied by the risk of degradation by RNases. The use of oligonucleotide analogues is one way of protecting an antisense molecule as there are no natural enzymes inside the cell that can use these synthetic molecules as a substrate. The introduction of peptide nucleic acids (PNA, Fig. 1) has attracted both scientific and commercial interest. The negatively charged sugar-phosphate backbone of DNA or RNA in PNA has been replaced by a polypeptide backbone, leading to enhanced stability and the formation of stronger hybrids with complementary RNA and DNA.

A number of antisense constructs which are used as pharmaceutical agents or as a tool for crop improvement have been developed and tested. However, only a few of these antisense products have got past the test phase to be used commercially (Table 1). Generally, this seems to be the major obstacle in antisense technology. Although the antisense theory seems to