Overview: Bioenergetics between Chemistry, Genetics, and Physics

The scientist is not that who gives right answers, but that who raises right questions.

C. Lévy-Strauss

L. Kováč

Institute of Animal Physiology
Slovak Academy of Sciences
900 28 Ivanka pri Dunaji, Czechoslovakia

I. Introduction
II. Proteins Are Molecular Engines
  A. The Scope of Bioenergetics
  B. What Is an Engine?
  C. Molecular Engines
  D. Internal Fluctuations in Proteins
  E. Ligands as Inputs
  F. Energy Flow through the Protein Machine
  G. Environmental Fluctuations
  H. Rotational Fluctuations
  J. Amino Acid Sequences and Tertiary Structures
III. How Engines Evolved
  A. Evolution as Tinkering
  B. Genome Organization
  C. Evolution of Bioenergetic Systems
  D. Energy and Evolution
  E. Evolution and Cognition
IV. How Engines Are Assembled
  A. Protein Folding
  B. Intracellular Protein Topogenesis
V. How Engines Are Maintained
  A. The Problem of Maintenance
  B. Proteolysis and Turnover
  C. Energy Costs of Maintenance
VI. Future Prospects: Bioenergetics beyond Chemistry, Genetics, and Physics
References

I. Introduction

The growth of science, ever accelerating, is both imposing and appalling. The meaning and goals of research are changing, and integration of knowledge is becoming more and more difficult. Overspecialization is maiming the intellectual ambitions of scientists.

It would appear that somewhere at the periphery of this rush, outside great research centers, better conditions for calm contemplation and for escape from conformity may exist. However, this is dubious. At such remote monasteries, all invention must often be invested in solving technical problems which elsewhere would be trivial or nonexistent. Sources of information are mostly restricted, and both competition of ideas and the necessary feedback between theory and experiment are lacking. The risk of superficial thinking and of simplification is overwhelming.

With such a risk in mind this essay has been conceived, but also in the hope that it may resound a major theme that has been implicit in the polyphony of articles presented in these two volumes.

Bioenergetics arose as part of biochemistry. Chemical thinking, based on the classical dictum that "substances do not react until dissolved," gave bioenergetics its initial impulses—in the chemical theory of oxidative phosphorylation, as well as in the diversity of methods. Phenomenological equilibrium thermodynamics long served as an elegant theoretical framework for bioenergetics.

The establishment of the chemiosmotic paradigm has been linked with the appreciation of the limits of classical chemical thinking and of the need for physical notions and theories. Although physical methods are rapidly permeating bioenergetics, physical thought is generally lagging behind. It is symptomatic that it is, again, the inextinguishable imagination of Peter Mitchell (1981, 1985b) which opens new horizons for the "physicalization" of bioenergetics. As a consequence, thermodynamics, in the meantime enlarged to a description of nonequilibrium phenomena, may become more and more complemented by statistical mechanics.

Genetic approaches were introduced into bioenergetics relatively late. The possibilities offered by mutants have not yet been fully exploited. On the other hand, the rapid application of techniques of gene cloning and DNA sequencing represents a major breakthrough in bioenergetics, so that it would not be exaggerating to say that in the years to come genetics will dominate bioenergetics.

However, as aptly stated by Harold (1982), "to find the secret of life we shall have to marry the study of macromolecules and genes with that of the vectorial flux of ions, molecules and chemical groups in the field of force." This brings us back to physics and allows us to extrapolate the interdisciplinary path of bioenergetics (Fig. 1).

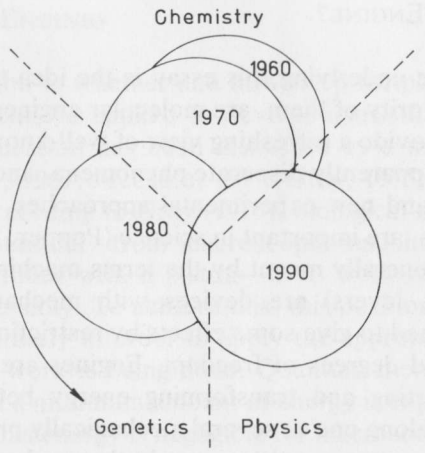

FIG. 1. Bioenergetics is interdisciplinary. Its center of gravity moves from chemistry, through genetics, to physics. (Vacancies remain in humanistic and social sciences.)

If we inquire after the meaning of all this research, we get to humanity, society, and their problems. It is therefore feasible that the last extension of bioenergetics will be to humanistic and social sciences.

II. Proteins Are Molecular Engines

A. The Scope of Bioenergetics

Bioenergetics was originally defined as "the study of energy transformations in living organisms" (Lehninger, 1965). The first textbook of bioenergetics (Lehninger, 1965) dealt with the energetic aspects of all biochemical processes, including replication of DNA, protein synthesis, and muscle contraction. Since then it has become customary to restrict bioenergetics to studies of energy transformation in biomembranes. This has been a useful restriction which has accelerated the general acceptance of the chemiosmotic paradigm.

The time may be ripe for a new transitory period in which emphasis is placed on the energetics of the protein molecule. The basic tenet of the chemiosmotic reasoning refers, not to membranes, but to the intrinsically vectorial nature of chemical transformations taking place on the enzyme molecule (Mitchell, 1976).

In Mitchell's words (1981), "catalytic connective and transformative ligand-conducting protein molecules or complexes of vectorial metabolism—enzymes, catalytic carriers, porters, and osmoenzymes—"can be treated as "miniature machines or engines."

B. What Is an Engine?

The main theme underlying this essay is the idea that many proteins, and perhaps a majority of them, are molecular engines. Treating proteins as engines may provide a refreshing view of well-known facts, disclose a unity underlying apparently disparate phenomena, and stimulate a search for new theories and new experimental approaches. Although theories, and not definitions, are important in science (Popper, 1982), it is useful to indicate what is generally meant by the terms machines and engines.

Machines (e.g., levers) are devices with mechanically constrained parts, predetermined to give some effects by restricting motion along one or several selected degrees of freedom. Engines are machines working cyclically, conducting and transforming energy between sources and sinks of potential along one or several mechanically predetermined paths.

It is immediately apparent that a purely thermodynamic description is not sufficient to understand the work of an engine. In an internal combustion engine, the chemical process of burning is dependent on such macroscopic parameters as temperature and pressure, which represent an averaging over an enormous number of microscopic degrees of freedom of molecules and atoms. The chemical process is an appropriate subject for thermodynamic analysis. In the engine, the burning is spatially controlled and guided to do work by mechanical parts of the engine, cylinders, pistons, valves. These parts also consist of molecules and atoms characterized by an enormous number of degrees of freedom, but the parts themselves have only a few mechanical degrees of freedom. It is the latter that are relevant for the performance of the engine.

The fact that a large system has only a few degrees of freedom means that only a few regions in its entire phase space are accessible and that the system can be found in relatively few possible microstates (Blumenfeld, 1977). This is not because the inaccessible regions of the phase space have very large free energies, but because they are separated from the accessible regions kinetically by very high barriers. These barriers correspond to the mechanical constraints present in the construction of the engine. The engine would spread over its entire phase space only if disintegrated completely into atoms and molecules. Were it not for corrosion, a process which itself has a large activation energy and is relatively slow, an automobile, left immobile, would need centuries to disintegrate into its parts and still much more time to disintegrate into molecules. Only then will the system achieve a complete thermodynamic equilibrium with its environment. Thus, although the constituent parts of an engine are in thermal equilibrium with the environment, having the same temperature, the engine as a whole is not, because of the kinetic barriers and long relaxation times imposed on the engine by its design.

C. Molecular Engines

A crucial question is whether and how far principles valid for macroscopic machines can be applied to devices consisting of one or a few molecules. The question has been discussed by a number of scientists (Chernavski *et al.*, 1967; McClare, 1971; Gray, 1975; Blumenfeld, 1977; Cooper, 1984). According to Gray (1975), biological molecular machines should differ substantially from macroscopic machines. If a machine—for instance a cylinder with a piston—is to work efficiently, it should perform work reversibly. To achieve this, this position of the piston must be measured accurately in order to apply the appropriate external force against which the work is being done. Quantum theory and information theory require that a minimum amount of energy is expended in ascertaining the position. This energy is negligible for macroscopic systems, but its relative value is so large for microscopic systems that, according to Gray, molecular machines cannot operate reversibly; they can operate cyclically and repeatedly in one direction only. This concept was used in an analysis of muscle contraction, but it is not clear how it could be applied to other systems, for instance to the reversible operation of ATP-driven ion pumps. Perhaps the actual work done by molecular engines needs no continual measurement of distance and force, as the optimization of performance has been established by natural selection and the energy necessary for recognition and measurement has been "paid for" in the course of evolution. However, systems assigned to perform continual measurement, such as receptors and associated proteins, seem indeed to work under conditions out of equilibrium and continually expend free energy (Kováč, 1986).

Blumenfeld (1977) sees no principal differences between conventional and molecular machines. Any machine exhibits a particular degree of freedom which, when excited by an input of energy, exchanges its energy with other degrees of freedom very slowly; in other words, its rate of relaxation is low. In a cylinder with a piston, the motion of the piston lifting a weight is the selected degree of freedom, excited by the expanding gas. In a cell, the selected degree of freedom which relaxes slowly is the chemical synthesis of ATP from ADP and P_i. In an enzyme molecule, a specific conformational change ensuing from the catalyzed chemical reaction is, according to Blumenfeld, the slowly relaxing step. It is important that the processes run slowly enough so that, at any moment, equilibrium distribution of energy over all the other degrees of freedom takes place.

The best insight into differences between macroscopic and molecular machines can be gained if one proceeds, in imagination, from a familiar macroscopic machine down to its smallest miniature. An internal combus-

tion engine, scaled down to the size of a protein molecule and operating by burning one gasoline molecule at a time, would not work. In the macroscopic version, fluctuations of molecules of the cylinder or the piston, which are in thermal equilibrium with the surroundings, would not affect the working cycle of the engine. In the molecular version, however, random fluctuations would probably entirely prevent the "purposeful" operation. Accordingly, any analysis of mechanical devices of molecular dimensions should pay prime attention to thermodynamic fluctuations, both of the device and of its surroundings. This also holds for protein molecules if they are molecular engines.

D. Internal Fluctuations in Proteins

Protein molecules are dynamic structures; the atoms and groups that make up a protein molecule are in a state of perpetual motion fueled by thermal excitations. The static crystallographic image of the protein molecule is only a first approximation to describing the actual physical state of the molecule (Schulz and Schirmer, 1979; Karplus and McCammon, 1981; Wagner, 1983; Cooper, 1984).

In contrast to the structural dynamics of other polymers, the dynamics of proteins combine structural flexibility with structural precision and stability. A protein, for example an enzyme, must have a large internal mobility to fit in structurally with both its substrate and its product, which can differ substantially in their space structure. At the same time, it must also have a structural rigidity sufficient to force the substrate to follow the preprogrammed path. Also, proteins must exhibit a high resistance to irrelevant effects of their environment. This is one of the reasons why the properties and kinetic parameters of purified enzymes are not much different from those found in a crude homogenate or *in situ*. A good example is bacterial and mitochondrial F_1-ATPases, which when isolated, were found to exhibit kinetic characteristics similar to those seen when present in membrane-bound form (Wise *et al.*, 1984; Penefsky, 1985).

Energetic factors are apparently decisive in determining the stable conformation of a native protein (Anfinsen and Scheraga, 1975), but kinetic factors are equally important. The difference in free energy between native and denatured proteins at physiological temperatures is only about 500 J per amino acid residue, which is five times less than the energy of thermal motion. The very existence of a stable native structure thus appears paradoxical. It is, however, accounted for by the fact that a protein is a cooperative system, and its elements can change their states only in a collective manner (Gō and Taketomi, 1978; Privalov, 1982). This is also

the reason why denaturation of proteins is a first-order phase transition, the single case of an intramolecular first-order transition in nature (Ptitsyn, 1984).

The possibility that other conformational states of proteins may also be separated by high kinetic barriers has not been rigorously disproved (Hantgan *et al.*, 1974). This implies that the most frequent states need not necessarily be states with the lowest possible energy. The multidimensional hypersurface of the conformational space may have a number of local minima in conformational energy (Anfinsen and Scheraga, 1975; Scheraga, 1984; Mutter, 1985), and the protein may reside in one of them as a result of a stepwise construction process. This does not mean that the secondary and tertiary structures of proteins are not determined by their amino acid sequences and thus dictated by structural genes. The genetic program, however, carries implicit working instructions for the construction of a protein and is, thus, a one-dimensional layout of the *four*-dimensional structure of the protein.

This also means that the internal fluctuations in the protein molecule are also partly "prescribed" by the gene. The extent of the displacement of various atoms under thermal excitation of the molecule depends on the nature and steepness of local gradients of potential energy. In the highly inhomogeneous protein molecule the distribution of such local potentials should also be highly inhomogeneous, so that fluctuations need not be spatially random. Relaxation times of such fluctuations must be widely disparate (Shaytan and Rubin, 1982). The faster and relatively short fluctuations belong to the side chains. They form a liquidlike layer around the core and have the role of a damping medium. Over these fluctuations, additional larger conformational fluctuations occur within the "conformational potentials" which are determined by the elastic properties of specific parts of the protein chain. Again, these "conformational potentials" are distributed inhomogeneously within the molecule so that the fluctuations are energetically biased (Fig. 2).

It is now firmly established that protein monomers consist of relatively independent units made of 100–200 amino acid residues, the domains (Schulz and Schirmer, 1979). Each domain is an independent cooperative system (Privalov, 1985). There is good evidence that interdomain interactions are weaker than intradomain ones (Wetlaufer, 1973; Rossmann and Liljas, 1974). In machine terminology, the domains can be equated to modules from which a functional machine is assembled (Schulz and Schirmer, 1979). As in macroscopic machines, similar modules carrying a single characteristic are present in different proteins having different functions. For instance, a domain which binds adenine is present in a number of dehydrogenases and kinases (Rossmann and Liljas, 1974; Walker *et al.*,

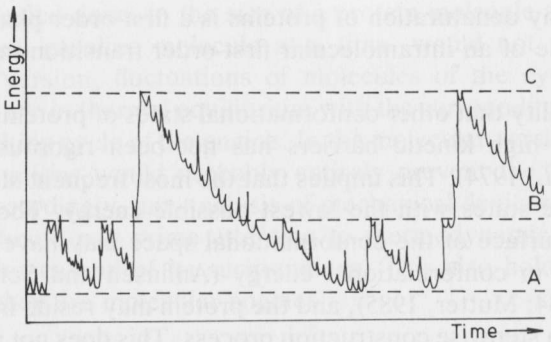

FIG. 2. Fluctuations in a protein molecule. Small, fast fluctuations of atoms and chemical groups are superimposed over larger conformational fluctuations within "conformational potentials" exhibiting longer relaxation times.

1982). Sequence homologies point to a common evolutionary origin for this domain in all of these enzymes.

To avoid confusion it should be added that, in protein chemistry, the term "module" has also been used in a different sense. It defines a compact structural unit as the least extended conformation in a globular protein (Gō, 1983). The lengths of modules are in the range of 20–40 residues, so that a domain can consist of several modules. In contrast to domains, a module has apparently no rigid specific conformation and, in a native protein, may be stabilized only by interactions with other modules. Yet, the modules may have an important role in protein folding and, as will be shown later, modules, rather than domains, may have been ancient units of protein evolution.

E. Ligands as Inputs

The most prominent characteristic of the majority of proteins is the capacity to recognize and bind specific ligands and to respond to them by a specific conformational change (Citri, 1973; Weber, 1975). It should be stressed that this recognition is accomplished against a background of enormous environmental noise. To achieve such exquisite specificity, the ligand must be attached at multiple points. At each point it is "checked" by a corresponding conformational adjustment of the protein. It may be this feature that directs the conformational transitions of proteins to proceed along one or a few selected degrees of freedom. This does not mean that the ligand "instructs" the protein how to proceed; the design of the machine and the working instructions are specified by the structural gene.

One cannot exclude the possibility that the minimum free energy, reached upon equilibrium binding of a ligand, is not the absolute minimum but rather a local minimum dictated by the preprogrammed pathway of the conformational transition.

The new conformational state assumed by the protein upon binding of the ligand need not necessarily be visualized as a gross change in conformation. It may, in some cases, only reflect a modification in the frequencies and amplitudes of the dynamic fluctuations of constituent atoms which may not have changed their space coordinates by much (Cooper, 1984). Ligand binding to proteins is almost invariably associated with a decrease in the overall heat capacity of the protein–ligand complex (Sturtevant, 1977). Since the heat capacity of a system is the measure of internal energy fluctuations, the energy fluctuations in the ligand–protein complex are smaller than that in the free protein. This means that the proteins are "stiffened" by specific ligand attachments. We may say that by fluctuating, a protein is rapidly generating hypotheses on the properties of the environment, and that the rate of hypothesizing becomes diminished when one of the hypotheses finds a positive response (Kováč, 1986).

Ligand-binding sites are almost universally positioned at the boundaries of two structural domains (Schulz and Schirmer, 1979), and the binding of ligand is accompanied by movements of domains relative to one another (Ptitsyn, 1984; Lesk and Chotia, 1984). A common molecular structure of many proteins is represented by two or more relatively rigid domains which, like lobes, are connected by a relatively flexible, so-called hinge region (Schulz and Schirmer, 1979; Anderson *et al.*, 1979; Janin and Wodak, 1983; Bennet and Huber, 1984). The binding of a suitable ligand is followed by a rapid hinge-bending and cleft-closing, so that the ligand is entrapped in the cavity, virtually inaccessible from the aqueous environment. In enzymes, this closing may be followed by an additional conformational change of the protein which stretches the substrate creating a dense packing of the enzyme–substrate complex and the achievement of the transition state configuration. Scarborough (1985) assumed that the hinge-bending motif underlies all mechanisms of solute transport across biological membranes. The transport protein, embedded in the membrane, would pick up a ligand at one side of the membrane; this would be followed by a hinge-bending cleft closure, and a displacement reaction would result in release of the ligand at the opposite side of the membrane by reopening the cleft toward that side. Random fluctuations between different conformational states of the transporter enable facilitated diffusion, while the input of energy affects the relative population of different states and results in active transport of ligands. Scarborough's idea is just

a variation of the theme present in almost all recent models of membrane transport, which describe it as a two-stroke cyclic process.

It should be reemphasized that kinetic factors, specified by the machinelike character of proteins, may have a major role in determining the specificity of ligand binding. The binding itself provides energy for the work of the machine. Ligand binding is always accompanied by a decrease of free energy, which sometimes is very large, leading to the binding of a ligand with very high affinity. As analyzed by Jencks (1975), the intrinsic binding energy is usually larger than is observed, and a considerable part of this energy is utilized for other purposes. If the binding energy were not used it would be wasted as heat.

In enzymes, part of the binding energy is used to overcome the activation barrier of the chemical reaction and, thus, to accelerate the process (Jencks, 1975; Fersht, 1977). In receptors, the binding energy is probably used to transfer the message, carried by the ligand, to evaluating and executing devices. In many translocators, the binding energy of one ligand, bound at one point in the reaction time-space, is used to release another bound ligand at a different point of the space (Boyer, 1975; Jencks, 1980; Tanford, 1981; Eisenberg and Hill, 1985; Penefsky, 1985). This is the basic principle allowing simple and elegant unifying explanations of energy transformation in biology, including the operation of ion-translocating ATPases, muscle contraction, and respiration-linked ATP synthesis (Fig. 3).

F. Energy Flow through the Protein Machine

To work continually, protein engines, like macroscopic engines, must work in cycles. They have to obey a set of strict rules that specify which binding and which changes are allowed or prohibited at particular stages of the cycle (Jencks, 1980; Hill and Eisenberg, 1981; Tanford, 1983). As in macroscopic engines, these rules of operation are built into the construction of the engine, in the geometric specificity of the fluctuations and conformational transitions of the protein.

Different opinions exist with respect to the "flow" of free energy in such cycles. According to Tanford (1981, 1983), free energy is essentially transferred in one step of the cycle, when the standard free energy of one ligand drops and simultaneously the standard free energy of another ligand increases by approximately the same amount. The protein itself is energetically inert: different protein conformations have about the same energy. This opinion has been questioned by Hill and Eisenberg (1981)

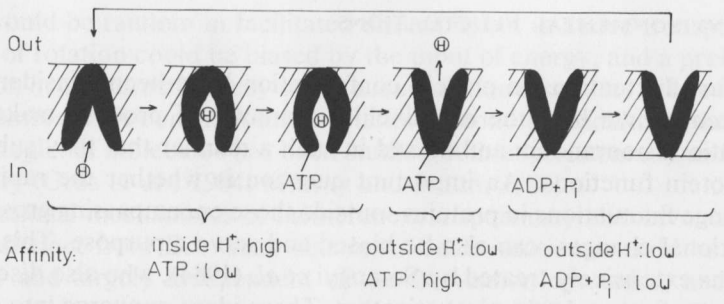

FIG. 3. A principle of free-energy transduction by H^+-ATPase. An ATPase, pictured as a two-lobe hinge-bending protein, is embedded in a membrane. The membrane separates two phases with high (out) and low (in) concentrations of protons. In an "inside conformation" the protein has a high affinity for H^+ and a low affinity for ATP. The binding of ATP to the "inside conformation" is followed by a conformational transition into an "outside conformation" exhibiting a low affinity for H^+ and a high affinity for ATP. H^+ has been transferred from inside to outside against its concentration gradient. To close the cycle, strongly bound ATP must be released from the protein. This is done by hydrolysis of ATP to ADP + P_i which, with the ligands bound to the "outside conformation," proceeds with no change of free energy. The couple ADP + P_i has a low affinity to the "outside conformation" and is released. The "outside conformation," with low affinity for outside H^+, spontaneously reverts to the starting "inside conformation." At high $H^+_{outside}/H^+_{inside}$ and low ATP/ADP + P_i ratios the system operates in the opposite direction, synthesizing ATP. The outline does not intend to picture mechanistic details of F_0F_1-ATPase.

because, according to them, it implies that the enzyme molecule, like the solvent water when the ligands are in solution, can be regarded as simply the background environment for the bound ligands. If this is so, it can be formally ignored, just as water (as solvent) can be ignored in many aqueous processes. These latter authors consider this anology to be untenable: During the transduction cycle, the specific properties of the enzyme molecule and of its complex with ligand are just as important as the specific properties of the ligand molecules. Enzyme and ligand represent a single system and should be analyzed as such. This opinion seems to be shared by Jencks (1980) and also by Mitchell (1981). As another extreme, Lumry (1974) proposed that a ligand can pull a protein into a thermodynamically unfavorable conformation, and the protein can store the free energy and use it in a separate step to increase the free energy of another ligand. Such a two-step transfer of free energy was analyzed as a plausible one by Hill and Eisenberg (1981).

Obviously, molecular machines can hardly be considered to be energetically inert when interacting with ligands. They may store free energy (like a spring) and not dissipate it if some degrees of freedom exhibit long relaxation times.

G. Environmental Fluctuations

So far, fluctuations in protein conformation have been considered. It has been shown how the machinelike character of proteins makes the fluctuations nonrandom and biased in such a manner that they subserve the protein functions. An important question is whether the rapid and low-range fluctuations in proteins, outside those encompassing gross conformational changes, can also be biased and serve a purpose. This question was extensively treated by Somogyi *et al.* (1984), who also discussed the ideas of several other investigators. These ideas converge into a general picture which indicates that, indeed, the protein is designed in such a manner that, interacting with the random thermal fluctuations of solvent molecules in the surrounding medium, it is able to select from them according to specific excitation patterns and conduct the excitations, along effective degrees of freedom, to active sites in the protein. In terms of enzyme action, this concept means that the enzyme molecule can utilize the energy of impinging solvent molecules to increase the rate of the catalyzed reaction.

This is not a violation of the second law of thermodynamics; the free energy of the protein is not changed. An increase in the enthalpy that is being delivered, via fluctuations, to the active site is "paid for" by an entropy change elsewhere in the macromolecule. These changes are not rigidly coupled, but it has been supposed that there is a statistical correlation in their rate of change around equilibrium (Careri *et al.*, 1979).

These interesting ideas, supporting the machine concept of proteins, await direct experimental corroborations.

H. Rotational Fluctuations

The last type of fluctuation to be considered is the Brownian motion of protein molecules. We can again ask whether these fluctuations have not been exploited by evolution to serve a biological purpose. Translational movements of proteins in membranes seem indeed to have a role in signal transduction in the plane of the membrane. The rotational motion of membrane proteins, though experimentally well explored (Cherry, 1979), has not as yet been linked with any biological function. The rotation of translocators around the axis in the plane of the membrane is thermodynamically so unfavorable that the theories assuming such a rotation as part of translocation mechanisms have been abandoned. Rotations around the axis normal to the plane of the membrane do take place, however, and they may be relevant for translocation. The sense of rotation would be random in facilitated diffusion, but in active transport the sense of rotation could be biased by the input of energy, and a prolonged rotation in one direction may cause accumulation of solutes at one side of the membrane. A contribution by rotational motion to facilitated diffusion in hemoglobin molecules has been studied both experimentally and theoretically (Gros *et al.*, 1984; Murray and Smith, 1986).

Rotational mechanisms of membrane transport may also involve rotations distinct from Brownian rotations. Such rotations would be much slower and largely independent of the fluctuation dynamics, and have been considered in various rotational models of ATP synthase (Boyer *et al.*, 1982; Cox *et al.*, 1984; Hayashi and Oosawa, 1984; Mitchell 1985a), inspired by studies of flagellar rotations in bacteria. Such models are, obviously, explicit in considering rotational devices as molecular machines.

J. Amino Acid Sequences and Tertiary Structures

Every protein has its unique amino acid sequence. On the other hand, extensive studies of the recurring features of protein structure indicate that the proteins so far investigated can be divided into a small number of groups that exhibit similar patterns of tertiary structures (Schulz and Schirmer, 1979; Richardson, 1981; Chothia, 1984). Tertiary structures are essentially determined by assemblies of secondary structure blocks (supersecondary structures, Schulz and Schirmer, 1979) which, in turn, are largely independent of the primary sequences. Accordingly, in many cases the tertiary structures of proteins are unaffected by exchange of amino acids. How much, then, can the primary sequences which, thanks to the rapid progress of gene cloning and nucleic acid sequencing, will soon be known for most of the proteins help us to reveal the three-dimensional construction of protein machines? This is a critical question and no clear answer is yet available.

Several methods of predicting secondary structures from amino acid sequences have been developed (Tanaka and Scheraga, 1977; Chou and Fassman, 1978; Garnier *et al.*, 1978; Cid *et al.*, 1982; Eisenberg, 1984; Busetta and Barrans, 1984; Nishikawa and Ooi, 1986). Tentative conclusions can be derived from them concerning the possible folding and, in particular, placement of the polypeptide segments in membranes. However, a general "folding code," if it exists at all, has not yet been deciphered (Jaenicke, 1980; Mutter, 1985). The genetic code may carry specifications concerning not only the nature of the construction materials and

of the modules that are to be assembled, but also concerning the timing and succession of the construction acts. The latter specifications may be inscribed in quite short sequences and yet may be decisive for the shape and the function of the finished product.

It can be taken for granted that not all possible sequences of amino acids provide polypeptides capable of assuming a single native conformation. From $\sim 10^{45}$ possible sequences for polypeptides consisting of 150 amino acids, only a vanishingly small fraction occur in nature, not only because evolution has not had enough time to deploy all the sequences, but also because only a subset of them are allowed to become proteins. This is why most synthetic peptides do not fold into unique structures but exist as random coils (Mutter, 1985). If organic chemists can now approach the construction of artificial proteins by reasoning and insight, evolution had no such insight, and constructed proteins by haphazard permutations of amino acids.

Privalov (1985) has hypothesized that, as the probability of achieving a polypeptide capable of folding into a compact and stable structure may be low, once successfully discovered, a block of sequences is used repeatedly, on different occasions and for different purposes. And, even though mutations may have successively modified the primary structure of such blocks to such an extent that the common origin is almost unrecognizable, the basic capacity to fold into a stable structure has been guarded and employed in different proteins.

From what has been said, it is rather surprising that averaging of primary structures of proteins does not provide statistically significant differences from random distribution of amino acids in the chain (Finkelstein and Ptitsyn, 1971; Ptitsyn, 1984; Poroykov *et al.*, 1984). This led Ptitsyn (1984) to a somewhat different view than that expounded above. According to this view, the formation of stable polypeptide structures may not need a long biological evolution in that they apparently can arise from random amino acid sequences. It is only the active site of a protein that presupposes a very strict and specific amino acid sequence. The active site should either be rigid or be capable of a very specific conformational transition which consists of moving rigid parts of the site against each other along selective paths. As the rigid structure of the site or of its parts is maintained by weak, noncovalent interactions, the only possible way to ensure the stability of the rigid site is by embedding it into a large cooperative structure capable of disintegrating only as a whole. The architectural details of the large supporting structure are irrelevant. Accordingly, a functional protein evolves from a statistic copolymer exhibiting cooperative capacity by "editing" at those specific positions which will give rise to the active site.

Such a view has important implications. Attempts to develop synthetic macromolecules in which active sites similar or identical to the active sites of contemporary proteins would be spatially fixed by supporting blocks of polymers (which might not even be made up from amino acids) seem justified and should be encouraged. Promising studies have already been undertaken aiming at synthetic enzymes (Mutter, 1985; Lehn, 1985), synthetic translocators (Lehn, 1985), and synthetic receptors (Hayward, 1983; Kaiser and Kezdy, 1984; Lehn, 1985). In addition, artificial protein mosaics (fused proteins) prepared by genetic engineering, which have proved useful in studies on *biogenesis* of proteins and protein complexes (see Section IV,B), will undoubtly soon render outstanding service in analyses of the *mechanism of action* of enzymes, translocators, and receptors.

It can be concluded that the most probable access to the tertiary and higher order structures of proteins will be through direct visualization by means of physical techniques, in particular X-ray crystallography and high-resolution electron microscopy. The importance of calculations of both static structures and protein dynamics should not be underestimated, taking into account the rapidly growing capacity of modern computers. However, delving into primary sequences, which should soon be completely known for practically all proteins, may unravel rules of protein design of which we are not as yet aware. The scrutiny of primary sequences may be a most productive way to gain insight into the succession of acts by which a protein molecule is assembled.

III. How Engines Evolved

A. Evolution as Tinkering

The engine concept can be applied to proteins not only in regard to their structure and function but also in regard to the way that they are constructed. In fact, the biogenesis of proteins has often been described in engineering terms, comparing the genome to a construction layout, messenger RNA to a tape, ribosomes to an assembly line, and so on. Such a description may have some explanatory value when picturing protein ontogeny, but would be misleading in explaining phylogeny.

Jacob (1977, 1981) pointed out that comparison of the action of natural selection to that of an engineer is not a suitable one. In contrast to an engineer, evolution does not "work" according to a preconceived plan, it does not have at its disposal materials and machines prepared for its tasks, and its products are far from the perfection which may characterize

up-to-date products of engineering work. Natural selection is not an engineer, but "it works like a tinkerer—a tinkerer who does not know exactly what he is going to produce but uses whatever he finds around him, whether it be pieces of string, fragments of wood, or old cardboards; in short it works like a tinkerer who uses everything at his disposal to produce some kind of workable object" (Jacob, 1977). Like a tinkerer making a roulette wheel from an old bicycle wheel or the cabinet of a radio from a broken chair, evolution makes a wing from a leg, a part of an ear from a piece of jaw, a lung from an esophagus.

Jacob's notion of "evolution as tinkering" is a most telling shorthand description of the evolutionary mechanisms that can be deduced from our present knowledge of the organization of genomes.

B. Genome Organization

The genome size of contemporary mammalian species is represented by roughly 3.2×10^9 base pairs of DNA (Ohno, 1982). It is generally assumed that the number of genes in this genome is ~50,000. If one takes the average size of individual genes (coding sequences) to be 1000 base pairs long, the 50,000 genes represent 5×10^7 base pairs. As pointed out by Ohno (1982), even if we double this number to make room for multiple copies of specialized genes as well as for putative regulatory elements, 97% of the genomic DNA of mammals remains unaccounted for. This huge quantity of DNA with no apparent function may not be placed under the vigilant surveillance of natural selection, and has been designated by Ohno as "junk DNA." However, in tinkerer's terms, this DNA may be considered as a stock of various materials from which new objects can be assembled and "displayed" to natural selection. Indeed, it is very heterogeneous, containing simple sequences that a priori cannot have any sense, as well as sequences that closely resemble functional genes and have probably originated from them by duplication, unequal crossing over, defective processing, etc. (e.g., reviewed by Jelinek and Schmid, 1982; Hardman, 1986). While the former can be compared to nails and screws, the latter represent broken parts that can be repaired and assembled for new purposes. This is done not only by accumulating random point mutations, which were long considered to be the main generator of genetic variations, but also by transposition and other rearrangement of genetic material (Wilson *et al.*, 1977).

In addition, eukaryotic genomes contain DNA which can properly be named "selfish" or "parasitical" (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). This DNA is replicated along with the rest of the DNA

simply because there is no means to get rid of it. It may contain coding sequences the only function of which is to assure their own replication. And yet, these sequences may also be instrumental in tinkering with the rest of the genome, since their transposition from one place to another in the genome can substantially affect the transcription of flanking structural or regulatory genes (Syvanen, 1984).

A considerable fraction of eukaryotic DNA represents introns, intragenic DNA sequences which, unlike exons, are cut off of the transcripts and do not appear in the final gene product (Breathnach and Chambon, 1981). The presence of split genes, mosaics consisting of exons and introns, is quite common in eukaryotic organisms. Introns are also usually considered to be selfish DNA, but a more balanced view would be to see in them a set of heterogeneous genetic elements, some senseless, others with coding capacity, still others with possible regulatory functions (Crick, 1979).

From all that has been said, a picture of the eukaryotic genome reappears as a busy workshop, overfilled with raw materials, spare parts, broken products, and junk of all kinds, and furnishing a variety of finished handiworks to the environmental customer. Early in evolution it must have been worse: junk everywhere, random sequences, heaps of nonsense, and, very rarely short sequences with simple biological meanings. Ohno (1982) has attempted to identify the primordial meaningful sequence and has proposed that it consisted of 20 bases. Cantor and Jukes (1966) hypothesized that the primordial protein sequences may have contained ~15 amino acid residues.

Longer polypeptides were almost certainly created by tandem fusions of genes coding for two or more shorter, preexisting peptides. This was achieved by placing short coding sequences close to each other in the genome (Fig. 4). However, to place them side by side with no noncoding spacers would be highly demanding engineering work, so that most of them remained separated by spacer sequences. The present-day introns may have originated in this way. Indeed, in many cases of split genes the exons closely correspond, by number and length, to structural modules of the proteins coded by the genes (Privalov, 1982; Gō, 1983). This supports the idea that introns have had an important role in evolution (Gilbert, 1978); unequal crossing over of introns enabled rapid reshuffling of functional units and, thus, evolutionary construction of proteins endowed with new functions by reassembly of the preexisting modules.

The combinatorial rush of evolution is apparent at all levels of biological organization. One of the best examples is the origin of mitochondria and chloroplasts. The well-founded and productive hypothesis assumes that these organelles are descendants of primitive prokaryotes (Margulis,

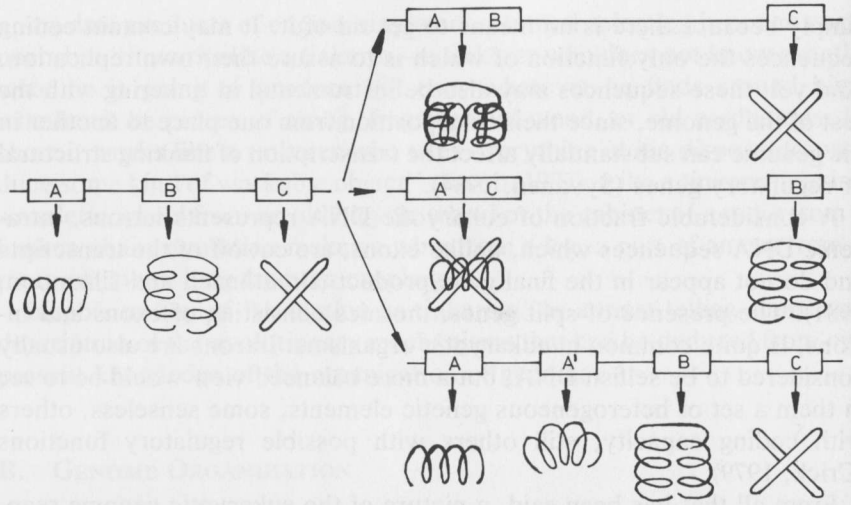

FIG. 4. Evolution of proteins. Genes A, B, and C, dispersed over the primordial genome between "junk" DNA, could fuse together and a fused gene would then code for a protein consisting of two domains, which were originally two independent proteins. Alternatively, gene A duplicated; its second copy was then relaxed from the control by natural selection and could evolve by mutations into a modified copy A′ coding for a new protein.

1981) which combined with organisms that may have resembled the present-day archaebacteria (Doolittle, 1982) to form the symbiotic protoeukaryotic organisms.

The picture outlined complies with current neo-Darwinian thought, the notions of "selfish genes," or "a gene as a unit of natural selection" (Dawkins, 1976). The virtue of the tinkering metaphore is that it also complies with those views which pretend to go "beyond neo-Darwinism" (Ho and Saunders, 1979). They stress the internal autonomy of evolving systems. Perhaps, using a comparison of Brooks (1983), living systems can better be described as motorboats, which can proceed even in the face of a wind and will continue to move in the absence of wind, rather than as sailboats, which have some ability to proceed in a directional manner, but always in a way determined by the wind, an external force. A tinkerer does not usually work for the market. He has an internal drive to work, to manipulate, to invent. If some of his products suit the market, so much the better. How else to account for the appearance of secondary metabolites, which are usually synthesized through intricate metabolic pathways and which, if the final product is advantageous to the producer (even this may not be always the case), the intermediates are usually of no avail? Wong (1981) coined the name "inventive evolution" for a haphazard

generation of catalytic sequences yielding a product that sometimes fulfills a useful function. This includes a recruitment of enzymes from other areas of metabolism.

An organism not only works; it also plays. As everywhere, redundancy is the main playground of creativity.

In a somewhat different linguistic analogy, the first phase of evolution, when short-coding DNA strings were emerging by mutations from the babble of random sequences, can be viewed as a selection of meaningful words from combinations of phonemes. Later, when transposition became prominent, a higher level of linguistic operations, the formation of sentences, set in. The complicated interplay of regulatory circuits in the genome of higher eukaryotes, which we have only started to grasp, may correspond to a still higher hierarchical level of selection, a selection for paragraphs. Evolution is thus a hierarchical process and, indeed, there is a continual evolution of evolution. It has been pointed out that such a hierarchy of selection levels enables continual speeding up of evolution (Ivanitski *et al.*, 1985).

The evolution of biochemical pathways would be placed near the bottom of the evolutionary hierarchy. This is why we face such an amazing biochemical unity of all living forms. Morphological diversification, behavioral patterns, and in particular, the brain, are products of selection at higher hierarchical levels, when no longer were chemical structures and pathways, but rather their regulation, subjected to the evolutionary tinkering (Wilson *et al.*, 1977).

This means that one subset of the set of protein engines, the enzymes, must also have been largely molded quite early in evolutionary history (Koch, 1972; Wong, 1981; Kacser and Beeby, 1984).

It has been calculated that, according to known sequences, proteins belong to 181 superfamilies, and conjectured that this figure may reach 500 as more data become available (Dayhoff *et al.*, 1978). But it is also possible that the original number of proteins was lower and that they served as a basis for enzyme divergence (Kacser and Beeby, 1984). Duplication of genes, the well-known source of evolutionary innovations (Ohno, 1970), liberated redundant copies of a gene from the selection pressure and allowed "mutational adventures" (Kacser and Beeby, 1984) to take place.

The analysis of the evolution of enzymes showed that the evolutionary maximization of catalytic power—that is, of the rate of product formation—demanded a maximization of the constant k_{cat}/K_m and simultaneously a progressive weakening of substrate binding (Cornish-Bowden, 1976; Albery and Knowles, 1976; Fersht, 1977; Brocklehurst, 1977). Hence, in the optimal state, both the catalytic turnover number k_{cat} and

the Michaelis constant K_m are high relative to the ambient substrate concentrations. The optimization of individual enzymes was probably attained quite early in the history of life and, later, there was an evolution toward the optimization of the catalytic power of multienzyme systems (Brocklehurst, 1977; Welch and Keleti, 1981; Hartl *et al.*, 1985).

C. Evolution of Bioenergetic Systems

The idea of evolutionary tinkering is implicit in most theories on the evolution of bioenergetic systems (Broda, 1975; Gest, 1980; Wilson and Lin, 1980; Raven and Smith, 1981; Kováč, 1982; Smith and Morowitz, 1982). The most ancient processes furnishing free energy may have been catabolism of amino acids by a kind of Stickland's reactions and simple phosphoroclastic breaking of organic acids. Ferredoxin, which, in *Clostridia,* takes part in a phosphoroclastic reaction, consists even today of 91% amino acids that had been synthesized abiogenically from simple gases, and can be justly considered to be one of the most ancient enzymes (Hall *et al.*, 1971). Yet, different ferredoxins participate in many other, especially redox, reactions; they were apparently adapted for these more recent reactions by tinkering.

A serious problem that faced protocells encompassing replicating nucleic acids was the necessity to get rid of protons which are produced in the course of the polymerization process (Kováč, 1982). Gramicidinlike pores or channels may have evolved to facilitate diffusion of protons across the lipid protomembrane down their osmotic gradient. The process became more efficient when an ATPase associated itself with the membrane, functioning both as a gate and as a pump for protons. Several scientists have elaborated the idea, first proposed by Mitchell (1968), that an ATPase, associated with the membrane and pumping protons, may have later become an ATP synthase when independent membrane proton generators enabled the reversal of proton flow (Wilson and Lin, 1980; Raven and Smith, 1981; Koch, 1985).

This, indeed, may have been the evolutionary scenario of the F_0F_1-type systems present in bacterial, mitochondrial, and chloroplast membranes. On the other hand, proton-pumping ATPases of plant and fungal plasma membranes may have evolved independently and in a different manner. There is no sequence homology between yeast F_1-ATPase and the H^+-pumping ATPase of yeast plasmalemma (Serrano *et al.*, 1986). The latter, however, has sequences similar to the Ca^{2+}-ATPase and Na^+K^+-ATPase of animals and the K^+-ATPase of bacteria (Hesse *et al.*, 1984; Shull *et al.*, 1985; MacLennan *et al.*, 1985). All these ATPases apparently evolved

from a common ancestor, which may have originally exhibited little specificity and no discrimination between H^+ and alkali cations. By implication, the mechanism of function of the F_0F_1-type ATPase and plasma membrane H^+-ATPase may be substantially different and, in addition, the mechanism of H^+ pumping by the plasma membrane H^+-ATPase must be related to that of Ca^{2+} and K^+ pumping and, thus, not similar to a Grottus-type H^+ conduction or other mechanisms exclusive for H^+.

Respiratory chain-linked proton pumping probably also evolved by such a piecemeal process. Hydrogenase, utilizing molecular hydrogen, may have been accommodated in the membrane in such a manner that separation of charges and generation of membrane proton electrochemical potential may have ensued. This system is considered to be the simplest chemiosmotic device (Koch, 1985; Hooper and DiSpirito, 1985). Since the common fermentation pathway with lactate or ethanol as end products is internally balanced with respect to oxidation–reduction and no pyruvate is available for biosynthesis, Gest (1981) hypothesized that, to spare pyruvate for biosynthesis, fumarate was utilized as a hydrogen acceptor. The reduction of fumarate was (and still is in some bacterial species) catalyzed by a soluble fumarate reductase. Later, fumarate reductase became associated with membranes, and still later the electon flow to fumarate became associated with proton pumping. The NADH–fumarate couple may be the most ancient predecessor of the respiratory chain.

Comparison of the evolutionary path of cytochrome *c* led to a somewhat different picture of the evolution of respiration (Dickerson *et al.*, 1976). It was assumed that bacterial and eukaryotic oxygen respiration arose from the dual-function photosynthetic and respiratory electron transport chain in purple nonsulfur bacteria by the loss of photosynthetic capabilities.

Cytochrome oxidase may have been the latest innovation in the construction of the respiratory chain, but even this latest innovation must have appeared rather early in evolution, since proton-pumping cytochrome oxidase is present in *Paracoccus,* which may be similar to a bacterium which gave rise to mitochondria, just as it is in mammalian mitochondria (Poole, 1983).

D. Energy and Evolution

The concept of selfish, parasitical, junk DNAs was brought about by molecular and evolutionary biologists, and has not been subjected yet to a bioenergeticist's scrutiny. Is the presence of huge amounts of apparently

useless material compatible with strict economy an organism has to observe in order to survive in competition with other species? Is not the energetic burden of maintaining and replicating (and sometimes even transcribing) this "luxury" DNA too excessive? It should also be borne in mind that cells carrying large genomes need a longer time for division than cells with small genomes. In addition, their sluggishness is reinforced by the fact that, being large, they have an unfavorable surface–volume ratio and thus a sluggish metabolism. To answer these questions we have to return to the evolution of bioenergetic mechanisms.

As Doolittle (1982) put it, "we will never understand the functions of all structural elements present in the eucaryotic genome until we understand the selection pressures which produced them, pressures which may operate at levels with which we are neither familiar nor comfortable." Intuitively, we are inclined to believe that competition for energy resources may have been a major evolutionary factor, rapidly selecting organisms with the highest possible efficiency of energy transformation and utilization. Indeed, all the existing organisms exhibit very efficient mechanisms of energy transformation, including such "living fossils" as methanogenic bacteria (Daniels *et al.*, 1984). It is feasible that the "energetic perfection" was achieved rather early in evolution, in parallel with the elaboration of metabolic pathways.

A parallelism between the evolution of the efficiency of energy conversion and enzyme catalysis follows from the following consideration: if an exergonic process were rapid and not coupled with an endergonic process, such as synthesis of ATP, the system would heat up considerably. Oxidation of 1 mol of glucose at pH 7.0 and 25°C is accompanied by a decrease in free energy of 2880 kcal. This amount of glucose would be oxidized by 1 kg yeast cells in ~30 min. If the oxidation of glucose were not coupled, 1 kg of yeast would be heated from 20 to 706°C, or, if the yeast was present as a 1% suspension in water, this still would bring about heating of the suspension from 20 to 27°C.

Yet, serious complications blur a simple reasoning. According to a commonly held scenario of the origin of life, the original atmosphere on earth contained gases with a high degree of reduction. By the input of energy, the reduced substances were transformed into amino acids, nucleic acid bases, and other substances with a reduction degree and energy content similar to the present-day biomass. The only energy required was for polymerization of the monomer units and possibly for their concentration within the protocells. As the "primordial soup" was probably rich in such monomers, energy could not be scarce and limiting in the early phases of evolution. As in the classical experiment of Spiegelman on *in vitro* Q_β phage evolution, there may essentially have been selection for the speed of replication (Mills *et al.*, 1967).

If later some pigments akin to bacteriorhodopsin started to furnish energy by harnessing solar radiation (Smith and Morowitz, 1982), this energy source again was not limiting.

However, even under conditions in which the energy resources are scarce, it is not the efficiency of energy utilization but rather the speed of growth that is advantageous. A rapidly metabolizing and rapidly growing organism would win competition for energy resources over a sluggish one even if the latter were using energy more efficiently and the former wasted it.

In analyzing the thermodynamics of bacterial growth, Westerhoff *et al.* (1982, 1983) reached the conclusion that present-day microorganisms are optimized with respect to maximum growth rate. In organisms growing on substrates with a high degree of oxidation, the thermodynamic efficiency (defined as the ratio between the rate of free-energy output and the rate of free-energy input) is optimized to match the requirement for maximal growth rate. In organisms growing on highly reduced substrates, such as methanol or alkanes, the thermodynamic efficiency of energy transformation is even negative: the problem for them is to get rid of the excess free energy (the highly reduced substrates contain more free energy than is necessary for the formation of biomass) rather than to be economical with it.

The selection for maximal growth rate may have been the reason why the genome of prokaryotes, in contrast to eukaroytes, does not contain substantial amounts of "junk" DNA and is as short as possible (Kingsbury, 1969).

The efficiency of energy conversion and the rate of processes are linked together. If the efficiency of energy conversion were maximal (100%), there would be no flow through the system (Kedem and Caplan, 1965; Stucki, 1980). Any net flow implies decrease in the capacity to work, dissipation of free energy. The optimal performance is determined by the nature of the output parameter that should be optimized. In power plants, for instance, it is usually provision of power that should be maximal; in this case, the optimal state of the system corresponds to 50% efficiency. However, if resources became scarce it would be necessary to minimize entropy production, which again would change the optimal efficiency (Andersen *et al.*, 1984).

The "survival of the fastest" (Kacser and Beeby, 1984) is, of course, not general. The diversity of the eukaryotic world is so rich that there is certainly no universal parameter that has been optimized in all species. This is obvious if we consider the variety of survival strategies. Maintenance of the large "experimenting" genome, dispersed in several genophores, seems to be a common denominator of all these strategies. One of the reasons is that, by possessing this device, hereditary innova-

tions can be brought about rapidly. A more subtle reason is that it is essentially the regulatory functions that are being subjected to evolution in eukaryotes. These can be refurbished mainly by genome rearrangement (Wilson *et al.*, 1977). The coupling device itself can also be subjected to regulation; in *Neurospora,* the plasma membrane proton-translocating ATPase can change its pump stoichiometry according to conditions. The H^+/ATP stoichiometry was found to be 1 under energy replete conditions, corresponding to 50% efficiency (i.e., optimized for maximal power output). Under conditions of energy restriction, the pump stoichiometry changed to 2, thus operating with maximal energy efficiency (Warncke and Slayman, 1980). There is a possibility that he H^+/O stoichiometry of cytochrome oxidase in mitochondria may also vary, being either 2 or 4 H^+ per O reduced, depending on the intracellular conditions (Lehninger *et al.*, 1985). Other examples may be found in the future.

The diversification and perfecting of regulatory toys has been the main preoccupation of the evolutionary tinkerer ever since the basic biochemical evolution was almost finished and in a state of excellence. Ricard (1978) showed in a theoretical study that an enzyme, when it has achieved a "kinetic perfection," cannot be regulated if composed of a single subunit. Only polymeric enzymes can exhibit both regulatory power and catalytic efficiency. This may be the reason why some eukaryotic enzymes contain more subunits than their prokaryotic counterparts, although their catalytic efficiency may not be different.

An instructive example was provided by Kadenbach (1986): cytochrome oxidase of prokaryotes contains 2 or 3 subunits; from *Dictyostelium discoideum,* 6; from yeast, 9; and from mammalian sources, 13 different subunits. The higher a species is placed on the evolutionary ladder, the more subunits it contains. The catalytic perfection of cytochrome oxidase is apparently the same in bacteria (it is also a proton pump) as it is in mammals, but, according to Kadenbach, the additional subunits present in mammals are involved in the allosteric regulation of cytochrome oxidase.

Another way of improving regulation has been a "dressing" of the existing enzymes by mechanisms of posttranslational modification. Inventive evolution goes on, and our own inventions can be conceived of as a continuation of the evolutionary process. "Rediscovery" of these evolutionary inventions by scientific research repeats the same path: first, we disclosed the basic mechanisms of energy transformation, and we are now in a phase in which we attempt to get an insight into the regulatory devices. This is the ubiquitous logic of Haeckel's law.

E. Evolution and Cognition

Boltzmann (1975) has made an ingenious statement that "the struggle for life among living beings is not the struggle for building materials or for energy ... but the struggle for entropy." Although he may have had something else in mind, the following meaning may be given to his statement: living beings strive to dissipate available energy as rapidly as possible. Life in its entirety is a big catalyst accelerating the dissipation of free energy in its surroundings, removing the "hang-ups" that retard the world's running down the potential of increasing entropy.

This idea is not a new one. In his book, "Elements of Mathematical Biology" Lotka (1956) pointed out the tendency of the living world "to increase the rate of energy flux through the system of organic nature, with a parallel increase in the total mass of the great world transformer, of its rate of circulation, or both." With a caution, he was inclined to see in it a law of evolution. The same idea was elaborated by Black (1973, 1978) in his thermodynamic theory of the origin and evolution of life. Por (1980) discussed the progress in the Animal Kingdom in similar terms. Zotin and Krivolutski (1982) plotted the relation between the specific rate of respiration of animals and their position on the evolutionary scale and showed that, in the course of evolution, there was a continual increase in the specific rate of respiration (Fig. 5). This rate may be considered as a measure of the density of processes which, in a living system, are displaced from equilibrium. Their sum would then express the extent of the departure of the living system from equilibrium. Hence, the increase in the specific rate of respiration indicates that in the course of evolution the newly evolving forms were increasing their departure from equilibrium.

The increase in departure from equilibrium has important implications. First, as we know now, the world far from thermodynamic equilibrium, sustained by continuous dissipation of free energy, is a world of dynamic structuring, a world rich in forms and dissipative structures (Prigogine, 1980). The amazing morphological diversity of higher organisms may have ensued from these thermodynamic principles. Still more importantly, the departure of a system from equilibrium may be equated with the distinction of the system from its environment. The farther the system is from equilibrium, the more distinct it is from its environment. This distinction does not mean isolation; to survive, the living system has to record and evaluate continuously the properties and changes of the environment, to accommodate to the environment, to exploit the properties of the environment—in short, to know the environment. Hence, biological evolution is the evolution of cognition, and organisms are cognitive structures (Goodwin, 1976). The main function of cognition is to find resources

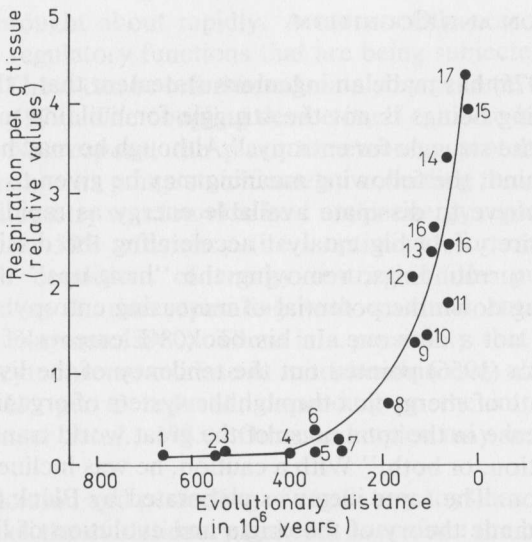

FIG. 5. The relationship between the specific rate of respiration and evolutionary distance of classes of organisms (adapted from Zotin and Krivolutski, 1982). The later a class appeared in evolution the higher is its specific rate of respiration. 1, Coelenterata; 2, Crustacea; 3, Mollusca; 4, Teleostei; 5, Amphibia; 6, Insecta; 7, Reptilia. 8–15, Mammalia: 8, Monotremata; 9, Marsupialia; 10, Edentata; 11, Chiroptera; 12, Insectivora; 13, Rodentia; 14, Perissodactyla; 15, Primates. 16,17, Aves: 16, Nonpasseriformes; 17, Passeriformes.

of free energy that can be dissipated, to reduce the entropy that accompanies the search for the free energy. Thus, cognition is both the result and the cause of increasing the rate of free-energy dissipation, which makes life a natural phenomenon in a universe subject to the second law of thermodynamics. A consistent attempt to analyze biological phenoma from the cognitive point of view, which has been named cognitive biology (Kováč, 1986), is thus a logical outgrowth of bioenenergetics.

In this view, achievements of human cognition are logical extensions of the universal evolutionary tendency. The advent of recombinant DNA technology, artificial translocations and recombinations of genes, construction of fused proteins, and preparation of synthetic genes signifies an enormous increase in the rate of evolution, but also, by necessity, in the rate of dissipation of free energy.

IV. How Engines Are Assembled

A. Protein Folding

The primary sequence of a protein will be considered here as a mere blueprint obtained by copying the original design imprinted in the gene. It

is the successive formation of secondary and higher order structures as well as the correct installation of products in the cell factory that will be treated as the process of construction of protein engines.

Since the classical studies of Anfinsen (1973) on ribonuclease, two principles have been generally accepted: that the information needed to specify the native three-dimensional structure of a protein is contained in its amino acid sequence, and that the folding of the unfolded polypeptide chain into the proper native conformation is a spontaneous process driven by a decrease of free energy. The prevailing view is that the folding is a kinetically controlled multistep process (Anfinsen and Scheraga, 1975; Jaenicke, 1980; Richardson, 1981; Scheraga, 1984).

In the first step, fluctuating nucleation centers, consisting of short segments of α helices, β sheets, and β bends, are formed as a result of short-range interactions. The nucleation centers are not stationary; they form and disintegrate, and they are distributed along the entire peptide chain. Subsequently, the transient secondary structures associate to form supersecondary structures (folding units) under the influence of medium interactions. In the third step, readjustments are made to achieve the final tertiary structure. During this step, areas of secondary structure formed originally may disappear (Kolaskar and Ramabrahman, 1984).

Privalov (1985) has assumed that the substructures which are formed as kinetically relatively stable intermediates in the folding process correspond to those structures which, in the evolutionary past, existed independently as small ancestral peptides and were used in varying combinations as construction blocks for elaborating larger proteins. Hence, the contemporary proteins inherited from their ancestors their technology of folding and, in the process of folding, "recapitulate" their own phylogeny. In Privalov's expression, this is Haeckel's law at the molecular level.

Incidentally, organic chemists, attempting the synthesis of artificial proteins, also try to learn from evolution and to reach their goals by recapitulating protein evolution in their laboratories (Mutter, 1985).

It is probably in this feature of protein folding that molecular machines the least resemble macroscopic machines. The latter can be assembled by processes involving only the sequential addition of components. The completed assembly can be dismantled by removing one component after another in the reverse order to which they were assembled. In contrast, the early steps in protein folding involve apparently the entire chain, and the final product is achieved via rearrangements of the intermediary structures late in the pathway. The final product, native protein or a protein domain, represents a cooperative unit which only falls apart in an all-or-none manner, as a first-order transition.

The distinction may not be as strict, at least not in the assembly process, if we consider protein folding as it takes place not in the laboratory,

but in the cell. Unfortunately, data on protein folding *in vivo* are scarce and not easy to obtain. An analysis of the folding of nascent protein was published by Spirin (1984). The synthesis of a protein on a ribosome is sequential, starting from the NH_2 terminus. In the process of elongation, the growing COOH terminus is covalently fixed in the peptidyltransferase center, while the NH_2 terminus is free. Spirin hypothesized that the peptidyltransferase center already generates a helix conformation of the nascent peptide, probably an α helix. The sequence of the first 30–40 amino acids from the NH_2 terminus is buried in the ribosome and, again, the ribosome imposes upon it an α-helical conformation. This conformation would allow a smooth sliding of the peptide in the channel of the ribosome.

Once the peptide is longer than 30–40 amino acid residues, it gets off the ribosome and can fold in a way similar to the way it does *in vitro*. However, the initial α helix would already represent a preset nucleation center, in contrast to folding in solution. In addition, the COOH terminus of the peptide remains fixed to the ribosome and this in turn may affect the folding. In any case, the cotranslational folding must be the proper one, since the growing, incomplete chain of β-galactosidase can be recognized by antibodies directed against the mature enzyme (Hamlin and Zabin, 1972), and it can also associate with other complete subunits to make a protein exhibiting β-galactosidase activity (Zipser and Perrin, 1963; Kiho and Rich, 1964).

We can conclude that cotranslational folding of a nascent polypeptide may be a less random process than is the folding of the complete polypeptide *in vitro* and may have some features of an orderly, successive process. Also, it is evident that, although the folding is prescribed by the sequence of amino acids, it depends on the environment in which the folding process takes place. We can speak of "the genotype" and of "the phenotype" of a protein.

B. Intracellular Protein Topogenesis

The protein that has left the assembly line of the ribosome need not be a finished product. It must be transferred to its proper place in the cell and there, or during the transfer, be completed into a functional machine. The synthesis of the protein is followed by intracellular protein topogenesis (Blobel, 1980). The latter process has been intensively studied since the mid-1970s and results, as well as speculations, have been reviewed in a number of papers (e.g., Wickner, 1979; Neupert and Schatz, 1981; Wickner and Lodish, 1986; Hurt and van Loon, 1986). The variety of topogenic

processes may be a suitable example of an opportunistic evolutionary tinkering. Yet, some universal principles, outlined below, may underlie the apparent diversity.

If a protein synthesized on cytosolic ribosomes is not assigned to remain in the cytosol, it must carry, in its amino acid sequence, the information which determines its next fate. In many nascent proteins, this information is represented by a short signal sequence at the NH_2 terminus (Watson, 1984). Some proteins do not have the signal sequence at the NH_2 terminus, but internally.

The signal sequence must be recognized and its meaning decoded by a device which belongs to the compartment to which the particular protein is assigned. These devices are protein receptors which can be specific for a single protein or for a group of proteins (Korb and Neupert, 1978; Walter and Blobel, 1981; Zwizinski *et al.*, 1985; Yoshida *et al.*, 1985). The translation code has not been deciphered yet, and its intricacy is one of the challenging problems in research on topogenesis.

In addition to its "addressing" function, the signal sequence has another role: it facilitates translocation of the nascent protein across membranes (Wickner, 1979; Von Heijne, 1986). Studies with synthetic peptides formed by amino acid sequences corresponding to signal sequences of bacterial (Briggs *et al.*, 1985) and mitochondrial (Roise *et al.*, 1986) proteins indicated that the physicochemical properties of signal peptides enable them to interact with artificial lipid membranes. Perturbation of phospholipid bilayers in membranes may be part of the mechanism by which the signal sequence translocates attached proteins across the membranes (Roise *et al.*, 1986).

Interaction of the nascent protein with the receptor can be accomplished in two different ways. In the first case, the receptor ("signal recognition particle") freely moves in the cytosol and searches for the corresponding signal sequence. After finding it, still in a phase when only part of the nascent protein has been formed, the signal recognition particle, with the nascent protein attached to it, interacts with a specific protein ("docking protein") in the membrane into which the nascent protein is to be inserted (Walter and Blobel, 1981; Meyer *et al.*, 1982). In the second case, the receptor is a membrane protein located in the membrane belonging to the compartment into which the protein is to be placed. The nascent protein itself searches for its receptor, probably by free diffusion. However, cytoplasmic components may be instrumental in this (random?) search (Miura *et al.*, 1983; Argan *et al.*, 1983; Ohta and Schatz, 1984).

After the attachment to the membrane, the nascent protein translocates across the membrane. It is not yet known whether the translocation is

freely, through lipid mediated by a protein translocator, or through a proteinaceous pore. Additional topogenic signals may exist which decide whether the protein will cross the membrane entirely, be embedded in it, or anchored to the membrane at its NH_2 or COOH terminus (Blobel, 1980). It has been assumed that these signals are inscribed as some specific amino acid sequences. It is, however, also possible that, except for the signal sequence, other topogenic sequences do not exist and it is the thermodynamics of the folding of a protein in the membrane that determines its final fate (Engelman and Steitz, 1981; Rapoport, 1985; Wickner and Lodish, 1986).

Approaching the end of the topogenic process, the NH_2-terminal signal sequence can be split off by a specific protease in most cases, but the signal sequence can also be left permanently attached to the final product (Hurt and van Loon, 1986). Posttranslational chemical modifications of proteins are accomplished either en route to or at their final location.

A provoking bioenergetic problem, largely unresolved, is the energetics of topogenesis (Kováč, 1972; Copeland *et al.*, 1984). In most cases, but not always, energy is required for protein translocation across membranes. If this energy were simply in the form of a phase-specific membrane potential, as indicated by a number of experimental data (e.g., Pfanner and Neupert, 1985; Roise *et al.*, 1986) one could envisage that proteins move electrophoretically across the membrane. The simple electrophoretic model is incompatible with the fact that proteins translocate in the same direction in bacterial and mitochondrial membranes, although the membrane potentials have opposite orientations across the two membranes (Bakker and Randall, 1984). In some cases, not the membrane potential but ATP was identified as the driving force for translocation (Grossman *et al.*, 1980; Chen and Tai, 1985). The possibility remains that in the cases in which neither the membrane potential nor ATP is needed for translocation of a protein, the process may still be energy-requiring and the energy may be provided by the proper folding of the translocated chain of the protein itself.

The last statement leads to an essential question that cannot be answered so far: what are the secondary and tertiary structures of nascent proteins with NH_2-terminal signal sequences attached to them (preproteins) before, and in the course of, translocation across membranes? It is doubtful that preproteins would exist as random coils. According to one hypothesis (Engelman and Steitz, 1981), during synthesis, the growing polypeptide chain folds in an aqueous environment to form an antiparallel pair of helices. The signal peptide forms one of two helices. The precursor form of the mitochondrial ATP/ADP translocator, which does not contain a NH_2-terminal signal sequence, exists in the cytosol in the form of aggregates (Zimmerman and Neupert, 1980). The folding into a conformation favoring the aggregation may be dictated by the water environment of the cytosol, while the final conformation is assumed in the mitochondrial membrane and is apparently automatically determined by the thermodynamics of the membrane environment. Synthetic peptides corresponding to a signal sequence were found to have little secondary structure in water, but became partly α-helical in the presence of detergent micelles simulating the lipid environment of the membrane (Roise *et al.*, 1986).

Methods of genetic engineering, which have already furnished most valuable data in the field of protein topogenesis (Wolfe *et al.*, 1983; Silhavy *et al.*, 1983; Douglas *et al.*, 1984; Benson *et al.*, 1985; Yaffe *et al.*, 1985; Hurt and van Loon, 1986), will certainly make a major contribution to the solution of the aforementioned problems. The answer to the last question would apparently need other approaches.

V. How Engines Are Maintained

A. The Problem of Maintenance

It is the recurrent idea of this essay that molecular engines, in contrast to macroscopic engines, are permanently exposed to enormous random fluctuations. The fact that in the disordered ocean of thermal noise they can be accurately assembled, maintain stability, and perform precise functions is one of the greatest miracles of the living world—a miracle that has not been appreciated enough.

Imagine a factory in which, besides workers engaged in the construction work, there are a huge number of people who randomly run back and forth and interfere with the work, and who incessantly pull at the machines with forces comparable in strength to those which hold the machines together. Is it not admirable that, in such chaos, the work goes on smoothly, with great economy and precision?

One of the arrangements which counteract the disordering effects of thermal fluctuations is the cooperativity of biological structures. The cooperativity of structure both in double-stranded DNA and in proteins protects the macromolecules from easy denaturation. In addition, as already discussed (Section II,G), fluctuations which "shake" these molecules from within may be correlated and endowed with functional meaning.

Another arrangement is the unceasing correcting, proofreading activity. In various processes and at various levels, products that have been synthesized by the enzyme machinery are being checked either by the

same machinery or by other devices (e.g., as reviewed by Fersht, 1980; Ruusala *et al.*, 1982; Englisch *et al.*, 1985). Proofreading not only corrects errors arising from interfering thermal noise, but also enables a high discrimination between correct and wrong substrates which is far beyond the precision allowed by differences in equilibrium binding of the two substrates (beyond the "lock-and-key" discrimination). Proofreading assures a low error rate of DNA replication, aminoacylation of tRNAs, codon–anticodon recognition, peptide formation (Fig. 6).

But even the continual correcting activity cannot entirely prevent formation of defective products. Neither can it prevent the functional products from being worn out. Protein machines, being cooperative structures, differ from macroscopic machines in one important characteristic: in a macroscopic machine, a breakdown of a single component, a loss of a single screw, may put the machine out of operation and the machine can be restored by replacing the broken or missing part. A protein, however, once assembled represents a single unit. A functional derangement can therefore only be repaired by removing the broken machine and replacing it by a new one. This is probably why there exists continual proteolysis and turnover of cell constituents.

B. Proteolysis and Turnover

The fact that the significance of proteolysis in the construction work of cells has not been sufficiently recognized may be one of the reasons why less is known of intracellular protein degradation than of intracellular protein synthesis and assembly (e.g., see Herschko and Ciechanover,

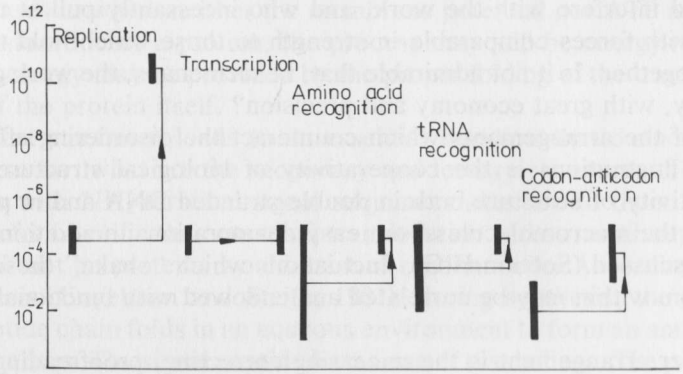

FIG. 6. Decrease in errors of various processes due to proofreading activity. Note that transcription of DNA is not proofread.

1982; Wheatley, 1984; Mayer and Doherty, 1986 for reviews). The proteolytic apparatus can be seen as an intracellular "sanitation system" (Goldberg and Dice, 1974) or "surveillance machinery," a kind of "intracellular police" that suppresses any defective products and any deviation from the norm.

A system degrading abnormal proteins in bacteria is probably the best known (Goldberg and Goff, 1985) of these. A similar system seems to exist in mitochondria (Rapoport *et al.*, 1982; Desaultels and Goldberg, 1982a) and chloroplasts (Schmidt and Mishbind, 1983; Matto *et al.*, 1984; Malek *et al.*, 1984). In these organelles, where protein engines generally consist of several subunits which may be synthesized in different cell compartments, the proteolytic system apparently ensures the balance between the products of organelle and nuclear genomes by degrading excess polypeptides (Luzikov, 1980; Desaultels and Goldberg, 1982b). An interesting hypothesis has been put forward which states that intramitochondrial proteolysis represents a selection device by which "superfluous" components, inappropriately synthesized or assembled, are continually broken down (Luzikov, 1980; Luzikov *et al.*, 1983). Idle, nonworking, though correctly assembled, mitochondrial functional units were supposed to be eliminated in a similar way. The hypothesis presupposes (and some experimental data support it) that working molecular engines are less susceptible to proteolysis than are the idle ones.

How does the proteolytic surveillance system distinguish between the proper and "wrong" protein? This is still a puzzle, one of the most provoking in biology. No intracellular analogy of the immune system is feasible.

Correlations between the general physicochemical properties of proteins (such as global size, isoelectric point, charge, hydrophobicity, denaturability) and the susceptibility to degradation have been searched for and asserted to be found. Such claims have been largely refuted and, as stated by Mayer and Doherty (1986), such correlations seem inherently unlikely. It is possible that a protein need only be tagged by a short sequence to be either protected (e.g., preproteins tagged with the signal sequence) or (as in the ubiquitin proteolytic system—see Herschko and Ciechanover, 1982; Ciechanover *et al.*, 1984) exposed to proteolysis. This, however, would not obviate the necessity to recognize the protein properly before its tagging.

Is such a recognition necessary? Wheatley (1984) expressed a view that the intracellular basic proteolytic system destroys proteins randomly, without distinguishing young from old, aberrant from normal. In his opinion, two factors are relevant: (1) the accessibility of proteinases to the peptide bonds of a protein, which is determined by its conformation and,

hence, in the end by its primary sequence; and (2) the frequency with which the existing proteins move in and out of protected sites or conformations in their associations with other molecules. A protein that cannot find its proper way or its "partner" protein is degraded, while a protein which assumes its role as soon as possible is largely protected.

A consequence of such a policy is almost incredible and, yet, experimentally substantiated: to go from one unit size to two units, HeLa cells synthesize more than twice as much protein as is retained (Wheatley, 1984). Half of all the proteins actually synthesized are degraded, and as much as one-third of the proteolysis is directed at nascent proteins. When the cells cease to grow, proteolysis continues and protein synthesis has to carry on to counterbalance the degradation. Wheatley supposes that the situation in microorganisms may not be much different and that protein turnover in microorganisms, which was studied mainly in the 1950s, was grossly underestimated. This need not be the case: microorganisms may be able to afford "wrong" proteins, in that they lose them anyhow in the rush of rapid growth.

From a purely energetic point of view continual proteolysis seems like an enormous waste of energy, since protein synthesis is a very costly process. This is all the more true in that intracellecular proteolytic processes are themselves ATP-requiring (Goldberg and Dice, 1974; Herschko and Ciechanover, 1982; Mayer and Doherty, 1986). Admitting that complete proteolytic degradation is the only way to "repair" defective protein engines, why should correct products be degraded as well? If protein turnover assures metabolic flexibility, one would wonder why evolution has not invented a less expensive mechanism for this purpose.

Yet, the reason may be a principal one involving the "cognitive limits" of molecular machines, discussed in a previous section (II,C). Any actual recognition at the molecular level would be so expensive in terms of energy that, unless "paid for" in evolution and firmly fixed, it could only rarely be afforded by the cells. This implies that even labeling by ubiquitin, which sorts out a protein for degradation, may not be based on recognition but only on the statistical accessibility of the protein.

C. Energy Costs of Maintenance

The energy costs of other, less drastic, correcting and maintaining processes are certainly lower than those of proteolysis, but their values under *in vivo* conditions cannot be assessed at present (Yamane and Hopfield, 1977; Mulvey and Fersht, 1977; Savageau and Freter, 1979). Proofreading mechanisms generally require conditions far from equilibrium and are

thus accompanied by dissipation of free energy (Hopfield, 1974; Ninio, 1975; Ehrenberg and Kurland, 1984). For instance, 270 molecules of ATP are hydrolyzed before one molecule of Val-tRNA^{Ile} (of *E. coli*) is formed, and even the formation of the cognate Ile-tRNA^{Ile} requires 1.5 ATP molecules (Englisch *et al.*, 1985). This, however, provides no hint of how much energy would be spent *in vivo*. At the limit, infinite accuracy would require infinite amounts of energy (Fersht, 1984; Englisch *et al.*, 1985).

Theoretical and experimental analysis indicates that, in wild-type bacteria, the costs of errors and accuracy are optimized and determined by growth rates (Ehrenberg and Kurland, 1984). Mutants can be prepared exhibiting greater accuracy of protein translation on ribosomes, but the high energetic cost of the excessive "pedantry" is reflected in lower growth rates, so that the mutants experience an "accuracy catastrophe". The analysis also indicates that ribosomes themselves, consisting (in bacteria) of 10^4 amino acids, cannot escape errors in their own construction that affect their actual performance. The corollary, which can be generalized (and has been, in fact, implicit all through the present essay) is that "the devices of a cell are ensembles with performance characteristics that are distributed, and it is not just the means, but also the variance of the distributions that are optimized during evolution" (Ehrenberg and Kurland, 1984).

It may be expected that some guesses about energy costs can be derived from measurements of energy investments into the synthesis of biomass and from growth yields. About 60% of the enthalpy of nutrients has been generally found to be conserved in the biomass of growing cells, indicating that the rest may have been dissipated during processes providing no useful work (Payne, 1970). In the past, it was calculated that 30–36 mmol ATP are needed for synthesis of 1 g (dry weight) of cells (Gunsalus and Shuster, 1961; Forrest and Walker, 1971), whereas, experimentally, a value of 95.2 mmol ATP was generally measured (Beauchop and Elsden, 1960). For some time, the latter value was considered as almost a biological constant and the comparison of the two values indicated that two-thirds of the ATP generated in growing cells was used for processes other than synthesis of cell materials.

Unfortunately, subsequent theoretical and experimental studies (e.g., reviewed by Stouthamer and Bettenhaussen, 1973; Stouthamer, 1979; Thauer *et al.*, 1977) on growth yields have blurred the simple picture, as did the analysis of the thermodynamic efficiencies of energy transformation in growing microorganisms discussed briefly in a previous section (III,D). An interesting statement to the effect that in growing yeast as much as 75–85% of the total energy requirement is used for the "development of cellular organization" (Oura, 1973) would need a novel theoretical substantiation.

It is not known how much energy is required for other "construction works" which have not been considered in theoretical calculations of maximal growth yield, such as energy required for regulating the topology of DNA, for intracellular transport of mRNAs, proteins, and lipids, for the cytoskeleton, for metabolic regulation (e.g., by using futile cycles). It is also possible that many cellular structures are dissipative structures (Prigogine, 1980), depending on continual dissipation of energy. This may hold for spatial two-dimensional patterns in membranes, which have not hitherto been considered much, and which may be of major importance in morphogenesis.

In conclusion, the bioenergetic aspects of proofreading and maintenance processes represent an underdeveloped area of bioenergetics. It is probably not far-fetched to expect that research in this area will bring exciting new discoveries. It encompasses the important problem of the energy costs of recognition and information processing by molecular machines.

VI. Future Prospects: Bioenergetics beyond Chemistry, Genetics, and Physics

Jacob's idea of "evolution as tinkering" (Section III,A) applies not only to biological evolution. The evolution of science is also essentially tinkering. Scientists in their laboratories are tinkerers: they use the chemicals, machines, and above all the ideas they have at their disposal to construct new ideas, as hypotheses and as interpretations of their experimental data. But, in a sense, scientists themselves are but pieces of material which the evolution of science finds useful in its tinkering activity. If biological evolution has no prescribed goal, the evolution of science, as long as it is conceived as a suprahuman activity, may also have no prescribed goal.

In any case, this activity is generated by the latest evolutionary acquisition, the human brain: that "bipartite" brain of McLean (1949), the one part of which—the newer one, the neocortex—directs our intellectual and cognitive functions, but would be idle, like a computer disconnected from the power, if not driven by the ancient part, the "visceral brain," which governs our emotions. Apparently, the two parts have not been hierarchized or coordinated completely. The human brain appears to Jacob (1981) to be a typical product of evolutionary tinkering, a sort of installation of a jet engine on an ancient horse cart. As Jacob (1981) put it, accidents should occur with little surprise using such an arrangement.

It may be revealing, but it is also becoming more and more mandatory,

to pause for a while in the rush of our ultracentrifuges and scintillation counters—to pause with a question: what is this for? How is this relevant? And to see for a while the overwhelming problems that face humanity. It is not hard to grasp that their solution is tightly linked to the understanding of human behavior and of social structures and dynamics.

There is no reason why bioenergetics should not endeavor, once we will have understood the least details of molecular engines, to approach these problems. The notions of energy and entropy are the most basic notions of the entire living world, from molecules to societies.

We have to take the lesson of evolution with the greatest care. We soon may become its principal tinkerers.

ACKNOWLEDGMENTS

This essay is marked by difficulties which the author had in procuring relevant sources of literature. The unique support of G. Schatz, who for years has been furnishing the author and his collaborators with precious journals and books, is greatly appreciated. Other colleagues were also generously helpful: A. Goffeau, S. Henry, B. Hess, J. Howland, Y. Kagawa, C. P. Lee, W. Neupert, J. Rydström, P. P. Slonimski, M. Somlo, B. Trumpower, P. V. Vignais. M. Greksák, P. Šmigáň, and E. Böhmerová read the manuscript and provided useful comments. Thanks are due to L. Ernster for his lasting moral support and his suggestion to write this essay, and to K. Boďa for his favor that allowed the preparation of the manuscript.

REFERENCES

Albery, W. R., and Knowles, J. R. (1976). *Biochemistry* **15,** 5631–5640.
Andersen, B., Berry, R. S., Ondrechen, M. J., and Salamon, P. (1984). *Acc. Chem. Res.* **17,** 266–271.
Anderson, C. M., Zucker, F. H., and Steitz, T. A. (1979). *Science* **204,** 375–380.
Anfinsen, C. B. (1973). *Science* **181,** 223–230.
Anfinsen, C. B., and Scheraga, H. A. (1975). *Adv. Protein Chem.* **29,** 205–300.
Argan, C., Lusty, C. J., and Shore, G. C. (1983). *J. Biol. Chem.* **258,** 6667–6670.
Bakker, E. P., and Randall, L. L. (1984). *EMBO J.* **3,** 895–900.
Beauchop, T., and Elsden, S. R. (1960). *J. Gen. Microbiol.* **23,** 457–469.
Bennett, W. S., and Huber, R. (1984). *Biopolymers* **22,** 261–279.
Benson, S. A., Hall, M. N., and Silhavy, T. J. (1985). *Annu. Rev. Biochem.* **54,** 101–134.
Black, S. (1973). *Adv. Enzymol.* **38,** 193–233.
Black, S. (1978). *Persp. Biol. Med.* **21,** 348–356.
Blobel, G. (1980). *Proc. Natl. Acad. Sci. U.S.A.* **77,** 1496–1500.
Blumenfeld, L. A. (1977). "Probleme der molekularen Biophysik." Akademie-Verlag, Berlin.
Boltzmann, L., quoted in Broda, E. (1975). "The Evolution of Bioenergetic Processes." Pergamon, Oxford.

Boyer, P. D. (1975). *FEBS Lett.* **58,** 1–6.
Boyer, P. D., Kohlbrenner, W. E., McIntosh, D. B., Smith, L. T., and O'Neal, C. C. (1982). *Ann. N.Y. Acad. Sci.* **402,** 65–83.
Breathnach, R., and Chambon, P. (1981). *Annu. Rev. Biochem.* **50,** 349–383.
Briggs, M. S., Gierasch, L. M., Zlotnick, A., Lear, J. D., and DeGrado, W. F. (1985). *Science* **228,** 1096–1099.
Brocklehurst, K. (1977). *Biochem. J.* **163,** 111–116.
Broda, E. (1975). "The Evolution of Bioenergetic Processes." Pergamon, Oxford.
Brooks, D. R. (1983). *Can. J. Zool.* **61,** 2637–2645.
Busetta, B., and Barrans, Y. (1984). *Biochim. Biophys. Acta* **790,** 111–124.
Cantor, C. R., and Jukes, T. H. (1966). *Proc. Natl. Acad. Sci. U.S.A.* **56,** 177–1.
Careri, G., Fasella, P., and Gratton, E. (1979). *Annu. Rev. Biophys. Bioeng.* **8,** 69–97.
Chen, L., and Tai, P. C. (1985). *Proc. Natl. Acad. Sci. U.S.A.* **82,** 4384–4388.
Chernavski, D. C., Khurgin, Ju. I., and Shnol, S. E. (1967). *Mol. Biol.* **1,** 419–424.
Cherry, R. J. (1979). *Biochim. Biophys. Acta* **559,** 289–327.
Chothia, C. (1984). *Annu. Rev. Biochem.* **53,** 537–572.
Chou, P. Y., and Fasman, G. D. (1978). *Adv. Enzymol.* **47,** 45–148.
Cid, H., Bunster, M., Arrigiada, E., and Campos, M. (1982). *FEBS Lett.* **150,** 247–254.
Ciechanover, A., Finley, D., and Varshavsky, A. (1984). *J. Cell. Biochem.* **24,** 25–73.
Citri, N. (1973). *Adv. Enzymol.* **37,** 397–647.
Cooper, A. (1984). *Prog. Biophys. Mol. Biol.* **44,** 181–214.
Copeland, B. R., Landick, R., Nazos, P. M., and Oxender, D. L. (1984). *J. Cell. Biochem.* **24,** 343–356.
Cornish-Bowden, A. (1976). *J. Mol. Biol.* **101,** 1–9.
Cox, G. B., Jans, D. A., Fimmel, A. L., Gibson, F., and Hatch, L. (1984). *Biochim. Biophys. Acta* **768,** 201–208.
Crick, F. M. C. (1979). *Science* **204,** 264–271.
Daniels, L., Sparling, R., and Sprott, G. D. (1984). *Biochim. Biophys. Acta* **768,** 113–163.
Dawkins, R. (1976). "The Selfish Gene." Oxford Univ. Press, Oxford.
Dayhoff, M. O., Barker, W. C., Hunt, C. T., and Schwartz, R. M. (1978). *In* "Atlas of Protein Sequence and Structure" (M. O. Dayhoff, ed.), Vol. 5, Suppl. 3, pp. 9–10. National Biomedical Research Foundation, Washington, D.C.
Desaultels, M., and Goldberg, A. L. (1982a). *J. Biol. Chem.* **257,** 11673–11679.
Desaultels, M., and Goldberg, A. L. (1982b). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 1869–1873.
Dickerson, R. E., Timkovitch, R., and Almassy, J. (1976). *J. Mol. Biol.* **100,** 473–491.
Doolittle, W. F. (1982). *Can. J. Biochem.* **60,** 83–90.
Doolittle, W. F., and Sapienza, C. (1980). *Nature (London)* **284,** 601–603.
Douglas, M. G., Geller, D. L., and Emr, S. D. (1984). *Proc. Natl. Acad. Sci. U.S.A.* **81,** 3983–3987.
Ehrenberg, M., and Kurland, C. G. (1984). *Q. Rev. Biophys.* **17,** 45–82.
Eisenberg, D. (1984). *Annu. Rev. Biochem.* **53,** 595–623.
Eisenberg, E., and Hill, T. L. (1985). *Science* **227,** 999–1006.
Engelman, D. M., and Steitz, T. A. (1981). *Cell* **23,** 411–422.
Englisch, U., Gauss, D., Freist, W., Englisch, S., Sternbach, H., and von der Haar, F. (1985). *Angew. Chem. Int. Ed. Engl.* **24,** 1015–1025.
Fersht, A. R. (1977). "Enzyme Structure and Mechanism." Freeman, San Francisco.
Fersht, A. R. (1980). *Trends Biochem. Sci.* **5,** 262–265.
Fersht, A. R. (1984). *Trends Biochem. Sci.* **9,** 145–147.
Finkelstein, A. V., and Ptitsyn, O. B. (1971). *J. Mol. Biol.* **62,** 611–624.
Forrest, W. W., and Walker, D. J. (1971). *Adv. Microbiol. Physiol.* **5,** 213–274.

Garnier, J., Osguthorpe, D., and Robson, B. (1978). *J. Mol. Biol.* **120,** 97–120.
Gest, H. (1980). *FEMS Microbiol. Lett.* **7,** 73–77.
Gest, H. (1981). *FEMS Microbiol. Lett.* **12,** 209–215.
Gilbert, W. (1978). *Nature (London)* **271,** 501.
Gō, M. (1983). *Proc. Natl. Acad. Sci. U.S.A.* **80,** 1964–1968.
Gō, M., and Taketomi, H. (1978). *Proc. Natl. Acad. Sci. U.S.A. 75,* 559–563.
Goldberg, A. L., and Dice, J. F. (1974). *Annu. Rev. Biochem.* **43,** 835–869.
Goldberg, A. L., and Goff, S. A. (1985). *Cell* **41,** 587–595.
Goodwin, B. (1976). "Analytical Physiology of Cells and Developing Organisms." Academic Press, London.
Gray, B. F. (1975). *Nature (London)* **253,** 436–437.
Gros, G., Lavalette, D., Molls, W., Gros, H., and Pochon, F. (1984). *Proc. Natl. Acad. Sci. U.S.A.* **81,** 1710–1714.
Grossman, A., Bartlett, S., and Chua, N. H. (1980). *Nature (London)* **285,** 625–628.
Gunsalus, I. C., and Shuster, C. W. (1961). *In* "The Bacteria" (I. C. Gunsalus and R. Y. Stanier, eds.), Vol. II, pp. 1–58. Academic Press, New York.
Hall, D. O., Cammack R., and Rao, K. K. (1971). *Nature (London)* **233,** 136–137.
Hamlin, J., and Zabin, I. (1972). *Proc. Natl. Acad. Sci. U.S.A.* **69,** 412–416.
Hantgan, R. R., Hammes, G. G., and Scheraga, H. A. (1974). *Biochemistry* **13,** 3421–3431.
Hardman, N. (1986). *Biochem. J.* **234,** 1–11.
Harold, F. M. (1982). *Curr. Top. Membr. Transp.* **16,** 485–516.
Hartl, D. J., Dykhuizen, D. E., and Dean, A. M. (1985). *Genetics* **111,** 655–674.
Hayashi, S., and Oosawa, F. (1984). *Proc. Jpn. Acad. Ser. B* **60,** 161–164.
Hayward, R. C. (1983). *Chem. Soc. Rev.* **12,** 285–308.
Hershko, A., and Ciechanover, A. (1982). *Annu. Rev. Biochem.* **51,** 335–364.
Hesse, J. E., Wieczorek, L., Altendorf, K., Reicin, A. S., Dorus, E., and Epstein, W. (1984). *Proc. Natl. Acad. Sci. U.S.A.* **81,** 4746–4750.
Hill, T. L., and Eisenberg, E. (1981). *Q. Rev. Biophys.* **14,** 463–511.
Ho, M. W., and Saunders, P. T. (1979). *J. Theor. Biol.* **78,** 572–591.
Hooper, A. B., and DiSpirito, A. A. (1985). *Microbiol. Rev.* **49,** 140–157.
Hopfield, J. J. (1974). *Proc. Natl. Acad. Sci. U.S.A.* **71,** 4135–4139.
Hurt, E. C., and van Loon, A. P. G. M. (1986). *Trends Biochem. Sci.* **11,** 204–207.
Ivanitski, G. R., Esipova, N. G., Abagyan, R. A., and Shnol, S. E. (1985). *Biofizika* **30,** 418–421.
Jacob, F. (1977). *Science* **196,** 1161–1166.
Jacob, F. (1981). "Le Jeu des Possibles." Fayard, Paris.
Jaenicke, R., ed. (1980). "Protein Folding." Elsevier, Amsterdam.
Janin, J., and Wodak, S. J. (1983). *Prog. Biophys. Mol. Biol.* **42,** 21–78.
Jelinek, W. R., and Schmid, C. W. (1982). *Annu. Rev. Biochem.* **51,** 813–844.
Jencks, W. P. (1975). *Adv. Enzymol.* **43,** 219–400.
Jencks, W. P. (1980). *Adv. Enzymol.* **51,** 75–106.
Kacser, H., and Beeby, R. (1984). *J. Mol. Evol.* **20,** 38–51.
Kadenbach, B. (1986). *J. Bioenerg. Biomembr.* **18,** 39–54.
Kaiser, E. T., and Kezdy, P. J. (1984). *Science* **223,** 249–255.
Karplus, M., and McCammon, J. A. (1981). *Crit. Rev. Biochem.* **9,** 293–349.
Kedem, O., and Caplan, S. R. (1965). *Trans. Faraday Soc.* **21,** 1897–1911.
Kiho, Y., and Rich, A. (1964). *Proc. Natl. Acad. Sci. U.S.A.* **51,** 111–118.
Kingsbury, D. T. (1969). *J. Bacteriol.* **98,** 1400–1410.
Koch, A. L. (1972). *Genetics* **72,** 297–316.
Koch, A. L. (1985). *J. Mol. Evol.* **21,** 270–277.

Kolaskar, A. S., and Ramabrahman, V. (1984). *Int. J. Pept. Protein Res.* **24,** 392.
Korb, H., and Neupert, W. (1978). *Eur. J. Biochem.* **91,** 609–620.
Kováč, L. (1972). *FEBS Lett.* **22,** 270–274.
Kováč, L. (1982). *In* "Liquid Crystals and Models of Biological Membranes." SAV, Smolenice.
Kováč, L. (1986). *Biol. Listy* **51,** 172–190.
Lehn, J. M. (1985). *Science* **227,** 849–856.
Lehninger, A. L. (1965). "Bioenergetics." Benjamin, New York.
Lehninger, A. L., Reynafarjee, B., and Costa, L. (1985). *J. Inorg. Chem.* **23,** 335–340.
Lesk, A. M., and Chothia, C. (1984). *J. Mol. Biol.* **174,** 175–191.
Lotka, A. J. (1956). "Elements of Mathematical Biology." Dover, New York.
Lumry, R. (1974). *Ann. N.Y. Acad. Sci.* **227,** 46–75.
Luzikov, V. N. (1980). "Regulyatsiya formirovaniya mitochondriy." Nauka, Moscow (an English translation is available).
Luzikov, V. N., Novikova, L. A., Tikhonev, A. N., and Zubatov, A. S. (1983). *Biochem. J.* **214,** 785–794.
McClare, C. W. F. (1971). *J. Theor. Biol.* **30,** 1–34.
McLean, P. (1949). *Psychosom. Med.* **11,** 338–353.
MacLennan, D. H., Brandl, C. J., Korczak, B., and Green, N. M. (1985). *Nature (London)* **316,** 694–698.
Malek, L., Bogorad, L., Ayers, A. R., and Goldberg, A. L. (1984). *FEBS Lett.* **166,** 253–257.
Margulis, L. (1981). "Symbiosis in Cell Evolution." Freeman, San Francisco.
Mattoo, A. K., Hoffman-Faulk, H., Marder, J. B., and Edelman, M. (1984). *Proc. Natl. Acad. Sci. U.S.A.* **81,** 1380–1384.
Mayer, R. J., and Doherty, F. (1986). *FEBS Lett.* **198,** 181–193.
Meyer, D. I., Krause, E., and Dobberstein, B. (1982). *Nature (London)* **297,** 647–650.
Mills, D. R., Peterson, R. L., and Spiegelman, S. (1967). *Proc. Natl. Acad. Sci. U.S.A.* **58,** 217–224.
Mitchell, P. (1968). "Chemiosmotic Coupling of Energy Transduction." Glynn Research Lab. Bodmin.
Mitchell, P. (1976). *Biochem. Soc. Trans.* **4,** 14–30.
Mitchell, P. (1981). *In* "Chemiosmotic Proton Circuits in Biological Membranes" (V. P. Skulachev and P. C. Hinkle, eds.), pp. 613–633. Addison-Wesley, London.
Mitchell, P. (1985a). *FEBS Lett.* **182,** 1–7.
Mitchell, P. (1985b). *J. Biochem.* **97,** 1–18.
Miura, S., Mori, M., and Tatibana, M. (1983). *J. Biol. Chem.* **258,** 6671–6674.
Mulvey, R. S., and Fersht, A. R. (1977). *Biochem. Soc. Trans.* **5,** 672–675.
Murray, J. D., and Smith, D. A. (1986). *J. Theor. Biol.* **118,** 231–246.
Mutter, M. (1985). *Angew. Chem. Int. Ed. Engl.* **24,** 639–653.
Neupert, W., and Schatz, G. (1981). *Trends Biochem. Sci.* **6,** 1–4.
Ninio, J. (1975). *Biochimie* **57,** 587–595.
Nishikawa, K., and Ooi, T. (1986). *Biochim. Biophys. Acta* **871,** 45–54.
Ohno, S. (1970). "Evolution by Gene Duplication." Springer-Verlag, Berlin.
Ohno, S. (1982). *Persp. Biol. Med.* **25,** 559–572.
Ohta, S., and Schatz G. (1984). *EMBO J.* **3,** 651–657.
Orgel, G. E., and Crick, F. H. C. (1980). *Nature (London)* **284,** 604–607.
Oura, E. (1973). *Biotechnol. Bioeng. Symp.* **4,** 117–127.
Payne, W. J. (1979). *Annu. Rev. Microbiol.* **24,** 17–52.
Penefsky, H. S. (1985). *J. Biol. Chem.* **260,** 13728–13734.

Pfanner, N., and Neupert, W. (1985). *EMBO J.* **4,** 2819–2825.
Poole, R. K. (1983). *Biochim. Biophys. Acta* **726,** 205–243.
Popper, K. (1982). "Unended Quest," p. 19. Collins, Glasgow.
Por, F. D. (1980). *Persp. Biol. Med.* **23,** 389–399.
Poroykov, V. V., Esipova, N. G., and Tumanyan, V. G. (1984). *Mol. Biol.* **18,** 541–546.
Prigogine, I. (1980). "From Being to Becoming." Freeman, San Francisco.
Privalov, P. L. (1982). *Adv. Protein Chem.* **35,** 1–104.
Privalov, P. L. (1985). *Biofizika* **30,** 722–733.
Ptitsyn, B. O. (1978). *FEBS Lett.* **93,** 1–4.
Ptitsyn, B. O. (1984). *Mol. Biol.* **18,** 574–590.
Rapoport, T. A. (1985). *FEBS Lett.* **187,** 1–10.
Rapoport, S., Dubiel, W., and Muller, M. (1982). *FEBS Lett.* **147,** 93–96.
Raven, J. A., and Smith, F. A. (1981). *BioSystems* **14,** 95–111.
Ricard, J. (1978). *Biochem. J.* **175,** 779–791.
Richardson, J. S. (1981). *Adv. Protein Chem.* **34,** 167–339.
Roise, D., Horvath, S. J., Tomich, J. M., Richards, J. H., and Schatz, G. (1986). *EMBO J.* **5,** 1127–1134.
Rossmann, M. G., and Liljas, A. (1974). *J. Mol. Biol.* **85,** 177–181.
Ruusala, T., Ehrenberg, M., and Kurland, C. G. (1982). *EMBO J.* **1,** 741–745.
Savageau, M. A., and Freter, R. F. (1979). *Proc. Natl. Acad. Sci. U.S.A.* **76,** 4507–4509.
Scarborough, G. A. (1985). *Microbiol. Rev.* **49,** 214–231.
Scheraga, H. A. (1984). *Carlsberg Res. Commun.* **49,** 1–55.
Schmidt, G. W., and Mishbind, M. L. (1983). *Proc. Natl. Acad. Sci. U.S.A.* **80,** 2632–2636.
Schulz, G. E., and Schirmer, R. H. (1979). "Principles of Protein Structure." Springer-Verlag, Berlin.
Serrano, R., Kielland-Brandt, M. C., and Fink, G. R. (1986). *Nature (London)* **319,** 689–693.
Shaytan, K. B., and Rubin, A. B. (1982). *Biofizika* **27,** 386–390.
Shull, G. E., Schwartz, A., and Lingrel, J. B. (1985). *Nature (London)* **316,** 691–695.
Silhavy, T. J., Benson, S. A., and Emr, S. D. (1983). *Microbiol. Rev.* **47,** 313–344.
Smith, T. F., and Morowitz, H. J. (1982). *J. Mol. Evol.* **18,** 265–282.
Somogyi, B., Welch, G. R., and Damjanovich, S. (1984). *Biochim. Biophys. Acta* **768,** 81–112.
Spirin, A. S. (1984). *Mol. Biol.* **18,** 1445–1460.
Stouthamer, A. H. (1979). *Int. Rev. Biochem.* **21,** 1–47.
Stouthamer, A. H., and Bettenhaussen, C. (1973). *Biochim. Biophys. Acta* **301,** 53–70.
Stucki, J. W. (1980). *Eur. J. Biochem.* **109,** 269–283.
Sturtevant, J. M. (1977). *Proc. Natl. Acad. Sci. U.S.A.* **74,** 2236–2240.
Syvanen, M. (1984). *Annu. Rev. Genet.* **18,** 271–293.
Tanaka, S., and Scheraga, H. A. (1977). *Macromolecules* **10,** 305–316.
Tanford, C. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78,** 270–273.
Tanford, C. (1983). *Annu. Rev. Biochem.* **52,** 379–409.
Thauer, R. K., Jungerman, K., and Decker, K. (1977). *Bacteriol. Rev.* **41,** 100–180.
Von Heijne, G. (1986). *EMBO J.* **5,** 1335–1342.
Wagner, G. (1983). *Q. Rev. Biophys.* **16,** 1–57.
Walker, J. E., Saraste, M., Runswick, M. J. and Gay, N. J. (1982). *EMBO J.* **1,** 945–951.
Walter, P., and Blobel, G. (1981). *J. Cell Biol.* **91,** 557–561.
Warncke, J., and Slayman, C. L. (1980). *Biochim. Biophys. Acta* **591,** 224–233.
Watson, M. E. E. (1984). *Nucleic Acids Res.* **12,** 5145–5164.
Weber, G. (1975). *Adv. Protein Chem.* **29,** 1–83.
Welch, G. R., and Keleti, T. (1981). *J. Theor. Biol.* **93,** 701–735.

Westerhoff, H. V., Lolkema, J. S., Otto, R., and Helingwerf, K. J. (1982). *Biochim. Biophys. Acta* **683,** 181–220.
Westerhoff, H. V., Hellingwerf, K. J., and Van Dam, K. (1983). *Proc. Natl. Acad. Sci. U.S.A.* **80,** 305–309.
Wetlaufer, D. A. (1973). *Proc. Natl. Acad. Sci. U.S.A.* **70,** 697–701.
Wheatley, D. N. (1984). *J. Theor. Biol.* **107,** 127–149.
Wickner, W. (1979). *Annu. Rev. Biochem.* **48,** 23–45.
Wickner, W., and Lodish, H. F. (1986). *Science* **230,** 400–407.
Wilson, T. H., and Lin, E. C. C. (1980). *J. Supramol. Struct.* **13,** 421–446.
Wilson, A. C., Carlson, S. S., and White, T. J. (1977). *Annu. Rev. Biochem.* **46,** 573–639.
Wise, J. G., Latchney, L. R., Ferguson, A. M., and Senior, A. E. (1984). *Biochemistry* **23,** 1426–1432.
Wolfe, P. B., Silver, P., and Wickner, W. (1983). *J. Biol. Chem.* **257,** 7898–7902.
Wong, J. T. (1981). *Trends Biochem. Sci.* **6,** 33–36.
Yaffe, M. P., Ohta, S., and Schatz, G. (1985). *EMBO J.* **4,** 2069–2074.
Yamane, T. and Hopfield, J. J. (1977). *Proc. Natl. Acad. Sci. U.S.A.* **74,** 2246–2250.
Yoshida, Y., Hashimoto, K., Kimura, T., Sakakibara, S., and Tagawa, K. (1985). *Biochem. Biophys. Res. Commun.* **128,** 775–780.
Zimmermann, R., and Neupert, W. (1980). *Eur. J. Biochem.* **109,** 217–229.
Zipser, D., and Perrin, D. (1963). *Cold Spring Harbor Symp. Quant. Biol.* **28,** 533–537.
Zotin, A. I., and Krivolutski, D. A. (1982). *Zh. Obshtch. Biol.* **43,** 3–13.
Zwizinski, C., Schleyer, M., and Neupert, W. (1984). *J. Biol. Chem.* **259,** 7850–7856.

Index

A

ADP/ATP carrier
  cytoplasmic synthesis without presequence, 186–187, 211
  transport into mitochondria
    cytosolic cofactor and, 188, 211
    receptors
      high- and low-affinity, 191
      inhibition by trypsin, 191
    signal sequences and, 187
    translocation contact sites and, 196
ADP/O ratio, mitochondrial translation and, 241
Aedes albopictus, mitochondrial, tRNA genes, unusual structure, 310–311
Amino acid sequences
  complex II SDH, bovine and bacterial, homology, 40, 47–50
  complex IV subunits
    bovine heart, 95–98, 126, 128, 131–135
    yeast, 95–98, 124, 132–133
  mitochondrial precursor polypeptide presequences, species differences, 246–248
  tertiary structure prediction from, 343–345
Anions, complex IV kinetics and, 144–145
Antigens, mitochondrial
  maternally inherited (Mta), mouse, 262–263
    expression prevention by Rhodamine 6G, 262
    regulation by mitochondria-transmitted factor (Mtf), 262
  in primary biliary cirrhosis, human, 261–262
Antimycin
  binding to complex III, 82–84
  protein import into mitochondria and, 197
Apocytochrome *c*
  import into mitochondria
    conversion to holycytochrome *c,* 189, 191
      inhibition by deuterohemin, 189–190
    pathway, hypothetical, 190, 209
    receptors for, 190, 208–209
  synthesis on free cytoplasmic polysomes, 208
Ascaris suum, mitochondrial tRNA genes
  codon recognition pattern, 303
  numbers and isoacceptors, 304 (table)
  structure, 310–311
  translation, 306
Aspergillus nidulans, mitochondria
  discontinuous genes type I for LSU rRNA, 299
  tRNA translation, 307
ATPase, proton-pumping
  evolution, fungi, higher plants, 350–351
  stoichiometry, energy supply effect, *Neurospora crassa,* 354
CF_1CF_0-ATPase, in chloroplasts
  CF_0 sector, membrane-embedded, subunits I-V, 278–280
  CF_1 sector, soluble
    distances between sites
      fluorescent probes, 281–282
      schematic model, 283
    reconstitution from isolated subunits, 283–286
      removal from thylakoid by NaBr and EDTA, 284
    subunits
      α and β, required for catalysis, 277
      δ, catalytic unit binding to membrane, 278
      ε, reversible inhibition in dark, 278, 285
      organization, model, 284
      stoichiometry, 279
  comparison with bacterial and mitochondrial complexes, 273–274
  future directions, 290