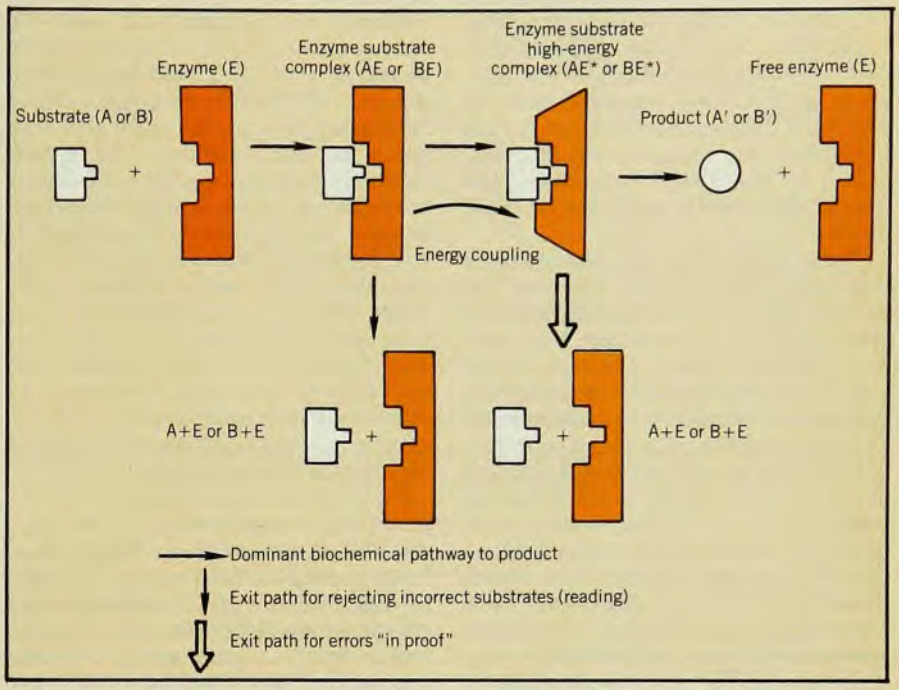
search & discovery

Proofreading by biological molecules detects "typos"

How does an enzyme know when it is making a mistake? The problem of accuracy in biosynthesis has been studied for a number of years. In DNA synthesis, for example, if too many errors occur a system cannot reproduce viable copies of itself. Too few and the system can never evolve. Solid-state theorist John Hopfield at Princeton has proposed[1] that many biological systems do "kinetic proofreading," a method of replication in which the system looks twice so that it can correct an initial mistake. Recently several groups have shown experimentally that such proofreading does appear to be taking place.

DNA is a long molecule with about 10^8 bases, which can be G,C,A or T. The instructions to replicate the molecule can be thought of as a sentence made of a four-letter alphabet in which there are 10^8 letters. Experimentally it is found that, in replicating, errors in the letters are made about 1 time in 10^8.

Proteins, like DNA, are polymers. Each polymer unit is chosen from among 20 different amino acids. The protein is about 100 amino acids long, arranged in a particular sequence. The experimentally observed error rate in putting the right amino acids in the right place is about 1 in 10^4.



A typical "kinetic proofreading" scheme for increasing fidelity, as proposed by John Hopfield. Discrimination is provided by the two exit paths, which have rates that are much faster for incorrect substrates than for correct ones. The energy coupling is essential, for without it, the reverse reaction from AE* to A+E becomes important, and the system ceases to proofread.

Hopfield became interested a couple of years ago in the question of what causes the observed level of mistakes in biosynthesis. To distinguish between two substances A and B, one can think of a chemical reaction in which the error rate will vary as exp $(-\Delta G/kT)$ where ΔG is the difference in free energy, k the Boltzmann constant and T the temperature. Because the true error rate appears to be smaller than this quantity, the possibility of proofreading seems possible. While wondering about the problem, Hopfield, who was a student of Albert

continued on page 20

Magnetic-mirror performance challenges tokamaks

Results with the Lawrence Livermore Laboratory magnetic-mirror experiments over the past year and a bit are encouraging ERDA to push much harder in that direction, so that mirrors might share some of the funds tokamaks have been attracting. The results on 2XIIB are showing how to stabilize the mirror plasma, increase its value of beta and raise $n\tau$, the product of density and confinement time, considerably. Now Livermore has proposed building a new, $94-million Mirror Experiment, MX for short. It is rumored that ERDA has requested the Office of Management and Budget to include MX in the FY 1978 budget.

The 2XIIB machine is a pulsed-field mirror experiment, in which the mirror separation is 1.5 meters. The typical central field is 5–7 kG, and the field at the mirrors is twice that; that is, the mirror ratio is 2:1.

In July 1975 the first of three significant experiments with 2XIIB was completed. Prior to that time, the initial plasma in the predecessor experiment, 2X, was created by capture in time-of-flight of a hot plasma injected by a gun. With the installation of neutral beams, which were developed over several years by a joint Livermore–Lawrence Berkeley Laboratory collaboration, the approach changed. Deuterium ions are accelerated across a three-grid structure operating at 20 kV. The ions pass through deuterium gas and undergo charge-exchange reactions. Because the beam is a mixture of atoms and molecules (and ions), the resulting beam is a mixture of 6-, 10- and 20-keV neutral atoms. The beam is focused to a 10 × 20 cm spot, three meters away. Twelve such beams are used, six on each side, stacked in a vertical array in a magnetic field running horizontally. The twelve beams have a total equivalent current capability of about 600 A. However, most of the experiments have been run at 200–300 A.

Earlier experiments had produced a stable plasma with an average ion energy of 1–2 keV and an $n\tau$ of 10^{10} cm^{-3} sec. In

crons. The competition is optically pumped molecular lasers, which are inherently less efficient, he says. —GBL

References

1. G. Bekefi, T. J. Orzechowski, Phys. Rev. Lett. **37**, 379 (1976).
2. V. Granatstein, P. Sprangle, R. K. Parker, J. Pasour, M. Herndon, S. T. Schlesinger, J. L. Seftor, Phys. Rev. **A 14**, 1194 (1976).

Proofreading

continued from page 17

Overhauser (Purdue University), had to write a letter of recommendation for the Buckley Prize describing the Overhauser effect. In this nmr effect, electron spins are coupled to nuclear spins. By applying a large microwave signal to the electrons, the nuclei acquire an induced polarization, their population ratio going from a small value to a large one. The system acts as though the energy difference between spin up and spin down is very different from the actual value.

Reasoning that chemical systems can also undergo an Overhauser effect, Hopfield searched for a system of chemical kinetics that could enhance the population ratio of an enzyme, discriminating between two molecular species A and B. If the enzyme were a Maxwell demon, it would wait to act, regardless of what substance was present. This delay would allow B to decay, thus discriminating in favor of A.

So the scheme would require the enzyme E, when reacting with A, to produce an intermediate chemical, AB*, before eventually producing the final product A′. In addition to this time delay, an alternative path in which AE* breaks up into A + E must be included.

The reaction Hopfield postulates is

$$A + E \rightleftharpoons AE \rightleftharpoons AE^* \longrightarrow A' + E$$
$$\uparrow\downarrow$$
$$A + E$$

Then one introduces an energy source (the chemical analog of the Overhauser-effect microwaves) to drive the reaction of A + E going to AE*; this allows one to make AE* a very high-energy compound; so one can make the probability very small of its formation from below.

Having arrived at AE* the system can go forward, or it can abort. The probability of aborting is determined by ΔG. The high-energy intermediate can be formed and discriminate once in a certain ΔG. Then the same ΔG can be used a second time, to decide whether the system will go forward and form A′ or go from AE* to form A + E again. Thus the same kinetic discrimination can be used twice because of the essentially irreversible energy driving source.

The commonest energy source for biological reactions is the hydrolysis of ATP. When AE goes to AE*, at the same time a molecule of ATP hydrolizes, being converted to ADP and phosphate. For all practical purposes, there is very little ADP around; so the reaction cannot go backward. The ATP acts as a driving force on the forward reaction.

What systems use kinetic proofreading? We asked Hopfield. One example is the choice of what base will be added to DNA. In DNA replication there is a chemical reaction that is isomorphic to the kinetic proofreading.

A second application is discrimination in building proteins. The protein is told what it is supposed to be making by a piece of messenger RNA, which consists of a sequence of bases like DNA has. The first three bases specify the first amino acid, the next three the second amino acid, and so on.

A third application is discrimination in using transfer RNA. A particular molecule of transfer RNA recognizes a particular three-letter code word in protein synthesis and must therefore have the corresponding amino acid bound to it, ready for insertion in a growing protein.

To observe experimentally whether or not proofreading occurs, one can look for a one-to-one relation between the amount of ATP used and the amount of A′ formed. After the ATP hydrolysis, if the correct product occurs, the system gets to AE*, and the amount of ATP hydrolized would be comparable to the amount of A′ formed. If on the other hand, an incorrect match occurs, BE* is formed. If effective proofreading is going on, BE* should chiefly be rejected. The ratio of the amount of product formed to the amount of ATP used for the correct material should be near one. For the incorrect material, the ratio should be far different than one. To the extent the ratios differ, they represent the benefit obtained from proofreading.

Because A and B are competing, one has an amount of AE and BE that greatly favors A. When material arrives at AE*, it has already read the enzyme once. Now two paths are available: One goes to the product. The other is a downward path that does not form the product—the exit path for errors in proof. Without the downward path, everything arriving at AE* would belong to A′ + E so that the net accuracy would be the accuracy with which AE* was formed compared to BE*. However, with the downward path, one can throw out most of the time that B is produced. This second discrimination is the proofreading. And the efficiency of the proofreading is found from counting how many times the reaction goes forward compared with the times it goes down.

Experiments. Recently Hopfield and Tetsuro Yamane (Bell Labs) and their collaborators studied[2] the matching of correct amino acids to correct transfer RNA. This matching is done enzymatically—the enzymes first recognize a particular amino acid out of a choice of 20. Then the enzymes must recognize the correct transfer RNA. They found that the discriminations have an error of one in 100 to 400, depending on the system they studied. That is, the system is proofreading on a scale of 1 in 100–400. Combined with an initial reading precision of 1 mistake in 100, the net error rate of 1 in 10^4 for protein synthesis is reached.

Before the Hopfield work, Arthur Kornberg (Stanford University) had found an editing function in DNA polymerase in E. coli. Nancy Nossal (National Institutes of Health) and independently Maurice Bessman (Johns Hopkins University) had studied mutants in bacteriophage, which has its own DNA polymerase that regulates how DNA is copied. These mutants can be qualitatively understood, Hopfield told us, in terms of the kind of mechanistic description that Kornberg gave, but the energetics of discrimination become clear only in terms of the kinetic proofreading scheme.

Other work that preceded Hopfield's was done by Paul Berg (Stanford University). In studying transfer RNA, Berg noted that if the wrong material is formed, in some cases it is rapidly hydrolized by the enzyme. So once wrong matches are made in the solution, they do not persist. Hopfield notes that Berg's experiments and those by Paul Schimmel (MIT), although not actually demonstrations of proofreading, did suggest what systems should be looked at.

Meanwhile, independently and at the same time that Hopfield was doing his work, Jacques Ninio (University of Paris) studied DNA and developed a mathematical formulation[3] isomorphic to the one Hopfield found.

A different example of proofreading was recently studied by Robert Thompson (Harvard Medical School), who studied the recognition of transfer RNA on a messenger ribosome complex. He found a proofreading improvement on a scale of 50–100 in this case. —GBL

References

1. J. J. Hopfield, Proc. Nat. Acad. Sci. **71**, 4135 (1974).
2. J. J. Hopfield, T. Yamane, V. Yue, S. M. Coutts, Proc. Nat. Acad. Sci. **73**, 1164 (1976).
3. J. Ninio, Biochimie **57**, 587 (1975).

in brief

The latest generation of atomic clock at the National Bureau of Standards has been used to determine the length of the second to within 0.85 parts in 10^{13}, according to a recent Bureau announcement. The NBS determination implies that the international atomic second, maintained by the International Time Bureau in Paris, is too short by about 11 parts in 10^{13}. □