

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

References

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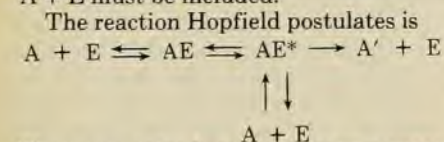
Proofreading

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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, AE*, 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.



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 hydrolyzes, 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 hydrolyzed 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 studied² 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 10⁴ 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 hydrolyzed 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 formulation³ 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

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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 10¹³, 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 10¹³. □