perovskite phase transition is expected to have an important mechanical effect on the downwelling flow. Because cold material must sink to depths greater than 660 km before it undergoes the transition, material less dense than that lying on either side is found in the region above the depressed boundary, which creates a buoyant force resisting the downward flow. This resistance may cause the sinking slab to deflect horizontally or to buckle and then pile up instead of penetrating into the lower mantle. The compositional buoyancy of the chemically differentiated rock in the slab and any viscosity increase or additional density increase due to possible differences between upper mantle and lower mantle bulk chemistry also produce resistance to the sinking slab. Thus, determination of the depth to which the subducting material sinks requires careful analysis.

Three-dimensional flow models with high Rayleigh numbers (*7*) suggest that downwelling material piles up above the 660-km boundary and then overturns catastrophically with large-scale downwellings. This unsteady behavior may be responsible for the variable character of the downwellings imaged by seismology (Figs. 1 and 2). Partly stratified convection may also help to explain long-term plate tectonic motion periodicities and geological cycles (*8*). However, the effects of strong temperature dependence of viscosity, which must be present to produce realistic slab-like behavior, are not yet fully known.

Another approach is to use global seismic velocity models in conjunction with phase-boundary undulations to develop mantle flow models. Morgan and Shearer (*9*) recently completed such an exercise. The density contrasts, caused by lateral temperature differences, drive the convective flow, whereas the viscosity and 660-km boundary deformation oppose the flow. They find little evidence for the inhibition of the vertical flow by phase boundary deformation and thus favor simple whole-mantle convection. A similar conclusion is reached by Jordan and co-workers (*10*), who argue that the seismic velocity anomalies above and below the 660-km depth are too well correlated to allow strongly stratified mantle convection.

Major questions remain unresolved. Seismologists are striving to sharpen images of deep velocity anomalies and discontinuity topography. Mineral physicists are obtaining better experimental data for phase transitions and elastic properties of mantle materials for the evaluation of differences in chemistry between the upper and lower mantle. Geodynamicists are investigating the viscosity structure, effects of phase transitions, and the role of plates in mantle convection. Multidisciplinary efforts drawing on the latest results from each of these fields are likely to be fruitful, as we try to find out how Mother Earth keeps her cool.

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Catalysis: Design Versus Selection

Steven A. Benner

There are two fundamentally different ways to create biological macromolecules that catalyze chemical reactions. The first involves rational (or at least rationalized) design of a biomolecule that folds in solution and presents an array of catalytic functional groups to a substrate. The second requires a system for selecting from a large pool of randomly generated biomolecules a few that present an effective array of catalytic functional groups to a substrate.

Each approach has its merits. Creation by design requires a detailed understanding of and control over biomolecular conformation and reactivity. Thus, examples are few (*1*, *2*) and are even fewer in cases where the microscopic rate constant of the catalytic system is at least partly understood (*3*). Nevertheless, the process of design forces the biochemist to address and solve problems fundamental to biological catalysts generally: What is the rate-limiting microscopic transformation in the reaction sequence? Where must functional groups be placed in space to catalyze the transformation? What biomolecules will fold correctly to deliver this functionality?

Selection strategies avoid these questions and may be viewed as technologically useful alternatives to true design. At present, the immune system is the best known tool for selecting catalytically active proteins from a random pool (*4*). As technology, catalytic antibodies can be viewed either as exciting (rate enhancements are typically two to six orders of magnitude) or not (rate enhancements are *only* two to six orders of magnitude). However, this and other selection technologies allow the discovery of fundamentals of catalysis that might later support rational design (*5*). Further, they raise scientific questions of their own. What can selection experiments in the laboratory reveal about the probability of a random biostructure having catalytic activity? What do they tell us about how biocatalysts arose naturally on Earth? How should the catalytic power of a selected enzyme be evaluated?

Appearing today in *Science* is a landmark paper by Bartel and Szostak (*6*) that touches on these and other questions. Starting from a pool of some 10^{15} different RNA sequences, a small number of RNA molecules were selected that covalently attached (ligated) a short RNA segment to their own 5' ends. The additional segment contained a binding site for a primer that selectively allowed these RNA molecules to be amplified by the polymerase chain reaction. The pool of amplified molecules was then subjected to random variation and repeated selection. This process yielded RNA molecules capable of effecting a ligation with a rate constant of 0.06 min^{-1}. These RNA molecules are not, of course, true catalysts; they do not remain unchanged at the end of the reaction cycle. Nevertheless, they offer insight into scientific issues relevant to selection processes that might ultimately yield true RNA catalysts, perhaps including those that created the first forms of life on Earth over 3 billion years ago.

The last point relates to the hypothesis that the first form of life on Earth was an RNA molecule catalyzing the template-directed polymerization of RNA, that is, it made copies of itself (*7*). Evidence for the existence of an "RNA world," in which a complex metabolism was sustained in life forms having RNA as the sole genetically encoded catalysis, can be found throughout contemporary biochemistry (*8*). Yet the problem remains: Is it plausible that the

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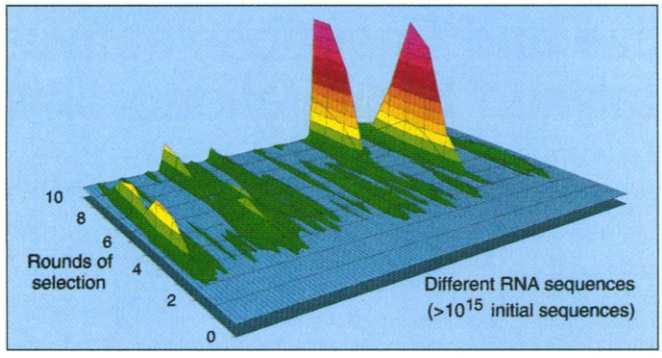
first RNA molecule arose spontaneously from a random pool of RNA molecules?

The Bartel-Szostak RNA ligases do not, of course, catalyze template-directed polymerizations. However, the ligation reaction can be viewed as a simplified polymerization reaction. By estimating how many RNA ligases were obtained from the random pool, Bartel and Szostak suggest that fewer than one in 10^{13} random RNA sequences can catalyze such ligation reactions. As polymerization is (presumably) more difficult than ligation, the number of active polymerases was estimated to be even smaller.

This estimate could suggest that a self-replicating RNA molecule could not have emerged spontaneously from a prebiotic soup, as it is difficult to believe that larger pools of random RNA emerged spontaneously without the gentle coaxing of a graduate student desiring a completed dissertation. This would imply that the RNA world model for the origin of life is wrong. This implication would be premature, however. The initial RNA pool on Earth may not have been random (*9*). The RNA world may have begun with a larger number of nucleoside building blocks (*10*), thereby increasing the catalytic versatility of primordial RNA and the fraction of molecules that displayed RNA polymerase activity. The initial substrates may not have been nucleoside triphosphates but rather substrates that are easier to polymerize. Or there might have been additional cofactors (in particular, metals other than magnesium) that enhanced the RNA's catalytic effectiveness. Efforts to reconcile the results of Bartel and Szostak with the RNA world model should lead to still more discovery. Betting odds are that a self-replicating RNA molecule will be prepared in the laboratory in the coming decade.

How good is the Bartel-Szostak RNA ligase? To answer this question, we must compare the rate of the ligation with an appropriate reference reaction. An important part of the contribution of Bartel and Szostak is their measurement of the rate of an excellent reference reaction, the ligation reaction of the identical molecules lacking the selected RNA appendage. The reference reaction is of considerable interest in its own right, in part because it yields the 3',5'-linkage found in natural oligonucleotides (*11*). But more important characteristics are that the reaction has the same first-order kinetic behavior as can be measured under the same conditions and therefore can be directly compared with the ligation reaction effected by the selected portion of their RNA molecules.



Evolution in a test tube. A pool of 10^{15} different random sequence RNA molecules, displayed along the left to right dimension, was subjected to succesive rounds of selection (front to back dimension). About 30 sequences (green) initially increase in abundance above the background level (blue). With mutation and continued selection, some species become extinct, while others come to dominate the population. [Illustration from D. P. Bartel]

When compared to this reference, the selected RNA ligase achieves a rate enhancement of seven orders of magnitude. Is this good or bad? It is three or four orders of magnitude less than the rate enhancements displayed by catalytic RNA molecules found naturally (*12*). And these are generally three to six orders of magnitude below those displayed by analogous protein catalysts. By these standards, the rate enhancement might appear poor.

But not by other standards. First, it is almost certain that the Bartel-Szostak ribozymes do not represent a maximum rate for an RNA-catalyzed ligation, but rather the maximum rate of an RNA enzyme that could be selected by the mechanics of the selection system that they used. It is conceivable that a still more clever selection system (if that were possible) would yield a still better RNA ligase.

Second, the rate enhancement achieved by the Bartel-Szostak ribozyme is better than that generally achieved by the immune system. Rate enhancements achieved by catalytic antibodies are typically two to six orders of magnitude (*4*), roughly the same as rate enhancements obtained in peptides produced by design (*1–3*). Further, selection for catalytic antibodies begins not with random sequences, but with sequences that fold. Catalytic antibodies also have access to the full range of catalytic groups of the 20 natural amino acids, instead of only the four nucleoside-building units of RNA. By these standards, the catalytic power of the Bartel-Szostak ligase is remarkable.

The work therefore allows comparison of two different selection-based approaches for obtaining catalytic biomolecules and underscores two limitations inherent in the catalytic antibody strategy: Good transition state analogs are often difficult to obtain, and the antibody is selected for binding to a transition state analog, rather than directly for its catalytic power. However, both catalytic antibodies (*4*) and designed peptide catalysts (*1–3*) solve a problem not solved by the Bartel-Szostak ligase: the release of product. As Bartel and Szostak point out, this is often a difficult problem in designing a true catalyst.

It is interesting to note that the rate enhancements achieved in the first round of selection of ligating RNA molecules (10^4) are similar to those obtained both with typical catalytic antibodies (*4*) and by design (*1–3*). This may not be a coincidence. Even simple reactions (such as the ligation) often involve multiple steps, one of which is rate-limiting at the outset. Increasing the rate of the rate-limiting step can improve overall efficiency, but only to the point where this step no longer remains rate-limiting. Successive rounds of variation and selection may be necessary to obtain high overall rates for multistep reaction sequences, with each round lowering a different kinetic barrier. Thus, as the detailed kinetics of the reaction catalyzed by the Bartel-Szostak ligase is worked out [as is beginning to be the case with catalytic antibodies (*13*)], this selection experiment will also contribute much to an understanding of catalysis needed for design.

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