



**STRUCTURAL BIOLOGY: A Glimpse of Biology's
First Enzyme**

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even greater consequence than for HIV.

What does this mean for HIV or HCV vaccine design? It might be possible to design vaccines that anticipate the frequently selected escape mutations by using more than one viral amino acid sequence in the vaccine (18), though this would require very complex vaccines. An alternative would be to use vaccines to redirect the immune response to the most conserved regions of the virus, which may be invariant because of functional or structural constraints. However, the extraordinary power of viruses

like HIV and HCV to escape almost any means of host attack remains a daunting hurdle to overcome. The phylogenetic trees may have eliminated some T cell footprints, but they reveal the enormous complexity of the viral epidemic both within individuals and within populations.

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STRUCTURAL BIOLOGY

A Glimpse of Biology's First Enzyme

Gerald F. Joyce

Biology uses DNA genomes and protein enzymes to carry out the operations of the cell. RNA plays many important roles, but it is subservient to DNA and proteins. In the distant evolutionary past, though, things may have been very different. Almost 40 years ago, it was first suggested that both genomic information and enzymatic function once resided exclusively in RNA (1–3). Crick speculated that the first enzyme may have been an RNA molecule that catalyzed the replication of other RNA molecules (2). After catalytic RNA was discovered, Gilbert coined the term “RNA world”, which he similarly envisioned as being based on RNA molecules that catalyze the synthesis of copies of themselves (4).

We now have the first glimpse of what such an RNA replicase might have looked like. On page 1549 of this issue, Robertson and Scott report the crystal structure of an RNA enzyme obtained by test-tube evolution that catalyzes the joining of two RNA molecules that are bound at adjacent positions along an RNA template (5).

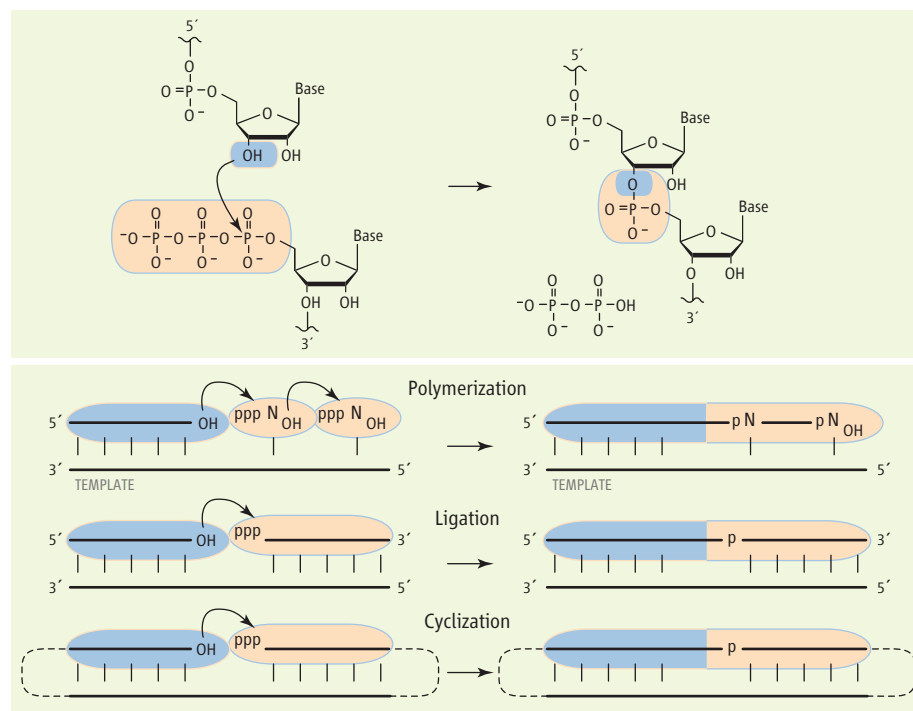
Protein enzymes that catalyze the copying of RNA use as building blocks the four nucleoside 5'-triphosphates (NTPs). These are bound to an RNA template and joined through the reaction between the 3'-hydroxyl group of one NTP and the 5'-triphosphate of the next, forming a 3',5'-phosphodiester linkage (see the figure, top panel). It is not clear whether the hypothetical replicase enzyme of the RNA world operated by the

same mechanism, but it is a reasonable bet. The template provides a favorable orientation for the reaction, and the 5'-triphosphate offers a desirable combination of being very stable in water yet thermodynamically highly activated for the reaction. All that is needed is the right catalyst to facilitate the reaction.

No known RNA enzyme in biology cat-

The structure of an RNA enzyme that catalyzes the joining of RNA has been solved, providing insight into what may have been the most ancient enzyme of biology.

alyzes the polymerase-like joining of RNA. However, the powerful methods of in vitro evolution have made it possible to generate such enzymes from scratch, starting from a large population of RNAs with random sequences (6). The usual approach is first to evolve an RNA enzyme that is an RNA ligase, which can join two oligonucleotides in a



RNA-templated joining of RNA. (Top) The 3'-hydroxyl of one nucleotide (blue) attacks the 5'-triphosphate of another (orange) to form a 3',5'-phosphodiester. This reaction is the basis for RNA polymerase proteins and for in vitro evolved RNA enzymes with RNA ligase or RNA polymerase activity. **(Bottom)** Somewhat different reaction formats are used for the polymerization of NTPs, the joining of RNAs by a ligase, and the formation of a circular RNA as carried out by Robertson and Scott (5). pppN_{OH} indicates an NTP, OH the attacking 3'-hydroxyl, ppp the reactive 5'-triphosphate, and pN the site of the newly-formed phosphodiester.

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template-directed manner. Then, through further evolution, the researcher attempts to coax the ligase to accept NTPs as substrates and to add multiple NTPs in succession.

Bartel and colleagues (7) have used one such in vitro evolved ligase, the class I ligase, and evolved it further to polymerize as many as 14 successive NTPs with high fidelity. Despite valiant efforts, however, it appears unlikely that this particular polymerase enzyme will ever be evolved to the point that it can copy RNA molecules as long as itself (~200 nucleotides). Nonetheless, it is likely that scientists will eventually apply a similar approach to a different set of RNA molecules to achieve more extensive polymerization and ultimately complete replication.

The class I ligase is the Ferrari of in vitro evolved ligase enzymes. Under optimal reaction conditions, it has a catalytic rate of up to 360 per minute. Like a Ferrari, however, it has very narrow tolerances and has been tweaked in imaginative ways to extract maximum performance. Three other RNA enzymes, the L1 ligase (8), R3C ligase (9), and DSL ligase (10), have substantial structural and biochemical similarity and may be regarded as three different versions of the family sedan. All catalyze the template-directed joining of an oligonucleotide 3'-hydroxyl and oligonucleotide 5'-triphosphate, but at a rate of only about 0.3 per minute. All have a simple three-helix junction architecture, in which the nucleotides that are essential for catalysis surround the junction and the site of ligation is offset from the junction by several base pairs. It is the L1 ligase that has been crystallized by Robertson and Scott, who

solved its structure at a resolution of 2.6 Å (5).

Robertson and Scott crystallized the product of an autoligation reaction, in which the L1 ligase was configured to join its own 3'-hydroxyl to its own 5'-triphosphate (see the figure). Two different forms of the circular product were present in the asymmetric unit of the crystal: one in an "undocked" conformation, with the three-helix junction splayed out and no contact between the catalytically essential nucleotides and the ligation site, and the other in a "docked" conformation, with many of these essential nucleotides held near the ligation site. The docked conformation (which can reasonably be interpreted as the active form of the enzyme) is stabilized by tertiary contacts involving a guanine-adenine-uracil base triple and by ionic interactions between a single Mg²⁺ ion and three phosphate groups.

The crystal structure reflects the product of ligation rather than the reactants. One must therefore be cautious in drawing conclusions about the reaction mechanism. Rather than a free 3'-hydroxyl and a reactive 5'-triphosphate bearing four negative charges, the 3',5'-phosphodiester is already in place. Nonetheless, some inferences regarding the reaction mechanism may be drawn from the structure, which reveals a network of hydrogen bonding and ionic interactions centered about the ribose sugar that bears the attacking 3'-hydroxyl. The adjacent 2'-hydroxyl appears to be kept out of the fray by its interaction with a tightly bound water molecule. If this were not the case, the reaction might instead result in formation of an unnatural 2',5'-phosphodiester,

rather than the 3',5'-phosphodiester of RNA.

The L1 ligase is not a polymerase, let alone a replicase, but it performs the same chemistry that would be expected for an RNA molecule with RNA replicase properties. Its crystal structure gives us a view toward what may have been the first enzyme of biology, or at least the central enzyme of the RNA world. In the years ahead, we can expect to see the structure of other ligases, and eventually of polymerase and replicase RNA enzymes. These laboratory mimics of our deepest evolutionary ancestors will not appear to be alien objects. Like the L1 ligase, they will have comfortably familiar structural features and an active site built of the usual stuff of biochemistry: hydrogen bonding, ionic, and hydrophobic interactions that have been crafted by processes of Darwinian evolution. Unlike the ancient RNA replicase enzymes that likely became extinct more than 3.5 billion years ago, these modern recreations will be available for detailed investigation.

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CLIMATE CHANGE

Rethinking Ice Sheet Time Scales

Martin Truffer and Mark Fahnestock

According to glaciology textbooks, glaciers respond to climate change on time scales that vary from a decade or more for nonpolar glaciers to millennia for polar ice sheets. These numbers have lured the scientific community into thinking that while small glaciers undergo rapid changes, the big ice sheets adjust at a leisurely pace.

Lately, the ice sheets have been teaching us differently. Recent reports documented rap-

idly increasing discharge of Greenland's outlet glaciers (1–3). These glaciers are responsible for most of the ice sheet's mass loss, acting as "bathtub drains" to the vast interior ice mass (see the figure). On page 1559 of this issue, Howat *et al.* (4) report that ice discharge can also decrease at a high rate: Two of the major outlet glaciers in southeastern Greenland—Helheim and Kangerdlugssuaq—doubled their discharge of ice into the ocean within 1 year in 2004. Two years later, the discharge quickly dropped back close to its former rate.

Near the other pole, Fricker *et al.* [page 1544 (5)] report changes in ice surface eleva-

Satellite data show that ice sheets can change much faster than commonly appreciated, with potentially worrying implications for their stability.

tion from data recorded by NASA's Ice, Cloud, and land Elevation Satellite (ICESat). These observations are interpreted as a sign of moving subglacial water under a large ice stream. At one ice stream location, the surface drop can be explained by the drainage of 2 km³ of subglacial water. Elsewhere, the ice surface rose sufficiently to account for the storage of this water. Earlier studies had shown the existence of such elevation changes (6, 7), but Fricker *et al.*'s analysis reveals a surprisingly active system of subglacial hydrology in a part of the world where little or no surface melting occurs.

Today, we can monitor ice sheets with unprecedented spatial and temporal resolu-

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