PERSPECTIVES: RNA STRUCTURE

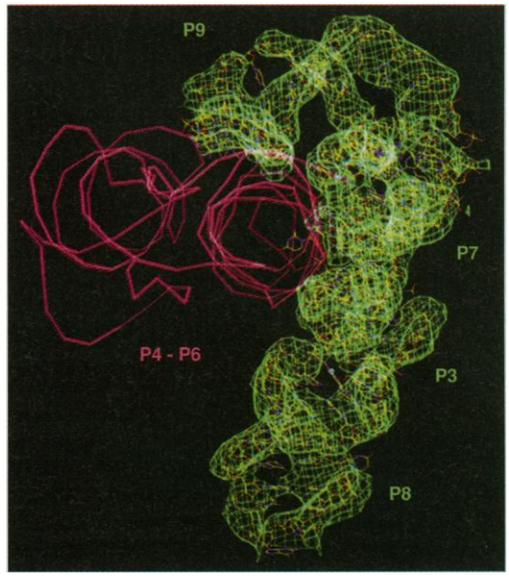
Ribozyme Architectural Diversity Made Visible

Eric Westhof and François Michel

Few RNA molecules with catalytic activity—also called ribozymes—are known in the contemporary biological world (*1*). Yet these natural ribozymes are considerably diverse in size and sequence, and they differ as well in the detailed molecular mechanisms they use for catalysis. Such variety has long been suspected to correspond to a diversity of shapes, but unfortunately ribozyme structures have been rather slow to come along. It took 12 years after the discovery of RNA catalytic activity for the structure of the smallest member of the family, the hammerhead ribozyme, to be solved at atomic resolution (*2*), and two more years for the 160-nucleotide P4-P6 subdomain of the *Tetrahymena* group I intron ribozyme to be unveiled (*3*).

Two new structures now broaden our view of ribozyme structural diversity. The structure of a functional 247-nucleotide ribozyme derived from the *Tetrahymena* intron, on page 259 of this issue, has been determined at 5 Å resolution (*4*). And a letter in this week's *Nature* reports the structure of the 72-nucleotide hepatitis delta virus (HDV) ribozyme at 2.3 Å resolution (*5*). Both molecules already had claims to fame. The HDV RNA is the only ribozyme to be part of a human pathogen, and the *Tetrahymena* intron—the first RNA ever shown to display catalytic activity—is a cult molecule and a favorite testing ground for technological innovations in the RNA field. The new structures illustrate the diversity of strategies used by nature to build stable RNA scaffolds, the rapid progress of RNA crystallography, and the power and hazards of RNA modeling.

Because of their uniformly charged backbone, RNA molecules constitute a challenge to crystallographers. How can such electrostatic monsters be packed into the regular arrays of a well-diffracting crystal? We now know that the same strong base-stacking and hydrogen-bonding interactions that allow a ribozyme to overcome electrostatic repulsion and fold back into a compact three-dimensional shape can also ensure crystal packing. This was serendipitously demonstrated by the hammerhead ribozyme crystals, which are held together by the intermolecular interaction of a GAAA "tetraloop" with an RNA receptor (*6*). Similar tetraloop-receptor interactions had previously been shown to contribute to the self-assembly of group I introns (*7*) and are now being deliberately engineered into RNA molecules to ensure crystallization (*8*). Another option for ensuring orderly packing, as illustrated by the *Tetrahymena* ribozyme crystal, is intermolecular Watson-Crick pairing. However, the strategy used to obtain HDV crystals has been an entirely different one: the introduction of a protein player into the game. The HDV RNA was reengineered to include a small hairpin structure that was known to form a tight complex—the structure of which had been determined at atomic resolution (*9*)—with a protein named U1A. The advantages of RNA-protein cocrystallization are twofold. First, the diversity of functional groups at the surface of proteins can sustain a greater variety of crystal contacts than would be the case with RNA alone. Second, the presence of a protein makes it possible to use selenium substitution to obtain the necessary crystal phases (*5*).



The group I intron ribozyme crystal structure. A view down the helical axis of domain P4-P6 (in red). Helical domains P9, P7, and P3 wrap around the P4-P6 subdomain. The green net represents the electron density map [calculated at 5 Å with $(2F_{obs} - F_{calc})$ Fourier coefficients and contoured at 1σ]. The RNA atoms of the fitted model are in yellow.

Both the group I intron and HDV ribozyme structures reveal a compact core formed by side-by-side associations of coaxial helical domains. In both structures, a "pseudoknot"—a short double-stranded helix that joins distinct loops of the planar, treelike secondary structure—is central to the architecture. The HDV ribozyme pseudoknot is in fact a convoluted one, with two separate helical segments and short interconnections, which probably explains the unusual thermal stability of this molecule. In contrast, the larger group I intron (including its previously crystallized P4-P6 subdomain) relies more on a variety of intricate RNA-RNA anchors, such as extensive triple-helical scaffolding and tetraloop-receptor contacts, as well as helical distortions, partly promoted by non–Watson-Crick pairings, to ensure close backbone-backbone contact.

Both ribozymes were crystallized without their RNA substrate, so that their structures do not provide clues to the chemical processes involved in catalysis beyond what was already known. Nevertheless, the structures, which agree with a large body of independent data, appear largely preorganized for substrate recognition and, in the case of the HDV ribozyme, the resolution is sufficient for inferences about the active site to be drawn. Such preorganization stands in sharp contrast to the adaptive binding observed in complexes between in vitro selected RNA "aptamers" and their ligands (*10*). Nevertheless, whether local rearrangements occur upon substrate recognition remains an open question, especially in the J8/7 single-stranded segment, one recognition element of the helical substrate in the *Tetrahymena* ribozyme.

Because models had been published for both the group I intron core (*7*) and HDV ribozyme (*11*), and their coordinates distributed and used widely, the merits and future of RNA modeling can be evaluated. Modeling of macromolecular structures is a paradoxical activity with elements of both the Prometheus and Sisyphus myths. Models are seen by many as rash attempts to look into the unknown; they can, nevertheless, be useful in organizing and integrating disparate data and, in doing so, they stimulate the design of

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experiments capable of disproving them. However, whatever their merits, all models are ephemeral, state-of-the-art creations destined to be ultimately replaced by those models of another sort that we call structures, because they were obtained by crystallography or nuclear magnetic resonance and usually can claim a much greater accuracy. Although modeling of RNA is most efficient and rigorous when performed at the atomic level, where full use can be made of stereochemical constraints, the accuracy of the final product is still inevitably much lower than the apparent resolution.

The architecture of the model for the group I intron core was derived on the basis of sequence comparisons, with limited experimental data, whereas the HDV ribozyme model rested more on chemical probing and mutagenesis experiments. As was already known for secondary structure prediction, the superiority of comparative sequence analysis, which looks for coordinated events in sequence evolution to infer spatial relationships, is again clearly established for three-dimensional modeling. However, the recent development of "chemogenetic" methods (*12*), which allow the binding partners of individual RNA chemical groups to be readily identified in a single experiment, could soon tip the balance in favor of hard-core biochemistry—that is, unless technical advances in RNA crystallography should make all other structural approaches accessory.

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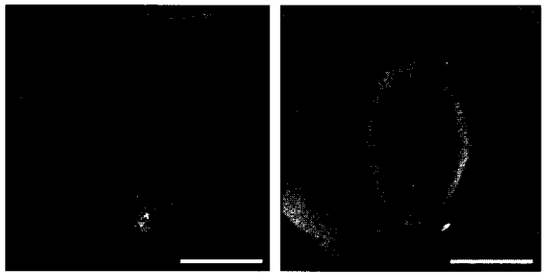
PERSPECTIVES: BOTANY

A Plant's Dilemma

Erwin Grill and Hubert Ziegler

According to a recent United Nations resolution, water will become an increasingly scarce resource for humankind in the next century. Plants have faced this same problem ever since they conquered land some 450 million years ago. To protect themselves from excess water loss they have adopted several strategies, including a waxy cuticula that coats the plant, and closable stomata, specialized cells within the epidermis that form pores for gas exchange (*1*). Now a report by Pei *et al.* (*2*) on page 287 of this issue points to a way in which plants can be assisted in conserving water, by harnessing the molecular mechanism that closes the stomata.

The stomatal aperture is controlled by osmotic adjustment in the surrounding cells. In a sophisticated regulatory mechanism, light, the carbon dioxide required for photosynthesis, and the water status of the plant are integrated to regulate stomatal aperture for optimization of the plant's growth and performance. In most plants, the stomatal pore is formed by two parallel, longitudinal guard cells whose flanking sides are physically linked just at the ends of the cells (see the figure at right). Opening of the stomata is brought about through swelling of the guard cells by solute and water uptake, which are then stored in the vacuole. Solute uptake—primarily ions such as potassium and chloride—from the apoplast of the 15-carbon group is driven by proton pumping and by the generation of osmolytes such as malate and sucrose within the cell. Closure of the stomata is mediated by solute efflux and is triggered by the plant hormone abscisic acid (ABA). Environmental cues such as drought, heat, and cold stress trigger the ABA-induced stomatal response, which is then executed by the orchestration of several ion channels located at the plasmalemma and the tonoplast of the guard cells (*3*). Several components of this signaling pathway have been identified, including cADP ribose (cADPR), Ca^{2+}, pH, two homologous type 2C protein phosphatases (PP2C) ABI1 and ABI2, as well as several ion channels (*3*, *4*).



Stomata, open and closed. Scanning electron micrographs of stomata from hydrated leaves of *Arabidopsis thaliana*, fully open (left) and largely closed (right). Bar, 10 μm.

Pei and his colleagues add another facet to ABA signal transduction by demonstrating control of stomatal aperture by farnesylation (*2*), enzymatic addition of the 1-carbon group farnesyl to a protein. In mammalian cells, farnesylation of the small G protein (GTP-binding protein) Ras is required for activation of the mitogenic response, which Ras accomplishes by recruiting the protein Raf to the membrane (*5*). In plants, the role of farnesylations is less well defined, but it has been linked to the control of cell division and ABA signaling (*6*, *7*).

Pei *et al.* now show that a farnesyltransferase negatively regulates the S-type (slow) anion channel activity in guard cells of *Arabidopsis*. When this channel—located in the plasmalemma—opens, the concomitant loss of cytoplasmic anions leads to a sustained decline in the membrane potential that subsequently activates outward-rectifying K^+ channels, a necessary step in stomata closure (*8*).

The analysis of a mutant strain of *Arabidopsis* defective in its ABA response paved the way for this achievement. The mutant *era1-2* is hypersensitive to inhibition of seed germination by ABA, owing to the deletion of a gene that encodes the essential β subunit and may signal through ERA1 by inhibiting the farnesyltransferase (*7*). Guard cells from *era1* mutants are hypersensitive to ABA in the stomatal response as well. The conductance of the S-type anion channel is enhanced in the presence of ABA in guard cells of the mutant or after farnesyltransferase inhibition (*2*). Genetic analyses with double mutants placed the action of the farnesyltransferase downstream of or parallel to the PP2C phosphatases ABI1 and ABI2.

A picture of ABA signal transduction in guard cells is beginning to emerge (see the figure on next page). Although it is not exactly clear where and how ABA is per-

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