scaled down to nearer 670 million years[5], bringing the figures more into line with the fossil record. A 'late arrival' model would also imply that the evolution of the main animal groups took place both late in Earth's history, and rapidly — perhaps in response to the evolution of Hox genes[6] or to the lifting of some external constraint such as insufficient atmospheric oxygen[2].

So should these supposedly ancient markings be accepted as new evidence for the 'deep time' model? When a respected researcher such as Seilacher, who has a good track record in the field of trace fossils, makes such a claim as this, it has to be taken seriously. But extraordinary claims, of course, require extraordinary evidence.

The first claim[1] is that these are the traces of 'triploblastic' animals. This means that the trace-makers had a gut and a fluid-filled coelom. Such an organization is found only in complex animals from worms to chordates. Seilacher and co-authors' argument is that, at 5 mm, the burrow diameters are too large to have been made by single-celled protists; but unicellular protists, including early Cambrian forms, can be that large[7] and can make burrows. Moreover, at 5 mm diameter, how could such organisms have remained hidden from the fossil record for over 500 million years? Finally, how come the tunnels are branched? Branching implies a behavioural sophistication which is thought to have appeared about 560 million years ago[8].

These seeming paradoxes may yet be explained by a fresh look at the age of the lower Vindhyan rocks (Fig. 1). Until now, they have broadly been taken to be 1,100 million years old on the basis of potassium–argon and fission-track dates. But these dates were mostly obtained in the 1960s, and no further particulars are given by the authors[1]. Unfortunately, the data they cite must now be regarded as a very doubtful guide to the age of the Vindhyan rocks[9,10], for which modern studies of geochronology are urgently required.

In fact, we may be in for a big surprise regarding the age of the Vindhyan supergroup, including, of course, the Chorhat sandstone. Acid digestion of the immediately overlying Rohtasgar limestones, undertaken in India by R. J. Azmi, has yielded a rich, well-preserved and typical early Cambrian skeletal fauna, including certain types of brachiopod[10]. This astounding discovery means that supposed trace-fossil markings from the Chorhat sandstone may be little more than 540 million years old, close to the beginning of the Cambrian 'explosion'. Branched burrow systems up to 5 mm wide are certainly well known from the Ediacaran and Cambrian[8]. Unless there is a major break between the Chorhat sandstone and the Rohtasgar limestone, this new interpretation[10] would mean that there is still no reliable fossil evidence to support the 'deep time' view of the emergence of animals. □

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RNA structure

Ribozyme crevices and catalysis

Daniel Herschlag

Since the X-ray crystallographic structure of the hammerhead ribozyme — a catalytic RNA molecule — was solved[1] in 1994, the RNA community has been waiting for a view of a second RNA enzyme. We are now rewarded for our patience by not one but two ribozyme structures: a 2.3-Å structure of the ribozyme from the hepatitis delta helper virus (HDV), reported by Ferré-D'Amaré *et al.*[2] on page 567 of this issue; and a 5-Å structure of the group I intron from *Tetrahymena thermophila*, published by Golden *et al.*[3] in this week's *Science*. The most striking feature of these RNAs is that they look like enzymes — that is, both have active sites that seem to be preorganized and located in crevices, as we are accustomed to seeing in protein enzymes. This is in marked contrast to the hammerhead ribozyme, which exposes the cleavage site in solution, rather than sequestering it in a cavity, and seems to require a substantial conformational rearrangement to perform its catalytic function[1,4,5].

Despite their general similarities, the HDV and *Tetrahymena* ribozymes are preorganized very differently. The HDV ribozyme is only about one-fifth the size of the *Tetrahymena* ribozyme, owing to a remarkable intertwining of secondary structural elements in what can be referred to as a 'double pseudoknot'. The positioning of secondary structure (that is, base-pairing) — which accounts for three-quarters of the HDV residues — within the double pseudoknot establishes the overall position of the helices with respect to each other. This allows a complex, three-dimensional architecture. Several tertiary connections then establish the precise tertiary fold, positioning the functional groups that are required for catalysis, despite the ribozyme's modest size.

In contrast, group I ribozymes are constructed from domains, some of which constitute independent folding units. The structure of one of these domains, the P4–P6 domain, has already been solved to high resolution[6], and this domain is largely unperturbed in Golden and colleagues' structure. The second domain, P3–P9, was not present in the previous structure, but it can now be seen to wrap around the P4–P6 domain. This observation provides a structural explanation for kinetic-folding results[7,8], which show that the P4–P6 domain forms earlier than the P3–P9 domain.

This structure, along with the many functional data for the *Tetrahymena* ribozyme, shows how the coming together of these domains is critical for forming the substrate binding sites. The binding site for one substrate, guanosine, was previously localized biochemically[9] to the P7 helix, which is found within the P3–P9 domain. The structure reveals that the P7 helix is distorted when P3–P9 interacts with the P4–P6 interface, presumably allowing P7 to bind guanosine. Distortion of the P7 helix explains an observation from my own laboratory (M. Engelhardt and D. H., unpublished) that removal of the P5abc subdomain of P4–P6 weakens guanosine binding, even though P5abc and P7 are not in direct contact. The intersection of the P4–P6 and P3–P9 domains also creates a shallow crevice. This is lined with residues that have been implicated in binding of the substrate domain P1–P2, which is absent from the molecule crystallized by Golden *et al.*[3]. Further experiments will reveal whether the 5-Å limit to resolution comes from limits that are specific to this crystal, or whether it is due to inherent flexibility in the absence of the P1–P2 substrate domain.

The two new structures provide an opportunity to evaluate what works in structural prediction, what doesn't work, and why. Because RNA secondary structure is determined locally, most secondary-structure elements can be identified through evolutionary relationships and mutagenic tests involving compensatory changes of putative pairing partners. A remarkably accurate three-dimensional model of the group I intron was derived from such analysis[10,11]. Similarly, secondary-structure prediction turns out to have been very good for

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HDV. In addition, the global placement of helices was correctly predicted from the constraints on the secondary structure, and groups identified as critical in functional assays are now seen to make central structural connections or inhabit the active-site crevice[12]. However, helix P1.1, which was revealed in Ferré-D'Amaré and colleagues' structure, was not identified previously. Moreover, a three-dimensional structure obtained by modelling does not fully reflect the observed tertiary structure.

One practical approach towards understanding the value and limits of a model would be to adopt, in three dimensions, what RNA modellers are already doing in two — deriving a family of possible low-energy structures. Regions of similarity in the structures would suggest interactions with strong constraints, whereas regions with substantial differences might highlight important features that require additional constraints. Biochemical tests might then distinguish between the possibilities.

Moderate- and high-resolution structures allow us to identify motifs and the overall architecture of an RNA molecule, and can also provide a powerful framework for interpreting biochemical results. In addition, the atomic-level pictures from high-resolution structures, such as that of the HDV ribozyme and the P4–P6 domain of the *Tetrahymena* ribozyme[2,6], mean that we can use biochemical experiments to probe the energetics of individual interactions and address specific questions about structure and folding.

But what about catalytic mechanism? What are the interactions of the ribozyme with the transition state, the highest-energy structure along the reaction pathway that must be stabilized by a catalyst? The active sites for protein enzymes are typically found in crevices. This allows the protein to position functional groups precisely, through networks of interactions, and to interact with substrates. Although different in molecular detail, RNA enzymes seem to follow the same strategies[13]. The preorganized functional groups within the crevice can provide catalysis by positioning substrates and catalytic groups with respect to one another, and by electrostatic interactions that are strengthened in the reaction's transition state. These groups may also facilitate bond rearrangements, acting, for example, as general acid and base catalysts.

The functional groups that line the HDV and *Tetrahymena* ribozyme crevices are implicated as candidates for catalysis. But the nature of their transition-state interactions and catalytic roles is not clear, even for HDV where the structural certainty is greatest. In the HDV-catalysed reaction, do metal ions interact with the nucleophilic 2′-oxygen atom, with the departing 5′-oxygen atom or with the non-bridging oxygens of the transferred phosphoryl group? Are there hydrogen-bonding interactions with ribozyme functional groups or associated water molecules that position the reacting groups and stabilize charge redistribution in the transition state? In this regard, even for protein ribonucleases, where many structures to resolution beyond 2 Å are known (including structures with bound transition-state analogues), we still do not completely understand the catalytic mechanisms and contributions. Indeed, there is still discussion about the pathway of proton transfers within these reactions. In this light, it would be far too much to expect a single atomic-resolution structure to solve a mechanism. Nevertheless, the HDV structure narrows the list of suspects for direct catalytic interactions, and provides unrivalled power to suggest potential mechanisms. Future structural studies that locate the reactive phosphoryl group and the metal ions required for efficient catalysis, and probe the extent to which functional groups are rearranged within the active-site crevice, will help to direct mechanistic proposals and tests of these proposals.

The mysteries of function are brought into much sharper focus through the atomic detail that structure provides. Mechanistic investigations and structural studies will accompany one another well into the next millennium, on the road to unravelling these mysteries. □

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Lunar exploration

Water amongst the rock

Timothy D. Swindle

The Moon isn't wet, but there is at least some water there. The latest and best evidence for water ice at the poles of the Moon comes from Feldman *et al.*[1] in a paper in *Science*. These findings may or may not make lunar bases more feasible, but they do represent a success for a new type of exploration for the US space agency NASA.

Although early astronomers called the vast, dark, circular patches of the Moon 'maria', thinking that they might be seas, water is scarce on the Moon. The amount of water in the samples returned by the Apollo astronauts is as low as 1 part per million, and most of that is probably contamination introduced during collection and processing[2]. Some water should be brought to the Moon in asteroids and comets, and some should be created near the surface by the reduction of iron-bearing minerals by hydrogen in the solar wind. But these processes would put the water near to the lunar surface. With daytime temperatures approaching 400 K in the regions spacecraft have visited, that water has little chance of staying there.

There are, however, areas of the Moon that are permanently shadowed from sunlight. These regions of perpetual darkness, primarily the interiors of impact craters at very high latitudes, can stay very cold (<120 K), so water might be retained there[3,4]. Such regions should also act as cold traps, and collect water that is released from other places on the Moon. The idea that the lunar poles might harbour ice gained more attention with radar studies in the early 1990s that presented evidence for the presence of ice in polar regions of Mercury[5–7], the closest planet to the Sun, which experiences even higher equatorial daytime temperatures than the Moon. But the permanently shadowed regions of the Moon have never been visited by spacecraft, and are difficult to observe from Earth because the same crater walls that block the sunlight often block the view from Earth.

Less than two years ago, the Clementine mission made headlines when data it had collected showed indications of ice at the lunar poles[8]. As in the case of Mercury, the evidence came from radar studies. Instead of looking at the radar return from a location, the Clementine studies consisted of analysing the scattering of the spacecraft's radio signal to Earth when it was bounced off the region of interest. Although they were exciting, the Clementine results were controversial, and later ground-based radar studies failed to confirm the presence of ice[9].

Into the breach came the Lunar Prospector. It was built for only $63 million, one to two orders of magnitude less than the planetary exploration spacecraft of the 1970s and

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