tors than the C-terminal half-molecule, especially in the region of the active site, suggesting much greater flexibility.

So structural differences between the two halves of the molecule can be identified in great detail, and in some cases these can be clearly ascribed to specific sequence differences. But we are still very hazy about the molecule's biological role and hence cannot say what the two half-molecules contribute to this role, or rationalize the differences between them. The protein was first claimed to be glutaredoxin-like on sequence grounds; this would imply a function as a reductant. In the present work, the protein is claimed to be most similar to PDI since it contains two trx/grx-fold regions in the same polypeptide, but there is no functional evidence that it isomerizes protein disulfides or acts in protein folding. Is the protein an oxidant, a reductant or an isomerase? Does it act on a wide range of substrates or have very limited specificity? Do the active sites have quite distinct independent roles, or do they function in tandem? Do their structural differences lead to very different redox and catalytic properties, and if so what are those different properties? To date only very limited functional characterization has been carried out along these lines. The underlying problem is the paucity of discriminating enzyme assays and the difficulty of adapting them to an enzyme which probably functions optimally at above 50 °C.

The inadequacy of our current knowledge is all the more frustrating since it limits what can be said about evolutionary relationships within this significant superfamily. Thioredoxins and glutaredoxins are clearly closely related across both prokaryotes and eukaryotes, whereas DsbA is only known in prokaryotes. Protein disulfide-isomerases, with multiple trx/grx domains within a single polypeptide, are currently only known in eukaryotes and it is likely that the first step in their molecular evolution was the duplication of an ancestral trx/grx domain. It is tempting therefore to see the archaeal protein discussed here as a precursor of the eukaryotic PDIs but, as yet, there is no functional data to support this.

This brings us to a position that is remarkable and surprising to any biochemist who remembers back more than about 15 years: we know almost everything we could wish to know about the protein's structure without yet being able to interpret this high-resolution data in a biologically meaningful way. It appears that enzymologists and cell physiologists will not be put out of business for some time to come.

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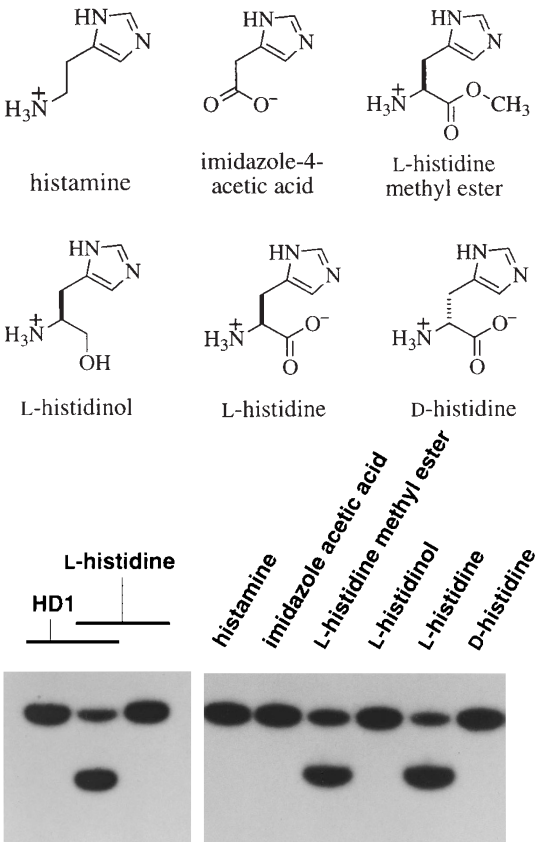
picture story

Clues to a patient revolution

Over the past few years, many groups have attempted to recreate the process of evolution in the test tube — by selecting DNA binding proteins, optimal DNA binding sites, RNA molecules that contort to bind a variety of ligands and so forth. A recent paper (Roth, A. & Breaker, R. *Proc. Natl. Acad. Sci. USA* **95**, 6027–6031, 1998) demonstrates that our appetite for *in vitro* evolution — and for understanding the origins of life — is not waning and reaffirms that nucleic acids can be much more than just storehouses of information.

Roth and Breaker devised a selection strategy to determine whether a DNA molecule could use an amino acid as a cofactor for cleaving an RNA phosphodiester bond. They chose to try histidine as a cofactor, since it is one of the most common residues in the active sites of protein enzymes. Starting with a random piece of DNA (40 nucleotides long) that had been embedded between two sets of fixed DNA pairing elements, they hoped to give some initial structure to a potential histidine binding site. They succeeded: a set of selected DNAs depend on histidine — and histidine very specifically — to perform the reaction. Under optimal conditions, these deoxyribozymes mediate catalytic rate enhancements of ~10^6-fold, on par with the activities of natural self-cleaving ribozymes. Moreover, the pH dependence of the reaction suggests that histidine plays a catalytic role. The pictures to the right show examples of cleavage reactions by HD1, a DNA molecule that was designed based on the results of the selection experiments. In the gel images, the upper band is the 5'-labeled substrate and the lower band is the cleaved product. A different histidine analog (depicted above the gels) was used in each reaction. These and more quantitative results demonstrate that HD1 discriminates effectively against many histidine-related compounds, using only specific histidine analogs as cofactors.



What does this tell us about the origin of life? To speculate wildly, perhaps nucleic acids used such adaptations to coerce protein components into performing chemical reactions. If so, little did they know that their clever cofactors would band together and gradually stage a revolution — taking over many of the duties suspected to be performed by nucleic acids in the prebiotic world. **TLS**