a dramatic recovery, leading to nearly perfect acoustic communication and language competence. The neural basis of this improvement was reported several weeks ago in *Science* (*10*) by Klinke and his colleagues. They fitted congenitally deaf kittens with cochlear implants, which conveyed acoustic stimulation directly to the brain, circumventing the damaged sensory hair cells of the inner ear. These previously acoustically deprived kittens showed dramatic improvements: Field potentials of higher amplitudes were produced during cortical activity, the activated area of the auditory cortex expanded, and long-latency neural responses (indicative of intracortical information processing) developed and showed more synaptic efficacy than they did in naïve, unstimulated deaf cats. A similar recruitment of the auditory cortex might form the basis of hearing acquisition in prelingually deaf infants after cochlear implantation. It is quite likely that a similar "awakening" of the visual cortex takes place in congenitally blind infants newly exposed to visual information after cataract removal and the fitting of an artificial lens.

The Maurer study demonstrates the amazing plasticity of the young human brain, and underscores the importance of complete, balanced early sensory input for guiding subsequent brain development. It also shows how a simple psychophysical test can be used as a powerful tool for early diagnosis and for monitoring subsequent therapy for a rare, but devastating, human ailment.

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RNA CATALYSIS

Chemical Diversity in RNA Cleavage

Eric Westhof

Ribozymes are RNA molecules that possess a catalytic activity and, thus, behave as enzymes. Those that occur in nature can catalyze the formation of a phosphodiester bond between two nucleotides or can break that bond. The ribozymes from plant pathogens or the human hepatitis delta virus (HDV) are self-cleaving RNAs that undergo an intramolecular reaction (called transesterification), leading to 5′-hydroxyl group and 2′,3′-cyclic phosphate products. The self-cleaving reactions are necessary for replication of the single-stranded RNA genome of viral pathogens (*1*). Despite the similarities in products, the three-dimensional architectures of the characterized self-cleaving ribozymes (*2, 3*) do not appear to be similar. The distinct dependence of cleavage efficiency on the presence of metal ions or other organic molecules (*4, 5*) suggests that there is also chemical diversity in the mechanisms of cleavage. Now, a report on page 123 of this issue by Perrotta *et al.* (*6*) presents clear evidence for a unique catalytic cleavage pathway in the HDV ribozyme. They show that this ribozyme is capable of a process called base catalysis during self-cleavage. This discovery sheds new light not only on the mechanisms of RNA catalysis, but also on the chemical evolution of RNA in a hypothetical prebiotic RNA world. Possibly, it could also lead to other yet undiscovered biological enzymatic activities effected by RNA molecules.

The mechanism for RNA self-cleavage entails activating a specific ribose 2′-hydroxyl group for attacking the adjacent phosphodiester bond (*7*). Such a nucleophilic reaction demands an increase in negative charge on the attacking oxygen atom and an increase in positive charge on the phosphorus atom to be attacked. This is the reason why RNA is randomly cleaved and degraded into its constituent nucleotides at basic pHs: Hydroxide ions abstract the proton from the ribose 2′-hydroxyl group, whereas protons are transferred to the departing 5′-group. This catalytic mechanism is called specific acid-base catalysis. In the protein universe, the ribonucleases cleave RNAs using amino acids such as histidine, which carries an imidazole side chain that has a nitrogen atom with a pK_a around 6 (where K_a is the acid constant). During acid-base catalysis, the nitrogen of a histidine attacks the ribose hydroxyl, whereas another histidine gives off a proton to the departing 5′-hydroxyl group (see the figure). Acid-base catalysis is most efficient when the catalysts have pK_a's around 7 (a pK_a similar to that of the imidazole ring) (*7*). However, nucleic acids do not possess chemical groups that can be ionized around neutral pH. The best candidates are the ring nitrogens N1 of adenine (A) or N3 of cytosine (C) (with pK_a values of 3.9 and 4.5, respectively). But nucleic acids, because of the negative charges they carry, regularly require metal ions for folding and stabilization, and metal ions can be a good source of hydroxide ions at neutral pH. Ribozymes have therefore been considered as metalloenzymes that use metal ion catalysis, with the magnesium ion (which has strong affinity for phosphate oxygens) being the most common catalytic metal ion (*8*). It is this latter consensus that Perrotta and co-workers (*6*) now fracture.

Indeed, following hints from crystallography (*3*), they convincingly suggest that in the HDV ribozyme, a C residue acts as a general base catalyst. Although previously debated (*5*), this extension of the repertoire of catalytic mechanisms that are open to RNA brings forth anew the amazingly efficient parsimony of biological evolution, which takes advantage of every physicochemical characteristic of the four natural nucleic acid bases. Furthermore, this observation also points to a meaningful functional partition of the four bases into two groups: the amino bases, A and C, which could sustain general base catalysis, and the keto bases, guanine (G) and uracil (U), which could not because they carry an imino proton on the pyrimidine ring. But RNA functions foremost for maintenance and exchange of genetic information and, therefore, a certain degree of chemical lethargy is a valuable asset. In this respect it is worth noting that, during the editing of RNA, the modifications occur essentially from amino to keto bases, C to U or A to inosine (I), a keto purine (*9*).

For their demonstration, Perrotta and colleagues (*6*) introduce a method that is sure to become popular in the RNA field. It has long been known that cytidine C75 of the genomic HDV ribozyme (an infectious RNA) is essential for catalysis (*10*). In the antigenomic HDV ribozyme, a replicative intermediate, the equivalent cytosine (C76) could be mutated into A with a loss in catalytic efficiency of three orders of magnitude. But it could not be

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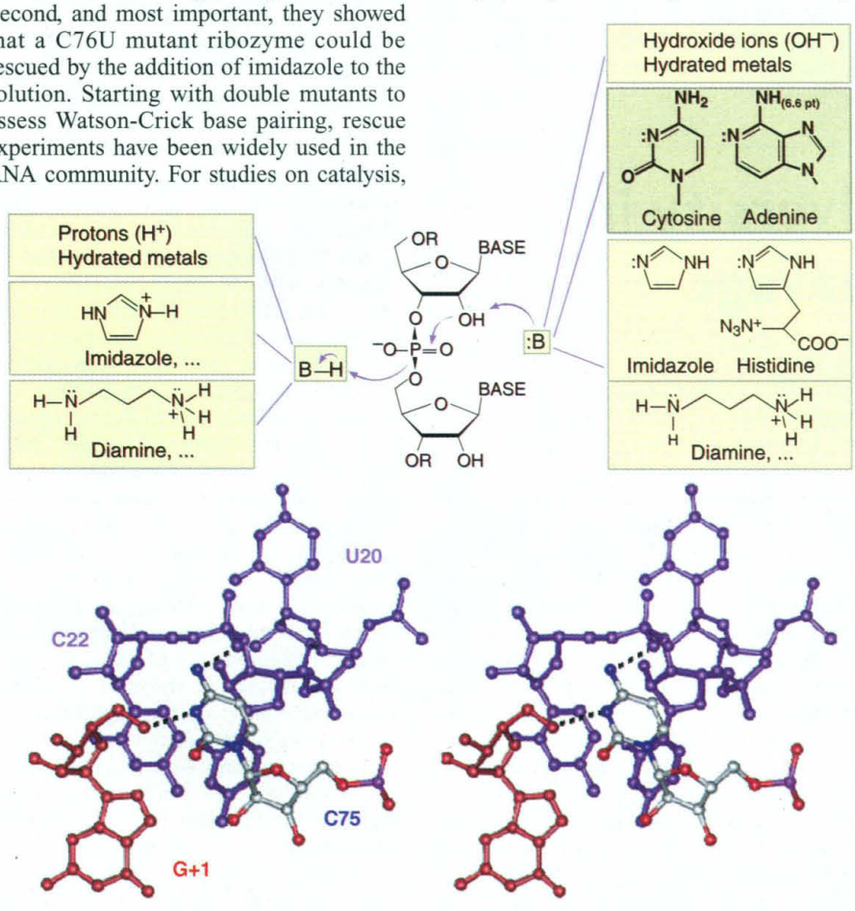
converted into G or U. Without discovering how to compensate for the loss in catalytic efficiency, one cannot prove very much. Perrotta *et al.* (*6*) found a very elegant way to repair the deficiency in the mutated ribozyme. First, they noticed that the curves for the pH dependence of the catalytic rate of the C76 wild-type ribozyme and that of the C76A mutant were separated by an amount corresponding to the pK_a difference between C and A (about 0.6 pH units), a clear indication that the residue at position 76 is acting as a general base. Second, and most important, they showed that a C76U mutant ribozyme could be rescued by the addition of imidazole to the solution. Starting with double mutants to assess Watson-Crick base pairing, rescue experiments have been widely used in the RNA community. For studies on catalysis, a most elegant rescue experiment consists (after exchanging a phosphate oxygen for a sulfur atom) of adding soft manganese ions (which interact favorably with a sulfur atom) rather than hard magnesium ions (which prefer oxygen atoms) (*11*). When cleavage activity is rescued, the most plausible explanation is that the exchanged oxygen interacts directly with the metal ion. Similarly, the imidazole rescue experiment introduced by Perrotta and co-workers (*6*) leaves little room for doubt, and the simplest explanation is general base catalysis by the RNA. The experiment, however, does not exclude the contribution of metal ion binding to stabilization of charge during catalysis. The results illustrate how the complex fold adopted by the ribozyme can create a local electrostatic environment capable of sufficiently perturbing the pK_a of a specific residue such that catalysis is promoted; an absence of tertiary structure therefore contributes to the stability of informational RNAs.



The complex chemistry of ribozymes. (**Top**) Possible acid-base catalysis of RNA cleavage. RNA cleavage requires a base for abstracting the proton of the 2′-hydroxyl group of the ribose and an acid for protonating the departing 5′-hydroxyl group (*7*). In specific acid-base catalysis, hydroxide ions attack the 2′-hydroxyl group and protons neutralize the 5′-departing group. This process is facilitated by the presence of divalent ions like lead or cadmium ions but also by magnesium ions, as a result of the equilibrium $M(aq)^{2+} \leftrightarrow M(OH)(aq)^{+} + H^{+}$, with a characteristic pK_a. Perrotta and co-workers (*6*) now show that C or A could act as a general base in the same way as imidazole. The nature of the proton donor for the 5′-departing group has not yet been identified. Most likely, a water molecule gives a proton to the 5′-departing group, whereas the hydroxide recaptures the proton from the C/A base. The two functions required for general acid-base catalysis of RNA cleavage can be performed by imidazole rings (oligoamines and polyamines) or aminoglycoside antibiotics, both of which possess proton donor and acceptor groups at neutral pH (*7, 13*). (**Bottom**) Stereo view of the environment around the cleavage site in the crystal structure of the self-cleaved form of the genomic HDV ribozyme (*3*), the closest atomic view available. The cytosine implicated in the cleavage mechanism, C75 in the genomic RNA, is shown (color code for atoms: C, white; N, blue; O, red). A potential contact between the N3 atom of C75 and the O5′ atom of G+1 (the departing group) is indicated by a dotted line. Notice how C75 is buried in a cavity formed by the loop residues U20 to C22 (*3*), forming H-bonds between the amino group N4 and acceptor atoms. Local conformational changes are expected in a C75U mutant that otherwise would place a keto group O4 close to an anionic oxygen of C22.

How comparable are the new findings to existing data in the literature? Imidazole, histidine, or polyamines have been used in the past for promoting or enhancing RNA cleavage (*7*). Because of the presence of histidine residues in the catalytic center of ribonuclease A, RNA hydrolysis in imidazole buffers has been extensively studied and discussed. Recently, an RNA-cleaving deoxyribozyme, which requires L-histidine as a cofactor, was selected by in vitro experimentation (*12*). Another ribozyme, the hairpin ribozyme, for which the presence of divalent ions is not absolutely required (*5*), is cleaved very efficiently by the polyamine spermine and by aminoglycoside antibiotics (*13*).

Thus, the findings of Perrotta *et al.* (*6*) fit well with a whole body of knowledge about RNA catalysis. We can now hope that this first set of experiments will be followed by many more on other natural and artificial ribozymes. But the present observations focus our attention on a new possibility in molecular biology—that RNA is capable of general base catalysis by itself.

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