groups of different ages and socioeconomic status (*6*). Conventional factor-analytical studies may find a *g* factor because they use only a relatively narrow range of tests (*7*). Moreover, tests of practical intelligence requiring real-world problem solving and decision-making—the kind in which Gore, Bush, and Bradley seem to excel—typically have been found to have only trivial correlations with tests of analytical intelligence, but to predict real-world job performance as well as or better than do analytical tests (*8*). In one study, the correlations were actually *negative* (*9*), suggesting that in societal circumstances in which practical skills are highly valued but academic ones are not, intelligent individuals may develop their practical skills at the expense of academic, analytical skills.

The frontal lobes certainly are important for many aspects of intelligence. However, although Duncan *et al.* propose that the neural circuitry of the frontal lobes is the basis of intelligence, they fail to show anything more than a correlational relationship. The fact that a dependent measure correlates with a biological event does not mean that it is caused by this event, because correlation does not imply causation. One cannot tell from a correlation between two variables whether the first variable causes the second variable, the second variable causes the first variable, or both variables are dependent on some third higher order variable. It is well established that learning alters the structure and function of the brain (*10*). Moreover, studies of individual differences in brain activation patterns as measured by PET scanning suggest that more intelligent people often show less, not more of certain kinds of frontal activation when they are performing analytical tasks (*11*), presumably because they find the tasks less challenging than do less intelligent people. Thus, the fact that areas of the brain are activated during some kinds of intelligent thought does not mean that their activation is the cause of these thought processes. Moreover, the Duncan study does not indicate whether these same areas are activated during creative or practical thought, or during the thought required for people to be intelligent in their everyday lives.

The mental-atlas approach taken by Duncan *et al.* (dating back to the time of the phrenologist Gall) implies that the understanding of intelligence depends upon finding the locus or loci of intelligence in the human brain. Their claim is similar to the weak claim that we understand the intelligence of a computer when we localize its artificial intelligence in a silicon chip embedded deep within the hardware. To understand human intelligence we must first unravel the functional significance of the frontal lobes and the elements contained therein and learn how operations in the designated areas are connected with the tasks that people perform.

Plato was among the first to recognize the brain as the seat of intelligence. It is sobering to realize that our progress since Plato is the alleged localization of intelligence to a certain part of the brain rather than an understanding of how the brain or anything else can produce the kind of achievements that the world has seen from Gore, Bush, or Bradley, or from Einstein, Darwin, or Galileo, for that matter. The results of Duncan *et al.* provide a holey grail rather than the Holy Grail, because as yet they have not provided the whole grail.

References

1. J. Duncan *et al.*, *Science* **289**, 457 (2000).
2. "Who's the dimmest dim bulb?" *U.S. News & World Report*, 3 April 2000, p. 20.
3. A. Binet and T. Simon, *The Development of Intelligence in Children* [Williams & Wilkins, Baltimore, MD, 1916 (Originally published in 1905)]; D. Wechsler, *The Measurement of Adult Intelligence* (Williams & Wilkins, Baltimore, MD, 1939).
4. E. G. Boring, "Tests test it," *New Repub.* (6 June 1923), p. 35.
5. R. J. Sternberg, *Beyond IQ: A Triarchic Theory of Human Intelligence* (Cambridge Univ Press, New York, 1985); *Rev. Gen. Psychol.* **3**, 292 (1999).
6. _____, E. L. Grigorenko, M. Ferrari, P. Clinkenbeard, *Eur. J. Psychol. Assessment* **15**, 1 (1999); R. J. Sternberg *et al.*, *Eur. J. Psychol. Assessment*, in press.
7. A. R. Jensen, *The g Factor: The Science of Mental Ability* (Praeger/Greenwoood, Westport, CT, 1998); J. B. Carroll, *Human Cognitive Abilities: A Survey of Factor-Analytic Studies* (Cambridge Univ. Press, New York, 1993).
8. R. J. Sternberg *et al.*, *Practical Intelligence in Everyday Life* (Cambridge Univ. Press, New York, 2000).
9. R. J. Sternberg *et al.*, *Intelligence*, in press.
10. A. Klintsova and W. T. Greenough, *Curr. Opin. Neurobiol.* **9**, 203 (1999).
11. R. J. Haier *et al.*, *Intelligence* **16**, 415 (1992).

PERSPECTIVES: RNA STRUCTURE

Ribozyme Evolution at the Crossroads

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It is a fundamental tenet of biology that the amino acid sequence of a protein determines its structure, which in turn determines its function. If two proteins have a similar sequence, then their structures and functions are likely to be similar. The same may be said of RNA molecules that behave as enzymes (called ribozymes). Sequence similarity between two such RNA molecules implies that they have the same structure and function. Or does it? On page 448 of this issue, Schultes and Bartel (*1*) present an example of one RNA sequence that can adopt two completely different structures, each having a distinct catalytic activity. Furthermore, they demonstrate that a continuum of mutations in this common RNA sequence leads in a stepwise manner to sequences that are optimized exclusively for one catalytic activity or the other. This shows that smooth evolutionary pathways exist between distinct ribozymes, facilitating the rapid evolution of RNA-based catalytic activities.

The two catalytic activities selected by the investigators have no evolutionary relationship. One activity is the cleavage of RNA catalyzed by the hepatitis delta virus (HDV) ribozyme, which assists in the replication of HDV viral RNA (*2*). The other is RNA ligation catalyzed by the class III ligase ribozyme, an activity obtained in the laboratory through "test-tube" evolution (*3*). The two catalyzed reactions have distinct mechanisms (see the figure). RNA is cleaved by the HDV ribozyme through attack by an internal 2′ hydroxyl on the adjacent phosphate, forming a 2′,3′-cyclic phosphate and releasing an oligonucleotide 5′-hydroxyl. RNA ligation by the class III ligase occurs through attack by the terminal 2′-hydroxyl group of an oligonucleotide substrate on the α-phosphate of an oligonucleotide 5′-triphosphate, forming a 2′,5′-phosphodiester linkage and releasing inorganic pyrophosphate. The two ribozymes have approximately 25% sequence similarity (no more than would be expected by chance) and adopt completely different secondary and tertiary structures.

After careful examination of the HDV and class III ligase ribozymes, Schultes and Bartel constructed an "intersection sequence" that simultaneously satisfied the requirements for both catalytic activities. This is no small feat. Imagine generating a string of text that, without changing the order of a single letter, could be grouped into different words so as to provide two paragraphs that have entirely different meanings. This would be a near-impossible task with an alphabet of 26 letters (or 20 amino acids), especially if the paragraphs (or proteins) had a complex structure. The task is less difficult with RNA molecules because they contain only four "letters"—A, U, G, and C. Furthermore, the letters are interchangeable in a pairwise fashion (maintaining Watson and Crick pairing) within stem structures or in-

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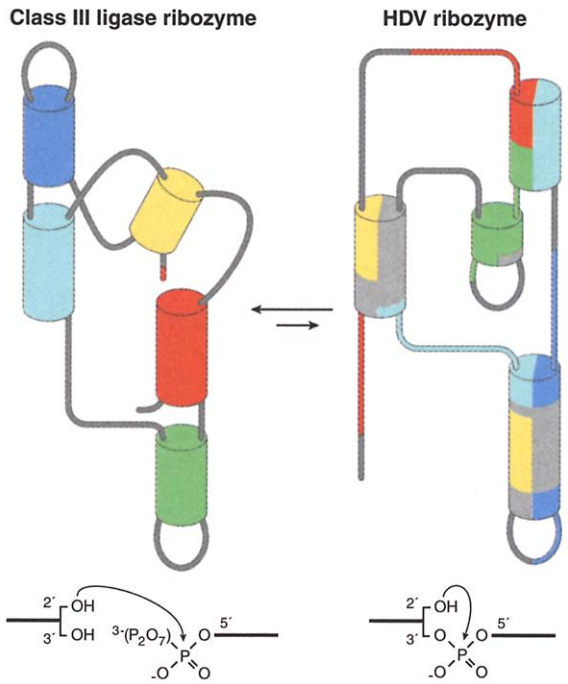
dividually within single-stranded regions of RNA. Another advantage of RNA is wobble pairing, which allows G to pair with either C or U, and U to pair with either A or G.

The HDV and class III ligase ribozymes studied by Schultes and Bartel contain 85 and 84 nucleotides, respectively. The secondary structure of these RNAs is well known, and a crystal structure of the HDV ribozyme has been reported (*4*). In converting the HDV ribozyme to the intersection sequence, the authors changed the nucleotide composition of 11 base pairs, altered 16 unpaired residues, converted one base pair to unpaired nucleotides, and replaced three Watson-Crick pairs by wobble pairs. In converting the class III ligase ribozyme to the intersection sequence, they changed the composition of 13 base pairs, altered 11 unpaired residues, converted one base pair to unpaired nucleotides, and replaced one Watson-Crick pair by a wobble pair. The resulting intersection sequence is both fish and fowl. Most of the time it folds into the shape of the class III ligase ribozyme and has a ligation rate that is about 750-fold greater than that of the uncatalyzed reaction. But some of the time it folds into the shape of the HDV ribozyme and has an RNA cleavage rate that is 70-fold greater than that of the uncatalyzed reaction.



A switch-hitting enzyme. A single RNA molecule has been engineered to adopt two different structures: that of the class III ligase ribozyme (**left**) or that of the HDV ribozyme (**right**). Each base-paired region in the ligase ribozyme is indicated by a different color (left). These regions are completely rearranged in the HDV ribozyme (right). The reaction catalyzed by each ribozyme is different, resulting in the formation of a 2′,5′-phosphodiester (left) or the cleavage of a 3′,5′-phosphodiester (right).

Schultes and Bartel then devised a series of RNA sequence mutants that represented all of the steps in the pathways from the intersection sequence to the standard form of either the HDV or class III ligase ribozyme. Each pathway contained 25 steps, with one or two mutations introduced per step and with either cleavage or ligation activity maintained throughout. The first few steps from the intersection sequence had the most impact. A single mutation resulted in an improvement in RNA-cleavage activity by a factor of 120 and a reduction in ligation activity by half. A second mutation provided another factor of 10 improvement in RNA-cleavage activity and reduced ligation activity to undetectable levels. A single mutation in the opposite direction improved ligation activity by a factor of 120 and reduced RNA-cleavage activity to undetectable levels; a second mutation in that direction resulted in another factor of 5 improvement in ligation activity.

It is not unusual for an RNA or protein molecule to exist in more than one conformation. Alternative conformations are a frequent source of interest (and aggravation) for enzymologists and structural biologists. Different conformations of a macromolecule may be associated with different biochemical properties, a notorious example being the soluble and insoluble forms of the prion protein (*5*). The intersection sequence of Schultes and Bartel, however, is the first example of a molecule that can adopt two different conformations, each associated with a distinct catalytic activity.

New enzymes can arise after a gene duplication event, with one gene copy retaining the original activity and the other diverging to adopt a new function. The new function is likely to be similar to the old one, perhaps involving a different substrate or a related reaction mechanism (*6*). In some cases the new activity is thought to derive from the "catalytic promiscuity" of the original enzyme, which has the ability to catalyze a reaction other than the one for which it evolved, albeit at a very low level (*7*). Combining the notion of catalytic promiscuity with that of alternative conformations, one can regard different conformations as providing an evolutionary opportunity similar to that afforded by duplicated genes. The dominant conformation retains the original function of the enzyme, whereas another conformation is free to evolve a new function. In this way, diversification might precede gene duplication, although a duplication event would eventually be needed to allow independent optimization of the two functions. An example of conformational opportunism in enzyme evolution is seen in the maturation of a catalytic antibody: The mature antibody takes on one of the several conformations in which the corresponding germ line antibody can exist (*8*).

More so than proteins, RNA molecules are amenable to the exploration of alternative conformations. The reason for this is that RNA has four subunits that are highly interchangeable. In addition, distinct conformations of an RNA molecule often are separated by only a few mutations. A computational analysis of the "neighborhood" of a typical RNA secondary structure demonstrated that these neighborhoods often overlap (*9*). Thus, a succession of modest sequence changes can give rise to a succession of structural and associated functional changes. An extreme example of this behavior is seen in the Schultes and Bartel study, which demonstrates that even without a sequence change, dramatic alterations in structure and function do occur.

Compared to proteins, RNA molecules are sorely lacking when it comes to the chemical diversity of their subunits. Ribozymes cannot match their protein counterparts in catalytic sophistication or the ability to sustain a complex biochemistry. No wonder that the "RNA world" (the presumed ancestral era during which life was based on RNA genes and RNA catalysts) was replaced by a genetic system based on DNA and protein. On the other hand, RNA appears to be built for speed in the arena of Darwinian evolution. Nearly every RNA sequence is soluble in aqueous solution. The secondary structural components of RNA are simple, modular, and highly tolerant of sequence variation. Moreover, evolutionary pathways exist that allow easy traversal between distinct structural and functional motifs. The time during which RNA-based evolution dominated life on Earth may have been brief, but it is likely to have been a fast ride.

References

1. E. A. Schultes and D. P. Bartel, *Science* **289**, 448 (2000).
2. M. Y.-P. Kuo, L. Sharmeen, G. Dinter-Gottlieb, J. Taylor, *J. Virol.* **62**, 4439 (1988).
3. E. Ekland, J. Szostak, D. Bartel, *Science* **269**, 364 (1995).
4. A. R. Ferré-D'Amaré, K. Zhou, J. A. Doudna, *Nature* **395**, 567 (1998).
5. K. Pan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10962 (1993).
6. R. A. Jensen, *Annu. Rev. Microbiol.* **30**, 409 (1976).
7. P. J. O'Brien and D. Herschlag, *Chem. Biol.* **6**, R91 (1999).
8. G. J. Wedemayer, L. Wang, P. A. Patten, P. G. Schultz, R. C. Stevens, *Science* **276**, 1665 (1997).
9. W. Fontana and P. Schuster, *Science* **280**, 1451 (1998).