cell contacts stimulated Notch activation. These findings suggest that contact-dependent Notch activation correlates with the inhibition of neurite outgrowth.

To investigate whether activation of Notch could directly inhibit neurite growth, neurons were transfected with plasmids encoding truncated forms of Notch1 and 2 comprising the entire intracellular domain. These forms of Notch lack the extracellular ligand-binding domain and are constitutively activated. Šestan *et al.* showed that transfection of the Notch intracellular domain stimulated transcription from reporter constructs that are normally activated by Notch, but inhibited the growth of neurites even in low-density cultures. In similar experiments in postmitotic primary mouse hippocampal and cortical neurons, Notch1 transfection caused the regression of preexisting neurites (*2*). In high-density cultures that already had a high level of Notch activity, the introduction of the Notch intracellular domain caused a retraction of neurites. Šestan and co-workers also showed that the exogenous addition of Notch ligands (Delta or Jagged) mimicked reporter gene activation and the inhibition of neurite growth.

Finally, inhibitors of Notch signaling were used to demonstrate that the effects observed were specific for Notch activation. Numb, Numb-like, and Deltex are thought to modulate Notch signaling and are also expressed in the developing cortex. It was observed that these mediators inhibited neurite extension, promoted by the Notch intracellular domain, and transcriptional activation to different degrees. But, when transfected into high-density cortical neuronal cultures, Numb, Numb-like, and Deltex promoted the extension of neurites, causing the neurons to behave as if they were in a low-density environment.

These findings suggest that Notch activation can alert neurons to the proximity of their neighbors' neurites. If there are lots of neurons around, neurites retract, becoming short, dense, and bushy. On the other hand, if neurons are isolated, they spread their neurites until they find somebody out there to compete with. These capabilities may be important because the whole nervous system is characterized by the careful spacing of neurons of similar type into mosaics, so that neighboring dendrites barely overlap, assuring even coverage by each neuronal type without holes or unnecessary overlap (*12*). The regular spacing of cell bodies can perhaps be attributed to the activity of Notch genes in controlling neuronal birth (*13*), but one can speculate that the regular spacing of dendrites and axons might reflect the operation of Notch signaling genes later on.

These studies raise many further questions about how and where Notch activation is regulated. Do all neurites (axons or dendrites) require Notch activity in vivo, and how is the activation of Notch limited? Does Notch signaling mediate activity-dependent neurite remodeling? Is Notch signaling influenced by synaptic activity? How does Notch interface with the neurotrophins that regulate neurite growth and neuronal survival?

The importance of Notch in maintaining normal adult neuronal function has been previously hinted at by a number of human neurological syndromes with derangements in Notch signaling. The CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) syndrome is associated with point mutations in Notch3 (*14*), and individuals with Alagille syndrome (where mild mental retardation is associated with multiple developmental disorders) have Jagged1 mutations (*15*). Moreover, genes that predispose to early Alzheimer's disease, called presenilins, are required for the normal intracellular processing and activation of Notch (*16–19*). The role of the Notch pathway in neurite outgrowth may ultimately lead to a better understanding of the abnormal neurite patterns that characterize Alzheimer's disease.

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PERSPECTIVES: MOLECULAR EVOLUTION

Do Proteins Predate DNA?

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The central dogma of molecular biology—that DNA makes RNA, which in turn makes protein—begs the question of the order in which these three fundamental biopolymers arose. The proposition that RNA came first has achieved wide popularity (*1, 2*). However, the concept of a primordial RNA world does not identify which molecule came next: Was it DNA (a more stable information storage medium) or protein (a more versatile catalyst)? An appraisal of the diverse and sophisticated catalytic potential of RNA oligomers (ribozymes) has led some to suggest that proteins came last, the final twist to a nucleic acid world (*3–6*). But new findings corroborate the view of an early RNA/protein environment with DNA evolving last. Establishing the correct chronology defines the context of genetic code evolution and allows predictions to be made about the distribution of RNA within the fundamental machinery of life.

Parsimony favors the notion that life first evolved through a single biological macromolecule that both stored genetic information and catalyzed the reactions required for self-replication. Today proteins perform sophisticated catalysis and DNA stores information, whereas RNA can do both. Intuitive early speculations (*7*) that RNA dominated some primordial biosphere reached mainstream theory (*1*) through two avenues of research. First, investigators have produced a diverse array of ribozymes that catalyze fundamental metabolic reactions and bind specific ligands. Second, identification of putative "molecular fossils" in extant metabolism (*8*) has inspired the "palimpsest" model of evolution (*3*) in which modern protein enzymes are postulated to have incompletely replaced earlier ribozyme equivalents. Indeed, patterns within present-day metabolism support the RNA-first model over any alternative. DNA probably arose as an RNA derivative because all organisms make deoxyribonucleotides by reducing ribonucleotides, and make thymine by methylating uracil (*9*). Proteins-first models cannot explain the presence of functional RNA in processes such as translation in extant organisms: The 20 "natural" amino acids are more chemically diverse than the four nucleotides, which suggests that proteins have greater

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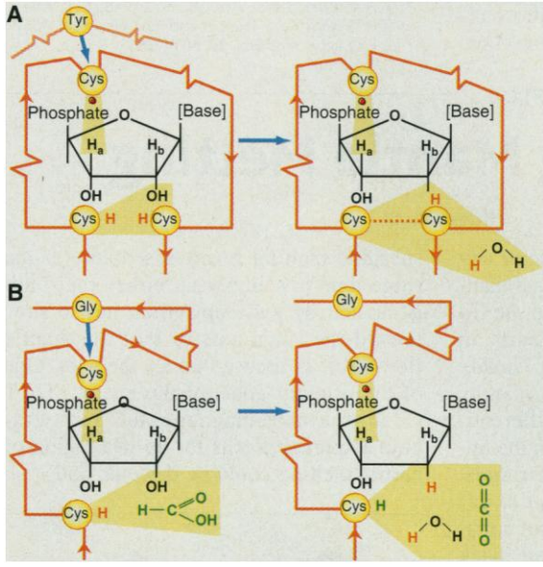
catalytic potential (*2*). If proteins came first, why should the evolution of nucleic acids (presumably for information storage) insinuate RNA into catalysis? Only the RNA-world model implies a logical, adaptive diversification from RNA into proteins (superior catalysts) and DNA (a more stable information store) (*9*).

If RNA came first, which came next: proteins or DNA? The mainstream view of an RNA/protein biosphere (*10, 11*) has until now been based on surprisingly little hard evidence. Indeed, the very findings that support an RNA world—namely the expanding repertoire of putative molecular fossils and of laboratory-produced ribozymes—have prompted speculation that proteins evolved last, once both types of nucleic acid were present (*3–6*). Furthermore, the ribonucleotide reductase enzymes (RNRs) responsible for producing deoxyribonucleotides group into three very different classes, distributed unevenly across taxa. Advocates of a proteins-last model have seized upon the lack of clear homology among the three classes to suggest that the enzymes are in fact polyphyletic, having evolved separately in different lineages to replace a primordial ribozyme equivalent (*3*). However, new research establishes the homology of all RNRs and places their biochemistry beyond the plausible scope of ribozyme catalysis, a view corroborated by the distribution of catalytic and functional RNA found within cells.

DNA biosynthesis occurs in two stages: An RNR reduces the 2′ hydroxyl group of ribonucleotides to form deoxyribonucleotides; a DNA polymerase then links these monomers into DNA (see the figure). Two aspects of RNR-catalyzed reduction inform the relative timing of DNA evolution. First, new insights into tertiary structural similarity (*12*) demonstrate RNR homology and strengthen the argument that subsequent divergence resulted from oxygenation of the atmosphere after the evolution of photosynthesis (*10*). Second, all RNRs use an unusual and energetically difficult reaction mechanism based on free-radical chemistry and the conserved spatial arrangement of two thiol groups at the active site (see the figure).

That the extraordinary sulfur-based chemistry of RNRs has been absolutely conserved, despite divergence in nearly every other aspect of the enzymes, implies that nature has only discovered this one mechanism for ribose reduction. Perhaps no others are biologically feasible or accessible. Why otherwise would all known organisms share this energetically costly and biochemically unusual mechanism? If ribonucleotide reduction requires such difficult chemistry, is it even conceivable that DNA arose in a world of ribozyme catalysis?

Two further ways in which DNA could exist in an RNA world without proteins deserve consideration. First, if the RNA world used abiotically synthesized DNA, ribonucleotide reduction would initially be unnecessary. Second, ribozymes might have augmented their capabilities by using sulfur-containing peptides as cofactors. However, prebiotic synthesis experiments indicate that deoxyribose was probably vanishingly rare on the early Earth, and that sulfur-containing amino acids were too unstable to have attained "useful" concentrations (*13*). The specific prediction of this argument is that future research will fail to produce a prebiotically plausible ribozyme capable of ribonucleotide reduction.



Contorted catalysis. Active sites and proposed reaction mechanisms in DNA biosynthesis. The orange lines indicate the enzyme, with important residues shown in yellow. Chemical structures represent substrates. Red dots indicate free radicals. **(A)** RNR class I enzymes. **(B)** RNR class III enzymes. Only two cysteine residues are used, the cysteine radical is generated from a glycyl radical further along the protein, and formate is reduced to carbon dioxide and water during the reaction.

This biochemical argument is corroborated by simple observations concerning the role of catalytic and functional RNA molecules in extant metabolism. Modern organisms process genotype into phenotype through two distinct stages. First, DNA genes are transcribed into RNA messages (mRNA); these messages are then translated into proteins. Functional RNA molecules are intimately associated with every aspect of translation, from tRNA (the "adapter" molecule that translates each mRNA codon into the appropriate amino acid) through ribonuclease P (involved in tRNA maturation) to the ribosome itself (an RNA/protein complex that coordinates translation). Indeed, the very latest insight into ribosome structure (*14*) emphasizes the crucial role of the ribosomal RNA subunits in coordinating the translation machinery. Other functional RNA molecules modify RNA messages before their translation (*1*).

In stark contrast, unique functional RNA is completely absent from transcription (*1, 15*) and from ribonucleotide reduction (*16*). Indeed, within DNA-related metabolism, ribonucleotides only appear in the form of RNR cofactors (shared with many other enzymes) and RNA oligomers used to prime DNA replication. The sheer diversity of functional RNA associated with the translation machinery imbues this conspicuous absence with meaning. Thus, another novel prediction is that any functional RNA molecules found to be associated with transcription, ribonucleotide reduction, or DNA replication will be clear secondary derivatives of other cellular machinery.

Class III RNRs, which use the cofactors *S*-adenosylmethionine (to generate the radical) and formate (as a reductant), are the best extant model for the primordial RNR (*10, 16*). Interestingly, class III RNRs appear homologous to other enzymes that use glycyl radicals in pathways predating atmospheric oxygen, such as anaerobic fermentation (*17*). Pyruvate formate lyase (PFL), for example, uses a glycyl radical to generate a cysteine radical, which then lyses pyruvate into formate and helps to create *S*-adenosylmethionine. Both PFL products are used in class III RNR reduction; the glycyl radical of PFL and class III RNRs is generated using an iron-sulfur cluster and both show sequence homology. If PFL is representative of early protein catalysis, then proteins may have initially evolved to perform tasks beyond the catalytic range of RNA and only later usurped the function of ribozymes.

The distribution of catalytic RNA within extant metabolism, together with the difficult biochemistry of ribonucleotide reduction, implies that sophisticated proteins probably predate DNA: Transcription machinery lacks ribozyme relics simply because ribozymes never performed this function. This restricts the latest point at which the genetic code could have arisen, because proteins (that is, translation) must have evolved before DNA. Yet life probably did not originate with RNA.

Abiotic synthesis of both ribose and bases is problematic, and linking the two into nucleotides is more difficult still (*18*). Increasing acceptance of an RNA world has actually stimulated research into plausible models for a pre-RNA world (*1*). Could translation have arisen even before RNA? Ribozyme relics argue against such a model: If translation evolved in a pre-RNA biosphere, why would the subsequent evolution of RNA introduce functional RNA components into preexisting (proteinaceous) ribosomes?

Finally, if proteins evolved in an RNA world, then this informs theories about the fixation of the canonical genetic code. RNA templates are significantly more error-prone (in terms of point mutation) than their DNA equivalents (*5*). An RNA-world origin for the genetic code thus adds significance to the finding that the arrangement of canonical codon assignments appears to minimize the phenotypic impact of errors (*19*).

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PERSPECTIVES: GEOPHYSICS

Mapping Mantle Melting

Roger Buck

Most of the volume of Earth, or any of the terrestrial planets, is a mantle made of silicate. Yet we see little of it at the surface because early in Earth's evolution a crust of lower density material floated to the top of the mantle. Present-day partial melting and differentiation of the mantle mostly occur in the upwelling region below mid-ocean ridges. On page 752 of this issue, Evans *et al.* (*1*) present results from the last phase of a major collaborative experiment to pull back the veil obscuring our view of this crucial geophysical process.

In the picture provided by plate tectonics, ridges are now seen as primary sites of mantle upwelling, whereas subduction zones are centers of downward flow. We know more about the downwelling regions because earthquakes occur within the brittle subducting plates and the seismic activity allows us to track the plate position. At the same time, seismic velocities are very different in the cold plates and the hotter mantle through which they plunge. Our view of the region of upwelling is not so clear; no deep seismicity marks the upwelling of hot weak mantle, and lateral temperature gradients there are small.

Whereas ~100-km-thick plates move down at subduction zones, the region of upwelling and pressure release melting under ridges could be as much as a thousand kilometers wide according to simple "passive" models of mantle flow driven by plate motion. The crust produced by that subridge melting forms essentially at the ridge axis (*2*), implying either that melt generated by wide upwelling has to flow toward the ridge axis or that the mantle flow itself is focused under the axis. One of the primary goals of the recent MELT (Mantle Electromagnetic and Tomography) Experiment was to see if a region of partial melting could be detected and if so to map the size of that region. This involved the largest deployment of ocean bottom instruments for the purpose of answering geological questions. It was centered on the fastest spreading and straightest part of the global mid-ocean ridge system—the East Pacific Rise around 17°S.

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Data from the deep. Magnetometer being deployed in the area of the MELT Experiment from the research vessel *Thompson* showing Rob Evans (of Woods Hole Oceanographic Institution) on the left and Helmut Moeller (of Scripps Institution of Oceanography).

Evans *et al.* (*1*) report results from the MELT Experiment involving interpretation of electromagnetic measurements. The new results build on what was learned from the earlier seismic part of the experiment, presented in a series of articles in 1998 (*3*). The two methods should indicate different things about melt in the mantle. Seismic velocities depend on melt volume and on the aspect ratio of melt inclusions. The electromagnetic experiments measure conductivity and are sensitive to how interconnected pockets of melt are, as well as to the water abundance in mantle minerals. The deployment of one of the magnetometers used in the experiment is shown in the figure.

Both the seismic and the electromagnetic experiments indicate the presence of a small amount (perhaps less than a few percent) of melt over a region several hundred kilometers wide and more than 100 km deep under the ridge. The inferred region of partial melt is very asymmetric, extending much farther to the west than to the east, under this roughly north-south–oriented ridge. The seismic low-velocity region extends more than a hundred kilometers to the east of the ridge with a gradual change to higher velocities. In contrast, the electromagnetic results show a sharper transition between a very conductive, inferred melt-rich region to the west and less conductive regions to the east of the ridge axis. The conductivity transition occurs at the spreading axis.

The MELT results preclude even moderate focusing of upwelling as predicted by some "dynamic" mantle flow models (*4*). They imply that melting-related density changes do not drive substantial mantle upwelling and that most flow is "passive" or driven by plate motion. Dynamic flow, driven by buoyancy, may be insignificant

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