How Did Replicating and Coding RNAs First Get Together?

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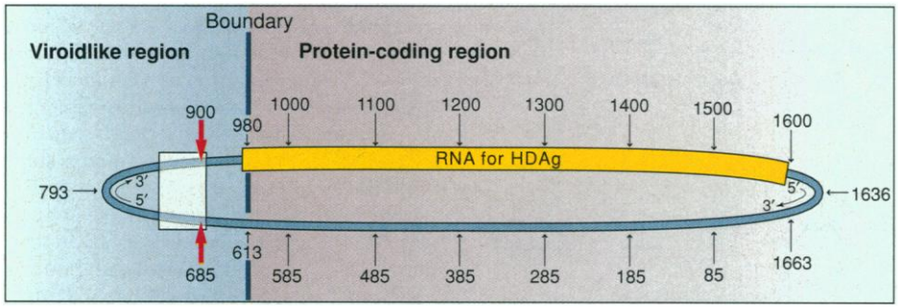
One of the most puzzling features of eukaryotic cells and viruses is the persistence of split genes—sequences that code for part of a protein and that are interspersed with intervening noncoding sequences that must be spliced out before production of mature messenger RNA (mRNA) (*1*, *2*). This elaborate and widespread genome structure may be a "living fossil" left over from the "RNA world," a hypothetical, early pre-DNA environment in which RNA replicated by itself and the genetic code and protein synthesis arose (*3*). Alternatively, such RNA mosaics may result from an ongoing process in informational RNA molecules, including those active in present-day cells. Clues to the origin of these structures now come from the causative agent of delta hepatitis, as reported by Brazas and Ganem (*4*) in this issue of *Science*.

These authors found in uninfected liver cells an mRNA encoding a protein that can strongly influence delta hepatitis RNA replication and that is related to a delta hepatitis protein. They then go on to suggest that delta RNA may have arisen when a free-living, self-replicating RNA "captured" a cellular mRNA encoding this protein. If they are correct, we have now acquired an experimental system in which to test ideas about molecular evolution in eukaryotic cells and their viruses, previously the exclusive province of theoreticians.

The genetic material of the hepatitis delta agent or virus (sometimes abbreviated HDV) is a circular, highly structured single-stranded RNA, a signpost at an important evolutionary crossroads. As shown in the figure, there are two domains of delta RNA, each with features of two quite different RNA types. On the left, one-quarter of the bases comprise the viroidlike region, named after a class of circular pathogenic RNAs about 350 bases long that infect plants (*5*). Like delta RNA, viroids replicate by a rolling circle pathway in which multiple end-to-end copies of the RNA are made by a host RNA polymerase that rolls around and around a complementary unit-length circular RNA template. Built-in RNA cleavage activities and ligation sites, in both plant viroids and delta's viroidlike region, then create monomeric circular offspring (*6*).



Double ancestral lineage. The genomic RNA of the delta hepatitis agent comprises two separate domains, the viroidlike region (left) and the protein-coding region (right), possibly a result of early "capture" of coding RNA by the viroidlike RNA. Residues 613 and 980 form the boundary between the regions. Yellow region, RNA encoding mRNA for the delta antigen protein; residue 685 (red arrow), site of ribozyme self-cleavage in the genomic strand; residue 900 (red arrow), site of ribozyme self-cleavage for the antigenomic strand; box, region of local tertiary structure and interaction.

The right-hand three-quarters of the delta RNA map, the protein-coding region, is very different: It encodes mRNA for a single protein, the delta antigen (HDAg), and is also much more divergent in sequence than is the viroidlike region. The extreme differences between these two domains suggest that delta RNA arose from two separate RNAs, one a viroid and one an mRNA (*2*, *7*). As we shall see, Brazas and Ganem provide a compelling candidate for the mRNA forebear.

Why is the newly described (*4*) cellular mRNA from liver a candidate for this ancestral role? Brazas and Ganem reasoned that HDAg might interact with cellular proteins to influence HDV replication. Using a series of ingenious assays, these authors isolated and cloned a gene that encodes such a 202–amino acid protein (called delta-interacting protein A or DIPA), with 56% of its amino acids identical to or very similar to the corresponding delta antigen residues. So DIPA mRNA may encode a host analog of HDAg. DIPA and HDAg RNA might thus share a common ancestor, which could have been captured by a free-living, viroidlike RNA some time in the past.

Such capture events would be rare and usually fatal to the "capturing" organism. But for the occasional RNA mosaic to survive and prosper, it must retain the viroidlike ability to replicate and the encoded protein must also contribute positively to the welfare of the organism. Brazas and Ganem show that DIPA occurs in a number of human tissues in addition to liver, and they speculate from sequence comparisons and other properties that it could be a nucleic acid–binding protein or possibly a transcription factor. RNA encoding a protein with these properties, which could help highly structured RNA molecules to simplify replication or expression, might well confer increased survival value to an RNA mosaic containing such RNA and explain that mosaic's survival.

Even if these events are theoretically possible, in practice can we propose testable mechanisms for the creation of viable RNA mosaics? The map of HDV RNA in the figure allows us to visualize the process of RNA conjunction that might accomplish this end. If a primitive viroidlike RNA occupies residues 613 to 980, reading 5′ to 3′ (clockwise) on the circular map, then there must have been a way either during or after synthesis to join residue 980 to the 5′ end of a copy of the cellular RNA to be captured. Brazas and Ganem suggest a copy-choice mechanism for this, in which the enzyme system copying the viroid's negative strand template switches, presumably at low frequency, to copying a cellular RNA such as DIPA mRNA. Alternatively, sites of RNA cleavage and ligation shown in the figure (whereby the multimeric RNA precursors produced by rolling circle replication yield monomers that are then circularized) could occasionally be misaligned, joining a linear negative viroid RNA strand to a cellular mRNA species. By either mechanism, surviving RNA mosaics would remain self-replicating, and the resulting deltalike RNA would encode an advantageous protein. As a test of this idea, the viroidlike domain of delta could be isolated and allowed to replicate in vitro or in cells containing various amounts of DIPA mRNA and protein. The formation of RNA mosaics would provide evidence in favor of the "capture" hypothesis.

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When would this scenario have happened? And how often? Are these merely viral anomalies, or do they have a more general lesson to teach? The most intriguing possibility would be one in which such events occurred in the RNA world (*3*), in which primitive self-replicating RNAs could have joined with equally primitive mRNA segments to form the first RNA genomes. If such mosaics survived until DNA arose, their preservation would lead to the presence of introns and split genes. This point of view could lead to the following: Today's intervening sequences or introns might have arisen from the viroidlike regions of early RNA mosaics from which modern DNA chromosomes may be descended. Although the independent replicating ability of these viroidlike sequences now embedded as introns in DNA-coded RNA precursors would soon have been lost, perhaps the RNA-catalyzed cleavage and ligation steps, characteristic of many viroidlike RNAs (*5*, *6*), have survived to become the mRNA splicing system now so widespread in eukaryotes (*8*).

References

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Hierarchical Control of Lymphocyte Survival

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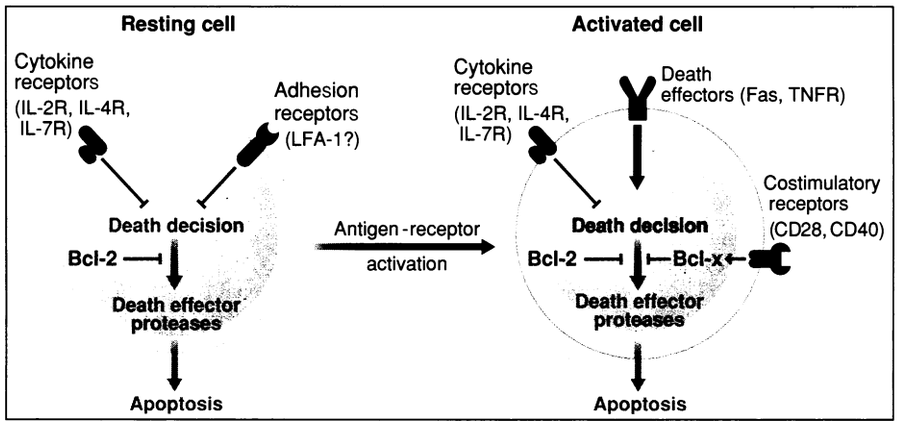
At rest, the lymphoid immune system consists primarily of quiescent cells—B and T lymphocytes—each potentially responsive to a unique, specific antigen. When an antigen is recognized, these lymphocytes proliferate into a clonal population of cells that directs an immune attack against the foreign antigen. To keep the number of lymphocytes manageable, the immune system balances this proliferation by the removal of excess cells once the antigen is successfully eliminated. This removal is accomplished by programmed cell death (apoptosis), which also kills potentially autoreactive lymphocytes and limits the clonal expansion of lymphocytes during an immune response. This carefully tuned homeostatic system is critical: When it goes awry, excess lymphocytes can cause lymphoid malignancies, lymphoproliferative diseases, and autoimmunity. Recent work reveals that this homeostasis is maintained by an extremely complex set of regulatory processes that differ markedly in quiescent and activated cells. But a sensible framework for the new information can now be assembled.

Apoptosis is mediated by the activation of intracellular proteases, of which the ICE/Ced-3 family of cysteine proteases is the best characterized. Once activated, several of these proteases can induce all the morphological and biochemical features of apoptosis (*1*). The apoptosis-controlling ICE/Ced-3 proteases are constitutively expressed as inactive precursors (zymogens) in lymphocytes. Therefore, for the lymphocyte to survive, the activation of these precursors must be carefully regulated. Lymphocytes can also control their own sensitivity to apoptosis through modulators of the apoptotic threshold. The best characterized of these regulators are members of the Bcl-2 family. Both ICE proteases and Bcl-2–related proteins are key controllers of apoptosis, as indicated by their conservation in metazoan cells. A third class of molecule also falls into this category—cell surface receptors.

The relative importance of these receptors and of the ICE proteases and Bcl-2 in regulating lymphocyte survival depends on the activation state of the lymphocyte (see figure). In a quiescent cell, survival depends primarily on the expression of Bcl-2. Animals without the *bcl-2* gene cannot sustain a peripheral immune system because they progressively lose their quiescent lymphocytes (*2*). In a quiescent cell, signal transduction through cytokine receptors can also promote lymphocyte survival. The best characterized of these receptors are members of the cytokine receptor family that share a common γ chain—interleukin-2 (IL-2), IL-4, and IL-7 receptors. Survival mediated through these receptors does not depend on the expression of Bcl-2, nor on the ability of these receptors to mediate progress through the cell cycle (*3*). This ability of cytokines to support the survival of cells that do not encounter antigen may help prevent the deletion of bystander cells during an inflammatory response, especially because lymphocytes can be recruited into an inflammatory lesion in the absence of antigen-receptor engagement. Adhesion receptors such as integrins also appear to regulate the survival of quiescent B and T cells (*4*). If a lymphocyte does not traffic to the usual peripheral immune sites—lymph nodes, Peyer's patches in the gut, and spleen—it will be deleted by apoptosis (*5*). These sites contain adhesion receptor ligands, which may allow long-term lymphocyte survival by signaling the posi-



The factors that regulate the survival of resting and activated lymphocytes. In this model, the survival information provided by cell surface receptors is hypothesized to prevent the cell from activating the proteolytic function of the effector proteases that mediate apoptosis. The factors that determine the conversion of ICE/Ced3 proteases from pro-enzymes to active enzymes have not been fully elucidated. ICE/Ced-3 proteases may be activated sequentially in a proteolytic cascade (*1*, *17*).

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