PERSPECTIVES: DEVELOPMENT

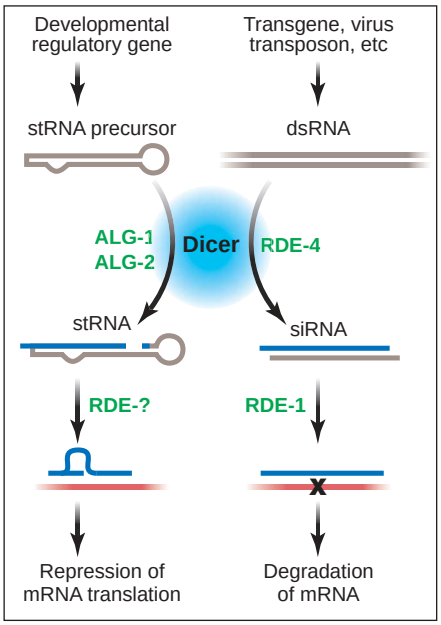
Dicing Up RNAs

Victor Ambros

RNA molecules are a constant source of joy to molecular biologists: They come in all shapes and sizes, and perform diverse informational, structural, and catalytic tricks in living cells. Perhaps less appreciated is their facility for regulating gene expression. Small regulatory RNAs figure prominently in two fascinating phenomena: gene inactivation by RNA interference (RNAi), and the control of gene expression during development. RNAi is implemented by short interfering RNAs (siRNAs), whereas developmental control depends on small temporal RNAs (stRNAs). Parallel research efforts have sought to understand the molecular and genetic basis for these ostensibly separate RNA-based systems of gene regulation. Discoveries reported on page 834 of this week's *Science* (*1*) and in a recent issue of *Cell* (*2*) provide evidence that gene inactivation by RNAi, and the control of developmental timing, are interconnected processes that share certain molecular components. The most prominent of these shared components is the highly conserved nuclease Dicer, which cleaves double-stranded RNA (dsRNA) precursor molecules into stRNAs and siRNAs. These new findings support the notion that siRNAs and stRNAs are different facets of one diversified system for RNA-mediated gene regulation.

In RNAi, a dsRNA triggers destruction of a homologous mRNA that has the same sequence as one of the dsRNA strands. In this way, expression of the gene encoding the mRNA is disabled after the gene has already been transcribed (*3*). RNAi is thought to be a primitive genetic surveillance mechanism that protects cells from intruders such as viruses that use dsRNA intermediates to replicate. In contrast, stRNAs regulate gene expression during normal development of the worm *Caenorhabditis elegans* (*4*, *5*), and probably of other animals as well (*6*). stRNAs are encoded within the genome and contain sequences complementary to specific target mRNAs (*4*, *5*), but unlike siRNAs, they do not destroy the mRNAs that they target (see the figure). For example, in *C. elegans*, the stRNA *lin-4* represses synthesis of the *lin-14* and *lin-28* developmental timing proteins without affecting the amount of *lin-14* and *lin-28* mRNAs, or even affecting their association with polyribosomes (the factories that translate mRNA into protein) (*7*).

Despite these differences, siRNAs and stRNAs share some intriguing similarities. First, they are both ancient in origin. Genetic and molecular analysis of proteins required for RNAi reveals that the core RNAi pathway (including members of the RDE family of RNA-binding proteins) evolved before the divergence of plants and animals (*3*). It is not yet known whether stRNAs are also present in plants and animals—so far, functional studies of stRNAs have been restricted to the worm. However, the worm stRNA *let-7* is conserved in sequence and in its temporal pattern of expression among bilateral animals (*6*), suggesting that worm stRNAs may be flag-bearers for an ancient legion of small regulatory RNA genes. *Lin-4* and *let-7* are called *s*mall *t*emporal RNAs (*st*RNAs) because they act at specific stages during worm development (*6*). It is conceivable that other small RNAs in this class (about 22 nucleotides in length with a strand that is complementary to a target mRNA) could regulate gene expression in response to other sorts of developmental or environmental signals.



Dicing with mRNA destruction. (**Left**) Double-stranded RNA molecules about 22 nucleotides in length (stRNAs) regulate the translation of specific mRNAs during development. The antisense strand of the stRNA (blue) forms a characteristic interrupted hybrid with the 3′-untranslated region of the target mRNA (red), which then cannot be translated into protein. (**Right**) Double-stranded siRNAs are also ~22 nucleotides long. The antisense strand of the siRNA (blue) forms a continuous hybrid with the mRNA target (red), which is then degraded. Both types of small RNA are formed by cleavage of double-stranded RNA precursor molecules by the enzyme Dicer (pale blue) (*1*, *2*). When processing the nonuniform dsRNA precursors of stRNAs, Dicer requires help from ALG-1 and ALG-2 (green), proteins of the RDE family (*2*). Dicer together with RDE-4 is needed to process long uniform dsRNA precursor molecules into siRNAs. The differing effects of mature stRNAs (*2*) and siRNAs (*14*) on the fate of target mRNAs depend on components specific to each pathway.

The most compelling similarity between RNAi- and stRNA-regulated gene expression is that both systems require small RNAs about 21 to 23 nucleotides in length. RNAi in both plants (*8*) and animals (*9*) requires the cleavage of dsRNAs into 21- to 23-nucleotide–sized siRNAs. Similarly, the *lin-4* and *let-7* stRNAs in the worm are also about 22 nucleotides in length, and are processed from longer precursor molecules, which are predicted to form a double-stranded stem-loop secondary structure (*4*, *5*). These similarities in the structure and production of siRNAs and stRNAs hint that they have molecular pathways in common (*3*).

Support for the notion of kinship between RNAi and developmental control by stRNAs comes from the recent reports by Zamore (*1*), Mello (*2*), and their colleagues. These studies indicate that the ribonuclease III–like enzyme Dicer is the central connection between RNAi and stRNAs. Dicer specifically recognizes the ends of dsRNA molecules and bites off ~22 nucleotide chunks as it moves (powered by ATP) along the molecule (*9*, *10*). These ~22-nucleotide Dicer products become siRNAs when processed from a long dsRNA precursor (*11*–*13*), or stRNAs when processed from a *let-7* or *lin-4* RNA precursor (*1*, *2*).

Proof that Dicer activity is required for RNAi comes from studies where loss of Dicer—by depleting *Drosophila* cells of the protein (*1*) or inactivating the *dcr-1* gene in worms (*2*, *14*)—results in the disabling of RNAi. The *dcr-1* worm mutant also displays developmental abnormalities: The hermaphrodites are sterile, suggesting that the Dicer protein is involved in oocyte development (*2*, *14*). Perhaps most remarkably, inactivation of the *dcr-1* gene in the worm causes developmental timing defects similar to those seen in *let-7* and *lin-4* worm mutants. In particular,

Department of Genetics, Dartmouth Medical School, Hanover, NH 03755, USA. E-mail: vambros@dartmouth.edu

like *lin-4* and *let-7* mutants, *dcr-1* animals fail to fully switch to adult morphology at the end of larval development, and instead repeat cell divisions and other events characteristic of much younger larvae (*2*). This suggests that Dicer could be required for the production or activity of *lin-4* and *let-7* stRNAs. Indeed, in worms with defective *dcr-1*, the *lin-4* and *let-7* ~22-nucleotide stRNAs are diminished and longer precursor molecules accumulate in their place (*2*). Similarly, when cultured mammalian cells are depleted of Dicer protein, mature *let-7* stRNA is also reduced, and its precursor dsRNA accumulates (*1*). Thus, the double-stranded stem-loop structures of the *lin-4* and *let-7* precursor RNAs seem to be processed by Dicer in worms, flies, and mammals to generate mature ~22-nucleotide stRNAs (see the figure on the previous page).

These new findings reveal that a common maturation cleavage step links the RNAi pathway and developmental gene regulation by stRNAs. Although further work is required to determine which other gene products and processing steps are common to both pathways, it is apparent that some gene products are relatively specialized. For example, whereas Dicer is required for the production of both siRNAs and stRNAs, RDE-4 is specific to RNAi and the production of ~22-nucleotide siRNAs (*15*). In addition, the RDE family proteins ARG-1 and ARG-2 are required for the production of stRNAs, and do not seem to operate in RNAi (*2*).

An emerging hypothesis is that the RNAi and stRNA systems represent different facets of an ancient and widespread strategy for controlling gene expression through small regulatory RNA molecules (*2*, *3*). This view is embodied in the notion of a dynamic ribonucleoprotein complex that carries out the steps of the overlapping RNAi and stRNA pathways (*2, 15*). According to this model, the 22-nucleotide siRNA or stRNA binds to the target mRNA to form a base-paired hybrid RNA. The fate of the mRNA depends on the nature of the hybrid: A continuous base-pair hybrid between siRNA and mRNA results in degradation of the mRNA (see the figure). In contrast, an interrupted hybrid in which stRNA binds to the 3′-untranslated region of the mRNA does not result in mRNA degradation but rather prevents it from being translated into protein (*2*).

Among the numerous fascinating issues arising from this work is the degree of interplay between the RNAi and stRNA pathways during normal development. For example, is Dicer activity regulated such that it could influence when and how stRNA-sensitive developmental programs are instigated? Are other genes associated with RNAi also involved in stRNA developmental pathways? Does RNAi directly control mRNA stability during normal development? What other classes of small RNAs are produced by enzymes in the RNAi and stRNA pathways? Undoubtedly, future research into small regulatory RNAs will uncover further layers of complexity. Yet, such work is also likely to reveal the unifying principles that underlie what seems to be an ancient and versatile system for controlling gene expression.

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PERSPECTIVES: GEOPHYSICS

Deep Diamond Mysteries

Catherine McCammon

Seismological studies indicate that a boundary at 660 km divides the transition zone from the lower mantle, but in the absence of direct samples from this enigmatic region, the nature of the boundary has remained controversial. In particular, the nature of convection—whether it involves the whole mantle or is layered with a boundary at 660 km—remains open. The tantalizing possibility that diamonds may contain tiny inclusions of lower mantle material was recognized in 1984 (*1*). It took another decade for more convincing evidence for such inclusions to be found (*2*). Similar evidence has now been reported from at least 12 localities on five continents (see the first figure). The inclusions provide insights into the 660-km boundary and, more generally, into mantle chemistry, diamond formation, and mantle dynamics.

The mineral ferropericlase accounts for more than half of the inclusions described so far. Other inclusions consist of enstatite and calcium silicate. They are believed to have existed in the perovskite structure at the higher pressures experienced in the lower mantle. To avoid confusion with their upper mantle counterparts, I will refer to these phases as Mg and Ca silicate perovskite, respectively. All three phases could occur elsewhere in the mantle, but their coexistence in individual diamonds (see the second figure) argues strongly for a lower mantle origin because at shallower depths the minerals would combine to produce different phases.

Ni concentrations provide an elegant means of distinguishing lower mantle mineral assemblages from those originating at shallower depths (*3*). Experiments show that Ni is incorporated preferentially into ferropericlase at high pressures, leaving Mg silicate perovskite depleted (*4*). Upper mantle enstatite normally contains 0.1 to 0.2% Ni by weight, but the enstatite (formerly Mg silicate perovskite) found in lower mantle inclusions contains less than 0.02% Ni (*4*). Rare earth elements are concentrated in Ca silicate perovskite lower mantle inclusions relative to ferropericlase and Mg silicate perovskite, which agrees with experimental work for lower mantle assemblages.

Phases identified in lower mantle assemblages also include quartz (likely converted from the high-pressure mineral stishovite) and the enigmatic tetragonal almandine pyrope phase (TAPP) (*5*). Although chemically similar to garnet, TAPP has a relatively open crystal structure and may have crystallized during the ascent of the host diamond (*6*).

We cannot determine the pressure and temperature conditions of the formation of the inclusions, but the composition of Mg silicate perovskite provides a means to estimate the depth of their origin (*2*, *3*). Mantle Al is concentrated in garnet, which persists to depths greater than 660 km. However, with increasing pressure, garnet eventually transforms to the perovskite structure and hence the concentration of Al in Mg silicate perovskite increases. Nearly all Mg silicate perovskite inclusions have low Al concentrations, which could occur only at the top of the lower mantle where garnet is also present. A small number of inclusions, however, suggest a much deeper origin. One Mg silicate perovskite grain was found to contain

The author is at the Bayerisches Geoinstitut, Universität Bayreuth, D-95440 Bayreuth, Germany. E-mail: catherine.mccammon@uni-bayreuth.de

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Victor Ambros

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