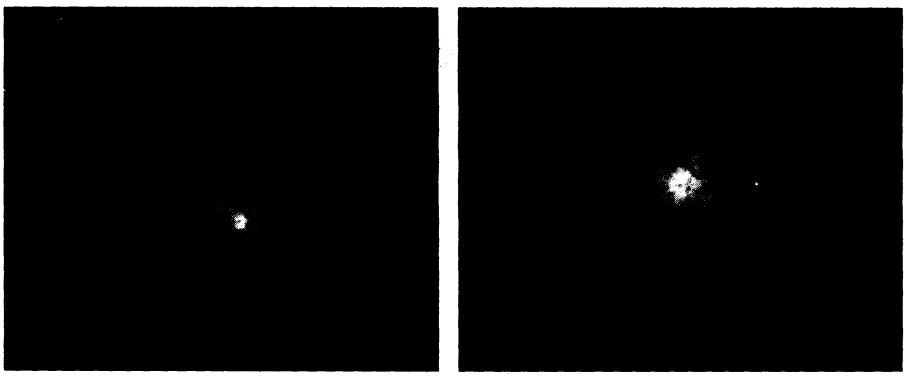
system. To obtain a perfect axial symmetry, the binary period should be short compared with the duration of the eruption.

The idea that binarity is relevant to LBV eruptions was recently revived by the discovery of a 5.5-year period in spectral properties of Eta Carinae (*8*). Spectroscopic and photometric variations on time scales of years are inherent to LBVs, but the very strict periodicity in Eta Carinae is striking and leaves little room for alternative explanations (*8*). The presence of a companion remains, however, difficult to relate to Eta Carinae's eruption or to the bipolar shape of its circumstellar matter. The observed period seems too long to generate axial symmetry and the orbital separation of the two stars too large to allow a companion to trigger an eruption.



A similar mechanism? The geometry of the Homunculus nebula ejected by the luminous blue variable (LBV) Eta Carinae (**left**) is strikingly similar to that of the planetary nebula Hubble 5 (**right**).

Clues may come from comparing the discussion about the mechanism of LBV eruptions and nebular geometry with the very similar debate on planetary nebulae, which form at the end of the evolution of low-mass stars (*9*). Planetary nebulae are mostly either axially symmetric (see the right panel of the figure) or spherical, indicating that the mechanism that produces the debris and the mechanism that shapes it are independent. In contrast, all known LBV nebulae are bipolar, suggesting that the LBV eruption mechanism is also responsible for their shape.

It thus appears reasonable to attribute particular features in Eta Carinae's debris—its knotty jets and streamers (*10*) and a massive cold torus (*11*), which are not known from other LBV nebulae—to binarity. This point of view is strengthened by the fact that similar features occur in some axially symmetric planetary nebulae. But the origin of the eruption is likely to be found in the erupting star itself, rendering the Eddington limit in rotating stars the most promising explanation.

The aftermath of an LBV phase is an almost bare stellar helium core, a so-called Wolf-Rayet (WR) star. WR stars have continuous winds so strong that they almost evaporate the star. It was recently found that their winds are considerably weaker than previously thought (*12*), but it remains true that the WR stars evolving from LBVs end up as objects of relatively low final mass (of 10 $M_\odot$ or less) (*13*).

The final stage of a massive star is determined by the mass of the iron core that it forms at the end of its life. Lower mass iron cores collapse to neutron stars, ejecting the stellar envelope in a supernova explosion. In contrast, massive iron cores collapse into black holes, which—if sufficient angular momentum resides in the core—may trigger an even more energetic event: GRB (*14*).

On average, stars with larger final mass develop more massive iron cores. It is thus conceivable that massive stars, which lose a lot of mass during an LBV and subsequent WR phase, end their lives in a supernova explosion, and that only those that avoid the LBV and WR stage may form GRBs. It then depends on the LBV eruption mechanism how many GRBs form. If the most massive single stars in the Milky Way become LBVs without the "help" of a binary companion, one may expect relatively few local GRBs. As the Eddington instability gets weaker when less heavy elements are present in the stellar envelope, one would expect most GRBs to occur in the early universe, which appears to be compatible with GRB observations (*4*).

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PERSPECTIVES: MOLECULAR BIOLOGY

RNA Interference

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The mechanical sounds of robots and the electronic hum of computers are features of 21st-century life. Despite the joys of increasing automation, simple experiments still produce wonderful new discoveries in molecular biology. The realization several years ago that double-stranded RNA (dsRNA) is a key player in one form of posttranscriptional gene silencing indicated that a new field of great importance was about to be created (*1*). The study of how RNA regulates gene expression—called RNA interference (RNAi)—has an exciting future, and the report by Grishok *et al.* on page 2494 of this issue (*2*) provides further support for such optimism.

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The RNAi phenomenon has been observed in a wide variety of organisms—plants, worms, flies, fungi, and vertebrates (including mouse embryos)—and is best considered a feature of nearly all eukaryotes. In most of these organisms, injection of dsRNA longer than 500 base pairs specifically suppresses the expression of a gene with a corresponding DNA sequence, but has no effect on genes unrelated in sequence. RNAi suppresses gene expression by a posttranscriptional process, although there is convincing evidence, at least in plants, that dsRNA can also regulate DNA methylation (another mechanism for silencing genes) (*3*). Thus, gene-specific regulation by RNA may also control transcription under certain circumstances.

The surprising nature of RNAi is highlighted by the recent discovery of small RNAs, about 25 nucleotides long, in plants displaying posttranscriptional gene silencing (*4*). These small RNAs are complementary to both the sense and antisense strands of the

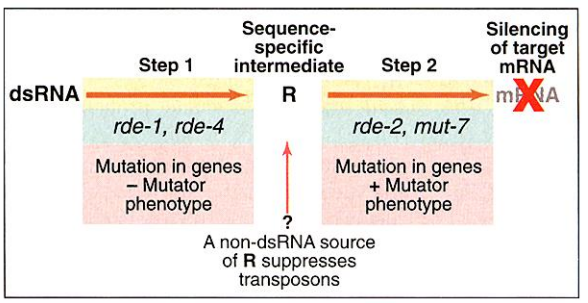
silenced gene, suggesting that they are derived from a dsRNA signal. They are reasonably abundant, perhaps indicating that they can replicate. The spread of gene silencing through the tissue of grafted plants also suggests that replication of a sequence-specific signal is possible.

Posttranscriptional gene silencing by dsRNA requires at least two steps: conversion of the dsRNA into an active species and the subsequent targeting of the mRNA for inhibition by this sequence-specific active species (see the figure). Experiments in vitro with lysates of *Drosophila* embryos—where the addition of dsRNA specifically suppresses the expression of a homologous mRNA but not of control mRNAs—have shown that the targeted mRNA is silenced because it is degraded (*5*). Furthermore, activation of the input dsRNA, as measured by an increase in specific mRNA silencing activity, is observed upon preincubation of the dsRNA in the lysate.

The genetic analysis of Grishok *et al.* assigns genes to these two steps in the RNAi process and predicts the existence of a sequence-specific intermediate, R, common to a variety of "triggers" for gene-specific silencing, including dsRNA. These investigators have previously identified a group of mutants in the worm *Caenorhabditis elegans* that do not show RNAi in response to a dsRNA signal (*6*). Mutations in some of these genes, for example, *mut-7* and *rde-2*, lead to mobilization of endogenous transposons (DNA elements that undergo duplication and insertion at new sites in the genome) in germ cells, the so-called mutator phenotype (*7*). This suggests that some aspect of the RNAi process is responsible for suppression of transposons in the *C. elegans* germ line. Two additional mutants, *rde-1* and *rde-4*, which have a defective RNAi response to injected dsRNA, do not mobilize transposons in the germ line.

Injection of dsRNA into a *C. elegans* hermaphrodite generates RNAi in the first generation (F_1) offspring, and this is usually lost by the F_2 generation. However, the inhibition of genes expressed in the maternal germ line by RNAi can occasionally be inherited by the F_2 and subsequent generations. Grishok and colleagues now show that if RNAi is initiated in the mother, it can be transferred through the sperm of the F_1 generation to the F_2 generation even if the sperm lack the targeted gene. Thus, male germ cells can transmit RNAi, once initiated, even in the absence of the endogenous gene that is silenced. Therefore, the RNAi trait is inherited epigenetically (that is, not through a change in the gene sequence).

With the availability of phenotypes in the F_2 generation, Grishok *et al.* were able to separate the tissue that initiates the inheritable RNAi state (in the mother) from that in which the target mRNA is degraded (in her F_2 descendants). This enabled them to test if each of the four mutations that do not yield an RNAi response are also defective for initiating heritable RNAi. They found that both *rde-1* and *rde-4* mutant worms can inherit RNAi-mediated silencing of specific genes, even though by themselves they cannot produce RNAi in response to injected dsRNA. In contrast, the *mut-7* and *rde-2* gene products are essential for producing inherited RNAi in the F_2 progeny, indicating that they are important for either initiation or maintenance of gene silencing, or both.



The RNAi two-step. Double-stranded RNA (dsRNA) is converted into a sequence-specific intermediate (R), which targets degradation of mRNA. Gene mutations and their transposon (mutator) activity are shown at each step. A possible source for R that does not involve dsRNA is indicated.

To resolve these possibilities, Grishok and colleagues generated RNAi by injection of dsRNA into a worm deficient in either *mut-7* or *rde-2*, but whose offspring were either wild type or mutant for these genes. By assaying RNAi in the offspring, the investigators showed that both *rde-2* and *mut-7* are required to carry out direct posttranscriptional silencing in an individual worm, but not to initiate heritable RNAi in the worm's forebears. These results suggest a scheme (see the figure) in which *rde-1* and *rde-4* are required to generate a sequence-specific intermediate (R) from dsRNA, whereas *rde-2* and *mut-7* are required to respond to R by silencing the targeted mRNA.

The possible functions of genes important for RNAi indicate an exciting biochemistry. The *rde-1* gene, a member of a large family (composed of 22 genes in *C. elegans*), encodes a homolog of the *Drosophila* sting protein, which is required to silence a repetitive gene family (*8*). The *mut-7* gene encodes a protein with homology to the nuclease domains of ribonuclease D and to the protein that is mutated in Werner syndrome (a disease of rapid aging) (*7*). Two genes of the fungus *Neurospora*, *qde-1* and *qde-3*, are required for "quelling," a phenomenon similar to RNAi. The *qde-3* gene is a homolog of a member of the DNA helicase gene family, whereas *qde-1* is homologous to a family of genes whose proteins have RNA-dependent RNA polymerase activity (*9*). The possibility that RNAi is involved in RNA replication has been strengthened by the recent discovery that *ego-1* mutants of *C. elegans* are also defective in RNAi (*10*). The *ego-1* gene also encodes a homolog of an RNA-dependent RNA polymerase.

Recall that the *rde-1* and *rde-4* mutants have an intact mechanism for transposon silencing despite a complete lack of dsRNA-induced gene silencing. This observation suggests that dsRNA is not a signal for suppressing transposon mobilization. In contrast, *rde-2* and *mut-7* mutants do show the mutator phenotype; these genes are important both for suppressing the movement of transposons and for the silencing of genes in response to dsRNA. The results suggest that if suppression of transposon movement is a response to a sequence-specific intermediate, R, then this intermediate can be generated from sources other than dsRNA (see the figure). As Grishok and co-workers suggest, the best guess for the nature of the intermediate R is a small RNA similar to that detected in plants (*4*). If this inference is correct, then RNAi may play a broader role in regulating gene expression than is now suspected. New findings strongly suggest that small RNAs (21 to 22 nucleotides long) are the sequence-specific intermediate, R, in RNAi. Hammond *et al.* (*11*) demonstrate that a complex that coelutes with RNAs of ~25 nucleotides produces degradation of a target mRNA in vitro. Meanwhile, Zamore and colleagues (*12*) show that 21- to 23-nucleotide RNAs are generated from dsRNA added to in vitro reactions and that the target mRNA is cleaved in patterns with a spacing of 21 to 23 nucleotides.

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