other crystallographic discontinuity forces it to deviate or stop. Processes to do so include thermomechanical treatments during hot-rolling from slab to plate, sheet, or bar (*6*), thermal cycling treatments that manipulate the transformations that occur during quenching (*7*), and tempering treatments that interpose fine distributions of nanosized second-phase particles to disrupt the intragranular crystallography (*7*).

The complexity of the many possible microstructures of steel often makes it difficult to decipher what the effective grain size really is. Recently, electron backscatter diffraction (EBSD) has been used to map crystallographic patterns in the microstructure of steels and to identify the effective grain sizes in several important microstructures (*4*, *8*). This information is being systematically exploited in steel research, particularly in various "supersteel" projects in Asia (*9*).

The second generic approach to lowering T_B is to decrease the effective yield stress at the crack tip. A simple way to do this is to replace thick beams with a laminate of thin sheets, a technique used by 19th-century engineers in early steels. A modern alternative is to design the microstructure so that it spontaneously delaminates in the stress field at the crack tip, effectively dividing itself into a laminate of thin sheets and lowering the effective stress at the crack tip by a factor of 2 or more (*10*). This approach was used (but, to my knowledge, never published) by manufacturers of steel line pipe more than 20 years ago. It lowers T_B but also lowers the toughness in the ductile mode, presumably because the weaker of the delaminated sheets fracture under small loads. Particularly in the case of ultrahigh-strength steels, high toughness is required for structural reliability in service.

Kimura *et al.* now describe a clever method for combining grain refinement and delamination to obtain a particularly promising combination of properties. The steel is processed to high strength through a combination of grain refinement and precipitation hardening. The grain refinement is accomplished by bar-rolling, producing a fibrous texture that delaminates ahead of and perpendicular to the propagating crack tip, thereby relaxing the crack-tip stress concentration without producing planes of weakness to lower toughness in the ductile mode.

Defeating the ductile-brittle transition in steel is always difficult, and as the figure makes clear, this becomes particularly challenging for an ultrahigh-strength steel. As Kimura *et al.* show, this challenge can be met with low-alloy steel by tailoring the microstructure to achieve high strength while resisting brittle fracture. As advances in the understanding of brittle fracture and the control of microstructure feed into the design of new structural steels, more exciting results of this kind can be expected.

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MOLECULAR BIOLOGY

Slicing and Dicing for Small RNAs

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A new type of small RNA and mode of gene regulation is discovered in fly and mammals.

Nearly two decades after gene silencing was first described (*1*), the field continues to reveal new and diverse roles for the small RNA moieties involved. These functions include modulating the translation of messenger RNA (mRNA) into protein, establishing chromosomal architecture, regulating stem cell renewal, and providing defense against viruses and mobile genetic elements (transposons) that could cause deleterious mutations (*2*). Three major classes of small RNAs have been defined in plants and animals: microRNAs (miRNAs), small interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs). Previous work has suggested that dedicated pathways generate each class of small RNA. Now, six recent studies, including one by Ghildiyal *et al.* on page 1077 of this issue, reveal an interplay of these canonical pathways in generating a new class of endogenous siRNAs (endo-siRNA) (*3*–*8*) (see the figure).

miRNAs are encoded by the genome and processed to 22–base pair products that interact with homologous mRNAs to modulate their translation and affect developmental processes. siRNAs are 21 base pairs long and generated from double-stranded RNA precursors such as those from viruses and endogenous transposons. Recently, piRNAs have been described in the germ line of flies and mammals (*9*). They are approximately 25 base pairs long with homology to transposable elements and other sequences.

In the fly *Drosophila melanogaster*, the enzyme Dicer-1 cleaves double-stranded miRNA precursors, and the resulting products bind to the protein Argonaute1 (Ago1). siRNAs are generated by Dicer-2 from double-stranded RNA precursors, and bind to Argonaute2 (Ago2), whose "slicer" activity cleaves homologous RNAs. piRNAs are generated by a dicer-independent mechanism that relies on the slicer function of a separate clade of argonaute proteins consisting of Piwi, Aubergine, and Argonaute3 (*9*). These proteins use antisense transcripts (complementary to mRNA) encoded by piRNA clusters in the genome that harbor transposable element fragments, and target the destruction of transposon sense transcripts. New studies report sequencing of extensive pools of small RNAs from various somatic and germline sources in flies and mice (*3*, *4*, *6*–*8*) and identify a new type of siRNA that is homologous to transposons and protein-encoding gene sequences.

Ghildiyal *et al.* found that although most small RNAs in fly somatic cells are miRNAs, some have characteristics of siRNAs—equal quantities of sense and antisense orientations, a 21–base pair length, and 2′-*O*-methyl modifications at the 3′ end. Most

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of these endo-siRNAs are homologous to transposable elements, and many originate from piRNA clusters. Curiously, mutations of the genes encoding Dicer-2 (*dcr-2*) and Ago2 (*ago2*) did not eliminate all of these small endo-siRNAs, suggesting that Dicer-1 and Ago1 can generate and bind, respectively, some siRNAs under these circumstances. Moreover, somatic pi-like RNAs, which exhibit characteristics of the germline namesake, were found in *ago2* mutants, raising the possibility of somatic activity of the Piwi family of argonautes. Mutations in *dcr-2* or *ago2* increased the expression of certain transposons, thus linking endo-siRNAs to the control of transposable elements in the soma.

Similarly, Czech *et al.* (*4*) found endo-siRNAs in somatic and germline cells of flies by analyzing sequences from three sources: those bound to Ago1 and Ago2, those present in small RNA libraries, and those from flies with mutations in the canonical miRNA and siRNA pathways. They observed that the protein Loquacious (Loqs), which associates with Dicer-1 for miRNA production, also interacts with Dicer-2 and is required for some endo-siRNA formation. Okamura *et al.* (*5*) analyzed the processing of products of endogenous long RNA hairpin (hp) genes in flies. These hpRNAs are processed by Dicer-2, in conjunction with Loqs, and then bind to Ago2. The siRNAs generated from these genes can silence selected mRNAs using the slicer function of Ago2 rather than by translational repression. Collectively, these new fly studies illustrate the interplay among mechanisms generating various classes of small RNAs.

In a complementary study, Kawamura *et al.* (*6*) sequenced small RNAs bound by Ago2 in cultured *Drosophila* cells. The endo-siRNAs recovered showed homology to a subset of transposons. Depletion of Dicer-2 and Ago2 in these cells reduced the quantities of endo-siRNAs and increased the expression of the corresponding transposons. Endo-siRNAs with homology to transposons were also found in fly ovaries, and their function depended on Dicer-2 and Ago2, revealing that the germ line has both siRNA- and piRNA-generating machineries.

The production of small RNAs by slicing and dicing has also been reported in mouse germline cells. Watanabe *et al.* (*7*) and Tam *et al.* (*8*) sequenced small RNAs from mouse oocytes and found both siRNAs and piRNAs. Depletion of Dicer and Ago2 in these cells reduced the number of siRNAs while increasing that of transposons and certain mRNAs. Because there is only one *Dicer* gene in mammals, these results demonstrate that this enzyme functions in both miRNA and siRNA production. The piRNAs found in oocytes were bound to MILI, a mammalian counterpart of Piwi. In this case, piRNAs and siRNAs sometimes correspond to distinct transposons, indicating preferred mechanisms for their generation, although transposon-rich loci could also give rise to both siRNAs and piRNAs.

It is not unexpected that endo-siRNAs to transposable elements would be found in animals, given their presence in plants (*10*) and fungi (*11*). Based on the sophisticated immune systems of vertebrates, early thinking posited that the small RNA–processing machinery involved Dicer primarily for the maturation of miRNAs. The discovery of a piRNA system that does not involve Dicer, but protects the germ line from transposon expression, reinforced this view. Although it is difficult to compare the strength of gene silencing across species, *Drosophila* somatic cells require multiple copies of a transgene to reduce the expression of homologous transcripts (*12*), and the effect is not as great as seen with a few copies of many transgenes in plants (*2*). Perhaps the evolution of robust immune systems in animals relaxed the selection pressure for a strong response of the siRNA defense against viruses. Thus, the piRNA system might represent a germline–accentuated mechanism to ameliorate the mobility of transposable elements, and thus reduce the occurrence of mutations. However, the finding that endo-siRNAs are present with homology to complementary pseudogenes and homologous genes as a newly recognized mode of gene regulation (*8*), and that long hpRNA genes produce siRNAs that can affect gene expression (*5*), might suggest that these roles for siRNAs have maintained the Dicer-dependent machinery at some level in both somatic and germline cells of animals. This machinery could still metabolize double-stranded viral and transposon RNAs.

Dual small RNA–processing mechanisms in animals might reflect a response to transposons that evolve to evade silencing by one or the other mechanism in the never-ending arms race between transposable elements and the host genome. Transposons in mouse oocytes have somewhat distinct profiles of siRNAs or piRNAs, indicating that certain elements can avoid one of the mechanisms of silencing. Any transposon that escapes both systems will likely proliferate into new genomic positions and produce substrates that could be subject to one or the other type of silencing. The piRNA loci are graveyards of transposable elements whose ghosts in the form of small RNAs commit fratricide against their homologous brethren. From a selfish DNA point of view, it is interesting to contemplate how the transposons are "tricked" by the host to insert into piRNA loci and in turn whether classes of transposons evolve mechanisms to avoid these locations for insertion.

SMALL RNAs

Germ line

Type	Length (nucleotides)	Mechanism of generation	Biological role
miRNA	21–23	Dicer dependent	Regulation of translation
piRNA	24–27	Dicer independent, Piwi clade dependent	Transposon regulation and unknown functions
endo-siRNA	21–23	Dicer dependent	Transposon regulation
Soma			
miRNA	21–23	Dicer-1 dependent (in *Drosophila*)	Regulation of translation
piRNA like	24–27	In Ago2 mutants (in *Drosophila*)	Transposon regulation
endo-siRNA	21–23	Dicer-2/Ago2 dependent (in *Drosophila*)	Regulation of transposons, mRNAs, and heterochromatin
hpsiRNA	21–23	Dicer-2/Ago2/Loqs dependent (in *Drosophila*)	Regulation of mRNAs

Small RNAs. A summary of small RNAs found in germline and somatic cells and their identified functions.

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