PERSPECTIVES: DNA EVENTS

An RNA Microcosm

David Baulcombe

Micro-RNA (miRNA) was first identified as a class of regulatory RNA in animals with perhaps hundreds of different members (*1*). It appeared to represent a previously unsuspected layer of regulation in higher organisms, but from the initial reports it was not clear what was being regulated by this RNA, or how. Now a number of studies, including two by Llave *et al.* (page 2053) and Hutvágner and Zamore (page 2056) published in this issue (*2*, *3*), are revealing that miRNAs and other similar tiny RNAs are involved at many different levels of genetic control in plants and animals.

The miRNA story started with the discovery of tiny RNAs, known as small temporal RNAs (stRNAs), in *Caenorhabditis elegans*. It now transpires that these stRNAs—*lin4* and *let7*—are a subclass of the many miRNAs discovered since. Like the miRNAs they are 21 nucleotides in length, are processed from a longer double-stranded precursor by a ribonuclease III known as Dicer (*1*), and are transcribed from inverted DNA repeats in intergenic regions.

A second class of short RNAs, the small interfering RNAs (siRNAs) (*4*), are also ~21 nucleotides in length and processed by Dicer from a longer double-stranded precursor. Initially the distinction between miRNAs and siRNAs appeared clear: miRNAs are specified by endogenous inverted DNA repeats, whereas siRNAs are derived from transgenes, exogenously introduced double-stranded RNA, or viruses. Moreover, in contrast to the single-stranded miRNAs, the siRNAs were thought to be double-stranded, with two unpaired nucleotides at each 3′ end.

Originally it was also thought that miRNAs and siRNAs have different modes of action. siRNAs were perfectly complementary to the target RNA that they regulate and associated with a ribonuclease complex (RNA interference specificity complex, or RISC). Base pairing between the siRNA and the target RNA sequence guides the RISC so that it cleaves precisely and specifically in the region of siRNA complementarity.

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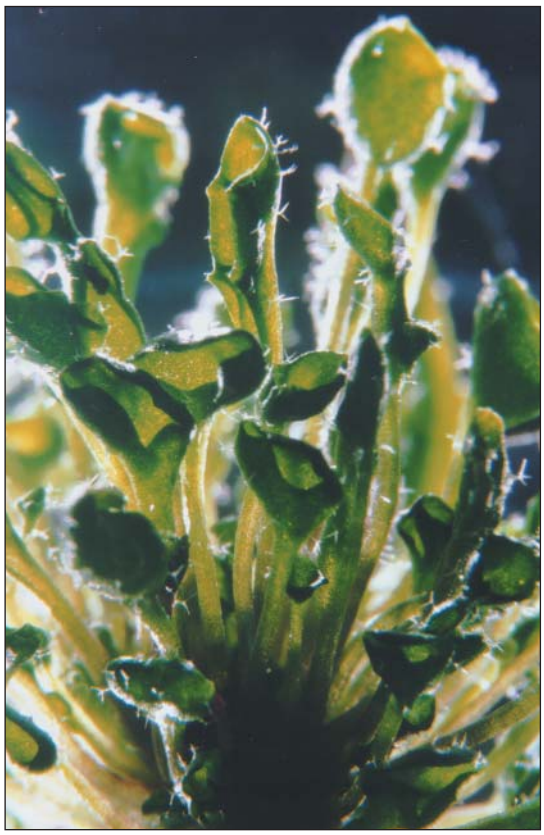
In contrast, as initially described, miRNAs matched their target RNA only imperfectly and caused either translational arrest, as with stRNAs, or directed some other, as yet undefined, mechanism of genetic regulation.

However, these tidy distinctions are now blurred. For example, in *C. elegans* the siRNAs, like miRNAs, can be single stranded (*5*). In addition, a *let7* stRNA acts like an siRNA by directing RNA cleavage in *Drosophila* and HeLa cells (*3*) if it encounters a perfectly complementary target RNA. Moreover, irrespective of whether the target is perfectly matched, the *let7* RNA occurs in a complex with RISC proteins. Thus, it seems that the same RISC is recruited by both siRNA and stRNA and that this complex mediates RNA cleavage if there is perfect complementarity with the target RNA, and translation arrest if there is a mismatch.

The properties of an *Arabidopsis* miRNA (miR171/miRNA39) also reveal functional similarity of miRNA and siRNA. This miRNA resembles an siRNA in that it is perfectly complementary to a family of mRNAs. In addition, it directs specific cleavage of the putative target RNA in a heterologous plant host (*2*) and, in *Arabidopsis*, the target mRNA cleavage product was detected in inflorescences when miR171/miRNA39 was abundant. It is likely therefore that miR171/miRNA39 directs developmentally controlled cleavage of a specific family of mRNAs.

Until now miR171/miRNA39 was the only example of an miRNA that perfectly matches a target. However, if up to three mismatches were allowed, putative mRNA targets were identified for 14 of 16 miRNAs in *Arabidopsis* (*6*). These partially mismatched plant examples are likely to be biologically relevant because randomized miRNA sequences matched much less frequently than the native sequences. Strikingly, most of the putative targets of these miRNAs, like the target of miR171/miRNA39, encode proteins that are implicated in meristem identity and cell division (*6*). It is likely therefore that the miRNAs coordinate regulation of growth and development in plants as illustrated in the figure. The computational method did not, however, identify potential targets of miRNA in animals even when mismatches were allowed (*6*).

Another potential function for miRNA/siRNA has been found in the ciliated protozoan *Tetrahymena*, which has two nuclei. The transcriptionally silent micronucleus has an intact genome, whereas the transcriptionally active macronucleus has extensive and defined DNA deletions. Short regulatory RNAs that correspond to the deleted DNA have been implicated in the genome



A mutant phenotype due to loss of miRNA regulation? *Arabidopsis PHB* mRNA is normally expressed on the adaxial (upper) leaf surface and is a potential target of miRNA because it has an miRNA complementary region (*6*). Dominant *phb* mutations disrupt this putative miRNA target and lead to ectopic expression of *phb* mRNA. Correspondingly, the leaves are rod-shaped or trumpet-shaped (shown here) with adaxial characteristics around their circumference (*9*). Accumulation of the wild-type *PHB* mRNA might be restricted by the presence of the corresponding miRNA in the abaxial (lower) parts of the leaf.

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rearrangement during macronucleus formation (*7*). These RNAs have the 5′-phosphorylated and 3′-hydroxylated termini that are characteristic of processing by Dicer-like enzymes, and so it is likely that they are members of the siRNA/miRNA family of molecules. However, they are not involved in mRNA turnover or translation arrest. A more likely role is in scanning the macronuclear genome and directing DNA deletion (*7*).

Intriguingly, the *Tetrahymena* RNAs implicated in macronuclear genome rearrangement are 28 nucleotides in length (*7*)—longer than the usual 21 nucleotides of miRNAs and siRNAs. This size difference may be significant because in *Arabidopsis* and tobacco there are also longer siRNAs/miRNAs that, like the macronuclear RNA, may influence genome rearrangements (*8*). These plant RNAs are up to 26 nucleotides in length, correspond to various retroelements, and might suppress transcription or transposition of these elements. Perhaps length determines the subcellular location of short regulatory RNAs. If they are 21 nucleotides in length they could be associated with RISC, irrespective of whether they are involved in RNA cleavage. Longer RNAs may be excluded from RISC and thus available to guide other complexes that target nucleic acids in a nucleotide sequence–specific manner.

Are tiny RNAs, as suggested (*1*), the biological equivalent of dark matter—all around us and important but difficult to detect? The answer is almost certainly yes, although simply knowing that this "dark matter" exists does not necessarily make it easy to study, as illustrated by the difficulty of identifying miRNA targets in animals (*6*). Nevertheless, within 12 months of the first miRNAs report, they and similar tiny RNAs have been implicated in control of translation, RNA cleavage, and genome rearrangement. I suspect that, in due course we will find out that base pairing of miRNA/siRNA family members affects many other layers of genetic regulation.

References

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PERSPECTIVES: SURFACE CHEMISTRY

Oxidation of Metal Surfaces

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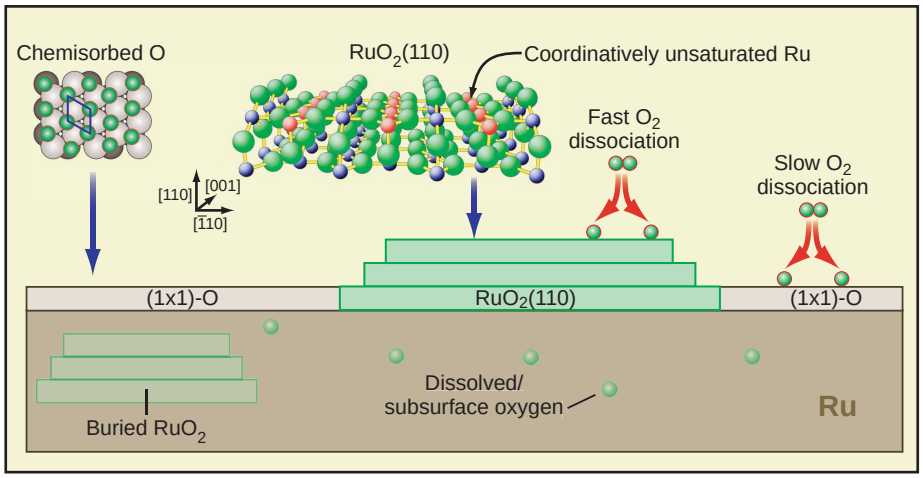
Oxidation is often associated with the unwanted corrosion of materials (for example, in automobiles). But under controlled conditions, oxidation may assist the production of catalysts, semiconductor devices, or protective and functional oxide films.

Despite this economic importance, knowledge of the atomic-scale processes behind metal oxidation remains limited (*1*). On page 2033 of this issue, Thürmer *et al*. (*2*) address this issue by investigating the oxidation of supported lead crystallites at 370 K. With time-lapse scanning tunneling microscopy, the authors show that ultrapure Pb clusters are not susceptible to corrosion when high doses of molecular oxygen from the gas phase are applied. In contrast, the presence of trace surface impurities initiates the growth of an oxide layer even at low oxygen exposures. The deliberate introduction of impurities may thus pave the way for the controlled structuring of metal surfaces by nanometer-sized oxide particles.

At the heart of metal oxidation is the complex interaction of oxygen with the metal surface. The initial stage of oxidation requires a supply of atomic oxygen, together with sufficient mobility of metal atoms on the surface. Therefore, breaking the internal bonding of molecular oxygen (dissociation) on the surface represents the first elementary step in the process of corrosion. Because ultrapure Pb crystallites are chemically resistant to molecular oxygen, the dissociation of O_2 requires the introduction of surface impurities (*2*), an effect that may be relevant to related metal systems. The subsequent oxide growth proceeds easily, as Pb atoms are very mobile at 370 K.

In contrast to Pb, transition metal systems readily dissociate molecular oxygen to provide atomic oxygen, which can form stable chemisorbed overlayers, the precursor to oxidation. The dissociation propensity of transition metal surfaces decreases steeply on approaching the saturation coverage of chemisorbed oxygen. Therefore, oxygen uptake beyond the saturation coverage [one monolayer in the case of Ru(0001) (*3, 4*) (see the figure)] becomes the rate-limiting step for oxidation. With increasing oxygen coverage, the binding energy per O atom also decreases, so that



The rich oxygen chemistry of ruthenium (0001). The oxidation of Ru(0001) is one of the best studied systems in the literature (*10*–*12*). Chemisorbed oxygen, surface oxide, buried oxides, and subsurface oxygen may coexist in the near-surface region. This complexity is characteristic of the oxygen chemistry of many transition metal surfaces.

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