Previews

MicroRNAs Guide Asymmetric DNA Modifications Guiding Asymmetric Organs

In plants and animals, microRNAs have been shown to regulate target genes by inhibiting translation or altering target mRNA stability. In this issue of *Developmental Cell*, Bao et al. extend the known mechanisms of action of microRNAs to RNA-directed DNA methylation, a mechanism previously associated only with siRNA-mediated gene silencing.

How much information can be stored in a short stretch of 20–22 ribonucleic acids? The emerging answer is quite a lot. The studies on mechanisms of target regulation by corresponding microRNAs continue to uncover novel modes of regulation. In this issue of *Developmental Cell*, Ying Bao and his colleagues in Kathy Barton's lab (Bao et al., 2004) provide compelling evidence for information transfer from processed mRNA:miRNA interactions into specific template DNA modifications.

It has been known for some time that a small group of transcription factors, termed class III HD-Zip proteins, regulate key steps in establishment of lateral organ asymmetry throughout flowering plants (McConnell et al., 2001; Emery et al., 2003; Jaurez et al., 2004). In *Arabidopsis* three (*PHB*, *PHV*, and *REV*) of the five members of this small gene family are expressed in a polar manner in all lateral organs, such as leaves and floral organs. Moreover, in each of the three genes, a single nucleotide change within a short sequence complementary to microRNAs miR165 and miR166 confer semidominant phenotypes with adaxial cell types developing in abaxial positions. Thus, base pairing between miR165/166 and its targets negatively regulates members of the class III HD-Zip gene family. How is such negative regulation achieved? Based on the action of other miRNAs, vastly different mechanisms could be proposed. Pioneering studies in worms demonstrated that miRNA (lin-4) binding to the 3′ UTR of target mRNAs resulted in translation attenuation (Olsen and Ambros, 1999). However, the near-perfect complementarity of plant miRNAs to their target mRNAs (Rhoades et al., 2002) suggested that they may act via mRNA cleavage in a manner similar to siRNAs. The detection of cleavage products for many miRNA-regulated targets, including *PHV*, supported this hypothesis (Tang et al., 2002). Yet, miR172, which exhibits extensive complementarity with a target (*AP2*), has been shown to act at the level of translation (Aukerman and Sakai, 2003), indicating that simple rules are unlikely to be universal.

The above mechanisms fail to explain the increased accumulation of *PHB* transcript in *Arabidopsis phb-1d* mutants, nor the accumulation of *RLD* transcript in maize *Rld-O* dominant mutants (orthologous to *REV* of *Arabidopsis*) (McConnell et al., 2001; Jaurez et al., 2004).

Seeking alternative mechanisms, Bao et al. examined another type of modification, DNA methylation, previously associated only with siRNA-mediated silencing mechanisms (Matzke et al., 2001). Using both methylation-sensitive restriction enzymes and bisulfite sequencing, differential methylation patterns among *PHB* exons were observed in wild-type plants. The great majority of molecules from exons 12 and 13 (3′ to the miRNA binding site at the junction of exons 4-5) were heavily methylated while exon 2 was unmethylated. In contrast, exons 12 and 13 remained predominantly unmethylated in *phb-1d* mutants, suggesting that miR165/166 may be responsible for the methylation patterns observed in wild-type. What could be the guide for such asymmetric methylation? miR165/166 has a 17–18 basepair match to its targets only after RNA processing, as the miR binding site is comprised of the end of exon 4 and the beginning of exon 5. Therefore, a mechanism by which miR-containing RISC complexes recognize the processed mRNA is implicated. When and where in the cell will such recognition take place? Bao and his colleagues take an elegant genetic approach to show that this interaction must occur at the transcription template site. When assaying for methylated DNA in the F1 between the *phb-1d* mutant in Ler background and a wild-type Col background, almost all methylated molecules originate from the wild-type Col DNA. How can the RISC complex recognize this specific DNA allele and not the mutant one? Bao et al. propose that during transcription, but following splicing, miR165/166 guides a RISC complex to the DNA and serves as a signal for DNA modifications (see Figure 6 of Bao et al.). Is methylation the primary and only modification that takes place? Many studies have shown that normal transcription can be carried out on heavily methylated DNA. Moreover, mutants deficient in primary components of the DNA methylation machinery often fail to display morphological alterations. Therefore, it is more likely that methylation provides a hallmark rather than a mechanism for chromatin configuration alterations that take place.

What are the implications of multiple pathways for negative regulation mediated by interactions between mRNAs and their corresponding microRNAs? It is becoming clear that not all targets are regulated in the same way and that regulation spans all levels of gene expression. Some types of regulation may be rapid and potentially transient, such as an inhibition of translation, while others, such as modification of chromatin, may last longer in a developmental sense. That *AGO1* and *DCL1* do not appear to alter miR165/166-mediated DNA methylation suggests that there may be unique RISC complexes for specific miR-mRNA pairs or activities. What is the relative importance of such specific complexes in specific cell types? The Barton group provides us with some initial indications. The DNA methylation phenomenon cannot be equally detected in all cell types. Inflorescence meristems of *ap1 cal* plants as well as wild-type siliques exhibit very low levels of methylation, in contrast to differentiating tissues that exhibit higher levels of methylation. This observation could be

explained either by a lack of miR165/166 expression in these tissues or by a lack of one or more of the specific RISC-mediating complexes. miRNA genes are standard RNA polymerase II-transcribed genes that can be expressed in complex spatial and temporal patterns (Aukerman and Sakai, 2003; Parizotto et al., 2004; Jaurez et al., 2004). In addition, the *Arabidopsis* genome encodes nine different Argonaute-like proteins, some of which are spatially and temporally regulated, suggesting that transcriptional regulation of specific RISC components also accounts for variation in miRNA-mediated gene regulation. This leads to the conspicuous questions of whether specific miR:mRNA pairs are recognized by specific RISC complexes or whether they can be recognized by multiple RISC complexes with different activities, and, in addition, whether certain cell types predominantly have specific types of RISC activities. It seems, as with many aspects of biology, if one can imagine a mechanism, it has evolved somewhere and we need only to look to find it.

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Mitochondrial Programmed Cell Death Pathways in Yeast

Whether or not yeast cell death is altruistic, apoptotic, or otherwise analogous to programmed cell death in mammals is controversial. However, growing attention to cell death mechanisms in yeast has produced several new papers that make a case for ancient origins of programmed death involving mitochondrial pathways conserved between yeast and mammals.

Defining Programmed Cell Death
In the early days when cell suicide was defined only in morphological terms (apoptosis), the field languished and was largely disbelieved until the discovery of a biochemical marker (DNA ladders resulting from endonuclease activity) and a genetic marker, the *bcl-2* genes of humans and *C. elegans* (*CED-9*) (Hengartner, 2000). The genetic death pathway constructed from landmark studies in *C. elegans* first connected the Bcl-2 family to a biochemical pathway involving Asp-cleaving cysteine proteases now known as caspases. Subsequently, many genes were identified in *C. elegans*, *Drosophila*, *Xenopus*, and mammalian model systems based on the ability of these genes to enhance or suppress cell death

and to alter the development or health of animals (Hengartner, 2000). Importantly, these models served to identify genes that evolved for the purpose of mediating autonomous altruistic cell death, thereby defining the term "programmed cell death" (apoptotic or nonapoptotic).

Is Programmed Cell Death in Yeast also Apoptotic Death?
Despite enormous progress, we still know fairly little about how the caspases of *Drosophila* and *C. elegans* actually mediate cell death and facilitate engulfment and destruction of cell corpses. Even in mammals, where more than a hundred caspase substrates have been identified, we have only begun to explain why apoptotic cells exhibit their characteristic features (e.g., DNA ladder formation, blebbing, chromatin condensation). But a few key observations, such as the finding that caspase-3 cleaves the inhibitor of the DNA-laddering endonuclease in mammals (Enari et al., 1998), have encouraged investigators to redefine the term "apoptosis" as a caspase-mediated death. The need to clearly articulate the nomenclature applied to cell death is emphasized by more recent discoveries of caspase-independent death involving cathepsins, autophagy, and potentially many other less well understood pathways.

Evidence that single-cell organisms have genetically programmed self-destruct mechanisms is based in part