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MOLECULAR BIOLOGY Hiding in Plain Sight

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The roles of RNA in protein synthesis are well known—as a template for translation of cellular proteins and as ribosomal and transfer RNA (tRNA). However, more recent studies have elucidated the intricate functions of other abundant types of RNAs, such as micro RNAs (miRNAs) and small inhibitory RNAs (siRNAs), which control the expression of target messenger RNAs (mRNAs) (1). Despite this extensive catalog of functional RNAs, many transcribed RNAs still lack an obvious protein product or the characteristic structure of known functional RNAs. Except for a few examples involved in gene silencing and imprinting (2,3), the importance of so-called long noncoding RNAs (lncRNA) remains controversial (4,5). On page 336 of this issue, Kondo *et al.* (6) show that an RNA called *polished-rice* (*pri*) has features of a lncRNA, but encodes tiny peptides that control gene expression during development in the fruit fly *Drosophila melanogaster*.

The *pri* gene was first identified as *mille-pattes* (*mlpt*) in the flour beetle, *Tribolium*, by virtue of its developmental role in body patterning (7). The same gene is responsible for the defects in leg, embryonic epidermis, and respiratory system of *pri* mutants [(also called *tarsal-less* (*tal*) in *Drosophila* (8,9)]. Surprisingly, the gene underlying these important functions does not encode a single large protein or functional RNA, but rather four or five small peptides of 11 to 32 amino acids that are highly conserved among insects (10). Unlike many bioactive peptides, Pri peptides are not processed from a longer precursor protein. Instead, the *pri* RNA is polycistronic and encodes several redundant units that are independently translated.

Kondo *et al.* used the epidermal phenotype of the *pri* gene to investigate the function of its peptide products in the *Drosophila* embryo. Specialized epidermal cells build apical projections (bristles) known as trichomes. A master gene, *shavenbaby* (*svb*), controls trichome formation: *svb* mutants lack trichomes, and *svb* expression determines where trichomes form. Indeed, most evolutionary changes in insect trichome patterning are due to modifications of *svb* cis-regulatory elements (11). *svb* encodes a multidomain transcription factor that activates expression of trichome-promoting genes (12). *pri* mutants also lack trichomes and exhibit altered expression of *svb* transcriptional targets. They do not, however, affect *svb* expression.

How does *pri* work with *svb* to differentiate trichomes in the epidermis? The critical interaction requires *pri* as a temporal switch during trichome formation. Pri peptides direct removal of a large amino-terminal fragment of the Svb protein that contains a transcriptional repression domain. This removal switches Svb from a repressor to an activator of transcription (see the figure). In the absence of *pri*, Svb retains its repression domain, and thus prevents trichome formation. The ability of Pri peptides to direct proteolytic cleavage

of a transcription factor within the same cell is a new function for bioactive peptides. How these peptides impart specificity to a protease (and to which protease) is still not clear.

Because the *pri/tal/mlpt* gene is highly conserved and is found in all insects, it is not merely a derived character of *Drosophila*. Furthermore, *pri* mutant flies have additional phenotypes that do not require *svb*, so Pri peptides likely act on other proteins as well. Remarkably, despite the sequence conservation of Pri peptides, their function in insects appears to have evolved rapidly: *pri/tal* does not seem to play a role in embryo segmentation in flies as *mlpt* does in beetles. This highlights the exciting possibility of a new class of regulatory molecules with diverse and dynamic functions that may be hiding in plain sight.

Because of their small size, Pri peptides cannot be identified by genome annotation or by protein prediction algorithms whose threshold of detection is about 100 amino acids. Like the miRNA genes *lin-4*, *let-7*, or *bantam*, the *pri/tal/mlpt* gene was identified by classical forward genetics (starting with a mutant phenotype and then identifying the underlying gene). One exciting question that remains unanswered is how many functional peptides might be hidden among RNAs. Some might be encoded by short open reading frames found in 5'-untranslated regions of mRNAs. An estimated 40% of *Drosophila* mRNAs contain such uORFs and some show signs of evolutionary conservation, suggesting that they are translated (13). Ribosome profiling, which sequences ribosome-bound mRNAs, has validated the translation of a number of uORFs in yeast (14), and vertebrate genomes have similar percentages of uORF-containing transcripts, which await similar analysis (15). When added to the lncRNAs of unknown function, there are many places to look for additional regulatory peptides like Pri. If only some of these transcripts encode active peptides that possess multiple protein substrates, then the scope of posttranslational regulation could quickly expand.

Small functional peptides could evolve rapidly. Random mutations that introduce start codons in existing lncRNAs (or within untranslated regions of coding mRNAs) could generate small peptides that are easily selected to perform a specific function. Many proteins (like Svb) have multiple functional domains, and proteolytic cleavage of one of these domains directed by small peptides may alter protein specificity, stability, localization, or function. A simple recognition code whereby small peptides act as adaptors to direct proteolytic machinery to different protein targets would make a small collection of peptides highly serviceable. Adapting such small peptides to target proteins might open the way to new therapeutic approaches (such as antivirals).

Why does nature need yet another mode of gene regulation? The same question was raised for miRNAs and their modulatory role. The ability of small peptides to quickly alter activities of target proteins without elaborate and time-consuming translation of large proteins suggests a niche for these tiny players as temporal switches. Thus, the discovery of a regulatory mechanism for small peptides highlights how a different reading of the same genomes could reveal additional surprises.

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