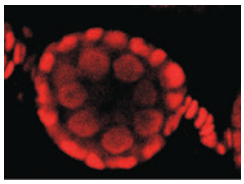
Piwi pathway for retrotransposon silencing



In the last few years, several pathways for RNA-mediated gene silencing have been defined in *Drosophila melanogaster*. The Argonaute (AGO) proteins AGO1 and AGO2, expressed ubiquitously throughout development, are essential in two of the pathways, with AGO1 binding microRNAs in one and AGO2 binding small interfering RNAs (siRNAs) in the other. Both activities lead to repression of transcripts complementary to the bound RNAs. The functions of other AGO-related proteins have remained relatively obscure, although mutants of Piwi, an AGO relative expressed primarily in germline stem cells, are known to affect the expression of retrotransposons. A new study by Siomi and colleagues further elucidates a silencing pathway involving specific interaction of Piwi with repeat-associated siRNAs (rasiRNAs) complementary to retrotransposons. The authors identified rasiRNAs bound to Piwi (but not to AGO1), whereas no miRNAs coimmunoprecipitated and siRNAs were not loaded onto Piwi in cell lysates. Unlike AGO1 and AGO2, *Drosophila* Piwi localizes in the nucleus; it also lacks the Asp-Asp-His catalytic motif shared by AGO1 and AGO2, yet has a similar ability to cleave target transcripts *in vitro*. The Piwi-bound rasiRNAs match transposable transcripts in both the sense and antisense directions. Chemical end groups on these RNAs imply that they are products of RNase III activity similar to that of the Dicer enzymes, which generate siRNAs and microRNAs and load them onto AGO proteins. As no such loading protein was found in Piwi immunoprecipitates, the factors and mechanisms that underlie rasiRNA processing remain to be determined. (*Genes Dev.* **20**, 2214–2220, 2006) *AB*

Giving G1 feedback

In yeast, two transcription factor complexes, SBF and MBF, affect the expression of G1-specific genes. How MBF regulates transcription is unclear. Some studies in budding yeast have suggested that MBF, a heterodimer of Swi6 and Mbp1, might repress rather than facilitate transcription of its target genes, but other studies in fission yeast have found that the MBF complex might also be involved in G1-specific transcriptional activation. A recent study by Wittenberg and colleagues provides insight into MBF-mediated regulation by identifying a new MBF-interacting factor, Nrm1, in budding yeast. Nrm1 localizes to the MBF-bound promoters of genes repressed outside of G1. Deletion of *NRM1* disrupts the cell's ability to repress G1-specific genes upon G1 exit. One of these genes is *NRM1* itself, which is expressed late in G1. When the *MBP1* gene is mutated, transcription of *NRM1* (as well as other MBF targets) increases in late G1 and is prolonged. SBF is responsible for repression of *NRM1* in early G1, but, surprisingly, maximal transcription of *NRM1* in late G1 also requires SBF. Nrm1 protein stabilization reduces G1 transcription of MBF-dependent, G1-specific genes, confirming that its function is primarily repressive. Nrm1 is also present in fission yeast MBF, where it also acts with MBF to repress the transcription of G1-specific genes in late G1, as cells are transitioning into S phase. These studies reveal a Nrm1 negative feedback loop that, together with MBF, controls the downregulation of genes expressed in G1. However, what counteracts MBF's repression during G1 to allow these genes to be expressed remains unknown. (*Mol. Cell* **23**, 483–496, 2006) *AKE*

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During the process of translation, eukaryotic elongation factor eEF1a brings an amino-acyl transfer RNA to the acceptor (A)-site of the ribosome. Once the peptidyl transferase reaction takes place, eEF2 catalyzes the movement of messenger RNA and tRNA from the A- and peptidyl (P)-sites to the P- and exit (E)-sites. eEF3 is an ATP-binding cassette (ABC) protein that converts chemical energy from ATP binding or hydrolysis into a 'power stroke' of mechanical energy. It was not clear how eEF3 interaction with the ribosome was correlated with its dynamic properties as an ABC protein and how the energy from binding or hydrolyis of ATP was used for its function in tRNA release. The crystal and cryo-EM structures of eEF3, determined by Beckmann and coworkers, begins to address these questions. The protein is organized into five structural domains: an N-terminal HEAT repeat, a four-helix bundle, two ABC-type ATPase domains and a chromodomain inserted within the second ABC domain. The cryo-EM structure of ATP-bound eEF3 is complexed with a post-translational ribosome with a peptidyl-tRNA in the P-site. In this complex, the eEF3 chromodomain lies near the ribosomal E-site, where it may interact with ribosomal regions which influence the affinity of tRNA for the E-site. This new information, combined with knowledge gained from previous studies, suggests that a conformational switch in the eEF3 chromodomain upon binding to the ribosome could stabilize an open conformation that allows tRNA release. (*Nature*, advance online publication 23 August 2006, doi:10.1038/nature05126) *BK*

Kip3p is a plus

The kinesin superfamilies are important regulators of microtubule dynamics. Most kinesins are motor proteins that transport microtubule-binding proteins to microtubule tips, promoting microtubule stability. Kinesins 13 and 8, however, have been implicated in microtubule destabilization. Kinesin-13 lacks motor activity but behaves as a microtubule depolymerase, actively removing tubulin from microtubule ends. Although kinesin-8 is closely related to kinesin-13, it is unclear whether it shares similar depolymerase activity. Kinesin-8 defects or deletions lead to longer cytoplasmic microtubules and mitotic spindle mispositioning, suggesting significant roles in cytoskeletal dynamics. Two related papers from the Pellman and Howard laboratories help clarify these roles. Using budding yeast kinesin-8 (Kip3p), these groups show that the protein has both plus end–directed motor activity and plus end–specific depolymerase activity, making it unique in the kinesin families. Data from the Howard group show length-dependent Kip3p depolymerase activity, with maximal depolymerase activity targeted to the plus ends of longer microtubules by the highly processive plus end–directed Kip3p kinesin motor. This activity may help maintain average cellular microtubule length. The Pellman group found that, similar to kinesin-13, Kip3p has tubulin dimer–dependent ATPase activity. Analysis of *kip3*Δ cells indicates that Kip3p maintains appropriate microtubule lengths needed for spindle positioning at the bud neck during preanaphase. Together, these studies suggest roles for Kip3p depolymerization activity that may be common to all kinesin-8 proteins. It will be interesting to see how the depolymerase and motor activities are involved in the several other microtubule functions in which kinesin-8 has been implicated. (*Nat. Cell Biol.*, advance online publication 13 August 2006, doi:10.1038/ncb1457 and doi:10.1038/ncb1462) *MM*