kinase loop domains? Or is p300 constitutively acetylated and therefore regulated by other mechanisms? With a conceptual framework now laid out, answers to many of these questions are within reach.

The apparent parallel relationship between phosphorylation and acetylation regulatory switches has been discussed by Kouzarides[8], but the question of an acetylation cascade has remained open. Kinases phosphorylate other kinases, which phosphorylate other proteins (**Fig. 2a**). Phosphorylation sites are bound by specific recognition motifs such as SH2 domains. So protein phosphorylation minimally serves two essential purposes, regulation of enzyme activity and creation of protein docking sites. Does a similar regulatory cascade exist for acetylation (**Fig. 2b**)? Several pieces of this puzzle are now in place. We know that an acetyltransferase can be regulated by acetylation, and have known that acetylation provides docking sites for bromodomain proteins. An important missing piece in this puzzle is whether another acetyltransferase activates p300 via acetylation of the proposed looped domain, or whether autoacetylation simply reinforces the activation decision.

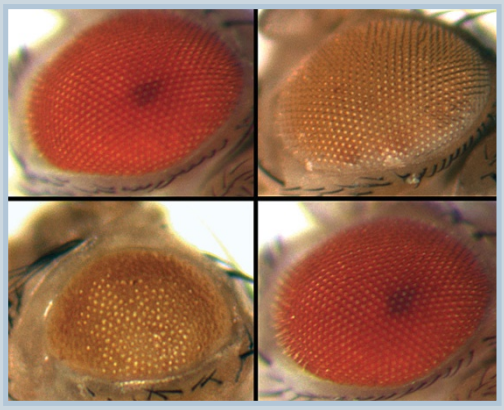
ACKNOWLEDGMENTS

I thank J. Adams for advice and S. Tan for generating the structures in Figure 1. Research support comes from the US National Institutes of Health (GM059055).

1. Marmorstein, R. & Roth, S.Y. *Curr. Opin. Genet. Dev.* **11**, 155–161 (2001).
2. Thompson, P.R. *et al. Nat. Struct. Mol. Biol.* **11**, 308–315 (2004).
3. Adams, J.A. *Biochemistry* **42**, 601–607 (2003).
4. Hubbard, S.R. *EMBO J.* **16**, 5572–5581 (1997).
5. Johnson, L.N., Noble, M.E. & Owen, D.J. *Cell* **85**, 149–158 (1996).
6. Morgan, D.O. *Nature* **374**, 131–134 (1995).
7. Marmorstein, R. *J. Mol. Biol.* **311**, 433–444 (2001).
8. Kouzarides, T. *EMBO J.* **19**, 1176–1179 (2000).

RISCy business

Two classes of small RNAs influence a wide variety of biological processes by silencing gene expression. MicroRNAs (miRNAs) are produced from stem-loop RNA precursors encoded in the genome and are incorporated into miRNA-induced silencing complexes (miRISCs). These complexes appear to silence gene expression by blocking mRNA translation and play many roles in growth and development. In contrast, small interfering RNAs (siRNAs) are generated from longer double-stranded RNA precursors. siRNAs are incorporated into and guide siRNA-induced silencing complexes (siRISCs) to target messenger RNAs for degradation.



The extent of base-pairing between the small RNA and the mRNA appears to determine the outcome of silencing. Specifically, translation inhibition occurs if the target mRNA is partially complementary to the silencing trigger (miRNA or siRNA). In contrast, direct cleavage of the message requires perfect complementarity. It is unclear whether a single silencing complex can cleave mRNA and block its translation, or whether the silencing triggers assemble into distinct RISCs each with its own activity.

Both mi- and siRNAs are generated by an RNase III enzyme called Dicer. *Drosophila melanogaster* contains two Dicer proteins, Dicer-1 and Dicer-2, which might work together to carry out silencing. In two papers published in a recent issue of *Cell*, the Carthew and Sontheimer laboratories show that in *Drosophila*, Dicer-1 and Dicer-2 have distinct but overlapping roles in the two silencing pathways. Furthermore, they show that Dicer-2 and siRNAs assemble into a RISC intermediate that subsequently forms the functional RISC involved in mRNA cleavage.

Lee *et al*. (*Cell*, in the press, 2004) developed a sensitive way to screen for mutations that affect siRNA-mediated silencing in the eyes of *Drosophila*. The normal red color of the eye (upper left panel) was changed to light orange (upper right panel) by silencing of a pigmentation gene. Mutations in Dicer-1 and Dicer-2 were discovered and these altered the eye color back toward normal (lower panels). Lee *et al*. show that flies with mutant *dicer-1* have darker orange eyes (lower left). Furthermore, the size and morphology of the eyes were different from that seen with wild-type Dicer-1(compare upper right and lower left panels). These developmental differences suggest that the miRNA pathway is somehow affected in *dicer-1* mutants. Further analysis showed that siRNA levels were comparable to those in the wild-type flies but no mature miRNAs were detected. These and other data suggest that Dicer-1 is critical for miRNA production, but the mechanism by which this protein functions remains unclear.

In contrast, Dicer-2 is dispensable for miRNA production. Furthermore, flies with a mutated Dicer-2 protein (lower right panel) have very low levels of siRNA suggesting that Dicer-2 is important for siRNA processing. Mutations of single residues in the active site of Dicer-2 confirmed that RNase III activity is required to generate siRNA. Further analyses showed that both Dicer-1 and Dicer-2 are required for the formation of functional RISCs.

Pham *et al*. (*Cell*, in the press, 2004) used biochemical techniques to show that Dicer-2 facilitates multiple steps in siRISC assembly. The data are consistent with a model in which these complexes form sequentially in an ordered assembly pathway. Dicer-2 interacts with siRNAs in an ATP-independent complex that initiates siRISC assembly, and remains associated with the siRNA in the functional siRISC. They also identify a form of active siRISC that is much larger (~80S) than those characterized previously. These results indicate that Dicer-2 not only processes double-stranded RNA and transfers siRNAs to a distinct complex, but also assembles into a very large siRISC along with the siRNAs.

Is the miRISC assembled using a similar process? Dicer-1 co-immunoprecipitates with miRNA and could initiate miRISC assembly in much the same way that Dicer-2 initiates siRISC assembly. The genetic data from Lee *et al*. showing that Dicer-1 and Dicer-2 play distinct roles in miRNA and siRNA processing lend support to such a model.

Evelyn Jabri