clumps of material whose radiative characteristics stand out above the mean flux of the system—would appear to emit pulsed radiation because of the aberration of light away from the direction of Earth when they orbit on the far side of the black hole (see the figure). The separation between pulses should decrease as the material spirals into the event horizon. The peak intensity of the pulses should also decrease as the material approaches the event horizon because of the Doppler effect in the gravitational potential well of the black hole. The last visible pulse should thus be the weakest. In contrast, if the accreting object had a solid surface, as in a neutron star, the last pulse would be the largest as the material impacted the surface.

Dolan (*10*, *11*) has analyzed high-speed photometer (HSP) data from Cyg XR-1 obtained with the Hubble Space Telescope (HST) (*12*). He detected two series of pulses in the ultraviolet (UV) that bear the characteristics of a dying pulse train and thus the signature of an event horizon. The statistical confidence level is not high enough, however, to prove that the pulses are not stochastic variations in the flux (*13*). Further observational studies of Cyg XR-1 in the x-ray and UV are needed to confirm the presence of dying pulse trains.

Other theories of gravity that are also consistent with the three classical tests of general relativity (*14*–*16*) do not predict the existence of black holes and event horizons, which may provide a fourth test of general relativity. In these theories, collapsed objects that are not point singularities can exist, but only radiation directed nearly radially outward can escape from their surface. If the impact of accreting material occurs on a part of their surface not visible from Earth, no radiation would reach us. If the orbital topography near compact objects in these competing theories cannot produce a way to reproduce these observations, the detection of event horizons may be another successful test of the validity of general relativity.

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PERSPECTIVES: DEVELOPMENT

Legs, Eyes, or Wings—Selectors and Signals Make the Difference

Markus Affolter and Richard Mann

People naturally like to compare individuals. Biologists, too, are especially keen to do this and have spent decades cataloging the differences between organisms that display interesting variations in their basic body plans. For the many cases in which such body plan differences are inherited, variations have been traced to specific genes, first in the fruit fly *Drosophila melanogaster* and later in vertebrates. Most of these genes encode evolutionarily conserved transcription factors that control the development of morphogenetic fields, discrete sets of cells that give rise to specific structures in the adult. Such genes are generally referred to as "selectors" because they select distinct developmental pathways that ultimately give rise to structures such as eyes, antennae, legs, or wings in the fly (*1*). Although these selector genes are known to encode transcription factors, exactly how they orchestrate the development of morphogenetic fields remains something of a mystery. The study by Guss *et al.* (*2*) on page 1164 of this issue, provides us with a more detailed understanding of how selector genes might work.

To build a leg or a wing, cells need to know not only which structure they are making, but also where they are located within that structure. A large body of beautiful work has given us an understanding of how a cell knows where it is. Specifically, the generation of positional information within morphogenetic fields is controlled by a small, evolutionarily conserved set of intercellular signaling pathways. In *Drosophila*, these include the epidermal growth factor, Decapentaplegic (Dpp), Wingless, Hedgehog, and Notch signaling pathways. Importantly, for each of these pathways, one of the last steps is the activation of a pathway-specific transcription factor (the signaling effector). Not surprisingly, these same pathways are used repeatedly during development to provide positional information to most, if not all, of the structures in developing animals. Thus, to generate a leg or a wing, the selector transcription factors must somehow interpret the positional information laid down by these signaling pathways (see the figure). How do signaling pathways and selector proteins interact with each other in vivo? Is specificity generated during transcription or at a later, post-transcriptional step? And what are the exact contributions of signaling pathways and selector proteins to the final structure?

There are two models to explain how selector transcription factors interpret positional information laid down by signaling pathways. The first model suggests that the target genes of selector proteins and the target genes of signaling cascades are largely distinct. According to this model, the products of the two sets of target genes would interact with each other to provide a cell with both its positional address and its identity. An alternative view posits that signaling pathways and selector proteins share many of the same target genes. According to this model, signaling effector proteins and selector transcription factors would interact with the regulatory DNA sequences (enhancers) that control the expression of these target genes. Several pieces of evidence support the second model. For example, in *Drosophila* a target gene enhancer activated by the Labial (Lab) selector protein also requires input from the Dpp signaling pathway (*3*, *4*). Similarly, a target gene enhancer that is activated in the developing heart is coregulated by the selector protein Tinman (Tin) and the Dpp pathway (*5*).

The study by Guss *et al.* (*2*), together with previous work from this group (*6*), extends these ideas to the fly wing. They show that Scalloped, the DNA binding component of the selector protein complex

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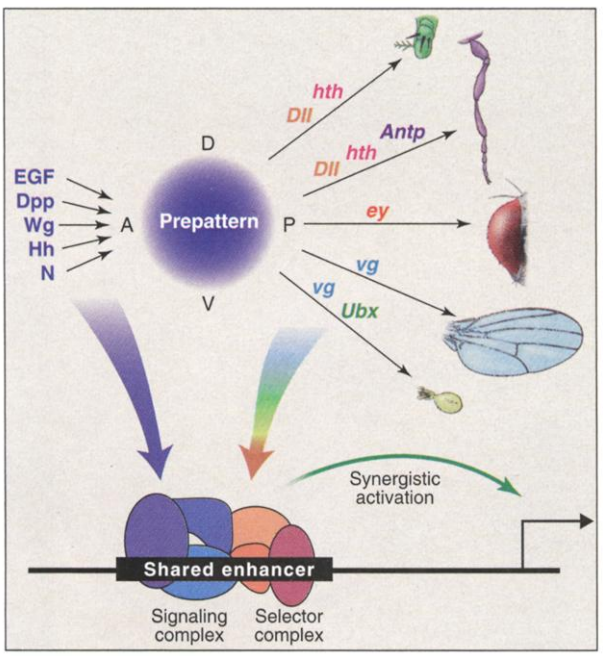
for the *Drosophila* wing, binds to and directly controls the regulatory enhancers of several target genes known to be required for wing development. They further provide evidence that these enhancers are also regulated by signaling pathways, suggesting that, as with the Lab and Tin target gene enhancers, selector and signaling inputs are integrated by commonly regulated enhancer elements.

As most biologists who study gene regulation know, real enhancers tend to bind to many proteins and can be a pain to work with. Guss *et al.* get around this problem by constructing minimal enhancers that contain binding sites for both a selector protein and a signaling effector. Strikingly, these synthetic enhancer elements are able to activate transcription in specific and predictable patterns in developing flies. In the two examples that Guss *et al.* describe, the enhancer binding site for the wing selector protein Scalloped was combined with binding sites either for the Notch pathway effector, Suppressor of Hairless, or for the Dpp pathway factor, Mad. Both of these artificial enhancers drove expression of a reporter gene in the wing, presumably because they contained binding sites for Scalloped. The two reporter gene expression patterns generated within the wing, however, were different and correlated with the activity of either the Notch or Dpp signaling pathway. Importantly, enhancer elements containing only a single class of binding site, for either the selector or the signaling effector, were unable to drive reporter gene expression in the developing fly wing. It is likely that the two classes of binding site recruit selector and signaling protein complexes to the DNA (see the figure). Apparently, these two complexes contribute complementary activities because both appear necessary for the integration of wing-specific and signal-specific inputs. The interaction between selector and signaling protein complexes attached to the same regulatory DNA element might be a common mechanism by which ubiquitously deployed signaling pathways can elicit field-, tissue-, and cell type–specific responses. Three other fly development studies provide compelling evidence that the specificity of Ras signaling arises from the integration of signaling and cell type–specific transcription factors at shared enhancers (*7*–*9*).



Sharing the work of making body parts. Signaling pathways between cells in the developing fly include the epidermal growth factor (EGF), Decapentaplegic (Dpp), Wingless (Wg), Hedgehog (Hh), and Notch (N) pathways. These signaling pathways provide positional information to morphogenetic fields in developing structures such as wings, legs, and eyes, resulting in a "prepattern" that has anterior (A)–posterior (P), dorsal (D)–ventral (V), and proximal-distal (not shown) information. Selector genes such as *Distal-less* (*Dll*), *homothorax* (*hth*), *Antennapedia* (*Antp*), *eyeless* (*ey*), *vestigial* (*vg*), and *Ultrabithorax* (*Ubx*) encode transcription factors that act combinatorially to interpret this positional information and to generate specific structures such as antennae (green), legs (purple), eyes (red), wings (blue), or halteres (yellow). One mechanism (**bottom**) by which selector and signaling inputs could interact with each other is shown. In this model, signaling (**left**) and selector (**right**) protein complexes interact with each other on shared enhancer elements to regulate the expression of a common set of target genes.

What is the molecular basis for the obligate synergy between selector and signaling protein complexes observed by Guss and colleagues? In one scenario, protein-protein interactions between signaling and selector complexes might be necessary for the stable assembly of a DNA-bound enhanceosome-like structure that then recruits an essential coactivator complex. Several examples of enhanceosomes have been described, and the formation of these multiprotein complexes appears to be necessary, and in some cases sufficient, to orchestrate the ordered recruitment of coactivators and the basal transcription machinery (*10*, *11*). Alternatively, because there are several qualitatively different classes of transcription coactivators (*12*), signaling and selector protein complexes could each recruit different but complementary coactivators from different classes. In both models, the dual requirement for signaling and selector protein complexes could underlie the regulation of many enhancers whose job it is to integrate cell type and positional information in developing structures.

What remains to be done is the rigorous testing of these ideas in native, wing-specific enhancers, which will undoubtedly be more complex than the synthetic elements characterized thus far. In particular, for most of the known native wing-specific enhancers, the binding sites that receive the signaling input have not been clearly defined. Nevertheless, the minimal enhancer elements described by Guss and colleagues may prove useful in other experiments. For example, if these simple elements synergistically activate transcription in cell culture when selector proteins and signaling factors are coexpressed, then the detailed dissection of protein-protein interactions between selector and signaling complexes should be possible. Such a system may also provide a way to test the enhanceosome model and to identify the coactivators that these complexes recruit to target gene enhancers. The in vivo confirmation of such interactions could lead to a more mechanistic view of the transcriptional synergy existing between selector proteins and signaling pathways. We will also need to discover how general this mechanism is, and if most developmentally regulated enhancers depend on synergistic inputs to enable them to integrate positional, tissue, and cell type–specific information.

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