

Nonetheless, there are reports that mouse homolog of Blimp-1 is expressed in the myotome¹⁵, and it will be interesting to determine whether it functions as a slow muscle selector there as well.

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Two Pax are better than one

Richard S Mann

In fly eye development, new experiments suggest that two distinct Pax genes control tissue growth and identity, respectively. Notably, these two functions may be encoded by distinct isoforms of the human gene PAX6

Most biologists, and many nonbiologists, are probably familiar with the experiment in which scientists generated fruit flies that had more than the normal pair of compound eyes¹. This result, produced by the forced misexpression of the gene *eyeless* (*ey*; called *PAX6* in humans), was so notable that it found its way to the front page of *The New York Times* with the headline “Science Outdoes Hollywood”, an accolade that has set a new standard for genetics research². Since this first set of discoveries, the mechanism underlying the control of eye development by the master control gene *ey* has been the subject of intense research. How can a single gene instruct the development of such a complex structure as the fly’s compound eye? On page 31 of this issue, a report by Maria Dominguez and her colleagues³ provides new insight into this problem.

Sizing up the eyes

To generate a complex organ, such as a compound eye, the correct cell types must be formed and these cell types must be correctly organized. In other words, an organ-specific identity must be generated. *ey* (or *Pax6*), together with a cohort of subordinate transcription factors, is essential for this aspect of eye development⁴. Organ size must also be controlled, and size control has to be coordinated with the process of organ-identity specification. For the fly eye, there is some

controversy as to whether growth and identity are controlled by the same pathway or by different pathways. Most researchers agree that growth of the eye is controlled by the Notch signal transduction pathway, which is activated during eye development at the interface between the dorsal and ventral halves of the developing eye, also called the dorsal-ventral organizer^{5–7}. Some researchers have suggested that Notch also controls eye identity and *ey* expression^{8,9}. Others, however, argue that Notch does not control *ey* expression or eye identity, only the growth of the eye¹⁰. That Notch is not responsible for eye identity makes more sense, because this pathway is used not only in the eye but repeatedly during

development in a wide variety of contexts. But these results raise an important question: if not *ey*, what are the Notch targets that mediate its growth-promoting activity in the eye?

The new work by Dominguez *et al.*³ answers this question. Their experiments suggest that growth and identity specification are separable pathways in fly eye development. They identify the Pax6-like gene *eyegone* (*eyg*)¹¹ as the Notch target responsible for mediating its growth-promoting effects in the eye (Fig. 1). Consistent with this notion, in the absence of *eyg*, the eye primordium does not grow, even though *ey* is still expressed. This is notable, in part because *eyg* is not expressed throughout the eye but seems to be

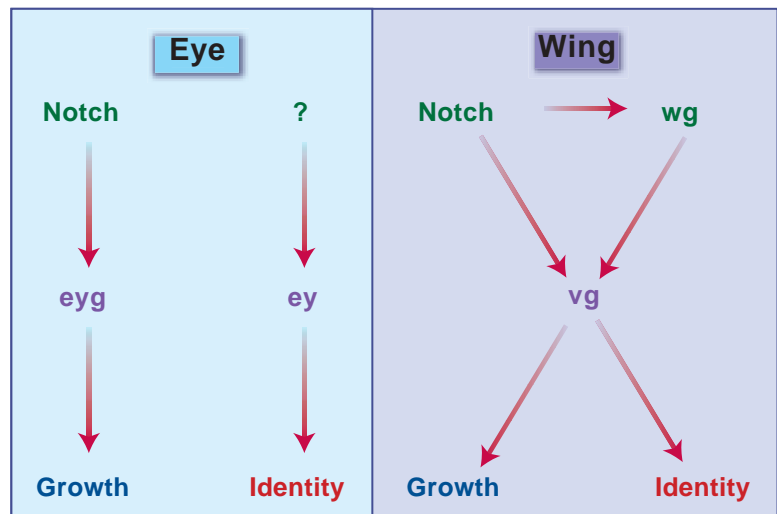


Figure 1 In the fly eye, tissue growth is regulated by Notch and its target *eyg*. Eye identity is regulated by *ey*. In the wing, the gene *vg* carries out both functions and is regulated by both the Notch and Wingless (*Wg*) pathways.

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specifically induced at the dorsal-ventral organizer. Also, the analysis of mosaic eyes indicates that the requirement for *eyg* function is not evenly distributed throughout the eye: *eyg*⁻ clones grow poorly when they arise close to the organizer but grow better when they arise far from the organizer. In addition, Dominguez *et al.*³ show that a block in eye growth caused by compromising the Notch pathway can be rescued by forcing *eyg* expression. Notably, *ey* was relatively ineffective at rescuing growth in this assay. Thus, *eyg* seems to be specifically induced by Notch at the dorsal-ventral organizer but required for the growth of the entire eye.

A pair of paired domains

The story gets even more interesting. Both *ey* and *eyg* encode transcription factors of the paired family, which means they have two DNA-binding domains: a homeodomain and a paired domain¹¹. Most paired domains, like the one in *ey*, have two subdomains that each have DNA-binding potential. The *eyg* paired domain, however, has only one of these two subdomains. There does not seem to be an *eyg* ortholog in humans. But the single PAX6 gene in humans produces two isoforms by alternative splicing¹². One isoform, PAX6, is similar to Ey, with a homeodomain and a complete paired domain. The other isoform, PAX6(5a), is similar to *Eyg*, with an intact homeodomain but only one of the two paired subdomains. Dominguez *et al.*³ show that PAX6(5a) induces extensive proliferation without eye differentiation when expressed in the fly wing primordium. The PAX6 isoform induces eye differentiation but not growth. Thus, Dominguez *et al.*³ suggest a model with a division of labor between these two paired family members. *Eyg* and PAX6(5a), which have incomplete paired domains and, therefore, probably distinct DNA-binding preferences, mediate tissue growth, whereas Ey and PAX6, which have complete paired domains, are responsible for eye tissue identity. These

results contrast with how wing development is thought to occur in the fly⁶. As in the eye, growth of the wing primordium depends on Notch signaling activated at a dorsal-ventral organizer. In the case of the wing, however, the Notch target is the gene *vestigial* (*vg*), which probably mediates both tissue growth and wing identity (Fig. 1). Thus, these functions seem to be mediated by a single gene in the wing but by two genes in the eye.

Filling in the blanks

Although the results presented by Dominguez *et al.*³ support the proposed division of labor by *eyg* and *ey*, several questions persist. Complicating the situation is the fact that both *eyg* and *ey* have very similar and closely linked sister genes, twin of *eygone* (*toe*) and twin of *eyeless* (*toy*), respectively^{4,11}. These gene duplications, together with a lack of mutations that remove their functions, make it difficult to be certain that the sister genes do not also contribute to growth or eye identity. For example, as shown by Dominguez *et al.*³, some *eyg*⁻ clones grow quite large, raising the possibility that *toe* can partially take its place in some circumstances. In addition, paired family proteins can form homo- and heterodimers¹³, so it is possible that *Eyg* could function together with Ey and/or Toy to mediate eye growth. One argument against this is the experiment showing that PAX6(5a) can induce overgrowth of the wing. As this happens without eye differentiation, it is unlikely that Ey or Toy is present. But *eyg* is normally expressed in and required for the development of another part of the fly, a portion of the notum¹⁴. Thus, there must be other factors, perhaps Ey, Toy or one of their downstream targets, that help distinguish the functions of *Eyg* in the notum from its growth-promoting functions in the eye.

Despite these complications, Dominguez *et al.*³ make a good case for *eyg* and *ey* carrying out two distinct functions in eye development.

Their results suggest that *eyg* is an organizer-induced transcription factor important for the growth of the entire eye. How exactly *eyg* promotes growth, especially in cells away from the organizer, is still not clear. One possibility is that *eyg* is responsible for activating another, still to be identified secreted signal. Another possibility is that the requirement for *eyg* is transient or that small amounts of *Eyg* are sufficient to carry out its functions. Ey, on the other hand, together with more downstream transcription factors⁴, would be responsible for specifying tissue identity. The Ey and *Eyg* pathways probably converge at some point, however, perhaps at some of the same downstream targets. Consistent with this notion, blocking some of the factors downstream of Ey both prevents differentiation and induces proliferation¹⁵, suggesting that there is communication between the identity- and growth-specifying pathways. Tests of these ideas must wait for the identification of Ey and Ey targets and an analysis of the genetic network downstream of these two Pax genes.

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