Eyeing *hedgehog* and *decapentaplegic*

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Cells of the eye–antennal disc proliferate in the larva, begin to differentiate into photoreceptors in the late third instar, and mature to form the complete eye during pupal division. The differentiation of the cells of the eye–antennal imaginal disc takes place by means of a morphogenetic furrow (MF) that sweeps across from the posterior margin to the anterior margin[1,3] (Figure 1). This MF forms a mobile boundary between the differentiated photoreceptor cells posterior to it and the eye progenitor cells anterior to it. It is not clear whether the formation of the furrow is responsible for initiation of differentiation or differentiation causes the formation of the furrow. This MF is ultimately responsible for the formation of the numerous ommatidia that make up the compound eye. This is later followed by rotation of the ommatidia so that the ommatidia in the dorsal half are 180° opposite to the ommatidia in the ventral half, thus forming a mirror image with respect to the midline.

Early eye genes

Early gene determination of the eye primordium has been found to require the expression of at least six genes at various times with varied spatial expression[5,6]. These include *eyeless* (*ey*), *twin of eyeless* (*toy*), *eyes absent* (*eya*), *sine oculis* (*so*), *dachshund* (*dac*), and *eye gone* (*eg*), mutations in each of which result in an eyeless or reduced eye phenotype. *ey* and *toy* encode Pax-6 proteins with paired domain and homeodomain DNA-binding motifs[7,8]. *eya*[9] and *dac*[10] are novel nuclear proteins, *eyg*[9] encodes a Pax-like protein and *so*[11], a homeodomain protein.

toy, ey and *eyg* are the first genes expressed in the embryonic primordium of the eye–antennal disc. Ectopic expression of *toy* results in ectopic expression of *ey*, while ectopic expression of *ey* does not induce expression of *toy*. Also Toy, which is present throughout the eye disc can bind to essential sites in the *ey* enhancer. This clearly places *toy* as the upstream activator of *ey*[8]. *ey* and *eyg* do not require each other for their expression and so are hypothesized to be activated by different factors[7,8,12]. *ey* is responsible for the activation of *eya* and *so* and the combination of *eya* and *so* is required for expression of *dac*. The expression of these genes begins before initiation of the MF and is highest at the posterior margin of the eye disc where the MF initiates[5]. Later their expression is seen strongly in the region anterior to the MF, suggesting a role in the control of expression of genes involved in the MF initiation as well as in the MF progression. In addition to this, *eya* and *dac* may also regulate the expression of *ey* through a feedback loop[6,13,14].

Eya, which has a transcriptional activation homeodomain, interacts *in vitro* with So which has a DNA-binding domain[15]. Dac, which has a transcriptional domain, may bind to this complex and modify its specificity. Interestingly, ectopic expression of most of these genes, both alone and in combination with each other, induces formation of ectopic eyes. Given that all the six genes mentioned above are expressed in various other sites, there must be some additional factors, which in connection with these are responsible for the ultimate development of the eye[6].

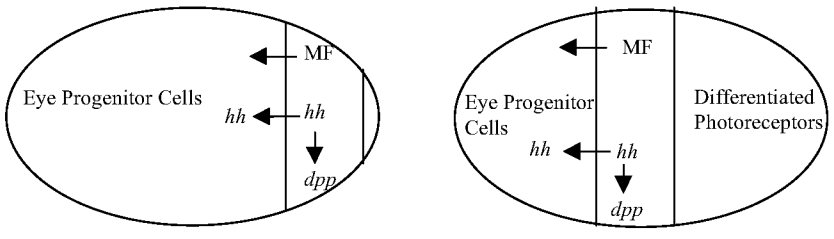

Figure 1. Schematic of the initiation and progression of the Morphogenetic Furrow (MF).

hedgehog pokes its nose

Differentiation of cells in the developing *Drosophila* eye starts at the posterior during the third instar larval stage. It then proceeds to the anterior region and is completed in about two days time. Differentiating photoreceptor cells have been found to express the product of the gene *hedgehog* (*hh*), a diffusible molecule, which is involved in initiation and progression of the MF[4,16–18]. The action of *hh* is primarily mediated by the transforming-growth-factor-β (TGFβ) family member *decapentaplegic* (*dpp*)[2,3,14]. Unlike the wing disc, where *hh* is expressed in the entire posterior compartment, in the eye disc, *hh* is expressed in the posterior-most cells. *hh* induces expression of *dpp* in the MF and in the cells secreting Atonal, which are anterior to the developing photoreceptor cells. *hh* also produces a short-range signal, which induces the cells immediately anterior to the MF to differentiate and so secrete *hh*. This progressive induction of *hh* towards the anterior portion is thought to be responsible for the movement of the MF, causing patterning of the eye. In the eye, *hh* also functions through a *dpp*-independent pathway.

The *hh* signal is transduced by two transmembrane receptors Patched (Ptc) and Smoothened (Smo)[2]. Ptc is a twelve pass transmembrane protein that binds to Smo, a seven-transmembrane protein, repressing its activity. When Hh is secreted, it binds to Ptc, and so Smo is derepressed resulting in the transduction of the signal. Protein Kinase A (PKA) is also another component of the *hh* signalling pathway. The gene for the catalytic subunit of PKA, *pka-C1* is required for spatiotemporal regulation of MF progression. Mutants in this gene cause *dpp* production in cells anterior to the MF, resulting in ectopic MF initiation. The involvement of PKA raises the possibility of a G-protein coupled receptor producing cAMP in the *hh* pathway[5].

The expression of two genes, *atonal* (*ato*) and *hairy* (*h*), parallels furrow progression. *ato* is required for neural development while *h* is thought to function by repressing neural differentiation anterior

to the MF. Both these genes are activated by *hh* and so *hh* apparently induces both an activator and an inhibitor of neural development. *hh* also induces cell-cycle synchronization, cell-cycle arrest in the MF and cell proliferation anterior to the MF. The induction of *hh* signal in response to the *hh* signal ensures gradual propagation of the morphogenetic wave across the eye. Ectopic expression of *hh* in the anterior region of the disc results in ectopic MF initiation[18].

decapentaplegic pitches in

dpp mediates *hh* signals by regulation of expression of tissue-specific genes[2,3,17]. It is expressed along the posterior margin of the eye disc and along the lateral margins prior to the MF initiation. The *dpp* signal is transduced by Thick veins (Tkv), a receptor, which phosphorylates Mothers against *dpp* (Mad) which in turn, being a cytoplasmic transducer of the *dpp* signal, migrates into the nucleus and acts on the specific target genes. While it is known that *dpp* plays a role in MF initiation, the role of *dpp* in progression of the MF is not very clear. It is expressed in the MF as it traverses across the eye disc, where it is required for cell-cycle regulation. Further, *dpp* can ectopically induce initiation of the MF when expressed in the anterior margin of the eye disc[21].

ey, *eya*, *so*, *dac* and *dpp*

It is well known that the early eye genes, *hh*, *dpp*, *pka-C1* and *h* are some of the many players involved in the development of the eye. The nature of their interactions and their hierarchy is unclear. In order to understand the relationship between *dpp*, *hh* and the early eye genes, mutants defective for *dpp* signalling and mutants defective for both *dpp* and *hh* signalling were studied[19].

Mad^{1-2} (ref. 20) homozygous mutant tissue, which is defective in *dpp* signalling, fails to initiate MF when present in the posterior margin. When present as clones in the internal region, MF induction is normal pointing to a role for *dpp* only in the initiation and not in the progression or maintenance of the MF. These clones were used to study the relationship between Eya, Dac, Ey, *so* mRNA and *dpp*[19].

Both prior to and after MF initiation, Dpp does not influence the expression of Ey. On the other hand, it affects the expression of Eya, Dac and *so* mRNA. Prior to MF initiation, in wild type cells, Eya, Dac and *so* mRNA expression is limited to the posterior half with strong expression in the cells close to the posterior margin. Prior to initiation of the MF, Mad^{1-2} homozygous mutant clones in the posterior margin, do not express Eya, Dac and *so* mRNA. In the case of Mad^{1-2} homozygous mutant clones just anterior to the posterior margin, Eya and Dac are more strongly expressed when compared to wild type. As expected, Mad^{1-2} homozygous mutant clones in the anterior region of the eye disc show either faint or no Eya and Dac expression prior to MF initiation. After MF initiation, Eya and Dac expression are not dependent on *dpp* signalling. Their expression depends only on the presence or absence of a MF. These results seem to show that Eya, Dac and *so* require Dpp signalling prior to initiation of the MF and once the MF has been initiated they do not require Dpp signalling. Also Dpp is not required for Ey at any stage. Interestingly, once initiated, MF progression is not dependent on expression levels of *dpp*.

dpp loss of function and loss of ability to transduce the *dpp* signal have both been assayed for rescue. The *dpp* allele used was a regulatory loss of function allele, which resulted in greatly reduced eyes. When Eya is overexpressed using the GAL4 system, in these *dpp* mutant systems, it has been found that there is a rescue on the dorsal side alone. The expression levels of Dac and *so* are comparable to wild type in this case, suggesting that Eya can induce Dac and *so* and lead to the initiation of MF in the absence of Dpp signalling. In both stronger *dpp* loss of function allele background and Mad^{1-2} clones, exogenous *eya* expression rescued the *dpp* mutant phenotype. On the other hand, exogenous expression of Dac and *so* enhanced the small eye phenotype. Also, consistent with its place upstream of *dpp*, or in parallel with *dpp*, exogenous Ey did not produce any change in the phenotype of these mutants. Overexpression of *ey* variably interfered with eye development in *dpp* mutant backgrounds, resulting in an occasional enhancement of the small eyes phenotype.

The various phenotypes observed on overexpressing the early eye genes in a mutant *dpp* background suggest that the regulation of the early eye genes is important for proper development of the eye. Overexpression of *ey*, *eya*, *dac* and *so*, in the wild type using a *ey*-GAL4 driver, interfered with the eye development to varying extents. On overexpression using a *dpp*-GAL4 driver, the effect was the same. Of the four, Eya showed the weakest effects (least reduction in eye size) while So and Ey showed the greatest effects (greatly reduced eyes). All of the above four, with the exception of the UAS-*so* construct were capable of inducing ectopic eyes.

hedgehog is back

Since *hh* has been implicated in progression of MF, mutants in *smoothened* were studied to test the hypothesis that *hh* is the factor that regulates gene expression of the early eye genes during this stage of eye development. The studies indicated that *hh* and *dpp* play similar roles in eye development. Both are essential for regulating MF initiation-associated expression of Eya and Dac, but not Ey. Both did not regulate the MF progression-associated expression of the early eye genes. A double mutant, defective for

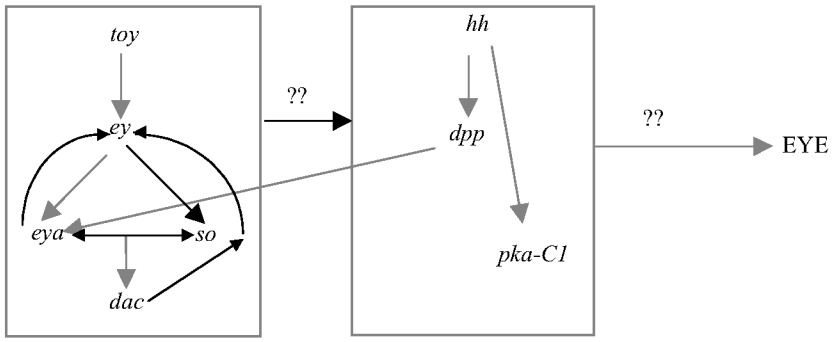

Figure 2. Schematic of the interaction of the early eye genes with *hh* and *dpp* signalling.

both *hh* and *dpp* resulted in no development of the eyes[19]. Figure 2 shows the interactions of the early eye genes, *hh* and *dpp*.

The interaction of *hh* and *dpp* is still a black box. The two genes seem to play equivalent roles with apparent redundancy. Unlike in the wing and leg discs, *dpp* does not participate very actively in patterning the eye in response to *hh* signals. It is mainly involved only in the initiation of MF. The expression of *hh* and *dpp* seems to be important for the regulation of spatiotemporal expression patterns of the early eye genes. The factors responsible for turning on the expression of *hh* are still unknown. The role of PKA suggests a G protein coupling resulting in the production of cAMP. It would also be interesting to find out how *hh* and *dpp*, both being diffusible molecules are regulated in their spatial expression patterns.

dpp expression in a mutant *ey* clone would help to place *dpp* either downstream of *ey* or alongside *ey* in a parallel pathway. Study of Eya, Dac and *so* expression on overexpressing *dpp* would give a little more insight into the interactions between these early genes and *dpp*. Understanding of the functioning of the *hh–dpp* signalling pathway in the eye would also help to understand some of the general principles underlying its role in patterning.

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