Running Title: **Early Retinal Development in *Drosophila***

Eye Development in *Drosophila*: Formation of the Eye Field and Control of Differentiation

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I. Introduction

The value of vision as a way for organisms to obtain long-range information about their environment is clear from the wide variety of species that have evolved systems for detecting light or processing more complex visual information. Recent results have suggested that the basic mechanisms regulating the development of these systems may have been conserved throughout evolution. The insect compound eye, although very different in structure from the vertebrate eye, is also a complex and sophisticated visual organ. Insect eyes accomplish spatial resolution by using multiple copies of a simple unit eye, precisely aligned on a curved surface such that each has a slightly different orientation. In *Drosophila melanogaster* each eye consists of approximately 800 of these unit eyes, called ommatidia. Each ommatidium contains 8 photoreceptor cells, 4 lens-secreting cone cells, 7 pigment cells, and a mechanosensory bristle (for a description of the structure of the adult retina see Wolff and Ready, 1993). The photoreceptors project to two optic ganglia of the brain, the lamina and medulla; two additional optic lobes, the lobula and lobula plate, are involved in higher-order visual processing.

Most adult *Drosophila* structures, including the eye, develop from imaginal discs. These are groups of cells set aside in the embryo that grow and differentiate inside the larva and evert to become functional during metamorphosis. Differentiation in the eye disc is progressive, moving across the disc in a wave-like manner from posterior to anterior. The front of the wave is marked by an indentation in the disc known as the morphogenetic furrow (MF). Most cell division occurs in the unpatterned cells anterior to the furrow, while on the posterior side of the furrow the cells are organized into clusters which develop into the ommatidia (for review see Wolff and Ready, 1993; Fig. 1). Within each cluster, the photoreceptors differentiate in a defined sequence; signals produced by cells that differentiate early recruit the cells that differentiate later. The best example of this is the induction of the R7 cell, the last photoreceptor to differentiate, by an

interaction between the sevenless receptor on its surface and the bride of sevenless ligand produced by the R8 cell, which is the first cell to differentiate (reviewed by Zipursky and Rubin, 1994). There is no lineage relationship between the cells of an ommatidium (Ready *et al.*, 1976; Lawrence and Green, 1979); thus cell fate and position are determined after cells complete their last division by local cell-cell interactions.

In this review, we will concentrate on the early stages of eye development, focusing on the mechanisms that determine global pattern rather than those involved in the establishment of specific cell fates. We will discuss the specification of the eye disc to form an eye, the control of morphogenetic furrow initiation and propagation, and the coordination of growth with differentiation.

II. Determination of the Eye Primordium

The adult eye develops from a monolayer epithelium, the eye imaginal disc, which is part of the eye-antennal disc complex from which most of the head is formed (Haynie and Bryant, 1986). This eye-antennal disc has a complex embryonic origin, containing cells derived from at least three of the head segments, the acron, antennal and maxillary segments. These cells are initially arranged in elongated groups in the epidermis; they are compressed late in embryogenesis and then invaginate into the embryo to give the disc its characteristic shape (Green *et al.*, 1993; Jurgens and Hartenstein, 1993). The region of the disc which forms the eye is primarily derived from the acron, the anterior-most region of the embryo (Jurgens and Hartenstein, 1993). A group of genes which distinguish the eye disc from the other imaginal discs, giving it the potential to form an eye, are described below.

A. *eyeless*

Mutations in several genes result in the partial or complete loss of the eye. Of those that have been analyzed, *eyeless* (*ey*) seems to act earliest in eye determination. *ey* is

expressed in the eye primordium during embryogenesis. Later, in the third larval instar, *ey* expression is seen in the anterior, undifferentiated, region of the eye disc (Quiring *et al*., 1994). The identified alleles of *ey* cause only a partial loss of the eye; they are transposable element insertions into an eye-specific enhancer (Quiring *et al*., 1994) and do not completely remove the function of the gene (Renfranz and Benzer, 1989; Lindsley, 1992). *ey* has been called "the master control gene for eye morphogenesis" because of its striking ability to induce eye formation when ectopically expressed in other imaginal discs such as wing and leg discs (Halder *et al*., 1995a) (Fig. 2F, G). These ectopic eyes are surprisingly well formed, containing large numbers of normally constructed ommatidia (Halder *et al*., 1995a). It is not known what other factors must be present to allow *ey* to induce an eye; however, *ey* is also expressed in the embryonic central nervous system (CNS) and does not transform that into an eye.

Intriguingly, *ey* is homologous to the mouse *Small eye* gene and the human *Aniridia* gene; all three encode Pax-6 proteins, which contain DNA-binding paired domains and homeodomains (Hill *et al*., 1991; Ton *et al*., 1991; Quiring *et al*., 1994). Both the mouse and human counterparts of *ey* are required for normal eye formation (Glaser *et al*., 1992; Jordan *et al*., 1992; Glaser *et al*., 1994; Grindley *et al*., 1995; Quinn *et al*., 1996), as well as for the development of the nose, forebrain and midbrain (Matsuo *et al*., 1993; Grindley *et al*., 1995; Stoykova *et al*., 1996). Mouse Pax-6 is sufficient to induce ectopic eyes in *Drosophila*, demonstrating that the regulatory properties of the protein have been conserved (Halder *et al*., 1995a). This result is surprising since the fly compound eye and the mammalian simple eye are structurally so different that they were thought to have evolved independently (Halder *et al*., 1995b). A likely explanation is that Pax-6 was employed for regulating the light-responsive properties of the first photoreceptor cell, and was subsequently co-opted for building the variety of structures which contain these cells in different species (Zuker, 1994). Support for this idea is provided by the observation that *ey* is expressed again late in eye development, during

the pupal period, at the time of rhodopsin gene activation, and that the promoters of *Drosophila* and mammalian rhodopsin genes contain essential binding sites recognized by Pax-6 (Sheng *et al*., in press). Rhodopsin genes may have been the primary targets of Pax-6 early in evolution. Vertebrate lens crystallin genes also contain Pax-6 binding sites (Cvekl *et al*., 1994; Cvekl *et al*., 1995a; Cvekl *et al*., 1995b; Richardson *et al*.,1995b), and *Small eye* is intrinsically required for lens formation (Fujiwara *et al*., 1994; Grindley *et al*., 1995); a corresponding function has not been demonstrated in *Drosophila*. Pax-6 appears to predate the appearance of photoreceptors, as a homolog is present in *C. elegans*, which has no photoreceptors; this homolog, *vab-3*, is used to determine the anterior region of the head (Chisholm and Horvitz, 1995).

A second *Drosophila Pax-6* homolog, *twin of eyeless* (*toy*), is more closely related to vertebrate *Pax-6* genes than *ey* is. *toy* is expressed earlier in embryogenesis than *ey*, and its ectopic expression induces both ectopic eyes and ectopic *ey* expression. This suggests that *toy* normally acts upstream of *ey* to initiate eye development (T. Czerny, personal communication).

B. *eyes absent*

As its name suggests, the eyes are missing in some *eyes absent* (*eya*) mutants (Sved, 1986) (Fig. 2B). However, stronger *eya* alleles cause lethality or sterility, and expression of the gene is not restricted to the eye, showing that *eya* has other functions in addition to eye determination (Bonini *et al*., 1993; Boyle *et al*., 1997). Larvae carrying *eya* alleles which cause complete loss of the adult eye have eye discs of reduced size, in which no photoreceptors differentiate and the level of cell death is greatly increased (Renfranz and Benzer, 1989; Bonini *et al*., 1993). *ey* is still expressed in these discs, indicating that *eya* does not act upstream of *ey* (Halder *et al*., 1995a). The cell death observed does not seem to be the primary consequence of loss of *eya* function, as clones of cells mutant for *eya*, analyzed in genetic mosaics, survive and proliferate (Fig. 3A, B; J. E.

Treisman, unpublished data). However, the mutant cells fail to differentiate as photoreceptors and instead form head cuticle. Perhaps *eya* mutant cells in mosaic discs are rescued from death by an *eya*-regulated survival factor emanating from neighboring wild type cells; this putative factor would be missing when the entire disc is mutant for *eya*. *eya* encodes a novel nuclear protein which is first detected in eye discs from second instar larvae; it forms a gradient with its highest levels at the posterior and lateral margins prior to the initiation of differentiation. Later, *eya* is expressed just ahead of the wave of differentiation and in differentiating photoreceptors (Bonini *et al*., 1993). Interestingly, three mouse homologs of *eya* have been recently shown to be expressed during eye development. Two of them are regulated in the lens and nasal placode by Pax-6 (Xu *et al*., 1997), suggesting that other conserved factors besides Pax-6 drive the early stages of eye development in vertebrates. Haploinsufficiency of a human homolog of *eya* leads to defects in the ear, kidney and branchial arches (Abdelhak *et al*., 1997); the homozygous mutant phenotype has not been described in vertebrates.

C. *sine oculis*

The eye is also completely or partially missing in *sine oculis (so)* mutants (Heitzler *et al*., 1993) (Fig. 2C); as for eya^1, the eyeless so^1 mutation appears to disrupt an enhancer driving expression in the eye disc, while complete loss of *so* function is lethal (Heitzler *et al*., 1993; Cheyette *et al*., 1994). The so^1 phenotype is very similar to the eya^1 phenotype: the eye disc is reduced in size, no photoreceptors form, and large numbers of cells undergo apoptosis (Cheyette *et al*., 1994). Unlike *eya*, clones of cells mutant for *so* do not appear to survive to the adult stage (Cheyette *et al*., 1994). *so* is also required for development of the ocelli and larval photoreceptor organ, and for the invagination of the optic lobes, making it an essential gene throughout the visual system (Cheyette *et al*., 1994; Serikaku and O'Tousa, 1994). *so* encodes a homeodomain protein first expressed in the eye disc in the early third larval instar, slightly later than the eya

protein, but in a similarly graded pattern (Cheyette *et al*., 1994; Serikaku and O'Tousa, 1994). Both *eya* and *so*, unlike *ey*, continue to be expressed in differentiating photoreceptors posterior to the morphogenetic furrow (Bonini *et al*., 1993; Cheyette *et al*., 1994; Quiring *et al*., 1994; Serikaku and O'Tousa, 1994). Of three mouse *sine oculis* homologs, only one, *Six3*, is expressed in the developing eye; it is also present in anterior neural structures (Oliver *et al*., 1995). Although *Six 3* expression in the brain is not altered in *Small eye* mutants (Oliver *et al*., 1995), regulation of its expression in the eye cannot be addressed as the eyes are not formed. Again, this indicates conservation of the mechanisms of eye determination.

D. *dachshund*

Another mutation which has been shown to prevent eye formation affects the *dachshund (dac)* gene (Mardon *et al*., 1994). In flies carrying strong *dac* alleles, few or no ommatidia are produced (Fig. 2D), although the eye disc grows to its normal size. *dac* encodes a novel nuclear protein first expressed in eye discs of second instar larvae, in a gradient with its high point at the posterior margin (Mardon *et al*., 1994). Targeted misexpression of *dac* in the ventral region of the antennal disc is able to induce eye development there (Shen and Mardon, 1997), leading to adult flies with ectopic eyes (Fig. 2H), although this effect is much less robust than the eyes induced in multiple discs by ectopic *ey* expression. By several criteria *dac* appears to act downstream of *ey*: first, *dac* is induced by *ey*; second, *dac* is required for the function of ectopic *ey*; and third, *ey* is still expressed in *dac* mutant discs (Shen and Mardon, 1997). However, *dac* is also able to induce *ey* expression in the antennal disc (Shen and Mardon, 1997), suggesting that there may be a positive feedback loop through which *dac* maintains the expression of *ey*. It is not clear why the antennal disc is particularly sensitive to the effects of ectopic *dac*; perhaps dac cofactors are present in both the eye and antennal discs due to their close embryonic origin (Jurgens and Hartenstein, 1993). *eya* and *so*

have only been overexpressed in flies using the heat shock inducible *hsp70* promoter, which allows rescue of their respective mutant phenotypes but does not induce ectopic eye formation (Bonini *et al*., 1993; Cheyette *et al*., 1994; Serikaku and O'Tousa, 1994). It remains to be seen whether stronger and/or localized ectopic expression of these genes would be sufficient to induce *ey* expression and/or the formation of ectopic eyes.

E. Regulation and Other Genes

The regulatory relationships between the four genes discussed above have not been explicitly defined, except that *dac* acts downstream of *ey* (Shen and Mardon, 1997). Based on their times of expression, one would expect *ey* to act first, *eya* second and *dac* and *so* third. Consistent with this, *ey* expression is not affected in *eya* or *so* mutants (Halder *et al*., 1995a), although the converse has not yet been demonstrated. The purpose of this cascade of potential transcription factors is not clear; it may amplify or stabilize the initial decision to form eye tissue, or each factor may have functions not mediated by the others. Several additional genes, including *eyegone* (*eyg*), *eyelisch*, *eye missing* and *Lobe*, can mutate to eyeless phenotypes (Lindsley, 1992). The eye disc is reduced in size in *eyg* and *Lobe* mutants, placing them early in the process of eye determination. However, *decapentaplegic* (*dpp*; see Section III. A) is still expressed in these mutants (Heberlein *et al*., 1993) (J. E. Treisman, unpublished data), suggesting that they may act further downstream than *eya* and *so*, which are required for *dpp* expression in third instar discs (Fig. 3C, D; J. E. Treisman, unpublished data). *dpp* is also expressed in *dac* mutant discs (Mardon *et al*., 1994), suggesting that *dac* acts relatively late in eye determination, and making its ability to induce ectopic eyes all the more surprising. Since most of the above genes are required for viability and were found to act in eye formation due to the fortuitous isolation of eye-specific alleles, many other essential genes may also contribute to the determination of the eye.

III. Initiation of Differentiation

During the third larval instar, cells in the eye disc begin to differentiate and assemble into photoreceptor clusters. This process initiates at the posterior tip of the disc and advances anteriorly row by row (Ready *et al*., 1976). The basis for the temporal regulation of initiation is completely unknown, although it has been speculated that the levels of hormones such as ecdysone may play a role (Mardon *et al*., 1994). The spatial regulation of initiation appears to depend on the interaction between a positive regulator encoded by the *decapentaplegic (dpp)* gene and a negative regulator encoded by the *wingless (wg)* gene. The evidence for this is discussed below.

A. Function of *dpp*

The *dpp* gene encodes a member of the TGF-β family of secreted proteins (Padgett *et al*., 1987), which has been shown to act at a distance to activate target gene expression in the wing disc (Lecuit *et al*., 1996; Nellen *et al*., 1996). *dpp* is expressed in the eye disc prior to any differentiation, around the posterior and lateral margins of the disc; subsequently, expression becomes restricted to a narrow stripe within the morphogenetic furrow (Masucci *et al*., 1990) (Fig. 4A). The first indication that *dpp* is required for normal retinal morphogenesis came from the observation that the viable allele $dpp^{d\text{-}blk}$ has a specific effect on the eye; $dpp^{d\text{-}blk}$ flies have severely reduced eyes (Masucci *et al*., 1990) (Fig. 2E). In $dpp^{d\text{-}blk}$ eye discs, differentiation is restricted to a central dorsal region; although differentiation in this region advances normally in the anterior direction, it fails to spread laterally (Wiersdorff *et al*., 1996; Chanut and Heberlein, in press ;Fig. 4B). This mutation is a deletion of an eye-specific enhancer element (St. Johnston *et al*., 1990; Blackman *et al*., 1991), resulting in a lack of detectable *dpp* expression in the third instar eye disc (Masucci *et al*., 1990). The mutant phenotype can be rescued by expressing *dpp* along the margins of the eye disc (Masucci *et al*., 1990; Staehling-Hampton *et al*., 1994). Interestingly, the mouse BMP-7

protein, a dpp homolog, is essential for development of the eye (Dudley *et al*., 1995; Luo *et al*., 1995). Another *dpp* homolog, BMP-4, is expressed in retinal progenitors cells at the dorsal ciliary margin of the eye (Papalopulu and Kintner, 1996).

It has been difficult to examine the effect of complete loss of *dpp* function, as the mutation is lethal and the gene acts highly non-autonomously, so that most clones of homozygous mutant tissue are rescued presumably by dpp protein diffusing from the surrounding wild type cells. However, large mutant clones including the posterior margin were shown to prevent retinal differentiation (Heberlein *et al*., 1993). A specific heteroallelic combination of embryonic lethal *dpp* mutations, dpp^{hr4}/dpp^{hr56}, that is temperature sensitive for viability (Wharton *et al*., 1996), has recently been used to show that early loss of *dpp* results in a complete failure of differentiation, while a slightly later loss prevents differentiation from spreading laterally, in a manner that is similar to $dpp^{d\text{-}blk}$ (Chanut and Heberlein, 1997) (Fig. 4C, D). Finally, the analysis of clones of cells mutant for autonomously acting downstream components of the *dpp* signaling pathway (Fig. 5A) have confirmed that *dpp* signaling is absolutely required for the initiation of differentiation; cells mutant for the type II receptor *punt* (Letsou *et al*., 1995; Ruberte *et al*., 1995), the type I receptor *thick veins (tkv)* (Affolter *et al*., 1994; Brummel *et al*., 1994; Penton *et al*., 1994; Ruberte *et al*., 1995) or the cytoplasmic component *Mothers against dpp (Mad)* (Raftery *et al*., 1995; Sekelsky *et al*., 1995) are unable to initiate photoreceptor development (Burke and Basler, 1996; Wiersdorff *et al*., 1996).

Experiments in which *dpp* was expressed ectopically support the idea that it is involved in patterning at the margins of the disc. Randomly positioned clones of cells that express *dpp* ectopically have an effect only on the anterior and lateral margins of the disc, where they induce ectopic differentiation and the generation of a second morphogenetic furrow that moves towards the posterior (Pignoni and Zipursky, 1997). In some cases, this leads to a duplication of the entire eye disc (Pignoni and Zipursky, 1997). This effect can be induced non-autonomously by expression of *dpp* in cells close

to but not including the anterior margin (Chanut and Heberlein, 1997; Pignoni and Zipursky, 1997), showing that this region of the margin responds to low levels of *dpp* while other regions of the disc are insensitive even to higher levels. It is not known what factors differentiate the margins from internal tissue and give them their unique sensitivity to *dpp*. However, some cells at the margin of the disc develop into head cuticle (Haynie and Bryant, 1986), so a choice between the two cell fates must be made in this region. Both loss of function and ectopic expression conditions have also shown that *dpp* positively autoregulates its own expression: *dpp* expression is lost from *Mad* clones at the posterior margin (Wiersdorff *et al*., 1996) and from dpp^{ts} discs grown at the restrictive temperature (Chanut and Heberlein, 1997), and the endogenous *dpp* gene is activated at the anterior margin by nearby cells expressing *dpp* (Chanut and Heberlein, 1997; Pignoni and Zipursky, 1997).

Clones of cells mutant for *dac* fail to initiate photoreceptor differentiation when located at the posterior margin, while internal clones have a much less severe effect (Mardon *et al*., 1994). This is very similar to the phenotype of *punt*, *tkv* or *Mad* clones, suggesting that *dac* could also act downstream of *dpp*. One difference is that *dpp* expression is not lost from *dac* clones (Mardon *et al*., 1994), indicating that *dac* is not required for *dpp* autoregulation. However, ectopic *wg* is observed at the posterior margin of *dac* mutant discs (Treisman and Rubin, 1995); thus *dac* is required for the ability of *dpp* to repress *wg* expression (see Section III. C). Since *dac* has only been ectopically expressed in regions where *dpp* is normally expressed (Shen and Mardon, 1997), its ability to induce ectopic eyes may require the simultaneous presence of *dpp*.

B. Function of *wg*

Although *dpp* is initially expressed at the lateral margins as well as the posterior margin, differentiation initiates only at the posterior tip of the disc. One reason for this seems to be that the *wg* gene is expressed at the lateral margins (Fig. 6A, B) where it acts as an

inhibitor of photoreceptor differentiation (Ma and Moses, 1995; Treisman and Rubin, 1995). *wg* is a member of the *Wnt* gene family (Nusse and Varmus, 1992), and its protein product appears to act as a secreted morphogen (Zecca *et al*., 1996; Neumann and Cohen, 1997). Removal of *wg* function using a temperature-sensitive allele results in precocious differentiation, starting from the dorsal and, more weakly, the ventral margins (Ma and Moses, 1995; Treisman and Rubin, 1995) (Fig. 6C, D). The cells that normally express *wg* go on to form regions of the head capsule (Royet and Finkelstein, 1996); it is not clear whether the primary effect of *wg* is to prevent photoreceptor differentiation or to promote head capsule fate. Clones of cells mutant for *dishevelled* (*dsh*), a downstream component of the *wg* pathway (Fig. 5B), can form ectopic eye tissue on the top of the head (Heslip *et al*., 1997), showing that those regions of the disc normally fated to become head capsule are competent to form eye in the absence of *wg* signaling. The weaker effect of loss of *wg* on the ventral margin of the disc suggests that an additional inhibitor, perhaps another *Wnt* gene, could be present in this region.

Ectopic expression of *wg* in randomly positioned clones of cells in the eye disc interferes with both the initiation and progression of differentiation (Treisman and Rubin, 1995). Loss of function of *shaggy/zeste-white3* (*sgg*), which encodes a kinase normally inhibited by *wg* signaling (Fig. 5B), has a similar effect, although ectopic *wg* appears to cause overproliferation and subsequent cell death, while loss of *sgg* transforms the tissue to head cuticle (Treisman and Rubin, 1995; Heslip *et al*., 1997). Expression of the transcription factor encoded by *optomotor-blind* (*omb*) is activated in response to *wg* pathway activity in the eye disc, and may mediate its effects (Zecca *et al*., 1996).

C. Interaction Between *dpp* and *wg*

wg and *dpp* are expressed along the margins of the eye disc in apparently non-overlapping domains (Figs. 3C, 6A, 6B, 7). As mentioned above, loss of *wg* function or ectopic expression of *dpp* along the disc's margins leads to inappropriate initiation of

differentiation, implying that *wg* and *dpp* have antagonistic roles in the eye. Some insight into the mechanisms underlying this functional antagonism comes from experiments where the effect of each gene on the other's expression and function was studied. Loss of *wg* function, in wg^{ts} eye discs, leads to ectopic expression of *dpp* along the anterior margin prior to the start of ectopic differentiation (Ma and Moses, 1995). On the other hand, ectopic expression of *wg* can inhibit differentiation at the posterior margin without obvious reduction in the levels of *dpp* expression (Treisman and Rubin, 1995), suggesting that *wg* signaling may inhibit the function of the dpp protein rather than its expression (Fig. 7). However, ectopic expression of high levels of *wg* along the margins causes a loss of *dpp* expression (U. Heberlein, unpublished). It is possible that low levels of wg inhibit dpp function, whereas high levels inhibit *dpp* transcription; as the function of *dpp* is required for its own expression, these effects may be related. Activation of the *wg* pathway by loss of *sgg* function is sufficient to prevent *dpp* expression (Heslip *et al*., 1997), suggesting that complete inhibition of sgg kinase activity may require high levels of wg signaling. Thus, *wg* is necessary and sufficient to block expression of *dpp*. Conversely, *dpp* is necessary and sufficient to repress inappropriate expression of *wg.* Clones of cells mutant for *punt* or *Mad*, which presumably cannot respond to the *dpp* signal, display ectopic *wg* expression when the clones are located at the posterior margin (Wiersdorff *et al*., 1996) (J. E. Treisman, unpublished data). In addition, ectopic *wg* expression is observed in dpp^{blk} eye discs (Wiersdorff *et al*., 1996; Chanut and Heberlein, 1997). Finally, overexpression of *dpp* can also repress *wg* in its normal expression domain (Chanut and Heberlein, 1997; Pignoni and Zipursky, 1997). Thus, as has been demonstrated for the leg disc (Brook and Cohen, 1996; Jiang and Struhl, 1996; Penton and Hoffmann, 1996; Theisen *et al*., 1996), *wg* and *dpp* display antagonistic functions during the development of the eye. This mutual antagonism may regulate the proper balance between their activating and inhibiting functions, which is crucial for normal retinal differentiation.

Whether *dpp* plays a positive role in photoreceptor differentiation, or merely acts to prevent *wg* from inhibiting this differentiation, is currently uncertain. However, the analysis of double mutant eye discs revealed that precocious differentiation initiating from the dorsal and ventral margins in wg^{ts} mutants is prevented by simultaneously reducing *dpp* expression using the dpp^{blk} mutation (Treisman and Rubin, 1995). This suggests that *dpp* does have a function in promoting differentiation in addition to inhibiting *wg* expression. Another unanswered question is how the initial expression domains of *wg* and *dpp* are established. This may be one of the functions of genes such as *ey*, *eya* and *so*, or it may be achieved by as yet unidentified factors. The target genes activated by the *dpp* pathway have not been identified in the eye, and it is unclear how dpp activity ultimately leads to neural differentiation.

IV. Progression of Differentiation

Once neuronal differentiation begins at the posterior tip of the eye disc, it progresses across the epithelium, one row at a time, reaching the anterior margin approximately two days later. The anterior edge of this differentiation wave is marked by an indentation in the apical surface of the epithelium, the morphogenetic furrow (MF), that spans the disc along its dorsoventral axis (Ready *et al*., 1976) . The MF is the consequence of transient and localized changes in cell shape; cells have reduced apical-basal dimensions and greatly diminished apical surfaces (Wolff and Ready, 1991; Fig. 8). The mechanism and purpose of these cell shape changes are not known.

The MF coincides temporally and spatially with several important events in retinal morphogenesis. Ahead of the furrow, cells are unpatterned and undifferentiated, and they divide asynchronously. In the furrow, cells become synchronized in the G1 phase of their cell cycle as they begin to associate into evenly spaced clusters which will eventually form the individual ommatidia (Ready *et al*., 1976; Tomlinson and Ready, 1987; Wolff and Ready, 1991). Some cells exit the cell cycle and begin differentiating as

neurons immediately posterior to the MF (Fig. 4A). Photoreceptor R8 differentiates first and is followed by the pairwise addition of photoreceptors R2/5 and R3/4. The remaining undifferentiated cells undergo another synchronous round of mitosis, after which they differentiate sequentially into photoreceptors R1/6 and R7 and the cone cells. The pigment cells and the interommatidial bristle group also develop from this set of cells during the pupal stage (for review see Wolff and Ready, 1993). The temporal sequence of eye development is laid out spatially in a single eye disc, with rows becoming successively older from the furrow to the posterior margin.

A. Mutations That Disrupt the Progression of Differentiation

Several mutations that specifically arrest the progression of the differentiation wave have been identified. In these so-called "furrow-stop" mutants the adult flies lack the anterior portion of the retina (Heberlein *et al.*, 1993; Fig. 9). The furrow-stop group includes several dominant mutations, such as *Bar (B)* and *rough*^{DOM} *(ro*^{DOM}*)* and one recessive mutation, *hedgehog*^{1} *(hh*^{1}*)*, an eye-specific mutation in the segment polarity gene *hedgehog (hh*; Mohler, 1988; see Section IV. C). The analysis of eye discs from furrow-stop mutants revealed that differentiation of new ommatidial rows stops after the furrow has moved part-way across the disc. Ommatidia that had begun differentiating prior to this block continue to develop normally causing the typical furrow-stop phenotype, in which the most anterior row of ommatidia is formed by mature clusters containing a full complement of photoreceptor neurons (Heberlein *et al.*, 1993; Chanut and Heberlein, 1997) (Fig. 10). Thus, the process by which the wave of differentiation moves across the eye disc is blocked in these mutants without an effect on ommatidial maturation or neuronal differentiation per se. An indistinguishable furrow-stop phenotype is obtained when eye discs from larvae carrying a temperature sensitive allele of *hh, hh*^{ts2}*,* are shifted to the non-permissive temperature (Ma *et al.*, 1993). While reduction of *hh* function across the whole eye disc, as achieved in *hh*^{1} and *hh*^{ts2} larvae,

leads to MF arrest, clones of cell homozygous mutant for complete loss-of-function *hh* alleles display only mild defects (Heberlein *et al*., 1993; Ma *et al*., 1993; Heberlein and Moses, 1995; Fig. 11). This non-autonomous function of *hh* could be explained if *hh* acts directly as a long range diffusible molecule, if *hh* induces a secondary diffusible signal, or both.

Both *Bar* and *rough* encode homeobox proteins normally expressed posterior to the MF (Tomlinson, 1988; Kimmel *et al*., 1990; Higashijima *et al*., 1992); the eye phenotype of the dominant alleles is caused by increased or inappropriate expression of the respective genes (Kojima *et al*., 1991; Heberlein *et al*., 1993) It has recently been shown that ectopic expression of *rough* inhibits the expression of *atonal (ato)* (Dokucu *et al*., 1996), a gene required for neuronal differentiation in the retina (see Section IV. E), providing a likely explanation for the furrow-stop phenotype of ro^{DOM}.

B. *hedgehog* Function and Furrow Progression

hedgehog (hh), originally identified as a mutation that disrupts embryonic segmentation in *Drosophila* (Nusslein-Volhard and Wieschaus, 1980), encodes a secreted protein (Lee *et al*., 1992; Mohler and Vani, 1992; Tabata *et al*., 1992; Tashiro *et al*., 1993) that also plays crucial roles in patterning various imaginal structures such as the wing and the leg (Basler and Struhl, 1994; Kojima *et al*., 1994; Tabata and Kornberg, 1994; Felsenfeld and Kennison, 1995; Ingham and Fietz, 1995). Several vertebrate *hh* homologs have been identified and shown to function in multiple developmental events (for review see Fietz *et al*., 1994; Ingham, 1994; Pownall, 1994; Ingham, 1995; Johnson and Tabin, 1995).

In the developing fly eye, *hh* is expressed in cells posterior to the MF (Lee *et al*., 1992; Fig. 12). It has been assumed that these cells are the differentiating photoreceptors based on two observations: First, an enhancer-trap insertion in the *hh* gene, in which a LacZ reporter (encoding bacterial β-galactosidase) is transcribed under

the control of *hh* regulatory sequences, is expressed in photoreceptors as they differentiate (Ma *et al*., 1993). Second, this expression requires that cells differentiate as neurons (Heberlein *et al*., 1993). However, it cannot be excluded that additional cells located immediately posterior to the MF also express *hh*.

A possible clue about how *hh*, expressed posterior to the MF, might act in furrow progression came from the observation that expression of *dpp* in the MF requires normal *hh* function; *dpp* expression is absent in the MF of hh^{1} and hh^{ts2} mutant eye discs (Heberlein *et al*., 1993; Ma *et al*., 1993; Fig. 10). This, together with the fact that loss of *dpp* function disrupts retinal morphogenesis (Spencer *et al*., 1982; Masucci *et al*., 1990; Heberlein *et al*., 1993), led to the proposal that *hh* functions by inducing *dpp* as a secondary signal (Heberlein *et al*., 1993). Ectopic expression of *hh* in clones of cells located anterior to the MF leads to ectopic induction of *dpp*, the generation of ectopic furrows and precocious neuronal differentiation (Heberlein *et al*., 1995). These ectopic neurons begin expressing their endogenous *hh* gene thus setting in motion waves of differentiation that can propagate in any direction across the disc (Fig. 13). Thus, *hh* is both necessary and sufficient to induce expression of *dpp* and to propagate the furrow across the eye disc (Heberlein and Moses, 1995). Recent evidence that suggests that *dpp* does not execute all the functions of *hh* will be discussed below.

hh signaling across the embryonic parasegmental boundary and the anterior/posterior compartment boundary of wing and leg discs has been studied extensively (for reviews see (Ingham, 1995; Kalderon, 1995; Perrimon, 1995; Fig. 14). Hh protein, expressed by cells lying posterior to both these boundaries, diffuses anteriorly across the boundary where it induces the expression of target genes such as *dpp*, *wg* and *ptc*. In addition to being a target of *hh* signaling, *ptc* encodes a multiple-pass membrane protein (Hooper and Scott, 1989; Nakano *et al*., 1989) which, based on genetic arguments, was postulated to be the *hh* receptor (Ingham *et al*., 1991). The recently reported observation of a physical interaction between ptc and hh proteins

lends strong support to the hypothesis (Stone *et al*., 1996). *ptc* acts through a series of additional components to constitutively repress the expression of *hh* target genes (Fig. 5C). These target genes are also repressed by *DCO* which encodes the major catalytic subunit of protein kinase A (*pka)* (Lane and Kalderon, 1993). *hh* relieves this inhibition by antagonizing *ptc* function (Fig. 5C). Groups of cells that lack either *ptc* or *pka* function in wing, leg or eye discs, act as if they have received the *hh* signal although they are not located near a source of hh (Chanut and Heberlein, 1995; Jiang and Struhl, 1995; Johnson *et al*., 1995; Li *et al*., 1995; Ma and Moses, 1995; Pan and Rubin, 1995; Sanicola *et al*., 1995; Strutt and Mlodzik, 1995; Strutt *et al*., 1995; Wehrli and Tomlinson, 1995). Thus, the molecules and signaling cascades that are activated (or prevented from inhibiting) by *hh* are very similar whether the signaling occurs across the parasegmental boundary in the embryo, the compartment boundary in the wing and leg disc, or the MF in the eye disc. A striking difference among these systems is, however, that the boundary in the eye, the MF, is transient, it moves, and it does not involve stably inherited cell fates as the other boundaries do. Consistent with this, the engrailed transcription factor, which is required to activate *hh* transcription in the posterior compartment of wing and leg discs (Tabata *et al*., 1992; Tabata *et al*., 1995; Zecca *et al*., 1995), does not control *hh* expression in the eye disc (Strutt and Mlodzik, 1996). Implicit in this “moving boundary” concept is the fact that cells that receive the *hh* signal have to become cells that send the *hh* signal (Fig. 15). Because this transition from a recipient to sender of the *hh* signal requires that the receiving cell differentiate as a photoreceptor neuron, the process of neuronal differentiation is central to furrow progression. Conversely, *hh* signaling is essential for progression of the neuronal differentiation wave. This tight linkage between differentiation and furrow progression, together with the repetitive nature of the process, often makes it difficult to establish clear causal relationships.

C. *dpp* function and MF Progression

While the available data supports the notion that *hh* is necessary and sufficient to induce the expression of *dpp* and to propagate the furrow across the eye disc, the role of *dpp* in this process is less clear. Recently, mutations in the genes that act cell-autonomously to receive and transduce the *dpp* signal (Fig. 5A) have been used to address the role of *dpp* signaling in MF progression. These include mutations in the type I receptors *tkv* (Burke and Basler, 1996; Penton *et al*., 1997) and type II receptor punt (Burke and Basler, 1996) (J. E. Treisman, unpublished data), and the cytoplasmic signal transducer *Mad* (Wiersdorff *et al*., 1996). This analysis, however, was complicated by the fact that these genes are not only required for viability of the animal, but also for cell proliferation and/or survival in clones of homozygous mutant tissue in mosaic animals. Therefore, the experiments have been limited to the analysis of small clones (for null alleles of *tkv*), or hypomorphic alleles (for *punt* and *Mad).* Adult eyes containing internal *tkv*, *punt* or *Mad* clones display only minor irregularities (Burke and Basler, 1996; Wiersdorff *et al*., 1996; Penton *et al*., 1997). In third instar eye discs, progression of the MF is slightly retarded while crossing mutant tissue. These data suggest that only low levels of *dpp* signaling are needed for normal MF progression, or that *dpp* acts through additional, yet unidentified, downstream components in this process. The latter possibility was addressed by generating very large clones of cells completely devoid of dpp production (Burke and Basler, 1996) using the *Minute* technique to give the mutant cells a growth advantage relative to the neighboring wild type cells (Morata and Ripoll, 1975). Adult eyes carrying such large *dpp* clones are normally patterned. It was argued that the large size of these clones effectively rules out the possibility of rescue from surrounding wild type tissue. However, the fact that these clones are able to proliferate suggests that they are indeed rescued, at least with regard to growth, by dpp presumably secreted by their wild type neighbors. Finally, it has been shown recently that removal of *dpp* by shifting a temperature-sensitive (ts) allelic combination to the

non-permissive temperature arrests, or at least slows, furrow progression (Chanut and Heberlein, 1997). This effect, however, is only observed after the furrow has progressed approximately half-way across the disc. Curiously, eye discs from larvae carrying a ts allele of *punt* show ectopic expression of *wg* ahead of the MF when grown at the restrictive temperature (Theisen *et al.*, 1996). While it has not been determined whether the MF is arrested in these discs, ectopic expression of *wg* has been shown to block furrow progression (Treisman and Rubin, 1995). This raises the interesting possibility that one of the functions of *dpp* in the furrow is to block inappropriate expression of *wg*; whether this ectopic *wg* expression is induced by *hh* remains untested.

While it is difficult, at this time, to reconcile all these data, it seems quite clear that the function of *hh* in the eye is not solely mediated by *dpp*. Consistent with this is the observation that, while ectopic expression of *dpp* along the disc's margin can induce ectopic differentiation (Chanut and Heberlein, 1997; Pignoni and Zipursky, 1997), ectopic expression of *dpp* or activation of the *dpp* signaling pathway (by ectopic expression of an activated form of the *tkv* receptor) in internal clones of cells ahead of the MF does not lead to precocious differentiation or the generation of ectopic furrows (Burke and Basler, 1996; Pignoni and Zipursky, 1997). Thus, *dpp* may play a permissive rather than instructive role in MF progression, by ensuring that conditions necessary for normal furrow progression, such as proper cell cycle control (see Section V. A), occur in synchrony.

D. Coordination of Initiation and Progression

As already discussed, *dpp* and *wg* have antagonistic effects on differentiation; *dpp* promotes the initiation of differentiation from the disc's margins, while *wg* inhibits it. Differentiation starts at a point near the optic stalk. As the furrow moves anteriorly across the disc, and the disc continues to grow, differentiation must continuously reinitiate along the margins, a process that requires *dpp* function and signaling. In

conditions of reduced *dpp* function, differentiation fails to spread laterally and this is correlated with ectopic expression of *wg* at the margins (Wiersdorff *et al*., 1996; Chanut and Heberlein, 1997). Because *wg* is expressed ahead of the MF along the dorsal and ventral margins, the advancing furrow has to continuously counteract the inhibitory effect of *wg*. Normally, the lateral margins do not support differentiation until the MF reaches them. It is therefore possible that *dpp* expressed in the MF near the margin is able to inhibit *wg* expression at the margin, allowing differentiation to initiate de novo at each step of MF progression. This inhibition could perhaps be achieved through a non-autonomously induced increase of *dpp* expression at the margin. Thus, the role of *dpp*, through positive autoregulation and inhibition of *wg*, would be to coordinate the progression of differentiation at the center and the edges of the retinal field.

E. Proneural Genes: *atonal* and *daughterless*

Neuronal differentiation in the retina requires the function of the proneural gene *atonal (ato)*. Flies carrying the loss-of-function ato^1 mutation occasionally survive; these survivors lack chordotonal organs (stretch receptors) and are nearly eyeless (Jarman *et al*., 1993; Jarman *et al*., 1994). The remaining eye is very narrow and contains no photoreceptor neurons; only some pigment cells and bristles remain. The failure of photoreceptor differentiation in *ato* mutants is due to a defect in the specification of photoreceptor R8, the so-called ommatidial founder cell (Jarman *et al*., 1994). It is believed that in the absence of an R8, the remaining photoreceptors fail to be recruited and induced to differentiate as neurons.

Eye discs from young ato^1 larvae appear indistinguishable from wild type: the discs are normal in size and express *dpp* along the posterior and lateral margins, the MF forms and even starts moving away from the posterior margin. However, neuronal differentiation is absent, *dpp* expression decays, and MF progression aborts (Jarman *et al*., 1995). While this has not been tested directly, it is likely that *hh* expression is

missing in *ato* eye discs, leading to the observed failure in MF progression. Thus, the analysis of *ato* mutants revealed that initiation and progression of the MF can be dissociated and that neuronal differentiation is crucial for continued furrow progression.

ato is expressed in a dynamic pattern prior to and during MF progression. Before initiation, *ato* expression is seen along the posterior margin in a pattern similar to that of *dpp*. During MF progression *ato* is expressed in a stripe of cells immediately anterior to the furrow. In the furrow, expression is restricted to evenly spaced groups of cells (called intermediate groups) and, shortly thereafter, to single R8 precursors (Jarman *et al*., 1994) (Fig. 16A). *ato* expression is completely dependent upon normal *eya* and *so* function (Fig. 16B, C), suggesting that *ato* acts downstream of the early functions of these genes. In addition, normal *ato* function is required for *ato* expression in the intermediate groups and in R8, revealing a positive autoregulatory loop (Jarman *et al*., 1995). The restriction of *ato* expression from the intermediate groups to single R8s requires signaling by the *Notch (N)* pathway; in eye discs from larvae carrying a temperature sensitive allele of *N*, all cells of the intermediate group differentiate as R8s at the restrictive temperature (Baker and Zitron, 1995; Baker *et al*., 1996).

ato encodes a basic helix-loop-helix (bHLH) protein and is thus a potential transcriptional regulator (Jarman *et al*., 1993). In vitro, ato protein forms heteromultimers with another bHLH protein encoded by the *daughterless (da)* gene; these ato-da multimers act as sequence-specific DNA-binding complexes (Jarman *et al*., 1993). *da* is required for numerous processes throughout development, and is thought to function as a partner for *ato* and other spatiotemporally restricted bHLH proteins such as *achaete (ac)* (Murre *et al*., 1989). Loss of *da* function during eye development, analyzed in mosaic flies due to the embryonic lethality of *da* mutants, results in a failure of neuronal differentiation similar to that observed with loss of *ato* function (Brown *et al*., 1996). However, while *ato* is only required in R8, *da* function is required, in addition to R8, in photoreceptors R2/5 and R3/4, which are the next cells to differentiate after R8. This

requirement, while not absolute, suggests that *da* may act alone or together with a yet unidentified partner in specifying R2/5 and R3/4. .

While the expression of *da* is low throughout most of the eye disc, higher levels of expression are observed just ahead of the MF (Fig. 17). The cells that express high levels of *da* correspond exactly with the cells expressing *ato* (Brown *et al*., 1996). In addition, *da* and *ato* regulate each other's expression patterns in a complex manner. These data, taken together, support the proposal that *ato* and *da* act as partners during proneural specification in the eye. However, *da* also appears to have functions that are independent of *ato*.

F. Antineural Genes: *hairy* and *extramacrochaetae*

hairy (h), like *ato* and *da,* encodes a bHLH protein; *extramacrochaetae (emc)* encodes a HLH protein lacking the basic DNA-binding domain. *h* is expressed in a dorsoventral stripe located immediately anterior to the stripe of *ato* expression (Brown *et al*., 1991). *emc* expression, while fairly ubiquitous, is highest near the anterior margin of the disc (Brown *et al*., 1995; Fig. 17). Loss of *h* or *emc* function in the eye, analyzed in mosaic retinae as both genes are required for viability, has little effect on normal development. However, loss of function of both *h* and *emc* causes the MF and the front of neuronal differentiation to accelerate by as much as eight ommatidial rows while traversing clones of doubly mutant tissue (Brown *et al*., 1995).

While the exact mechanism of *h* and *emc* function during eye development is not known, their roles as negative regulators of proneural function elsewhere in the fly's peripheral nervous system are better understood. *h* and *emc* use different strategies to block proneural gene function. h binds to promoter sequences in the proneural gene *achaete (ac)* that are required for proper repression of *ac* transcription, suggesting that h acts directly as a transcriptional repressor (Ohsako *et al*., 1994; Van Doren *et al*., 1994). Emc, on the other hand, binds in vitro to several bHLH proteins, including ac and

da, forming heterodimers (Van Doren *et al*., 1991; Van Doren *et al*., 1992) that do not bind to DNA and are thus nonfunctional. It is currently unclear whether in the retina *h* and *emc* have redundant actions on the same target, presumably *ato*, or whether they act on different targets and have a synergistic effect.

G. Coordination of Proneural and Antineural Gene Function During Furrow Progression

Orderly progression of the MF requires the adoption of neuronal potential and subsequent differentiation to be tightly regulated both spatially and temporally, such that one row of ommatidia after another develops in exact synchrony. One logical way to achieve this is by coupling differentiation to *hh* signaling. Indeed, *hh* has been shown to induce the expression of *ato* when expressed ectopically ahead of the MF (Heberlein *et al*., 1995). These *ato*-expressing cells then differentiate neuronally and begin expressing their endogenous *hh* gene, which in turn leads to the induction of *dpp* and *ato* in neighboring cells. The repetition of this cycle allows the furrow to move across the disc. Curiously, ectopic expression of *hh* or loss of *pka* function ahead of the MF also leads to ectopic expression of *h* in nearby cells (Heberlein *et al*., 1995; Pan and Rubin, 1995). This suggests that the stripe of *h* expression ahead of the MF is normally induced, directly or indirectly, by *hh*. Thus, *hh* seems to carry out two apparently paradoxical functions; it induces the expression of not only of an activator of neuronal competence, *ato*, but also an inhibitor, *h*. It is likely that this mechanism ensures that *ato* expression and the adoption of neuronal competence are not achieved prematurely, allowing only one new row of ommatidia to be induced at a time. This in turn may be important for orderly patterning of each row of ommatidia and correct projections of photoreceptor axons to the optic ganglia. Another curious observation is that expression of *h* is expanded anteriorly in eye discs from ato^{1} (Jarman *et al*., 1995) and other furrow-stop mutants (U. Heberlein, unpublished), implying that the anterior limit of *h* expression is

also normally defined by signals emanating from the furrow. It is currently unclear how *hh* regulates *ato* and *h* expression, and how *ato* and *h* may regulate each others' expression. However, it appears quite clear that the advancing front of differentiation has long range effects on cells located far ahead of the MF (Fig. 18).

H. A Missing Signal?

It is inferred from loss-of-function and ectopic expression experiments that *hh* not only induces the expression of *dpp*, but also the expression of the proneural gene *ato* (Heberlein et al., 1995*).* While it has not been tested directly, *ato* expression does not appear to be induced by *dpp*: first, *ato* expression is present in *tkv* mutant clones (Penton *et al*., 1997), and second, neurons differentiate in cells that, in theory, cannot receive or transduce the *dpp* signal (Burke and Basler, 1996; Wiersdorff *et al*., 1996; Penton *et al*., 1997). This suggests that either *hh* induces *ato* directly, or that another *hh*-induced signal is responsible for *ato* induction. It has been shown recently that the *N* pathway is not only required to single out R8 cells from the intermediate groups (Baker *et al*., 1996), but also to obtain high levels of *ato* expression in the intermediate groups and for neuronal differentiation (Baker and Yu, 1997). The exact mechanisms by which *N* induces *ato* and neuronal differentiation are unknown. It is possible that *N* is required for cells to respond to *hh* or to another *hh*-induced signal; conversely, *N* may play a more direct role in neuronal differentiation.

V. Cell Cycle Regulation

As the furrow progresses from the posterior tip to the middle of the eye disc, the epithelium grows extensively, increasing in size by approximately six- to eightfold. Thus, patterning, differentiation and cell division have to be tightly regulated both temporally and spatially. This is particularly important in and near the MF, where the transition from asynchronous to synchronous cell division occurs (Ready *et al*., 1976; Wolff and Ready,

1991; Thomas *et al*., 1994). Far ahead of the MF cells divide asynchronously and are therefore found in either the M, G1, S or G2 stages of the cell cycle. Immediately anterior to the MF cells begin to become synchronous; there is an increase in mitosis and an arrest in the ensuing G1 (Thomas *et al*., 1994). In the MF cells are arrested in G1 (Thomas *et al*., 1994). Behind the furrow the precursors of R2-5 and R8 exit the cell cycle and begin differentiation, while all other cells undergo one additional mitosis before differentiating (Wolff and Ready, 1991; Wolff and Ready, 1993). The role of the latter cell division is to generate enough precursor cells to complete the assembly of ommatidia (Wolff and Ready, 1991; Wolff and Ready, 1993; de Nooij and Hariharan, 1995).

Progression through the different stages of the cell cycle requires the activity of different cyclin-dependent kinases (Cdks) and their associated positive regulatory subunits, the cyclins (Cycs) (Norbury and Nurse, 1992; Morgan, 1995). Both positive and negative regulation ensures that this activation is specific for the appropriate stage of the cell cycle. Several Cycs and Cdks have been identified in *Drosophila* (for recent reviews see (Edgar, 1994; Edgar and Lehner, 1996; Follette and O'Farrell, 1997). While their domains of expression in the developing retina are mostly known, the assessment of their functions in the eye is still incomplete and an area of intense research. The expression patterns of several genes that play a role in cell cycle regulation near the MF, and that will be discussed below, are shown in Figure 19.

A. Regulation of G2-M Transition

Synchronization of cells leading to G1 arrest in the MF is thought to occur in two steps: cells in G2 are driven into mitosis, while cells in G1 are prevented from re-entering S-phase. It has been proposed recently that one possible role of *dpp* in the MF is to promote the G2-M transition in cells located just ahead of the MF (Penton *et al*., 1997). Clones of cells mutant for the *dpp* receptors *tkv* or *saxophone (sax)*, and which

encompass the MF, display persistent expression of the G2 cyclin, CycB, in the anterior region of the MF suggesting that cells are arrested in G2. This arrest is transient as mutant cells enter delayed mitosis in the posterior region of the MF. Mutations in the *division abnormally delayed (dally)* gene display a similar phenotype; the domain of CycB expression and the domain containing mitotic cells are shifted posteriorly in the MF (Nakato *et al*., 1995). *dally* encodes an integral membrane proteoglycan that may act as a coreceptor for *dpp* in the eye (Nakato *et al*., 1995).

The product of the *string (stg)* gene, a phosphatase homologous and functionally equivalent to the mitotic inducer Cdc25 (Edgar and O'Farrell, 1989; Kumagai and Dunphy, 1991), is also believed to drive the transition from G2 to M in cells located just anterior to the MF. *stg* is expressed in a band of cells just ahead of the MF (Alphey *et al*., 1992) that coincides with a domain of increased mitosis (Thomas *et al*., 1994). However, the effect of loss of *stg* function in the developing eye has not yet been established.

In conclusion, the three genes described above are believed to help drive cells located just ahead of the MF into mitosis as a first step towards G1 synchronization in the MF. It is tempting to speculate that the role of *dpp* signaling in the MF is to regulate the expression of *stg*, which in turn would activate a CycB/Cdk complex thus driving cells into mitosis.

B. Control of G1

Cells in the MF are arrested in G1; this arrest is thought to be necessary for proper cell-cell communication. In *roughex (rux)* mutants, cells in the MF fail to arrest in G1 and proceed prematurely into S phase and mitosis leading to patterning defects (Thomas *et al*., 1994). *rux* encodes a novel protein that is believed to suppress entry into S phase by preventing the accumulation of CycA: loss of *rux* function causes increased accumulation of CycA, while ectopic expression of *rux* leads to CycA degradation

(Thomas *et al*., in press). As cells exit the furrow, some cells need to be relieved from G1 arrest in order to enter their final mitosis. Cyclin E, which is expressed in these cells (Richardson *et al*., 1995a), has been shown recently to bind to rux protein in vitro and in a yeast two-hybrid assay (Thomas *et al*., in press). The binding of CycE is believed to destabilize rux; loss of rux leads to stabilization of CycA, which in turn promotes cells to complete S phase and to enter the ensuing final mitosis (Thomas *et al*., in press).

The progression into S phase is also regulated by the product of the *regulator of cyclinA (rca1)* gene (Dong *et al*., 1997). *rca1* mutants, which were isolated as dominant suppressors of *rux*, have an embryonic phenotype very similar to that of mutations in *cycA*. Ectopic expression of *rca1* promotes CycA accumulation and drives cells into S phase. This effect is reversed by reducing *cycA* dosage, suggesting that *rca1* functions as a positive upstream activator of *cycA*. Thus, the antagonistic functions of *rca1* and *rux* may limit CycA activity. In the furrow, a critical balance between *rux, rca1* and *stg* activities might lead to G1 arrest by preventing activation of a CycA/cdk complex during G1.

C. Coordination of Cell Cycle Regulation and Furrow Progression

The tight spatial and temporal linkage between patterning and cell cycle synchronization in the eye disc suggests that both events are under the same genetic control. Indeed, ectopic expression of *hh* ahead of the MF leads to ectopic expression of *stg* (Heberlein *et al*., 1995).This is similar to the situation in the embryo, where *stg* expression is controlled by patterning genes which regulate the location of mitotic domains (Edgar *et al*., 1994). High levels of expression of a *rux-lacZ* reporter is also seen in a band of cells just anterior to the MF (Thomas *et al*., in press). It will be interesting to determine whether *rux* expression is also regulated directly or indirectly by *hh*.

Another gene, *yan*, is required for cells to execute the choice between cell division and differentiation. In the eye disc, clones of cells mutant for *yan* overproliferate

and fail to begin neuronal differentiation (Rogge *et al*., 1995). It was postulated that, in wild type development, inactivation of *yan* in/near the MF is required to release certain cells from G1 arrest, driving them into their last mitosis (Rogge *et al*., 1995). Inactivation of *yan* is thought to occur via signaling through the *Drosophila* EGF receptor (Egfr). Consistent with this is the observation that in *Ellipse,* a gain-of-function mutation in *Egfr*, too many cells behind the MF reenter S-phase instead of differentiating neuronally (Baker and Rubin, 1992) (for a recent review on Egfr signaling in the eye see Freeman, 1997). How activation of Egfr and inactivation of *yan* regulate the choice between cell division and differentiation, and how this regulation is coordinated with progression of the MF is not known.

VI. Concluding Remarks

The *Drosophila* eye disc develops in a remarkably coordinated manner; its differentiation begins at a precisely defined point and expands at a steady rate, laying down rows of evenly spaced ommatidial founder cells. Their further development results in the highly ordered structure of the adult compound eye. In this review we have discussed only the first phases of development, from the specification of the eye field to the establishment of ommatidial preclusters. There has been much recent progress in this area of research, and we now have some understanding of the signals used to impose pattern on an apparently uniform tissue. However, many questions still remain unanswered. Although a number of apparent transcription factors encoded by *ey, eya, so* and *dac*, are required to allow the eye to form, the interactions between them and the target genes they regulate have not been well defined. The disc is then spatially organized by the interaction between *dpp* and *wg*; but the molecular basis for this interaction, the initial establishment of the patterns of *dpp* and *wg* expression, and the temporal control of differentiation are not understood. The progressive pattern of development is controlled by *hh* and its target proneural, antineural and cell cycle

genes, but we are far from a mechanistic understanding of this process. The transition from *dpp*-controlled initiation to *hh*-controlled progression is also not understood. We thus anticipate many additional insights into these processes in the future. As the mechanism of eye development appears to be conserved to an unexpected degree between flies and vertebrates, some of these insights will probably also be applicable to other organisms.

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Figure Legends

Fig. 1 Schematic representation of the retina at different stages of development. From left to right are shown cartoons of eye discs of gradually more mature stages, from early to late third instar larvae, and an adult retina, viewed from the top. Below is shown a lateral view of a portion of an eye disc in which the morphogenetic furrow has progressed part way across the disc. Posterior is to the right.

Fig. 2 Scanning electron micrographs of wild type, mutant, and ectopic eyes. A) Wild type. B) *eyes absent*^{E1} *(eya*^{E1}*)*, a strong *eya* allele. C) *sine oculis*^{1} *(so*^{1}*)*. D) *dachshund*^{4}*(dac*^{4}*)*. E) *decapentaplegic*^{blk}*(dpp*^{blk}*)*. F) and G) Ectopic eyes induced by misexpression of *eyeless* located on the antenna and under the wing, respectively. F) Ectopic eye on the antenna induced by misexpression of *dachshund*. Magnification in panels F-H is approximately 4 to 5 times that in panels A-E. B) reprinted with permission from Bonini et al., 1993; C) reprinted with permission from Cheyette et al., 1994; F) and G) reprinted with permission from Halder et al., 1995a; D and H) reprinted with permission from Shen and Mardon, 1997. Posterior is to the right.

Fig. 3 Photomicrographs of eye discs displaying phenotypes associated with loss of *eyes absent (eya)* function. A) and B) Eye discs carrying clones of cells mutant for *eya* located at the posterior margin and the middle of the disc (arrows), respectively. Clones are marked by the absence of *β–galactosidase* expression (gray); differentiating photoreceptors are marked using an anti-Elav antibody (black staining), which recognizes a neuronal nuclear protein (Robinow and White, 1991). C) and D) Eye discs from early and mid third instar larvae carrying a *dpp-lacZ* reporter (Blackman et al., 1991). C) A wild type eye disc displays *dpp* expression along the posterior and lateral margins. D) Eye disc from an *eya* mutant larva; expression of *dpp* is abolished.

Magnification in panels C and D is approximately 4 times that in A and B. Posterior is to the right.

Fig. 4 Photomicrographs of eye discs displaying phenotypes associated with loss of *dpp* function. All discs are derived from late third instar larvae and are stained with anti-Elav to mark differentiating photoreceptors posterior to the furrow (arrowheads) and with X-GAL to show the expression of the *dpp-lacZ* reporter in the furrow. A) Wild type. B) dpp^{blk}. C) and D) dpp^{hr4}/dpp^{hr56} dissected 48 hours after a shift to the restrictive temperature. Differentiation is completely blocked in C), and is restricted to the medial portion of the disc in D). Posterior is to the right. Reprinted with permission from Chanut and Heberlein, 1997a, b.

Fig. 5 Diagrams of the signal transduction pathways activated by decapentaplegic (dpp), wingless (wg) and hedgehog (hh). Positive genetic interactions are shown as arrows, and negative interactions are shown as two intersecting lines. See text for details.

Fig. 6 Photomicrographs of eye discs showing the normal *wg* expression domains (A and B) and the consequences of loss of *wg* function (C and D). A) and B) Eye discs from wild type mid and late third instar larvae, respectively, carrying a *wg-lacZ* reporter. Discs are stained with anti-Elav to localize differentiating neurons and X-GAL to identify *wg-lacZ* expression domains. *wg* is expressed along the dorsal and ventral margins of the disc (arrowheads) ahead of the morphogenetic furrow (arrows). C) and D) Eye discs from wg^{ts} larvae dissected approximately 48 hours after having been shifted to the restrictive temperature are stained with anti-Elav. Differentiation initiates precociously from the dorsal and ventral margins. Magnification in panel D) is 3-fold higher than that in the other panels. Posterior is to the right and dorsal is up.

Fig. 7 Diagram summarizing the interactions between *wingless (wg)* and *decapentaplegic (dpp)*. Expression of *dpp* is shown as horizontally hatched areas and expression of *wg* as vertically hatched areas. Positive interactions are shown with arrows, negative interactions with two intersecting lines. Posterior is to the right.

Fig. 8 Confocal photomicrograph of a portion of an eye disc stained with phalloidin to highlight the shape of cells. Cells in the furrow (arrow) have reduced apical surfaces. Cells posterior to the furrow begin to associate into evenly spaced clusters. Posterior is towards the top right corner. Reprinted with permission from Heberlein et al., 1993.

Fig. 9 Scanning electron micrographs of eyes of furrow-stop mutants. Genotypes shown are: A) hh^{1}/hh^{1}. B) $ro^{DOM}/+$. C) *Bar/Bar*. The anterior portion of the retina is missing in these mutants. Posterior is to the right. Reprinted with permission from Heberlein et al., 1993.

Fig. 10 Photomicrographs of third instar eye discs carrying the *dpp-lacZ* reporter stained with anti-Elav (to identify differentiating neurons posterior to the furrow) and X-GAL (to identify *dpp-lacZ*-expressing cells). A) In a wild type disc Elav-positive cells lie behind the furrow (arrow), where *dpp* is expressed. B) In a hh^{1} eye disc expression of *dpp* in the furrow (arrow) is absent, and ommatidia in the first row (black arrowhead) contain a full complement of photoreceptors; in wild type this would only be seen several rows posterior to the furrow (white arrowhead). Posterior is to the right. Reprinted with permission from Chanut and Heberlein, 1997a.

Fig. 11 Phase contrast photomicrograph of tangential sections through adult retinae. A) Wild type retina. B) Retina containing a clone of cells homozygous mutant for a null *hh*

allele. The mutant tissue is identified by the lack of the *white*$^{+}$ marker, which leads to the absence of pigment granules. The approximate location of the posterior border of the clone is highlighted with a dotted line. Most ommatidia within the mutant clone are normally structured; one exception is highlighted with the arrowhead. Posterior is to the right.

Fig. 12 Photomicrograph of an eye disc from a third instar larva carrying a *lacZ* enhancer-trap insertion in the *hh* locus stained with X-GAL. Line P30 (Lee *et al*., 1992) faithfully reflects the expression pattern of the *hh* gene. Expression of β-galactosidase is seen posterior to the furrow (vertical arrow), in the presumptive ocellar region (arrowhead), and in the antennal disc (horizontal arrow). Posterior is to the right.

Fig. 13 Photomicrographs of eye discs carrying clones of cells that ectopically express *hh* under the control of the *Tubulin α2* promoter generated using the "flp-out" technique (Struhl and Basler, 1993). Expression of the *dpp-lacZ* reporter is visualized by X-GAL staining (blue), and neuronal differentiation is marked by staining with the anti-Elav antibody (brown). A) Disc carrying a group of precociously differentiating neurons (arrow) and groups of cells showing ectopic expression of *dpp* (arrowheads). B) Disc in which an ectopic furrow (arrow) progressed from the ventral margin. Posterior is to the right. Reprinted with permission from Heberlein et al., 1995.

Fig. 14 Diagram of a "stable boundary". The signaling process across the "stable" anterior/posterior (A/P) compartment boundary in a wing disc is summarized. hh, expressed under the control of *en* in the posterior compartment, diffuses across the A/P boundary where it antagonizes *ptc* function; this leads to the derepression of *dpp* and *ptc* expression. dpp protein acts as a morphogen patterning both compartments. For details see text. Posterior is to the right.

Fig. 15 Diagram of a "moving boundary". Signaling in the morphogenetic furrow is very similar to signaling across the A/P compartment boundary (see Figure 14). However, in contrast to the "stable" compartment boundary, the furrow is a transient boundary that moves across the disc. Thus, cells that receive the *hh* signal become in time cells that send the *hh* signal. See text for details. Posterior is to the right.

Fig. 16 Photomicrographs of wild type and mutant eye discs displaying expression of *ato*, assayed by in situ hybridization. A) Eye disc from a wild type larva. *ato* is expressed in and near the furrow (large arrowhead), in the presumptive ocellar region (small arrowheads), and in the antennal disc (arrow). B) and C) Eye discs from *eya* and *so* mutant larvae, respectively, stained as for A). Posterior is to the right. Reprinted with permission from Jarman et al., 1995.

Fig. 17 Diagram of the expression patterns of HLH proteins in the eye disc relative to the position of the morphogenetic furrow (MF). See text for details. Posterior is to the right. Adapted with permission from Brown et al., 1995.

Fig. 18 Diagram summarizing the interactions between *hh* and the genes encoding HLH proteins as deduced from ectopic expression and loss-of-function experiments. *hh* induces the expression of *ato* and *h* in non-overlapping domains ahead of the furrow. *hh* also appears to repress *h* expression in more anterior cells. *emc* and *da* block furrow progression and the expression of *ato*. *ato* and *da* regulate each others' expression. Positive interactions are shown with arrows, negative interaction with two intersecting lines. It is unclear whether the interactions shown are direct or indirect. The position of the furrow coincides with the region of *ato* and *da* expression. Posterior is to the right.

Fig. 19 Diagram summarizing the expression patterns of cell cycle regulated genes in the eye disc. The disc can be divided into five domains (I-V), each domain containing cells in different stages of the cell cycle. Adapted with permission from Thomas et al., 1994 and 1997.