Cell Death and Differentiation (1997) 4, 4–11
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Surviving *Drosophila* eye development

Nancy M. Bonini

Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018, USA; tel: 215-573-9267; fax: 215-898-8780 e mail: nbonini@sas.upenn.edu

Received 17.6.96; revised 30.8.96; accepted 6.9.96
Edited by H. Steller

Abstract

During eye development, cell death interplays dynamically with events of differentiation to achieve the remarkably patterned structure of the fly compound eye. Mutations in genes that affect the normal developmental process can lead to excessive death of progenitor cells, or, alternatively, to the differentiation of supernumerary neurons, pigment and cone cells due to survival of cells that would normally be eliminated. These data reveal that eye development contains cell selection processes: only certain cells are selected to undergo differentiation, and supernumerary cells are actively eliminated by cell death pathways to achieve the highly ordered lattice of the eye. The final number of cells that comprise the eye is controlled through a balance of cell proliferation with proper cell differentiation and removal by cell death.

Keywords: cell death, cell differentiation, cell selection, apoptosis, *Drosophila*, compound eye

Abbreviations: *dpp*, decapentaplegic; EGF, epidermal growth factor; emc, extra macrochaetae; hid, head involution defective

Introduction

The *Drosophila* eye provides a system approachable with genetic and molecular tools for understanding signal transduction pathways and cell-cell interactions involved in differentiation. During the developmental process, cell survival interplays crucially with cell differentiation to generate the highly precise array of photoreceptor neurons and accessory cells of the eye. The adult compound eye comprises approximately 800 individual ommatidia, each consisting of an ordered array of eight photoreceptor neurons with a complement of accessory cells including four cone cells, two primary pigment cells, shared secondary and tertiary pigment cells and bristle cell (reviewed in Wolff and Ready, 1993) Differentiation commences in the mid-third larval instar with a wave of development marked by the morphogenetic furrow, which progresses from posterior to anterior across the epithelial field of progenitor cells (Ready *et al*, 1976). Anterior to the furrow, progenitor cells are dividing; posterior to the furrow clusters of cells destined to become the adult ommatidial units undergo cell-cell interactions and cell selection events of differentiation.

Although much attention has focused on target-dependent survival (reviewed by Cowan *et al*, 1984; Oppenheim, 1991; Raff *et al*, 1993), a cell's developmental history is comprised of multiple stages when survival is critical. In the fly eye, cell death functions at several times to sculpt the selection of cells (Figure 1): one selection process occurs prior to the major differentiation hurtle of the furrow; a second occurs posterior to the furrow during cellular interactions involved in differentiation of the clusters (Wolff and Ready, 1991; Bonini *et al*, 1993; Hay *et al*, 1994); and a third in final stages of pattern formation to eliminate supernumerary cells associated with each cluster and establish the highly ordered lattice of the adult eye (Cagan and Ready, 1989a). Exquisite coordination of events of differentiation, including cell cycle regulation, is required for appropriate cell development; loss of coordination can lead to loss of cells by death. Here, we focus on the integration of cell survival strategies with the cell differentiation process at various stages of eye development, emphasizing genes whose altered function leads to changes in cell death patterns of cells in the eye (Table 1). Given the many striking examples of conservation of gene sequence and function between *Drosophila* and humans (e.g. Halder *et al*, 1995; Banfi *et al*, 1996), these studies have application to vertebrate development mechanisms (see accompanying reviews). Recent reviews emphasizing other aspects of *Drosophila* eye development include Thomas and Zipursky (1994), Zipursky and Rubin (1994), Bonini and Choi (1995), Heberlein and Moses (1995). Recent coverage of genes of *Drosophila* cell death pathways is found in White and Steller (1995).

Interweaving cell survival with pattern formation

Prior to the furrow

Key genes that function anterior to the furrow in eye development include *eyeless*, *sine oculis* and *eyes absent*. The *eyeless* gene encodes a Pax-6 homeobox homolog (Quiring *et al*, 1994), *sine oculis* a homeobox gene (Cheyette *et al*, 1994; Serikaku and O'Tousa, 1994), and *eyes absent* a novel nuclear protein (Bonini *et al*, 1993). Ectopic expression of *eyeless* in other tissues can induce the formation of eyes; *eyeless* is thus thought to be a fundamental control gene for eye formation and is highly conserved from flies to mammals (Halder *et al*, 1995; see Zuker, 1994). Given this role of *eyeless*, the *sine oculis* and *eyes absent* genes may function in concert with or as targets of *eyeless* activity, both genes being essential for eye formation with their loss of function leading to loss of the adult compound eye. They may function in a number of biological events, including to determine which cells are competent to form eye, cell survival and initiation of pattern formation with the furrow (below).

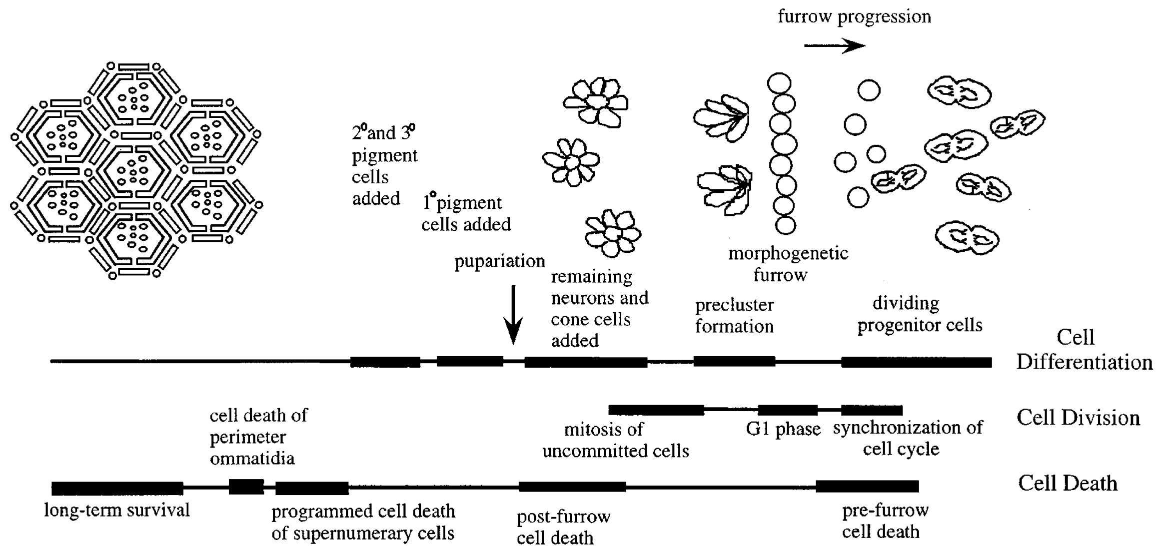

Figure 1 *Drosophila* eye development from the third instar larval stage (right) to the neurocrystalline lattice of the adult eye (left), with The approximate relative timing of events of cell differentiation, cell division, and cell death. Anterior to the furrow, cells are dividing (Ready *et al*, 1976). Close to the furrow, cell cycle synchronization occurs (Thomas *et al*, 1994), and a phase a cell death takes place (Wolff and Ready, 1991; Bonini *et al*, 1993; Hay *et al*, 1994). In the furrow, cells are in the G1 phase of the cell cycle (Thomas *et al*, 1994). Posterior to the furrow, the first events of cell differentiation are seen with preclusters of photoreceptor neurons forming (reviewed in Wolff and Ready, 1993). Subsequently, a final round of mitosis takes place (Ready *et al*, 1976) and additional cells are added to the clusters, including the remaining photoreceptor neurons and the cone cells. In the more posterior region of the disc beginning around 12 rows behind the furrow, another phase of cell death occurs (Wolff and Ready, 1993). During the pupal period, the primary, secondary and tertiary pigment cells are added, followed by a phase of cell death which removes supernumerary cells to sculpt the final pattern of the neurocystalline lattice (Cagan and Ready, 1989a; Wolff and Ready, 1991). This phase is followed by the death of cells of perimeter ommatidia (Wolff and Ready, 1991). Mitosis and development of the bristle cells occurs during the pupal period, and is not indicated here (see Wolff and Ready, 1993). In the adult, the compound eye is maintained by trophic interactions, due to connections between the photoreceptor neurons and the brain (Campos *et al*, 1995).

Loss-of-function mutations of the *eyeless*, *eyes absent* or *sine oculis* genes increases the amount of cell death that occurs anterior to the furrow (Fristrom, 1969; Bonini *et al*, 1993; Cheyette *et al*, 1994; Serikaku and O'Tousa, 1994). This cell death, as with most other cell death referred to here, has morphological and histological features characteristic of programmed cell death (Kerr *et al*, 1972; Wolff and Ready, 1991; Abrams *et al*, 1993), although which programmed cell death genes are activated in these mutants has not yet been addressed. The timing of excessive cell death in these mutants overlaps the period of normal death of some cells ahead of the furrow (Spreij, 1971; Wolff and Ready, 1991; Bonini *et al*, 1993). This spatiotemporal overlap suggests that a cell selection event occurs just anterior to the furrow: for example, at this time, cells with the right set of factors and at the appropriate stage of the cell cycle will proceed into the differentiation process marked by the furrow, whereas others, developmentally or otherwise amiss or deemed extraneous, become eliminated by activation of programmed cell death.

Although the reason why cells are normally eliminated ahead of the furrow is unknown, the time in development provides a logical point in eye formation at which cell selection should occur. At this time, the preliminary number of cells that will comprise the adult eye is defined. The total cell number will reflect the number generated by division (ahead of the furrow and in one additional wave of mitosis posterior to the furrow), minus the number eliminated by cell death. Instead of controlling precise cell number by the number of cell divisions (cell lineage), the animal appears to have acquired a degree of plasticity in that progenitor cells continually divide and may compete for differentiation. The appropriate number of cells is selected in parallel with the progression of cell differentiation. Such developmental flexibility incorporates plasticity required to generate a structure of the appropriate size to match the size of the animal which may depend upon environmental conditions, nutritional state, and other criteria: for any one fly, the total number of ommatidia is distinct, although falls in a range of 745 to 828 for females, with an average of 776 (Ready *et al*, 1976). Analysis of mutants like *eyeless*, *sine oculis* and *eyes absent* indicates that proper function of these genes is one checkpoint for cell differentiation at the furrow.

Distinctions exist between *sine oculis* and *eyes absent* mutants in positional information of cells that survive, suggesting that the two genes are involved in at least partially distinct aspects of the differentiation process. In *sine oculis* mutants, islands of eye progenitor cells that survive in weak alleles (which generate partial eyes) maintain their dorsoventral positional information within the eye disc, such that their photoreceptor axons make the appropriate dorsoventrally positioned contacts in the brain (Kunes *et al*, 1993). In contrast, in weak *eyes absent* mutations, cells that develop are always located at the most posterior tip of the eye field (Bonini *et al*, 1993), and are therefore not anticipated to retain prior positional information.

Table 1 Listing of some eye developmental genes that influence cell survival

Gene	Mutant phenotype and influence on cell survival	Gene product and function	References
Early genes			
eyeless	Reduced eye/eyeless; cell death ahead of the furrow	Pax-6 homeodomain homolog; can induce ectopic eye formation	Fristom, 1969; Quiring *et al*, 1994; Halder *et al*, 1995
eyes absent	Reduced eye/eyeless; cell death ahead of the furrow	Novel nuclear protein; functions ahead of the furrow	Bonini *et al*, 1993
sine oculis	Reduced eye/eyeless; cell death ahead of the furrow	Homeodomain protein; functions ahead of the furrow	Cheyette *et al*, 1994; Serikaku and O'Tousa, 1994
dachshund	Reduced eye/eyeless; cell death and transformation of eye tissue to cuticle	Novel nuclear protein; role in furrow initiation	Mardon *et al*, 1994
Furrow progression			
dpp	Reduced eye/eyeless; cell death	TGFβ homolog; functions non-autonomously in furrow propagation	Bryant, 1988; Heberlein *et al* 1993b
hedgehog	Reduced eye; cell death ahead of the furrow	Functions in non-autonomous signalling for propagation of the furrow	Ma *et al*, 1993
Cell cycle control			
roughex	Failure of G1 arrest in the furrow; extensive cell death after the furrow	Novel protein; functions with *string* to synchronize cells in the furrow in G1	Thomas *et al.,* 1994
string	G2 arrest	Homolog of mitotic inducer cdc25; functions with *roughex* to synchronize cells in the furrow in G1	Alphey *et al*, 1992; Thomas *et al*, 1994
Spacing of the clusters			
atonal	Eyeless; cell death after the furrow	Proneural gene; required for establishing founder cell of each ommatidial cluster	Jarman *et al*, 1994, 1995
Ellipse	Reduced eye; influences mitosis and differentiation of progenitor cells; increases cell death after the furrow	EGF receptor homolog; affects precluster formation	Baker and Rubin, 1989, 1992
Pigment cell selection			
roughest (also *irregular chiasm C*)	Rough eye due to survival of supernumerary pigment cells	Transmembrane protein with immunoglobulin-like domains	Wolff and Ready, 1991; Ramos *et al*, 1993; Schneider *et al*, 1995
echinus	Rough eye due to survival of supernumerary cells		Wolff and Ready, 1991
argos (also *giant lens* or *strawberry*)	Extra photoreceptor, pigment and bristle cells due to survival and differentiation of supernumerary cells in the larval eye disc and during pupal stages	Protein with EGF motif that functions non-autonomously; inhibits neighboring cells from adopting identical fates	Freeman *et al*, 1992; Kretzschmar *et al*, 1992; Okano *et al*, 1992; Brunner *et al*, 1994; Schneider *et al*, 1995
Genes of programmed cell death pathways			
reaper	Within 300 kb deletion that is required for programmed cell death in the embryo	Novel; expressed in cells that will undergo cell death; ablates the eye when ectopically expressed	White *et al*, 1994, 1996; Hay *et al*, 1995
grim	Within 300 kb deletion that is required for programmed cell death in the embryo	Novel; ablates the eye when ectopically expressed	Chen *et al*, 1996
hid	Within 300 kb deletion that is required for programmed cell death in the embryo; mutations in *hid* fail to undergo embryonic head involution and have supernumerary cells	Novel; ablates the eye when ectopically expressed	Grether *et al*, 1995
P35	Viral protein; inhibits ICE/ced-3 cysteine protease activity	Blocks normally occurring, *reaper* and *hid* induced cell death in the eye	Hay *et al*, 1994, 1995; Grether *et al*, 1995; White *et al*, 1996; Xue and Horvitz, 1995; Bump *et al*, 1995
DIAP1/*thread*	Lethal	Cellular homolog of baculovirus inhibitor of apoptosis; blocks normally occurring, *reaper* and *hid* induced cell death in the eye	Hay *et al*, 1995
DIAP2		Cellular homolog of baculovirus inhibitor of apoptosis; blocks normally occurring, *reaper* and *hid* induced cell death in the eye	Hay *et al*, 1995

The progression of differentiation: Moving the furrow

Pattern formation of the eye involves the function of a number of genes, some required for differentiation of specific cell subtypes, whereas others function to initiate, push and/or pull the morphogenetic furrow (as noted, *sine oculis* and *eyes absent* may function in furrow initiation or progression as well as other events, given that their expression initiates prior to furrow formation (Bonini *et al*, 1993; Cheyette *et al*, 1994; Serikaku and O'Tousa, 1994)). The fly homolog of transforming growth factor β (*decapentaplegic (dpp)*), and *hedgehog* are key players in movement of the furrow. *dpp* is expressed at the posterior margin and lateral edges of the eye progenitor field prior to furrow progression, and expression is maintained in the furrow as it moves (Heberlein *et al*, 1993b; Ma *et al*, 1993). Maintenance of *dpp* expression within the furrow is achieved by *hedgehog* activity: *hedgehog* is expressed in the differentiating clusters posterior to the furrow, but functions to signal progenitor cells ahead of the furrow to initiate events of differentiation, including *dpp* expression (Heberlein *et al*, 1993b, 1995; Ma *et al*, 1993). Thus, whereas *hedgehog* is expressed spatially in differentiating clusters posterior to the furrow, it functions in furrow progression to induce cells ahead of the furrow into pattern formation events. Loss of *hedgehog* or *dpp* activity increases the amount of cell death ahead of the furrow, typically in a band of death just prior to the furrow (Heberlein *et al*, 1993b; Ma *et al*, 1993). One way to account for these observations is that inappropriate differentiation leads to elimination of cells by programmed death prior to furrow formation. These data support the idea of an interplay between genes functioning within progenitor cells ahead of the furrow and those involved in non-autonomous events of the dynamic patterning process being important for cell selection events of differentiation.

Initiation of furrow progression from the posterior pole of the eye disc involves the actions of additional genes, such as *dachshund* (Mardon *et al*, 1994). Loss of *dachshund* function at the posterior margin leads to death of some cells, and a change in fate of other cells to that of cuticle. The temporal restriction of furrow movement to a dorsal-ventral band progessing across the progenitor epithelial field also involves interactions that prevent precocious differentiation of the progenitor cells. This includes inhibition from the diffusible factor *wingless* to restrict furrow initiation to the posterior end of the disc by inhibiting progression from the lateral margins (Ma and Moses, 1995; Treisman and Rubin, 1995). The *hairy* and *emc* (*extra macrochaetae*) genes encode helix–loop–helix proteins, and likewise prevent precocious differentiation within the progenitor field just ahead of the furrow (Brown *et al*, 1995). These genes are normally strongly expressed in bands several cell diameters wide anterior to the furrow, with strong *emc* expression preceding the band of *hairy* expression, and temporally regulate the rate of progression by restricting the advance of the furrow. cAMP-dependent protein kinase and *patched* activities are also important to regulate the rate of progression; loss of cAMP-dependent protein kinase function in clones anterior to the furrow allows precocious furrow formation, similar to ectopic *hedgehog* expression (Heberlein *et al*, 1995; Strutt *et al*, 1995; Pan and Rubin, 1995). Biologically, the region just anterior to the furrow is distinct from regions further anterior, since eye disc fragments that include this region can re-initiate furrow formation (Lebowitz and Ready, 1986). This region would appear to overlap the domain of strong expression anterior to the furrow of a number of the genes mentioned, including *eyes absent*, *sine oculis*, *hairy*, *emc* and *string* and correspond to the region where the loss of cAMP-dependent protein kinase activity or ectopic *hedgehog* expression can initiate furrow formation *de novo*.

The *hedgehog* gene functions in furrow progression in short-range diffusible interactions, however when expressed ectopically within progenitor cells far anterior to the furrow, it stumulates division dramatically (Heberlein *et al*, 1995). Extension of these observations to the normal situation *in vivo* suggests that cellular response to *hedgehog* in the eye progenitor field may be concentration-dependent and differ in short-range compared to long-range interactions. Moreover, the response to *hedgehog* or other signals likely depends on the levels of other factors expressed in the cells which receive the signal. Some genes are only expressed just anterior to the furrow, such as *hairy*, whereas the expression patterns of other genes, notably *eyes absent* and *sine oculis*, are graded with weaker expression further anterior to the furrow and strong expression just anterior to the furrow. Thus, the same signal may mediate different functions based on concentration-dependence and the expression levels of other genes within the cells.

When cells enter the furrow, several genes are critical for the cellular dynamics involved in generating an array of cell clusters of the right number and spatial pattern. These genes include *atonal* (Jarman *et al*, 1995), *Ellipse* (Baker and Rubin, 1992), *scabrous* (Baker and Rubin, 1990), *Notch* (Cagan and Ready, 1989b), among others, and play roles in restricting the number of clusters that are initiated, as well as regulating their spacing. Other genes are important for cellular interactions that define photoreceptor and accessory cell types. In some cases, loss of function or aberrant function of these genes leads to cell death, for example, as in mutations of *atonal* (Jarman *et al*, 1995), *Ellipse* (Baker and Rubin, 1992) and *glass* (Ready *et al*, 1986) (whereas others, such as *sevenless* mutations, result in cell fate transformations to another cell of the ommatidial cluster (Tomlinson and Ready, 1986)). The timing of cell death is distinct, however, from the earlier mentioned examples where cell death is increased ahead of the furrow. In the cases described here, cells die in the posterior region of the eye disc within the developing clusters, or even later as in *glass* mutations with presumptive photoreceptor cells dying during the pupal stages (Ready *et al*, 1986). In mutations in the proneural gene *atonal*, which is important for selecting the founding neuron of the ommatidial clusters, cell death is dramatically increased just after the furrow (Jarman *et al*, 1995). Hence, in *atonal* mutants, normal furrow progression occurs, but no cells survive to contribute to the adult eye due to loss of the cells just after the furrow.

During eye development, some cells are normally eliminated by cell death among the differentiated clusters, initiating about 12 rows after the furrow and continuing as a diffuse band of death (Wolff and Ready, 1991; Bonini *et al*, 1993; Hay *et al*, 1994; also Wolff and Ready, 1993). This cell death may be associated with failure of proper cell fate specification of cone and primary pigment cells, or cell cycle events. In *Ellipse* mutants, which represent hyper-active alleles of the *Drosophila* epidermal growth factor (EGF) receptor, some cells that die are presumably in the G2 phase of the cell cycle and have failed to undergo mitosis to proceed to G1 where they can undergo differentiation (Baker and Rubin, 1989, 1992). Altered function of additional genes required for patterning of the eye can also lead to increased cell death in the differentiating region of the disc, for example, as in *Star* mutations (Heberlein *et al*, 1993a), or flies expressing an activated form of yan, an ETS DNA binding domain protein modulated by the MAP kinase pathway (Lai and Rubin, 1992; Rebay and Rubin, 1995). The normal function of the *yan* gene is to inhibit progenitor cell differentiation. Expressing an activated form of yan ectopically in differentiating cells can lead to cell death (Rebay and Rubin, 1995), suggesting that if a cell receives conflicting signals of whether or not to differentiate, the net result can be to trigger programmed cell death pathways. Thus, cells may die during the differentiation process for many reasons, including failure to make appropriate contacts required to stimulate differentiation, failure to obtain sufficient levels of factors required for differentiation and/or survival, abnormal cell cycle events, conflicting differentiation and mitotic signals, conflicting inhibitory and stimulatory signals for differentiation, among others. In sum, it may be that if a cell fails to receive an appropriate mitotic or differentiation signal by a specific time, or if it receives conflicting signals, death is the necessary outcome.

Integration of cell cycle control with the differentiation process

As noted, appropriate regulation of the cell cycle is integral to the cell differentiation process. Anterior to the furrow progenitor cells are dividing asynchronously, but just prior to pattern formation cell cycle becomes synchronized such that cells enter the furrow in the G1 phase (Ready *et al*, 1976; Thomas *et al*, 1994a). Cells recruited into clusters posterior to the furrow begin differentiation and become postmitotic, whereas the remaining progenitor cells proceed through an additional round of division (Ready *et al*, 1976). Cell cycle control genes and regulators, including *string* (homolog of the mitotic inducer *cdc25* (Edgar and O'Farrell, 1989)), cyclins (Thomas *et al*, 1994b; Finley *et al*, 1996; Richardson *et al*, 1995), EGF signalling molecules (Baker and Rubin, 1992; Tio *et al*, 1994), among others to be defined (e.g. de Nooij and Hariharan, 1995), contribute to synchronization of cell cycle events with differentiation.

string is expressed in a domain anterior to the furrow that may be critical to coordinate events of cell cycle control with differentiation (Alphey *et al*, 1992; Thomas *et al*, 1994a). Within this domain, two cell cycle events are thought to occur: those cells in G1 are prevented from re-entering the cell cycle, whereas those in G2 are stimulated by *string* function to progress through mitosis to arrest in G1 (Thomas *et al*, 1994b). The *roughex* gene encodes a novel protein whose activity is critical to synchronize the cell cycle in G1 within the furrow; with loss of *roughex* activity, cells fail to undergo G1 arrest prior to the furrow and continue division as they proceed through the furrow (Thomas *et al*, 1994a). *roughex* mutants develop eyes with fewer than the normal number of cells and display ommatidial patterning defects. In the eye disc, an increase in the numbers of cells dying within and posterior to the furrow occurs. Failure of cell cycle synchronization may lead to death of cells that receive conflicting signals of differentiation and cell division, or of cells that find themselves in the wrong phase of the cell cycle for differentiation (such as G2) but fail to receive a signal for mitosis (such as described for *Ellipse* mutants, above). The normal cell death that occurs ahead of the furrow overlaps the *string* expression domain in which cell cycle synchronization begins. One reason to eliminate a cell at this time may be its inability to undergo cell cycle synchronization events required for progression into the furrow. Thus, genes that lead to increased cell death at this time, such as *eyes absent*, *sine oculis*, *dpp*, and *hedgehog*, may influence this event as well as others.

Sculpting the neurocrystalline lattice of the eye

During pupation, two phases of cell death occur in the eye field to eliminate extraneous cells associated with the ommatidia, generating the highly ordered array characteristic of the adult compound eye. One phase occurs between 35 and 50 h post pupariation, and results in elimination of two or three extra secondary and tertiary pigment cells per ommatidium; these cells form the outer part of the ommatidial lattice and are shared between neighboring clusters (Cagan and Ready, 1989a; Wolff and Ready, 1991). A second burst of death occurs between 60 and 70 h after pupariation to eliminate perimeter ommatidia, which are frequently observed to be stunted in their position in the array (Wolff and Ready, 1991). The death of perimeter clusters occurs simultaneously across the entire epithelium, suggesting the possibility of a coordinated signalling mechanism to effect this cell loss.

Mutations in the genes *roughest* (also called *irregular chiasm C*), *echinus* and *argos* (also called *giant lens* and *strawberry*) prevent proper elimination of extraneous pigment cells during the first phase of pupal cell death, leading to adult eyes of disrupted pattern (Wolff and Ready, 1991; Brunner *et al*, 1994). In *roughest* and *echinus* mutants, the perimeter ommatidial deaths occur normally. The *roughest* gene encodes a transmembrane protein with extracellular immunoglobulin-like domains that displays homophilic adhesive interactions *in vitro* (Ramos *et al*, 1993; Schneider *et al*, 1995). Mutations in *roughest* also affect axon targeting. The *roughest*^{CT} mutation that specifically affects the eye lattice results from truncation

of the intracellular domain of the protein and leads to altered subcellular protein localization (Schneider *et al*, 1995; Reiter *et al*, 1996). These data suggest that proper placement of the protein is critical for the selection of certain pigment cells over others for the shared lattice network. Mutants in *argos* also show a complete lack of cell death during the first phase of pupal cell death; the amount of cell death during differentiation of the third instar larval disc is also lower than normal (Brunner *et al*, 1994). The adult eye has excess cells, including photoreceptor neurons, cone cells, and pigment cells (Freeman *et al*, 1992; Kretzschmar *et al*, 1992; Okano *et al*, 1992; Brunner *et al*, 1994). The *argos* gene encodes a secreted protein with an EGF motif that functions over long range, and that interacts with other genes of EGF pathways (Freeman *et al*, 1992; Kretzschmar *et al*, 1992; Okano *et al*, 1992; Schweitzer *et al*, 1995). The additional cells in *argos* mutant eyes appear recruited from extra cells which would normally be eliminated by cell death in the larval disc and pupal eye; *argos* thus encodes a factor whose normal function is to inhibit neighboring cells from undergoing identical differentiation events. With more attention being paid to cell death processes, additional genes whose function is involved in survival of supernumerary cells in the eye is likely to become emphasized.

Together, these data suggest that the appropriate cell interactions and signals are important for the selection of the right number and type of cells for the ommatidial lattice. The signalling mechanisms for pigment cell selection, for example, are coupled to survival pathways. Multiple cells make the right contacts, but still only select cells survive, suggesting a competition for unknown elements (Cagan and Ready, 1989a). Genes that prevent cell death in the animal at embryonic stages and during formation of the eye, such as the baculoviral P35 protein and cellular homologs of inhibitors of apoptosis DIAP1 and DIAP2, can prevent both pigment cell and perimeter cell death in the pupal eye (Hay *et al*, 1994, 1995). This indicates that common molecular mechanisms of cell death pathways are activated during these cell selection processes. Moreover, cells that survive as a result of these manipulations, as well as in mutant situations where supernumerary cells differentiate – as in *argos* mutants – show features of normally differentiated cells. This supports the idea that supernumerary cells will undergo normal differentiation events when they are protected from elimination by cell death.

Maintenance in the adult

Selection of the appropriate cells and their correct differentiation to comprise the compound eye is only the first step toward a functioning neural structure. With the eye properly formed, the fly must then maintain it appropriately. Aberrant photoreceptor function can lead to loss of the differentiated cells. Notably, mutations in genes that affect products involved in the phototransduction cascade can lead to loss of photoreceptor cells by light-dependent or -independent manners (reviewed in Smith *et al*, 1991; Zuker, 1992). Such mutants include those in genes involved in phosphatidyl inositol pathways (*retinal degeneration A*, *B*, and *D*), and protein phosphatase which results in rhodopsin activation (*retinal degeneration C*; Steele and O'Tousa, 1992). Presumably, these mutations result in metabolic defects due to aberrant regulation of phototransduction, leading to subsequent death of the cells. Although these mutations have not yet been characterized for cell loss by programmed cell death versus degeneration by necrosis, vertebrate mutations in rhodopsin in forms of retinitis pigmentosa lead to death by apoptosis (Portera-Cailliau *et al*, 1994; Chang *et al*, 1994; see accompanying reviews). Thus, it is likely that cell loss occurs in at least some of these mutations through activation of programmed cell death pathways.

Trophic interactions between the eye and the brain are critical to long-term survival of the neural structure. Eyes that develop ectopically due to select mutations (for example, *extra eye*) or that develop in the absence of proper synaptic connections with the brain (*disconnected* mutants), develop relatively normal compound eye structures (Marcey and Stark, 1985; Steller *et al*, 1987). However, in such cases, since the axons of the photoreceptor neurons fail to connect with the optic centers of the brain, the photoreceptor cells fail to be maintained in the adult (Campos *et al*, 1992). Again, whether this cell loss is through programmed cell death pathways or degeneration by necrosis remains to be defined. Nevertheless, these studies indicate trophic interactions between the eye and brain, similar to such interactions fundamental to vertebrate cell selection (Cowan *et al*, 1984; Oppenheim, 1991; Raff *et al*, 1993), are critical to long-term neural maintenance in the fly.

The eye as a probe to dissect molecular components of cell death pathways

Genes involved in triggering cell death or effecting cell survival have been identified in *Drosophila*. One chromosomal region critical for all normally occurring cell death in the embryo is defined by an approximately 300 kb deletion that includes at least three genes, *reaper*, *head involution defective* (*hid*) and *grim*, known to be important to effect cell death in the animal (White *et al*, 1994; Grether *et al*, 1995; Chen *et al*, 1996). The *Drosophila* eye provides a powerful selection assay to reveal additional genes that function upstream or downstream of cell death genes, or otherwise modify the activity of these genes to kill cells. Ectopic expression in eye cells of genes that prevent cell death disrupts the highly regular lattice of the compound eye (Hay *et al*, 1994). Moreover, similar expression of genes that effect killing can ablate the eye (Grether *et al*, 1995; Hay *et al*, 1995; Chen *et al*, 1996; White *et al*, 1996); these effects are dominant and sensitive to gene dosage. Hence, regions of the genome that contain new genes that may modify the function of cell death genes can be detected by altering gene dosage with the large number of available *Drosophila* chromosomal deletions, among other techniques. In this way, cellular homologs of baculoviral inhibitor of apoptosis genes have already been defined by the ability to enhance ablation of the eye by ectopic *hid* or *reaper* when present in one versus two copies (Hay *et al*, 1995). The eye can thus be used as a

window into cell death pathways of the fly, to define molecular components of cell death regulation in the organism. Once such genes are identified, their normal function in the organism in development, cell survival and maintenance are of issue.

Conclusions/future focus

Cell death is integral to normal differentiation of the fly eye. It occurs at multiple points in the developmental process, and is essential for the appropriate differentiation of the structure (see Figure 1). A common theme that characterizes the results of a number of mutations in genes essential for proper eye differentiation and/or patterning is to lead to death of cells (see Table 1). Cell death may be the default fate of eye cells should they develop at inappropriate times or receive inappropriate amounts of factors or signals required for differentiation, receive conflicting signals such as for division and differentiation, or fail to make appropriate contacts with target cells for survival. Thus, the activities of genes involved in eye patterning are integrated into survival pathways. Moreover, hormonal interactions are of critical importance to the differentiation of many adult structures, including the eye. Hormones such as ecdysone influence the survival of specific cells within the nervous system through regulation of programmed cell death pathways (Truman *et al*, 1992). Thus, hormonal signals, local diffusible molecules, as well as cell autonomous factors all dynamically integrate to make the exquisitely organized structure of the fly compound eye. How this is achieved in molecular detail is a key to understand cell selection during the differentiation process. Are signals for survival the same as those for specific aspects of cell differentiation? How the signalling processes of survival interplay with differentiation genes will be a key focus for the future. The *Drosophila* eye provides an experimental system in which to dissect the interwoven pathways of cell differentiation and cell death to define how groups of cells are sculpted to generate a functioning neural structure.

Acknowledgements

Thanks to A. Cashmore, L. Lillien, K. Zinsmaier, and members of the Bonini laboratory for comments. N.B. is supported by grants from the John Merck Fund, March of Dimes, NIH, and University of Pennsylvania Research Foundation.

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