**glass** encodes a site-specific DNA-binding protein that is regulated in response to positional signals in the developing *Drosophila* eye

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The *glass* gene encodes a zinc finger protein required for normal photoreceptor cell development in *Drosophila*. We show that *glass* transcripts are present in the third-instar eye-imaginal disc starting in the morphogenetic furrow and extending to the posterior margin of the disc; *glass* protein is detected in the nuclei of all cells in this region. We also show that *glass* encodes a site-specific DNA-binding protein. A 27-bp *glass*-binding site can confer *glass*-dependent expression on a reporter gene in developing photoreceptor cells, the particular subset of *glass*-expressing cells known to require *glass* function. This specificity may represent a regulation of *glass* protein activity after cells are recruited to the photoreceptor cell fate.

[Key Words: *glass*; eye development; *Drosophila*; transcription factor]

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During the development of multicellular organisms, the fate of a cell is often determined by the influence of neighboring cells or tissues. The molecular mechanisms by which such inductive signals cause changes in the genetic program of the responding cell remain largely unknown. In the early stages of the response, signals from the cell surface must lead to modifications in the activity of one or more pre-existing transcription factors, which then set in motion the appropriate cascade of gene activation.

Post-translational activation of transcription factors has been demonstrated in a number of cases, including steroid hormone receptors (Glineur et al. 1990), the yeast heat shock response factor (Sorger and Pelham 1988), and the mammalian factor AP-1 [Angel et al. 1987; Lee et al. 1987]. The activation of transcription factors in response to inductive signals during development has proved more difficult to demonstrate, largely because the critical transcription factors have not been identified. Cell identities in the developing eye of *Drosophila* are determined by induction, and mutations in several genes that encode putative transcription factors have been shown to disrupt normal eye development [for reviews, see Tomlinson 1988; Banerjee and Zipursky 1990]. Here, we show that one of these genes, *glass*, encodes a site-specific DNA-binding protein and that *glass* function, in its broadest sense, is regulated at the protein level.

The *glass* gene is required for the normal development of photoreceptor cells in all three organs in which they occur: the adult compound eye (Johannsen 1924; Garen and Kankel 1983), the adult simple eyes or ocelli (Stark et al. 1984; Moses et al. 1989; Stark and Sapp 1989), and the larval photoreceptor or Bolwig's organ (Moses et al. 1989). In the retina, we demonstrated that only the photoreceptor cells have a cell-autonomous requirement for *glass* function (Moses et al. 1989). Developing *glass* mutant photoreceptor cells in the eye-imaginal disc express neural antigens and extend axons; however, they show early morphological defects, fail to express the photoreceptor cell-specific protein chaoptin [Ready et al. 1986; Van Vactor et al. 1988; Moses et al. 1989], and die after ~60 hr of pupal development [Ready et al. 1986]. We cloned the *glass* gene and found that it can encode a 604-amino-acid protein with five consecutive Cys$_2$-His$_2$ zinc finger domains [Moses et al. 1989], suggesting that *glass* may encode a transcription factor required for the normal development of photoreceptor cells.

Here, we show that the *glass* protein is expressed in all cell types in the developing eye. However, its ability to activate transcription differs between cell types. In particular, a heterologous promoter construct containing five tandem copies of a *glass*-binding site can confer *glass*-dependent expression on a reporter gene in photoreceptor cells in the developing larval eye-imaginal disc.
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Results

glass expression in the eye-imaginal disc

Given the genetic specificity of glass [Moses et al. 1989], one might expect glass expression to be limited to the photoreceptor cells; however, we found that this is not the case. The photoreceptor cells of the compound eye begin their development in the third-larval instar when a coordinated series of cell shape changes, known as the morphogenetic furrow, progresses across the eye-imaginal disc from posterior to anterior, and in this furrow the first signs of the development of the eye facets (or ommatidia) can be seen. Posterior to the furrow, ommatidia are assembled by a process of sequential cell recruitment beginning with the eight photoreceptor cells, followed by the four lens-secreting cone cells and the other accessory cells (Ready et al. 1976; Tomlinson and Ready 1987). These recruitment events are independent of cell lineage [Hotta and Benzer 1970; Ready et al. 1976, Lawrence and Green 1979] and are thought to be mediated by specific instructions that an uncommitted cell receives from its immediate neighbors [Tomlinson 1985; Tomlinson and Ready 1987].

We found that glass RNA is detectable in the eye-imaginal disc beginning in the morphogenetic furrow, where the leading edge of expression is serrated [Fig. 1A,B]. This initial expression pattern appears to be coincident with or closely following the pattern of cells seen in the furrow by cobalt sulfide staining [Tomlinson and Ready 1987]. RNA expression is strongest in the furrow and persists at a lower level in more posterior regions of the disc, the resolution of the digoxygenin in situ hybridization method was insufficient to distinguish variations in expression between individual cells. The expression of glass RNA in the furrow is consistent with the onset of the glass mutant phenotype, as some abnormalities are seen in mutant discs at the earliest stages of ommatidial assembly [Moses et al. 1989]. We did not conduct an extensive survey of other tissues and developmental stages; however, other evidence [see below] indicates that glass expression is limited to the visual system and a small number of cells in the central nervous system.

To examine glass protein expression we raised polyclonal sera against three different glass fusion proteins [see Materials and methods], and all produced a similar signal when used to stain imaginal discs. The glass protein is present in the nuclei of all cells posterior to the morphogenetic furrow in the eye-imaginal disc. To establish that the signal was due to specific glass protein detection, discs from a null allele of glass (glass600, Moses et al. 1989) were also stained. Thus, we were able to unambiguously distinguish signal from background by directly comparing wild-type to glass–null control discs [Fig. 1C,D]. It is intriguing that glass protein is not restricted to the nuclei of the photoreceptor cells, because we have shown previously [Moses et al. 1989] that the accessory cells have no detectable requirement for glass function. These sera do not detect any glass protein in the embryo, larval brain, or the adult head; however, we believe that this negative result is due to the limited sensitivity of these reagents, because we have other evidence [see below] that glass is expressed in these tissues.

To determine whether the glass expression pattern is altered in discs when some cells adopt abnormal fates, we used the anti-glass sera to stain eye-imaginal discs mutant for four genes known to affect eye development: sevenless [Tomlinson and Ready 1986], rough [Tomlinson et al. 1988], scabrous [Mlodzik et al. 1990a, Baker et al. 1990], and Ellipse [Baker and Rubin 1989]. None of these mutations affects the progress of the morphogenetic furrow. In all cases, glass protein was detected in Figure 1. The glass gene is expressed in all cells posterior to the morphogenetic furrow in the eye-imaginal disc. (A–F) Portions of third-instar eye-imaginal discs (magnification, 163×). The arrow in A marks the position of the morphogenetic furrow for A–F; posterior is down. (A and B) In situ hybridizations of glass cDNA sequences to wild-type eye-imaginal discs. The specimen in A was developed for a short period to make apparent the high concentration of glass RNA near the furrow. In B, glass RNA can be seen to persist to the back of the disc. (C) A wild-type eye-imaginal disc stained with polyclonal anti-glass serum is shown. The nuclei of all cells posterior to the furrow stain. In D, a glass600 eye-imaginal disc stained as in C is shown; glass600 is a protein null allele. (E) A genetically glass+ eye-imaginal disc bearing the B172 insertion of the P-lacW element within the glass promotor has been stained with an anti-β-galactosidase antibody. The nuclei of all cells posterior to the furrow stain. (F) An eye-imaginal disc bearing the B172 insertion in a glass– (glass827) genetic background was stained as in E. Most nuclei posterior to the morphogenetic furrow do not stain. (G–L) High-power (magnification, 1300×) views of individual ommatidia. (C) An ommatidium from the glass600 eye disc shown in D illustrates the level of nonspecific staining. (H–L) Progressively more mature ommatidia from the disc shown in C are presented; the ommatidium in L is 15 rows behind the furrow [early four-cone cell stage; Tomlinson 1986]. Individual photoreceptor cell nuclei are numbered; the arrows in L mark cone cell nuclei. glass can be seen to be expressed in each photoreceptor cell type and the four cone cells.
the nuclei of all cells posterior to the morphogenetic furrow, although many of these mutants affect the positions and numbers of these nuclei.

glass autoregulation

We used a lacZ enhancer-trap element (O'Kane and Gehring 1987; Bellen et al. 1989; Bier et al. 1989) inserted at the glass locus to examine whether glass plays a role in regulating its own transcription. The B172 line carries an insertion of the P-lacW element (Bier et al. 1989) located between 2 and 3 kb upstream of the start site of glass transcription. In the B172 line β-galactosidase expression was evident posterior to the furrow (Fig. 1E) and in some cells in the larval brain (data not shown). The pattern of the β-galactosidase expression in B172 eye-imaginal discs was indistinguishable from that of glass protein. β-Galactosidase was also expressed in the nuclei of the Bolwig's organ in the late embryo and in some cells of the embryonic and third-instar larval brain (data not shown). No expression was seen in other larval tissues or in the adult head, although glass RNA is detectable in the adult head (Moses et al. 1989).

Because the B172 line reflects the pattern of glass transcription in the eye-imaginal disc, we used the expression of β-galactosidase in B172 as a marker to ask if glass protein plays a role in regulating glass expression. To be able to examine expression from the B172 insertion in the absence of glass function, we induced three strong glass alleles on the B172 chromosome using the chemical mutagen ethylmethane sulfonate. We stained the glass-binding sites confer glass-dependent regulation on a reporter gene

The observation that glass protein can bind to rhodopsin gene enhancers supports a role for glass in the direct regulation of genes in photoreceptor cells. To demonstrate a function for these sites in vivo we inserted a pentamer of a 38-bp oligonucleotide that includes the 27-bp glass-protein-binding site from the Rhl element II enhancer (see Fig. 2) into two different heat shock promoters–lacZ reporter constructs (see Fig. 3A and Materials and methods). In two corresponding control constructs similar oligonucleotides were used, but the 27 bp of the glass-binding site are in a random sequence. Construct A includes the glass-binding site pentamer upstream of a heat shock promoter that is truncated to position –250 bp; this 250 bp is sufficient for heat inducibility. Construct B is the random site control to construct A. Construct C is similar to construct A, except that the heat shock promoter has been truncated to –50 bp, and construct D is the random site control to construct C.

Six of the nine independent construct A transformant lines showed a common and striking pattern of lacZ expression (see Fig. 3E). In the eye-imaginal disc, these lines display a three-zone pattern of expression: no expression anterior to the furrow; weak expression beginning in and just posterior to the furrow; and, finally, strong expression from about the eighth column of developing ommatidia (~16 hr after the first photoreceptor cell, R8, begins to express neural antigens; Tomlinson and Ready 1987) to the back of the disc. Use of an anti-β-
Figure 2. The glass gene encodes a sequence-specific DNA-binding protein. (Left) DNase I protection of sequences from the Rhl and glass promoters, using protein extracts (3 µg/ml) from glass-expressing or control E. coli (see Materials and methods). Lanes: (M) Purine cleavage products used as size markers; (±) no extract; (5 or 10) the volume (in µl) of bacterial extract added. Position numbers are from the start of transcription. [Rhl enl] DNase I protections of sequences from the Rhl promoter in the region of element II with control [left] and glass-containing extracts [right]. [Rhl enl] DNase I protections of sequences from the Rhl promoter in the region of element I with glass-containing extract. The protected regions seen with the glass-containing extract are indicated by brackets; no specific protection was observed with the control extract. (glass) DNase I protections of sequences from glass genomic DNA with glass-containing extract are shown. [Right] The relationship of glass-protected sequences to features of the structure of the Rhl promoter and their DNA sequence conservation in Drosophila virilis (M. Fuchs and G.M. Rubin, unpubl.). The DNA sequence protected in element II is highlighted. The DNA sequences including the glass-binding site oligonucleotide (experimental), which was based on this protected site, and a corresponding random sequence control oligonucleotide (control), are shown. These oligonucleotides were used to make the glass-binding site pentamer and control pentamer constructs (see Materials and methods). 

Figure 3. A glass-binding site pentamer can confer glass-regulated expression on a β-galactosidase reporter gene in the larval and adult visual systems. [A] A schematic representation of constructs A and C. In construct A, the glass-binding site pentamer has been inserted 250 bp upstream of the transcriptional start site of a truncated hsp70 promoter driving lacZ expression. In construct C, the hsp70 promoter has been truncated to position 50 bp. Two corresponding control constructs were also made in which a pentamer of random sequence, but of the same base composition as the glass-binding site (see Fig. 1), replaced the glass-binding site pentamer; the control constructs corresponding to constructs A and C are constructs B and D, respectively. [B] A genetically glass + larva carrying construct C has been dissected to show the visual system after staining for β-galactosidase activity (magnification, 80×). [b] Bolwig’s nerve, [open arrow] the morphogenetic furrow; [os] the optic stalk; [hb] brain hemisphere. (C) The brain hemispheres from a construct C transformant line in a glass + genetic background, stained for β-galactosidase activity (magnification, 100×). The afferent axons from the retina [ax] stain, as do several groups of cell bodies [cb] in the more central brain, which show commissural connections [com] across the midline. [D] The brain hemispheres from the same construct C transformant line shown in C, but now in a glass 6° genetic background, have been stained for β-galactosidase activity (magnification, 100×). No staining was observed, indicating that expression of construct C is glass dependent. [E–I] Portions of eye-imaginal discs [magnification, 250×]. The morphogenetic furrow is indicated by the arrow, and posterior is down. [E] A disc from a construct A transformant line in a glass + genetic background is shown after staining for β-galactosidase activity. No staining is seen anterior to the furrow, low-level staining is seen for the first seven rows of developing ommatidia, and then the level of staining greatly increases and extends to the posterior of the disc. [F] A disc from the same construct A transformant line as shown in E in a glass 6° genetic background, stained for β-galactosidase activity. No staining was observed. [G] A disc from a construct C transformant line in a glass + genetic background, stained for β-galactosidase activity. No staining is seen until the eighth column of developing ommatidia. [H] A disc from the same construct C transformant line as shown in G in a glass 6° genetic background stained for β-galactosidase activity. No staining was observed. [I] A disc from the same construct C transformant line as shown in G in a glass + genetic background stained with an anti-β-galactosidase antibody. From the eighth column of developing ommatidia, the photoreceptor cells appear in their normal order of recruitment. The cone cells and basal nuclei do not stain. [J] A section from the head of an adult of a construct C transformant line in a glass + genetic background, stained for β-galactosidase activity (magnification, 50×). Strong staining is seen in the retina [re], the weak staining visible in the lamina [la] most likely is due to β-galactosidase activity in the photoreceptor axons. [K] A section from the head of an untransformed glass + adult stained as in J. No staining is seen. [L] An apical adult head section of the same construct C transformant line stained for β-galactosidase activity (magnification, 100×). Staining is seen in the ocelli [asterisks]. [M] An apical section from the head of an untransformed glass + adult stained as in J. No staining is seen in the ocelli [asterisks]. Staining for β-galactosidase activity was carried out as described in Materials and methods; the stainings shown in C, D, G, and H were carried out in the presence of SDS.
glass function is modulated in photoreceptors

Figure 3. [See facing page for legend.]
galactosidase antibody showed that this high-level expression is only in photoreceptor cells and that they initiate this expression in the same order as that in which they express neural antigens [see Fig. 4]. No such staining is seen in the cone cells, although they express high levels of glass protein. The early low-level expression is more diffuse and may be in all cells. To determine whether this pattern of expression is regulated by glass, three of these lines were crossed into a glass mutant genetic background. In all three lines, β-galactosidase expression was abolished, suggesting that the pattern was generated by the binding of the glass protein to the pentamer site in vivo [Fig. 3F]. The other three construct A lines showed patterns that may be most simply interpreted as chromosome position effects on the inserted construct; in the one example tested in a glass mutant background, the expression pattern was unaffected, indicating that this pattern is not controlled by glass. Similarly, seven independent lines were recovered for construct B, the random site control to construct A, of these, three were tested in a glass mutant background. Various weak expression patterns were observed in the third-larval instar eye-imaginal discs and brain, none of these patterns are glass dependent.

Ten independent construct C lines showed a much stronger and more tightly restricted expression pattern than those of construct A [Fig. 3, B, G, and I]. No expression was seen anterior to the furrow or in the first seven rows of developing ommatidia, but intense staining was seen in the posterior part of the disc where it was restricted to the photoreceptor cells. As for construct A, staining appeared in the normal sequence of photoreceptor cell recruitment (see Fig. 4) and was absent from the cone cells. This expression was abolished in all four lines tested in a glass mutant background [Fig. 3H]. Eight independent lines were recovered for construct D, the random site control for construct C; of these, six were tested in a glass mutant background. Although all eight showed strong and widespread lacZ expression in the larva, including both anterior and posterior to the furrow in the eye-imaginal disc, no part of this lacZ expression pattern was glass dependent.

In the third-instar larval brain, all 10 construct C lines showed strong staining in the photoreceptor cell axons and also in several groups of cell bodies deeper in the brain. In these cells, the stain permeated the cytoplasm and revealed that their axons form commissural paths crossing the midline [Fig. 3C]. All of this staining was glass dependent [Fig. 3D], and the positions of the lacZ-expressing cells in the brain are consistent with the positions of cells that weakly stain in the enhancer trap line B172. These data support a role for glass in the regulation of gene expression in the developing brain as well as in the retina. In the embryo, constructs A and C showed similar patterns of glass-dependent staining, as in the eye disc, construct C displayed more intense staining [Fig. 5]. With this intense expression, the Bolwig's nerve can be traced for its entire length, from the cell bodies to the growth cone; this can be seen just above a small group of the cells that stain in the brain, which may include the optic lobe precursor cells [Tix et al. 1989]. A similar but less intense staining was seen in B172 embryos. In the adult head, both constructs are expressed in the retinas and the ocelli but not in the brain [construct C is shown in Fig. 3J, L]. However, unlike for the larva, for the adult, a glass mutant control is not informative because in glass- adults the retina, ocelli, and brain are degenerate.

glass function, but not expression, is downstream of sevenless in the R7 cell alone

That glass function is activated in the photoreceptors and not the cone cells implies that this activation is downstream of the signals and receptors that mediate photoreceptor cell recruitment. The sevenless mutation prevents the presumptive R7 cell from developing as a photoreceptor by blocking an incoming inductive signal at the cell surface; therefore, we might expect this mutation to block the activation of glass in this cell. We stained sevenless eye-imaginal discs with the glass antibody and found the pattern of glass protein expression to be unaltered. We also crossed a construct A and a construct C line into a sevenless background and stained the eye-imaginal discs for β-galactosidase antigen. In both cases, the pattern was the same as in sevenless+, with the exception of the presumptive R7 cells, in which the reporter was silent (see Fig. 4K, L). This result implies that although the expression of glass protein is unaffected by sevenless, the function of the glass protein is affected specifically in the presumptive R7 cell.

Discussion

We reported previously that the glass gene encodes a zinc finger protein that is required for photoreceptor cell development (Moses et al. 1989). Here, we show that glass protein can bind in vitro to specific enhancer sequences from the Rh1 rhodopsin gene, as well as to the promoter of the glass gene itself. glass RNA is expressed posterior to the morphogenetic furrow in the developing eye-imaginal disc, and glass protein shows a parallel distribution, being found in the nuclei of all cells posterior to the furrow. A high level of glass RNA is observed in the morphogenetic furrow; glass shares this early and general expression pattern with the rough gene (Kimmel et al. 1990), and both genes may be responding to similar cues. We identified a lacZ enhancer trap insertion close to glass, B172, that accurately mimics the glass antibody pattern in the eye disc and reveals sites of expression in the larval visual system and brain. Moreover, aspects of the B172 expression pattern are glass dependent, suggesting that glass plays a role in its own regulation. Our data also reveal a novel function for glass in the activation of gene expression in a few cells of the embryonic and larval brain. The identity and function of these cells is unknown. However, there is evidence indicating that the brain can detect light by a mechanism independent of...
part of C corresponds to the apical cytoplasm of the photoreceptor cells as they project between the cone cell nuclei. In a diagram summarizing these data, photoreceptor cells are shown as they are assembled into the ommatidia: R8, R8/R2/R5, R8/R2/R5/R3/R4/R1/R6, and, finally, all eight photoreceptors, R1–R8. Developmental time is indicated as hours following the passage of the morphogenetic furrow or as the number of columns behind the furrow. The symmetrical cluster, two-cone cell stages, are shown as unshaded from the time at which they first express the neural antigen recognized by mAb 22C10 [Tomlinson and Ready 1987] and as solid from the time at which they first express high levels of β-galactosidase from construct A. At this stage, photoreceptors R8, R2, R5, R3, and R4 are expressing high levels of β-galactosidase, whereas no β-galactosidase is detected in the cone cells. However, the axons projecting down from the photoreceptor cell bodies contain β-galactosidase. (A) A diagram summarizing these data. Photoreceptor cells are shown as they are assembled into the ommatidia as either shaded gray (if the cell is expressing high levels of β-galactosidase) or black (if the cell is expressing high levels of β-galactosidase).

The known photoreceptors [e.g., see Dushay et al. 1989], and it is possible that these glass-expressing cells are involved in this process. We have shown that glass protein distribution and glass functional activity are not coincident and that in certain promoter constructs glass function is observed only in photoreceptor cells. A pentamer of the glass-binding site from an Rhl enhancer confers glass-dependent expression on a heat shock–lacZ reporter gene in two different constructs in vivo in the developing compound eye. The differences in β-galactosidase expression pattern between the two heat shock–lacZ reporter genes may reflect differences in the context or length of the promoters, which affect their sensitivity to different functional states of the glass protein. Indeed, the Rhl gene from which the glass-binding site was obtained is not active at all in the eye disc. Clearly, natural promoters are more complex in their regulation than our artificial constructs and may reflect the interplay of many different transcription factors. However, constructs A and C provide an in vivo assay that allows us to distinguish between states of glass function in isolation from the effects of other factors that may act on natural promoters.

Beginning in the morphogenetic furrow, glass protein is expressed in all cells; glass appears to be able to positively regulate its own promoter and is required to induce the low-level expression of construct A seen in the first seven rows of developing ommatidia. In addition, ommatidial development is abnormal in glass mutants even at these very early stages [Moses et al. 1989]. Thus, the glass protein appears to be an active transcription factor in the first seven rows of developing ommatidia yet is unable to activate more stringent promoters such as that of construct C. From about the eighth column of developing ommatidia (~16 hr after the first photorecep-
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Figure 5. A glass-binding site pentamer can confer glass-regulated expression on a ß-galactosidase reporter gene in the embryonic nervous system. A late embryo of a construct C transformant line in a glass + [A] or glass − [B] genetic background is shown after staining with an anti-ß-galactosidase antibody [magnification, 75 x]. In a glass + background [A] staining is seen in the Bolwig’s organs (upper circle), the Bolwig’s nerve growth cone and a small group of cells in the brain hemispheres (lower circle), and two central structures (arrows). Unlike the other observed staining, the staining in these central structures is not glass-dependent as they remain stained when this transformant line is crossed into a glass606 genetic background [B]. (C) A high-magnification view (750 x) of the Bolwig’s organ circled in A. (D and E) Two planes of focus of the same area showing high-magnification views (750 x) of two groups of cells in the brain hemisphere (arrows).

tor cells express neural antigens) glass is capable of activating the photoreceptor-specific promoter of construct C and the high-level photoreceptor-specific expression of construct A. The expression of construct C closely resembles that of the chaoptin gene, a glass-dependent gene [Moses et al. 1989] that is the earliest gene known to be expressed specifically in photoreceptors [Zipursky et al. 1984]. There is no apparent increase in the levels of glass protein in photoreceptors from column eight, implying that this functional change occurs post-translationally.

An early step in the modification of the developmental program of a target cell by an inductive signal must be the activation of one or more pre-existing transcription factors. However, it has been possible to demonstrate the post-translational activation of a particular factor in few developing systems. One such example is the Drosophila dorsal protein, which becomes localized to the nucleus in response to inductive signals in the ventral part of the developing embryo [for review, see Gilmore 1990]. dorsal has recently been shown to be related to the mammalian factor NF-xB, which can be released from a cytoplasmic inhibitor by various extracellular signals, move to the nucleus, and act as a transcription factor [for review, see Lenardo and Baltimore 1989]. The change in function of the glass protein that we observe does not involve any apparent change in its subcellular localization as it is already present in the nucleus. Thus, any modulation of glass protein function in photoreceptor cells must operate by a different mechanism.

Our results do not allow us to determine whether modulation of glass function results from a change in binding or a difference in the activity of the bound factor. Nor do they address the molecular nature of the modulating mechanism or clarify whether the glass protein itself or the level or activity of a coactivator protein is altered. Moreover, we cannot rule out the possibility that another factor acts on the glass DNA-binding sites, but our data show that the presence or activity of such a factor would itself then have to be dependent on glass function because the change in the activity mediated by the DNA-binding sites is under the genetic control of the glass gene.

Although glass function is required in all of the photoreceptor cell subtypes, the extracellular signals leading to its functional modulation may differ in each. Indeed, glass may be the point at which these signals converge, leading to the initiation of those aspects of cellular development common to all photoreceptors. An understanding of the mechanism(s) by which the function of the glass protein is controlled may lead to a wider understanding of the ways in which transcription factors respond to intra- and intercellular signals during development.

Materials and methods

Drosophila stocks

Wild-type Canton-S flies were used. The transformation recipient was rosy606. The glass alleles used were gl1, gl2, gl3 T[2;3]gl606 (Baker et al. 1990; Mlodzik et al. 1990a), and gl606 (Moses et al. 1989). The last two were found to be protein nulls, and gl606 was used as the null allele in all experiments. Other mutations used were sevenless2 [Banerjee et al. 1987], rough603 [Kimmel et al. 1990], scabrous1 [Baker et al. 1990, Mlodzik et al. 1990a], and Ellipse1 [Baker and Rubin 1989]. Chromosomes bearing an insertion of constructs A-D were either balanced [for the lethals and steriles] or made homozygous. Insertions on the X or second chromosomes were introduced into a glass mutant background by means of standard crosses. The position of insertion in the enhancer trap line B172 was determined by genomic DNA blots to lie between 3148 and 2068 bp 5′ to the start of glass transcription.

DNA-binding assays

The cDNA clone 5A6 [Moses et al. 1989] was mutated in vitro to introduce an Ndel site at the 5′ end of the glass open reading frame.
frame. A 2056-bp fragment from this NdeI site to a BamHI site in the 3'-untranslated region was inserted between the NdeI and BamHI sites of pAR3038 (Rosenberg et al. 1987). glass protein was expressed from this plasmid in Escherichia coli strain BL21 DE3 (Studier and Moffatt 1986) after IPTG induction. The protein was recovered from the bacteria as described by Hoey et al. [1988]. The protein was found to be highly insoluble and was partially solubilized by treatment with 4 M urea followed by slow dialysis. This glass protein migrates as 62 kD on an SDS-PAGE gel, consistent with the size predicted from the cDNA sequence, and its final concentration was ~3 μg/ml. A parallel preparation was made from cells containing the vector pAR3038 with no glass-coding insert for use as a negative control.

DNase I protection reactions were carried out as described by Heberlein and Tjian [1988], in a total reaction volume of 50 μl with no competitor or with 40 μg/ml of sonicated calf thymus DNA or 400 μg/ml of poly[d(I-C)]. The DNA substrates for the Rhl elements II and III were a 319-bp fragment from the subclone pFRy: Rhl-1-252/+67-CAT [Mismsner and Rubin 1987], labeled at the left-flanking NotI site and recut with KpnI for the coding strand or the right-flanking NotI site and recut with Xhol for the noncoding strand. The DNA substrate for the Rhl element I region was a 282-bp XhoI–BamHI fragment from pFRy: Rhl-1-604 (the entire gene was an 845-bp glass-coding sequence, accession number X15400) to pFRy: Rhl-1-252/+67-CAT [Mismer and Rubin 1987], labeled at the BamHI site for the coding strand and the Xhol site for the noncoding strand. The DNA substrate from the glass gene was an 845-bp Nael [position 1626 in the glass genomic sequence, accession number X15400] to ClaI [position 2471] fragment inserted into the vector bpluescript KS M13(+) [Stratagene] between the EcoRV and the ClaI sites. This fragment was excised by cutting with Xhol and BamHI and labeled at the Xhol site for the coding strand and at the BamHI site for the noncoding strand.

In situ hybridization, histochemistry, and immunocytochemistry

In situ hybridization to imaginal discs was performed as described by Tautz and Pfeifle [1989], as modified by Mlodzik et al. [1990b]. The probe was the trpE–glass fusion proteins and goat anti-mouse secondary antibody conjugated to horseradish peroxidase (1:200; Bio-Rad). Three fusion proteins were generated and expressed in E. coli strain HB101 and given boosts of 25 μg of the antigens in Freund’s incomplete adjuvant at 2-week intervals. Mice were bled from the tail vein and 50 μg of the antigens in Freund’s complete adjuvant with 50 μg of the antigens in Freund’s complete adjuvant and 0.5 μg/ml of construct and 0.4 μg/ml of helper plasmid pUC8IIΔ2-3wc, and G1-transformed flies were selected on the basis of their rosy+ phenotype (Rubin and Spradling 1982).

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The oligonucleotides were end-labeled by treatment with T4 polynucleotide kinase and γ-32P[ATP]. Oligo 1 was annealed to oligo 2 to produce a double-stranded fragment including a glass protein-binding site, and oligo 3 was annealed to oligo 4 to produce a double-stranded fragment without a glass protein-binding site. Each fragment type was then ligated to form concatemers in the presence of both SalI and XhoI restriction enzymes. The ligation products were run on a nondenaturing acrylamide gel, and the pentamers were eluted and inserted into the vector bpluescript KS M13(+)[Stratagene] between the XhoI and SalI sites. Clones were selected that had inserted in the orientation that restores both the XhoI and SalI sites, and the head–tail pentamer structure was confirmed by sequencing. The glass-binding site and control pentamers were then cloned into the XhoI site of the kanamycin-resistant NotI shuttle vector pHSX [a gift of K. Jones] and then as NotI fragments into the NotI site of the P-element transformation vectors pDM300hsacZ [Bowell et al. 1989] to generate constructs A and B, and into HZ50PL [Hiromi and Gehring 1987] to generate constructs C and D. In each case, the orientation of the pentamer was confirmed by DNA sequencing to be in the same orientation relative to transcription as this sequence is in the natural Rhl promoter [Mischer and Rubin 1987]. rosy+666 embryos were injected with 1.2 mg/ml of construct and 0.4 mg/ml of helper plasmid pUC8IIΔ2-3wc, and G1-transformed flies were selected on the basis of their rosy+ phenotype (Rubin and Spradling 1982).

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