

# Lozenge is expressed in pluripotent precursor cells and patterns multiple cell types in the *Drosophila* eye through the control of cell-specific transcription factors

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## SUMMARY

In the developing *Drosophila* eye, individual cell fates are specified when general signaling mechanisms are interpreted in the context of cell-specific transcription factors. Lozenge, a Runt/AML1/CBFA1-like transcription factor, determines the fates of a number of neuronal and non-neuronal cells by regulating the expression of multiple fate-determining transcription factors. The Lozenge protein is expressed in the nuclei of the cells that it patterns and also in their undifferentiated precursors. An enhancer

element located within the second intron of the *lozenge* gene is responsible for its eye-specific expression. Lozenge is not itself a cell-specific transcription factor, rather it prepatterns the eye disc by positioning cell-specific factors in their appropriate locations.

Key words: *lozenge*, AML1, Pattern formation, *Drosophila*, Eye development, Runt, Signal transduction

## INTRODUCTION

During animal development, cells acquire specific fates via extracellular cues transduced through signaling pathways. A single class of cell surface receptors can often elicit different cellular responses ranging from cell division and differentiation to migration and programmed cell death. These differing outcomes frequently depend on the developmental context of the cell receiving the signal. In general, a specific cell that expresses a set of transcription factors or cytoplasmic determinants will respond uniquely to a given signal. For example, the signal transduced through the *Drosophila* Epidermal Growth Factor Receptor (EGFR) is reiteratively used for the differentiation of each of the eight neuronal and five non-neuronal cell types in the eye disc (Freeman, 1996; Tio and Moses, 1997). The signal itself relays no fate-determining information but rather acts as a trigger that allows a cell to follow a predisposed differentiation program. Genetic analysis in this system affords a unique opportunity to study the origins of such cell-specific responses and the basis for the predisposition of the different cell types.

Unpatterned cells in the larval eye imaginal disc of *Drosophila* undergo spatially and temporally restricted cell-cell interactions that ultimately specify the fates of the different cell types constituting the approximately 800 ommatidia of the adult compound eye (Tomlinson and Ready, 1987). Each ommatidium contains eight photoreceptor neurons (R1-R8),

four lens-secreting cone cells, and a number of pigment and bristle cells. In the third instar of larval development, differentiation begins at a morphogenetic furrow that sweeps across the eye disc from posterior to anterior (Ready et al., 1976). Coincident with the furrow, cells arrest in the G<sub>1</sub> phase of the cell cycle (Wolff and Ready, 1991). Immediately posterior to the furrow, the cells that are destined to become photoreceptors R8, R2/R5 and R3/R4 exit the cell cycle and emerge as 5-cell preclusters, while all cells that do not become part of the precluster undergo a synchronized round of mitosis and give rise to a pool of precursor cells that eventually differentiates into R1/R6, R7, cone, pigment and bristle cells (Ready et al., 1976; Wolff and Ready, 1991). How the equipotent cells within this undifferentiated pool achieve their distinct fates has not yet been fully elucidated and is a central focus of this study.

A number of transcription factors are expressed in cell-specific patterns and function exclusively in determining the fates of the cells that arise from the synchronized round of mitosis at the furrow (reviewed in Kumar and Moses, 1997). These include Seven-up (Svp), a steroid hormone receptor expressed in R1/R6 (as well as in the earlier differentiating R3/R4; Mlodzik et al., 1990), the homeodomain protein Bar, expressed in R1/R6 and primary pigment cells (Higashijima et al., 1992), Prospero (Pros), a homeodomain protein found in R7 and cone cells (Kauffman et al., 1996), and the Pax-2 homolog Sparkling (Spa), which is expressed in cone, primary pigment

and bristle cells (Fu and Noll, 1997). The developing *Drosophila* eye disc has thus been described as a mosaic of transcription factors expressed in different yet sometimes overlapping subsets of cells (Kumar and Moses, 1997). To understand the source of specificity of pattern formation, it is important to determine how these cell-specific factors achieve their appropriate expression patterns. Genetic analysis has shown that the transcription factor Lozenge (Lz; Green and Green, 1949) plays a pivotal role in this process (Daga et al., 1996). Lz encodes a transcription factor that negatively regulates *svp* in R7 and cone cells and positively regulates *Bar* in R1/R6 in the developing eye disc (Daga et al., 1996; Crew et al., 1997). Recent studies suggest that Lz also positively regulates *pros* in R7 and cone cells (R. Carthew, personal communication) and *spa* in cone and primary pigment cells (G. V. F., H. Duan, M. Noll and U. B., unpublished results). Thus, Lz controls the expression of all known cell-specific transcription factors expressed in cells that differentiate after the formation of the 5-cell precluster. Consistent with this role, Lz function has been shown to be required for the proper specification of photoreceptor neurons R1/R6, R7 and for determination of cone and pigment cells (Daga et al., 1996; Batterham et al., 1996). It is not clear from these previous studies, however, whether Lz is a cell-specific transcription factor like the ones that it regulates, or whether it is a ubiquitously expressed transcriptional regulator that responds differentially to incoming signals in different cell precursors. In this paper, we use immunolocalization, promoter analysis and germline-mediated transformation to show that Lz expression is controlled by an intronic enhancer and is exclusively required in the pool of undifferentiated cells posterior to the morphogenetic furrow for the proper specification of all cell types that are derived from this pool. Lz therefore functions as a fundamentally important pre patterning molecule during eye development.

## MATERIALS AND METHODS

### Scanning electron microscopy

Adult flies were anesthetized with carbon dioxide and attached to metal mounts as wet, uncoated samples using fingernail polish (Wet 'n' Wild #401). Images were acquired digitally using a Hitachi S-2460N Scanning Electron Microscope at a 'high pressure' setting of 30 Pa using a Robinson detector.

### Immunohistochemistry

A rabbit polyclonal antibody was raised against a peptide including residues 730-741 in the C-terminal portion of the predicted Lozenge amino acid sequence (Daga et al., 1996). The  $\alpha$ -Lz antibody was affinity-purified using the peptide conjugated to a Biorad Affigel 10 column. The purified antibody was preabsorbed against fixed *lz<sup>r1</sup>* larval tissue before use. Eye discs were stained as described in Rogge et al. (1995), except that the peripodial membranes were removed to facilitate visualization of individual cell types.

### Genetics and generation of transformants

All crosses were performed at 25°C. Oregon-R flies were used as wild type. The following fly strains were used for genetic interactions: *lz<sup>r1</sup>*, *lz<sup>r7</sup>*, *lz<sup>r8</sup>* and *lz<sup>Sprite</sup>* (Daga et al., 1996), *dpp-LacZ* (U. Heberlein and G. Rubin), *pGMR-p21* (I. Hariharan), *lz<sup>77a7</sup>* and *lz<sup>hb</sup>* (M. Green), and *lz<sup>50e</sup>* (Kathy Matthews). Transformation was performed as described in Daga et al. (1996). PCR primers 5'-GTCGAATTCGCGATTGG-TACAGAAGCGACAACAG-3' and 5'-GTCGAATTCGGCGGAG-CACAGGAAGTACGGATTG-3' were used to amplify intron II from

wild-type and mutant genomic fly DNA. All constructs for rescue of the *lz<sup>r1</sup>* phenotype were subcloned into the transformation vector HS-Casper. Construct 1 was generated by fusing an 11 kb *EcoRI-SalI* genomic DNA fragment to the *SalI* site in position 216 of the cDNA. Construct 2 was made by ligating an *EcoRI-NotI* genomic fragment containing the endogenous promoter and introns I, II, III and IV into the *NotI* site in position 1632 of the cDNA. Construct 3 was engineered by cloning the PCR amplified intron II upstream of a 3.8 kb *EcoRI-SalI* genomic fragment fused to the *SalI* site in position 216 of the cDNA. Construct 4 was generated by placing the PCR amplified intron II ligated to a genomic fragment beginning at the *XmnI* site (position -1101 from the gene start) and containing the endogenous promoter upstream of the full-length cDNA.

## RESULTS

### Lz expression pattern

A polyclonal antibody raised against Lz was used to examine its expression pattern in wild-type third larval instar eye-antenna discs. At low magnification, Lz expression is seen to be restricted to cells posterior to the morphogenetic furrow (Fig. 1A). This antibody is specific since no staining is detectable in null alleles of *lz* (Fig. 1B). At higher magnification, Lz expression is visible basally in the nuclei of all undifferentiated cells posterior to the furrow (Fig. 1C). This is in contrast to the undifferentiated cells anterior to the furrow, in which Lz is not expressed (Fig. 1A). Immunolocalization of Lz and Decapentaplegic shows that Lz expression is initiated at the posterior edge of the morphogenetic furrow but not within it (not shown). An apical view reveals that Lz is also expressed in three cells within each ommatidium in positions consistent with their being R1/R6 and R7, as well as in cone cells (Fig. 1D). To confirm the identity of these Lz-expressing cell, a *svp* enhancer trap line, *AE127*, that expresses  $\beta$ -galactosidase in R3/R4 and R1/R6 (Mlodzik et al., 1990) was used. *AE127* eye discs double-stained with  $\alpha$ Lz and X-gal show that Lz and  $\beta$ -galactosidase indeed colocalize in R1/R6 but not in R3/R4 (not shown). Furthermore, Lz is expressed in a cell positioned between R1/R6 (Fig. 1D), which is consistent with its being the R7 precursor (Tomlinson and Ready, 1987). At 20-30 hours after puparium formation (APF), Lz expression is seen in primary pigment cells (Fig. 1E) and, at 30-40 hours APF, Lz is expressed in secondary and tertiary pigment cells (Fig. 1F). At this late stage, Lz ceases to be expressed in the other cells of the eye disc.

The above data show that all cells that express Lz share the property that they arise from the synchronous round of cell division at the furrow. However, when this mitosis is prevented by ectopic expression of the human cell-cycle inhibitor p21<sup>CIP1/WAF1</sup> under the control of the *pGMR* promoter (de Nooij and Hariharan, 1995), we observed that Lz is still expressed in a wild-type pattern (not shown), suggesting that Lz expression is not directly controlled by the cell cycle.

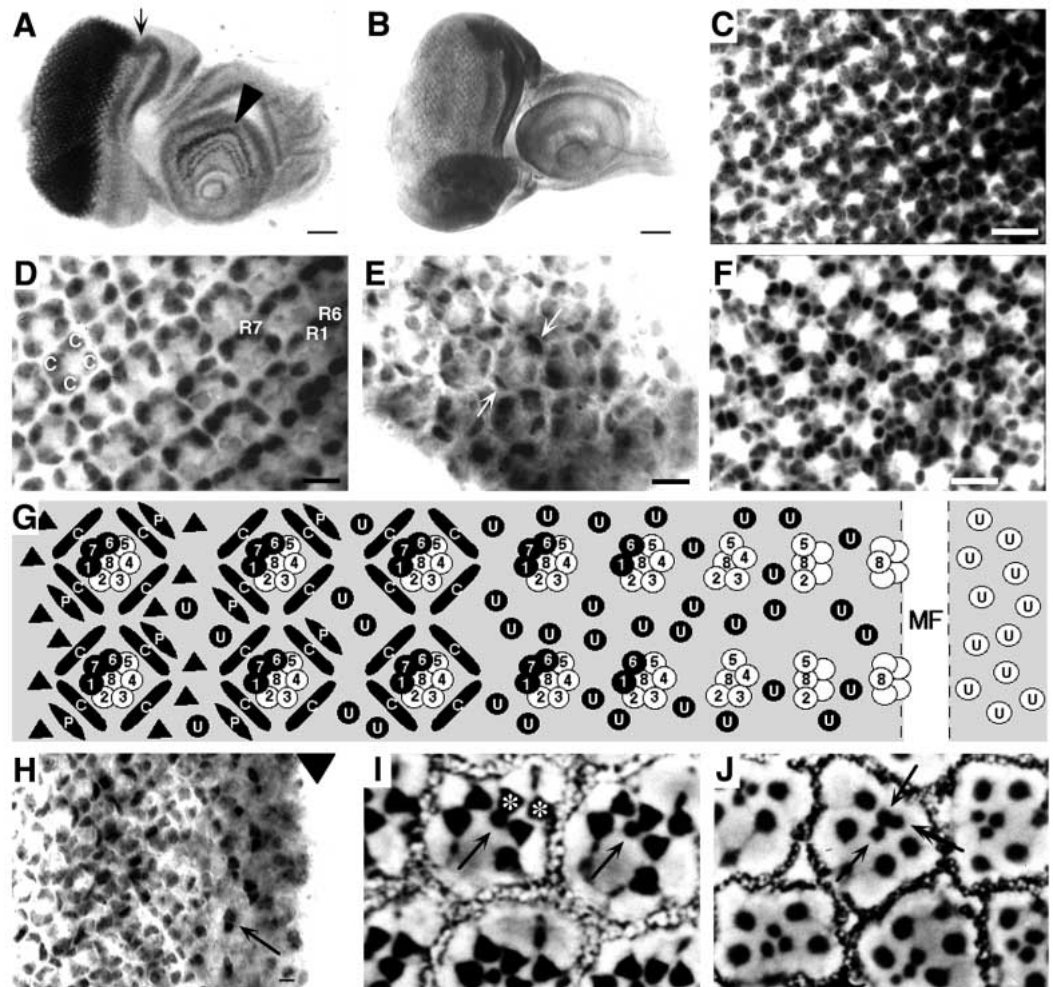
Lz is expressed in all cells posterior to the morphogenetic furrow except for those belonging to the initial 5-cell precluster. The exclusion of Lz from photoreceptor cells R8, R2/R5 and R3/R4 is functionally important for proper cell fate specification. Indeed, when Lz is misexpressed in R3/R4 in the *lz<sup>Sprite</sup>* allele (Fig. 1H), the presumptive R3/R4 cells differentiate as R7s (Fig. 1I,J). This phenotype results from the repression of *svp* transcription caused by the misexpression of Lz in R3/R4 (Daga et al., 1996). It is

interesting to note that, in wild-type flies, Lz is expressed in R1/R6 yet it does not repress transcription of *svp* in these cells, suggesting that Lz controls its target genes in a context-dependent manner.

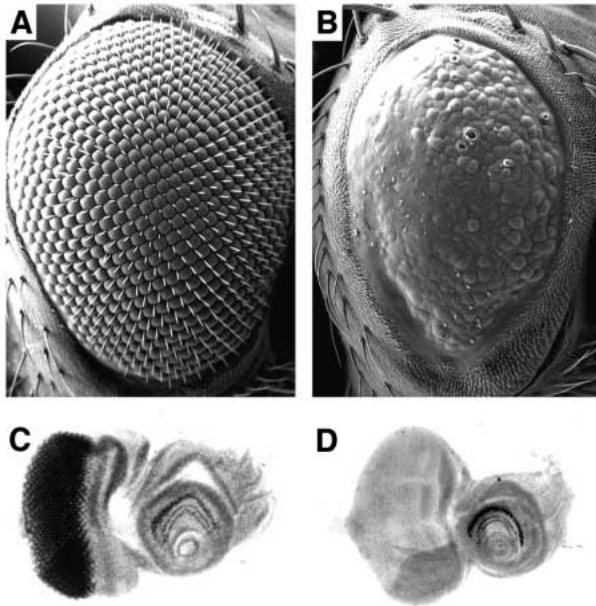
### Eye-specific enhancer of *lozenge*

A group of *lz* alleles including *lz*<sup>50e</sup>, *lz*<sup>hb</sup> and *lz*<sup>77a7</sup> exhibit eye phenotypes that are similar to that seen in *lz* null alleles (Fig. 2A,B) and fail to complement deletions of the *lz* locus as would normally be expected of all *lz* alleles. However, these alleles belong to a unique class in that they can fully complement the eye phenotype of many other *lz* alleles (Batterham et al., 1996). When stained with the Lz antibody, *lz*<sup>77a7</sup> shows no expression in the eye disc, yet expression in the antenna portion remains wild type (Fig. 2C,D). This suggests that *lz*<sup>77a7</sup> is a mutation in an eye-specific enhancer of the *lz* gene. We used a genetic approach that enabled us to map this enhancer. *lz*<sup>77a7</sup> was crossed to a series of nested *lz* deletions in which varying extents of the gene are missing. In *lz*<sup>r1</sup>, the entire coding region of the *lz* gene is deleted while, in *lz*<sup>r7</sup> and *lz*<sup>r8</sup>, the first two introns are intact while more 3' sequences are deleted (Fig. 3A). *lz*<sup>77a7</sup> can completely rescue the mutant phenotype of *lz*<sup>r7</sup> and *lz*<sup>r8</sup>, as *lz*<sup>77a7</sup>/*lz*<sup>r7</sup> and *lz*<sup>77a7</sup>/*lz*<sup>r8</sup> transheterozygotes have wild-type eyes (Fig. 3C,D,F,G). In contrast, *lz*<sup>77a7</sup>/*lz*<sup>r1</sup> flies have eyes resembling those of *lz* null alleles (Fig. 3B,E). This led us to infer that *lz*<sup>r7</sup> and *lz*<sup>r8</sup> retain an eye-specific enhancer element that can stimulate transcription from the *lz*<sup>77a7</sup> promoter in *trans*. Such somatic pairing-induced transvection events in *Drosophila* have been elegantly described for numerous loci including *yellow* (Geyer et al., 1990), *Ultrabithorax* (Martínez-Laborda et al., 1992), *eyes absent* (Leiserson et al.,

1994) and *vestigial* (Williams et al., 1994), and are likely to form the basis for the transvection phenomenon (reviewed in Müller & Schaffner, 1990). These results enabled us to map the putative eye-specific enhancer mutated in *lz*<sup>77a7</sup> to the region deleted in *lz*<sup>r1</sup> yet present within *lz*<sup>r7</sup> and *lz*<sup>r8</sup>, i.e. within the first 5 kb of the gene. Since the *lz*<sup>r1</sup> breakpoint does not remove sequences upstream of the start site, we reasoned that the



**Fig. 1.** Lz expression pattern and phenotypic consequences of misexpression. (A-H) Expression of Lz in eye-antenna disc. Posterior is to the left. (A) Wild type. At low magnification, Lz expression is seen in cells posterior to the morphogenetic furrow (arrow) in the eye disc and in three concentric rings in the antenna disc (arrowhead). Bar, 50  $\mu$ m. (B) *lz*<sup>r1</sup>. No staining with the antibody can be seen in this null allele of *lz*. Bar, 50  $\mu$ m. (C-F) Higher magnification of wild-type eye disc stained with  $\alpha$ -Lz. Bar, 15  $\mu$ m. (C) When focused at the basal level, Lz expression is seen in the nuclei of all undifferentiated cells posterior to the furrow. (D) Apically, Lz is expressed in R1/R6, R7 and cone cells (c). (E) Expression of Lz in primary pigment cells (arrows) in pupal disc. (F) Expression of Lz in secondary and tertiary pigment cells in pupal disc. (G) Schematic diagram of Lz expression pattern. The morphogenetic furrow (MF) moves from posterior (left) to anterior. Black shapes represent cells that express Lz, open circles represent cells that do not express Lz. Anterior to the furrow, undifferentiated cells (u) do not stain with  $\alpha$ -Lz, while all undifferentiated cells posterior to the furrow do stain. Cells within the precluster (R8, R2/R5, R3/R4) do not stain while photoreceptors and non-neuronal cells added to the cluster later do stain with Lz. c, cone cells; p, primary pigment cells; 1-8, R1-R8; triangles, secondary and tertiary pigment cells. (H) In the gain-of-function allele *lz*<sup>Sprite</sup>, Lz is ectopically expressed in R3/R4 (which belong to the precluster) (arrow). This view is focused immediately posterior to the morphogenetic furrow (arrowhead) to facilitate visualization of R3/R4 staining. Bar, 10  $\mu$ m. (I-J) Tangential section of adult eye. (I) Wild type. Each ommatidium has a single R7 cell (arrow). The R3/R4 rhabdomeres (asterisks) are significantly larger than that of R7. (J) *lz*<sup>Sprite</sup>. In this allele, misexpression of Lz causes cells in the position of R3/R4 to be converted to an R7 fate (arrows).

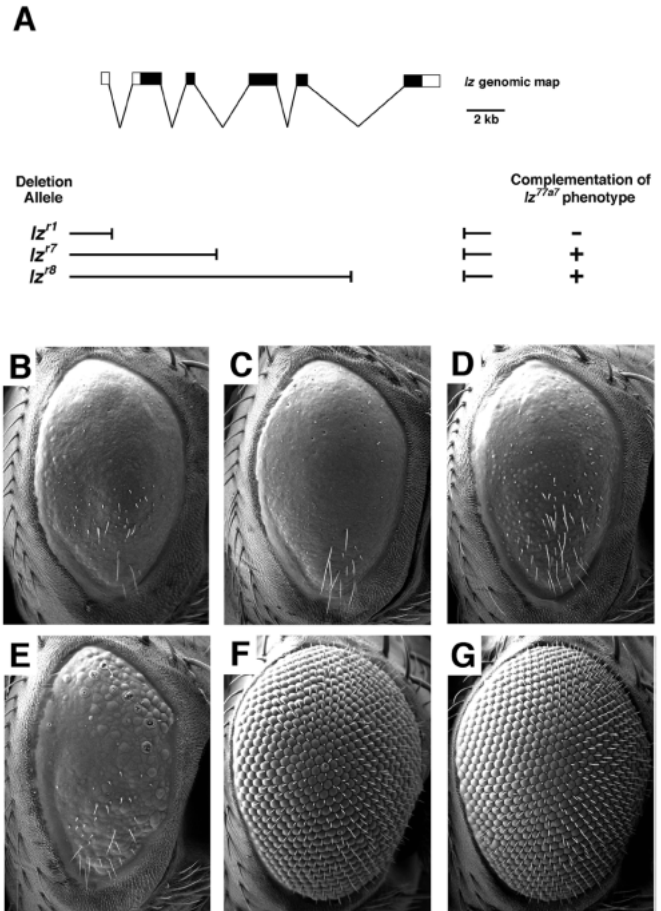


**Fig. 2.** Eye-specific alleles of *lz*. (A,B) Scanning electron micrograph (SEM) of adult eye. (A) Wild type. (B) *lz*<sup>77a7</sup>. The organized ommatidial lattice seen in wild type is disrupted giving rise to a smooth eye typical of *lz* alleles. (C,D) Expression of Lz in eye-antenna disc. (C) Wild type. (D) *lz*<sup>77a7</sup>. Expression of Lz is eliminated in the eye portion of the disc but is maintained in the antenna disc.

enhancer element will reside in one of the first three introns of the gene.

The first three intron sequences from wild type and *lz*<sup>77a7</sup> were PCR amplified. While amplification of introns I and III from *lz*<sup>77a7</sup> yielded PCR products of identical size to those amplified from wild type, amplification of intron II from the *lz*<sup>77a7</sup> template generated band approximately 150 bp in length, compared to an approximately 1550 bp band isolated from wild type. This suggested that a deletion within this intron may be responsible for the *lz*<sup>77a7</sup> phenotype. Sequence analysis of the PCR product confirmed that the *lz*<sup>77a7</sup> mutation is indeed a 1398 bp deletion of the second intron (Fig. 4A). In addition, amplification of intron II from *lz*<sup>50e</sup> and *lz*<sup>hb</sup> also generated PCR products shorter than that from wild type. Sequencing of these PCR products demonstrated that *lz*<sup>50e</sup> is molecularly identical to *lz*<sup>77a7</sup> and that *lz*<sup>hb</sup> contains a smaller deletion of 880 bp within intron II (Fig. 4A). The existence of three independently isolated eye-specific alleles harboring deletions of intron II strongly supports our hypothesis that an eye-specific enhancer lies within this intron.

These results do not rule out the possibility that other regions of the *lz* gene may additionally be required for eye-specific expression. To identify all of the regions necessary for proper *lz* expression in the eye, a number of *lz* minigene constructs were engineered, transformed into flies and assessed for their ability to rescue the *lz*<sup>r1</sup> mutant eye phenotype. Construct 1, which contains 10 kb of upstream genomic sequences, the transcription start site and intron I fused to the cDNA (Fig. 4B), was not able to rescue the *lz*<sup>r1</sup> mutant eye phenotype. Full rescue was obtained with construct 2, which includes introns I-IV, construct 3, which included introns I and II, and most importantly, construct 4, in which intron II alone was fused to the promoter region and the



**Fig. 3.** Genetic mapping of the eye-specific enhancer of *lz*. (A) Genomic map of *lz* excision alleles *lz*<sup>r1</sup>, *lz*<sup>r7</sup> and *lz*<sup>r8</sup> based on Daga et al. (1996). Open boxes represent untranslated regions, black boxes represent protein coding sequences. The results from complementation analysis are summarized as (+) for the ability of *lz*<sup>77a7</sup> to complement the eye phenotype of the deletion allele and (-) for failure to complement. (B-G) SEM of adult eye. (B-D) *lz*<sup>r1</sup>/*lz*<sup>r1</sup>, *lz*<sup>r7</sup>/*lz*<sup>r7</sup> and *lz*<sup>r8</sup>/*lz*<sup>r8</sup>, respectively. (E) *lz*<sup>r1</sup>/*lz*<sup>77a7</sup>. The eye looks mutant as in B-D demonstrating failure of *lz*<sup>77a7</sup> to complement the null allele *lz*<sup>r1</sup>. (F,G) *lz*<sup>r7</sup>/*lz*<sup>77a7</sup> and *lz*<sup>r8</sup>/*lz*<sup>77a7</sup>, respectively. These eyes resemble wild type (Fig. 2A) suggesting that *lz*<sup>77a7</sup> function is complemented by regions of the gene that are not deleted in *lz*<sup>r7</sup> and *lz*<sup>r8</sup>.

*lz* cDNA (Fig. 4B-D). Staining with the  $\alpha$ Lz antibody revealed that Lz is expressed at high levels behind the morphogenetic furrow in *lz*<sup>r1</sup> eye discs carrying the rescuing constructs, and that there is no expression in the antenna discs (Fig. 4E). Interestingly, expression of Lz in these transformants is robust in undifferentiated cells, yet appears to be absent from R1/R6, R7 and cone cells (Fig. 4F). Since these constructs completely rescue the *lz* null eye phenotype, we conclude that Lz is only required in undifferentiated cells at the time of cell fate specification. In wild type, Lz expression is maintained in cells whose identities have already been established; however, this later expression does not appear to be required for the proper patterning of the ommatidium. Taken together with the molecular and genetic data, these rescue experiments demonstrate that intron II is necessary and sufficient for Lz function in the eye.



of the fate of a large number of cells. In *lz* mutants, *svp* is de-repressed (Daga et al., 1996), and the expression of *Bar* (Daga et al., 1996), *pros* (R. Carthew, personal communication) and *spa* (G. V. F., H. Duan, M. Noll and U. B., unpublished) is not initiated, leading to the differentiation of cone cell precursors as neurons and an overall collapse of the ommatidial lattice (Daga et al., 1996). The mammalian proteins that are similar to Lz include AML1 (acute myeloid leukemia 1) and CBFA1 (core binding factor A1), and, like Lz, are expressed in pluripotent cells in specific tissues and regulate multiple target genes during development. Mutations in these genes lead to severe alterations in the fates of multiple cell types in these tissues (reviewed in Ito, 1997; Rodan and Harada, 1997). For example, AML1 regulates the expression of T cell receptor and cytokines such as GM-CSF and IL3, and represents the most frequent target of translocations that lead to acute myeloid leukemia in humans (Ito, 1997). In mice, a complete knockout of the AML1 homolog *PEBP2a* results in animals that do not undergo definitive hematopoiesis and lack all cell types that result from this process (Okuda et al., 1996; Wang et al., 1996). CBFA1 is required for the proper expression of osteoblast-specific genes such as *Osteocalcin gene 1* and *al(I) collagen*, and mouse knock-outs lack all bone structures (Rodan and Harada, 1997 and references therein). Thus, Lz, AML1 and CBFA1 share the property that they differentially regulate the expression of several genes within a pool of equipotent cells thereby functioning as pre-patterning molecules.

The developmental events in the eye disc can be separated into two stages of patterning. The first occurs within the morphogenetic furrow and leads to the formation of the 5-cell precluster, while the second occurs in the undifferentiated cells posterior to the furrow that gives rise to the remainder of the cells of the mature ommatidium. The first pre-patterning event is controlled by transcription factors such as Atonal (Jarman et al., 1995) and Rough (Dokucu et al., 1996). Lz plays no role in this process as it is not expressed in the 5-cell precluster and *lz* mutants show no disruption in the patterning of these cells (Daga et al., 1996). In fact, our results show that misexpression of Lz at the 5-cell stage leads to a re-programming of cell fates within the precluster. In contrast, proper expression of Lz is crucial for the second phase of pre-patterning that completes the ommatidium by adding the last three photoreceptor cells and the non-neuronal cell types to the precluster. Other transcription factors that play a role in this process include the zinc-finger protein Tramtrack (Ttk) (Xiong and Montell, 1993; Li et al., 1997; Tang et al., 1997) and the Ets domain proteins Yan (Rebay and Rubin, 1995; Rogge et al., 1995) and Pointed (Brunner et al., 1994; O'Neill et al., 1994). The activity of these three proteins is modulated by the EGFR and Sevenless receptor tyrosine kinase (RTK) signaling pathways (Rebay and Rubin, 1995; Brunner et al., 1994; O'Neill et al., 1994; Li et al., 1997; Tang et al., 1997). It seems likely that Lz may function combinatorially with these transcription factors in order to differentially regulate its target genes in different cells. It is interesting to note that the mammalian homolog of Pointed (Pnt), Ets-1, directly binds to AML1 and, together, they cooperatively activate transcription of the T cell receptor (Wotton et al., 1994; Giese et al., 1995).

While it is clear that Lz regulates the expression of many different transcription factors, it has not yet been determined how the expression of Lz itself is controlled. We have shown

that an enhancer sequence located within the second intron of *lz* is necessary and sufficient for proper eye-specific function. The transcomplementation seen at the *lz* locus has been previously described as involving two cistrons, in which 'Cistron A' alleles (*lz*<sup>50e</sup>, *lz*<sup>hb</sup> and *lz*<sup>77a7</sup>) can complement the phenotype of 'Cistron B' alleles (*lz*<sup>r7</sup> and *lz*<sup>r8</sup>) and vice versa (Batterham et al., 1996). Our studies suggest that Lz is encoded by a single transcriptional unit, with the so-called 'Cistron A' alleles representing mutations in regulatory sequences in the eye-specific enhancer located in intron II. 'Cistron B' alleles define mutations in the protein-coding region of the gene. Deletions such as *lz*<sup>r1</sup> eliminate both the eye-specific enhancer and the coding regions and therefore fail to complement mutations of either class. Further dissection of the eye-specific enhancer element should allow the identification of both the signaling mechanism as well as the specific transcription factors that are responsible for the initiation and maintenance of Lz expression. We have identified seven consensus AP-1 binding sites within the second intron (Quandt et al., 1995), although the significance of this observation is as yet unclear since *Drosophila* Jun is either not functional or redundant during eye development (Riesgo-Escovar et al., 1996; Hou et al., 1997; Kockel et al., 1997), and no eye phenotype has yet been reported for *Drosophila fos* mutants (Riesgo-Escovar and Hafen, 1997; Zeitlinger et al., 1997).

The determination of a cell with a distinct fate from amongst an initially equipotent group is a fundamental problem in all developing systems. Although such processes have been extensively studied in vertebrate as well as in invertebrate development, the precise mechanisms by which common signaling pathways trigger cells to assume specific and distinct fates has not yet been determined. In the *Drosophila* eye, RTK signaling pathways have been well described, but the mechanisms by which such signals trigger the differentiation of one cell type rather than another is not well understood. In this study, we have identified Lz as a transcription factor that is expressed in a population of pluripotent cells and causes the segregation of specific cell types by controlling the expression of different genes in different cells. The mechanism by which Lz differentially regulates the expression of cell-specific factors is not yet known, but a model in which Lz and other transcriptional regulators expressed in undifferentiated cells, including Ttk, Pnt and Yan, combine to create unique contexts in which to interpret common signals such as those triggered by the RTK and Notch pathways is appealing. Future studies will begin probing the molecular basis for generating predisposition and patterning in this system. The molecular similarity of Lz with AML1 and CBFA1, which serve similar functions during mammalian hematopoiesis and bone morphogenesis, furthermore suggests that the pathways elucidated in *Drosophila* eye development will be generally applicable in understanding mechanisms that underlie patterning of cells in vertebrate systems.

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