The division abnormally delayed (dally) gene: a putative integral membrane proteoglycan required for cell division patterning during postembryonic development of the nervous system in Drosophila

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SUMMARY

We have devised a genetic screen to obtain mutants affecting cell division patterning in the developing central nervous system of Drosophila. The division abnormally delayed (dally) locus was identified using a combination of “enhancer trap” and behavioral screening methods. The ordered cell cycle progression of lamina precursor cells, which generate synaptic target neurons for photoreceptors, is disrupted in dally mutants. The first of two lamina precursor cell divisions shows a delayed entry into mitosis. The second division, one that is triggered by an intercellular signal from photoreceptor axons, fails to take place. Similar to lamina precursors, cells that generate the ommatidia of the adult eye show two synchronized divisions found along the morphogenetic furrow in the eye disc and the first division cycle in dally mutants displays a delayed progression into M phase like that found in the first lamina precursor cell division. dally mutations also affect viability and produce morphological defects in several adult tissues, including the eye, antenna, wing and genitalia. Sequencing of a dally cDNA reveals a potential open reading frame of 626 amino acids with homology to a family of Glypican-related integral membrane proteoglycans. These heparan sulfate-containing proteins are attached to the external leaflet of the plasma membrane via a glycosylphosphatidylinositol linkage. Heparan sulfate proteoglycans may serve as co-receptors for a variety of secreted proteins including fibroblast growth factor, vascular endothelial growth factor, hepatocyte growth factor and members of the Wnt, TGF-β and Hedgehog families. The cell division defects found in dally mutants implicate the Glypican group of integral membrane proteoglycans in the control of cell division during development.

Key words: cell division, dally, proteoglycan, Drosophila, nervous system, cell cycle

INTRODUCTION

Understanding the control of cell division is a central problem in biology. In recent years, a highly conserved set of proteins required for cell cycle progression in organisms from yeast to humans have been identified. The two central components of this conserved machinery are cyclins, which show cycles of synthesis and degradation through the division cycle and the cyclin-dependent kinases (cdk), enzymes regulated by their association with cyclins and whose activity governs passage through the division cycle (reviewed in Norbury and Nurse, 1992; Kirshner, 1992). The progress in our understanding of cell division has served to emphasize the common features of cell cycle control in eukaryotic cells but has left largely unanswered how the specificity of cell division pattern is achieved in multicellular organisms. What are the mechanisms that ensure that cell division takes place only at the right time and place during development or tissue regeneration, or in adult tissues where cell turnover is high? In all of these cases, the spatial and temporal control of division must be precisely regulated to maintain normal tissue architecture and function.

We are studying a set of patterned divisions in the developing central nervous system (CNS) of Drosophila. During visual system development the assembly of the peripheral and CNS components must be precisely coordinated (reviewed in Meinertzhagen and Hanson, 1993). Each sensory unit, or ommatidium, of the compound eye is composed of eight photoreceptors. During the late third instar larval stage, photoreceptor axons grow across the optic stalk to reach their synaptic target cells in the brain. Six photoreceptors (R1-6) synapse with lamina neurons, which constitute the first relay station for visual information in the CNS. The generation of lamina neurons from their precursor cells (lamina precursor cells or LPCs) is coordinated with the arrival of photoreceptor axons and LPC divisions take place in a stereotyped and highly ordered pattern (Selleck and Steller, 1991). Earlier studies have shown that LPC divisions are controlled by an intercellular signal delivered by photoreceptor axons (Selleck and Steller, 1991). This signal is required for LPCs to enter their final S phase from the preceding G1 (Selleck et al., 1992). The number and location of LPC divisions is dictated by the number and placement of photoreceptor axons arriving in the CNS.
In addition to the interaction between LPCs and photoreceptors providing a system for studying intercellular control of cell division, the anatomy of LPC divisions displays some very useful features. LPCs are part of an epithelial sheet where the successive phases of two division cycles are found in sequence along the epithelium (Selleck et al., 1992). This organization allows: (1) tracking of LPC cell cycle progression, (2) the identification of genes expressed during restricted segments of their division cycles, and (3) the analysis of LPC cell cycle progression in mutants affecting the normal patterning of these divisions. These features permit the ready identification of genes that are required for patterning of LPC division and their ordered progression through the cell cycle. This class of genes is likely to provide links between the signaling systems that control cell division pattern and the apparatus that orchestrates progression through the cell cycle. In this report, we describe the isolation and characterization of a novel gene, division abnormally delayed (dally), that affects cell division control of LPCs. Molecular cloning of the dally cDNA shows it encodes a putative integral membrane proteoglycan of the Glypican family.

MATERIALS AND METHODS

Fly stocks
dallyP1 is a homozygous viable enhancer trap insertion obtained from a collection generously provided by Tim Tully (Boynton and Tully, 1992). dallyP2 is a semi-lethal P-element insert [sli(3)06464] from a collection generated in the laboratory of A. Spradling (Karpen and Spradling, 1992). Df(3L) h, i22, Kit/TM3 Ser [deficiency breakpoints at 66D10 and 66E1.2: Ingham et al., 1985] was obtained from the Bloomington Stock center. Canton-S and Oregon-R were used as wild-type stocks. Two excision alleles (dallyP1 and hairy1) were used during the course of this project. Canton-S and Oregon-R were used as wild-type stocks.

Enhancer trap and phototaxis screening
A total of approximately 600 independent homozygous viable enhancer trap insertions (Boynton and Tully, 1992) were examined for ß-galactosidase (ß-gal) activity in whole-mount third instar larval brains (O’Kane and Gehring, 1987; Mlodzik and Hiromi, 1992). Lines with expression in dividing cells of the visual system were tested as adults for fast phototaxis using a counter-current phototaxis apparatus (Benzer, 1967; Luo et al., 1992). Phototaxis scores were calculated using the formula: \( \Sigma N_i / \Sigma N \), where \( i \) is the tube number from 0-10 and \( N \) is the number of flies in the \( i \)th tube (Luo et al., 1992).

Immunohistochemistry and microscopy
Anti-cyclin B antibody and propidium iodide staining was carried out as previously described (Whitfield et al., 1990). Anti-ß-gal antibodies (Cappel) were used at 1:1000 dilution, with FITC-linked secondary at 1:200. Anti-Elav monoclonal antibody 5D3C5 and monoclonal antibody 22C10 (Fujita et al., 1982) were used at 1:2 dilution. Bro-mouridine deoxyriboside (BUdR) labelling and detection in whole-mount third instar larval CNS preparations was carried out according to published protocols (Truman and Bate, 1988; Selleck and Steller, 1991). Confocal microscopy and image analysis was performed on a Biorad MRC 600 using software provided by Biorad, Adobe Photoshop, Adobe Illustrator and NIH Image. Adult heads were prepared for scanning electron microscopy according to Restifo and Merril (1994), and examined in an ISI DS-130 scanning electron microscope. Hybridization of digoxigenin-labelled probes to whole-mount larval CNS preparations was performed according to protocols described by Ebens et al. (1993). Defects in adult structures were examined and photographed using a Zeiss Axioplan microscope following mounting of the tissues in Aquamount (Lerner Laboratories).

Excision mutagenesis
The dallyP1 P-element was excised using the following genetic scheme. First, w1118; dallyP1[P, w+] virgin females were crossed to males bearing the Ð2-3 transposon, Cy0/Sp; P[∆2-3, ry+] Sb/TM6, Ubx (Robertson et al., 1988). The w+; Sb male progeny (F1) (w1118/Y; P[∆2-3, Sb/dallyP1 P[w+]]) were then crossed to virgin females of the genotype w1118; TM3, Sb/CXD. Individual male progeny from this cross (F2) that were phenotypically w, Sb+ and D had lost the dallyP1 insertion on the third chromosome (loss of w+ phenotype) and the transposase-bearing chromosome (marked with Sb). 569 males of this class (w1118; ∆P (loss of P1 insert)/CXD were mated individually to dallyP2 (P [ry+] ry/ TM3, Sb; ry+ virgin females. Sb+, D+ flies from this cross were evaluated for defects in antenna, wing, genitalia and eye. ∆P/TM3, Sb males and virgin females were collected for those lines that failed to complement dallyP2 in order to establish balanced stocks. Two excision alleles (dallyP1 and dallyP2) were obtained from a separate excision mutagenesis where 145 excision-bearing chromosomes were scored for lethality as homozygotes.

Molecular analyses
Plasmid rescue of dally P-element insertion lines (dallyP1 and dallyP2) were performed as described previously (Ashburner, 1989). A Drosophila genomic DNA library was kindly provided by J. Tamkun (Tamkun et al., 1992) and screened according to published methods (Maniatis et al., 1978). The cDNA clone was obtained by screening an embryonic cDNA library (Brown and Kafatos, 1988) with a 10 kb Sacl fragment that encompassed the two P element inserts in the dally locus. Sequencing was performed by primer extension using the Applied Biosystems 373A DNA Sequencer at the W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University Sequencing Facility. Both strands were sequenced and any ambiguities were resolved by an additional sequencing run. DNA sequences were aligned and homology searches performed using September 1994, version 8 of the Wisconsin package, National Center for Biotechnology Information and the BLAST network service. For the northern blot analysis, total RNA was extracted from mutant and wild-type (Or-C) third instar larval brains and imaginal disc complexes. 10 µg total RNA was loaded into each lane and probed with c1 (dally cDNA clone) and rp49 riboprobes simultaneously. c1 and rp49 riboprobes were transcribed using the Genius system (Boehringer Mannheim). Hybridization was performed overnight at 65°C in modified Church buffer (Trayhurn et al., 1994) at a concentration of 75 ng/ml for c1 and 15 ng/ml for rp49. Washes and chemiluminescence detection was performed according to the Genius system protocol. All other procedures for molecular studies were essentially as described by Sambrook et al. (1989) and Ausubel et al. (1994).

RESULTS

The anatomy of lamina precursor divisions
The utility of LPCs as a model for understanding the control of cell division patterning is in large part due to their anatomical organization. Lamina neurons receive synaptic input from photoreceptors R1-6. LPCs divide late in the third instar larval stage, when photoreceptor axons reach the brain from the developing eye. The coordination between eye and lamina...
assembly is achieved in part at the level of cell division; an intercellular signal from photoreceptor axons entering the brain induces the final cell division of LPCs (Selleck and Steller, 1991).

LPCs are derived from a set of neighboring neuroblasts in the anterior segment of the outer proliferative center (aOPC) (White and Kankel, 1978; Hofbauer and Campos-Ortega, 1990; Selleck and Steller, 1991). The aOPC and LPCs form an epithelial sheet on the surface of the brain (Fig. 1). OPC neuroblasts that produce LPCs are at the anteriormost extent of this epithelium, with the lamina marking its posterior limit (Fig. 1A-B). LPCs are produced continuously from OPC neuroblasts and complete two cell cycles before differentiating into neurons (Selleck et al., 1992). LPCs therefore enter the cell cycle as they are produced from aOPC neuroblasts and exit after the second division to differentiate into lamina neurons (Fig. 1D). As a result of this “assembly line” organization, LPCs in successive phases of the cell cycle are found in sequence along the proliferative epithelium (Fig. 1B-D).

LPCs in different phases of the cycle are found at discrete positions relative to anatomical landmarks (Selleck et al., 1992). Most notable of these is a furrow in the aOPC/LPC epithelium, located at the anterior boundary of the developing lamina (Fig. 1A-C). The lamina furrow, like the morphogenetic furrow (MF) in the eye disc, sweeps forward as neurons are added to the differentiating lamina. Previous work using a variety of cell cycle-specific markers established that LPC divisions are synchronized across the furrow, with cells in different phases of the cell cycle at specific positions (summarized in Fig. 1C; Selleck et al., 1992). Fig. 1B shows the distribution of LPCs using two markers, antibodies to cyclin B, which is expressed at highest levels in late G2−early M phase, and propidium iodide, a fluorescent dye that binds to DNA and permits the visualization of mitotic chromosomes (Whitfield et al., 1990). The two LPC division cycles are evident as two regions of peak cyclin B expression and two domains of mitotic figures, along the anterior and posterior segments of the lamina furrow respectively (Fig. 1B). Cells between the two cyclin B-expressing domains reside in G1, (low levels of cyclin B) and the subsequent S phase (Fig. 1C, and Selleck et al., 1992). Newly arrived photoreceptor axons specifically run along the base of the G1-phase LPCs (Selleck et al., 1992, shown in cartoon form, Fig. 1C) and trigger their entry into S phase.

A genetic screen to isolate mutations affecting the control of LPC division

To investigate the molecular mechanisms that govern the patterning of LPC division, we devised a two-part screening procedure to identify genes that are expressed in LPCs and required for their normal division. First, we examined a set of homozygous viable enhancer trap insertions for expression of the marker gene, β-gal, in the aOPC/LPC epithelium of third instar larvae. Second, to determine if any of the enhancer trap insertions may have disrupted the organization of the visual system, we tested adult flies for a visual system-dependent behavior, phototaxis. Wild-type adult flies display a positive phototaxis response that can be measured using a simple apparatus (Benzer, 1967; Luo et al., 1992). Enhancer trap lines with expression in the aOPC/LPC epithelium and abnormal phototaxis were subsequently examined for morphological defects in cell division.

Approximately 600 independent, homozygous viable enhancer trap insertions were screened by histochemical staining for β-gal activity in third instar larval CNS. More than a dozen showed β-gal expression in the aOPC/LPC epithelium and adult flies from these homozygous lines were then scored for phototaxis. Of the 15 lines tested, 6 showed abnormal phototaxis responses (phototaxis index 6.5 or below, where wild-type flies typically have phototaxis index scores over 8.5; Luo et al., 1992). Two lines, P1 and H142, gave phototaxis index scores below 5 on at least three separate trials and were chosen for morphological analysis. Approximately 10% of P1 homozygous larvae showed morphological abnormalities in the aOPC/LPC epithelium (data not shown). [Note: The H142 insert is located at 67D1-3, shows a rough eye phenotype and fails to complement Gap1H2 (a severe EMS-induced allele, U. Gaul, personal communication). This enhancer trap line shows expression in the OPC, in addition to expression in the lamina and photoreceptors as previously described for enhancer trap insertions in the Gap1 locus (Gaul et al., 1992, Rogge et al., 1992; Buckles et al., 1992).]

The P1 enhancer trap insertion shows expression in G2-M of the first LPC division

The P1 enhancer trap insertion showed a severe phototaxis deficit and disorganization of the aOPC/LPC epithelium in a fraction of P1 larvae. On the basis of these phenotypes, this line was chosen for further morphological and genetic analysis. We began by examining in detail the expression of the P1 enhancer trap line through the division cycles of LPCs in third instar larval brains using anti-β-gal antibody and digoxigenin-labelled DNA probes complementary to lacZ mRNA. The highest levels of β-gal immunoreactivity are found in LPCs along the anterior segment of the lamina furrow (Fig. 2A). Cells in this region are in G2 and M phase of the first division (Fig. 1). The lacZ mRNA shows a more limited distribution, presumably because of β-gal protein perdurance and is restricted to the G2 and M phase domains of LPC division one (Fig. 2B).

We examined the cell cycle-dependent expression of enhancer trap insertions in this locus by staining third instar larval brains with both anti-β-gal and anti-cyclin B antibodies. The preparation shown in Fig. 2C is from a larva heterozygous for another enhancer trap insertion in the locus [sli(3)06464, described in next section] that shows stronger β-gal expression, although in the same pattern as P1. There is overlap of expression of the enhancer trap insertion with cyclin B in several groups of dividing cells including the inner proliferative center (IPC) (Fig. 2C), suggesting that the cell cycle-restricted expression is not limited to LPCs (Fig. 2C). However, some cells of the central brain complex express the enhancer trap marker but do not show high levels of cyclin B immunoreactivity (Fig. 2C).

Genetics of the daily locus and adult phenotypes

Larvae homozygous for the P1 insertion showed disorganization of the aOPC/LPC epithelium in approximately 10% of the CNS preparations examined. Disordering of the eye and reductions or duplications of the antenna, also with low penetrance [for one experiment at 25°C, 48/325 showed antennal defects, (14%), 61/325 showed eye defects (19%)] were found in P1 homozygous adults. The low penetrance found in the P1
mutant made analysis of the cell division defect difficult. We therefore searched for more severe alleles within existing collections of P-element-induced mutants.

*In situ* hybridization of a P-element probe to salivary gland chromosomes localized the P1 insert to a single site on the left arm of the third chromosome, at the 66D/E junction. One semilethal enhancer trap insertion in the region of 66D/E, sl(3)06464 (kindly provided by A. Spradling, see Karpen and Spradling, 1992) showed a β-gal-staining pattern in the larval brain like that of line P1. Homozygous sl(3)06464 adults exhibited abnormalities in several adult tissues including reductions or complete loss of genitalia, disordering and reduction in the number of ommatidia, reductions and duplications of the antenna, and incomplete wing vein V and wing notching (Table 1; Fig. 3). P1 and sl(3)06464 enhancer trap expression patterns in other tissues (antenna, eye, leg and wing discs) and developmental stages (embryo) were also coincident (data not shown). Genetic and molecular experiments (see below) have provided further evidence that the sl(3)06464 insert affects the same gene as P1. On the basis of the cell division defects described below, we have named the gene, *division abnormally delayed* (dally). The two P-element alleles are henceforth referred to as dallyP1 (the P1 insertion described above) and dallyP2, the sl(3)06464 insertion.

We have conducted a series of genetic experiments to: (1) determine the range of phenotypes produced from defects in dally function, (2) establish that these phenotypes result from mutations in a new gene, and (3) demonstrate that the abnor-

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Fig. 1. Organization of lamina precursor cell divisions. A and B are horizontal optical (confocal) sections of third instar larval CNS preparations, stained with anti-cyclin B antibody (red) and propidium iodide, a fluorescent DNA stain used to visualize mitotic chromosomes (grey tones). (Anterior to the left; lateral down.) (A) Cross section of entire brain lobe and eye disc. Regions with high levels of anti-cyclin B staining define the outer (marked aOPC and pOPC for anterior and posterior segments, respectively) and inner proliferative centers (IPC). LPCs are continuous with the aOPC. (B) Region comparable to that in rectangle in A at higher magnification, showing aOPC and LPCs. At the anterior limit of the lamina (LA) is the lamina furrow (asterisk). Along the length of the furrow LPCs complete two division cycles. G2 and M phase of the first division are seen as domains of peak anti-cyclin B staining and cells with condensed propidium iodide-stained chromosomes (arrow marked 1), respectively, along the anterior portion of the furrow. The G2 and mitosis of the second division (marked G2/M) are found along the posterior segment of the furrow (arrows labelled 2 indicate two mitotic figures of second division). (C) Cartoon showing approximate locations of LPC cell cycle domains along the lamina furrow (Selleck et al., 1992). Photoreceptor axons (in green) are also shown, with the most anterior axon bundles running along the base of G1-phase LPCs. (D) Cartoon of the two LPC division cycles. A signal from photoreceptor axons is required for LPCs to enter their final S phase from G1 (Selleck et al., 1992).
**Fig. 2.** Expression of enhancer traps P1 and sl(3)06464 in late third instar larval brain. All panels show horizontal optical sections of third instar larval brain preparations, with anterior to the left and the lateral margin toward the bottom. The lamina furrow is indicated with an arrow in all panels. (A,B) P1 homozygous larval brains that have no apparent morphological defects. (C) sl(3)06464/TM6B larval CNS preparation. (A) LPCs and lamina (LA) stained with anti-β-gal antibody (red) and propidium iodide (grey tones), showing the highest level of enhancer trap P1 expression in the anterior segment of the lamina furrow. The absence of mitotic figures in this image does not indicate a cell division defect as not every optical section contains cells in M phase, even in wild-type. (B) Distribution of lacZ mRNA in LPCs and lamina. The dark region (bracket) indicates the cells with the highest signal, where G2- and M-phase LPCs of the first division are found. Note the more restricted distribution of the lacZ mRNA compared to anti-β-gal antibody staining. (C) Horizontal optical section of an entire brain lobe stained with both anti-β-gal (green signal in nuclei) and anti-cyclin B antibodies (red). Note that the staining is largely coincident for several groups of dividing cells, including the IPC, the pOPC and the first division G2-M domain of LPCs (cells to the left of the lamina furrow, indicated with arrow). A group of β-gal-expressing cells in the central brain complex however, do not show high levels of cyclin B immunoreactivity.

Abnormalities in dally mutants are a consequence of loss, or partial loss, of dally function. Genetic complementation tests were performed with dallyP1 and dallyP2 alleles for the antennal phenotypes since these are easy to score and of fairly high penetrance in dally P1. For antennal defects, dallyP1 and dallyP2 failed to complement, supporting the conclusion that these two P-element insertions affect the same gene [34/355 (9%) with antennal defects]. To demonstrate that the phenotypes observed in dally mutants are due to P-element inserts in the interval 66D10-66E1,2, complementation analysis of dallyP2 with a deficiency spanning the interval 66D10 to 66E1,2 was undertaken. As expected, the deficiency fails to complement dallyP2 (Table 1). Thus dally phenotypes genetically map to the same region as the insertion site. dally mutants do complement the only characterized locus in this region, hairy (data not shown).

To confirm that dallyP1 and dallyP2 are responsible for the phenotypes described above and that these phenotypes are caused by loss-of-function alleles, the P-element insert in dallyP1 was mobilized. Loss of a P element can result in: (1) precise excision that restores the wild-type sequence, (2) partial excision that removes sequences within the P element only, or (3) imprecise excision that removes genomic sequences adjacent to the site of insertion (Daniels et al., 1985). Over 700 excision lines were generated and sixteen of these were selected at random to evaluate their ability to complement the antennal phenotype of dallyP2. 9 of the 16 showed complete reversion of the dally antennal phenotype. The high reversion rate associated with loss of the dallyP1 insert provides conclusive evidence that the P-element insertion is responsible for this phenotype.

The imprecise excision class creates small deletions that can potentially remove, completely or partially, the normal function of the targeted gene. 23 independent excision alleles failed to complement the adult phenotypes of dallyP2. These alleles, either as homozygotes or in combination with dallyP2 show phenotypes with a range of expressivity and penetrance; the more severe mutants affecting the same tissues and to the same degree as observed for dallyP2 homozygous adults (Fig. 3; Tables 1, 2). For example, dallyΔP-188 shows abnormalities in the eye (Fig. 3C), antenna (Fig. 3F), genitalia (Fig. 3I) and wing (Fig. 3L), similar to dallyP2 (Fig. 3B,E,H,K). Molecular characterization of four excision alleles (ΔP-188, ΔP-527, ΔP-75, ΔP-305) have confirmed that genomic DNA adjacent to the dallyP1 insertion site has been deleted (Fig. 6). The results from
the excision alleles show that the adult phenotypes are due to loss or partial loss of dally function.

**division abnormally delayed (dally) mutations affect cell cycle progression of lamina precursors**

Third instar larvae homozygous for several dally alleles were evaluated for the organization and cell cycle progression of LPC divisions. All dally mutants examined (dallyP2, dallyP-14, dallyP-48, dallyP-188) showed the same constellation of defects, with varying degrees of abnormalities either as homozygotes, or in combination with dallyP2. Presented here is the analysis of dallyP2, which shows complete penetrance of the defects in the larval brain. Molecular analysis of dallyP2 shows that it severely affects the level and size of the dally mRNA (see below). Larval brains were stained with both anti-cyclin B antibodies and propidium iodide, allowing for the simultaneous visualization of G2- and M-phase cells. In every brain examined the G2- and M-phase cells of the second LPC division were absent (compare Fig. 4A and B; F and G). In wild-type and dally/TM6B, Tb larvae, G2- and M-phase cells of the second division are located near the surface of the brain, at the posterior limit of the proliferative epithelium (Figs 1B, 4A). cyclin B-expressing cells and mitotic figures are not found in this region of dallyP2 homozygous larval brains (absence of division 2 in Fig. 4B,C). The complete absence of the second LPC division in dally homozygotes, as evidenced by the loss of the second cyclin B-expressing domain, is particularly clear from lateral views of brain lobes (compare dally heterozygote and homozygote in Fig. 4F and G).

Anti-cyclin B antibody and propidium iodide staining also revealed abnormalities in the first LPC division cycle. Normally, G2- and M-phase cells of the first division are found exclusively along the anterior segment of the lamina furrow (Figs 1B, 4A). In dallyP2 homozygous larvae, mitotic figures are frequently found in the posterior part of the furrow (Fig. 4C). The domain of cyclin B immunoreactivity marking the G2 phase of the first division extends up to these abnormally positioned mitotic cells. The extended cyclin B domain, and the misplacement of the M phase cells of the first division, suggested that this division cycle is delayed somewhere along the G2-M transition in dallyP2 mutants. To confirm that the change in position of the G2- and M-phase LPCs reflects changes in their cell cycle progression and not a shift in the position of the furrow, we examined the furrow location relative to a neighboring anatomical landmark. Ganglion mother cell divisions that produce elements of the second optic ganglion, the medulla, are seen with anti-cyclin B antibody staining along the medial face of the aOPC, just anterior to the lamina furrow (insets, Fig. 4A,B). Since dally affects eye development, it was important to choose an anatomical feature that is not affected by interactions with the eye. Available evidence indicates that medullary divisions are not affected by signals from the eye (Fischbach and Technau, 1984; Selleck et al., 1992) and therefore provide an appropriate anatomical landmark to assess the location of the lamina furrow. In dally mutants, these divisions are found at the same distance from the furrow as in the controls, indicating that the furrow position is not altered in dally larvae.

Given the complete absence of the second LPC division and the dependence of this division on an intercellular signal from photoreceptor axons (Selleck and Steller, 1991; Selleck et al., 1992), we examined whether axons do in fact arrive from the

### Table 1. Complementation analysis of dallyP2

<table>
<thead>
<tr>
<th>Cross</th>
<th>Lethality</th>
<th>Genitalia</th>
<th>Wing vein</th>
<th>Eye</th>
<th>Antenna</th>
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<tr>
<td>dallyP2/TM3 Sb × dallyP2/TM3 Sb</td>
<td>78 (n=1016)</td>
<td>57</td>
<td>50</td>
<td>61</td>
<td>9</td>
</tr>
<tr>
<td>dallyP2/TM3 Sb × Df(3L)h-122, Kit/TM3 Ser</td>
<td>35 (n=716)</td>
<td>34</td>
<td>80</td>
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<td>1</td>
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<td>dallyP2/TM3 Sb × AP-14/TM3 Sb</td>
<td>0 (n=583)</td>
<td>0</td>
<td>14</td>
<td>13</td>
<td>5</td>
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<tr>
<td>× AP-48/TM3 Sb</td>
<td>0 (n=279)</td>
<td>0</td>
<td>32</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>× AP-75/TM3 Sb</td>
<td>49 (n=708)</td>
<td>39</td>
<td>38</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>× AP-179/TM3 Sb</td>
<td>36 (n=613)</td>
<td>79</td>
<td>57</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>× AP-188/TM3 Sb</td>
<td>61 (n=561)</td>
<td>56</td>
<td>62</td>
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<td>13</td>
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<td>× AP-199/TM3 Sb</td>
<td>29 (n=638)</td>
<td>69</td>
<td>29</td>
<td>0</td>
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<td>× AP-234/TM3 Sb</td>
<td>12 (n=659)</td>
<td>80</td>
<td>11</td>
<td>0</td>
<td>10</td>
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<td>× AP-238/TM3 Sb</td>
<td>17 (n=600)</td>
<td>51</td>
<td>61</td>
<td>1</td>
<td>4</td>
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<tr>
<td>× AP-301/TM3 Sb</td>
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<td>0</td>
<td>56</td>
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<td>3</td>
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<td>× AP-305/TM3 Sb</td>
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<td>46</td>
<td>0</td>
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<td>× AP-364/TM3 Sb</td>
<td>57 (n=481)</td>
<td>95</td>
<td>72</td>
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<td>7</td>
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<tr>
<td>× AP-527/TM3 Sb</td>
<td>47 (n=580)</td>
<td>99</td>
<td>66</td>
<td>14</td>
<td>11</td>
</tr>
</tbody>
</table>

1. Lethality was calculated as follows: % lethality = expected number of dally/dally flies (one half of dally/TM3 Sb flies obtained) – observed number of dally/dally flies/expected×100. For dallyP2/TM3 Sb × Df(3L)h-122, Kit/TM3 Ser crosses % lethal = expected (number of dally/TM3 Sb Ser flies) – observed (dally/dally)×expected×100. The values for lethality may be underestimated since dally/TM3, which is used for comparison, is not equivalent to dally/+.

2. Percent of dally/dally flies with defects in this tissue.

### Table 2. Phenotypes of homozygous dally excision lines

<table>
<thead>
<tr>
<th>Excision line self cross</th>
<th>Lethality</th>
<th>Genitalia</th>
<th>Wing vein</th>
<th>Eye</th>
<th>Antenna</th>
</tr>
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<tbody>
<tr>
<td>AP-75/TM3 Sb</td>
<td>59 (n=419)</td>
<td>17</td>
<td>13</td>
<td>0</td>
<td>1</td>
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<tr>
<td>AP-179/TM3 Sb</td>
<td>86 (n=506)</td>
<td>88</td>
<td>33</td>
<td>9</td>
<td>3</td>
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<tr>
<td>AP-188/TM3 Sb</td>
<td>49 (n=630)</td>
<td>84</td>
<td>61</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>AP-199/TM3 Sb</td>
<td>70 (n=729)</td>
<td>85</td>
<td>0</td>
<td>0</td>
<td>4</td>
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<tr>
<td>AP-234/TM3 Sb</td>
<td>61 (n=568)</td>
<td>74</td>
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<td>0</td>
<td>24</td>
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<tr>
<td>AP-238/TM3 Sb</td>
<td>91 (n=512)</td>
<td>62</td>
<td>48</td>
<td>0</td>
<td>0</td>
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<tr>
<td>AP-301/TM3 Sb</td>
<td>61 (n=527)</td>
<td>2</td>
<td>27</td>
<td>1</td>
<td>2</td>
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<tr>
<td>AP-305/TM3 Sb</td>
<td>56 (n=697)</td>
<td>4</td>
<td>16</td>
<td>4</td>
<td>0</td>
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<tr>
<td>AP-364/TM3 Sb</td>
<td>99* (n=443)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>AP-527/TM3 Sb</td>
<td>92 (n=558)</td>
<td>100</td>
<td>10</td>
<td>14</td>
<td>19</td>
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1. Lethality was calculated as follows: % lethality = expected number of dally/dally flies (one half of dally/TM3 Sb flies obtained) – observed number of dally/dally flies/expected×100.

2. Percent of dally/dally flies with defects in this tissue.
eye disc in dally mutants using an antibody that recognizes axonal membranes. Double-staining of dally homozygous brains with mAb22C10 and anti-cyclin B antibody shows that axons do reach the lamina, and yet the second LPC division fails to take place (Fig. 4D). The absence of the second LPC division is therefore not a consequence of photoreceptor axons failing to reach the CNS. To further define when during the second cycle LPC division fails in dally mutants, we have examined the distribution of S-phase cells by labelling larvae with the thymidine analogue BUdR. We found that in dallyP2 homozygous larvae LPCs do not enter the S phase of the division cycle triggered by photoreceptor axons (data not shown). Therefore, despite the presence of photoreceptor axons in dally mutants, the second LPC division does not take place as assessed by the absence of the S, G2 and M phases of this division cycle.

dally mutants clearly have defects in LPC division but is it possible that these abnormalities are secondary to a gross morphological disorganization of the tissue? Several results show this is not the case. First, anti-cyclin B antibody staining of whole brain lobes reveals the overall CNS morphology to be intact; for example, the lamina furrow and lamina remain (Fig. 4B,C,F,G). The defect apparent in these brains is a selective loss of the second LPC division cycle. We have also examined the expression of a neuronal marker gene in the developing lamina as another measure of the specificity of cell division defects in dally mutants. Elav expression is found in lamina neurons of dally mutants, with the highest level of expression in the posterior elements of the lamina as has been described for wild-type larvae (Fig. 4E) (Robinow and White, 1991; Selleck and Steller, 1991). Together these findings show that cell division defects in dally mutants are not secondary to a gross morphological abnormality.

dally affects cell division patterning in the eye disc

Cell cycle progression along the morphogenetic furrow in the wild-type eye disc

Given the cell division defect in LPCs, we examined the patterning of cell division in another tissue affected in dally mutants, the eye. In the wild-type eye disc, the morphogenetic furrow (MF) serves as an anatomical marker for the assembly of the repeated sensory units, the ommatidia. As originally described, the MF is a broad indentation in the eye disc epithelium, which moves from the posterior to the anterior, marking the wave of differentiation that sweeps forward (Ready et al., 1976; Tomlinson, 1985, 1988). Associated with the assembly of ommatidia within the MF cell division also becomes synchronized. Cells in different parts of the cell cycle occupy specific positions relative to the MF (Ready et al., 1976; Tomlinson, 1985, 1988; Thomas et al., 1994). We have used anti-cyclin B and propidium iodide staining to identify G2, and mitotic prophase, metaphase, anaphase and telophase cells in the eye disc.

Cell division in the developing eye disc epithelium anterior to the MF is asynchronous as seen by the unpatterned cyclin B expression (Tomlinson, 1985) (Fig. 1A, note cyclin B expression ahead of MF). However, we observed an increase in the level of anti-cyclin B immunoreactivity anterior to the MF, providing evidence of cell cycle synchrony beginning in G2 as cells approach the furrow (Fig. 5E). Immediately posterior to this G2 domain mitosis occurs. Mitotic cells in metaphase, anaphase and telophase are found immediately ahead of the MF (Fig. 5A). This mitotic domain has been noted previously (Tomlinson, 1985; Thomas et al., 1994), and reflects the cell cycle synchronization taking place just ahead of the MF.

Cells complete mitosis as they enter the furrow, and become synchronized in G1 in the MF (Thomas et al., 1994, and Fig. 5A). Progression into the subsequent S phase takes place within the furrow, (Tomlinson, 1985) followed by G2 (seen as another zone of high cyclin B expression, Fig. 5A,C,E). A second coordinate mitosis follows, completing the two division cycles distributed across the MF (Fig. 5C,E). This is precisely the organization seen for LPC divisions along the lamina furrow; two division cycles with successive phases found in sequence along the anterior-posterior length of the furrow.

Disruption of G2-M progression in the eye disc of dally mutants

In LPCs of dallyP2 mutants, the mitosis of the first division is displaced toward the posterior, suggesting a delay in cell cycle progression. Anti-cyclin B antibody and propidium iodide staining of dallyP2 eye discs reveals three features that indicate a similar delay in the first division cycle. First, in dally mutants, the first zone of cyclin B expression, which normally ends approximately 3-5 cell dimensions anterior to the MF (Fig. 5A,C,E), extends too far posterior, to the beginning of the MF (Fig. 5B,D,F). Second, the M phase of this first division is also displaced toward the posterior in dally mutants (compare Fig. 5A,B and E,F). Third, the mitotic cells found at the edge of the MF in dallyP2 mutants are in earlier stages of mitosis (note prophase cells (p), Fig. 5B) compared to wild-type (note metaphase and anaphase cells, Fig. 5A). In contrast to LPC divisions, however, the second division along the eye disc MF does taken place in dallyP2 mutants (Fig. 5B,D,F). The cell division defects observed for dallyP2 are also found in two other dally alleles (dallyAp-14, dallyAp-48), both as homozygotes and in combination with dallyP2 (data not shown).

As is the case for the lamina, the cell division defects in the eye disc are found without an overall disruption of normal morphology. In the wild type, the neuronal marker Elav is expressed in the assembling ommatidia posterior to the MF (Robinow and White, 1991). dally mutant discs show this pattern of Elav expression as well, indicating that the cell division defects are not secondary to a gross disruption of eye development (Fig. 5G).

We have also conducted an experiment designed to measure the timing of cell cycle progression in dally mutants directly, by pulse-labeling S-phase cells with BUdR and chasing the label into the subsequent phases of the cell cycle. If dally mutations delay cell division, the time required for cells labelled in the S phase of the first division to traverse into the following G1 should be slower in dally mutants compared to heterozygous control larvae. In the wild-type eye disc, mitotic cells of the first division are found at the apical surface of the epithelium in front of the MF and, following M phase, nuclei migrate basally into the MF (Tomlinson, 1988). The position of a nucleus labelled in the previous S phase can therefore be used as a measure of cell cycle progression; more basally located cells being further along. In dally mutants 4 hours after pulse-labeling, nuclei were delayed in their descent into the furrow compared to labelled cells from the heterozygous
control (data not shown). These findings support the conclusion that the first division cycle is delayed as a consequence of compromised dally function. We do observe some mortality in dally larvae labelled with BUdR and this experiment must therefore be interpreted with caution, while recognizing that the findings support conclusions drawn from morphological studies.

Molecular analysis of the dally gene

To characterize the dally gene molecularly, we obtained genomic DNA flanking the two dally P-element insertions by plasmid rescue. Plasmid rescue clones from dallyP1 and dallyP2 showed a large region of overlap. A probe derived from this common region (Probe A, Fig. 6A) hybridized to the site of the P-element inserts at position 66D/E and was used to screen a Drosophila genomic DNA library. Genomic clones covering approximately 20 kb of DNA surrounding the two P-element insertions in the dally locus were obtained and a restriction map assembled. Southern blot analysis and restriction site mapping showed the two P-element insertions to be separated by less than 1 kb (Fig. 6A).

To identify genomic sequences critical for dally function, we characterized the molecular organization of several dally excision alleles. The sequences deleted in dallyΔP-75, dallyΔP-188, dallyΔP-305 and dallyΔP-527 were determined by a combination of Southern blot and PCR analyses. Fig. 6B shows a set of Southern blots using genomic probes surrounding the P-element insertions. Molecular lesions were limited to a PstI restriction fragment that encompasses the P-element insertion sites. PCR analyses using a series of primers from the probe B region defined the breakpoints of four excision alleles (Fig. 6A). dallyΔP-188 and dallyΔP-527 show more severe phenotypes and larger deletions compared to dallyΔP-75 and dallyΔP-305 (Tables 1, 2; Fig. 6A).

**Fig. 3.** Adult phenotypes of dally mutants.
(A-C) Heads from wild-type (A), dallyP2 (B) and dallyΔP-188 (C) homozygous flies, respectively. Note the reduction in the size of the eye in dally mutants. SEM photomicrographs show the complete loss of ommatidia found in some dallyP2 individuals (wild-type, M; dallyP2, N). (D-F) Antennal defects (arrows) of dallyP1, dallyP2 and dallyΔP-188 homozygous adults, respectively. These photomicrographs show antennal reductions but duplicated structures are also observed (N). Disorganized rows of ommatidia can also be seen in E and F. (G-I) Comparison of the male genitalia in wild-type (G), dallyP2 (H) and dallyΔP-188 (I) homozygous adults. Genitalia structures are completely missing (H), or abnormal (I, arrow). (J-L) The wing phenotypes observed in dallyP2 (K) and dallyΔP-188 (L) mutants, with a wild-type wing for comparison (J). dally mutants show wing notching with loss of wing margin structures (K) and incomplete wing vein V formation (K,L).
Fig. 4. Cell division defects in LPCs of *dally* 

**dally**, a putative integral membrane proteoglycan, affects cell division

(A-C) Horizontal optic sections stained with anti-cyclin B (red) antibody and propidium iodide (grey tones). The lamina furrow is marked with a long arrow in A–E. (A) LPCs and lamina (LA) of *dally* 

**dally* 

P2/TM6B larva, showing normal pattern of LPC division. Note the presence of two domains of peak cyclin B immunoreactivity, marking the late G2, early M-phase cells of the two LPC divisions (numbered 1 and 2; the thick arrows mark mitotic figures of first and second division). Insert in the top left corner shows the anti-cyclin B signal from this preparation, revealing ganglion mother cells that contribute to the medulla, along the medial face of the OPC (arrowhead). Note the position of these cells relative to the lamina furrow (long arrow). (B, C) Preparations from *dally* 

P2 homozygous larvae. (B) Note the presence of the first cyclin B domain, while the second is entirely lacking, reflecting the loss of the second LPC division. No mitotic figures are found for the second LPC division. The insert shows the cyclin B immunoreactivity alone, with the location of the medullary ganglion mother cells (arrowhead) in the same position relative to the furrow (marked with arrow) as in the heterozygous brain (A). There is a mitotic figure seen at the anterior edge of the furrow. Mitotic figures can be found in this region of the aOPC in wild-type. (C) *dally* 

P2 larval brain showing an abnormally placed mitotic figure of the first LPC division, located in the posterior portion of the furrow (arrow labelled 1). Although the OPC is abnormal in this particular preparation (cells are “piled up” entering the furrow), the position of the furrow remains unchanged relative to the ganglion mother cell divisions seen at the medial face. (D) Horizontal section of *dally* 

P2 homozygous larva stained with mAb 22C10, to detect photoreceptor axons (green) and anti-cyclin B antibody (grey tones). Photoreceptor axons enter the lamina yet the second LPC division does not take place in *dally* mutants. (E) Horizontal optical section showing Elav expression (green) in the developing lamina (propidium iodide staining shown in grey tones) of a *dally* 

P2 homozygote. Elav expression is detected at highest levels in the posterior, more differentiated region of the lamina, as has been described for wild-type. No gross disruption of lamina development is found in *dally* bearing larval brains. An abnormally placed mitotic figure is indicated (short arrow) located along the posterior segment of the lamina furrow (long arrow). F and G show lateral views of brain lobes from *dally* 

P2/TM6B (F) and *dally* 

P2 (G) homozygous larvae, stained with anti-cyclin B antibody. The two LPC divisions are represented as two bands of cyclin B immunoreactivity (labelled 1 and 2 in F). Note the lack of the second cyclin B domain along the entire length of the lamina (LA) in the homozygote (G).
Fig. 5. Cell division defects in dallyP2 eye discs. (A-F) Late third instar larval eye discs stained with anti-cyclin B antibody (red) and propidium iodide (grey). All scale bars mark 10 µm. A-D and G provide a view looking down on the eye disc, “into” the MF, with anterior to the left. Vertical two-headed arrows mark the anterior edge of the MF. (E,F) Cross-sectional views of eye discs, with anterior to the left. (A) dallyP2/TM6B eye disc, with the optical plane very near the surface. A region of high cyclin B immunoreactivity is located 3-5 cell dimensions anterior to the beginning of the MF (vertical arrow marks MF edge, seen by low level of background anti-cyclin B staining). Immediately adjacent to that cyclin B domain mitotic cells are seen, in metaphase (m), anaphase (a) and telophase (t). (B) Eye disc from a dallyP2 homozygous larva. Cells with high levels of cyclin B immunoreactivity are found all the way to the edge of the MF (vertical arrow marks MF edge, seen by low level of background anti-cyclin B staining). Immediately adjacent to that cyclin B domain mitotic cells are seen, in metaphase (m), anaphase (a) and telophase (t). (C) dallyP2/TM6B control eye disc, with focal plane approximately 4 µm into the MF. Cyclin B-expressing cells are found anterior to the edge of the MF. Numbers 1 and 2 mark the first and second divisions seen with anti-cyclin B antibody staining (also in D). (D) A dallyP2 homozygote disc at the same level of focus as for C. Cyclin B expression at this depth is also too far posterior (to the right) and extends to the furrow edge. (E) Anti-cyclin B and propidium iodide-stained dallyP2/TM6B eye disc, cross-sectional view. Note the placement of the first cyclin B domain (labelled 1) and associated mitoses (arrow) relative to the base of the MF (square bracket). (F) The comparable view of a dallyP2 homozygous eye disc. The cyclin B domain and mitotic figures of the first division are located too far posterior, adjacent to the base of the MF (bracket). The second division cycle is observed in dally eye discs (cyclin B-expressing domain and mitosis marked with 2). (G) Elav expression in a dallyP2 homozygous eye disc, showing the assembly of ommatidial clusters posterior to the MF.
**dally**, a putative integral membrane proteoglycan, affects cell division

All six **dally** alleles that we have examined molecularly show lesions confined to a region of approximately 2 kb. Cloned genomic DNA fragments surrounding the sites of molecular lesions in the **dally** locus were used to search for the **dally** transcript. A 10 kb SauI DNA probe that encompasses the sites of **dally** mutations (Fig. 6A) detects a single transcript of approximately 4.4 kb from larval brain RNA (data not shown). This same genomic fragment was used to identify a cDNA probe that detects a single transcript of approximately 4.4 kb from larval brain RNA (data not shown).
clone, c1, from an embryonic cDNA library that hybridizes to a transcript of identical size (Fig 6C). Several lines of evidence indicate that c1 encodes the dally message. First, all five dally mutants that we have examined show abnormalities in the level or size of the c1 transcript (4 dally mutants shown in Fig. 6C). Second, dally mutants with mild, moderate and severe phenotype types show corresponding degrees of effects on the size and abundance of the c1 mRNA (Fig. 6C). Third, no other transcripts from this region were detected in RNA from larval brains by northern analysis (data not shown). Fourth, the expression pattern of the c1 mRNA in embryos and third instar larval brains detected by whole-mount in situ hybridization.
the dally, a putative integral membrane proteoglycan, affects cell division 3699

**B**

<table>
<thead>
<tr>
<th>DALLY</th>
<th>CO−5</th>
</tr>
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<td>AARSVRLA..LLLFTGCCFVGLSAAKHDLDDGHQHHLHSATTHRRRQDSRARKAVGSSTQCAVSVET.ESIDKSSGT</td>
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The dally gene encodes a putative integral membrane proteoglycan

Sequencing of the dally cDNA clone revealed a potential open reading frame of 626 amino acid residues with a predicted protein sequence homologous to a family of mammalian integral membrane proteoglycans of the glypicann type (Grancar-Films et al., 1974; Stipp et al., 1994). The entire predicted Dally protein sequence shows 23.9-25.9% identity and 45.8-48.3% similarity to members of the GRIP family; rat and human Glypican, OCI-5, and Cerebroglycan (Fig. 7B) (David et al., 1990; Films et al., 1988; Stipp et al., 1994). Dally shows a similar degree of homology to the different GRIPs as they do to each other. The putative Dally protein also shows several features characteristic of this protein family including (1) a set of 14 conserved cysteine residues found at specific positions in all vertebrate GRIPs; (2) a potential signal peptide at the amino terminus, (3) glycosaminoglycan attachment site consensus sequences and (4) a stretch of hydrophobic amino acid residues at the carboxy terminus required for GPI-anchoring to the cell membrane (Fig. 7B).

**DISCUSSION**

**dally affects cell division in the developing lamina and eye**

dally is a new gene required for the normal patterning of cell division in at least two larval tissues, the eye disc and lamina. dally mutants show specific cell division defects in these tissues in the absence of any gross morphological disorganization. Furthermore, we observe the same type of defect in the dally locus (data not shown). Finally, the two most severe excision-generated alleles remove segments of the untranslated leader of the c1 message (summarized in Fig. 6A). On the basis of these findings, we conclude that the c1 cDNA clone encodes the dally message.

**The dally gene encodes a putative integral membrane proteoglycan**

Matching that observed for the two enhancer trap insertions in the dally locus (data not shown). Finally, the two most severe excision-generated alleles remove segments of the untranslated leader of the c1 message (summarized in Fig. 6A). On the basis of these findings, we conclude that the c1 cDNA clone encodes the dally message.

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division cycles in the eye and lamina. In both tissues, precursor cells go through two division cycles along a morphogenetic furrow and, in both the eye and lamina, the first of these divisions is abnormal. Morphological analysis shows that entry into mitosis is delayed in *dally* mutants. We do not know, however, if this is the only defect in the division cycle affected by *dally*. While the genetic analysis has shown that the *dally* alleles that we have characterized are hypomorphs and the molecular analysis shows the *dally* mRNA to be severely affected, we do not yet know if any of these mutants is a complete loss-of-function allele. Analysis of amorphic alleles will be required to determine the entire range of functions served by the *dally* gene.

In LPCs of *dally* mutants, two division defects are found, a delay in the first division, and failure of the second division to enter S phase. Earlier studies of eyeless mutants documented that signals from the eye are not required for the first LPC division to proceed through G1 (Selleck et al., 1991). We can therefore conclude that the cell division defect in the first LPC division is not the result of defects in eye-lamina signaling and strongly suggests that *dally* is required in LPCs, where it is in fact expressed.

The second division of LPCs is triggered by photoreceptor axons arriving in the brain (Selleck and Steller, 1991). Photoreceptor axons do reach the CNS in *dally* mutants, however, indicating that a lack of photoreceptor axons does not account for the failure of the second LPC division. Since *dally* mutants have eye defects, it is possible, however, that photoreceptors are defective in providing the mitogenic trigger required for the second LPC division. Several other mechanisms could account for the lack of the second LPC division. *dally* could be required for events triggered by the signal from photoreceptor axons. The expression of *dally* in cells preceding this division leaves open the possibility that Dally protein plays a direct role in the response of LPCs to arriving photoreceptor axons. Alternatively, the disruption of G2-M progression in the first LPC division could prevent the proper signaling between photoreceptors and LPCs. If LPCs are not in G1 at the time that they normally receive instructions from photoreceptor axons, they may not respond to the mitogenic signal. In this scenario, the absence of the second LPC division is entirely the consequence of the earlier cell division defect.

LPCs fail to enter the second division cycle in *dally* mutants, as is observed in several eyeless mutants (Selleck and Steller, 1991). Despite this defect, lamina neurons do differentiate in *dally* larvae as evidenced by the expression of the neuron-specific protein, Elav. This contrasts to the phenotype observed in eyeless mutants, where the second division fails to enter the final S phase and Elav-expressing lamina neurons never appear. These observations argue that the complete absence of lamina neurons in eyeless mutants is not a direct consequence of the failed second division, but rather the loss of differentiation signals normally delivered by photoreceptor axons. Our findings support earlier studies of *Daphnia* (Macagno, 1979), which documented the role of photoreceptor axons in promoting the differentiation of lamina neurons.

**dally is required for patterning of several adult structures**

In addition to the cell division defects in LPCs and eye disc, *dally* mutants display morphological defects in several other adult tissues, including the antenna, wing and genitalia. We do not know if cell division defects are responsible for the abnormal development of antenna, wing and genitalia in *dally* mutants. The wing notching defects found in *dally* adults are reminiscent of phenotypes affected by *wingless, Notch* and *Serrate* and suggests the possibility that *dally* plays a role in these signaling pathways (Baker, 1988; Couso and Martinez Arias, 1994; Thomas et al., 1991; Fleming et al., 1990). A recent report provides evidence that *wingless* and *Notch* functionally interact in patterning decisions throughout development and experiments are underway to assess if *dally* participates in these events (Couso and Martinez Arias, 1994).

**dally encodes a putative integral membrane proteoglycan**

Cloning and sequencing of the *dally* cDNA reveals homology to a family of integral membrane proteoglycans of the Glycan-type. The putative Dally protein contains several structural features characteristic of this class of molecules (David, 1993); a set of 14 precisely positioned cysteine residues, consensus glycosaminoglycan attachment sites, a potential N-terminal signal sequence and a carboxy-terminal hydrophobic region that is required for processing and attachment of GRIPs to the external leaflet of the plasma membrane via a glycosylphosphatidylinositol linkage. The degree of sequence homology of Dally to each member of this vertebrate family is similar to their homology to one another, supporting the assignment of Dally to this gene family.

The cell division defects in *dally* mutants argues that integral membrane proteoglycans can affect cell division patterning during development. The characterization of heparin-like cell surface molecules as potential co-receptors for secreted growth factors provides a model for how Dally may work. FGF binds to heparan sulfate proteoglycans (HSPGs) at the cell surface and heparin has been shown to affect the biological responses induced by FGF (reviewed by David, 1993; Rapraeger et al., 1991; Ornim et al., 1992). Recently it has been demonstrated that heparin can mediate FGF receptor dimerization, an event obligatory for transmembrane signaling and FGF-mediated cell effects, including proliferation (Spivak-Kroizman et al., 1994). These findings suggest a model where Dally serves as a coreceptor for extracellular factors affecting regulation of cell cycle progression.

Many patterning events during development are controlled by extracellular protein factors, including members of the TGF-β, Wnt-1/Wingless and Hedgehog gene families. Members of all these secreted protein families have been shown to bind heparin and associate with the extracellular matrix (reviewed in David, 1993; and see for examples Bradley and Brown, 1990; van Leeuwen et al., 1994; Lee et al., 1994). Binding to HSPGs may be required for the signaling mediated by these molecules. Given the diverse tissues affected in *dally* mutants including the wing, genitalia, eye and antenna, it is possible that Dally, a putative HSPG, may serve as a coreceptor for some of these secreted effectors of patterning decisions. We are in the process of investigating whether Dally interacts with known secreted growth and pattern regulators.

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References


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