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The Functions of Klarsicht and Nuclear Lamin in Developmentally Regulated Nuclear Migrations of Photoreceptor Cells in the *Drosophila* Eye

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Photoreceptor nuclei in the *Drosophila* eye undergo developmentally regulated migrations. Nuclear migration is known to require the perinuclear protein Klarsicht, but the function of Klarsicht has been obscure. Here, we show that Klarsicht is required for connecting the microtubule organizing center (MTOC) to the nucleus. In addition, in a genetic screen for *klarsicht*-interacting genes, we identified *Lam* Dm_0, which encodes nuclear lamin. We find that, like Klarsicht, lamin is required for photoreceptor nuclear migration and for nuclear attachment to the MTOC. Moreover, perinuclear localization of Klarsicht requires lamin. We propose that nuclear migration requires linkage of the MTOC to the nucleus through an interaction between microtubules, Klarsicht, and lamin. The Klarsicht/lamin interaction provides a framework for understanding the mechanistic basis of human laminopathies.

INTRODUCTION

The position of the cell nucleus is critical to many developmental processes. For example, in *Drosophila*, both the A/P and D/V axes of symmetry are defined by the position of the oocyte nucleus. First, signaling by the nucleus at the oocyte posterior defines the A/P axis. Subsequently, the oocyte nucleus migrates anteriorly to a random side of the oocyte and signals there to define the D/V axis of symmetry (van Eeden and St. Johnston, 1999). Later in *Drosophila* development, migration of syncitial nuclei to the cell cortex is a defining event in embryogenesis, which results in syncitial blastoderm formation, a phenomenon preceding cellularization (Foe *et al.,* 1993). In *Caenorhabditis elegans*, nuclear migrations in P-cells, which give rise to neurons and the vulva, are important for cell viability (Malone *et al.,* 1999).

Nuclear migration in the developing *Drosophila* eye is critical for shaping each individual cell and thus for normal morphology of the entire compound eye (Fischer-Vize and Mosley, 1994). The *Drosophila* compound eye develops within the larval eye imaginal disc, an epithelial monolayer (Figure 1; Wolff and Ready, 1993). Within the eye disc, the morphogenetic furrow marks the initiation of eye assembly. Rows of identical facets, or ommatidia, assemble posterior to the furrow, starting with the eight photoreceptors (R-cells), followed by the lens-secreting cone cells, and finally the pigment cells. Anterior to the furrow, cells are undifferentiated and their nuclei are positioned randomly within the monolayer. The nuclei dive basally within the furrow and posterior to the furrow, migrate apically as they are recruited into ommatidia (Tomlinson and Ready, 1987).

Two *Drosophila* genes, *klarsicht* (previously known as *marbles*) and *Glued*, are essential for the apical migration of nuclei in differentiating R-cells (Fischer-Vize and Mosley, 1994; Fan and Ready, 1997). *Glued* encodes the large subunit of dynactin, a protein complex that regulates the minus-end–directed microtubule motor dynein (Holzbauer *et al.,* 1991). The requirement for dynactin suggests that R-cell nuclear migration is a dynein- and microtubule-dependent process. Consistent with this idea, two other *Drosophila* genes, *Bicaudal-D* and D-Lis1, both of which may regulate dynein (reviewed in Reiner, 2000; Wynshaw-Boris and Gambello, 2001; Vallee *et al.,* 2001), are implicated in R-cell nuclear migration (Swan *et al.,* 1999), although their mutant phenotypes are weak compared with *klarsicht* and *Glued* (C.R. and J.A.F., unpublished observations). *DLis-1*, a WD40 repeat protein, is the homolog of the human disease gene *Lissencephaly-1* (Reiner *et al.,* 1993). Lissencephaly, or smooth brain, is a disorder resulting from defects in neuronal migrations essential for normal human brain development (reviewed in Morris *et al.,* 1998; Morris, 2000; Wynshaw-Borris and Gambello, 2001). Neuronal migration requires nuclear migration, and the involvement of *DLis-1* in *Drosophila* R-cell nuclear migration suggests that the two processes may be in part analogous.

klarsicht (*klar*) encodes a large protein, unique except for its small N-terminal KASH (Klarsicht, Anc-1, Syne-1 homology) domain, which localizes proteins to the nuclear membrane (Mosley-Bishop *et al.,* 1999; Apel *et al.,* 2000; Zhang *et al.,* 2001; Starr and Han, 2002; Zhen *et al.,* 2002; J.A.F., C.C., S.A., C.R. *et al.,* unpublished results). The KASH-domain–containing protein Anc-1 and its vertebrate homolog, Syne-1 (also known as Myne-1, Nesprin, and NUANCE) are dystrophin-related proteins that anchor the nucleus to the actin cytoskeleton (Apel *et al.,* 2000; Mislow *et al.,* 2001, 2002;

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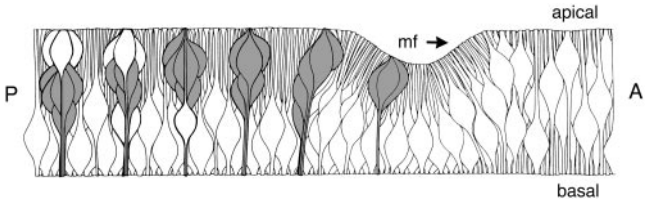


Figure 1. Nuclear migration in the *Drosophila* eye disc. A longitudinal section of a wild-type larval eye disc is diagrammed with anterior right (A) and posterior left (P). Most of the cell volume surrounds the nucleus. Nuclei of undifferentiated cells are randomly positioned in the monolayer anterior to the morphogenetic furrow (mf), which is moving anteriorly, in the direction of the arrow. In the furrow, the nuclei are basal, and posterior to the furrow, nuclei rise as cells are recruited into ommatidia. Ommatidial clusters are progressively more mature from anterior to posterior. R-cells are gray, and cone cells are white. (Adapted from Tomlinson and Ready, 1986.)

Zhang *et al.*, 2001; Starr and Han, 2002; Zhen *et al.*, 2002; Starr and Han, 2003). In addition to its role in nuclear migration in the eye, *klar* is required for the developmentally regulated migrations of lipid droplets during embryogenesis. In this role, it has been proposed that Klar regulates dynein and also the plus-end–directed motor kinesin (Welte *et al.*, 1998; Gross *et al.*, 2000).

Here, we use genetics and immunohistochemistry to investigate the role of Klar in R-cell nuclear migration. The results suggest that a connection between the MTOC and the nucleus is necessary for nuclear migration and that this connection is mediated by Klar and nuclear lamin. In addition to suggesting a specific role for Klar in nuclear migration, the results propose a general mechanistic explanation for the cytoplasmic effects of nuclear lamin, including human laminopathies.

MATERIALS AND METHODS

Drosophila Strains and Genetics

The following mutant lines were used in this work:

klar^{mBP} and klar^{mBX3} are strong *klar* mutant alleles described in Fischer-Vize and Mosley (1994) and Mosley-Bishop *et al.* (1999).

Lam^{4643} (Spradling *et al.*, 1999) was obtained from the Bloomington Stock Center.

Lam^{P} (Lenz-Bohme *et al.*, 1997) was obtained from B. Schmitt.

Df(3L)emc^{E12} (61A–61D3), described in Lindsley and Zimm (1992) and Mosley-Bishop *et al.* (1999), was obtained from the Bloomington Drosophila Stock Center.

Df(2L)cl-h4 (25D6–25F3/4), described in Kotarski *et al.* (1983) and Lindsley and Zimm (1992), was obtained from the Umea Drosophila Stock Center.

Tw2-Lam^{P}, the *Lam^{+}* genomic rescue line described in Lenz-Bohme *et al.* (1997), was obtained from B. Schmitt.

P{w^{+}, UAS-nod-lacZ}B3.3, a third chromosome insertion described in Giniger *et al.* (1993) and Clark *et al.* (1997), was obtained from I. Clark.

P{w^{+}, elav-Gal4}H19 was hopped to chromosome 3. The original line was obtained from B. Zhang.

P{w^{+}, glrs-klar} on X is described in Mosley-Bishop *et al.* (1999).

P{ry^{+}, neoFRT}40A (Xu and Rubin, 1993) was obtained from the Bloomington Stock Center (Abbreviated *FRT40A*).

*P{w^{+}, GMR-hid}G1, l(2)*CL-L^{1}, *P{ry^{+}, neoFRT}40A/CyO; P{w^{+}, ey-Gal4.H}SS5, P{w^{+} UAS-FLP1.D}JD2* (Stowers and Schwarz, 1999) was obtained from the Bloomington Drosophila Stock Center. (Abbreviated *GMR-hid, CL2, FRT40A/CyO; EGUF.*)

P{w+, UAS$_{p}$-6Xmyc-klar}NP5, a third chromosome insertion, was newly generated here. An *Asc*I fragment containing 6Xmyc-klar (from pGLRS-6Xmyc-klar1; Mosley-Bishop *et al.*, 1999) was ligated into a derivative of pUASp (Rorth, 1998) in which the *Not*I site was changed to *Asc*I.

P element transformation of *w^{1118}* flies was as described (Fischer-Vize *et al.*, 1992a). A *CyO,GFP* balancer (Flybase, 2003) was used to identify *Lam* homozygous mutant larvae, and a *TM6B, Tb* (Lindsley and Zimm, 1992) balancer was used to identify *klar* homozygous mutant larvae. Common marker strains used are described in Lindsley and Zimm (1992). All experimental flies were grown at 25°C.

Lam^{4643} homozygous eyes were generated in *Lam^{4643}*/+ heterozygotes using the GMR-hid technique (Stowers and Schwarz, 1999). First, two stocks were generated: *Lam^{4643} FRT40A/CyO,GFP* and *GMR-hid, CL, FRT40A/CyO, GFP*; *EGUF*. Flies from the two stocks were intercrossed, and among the progeny, *Lam^{4643} FRT40A/GMR-hid CL FRT40A; EGUF/+* larvae were detected by the absence of GFP expression. The eye discs that of these larvae are homozygous for *Lam^{4643}*.

Modifier Screen

Males (*pr; st*) were mutagenized with EMS (Lewis and Bacher, 1968) or X-rays (4000 rads) and mated with *w; P{w+; glrs-klar}* females. The F1 males (14,500 with EMS-mutagenized autosomes and 35,000 with X-ray–mutagenized autosomes) were screened for enhanced roughness of the external eyes. The EMS screen yielded two *egk1* alleles (Ari3 and Ari7), and the x-ray screen yielded seven *egk1* alleles: A25, K2, 83, and four stronger alleles that were lethal *in trans* to *Df(2L)cl-h4.* (The lethal *egk1* alleles have been lost.)

Genetic Mapping of egk1 Alleles

The *egk1* alleles Ari3 and Ari7 were mapped meiotically between *dp* and *b* using the multiply marked chromosome *al dp b pr Bl c px sp* and by scoring the mutant eye phenotype of the *egk1* homozygotes. Females that were *al dp b pr Bl c px sp/egk1* were crossed with *al dp b pr c px sp* males, and ~20 male progeny of each single recombinant class were collected. To determine which of the recombinant chromosomes contained *egk1,* each male was individually crossed to *egk1/CyO* females, and progeny were examined for the *egk1* homozygous eye phenotype. Ari3 and Ari7 were subsequently tested for complementation with a variety of deficiency chromosomes spanning the region between *dp* and *b*, and *Df(2L)cl-h4* (25D6–25F3/4) failed to complement *egk1*.

Molecular Analysis of Lam Alleles

Lam alleles were amplified by PCR using total genomic DNA prepared from a single fly homozygous or hemizygous (*in trans* to *Df(2L)cl-h4*) for each of the five viable or semiviable *egk1* alleles. Genomic DNA was prepared as described in Chen and Fischer (2000). Six primer pairs (sequences available upon request) and standard PCR conditions were used. The DNA sequence of the PCR products were sequenced directly using automated fluorometric sequencing. Sequences were analyzed with MacVector (Accelrys, San Diego, CA) software.

Phenotypic Analysis of Eyes

Scanning electron micrographs (Huang *et al.*, 1995) and plastic sections of adult eyes (Fischer-Vize and Mosley, 1994) were prepared as described previously. Light microscope analysis of eye discs immunostained with anti-Elav was performed exactly as described in Fischer-Vize and Mosley (1994). Light microscope images were produced with a Zeiss Axioplan microscope and a Zeiss AxioCam (Thornwood, NY), and processed with Adobe Photoshop software (Adobe, San Jose, CA). For confocal microscopy, immunostaining of eye discs was performed with PEMS fixation and PBST washes as described (Fischer-Vize *et al.*, 1992a, 1992b). Eye discs were mounted in VectaShield (Vector Laboratories, Burlingame, CA). The primary antibodies used were as follows: mouse anti-Lam at 1:100 (mAbADL84; Stuurman *et al.*, 1995), obtained from P. Fisher; mouse anti-Futsch at 1:5000 (mAb22C10; Fujita *et al.*, 1982), obtained from the Developmental Studies Hybridoma Bank (DSHB, Iowa); rabbit anti-Myc at 1:500 (Santa Cruz Biochemicals, Santa Cruz, CA; sc-789 [c-myc/A-14]); rat anti-Elav at 90:100 (7E8A10, DSHB); mouse anti-βgal at 1:10 (40-1a, DSHB); rabbit anti–γ-tubulin (Rb 1015) at 1:40 (Tavosanis *et al.*, 1997), obtained from C. Gonzalez. Secondary antibodies were from Molecular Probes (Eugene, OR; Alexa) and Jackson ImmunoResearch (West Grove, PA; Cy) and were used at 1:600. When double-labeling with rat and mouse primary antibodies, preadsorbed Jackson secondary antibodies were used. Green:Alexa^{488}-goat anti-rabbit, Alexa^{488}-goat anti-rat. Blue: Cy5-goat anti-rat, Alexa^{633}-goat anti-rat. Red: Cy3-goat anti-mouse, Alexa^{568}-goat anti-rabbit, or Alexa^{568}-phalloidin.

Images were produced with a Leica TCS SP2 confocal microscope (Deerfield, IL) and processed with Adobe Photoshop software.

RESULTS

Klar Is Perinuclear and also Is Associated with Apical Microtubules

It was shown previously using light microscopy that Klar is associated with the nuclear membrane (Mosley-Bishop *et al.*, 1999). Here, we investigate the subcellular localization of Klar in greater detail and at higher resolution using confocal microscopy. To visualize Klar protein, an epitope-tagged form of Klar, 6Xmyc-Klar, was expressed in R-cells by using

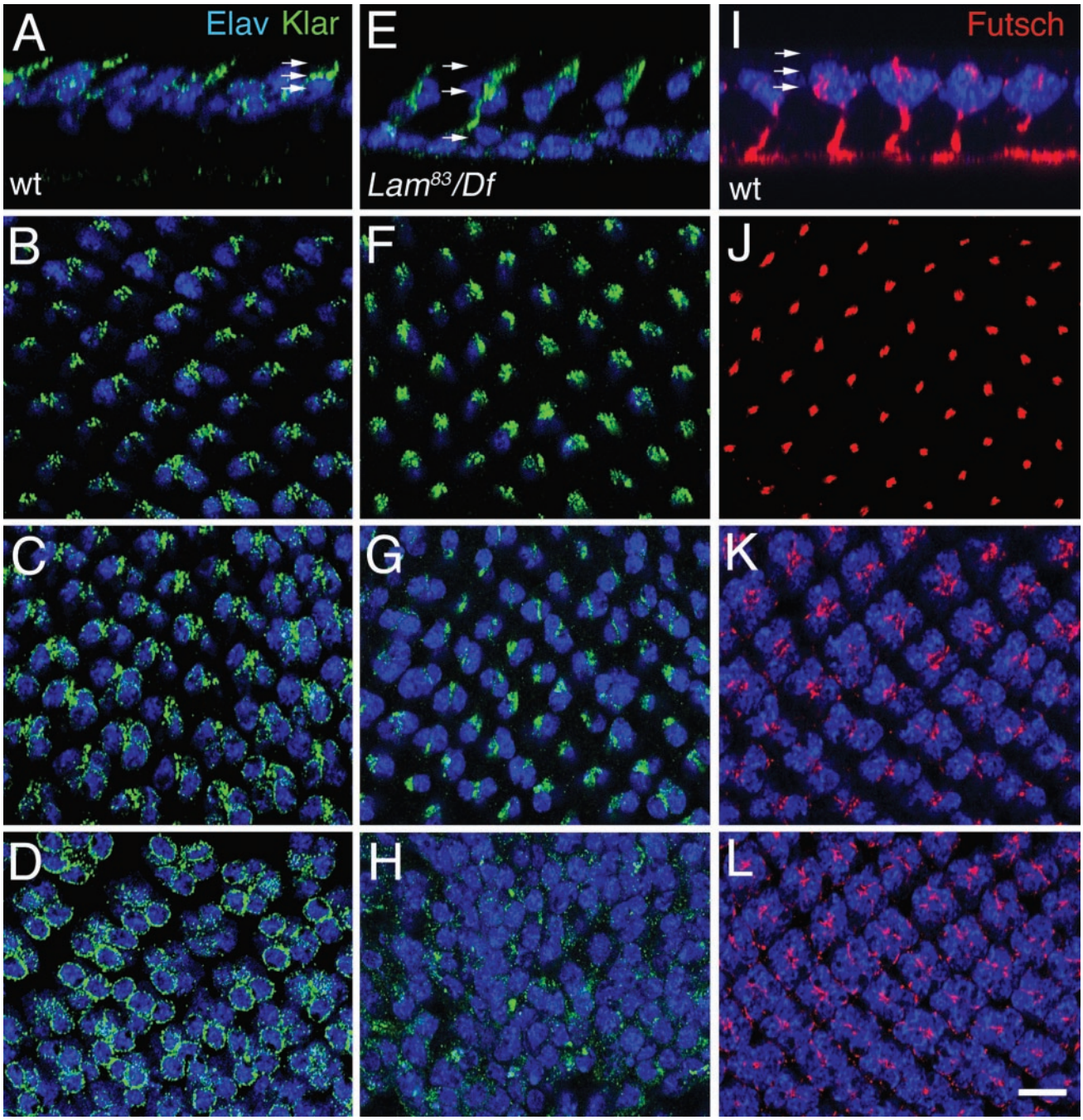


Figure 2. Klar localization in eye discs. Shown are confocal images of eye discs double-labeled to reveal R-cell nuclei (anti-Elav; blue) and either 6Xmyc-Klar (anti-myc; green) or Futsch (mAb22C10; red), a microtubule-associated protein. (A, E, and I) Z-sections; (B–D, F–H, and J–L) progressively more basal XY-sections, whose positions correspond to the arrows in A, E, and I, respectively. (A–D) Wild-type eye discs expressing 6Xmyc-Klar in R-cells (*elav>6Xmyc-klar*). Klar is localized to microtubules apical to the nuclei (A–C) and is also perinuclear (D). (E–H) *Lam*^{83}*/Df(3L)cl-h4* eye discs expressing 6Xmyc-Klar. Klar is localized to apical microtubules (E and F), but is not present in perinuclear rings in the apical R-cell nuclei (G) or the basal ones (H). (I–L) Wild-type eye discs revealing Futsch protein localization on microtubules is shown. Futsch is apical to the R-cell nuclei (I–K) and extends around the nuclei (I and L), to the basal surface of the disc (I). Size bar in L is ~10 μm and applies to all panels.

a *UAS-6Xmyc-klar* transgene and an *elav-Gal4* driver (*elav>6Xmyc-klar*). (The 6Xmyc-Klar protein is functional; Mosley-Bishop *et al.*, 1999.) Otherwise wild-type eye discs expressing *elav>6Xmyc-klar* were labeled with anti-Myc and also with anti-Elav (Robinow and White, 1991), which marks R-cell nuclei after they have risen apically. As observed before, Klar is associated with the nuclear membrane (Figure 2D). In addition, dots of Klar are seen to extend from the nuclei toward the apical cell surface (Figure 2, A–C). The apical dots resemble the apical expression pattern of Futsch, also known as 22C10 (Figure 2, I–L), a neural-specific microtubule-associated protein (Hummel *et al.*, 2000).

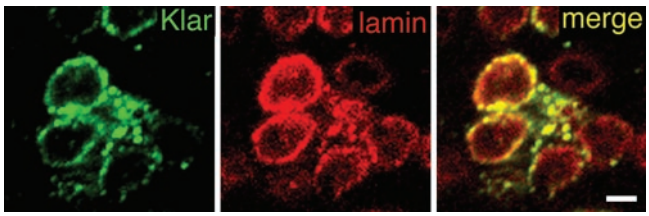


Figure 3. Klar colocalizes with nuclear lamin. Shown are confocal images of a single developing ommatidium from otherwise wild-type eye discs that express 6Xmyc-Klar in R-cells (*elav >6Xmyc-klar*). The eye discs were double-labeled with anti-Myc and anti-Lam. Size bar, ~2 μm.

There is a variety of experimental evidence that the KASH domains of Anc-1 and Syne-1 localizes those proteins to the nuclear envelope (Mislow *et al.*, 2001, 2002; Starr and Han, 2002; Zhang *et al.*, 2001; Zhen *et al.*, 2002). Similarly, we find that Klar is genuinely associated with the nuclear membrane, rather than appearing perinuclear only because it is associated with microtubules that extend around the nucleus. First, immunostaining with anti-Futsch reveals the microtubule cytoskeleton as it extends from the apical to basal cell surfaces, weaving around the nucleus (Figure 2I). Although Futsch is bound to microtubules, it does not appear perinuclear as does Klar (Figure 2L). Second, 6Xmyc-Klar colocalizes with the nuclear envelope protein lamin (Figure 3). Finally, the two aspects of Klar localization are separable: when an isolated Klar KASH domain is expressed, only nuclear membrane localization, not apical microtubule localization, is observed (J.A.F., C.C., S.A., C.R. *et al.*, unpublished data). We conclude that 6Xmyc-Klar localizes to the apical microtubules and to the nuclear envelope in R-cells.

The MTOC Detaches from R-cell Nuclei in klar Mutants

To probe the function of Klar in nuclear migration, we asked whether the cytoskeleton is organized differently in *klar* mutants than in wild-type eye discs. The MTOC was marked by expressing a Nod-βgal fusion protein, which accumulates at microtubule minus ends (Giniger *et al.*, 1993; Clark *et al.*, 1997). The MTOC is the point in the cell from which the microtubules grow: the slow-growing minus ends gather at the MTOC and the rapidly growing plus ends emanate from it. Nod-βgal was expressed using an *elav-Gal4* driver and a *UAS-nod-lacZ* transgene. Otherwise wild-type and also *klar* mutant eye discs expressing *elav>nod-lacZ* were double-labeled with anti-Elav and anti-βgal. In wild-type, Nod-βgal is closely associated with the R-cell nuclei, and just apical to them (Figure 4A).

In *klar* mutants, most of the R-cell nuclei are basal, but all of the Nod-βgal is apical (Figure 4C). There are two possible interpretations of this result: 1) The *klar* mutant R-cells with basal nuclei (most of the cells) do not form an MTOC, or 2) the MTOC is separated from the abnormally basal nuclei of *klar* mutant R-cells. If most of the R-cells in *klar* mutant eye discs do not form an MTOC, then in *klar* discs we would expect to observe fewer apical dots of Nod-βgal in each ommatidial cluster than in wild-type. By contrast, we observe that the Nod-βgal dots in *klar* mutant discs appear wild-type in number and pattern (Figure 4, B and D). We conclude that in *klar* mutant R-cells the MTOC forms normally, but usually separates from the nucleus.

Identification of egk1 as a Modifier of the Overexpressed klar Phenotype

A transgene called *glrs-klar* overexpresses $klar^+$ in the developing eye, resulting in defects in eye morphology (Mosley-Bishop *et al.*, 1999; Figure 5A). To identify additional genes that function in nuclear migration in the *Drosophila* eye, we performed a mutagenesis screen for dominant enhancers of the *glrs-klar* rough eye phenotype (Figure 5B). Nine mutant alleles of a complementation group that we named *egk1* (*e*nhancer of *g*lrs-*k*lar) were isolated (Figure 5A). The nine *egk1* alleles were divided into three groups based on the severity of their mutant phenotype: 1) four alleles are lethal as homozygotes or *in trans* to each other, 2) four alleles (Ari3, Ari7, K2, 83) are semiviable as homozygotes, and 3) one allele (A25) is homozygous viable. Initial observation of the *egk1* mutants suggested that the *egk1* gene has an essential role in eye development; adults homozygous for any of the semiviable or viable alleles have external eye defects (Figure 5A). Meiotic mapping localized *egk1* between the markers *dp* and *b* on chromosome 2, and subsequent physical mapping localized *egk1* to polytene position 25E3–6, the region uncovered by the deletion chromosome *Df(2L)cl-h4*. *In trans* to *Df(2L)cl-h4*, the lethal *egk1* alleles are lethal and the semiviable or viable *egk1* alleles are semiviable. Below we show that the weak *egk1* alleles are loss-of-function mutants and that they display nuclear migration defects. Because *egk1* loss-of-function mutants have a similar mutant eye phenotype to *klar* mutants and *egk1* interacts genetically with *klar*, we conclude that the *egk1* gene is likely to function in the *klar* pathway.

egk1 Is Lamin Dm_0

Among the ~25 genes in 25E3–6 (Flybase, 2003), *Lam* Dm_0 (*Lam*) was chosen as a candidate for *egk1*. *Lam* encodes type B nuclear lamin, an intermediate filament protein that is a major component of the inner nuclear envelope (Lenz-Bohme *et al.*, 1997; Stuurman *et al.*, 1998; Gruenbaum *et al.*, 2000). To determine if *egk1* is *Lam*, we tested several of the *egk1* alleles for complementation with two previously identified homozygous lethal *Lam* mutants, Lam^{4643} and Lam^{P}. Neither *Lam* mutant complements any of the *egk1* alleles tested. In addition, we determined the DNA sequences of the *Lam* genes in flies homozygous for each of the semiviable or viable *egk1* alleles. In each case, a nonsense or frameshift mutation was found within the *Lam* coding region (Figure 5, C and D). An antibody to Lam (mAbADL84) recognizes no protein in immunostained eye discs carrying any one of the semiviable alleles *in trans* to *Df(2L)cl-h4*. This result is consistent with the DNA sequence analysis of the four semiviable *Lam* alleles, which predicts that severely truncated Lam proteins are the most likely gene products. Even if these truncated proteins are produced and stable, they need not contain the epitope recognized by mAbADL84. The weakest allele, A25, has a frameshift that results in the deletion of the C-terminal CaaX box, which localizes lamin to the inner nuclear membrane (Holtz *et al.*, 1989; Kitten and Nigg, 1991). Consistent with this observation, A25/*Df(2L)cl-h4* eye discs immunostained with mAbADL84 reveal that Lam protein does not localize to the membrane, but instead is found throughout the nucleus (Figure 6). Finally, a P element containing Lam^+ genomic DNA ($Tw2\text{-}Lam^P$) rescues the lethality and eye phenotypes of the *egk1* alleles. We conclude that *egk1* is *Lam*.

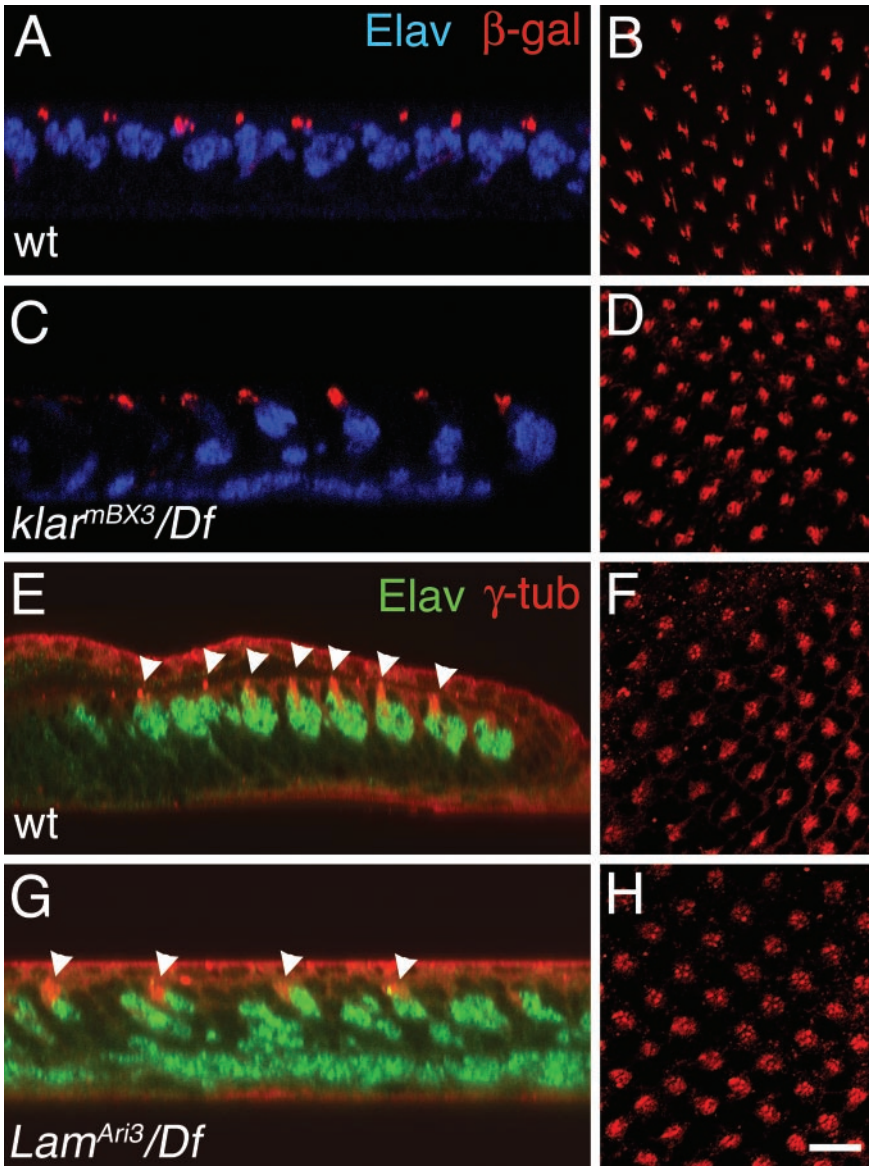
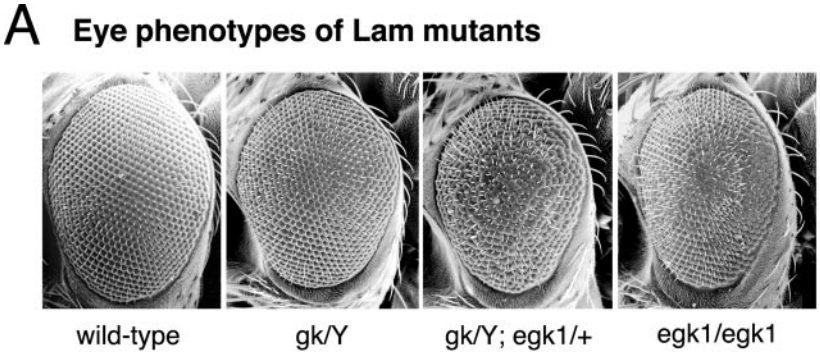


Figure 4. Position of the MTOC and R-cell nuclei in eye discs. Confocal images of eye discs labeled to reveal R-cell nuclei (anti-Elav; blue or green) and the MTOC (anti–β-gal or anti-γ-tub; red) are shown. (A, C, E, and G) Z-sections; (B, D, F, and H) corresponding apical XY-sections, respectively. (A and B) Wild-type eye discs expressing Nod-β-gal in R-cells (*elav>nod-lacZ*). (C and D) $klar^{BX3}/Df(3L)emc^{E12}$ discs expressing Nod-β-gal in R-cells. (E and F) Wild-type eye discs. (G and H) $Lam^{Ari3}/Df(3L)cl\text{-}h4$ eye discs. The arrowheads in E and G indicate the MTOC. (Anti–γ-tub has some background membrane staining.) Size bar in H is ~10 μm and applies to all panels.

Photoreceptor Nuclear Migration Fails in Lam Loss-of-Function Mutants

To determine if the eye morphology flaws in *Lam* mutants are due to nuclear migration defects, anti-Elav was used to label R-cell nuclei in *Lam* eye discs. All of the semiviable and viable *Lam* mutants (Figure 5, C and D) were analyzed; Lam^{A25}, Lam^{Ari3}, and Lam^{Ari7} homozygotes were assayed, and all five weak *Lam* alleles were analyzed *in trans* to *Df(2L)cl-h4*. With the exception of the weakest allele, Lam^{A25}, all homozygotes and hemizygotes showed similar phenotypes; as in *klar* mutants, R-cell nuclei are present throughout the apical/basal axis of the eye disc, and most of them are basal (Figure 4G and Figure 7, H and J). In Lam^{A25} homozygous discs the R-cell nuclear positions are indistinguishable from wild type (Figure 7, B and D). By contrast, $Lam^{A25}/Df(2L)cl\text{-}h4$ discs show nuclear migration defects, but they are less severe than those of the other alleles analyzed (Figure 7F). The difference in severity of the nuclear migration defects in the different alleles is mirrored in their adult eye morphology. Adult retinas with R-cell nuclear migration defects, like those of *klar* mutants, typically have misshapen rhabdomeres (Fischer-Vize and Mosley, 1994; Mosley-Bishop *et al.*, 1999). Rhabdomeres are light-gathering organelles that project from each photoreceptor cell throughout the apical/basal plane of the eye disc. When R-cell nuclei fail to migrate apically, the cell shapes are aberrant, resulting in oddly shaped or missing rhabdomeres in tangential retinal sections. The retinas of Lam^{A25} homozygotes are nearly wild type (Figure 7, A and C), Lam^{A25} hemizygotes are defective (Figure 7E), and the eyes of Lam^{Ari3} homozygotes or hemizygotes have more severe defects (Figure 7, G and H).

Several results described above indicate that the five weak *Lam* mutant alleles are partial loss-of-function mutants, as opposed to gain-of-function mutants: 1) Both lethal and viable *Lam* alleles were isolated as enhancers of *glrs-klar*. This result indicates that all classes of *Lam* alleles have a similar (detrimental) effect on nuclear migration. 2) Lam^{A25} homozygotes have a weaker phenotype than $Lam^{A25}/Df(2L)cl\text{-}h4$ hemizygotes. 3) All phenotypes of the weak and strong *Lam* mutants (lethality and eye phenotypes) are complemented completely by one copy of the transgene Tw2-



D Lam mutations (viable or semi-viable in trans to Df(2L)cl-h4)

allele	induced	amino acid changes	nucleotide changes
Ari3	EMS	$Q^{274} \rightarrow$stop	^{820}CAG $\rightarrow$TAG
Ari7	EMS	$Q^{208} \rightarrow$ stop	^{622}CAG $\rightarrow$TAG
A25	X-rays	frameshift after G^{610} $\Delta[D^{611} \rightarrow M^{622}]$ + 27 aas	$\Delta(^{1831}$GATCC)+(TCTACCA)
K2	X-rays	frameshift after A^{153} + 29 aas	G^{460} (CTGC) A^{461}
83	X-rays	frameshift after A^{234} + 28 aas	$\Delta(^{700}$CATTCGCAGGAGATCAAT) + (AGATCAGATCA)

Figure 5. Identification and characterization of *egk1* mutants. (A) Scanning electron micrographs of eyes of the genotypes indicated are shown. gk is *glrs-klar*. Y is the Y chromosome. The *egk1* allele shown is Lam^{Ari3}. (B) The cross scheme used in the F1 mutagenesis screen for enhancers of the *glrs-klar* rough eye phenotype is shown. (C) The positions of the nonsense or frameshift mutations in each of the five homozygous viable *Lam* alleles isolated as *egk1* mutations are shown. The allele names are at the top, and the number beneath each indicates the first amino acid affected. (The M residue of the start codon is 1.) NLS is the nuclear localization signal, and CaaX refers to the motif used to localize lamin to the cytoplasm at the inner nuclear membrane. (D) The precise nucleotide and predicted amino acid changes of the five mutant *Lam* alleles in C.

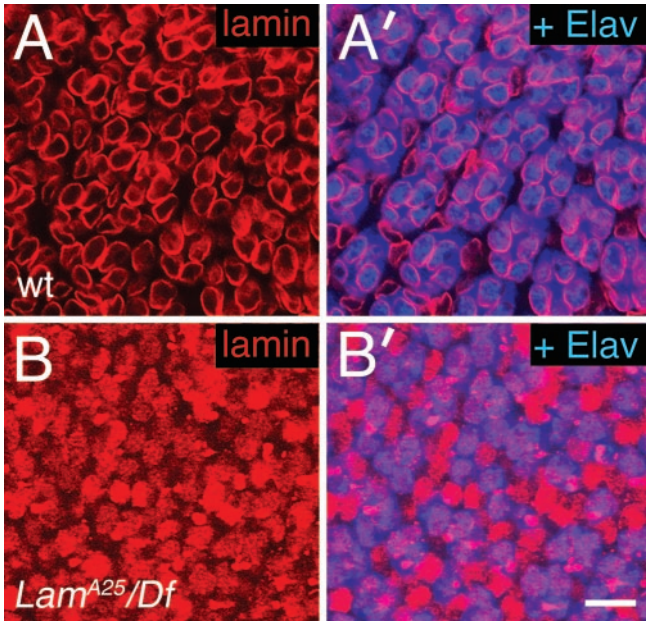


Figure 6. Lamin localization in eye discs. Confocal images of eye discs double-labeled to reveal R-cell nuclei (anti-Elav; blue) and nuclear lamin (anti-Lam; red) are shown. (A and A′) Wild-type discs. (A) Lamin expression in apical nuclei; (A′) a merge of lamin and Elav. The apical nuclei in A′ that have lamin but no Elav are cone cell nuclei. The pink appearance of the R-cell nuclear lamin in A′ is due to colocalization of lamin and Elav within the nucleus. (B and B′) $Lam^{A25}/Df(3L)cl\text{-}h4$ discs. (B) Lamin expression; (B′) a merge of lamin and Elav. The pink is where lamin and Elav overlap. The purely red nuclei are of cone cells. The plane in B and B′ is more basal than in A and A′, in order to detect *Lam* mutant R-cell nuclei that are not as apical as in wild-type discs. Size bar in B′ is ~10 μm.

Lam^P, which contains a Lam^+ gene (see above). Nevertheless, to be certain that the nuclear migration defects represent loss-of-function phenotypes, we observed eye discs homozygous for Lam^{4346}, a strong lethal, loss-of-function mutation caused by P element insertion (Guillemin *et al.*, 2001). We find that Lam^{4346} eye discs have nuclear migration defects similar to those of Lam^{Ari3} homozygotes (Figure 7, H and K) or $Lam^{Ari3}/Df(2L)cl\text{-}h4$ (compare Figure 7K with Figure 4G). We conclude that like $klar^+$, the Lam^+ gene is normally required for R-cell nuclear migration.

Nuclei Disconnect from the MTOC in Lam Mutants

The position of the MTOC was monitored in wild-type and *Lam* mutant eye discs with antibodies to γ-tubulin, a constituent of a protein complex that binds the MTOC (Joshi, 1993). In wild-type discs, we observe dots of γ-tubulin just apical to the R-cell nuclei. Moreover, the γ-tubulin dots are present only in differentiating cells, whose nuclei are normally apical (Figure 4E). Undifferentiated cells that have not yet been recruited into ommatidial clusters surround the developing facets and their nuclei are basal (Figure 1). No γ-tubulin is observed associated with the basal nuclei or at the apical surface of the undifferentiated cells (Figure 4F); all of the γ-tubulin dots are within the developing clusters (Figure 4F; see also Swan *et al.*; 1999.) This result suggests that the cytoskeleton becomes organized and an MTOC forms in differentiating cells as they are recruited into the ommatidia.

As in *klar* mutants, in *Lam* mutant discs the MTOCs of all of the R-cells are apical, even though most of the R-cell nuclei are basal (Figure 4, G and H). We conclude that like Klar, lamin is required for nuclear migration and to link the MTOC to the nucleus.

Lamin Is Required for Perinuclear Localization of Klar

To determine if lamin and Klar function together, the localization of each protein was monitored in the mutant background of the other. In *Lam* mutant eye discs that express *elav>6Xmyc-klar*, Klar localization on microtubules apical to the nucleus appears normal (Figure 2, A–C and E–G). Perinuclear Klar, however, is largely absent in *Lam* mutants (Figure 2, C, D, G, and H). In contrast, lamin localization appears normal in *klar* mutant eye discs (our unpublished results). We conclude that localization of Klar to the nuclear envelope requires nuclear lamin.

DISCUSSION

A Model for the Roles of Klar and Lam in R-cell Nuclear Migration

To understand the role of Klar in R-cell nuclear migration, we have investigated Klar subcellular localization and the position of the MTOC in *klar* mutant eye discs. In addition, we have used genetics to identify another protein, nuclear lamin, that functions in the same pathway with Klar. We find that Klar is perinuclear and also is associated with microtubules apical to the nucleus. In addition, we find that in *klar* and *Lam* mutant discs, MTOCs form normally in R-cells, but are often not associated with the nucleus as they are in wild-type eyes. Finally, we find that Lam^+ is required for Klar localization to the nuclear membrane. These observations, taken together with previous results, suggest a model for the function of Klar in nuclear migration where Klar, held in the nuclear envelope by nuclear lamin, links the nucleus to the MTOC (Figure 8).

The interaction between Klar and lamin may be indirect, but it is likely to be specific, rather than a generalized failure of nuclear envelope assembly in *Lam* mutants. Although we observe that most R-cell nuclei fail to migrate apically even in weak, viable *Lam* mutants, >90% of nuclear envelopes are intact even in stronger, lethal *Lam* mutants (Lenz-Bohme *et al.*, 1997; Guillemin *et al.*, 2001).

We propose (Figure 8) that one or more proteins may form a bridge between the KASH domain of Klar, present in the outer nuclear membrane, and nuclear lamin, in the inner nuclear envelope. The observation that in addition to its perinuclear localization, Klar is cytoplasmic (on apical microtubules) supports the idea that Klar is in the outer, as opposed to the inner, nuclear membrane. Similarly, *C. elegans* Anc-1 is present in the cytoplasm as well as the nuclear membrane, and a model has been proposed where the Anc-1 KASH domain is held in the outer nuclear membrane by an inner nuclear membrane protein, Unc-84 (Malone *et al.*, 1999; Starr and Han, 2002). Although nuclear lamin has not been shown directly to be required for Anc-1 nuclear membrane localization, nuclear envelope localization of Unc-84 requires lamin (Lee *et al.*, 2002). For Syne-1, the vertebrate homolog of Anc-1, experiments where the detergent digitonin was used to allow antibody access to the outer but not the inner nuclear membrane provide direct evidence that the KASH domain is in the outer nuclear membrane (Zhen *et al.*, 2002). There is, however, some conflicting data (Zhang *et al.*, 2001; Mislow *et al.*, 2001, 2002).

We speculate that the N-terminal portion of Klar is linked to microtubules by dynein. At present, we cannot test for colocalization of Klar and dynein because there are no avail-

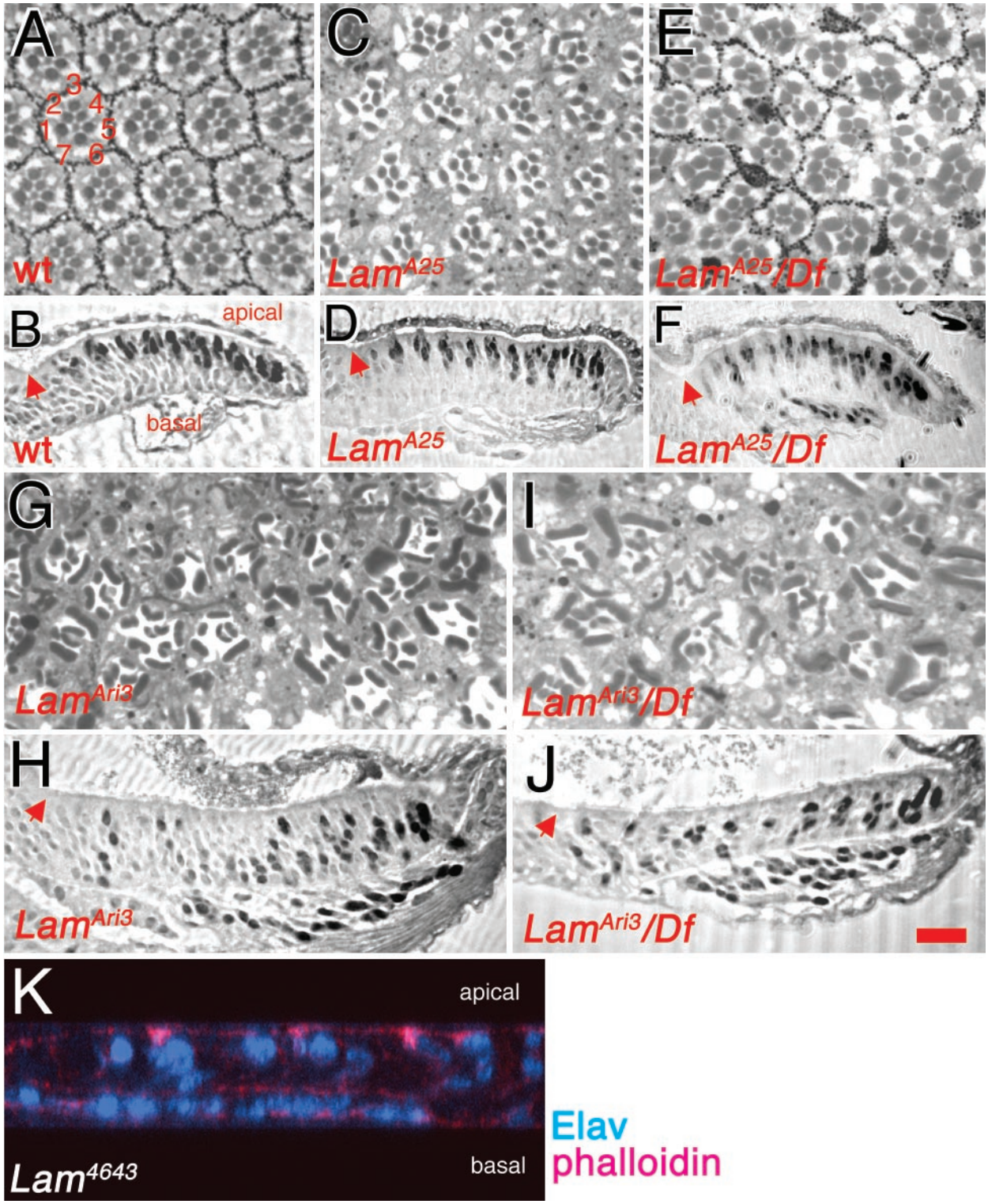


Figure 7. Eye phenotypes of *Lam* mutants. Light micrographs of apical tangential sections through adult compound eyes are shown in A, C, E, G, and I, and of sections through third instar larval eye discs immunostained with anti-Elav to label R-cell nuclei in B, D, F, H, J, and K. (A and B) Wild-type eyes and discs. The numbers in A refer to the seven R-cells in each ommatidium visible in apical sections. The R-cell nuclei are apical in B. (C and D) Lam^{A25} homozygotes. In C, some ommatidia are defective. The disc in D is indistinguishable from wild-type. (E and F) $Lam^{A25}/Df(2L)cl\text{-}h4$ hemizygotes are shown. Severe eye morphology (E) defects and nuclear migration (F) defects are observed. (G and H) Lam^{Ari3} homozygotes. The eye morphology (G) and nuclear migration (H) defects are more severe than in Lam^{A25} hemizygotes. (I and J) $Lam^{Ari3}/Df(2L)cl\text{-}h4$ hemizygotes. The adult eye and disc defects are similar to those of Lam^{Ari3} homozygotes. The red arrows in B, D, F, H, and J indicate the morphogenetic furrow. (K) A confocal image of a Lam^{4643} homozygous eye disc generated by mitotic recombination is shown, labeled with anti-Elav to mark R-cell nuclei, and phalloidin to mark apical and basal cell membranes. Size bar in J is ~20 μm in all panels except B, D, and F, where it is ~25 μm.

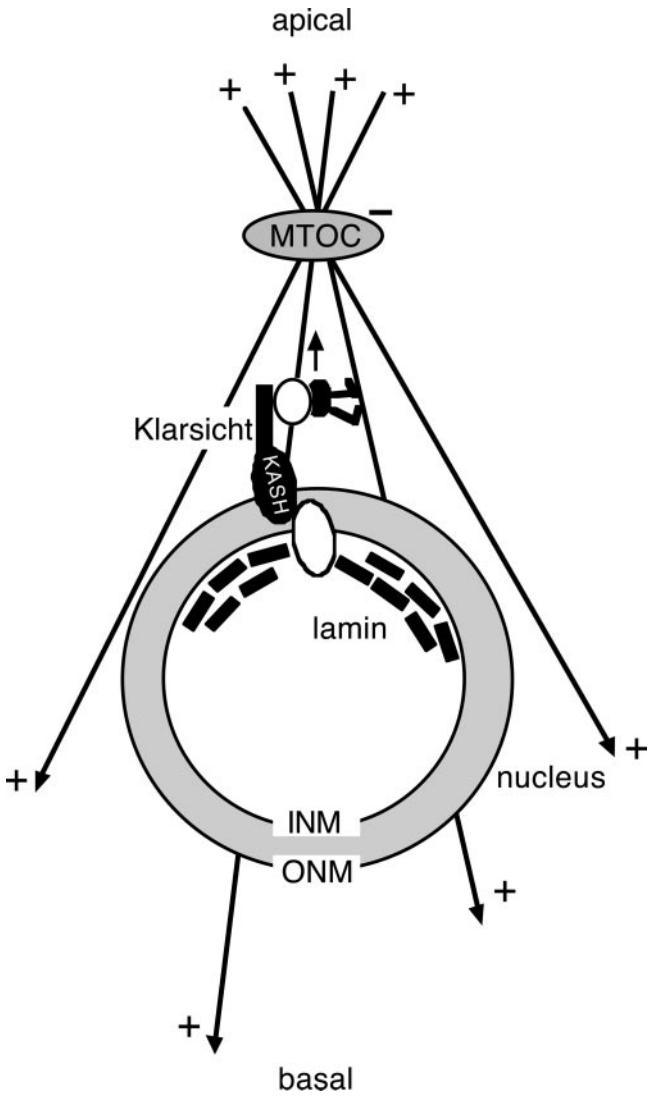


Figure 8. Model for the roles of Klar and lamin in R-cell nuclear migration. A diagram showing how Klar links the nucleus to the MTOC is shown. INM and ONM are the inner and outer nuclear membranes, respectively. The two unfilled ovals indicate possible intermediate proteins that link the C-terminal KASH domain of Klar to lamin, and also link Klar to dynein. Dynein, in black, is walking in the direction of the arrow. (See text for details.)

able reagents that allow detection of dynein or dynactin in the eye disc. Nevertheless, there is much evidence to support an essential role for dynein in R-cell nuclear migration and Klar function. As mentioned above, dynactin, a regulator of dynein, is essential for R-cell nuclear migration in the eye; mutants in the p150 dynactin subunit (*Glued*) have a phenotype similar to that of *klar* mutants in the eye disc (Fan and Ready, 1997). In addition, dynein linkage could explain why Klar is localized to microtubules only apical to the nucleus; Klar that escapes the hold of the nuclear envelope, still attached to dynein, would walk along microtubules to the MTOC. Finally, Klar has been implicated as a regulator of dynein in *Drosophila* embryos (Welte *et al.,* 1998). In addition to its role in R-cell nuclear migration, Klar is required for developmentally regulated migration of lipid storage vesicles during embryogenesis. Lipid droplets at the center of the cellular blastoderm embryo normally migrate cortically during gastrulation. In embryos from *klar* mutant mothers, the lipid droplets fail to migrate. A variety of data support a model where dynein transports the lipid droplets along microtubules, whose minus ends are at the cell periphery. The results of biophysical experiments led to a model where Klar may attach the appropriate types of motor to lipid droplets, control the number of actively engaged motors on a droplet, or coordinate the activities of kinesins and dyneins bound simultaneously to the same droplet (Jackle and Reinhard, 1998; Welte *et al.,* 1998; Gross *et al.,* 2000). Notably, dynein is required for nuclear attachment to centrosomes (the MTOCs) during mitosis in *Drosophila* embryos (Robinson *et al.,* 1999). Klar, however, is not essential for this process (Fischer-Vize and Mosley, 1994).

Migration versus Anchoring

The observation that the MTOC is normally apical to the R-cell nuclei, at the leading edge of nuclear movement, suggests that a force pulls on the MTOC from above. We speculate that the mechanism for this force could be analogous to the means by which the nucleus of budding yeast are pulled into the bud neck before cell division. One pathway for migration of the nucleus into the bud neck involves dynein, anchored at the cell cortex to which the nucleus is moving. Cortically tethered dynein "reels in" the nucleus by walking along microtubules whose plus ends are at the cortex, toward the MTOC, which is anchored to the nucleus (reviewed in: Morris *et al.,* 1995; Beckwith *et al.,* 1995; Bloom, 2000, 2001, Segal and Bloom, 2001; Morris, 2003). In support of this idea, microtubule plus-ends are present apically in R-cells (Mosley-Bishop *et al.,* 1999), and as discussed above, dynactin is essential for R-cell nuclear migration (Fan and Ready, 1997).

Whether a force emanating from the apical membrane pulling on the MTOC would drive nuclear migration or serve as an anchor after the nucleus has migrated depends on where the MTOC initially forms. The γ-tubulin antibody detects MTOCs only apically in differentiating cells. Transiently basal MTOCs associated with nuclei that are about to rise could have escaped detection. However, if the MTOC does form apically, then the force that drives nuclear migration would come from below the nucleus, that is, dynein, linked to the nuclear membrane by Klar and lamin, walking on microtubules up toward the MTOC.

The Klar/lamin Interaction, Cytoplasmic Phenomena, and Human Laminopathies

The model we propose whereby Klar forms a bridge between nuclear lamin in the inner nuclear membrane and cytoplasmic microtubules provides a general framework for explaining how nuclear lamin affects cytoplasmic events. Guillemin *et al.* (2002) showed that *Drosophila Lam* mutations result in D/V polarity defects in eggs, and tracheal branching defects in embryos. Moreover, a variety of human diseases are the result of mutations in the LMNA gene, which encodes lamin A (reviewed in Hutchison, 2002; Burke and Stewart, 2002; Goldman *et al.,* 2002; Holaska et al., 2002). The *Drosophila Lam* Dm_0 gene encodes type B lamin, whereas the *Drosophila LamC* gene encodes lamin C, which is most similar to human lamin A (Riemer *et al.,* 1995). The A/C- and B-type lamins are similar proteins, with some different structural features, and some expression pattern differences (Gruenbaum *et al.,* 2000). LMNA-associated human diseases affect the heart, skeletal muscles, and the nervous system (Emery-Dreifuss muscular dystrophy, limb-girdle muscular dystrophy, cardiomyopathy, and Charcot-Marie-Tooth disorder), and metabolism (Dunnigan-type lipodystrophy). The

two main hypotheses as to how nuclear lamin defects can result in these disease phenotypes are that the mutations result in nuclear envelope fragility or result in changes in gene expression. An alternative hypothesis is that the inner nuclear envelope interacts with the cytoplasm through proteins like Klar or Anc-1/Syne-1, which connect the inner nuclear envelope to the microtubule, or actin cytoskeletons, respectively.

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