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 Dm0, 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; Wolf 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 dynactin- 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-Borris 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;
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. Here, the furrow is 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 dynenin 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:

- \textit{klar}\textsuperscript{9} (Spradling et al., 1999) was obtained from the Bloomington Stock Center.
- \textit{Lam}\textsuperscript{4643} (Spradling et al., 1999) was obtained from B. Schmitt.
- \textit{Ari3} and \textit{Ari7} were subsequently tested for complementation with a variety of flies spanning the region between \textit{dpp} and \textit{egk1}.

\textbf{Modifier Screen}

The \textit{egk1} alleles \textit{Ari3} and \textit{Ari7} were mapped meiotically between \textit{dp} and \textit{egk1} using the multiply marked chromosome \textit{al dp b pr B cx px} and by scoring the mutant eye phenotype of the \textit{egk1} homozygotes. Females that were \textit{al dp b pr B cx px sp} were crossed with \textit{al dp b pr B cx px sp male}, and ~20 male progeny of each single-recombinant class were collected. To determine which of the recombinant chromosomes contained \textit{egk1}, each male was individually crossed to \textit{egk1/CyO} females, and progeny were examined for the \textit{egk1} homozygous eye phenotype. \textit{Ari3} and \textit{Ari7} were subsequently tested for complementation with a variety of deficiency chromosomes spanning the region between \textit{dp} and \textit{egk1}.

\textbf{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 \textit{Df(2L)cl-h4}) for each of the five viable or semiviable \textit{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.

\textbf{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 Axiosplan microscope and a Zeiss AxiosCam (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., 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); rabbit anti-p-tubulin (B5 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, predesorbed Jackson secondary antibodies were used. Green:Alexa\textsuperscript{488}-goat anti-rabbit, Alexa\textsuperscript{488}-goat anti-rat. Blue: Cy5-goat anti-rat, Alexa\textsuperscript{555}-goat anti-rat. Red: Cy3-goat anti-mouse, Alexa\textsuperscript{594}-goat anti-rabbit, or Alexa\textsuperscript{488}-phalloidin.

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

\textbf{RESULTS}

\textbf{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

strains used are described in Lindsley and Zimm (1992). All experimental flies were grown at 25°C.

\textit{Lam}\textsuperscript{4643} homozygous eyes were generated in \textit{Lam}\textsuperscript{4643} heterozygotes using the GMR-hid technique (Stowers and Schwarz, 1999). First, two stocks were generated: \textit{Lam}\textsuperscript{4643} \textit{FRT40A/CyO.GFP} and \textit{GMR-hid, CL, FRT40A/CyO, GFP}. \textit{EUG1}. Flies from the two stocks were intercrossed, and among the progeny, \textit{Lam}\textsuperscript{4643} \textit{FRT40A/CyO.GMR-hid CL FRT40A, GFP+} larvae were detected by the absence of GFP expression. The eye discs of these larvae are homozygous for \textit{Lam}\textsuperscript{4643}.
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).
Klar colocalizes with the nuclear envelope protein 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, \( \sim 2 \mu m \).

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 2I). 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-\( \beta \)gal 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-\( \beta \)gal 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-\( \beta \)gal. In wild-type, Nod-\( \beta \)gal 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.

**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 (enhancer of glrs-klar) 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 Dm0**

Among the \( \sim 25 \) genes in 25E3–6 (Flybase, 2003), Lam Dm0 (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\(^{A463}\) and Lam\(^{A}\). 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 egk1 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\(^{A}\) genomic DNA (Tu2-Lam\(^{A}\)) rescues the lethality and eye phenotypes of the egk1 alleles. We conclude that egk1 is Lam.

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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; LamA25, LamAri3, and LamAri7 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, LamA25, 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 LamA25 homozygous discs the R-cell nuclear positions are indistinguishable from wild type (Figure 4G and Figure 7, H and J). In LamA25/Df(2L)cl-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 LamA25 homozygotes are nearly wild type (Figure 7, A and C), LamA25 hemizygotes are defective (Figure 7E), and the eyes of LamAri3 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) LamA25 homozygotes have a weaker phenotype than LamA25/Df(2L)cl-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-
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 LamAri3. (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.
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>6xynp-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 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$^{A25/DF}$ 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.
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-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-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.
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 apically to the nucleus; Klar that escapes the hold of the nuclear envelope, why Klar is localized to microtubules only apically to the nucleus; Klar that escapes the hold of the nuclear envelope, and also link Klar to dynein. Dynein, in black, is walking in the direction of the arrow. (See text for details.)

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 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 Dm0 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 lamin 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 cally 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 centrobesomes (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).

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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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