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Interactions among *Drosophila* Nuclear Envelope Proteins Lamin, Otefin, and YA

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The nuclear envelope plays many roles, including organizing nuclear structure and regulating nuclear events. Molecular associations of nuclear envelope proteins may contribute to the implementation of these functions. Lamin, otefin, and YA are the three *Drosophila* nuclear envelope proteins known in early embryos. We used the yeast two-hybrid system to explore the interactions between pairs of these proteins. The ubiquitous major lamina protein, lamin Dm, interacts with both otefin, a peripheral protein of the inner nuclear membrane, and YA, an essential, developmentally regulated protein of the nuclear lamina. In agreement with this interaction, lamin and otefin can be coimmunoprecipitated from the vesicle fraction of *Drosophila* embryos and colocalize in nuclear envelopes of *Drosophila* larval salivary gland nuclei. The two-hybrid system was further used to map the domains of interaction among lamin, otefin, and YA. Lamin's rod domain interacts with the complete otefin protein, with otefin's hydrophilic NH_2-terminal domain, and with two different fragments derived from this domain. Analogous probing of the interaction between lamin and YA showed that the lamin rod and tail plus part of its head domain are needed for interaction with full-length YA in the two-hybrid system. YA's COOH-terminal region is necessary and sufficient for interaction with lamin. Our results suggest that interactions with lamin might mediate or stabilize the localization of otefin and YA in the nuclear lamina. They also suggest that the need for both otefin and lamin in mediating association of vesicles with chromatin might reflect the function of a protein complex that includes these two proteins.

The nuclear envelope is a complex structure with multiple functions, including regulation of nucleocytoplasmic transport, organization of chromatin, gene regulation, DNA replication, and determination of nuclear morphology (for reviews, see references 14, 19, 21, 28, 36). The inner nuclear membrane and the nuclear lamina are two nuclear envelope components that are closely associated and are important for the organization of nuclear structure and regulation of nuclear processes (17, 18, 21, 42). In *Drosophila melanogaster*, several components of the inner nuclear membrane and lamina have been identified and cloned. These include derivatives of the lamin Dm_0 protein (22, 52), YA (34, 37, 39), otefin (1, 2, 23, 43), and lamin C (8, 46, 47). These proteins are present in the insoluble nuclear matrix-pore-complex-lamina (NMPCL) fraction after salt and Triton X-100 extraction, making it difficult to dissect biochemically the relationships among these proteins inside *Drosophila* cells.

The nuclear lamina is a dynamic structure whose composition is developmentally controlled (for a review, see reference 36). In *Drosophila*, lamin Dm_0 derivatives are major components of the lamina and are present in all cells except mature sperm (22, 35, 46, 51, 62). The primary translation product lamin Dm_0 is processed posttranslationally to produce three isoforms, Dm_1, Dm_2, and Dm_{mit} (51, 52, 56). Lamins Dm_1 and Dm_2 exist as insoluble lamin polymers in the interphase nuclear envelope (52). They are converted to soluble lamin Dm_{mit} during mitosis and meiosis (48, 51). Lamin C is a developmentally regulated *Drosophila* lamin that is detected only after stage 12 of embryogenesis and in differentiated cells (46); since this paper concerns proteins present in early embryos, lamin C will not be discussed further and we will use the designation lamin to refer solely to lamin Dm_0 and its derivatives.

Lamin is an essential protein which is required for nuclear organization. Flies homozygous for mutations that abolish all apparent function of the *lamin Dm_0* gene have an aberrant nuclear structure and die following 9 to 14 h of development (21a). A weaker mutation in the lamin Dm_0 gene (<20% of normal lamin expression levels) causes reduced viability, defective nuclear envelopes and accumulation of annulate lamellae (33). As part of its role in organizing the nucleus, lamin is thought to associate chromosomes with the nuclear envelope, given the ability of lamin to bind chromatin, DNA and RNA sequences in vitro (4, 48, 63, 64). Lamin and otefin are also involved in nuclear envelope assembly, since antibodies against either protein block the interaction between vesicles and chromatin in a *Drosophila* cell-free system (1, 60, 61).

Otefin is a 45-kDa peripheral nuclear envelope protein with no apparent sequence similarity to other known proteins (43). It contains a large hydrophilic domain, a single COOH-terminal hydrophobic sequence of 17 amino acids (aa), and a high content of serine and threonine. With the exception of sperm cells, otefin is present, like lamin, in the nuclear envelopes of

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all cells examined during *Drosophila* development. In eggs and young embryos, otefin is associated with the maternal fraction of membrane vesicles (1). The COOH-terminal, 17-aa hydrophobic sequence of otefin is essential for targeting otefin to the nuclear periphery. Amino acids between positions 173 and 372 of otefin are required for efficient targeting of otefin to the nuclear envelope, while sequences between positions 35 and 172 are required for further stabilizing otefin's interaction with the nuclear envelope (2).

YA (young arrest) protein is a developmentally regulated *Drosophila* nuclear lamina component (34, 39). It is essential for the function of the nuclear lamina during egg activation and fertilization; in its absence, development arrests during the transition from meiosis to the initiation of the mitotic divisions by the zygote (29, 34, 39). YA is provided maternally and found only in eggs and in early embryos (34, 53). In the first 2 h of zygotic development, YA is localized to the nuclear lamina from interphase to metaphase in a cell cycle-dependent manner (34). YA is not needed for nuclear assembly, but instead appears to mediate association of chromosomes with the lamina, thus contributing to organizing the nucleus in a developmental stage-specific way (37, 40). This hypothesis is based on the observations that nuclei form in YA-deficient eggs and embryos but have abnormal chromosome condensation states (37), that ectopically expressed YA associates with polytene chromosomes in vivo (40), and that YA can associate with chromatin in vitro (40). However, it is not clear how YA associates with the lamina. Mutagenesis of YA shows that a serine-threonine-rich region and a polar COOH terminus, which could be involved in protein-protein interactions (26, 45), are important for YA function (38).

Here, we show that both otefin and YA interact with lamin in the yeast two-hybrid system. No interaction between otefin and YA was observed in this system. Interaction between otefin and lamin was then confirmed in *Drosophila* cells in vivo by observing an almost completely overlapping pattern of localization in salivary gland cells stained with antilamin and antiotefin antibodies. Extraction of maternal membrane vesicles with buffer containing 0.5 M NaCl, followed by immunoprecipitation with either antilamin or with antiotefin antibodies, also revealed that lamin and otefin interact with each other. Deletion constructs were used to delineate interaction domains in lamin, YA, and otefin. Our results, together with data reported previously, suggest that (i) lamin interacts with both otefin and YA and that these interactions require specific domains in each of the proteins and (ii) interaction with lamin could be a general means for targeting proteins to the nuclear lamina and/or envelope.

MATERIALS AND METHODS

The yeast two-hybrid system. The Matchmaker yeast two-hybrid system (Clontech) was used for interaction assays. Yeast strain SFY526 (6) contains a *lacZ* reporter gene under the control of the upstream activating sequence (UAS) and the TATA portion of the *GAL1* promoter. Yeast strain HF7c contains a *GAL1-HIS3* reporter gene and a *lacZ* reporter gene under the control of the *GAL4* 17-mer consensus sequence and the TATA portion of the *CYC1* promoter. The yeast two-hybrid vectors for the Matchmaker system are pGBT9 and pGAD424 (6). Open reading frames (ORFs) cloned into pGBT9 (which we abbreviate as the B vector, since the fusion is to the GAL4 DNA binding domain) were fused to GAL4 between aa 1 and 147 [$GAL4_{(1–147)}$]; ORFs cloned into pGAD424 (the A vector, since the fusion is to the GAL4 activation domain) were fused to $GAL4_{(768–881)}$. Yeast cells cotransformed with pGBT and pGAD derivatives were plated on Trp^- Leu^- synthetic medium (SM) to select for the plasmids. Yeast transformations and β-galactosidase (β-Gal) activity assays were performed according to standard protocols (3, 9) on media containing either 2% glucose or sucrose as the carbon source. Comparable levels of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) staining were observed on both carbon sources in all but two cases (lamin-lamin and lamin-B–otefin-A), in which darker X-Gal staining was seen for sucrose-grown cells relative to glucose-grown cells.

Interpreting two-hybrid data. We emphasize a plus or minus interpretation over one based on relative strengths of β-Gal signals, and we consider positives (blue rather than white colonies and his-independent rather than -dependent growth) to be more significant than negatives. Our reasoning is based on the following considerations. Rather than necessarily indicating a failure of proteins to interact, diminution or loss of signal in two-hybrid assays can result from abnormal conformation, decreased stability, or differing amounts of the fusion proteins (11); there is variability in expression levels among some constructs (e.g., see reference 57). Moreover, the DNA binding domain vectors (B vectors) used in this study produce significantly more fusion protein than the activation domain vectors (A vectors) (reference 57 and data not shown). The amount of DNA binding domain fusion protein that they produce is more than sufficient to occupy the GAL4 UAS close to 100% of the time (10). This can result in competition for UAS occupancy between the DNA binding domain fusion protein alone and the DNA binding domain fusion complexed with the activation domain fusion (10). In addition, most of the lamin fusion proteins used in this study form dimers and likely also higher-order structures within the yeast cell (57). In the context of a two-hybrid experiment, such structures can prevent lamin from entering nuclei (15a) or otherwise interfere with interactions allowing for activation of transcription at the GAL4 UAS (13). Finally, in several cases (e.g., see references 10 and 11), including some reported here, the same pair of proteins that are known to interact can give greatly differing levels of β-Gal in the two different vector pair combinations, including positive in one vector pair and negative in the reverse.

Library screening. A *Drosophila melanogaster* 0- to 18-h-old embryo cDNA library made in pGAD10 was purchased from Clontech. pGBT9-YA either was transformed first into the yeast strain HF7c, followed by the library DNA, or was cotransformed with the library DNA into HF7c. Positive candidates were selected on His^- Trp^- Leu^- SM plates and were further checked on His^- Trp^- Leu^- SM plates supplemented with 3 mM 3-aminotriazole (3-AT) as well as colorimetrically for β-Gal expression. Crude plasmid extracts were obtained from the candidate yeast colonies and transformed into *Escherichia coli* HB101 for plasmid amplification (3). The rescued plasmids were transformed back into HF7c, either with the empty pGBT9 vector or with pGBT9-YA, to verify that the interaction depended on the presence of both YA and the candidate protein.

Southern blots and sequencing of YA-interacting clones. Southern blots were performed to check if the candidate interacting plasmids from the screen of the embryo cDNA library contained lamin Dm_0. cDNAs in the candidate plasmids were excised with *Eco*RI and subjected to agarose gel electrophoresis. Full-length lamin Dm_0 cDNA (22) was used for probe synthesis by random priming. The blots were generated and probed as described previously (37). To confirm that the clones contained a lamin Dm_0 insert in frame with the pGAD vector, we sequenced the ends of the inserts of five of the clones. We used primers within the vector or from bases 1771 to 1788 within the lamin sequence.

Plasmid constructions. The full-length coding regions of YA (34, 37), lamin Dm_0 (22), and otefin (43) were amplified by PCR (3, 59) and cloned into pGBT9 and pGAD424 with *Eco*RI sites engineered into our PCR primers. To delete the COOH-terminal 179 aa from YA, primers HL6 (5′-TCG CCG GAA TTC ATG TCG TTT TCC AAT G-3′) and HL7 (5′-CAT CAC CGC CAG CCC CTG CAG CTA CGG CTT-3′) were used to amplify the NH_2-terminal 517-aa region, which was then cloned into pGBT9 by using the *Eco*RI and *Pst*I sites, yielding plasmid pGBT9-YA1-517. pGBTT-YA1-638 was constructed by inserting the 1.94-kb *Eco*RI-*Nco*I fragment of YA from pGBT9-YA into pGBTT. pGBT9-YA1-649 was obtained by self-ligating the 7.4-kb *Bam*HI fragment of pGBT9-YA, leaving out the 0.2-kb fragment which encodes YA's COOH-terminal 47 aa. pGBTT-YA506-696 was constructed by deleting from pGBTT-YA the *Eco*RI-*Esp*I fragment that encodes YA's aa 1 to 505. Sequence analysis verified that this resulted in an in-frame fusion.

Fragments lam57-622 (that is, sequences in lamin Dm_0 from aa 57 to 622 inclusive; also called headless), lam57-411 (rod), and lam1-411 (tailless) were cut out of pETL57-622, pETL57-411, and pETL1-411 (56, 57), respectively, by using the *Nde*I-*Nco*I restriction enzymes and were ligated into the corresponding sites in pGBTT and pGADT (57). The resulting plasmids were named pGBTT-lam57-622, pGBTT-lam57-411, pGBTT-lam1-411, pGADT-lam57-622, pGADT-lam57-411, and pGADT-lam1-411. pGBTT-lam1-179, pGBTT-lam180-452, pGADT-lam1-179, and pGADT-lam180-452 were described previously (57). Construction of vectors encoding fusions with the lamin tail domain (lam385-622) involved the following steps. First, the vector pGBT-lam385-452 was made by inserting the *Nde*I-*Sal*I fragment of the product of a PCR with pETL1-622 (56) as a template, the T7 terminator primer (Novagen, Madison, Wis.), and primer 5′-TAC CAG GAC CAT ATG GAC ATC AAG GTC TC-3′. The correctness of this insert was confirmed by sequencing. pGBT-lam385-622 resulted from insertion of a *Sal*I fragment of pGBTT-lam57-622 into the unique *Sal*I site of pGBTL385-452 and selection of a clone with the correct orientation by restriction digestion and sequencing. pGADT-lam385-622 was constructed by insertion of the lamin coding sequence of pGBT-lam385-622 into pGADT.

pGBTT-ote1-387 and pGADT-ote1-387 were obtained by subcloning otefinΔ388-406 from pET20 m2-UAA (43) into pGBTT and pGADT by using *Nde*I and *Not*I sites. Primers HL4 (5′-TAT GGC CCC G-3′) and HL5 (5′-GAT CCG GGG CCA-3′) were annealed to produce a linker with *Nde*I and *Bam*HI sticky ends. The linker was used to ligate the *Bam*HI-*Sac*I fragment of otefin from pCA1038 otefinΔ35-172 and the *Nde*I-*Sac*I-digested vectors, pGBTT and

pGADT, resulting in two new plasmids, i.e., pGBTT-ote1-34;173-406 and pGADT-ote1-34;173-406. Plasmids pGBTT-ote1-34;173-387 and pGADT-ote1-34;173-387 were obtained from pCA1038 otefinΔ35-172Δ388-406 by the same strategy. Plasmids pET20 m2-UAA, pCA1038 otefinΔ35-172, and pCA1038 otefinΔ35-172Δ388-406 were described elsewhere (2). Primers HL12 (5′-GGA ATT CGC TAG TCC CAA GAA GAC C-3′) and HL13 (5′-ACG GAT CCG TTG ACC TTA AGA TAT TTC TC-3′) were used to amplify the region encoding aa 35 to 172 of otefin. The resulting products were cloned into pGBT9 and pGAD424 by using the *Bam*HI and *Eco*RI sites. All constructs were designed so that the GAL4 binding domain and the activation domain encoded in the vector were in frame with the YA, lamin, and otefin coding sequences. Expression of all fusion proteins with predicted sizes was confirmed by Western blots (not shown), with the exception of the YA506-696 and lam385-622 fusions, which were not tested.

Antibodies. A monoclonal antibody (MAb) specific for *Drosophila* lamin (611A3A6) and a MAb specific for *Drosophila* otefin (618A2O7) were previously described (41), as were polyclonal antibodies specific for lamin (60, 61). Purified immunoglobulin G (IgG) was obtained by chromatography on a protein A-Sepharose CL-4B (Pharmacia) column. Affinity-purified antilamin antibodies were prepared from IgG fractions as described elsewhere (60). Affinity-purified polyclonal anti-*Drosophila* otefin antibodies have also been described previously (1). Fluorescein isothiocyanate (FITC) conjugated goat-anti-mouse IgG, Cy3A-conjugated goat-anti-rabbit IgG, and horseradish peroxidase-conjugated goat-anti-mouse antibody were purchased from Jackson ImmunoResearch Labs, West Grove, Pa. Anti-*Drosophila* tubulin antibody (30) was a kind gift of T. Karr. RL1 antibody (55) was a kind gift of L. Gerace. Goat-anti-mouse Ig was purchased from Southern Biotechnology Associated, Birmingham, Ala.

Immunoprecipitation of otefin and lamin from membrane vesicles. Complete cytoplasmic homogenate, membrane vesicles, and soluble membrane-free cytoplasm were prepared from 0- to 6-h-old *Drosophila* embryos (Canton S) as described elsewhere (61). Vesicles derived from 0.5 ml of the complete cytoplasmic homogenate (61) were resuspended in 300 μl of buffer MG (250 mM sucrose, 0.5 M NaCl, 50 mM KCl, 1 mM dithiothreitol, 50 mM HEPES [pH 7.3], 10 μM leupeptin, 10-μg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, 2.5 mM benzamidine, 1 μM pepstatin A), and the suspension was incubated for 30 min on ice with constant shaking and was centrifuged for 40 min at 150,000 × *g*. The supernatant was saved, and the pellet was resuspended in 100 μl of buffer MG and centrifuged. The supernatant from this centrifugation was combined with the previously saved supernatant. A 200-μl volume of supernatant was incubated for 1 h at 4°C with 50 μg of either affinity-purified antilamin antibodies, with affinity-purified antiotefin antibodies or, as a control, with 50 μg of goat-anti-mouse Ig. Ten milligrams of protein A-Sepharose CL-4B beads (Pharmacia), which was equilibrated and washed in buffer MG containing 10% bovine serum albumin, was incubated with the antibody-containing supernatant for 1 h at 4°C with constant agitation. The beads were separated from the supernatant by a 15-s centrifugation in a microcentrifuge and washed twice with 300 μl of buffer MG, and the proteins on the beads were extracted by boiling for 5 min in 50 μl of 1× buffer SB (80 mM Tris-HCl [pH 6.8], 2.4% SDS, 140 mM β-mercaptoethanol, 10% glycerol). The liquid was separated from the beads by centrifugation at 12,000 × *g*, loaded (12 μl per lane) on an SDS-10% polyacrylamide gel, and subjected to electrophoresis and immunoblot analysis. Immunoblots of material immunoprecipitated with affinity-purified polyclonal antilamin antibodies were probed with MAb 611A3A6 (antiotefin). Immunoblots of material immunoprecipitated with purified polyclonal antiotefin antibodies were probed with MAb 618A2O7 (antilamin). As controls, some immunoprecipitated material was subjected to immunoblot analysis with MAb specific for *Drosophila* tubulin. The secondary antibody was horseradish peroxidase-conjugated goat-anti-mouse antibody. Detection of horseradish peroxidase was with an enhanced chemiluminescence kit (Amersham).

Immunofluorescence analysis and confocal microscopy. Salivary glands were dissected from third-instar larvae (Canton S) and fixed for 15 min at 22°C in phosphate-buffered saline (PBS) containing 3.7% formaldehyde and 1% Triton X-100. The glands were incubated for 2 h at 22°C either with affinity-purified polyclonal antiotefin antibodies and antilamin MAb 611A3A6 or with affinity-purified polyclonal antiotefin antibodies and MAb RL1 against O-linked sugar-containing glycoproteins (55) or with each antibody alone. Staining was by incubation with Cy3-conjugated goat-anti-rabbit IgG, followed by several washes with PBS containing 0.1% Triton X-100 and incubation with FITC-conjugated goat-anti-mouse IgG. Imaginal disk cells were stained in the same incubation mixture to confirm that the antibodies gave their previously reported staining patterns (data not shown).

A Bio-Rad MRC-1024 confocal scanhead coupled to a Zeiss Axiovert 135M inverted microscope was used to acquire images of the stained cells, with a ×63/numerical aperture = 1.4 oil immersion objective. Excitation light was provided by a 100-mW air-cooled argon ion laser run in the multiline mode. Both 488- and 514-nm excitation wavelengths were used, as described below. The emission filter in the Cy3 detection channel was a D580/32 interference filter (32-nm bandpass, centered around 580 nm). In the FITC channel, a D522/35 interference filter (522-nm-center wavelength and 35-nm bandwidth) was used with 488-nm excitation and a D540/30 interference filter (540-nm-center wavelength and 30-nm bandwidth) was used with 514-nm excitation. The confocal iris diameter was 2.5 to 3 mm, with the larger opening used for weaker signals. Vertical resolution was approximately 1 μm. If necessary, two to four images were averaged in order to reduce noise. Images (512 by 512 pixels) were acquired by using a hardware zoom of 1.0 (0.308 μm/pixel) or 1.8 (0.175 μm/pixel).

Since the confocal data were acquired for the purpose of determining colocalization of the two labeled species, careful calibration was performed with single-labeled samples to ensure that the signal in a given channel was not caused by bleedthrough (crossover) from the second channel. Singly labeled samples were tested to measure the degree of crossover under the experimental conditions used for the double-labeled cells. Under these conditions, there was no measurable detection of Cy3 in the FITC channel when 488-nm excitation and the D522/35 emission filter were used. Therefore, 488-nm excitation and the D522/35 filter were used to acquire FITC fluorescence. FITC emission appeared in the Cy3 channel at about one-third of the level of the FITC channel. Therefore, to verify that a signal in the Cy3 channel was caused by Cy3, one-third of the level of the signal in the FITC channel was subtracted. However, in order to further enhance the Cy3 signal, Cy3 emission was in fact acquired by excitation at 514 nm. This reduced the FITC signal (although it did not eliminate it) and enhanced the Cy3 signal. Thus, the images of Cy3 were shown to be due to Cy3 emission, with a negligible FITC contribution under the conditions of measurement.

To assess colocalization visually, the images from the Cy3 channel and the FITC channel were combined into a 24-bit red-green-blue (RGB) image, with the Cy3 image as the red component and the FITC image as the green component, by using the Image Pro Plus image processing package (Media Cybernetics, Silver Spring, Md.).

RESULTS

In the two-hybrid system, lamin interacts with otefin and YA but YA and otefin do not interact with one another. To test directly for interaction among the three *Drosophila* nuclear envelope proteins, full-length cDNAs encoding lamin, otefin, or YA were introduced into the yeast two-hybrid vectors (see Materials and Methods for experimental design and intepretational considerations). Lamin, otefin, and YA fusion constructs made in vector A (pGAD424 [vector with DNA activation domain]) and vector B (pGBT9 [vector with binding domain]) were transformed separately into yeast hosts with the other vector (B and A, respectively). None of the fusion constructs paired with the other empty vector induced β-Gal activity in SFY526 or permitted growth of HF7c on plates lacking histidine (data not shown). Thus, these fusion proteins have no detectable intrinsic transcriptional activation activity on GAL4-responsive promoters, making it feasible to use them for testing interacting proteins.

Table 1 shows that otefin interacts with lamin in both vector pairs, as indicated by measurement of β-Gal activities on filters and in liquid culture and growth of HF7c in the absence of histidine. YA interacts with lamin when YA is fused to the binding domain and lamin is fused to the activation domain. We did not detect YA-lamin interaction in the reverse vector-protein pair. Since we also did not detect YA-YA interaction in this system (Table 1) but have biochemical evidence that YA interacts with itself (38, 63a), we reasoned that the YA activation domain hybrid protein may be unable to adopt the right conformation for interaction (11) and is, therefore, unable to interact with YA or lamin fused to the DNA binding domain. Thus, lamin can interact with both of the other nuclear envelope proteins that we tested.

As shown in Table 1, we observed no interaction between YA and otefin, nor did we detect self-interaction of otefin. In agreement with a previous report (57), we observed lamin self-interacting in the two-hybrid system.

Lamin and otefin can be coimmunoprecipitated from salt extracts of oocyte-derived vesicles. The biological significance for *Drosophila* cells of the interaction between lamin and otefin in yeast cells was demonstrated by coimmunoprecipitation of lamin and otefin from vesicles of early embryos. The vesicle-enriched fraction was isolated from 0- to 6-h-old *Drosophila* embryos (61) and was extracted with buffer MG. Under these conditions, most lamin and otefin molecules partition into the

TABLE 1. Interactions among nuclear envelope proteins as measured by the yeast two-hybrid assay

Gene fused to vector B/gene fused to vector A[a]	β-Gal color (filter assay)	β-Gal activity (U)[b]	Growth without histidine[c]
Otefin/lamin	Blue	1.92	+
Lamin/otefin	Pale blue	0.02	+
YA/lamin	Pale blue	0.22	+
Lamin/YA	White	$<10^{-3}$	−
YA/YA	White	$<10^{-3}$	−
YA/otefin	White	$<10^{-3}$	−
Otefin/otefin	White	$<10^{-3}$	−
Lamin/lamin	Pale blue	0.01	+

[a] Fusion constructs transformed into the yeast hosts. A and B represent the activation domain and DNA binding domain two-hybrid vectors, respectively, used for making fusion proteins. Their ORFs were fused in frame to full-length ORFs of lamin, otefin, or YA as described in Materials and Methods. None of the fusion constructs listed was able to induce detectable transcription of reporter genes when cotransformed with the other empty vector into yeast hosts (data not shown).

[b] β-Gal activity in cells grown with glucose as a carbon source was determined both by X-Gal analysis of cells immobilized on filters and by assays for enzyme activity in cultures. Values are averages of duplicate determinations (3, 9).

[c] +, ability of yeast strains carrying the *GAL1-HIS*$_3$ reporter gene to grow on plates lacking histidine in the presence of the two hybrid plasmids. All of the fusion constructs produced fusion proteins of the predicted size and antigenicity in the host yeast cells (data not shown).

supernatant following centrifugation at 150,000 × *g* (1). Proteins were immunoprecipitated from the supernatant either with affinity-purified polyclonal antiotefin antibodies or with affinity-purified polyclonal antilamin antibodies and were subjected to immunoblot analysis (Fig. 1). Lamin Dm_{mit} (75 kDa) was found to coimmunoprecipitate with otefin by using otefin-specific polyclonal antibodies (left panel in Fig. 1). Likewise, otefin was detected among proteins that were immunoprecipitated with polyclonal antibodies specific for the lamin protein (right panel in Fig. 1). These results were reproducible in two (left panel) or three (right panel) completely independent experiments, starting with different batches of embryos. As a control, proteins immunoprecipitated from the vesicle extract with goat-anti-mouse Ig were analyzed by immunoblotting. The signals obtained for lamin or otefin in these controls were less than 10% of those seen following immunoprecipitation with polyclonal antibodies specific for lamin or otefin (Fig. 1). In contrast, when the immunoblots were probed with antitubulin antibodies, the levels of tubulin in all precipitations (control, antilamin, and antiotefin) were the same (data not shown). These data indicate that in the maternal vesicle fraction of *Drosophila* early embryos, lamin and otefin interact with each other and may be part of a protein complex.

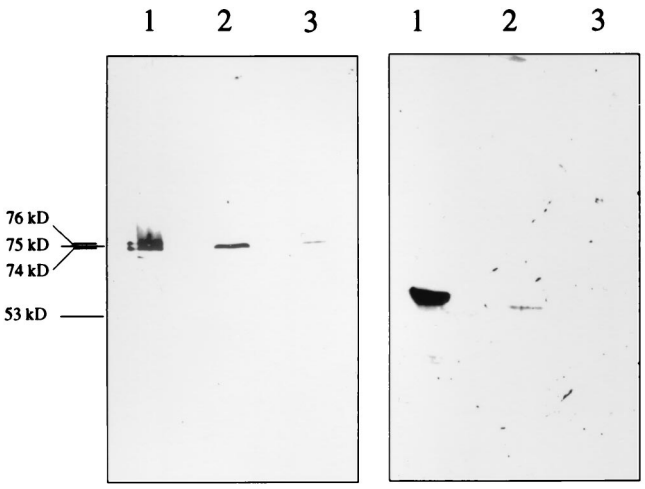


FIG. 1. Coimmunoprecipitation of lamin and otefin from extracts of membrane vesicles. Proteins were extracted with buffer containing 0.5 M NaCl from membrane vesicles prepared from 0- to 6-h-old *Drosophila* embryos. Immunoprecipitation of proteins from the extract was with affinity-purified polyclonal antiotefin antibodies (left panel) or with affinity-purified antilamin antibodies (right panel). Lanes 1, total nuclear extracts from 0- to 6-h-old embryos (lane 1 in the right panel contains 3.5 times more extract than lane 1 in the left panel; lanes 2, proteins immunoprecipitated with affinity-purified antibodies as noted above; lanes 3, proteins immunoprecipitated with total Ig. The proteins were separated by SDS-10% PAGE and subjected to protein blot analysis with MAb 611A3A6 antilamin (left panel) and MAb 618A2O7 antiotefin (right panel). The positions of lamin isoforms Dm_1 (76 kDa), Dm_{mit} (75 kDa), and Dm_2 (74 kDa) and the position of otefin (53 kDa) are indicated.

Immunofluorescence reveals colocalization of lamin and otefin in salivary gland cell nuclei. To investigate the subcellular localization of otefin and lamin with respect to each other, salivary glands were dissected from third-instar larvae, fixed, and reacted with both affinity-purified polyclonal rabbit-antiotefin antibodies and mouse-antilamin MAb 611A3A6, followed by fluorochrome-labeled secondary anti-rabbit or anti-mouse IgG. Observation of fluorescence by confocal microscopy showed that as expected, lamin immunoreactivity was localized along the nuclear rim. Lamin staining was not smooth but displayed areas (spots) of higher-intensity and other areas with lower-intensity fluorescence (Fig. 2B). Very similar patterns of lamin distribution along the nuclear envelope have been demonstrated in a variety of cell types (7, 44). Otefin was distributed in a comparable fashion in these cells (Fig. 2A); the staining of lamin and otefin largely overlapped (yellow in Fig. 2C), indicating that the proteins colocalize in salivary gland cell nuclei. To demonstrate that the overlap of spots in the lamin and otefin staining was not due to an experimental artifact, salivary glands were also reacted with both affinity-purified anti-otefin polyclonal antibodies and MAb RL1 directed against glycoproteins containing O-linked *N*-acetylglucosamine (55); RL1 recognizes many nuclear pore proteins. It has previously been shown that at this level of analysis, lamin staining and nuclear pore staining largely exclude each other in CHO cells (7). Staining for otefin and RL1 antigens did not overlap to a significant extent (Fig. 2D to F), showing that otefin and RL1 antigens do not colocalize in these cells.

Otefin interacts with the lamin rod domain. To identify lamin domains needed for interaction with otefin, we tested the combination of two-hybrid fusions of full-length otefin and of a series of lamin deletion constructs (57) transformed into yeast strains SFY526 and HF7c. As with full-length lamin, none of the lamin deletions in two-hybrid vectors could induce β-Gal activity or allow histidine-independent growth when cotransformed with the other empty vector (data not shown). Figure 3A shows that full-length otefin can interact with activation domain fusions of headless lamin, tailless lamin, and lamin rod (lamin Dm_0 aa 57 to 622, 1 to 411, and 57 to 411, respectively). Interaction with the last two was observed only in one otefin-lamin vector direction; in these cases, we consider the positive interaction the meaningful one, for reasons detailed in Materials and Methods. We did not observe otefin interacting with lamin's head plus partial rod, partial rod plus partial tail, partial rod only, or partial rod plus complete tail (lamin Dm_0 aa 1 to 179, 180 to 452, 57 to 179, and 385 to 622, respectively) in either vector pair combination.

Taken together, these data indicate that the lamin Dm_0 protein sequence from 57 to 411 (lamin rod domain) is sufficient for interaction with otefin.

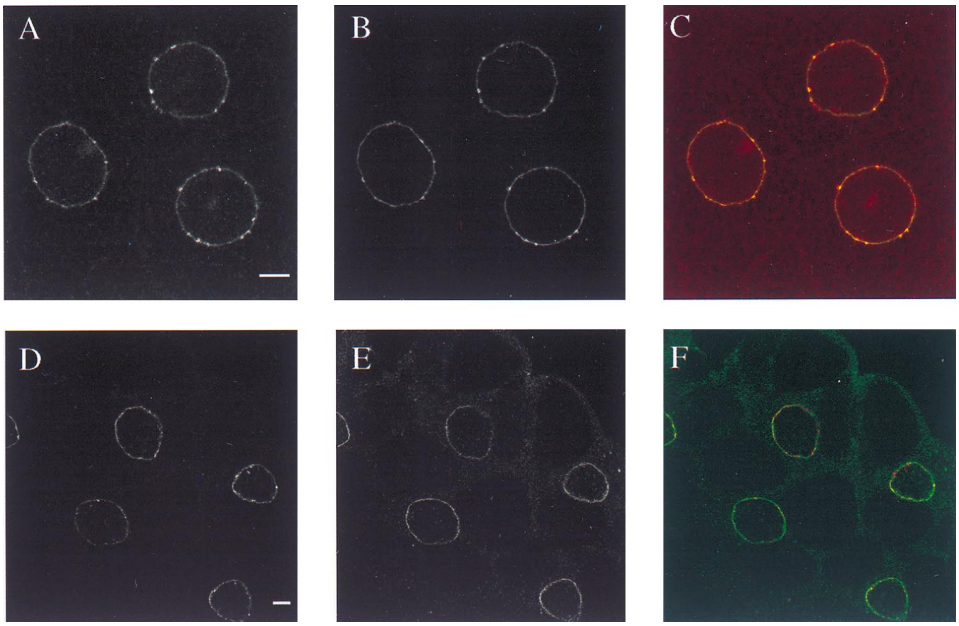


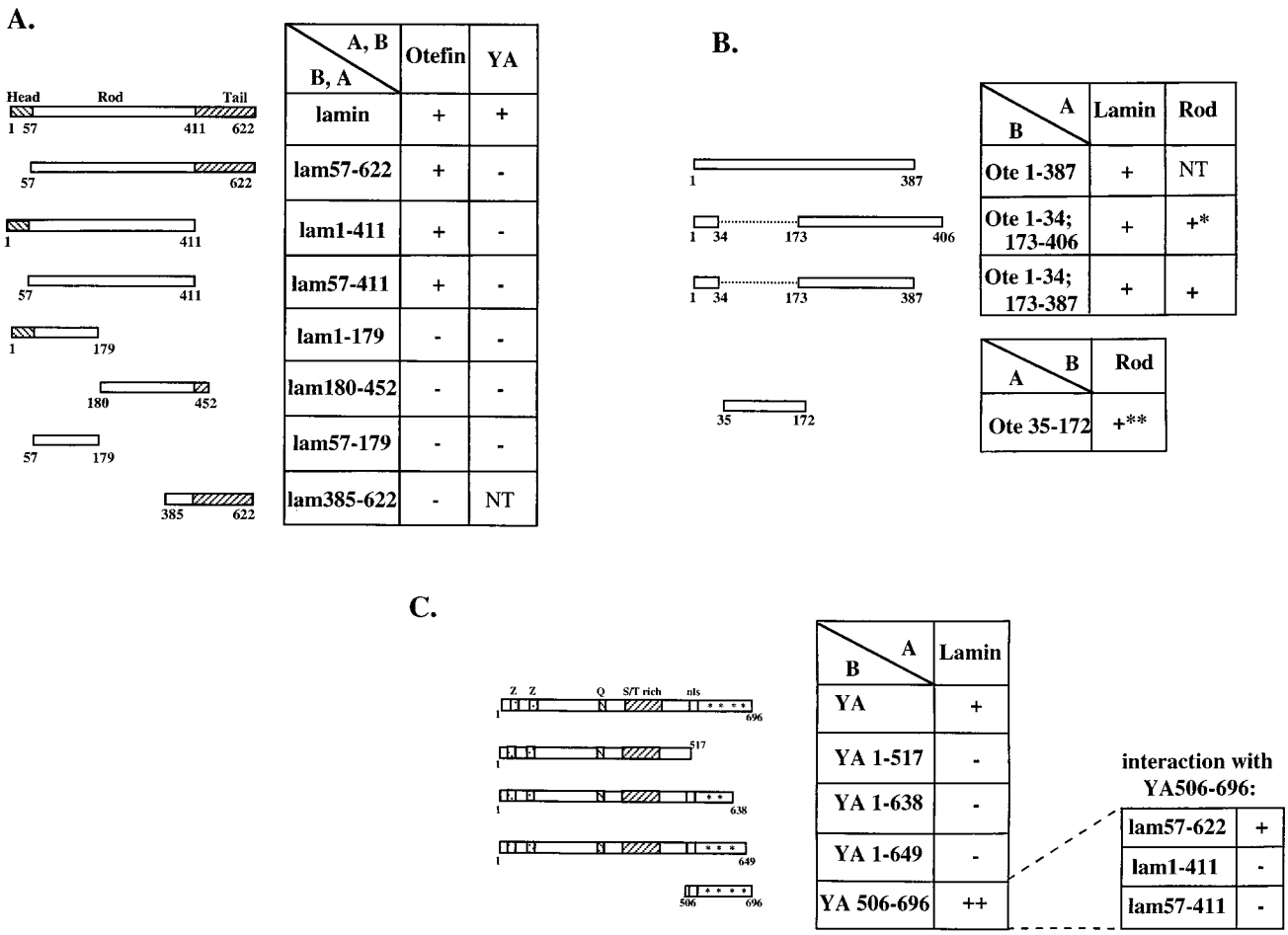
FIG. 2. Lamin and otefin colocalize in salivary gland nuclei. Salivary glands were dissected from third-instar larvae, fixed, and probed with affinity-purified polyclonal antibodies specific for otefin (A and D), monoclonal antilamin antibody 611A3A6 (B), and monoclonal antibody RL1 against O-linked sugar-containing glycoproteins (E). FITC-conjugated goat-anti-mouse IgG and Cy3-conjugated goat-anti-rabbit IgG were used as the secondary antibodies for the monoclonal and polyclonal antibodies, respectively. To assess colocalization visually in double-labeled salivary gland cells (panels A and B, and panels D and E), the images from the Cy3 channel and the FITC channel were combined into a 24-bit RGB image as the red and green components, respectively (C and F). The resulting color image was yellow where the red and green features overlapped. Bar, 10 μm.

Lamin interacts with the hydrophilic region of otefin. Otefin's hydrophobic region (aa 388 to 406) is essential for targeting otefin to the nuclear periphery, yet is not sufficient for high-efficiency targeting (2). This result could be explained if the hydrophobic region directly associated with the inner nuclear membrane and the large hydrophilic domain of otefin interacted with other proteins, such as lamin and/or proteins in the inner nuclear membrane, to stabilize otefin's envelope localization.

To test this hypothesis, we cloned otefin's hydrophilic region, ote1-387, into the pGBTT vector. When transformed into the yeast hosts, this region did not induce transcription of the reporter genes in the presence of empty pGAD424; however, as shown in Fig. 3B, it did interact with lamin cloned in pGAD vectors. This indicates that otefin's hydrophilic region can interact with lamin.

Within this hydrophilic region of otefin, aa 35 to 172 have been suggested to stabilize otefin's association with the nuclear envelope. This is based on the observation that although otefin from which aa 35 to 172 have been deleted targets to the nuclear envelope in *Drosophila* cells, its localization there is more sensitive to extraction than is full-length otefin (2). To test whether these amino acids also participate in the interaction with lamin, we PCR amplified the sequences encoding otefin's aa 35 to 172 and cloned this domain into the yeast two-hybrid vectors. We also cloned otefin from which these amino acids were deleted (ote1-34; 173-406) or otefin's hydrophilic domain from which these amino acids were deleted (ote1-34; 173-387 [a double deletion] [2]) into two-hybrid vectors. None of these constructs induces detectable transcription of the reporter genes when cotransformed with the other empty vector into the yeast hosts. In contrast, when HF7c was cotransformed with pGAD424-ote35-172 and pGBTT-lamin rod domain, the resulting colonies grew on plates lacking histidine, indicating an interaction between the otefin fragment and lamin rod (Fig. 3B). However, this interaction was weak. Colony growth was slower than that of colonies containing full-length otefin and lamin rod in the same vector combinations, and no β-Gal activity was detectable in SFY526 containing pGAD424-ote35-172 and pGBTT-lamin rod. Since the *his* reporter gene is more sensitive than the *lacZ* reporter gene, we conclude that the otefin's region from aa 35 to 172 can interact with the lamin rod domain weakly. Both constructs that contained otefin from which aa 35 to 172 were deleted also interacted with full-length lamin and with lamin's rod domain in two-hybrid tests (Fig. 3B). Taken together, our results suggest that at least two subfragments of the hydrophilic region can interact with lamin, although whether they do so completely independently or as a more stable whole remains to be determined.

Removal of head or tail from lamin abolishes detectable interaction with full-length YA. Although YA fused to the binding domain could interact with full-length lamin fused to the activation domain, the YA fusion was not able to interact with any lamin deletions fused to the activation domain (Fig. 3A). This suggested that YA's interaction with lamin requires both lamin's head (aa 1 to 57) and tail (aa 411 to 622) domains, because none of the lamin deletions that we tested preserves both domains simultaneously. In agreement with this result, when we screened 10^7 clones of an interaction trap cDNA library made from 0- to 18-h-old embryo mRNA (Clontech) for clones of proteins that interacted with YA, we obtained 13 positive clones, all of whose inserts hybridized to ^{32}P-labeled lamin Dm_0 cDNA probes with signals as strong as that of the positive control (Fig. 4), indicating that the inserts were all lamin cDNAs. All of the clones had insert sizes of 2.3 kb, which is sufficient to encode the lamin ORF. To confirm that they encoded lamin, we sequenced the ends of five of the clones. All contained nearly full-length lamin coding sequences in the pGAD vector, fused in frame. Lamin sequences in the clones



A, B / B, A	Otefin	YA
lamin	+	+
lam57-622	+	-
lam1-411	+	-
lam57-411	+	-
lam1-179	-	-
lam180-452	-	-
lam57-179	-	-
lam385-622	-	NT

B / A	Lamin	Rod
Ote 1-387	+	NT
Ote 1-34; 173-406	+	+*
Ote 1-34; 173-387	+	+

A / B	Rod
Ote 35-172	+**

B / A	Lamin
YA	+
YA 1-517	-
YA 1-638	-
YA 1-649	-
YA 506-696	++

interaction with YA506-696:	
lam57-622	+
lam1-411	-
lam57-411	-

FIG. 3. Characterization of the interaction domains in lamin, otefin, and YA. A and B, vectors containing the GAL4 activation domain and binding domain, respectively. The otefin and lamin constructs were cloned in frame in either A or B as indicated; YA was fused in frame to B. All constructs were tested for induction of β-Gal, and all except YA506-696 and lam385-622 were tested for histidine-independent growth as described in Materials and Methods. +, detection of *lacZ* and (when tested) *his* reporter gene expression with levels as shown in Table 1; −, lack of detectable signals (again as shown in Table 1); +*, histidine-independent growth of transformed HF7c, but no detectable activity of *lacZ* in transformed SFY526; +**, weak *his* reporter gene expression, as indicated by reduced, but still detectable, ability of transformed HF7c to grow on plates lacking histidine, as compared to a positive control; NT, not tested. All fusion constructs in either vector are not able to induce β-Gal activity or permit histidine-independent growth ability when cotransformed with the other empty vector, and all fusion constructs (except for lam385-622 and YA506-696, which were not tested) resulted in the production of fusion proteins of the correct size and antigenicity (data not shown). (A) To define the region of lamin that interacts with otefin, combinations of full-length otefin constructs in vector A were cotransformed into yeast hosts SFY526 and HF7c with lamin deletion constructs (diagrammed on the left; lamin's head, rod, and tail are marked) in vector B. The same results were obtained with the reciprocal construct-vector pairs, except that interaction was not detected between otefin fused to the binding domain and lam 57-411 or lam 1-411 fused to the activation domain. As described in Materials and Methods, in the case of such conflicts, we consider the positive results significant, since changes in the conformation of the fusion protein could have abolished interaction in the negative cases (11). To test lamin regions needed for interaction with YA, full-length YA in vector B was cotransformed with the lamin deletion constructs in vector A. (B) To define the region of otefin that interacts with lamin, two-hybrid fusions of otefin deletions (diagrammed on the left) containing aa 1 to 387, aa 1 to 34 and 173 to 406, or 1 to 34 and 173 to 387 were introduced into the yeast strains along with full-length lamin fusions. Results with the otefin deletions in vector B and lamin in vector A are shown. In the reciprocal vector-insert pairs, the doubly deleted otefin (ote1-34; 173-387) interacted with full-length lamin, although the singly deleted otefins (ote1-387 or ote1-34; 173-406) did not. In the conflicting cases, we consider the positive results significant, as discussed in Materials and Methods. We tested for interaction between ote35-172 and the lamin rod domain using only the vector-insert combination shown in the figure. (C) To determine whether YA's COOH terminus is needed for interaction with lamin, we tested the YA deletions or fragments diagrammed on the left, in vector B, for interaction with full-length lamin and, for YA506-696, with the lamin deletions shown, cloned in vector A. For this panel only, relative signal strengths are indicated (by the number of plus signs) to indicate why we could detect an interaction between the lamin deletions and the YA C terminus but not between those deletions and full-length YA (in panel A). The diagrams show YA's two potential zinc fingers (Z), glutamine-rich (Q) and serine/threonine-rich (S/T rich) regions, its two putative nuclear localization signals (nls), and its polar COOH terminus (***) (34, 37).

began at aa 10 or 26 (depending on the clone) and extended through aa 622.

Although one must use caution in interpreting negative results of two-hybrid assays (references 5, 6, 10, 11, and 13 and the present study), the capability of the tested lamin deletions to interact with otefin and the isolation of nearly full-length lamin clones in two-hybrid screening suggest that both the tail and at least part of the head domain (aa 26 to 56) contribute to the ability of lamin to bind to YA.

YA's carboxyl-terminal region interacts with lamin. Ectopically expressed YA lacking the COOH-terminal 179 aa of YA (aa 518 to 696) is not able to localize to the nuclear lamina, although it can enter the nucleus (38). The interaction between YA and lamin described above suggested that YA might be targeted to, or retained by, the nuclear lamina by virtue of its interaction with lamin. If this is the case, then this mutation which abolishes lamina targeting should abolish YA's interaction with lamin.

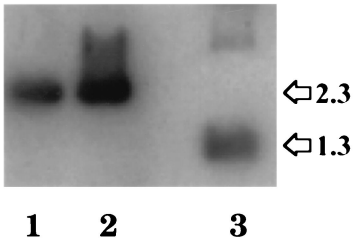


FIG. 4. YA interactor candidates hybridize to ^{32}P-labeled lamin probes on Southern blots. Plasmids retrieved from candidate yeast colonies were digested with *Eco*RI to release their cDNA inserts. Examples of two representative isolates are shown in lanes 1 and 2 (100 ng/lane). ^{32}P-labeled lamin Dm_0 probes were used to probe the inserts (see Materials and Methods). As a positive control, we loaded the same amount of plasmid pGBTT-lam57-411 (lamin rod domain; insert size, 1.3 kb) digested with *Eco*RI (lane 3).

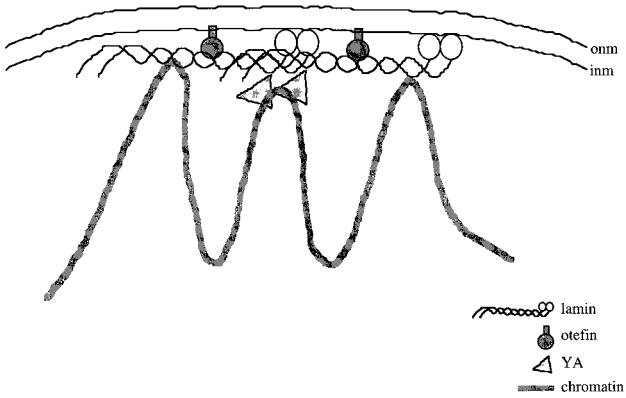


FIG. 5. A model for the organization of lamin, otefin, and YA in the nuclear envelope. Otefin interacts with both lamin (this study) and the inner nuclear membrane (1, 2) and hence is shown on the membrane-facing side of the nuclear lamina. The contacts of otefin with lamin require only lamin's rod domain. YA associates with lamin (this study) and chromatin (40) and hence is placed at the nucleoplasmic side of the nuclear lamina and in contact with chromatin; YA is also shown as self-associating as reported previously (38). Lamin is shown associating with itself, with chromatin and, via its C terminus, with the inner nuclear membrane as reported elsewhere (4, 7, 57, 58, 64). onm, outer nuclear membrane; inm, inner nuclear membrane.

To test whether YA's lamina-targeting region was essential for interaction with full-length lamin, we cloned YA deleted for the COOH-terminal 179 aa into pGBT9 and tested this deletion for interaction with lamin (Fig. 3C). We also tested two smaller COOH-terminal deletions of YA (from which aa 639 to 696 or 650 to 696 were deleted) for interaction with lamin (Fig. 3C). Upon cotransformation with empty pGAD424 or with pGAD424-lamin into the yeast host SFY526, the YA fusion plasmids caused no detectable β-Gal activity in the filter assay. Similarly, HF7c transformed with any of the YA deletion clones and pGAD424-lamin could not grow without histidine, in contrast with the histidine-independent growth of the positive control (HF7c carrying full-length YA in pGBT9 and pGAD424-lamin). We detected expression of YA fusion proteins of the predicted molecular weights in the transformed yeast hosts (data not shown). These results suggested that YA's COOH terminus is needed for interaction with lamin, since its removal abolishes this interaction.

To determine whether the COOH terminus of YA contained a lamin-interaction domain, we tested directly for interaction between aa 506 to 696 of YA (in pGBTT) and lamin (in pGAD424) (Fig. 3C). The YA COOH terminus interacted strongly with full-length lamin. The interaction was stronger than that seen with full-length YA, suggesting that YA's lamin interaction domain is partially masked in the full-length fusion. The stronger signal also permitted retests of interaction between YA and lamin deletions. YA506-696 interacted with lam57-622, although less strongly than with full-length lamin. The YA COOH terminus did not interact with lam1-411 or lam57-411. These data suggest that lamin's tail domain mediates some interaction with YA but that full interaction also requires sequences in lamin's head domain.

DISCUSSION

We report interactions among three *Drosophila* nuclear envelope proteins present in early embryos: lamin Dm, a major component of the nuclear lamina (22, 52), interacts with otefin, a peripheral inner nuclear membrane protein (1, 2, 23, 43), and with YA, a developmentally regulated lamina protein that is essential for early embryogenesis (34, 37, 39). Interaction with lamin may be important for the stable association of these latter proteins within the nuclear envelope and suggests a relative organization of the proteins in the nuclear envelope (Fig. 5). Lamin is associated with both inner nuclear membrane and chromatin (7, 58). Otefin is likely to be oriented toward the inner nuclear membrane, and YA is likely to be oriented toward chromatin: otefin's association with the inner nuclear membrane is supported by its resistance to salt extraction, which is greater than that of lamin, and by the fact that otefin's 17 COOH-terminal aa target proteins to cellular membranes (2). YA's association is more likely to be with the nucleoplasmic side of the lamina, since YA can associate with chromatin and since YA is seen primarily at this inner side in immunoelectron microscopy (39, 40). The lack of interaction between otefin and YA supports this model of organization of these three proteins in the nuclear envelope.

Interaction between lamin and otefin. Previous studies (1, 2, 23) provided indirect evidence for an in vivo interaction between lamin and otefin. This evidence included the peripheral nucleoplasmic localization of both otefin and lamin, the similar levels of resistance of otefin and of lamin to extraction with Triton X-100, the finding that in early embryos both proteins remain associated with the spindle envelope during mitosis, and the finding that both lamin and otefin are required during nuclear assembly for the attachment of membrane vesicles to chromatin. In addition, in the maternal pool, otefin is associated with the same membrane vesicle fractions as lamin Dm_{mit} (2a). Our present study has provided in vitro and in vivo evidence for such an interaction and has shown that otefin and lamin colocalize in salivary gland cell nuclei.

The two-hybrid experiments in yeast cells revealed that otefin and lamin can interact with one another in the absence of any other *Drosophila* proteins. Interaction domains delineated in the yeast two-hybrid assay suggest that otefin interacts with the lamin rod domain through otefin's hydrophilic NH_2 terminus, including otefin's aa 35 to 172, which have been shown previously to stabilize otefin's localization to the nuclear envelope (2). Since the hydrophobic COOH terminus of otefin is required for targeting to the inner nuclear membrane (2), otefin may connect with the inner nuclear membrane through its COOH terminus and with the nuclear lamina through other regions of otefin. Interaction between otefin and lamin may stabilize the localization of otefin. This could be similar to the case of the lamin B receptor (LBR) in vertebrates, which has a hydrophilic NH_2 terminus and a hydrophobic COOH terminus that is capable of targeting LBR to the inner nuclear membrane (54). Since the NH_2 terminus of LBR alone targets

a cytosolic protein to the nucleus but a type II integral protein to the inner nuclear membrane (54), this suggests that targeting a protein to the inner nuclear membrane requires a special domain, such as one mediating interaction with other nuclear envelope proteins.

In vitro studies have shown that the rod domain of lamin has several biological activities. The heptad repeats in the rod domain are involved in coiled-coil interactions, and sequences at both ends are involved in the head-to-tail organization of lamin filaments (57). The rod domain contains a chromatin binding site (20), and it can bind M/SAR sequences with high affinity (64). Our results show that the rod domain can also serve as a binding domain for otefin.

Interaction between lamin and YA. Previous reports showed that lamin Dm_0 derivatives and YA are present at the same time and in the same cells (developing oocytes and eggs and early embryos [34, 37, 39, 46, 51]) and that YA and lamin colocalize, at the electron microscopy level, in the nuclear lamina from interphase to metaphase (34, 39). These results make it reasonable to postulate that the interaction seen with the two-hybrid system in yeast cells also occurs in *Drosophila* cells. Also in agreement with such an interaction, YA and lamins Dm_1 and Dm_2 copurify in the NMPCL fraction (16, 53). Yet we were unable to obtain coprecipitation of soluble lamin isoforms (lamin Dm_{mit}) with YA from cytoplasmic extracts (37a), suggesting that YA can interact only with polymerized forms of lamin, which are present in the insoluble NMPCL but not in the soluble fraction. These results, coupled with the failure of YA to interact fully with lamin rod or with headless lamin in the two-hybrid system, suggest a model in which full-length lamin Dm_1 and/or Dm_2 interacts with YA. More specifically, YA may be targeted to or retained in the nuclear lamina by associating with assembled lamin polymers.

A number of additional results are consistent with the hypothesis that YA can interact only with polymerized lamins, which are normally found in the context of a nuclear lamina. First, all of YA, including its COOH-terminal region which reacts with lamin in two-hybrid tests, lacks any of the known lamina-targeting sequences (12, 15, 25, 27, 31, 32, 50) or a hydrophobic sequence that could associate it with the inner nuclear membrane. Instead, the COOH terminus of YA is necessary to target or retain it in the *Drosophila* nuclear lamina (38), and we observed here that this region of YA is required and sufficient for interaction with lamin in the two-hybrid system. Moreover, as we report here, the head domain of lamin is likely necessary for full lamin interaction with YA in the two-hybrid system. The head domain of lamin is essential for formation of head-to-tail polymers in vitro and most likely for the assembly of polymerized lamin such as that which occurs in the nuclear lamina in vivo (49, 57 [see also references 24 and 28]). In this context, it is worth noting that certain lamin-lamin interactions underlying filament formation can be reconstituted in the yeast two-hybrid system, suggesting that lamin in yeast cells can also form multimers and that these are functional in two-hybrid assays (57). Second, during embryonic mitotic cycles, lamin is seen at the nuclear periphery before YA (telophase for lamin and interphase for YA [34]). This relative timing is as expected if assembled lamin is indeed necessary to recruit or retain YA in the nuclear lamina. Third, since ectopically expressed YA enters nuclei and is retained in the nuclear lamina at postembryonic stages (39), YA's localization is not dependent on an embryo-specific condition but rather on something found in all somatic nuclei; lamin is a good candidate, given its ubiquitous presence in somatic cell nuclei (23, 46, 62).

In summary, the results that we have reported have allowed us to build a picture of the interactions among three *Drosophila* nuclear envelope proteins and lead to the hypothesis that interactions among these proteins mediate or stabilize their residence at the nuclear periphery. Now that we have defined these interactions, future work can exploit mutants in the *fs(1)Ya* (34, 37) or *lamin* Dm_0 genes (21a, 33) to define the nature and consequence of these interactions in *Drosophila* embryos.

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Michal Goldberg and Huihua Lu contributed equally to this work.

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REFERENCES

1. **Ashery-Padan, R., N. Ulitzur, A. Arbel, M. Goldberg, A. M. Weiss, N. Maus, P. A. Fisher, and Y. Gruenbaum.** 1997. Localization and posttranslational modification of otefin, a protein required for vesicle attachment to chromatin, during *Drosophila melanogaster* development. Mol. Cell. Biol. **17:**4114–4123.
2. **Ashery-Padan, R., A. M. Weiss, N. Feinstein, and Y. Gruenbaum.** 1997. Distinct regions specify the targeting of otefin to the nucleoplasmic side of the nuclear envelope. J. Biol. Chem. **272:**2493–2499.

2a. **Ashery-Padan, R., and Y. Gruenbaum.** Unpublished observations.

3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1994. Current protocols in molecular biology. John Wiley & Sons, Inc., New York, N.Y.
4. **Baricheva, E. A., M. Berrios, S. S. Bogachev, I. V. Borisevich, E. R. Lapik, I. V. Sharakhov, N. Stuurman, and P. A. Fisher.** 1996. DNA from *Drosophila melanogaster* beta-heterochromatin binds specifically to nuclear lamins *in vitro* and the nuclear envelope *in situ*. Gene **171:**171–176.
5. **Bartel, P. L., C. T. Chien, R. Sternglanz, and S. Fields.** 1993. Elimination of false positives that arise in using the two-hybrid system. BioTechniques **14:**920–924.
6. **Bartel, P. L., C. T. Chien, R. Sternglanz, and S. Fields.** 1993. Using the two-hybrid system to detect protein-protein interactions, p. 153–179. *In* D. A. Hartley (ed.), Cellular interactions in development: a practical approach., Oxford University Press, Oxford, United Kingdom.
7. **Belmont, A. S., Y. Zhai, and A. Thilenius.** 1993. Lamin B distribution and association with peripheral chromatin revealed by optical sectioning and electron microscopy tomography. J. Cell Biol. **123:**1671–1685.
8. **Bossie, C. A., and M. M. Sanders.** 1993. A cDNA from *Drosophila melanogaster* encodes a lamin C-like intermediate filament protein. J. Cell Sci. **104:**1263–1272.
9. **Breeden, L., and K. Nasmyth.** 1985. Regulation of the yeast HO gene. Cold Spring Harbor Symp. Quant. Biol. **50:**643–650.
10. **Brent, R., and R. L. Finley, Jr.** 1997. Understanding gene and allele function with two-hybrid methods. Annu. Rev. Genet. **31:**663–704.
11. **Brown, N. G., M. C. Costanzo, and T. D. Fox.** 1994. Interactions among three proteins that specifically activate translation of the mitochondrial COX3 mRNA in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:**1045–1053.
12. **Dalton, M., and M. Sinensky.** 1995. Expression systems for nuclear lamin proteins: farnesylation in assembly of nuclear lamina. Methods Enzymol. **250:**134–148.
13. **Estojak, J., R. Brent, and E. A. Golemis.** 1995. Correlation of two-hybrid affinity data with *in vitro* measurements. Mol. Cell. Biochem. **15:**5820–5829.
14. **Fabre, E., and E. C. Hurt.** 1994. Nuclear transport. Curr. Opin. Cell Biol. **6:**335–342.
15. **Firmbach-Kraft, I., and R. Stick.** 1993. The role of CaaX-dependent modifications in membrane association of *Xenopus* nuclear lamin B3 during meiosis and the fate of B3 in transfected mitotic cells. J. Cell Biol. **123:**1661–1670.

15a. **Fisher, P. A.** Unpublished data.

16. **Fisher, P. A., M. Berrios, and G. Blobel.** 1982. Isolation and characterization of a proteinaceous subnuclear fraction composed of nuclear matrix, peripheral lamina, and nuclear pore complexes from embryos of *Drosophila melanogaster*. J. Cell Biol. **92:**674–686.

17. **Gerace, L.** 1986. Nuclear lamina and organization of nuclear architecture. Trends Biochem. Sci. **11:**443–446.
18. **Gerace, L., and B. Burke.** 1988. Functional organization of the nuclear envelope. Annu. Rev. Cell Biol. **4:**335–374.
19. **Gerace, L., and R. Foisner.** 1994. Integral membrane proteins and dynamic organization of the nuclear envelope. Trends Cell Biol. **4:**127–131.
20. **Glass, C. A., J. R. Glass, H. Taniura, K. W. Hasel, J. M. Blevitt, and L. Gerace.** 1993. The alpha-helical rod domain of human lamins A and C contains a chromatin binding site. EMBO J. **12:**4413–4424.
21. **Goldberg, M. W., and T. D. Allen.** 1995. Structural and functional organization of the nuclear envelope. Curr. Opin. Cell Biol. **7:**301–309.
21a. **Gruenbaum, Y.** Unpublished data.
22. **Gruenbaum, Y., Y. Landesman, B. Drees, J. W. Bare, H. Saumweber, M. P. Paddy, J. W. Sedat, D. E. Smith, B. M. Benton, and P. A. Fisher.** 1988. *Drosophila* nuclear lamin precursor Dm_0 is translated from either of two developmentally regulated mRNA species apparently encoded by a single gene. J. Cell Biol. **106:**585–596.
23. **Harel, A., E. Zlotkin, S. Nainudel-Epszteyn, N. Feinstein, P. Fisher, and Y. Gruenbaum.** 1989. Persistence of major nuclear envelope antigens in an envelope-like structure during mitosis in *Drosophila melanogaster* embryos. J. Cell Sci. **94:**463–470.
24. **Heitlinger, E., M. Peter, A. Lustig, W. Villiger, E. A. Nigg, and U. Aebi.** 1992. The role of the head and tail domain in lamin structure and assembly: analysis of bacterially-expressed chicken lamin A and truncated B2 lamins. J. Struct. Biol. **108:**74–89.
25. **Hennekes, H., and E. A. Nigg.** 1994. The role of isoprenylation in membrane attachment of nuclear lamins. J. Cell Sci. **107:**1019–1029.
26. **Hoey, Y., R. O. J. Weinzierl, G. Gill, J. Chen, B. D. Dynlacht, and R. Tjian.** 1993. Molecular cloning and functional analysis of *Drosophila* TAF110 reveal properties expected of coactivators. Cell **72:**247–260.
27. **Holtz, D., R. A. Tanaka, J. Hartwig, and F. McKeon.** 1989. The CaaX motif of lamin A functions in conjunction with the nuclear localization signal to target assembly to the nuclear envelope. Cell **59:**969–977.
28. **Hutchison, C. J., J. M. Bridger, L. S. Cox, and I. R. Kill.** 1994. Weaving a pattern from disparate threads: lamin function in nuclear assembly and DNA replication. J. Cell Sci. **107:**3259–3269.
29. **Judd, B. H., and M. W. Young.** 1973. An examination of the one cistron: one chromomere concept. Cold Spring Harbor Symp. Quant. Biol. **38:**573–579.
30. **Karr, T. L., and B. M. Alberts.** 1986. Organization of the cytoskeleton in early *Drosophila* embryos. J. Cell Biol. **102:**1494–1509.
31. **Kitten, G. T., and E. A. Nigg.** 1991. The CaaX motif is required for isoprenylation, carboxyl methylation, and nuclear membrane association of lamin B2. J. Cell Biol. **113:**13–23.
32. **Krohne, G., I. Waizenegger, and T. H. Hoeger.** 1989. The conserved carboxy-terminal cysteine of nuclear lamins is essential for lamin association with the nuclear envelope. J. Cell Biol. **109:**2003–2011.
33. **Lenz-Böhme, B., J. Wismar, S. Fuchs, R. Reifegerste, E. Buchner, H. Betz, and B. Schmitt.** 1997. Insertional mutagenesis of the *Drosophila* nuclear lamin Dm_0 gene results in defective nuclear envelopes, clustering of nuclear pore complexes, and accumulation of annulate lamellae. J. Cell Biol. **137:**1001–1016.
34. **Lin, H., and M. F. Wolfner.** 1991. The *Drosophila* maternal-effect gene *fs(1)Ya* encodes a cell cycle-dependent nuclear envelope component required for embryonic mitosis. Cell **64:**49–62.
35. **Liu, J., H. Lin, J. M. Lopez, and M. F. Wolfner.** 1997. Formation of the male pronuclear lamina in *Drosophila*. Dev. Biol. **184:**187–196.
36. **Liu, J., J. M. Lopez, and M. F. Wolfner.** 1996. Developmental modulation of the nuclear envelope. Curr. Top. Dev. Biol. **35:**47–70.
37. **Liu, J., K. Song, and M. F. Wolfner.** 1995. Mutational analyses of *fs(1)Ya*, an essential, developmentally regulated, nuclear envelope protein in *Drosophila*. Genetics **141:**1473–1481.
37a. **Liu, J., and M. F. Wolfner.** Unpublished data.
38. **Liu, J., and M. F. Wolfner.** 1998. Functional dissection of YA, an essential, developmentally regulated nuclear lamina protein in *Drosophila*. Mol. Cell. Biol. **18:**188–197.
39. **Lopez, J., K. Song, A. Hirshfeld, H. Lin, and M. F. Wolfner.** 1994. The *Drosophila fs(1)Ya* protein, which is needed for the first mitotic division, is in the nuclear lamina and in the envelopes of cleavage nuclei, pronuclei and nonmitotic nuclei. Dev. Biol. **163:**202–211.
40. **Lopez, J. M., and M. F. Wolfner.** 1997. The developmentally regulated *Drosophila* embryonic nuclear lamina protein 'Young Arrest' (fs(1)Ya) is capable of associating with chromatin. J. Cell Sci. **110:**643–651.
41. **Miller, K. G., T. L. Karr, D. R. Kellogg, J. I. Mohr, M. Walter, and B. M. Alberts.** 1985. Studies on the cytoplasmic organization of early *Drosophila* embryos. Cold Spring Harbor Symp. Quant. Biol. **50:**79–90.
42. **Nigg, E. A.** 1989. The nuclear envelope. Curr. Opin. Cell Biol. **1:**435–440.
43. **Padan, R., S. Nainudel-Epszteyn, R. Goitein, A. Fainsod, and Y. Gruenbaum.** 1990. Isolation and characterization of the *Drosophila* nuclear envelope otefin cDNA. J. Biol. Chem. **265:**7808–7813.
44. **Paddy, M. R., A. S. Belmont, H. Saumweber, D. A. Agard, and J. W. Sedat.** 1990. Interphase nuclear envelope lamins form a discontinuous network that interacts with only a fraction of the chromatin in the nuclear periphery. Cell **62:**89–106.
45. **Pascal, E., and R. Tjian.** 1991. Different activation domains of SP1 govern formation of multimers and mediate transcriptional synergism. Genes Dev. **5:**1646–1656.
46. **Riemer, D., N. Stuurman, M. Berrios, C. Hunter, P. A. Fisher, and K. Weber.** 1995. Expression of *Drosophila* lamin C is developmentally regulated: analogies with vertebrate A-type lamins. J. Cell Sci. **108:**3189–3198.
47. **Riemer, D., and K. Weber.** 1994. The organization of the gene for *Drosophila* lamin C: limited homology with vertebrate lamin genes and lack of homology versus the *Drosophila* lamin Dm_0 gene. Euro. J. Cell. Biol. **63:**299–306.
48. **Rzepecki, R., S. Bogachev, E. Kokoza, N. Stuurman, and P. A. Fisher.** 1998. *In vivo* association of lamins with nucleic acids in *Drosophila melanogaster*. J. Cell Sci. **111:**121–129.
49. **Sasse, B., A. Lustig, U. Aebi, and N. Stuurman.** 1997. *In vitro* assembly of *Drosophila* lamin Dm_0: lamin polymerization properties are conserved. Eur. J. Biochem. **250:**30–38.
50. **Schmidt, M., and G. Krohne.** 1995. *In vivo* assembly kinetics of fluorescently-labeled *Xenopus* lamin A mutants. Eur. J. Cell Biol. **68:**345–354.
51. **Smith, D. E., and P. A. Fisher.** 1989. Interconversion of *Drosophila* nuclear lamin isoforms during oogenesis, early embryogenesis, and upon entry of cultured cells into mitosis. J. Cell Biol. **108:**255–265.
52. **Smith, D. E., Y. Gruenbaum, M. Berrios, and P. A. Fisher.** 1987. Biosynthesis and interconversion of *Drosophila* nuclear lamin isoforms during normal growth and in response to heat shock. J. Cell Biol. **105:**771–790.
53. **Song, K.** 1994. Developmental, genetic, and biochemical studies of fs(1)Ya, a nuclear envelope protein required for embryonic mitosis in *Drosophila*. Ph.D. thesis, Cornell University, Ithaca, N.Y.
54. **Soullam, B., and H. J. Worman.** 1995. Signals and structural features involved in integral membrane protein targeting to the inner nuclear membrane. J. Cell Biol. **130:**15–27.
55. **Sterne-Marr, R., J. M. Blevitt, and L. Gerace.** 1992. O-linked glycoproteins of the nuclear pore complex interact with a cytosolic factor required for nuclear protein import. J. Cell Biol. **116:**271–280.
56. **Stuurman, N., N. Maus, and P. A. Fisher.** 1995. Interphase phosphorylation of the *Drosophila* nuclear lamin: site-mapping using a monoclonal antibody. J. Cell Sci. **108:**3137–3144.
57. **Stuurman, N., B. Sasse, and P. A. Fisher.** 1996. Intermediate filament protein polymerization: Molecular analysis of *Drosophila* nuclear lamin head-to-tail binding. J. Struct. Biol. **117:**1–15.
58. **Taniura, H., C. Glass, and L. Gerace.** 1995. A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones. J. Cell Biol. **131:**33–44.
59. **Turner, S. G.** 1996. Interactions and posttranslational modifications of YA, a *Drosophila* nuclear envelope protein. M.S. thesis, Cornell University, Ithaca, N.Y.
60. **Ulitzur, N., A. Harel, N. Feinstein, M. Goldberg, and Y. Gruenbaum.** 1997. Nuclear membrane vesicle targeting to chromatin in a *Drosophila* embryo cell-free system. Mol. Biol. Cell **8:**1439–1448.
61. **Ulitzur, N., A. Harel, N. Feinstein, and Y. Gruenbaum.** 1992. Lamin activity is essential for nuclear envelope assembly in a *Drosophila* embryo cell-free extract. J. Cell Biol. **115:**941–948.
62. **Whalen, A. M., M. McConnell, and P. A. Fisher.** 1991. Developmental regulation of *Drosophila* DNA topoisomerase II. J. Cell Biol. **112:**203–214.
63. **Yang, L., T. Guan, and L. Gerace.** 1997. Integral membrane proteins of the nuclear envelope are dispersed throughout the endoplasmic reticulum during mitosis. J. Cell Biol. **137:**1199–1210.
63a. **Yu, J., and M. F. Wolfner.** Unpublished data.
64. **Zhao, K., A. Harel, N. Stuurman, D. Guedalia, and Y. Gruenbaum.** 1996. Binding of matrix attachment regions to nuclear lamin is mediated by the rod domain and depends on the lamin polymerization state. FEBS Lett. **380:**161–164.