The JIL-1 kinase interacts with lamin Dm_0 and regulates nuclear lamina morphology of *Drosophila* nurse cells

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Summary

We have used a yeast two-hybrid screen to identify lamin Dm_0 as an interaction partner for the nuclear JIL-1 kinase. This molecular interaction was confirmed by GST-fusion protein pull-down assays and by co-immunoprecipitation experiments. Using deletion construct analysis we show that a predicted globular domain of the basic region of the COOH-terminal domain of JIL-1 was sufficient for mediating the molecular interactions with lamin Dm_0. A reciprocal analysis with truncated lamin Dm_0 constructs showed that the interaction with JIL-1 required sequences in the tail domain of lamin Dm_0 that include the Ig-like fold. Further support for a molecular interaction between JIL-1 and lamin Dm_0 in vivo was provided by genetic interaction assays. We show that nuclear positioning and lamina morphology were abnormal in *JIL-1* mutant egg chambers. The most common phenotypes observed were abnormal nurse cell nuclear lamina protrusions through the ring canals near the oocyte, as well as dispersed and mislocalized lamin throughout the egg chamber. These phenotypes were completely rescued by a full-length *JIL-1* transgenic construct. Thus, our results suggest that the JIL-1 kinase is required to maintain nuclear morphology and integrity of nurse cells during oogenesis and that this function may be linked to molecular interactions with lamin Dm_0.

Key words: Lamin, Oogenesis, Nuclear lamina, JIL-1, *Drosophila*

Introduction

Control of gene expression is a complex process involving regulatory mechanisms that are integrated at multiple hierarchical levels ranging from primary regulatory DNA sequences to interactions of chromatin with the nuclear lamina (reviewed by Goldberg et al., 1999a; Wilson et al., 2001; Goldman et al., 2002; van Driel et al., 2003). In *Drosophila* we have recently identified a chromosomal tandem kinase, JIL-1, that regulates chromatin structure (Jin et al., 1999; Wang et al., 2001; Deng et al., 2005) and has been implicated in transcriptional regulation (Zhang et al., 2003a; Zhang et al., 2003b) – it localizes to interband regions of polytene chromosomes and is a member of the MSL (male specific lethal) dosage compensation complex (Jin et al., 2000). JIL-1 is an essential kinase and mutational analysis has shown that it is the predominant kinase regulating histone H3 Ser10 phosphorylation in the interphase nucleus (Wang et al., 2001). However, it is not known whether physiological substrates of JIL-1 may include other nuclear proteins or whether there are proteins directly regulating the function of JIL-1. To identify such proteins that interact with JIL-1 we carried out yeast two-hybrid screens using different JIL-1 regions as bait. Here we report that the *Drosophila* nuclear lamin Dm_0 directly interacts with the COOH-terminal domain of JIL-1.

Lamins are intermediate filament proteins that are constituents of the inner nuclear membrane (Goldman et al., 2002). In *Drosophila* there are two lamins, lamin Dm_0 and lamin C (Riemer et al., 1995). Lamin Dm_0 is present in all *Drosophila* cells except mature sperm, whereas lamin C is expressed only after stage 12 of embryogenesis (Riemer et al., 1995; Lopez and Wolfner, 1997). Lamins and associated proteins have recently been implicated in multiple functions including maintenance of nuclear structure and regulation of chromatin organization, and they may have structural roles in the elongation phase of DNA replication (reviewed in Wilson et al., 2001). Defects in one or more of these functions are likely to be responsible for the majority of clinical symptoms and tissue specific pathologies found in human laminopathies (Wilson et al., 2001). In addition, in *Drosophila*, nuclear lamins have been shown to be involved in determining aspects of cytoplasmic organization and egg polarity (Guillemin et al., 2001), as well as in regulating nuclear migration of photoreceptor cells through links to the cytoskeleton (Patterson et al., 2004).

In this study we have used deletion construct analysis of lamin Dm_0 to map the regions required for binding to the JIL-1 COOH-terminal domain. We show that the binding activity is localized to a region within the tail-domain of lamin Dm_0 that includes the Ig-like fold. Furthermore, we show that nuclear positioning and lamina morphology are abnormal in *JIL-1* mutant egg chambers. The most common phenotypes observed were abnormal nurse cell nuclear lamina protrusions

through the ring canals near the oocyte as well as dispersed and mislocalized lamin throughout the egg chamber. These phenotypes were completely rescued by a full-length *JIL-1* transgenic construct. Thus, our results suggest that JIL-1 kinase activity is required to maintain nuclear morphology and integrity of nurse cells during oogenesis and that this function may be linked to molecular interactions with lamin Dm_0.

Materials and Methods

Drosophila stocks

Fly stocks were maintained according to standard protocols (Roberts, 1986). Oregon-R or Canton-S was used for wild-type preparations. The *JIL-1*^{EP3657} (*JIL-1*^{3657}), *JIL-1*^{h9} and *JIL-1*^{z2} alleles have been previously described (Wang et al., 2001; Zhang et al., 2003a). Balancer chromosomes and mutant alleles are described in Lindsley and Zimm (Lindsley and Zimm, 1992). The *Lam*^{4643} allele was obtained from the Bloomington *Drosophila* Stock Center and the *Lam*^{A25} allele was the generous gift of J. A. Fischer (University of Texas at Austin). The relative viability of double mutant flies was determined by performing crosses and dividing the numbers of eclosed flies of each genotype with the total number of eclosed flies. For rescue experiments a line carrying a full-length *JIL-1* transgene on the second chromosome was crossed into *JIL-1*^{z2}/*JIL-1*^{h9} heterozygous flies as previously described (Wang et al., 2001). All genetic crosses and interaction assays were conducted at 23°C.

Yeast two-hybrid interaction assays

JIL-1 cDNA sequence encoding a 314 amino acid (aa) fragment comprising JIL-1's COOH-terminal domain (JIL-1 CTD) was subcloned in-frame into the yeast two hybrid bait vector pGBKT7 (Clontech) using standard methods (Sambrook and Russell, 2001) and verified by sequencing (Iowa State University Sequencing Facility). The JIL-1 CTD bait was used to screen a *Drosophila* 0-2 hour embryonic yeast two-hybrid library (the generous gift of L. Ambrosio, Iowa State University, Ames) as previously described (Zhang et al., 2003b; Rath et al., 2004). Two positive cDNA clones were isolated, retransformed into yeast cells containing the JIL-1 CTD bait to verify the interaction, and sequenced. Homology searches identified the clones as two independent isolates of lamin Dm_0 of different lengths containing residues 216-622 and 270-622, respectively.

Antibodies

The lamin Dm_0 mAb HL1203 was provided by M. Paddy (University of California at Davis) and H. Saumweber (Humboldt University, Berlin, Germany) and has been previously characterized (Gruenbaum et al., 1988); the anti-lamin polyclonal antiserum R836 (Stuurman et al., 1996) was the gift of P. Fisher (State University of New York at Stony Brook). The affinity purified Hope rabbit anti-JIL-1 polyclonal antibody was described by Jin et al. (Jin et al., 1999) and the anti-GST mAb 8C7 by Rath et al. (Rath et al., 2004).

Biochemical analysis

SDS-PAGE and immunoblotting

SDS-PAGE was performed according to standard procedures (Laemmli, 1970). Electroblot transfer was performed as described previously (Towbin et al., 1979) with transfer buffer containing 20% methanol and in most cases including 0.04% SDS. For these experiments we used the Bio-Rad Mini PROTEAN II system, electroblotting to 0.2 μm nitrocellulose, and using anti-mouse or anti-rabbit HRP-conjugated secondary antibody (Bio-Rad) (1:3000) for visualization of primary antibody diluted 1:1000 in Blotto. The signal was visualized using chemiluminescent detection methods (ECL kit, Amersham). The immunoblots were digitized using a flatbed scanner (Epson Expression 1680). Lamin Dm_0 levels in *Lam*^{4643}/*CyO*; *JIL-1*^{3657}/*JIL-1*^{3657} flies were compared with the levels in *Sp/CyO*; *JIL-1*^{3657}/*JIL-1*^{3657} flies by homogenization of single adult flies of the appropriate genotype in 25 μl of ip buffer before boiling in SDS-sample buffer. For quantification of immunolabeling, digital images of exposures of immunoblots on Biomax ML film (Kodak) were analyzed using the ImageJ software as previously described (Wang et al., 2001). In these images the grayscale was adjusted such that only a few pixels in the control lanes were saturated. The area of each band was traced using the outline tool and the average pixel value determined. Levels in mutant larvae were determined as a percentage relative to the level determined for control larvae using tubulin as a loading control.

Immunoprecipitation assays

For co-immunoprecipitation experiments, anti-lamin (mAb HL1203) or anti-JIL-1 antibody (affinity purified Hope-antiserum) was bound to 10 μl protein-G Sepharose beads (Sigma) for 2.5 hours at 4°C on a rotating wheel in 50 μl ip buffer (20 mM Tris-HCl pH 8.0, 10 mM EDTA, 1 mM EGTA, 150 mM NaCl, 2 mM Na_3VO_4, 0.2% Triton X-100, 0.2% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride and 1.5 μg/ml Aprotinin). Protein extracts prepared from S2 cells (10^7 cells/ml) were homogenized in immunoprecipitation (ip) buffer, sonicated three times, and the supernatant cleared by centrifugation at 16,000 ***g*** for 10 minutes at 4°C. The appropriate antibody-coupled beads or beads only were washed and incubated overnight at 4°C with 250 μl of S2 cell lysate on a rotating wheel. Beads were washed four times for 10 minutes each with 1 ml of ip buffer with low speed pelleting of beads between washes. The resulting bead-bound immunocomplexes were analyzed by SDS-PAGE and western blotting according to standard techniques (Harlow and Lane, 1988) using mAb HL1203 to detect lamin Dm_0 and pAb Hope to detect JIL-1. In addition, control ips were performed with Hope preimmune serum and with the leech anti-Tractin mAb 1H4 (Xu et al., 2000).

Pull-down assays

In pull-down assays GST-fusion proteins were used to pull down endogenous lamin Dm_0 and JIL-1 proteins from S2 cell lysates. Initially the JIL-1 GST-fusion proteins NTD (aa 1-211) and CTD (aa 927-1207), which have been previously described (Jin et al., 2000), and a lamin Dm_0 fragment containing residue 260-622 that was RT-PCR amplified from mRNA extractions from S2 cells were cloned into the pGEX4T vector and expressed in *Escherichia. coli* using standard techniques (Sambrook and Russell, 2001). For subsequent experiments GST-fusion proteins with various truncations of the COOH-terminal domains of both lamin Dm_0 and JIL-1 were generated from PCR amplification and insertion into the pGEX4T vector. For the in vitro protein-protein interaction assays, approximately 2 μg of GST or the appropriate GST-fusion protein were coupled with glutathione agarose beads and incubated with 300 μl of S2 cell lysate (from ~10^7 cells) at 4°C overnight on a rotating wheel. The beads were washed four times for 10 minutes each in 1 ml PBS with 0.5% Tween-20, and proteins retained on the glutathione agarose beads were analyzed by SDS-PAGE and western blotting with signals detected by ECL chemiluminescence (Amersham).

Immunohistochemistry

Antibody labelings of ovaries, embryos, imaginal discs and polytene salivary glands were performed as previously described (Wang et al., 2001; Zhang et al., 2003a; Johansen and Johansen, 2003). The fixative was either 4% paraformaldehyde in phosphate buffered saline (PBS) or Bouin's fluid (0.66% picric acid, 9.5% formalin, 4.7% acetic acid). Single, double and triple labelings employing epifluorescence were

performed using various combinations of antibodies against lamin (mAb HL1203; pAb R836), JIL-1 (Hope pAb), Hoechst to visualize the DNA, and rhodamine-conjugated phalloidin (Molecular Probes) to visualize the actin cytoskeleton. The appropriate species- and isotype-specific Texas Red-, TRITC- and FITC-conjugated secondary antibodies (Cappel/ICN, Southern Biotech) were used (1:200 dilution) to visualize primary antibody labeling. Confocal microscopy was performed with a Leica confocal TCS NT microscope system equipped with separate Argon-UV, Argon and Krypton lasers and the appropriate filter sets for Hoechst, FITC, Texas Red and TRITC imaging. A separate series of confocal images for each fluorophor of double labeled preparations were obtained simultaneously with z-intervals of typically 0.5 μm using a PL APO 100X/1.40-0.70 oil objective. A maximum projection image for each of the image stacks was obtained using the ImageJ software (http://rsb.info.nih.gov/ij/). In some cases individual slices or projection images from only two to three slices were obtained. Images were imported into Photoshop where they were pseudocolored, image processed and merged.

Results

JIL-1 COOH-terminal domain interacts with the lamin Dm_0 tail domain

To identify proteins that have direct interactions with the COOH-terminal domain (CTD) of the JIL-1 kinase, we performed a yeast two-hybrid screen using residues 893-1207 of the JIL-1 protein as bait. Positive clones detected in the primary screen were confirmed by β-galactosidase two-hybrid interaction assays on filter paper following retransformation of the candidate clones and JIL-1 CTD bait plasmid into the yeast strain AH109 (data not shown). From this screen, two independent clones containing lamin Dm_0 fragments of different lengths (residues 216-622 and 270-622, respectively) that both included the tail domain were identified. To further verify the interaction between lamin Dm_0 and JIL-1 that we observed in the yeast two-hybrid assays we performed pull-down assays with JIL-1 NH_2- and COOH-terminal GST-fusion proteins using protein extracts from the S2 cell line. The two JIL-1-GST fusion proteins were coupled with glutathione agarose beads, incubated with S2 cell lysate, washed, fractionated by SDS-PAGE and analyzed by immunoblot analysis using lamin Dm_0 specific antibody (Fig. 1A). Whereas the GST-JIL-1-NTD and beads-only controls showed no pull-down activity, GST-JIL-1-CTD was able to pull down lamin Dm_0 as detected by the lamin Dm_0 antibody. Owing to the SDS-PAGE conditions used the characteristic lamin Dm_0 interphase protein doublet of 74 kDa and 76 kDa (Smith and Fisher, 1989) has not been resolved and instead appears as a single band. Western blot analysis of the GST proteins purified in these experiments and detected with GST-antibody showed that approximately equivalent levels of GST-JIL-1-NTD and GST-JIL-1-CTD fusion proteins were present in these assays (Fig. 1B). In the reciprocal pull-down experiments a GST-lamin Dm_0 fusion protein composed of residues 260-622 (similar in length to the smaller of the two identified two-hybrid interacting clones) was incubated with total S2 cell lysate and analyzed by immunoblot analysis as described above (Fig. 1C). GST protein coupled to glutathione-agarose beads or beads-only incubated with total S2 cell lysate served as controls. Whereas the GST and beads-only controls showed no pull-down activity, the GST-lamin Dm_0 fusion protein was able to pull down JIL-1 as detected by JIL-1 antibody. Western

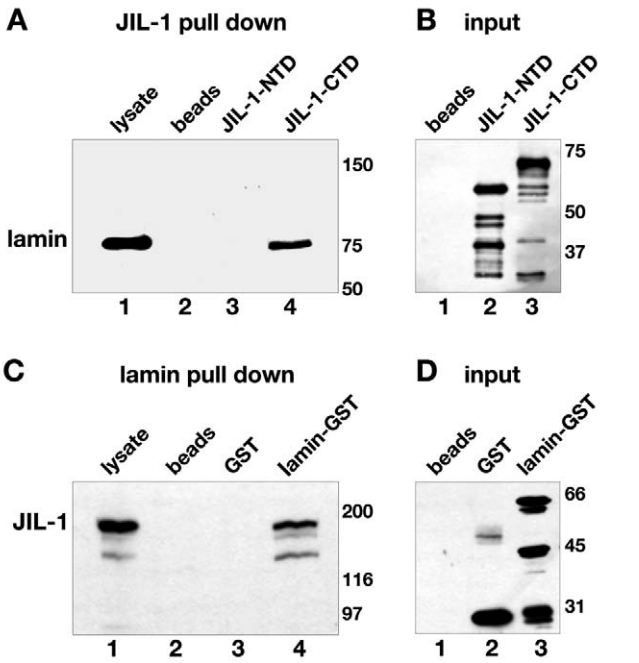

Fig. 1. Lamin Dm_0 interacts with JIL-1 in pull-down assays. (A) S2 cell lysate incubated with JIL-1-NTD (aa 1-211) or JIL-1-CTD (aa 927-1207) GST-fusion protein constructs or with a beads-only control was pelleted with glutathione-agarose beads and the interacting protein(s) fractionated by SDS-PAGE, western blotted, and probed with the lamin mAb HL1203. Unincubated S2 cell lysate was included as a control (lane 1). Only the JIL-1-CTD construct was able to pull down the 76 kDa lamin protein (lane 4) also detected in the cell lysate, whereas no interaction was observed with the GST-only control (lane 2) or the JIL-1-NTD construct (lane 3). (B) Immunoblot of the input GST-fusion proteins (JIL-1-NTD and JIL-1-CTD) used for the pull-down experiments in A detected with the anti-GST mAb 8C7. (C) S2 cell lysate incubated with a lamin-GST fusion construct (aa 260-622) or a GST-only control or with beads-only was pelleted with glutathione-agarose beads and the interacting protein(s) fractionated by SDS-PAGE, western blotted, and probed with affinity purified JIL-1 antiserum. Unincubated S2 cell lysate was included as a control (lane 1). The lamin-GST fusion protein construct was able to pull down the 160 kDa JIL-1 protein (lane 4) also detected in the cell lysate while no interaction was observed with the GST-only control (lane 3) or with the beads-only (lane 2). (D) Immunoblot of the input GST-fusion protein (lamin-GST) and the GST control used for the pull-down experiments in C detected with the anti-GST mAb 8C7. The relative migration of molecular weight markers is indicated to the right in kDa.

blot analysis of the GST proteins purified in these experiments and detected with anti-GST antibody showed that approximately equivalent levels of GST-lamin Dm_0 and GST protein were present in these assays (Fig. 1D). In addition, we performed immunoprecipitation (ip) experiments using S2 cell lysates. For these immunoprecipitation experiments proteins were extracted from S2 cells, immunoprecipitated using either JIL-1 or lamin Dm_0 specific antibodies, fractionated on SDS-PAGE after the ip, immunoblotted, and probed with antibodies to lamin Dm_0 and JIL-1, respectively. Fig. 2A shows an ip experiment using lamin Dm_0 antibody where the immunoprecipitate is detected by JIL-1 antibody as a 160 kDa band that is also present in the S2 cell lysate. This band was

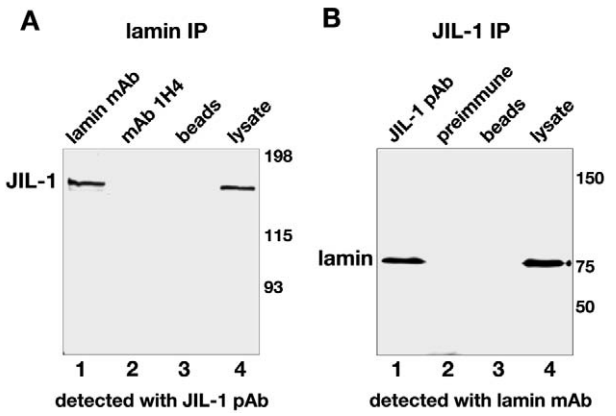

Fig. 2. JIL-1 and lamin Dm_0 immunoprecipitation assays. (A) Immunoprecipitation (ip) of S2 cell lysate was performed using the lamin mAb HL1203 (lane 1) and the leech tractin control mAb 1H4 (lane 2) coupled to immunobeads or with immunobeads only (lane 3). The immunoprecipitations were analyzed by SDS-PAGE and western blotting using JIL-1 antiserum for detection. JIL-1 antiserum staining of S2 cell lysate is shown in lane 4. JIL-1 is detected in the lamin immunoprecipitation sample as a 160 kDa band (lane 1) but not in the control samples (lanes 2 and 3). (B) Immunoprecipitation (ip) of S2 cell lysate was performed using JIL-1 antiserum (lane 1) as well as a pre-immune serum control (lane 2) coupled to immunobeads or with immunobeads only (lane 3). The immunoprecipitations were analyzed by SDS-PAGE and western blotting using lamin mAb HL1203 for detection. Lamin mAb HL1203 staining of S2 cell lysate is shown in lane 4. Lamin is detected in the JIL-1 antiserum immunoprecipitation sample as a 76 kDa band (lane 1) but not in the pre-immune or beads-only control samples (lanes 2 and 3). The relative migration of molecular weight markers is indicated to the right in kDa.

not present in lanes where a control mAb (mAb 1H4 is specific to an unrelated leech antigen) (Xu et al., 2000) or immunobeads-only were used for the ip (Fig. 2A). Fig. 2B shows the converse experiment: JIL-1 antiserum immunoprecipitated a 76 kDa band detected by lamin Dm_0 antibody that was also present in S2 cell lysate but not in control ips with immunobeads only. The lamin Dm_0 band was also not present in lanes immunoprecipitated with JIL-1 preimmune serum (Fig. 2B). These results strongly indicate that lamin Dm_0 and the JIL-1 kinase are present in the same protein complex.

Mapping of the JIL-1 and lamin Dm_0 interaction domains

The region of JIL-1 that was found to interact with lamin, the JIL-1 CTD-domain, can be further divided into two distinct regions: an acidic region from residue 887-1033 that has a predicted pI<4 and a basic region from residue 1034-1207 that has a pI>11 (Fig. 3A). Furthermore, using the program GlobPlot (Linding et al., 2003) we identified a putative globular domain present within the basic region that spans residues 1065-1187. Thus, to better define the sequences of JIL-1 responsible for the molecular interaction between JIL-1 and lamin Dm_0, we generated GST fusion proteins comprising these three regions: CTD-A, CTD-B and CTD-G (Fig. 3A) and performed pull-down experiments of proteins from S2 cell lysate as described above. As shown in Fig. 3B the CTD, CTD-

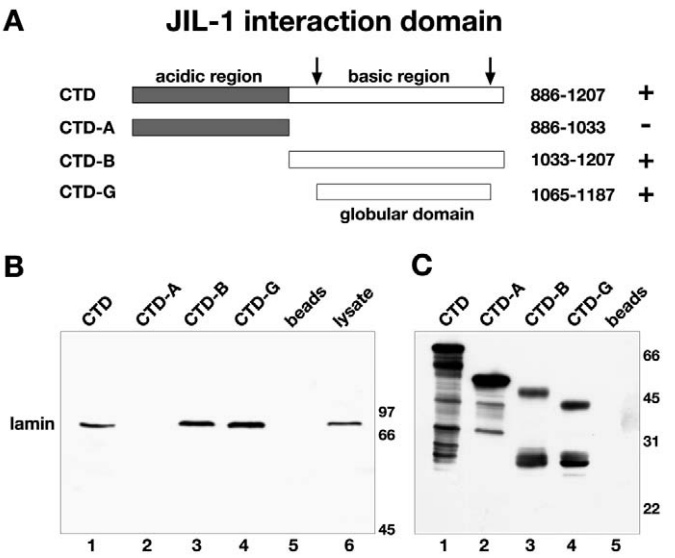

Fig. 3. Mapping of the JIL-1 interaction domain with lamin Dm_0. (A) The truncated COOH-terminal JIL-1 GST-fusion protein constructs used for domain mapping. The JIL-1 COOH-terminal domain can be divided into predominantly acidic and basic regions, with the basic region containing a predicted globular domain. (B) S2 cell lysate was incubated with the various truncated JIL-1 GST-fusion protein constructs shown in A or with a beads-only control and pelleted with glutathione-agarose beads. Interacting protein(s) were fractionated by SDS-PAGE, western blotted, and probed with the lamin mAb HL1203. Unincubated S2 cell lysate was included as a control (lane 6). The CTD, CTD-B and CTD-G were able to pull down the 76 kDa lamin protein (lanes 1, 3 and 4) also detected in the cell lysate (lane 6), whereas no interaction was observed with the CTD-A construct (lane 2) or with the beads-only control (lane 5). This defined the globular domain in the basic region as sufficient for mediating interactions with lamin Dm_0. (C) Immunoblot of the input GST-fusion proteins used for the pull-down experiments in (B) detected with the anti-GST mAb 8C7. The relative migration of molecular weight markers is indicated to the right in kDa.

B and CTD-G fusion proteins all pulled down a 76 kDa protein detected by lamin Dm_0 antibody also present in the S2 cell lysate. This band was not present in pull-down assays with the CTD-A fusion protein or in beads-only controls (Fig. 3B), providing strong support for the specificity of the assay as a negative control. Immunoblot analysis of each of the input GST fusion proteins probed with anti-GST antibody showed comparable levels of GST-fusion proteins in each of the pull-down assays (Fig. 3C). These results suggest that the prospective globular domain of the basic region of the COOH-terminal domain of JIL-1 is sufficient for mediating molecular interactions with lamin Dm_0.

To determine the corresponding region of lamin Dm_0 responsible for its molecular interaction with JIL-1, we generated GST fusion proteins with truncations of the lamin Dm_0 clone containing residues 260-622 that had been shown to interact with JIL-1 in the yeast two-hybrid assays and in the initial pull-down experiments (Fig. 1C). These constructs, lamin-D1, lamin-D2, lamin-D3 and lamin-D4, are illustrated in Fig. 4A and encompass various parts of the lamin Dm_0 tail- and rod-domain including the Ig-like fold in the tail domain (Dhe-Paganon et al., 2002). The constructs were used in pull-

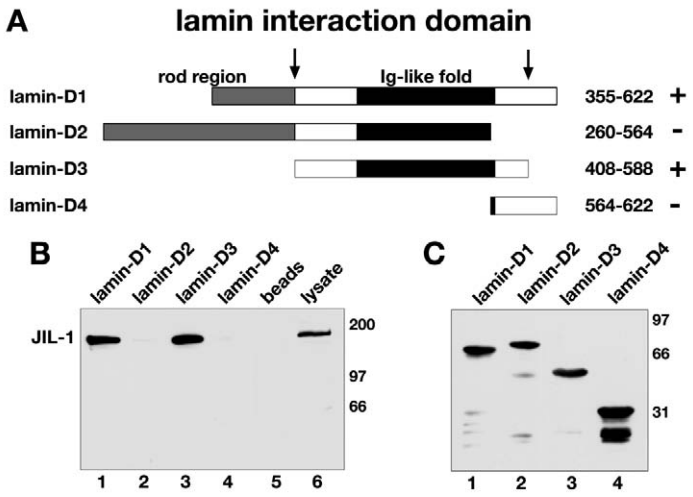

Fig. 4. Mapping of the lamin Dm_0 interaction domain with JIL-1. (A) The truncated COOH-terminal lamin Dm_0 GST-fusion protein constructs used for domain mapping. The Ig-like fold of the lamin Dm_0 tail domain (white) is shown in black. Some of the constructs also contained parts of the rod domain (shown in gray). (B) S2 cell lysate was incubated with the various truncated lamin GST-fusion protein constructs shown in A or with a beads-only control and pelleted with glutathione-agarose beads. Interacting protein(s) were fractionated by SDS-PAGE, western blotted and probed with JIL-1 antiserum. Unincubated S2 cell lysate was included as a control (lane 6). The lamin-D1 and lamin-D3 constructs were able to pull down the 160 kDa JIL-1 protein (lanes 1 and 3) also detected in the cell lysate (lane 6), whereas no interaction was observed with the lamin-D2 and lamin-D4 constructs (lanes 2 and 4) or with the beads-only control (lane 5). This defined the lamin-D3 domain in the tail region as sufficient for mediating interactions with JIL-1. (C) Immunoblot of the input GST-fusion proteins used for the pull-down experiments in B detected with the anti-GST mAb 8C7. The relative migration of molecular weight markers is indicated to the right in kDa.

down assays of *Drosophila* S2 cell extract and analyzed by immunoblot analysis with JIL-1 antibody (Fig. 4B). The results from these experiments showed that fusion proteins that contained residues from the beginning of the tail domain to just after the end of the Ig-like fold (lamin-D1 and lamin-D3) were able to pull down a 160 kDa protein band recognized by JIL-1 antibody that was also present in the S2 cell lysate (Fig. 4B). By contrast, fusion proteins truncated at the end of the Ig-like fold (lamin-D2) or the COOH-terminal sequences after the Ig-like fold (lamin-D4) were not able to pull down JIL-1 (Fig. 4B). Immunoblot analysis of each of the input GST fusion proteins probed with anti-GST antibody showed comparable levels of GST-fusion proteins in each of the pull-down assays (Fig. 4C). These results suggest that the interaction between JIL-1 and lamin Dm_0 requires sequences between residues 408 and 588 in the tail domain that include the Ig-like fold. Attempts to define a smaller binding domain within this region were unsuccessful (data not shown), which may indicate the presence of a discontinuous binding surface.

Abnormalities of nuclear lamina morphology in *JIL-1* mutant egg chambers

To determine whether JIL-1 was required for maintaining nuclear lamina organization we analyzed lamin Dm_0 distribution in *JIL-1* mutant backgrounds. For these experiments we examined fixed ovaries, embryos, imaginal discs and polytene salivary glands labeled with lamin Dm_0 antibody. No obvious morphological changes in the lamina of nuclei were evident in embryonic or larval tissues of the null $JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ genotype (data not shown). This included third instar larval salivary gland nuclei which, because of the absence of JIL-1 protein, have severely perturbed polytene chromosomes (Wang et al., 2001; Deng et al., 2005). We could not examine ovaries from $JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ flies because they die before eclosion; however, in egg chambers of ovaries from $JIL\text{-}1^{z2}/JIL\text{-}1^{h9}$ flies, which do eclose, we observed a high penetrance of abnormalities in nurse cell nuclear lamina morphology and lamin Dm_0 localization defects (Fig. 5). The $JIL\text{-}1^{h9}$ allele expresses a truncated JIL-1 protein that completely lacks the CTD (Zhang et al., 2003a) and the lamin Dm_0 interaction domain. Zhang et al. (Zhang et al., 2003a) previously showed that JIL-1 is important for early development and oogenesis as ovaries from $JIL\text{-}1^{z2}/JIL\text{-}1^{h9}$ flies are smaller than normal and the egg chambers frequently contain abnormal numbers of nurse cells. In normal egg chamber development, 15 of the 16 interconnected germline cells undergo multiple rounds of DNA replication in the absence of division (reviewed by Mahowald and Kambysellis, 1980), giving rise to large, polyploid nurse cell nuclei (Fig. 5A). A layer of somatically derived follicle cells surround the developing egg chamber, and the diploid germline-derived oocyte cell gradually enlarges during maturation with its nucleus localizing posteriorly (reviewed in Spradling et al., 1997). The most common phenotype in $JIL\text{-}1^{z2}/JIL\text{-}1^{h9}$ egg chambers was protrusions of the nurse cell nuclear lamina near the oocyte (Fig. 5C,D), as well as dispersed and mislocalized lamin throughout the egg chamber (Fig. 5B). Interestingly, double labelings with lamin antibody and phalloidin showed that the nurse cell nuclei were abnormally positioned relative to the actin cytoskeletal networks and that the nuclear lamina protrusions were extending through the ring canals (Fig. 5E,F). The lamina protrusion phenotype of the nurse cell nuclei had a penetrance of 42.8% (at least one such nurse cell nucleus was observed in 71 out of 166 mutant egg chambers examined), whereas diffused lamin Dm_0 was observed in 5.2% of mutant egg chambers (7/134). Neither of these phenotypes were observed in 200 wild-type egg chambers examined and the lamin phenotypes were completely rescued by a full-length JIL-1 rescue construct (Wang et al., 2001) (data not shown). The data presented was collected from five to seven different ovaries for each of the experimental conditions.

Genetic interactions between *lamin* Dm_0 and *JIL-1* alleles

To further study whether JIL-1 and lamin Dm_0 interact in vivo we explored genetic interactions between mutant alleles of *Lam* and *JIL-1* by generating double mutant individuals containing either of the *Lam* alleles Lam^{4643} or Lam^{A25} and $JIL\text{-}1^{3657}$. The Lam^{4643} allele contains a recessive lethal P element insertion 258 bp upstream of the translation initiation site that leads to reduced lamin Dm_0 protein levels (Guillemin et al., 2001; Patterson et al., 2004). The Lam^{A25} allele has a frameshift that results in the deletion of the 12 COOH-terminal amino acids

including the CaaX box which localizes lamin to the inner nuclear membrane (Patterson et al., 2004). Loss of the CaaX box leads to a diffuse nuclear distribution of the truncated protein; however, *Lam*^{A25} flies are homozygous viable suggesting that the truncated protein is still able to provide essential functions (Patterson et al., 2004). The *JIL-1*^{3657} P element insertion line is a hypomorphic allele that can be maintained in a homozygous stock for only a few generations due to the low hatch rate and recessive semi-lethality (Wang et al., 2001). The hatch rate of *JIL-1*^{3657} homozygous embryos produced by homozygous parents is as low as 4% when compared with the hatch rate of wild-type Oregon-R embryos (Wang et al., 2001). We generated double mutants of these alleles by crossing a chromosome containing either the *Lam*^{4643} allele or the *Lam*^{A25} allele into a homozygous hypomorphic *JIL-1*^{3657} background in otherwise identical crosses as indicated in Fig. 6. By immunoblot analysis we determined that lamin Dm_0 levels in *Lam*^{4643}*/CyO*; *JIL-1*^{3657}*/JIL-1*^{3657} third instar larvae were reduced to about half that in *Sp/CyO*; *JIL-1*^{3657}*/JIL-1*^{3657} larvae (0.54±0.11, n=4) (data not shown). To quantify the effect of the two lamin alleles on viability we compared the ratio of *JIL-1*^{3657} homozygous progeny with or without one of the *Lam* alleles (Fig. 6). The viability ratio in each cross was determined by dividing the number of eclosed flies of each genotype with the total number of eclosed flies. Fig. 6A compares the average ratio of eclosed *Lam*^{4643}*/CyO*; *JIL-1*^{3657}*/JIL-1*^{3657} flies with that of the *Sp/CyO*; *JIL-1*^{3657}*/JIL-1*^{3657} genotype (which has two wild-type *Lam* copies) from 13 independent crosses. The average ratio of viability for *Lam*^{4643}*/CyO*; *JIL-1*^{3657}*/JIL-1*^{3657} flies (0.36±0.03) was more than double that of *Sp/CyO*; *JIL-1*^{3657}*/JIL-1*^{3657} flies (0.16±0.06). This difference in the viability ratio between the two genotypes was statistically significant (P<0.0001, Student's two-tailed t-test) and suggests a genetic interaction where reduced levels of lamin Dm_0 can partly rescue the recessive semi-lethality of homozygous *JIL-1*^{3657} flies. This is in contrast to results from the same kind of cross where the only difference was that the *Lam*^{4643} allele was replaced by the weaker *Lam*^{A25} allele. In Fig. 6B the average ratio of eclosed *Lam*^{A25}*/CyO*; *JIL-1*^{3657}*/JIL-1*^{3657} flies is compared to that of the *Sp/CyO*; *JIL-1*^{3657}*/JIL-1*^{3657} genotype from 21 independent crosses. In these crosses the average ratio of viability between the two genotypes (0.30±0.03 and 0.29±0.03, respectively) was not statistically different (P>0.8, Student's two-tailed t-test). This suggests that the truncated protein made by this allele that still retains the JIL-1 binding sequences can maintain sufficient interactions with JIL-1 as to not affect viability.

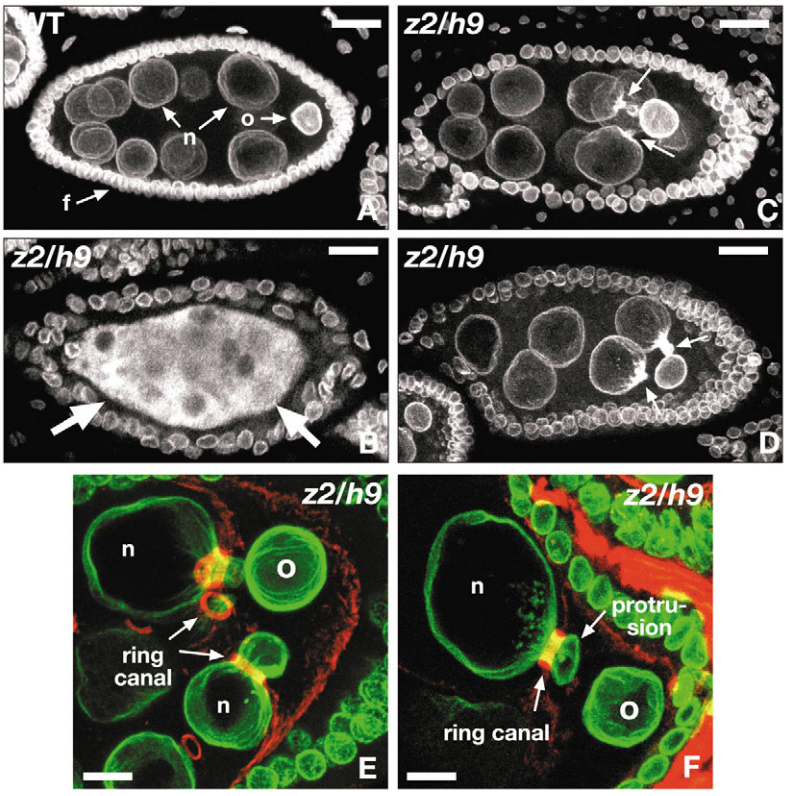

Fig. 5. Egg chambers from JIL-1 mutant flies labeled with lamin Dm_0 antibody. (A) Wild-type (WT) egg chamber labeled with lamin pAb R836. The location of follicle cells (f), nurse cells (n) and the oocyte (o) is indicated by arrows. (B) Egg chamber from a *JIL-1*^{z2}*/JIL-1*^{h9} (*z2/h9*) mutant fly labeled with lamin pAb R836. The arrows indicate dispersed and mislocalized lamin Dm_0 throughout the egg chamber. (C,D) Egg chambers from a *JIL-1*^{z2}*/JIL-1*^{h9} (*z2/h9*) mutant fly labeled with lamin pAb R836. The arrows point to protrusions of the nuclear lamina of nurse cells near the oocyte. (E,F) Egg chambers from a *JIL-1*^{z2}*/JIL-1*^{h9} (*z2/h9*) mutant fly double-labeled with lamin pAb R836 (green) and phalloidin (red). The protrusions from the nuclear lamina of the nurse cells extend through the ring canals. Bars, 25 μm in A-D and 10 μm in E-F.

Discussion

In this study we provide evidence that the JIL-1 tandem kinase molecularly interacts with lamin Dm_0. This interaction was first detected in a yeast two-hybrid screen and subsequently confirmed by pull-down and cross-immunoprecipitation assays. Using deletion construct analysis we show that a predicted globular domain of the basic region of the COOH-terminal domain of JIL-1 was sufficient for mediating the molecular interactions with lamin Dm_0. A reciprocal analysis with truncated lamin Dm_0 constructs showed that the interaction with JIL-1 required sequences between residues 408 and 588 in the tail domain of lamin Dm_0 that include the Ig-like fold. Further support for a molecular interaction between JIL-1 and lamin Dm_0 in vivo was provided by genetic interaction assays. These assays showed that reduced levels of lamin Dm_0 in double mutant flies with the hypomorphic *Lam*^{4643} allele could partly rescue the recessive semi-lethality of homozygous *JIL-1*^{3657} flies. By contrast, a truncated lamin allele which provides essential lamin function and retains the JIL-1 interaction domain did not rescue the JIL-1 mediated semi-lethality. This finding strongly supports the specificity of the observed genetic interaction between the *JIL-1*^{3657} and *Lam*^{4643} alleles.

The results from the yeast two-hybrid interaction assays suggest that the interaction between JIL-1 and lamin Dm_0 is direct. However, JIL-1 is localized to euchromatic regions of chromosomes and lamin Dm_0 is mainly a component of the inner nuclear membrane raising

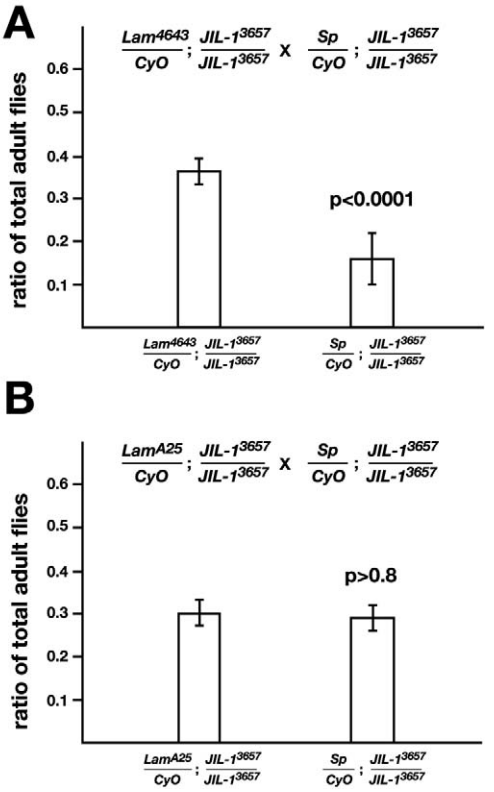

Fig. 6. Genetic interaction between *JIL-1*^{3657} and *Lam* alleles. (A) Presence of a heterozygous *Lam*^{4643} allele increases the viability of *JIL-1*^{3657} homozygous animals. *Lam*^{4643}*/CyO; JIL-1*^{3657}*/JIL-1*^{3657} males were mated with *Sp/CyO; JIL-1*^{3657}*/JIL-1*^{3657} females. Homozygous *JIL-1*^{3657} individuals that were also homozygous for the wild-type *Lam* allele (histogram to the right) eclosed at a rate only about half (0.44) that of *JIL-1*^{3657} homozygotes that contained one copy of the loss-of-function *Lam*^{4643} allele (histogram to the left). The average ratios of adult flies of each genotype to total eclosed flies are from 13 independent matings. The difference in numbers observed for the two classes was statistically significant (P<0.0001, Student's two-tailed t-test). (B) The presence of a heterozygous *Lam*^{A25} allele does not affect the viability of *JIL-1*^{3657} homozygous animals. *Lam*^{A25}*/CyO; JIL-1*^{3657}*/JIL-1*^{3657} males were mated with *Sp/CyO; JIL-1*^{3657}*/JIL-1*^{3657} females. Homozygous *JIL-1*^{3657} individuals that were also homozygous for the wild-type *Lam* allele (histogram to the right) eclosed at the same rate (0.97) as *JIL-1*^{3657} homozygotes that contained one copy of the *Lam*^{A25} allele (histogram to the left). The average ratios of adult flies of each genotype to total eclosed flies are from 21 independent matings. The difference in numbers observed for the two classes was not statistically significant (P>0.8, Student's two-tailed t-test).

the question of how this interaction occurs. Recently it has become clear that lamins and associated proteins in the nuclear envelope are involved in several nuclear activities apart from providing a barrier between the nucleoplasm and the cytoplasm (reviewed by Goldberg et al., 1999a; Gotzmann and Foisner, 1999; Wilson et al., 2001). One of these functions of the nuclear lamina is to serve as a scaffold that provides attachment sites for interphase chromatin directly or indirectly regulating many nuclear activities such as DNA replication and transcription, nuclear and chromatin organization, cell development and differentiation, nuclear migration, and apoptosis (reviewed by Mattout-Drubezki and Gruenbaum, 2003). In *Drosophila* it has been shown that direct interactions between the tail domain of lamin Dm_0 and histone H2A and H2B may mediate the attachment of chromosomes to the nuclear lamina (Goldberg et al., 1999b). Interestingly, the early embryonic nuclear lamina protein YA (Young Arrest), which is a lamin Dm_0 binding protein, when ectopically expressed in larval salivary gland cells, associates with interband regions of polytene chromosomes (Lopez and Wolfner, 1997). Thus, there is considerable evidence for direct interactions of lamins with chromatin associated proteins such as JIL-1. Furthermore, lamins have also been found in the nuclear interior (reviewed by Goldberg et al., 1999a; Wilson et al., 2001) and the possibility remains that there may be a hitherto undetected soluble pool of JIL-1 that potentially could provide additional avenues for direct interactions.

To determine whether disruptions in nuclear lamina organization could be detected in *JIL-1* mutant backgrounds we examined fixed ovaries, embryos, imaginal discs and polytene salivary glands labeled with lamin Dm_0 antibody. We observed abnormal lamin Dm_0 distribution only in ovaries of *JIL-1*^{z2}*/JIL-1*^{h9} flies. One phenotype which was found in about 5% of mutant egg chambers was dispersed and mislocalized lamin throughout the egg chamber. This is not likely to be a consequence of apoptotic events because lamins are degraded by proteolysis during apoptosis and do not show accumulation (Lazebnik et al., 1995; Takahishi et al., 1996; Rao et al., 1996). The phenotype may therefore reflect a destabilization of the integrity of the nuclear lamina leading to lamin Dm_0 dispersal. Thus, these experiments may provide evidence that the stability of the nuclear lamina in *Drosophila* egg chambers depends on JIL-1 kinase activity and phosphorylation of lamin Dm_0. Unfortunately, this hypothesis cannot be tested at the present time because of a lack of a functional in vitro JIL-1 kinase assay. The other phenotype we observed in *JIL-1* mutant egg chambers with high penetrance (42.8%) was abnormally positioned nurse cell nuclei which extended nuclear lamina protrusions through the ring canals near the oocyte. It is not clear how this phenotype arises. However, several morphogenetic processes such as anterior-posterior/dorso-ventral axis formation as well as cell and nuclear migration during oogenesis require reciprocal cell signaling between germline, oocyte and nurse cells, and somatic follicle cells (reviewed by Rotoli et al., 1998; Ghiglione et al., 2002; Fulga and Rørth, 2002; Hombria and Brown, 2002). In JIL-1 mutant backgrounds cell signaling pathways that normally prevent nurse cell nuclei from responding to posterior migration signals may be downregulated, resulting in a posterior dislocalization towards the oocyte. It has been shown that the nuclear lamina is involved in regulating nuclear migration in the developing eye through interactions of the lamin Dm_0-binding protein Klarsicht with the microtubule organizing center (Patterson et al., 2004). Furthermore, Bicaudal-D, a dynein-interacting protein required for control of nuclear migration and cytoskeletal organization in oogenesis (Oh and Steward, 2001) has been shown to interact with lamin Dm_0 in yeast two-hybrid assays (Stuurman et al., 1999). Thus, dynamic local interactions of cytoskeleton-associated motor proteins linked to lamin Dm_0 may be capable of providing the forces necessary for generating the observed deformations of the nuclear lamina. Nuclear lamins are generally considered to provide

stiffness and incompressibility to the nuclear envelope (Dahl et al., 2004) suggesting that the aberrations in nuclear morphology observed here may be linked to a weakening of the nuclear lamina. However, the present experiments cannot distinguish between the possibilities that JIL-1 may be involved in nuclear deformation by regulating nuclear lamina cytoskeletal interactions via direct modulation of lamin Dm_0 or indirectly by modulating a signal transduction pathway, or both.

Our results suggest that JIL-1 kinase is required to maintain nuclear morphology and integrity of nurse cells during oogenesis. It has recently been shown that some lamin Dm_0 interactions occur only during early development, indicating that special properties of the nuclear lamina may be required for regulating nuclear processes and morphology at specific developmental stages. For example, the lamin Dm_0 binding protein YA is expressed only in ovaries and pre-gastrulation embryos and is required for the interaction between chromatin and the nuclear envelope during early embryogenesis (Lopez and Wolfner, 1997). Previously, we have shown that the interaction between JIL-1 and Lola zf5, a splice variant of the complex *lola* locus encoding multiple different transcription factors, is developmentally regulated and restricted to early embryogenesis as well (Zhang et al., 2003b). Thus, it will be informative in future experiments to further explore the interaction between JIL-1 and lamin Dm_0 to clarify how this interaction contributes to nuclear lamina function in development.

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