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The tail domain of lamin Dm_0 binds histones H2A and H2B

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ABSTRACT **In multicellular organisms, the higher order organization of chromatin during interphase and the reassembly of the nuclear envelope during mitosis are thought to involve an interaction between the nuclear lamina and chromatin. The nuclear distribution of lamins and of peripheral chromatin is highly correlated *in vivo*, and lamins bind specifically to chromatin *in vitro*. Deletion mutants of *Drosophila* lamin Dm_0 were expressed to map regions of the protein that are required for its binding to chromosomes. The binding activity requires two regions in the lamin Dm_0 tail domain. The apparent K_d of binding of the lamin Dm_0 tail domain was found to be approximately 1 μM. Chromatin subfractions were examined to search for possible target molecules for the binding of lamin Dm_0. Isolated polynucleosomes, nucleosomes, histone octamer, histone H2A/H2B dimer, and histones H2A or H2B displaced the binding of lamin Dm_0 tail to chromosomes. This displacement was specific, because polyamines or proteins such as histones H1, H3, or H4 did not displace the binding of the lamin Dm_0 tail to chromosomes. In addition, DNA sequences, including M/SARs, did not interfere with the binding of lamin Dm_0 tail domain to chromosomes. Taken together, these results suggest that the interaction between the tail domain of lamin Dm_0 and histones H2A and H2B may mediate the attachment of the nuclear lamina to chromosomes *in vivo*.**

Underlying the inner nuclear membrane and abutting the chromatin is the filamentous protein meshwork of the nuclear lamina (reviewed in refs. 1–4). The nuclear lamina is involved in several biological activities, including the regulation of the size, shape, and assembly of the nuclear envelope (5–10); facilitation of higher order chromatin organization (7, 8, 11); and regulation of DNA replication (12–14). Changes in the nuclear lamina composition during development point toward a possible role for lamins, which are the major proteins of the nuclear lamina, in cell differentiation (reviewed in ref. 15). The nuclear lamina also is a major substrate for signals that control the cell cycle (16), and lamins are specifically degraded in apoptosis (17).

Lamins are classified as type V intermediate filament proteins, and like all intermediate filaments, they contain a helical rod domain flanked by amino (head) and carboxyl (tail) domains (reviewed in refs. 16 and 18). Different eukaryotes possess between one and six lamin genes. Mammalian lamins A and C result from alternative splicing of the same gene product, whereas lamins B1–B3 and C2 are coded for by separate genes (19). The two major lamins in chicken are lamins A and B2 (20). An additional minor species is termed lamin B1. *Xenopus laevis* has at least five different lamin genes (21, 22). *Drosophila melanogaster* has two lamin genes, termed lamin Dm_0 and lamin C (23, 24). *Caenorhabditis elegans* probably has only a single lamin gene, termed CeLam-1 (25).

Three-dimensional *in vivo* studies in *Drosophila* and mammalian cells revealed that lamin fibers are closely associated with chromatin fibers (26). *In vitro* studies have shown that lamins can specifically bind chromatin fragments and interphase chromatin (27–29), condensed *in vitro* assembled chromatin (9), or mitotic chromosomes (30, 31). Lamins can also bind chromosomal proteins (27–32) and specific DNA sequences, such as M/SARs (33–36) and telomeric sequences (37). The binding of lamins to chromatin is specific and depends on the integrity of the chromosomes. Human lamin A binds *in vitro* to polynucleosomes with a dissociation constant of about 1 nM (29). A binding site for mammalian lamins A and B to chromatin was localized at their tail domain (28). In the latter study, the dissociation constant of the tail domain binding to interphase chromatin was estimated to be in the range of 0.12–0.3 μM, and the binding was mediated by core histones. The actual association of the lamin filament may be stronger, because lamins form large polymers *in vivo*. A specific binding site to mitotic chromosomes was identified in human lamin A and C rod domain (31). However, the *in vivo* relevance of this binding is not yet clear, because the rod domain binding occurred only under acidic, nonphysiological, conditions.

We previously reported that interphase and bacterially expressed *Drosophila* lamin Dm_0 can specifically bind chromatin *in vitro* (9). In this study we show that this binding activity is localized within the tail domain of lamin Dm_0, requires two sequences for efficient binding, and we identify their putative target chromosomal proteins. Lamin Dm_0 tail domain can bind chromatin fragments with an apparent K_d ranging between 0.5 and 2 μM. The binding of lamin Dm_0 tail domain to chromosomes can be displaced with polynucleosomes, nucleosomes, histone octamer, histone H2A/H2B dimer, and histones H2A or H2B, but not with spermine, spermidine, or histones H1, H3, or H4. The relevance of these results to the roles of lamins in nuclear envelope assembly and higher order chromatin organization is discussed.

MATERIALS AND METHODS

Vectors, Antibodies, Constructs, and Bacterial Overexpression of Lamin Dm_0 and Lamin Dm_0-Derived Proteins, Histones, and Histone-Derived Proteins. *Drosophila ftz* S/MAR and yeast ARS (38, 39) were a kind gift of S. Gasser (Lausanne). Monoclonal and polyclonal anti-*Drosophila* lamin Dm_0 antibodies are described in ref. 10. Anti-his tag mAb, ^{RGS}His, was purchased from Qiagen (Germany). The cloning, expres-

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sion, and purification of the complete lamin Dm_0, lamin Dm_0 R64 → H, the isolated lamin Dm_0 rod domain (amino acids 55–413), and lamin Dm_0 tail domain constructs T411–462 and T425–622 in pET20b(+) (Novagen) are described in ref. 36. All other lamin Dm_0 tail domain constructs were derived from the T425–622 construct in pET20b(+) or in pET20b(+) into which the $RGS(H)_4$ epitope was added. *Escherichia coli BL21(DE3)* cells or *E. coli BL21(DE3)* pLysS cells were used to express the different lamin Dm_0 constructs. All constructs were purified to near homogeneity by a one-step affinity chromatography on a His-bind resin column (Novagen). The proteins were concentrated to 5–10 mg/ml and dialyzed against buffer TK (50 mM Tris·HCl, pH 7.5/70 mM KCl/1 mM DTT/2.5 mM benzamidine). Complete lamin Dm_0, headless lamin Dm_0, and T425–522 and T523–622 proteins were dialyzed against buffer TK containing 300 mM NaCl. Expression of *Xenopus* core histones and histone H1 (amino acids 1–142), purification to >95% purity and folding of histones to dimers, tetramers, and octamers are described in ref. 40. Nucleosome core particles were assembled with 146-bp fragment from pTJR2 (40, 41). All histone preparations were analyzed on 15% SDS/PAGE before their use.

***In Vitro* Binding of Lamin Dm_0 and Lamin Dm_0-Derived Polypeptides to Mitotic Chromosomes.** Mitotic chromosomes were isolated from mitotic Chinese hamster ovary (CHO) cells as described (30). Binding reactions were in total volume of 10 μl of buffer TK containing 0.085 μM isolated lamin Dm_0-derived polypeptides, 5–10% BSA, or 10% fetal calf serum and competitor substrates when used. CHO mitotic chromosomes (0.1–0.3 OD_{260} units/ml) were added after 5 min incubation at 22°C followed by a 45-min incubation at 22°C. When naked DNA was used in an attempt to displace the binding, it was added to a final concentration of 100 μg/ml. When different lamin Dm_0-derived polypeptides were used to compete with each other, the competitor protein was added at 4.5–8.5 μM. Competition experiments with nucleosomes, histone tetramers, histone dimers, and individual histones included 0.25 μM isolated T425–622 protein and 7–30 μM of the competitor. Competition experiments with histone octamers included 2–3 μM of the competitor. Competition experiments with spermine and spermidine included 1,000-fold molar excess of the competitor over the lamin Dm_0-derived polypeptides. Paraformaldehyde (0.05%) was added to the reaction mixture, which was immediately transferred to poly-L-lysine coated coverslips. After 5 min, the coverslips were washed twice with 100 μl of PBS followed by fixation for 20 min at 22°C with PBS containing 2% paraformaldehyde. In some experiments, the first fixation step was avoided, and the reaction mixture was transferred to a 6-well tissue culture plates containing poly-L-lysine-coated coverslips. The plates were centrifuged for 10 sec at 1,000 rpm, followed by the above wash and fixation steps. This procedure produced similar results to those of the two-step fixation method. The coverslips were washed twice with PBS and once with PBS containing 0.2% Tween-20 (PBST), each for 10 min, incubated for 45 min with PBST containing 10% spray dried skimmed milk 1.5% fat and 1% BSA (PBSTB), and washed for 10 min with PBST. Coverslips were then incubated for 30 min at 30°C with anti-lamin (monoclonal 611A3A6 or polyclonal) antibodies or with monoclonal ^{RGS}His antibody in PBST, washed twice with PBST, each time for 10 min, incubated for 45 min at 22°C in PBSTB, and washed for 5 min at 22°C with PBST. Coverslips then were incubated with PBST containing 1 μg/ml Cy_3A-conjugated anti-rabbit (for polyclonal antibodies) or anti-mouse (for monoclonal antibodies) (Jackson ImmunoResearch) and 1 μg/ml 4′,6′-diamidino-2-phenylindole (DAPI), and incubation proceeded for additional 30 min at 30°C. After two washes with PBST and one wash with PBS, each for 10 min, the coverslips were mounted on slides in PBS containing 50% glycerol and 2% *n*-propyl gallate and viewed under a Leitz microscope equipped with epifluoresence. The effectiveness with which various histones displaced T425–622 was assessed by measuring the average fluorescence emission of Cy3 from the chromosomes, using an electromechanical shutter, a D2 filter block (excitation filter: 355–425 nm; dichroic mirror: 455 nm cutoff; emission filter: 460 nm lowpass) for DAPI staining, a N2 filter block (excitation filter: 530–560 nm; dichroic mirror: 580 nm cutoff; emission filter: 580 nm lowpass) for detection of the Cy3 stain, and a charge-coupled device imager. The emission uniformity was ±5%, as measured by using sulforhodamine-101 dissolved in agar gel. For each exposure, the dark noise was measured and subtracted from the fluorescence image. The images were analyzed by using the IMAGE PRO for Windows package (Media Cybernetics, Silver Spring, MD).

Solid-Phase Chromatin Binding Assay. Purified 50–100 μg of T425–622 protein was ^{125}I-labeled by the chloramine-T technique to a specific activity of $0.5–1.4 \times 10^7$ cpm/μg. Free ^{125}I was blocked by potassium iodide and sodium disulfide, and the labeled protein was separated on Sephadex G-50 gel filtration column, equilibrated in TK buffer containing 1 mg/ml BSA, 5 mM benzamidine and 0.4 μg/ml aprotinin. Chromatin fragments were isolated from rat liver nuclei exactly as described (42). After digestion of isolated nuclei with micrococcal nuclease (Sigma), the polynucleosomes were separated on 10–50% linear sucrose gradient, and fractions containing DNA fragments of 1.6–6 kb (8–30 nucleosomes) were pooled and used for the binding assay. The chromatin fragments (≈400 ng per well) were immobilized in microtiter Immunolon-4 plates (Dynatech) as described (28). The wells were then incubated for 2 hr at 22°C with TK buffer containing 30 mg/ml BSA and washed once in the same buffer. The chromatin fragments were then incubated with radiolabeled T425–622 protein (2 μg/ml) in TK buffer containing 30 mg/ml BSA, in the presence or absence of unlabeled T425–622 protein, for 2 hr at 22°C. After three brief washes in TK buffer containing 30 mg/ml BSA, the radioactive protein was recovered from each well by two extractions with 0.2 M NaOH and measured by gamma counting. Nonspecific background was taken as the amount of radioactivity bound per well when 2 μg/ml of labeled T425–622 protein was incubated together with 250-1500 μg/ml unlabeled T425–622 protein (20–32% of total bound radioactivity). In each experiment, data points were taken in duplicates. To calculate the K_d, assuming a single-site competitive interaction, data was expressed in a linearized plot as described (28).

RESULTS

Lamin Dm_0 Binds Chromatin Through Two Separate Regions Within Its Tail Domain. Previous studies have shown that lamin Dm_0 can specifically bind decondensed sperm chromatin and nuclei assembled *in vitro* in *Drosophila* embryonic extracts (9). Here we show that lamin Dm_0 can also bind mitotic chromosomes, in a similar fashion to vertebrate lamins A/C (30, 31). The binding of lamin Dm_0 was mostly peripheral, with few lamin aggregates on each chromosome (Fig. 1). Such lamin aggregates could also be detected outside the chromosomes (data not shown). The binding of lamin Dm_0 to chromosomes did not require lamin polymerization, since lamin Dm_0 mutated in Arg-64 (R64 → H), which is unable to polymerize (36), could bind chromosomes with an overall pattern of binding similar to that of wild-type lamin Dm_0 (Fig. 1). To analyze whether the binding of lamin Dm_0 to chromosomes is mediated by its tail domain, 50- and 100-fold excess amounts of T425–622 were used to displace the binding of either wild-type lamin or lamin R64 → H to chromosomes, and lamin Dm_0 binding was analyzed with affinity purified polyclonal antibodies directed against the lamin Dm_0 rod domain (43). The binding of lamin R64 → H to chromosomes could be displaced by T425–622 (Fig. 1). With the exception of a few

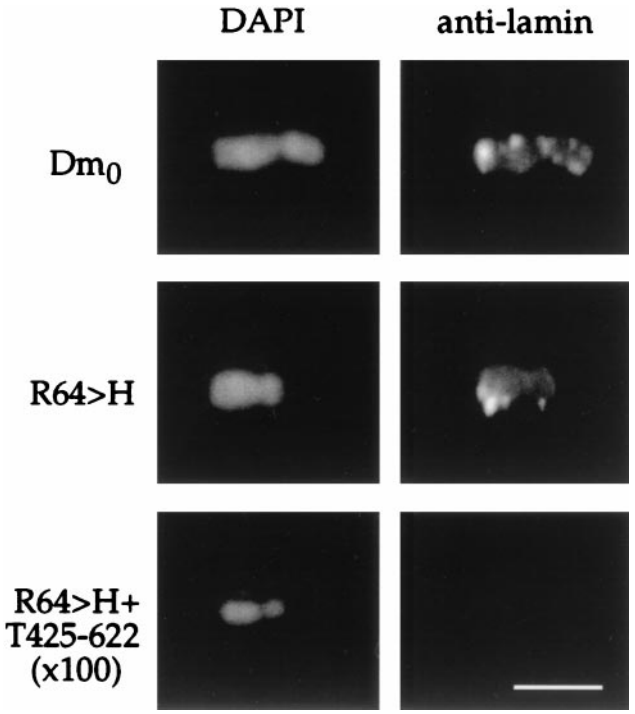

FIG. 1. The binding of lamin Dm_0 to chromosomes is mediated by its tail domain. Binding of wild-type lamin Dm_0 (*Top*) and lamin R64 → H, which is a mutant form of lamin Dm_0 that cannot polymerize *in vitro* (*Middle*) (36) to mitotic CHO chromosomes was performed in the presence of 10% BSA. Staining was with affinity purified polyclonal antibodies directed against lamin Dm_0 rod domain as primary antibodies and Cy3-conjugated anti rabbit antibodies as secondary antibodies. Staining was not observed when the chromatin was first incubated with a 100-fold molar excess of the tail domain (residues 425–622; *Bottom*). (Bar = 5 μm.)

aggregates, wild-type lamin Dm_0 was displaced from mitotic chromosomes by T425–622 with similar efficiency to that of R64 → H (data not shown).

To identify the regions in the Dm_0 lamin protein that are required for the interaction with chromatin, substitution and deletion mutants of lamin Dm_0 were expressed in bacteria and purified to near homogeneity, as analyzed by 15% SDS/PAGE (ref. 36; Fig. 2*A* for other lamin tail domain constructs). The purified lamin Dm_0 constructs were analyzed for their ability to bind mitotic chromosomes by using fluorescence (data not shown) as summarized in Fig. 2*B*. The headless lamin Dm_0 (rod + tail; amino acids 55–622) bound to chromosomes. The isolated lamin Dm_0 tail domain (amino acids 411–622) and T425–622 could bind to the periphery of the mitotic chromosomes with similar intensity to that of the wild-type lamin Dm_0 (Fig. 2*B*). In contrast, under similar conditions, the isolated lamin Dm_0 rod domain (amino acids 55–413) did not bind mitotic chromosomes (Fig. 2*B*). The T425–572 protein bound chromosomes with an intensity similar to that of the complete tail domain (Fig. 2*B*). The T425–522 protein bound chromosomes with lower immunofluorescence intensity than T425–622 protein, but with significantly higher intensity than T473–622, T523–622, and T473–572 proteins. A 50-fold molar excess of T473–622, T523–622, or T473–572 could not compete with the T425–622 protein for its binding to chromosomes (data not shown). In addition, the intensity of the binding of T473–572 to chromosomes was close to background levels (Fig. 2*B*). Taken together, these data indicate that sequences within amino acids 425–473 and amino acids 572–622 are required for efficient binding of lamin Dm_0 to chromosomes. It is worth noting that sequences within amino acids 425–473 share homology to sequences that are involved in the binding of *Xenopus* lamin B2 and human lamin A/C to chromatin (27, 28), as well as to *Drosophila* lamin C (ref. 23; Fig. 2*C*).

Lamin Tail Domain Binds Polynucleosomes with a $K_d \approx 1$ μM. The K_d for chromatin binding of the lamin Dm_0 tail construct, T425–622, was determined by the displacement assay described for human lamins A and C tail domains (28). These experiments were repeated >10 times, by using different preparations of the T425–622 protein and chromatin and always resulted in calculated K_d values between 0.5–2.1 μM, which are 3–7 times lower than the reported K_d values for human lamins A/C tail domains (28). An experiment that gave a K_d of 2.1 μM is shown in Fig. 3.

Lamin Binding to Chromosomes Can Be Displaced with Histones H2A and H2B. To identify the target molecules for the binding of lamin Dm_0 to chromosomes, we have tested DNA sequences, polyamines, nonrelevant proteins, and histones for their ability to displace the binding of the lamin Dm_0 tail domain to chromosomes. Previous reports have shown a strong affinity of lamin Dm_0 to S/MAR DNA sequences (33–36). However, these DNA sequences were not the target of lamin tail binding to chromosomes, because a 100-fold molar excess of the *Drosophila ftz* S/MAR sequence (Fig. 4) or yeast ARS sequences (ref. 39, and data not shown) (39), as well as sequences adjacent to the M/SARs and plasmid DNA, did not displace the binding of the T425–622 protein to chromosomes.

As shown above, the lamin Dm_0 tail domain could bind polynucleosomes (Fig. 3). Accordingly, a 100-fold molar excess of isolated nucleosome core particles displaced lamin Dm_0 tail from chromosomes, as judged by the lack of immunofluorescence signal following staining with a monoclonal anti-lamin antibody (data not shown). In addition, a 100-fold molar excess of commercially available crude preparation of core histones and histone H1 (Sigma) efficiently displaced the lamin tail's binding to chromosomes (Fig. 4). The displacement of the lamin tail from chromosomes was specific, because the binding of the lamin Dm_0 tail domain was always obtained in the presence of vast excess amounts of either BSA or fetal calf serum. Furthermore, a 1,000-fold molar excess of spermine and spermidine (Fig. 4) or a 100-fold molar excess of histidine-tagged β-galactosidase, expressed and purified by the same procedure used for lamin derivatives, did not displace the lamin tail's binding to chromosomes (data not shown).

To identify specific histone(s) that interact with the lamin Dm_0 tail domain, we have tested the individual core histones and histone H1 for their ability to displace the binding of the T425–622 protein to chromosomes (Fig. 5). A molar excess concentration of *in vitro*-assembled histone octamers blocked the binding of the T425–622 protein (Fig. 5). Rough estimation of the efficiency of the blocking showed that >95% of the binding was displaced with purified histone octamers. Because the binding of the T425–622 protein in the presence of histone octamers was performed at 70 mM KCl and 10–20 mM NaCl, these histone octamers must have disintegrated into H2A/H2B dimers and H3/H4 tetramers when placed in the binding reaction. Indeed, a 12-fold molar excess of purified H2A/H2B dimers also efficiently displaced the binding of the T425–622 protein to chromosomes (data not shown). Purified histones of both H2A or H2B could block the binding of the T425–622 protein to chromosomes (Fig. 5). In contrast, a 12-fold molar excess of H3/H4 histone tetramers (data not shown) or 30- to 120-fold molar excess of individual histones H3 or H4 did not displace the T425–622 protein's binding to chromosomes (Fig. 5). The role of histone H1 in lamin Dm_0 binding to chromosomes was investigated by performing the binding reaction in the presence of a 32-fold molar excess of purified histone H1 (amino acids 1–142). As shown in Fig. 5, this histone H1 fragment did not displaced the binding of lamin tail to chromosomes.

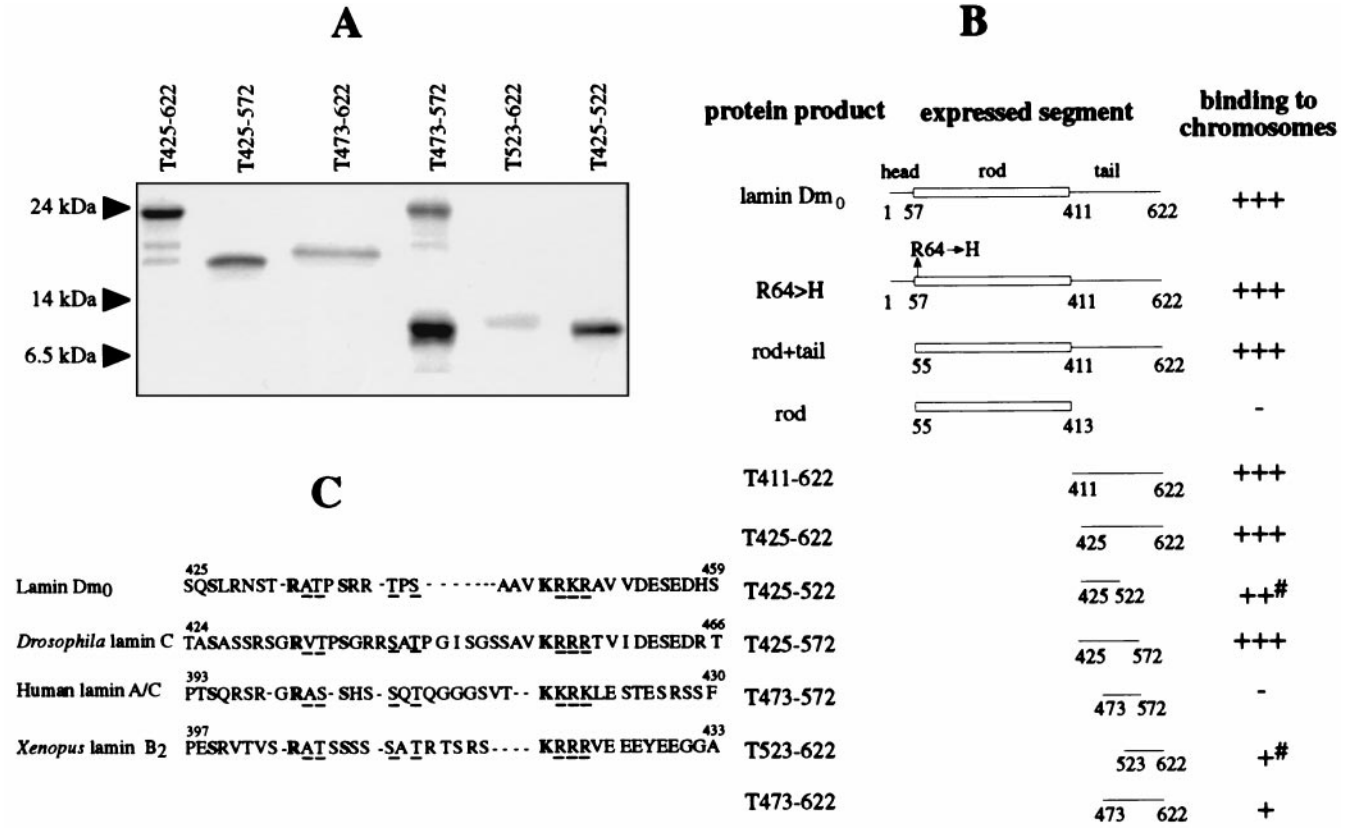

FIG. 2. The binding of lamin Dm_0 to chromosomes requires amino acids 425–473 and 572–622. (*A*) SDS/PAGE (15%) analysis of different bacterially expressed and purified constructs of the lamin Dm_0 tail domain. Proteins were stained with Comassie brillant blue. The names assigned to the different constructs are written above the lanes, and the positions of the size markers are indicated. (*B*) Summary of the binding of different lamin Dm_0 constructs to chromosomes. Examples of the binding of lamin Dm_0 and R64 → H to chromosomes are shown in Fig. 1, and an example of the binding of T425–622 to chromosomes is shown in Fig. 4. The position of the amino acid termini of each expressed segment are shown below the map of each construct. +++ indicates strong binding; ++ indicates medium binding; + indicates weak binding; − indicates lack of detectable binding; # indicates that the detection was performed with ^{RGS}His antibody and the intensity of the binding was compared with that of T425–622 staining with ^{RGS}His antibody. (*C*) Comparison between lamin Dm_0, *Drosophila* lamin C, human lamin A/C, and *Xenopus* lamin B2 sequences in the amino-terminal part of the tail domains that bind chromosomes. Conserved amino acids are in boldface; similar amino acids are underlined. The position of the amino acids termini of each lamin segment are shown above the sequence; − indicates a gap.

DISCUSSION

Two Sequences in the Tail Domain of Lamin Dm_0 Are Required for the Specific Interaction with Chromatin. In this study, we used bacterially expressed constructs of lamin Dm_0 and CHO mitotic chromosomes to map the binding regions in lamin Dm_0 and to determine their target chromosomal proteins. The specific binding of *Drosophila* lamin Dm_0 to CHO chromosomes implies that the interaction between nuclear lamins and chromatin is evolutionarily conserved. Evolutionary conservation was already anticipated on the basis of the following observations: (*i*) lamin Dm_0 can bind to rooster DNA assembled into chromatin in a *Drosophila* nuclear assembly system (45), (*ii*) human lamins can also bind CHO chromosomes (28), and (*iii*) histones H2A and H2B, which are the target chromosomal proteins for lamin binding, and sequences within the lamin tail region are conserved in evolution.

A 100-fold molar excess of the soluble, highly purified T425–622 protein was sufficient to exclude most or all of the binding of the wild-type lamin Dm_0 (>95%). Moreover, the intensity of the binding of the T425–622 protein to chromosomes was roughly similar to that of the complete molecule, and under physiological conditions the lamin Dm_0 rod domain could not bind chromosomes. We thus conclude that the major contribution to the binding of lamin Dm_0 to chromosomes *in vitro*, can be attributed to its tail domain. The existence of a chromatin binding site in the lamin tail domain also was reported for *Xenopus* lamins A and B2 (27) and for mammalian lamins (28).

By using a series of deletion constructs of the lamin Dm_0 tail domain we mapped two separate regions, containing amino acids 425–473 and 572–622, that are required for this binding. The binding of the T425–573 construct, which contains the first region, was similar to that of the complete tail domain, whereas the T523–622 and T473–622 constructs, which contain the second region, exhibited significantly weaker binding. The lower signal obtained with T425–522, as compared with T425–573, can be explained by a difference in protein folding or by the need for additional residues. Studies with the human lamin C mapped the binding activity to chromosomes to amino acids 396–430, which is a segment that is immediately adjacent to the rod domain and includes the nuclear localization signal (28), and studies with *Xenopus* lamin B2 revealed that the binding to chromatin requires both amino acids 404–419, which is a segment that begins 11 amino acids downstream to the end of the rod domain, and amino acids 432–467 (27). Although the amino-terminal sequence of the T425–622 protein has only low homology to these sequences, they all contain several conserved amino acids, including the R A/V S/T sequence (Fig. 2C). The sequences in the T425–622 protein that are required for chromatin binding also include the nuclear localization signal, which was found to be important for the binding of human lamin to chromosomes (28), and sequences with low homology to amino acids 432–467 in *Xenopus* lamin B2 (27).

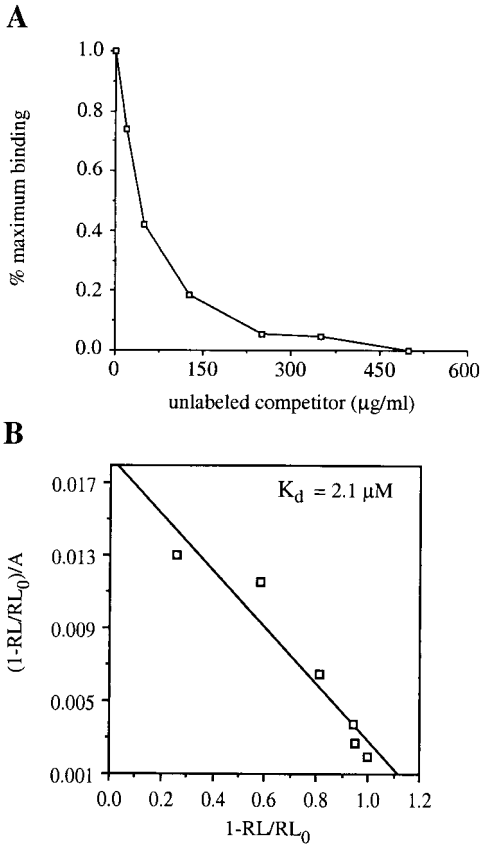

FIG. 3. Binding of the lamin Dm_0 tail domain to immobilized chromatin with an apparent K_d of 2.1 μM. (*A*) ^{125}I-labeled T425–622 protein (2 μg/ml) was incubated with immobilized polynucleosomes in the presence of increasing concentrations of unlabeled T425–622 protein. Correction for nonspecific binding was obtained by subtraction of values obtained with 250 μg/ml unlabeled T425–622. (*B*) Data for specific binding was analyzed as described (28). RL, amount of radioactive protein bound to chromatin at cold competitor concentration A; RL_0, amount of radioactive protein bound to chromatin in the absence of unlabeled competitor. Assuming a single-site competitive interaction (47), the slope given by this plot equals $-1/K_d$.

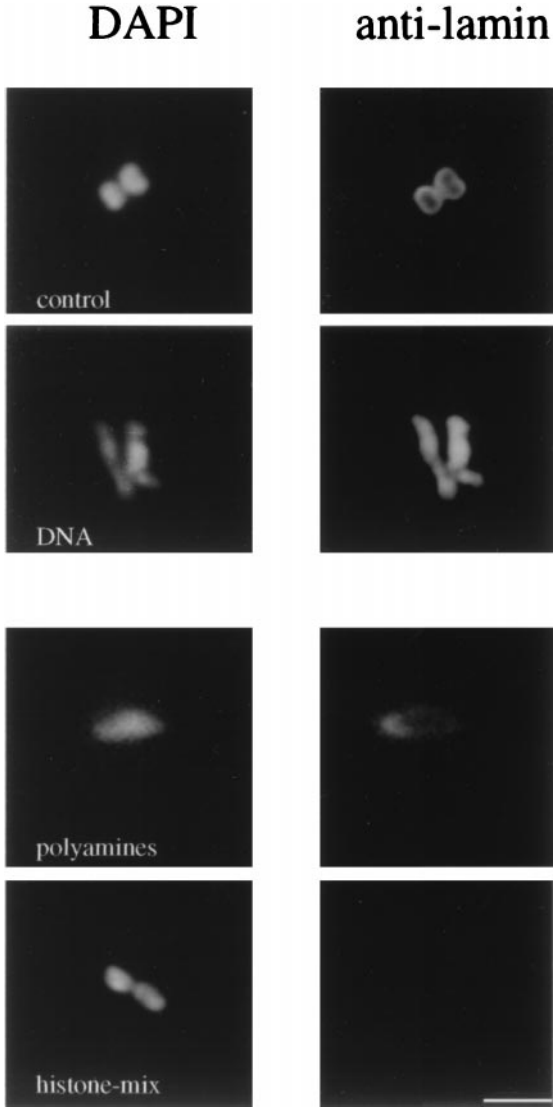

FIG. 4. The binding of lamin Dm_0 to chromosomes is mediated by histones. T425–622 protein was used to bind chromosomes in the presence of 10% BSA (control). Mitotic chromosomes were added 5 min after incubation of the reaction mixture with a plasmid containing the *Drosophila ftz* M/SAR DNA (38, 39), a mixture of spermine and spermidine (polyamines), or the commercial mixture of histone proteins (histone-mix). (Bar = 5 μm.)

The second region in lamin Dm_0 that is required for efficient binding is localized to the carboxyl-terminal part of its tail. The binding of *Xenopus* lamin A to chromatin was also mapped to sequences located at the carboxyl terminus of its tail domain (amino acids 598–641) (27). However, these regions in *Xenopus* lamin A and *Drosophila* lamin Dm_0 have only low homology with each other.

The Lamin Dm_0 Tail Domain Probably Interacts with Histones H2A and H2B. Lamin Dm_0 probably interacts specifically with the core histones H2A and H2B because these histones, both as homo-and heterodimers, can specifically displace the lamin tail's binding to chromosomes. This interaction does not result merely from the positive charge of these histones, because other positively charged molecules such as polyamines, core histones H3 and H4, and histone H1 cannot displace lamin binding to chromosomes. The specific displacement of the lamin tail domain from chromosomes by histones H2A and H2B is not an artifactual result of a change in chromatin structure caused by binding by these histones, because lamin can bind specifically to polynucleosomes, and nucleosome core particles could efficiently displace the binding of lamin tail to chromosomes. Rather, these results seem to implicate specific protein epitopes in histones H2A and H2B as sites that interact with the lamin tail domain. Although there is very low sequence homology between the four core histones, the specific competition with both histones H2A and H2B indicates that the lamin tail probably binds to a common histone motif such as the "histone fold" (41, 45). The specific inhibition of lamin tail's binding to chromosomes by the histone octamers and histone H2A/H2B dimer also indicates that these epitopes are exposed to lamin Dm_0. Taken together with previous observations that the binding of human lamin C to chromosomes can be displaced by a mixture of core histones (28), these results indicate that the interaction between lamin and histones H2A and H2B is evolutionarily conserved.

Like all core histones, histone H2A has a structurally defined central domain and a labile amino-terminal domain in which the structure and molecular interactions are not well defined (41). The amino-terminal domain of histone H2A is thought to be involved in the regulation of replication and transcription (46). It is, therefore, interesting to analyze the possible involvement of the histone H2A amino-terminal domain in the lamin tail binding. Preliminary results suggest that the binding of lamin Dm_0 tail domain to chromosomes does not require the histone H2A amino-terminal domain. Additional competition experiments utilizing mutant histones

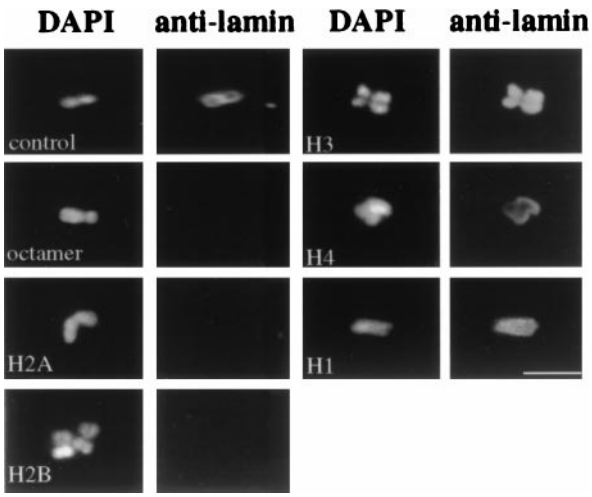

FIG. 5. The binding of lamin Dm_0 to chromosomes is mediated by histones H2A and H2B. T425–622 protein was used to bind chromosomes in the presence of 10% BSA (control). Chromosomes were added 5 min after incubation of the reaction mixture with histone octamers, purified histones H2A, H2B, H3, H4, or H1 (amino acids 1–142). (Bar = 6 μm.)

will be required to further map the lamin–histone interaction. Mutant core histones lacking specific domains, histone H2A/H2B heterodimer that lacks the amino terminal domains of both histones H2A and H2B, and histone octamers assembled from mutant and wild-type histone combinations should prove to be useful for this purpose.

Previous reports showed a strong interaction *in vitro* between lamin Dm_0 and M/SAR sequences (34, 35), which requires lamin polymerization and is mediated by the lamin Dm_0 rod domain (36). This interaction is different from the lamin–histones interaction, because the tail domain mediates the latter interaction with no apparent contribution from the rod domain. Further support to the specificity of the lamin–histones interaction came from the fact that a 100-fold molar excess of the *Drosophila ftz* S/MAR sequence could not displace the binding of the tail domain or R64 → H (not shown) to mitotic chromosomes. Because mitotic chromosomes and polynucleosomes represent a more native structure, as compared with isolated DNA sequences, it is likely that the interaction between lamin Dm_0 and chromatin *in vivo* involves its lamin Dm_0 tail domain and histones H2A and H2B.

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