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The α-helical rod domain of human lamins A and C contains a chromatin binding site

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We examined regions of human lamins A and C involved in binding to surfaces of mitotic chromosomes. An *Escherichia coli* expression system was used to produce full-length lamin A and lamin C, and truncated lamins retaining the central α-helical rod domain (residues 34-388) but lacking various amounts of the amino-terminal 'head' and carboxy-terminal 'tail' domains. We found that lamin A, lamin C and lamin fragments lacking the head domain and tail sequences distal to residue 431 efficiently assembled into paracrystals and strongly associated with mitotic chromosomes. Furthermore, the lamin rod domain also associated with chromosomes, although efficient chromosome coating required the pH 5-6 conditions needed to assemble the rod into higher order structures. Biochemical assays showed that chromosomes substantially reduced the critical concentration for assembly of lamin polypeptides into pelletable structures. Association of the lamin rod with chromosomes was abolished by pretrypsinization of chromosomes, and was not seen for vimentin (which possesses a similar rod domain). These data demonstrate that the α-helical rod of lamins A and C contains a specific chromosome binding site. Hence, the central rod domain of intermediate filament proteins can be involved in interactions with other cellular structures as well as in filament assembly.

Key words: chromatin binding/lamins/rod domain

Introduction

The nuclear lamina is a protein meshwork lining the nucleoplasmic side of the inner nuclear membrane (Franke *et al.*, 1981; Gerace and Burke, 1988; Dessev, 1992), which is thought to provide an architectural framework for the nuclear envelope and an anchoring site at the nuclear periphery for interphase chromosomes. In higher eukaryotes the lamina consists mainly of a polymer of one or more closely related proteins, termed lamins. Structural studies of purified lamins (Aebi *et al.*, 1986; Gieffers and Krohne, 1991; Heitlinger *et al.*, 1991; Moir *et al.*, 1991) and molecular cloning of lamin cDNAs has revealed that these proteins are members of the intermediate filament (IF) protein superfamily (Fisher *et al.*, 1986; McKeon *et al.*, 1986; Franke, 1987; Peter *et al.*, 1989; Hoger *et al.*, 1990). Similar to other IF proteins, lamins contain an internal α-helical 'rod' domain flanked at the amino- and carboxy-termini by 'head' and 'tail' domains of largely non-α-helical character. The rod domain of IF proteins consists mainly of heptad repeats that form a two stranded coiled-coil α-helix (Steinert and Roop, 1988; Stewart, 1990; Parry and Steinert, 1992). It is relatively conserved in size and sequence among different IF classes and forms the filament backbone. In contrast, the head and tail domains are highly divergent among different IF classes. Since these latter regions are thought to protrude from the IF surface (Steinert and Roop, 1988), they may mediate interactions of IFs with other cellular structures, consistent with *in vitro* binding studies (Georgatos *et al.*, 1985, 1987; Georgatos and Blobel, 1987).

Lamins of vertebrate cells have been classified in A and B subgroups based on sequence similarities (Gerace and Burke, 1988; Peter *et al.*, 1989). In mammalian somatic cells there are two major A-type lamins, termed lamins A and C. These two proteins are identical for the first 566 amino acids, but contain unique carboxy-terminal extensions after this common region that are probably the consequence of alternative splicing (Fisher *et al.*, 1986; McKeon *et al.*, 1986). In lamin A, the carboxy-terminal extension consists of 98 amino acids while in lamin C it comprises six residues. Two B-type lamins (termed lamin B_1 and lamin B_2) have been described in mammalian somatic cells, and are products of different genes (Hoger *et al.*, 1990).

The structure and assembly properties of vertebrate lamins have been analyzed *in vitro* utilizing proteins purified from animal tissues or bacterial expression systems (Aebi *et al.*, 1986; Gieffers and Krohne, 1991; Heitlinger *et al.*, 1991, 1992; Moir *et al.*, 1991). The basic lamin protomer is an ~50 nm long, rod-shaped dimer containing parallel and unstaggered monomers. *In vitro*, this protomer has a strong tendency to assemble in a head-to-tail fashion into linear polymers (Gieffers and Krohne, 1991; Heitlinger *et al.*, 1991), and also associates laterally to form filaments and paracrystals having a 23-25 nm axial repeat (Aebi *et al.*, 1986; Gieffers and Krohne, 1991; Heitlinger *et al.*, 1991; Moir *et al.*, 1991). A similar axial repeat is found in native lamin filaments (Aebi *et al.*, 1986), presumably reflecting an approximately half-staggered packing of protomers along the filament axis. Like other IF proteins (Stewart, 1990; Parry and Steinert, 1992), self-association of the lamin rod domain appears to be fundamentally important for its assembly. The lamin rod is sufficient for formation of paracrystals having a 23-25 nm transverse banding pattern, although the head and tail domains facilitate assembly of lamin protomers into higher order structures (Moir *et al.*, 1991; Heitlinger *et al.*, 1992).

Recent *in vitro* studies have shown that lamins can bind directly to chromatin (Glass and Gerace, 1990; Hoger *et al.*, 1991; Yuan *et al.*, 1991) and to purified DNA (Shoeman and Traub, 1990; Luderus *et al.*, 1992). In principle, lamin-chromatin binding could be important for attachment

of chromatin to the nuclear envelope in interphase and/or for lamin assembly at the end of mitosis. A useful situation for studying the interaction of lamins with chromatin occurs during higher eukaryotic mitosis, when lamins are disassembled to protomers during prophase by a process involving lamin phosphorylation (Gerace *et al.*, 1978; Gerace and Burke, 1988) and subsequently are reassembled into a polymeric structure at chromosome surfaces during telophase. We have developed an *in vitro* assay that mimics lamin reassembly around chromosomes during telophase. This involves dialysis of solubilized lamins into a buffer of physiological ionic strength and pH in the presence of mitotic chromosomes (Glass and Gerace, 1990). Under these conditions, lamins A and C assemble into a supramolecular structure in contact with the chromosome surfaces due to interactions with specific chromosomal binding sites (Glass and Gerace, 1990). In this study, we have used this assay to analyze the chromosome binding ability of lamin A, lamin C and truncated lamins lacking various amounts of the head and tail domains. Unexpectedly, we found that the rod domain alone of lamins A and C was sufficient for association with mitotic chromosomes. Hence, the central α-helical domain of lamins, in addition to being involved in filament formation, has the ability to interact specifically with another cellular structure (chromatin). This has general implications for IF structure and interactions.

Results

Expression and isolation of intact and truncated lamins A and C

To define a specific region of lamins A and C involved in chromosome binding, we have used an assay involving lamin association with mitotic chromosome surfaces (Glass and Gerace, 1990). These assay conditions lead to complete and uniform 'coating' of chromosomes with purified lamins A and C as visualized by immunofluorescence microscopy.

For the present study we prepared a variety of polypeptides related to human lamins A and C using an *Escherichia coli* expression system, including full-length lamin A and lamin C, and truncated versions of lamin A/C retaining the entire rod domain but lacking varying amounts of the 'head' and 'tail' domains. This truncation series was dictated by the nature of chromosome association in our assay, which is suggested to reflect a cooperative process involving both lamin−lamin and lamin−chromosome interactions (Glass and Gerace, 1990). Because of this cooperativity, mutant lamins lacking the ability to self-associate might be unable to associate with chromosomes under our conditions even if they retained the relevant chromosome binding site. To minimize this problem we restricted our analysis to constructs retaining the entire rod domain, since the latter region is essential for assembly of IFs, and removal of even short sequences at the amino- and carboxy-terminal ends of the IF rod is strongly deleterious to filament assembly (Parry and Steinert, 1992; Stewart, 1990). However, since the head and tail domains of lamins are dispensable for assembly into supramolecular structures (Moir *et al.*, 1991; Heitlinger *et al.*, 1992), it was possible to evaluate the chromosome association of lamin fragments lacking various parts of these regions.

Lamin constructs analyzed in this study are depicted in Figure 1A. In addition to lamin A (664 residues) and lamin C (572 residues), we examined seven versions of the common lamin A/C sequence truncated in the tail domain at positions 561, 548, 521, 500, 450, 431 and 399 (see Figure 1). Of particular interest was a stretch of highly charged residues found in both lamins A and C (residues 551−566) that contains a series of nine out of 10 sequential acidic residues followed by four sequential basic residues (histidines). This could be regarded as a candidate chromatin binding region of lamins, in analogy with other chromatin binding proteins which contain extended stretches of acidic and/or basic residues. Lamin A/C(1−561) lacks only the four histidines, while lamin A/C(1−548) lacks the clustered

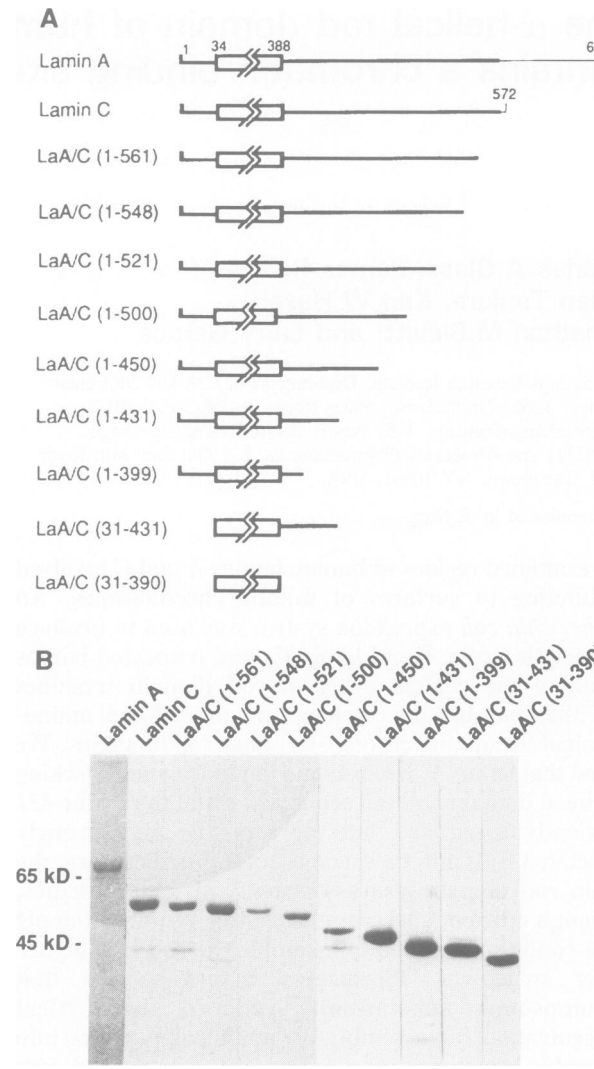

Fig. 1. Lamin polypeptides and fragments analyzed in this study. **(A)** Schematic diagram depicting cDNA constructs that were cloned into expression plasmids for producing lamins A and C and lamin A/C fragments in *E.coli* (see Materials and methods). The common rod domain extends from residue 34 to 388 (Fisher *et al.*, 1986; McKeon *et al.*, 1986). Other sequences common to lamins A and C are indicated by bold lines, while isotype-specific sequences in the tail domains are indicated by thin lines. Numbers in parentheses refer to the first and last residues of lamins in truncated constructs. **(B)** *E.coli* cells were transformed with expression plasmids and protein production was induced with IPTG (see Materials and methods). The expressed lamins and lamin fragments were solubilized from bacterial inclusion bodies in 8 M urea, purified by ion exchange chromatography and analyzed by 10% SDS−PAGE and Coomassie blue staining (see Materials and methods).

acidic residues as well. In addition to these deletions from the carboxy-terminus, we examined a construct containing the rod domain and only the first ~40 residues of the tail domain [lamin A/C(31−431)] and a construct containing only the rod domain [lamin A/C(31−390)].

Lamin polypeptides were expressed in *E.coli* using an expression system driven by bacteriophage T7 RNA polymerase (Studier *et al.*, 1990). Expressed polypeptides were solubilized from an inclusion body fraction of bacteria in 8 M urea, and were subsequently purified by two steps of ion exchange chromatography. As shown by SDS−PAGE (Figure 1B), the purified proteins were devoid of major contaminating bands. The constructs migrated with their expected gel mobility, and furthermore, they reacted with anti-lamin antibodies when analyzed by Western blotting (data not shown) and immunofluorescence microscopy (Figures 3 and 4).

Assembly of intact and truncated lamins into higher order structures

We first examined whether the expressed lamins and lamin fragments were able to assemble into paracrystals or filament bundles when dialyzed from high ionic strength/alkaline pH dissociating conditions into buffers at pH 7, pH 6 or pH 5 containing approximately physiological salt concentrations (see Materials and methods). This analysis allowed us to define conditions for efficient self-association of the lamin fragments. Representative constructs examined by electron microscopy (EM) after negative staining are shown in Figure 2. When dialyzed into pH 7 assembly buffer, full-length lamin A and lamin C (Figure 2) assembled into paracrystals with a transverse banding pattern consisting of alternating light and dark bands with longitudinal periodicity of ~25 nm, similar to a mixture of rat liver lamins A and C (Aebi *et al.*, 1986). All carboxy-terminally truncated versions of lamin A/C up to and including lamin A/C(1−431) yielded similar paracrystals, although with some constructs a thin dark band bisecting the light longitudinal bands was clearly evident in the paracrystals [e.g. Figure 2, LaA/C (1−431)]. In the case of the latter carboxy-terminal truncation, the amino-terminal head domain was not required for assembly of paracrystalline structures, since lamin A/C(31−431) efficiently formed similar paracrystals with these conditions (Figure 2).

In contrast to these results, lamin fragments representing further carboxy-terminal truncations required lower pH values to assemble into paracrystals or filament bundles with the salt conditions used for this assay. Lamin A/C(1−399) yielded filamentous structures that showed no strong tendency to associate laterally in pH 7.0 assembly buffer (Figure 2), while filament bundles were obtained in pH 6 assembly buffer (data not shown), and paracrystals displaying somewhat disordered transverse banding were obtained at pH 5 (Figure 2). Finally, lamin A/C(31−390) representing the rod domain displayed unassembled protomers at pH 7 (Figure 2) and filament bundles at both pH 6 (data not shown) and pH 5 (Figure 2). Hence our results suggest that assembly of lamins A and C into higher order structures in pH 7 buffer is particularly sensitive to amino acid truncations near the carboxy-terminus of the rod region proximal to residue 431. However, lowering the pH of the assembly buffer without changing the salt concentration strongly promotes the self-association of these shorter lamin fragments.

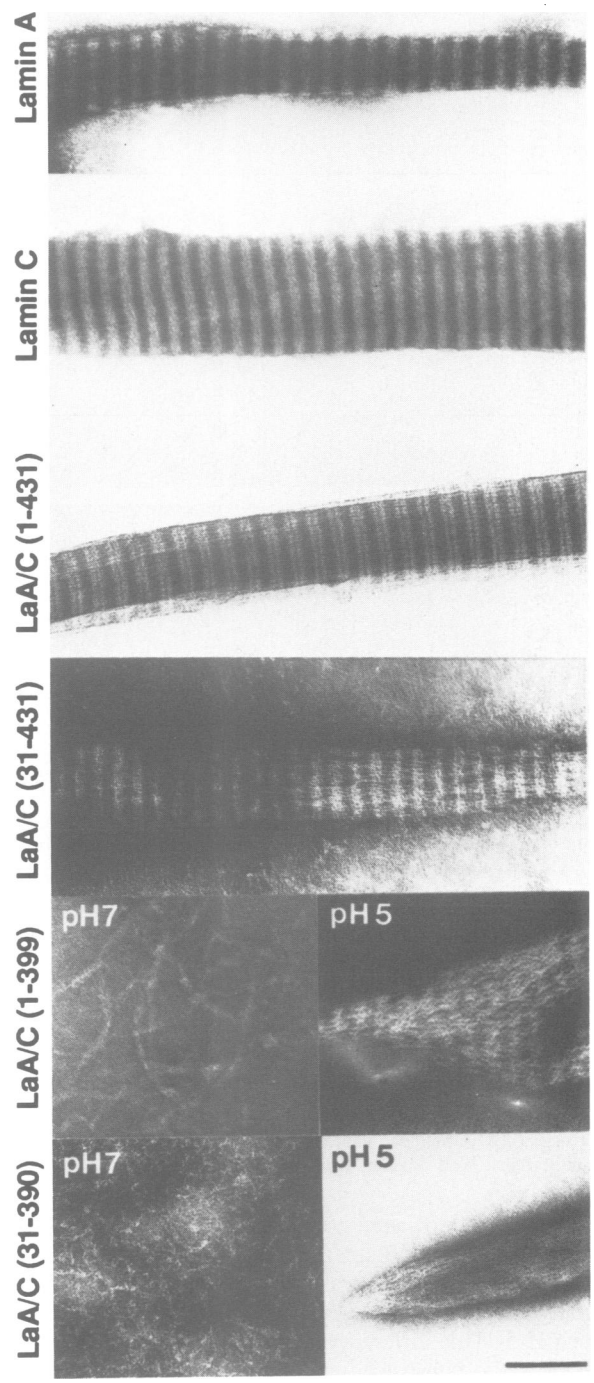

Fig. 2. *In vitro* assembly of paracrystals and related structures from expressed lamins and lamin fragments. Purified polypeptides were dialyzed from a solution containing 8 M urea into either pH 7 or pH 5 buffer (see Materials and methods). Following dialysis, samples were adsorbed to charged EM grids, negatively stained with 1% uranyl acetate and viewed by electron microscopy. Bar = 100 nm.

The results of these assembly studies in part agree with studies carried out by Moir *et al.* (1991) who showed that intact human lamin A and lamin C form paracrystals with ~25 nm transverse banding at pH 7, and that lower pH

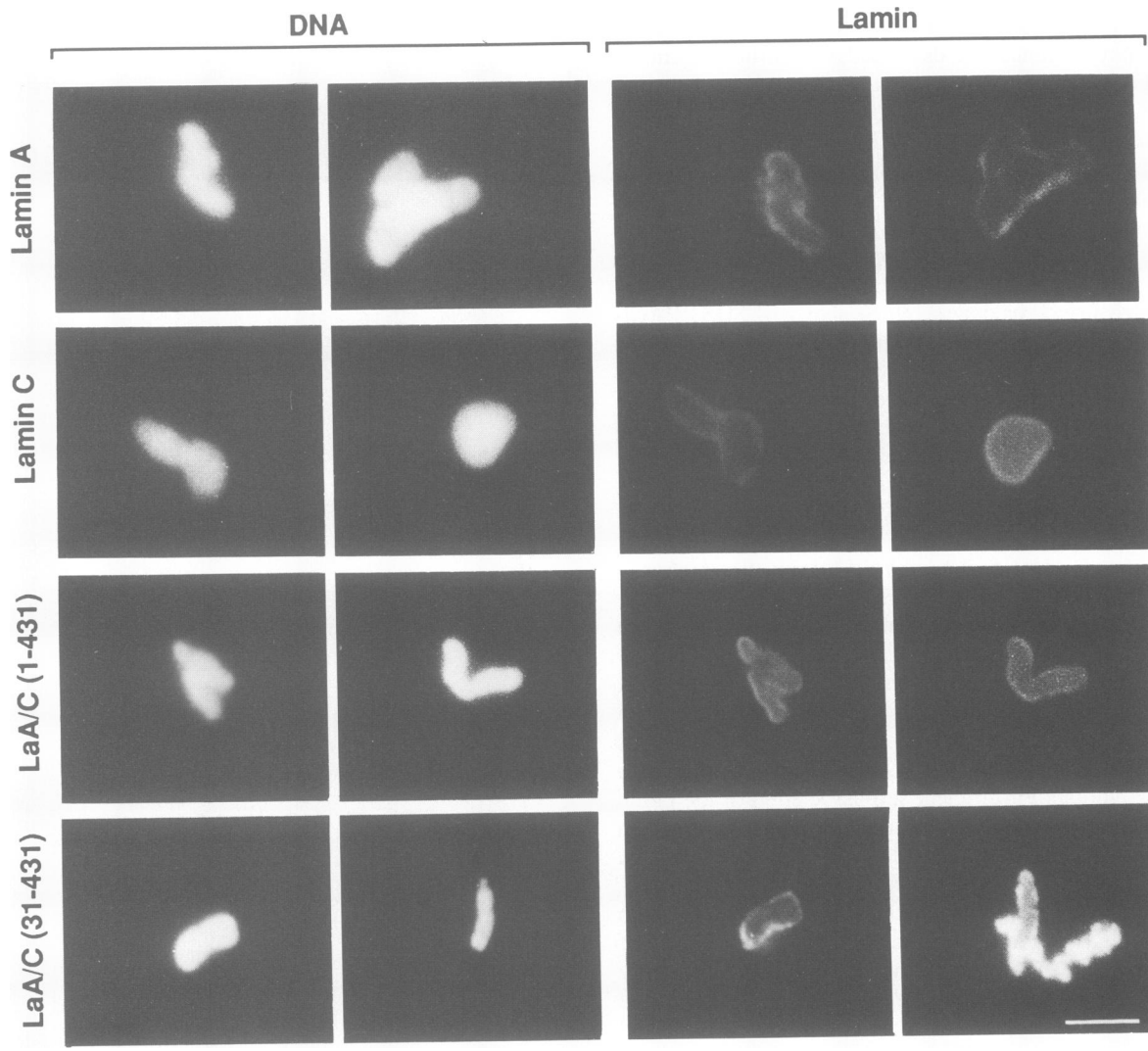

Fig. 3. Assembly of lamins and longer lamin fragments around mitotic chromosomes *in vitro* at pH 7. Samples of lamin A, lamin C, lamin A/C(1−431) and lamin A/C(31−431) were dialyzed out of a solution containing 8 M urea into lamin solubilization buffer, and were then mixed with mitotic chromosomes and dialyzed into pH 7 assembly buffer (see Materials and methods). Samples were then fixed, adsorbed on to coverslips and labeled with anti-lamin antibodies for indirect immunofluorescence microscopy (right two panels). Samples were also stained with Hoechst 33258 to visualize DNA (left two panels). Lamin A, lamin C and lamin A/C(1−431) were stained with a polyclonal antibody specific for a peptide in the amino-terminal head domain, while lamin A/C(31−431) was stained with a monoclonal antibody specific for the rod domain. Bar = 5 μm.

promotes self-association of a lamin rod fragment. However, the latter workers obtained paracrystals with a lamin rod construct at pH 6, in contrast to the filament bundles lacking apparent transverse banding that we obtained at either pH 6 or pH 5. This difference may be explained by different solutions and constructs used for *in vitro* assembly (Moir *et al.*, 1991).

Association of intact and truncated lamins with chromosomes

To evaluate the ability of these different lamin constructs to bind to chromosomes, purified proteins at concentrations ranging from 25 to 250 μg/ml were mixed with mitotic chromosomes in lamin solubilizing buffer and samples were then dialyzed into 'assembly buffer' of approximately physiological ionic strength (see Materials and methods) and examined by immunofluorescence microscopy (Figure 3). When full-length lamin A and lamin C polypeptides were dialyzed with chromosomes into pH 7 assembly buffer, essentially all chromosomes became uniformly coated with lamins, as observed previously for a mixture of purified rat liver lamins A and C (Glass and Gerace, 1990). (It should be noted that occasional chromosome preparations yielded incomplete lamin coating, but this was seen for both intact and truncated lamins and presumably resulted from steric interference by particulate material aggregated on the chromosome surfaces and/or by partial chromosome disruption.) Hence, lamin A and lamin C are individually able to bind to sites on mitotic chromosome surfaces. All of the lamin A/C constructs truncated from the carboxy-terminus up to and including residue 431 assembled around chromosomes in a fashion similar to intact lamins A and C, yielding complete chromosome coating [e.g. Figure 3, LaA/C(1−431); others not shown]. The same result was obtained with a construct lacking the lamin head domain [Figure 3, LaA/C(31−431)]. Together, these results indicate that the chromosome binding site for lamins A and C revealed in this assay does not involve the head domain or the tail domain distal to residue 431.

However, chromosome association obtained with a further

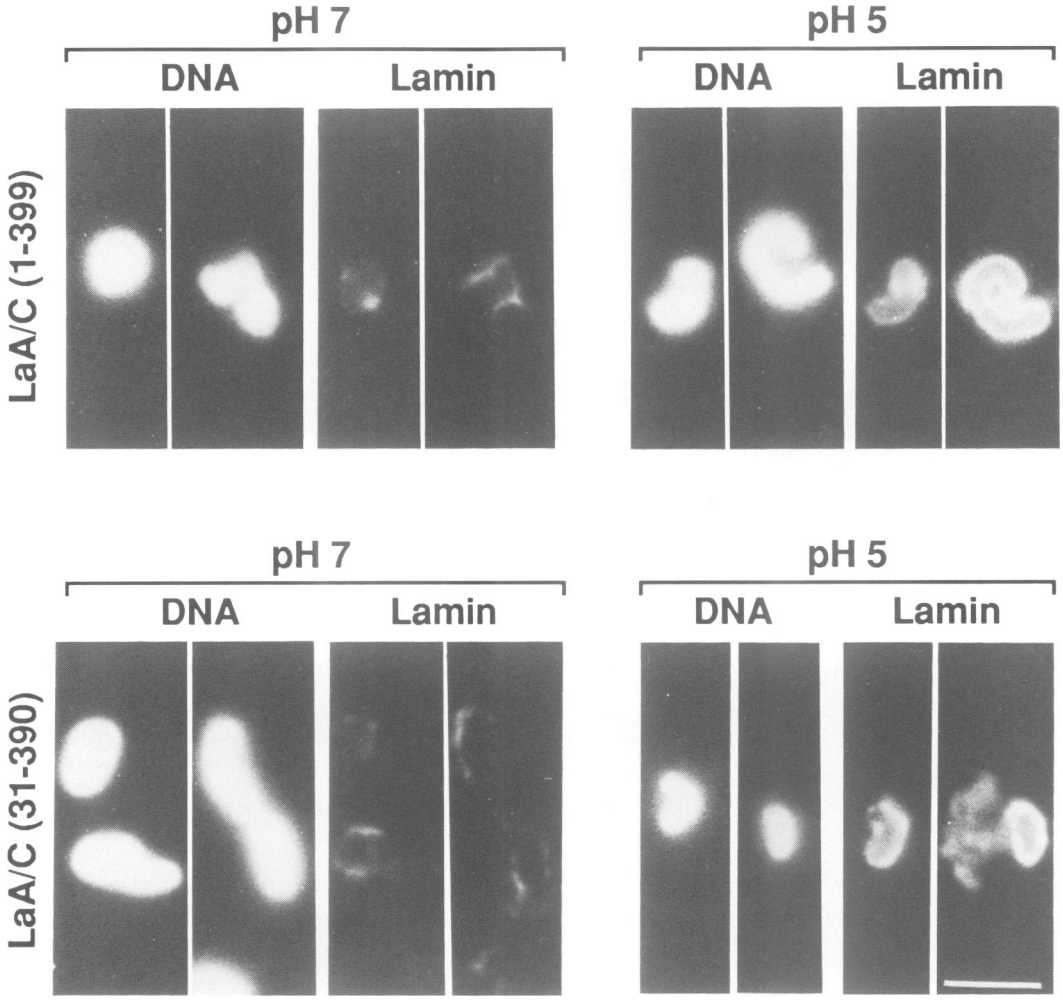

Fig. 4. Effect of pH on assembly of shorter lamin fragments around chromosomes. Samples of lamin A/C(1−399) and lamin A/C(31−390) were dialyzed into lamin solubilization buffer, mixed with mitotic chromosomes and then dialyzed into either pH 7 assembly buffer (left set of panels) or pH 5 assembly buffer (right set of panels). Samples were subsequently processed for immunofluorescence microscopy to visualize lamins and DNA as in Figure 3. Concentrations of lamin fragments were 22 and 44 μg/ml for lamin A/C(1−399) at pH 7, 35 μg/ml for lamin A/C(1−399) at pH 5, 256 μg/ml for lamin A/C(31−390) at pH 7 and 130 μg/ml for lamin A/C(31−390) at pH 5. Bar = 5 μm.

carboxy-terminal truncation of lamins A/C [lamin A/C(1−399)] was substantially impaired in pH 7 assembly buffer (Figure 4). In these cases, truncated lamins were not detectable around a substantial number of chromosomes at the lower protein concentrations tested (e.g. 25 μg/ml). At higher concentrations (e.g. 250 μg/ml), association with chromosomes was 'patchy' and reduced in intensity compared with the previous constructs [Figure 4, LaA/C(1−399) and LaA/C(31−390)].

The diminished chromosome association of these shorter lamin constructs could be due to loss of a lamin region that facilitates chromosome binding. Alternatively, this effect simply could be due to the fact that the shorter constructs have impaired ability to assemble into paracrystals or filament bundles at pH 7. To test this latter possibility, we analyzed the chromosome association of these shorter lamin constructs in assembly buffers of pH 5 and pH 6, where there was more self-association to yield higher order structures than at pH 7 (Figure 2). Indeed, when lamin A/C(1−399) and lamin A/C(31−390) were dialyzed with chromosomes into either pH 5 assembly buffer (Figure 4) or pH 6 assembly buffer (data not shown), they associated with most chromosomes to yield a strong and relatively continuous coating, in contrast to the faint and patchy chromosome coating obtained with the same lamin fragment concentration in pH 7 assembly buffer (Figure 4, compare pH 5 and pH 7 panels). This supports the notion that the diminished ability of lamin A/C(1−399) and lamin A/C(31−390) to coat chromosomes at pH 7 is due to impaired ability to self-associate, rather than loss of the chromosome binding site essential to this interaction. However, we cannot exclude the possibility that sequences immediately amino- and carboxy-terminal to the lamin rod also have an influence on the efficiency of the chromosome interaction.

When samples containing chromosomes coated with lamin A/C(31−390) were examined by thin section EM (Figure 5A), the chromosome surfaces appeared essentially identical to those from samples incubated in the absence of lamin (Figure 5B). Hence the lamin construct that coats chromosomes under these conditions does not occur in large assemblies such as filament bundles (e.g. Figure 2). Rather, it is present as an undetectably 'thin' coating similar to the native nuclear lamina, which cannot be visualized at the surfaces of Triton-treated liver nuclei by thin section EM

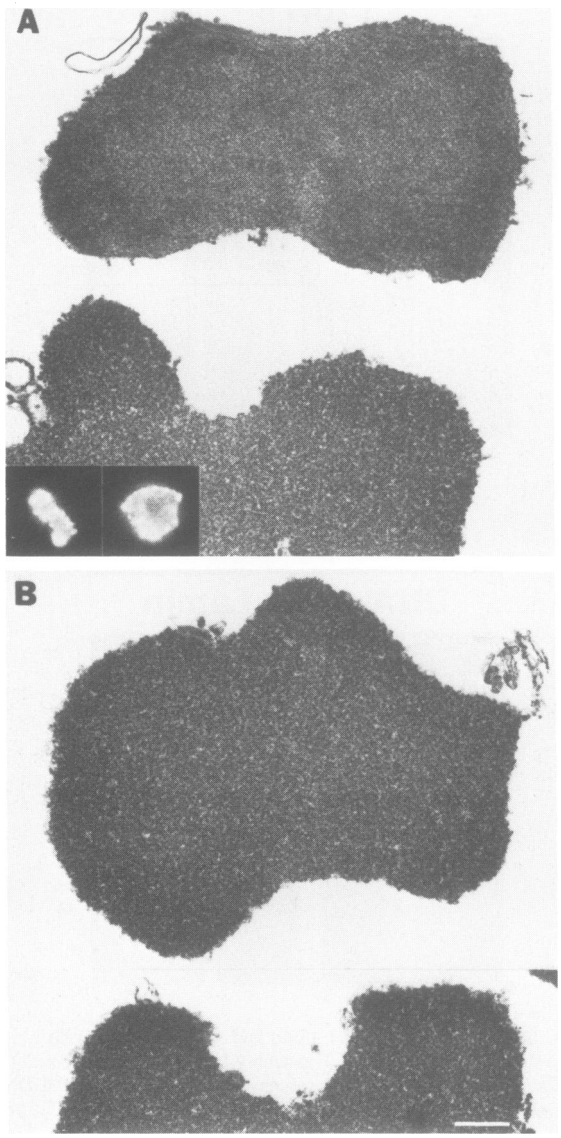

Fig. 5. Analysis of chromosomes coated with the lamin rod by electron microscopy. Samples of chromosomes were dialyzed into pH 6 assembly buffer in the presence (**A**) or absence (**B**) of lamin A/C(31−390). Following dialysis, aliquots were removed for immunofluorescence microscopy and the remaining material was fixed and processed for thin section electron microscopy. Shown are electron micrographs of representative chromosomes. Insets in panel A are fluorescence micrographs of representative chromosomes from the sample containing lamin A/C(31−390) labeled with anti-lamin antibodies. With this chromosome preparation, we obtained slightly discontinuous coating of chromosomes both with lamin A/C(31−390) and with full-length lamin A (not shown). Bar = 300 nm.

(Aaronson and Blobel, 1974). Similarly, chromosomes coated with a mixture of intact lamins A and C (Glass and Gerace, 1990) showed no visible structure corresponding to assembled lamins at the mitotic chromosome surfaces in thin section EM (J.Blevitt, J.Glass and L.Gerace, unpublished).

In our earlier studies on association of rat liver lamins A and C with chromosomes, we demonstrated that chromosomes substantially lowered the critical concentration for assembly of lamins into an insoluble structure (Glass and Gerace, 1990). This provided a clear biochemical indication of specific lamin binding sites on chromosome surfaces. We

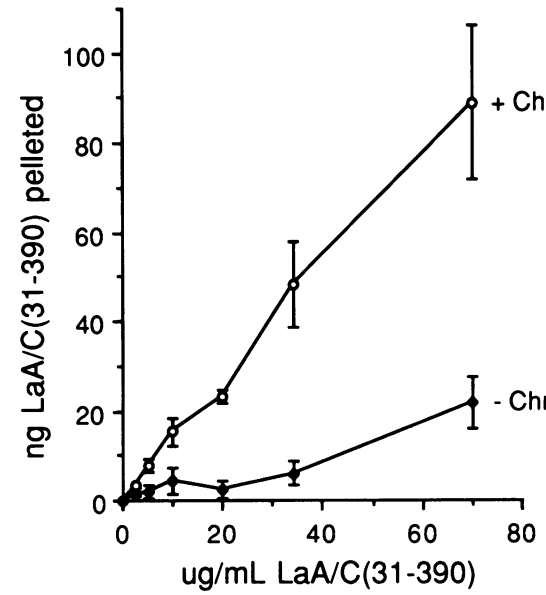

Fig. 6. Concentration dependence for assembly of the lamin rod domain in the absence or presence of chromosomes. Radiolabeled lamin A/C(31−390) was dialyzed into lamin solubilization buffer. Samples were then diluted to a range of different protein concentrations and dialyzed over a period of 120 min into pH 6 assembly buffer in the absence (−Chr.) or presence (+Chr.) of mitotic chromosomes. Samples were then pelleted (see Materials and methods) and the amount of radiolabeled lamin A/C(31−390) in the pellets was determined.

have performed a similar analysis with the lamin A/C rod domain (residues 31−390). As shown in Figure 6, in pH 6 assembly buffer, lamin A/C(31−390) first detectably assembled into a pelletable structure in the absence of chromosomes at 70 μg/ml. This value is substantially higher that the critical concentration for self-assembly of intact rat lamins A and C (~4 μg/ml; Glass and Gerace, 1990), consistent with the demonstration that the head and tail domains of lamins promote self-assembly (Gieffers and Krohne, 1991; Moir *et al.*, 1991; Heitlinger *et al.*, 1992). In the presence of mitotic chromosomes in pH 6 assembly buffer (Figure 6), the lamin A/C rod domain first detectably entered a pelletable structure at 5−10 μg/ml, and the amount of this construct in the pellet progressively increased above this value. Entry into a pelletable form in this concentration range was accompanied by association of this fragment with chromosome surfaces as detected by immunofluorescence microscopy (data not shown). Therefore, these data support the possibility that chromosome binding sites promote self-assembly of the lamin rod domain, similar to the effect seen with intact lamins. Furthermore, these data provide direct biochemical evidence for the presence of a chromosome binding region in the rod domain of lamins A and C.

Specificity of assembly around chromosomes

To demonstrate that association of the lamin A/C rod with chromosomes is specific, we evaluated whether the rod was still able to interact with chromosomes that had been briefly pretreated with trypsin. Previous studies showed that prior trypsinization of chromosomes completely abolished the ability of intact rat liver lamins A and C to bind to chromosomes. As shown in Figure 7, when chromosomes were preincubated with 0.5 μg/ml of trypsin for 2 min (+ trypsin), the patchy coating of untrypsinized chromosomes

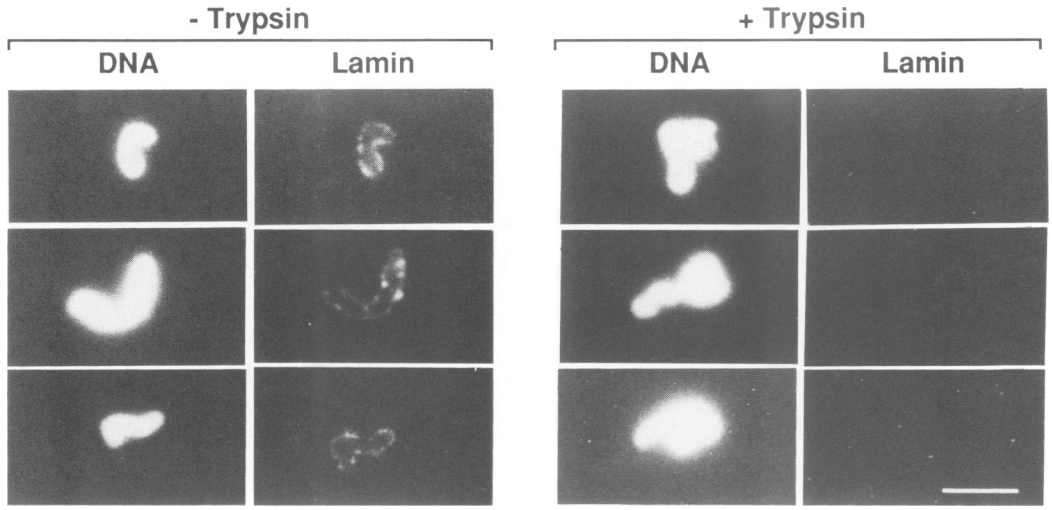

Fig. 7. Effect of trypsin pretreatment of chromosomes on chromosome association of the lamin A/C rod domain. Chromosomes were preincubated in the absence (− trypsin) or presence (+ trypsin) of trypsin. Subsequently, the trypsin was inactivated (see Materials and methods) and the chromosomes were added to lamin A/C(31−390) in lamin solubilization buffer. Samples were then dialyzed over a period of 90 min into pH 7 assembly buffer, and then processed for immunofluorescence microscopy and DNA labeling as in Figure 3. Bar = 5 μm.

obtained when the rod domain was incubated in pH 7 assembly buffer was almost completely abolished. These conditions of trypsin treatment cause slight chromosome swelling, but do not cause large scale degradation of histones (Glass and Gerace, 1990). We obtained similar results when assembly of the lamin A/C rod was analyzed at pH 6.0 as in Figure 6. When chromosomes were pretreated with 1 μg/ml trypsin and subsequently incubated in the presence of 20 μg/ml radiolabeled lamin A/C(31−390), only 20% of the lamin rod appeared in the pellet relative to a control sample containing untrypsinized chromosomes (data not shown). Hence, mild proteolysis of chromosomes destroys or significantly alters the chromosomal binding sites for the lamin A/C rod. This indicates that the chromosome surfaces are not simply providing sites for nonspecific aggregation of the rod domain. Rather, chromosome association of the lamin A/C rod depends on structural features or components of chromosomes that are destroyed by brief trypsin treatment.

In view of the overall sequence similarity between the rod domain of lamins and other IF proteins (see Introduction), we performed one additional experiment to validate that the chromosomal association of lamins and lamin fragments obtained in our assay has biologically relevant specificity. Since cytoplasmic IF proteins are absent from the nucleus *in vivo*, they should not associate with mitotic chromosome surfaces *in vitro*, even though cytoplasmic IF proteins also contain a structurally related α-helical rod domain.

To carry out this experiment, we analyzed assembly of bovine lens vimentin in the presence of mitotic chromosomes, both in the standard pH 7 lamin assembly buffer, and with a somewhat different buffer ('vimentin assembly buffer') previously described for *in vitro* assembly of vimentin (Figure 8). The vimentin preparation we isolated was highly enriched in vimentin as seen by SDS−PAGE and immunoblotting (Figure 8A), although it also contained a band migrating slightly more quickly than vimentin. This lower band also was reactive with anti-vimentin antibodies and thus must have been a proteolytic product of vimentin as observed previously (Nelson and Traub, 1982). When this preparation was used for *in vitro* assembly of vimentin IF in either vimentin assembly buffer or pH 7 lamin assembly buffer (see Materials and methods), it yielded distinct 10 nm filaments as seen by EM of negatively stained specimens (Figure 8B).

Immunofluorescence microscopy to detect vimentin assembly was carried out with a monoclonal antibody prepared against human fibroblast vimentin. This antibody recognizes both denatured and native bovine vimentin, since it binds to bovine vimentin on immunoblots (Figure 8A), stains the vimentin IF network present in a cultured bovine cell line (MDBK cells; Figure 8C), and decorates aggregates of bovine vimentin filaments adsorbed to polylysine-coated coverslips (Figure 8D). When bovine vimentin was dialyzed with mitotic chromosomes into pH 7 lamin assembly buffer (230 μg/ml) or into vimentin assembly buffer (165 μg/ml), no association of vimentin with the chromosome surfaces could be detected (Figure 8F). In contrast, a control lamin construct [lamin A/C(1−431)] strongly coated mitotic chromosome surfaces when dialyzed into either buffer (Figure 8E). Therefore, a cytoplasmic IF protein—vimentin—does not associate with mitotic chromosomes *in vitro* with our assay conditions, demonstrating that chromosome binding is not a general feature of the IF α-helical rod domain. This provides additional strong support for the possibility that the interaction measured *in vitro* is specific to lamins and is physiologically significant.

Discussion

We have isolated full-length and truncated lamins A and C from an *E.coli* expression system, and have used these proteins for identification of a region of human lamins A and C involved in binding to the surfaces of mitotic chromosomes *in vitro*. These expressed proteins included full-length lamin A and lamin C, and lamin fragments from which progressively increasing amounts of the carboxy-terminal tail domain and/or the amino-terminal head domain were removed from the common lamin A/C sequence (residues 1−566) by truncation. All expressed proteins contained the central α-helical rod domain of lamins A and C; the smallest fragment used in this study was essentially

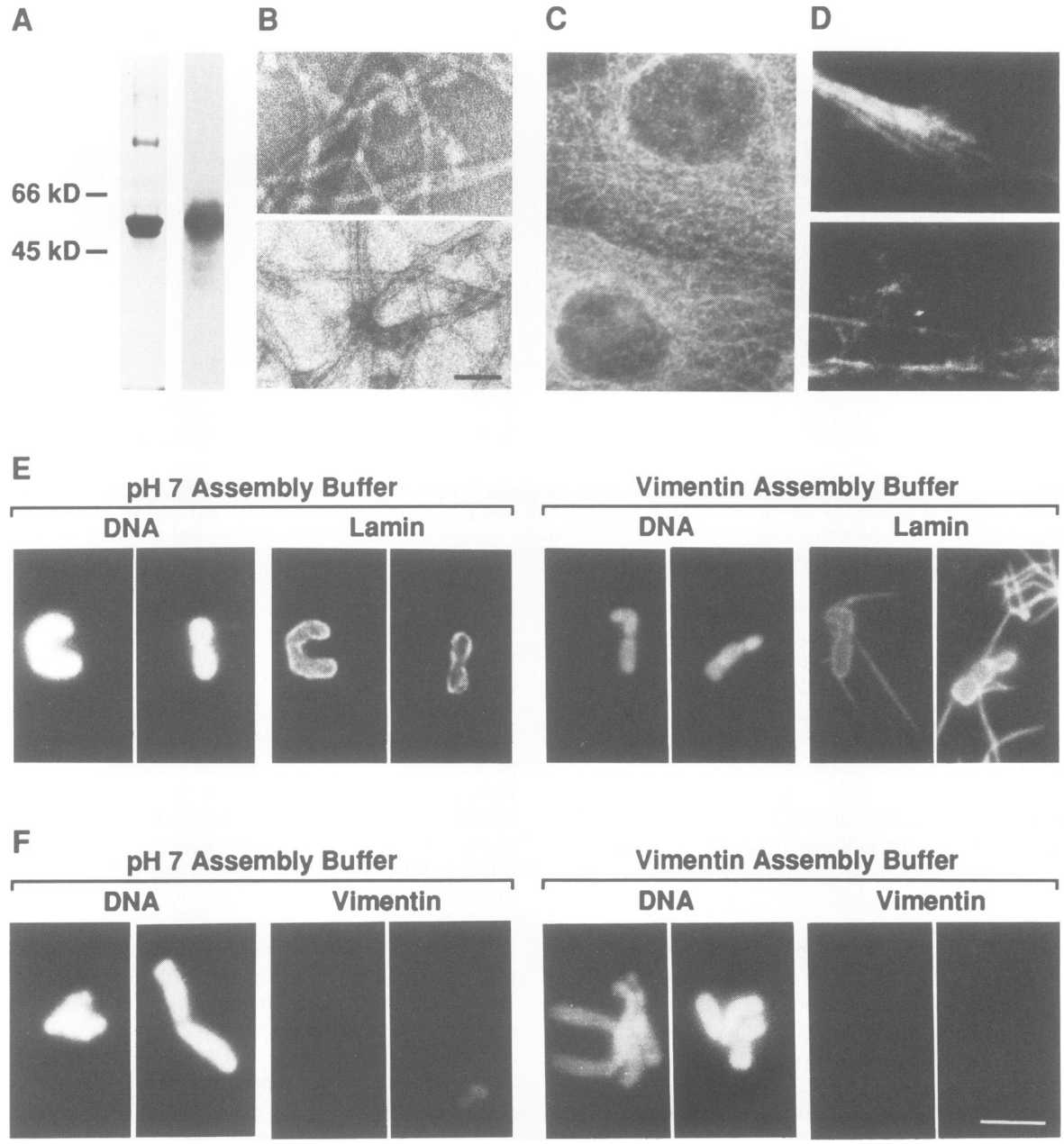

Fig. 8. Characterization of purified vimentin and assembly of vimentin around mitotic chromosomes. (**A**) Vimentin was purified from bovine lens (see Materials and methods) and analyzed by 10% SDS−PAGE followed by staining with Coomassie blue (lane 1) or immunoblotting with anti-vimentin antibodies (lane 2). (**B**) Electron micrographs of negatively stained specimens show filaments assembled from the purified vimentin fraction. Top panel is a sample dialyzed into 'vimentin assembly buffer' and bottom panel is a sample dialyzed into 'pH 7 assembly buffer'. Bar = 50 nm. (**C**) MDBK cells were stained for immunofluorescence microscopy with anti-vimentin antibodies. (**D**) Purified vimentin was assembled in vimentin assembly buffer, adsorbed on to poly-L-lysine coated coverslips and stained with anti-vimentin antibodies for immunofluorescence microscopy. (**E**) Lamin A/C(1−431) was assembled in the presence of mitotic chromosomes in pH 7 assembly buffer or vimentin assembly buffer as indicated. Left panels show DNA staining with Hoechst 33258. Right panels show the corresponding chromosomes stained with specific antibodies. (**F**) Vimentin was assembled and analyzed as in panel E. Bar = 5 μm.

the rod domain without flanking sequences (residues 31−390).

Since our previous work suggested that association of lamins A and C with mitotic chromosome surfaces *in vitro* may be a cooperative process dependent on both lamin assembly into a supramolecular structure and on lamin−chromosome binding, the purified proteins were first tested for their ability to self-assemble into higher order structures in buffers of approximately physiological ionic strength. In previous work, a number of different vertebrate lamins obtained by purification from tissues or expression in bacteria have been shown to readily form paracrystals displaying 23−25 nm longitudinal banding in EM (Aebi *et al.*, 1986; Gieffers and Krohne, 1991; Heitlinger *et al.*, 1991, 1992; Moir *et al.*, 1991). In pH 7 assembly conditions, we found that lamin A, lamin C and all carboxy-terminal truncations of the common lamin A/C sequence up to and including residue 431 [as well as the lamin A/C(31−431) headless variant] assemble into the characteristic lamin paracrystals with a 25 nm longitudinal repeat. However, when further residues were removed from the tail domain [in lamin A/C(1−399) and lamin A/C(31−390)], the ability

of these polypeptides to assemble into higher order structures in pH 7 buffer is lost or severely reduced. Hence the lamin tail domain between residues 399 and 430 exerts a significant effect on the ability of lamins to self-associate in a buffer of approximately physiological pH and ionic strength. However, lowering the pH of the assembly buffer to pH 5 or pH 6 can partially compensate for loss of this region, since these latter constructs show considerably enhanced ability to form higher order structures (filament bundles) under the more acidic conditions than at pH 7. An enhancement of lamin rod assembly at pHs at or below 6 has been reported previously for human lamins A and C (Heitlinger *et al.*, 1991; Moir *et al.*, 1991).

When the ability of these constructs to associate with mitotic chromosomes was evaluated in pH 7 assembly buffer, we found that lamin A, lamin C and all carboxy-terminal truncations of lamins A/C up to and including residue 431 [including the headless lamin A/C(31−431)] strongly and uniformly coated most chromosome surfaces as visualized by immunofluorescence microscopy, similar to a mixture of rat liver lamins A and C (Glass and Gerace, 1990). In contrast, constructs containing further truncations of the carboxy-terminus [lamin A/C(1−399) and lamin A/C(31−390)] associated with chromosomes weakly and in a 'patchy' fashion at pH 7. However, if assembly was carried out at pH 5 or pH 6 where self-association of these lamin fragments was enhanced compared with pH 7, these same constructs associated strongly with chromosomes. It is plausible that removal of the region between residues 391 and 430 of lamins A and C affects chromosome association of this construct indirectly, by diminishing the ability of lamins to self-associate (see above). It also is possible that residues 391−430 are part of a second chromosome binding site (see below). Nevertheless, these data demonstrate that a (major) site responsible for association of lamins A and C with chromosomes in our assay occurs within the α-helical rod domain.

In view of the extensive sequence similarity between the rod domain of lamins A and C and that of B-type lamins (e.g. Peter *et al.*, 1989; Hoger *et al.*, 1990), it is plausible that the rod domain is a chromatin binding region common to all lamins. The chromatin binding properties of lamin B have not yet been analyzed in detail, although binding of lamin B to isolated DNA has been reported (Luderus *et al.*, 1992).

It is of considerable interest that a lamin region consisting mainly of a coiled-coil α-helix is involved in association with chromatin. Interaction of coiled-coil α-helices with heterologous structures is not unprecedented. For example, tropomyosin, which is almost entirely a coiled-coil helix, apparently interacts at multiple sites with actin monomers when bound to actin filaments (McLachlan and Stewart, 1976). The precise nature of the chromosome binding site for the lamin rod domain is presently unclear, but may involve protein, DNA or a protein−DNA complex such as the nucleosome. While trypsin pretreatment of chromosomes abolishes the ability of the rod domain to associate detectably with chromosomes, mild trypsinization may disrupt the higher level organization of a hypothetical array of binding sites required for cooperative assembly of the rod, rather than destroy the binding site itself.

While the rod domain of lamins A/C is shown to contain a chromosome binding site in the present work, other regions of lamin molecules also may be directly involved in the interaction of lamins with chromatin. Recent studies measuring association of *in vitro* translated *Xenopus* lamins with minichromosomes assembled in *Xenopus* oocyte extracts (Hoger *et al.*, 1991) have provided evidence for a chromatin binding site in the tail domain of certain lamin isotypes, since chromosome association was not detected when part of the carboxy-terminal tail domain of these lamins was removed. We have recently obtained direct support for a second chromatin binding site in the lamin tail, since we have obtained saturable, specific and moderately high affinity binding of recombinant lamin A and lamin C tails to isolated chromatin fragments (H.Taniura, C.Glass and L.Gerace, manuscript in preparation). Hence lamins A and C appear to have at least two distinct chromatin binding regions.

Several lines of evidence indicate that the mitotic chromosome binding revealed by our assay is likely to be physiologically significant. First, the chromosome association that we measure *in vitro* (Glass and Gerace, 1990 and this study) closely resembles reassembly of lamins at the chromosome surfaces seen in cultured cells *in vivo* during telophase (Gerace *et al.*, 1978; Gerace and Burke, 1988). The associated lamins do not occur in paracrystals or filament bundles, but rather are undetectable by thin section EM, similar to the native lamina of rat liver nuclei. Second, our lamin binding studies involve a native substrate that occurs in cells (chromosomes), rather than isolated components derived from chromatin, which may be more prone to nonspecific interactions when their interactions with other chromatin components are disrupted. Third, mild nuclease or protease pretreatment of chromosomes abolishes lamin association (Glass and Gerace, 1990 and this study), indicating that lamins do not simply coat highly charged surfaces in a nonspecific fashion. Fourth, as shown in this study, a cytoplasmic IF protein (vimentin) which does not enter the nucleus *in vivo* does not associate with chromosomes under our conditions, further arguing for specificity of this assay. Nonetheless, direct functional studies will be required to determine the biological relevance of our findings, as well as the significance of lamin association with isolated DNA (Shoeman and Traub, 1990; Luderus *et al.*, 1992) and with chromatin fragments (Yuan *et al.*, 1991) measured in other assays.

A priori, chromosome binding of lamins could have a number of different functions. First, it could be important for mediating reassembly of the nuclear lamina at the end of mitosis, which in turn could be important for certain aspects of nuclear envelope reassembly (see Glass and Gerace, 1990). In addition, a subset of the binding interactions of lamins with chromosomes that may be established during telophase could persist during the subsequent interphase, and could be involved in anchoring specific chromosomal regions to the nuclear periphery. This could play a fundamental role in ordering the genome in the interphase nucleus and potentiating its functions (see Benavente and Krohne, 1986; Newport *et al.*, 1990; Meier *et al.*, 1991). Finally, it is possible that lamins are involved in contributing to cooperative assembly of heterochromatin at the nuclear periphery during cell differentiation (Franke, 1974) by providing an array of chromatin binding sites at the nuclear periphery.

Our results on the presence of a chromatin binding region in the lamin A/C rod have interesting implications for IF

proteins in general. The rod domain of IF polypeptides appears to function principally in formation of the IF backbone through several types of self-association (Steinert and Roop, 1988; Stewart, 1990). Our results suggest that this may not be the only function of this region. Based on the lamin–chromatin model, the IF rod in some circumstances may interact with other cellular components besides itself, and these heterologous interactions could in part contribute to the role of IFs in cellular architecture. Evidence for interaction of an IF rod domain with a heterologous protein has also been obtained in the case of vimentin binding to plectin (Foisner *et al.*, 1988). It will be important to investigate the possible existence of additional heterologous associations of IF rod regions.

Materials and methods

Construction of expression plasmids

Clones containing the full-length lamin A and lamin C cDNA sequences were generously given by Gunter Blobel's laboratory (Fisher *et al.*, 1986). Full-length and truncated regions of the lamin A sequence were amplified by the polymerase chain reaction (PCR) (Saiki *et al.*, 1988). Synthetic oligonucleotide primers for PCR contained sequences corresponding to the amino- and carboxy-termini of the desired polypeptide products, flanked sequentially by sequences for initiation and termination codons and by sequences for restriction enzyme sites (*Nde*I and *Eco*RI) compatible with subcloning into the expression plasmid pRK172 (McLeod *et al.*, 1987), which is driven by an inducible bacteriophage T7 RNA polymerase promoter. Ligation reactions were used to transform the XL1-blue strain of *E.coli* (Stratagene), and colonies containing plasmids with lamin sequences were identified by restriction enzyme analysis.

Expression of lamin constructs in bacteria

Polypeptides were expressed in the *E.coli* strains K38 (Tabor and Richardson, 1985) or BL21 (Studier *et al.*, 1990). When K38 cells were used, colonies from transformed cultures were grown to an OD_{600} of 0.2, at which point helper bacteriophage mGP1-2 (Tabor and Richardson, 1985) (an M13 derivative harboring the T7 RNA polymerase gene), was added at a multiplicity of infection of 20 together with 2 mM IPTG (isopropyl β-D-thiogalactopyranoside). Growth was continued for 2–3 h or overnight. Cells were then collected by centrifugation, washed once in 10 mM Tris–HCl pH 8, 100 mM NaCl, 1 mM EDTA, and pelleted cells were stored at −20°C. Alternatively, expression was carried out in the bacterial strain BL21. BL21 contains the T7 RNA polymerase gene under the control of the β-galactosidase promoter in a lambda lysogen, as well as a plasmid containing the PlysS gene to inhibit T7 polymerase arising from 'leaky' expression prior to induction (Studier *et al.*, 1990). Single colonies of transformed bacteria were grown to an OD_{600} of 0.5–0.8 at which point expression was induced by addition of 2.5 mM IPTG. Bacterial growth was continued for ~2 h after which cells were harvested as above.

Purification of expressed lamins and lamin fragments

Bacterial cell pellets from 500–1000 ml cultures were resuspended using a Dounce homogenizer in 9 vol of lysis buffer. Lysis buffer contained 50 mM Tris–HCl pH 8.0, 1 mM EDTA, 100 mM NaCl, 1 mM DTT, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin. Resuspended cells were then incubated at room temperature for 40 min with 10 mg/ml lysozyme. Following this incubation, deoxycholate was added to 1.3 mg/ml and the solution was incubated at 37°C for 15 min with continuous stirring. The solution then was made 2 mM in $MgCl_2$, DNase I was added to a final concentration of 1 μg/ml and the solution was incubated for 30 min at room temperature. Following DNase treatment, the solution was sonicated with a Branson sonifier equipped with a microtip at maximum power for two 1 min periods on ice, and then frozen and thawed twice using liquid nitrogen and a 37°C water bath. The solution was then spun at 12 000 *g* for 5 min at room temperature and the pellet resuspended in 9 vol of lysis buffer containing 0.5% Triton X-100 and 10 mM EDTA. After a 5 min incubation at room temperature, the solution was respun at 12 000 *g* for 5 min at room temperature and the pellet resuspended in 5 ml 'urea solubilization buffer' containing 8 M urea, 20 mM Tris–HCl pH 8.8, 1 mM DTT, 1 μg aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin. The urea solution was then centrifuged for 10 min at 3000 *g* at 4°C. Urea supernatants were stored at 4°C with no detectable lamin polypeptide breakdown.

Lamins or lamin fragments were isolated from this final urea supernatant by ion exchange chromatography under various conditions at room temperature. Lamin A, lamin C and truncations through lamin A/C(1–431) were purified by phosphocelulose ion exchange chromatography (P11, Whatman) followed by diethylaminoethyl cellulose ion exchange chromatography (DE52, Whatman) at pH 8.8. Lamin polypeptides were eluted from the P11 using a 0 to 0.5 M NaCl gradient and were recovered in the flow-through and wash fractions from DE52. Lamin A/C(31–431) and lamin A/C(1–399) were bound to P11 in urea solubilization buffer at pH 6.5 with added 20 mM sodium phosphate and at pH 5.5 with added 20 mM sodium acetate, respectively. Fractions were eluted using a 0 to 0.5 M NaCl gradient. Lamin A/C(31–431) was then purified with Q-Sepharose (Pharmacia) in urea solubilization buffer at pH 8 using a 0 to 0.5 M NaCl gradient while lamin A/C(1–399) was purified with DE52 in urea solubilization buffer at pH 8.8 using a 0 to 0.5 M NaCl gradient. Lamin A/C(31–390) was purified by chromatography on Q-Sepharose (Pharmacia) using a 0 to 0.5 M NaCl gradient in 8 M urea, 20 mM Tris–HCl pH 8 and 2 mM EDTA, followed by elution from S-Sepharose (Pharmacia) with a 0 to 0.5 M NaCl gradient in 8 M urea, 20 mM MES pH 6, 2 mM EDTA. Purified lamins and lamin fragments were stored at 4°C in a solution containing 8 M urea, 20 mM Tris–HCl pH 8.8.

In vitro lamin assembly on chromosomes

Mitotic chromosomes used in this assay were isolated from synchronized mitotic CHO cells and were stored frozen as described (Glass and Gerace, 1990). CHO cultures were incubated in nocodazole for only ~1.5 h prior to collection of mitotic cells by shake-off. With this condition, all mitotic cells are arrested in a pseudo-metaphase state where the lamina is completely disassembled, and no lamins can be detected at the surfaces of isolated chromosomes by immunofluorescence microscopy. We determined by quantitative immunoblotting that the mitotic chromosome sample contains ~3% of lamins A and C found in an equivalent number of interphase cells. All lamins A and C in this sample can be ascribed to contaminating interphase nuclei, since the mitotic cell population used for chromosome isolation contains 3–5% interphase cells.

In vitro lamin assembly on chromosomes was performed by methods similar to those described previously (Glass and Gerace, 1990). Lamins or lamin fragments were dialyzed from urea-containing storage buffer into 20 mM Tris–HCl pH 8.0, 250 mM KCl, 1 mM DTT, 1 μg/ml aprotinin, 1 μg/ml leupeptin and 1 μg/ml pepstatin. Next, 20 μl of chromosomes (stock solution at 0.8 OD_{260}/ml) was mixed with 80 μl of 20 mM Tris–HCl pH 8, 180 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 5 mg/ml BSA, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin and 1–10 μl of dialyzed lamin solutions. The final lamin concentration at this point was between 25 and 250 μg/ml, except for experiments shown in Figure 6. Samples were then loaded into individual wells of a microdialyzer unit (Pierce Chemical Co., Rockford, IL) and dialyzed against 'assembly buffer' of pH 7, pH 6 or pH 5 for 1–2 h at room temperature or 37°C. pH 7 assembly buffer contained 50 mM HEPES–KOH pH 7, 70 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 1 μg/ml aprotinin, 1 μg/ml leupeptin and 1 μg/ml pepstatin. pH 6 assembly buffer contained 50 mM PIPES–KOH pH 6, 70 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 1 μg/ml aprotinin, 1 μg/ml leupeptin and 1 μg/ml pepstatin. pH 5 assembly buffer contained 50 mM sodium acetate pH 5, 70 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 1 μg/ml aprotinin, 1 μg/ml leupeptin and 1 μg/ml pepstatin. Dialyzed samples were transferred to glass coverslips situated in 6-well culture plates and centrifuged at 1000 r.p.m. for 5 s in a model GPR centrifuge (Beckman Instruments). Samples on the coverslips were then fixed for 5 min in 3.7% formaldehyde in PBS (phosphate buffered saline) containing 5 mM $MgCl_2$ and washed with PBS. Coverslips were then stained for immunofluorescence microscopy by incubation with a rabbit antiserum raised against a synthetic peptide from the head domain of human lamins A and C (residues 18–32) followed by rhodamine-conjugated mouse anti-rabbit IgG (Cappel Laboratories, Melvern, PA), or with a mouse monoclonal antibody that recognizes the rod domain of rat and human lamins A and C (RL12) followed by rhodamine-conjugated goat anti-mouse IgG (Cappel Laboratories, Melvern, PA). Lastly, coverslips were stained with the DNA-specific dye Hoechst 33258 (Calbiochem-Behring Corp.) at a concentration of 10 μg/ml in PBS, washed in PBS and mounted in a Mowiol (Calbiochem) solution. Slides were examined using a Zeiss Axiophot microscope equipped with epifluorescence optics and photographed using Kodak T-MAX 400 film.

In some cases, chromosomes were pretreated with TPCK-trypsin for various time periods prior to use in assembly assays as described (Glass and Gerace, 1990), except that a 100-fold excess by weight of soybean trypsin inhibitor (Sigma Chemical Co.) was used following the digestion period.

Concentration dependence of rod domain assembly

Lamin A/C(31–390) was labeled with ^{125}I using Iodobeads (Pierce) by recommended procedures. Polypeptides were first dialyzed into 100 mM borate pH 8.5 containing 100 mM NaCl. Following a 15 min labeling period, polypeptides were separated from free iodide by dialysis into 15 mM Tris–HCl pH 8.8, 100 mM NaCl, 0.1 mM PMSF. Typical labeling yielded 1×10^6 c.p.m./μg. For assembly, various amounts of labeled polypeptides were mixed with or without chromosomes in a solution containing 10 mM HEPES–KOH pH 7, 90 mM NaCl, 5 mM $MgCl_2$, 2 mg/ml BSA. These solutions were dialyzed in microdialyzer wells for 2 h at room temperature against a solution containing 50 mM HEPES–KOH pH 6, 100 mM NaCl, 5 mM $MgCl_2$. Following dialysis, the samples were made 1% in Triton-X100 and layered on 150 μl solutions of 20% sucrose in dialysis buffer (pH 6) in narrow 400 μl microfuge tubes (Eppendorf). Tubes were centrifuged in a JS13.1 (Beckman) swinging bucket rotor with 4-place adaptors at 12 800 r.p.m. for 10 min at 10°C. Following centrifugation the tubes were frozen in a dry ice/ethanol bath and the bottom 0.5 cm excised for gamma counting.

Vimentin assembly

Vimentin was purified from bovine lenses as previously described (Georgatos *et al.*, 1985) with modifications (Georgatos and Blobel, 1987). For assembly of vimentin in the presence of chromosomes, chromosomes were pelleted and resuspended in 2 mM Tris–HCl pH 8.5, 5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 5 mg/ml BSA, and purified vimentin was dialyzed against 2 mM Tris–HCl pH 8.5, 1 mM DTT, 1 mM PMSF at 4°C. Next, 20 μl of chromosomes were mixed with 80 μl of buffer consisting of 2 mM Tris–HCl pH 8.5, 5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 5 mg/ml BSA, and 30 μl dialyzed vimentin was added. Samples were then dialyzed in a microdialyzer at 37°C for 1–2 h into either 'pH 7 assembly buffer' (see above) or 'vimentin assembly buffer' (100 mM imidazole-HCl pH 6.8, 10 mM EGTA, 5 mM $MgCl_2$). Low ionic strength solutions were used for initial buffers in this experiment (in contrast to the initial buffers used for lamin assembly around chromosomes) to promote solubility of unassembled vimentin.

As a control to monitor lamin assembly under these conditions, 10 μl of lamin A/C(1–431) was mixed with 20 μl of resuspended chromosomes and 80 μl of buffer containing 25 mM Tris–HCl pH 8, 225 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 5 mg/ml BSA, and samples were dialyzed into either pH 7 lamin assembly buffer or vimentin assembly buffer as above. Vimentin and lamin samples from each of the *in vitro* assembly procedures were fixed on coverslips as described above, stained with specific antibodies and Hoechst dye #33258, visualized by fluorescence microscopy, and photographed as described above.

Vimentin specific mouse monoclonal antibodies (IgM) obtained from Sigma (St Louis, MO) were used at a 1:200 dilution as recommended and visualized by rhodamine-conjugated goat anti-mouse IgG H+L (Pierce) antibodies. Antibody recognition of the purified vimentin was tested by immunofluorescence staining of *in vitro* assembled vimentin fixed after adsorbing to poly-L-lysine coated glass coverslips and by immunofluorescence staining of vimentin-containing MDBK (Madine–Darby bovine kidney) cells.

Electron microscopy

To examine the structures formed by *in vitro* assembly of lamins and lamin fragments at pH 5–7, 50–200 μg/ml of purified polypeptides were dialyzed from storage buffer into one of the following solutions: (i) 20 mM PIPES–KOH pH 7, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin; (ii) 20 mM PIPES–KOH pH 6, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin; 25 mM sodium acetate pH 5, 5 mM $MgCl_2$, 1 mM DTT, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin. Following dialysis, samples were adsorbed on to carbon-coated grids and negatively stained for 1.5 min in 1% uranyl acetate. Grids were observed using a Philips CM12 electron microscope.

For analysis of vimentin assembly, a 50–500 mg/ml solution was first dialyzed for 2 h into 10 mM Tris–HCl pH 8.5, 1 mM DTT, 1 mM PMSF, and particulate matter was removed by centrifugation at 100 000 *g* for 30 min at 4°C. Subsequently, 0.1 vol of 1 M imidazole-HCl pH 6.8, 10 mM EGTA was added and the solution incubated at 37°C for 30 min. Alternatively, the vimentin solution was dialyzed into the pH 7 buffer used for analysis of lamin filament assembly by electron microscopy (see above). Vimentin filaments were then adsorbed on to grids and visualized by EM as described for lamins.

For EM analysis of chromosomes coated with the lamin rod, chromosomes were diluted into 20 mM Tris pH 8, 180 mM KCl, 5 mM $MgCl_2$, 5 mg/ml BSA. Lamin A/C(31–390) was added to 60 μg/ml as in standard assembly assays (see above), and the mixture was dialyzed into pH 6 assembly buffer for 2 h at 37°C. After removal of small aliquots for immunofluorescence microscopy, the remaining samples were mixed with equal volumes of 4% glutaraldehyde in pH 6 assembly buffer and fixed for 1 h on ice. The samples were then pelleted and processed for thin section EM as described by Aaronson and Blobel (1974).

Protein concentration, SDS–PAGE and Western blotting

Protein concentrations for lamin polypeptides and vimentin were determined with a Bio-Rad protein assay kit using bovine gamma-globulins (Bio-Rad) to construct standard curves. SDS–PAGE of lamin polypeptide and vimentin samples was performed by the method of Laemmli (1970) using gel sample buffer containing 2% SDS, 150 mM Tris–HCl pH 8.8, 10% sucrose, 10 mM DTT. Electrophoretic transfer of protein was by the method of Burnette (1981) using a semi-dry blotting apparatus. Lamin polypeptides and vimentin were detected with anti-lamin and anti-vimentin antibodies followed either by ^{125}I-labeled protein A directly or following an intermediate incubation with rabbit anti-mouse IgM chain antibodies (Pierce, Rockford, IL).

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References

Aaronson,R. and Blobel,G. (1974) *J. Cell Biol.*, **62**, 746–754.
Aebi,U., Cohn,J., Buhle,L. and Gerace,L. (1986) *Nature*, **323**, 560–564.
Benavente,R. and Krohne,G. (1986) *J. Cell Biol.*, **103**, 1847–1854.
Burnette,W.N. (1981) *Anal. Biochem.*, **112**, 195–203.
Dessev,G. (1992) *Curr. Opin. Cell Biol.*, **4**, 430–435.
Fisher,D.Z., Chaudhary,N. and Blobel,G. (1986) *Proc. Natl Acad. Sci USA*, **83**, 6450–6454.
Foisner,R., Leichtfried,R., Herrmann,H., Small,J., Lawson,D. and Wiche,G. (1988) *J. Cell Biol.*, **106**, 723–733.
Franke,W.W. (1974) *Int. Rev. Cytol.*, Suppl. **4**, 71–236.
Franke,W.W. (1987) *Cell*, **48**, 3–4.
Franke,W.W., Scheer,U., Krohne,G. and Jarasch,E.-D. (1981) *J. Cell Biol.*, **91**, 39s-50s.
Georgatos,S. and Blobel,G. (1987) *J. Cell Biol.*, **105**, 105–115.
Georgatos,S., Weaver,D. and Marchesi,V. (1985) *J. Cell Biol.*, **100**, 1962-1967.
Georgatos,S., Weber,K., Geisler,N. and Blobel,G. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 6780–6784.
Gerace,L. and Burke,B. (1988) *Annu. Rev. Cell Biol.*, **4**, 335–374.
Gerace,L., Blum,A., and Blobel,G. (1978) *J. Cell Biol.*, **79**, 546–566.
Gieffers,C. and Krohne,G. (1991) *Eur. J. Cell Biol.*, **55**, 191–199.
Glass,J. and Gerace,L. (1990) *J. Cell Biol.*, **111**, 1047–1057.
Heitlinger,E., Peter,M., Haner,M., Lustig,A., Aebi,U. and Nigg,E. (1991) *J. Cell Biol.*, **113**, 485–495.
Heitlinger,E., Peter,M., Lustig,A., Villiger,W., Nigg,E. and Aebi,U. (1992) *J. Struct. Biol.*, **108**, 74–91.
Hoger,T., Zatloukal,K., Waizenegger,I. and Krohne,G. (1990) *Chromosoma*, **6**, 379–390.
Hoger,T., Krohne,G. and Kleinschmidt,J. (1991) *Exp. Cell Res.*, **197**, 280–289.
Laemmli,U.K. (1970) *Nature*, **227**, 680–682.
Luderus,M., de Graaf,A., Mattia,E., Blaauwen,J., Grande,M., Jong,L. and van Driel,R. (1992) *Cell*, **70**, 949–959.
McKeon,F.D., Kirschner,M.W. and Caput,D. (1986) *Nature*, **319**, 463–468.
McLachlan,A. and Stewart,M. (1976) *J. Mol. Biol.*, **103**, 271–298.
McLeod,M., Stein,M. and Beach,D. (1987) *EMBO J.*, **6**, 729–736.
Meier,J., Campbell,K., Ford,C., Stick,R. and Hutchinson,C. (1991) *J. Cell Sci.*, **98**, 271–279.
Moir,R.D., Donaldson,A.D. and Stewart,M. (1991) *J. Cell Sci.*, **99**, 363–372.
Nelson,W.J. and Traub,P. (1982) *J. Biol. Chem.*, **257** (10), 5536–5543.
Newport,J., Wilson,K. and Dunphy,W. (1990) *J. Cell Biol.*, **111**, 2247–2259.

Parry,D. and Steinert,P. (1992) *Curr. Opin. Cell Biol.*, **4**, 94–98.
Peter,M., Kitten,G., Lehner,C., Vorburger,K., Bailer,S., Maridor,G. and Nigg,E. (1989) *J. Mol. Biol.*, **208**, 393–404.
Saiki,R.K., Gelfand,D.H., Stoffel,S., Scharf,S.J., Higuchi,R., Horn,G.T., Mullis,K.B. and Erlich,H.A. (1988) *Science*, **239**, 487.
Shoeman,R. and Traub,P. (1990) *J. Biol. Chem.*, **265**, 9055–9061.
Stewart,M. (1990) *Curr. Opin. Cell Biol.*, **2**, 91–100.
Steinert,P. and Roop,D. (1988) *Annu. Rev. Biochem.*, **57**, 593–625.
Studier,F., Rosenberg,A., Dunn,J. and Dubendorff,J. (1990) *Methods Enzymol.*, **185**, 60–89.
Tabor,S. and Richardson,C.C. (1985) *Proc. Natl Acad. Sci. USA*, **82**, 1074–1078.
Yuan,J., Simos,G., Blobel,G. and Georgatos,S. (1991) *J. Biol. Chem.*, **266**, 9211–9215.

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