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DIFFERENTIAL ACCESSIBILITY OF THE TAIL DOMAIN OF NUCLEAR LAMIN A IN INTERPHASE AND MITOTIC CELLS

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Human autoantibodies reactive against the tail domain exclusive to lamin A and absent from lamin C have been used for immunofluorescence studies on human fibroblast and epithelial cells. These autoantibodies were seen to react on mitotic cells where lamin A is present in a soluble depolymerized form and to react against lamin A in assembled interphase nuclear lamina after *in situ* extraction of chromatin. Taken together, these results support the suggestion that the tail domain of lamin A may be involved in the putative interaction of lamin A with chromatin.

The nuclear lamina lining the nucleoplasmic face of the inner nuclear membrane is composed of A- and B-type lamins (1,2). While B-type lamins appear responsible for lamina attachment to the nuclear membrane (3-5), A-type lamins have been shown to interact with chromatin (6-8). *In vitro* reconstitution studies have shown that lamins A and C, the two highly related mammalian A-type lamins (9,10), reassociate with the surface of mitotic chromosomes to reassemble a lamina structure (6-8). Binding of lamin A was more rapid than that of lamin C (7). However, which portion of the lamin A and C molecules is responsible for this interaction with chromatin was not determined.

In this study we show that an epitope in the exclusive carboxyl-terminus of lamin A, located outside the central rod domain involved in lamin polymerization and absent from lamin C, was inaccessible or masked in interphase cells. This epitope was accessible in mitotic cells where lamin A was present in a depolymerized state (1) or after *in situ* extraction of interphase cells under conditions where chromatin was solubilized. These results constitute the first experimental evidence that the tail domain of lamin A interacts in interphase cells with another element of the nucleus, the extraction properties of which are compatible with its identification as chromatin.

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MATERIALS AND METHODS

Electrophoresis and immunoblotting

Conditions for SDS-polyacrylamide gel electrophoresis, electrophoretic transfer of proteins onto nitrocellulose sheets and immunodetection were as previously described (11). Rat liver lamins were purified using established techniques (12). Cleavage of individually purified lamins with N-chlorosuccinimide and subsequent separation and identification of the fragments have been described (11).

Cell culture

Human fibroblasts (WI38, ATCC CCL75) were cultivated in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) heat-inactivated fetal calf serum. Human epithelial cells (HEp-2, ATCC CCL23) were grown in Eagle's minimal essential medium in the presence of 10% (v/v) heat-inactivated fetal calf serum. Enrichment for mitotic cells was achieved by a 22h exposure of HEp-2 cell cultures to the microtubule polymerization inhibitor nocodazole (13) at a final concentration of 4 μg/ml.

Antisera and indirect immunofluorescence

A guinea pig antiserum reactive against lamins A and C has been previously described (14). Human autoantibodies reactive against lamins were identified during a survey of serum samples from patients seen at Notre-Dame Hospital (JLS and YR, manuscript in preparation). Human autoantibodies reactive against lamin B have been described (15). The three anti-lamin A serum autoantibodies used in this study were from a normal subject, and from two patients with osteoarthritis and ankylosing spondylitis, respectively (to be described elsewhere). The code numbers for these sera are 787, 312 and 896, respectively. Human autoantibodies reactive against lamins A and C (LS1, 16) were kindly provided by Dr. Frank McKeon (Harvard Medical School). All anti-lamin reactive antibodies were of IgG isotype.

Serum adsorption experiments were conducted using purified rat liver lamins (12) or purified human lamin A expressed in *E. coli* as described (9,17) from a plasmid kindly provided by Dr. Frank McKeon (Harvard Medical School). An aliquot of the serum (5 μl) diluted in phosphate-buffered saline containing 6% (w/v) bovine serum albumin was incubated for 60 min at 37°C in a humidified chamber with a 10-20 μg sample of lamins suspended in phosphate-buffered saline (5μl). Control experiments were run in parallel but without the addition of lamins. After centrifugation for 2 min at 12000 x g in a Beckman Microfuge the supernatant was used immediately for indirect immunofluorescence as previously described (15,18,19).

RESULTS AND DISCUSSION

Human autoantibodies were reacted against purified rat liver lamins. Fig. 1a shows that three of these autoantibodies reacted exclusively against lamin A under conditions where a lamin A and C reactive antiserum (14) recognized both polypeptides. According to the cDNA sequences determined for lamins A and C (9,10), the epitopes recognized by the autoantibodies must be located in the 98 amino acid carboxyl-terminal extension of lamin A over the otherwise identical sequence of lamin C. This epitope assignment was

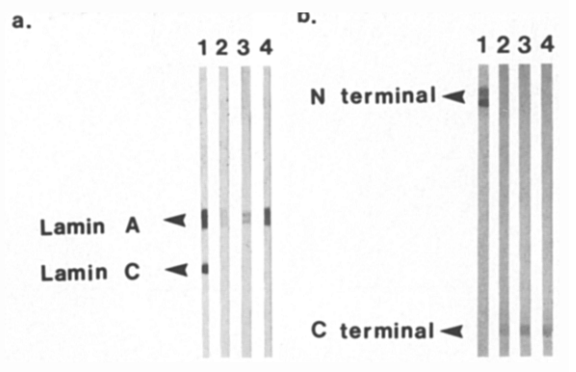

Figure 1. Human autoantibodies specific to the carboxyl-terminus of lamin A. Immunoblotting on purified rat liver lamins (panel a) or on N-chlorosuccinimide cleaved rat liver lamin A (panel b) using an antiserum reactive against both lamins A and C (lane 1) or with human sera 312 (lane 2), 787 (lane 3) and 896 (lane 4).

confirmed by reaction of all three autoantibodies on the carboxyl-terminal fragments of lamin A (Fig. 1b) resulting from N-chlorosuccinimide cleavage at tryptophan residues (11), while the lamin A and C antiserum reacted against the amino-terminal fragments shared by lamin A with lamin C. This constitutes the first report of autoantibodies from human sera reacting exclusively against lamin A.

Immunofluorescence experiments using the lamin A specific human autoantibodies on human fibroblast (Fig. 2) or epithelial (not shown, see below) cell cultures showed absence of reaction on the nuclear lamina of interphase cells and strong reaction over cells at various stages of mitosis. Fluorescence due to lamin A was dispersed in the whole volume of the mitotic cells surrounding dark masses occupied by the chromosomes (see below), a result compatible with the cytoplasmic dispersion of depolymerized lamins in mitotic cells (1). All three lamin A specific autoantibodies produced the same pattern of fluorescence. These results were extended and confirmed using human epithelial cell cultures enriched for mitotic cells by treatment with the microtubule polymerization inhibitor nocodazole (13). Figure 3 shows compact masses of chromosomes accumulated in mitotic cells (Fig. 3a) and diffuse fluorescence due to lamin B (Fig. 3b) or to lamins A and C (Fig. 3c), surrounding the chromosomes. Figure 3b and 3c also show normal (1) nuclear peripheral staining due to lamin B or to lamins A and C in the few interphase cells present in the population of nocodazole treated cells. Again, anti-lamin A autoantibodies reacted exclusively with mitotic cells (Fig. 4) with the same features as seen on mitotic cells not treated with nocodazole (Fig. 2).

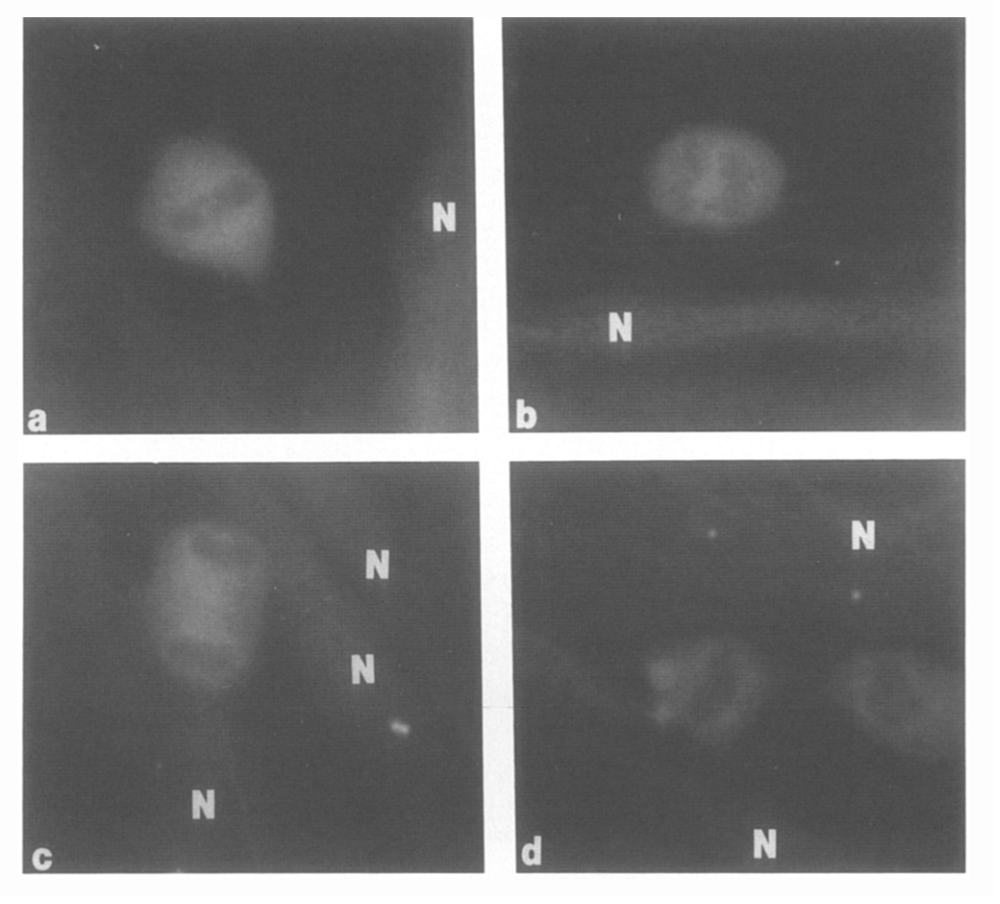

Figure 2. Anti-lamin A autoantibodies react positively on mitotic cells but not on interphase nuclei. Human serum 312 was tested by indirect immunofluorescence on human fibroblasts. Interphase nuclei (labeled N) as well as mitotic cells at metaphase (a), anaphase (b) and early (c) and late (d) telophase are shown. Identical results were obtained with human sera 787 and 896.

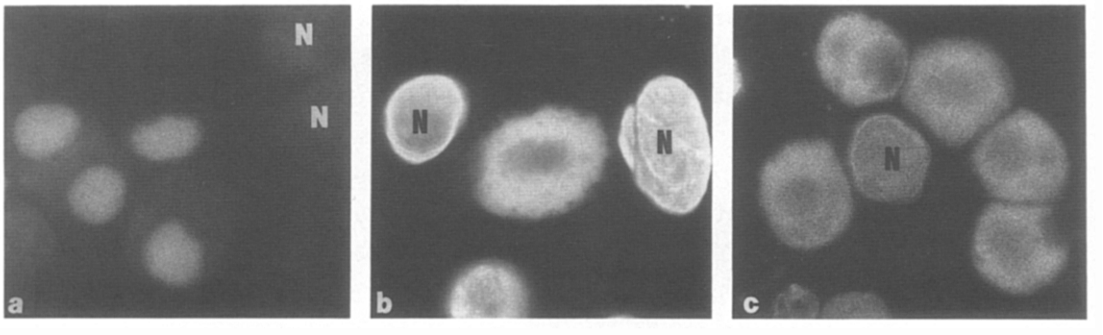

Figure 3. Distribution of DNA, lamin B and lamins A and C in nocodazole treated human epithelial cells. HEp-2 cultures were treated with nocodazole as described under Materials and Methods. Staining of DNA was with Hoechst dye 33258 (a). The distribution of lamin B (b) and lamins A and C (c) as detected by human autoantibodies (refs. 15 and 16, respectively) is shown on mitotic cells and on interphase cells (labeled N).

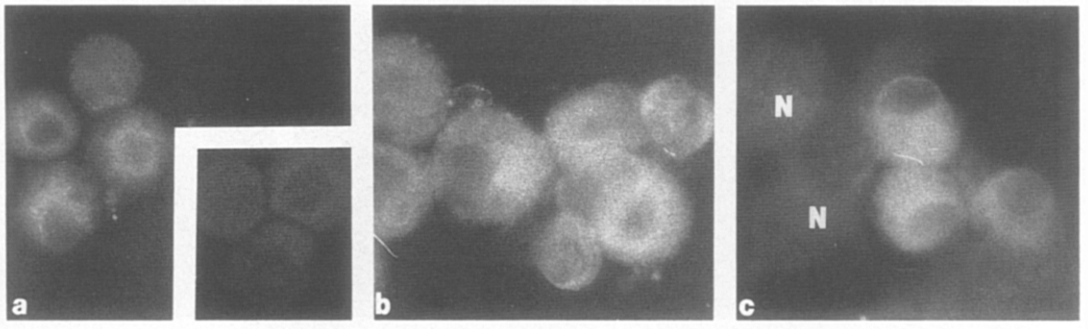

Figure 4. Anti-lamin A autoantibodies react on nocodazole treated epithelial cells in mitosis. Human sera 312 (a), 787 (b) and 896 (c) were tested by indirect immunofluorescence on nocodazole treated HEp-2 cell cultures. Interphase cells are labeled (N). The inset in panel (a) shows the fluorescence produced by serum 312 on mitotic cells after adsorption on rat liver lamins as described under Materials and Methods. Identical results were obtained with human sera 787 and 896.

Adsorption on purified rat liver lamins of the human sera which recognized only lamin A in this substrate (Fig. 1a) or on bacterially expressed purified human lamin A (not shown) completely abolished the fluorescence over mitotic cells (Fig. 4a, inset) indicating that the staining was due to lamin A specific antibodies present in the sera. The inset in Fig. 4a also illustrates the low background staining due to the fluorescein-conjugated second antibody.

The exclusive staining of lamin A in mitotic and not in interphase cells could be explained in two alternative ways: either the autoantibodies reacted exclusively against an epitope of lamin A that becomes phosphorylated during mitosis (1) or the epitope on lamin A was masked by a ligand in interphase cells and became accessible only after depolymerization of the lamina during mitosis. The former possibility appears unlikely for the following reasons. First, functionally important phosphorylation sites on lamins A and C during mitosis have recently been identified and found to be identical on both polypeptides (17,20), i.e. no mitotic phosphorylation has been shown to occur or to be required on the carboxyl-terminus exclusive to lamin A. Second, the anti-lamin A autoantibodies were reactive against lamin A prepared from rat liver in which the majority of cells are in interphase (Fig. 1a). Third, the reaction due to the lamin A specific autoantibodies could be adsorbed by lamin A from rat liver (Fig. 4a, inset) or by the presumably unmodified recombinant lamin A.

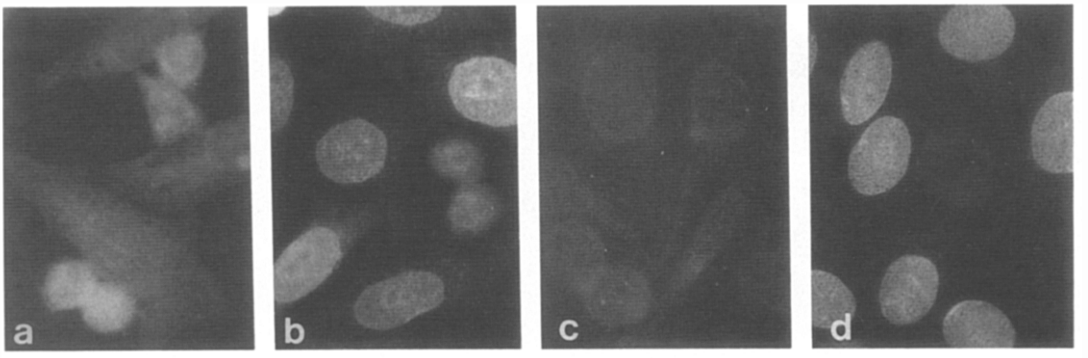

Figure 5. Anti-lamin A autoantibodies react on interphase nuclear lamina after extraction of chromatin. HEp-2 cultures were extracted *in situ* as described (21,22) with Triton and treated with nucleases (a,b) followed by extraction with concentrated salt solutions (c,d). Indirect immunofluorescence was performed with human serum 787 (a,c) or with a lamin A and C specific antiserum (b,d).

The alternative possibility of epitope masking in interphase cells was directly tested by extracting interphase cells *in situ* with the various solutions used in the biochemical isolation of the nuclear lamina (21,22). Extraction with Triton alone (not shown) or followed by treatment with nucleases (Fig. 5a) did not allow staining of interphase nuclear lamina by the anti-lamin A autoantibodies while still preserving reactivity over mitotic cells. The presence in these preparations of lamins A and C was confirmed by the use of an antiserum reactive against both polypeptides (Fig. 5b). Subsequent extraction with concentrated salt solutions that allow extraction of nuclease-digested chromatin (21-23), led to the following results. First, lamins A and C were seen in interphase cells but had disappeared from mitotic cells, presumably due to extraction of depolymerized and soluble lamins (Fig. 5d). Second, the lamin A specific autoantibodies were seen to react with the interphase nuclear lamina (Fig. 5c). Fluorescence intensity due to lamin A was lower, as expected, than the intensity seen with the lamin A and C specific antiserum but the periphery of salt-extracted interphase nuclei was clearly delineated (Fig. 5c) in contrast to the complete absence of nuclear rim fluorescence after Triton and nuclease treatment (Fig. 5a).

In summary, we have presented for the first time evidence that an epitope in the carboxyl-terminus or tail domain exclusive to lamin A was inaccessible in the interphase nuclear lamina but became accessible after *in vivo* depolymerization of the lamina during mitosis or after *in vitro* extraction of chromatin from interphase nuclei. These results, taken together with the available evidence

on chromatin interaction of lamin A (6-8), support the suggestion that lamin A may interact with chromatin via its tail domain.

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