Changes in Distribution of Nuclear Matrix Antigens during the Mitotic Cell Cycle

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ABSTRACT We examined the distribution of nonlamin nuclear matrix antigens during the mitotic cell cycle in mouse 3T3 fibroblasts. Four monoclonal antibodies produced against isolated nuclear matrices were used to characterize antigens by the immunoblotting of isolated nuclear matrix preparations, and were used to localize the antigens by indirect immunofluorescence. For comparison, lamins and histones were localized using human autoimmune antibodies. At interphase, the monoclonal antibodies recognized non-nucleolar and nonheterochromatin nuclear components. Antibody P1 stained the nuclear periphery homogeneously, with some small invaginations toward the interior of the nucleus. Antibody 11 detected an antigen distributed as fine granules throughout the nuclear interior. Monoclonals P11 and P12 stained both the nuclear periphery and interior, with some characteristic differences. During mitosis, P1 and 11 were chromosome-associated, whereas P11 and P12 dispersed in the cytoplasm. Antibody P11 heavily stained the periphery of the chromosome mass, and we suggest that the antigen may play a role in maintaining interphase and mitotic chromosome order. With antibody 11, bright granules were distributed along the chromosomes and there was also some diffuse internal staining. The antigen to 11 may be involved in chromatin/chromosome higher-order organization throughout the cell cycle. Antibodies P11 and P12 were redistributed independently during prophase, and dispersed into the cytoplasm during prometaphase. Antibody P12 also detected antigen associated with the spindle poles.

The nuclear matrix is a complex biochemical fraction consisting of nonhistone nuclear proteins and small quantities of DNA and RNA. It is obtained by sequential extraction of isolated interphase nuclei with low- and high-salt buffers, detergents, and DNase and RNase. Structurally, the nuclear matrix comprises the peripheral nuclear pore complex—lamina, an internal fibrogranular network and residual nucleoli. Part of the matrix has been envisaged as an interphase “nuclear skeleton” on which nuclear functions such as DNA replication, RNA transcription and processing, virus replication, and hormone response can be ordered (see references 1–3 for reviews).

Several studies have suggested that the nuclear matrix is involved in mitosis (4–7). Recent work (8, 9) has also indicated a potential role for the nuclear matrix in the organization of mitotic chromosomes per se. To examine the distribution of individual nuclear matrix polypeptides throughout the cell cycle, we used monoclonal antibodies against isolated nuclear matrices in an immunofluorescence study of mouse 3T3 fibroblasts.

MATERIALS AND METHODS

Cell Culture: Mouse 3T3 fibroblasts were cultured at 37°C with 5% CO₂ in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml fungizone. Mouse myeloma Sp2/0 cells were grown in the same conditions but in 10% CO₂.

Production of Monoclonal Antibodies: Nuclear matrices from resting mouse splenic or bovine lymph node lymphocytes were isolated as previously described (10). BALB/c-BYJ mice received a total of 400 µg/mouse of mouse or bovine lymphocyte nuclear matrix in four injections at 10-d intervals. Fusion, cloning, and ascites fluid production were carried out according to the protocol of Kennett et al. (11). Hybrids were screened by an indirect immunofluorescence assay. Mouse 3T3 cells were grown on coverslips (c.s.), 1 fixed in cold 95% ethanol, and air-dried. Small volumes of supernatant were

1 Abbreviations used in this paper: c.s., coverslip; FITC, fluorescein isothiocyanate.
applied for 30 min; the c.s. were rinsed in phosphate-buffered saline (PBS), pH 7.0, flooded with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse Ig for 30 min, washed in PBS, and mounted in 50% glycerol-PBS with 0.1% p-phenylenediamine to retard fluorescence bleaching.

**Immunoblotting:** Lymphocyte nuclear matrix proteins were separated in SDS polyacrylamide gels according to Brasch (12) and were transferred electrophoretically to nitrocellulose (Bio-Rad Laboratories, Richmond, CA) by a modification of the method of Burnette (13). The electrode buffer contained 19.5 M urea, and 25 mM Tris base. The total transferred proteins were visualized directly by staining a strip of the nitrocellulose sheet with amido black. Molecular weights were estimated by comparison with high- and low-molecular-weight standard kits (Bio-Rad Laboratories, Mississauga, Ont.).

For immunostaining, the nitrocellulose sheet was incubated at room temperature in 3% gelatin in PBS, pH 7.0, to block nonspecific staining. Nitrocellulose strips were then incubated with ascites fluid at 4°C for 16 h, after the following dilutions: P1, 1:1000; P11, 1:200; PI2, 1:200. Detection used horse-radish peroxidase-conjugated goat anti-mouse IgM (1:400) (Cappel Laboratories, Inc., Cochranville, PA) and diaminobenzidine (Polysciences, Inc., Warrington, Pa.).

**Immunofluorescent Staining:** Mouse 3T3 fibroblasts were grown on coverslip and fixed in 3% paraformaldehyde-PBS, pH 7.0, for 5 min at room temperature. They were reduced in sodium borohydride in PBS, permeabilized for 20 min in 0.2% Triton X-100 in PBS, washed in PBS, drained, and flooded with 1st antibody. Control samples were flooded with PBS. The c.s. were incubated for 45 min, drained, washed in PBS, flooded with 2nd antibody, and incubated for 45 min. After washing in PBS, the c.s. were placed in Hoechst 33258 (American Hoechst Corp., San Diego, CA) (1.5 μg/ml in PBS, pH 7.0) and then mounted in 50% glycerol-PBS with p-phenylenediamine.

**Double Immunofluorescence Staining:** After fixation, permeabilization and washing in PBS as above, the c.s. were placed for 20 min in 0.1% gelatin in PBS and washed three times for 10 min in PBS. The antibodies were then applied as follows: first 1st antibody, 45 min; three times for 4 min in PBS; first 2nd antibody, 45 min; three times for 4 min in PBS; second 1st antibody, 45 min; three times for 4 min in PBS; second 2nd antibody, 45 min; three times for 4 min in PBS; 2 min in Hoechst 33258; mount in glycerol-PBS.

The following antibodies were used, at the dilution indicated:

- **1st Antibodies:** Monoclonal antibodies to nuclear matrix, ascites fluid: P1, 1:250; P11, 1:100; PI2, 1:500; P12, 1:750.

- **2nd Antibodies:** FITC-conjugated goat anti-mouse IgG (H+L) (1:75-1:100) and tetramethylrhodamine isothiocyanate-conjugated goat anti-mouse IgG (H+L) (1:75) (Cappel Laboratories, Inc.); FITC-conjugated goat anti-mouse IgG, mu chain-specific (1:80), and FITC-conjugated goat anti-mouse IgG, gamma chain-specific (1:80) (Flow Laboratories, Inc., Mississauga, Ont.); tetramethylrhodamine isothiocyanate-conjugated rabbit anti-mouse IgG (H+L) (1:75) (Miles Laboratories, Ltd., Rexdale, Ont.); FITC-conjugated rabbit anti-human IgG (H+L) (1:560) and tetramethylrhodamine isothiocyanate-conjugated rabbit anti-human IgG (H+L) (1:125) (Cedar Laboratories Ltd., Hornby, Ont.).

**Microscopy:** Preparations were observed with a Zeiss Universal microscope (Carl Zeiss, Inc., New York) equipped for epifluorescent illumination and were photographed on Ilford XP-1 film.

**RESULTS**

**Selection of Monoclonal Antibodies**

We selected 17 hybrids producing antibodies that detected nuclear antigens. The staining patterns of interphase nuclei of 3T3 cells with these antibodies fell into three categories: peripheral (P), internal (I), or both (PI). In this study, we report observations using antibodies P1 and P11, produced against bovine retropharyngeal lymphocyte nuclear matrix, and antibodies P11 and P12, produced against mouse splenic lymphocyte nuclear matrix. In an earlier report (15), these antibodies were referred to as 3F5, 5H12, 1G11, and 2F8, respectively. All four antibodies were detected by FITC-conjugated goat anti-mouse IgG, but not by FITC-conjugated goat anti-mouse IgM.

The structures and the polypeptide compositions of the isolated lymphocyte nuclear matrices have been described previously (10, 16). Fig. 1 shows immunoblotting results with lymphocyte matrices for three of the four monoclonal antibodies used in this study. Antibody P1 detected a triplet of polypeptides in the range of M, 27,000–31,000 and P11 a single polypeptide of M, 60,000. Antibody P12 detected three components (M, 35,000, 70,000, and 140,000), which may be related subunits or degradation products. We have been unsuccessful so far in identifying the antigen to PI by immunoblotting.

**Interphase**

The antigen detected by P1 was exclusively peripheral in interphase (Fig. 2a–c). Focusing on the midplane of the nucleus showed a relatively uniform staining of the periphery (Fig. 2b), with some small irregular projections towards the interior of the nucleus. Focusing on the surface of the same nucleus (Fig. 2a) showed that the projections were uneven in size and apparently distributed at random.

The staining pattern with antibody P1 was compared to that with antilamin (Fig. 3, a and b). Peripheral staining was very prominent with antilamin, but showed no internal projections and was agranular. Furthermore, there was a significant fluorescence in the nuclear interior. The internal staining with antilamin consisted of diffuse non-nuclear fluorescence and some bright irregular streaks and/or spots.

Antibody P11 stained exclusively the internal region of the nucleus. The stain appeared as specks of fairly uniform size.
and intensity distributed in the regions between nucleoli and heterochromatin bodies, i.e., the interchromatinic region (Fig. 2, d and e). Occasionally, larger bright spots were seen associated with the edge of one or more of the nucleoli.

With antibody P11, the staining pattern in most nuclei was both peripheral and internal (Figs. 2, f–h, and 4, a–d). In some cases, however, only peripheral staining was observed (Fig. 4, c and d). The peripheral staining was similar in both situations. Viewed in the midplane of the nucleus (Fig. 2g), the peripheral staining consisted of a discontinuous layer of uniform thickness. In surface view (Fig. 2f), this staining was resolved into evenly distributed coarse granules of uniform size and brightness. The internal staining component was unevenly distributed throughout the nucleoplasm as numerous granules that varied greatly in size and brightness. There was no obvious staining of nucleoli or heterochromatin. A further characteristic of P11 staining was the presence in the cytoplasm of some cells (Fig. 4, a and b) of a cluster of brightly staining granules of variable size near the nuclear surface.

The interphase staining pattern with antibody P12 was similar to that with P11 (Fig. 2, i–k). However, there was little variation in the staining pattern from one cell to another, and the staining was exclusively nuclear in all interphase cells. The peripheral staining consisted of finer, more irregularly sized granules than with P11, as is clearly seen in Fig. 2i. The internal staining was also somewhat finer (Fig. 2j) and appeared to be more evenly distributed than with P11.

Mitosis

Cells were identified as to mitotic stage, using primarily DNA staining with Hoechst, according to the following criteria: (a) prophase (Figs. 5 and 6)—from the first sign of chromosome condensation up to the loss of a smooth nuclear outline resulting from the start of nuclear envelope breakdown; (b) prometaphase—from nuclear envelope breakdown to the formation of the metaphase plate; at mid-prometaphase (Fig. 7), chromosomes were oriented radially around a
nonstaining central space; (c) metaphase (Fig. 8, a–h)—chromosomes aligned at the metaphase plate; (d) anaphase (Fig. 8, i–p)—from the first signs of chromosome separation up to the formation of two chromosome sets at the spindle poles; (e) telophase (Fig. 8, q–x)—individual chromosomes difficult to distinguish, although the chromosome mass as a whole retained a “ropey” appearance; in many instances a midbody was visible by phase-contrast microscopy; (f) early G1 (Fig. 9)—the daughter cells were still connected through the midbody when viewed by phase-contrast microscopy. The paired nuclei were smaller than in surrounding interphase cells, but were otherwise similar in appearance, with a diffuse DNA fluorescence throughout the nucleoplasm and well-defined nucleoli. It should be noted that no squashing was applied to the cell preparations so that the arrangement of chromosomes remained largely undisturbed from that present in living cells.
Prophase

In prophase, the staining intensity with antibody P1 increased manyfold, so that adjacent interphase and prophase nuclei could not be photographed with the same exposure. This elevated staining intensity occurred throughout mitosis. The stained irregular inward projections from the nuclear periphery present in interphase were more numerous in early prophase (Fig. 5, a–c). This was seen most clearly when the inner surface of the nuclei was examined (Fig. 5a). In later prophase (Fig. 6, a and b), the projections were seen to coincide with the surface of short lengths of condensed chromosomes, coating the chromosomes where they lay at the nuclear periphery. Although condensed chromosomes were evident throughout the entire nucleus by DNA staining (Fig. 6b), only those portions at the periphery of the nucleus were coated with antigen to P1. All chromosomes at the nuclear periphery showed surface staining with P1.

The internal staining of interphase nuclei with antibody II was redistributed at prophase (Fig. 6, c and d), apparently associating itself with the condensing chromosomes. In contrast to the staining pattern of P1 (Fig. 6, a and b), all
chromosome segments throughout the nucleus were stained with 11. The stain was distributed as specks along the entire surface of each chromosome segment, and there also appeared to be some diffuse staining of the interior of each chromosome.

Early in prophase the staining of the nucleoplasm with antibody PI1 (Fig. 5, d–f) was patchy, indicating clustering of the interphase specks of stain between condensing chromosomes. There was no evidence of any direct association of stain with the chromosomes. Brightly staining granules were observed in the cytoplasm of some prophase cells, usually in two clusters near the surface of the nucleus (Fig. 5, d and e). The peripheral nuclear staining was accentuated later in prophase (Fig. 6, e and f), where two involutions of the nuclear periphery were readily visible (Fig. 6, e and f).

The internal component staining with PI2 became redistributed early in prophase (Fig. 5, g–i), lying in patches between condensing chromosomes. The peripheral staining was no longer granular (Fig. 5 h), and the surface appeared “wrinkled” in places. Later in prophase (Fig. 6, g and h), internal staining disappeared and the peripheral staining consisted of bright diffuse fluorescence. As observed with PI1 (Fig. 6, e and f), the nuclear periphery was involuted in two places at prophase (Fig. 6, g and h). No staining of cytoplasmic granules was seen with PI2.

Prometaphase

At prometaphase, P1 was detected only at the periphery of the chromosome mass (Figs. 7 a and 10, a, c, and e), closely following the outlines of projecting chromosome arms (Figs. 7 b and 10, b, d, and f). No stain was detected within the individual chromosomes or within the chromosome mass. This is illustrated in the through-focus series in Fig. 10, a–f. All chromosome arms at the surface of the configuration (Fig. 10, b and f) were coated (Fig. 10, a and e), but in the midplane of the mass, only those portions of the chromosomes at the periphery (Fig. 10 d) were coated by P1 (Fig. 10 c). In contrast, prometaphase chromosomes in preparations stained with antititin were unstained (Fig. 3, a and b). The cytoplasm showed considerable diffuse fluorescence, and in some cells granular bright lines could be seen in places in the cytoplasm around the chromosome mass.

To ascertain that peripheral staining of the chromosome mass by P1 was not simply due to exclusion of antibody, we performed double immunostaining with P1 and antihistone (Fig. 10, g–h). Although the chromosome mass was only outlined by P1 (Fig. 10 g), each chromosome was fully stained by antihistone (Fig. 10 h), resembling the pattern recently described with a monoclonal antibody to histone H2b (17).

With antibody 11, a finely granular stain was associated with the surface of the chromosomes at prometaphase (Fig. 7, c and d). There also appeared to be some diffuse staining of the interior of the chromosomes themselves, as had been seen in prophase nuclei (Fig. 6, c and d).

Antibodies PI1 and PI2 were not chromosome associated after nuclear envelope breakdown. Staining with both antibodies was greatly reduced at prometaphase (Fig. 7, e–h). Some specks of stain were visible between chromosomes and in the central space of the prometaphase configuration, perhaps corresponding to the sites of involution of the nuclear periphery observed in prophase.

Metaphase, Anaphase, and Telophase

At metaphase (Fig. 8, a and b), the periphery of the metaphase plate was precisely outlined by P1 (Fig. 8a), with no visible staining of the central portions of the chromosomes. However, any chromosomes lying outside the metaphase plate were individually delineated by P1 (Fig. 8a).

In early anaphase, if the two daughter sets of chromosomes were intertwined, P1 outlined them as one mass. At a more advanced stage of anaphase (Fig. 8, i–j), when the distal ends of the chromosome sets have moved past one another towards the poles, each daughter set of chromosomes was outlined separately by P1 (Fig. 8 i). During telophase, the distribution of P1 paralleled the surface irregularities of the condensing chromosome mass (Fig. 8, q and r). In many cells in telophase (Fig. 8 q) and in early G1, there were brightly staining globular masses in the cytoplasm.

With antibody 11, the metaphase (Fig. 8, c and d), anaphase (Fig. 8, k and l) and telophase (Fig. 8, s and t) chromosomes were associated along their entire length with bright specks of stain as described for prometaphase. The interior of the chromosomes also showed a diffuse fluorescence.

The P11 staining was reduced to a diffuse glow of the cytoplasm at metaphase (Fig. 8, e and f) and anaphase (Fig. 8, m and n). As the chromosomes began to lose their individual definition in telophase, a very faint stain could be seen at the periphery of the chromosome mass (Fig. 8, u and v), initially at the side of the mass nearer the spindle poles.
The chromosomes remained unstained with P12 during metaphase (Fig. 8, g and h), anaphase (Fig. 8, o and p), and telophase (Fig. 8, w and x) within a diffusely fluorescent cytoplasm. However, two stained spots located at sites apparently corresponding to the spindle poles were visible during these stages (Fig. 8, g, o, and w). At telophase, very faint staining was acquired at the periphery of the chromosome mass (Fig. 8 w).
Figure 10. (a–f) Through-focus series of cell in late prometaphase stained with antibody P1 (a, c, and e) and counterstained with Hoechst (b, d, and f). When viewed in midplane (c and d) the chromosome mass is closely outlined by P1-staining. The chromosome arms projecting at the top and bottom surfaces of the mass (a and b; e and f) are also fully outlined. The portions of the chromosomes in the center of the chromosome mass (d) are not stained (cf. c and d). (g and h) Cell in early prometaphase stained with P1 (g) and antihistone (h) antibodies. The chromosome mass is outlined by P1 (g), but, at the same plane of focus, each chromosome is fully stained by the antihistone (h). x 1,450.

Early G1

In early G1, the daughter cells were still joined at the midbody (Fig. 9, c, f, i, and l). Some cells showed the antibody-specific staining pattern characteristic of interphase nuclei. Most, however, presented staining patterns intermediate between telophase and interphase.

The staining with P1 was somewhat less bright than earlier in mitosis but was still brighter than after cytokinesis. It was once more peripheral and fairly even in distribution (Fig. 9, a–c).

In parallel with the reappearance of interphase chromatin organization in early G1, the staining by antibody II was gradually redistributed throughout the nucleus as bright specks of even size (Fig. 9, d–f).

The peripheral components detected by monoclonals PII1 (Fig. 9, g–i) and PII2 (Fig. 9, j–l) were clearly visible in all early G1 cells. In nuclei showing a relatively diffuse Hoechst stain, similar to that in interphase nuclei (cf. Figs. 9, i and l and 1c), the internal component was also evident (Fig. 9, g–i).

DISCUSSION

The monoclonal antibodies studied here were generated against a biochemically characterized nuclear fraction, the nuclear matrix (10, 16, 18). They were chosen because they localized antigens of interphase nuclei outside visible chromatin and nucleoli, in distributions paralleling the known location, from electron microscope studies, of different regions of the nuclear matrix, i.e., the pore complex–lamina (P1), the fibrogranular internal matrix (II), or both (P11, P12). Furthermore, we have demonstrated by immunoblotting that three of the four monoclonal antibodies detected polypeptides of the isolated nuclear matrix.

Reports dealing specifically with nuclear matrix antigens are relatively few (19–21), although the distribution of the lamins during the cell cycle and cell differentiation has been extensively investigated (e.g. 14, 22–27). Using the first monoclonal antibodies directed against nonlamin nuclear matrix antigens, we have demonstrated that, whereas the peripheral and internal regions of the nuclear matrix have some common antigens (detected by P11 and P12), others (detected by P1 and II) are restricted to one region or the other. We have also shown that, with the disruption or reorganization of the interphase nuclear matrix during mitosis, the antigens behave independently of one another. Some antigens are dispersed into the cytoplasm, as has been described for the lamins and most other nuclear antigens. Others, however, associate with the chromosomes in characteristic readily distinguishable patterns. These differences in behavior at mitosis may be a reflection of the particular nuclear matrix function in which the antigens are engaged during interphase. The observations with P1 and II support a role for the nuclear matrix in the spatial ordering of nuclear processes, a role maintained during mitosis as participation in the structuring of chromosomes.

Monoclonals P1 and II were produced against bovine lymphocyte nuclear matrices, whereas monoclonals PII1 and PII2 were made against mouse lymphocyte nuclear matrices. All four antibodies detected antigens in the mouse 3T3 fibroblast cell line, as well as antigens in mouse and bovine lymphocytes. Indeed, antibody P1, for example, detects antigens with similar interphase and mitotic distributions in a number of mammalian, insect, and plant tissues (manuscript in preparation). This suggests that the antigens under investigation are involved in basic nuclear functions common to many organisms.

The antigen detected by P1 is localized at the nuclear periphery during interphase, and numerous studies have shown by immunocytochemistry that the lamins are associated with the nuclear periphery (14, 22, 23, 26). However, from our observations of both interphase and mitotic cells, it is quite clear that the antigens detected by the two antibodies are not the same. The interphase staining with P1 is exclusively peripheral, while the antilamin reveals an internal component not only in our preparations but also in previously published reports (14, 22). At mitosis, whereas the P1 antigen becomes associated with chromosomes, the lamins disperse into the cytoplasm. Furthermore, we have shown by immu-
nol blotting that P1 detects an antigen of \( M_r 27,000-30,000 \); the laminas have been identified at \( M_r 60,000-70,000 \) (14, 22).

Perichromin, a conserved nuclear envelope-associated antigen localized at the periphery of mitotic chromosomes has been recently detected by an autoimmune antibody (28). The antigen has been identified as a single polypeptide of \( M_r 33,000 \). Although perichromin is strongly peripheral at interphase in Chinese hamster ovary cells, it is uniformly distributed throughout the nuclei of Drosophila cells. The P1 antigen appears to be distinct from perichromin. It is exclusively peripheral in all cell types examined, including insect cells (manuscript in preparation), and detects polypeptides of \( M_r 27,000-31,000 \). The monoclonal antibodies PI1 and PI2 also detect components of the nuclear periphery in 3T3 cells. Taken together these data indicate that the nuclear periphery is more complex than previously considered.

We propose that the antigen to P1 may function in the spatial ordering of DNA during interphase, and the spatial ordering of chromosomes during mitosis. Recently, a similar function has been proposed for perichromin (28). Numerous workers have proposed that DNA is spatially ordered during interphase (29-34), and that the order is established by anchoring of interphase DNA loops on the nuclear matrix (33-37). At least some of these loops are anchored at the nuclear periphery (for reviews, see references 32 and 33). The antigen detected by P1 may be involved in anchoring DNA loops, by itself or by interaction with other nuclear proteins, e.g., the lamins.

There is also considerable evidence for spatial order of chromosomes during mitosis, i.e., at the metaphase plate (32, 38). The behavior of the P1 antigen during mitosis is consistent with the notion that it may be implicated in translating interphase order into metaphase order. With the fragmentation of the nuclear envelope at the end of prophase, the P1 antigen, either by itself or in association with yet unknown factors, may remain chromosome associated so as to retain the spatial order present during interphase. It is unlikely that the P1 antigen interacts in a sequence-specific manner with the chromosomes. Considering only the regions of the centromeres, for example, it is clear that, whereas these are not coated by P1 at metaphase, they become associated with P1 during anaphase and telophase. The antigen to P1 may act merely as a physical constraint on the chromosomes, maintaining them in the relative positions established previously.

It is interesting to note that Paulson (39) has shown that all the chromosomes from a cell adhere to one another and are isolated as one cluster under appropriate isolation conditions. He suggests that this may reflect suprachromosomal organization in vivo.

The association of the II antigen with chromosomes during mitosis and its nonheterochromatin localization during interphase suggest that the antigen may be involved in structural organization of chromosomes both during interphase and mitosis. Since the II antigen appears to be localized largely at chromosome surfaces during mitosis, it is unlikely to be a constituent of the internal chromosome scaffold as prepared by Earnshaw and Laemmli (40). However, antigen II could be a component of the 25-nm granules recently demonstrated by Engelhardt et al. (41) in interphase nuclear matrices and chromosome scaffolds.

The antigens for antibodies PI1 and PI2 showed similar distributions throughout the cell cycle, but with some characteristic differences. At interphase, the intercellular staining pattern was relatively consistent with PI2, but with PI1, varied considerably in different nuclei. The type of staining displayed by a particular nucleus with PI1 could not be correlated with any unique structure of the nucleus. It may be related to the metabolic state of the cell, perhaps to its position within the cell cycle. Riley and Keller (42) have shown extensive reorganization of a nuclear matrix-like fraction as HeLa cells progress through interphase.

The interphase staining pattern as well as the dissolution and reacquisition of staining at prometaphase and telophase, respectively, suggest some association of the PI1 and PI2 antigens with the nuclear envelope (33). By mid-prophase, monoclonal PI2 showed only an agranular peripheral component. The altered appearance of the peripheral staining could be due to posttranslational modifications of the PI2 antigen similar to changes in the extent of phosphorylation of lamins and nuclear proteins which are known to occur at about this stage of mitosis (27, 43, 44). It may also, however, reflect changes in distribution resulting from the loss (e.g., the lamins) and/or gain (e.g., P1 antigen) of other peripheral proteins during prophase.

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