Biochemical and morphological characterization of the nuclear matrix during the synchronous cell cycle of *Physarum polycephalum*

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SUMMARY

We have investigated biochemical and ultrastructural aspects of the nuclear matrix during the naturally synchronous cell cycle of *Physarum polycephalum*. The morphology of the in situ nuclear matrix exhibited significant cell cycle changes as revealed by electron microscopic examination, especially during the progression of nuclei through mitosis and S-phase. In mitosis the interchromatin matrix was found to be retracted to the nuclear periphery; during S-phase this interchromatin matrix gradually reassembled, concomitant with the reconstruction of a nucleolar remnant structure. During the G₂-period no significant changes in matrix morphology were observed. The pattern of nuclear matrix proteins was invariant during the cell cycle; no cycle phase-specific proteins could be detected. In vivo labelling of plasmodia with [³⁵S]methionine/cysteine showed that only a few proteins are synthesized and assembled into nuclear matrix structures in a cell cycle-dependent way; the majority of proteins were synthesized almost continuously. This was also shown for nuclear lamins homologues.

In contrast to bulk nuclear histones, those histones that remain tightly bound to the nuclear matrix were synthesized and assembled into nuclear structures in the very first hour of S-phase; assembly was terminated in mid-S-phase, indicating that nuclear matrix-bound chromatin is replicated early in S-phase. Comparison of the acetylation pattern of matrix-bound histone H4 with bulk nuclear H4 revealed a largely elevated acetate content of matrix H4. The percentage of acetylated sub-species was entirely different from that in bulk nuclear H4, indicating that matrix-associated histones represent a subpopulation of nuclear histones with distinct properties, reflecting specific structural requirements of matrix-attached chromatin.

Key words: nuclear matrix, cell cycle, chromatin, mitosis, histones, histone acetylation, lamin, intermediate filament proteins, *Physarum polycephalum*, histone acetyltransferase, histone deacetylase

INTRODUCTION

The operational term ‘Nuclear Matrix’ defines a structure that resists treatment of nuclei with detergents, high salt buffers and nucleases and was first described by Berezney and Coffey (1974). The concept of a basic skeleton structure within the cell nucleus was attractive with respect to the topological organization of those nuclear processes that require a defined temporal accessibility of distinct areas among the highly ordered eukaryotic chromatin. Accordingly, it has been shown that DNA replication (Jackson and Cook, 1986), transcription (Jackson and Cook, 1985), RNA-processing (Agutter, 1985) and UV-induced DNA repair (McCready and Cook, 1984) are transiently associated with a nuclear remnant framework. Moreover, a variety of enzymes and regulatory proteins have been shown to be tightly associated with the nuclear matrix (e.g. see Kaufmann et al., 1986; Kirsch et al., 1986; Waitz and Loidl, 1991; Karwan et al., 1990; Li and Roux, 1992; Verheijen et al., 1988). A number of genes have been shown to be associated with the nuclear matrix in their actively transcribed configuration (e.g. see Jost and Seldran, 1984; Andreeva et al., 1992). Recently, some of the internal matrix proteins, the nuclear matrixins, have been characterized, cloned and sequenced (Nakayasu and Berezney, 1991; Belgrader et al., 1991; Hakes and Berezney, 1991); these studies demonstrate that matrixins have structural characteristics typical of regulatory DNA-binding proteins, which is a further indication of the essential role of matrix proteins in the regulation of nuclear processes.

One of the main problems of nuclear matrix research during the last few years has been the tendency of each laboratory to establish its own unique protocol for nuclear matrix isolation, depending on whether biochemical, morphological or DNA studies were being performed. For this reason discrepant results were often difficult to interpret. In
a systematic survey, using the currently available preparation protocols with mammalian cells and the lower eukaryote Physarum polycephalum, our laboratory has shown by morphological, biochemical and immunological criteria that the nuclear matrix represents a rather reproducible structural entity, largely independent of the preparation method (Waitz and Loidl, 1988; Grabher et al., 1992; Eberharter et al., 1993).

A further reason for discrepant results may be a cell cycle-dependent behaviour of nuclear matrix components, since the organisms and cell systems used differ from each other in their developmental and differentiation state, therefore representing different cell cycle stage distributions. Until now only a few data are available about the cell cycle dependence of nuclear matrix constituents (Chaly et al., 1984; Bludau et al., 1986; Noaillac-Depeyre et al., 1987; Todorov et al., 1988, 1991).

Here we report the morphology, composition and synthesis of proteins of the nuclear matrix, as well as the synthesis and modification of specific matrix-bound proteins during the cell cycle. The investigations were performed in a lower eukaryote, Physarum polycephalum, an organism that offers the unique advantage of a naturally synchronous cell cycle. Macroplasmodia of this organism contain up to $10^9$ nuclei in a common cytoplasm, which divide with perfect synchrony every 8-10 h. Not only nuclear division occurs synchronously, but also the intermitotic period is synchronous with respect to biochemical processes; therefore a distinction of DNA replication and transcription is possible. Our results demonstrate that the morphology of the nuclear matrix changes during the cell cycle; these changes closely reflect the structural changes of intact nuclei in whole plasmodia. In contrast, the protein composition of the nuclear matrix and the synthesis of the most abundant matrix proteins do not vary during the cycle. The assembly of newly synthesized core histones into nuclear matrix-bound chromatin during S-phase, and the post-translational acetylation of matrix-bound core histones, is entirely different from that of bulk nuclear chromatin, with a pronounced cell cycle periodicity. The data suggest that chromatin domains tightly bound to the nuclear matrix represent a unique functional entity replicating in early S-phase of the cell cycle.

MATERIALS AND METHODS

**Cultivation of Physarum polycephalum**

Macroplasmodia of Physarum polycephalum (strain M3b, a Wis isolate) were cultivated at 27°C in the dark, as suspension cultures in 500 ml Erlenmeyer flasks with reciprocal shaking in axenic nutrient medium, as described by Daniel and Baldwin (1964). Giant synchronous macroplasmodia were prepared after centrifuging suspension cultures for 30 s at 300 g by applying 4 ml of plasmodial pellet to sterile filter paper supported by glass beads. The plasmodial inoculum was prepared in an ‘S’-shape to avoid nutritional exhaustion of the inner parts of the plasmodium. After addition of nutrient medium macroplasmodia were incubated at 26°C in the dark. After 5 h the first mitosis (M1) could be observed. The ensuing completely synchronous nuclear divisions take place at 7- to 9-h intervals. Plasmodial smears from 1-2 mm² pieces of macroplasmodia were prepared, fixed with ethanol and inspected by phase-contrast microscopy to determine the mitotic and cell cycle stages of the nuclei.

**Isolation of nuclei and preparation of nuclear matrix**

All steps were carried out on ice; solutions contained 0.2 mM phenylmethylsulfonyl fluoride. Macroplasmodia on filter paper were carefully rinsed in distilled water before removing the plasmodium with a spatula; the slimy parts of the inoculum were omitted. Isolation of nuclei was carried out as described by Nöthacker and Hildebrandt (1985) with the modifications of Loidl and Griebner (1987a). The nuclear pellet was further processed for nuclear matrix preparation or lysed in SDS-sample buffer (Laemmli, 1970) for SDS-polyacrylamide gel electrophoresis.

Isolated nuclei were counted in a hemocytometer and adjusted to an optimal density of $7\times10^8$ nuclei per ml before encapsulation in agarose beads (Waitz and Loidl, 1988). After preincubation at 25°C for 5 min one volume of nuclei was mixed with 1/3 volume of liquid agarose (type IX, ultra low gelling temperature, Sigma Chem. Comp., St. Louis, USA). Immediately 7 volumes of liquid paraffin (Merck, Darmstadt, FRG) were added and the mixture was gently shaken by hand to obtain agarose beads of uniform size. The mixture was put on ice for 5 min and then repeatedly centrifuged (800 g, 5 min, 4°C) and washed in buffer I (20 mM Tris-HCl, pH 7.3, 20 mM KCl, 0.05 mM spermine, 0.125 mM spermidine, 0.5 mM EDTA, 0.2 mM PMSF, 0.1% (v/v) thioglycolate). After washing with buffer I containing 1% Triton X-100 the pelleted beads were resuspended and incubated in the same solution for 10 min. The beads were washed twice with buffer I before subsequent incubation in 2 M NaCl (in buffer I) for 15 min. After further centrifugation the pellet was washed twice with buffer II (buffer I without EDTA but containing 50 mM NaCl and 5 mM MgCl₂) and then incubated with DNase I (50 µg/ml) for 20 min at 25°C with occasional shaking. After 2 washing steps with buffer II, RNase A (250 µg/ml) was added and the beads were incubated for 15 min at 25°C. The final pellet was washed 5 times with buffer I and nuclear matrix proteins were lysed in SDS-sample buffer for subsequent electrophoretic analysis.

**Electron microscopy**

For electron microscope studies whole macroplasmodial explants (approx. 3 mm²) were encapsulated in agarose beads without nuclear isolation and processed as described by Waitz and Loidl (1988, 1991). Briefly, samples were fixed in 4% formaldehyde (freshly prepared from paraformaldehyde) and 0.25% glutaraldehyde in 0.125 M Sörensen’s phosphate buffer (pH 7.3) for 1 h at 4°C. Samples were then dehydrated with increasing concentrations of ethanol, up to 90%, and embedded in LR-white (hard grade) according to the cold catalytic method (Newman, 1989). Ultrathin sections were made using a Reichert OmU3 microtome.

**Pulse labelling of macroplasmodia with $[^{35}]$methionine/cysteine or $[^{3}H]lysin$**

Macroplasmodia at distinct stages of the cell cycle were rinsed with distilled water and drained on filter paper. Each macroplasmodium was then transferred to nutrient medium without yeast extract and peptone to minimize contamination with nonradioactive amino acids and thus enhance incorporation of labelled amino acids. After 15 min of preincubation plasmodia were placed in 4 ml of nutrient medium without peptone and yeast extract, and containing 0.4 µCi of a mixture of $[^{35}]$methionine and $[^{35}]$cysteine (Tran35S-Label-TM prepared from an E. coli hydrolysate), ICN, Irvine USA; specific activity 1100 Ci/mmol). After 45 min at 26°C in the dark the macroplasmodia were harvested for nuclear matrix isolation.

For measuring de novo biosynthesis of histones the same...
labelling protocol was applied using \([4,5,3^H]\)lysine monohydrochloride (aqueous solution; Amersham International plc, UK; specific activity 84 Ci/mmol). Labelling was performed for 1 h with 0.8 mCi \([3^H]\)lysine in 4 ml nutrient medium lacking peptone and yeast extract.

**Gel electrophoresis, western blotting, immunodetection and fluorography**

Nuclear lysates and nuclear matrices in SDS sample buffer were applied to 6 cm long SDS-10%, 13% or 15% polyacrylamide slab gels (Laemmli, 1970). Nuclear matrices suspended in 6 M urea were applied to 48 cm long acid-ura-Triton-polyacrylamide gels as described (Loidl et al., 1983). Acid-ura-Triton gels were stained with Coomassie blue, destained by diffusion and evaluated quantitatively by densitometry. The steady state acetate content \(N\) of histone H4 was calculated by the formula:

\[
\frac{\sum_{n=1}^{N} n \cdot P(n)}{\sum_{n=1}^{N} P(n)}
\]

where \(P(n)\) is the percentage of total H4 present as the subspecies with \(n\) acetates.

For subsequent immunodetection, proteins on SDS-gels were blotted onto nitrocellulose membranes using the method of Towbin et al. (1979). After blotting the membranes were rinsed with distilled water, washed in 0.1 M NaOH for 1 min and subsequently incubated in blocking solution (PBS + 2% (w/v) milk protein) for 2 h at room temperature. Incubation with primary antibodies (in PBS + 1% milk protein) was carried out for 3 h, with secondary antibody-alkaline phosphatase conjugates for 2 h at room temperature. Anti-cytokeratin-KL1 antibodies (monoclonal) were raised in guinea pigs (S. D. Georgatos, EMBL, Heidelberg, FRG). Autoimmune serum PBC-1 (W. Vogel, University of Innsbruck, Austria) was from a patient suffering from primary biliary cirrhosis, an autoimmune disease. Protein-antibody complexes were visualized by washing with barbital buffer (85 mM sodium barbital, 195 mM sodium acetate, 195 mM sodium barbital, pH 9.6) and subsequent incubation in detection solution (nitro blue tetrazolium chloride 0.0015%, 5-bromo-4-chloro-3-indolyl-disodium phosphate 0.003%, 4 mM MgCl\(_2\)) and prepared for fluorography as described. The fluorograms were exposed for 1-2 weeks. For subsequent immunodetection, proteins on SDS-gels were blotted onto nitrocellulose membranes using the method of Towbin et al. (1979). After blotting the membranes were rinsed with distilled water, washed in 0.1 M NaOH for 1 min and subsequently incubated in blocking solution (PBS + 2% (w/v) milk protein) for 2 h at room temperature. Incubation with primary antibodies (in PBS + 1% milk protein) was carried out for 3 h, with secondary antibody-alkaline phosphatase conjugates for 2 h at room temperature. Anti-cytokeratin-KL1 antibodies (monoclonal) were purchased from Becton Dickinson, Mountain View, California. Polyclonal antibodies against turkey erythrocyte lamin B were raised in guinea pigs (S. D. Georgatos, EMBL, Heidelberg, FRG). Autoimmune serum PBC-1 (W. Vogel, University of Innsbruck, Austria) was from a patient suffering from primary biliary cirrhosis, an autoimmune disease. Protein-antibody complexes were visualized by washing with barbital buffer (85 mM sodium acetate, 195 mM sodium barbital, pH 9.6) and subsequent incubation in detection solution (nitro blue tetrazolium chloride 0.0015%, 5-bromo-4-chloro-3-indolyl-disodium phosphate 0.003%, 4 mM MgCl\(_2\)) and prepared for fluorography as described. The fluorograms were exposed for 1-2 weeks.

**RESULTS**

**Structure and protein composition of the nuclear matrix during the cell cycle**

We harvested explants of macroplasmodia at various stages of the cell cycle, encapsulated them in agarose beads for nuclear matrix isolation and processed the specimens for inspection in the transmission electron microscope. Moreover, due to the fact that *Physarum* has a closed mitosis, we also analyzed plasmoidal pieces from different mitotic stages. The cell cycle of *Physarum* plasmodia consists of an S-phase (approximately 3 h), which follows immediately after mitosis (no G\(_1\)-period) and a G\(_2\)-period (approx. 6 h); mitosis itself lasts for about 0.5 h. Fig. 1 shows low magnification electron micrographs from 4 stages of the cell cycle: metaphase of the second macroplasmodial mitosis (Fig. 1A); 0.5 h after mitosis 2, an early stage of S-phase (Fig. 1B); 1.5 h after mitosis 2, a timepoint in mid-S-phase (Fig. 1C) and 6.5 h after mitosis 2, a timepoint in late G\(_2\)-period (Fig. 1D). In all stages the shape and the periphery of the nuclear matrices were well preserved against the surrounding cytoplasm. Nuclei in mitosis (metaphase) appeared empty, with internal matrix structures retracted to the nuclear periphery (Fig. 1A); in early S-phase the internal matrix gradually reorganized from the periphery (Fig. 1B) and in mid-S-phase this matrix structure already filled the whole nucleus except the nuclear center, where remnants of the nucleolus appeared (Fig. 1C); the nucleolus of *Physarum* is reconstructed from different centers in the first half of the S-phase. In later stages of the cell cycle the nucleolar matrix became prominent and was located in the center of the nucleus (Fig. 1D). Detailed investigations of different G\(_2\)-stages (from 4 until 8 h after mitosis) did not reveal significant differences in the morphology of the internal nuclear matrix, the nucleolar remnant or the nuclear lamina structure (results not shown in Fig. 1).

To detect cell cycle phase-specific abundant nuclear matrix proteins, we compared the protein composition of matrix preparations from different cell cycle stages. Fig. 2 shows that the amount of the most abundant matrix proteins (31,000, 35,000, 57,000, 67,000 and 71,000 \(M_r\)) as well as of most of the less prominent proteins and the matrix-bound core histones does not change significantly during the cell cycle, apart from slight quantitative changes of some proteins (e.g. 48,000 and 25,000 \(M_r\)). However, densitometric scanning of gels shows that the quantitative differences are within ± 15% (result not shown).

**Assembly of newly synthesized nuclear matrix proteins during the cell cycle**

To study the metabolic behaviour and to reveal subtle cell cycle changes of minor nuclear matrix proteins we labelled macroplasmodia with \([35S]\)methionine/cysteine at various intermitotic stages. After SDS-polyacrylamide gel electrophoresis the nuclear matrix proteins were analyzed by...
fluorography. None of the detected nuclear matrix proteins was synthesized in a strictly cell cycle-dependent mode, which would have meant that the synthesis was restricted to a particular cell cycle phase. Only slight quantitative changes in the rate of synthesis or assembly could be observed. Fig. 3A shows that two proteins (of 16,000 and 21,000 $M_r$) exhibit a biphasic synthesis pattern with maxima at 3 h, 7.5 h and at mitosis; these time points correspond to the end of S-phase, and the late G$_2$-period with the subsequent mitosis. Analysis of higher molecular weight proteins (Fig. 3B) also reveals invariant synthesis patterns during the cell cycle, except for two proteins of 46,000 and 48,000 $M_r$. The synthesis of the 46,000 $M_r$ protein has a clear maximum in late S-phase (S-G$_2$ transition), but very low levels during later stages of G$_2$ and mitosis. The synthesis of the 48,000 $M_r$ protein has a pronounced peak in mitosis with low levels during the rest of the cell cycle (Fig. 3B). Both bands comigrate with bands on autoradiograms on which proteins were analyzed after in vivo labelling with $[^{32}\text{P}]$orthophosphate (results not shown).

**Fig. 1.** Morphology of the nuclear matrix of macroplasmodia of *Physarum polycephalum* at different stages of the cell cycle. Small explants of macroplasmodia were encapsulated into agarose beads and processed for nuclear matrix isolation. Nuclear matrix preparations were fixed and prepared for electron microscopy as described in Materials and Methods. (A) metaphase of the second mitosis; (B) 0.5 h after mitosis 2 (early S-phase); (C) 1.5 h after mitosis 2 (mid-S-phase); (D) 6.5 h after mitosis 2 (late G$_2$-period). Arrows indicate remnants of the nucleolus. Bars, 1 $\mu$m.

**Assembly of newly synthesized histones into the nuclear matrix, and matrix core histone acetylation during the cell cycle**

To analyze the assembly of newly synthesized histones into the nuclear matrix during the cell cycle we labelled macroplasmodia with $[^3\text{H}]$lysine at metaphase of mitosis 2 and at time points during the subsequent S-phase and early G$_2$-period. To reveal possible differences in the incorporation of new histones between nuclear matrix structures and bulk nuclear chromatin, we compared total nuclei and isolated nuclear matrix in one and the same experiment. Fig. 4A shows that the assembly of newly synthesized histones into bulk nuclear chromatin decreased continuously from metaphase until the beginning of G$_2$-period, when histone synthesis has already reached a rather low level. This result just confirms earlier data (Loidl and Gröbner, 1987a). One has to consider that the *Physarum* cell cycle lacks a G$_1$-period, therefore S-phase starts immediately after mitosis. In contrast to total nuclei, the assembly of newly synthesized histones into nuclear matrix structures was restricted
to the first hour of S-phase, since at later time points of S-phase the incorporation of $[^3]$H]lysine into matrix histones was already at a very low level, roughly resembling the values seen in the G$_2$-period in total nuclei (Fig. 4B). Densitometric analysis of fluorograms of SDS-polyacrylamide gels shows that the time-course of synthesis did not differ among the individual histones (Fig. 4C, D). Since H2B and H3 of Physarum have similar molecular masses, it is not easy to resolve these histones in SDS-polyacrylamide gels. We could not detect H1 in the nuclear matrix, neither by electrophoretic nor immunological methods. It should be noted that results were also reproducible for different time points during S-phase (results not shown).

Our results demonstrate that matrix-associated core histones represent a distinct subpopulation of histones that differ considerably with respect to their synthesis from bulk chromatin histones. For this reason we also performed experiments to reveal possible differences in the post-translational acetylation between the two populations of core histones. We analyzed histones from nuclear matrices at different stages of the cell cycle on acid-urea-Triton gels. This electrophoretic system resolves the acetylated subspecies of the core histones, in particular those of histone H4. The percentages of acetylated H4 subspecies (H4Ac$_0$ - H4Ac$_4$) and the calculated overall acetate content (N) of H4 were compared to the corresponding values of total nuclear histones, following the methodology outlined previously (Loidl et al., 1983). Fig. 5 clearly shows that the steady state acetate content of H4 is much higher in the nuclear matrix-bound histone as compared to H4 of bulk nuclear chromatin, regardless of the cell cycle stage. The H4 acetate content (N) in the nuclear matrix is around 1.75 in the four cell cycle stages investigated, whereas the corresponding values in bulk chromatin fluctuate between 1 and 1.10 (Fig. 5). The percentages of acetylated H4 subspecies (H4Ac$_0$ - H4Ac$_4$), leading to the calculated values of N, are shown in Fig. 6. It can clearly be seen that the distribution of acetylated H4 subspecies is entirely different among total nuclei and nuclear matrix. Whereas the percentage of H4 subspecies in total nuclei follows the pattern published previously (Loidl et al., 1983) with the mono-acetylated subspecies (H4Ac$_1$) being the most abundant and the tetra- and di-acetylated forms (H4Ac$_4$, H4Ac$_2$) showing a moderate maximum in S-phase, the distribution of acetylated H4 subspecies in the nuclear matrix was characterized by extremely high amounts of highly acetylated forms (H4Ac$_4$, H4Ac$_2$), irrespective of the cell cycle stage (Fig. 6). During early S-phase almost 20% of nuclear matrix-bound H4 is in the tetra-acetylated form (H4Ac$_4$), which disappears from the matrix in later cell cycle stages. The tri-acetylated subspecies (H4Ac$_3$) represents approximately 25% of total H4...
in early S-phase, increases to almost 50% during progression through S-phase, but decreases to around 30% during the G2-period. The di-acetylated H4-subspecies (H4Ac2) exhibits the most dramatic changes in the nuclear matrix; it increases from around 10% in S-phase to almost 40% during the G2-period. The mono-acetylated H4 form (H4Ac1) increased from less than 10% in early S-phase to around 25% in late S, with a slight decrease during progression into G2-period (Fig. 6). The non-acetylated H4 (H4Ac0) decreased from 40% in early S-phase to less than 20% during later stages of the cycle.

Levels of intermediate filament type-related proteins (cytokeratin, lamin) during the cell cycle

We have recently identified proteins of the Physarum nuclear matrix that are related to cytokeratin and nuclear lamins (Lang and Loidl, 1993). To see whether the amount of these proteins fluctuates during the cell cycle, we blotted SDS-polyacrylamide gels of nuclear lysates or nuclear matrix preparations from various cell cycle stages onto nitrocellulose membranes and probed them with specific antibodies. Fig. 7A shows that immunodetection of a 65,000 M_r lamin B-related protein in nuclear lysates with an antibody raised against turkey lamin B did not reveal any cell cycle dependence. Immunodetection of isolated nuclear matrices with an autoimmune-serum (PBC-1, from a patient suffering from primary biliary cirrhosis), which detects a 67,000 M_r minor lamin or lamin precursor of Physarum (Lang and Loidl, 1993), also did not reveal a prominent cell cycle-dependent pattern, except a slightly decreased amount
during late S-phase and early G_2-period (Fig. 7B). Immunoblotting of nuclear matrices with an anti-cytokeratin antibody revealed a cell cycle periodicity of related *Physarum* proteins of molecular masses 50,000 and 65,000 (Fig. 7C); the amount of crossreactive protein reached a maximum in mitosis and early S-phase, decreased during late S and early G_2-period and reached a minimum (hardly detectable) during mid-G_2-period (Fig. 7C).

**DISCUSSION**

Despite almost 20 years of nuclear matrix research, one of the basic problems in this field still remaining is whether the operationally defined nuclear matrix structure indeed reflects an in vivo organization, or just represents the artificial coagulation of nuclear components as a result of the rather tough preparation procedure (commented on by Cook, 1988). In case the nuclear matrix indeed specifically serves as an active site for different nuclear processes, one would at least expect morphological differences when interphase nuclei are compared with nuclear structures during mitosis. On the other hand, an artificial precipitation of nuclear compounds during matrix isolation should always yield approximately the same morphological equivalent, regardless of the cell cycle stage, especially since the basic protein composition of the nuclear matrix does not significantly change during the cycle. The morphological data presented in this report clearly show pronounced differences, especially during mitosis and S-phase, which support the view of the nuclear matrix as a dynamic in vivo structure reflecting the functional cell cycle state of the nucleus.

Most nuclear proteins are characterized by the strong cell cycle dependence of their synthesis. For the myxomycete *Physarum polycephalum* it has been shown that core hist-

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**Fig. 6.** Percentage of acetylated H4 subspecies (H4Ac_0 - H4Ac_4) at various stages of the cell cycle. Nuclear matrices were prepared from isolated nuclei, suspended in 6 M urea and subjected to acid-urea-Triton polyacrylamide gel electrophoresis. The H4 region of the gel was evaluated by densitometry and the amount of acetylated H4 subspecies (non- to tetra-acetylated) was expressed as percent of total H4. Percentages are compared with the corresponding values of total nuclear H4. Intermitotic period between mitosis 2 and mitosis 3 was 8.5 h.
tones, like in vertebrate cells, are synthesized with a clear maximum during S-phase; during G2-period only H2A and H2B are synthesized at a basic rate (Loidl and Gröbner, 1987a). Most nonhistone proteins and also histone H1 are synthesized with a maximum in late S-phase and early G2-period (Loidl and Gröbner, 1987a). HMG-homologous proteins of Physarum (Smolarz et al., 1988) show a highly periodic pattern of synthesis with maxima in mid-S-phase (AS1, AS4) or mid-G2-period (AS2, AS3). It was therefore surprising that the most abundant nuclear matrix proteins are synthesized at a continuous rate during the cell cycle. One has to consider that our results show the assembly of newly synthesized proteins into the nuclear matrix structure rather than the actual synthesis process itself. However, since it is likely that newly synthesized matrix proteins are assembled into nuclear structures immediately after their synthesis, one may treat assembly as synthesis. The situation is even more complicated when studying the nuclear matrix, because periodic assembly may also be due to periodic association. There are only two polypeptides, with molecular masses of 46,000 and 48,000, which are synthesized and assembled in a clearly cell cycle-dependent way. Preliminary results with metabolic inhibitors (actinomycin, hydroxyurea, cycloheximide) indicate that the 46,000 and 48,000 Mr proteins are individual polypeptides with distinct cell cycle periodicities. The 48 kDa protein could be involved in cell cycle regulation, since it is specifically synthesized around mitosis and we have evidence that this protein is phosphorylated. Work is in progress to characterize and identify these proteins. An invariant level during the cell cycle was also seen for lamin-homologues, which build up the nuclear lamina structure. Such an invariant cell cycle pattern and the lack of phase-specific nuclear matrix proteins are in line with previous results that demonstrated the absence of phase-specific proteins among the 700 most abundant Physarum cellular proteins (Gröbner and Loidl, 1985). Obviously, the specific functional properties of the nuclear scaffold framework are regulated by the specific assembly and reconstruction of structural units rather than by the de novo synthesis of the individual components. This may be specially true for organisms with a closed mitosis, like Physarum plasmodia; in this case the nuclear membrane and most likely also the nuclear lamina persist during mitosis; therefore nuclear organization in general might be more stable and invariant as in cellular systems with nuclear membrane and lamina breakdown during nuclear division. Our results can also be interpreted in the sense of a high and continuous turnover of matrix proteins.

One of the striking findings of this investigation is that those newly synthesized histones, which remain tightly associated with the nuclear matrix, are assembled into matrix structures only during the early part of S-phase, thus differing considerably from histones assembled into bulk nuclear chromatin. Again, this finding argues against the possibility that proteins that are found to be matrix associated are artificially bound during nuclear matrix isolation; in this case one would expect an assembly pattern identical to the pattern of bulk chromatin. Since matrix-bound histones actually reflect matrix-bound chromatin domains, the most plausible explanation is that matrix-attached chromatin replicates very early during S-phase. This doesn’t necessarily rule out the possibility that replication of the whole genome takes place on a nuclear scaffold; there might be differences in the strength of matrix association between the replicating machinery of bulk nuclear chromatin and that of specific chromatin areas, which remain bound to the matrix throughout the entire cell cycle.

The assumption that matrix-bound histones are a distinct subpopulation of bulk nuclear histones, representing components of matrix-attached chromatin regions, is substantiated by the striking difference in the acetylation pattern of H4 between matrix-associated histones and bulk nuclear histones. This again presents evidence that nuclear matrix structures do not arise by artificial random precipitation of nuclear compounds; it emphasizes the fact that proteins remaining attached to the nuclear matrix indeed represent distinct functional entities. The acetate content, N, of matrix-bound H4, with an average value of 1.75, is extremely high (possible maximum value = 4) and even exceeds the value reached after butyrate-induced hyperacetylation (Loidl et al., 1983, 1984); almost 20% of total H4 are in the tetra-acetylated form during early S-phase, approximately 65% of H4 is in the di- and tri-acetylated form during G2-period. It seems as if matrix-attached chromatin represents a hotspot for histone acetylation.

There are several possible explanations for the local hyperacetylation in nuclear matrix-bound chromatin. It is possible that enzymes maintaining the dynamic equilibrium of histone acetylation, histone acetyltransferases and histone deacetylases, are specifically attached to the nuclear matrix. The only candidate, in this case, would be a hist-
tome acetyltransferase enzyme, but until now we have not been able to detect one of the multiple enzyme forms (López-Rodas et al., 1992) in the nuclear matrix fraction (P. Loidl, unpublished). The extremely high acetylation state of matrix-bound histones rules out the possibility that a histone deacetylase form is an integral part of the nuclear matrix, as proposed by Hendzel and Davie (1992). We have recently shown that nuclear matrix preparations of Zea mays and Physarum only contain less than 3% of the total histone deacetylase activity (Brosch et al., 1992). Moreover, the acetylation pattern of matrix H4 is not disturbed by the inhibition of histone deacetylase activity, since in vivo treatment of plasmodia with butyrate does not lead to significant hyperacetylation (Loidl, unpublished).

We explain the specific acetylation pattern of matrix-attached core histones in terms of our model (Loidl and Gröbner, 1987b; Loidl, 1988), which attributes a rather general function to histone acetylation in the modulation of chromatin structure. According to this model a distinct acetylation pattern would serve as a kind of signal for certain nuclear processes occurring on chromatin. In this sense the acetylation pattern of matrix H4 would reflect the structural requirements of matrix-attached chromatin regions to participate in certain nuclear processes, and to fulfill a function in the binding of regulatory proteins. Our results and interpretation are also compatible with a recent report on Drosophila chromatin (Turner et al., 1992), where it was shown that specific site acetylated H4 subspecies have distinct patterns of distributions in polytene chromosomes.

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