Periodic Fluctuations of Nuclear High Mobility Group Like Proteins during the Cell Cycle of *Physarum polycephalum*†

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ABSTRACT: High mobility group like (HMG-like) nuclear proteins were isolated from plasmodia of the lower eucaryote *Physarum polycephalum* and characterized by different types of polyacrylamide gel electrophoresis. The synthesis of these proteins was measured during the naturally synchronous cell cycle of *Physarum*. The four HMG-like proteins (AS1–4) exhibit a pronounced cell cycle dependent pattern of synthesis: AS1 and AS4 have a clear maximum of synthesis in mid S phase with a basal synthesis during the entire G_2 period. In contrast, AS2 and AS3 have little synthesis in S phase but a broad maximum in mid G_2 period. The four HMG-like proteins have a very low synthesis in early S phase and late G_2 period. In addition, other non-histone proteins, which are coextracted with the HMG proteins, exhibit distinct periodic synthesis patterns. A novel non-histone protein, which is the most abundant protein species in 0.35 M NaCl extracts, was detected. It exhibits a high rate of synthesis around the time of mitosis. In general, the results indicate that, in contrast to the main cytoplasmic proteins, most nuclear proteins are phase-specific with respect to their synthesis in the cell cycle.

Only a few proteins of eucaryotic cells are cell cycle specific proteins (Bravo & Celis, 1980). Even the synthesis of most of the abundant proteins of a cell occurs during the entire cell cycle (Elliott & McLaughlin, 1978; Milcarek & Zahn, 1978). However, the main structural proteins of chromatin, the histones, are synthesized mainly during the S phase of the cell cycle [see review by Maxson et al. (1983)]. Another class of nuclear proteins, which are tightly associated with chromatin, are the "high mobility group" ("HMG") proteins; these proteins are characterized by their solubility in 0.35 M NaCl and 2–5% trichloroacetic acid, by their high content of both basic and acidic amino acids, and by their relatively high mobility in acid–urea–polyacrylamide gels (Johns, 1982). The precise function of these proteins is still obscure. There are experimental indications that the function of the HMG proteins with higher molecular weight (HMG1 and 2) is different from the function of HMG14 and 17. While HMG1 and 2 have mainly been correlated with structural functions in chromatin, HMG14 and 17 may play an important role in the determination of active chromatin [see review by Einck and Bustin (1985)].

In order to progress in the understanding of the function of HMG proteins, it would be desirable to have information about the synthesis of these proteins during the cell cycle. For such cell cycle investigations the myxomycete *Physarum polycephalum* is the most suitable system, since mitosis as well as biochemical events during interphase occur with perfect natural synchrony in plasmodia of this organism. The cell cycle of *Physarum* consists of the S phase (duration approximately 3 h) and a G_2 period (approximately 6 h), which is followed by mitosis (0.5 h). S phase follows immediately after mitosis (no G_1 period in *Physarum* plasmodia). With respect to synthetic processes occurring on chromatin, a phase of DNA replication (S phase) is clearly separated from a phase of maximum overall transcription (G_2 period).

HMG-like proteins have recently been isolated and characterized from *Physarum* amoebae (Cotê et al., 1985). Four main HMG-like proteins were detected (AS1–4). We were able to isolate and characterize the same proteins from plasmodia of *Physarum* and studied the synthesis of these proteins throughout the synchronous cell cycle of macroplasmodia. The synthesis, or rather the uptake and assembly of newly synthesized HMG-like proteins, is highly periodic with two distinct cell cycle patterns. These patterns follow neither the synthesis patterns of core histones nor that of H1 (Loidl & Gröbner, 1987). Moreover, the results in general suggest that, contrary to most cytoplasmic proteins, non-histone nuclear proteins are cell cycle regulated with respect to their synthesis.

Experimental Procedures

Materials. These were purchased from the following sources: L-[4,5-^{3}H]lysine (88 Ci/mmol) and L-[2,3-^{3}H]-glutamic acid (25 Ci/mmol) from New England Nuclear, Boston, MA; Percoll from Pharmacia AB, Uppsala, Sweden.

Culture Techniques. Physarum polycephalum, strain M_3b (a Wis 1 isolate) was used. Microplasmodia were grown in submersed shake culture in semidefined medium (Daniel & Baldwin, 1964) supplemented with 0.013% hemoglobin. Exponentially growing cultures were diluted 1:7 with fresh nutrient medium (total volume per flask 30 mL) approximately 12 h before they were used for an experiment.

Giant S-shaped macroplasmodia were prepared by coalescence of 2.5 mL of microplasmodial sediment on filter paper in bioassay dishes (33 × 20 cm) as described (Affolter et al., 1979). At a time of 1.5 h after inoculation of macroplasmodia 240 mL of nutrient medium was added. Mitosis (telophase) was determined in ethanol-fixed smears of tiny plasmodial explants under phase contrast. Explants for the determination of the exact time of mitosis were always taken from various positions within the giant macroplasmodium to detect possible minor asynchronies. Telophase occurred within 5 min over the whole macroplasmodium. The second postfusion mitosis (M2) occurred 15–16 h after inoculation of the plasmodium.

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Labeling with ^3H Amino Acids. For labeling of HMG proteins a macroplasmodium at the desired phase of the cell cycle was washed 3 times in nutrient medium deficient in peptone and yeast extract. After washing, the plasmodium was carefully drained on filter paper and transferred to Petri dishes (15 cm in diameter) containing 1.0 mCi each of [^{3}H]glutamic acid and [^{3}H]lysine in medium deficient in peptone and yeast extract. After 1.0 h of incubation on radioactivity plasmodia were harvested for nuclear isolation and HMG protein extraction.

Nuclear Isolation and Extraction of HMG-like Proteins. Nuclei were isolated according to a published procedure (Nothacker & Hildebrandt, 1985) with slight, but essential, modifications (Loidl & Gröbner, 1987). Phenylmethanesulfonyl fluoride (0.2 mM) was included in all solutions for nuclear isolation. Isolated nuclei were counted in a hemocytometer.

Extraction of HMG-like proteins was performed following published procedures (Goodwin et al., 1973; Bhullar & Candido, 1981). Isolated nuclei were resuspended in extraction buffer (0.35 M NaCl, 10 mM Tris-HCl,[1] pH 7.5) and stirred on ice for 15 min. For 2×10^8 nuclei 20 mL of extraction buffer was used. A total of 0.2 mM phenylmethanesulfonyl fluoride was included. After centrifugation at 50000*g* for 25 min, the supernatant was saved and the pellet reextracted for 10 min in extraction buffer on ice (10 mL for 2×10^8 nuclei). After centrifugation at 50000*g* (25 min), both extraction supernatants were combined and adjusted to 2% trichloroacetic acid and stirred on ice for 10 min. Then the solution was adjusted to 15% ammonium sulfate saturation and stirred for another 15 min on ice. The solution was centrifuged for 30 min at 50000*g*. The pellet contains low mobility group proteins and some histones. The supernatant was adjusted to 25% trichloroacetic acid and left on ice for at least 2 h. The precipitated HMG-like proteins were then recovered by centrifugation (30 min at 50000*g*). Finally, the HMG-pellet was washed with ethanol and dried. For electrophoretic analysis the pellet was solubilized in 8 M urea–0.9 M acetic acid. The protein content was measured as described (Lowry et al., 1951). $CaCl_2$-extracted histones served as control in subsequent electrophoretic analyses.

Electrophoretic Techniques. Samples were analyzed on SDS–15% polyacrylamide slab gels (16 cm long) as described by Laemmli (1970) with slight modifications (Weintraub et al., 1975), on acid–urea–polyacrylamide slab gels (16 cm long) as described by Panyim and Chalkley (1969), or on two-dimensional (2-D) gels, where both types of electrophoreses were combined (first dimension, acid–urea gel; second dimension, SDS gel). Gels were stained with Coomassie Blue and destained by diffusion. Fluorography of 2-D gels was performed essentially as described (Bonner & Laskey, 1974; Laskey & Mills, 1975) by using preflashed Amersham Hyperfilm MP.

Quantitative Evaluation of Fluorograms. In order to quantitate the spots of fluorograms of 2-D gels, the following procedure was applied: Prior to exposure of 2-D gels the X-ray film was fixed with tape to the dried, PPO-impregnated gel. The X-ray film was then carefully cut to exactly the same shape as the dried gel. After exposure at −80 °C for the appropriate time X-ray sheets were developed. The developed film sheet was then placed on the congruent gel and spots on the fluorogram were carefully marked on the gel. These marked spots were then excised from the gel with a cork borer and counted by liquid scintillation spectrophotometry [counting cocktail, 32 mM 2,5-diphenyloxazole and 0.58 mM 2,2′-*p*-phenylenebis(5-phenyloxazole) in 100% toluene].

[1] Abbreviations: Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; SDS, sodium dodecyl sulfate; PPO, 2,5-diphenyloxazole.

Table I: Amino Acid Composition (mol %) of Protein PX

Cys	–[a]	Arg	3.5	Pro	–[a]
Asx	11.2	Ala	12.7	Ile	6.9
Glx	15.6	Tyr	2.7	Leu	12.2
Ser	6.7	Trp	–[a]	Lys	8.4
His	0.6	Met	0.6	Asx + Glx	26.8
Gly	–[a]	Val	8.0	basic amino acids	12.5
Thr	6.0	Phe	4.9	Lys/Arg ratio	2.4

[a] Not determined (see Experimental Procedures).

Amino Acid Analysis of Protein PX. The PX band was excised from unstained SDS–polyacrylamide slab gels. The gel slices were minced and incubated for 2 h in SDS electrophoresis running buffer (Laemmli, 1970) without glycine under stirring on ice. After centrifugation for 15 min at 3500*g*, the supernatant was dialyzed for 5 h against 5 mM Tris-HCl, pH 7.1 (repeated changes). Finally, the protein was recovered from the dialysate by ethanol precipitation and dried. All equipment and solutions were sterile. The protein was hydrolyzed in 6 N HCl for 24 h at 110 °C and analyzed in an amino acid analyzer from Waters by using the *o*-phthaldialdehyde method. Tryptophan, cysteine, and proline were not determined. Due to traces of contaminating glycine from the electrophoresis buffer we did not consider the measured percentage of glycine. Therefore, we corrected all amino acids by setting glycine to zero.

Results

HMG-like proteins (AS1–AS4) were isolated from exponentially growing microplasmodia of *P. polycephalum*. The isolated proteins were then analyzed electrophoretically on acid–urea–polyacrylamide gels (Figure 1a), on SDS–polyacrylamide gels (Figure 1b), and on two-dimensional gels (Figure 1c). For comparison of electrophoretic mobilities *Physarum* histones were run on the same gel (left lanes in Figure 1a,b). Figure 1c shows that histone H1 is present in our HMG preparation, being the most upper band in the acid–urea gel, as well as in the SDS gel. AS1 comigrates with H1 in the acid–urea gel but is clearly separated in the SDS gel. AS2 has the same mobility as H2B and H3 in acid–urea gels but moves considerably slower on SDS gels. AS2 shows a twin spot on two-dimensional gels. AS3 migrates in the H4 region in acid–urea gels but comigrates with H2A in SDS gels. AS4 is the fastest band in both gel systems; it migrates faster than H4 in acid–urea gels but slightly slower than H4 in SDS gels.

The H1 region in acid–urea gels is further contaminated with a protein, which is well separated from H1 in the SDS gel. We named this band PX; this band was not present in a previous study on HMG-like proteins in *Physarum* amoebae (Cotē et al., 1985). PX is one of the most abundant spots on Commassie Blue stained gels. Amino acid analysis of this protein (Table I) revealed that it has a high percentage of glutamic acid and aspartic acid (approximately 27%) but a relatively low content of basic amino acids (approximately 12%), with a lysine/arginine ratio of 2.4. The low lysine content is reflected in the relatively weak label of this protein (in relation to its abundance) in fluorograms of two-dimensional gels. On the other hand, the mean content of glutamic acid and aspartic acid of AS1–4 was determined to be 21.30%, the mean content of basic amino acids 23.20%, and the lysine/arginine ratio 4.55 (Cotē et al., 1985). It is therefore unlikely that protein PX represents another HMG-like protein in *Physarum* plasmodia.

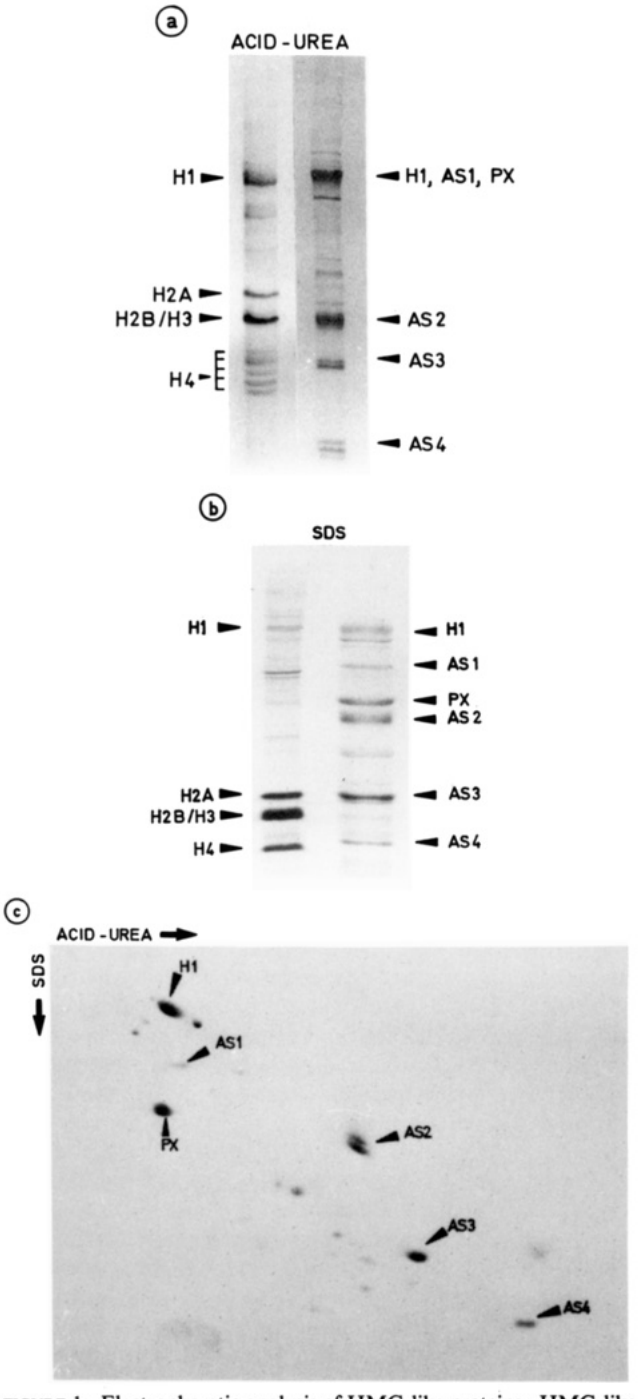

FIGURE 1: Electrophoretic analysis of HMG-like proteins. HMG-like proteins were prepared from exponentially growing *Physarum* microplasmodia as described under Experimental Procedures and analyzed by different types of gel electrophoresis: (a) acid–urea–polyacrylamide slab gel; (b) SDS–polyacrylamide slab gel; (c) two-dimensional gel (first dimension, acid–urea gel; second dimension, SDS gel). Gels were stained with Coomassie Blue. Left lanes of (a) and (b) contain *Physarum* histones as control, and right lanes, the HMG-like proteins.

The apparent molecular weights of the isolated proteins were calculated from their mobilities on SDS gels, using *Physarum* histones H1 and H4 as internal markers. The molecular weights of *Physarum* histones were taken from work by Mende et al. (1983). Molecular weights are as follows: AS1, 26 500; AS2, 21 000; AS3, 14 500; AS4, 12 000. Protein PX has a molecular weight of 22 500. It should be emphasized that the electrophoretic behavior of our plasmodial HMG-like proteins is identical with the electrophoretic properties of AS1–4 in *Physarum* amoebae (Cotē et al., 1985).

After characterization of plasmodial HMG-like proteins we investigated the synthesis of these proteins during the synchronous cell cycle of *Physarum* macroplasmodia. For this purpose plasmodia were transferred to radioactive glutamic acid and lysine at the desired cell cycle phases and incubated for 1 h. Then plasmodia were harvested for nuclear isolation and HMG extraction. After two-dimensional gel electrophoresis (first dimension, acid–urea gel; second dimension, SDS gel) of the samples fluorography was performed. Figure 2 shows the fluorograms of five time points of the cell cycle between mitosis 2 and 3. All four HMG-like proteins are heavily labeled. It can be immediately recognized that the HMG-like proteins exhibit high periodicity of their synthesis during the cell cycle. In order to exactly quantitate the intensity of the spots of the fluorograms, we excised the spots from the gels (see Experimental Procedures) and counted them in the liquid scintillation spectrophotometer. The results of this measurement (Figure 3) are in accordance with the visual estimation of the spots in the fluorograms (Figure 2). AS1 and AS4 have the same pattern of synthesis with a sharp maximum in S phase and relatively low levels during the G_2 period (Figure 3a). A completely different pattern is exhibited by AS2 and AS3. Both have a maximum in mid G_2 period with a sharp decline thereafter (Figure 3b). AS2 and AS3 have relatively low levels at the time of the maximum synthesis of AS1 and AS4. All four proteins have little synthesis in early S phase and late G_2 period, indicating low synthesis during mitosis.

Histone H1 is synthesized with a broad maximum in early G_2 period (Figure 3c), which is in line with recently published results on the synthesis of histones during the *Physarum* cell cycle (Loidl & Gröbner, 1987). The synthesis pattern of H1 is a valuable internal control, since unreproducibilities in the extraction of proteins would seriously disturb the pattern. The protein pattern on Coomassie Blue stained two-dimensional gels from different time points in the cell cycle revealed neither qualitative nor significant quantitative changes (result not shown). This also shows that there are no differences in the extraction behavior of the HMG-like proteins among different cell cycle stages. Interestingly, the opposite synthesis pattern in comparison to H1 is exhibited by protein PX, which is high in early S phase and late G_2 period, with a clear minimum in early G_2 period (Figures 2 and 3d). Thus PX has a maximum of uptake and assembly into the nucleus around the time of mitosis. Although this protein is abundant on Coomassie Blue stained gels, it is rather weakly labeled in fluorograms, due to its low lysine content.

The few remaining spots on the fluorograms do not exhibit a unique synthesis pattern. Some of the spots follow the pattern of histone H1, whereas others have a biphasic pattern with maxima in late S phase and mid G_2 period. It is noteworthy that none of the spots exhibits a constant label at different time points, reflecting the periodicity of synthesis of all nuclear proteins present on our two-dimensional gels during the cell cycle.

Discussion

Synthesis Patterns of Nuclear Proteins during the Cell Cycle. The cell cycle represents a well-defined sequence of events between the formation of a new cell and its division into two daughter cells. The interphase between is subdivided into distinct periods characterized by distinct biochemical events. Therefore, it is suggestive to postulate certain proteins that are specific for a particular cell cycle phase. However, most studies on the cell cycle synthesis of proteins did not verify the existence of such phase-specific proteins (Elliott &

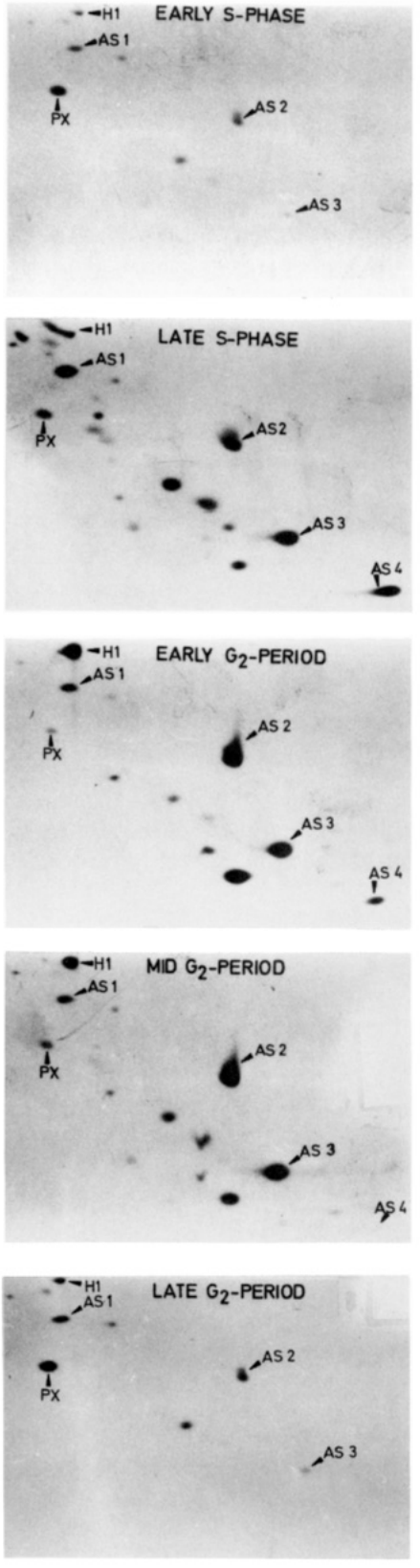

FIGURE 2: Incorporation of [^{3}H]glutamic acid and [^{3}H]lysine into HMG-like proteins during the cell cycle. HMG-like proteins were prepared from macroplasmodia of *Physarum* from different phases of the cell cycle after incubation (1 h) in [^{3}H]glutamic acid and [^{3}H]lysine as described under Experimental Procedures. The time points (half-time of incubation on radioactive amino acids) were as follows: early S phase, 0.75 h after mitosis 2 (M2); late S phase, 2 h after M2; early G_2 period, 4 h after M2; mid G_2 period, 6.5 h after M2; late G_2 period, 7.8 h after M2. The HMG fraction (25 μg) was subjected to two-dimensional gel electrophoresis with subsequent fluorography.

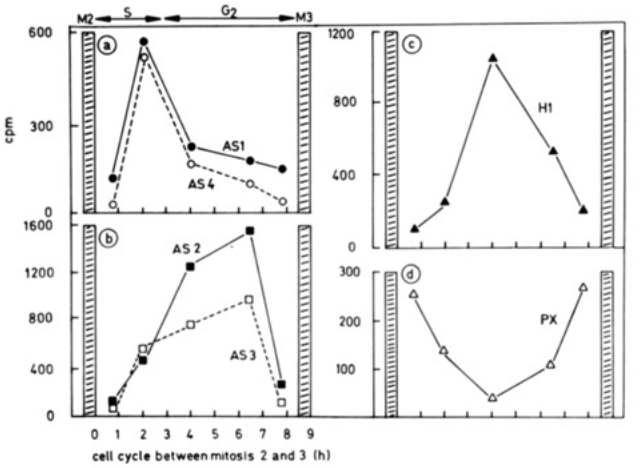

FIGURE 3: Quantitative evaluation of the fluorograms of Figure 2. After exposure of the dried PPO-impregnated gels the spots of the fluorograms were carefully marked on the dried gel and excised with a cork borer. The excised spots were counted for radioactivity. (a) Synthesis pattern of AS1 and AS4; (b) AS2 and AS3; (c) histone H1; (d) PX. The arrows in (a) indicate the duration of S phase and G_2 period; hatched columns indicate the time and duration of the second and third mitosis (M2, M3).

McLaughlin, 1978; Lutkenhaus et al., 1979; Bravo & Celis, 1980; Gröbner & Loidl, 1985). It has been shown that even the synthesis of most cellular proteins is not cell cycle dependent (Milcarek & Zahn, 1978; Elliott & McLaughlin, 1978; Lutkenhaus et al., 1979). Several groups have investigated cell cycle dependent protein synthesis in *Physarum*, since this organism offers the unique advantage of synchronous nuclear division (Turnock et al., 1981; Pahlic & Tyson, 1983, 1985; Gröbner & Loidl, 1985); these studies all agree that there are no phase-specific proteins and even only a few cellular proteins show detectable changes in the rate of synthesis through the cell cycle. For this reason it was surprising to see the pronounced periodicity of all nuclear proteins present in the HMG fraction. The synthesis of the main nuclear proteins, the histones, is well-known to be periodic during the cell cycle (Maxson et al., 1983). The present study reveals that HMG-like proteins exhibit a periodicity during the *Physarum* cell cycle, which is entirely different from the periodic synthesis pattern of histones in this organism (Loidl & Gröbner, 1987). We can distinguish at least five different synthesis patterns of nuclear proteins in the *Physarum* cell cycle: (1) maximum synthesis in early S phase, as shown for core histones (Loidl & Gröbner, 1987); (2) maximum in late S phase (AS1 and AS4); (3) maximum in mid G_2 period (AS2 and AS3); (4) maximum rate of synthesis at the S/G_2 boundary (mid-interphase), as shown for histones H1 and some non-histone proteins (Loidl & Gröbner, 1987); (5) main synthesis around mitosis with a minimum in mid-interphase (protein PX).

In previous investigations on cell cycle dependent protein synthesis these periodic nuclear proteins have not been investigated, since neither acidic nor basic proteins have been included in the analysis. Moreover, only unfractionated cellular homogenates have been studied; for this reason the sensitivity of current detection methods in combination with two-dimensional gel electrophoresis only allowed the analysis of the more abundant cellular proteins. For example, thymidine kinase and thymidylate synthase, both periodic enzymes in the *Physarum* cell cycle (Gröbner & Loidl, 1982, 1983), were not detected on two-dimensional gels (Pahlic & Tyson, 1983, 1985). A closer inspection of fractionated extracts or of certain classes of proteins, as in this study, may reveal the existence of highly periodic proteins apart from the main

"housekeeping" proteins of a cell. However, it should be noted that we measure the uptake of newly synthesized proteins into the nucleus and their assembly into nuclear structures rather than the true synthetic capacity; this is due to the fact that we analyzed non-histone proteins from isolated nuclei after in vivo labeling of plasmodia with radioactive amino acids.

In the present report we have detected the novel nuclear protein PX with the same solubility properties as HMG proteins, which was not observed in a previous study of *Physarum* amoebae (Cotê et al., 1985). The low content of basic amino acids does not suggest that this protein is another HMG-like protein. The cell cycle pattern of PX clearly correlates with mitosis. This unique correlation together with the abundance of this protein suggests that it may have an important function in nuclear division. The lack of this protein in amoebal preparations of *Physarum* could be due to the utilization of a novel nuclear isolation procedure (Nothacker & Hildebrandt, 1985; Loidl & Gröbner, 1987), which results in a better preservation of nuclei with a higher protein content in comparison to other nuclear isolation procedures. Alternatively, PX could only be expressed in *Physarum* plasmodia but not in amoebae. It should be noted that uninuclear amoebae undergo normal cell division, whereas multinuclear plasmodia undergo nuclear division only. Thus, PX could be a marker protein for closed mitosis, since the nuclear membrane persists throughout mitosis in *Physarum* plasmodia.

HMG-like Proteins during the Cell Cycle. We could show that the HMG-like proteins AS1–4, which were recently identified in *Physarum* amoebae (Cotê et al., 1985), are also expressed during the plasmodial stage of this organism. In an earlier report on *Physarum* HMG-like proteins, two protein bands with molecular weights higher than H1 were designated as HMG-1P and HMG-2P (Czupryn & Toczko, 1984). However, these two proteins were present neither in the study of Cotê et al. (1985) nor in our present investigation. The same report also showed a band comigrating with histone H2A on SDS–polyacrylamide gels (HMG14/17); this band could be identical with that of AS3, according to our data and previous results (Cotê et al., 1985).

The true functions of HMG proteins are still far from clear. The abundance of HMG proteins in mammalian cell nuclei suggests that these proteins are somehow involved in the structure of chromatin rather than having exclusively regulatory functions; it has been demonstrated that HMG proteins bind to DNA, histones, and nucleosomes [see Einck and Bustin (1985) and Reeck and Teller (1985)]. The majority of available data suggests that HMG proteins do not represent a unique protein population with respect to their distribution and function in chromatin. There is evidence that HMG1 and -2 are involved in DNA replication, since they exhibit a preferential affinity for single-stranded DNA [e.g., Isackson et al. (1979)] and are correlated with the proliferative activity of tissues [e.g., Seyedin and Kistler (1979) and Shastri et al. (1982)]. However, analysis of HMG protein synthesis following partial hepatectomy showed that the peaks of DNA synthesis and HMG protein synthesis are time shifted (Kuehl, 1979). This indicates that the interrelation between the two processes is not direct. The synthesis pattern during the cell cycle of AS1 and AS4, having a maximum in late S phase, suggests that these proteins may also serve some function during DNA replication. This function could be related to the maturation and stabilization of nucleosomes at later stages of S phase. Obviously, AS1 and AS4 are not essential for chromatin replication itself, because they are utilized at a relatively late time point in comparison to the maximum of DNA replication. In contrast, core histone synthesis already starts before mitosis and reaches a maximum in early S phase (Loidl & Gröbner, 1987).

In several experimental systems evidence was obtained for a close correlation between HMG14 and -17 and the transcriptional activity of chromatin. Weisbrod and Weintraub (1979) suggested that the presence of HMG14 and -17 causes the observed deoxyribonuclease (DNase) I sensitivity in active chromatin regions. This suggestion has been substantiated in several subsequent reports (Weisbrod & Weintraub, 1981; Gazit et al., 1980; Limas, 1982; Spiker et al., 1983). If HMG14 and -17 confer some specific structural features to potentially active chromatin, then one would expect the synthesis of these proteins in a cell cycle phase, when most of the transcription occurs. We find an increase of utilization of newly synthesized AS2 and -3 during late S phase and early G_2 period with a maximum in mid G_2; This pattern corresponds to the overall synthesis of RNA during the cell cycle (Turnock, 1979).

In a recent report (Bustin et al., 1987) HMG17 mRNA levels were followed in synchronized HeLa cells. HMG17 mRNA is most abundant at the end of S phase; thus HMG17 does not correlate with DNA replication and histone synthesis, as is also the case for AS2 and 3 during the *Physarum* cell cycle.

The characterization of plasmodial and amoebal (Cotê et al., 1985) HMG-like proteins in *Physarum* is based on extraction properties, electrophoretic behavior, and amino acid composition of the proteins. It is therefore difficult to estimate possible homologies to similar proteins of other organisms before the sequence of the proteins has been determined. As previously discussed in detail (Cotê et al., 1985), a weak similarity of amino acid composition between proteins does not necessarily rule out a phylogenetic relation of these proteins. It could be that HMG-like proteins of lower eucaryotes are not as highly conserved in structure, like histones, but may be conserved with respect to their function. To elucidate a possible functional homology between HMG proteins of higher eucaryotes and their *Physarum* counterparts will be subject of further investigations.

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Stereochemical Inversion at C-15 Accompanies the Enzymatic Isomerization of *all-trans*- to 11-*cis*-Retinoids†

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ABSTRACT: *all-trans*-Retinol (vitamin A) is processed by membranes from the pigment epithelium of the amphibian or bovine eye to form 11-*cis*-retinoids. When the isomerization reaction is performed with either [15(*S*)-^{3}H,^{14}C]-*all-trans*-retinol or [15(*R*)-^{3}H,^{14}C]-*all-trans*-retinol as substrate, the resultant 11-*cis*-retinals, formed by the in vitro enzymatic oxidation of the retinols, retain their ^{3}H in the former case and lose it in the latter. The ocular *all-trans*- (*pro-R* specific) and 11-*cis*-retinol (*pro-S* specific) dehydrogenases operate with different stereochemistries with respect to the prochiral methylene hydroxyl centers of their substrates. Inversion of stereochemistry at the prochiral retinol centers was shown to accompany the isomerization process in both the amphibian and bovine systems. The 11-*cis*-retinol formed from [15(*S*)-^{3}H,^{14}C]-*all-trans*-retinol was chemically isomerized with I_2 to produce [15(*R*)-^{3}H,^{14}C]-*all-trans*-retinol. The 11-*cis*-retinol formed from [15(*R*)-^{3}H,^{14}C]-*all-trans*-retinol was chemically isomerized with I_2 to produce [15(*S*)-^{3}H,^{14}C]-*all-trans*-retinol. The stereochemistry at the prochiral center of retinol is not affected by the I_2-catalyzed double-bond isomerization process and, hence, inversion of stereochemistry at C-15 must accompany isomerization. The same inverted stereochemistry was found with the associated retinyl palmitates. Possible mechanistic reasons for the observed inversion of stereochemistry during isomerization are discussed.

The absorption of light by rhodopsin results in the cis to trans isomerization of rhodopsin's 11-*cis*-retinal Schiff base chromophore, leading to the bleaching of the pigment (Hubbard & Wald, 1952). Hydrolysis of the resultant *all-trans*-retinal Schiff base results in the formation of free *all-trans*-retinal, which is rapidly reduced by specific retinol dehydrogenase enzymes in the rod outer segments to produce *all-trans*-retinol (Wald & Hubbard, 1949). The *all-trans*-retinol is transported to the pigment epithelium by specific binding proteins (Bok, 1985; Saari et al., 1984; Pfeffer et al., 1983; Adler & Evans, 1985), where it is esterified to form *all-trans*-retinyl esters (Hubbard & Dowling, 1962; Knowles & Dartnall, 1977). In

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