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SYNTHESIS OF NUCLEAR LAMINS IN BHK-21 CELLS SYNCHRONIZED WITH APHIDICOLIN

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Lamins A, B and C are the major proteins of mammalian nuclear lamina and have been well studied in BHK-21 cells [1, 2]. By synchronizing BHK-21 cells with aphidicolin, a potent inhibitor of DNA α-polymerase, we were able to detect a differential pattern of synthesis for nuclear lamins during the cell cycle. Lamin B starts to be synthesized only in S phase up to mitosis while synthesis of lamins A and C remain stable throughout the cell cycle. The precursor of lamin A [2] see its half-life increase from a reported 63 min in interphase cells to 103 min in G2/M cells.

The nuclear membranes of eukaryotic cells are underlined with a fibrillar meshwork called nuclear lamina [3-5]. The main proteins of this structure are known as lamins A, B and C in mammalian cells [3-5]. Lamins A and C are synthesized by two different mRNAs [6] but may came from a single gene by differential splicing [7,8]. Immunological cross-reactions show that lamin B is related to the other two lamins [9-16]. Lamin A is synthesized as a precursor of higher molecular weight [2,6,14]; lamin B is also the target of some post-translational modifications [17].

The lamins exhibit striking behavior differences in their distribution during the cell cycle. From G1 to G2 phase, the lamins are polymerized together into the nuclear lamina [5]. During mitosis, lamins A and C become soluble while lamin B is associated with nuclear membranes remains [18]. Although lamins are always present in the cell, the exact timing of their synthesis remains undefined. Conflicting reports have been published: synthesis only in S phase in HeLa cells [19], throughout the cell cycle in CHO cells [14,15], presence of mRNAs for lamins B and C only in S phase in Ehrlich ascites cells [20]. We report in this manuscript that in BHK-21 cells synchronized with aphidicolin, a specific inhibitor of DNA α-polymerase in eukaryotic cells [21-23], lamin B is synthesized only in S phase and afterward while levels of synthesis of lamins A and C remain stable in all phases. We also observe variation in the half-life of the lamin A precursor during the cell cycle.

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MATERIALS AND METHODS

Cell culture and synchronization

Baby hamster kidney cells (BHK-21) were grown at 37°C in α-medium (Gibco) supplemented with 10% fetal calf serum (Gibco) (standard medium). Cell synchronization was done according to Pedrali-Noy [22]: cells grown in standard medium for 24 h were washed with α-medium without serum and incubated for 24 h. After a second 24 h serum starvation period, cells were incubated for 24 h in standard medium containing 5 μg/ml aphidicolin (Sigma; freshly prepared at 5 mg/ml in DMSO), to block the cells in G1/S interphase. The cells were released from the aphidicolin block by washing with PBS prior to incubation in standard medium.

Cell cycle analysis

For cell cycle analysis, 24-well trays (Corning) were plated with 5 x 10^4 cells/well and the cells synchronized as described above. Each hour after release from the aphidicolin block, cells from 3 wells were labelled for 15 min with 10 μCi/ml of [^{3}H]thymidine (117 Ci/mmol, Amersham) in standard medium. The cells were washed twice with ice-cold PBS and scraped off with a rubber policeman in 500 μl of PBS. Labelled DNA was precipitated with 5% trichloroacetic acid and filtered on GF/A filters (Whatman Ltd). The filters were washed twice with cold 5% TCA, twice with ethanol and dried. The radioactivity was counted for each filter.

Cell cycle phases were established according to the [^{3}H]thymidine incorporation and data from other laboratories [22]: G1 and G1/S, respectively 5 h and 24 h after release from serum starvation, during aphidicolin block; S, G2 and G2/M, respectively 6 h, 9 h and 11 h after release from the aphidicolin block.

Protein synthesis studies

BHK-21 cells were plated in 60 mm culture dishes at 2 x 10^5 cells/dish and synchronized. At each pre-established cell cycle phase, the cells were washed twice with methionine-free α-medium containing 10% dialyzed fetal bovine serum, either with 5 μg/ml aphidicolin for G1 and G1/S phases or without for S, G2 and G2/M. After a 20 min incubation in the same medium, [^{35}S]methionine (1100 Ci/mmol, Amersham) was added at either 25 μCi/ml or 40μCi/ml for pulse-chase labelling experiments [2]. After labelling, the cells were washed twice with cold PBS, scraped off with a rubber policeman in cold PBS and microfuged at 15000 rpm at 4°C for 15 min. The pellets were frozen on dry ice and kept at -20°C prior to immunoprecipitation.

Immunoprecipitations were performed using a serum against BHK lamins [2]. Additional protease inhibitors were added to the immunoprecipitation buffer (1 mM benzamidine, 25 μg/ml leupeptin, 25 μg/ml aprotinin). The samples were frozen on dry ice and kept at -20°C prior to electrophoresis.

SDS-PAGE and autoradiographies were done as described in previous papers [1,2]. For the pulse-chase experiments, the same amount of radioactivity was applied in each well of the gel. Quantitative data was obtained with an UltroScan XL densitometer and GelScan XL densitometry analysis software (LKB, Uppsala) linked to a IBM XT-compatible computer.

RESULTS AND DISCUSSION

The cell cycle kinetics of BHK cells synchronized with aphidicolin was determined by [^{3}H]thymidine incorporation into DNA (fig. 1). A maximum of incorporation was observed 6 h after the removal of the aphidicolin block identifying the S phase. Synchronization by aphidicolin was spread over two full cycles since a second peak of synthesis of DNA was observed after 24 to

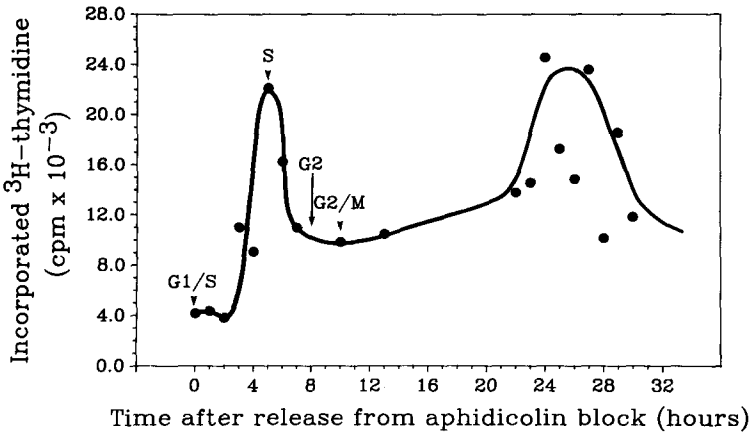

Fig. 1. Incorporation of [^{3}H]thymidine in DNA of synchronized BHK-21 cells. BHK-21 cells were plated in 24-well tissue culture plates, synchronized, labelled and their DNA precipitated as described in MATERIALS AND METHODS. Each point represents the average value from 3 wells.

26 h; this second maximum was however much broader indicating a progressive lost of the synchronization. With Pedrali-Noy's data [22], these results enabled us to establish four of the phases of the cell cycle: G0, during serum starvation; G1, when most of the cells are out of G0, 5 h after release from serum starvation with standard medium containing aphidicolin; G1/S, after a 24 h aphidicolin block; S, 6 h after the release from the aphidicolin block, at the first burst of DNA synthesis. G2 and G2/M phases were arbitrarily fixed at 3 h (G2) and 5 h (G2/M) after the S phase. That last point contains an increasing number of mitotic cells on the basis of the mitotic index (data not shown). Some difficulties to pinpoint an accurate position for G2 phase is in accordance with results from other groups [24].

Variations in lamins synthesis during the cell cycle were assessed by labelling of synchronized cells with [^{35}S]methionine for 10 and 60 min followed by immunoprecipitation of lamins with a specific antibody [2]. The newly synthesized lamins were visualized after autoradiography of SDS-PAGE gels. In G0 cells, no lamins were detected (data not shown) suggesting that the synthesis of lamins A, B and C is growth-regulated. As shown in Fig. 2, we observed an almost total lack of lamin B synthesis in G1 phase although newly synthesized lamins A and C were detected during this phase. All three lamins were detected in all phases afterward. Quantitative studies of SDS-PAGE autoradiograms were obtained by densitometry as shown in fig. 3. Since actin and vimentin, detected by our anti-lamin serum [1,2], are cell cycle dependant [25,26], no internal standard could be used. Therefore, the relative abundance of each lamin was calculated as the ratio of the total amount of lamins A, B and C. In normal non-synchronous cells, lamins are produced in almost equal amounts. During synchronization, we observed that the relative abundance of

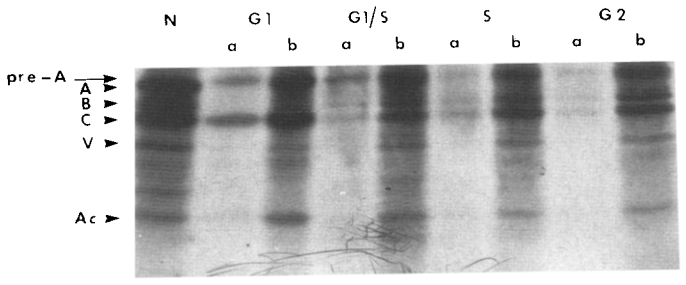

Fig. 2. Autoradiograph of SDS-PAGE of immunoprecipitated lamins labelled with [^{35}S]methionine in synchronized cells. BHK-21 cells were plated in 60 mm tissue culture petris, synchronized and labelled with [^{35}S]methionine as described in MATERIALS AND METHODS. Lane 1 represents normal non-synchronous cells labelled for 60 min. Lanes 2, 4, 6 and 8 represent immunoprecipitated lamins from cells labelled 10 min; lanes 3, 5, 7 and 9 represent immunoprecipitated lamins from cells labelled 60 min. A: lamin A; B: lamin B; C: lamin C; V: vimentin; Ac: actin.

pre-lamin A/lamin A and lamin C is fairly constant throughout the cell cycle; this is a very different situation from the ones previously reported by Jost and Johnson [19] on HeLa cells and Bludau et al. [20] on Ehrlich ascites cells but is in accordance with the results of Gerace et al. [14] and Ottaviano and Gerace [15]. In G1/S, we observed a low level of synthesis of lamin B with a maximum in S phase and a continuous synthesis at lower level in phases afterward. The presence of lamin B synthesis in G1/S phase can be explained by the fact that aphidicolin does not suppress in the G1/S interphase the

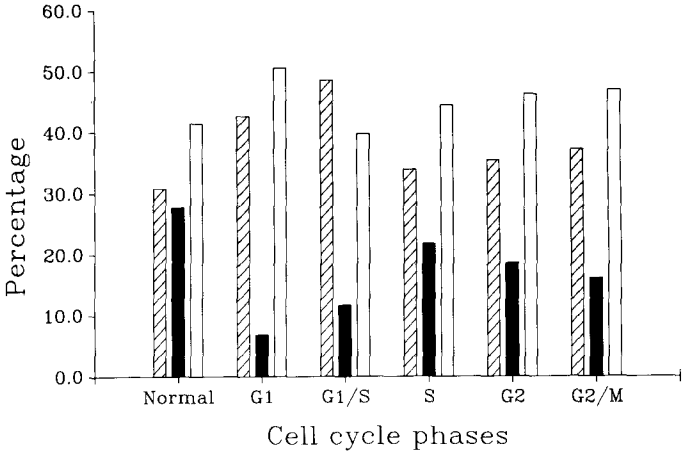

Fig. 3. Abundance of each lamin in the different phases of the cell cycle. Autoradiographs from SDS-PAGE of labelled immunoprecipitated lamins were scanned with a LKB Ultroscan XL and the lamins quantified. The value for each lamin represents its ratio of the total amount of immunoprecipitated lamins, for each phase of the cell cycle determined as described in MATERIALS AND METHODS. The hatched box represents pre-lamin A/lamin A, the open box, lamin B and the filled box, lamin C.

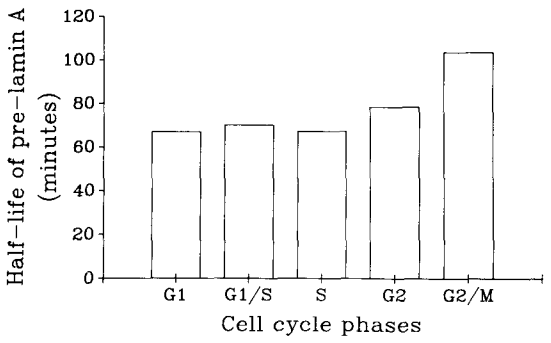

Fig. 4. Variation of the half-life of the precursor of lamin A during the cell cycle. BHK-21 cells plated in 60 mm tissue culture petri dishes were synchronized as described in MATERIALS AND METHODS. For each phase of the cell cycle, cells were pulse-labelled for 10 min with [^{35}S]methionine and chased with radioactivity-free medium for 0, 30, 60, 90 and 120 min. Lamins were immunoprecipitated and separated on SDS-PAGE, visualized by autoradiography and quantified using a LKB Ultroscan XL. The half-life was determined according to Dagenais et al. [2] by dividing the quantity of the pre-lamin A (74 kD) by the total amount of pre-lamin A (74 kD) and lamin A (72 kD).

synthesis of proteins normally synthesized in S phase [22]. The fact that lamin B is mainly produced in S phase may indicate that its synthesis is related to the expansion phase of the nucleus associated with the DNA synthesis that characterized that phase.

We examined also if there were variations in the half-time of the precursor of lamin A by pulse-chase experiments. Previous experiments using normal non-synchronized BHK-21 [2] cells have shown a half-life of 63 minutes, a very long time compared to other known precursors suggesting a post-transcriptional processing. The half-time determined for the conversion of pre-lamin A into lamin A at each phase is shown in fig. 4. We observed that the half-life of the pre-lamin A remains constant for most of the cell cycle. However, we observed a sharp increase in G2 to 78 min with a maximum in G2/M of 103 min. This increase suggests that the protease responsible for the processing of pre-lamin A is not present nor active prior to mitosis, allowing an accumulation of the precursor for lamin A in pre-mitotic cells. Although a precursor for lamin A has been observed in many different cells [27], no role has been suggested for this protein. This difference in half-life during the cell cycle is the first indication that pre-lamin A may play an important function during cell growth.

We report an effective approach to synchronize fibroblasts in culture by using aphidicolin to inhibit DNA synthesis therefore stopping the cells in a G1/S interphase. The analysis of the synthesis of lamins in such cells shows that general synthesis of lamins A, B and C is related to cell growth with differences in individual synthesis pattern. Lamin B is not synthesized in G1 cells but is produced in the phases afterward with a peak of synthesis in S

phase whereas pre-lamin A/lamin A and lamin C are produced throughout the cell cycle. We also observed that in cells synchronized with aphidicolin, the half-life of the precursor for lamin A is increased in pre-mitotic cells. The studies of differential patterns of synthesis and variations in half-life on other cell lines are underway.

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