Increased nuclear cyclin/PCNA antigen staining of non S-phase transformed human amnion cells engaged in nucleotide excision DNA repair

Julio E. Celis and Peder Madsen

Department of Medical Biochemistry, The University of Aarhus, Building 170, DK-8000 Aarhus C, Denmark

Received 20 October 1986

PCNA autoantibodies specific for cyclin/PCNA were used to determine the nuclear distribution of this protein in transformed human amnion cells (AMA) irradiated with ultraviolet light (254 nm) under conditions that induced nucleotide excision DNA repair synthesis. The results showed a striking increase in nuclear cyclin/PCNA antigen staining of non S-phase cells that was not abolished by cycloheximide (20 μg/ml, added 2 h before irradiation), and that is most likely due to a redistribution of pre-existing cyclin. These observations raise the possibility that cyclin/PCNA may play a role in nucleotide excision DNA repair synthesis in addition to its putative role in replicative DNA synthesis.

Cell cycle *DNA replication* *PCNA antibody* *Cyclin* *Cell proliferation*

1. INTRODUCTION

Studies in this laboratory have for some years been dedicated to the identification of cell cycle specific proteins that may participate in the control of cell proliferation in human cells ([1] and references therein). So far, two S-phase specific proliferation sensitive nuclear proteins (cyclin, M_r = 36000 [1–4], also termed PCNA [5–7] and dividin, M_r = 54000 [1,3,8,9]) have been identified. Increased synthesis of these proteins is first detected late in G_1 near the G_1/S transition border of the cell cycle and reaches a maximum in mid to late S-phase [8–11]. Cyclin/PCNA and dividin are present in all proliferating human cells so far studied (of both normal and transformed origin), and their levels are very low in non-proliferating cells ([1,4,8] and references therein). The precise function of these proteins is unknown although there is firm evidence mainly from immunofluorescence (PCNA/cyclin antibodies) and autoradiographic studies ([^{3}H]thymidine incorporation) [12–14] indicating that cyclin/PCNA may play a role in some specific aspect of DNA replication. Furthermore, early S-phase patterns of cyclin/PCNA antigen distribution resemble very much the granular pattern observed in cultured cells reacted with 5-bromodeoxyuridine (BrdU) antibodies [15]; these structures are believed to correspond to replicating replicon domains [15]. As far as we can judge cyclin/PCNA is different from DNA polymerases alpha, beta and gamma, DNA ligase, DNA primase, DNA topoisomerase I and DNA polymerase cofactors C_1 and C_2 ([13,16] and references therein).

Here we show that UV irradiation of asynchronously growing transformed human amnion cells (AMA) under conditions that induced nucleotide excision DNA repair synthesis, causes a significant change in the nuclear levels of cyclin/PCNA antigen staining that is particularly striking in non S-phase cells. These results strengthen the notion that cyclin/PCNA plays a role in some specific aspect of DNA replication.

Abbreviations: PCNA, proliferating cell nuclear antigen; UV, ultraviolet light

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

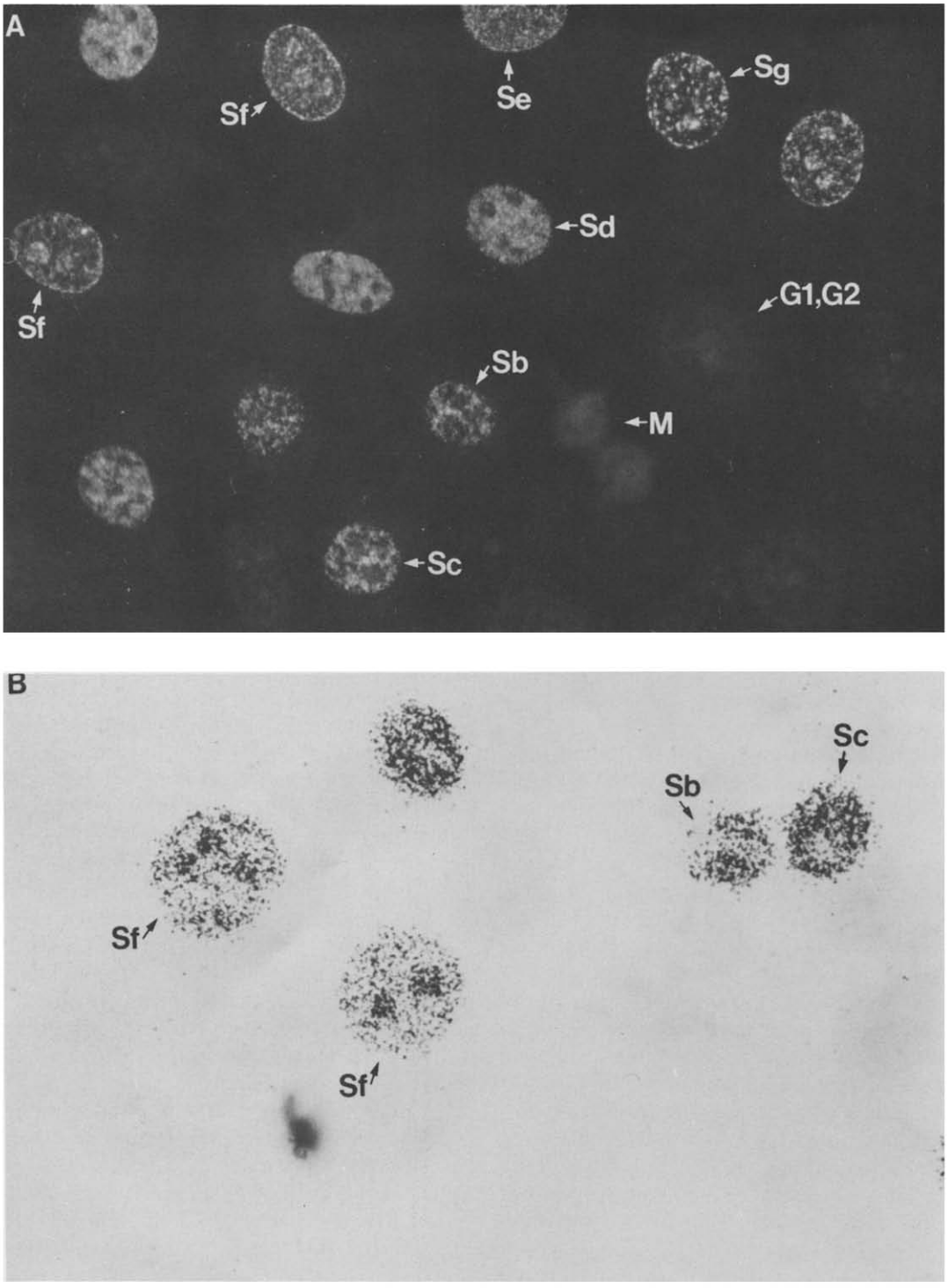

Fig.1. Nuclear patterns of cyclin/PCNA antigen distribution mimic topographical patterns of DNA replication. (A) AMA cells grown in coverslips were treated with methanol prior to immunofluorescence using PCNA antibodies specific for cyclin. (B) As above but labelled with [^{3}H]thymidine (2 μCi/ml) for 30 min.

2. MATERIALS AND METHODS

2.1. *Cells*

All the cell types used in this study were grown as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) and antibiotics (100 units of penicillin per ml, 50 μg of streptomycin per ml).

2.2. *Indirect immunofluorescence and autoradiography*

The procedures for indirect immunofluorescence and autoradiography have been detailed in [12]. PCNA antibodies specific for cyclin ([6,14,17] and references therein) were kindly provided by E.M. Tan.

2.3. *Ultraviolet light irradiation*

Cells grown in coverslips were placed in 5 cm petri dishes containing 2 ml of Hank's buffered saline and were irradiated with a 15-W Philips germicidal lamp (254 nm). The UV doses were monitored with a Black-Ray ultraviolet intensity meter (J-225, Ultraviolet Products Inc., USA). Following irradiation, the saline was aspirated and replaced with 10 ml of complete DMEM media. Cells were usually prepared for immunofluorescence and/or autoradiography following a 30 min post-irradiation incubation period to allow for expression of the damage.

2.4. *Other procedures*

The procedures for labelling somatic cells with [^{35}S]methionine (1 mCi/ml) [18,19] and two-dimensional gel electrophoresis [20] have been described in detail elsewhere.

3. RESULTS

3.1. *Nuclear patterns of cyclin/PCNA antigen distribution mimic topographical patterns of DNA replication*

Fig.1A shows an immunofluorescence micrograph of asynchronously growing AMA cells treated with methanol and reacted with PCNA antibodies specific for cyclin ([6,14,17] and references therein). As shown in [6,12–14,21–23] only S-phase cells react with the antibody to reveal variable nuclear staining patterns in terms of both the intensity and distribution of the antigen. Various S-phase patterns of cyclin/PCNA antigen staining are indicated in fig.1A according to the nomenclature proposed by Celis and Celis ([22], see also [14]). Patterns Sb and Sc (nucleolar exclusion) occur early in S-phase while patterns Sf and Sg (nucleolar staining) are observed in mid and late S-phase, respectively. Under favourable condi-

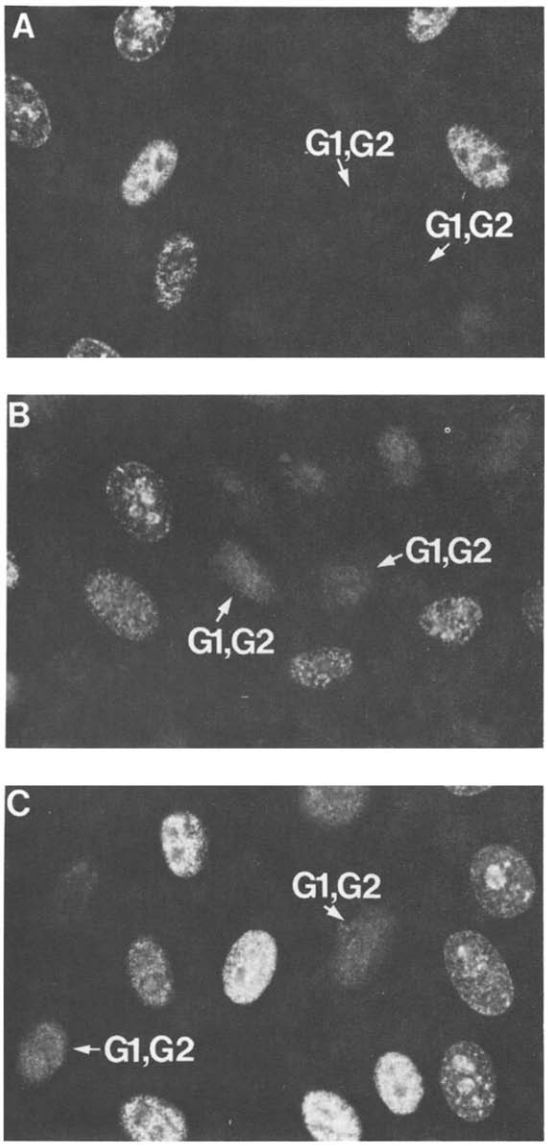

Fig.2. Cyclin/PCNA antigen staining in asynchronously growing AMA cells irradiated with increasing UV doses. (A) Control. (B) 16 J/m^2 and (C) 30 J/m^2. Irradiated control cells were incubated for 30 min in complete media prior to immunofluorescence.

tions, similar patterns to those shown in fig.1A are observed in [^{3}H]thymidine autoradiograms. As shown in fig.1B, there is a remarkable similarity between patterns of cyclin/PCNA antigen distribution and topographical patterns of DNA replication (see also [12–14]).

3.2. *Effects of UV irradiation on the nuclear patterns of cyclin/PCNA antigen staining (increased staining of non S-phase cells)*

Fig.2B and C shows immunofluorescence micrographs of asynchronously growing AMA cells irradiated with increasing doses of UV (16 J/m^2, fig.2B; 30 J/m^2, fig.2C), treated with methanol and reacted with PCNA antibodies 30 min after irradiation in order to allow for expression of the damage. The main effect of UV irradiation at the doses analyzed (3–30 J/m^2) was the striking increase in the nuclear cyclin/PCNA antigen staining of non S-phase cells (cf. fig.2B and C with figs 1A and 2A (unirradiated controls)). Increased nuclear staining of non S-phase cells was observed at 6 J/m^2 (not shown), but the effect was more striking at 30 J/m^2. Repair synthesis in these cells was monitored by [^{3}H]thymidine autoradiography (fig.3, 30 J/m^2, cf. fig.4A). About 40–50% inhibition of S-phase DNA synthesis was observed at 30 J/m^2 (not shown).

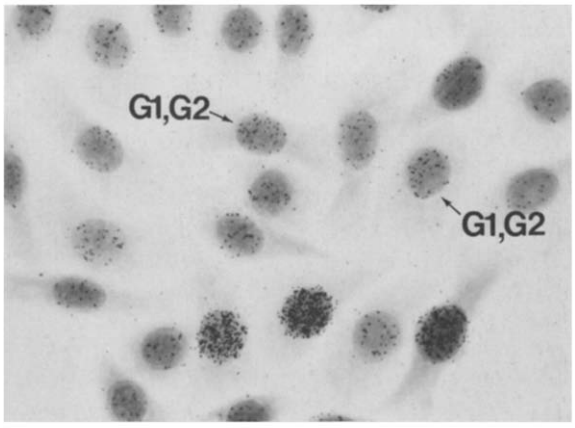

Fig.3. [^{3}H]Thymidine autoradiography of asynchronously growing AMA cells UV irradiated at 30 J/m^2. Following irradiation the cells were incubated for 30 min with [^{3}H]thymidine (5 μCi/ml). Autoradiograms were exposed for 1 day.

Evidence showing that G_1 and G_2 cells react positively with PCNA antibodies following UV irradiation was obtained by comparing the immunofluorescence distribution of cyclin/PCNA antigen in asynchronously growing AMA cells that were pre-labelled with [^{3}H]thymidine (2 μCi/ml; 30 min) prior to irradiation (30 J/m^2). After a 30 min post-irradiation incubation period, the cells were fixed with methanol, reacted with PCNA antibodies and prepared for autoradiography. Fig.4A and B shows autoradiographic (fig.4A) and immunofluorescence (fig.4B) micrographs of the same field of AMA cells. Clearly, cells that were

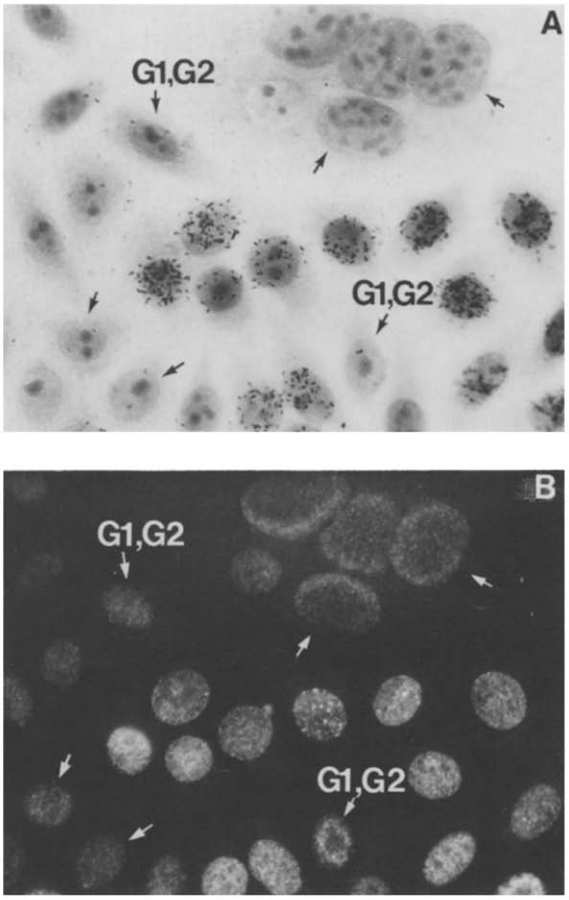

Fig.4. Autoradiographic and immunofluorescence micrographs of asynchronously growing AMA cells. (A) [^{3}H]Thymidine incorporation (2 μCi/ml; 30 min pulse given before irradiation) and (B) immunofluorescence (PCNA antibodies 30 min after irradiation at 30 J/m^2) of the same field of cells.

not synthesizing DNA at the time of irradiation (G_1 and G_2 cells; fig.4A and B) exhibit a positive nuclear staining with the antibody.

The increase in nuclear cyclin/PCNA antigen staining observed in G_1 and G_2 cells after irradiation 30 J/m^2 was not prevented by treatment with doses of cycloheximide that inhibited protein synthesis almost completely (20 μg/ml, added 2 h before irradiation and kept thereafter; not shown). These results suggest that the increased nuclear

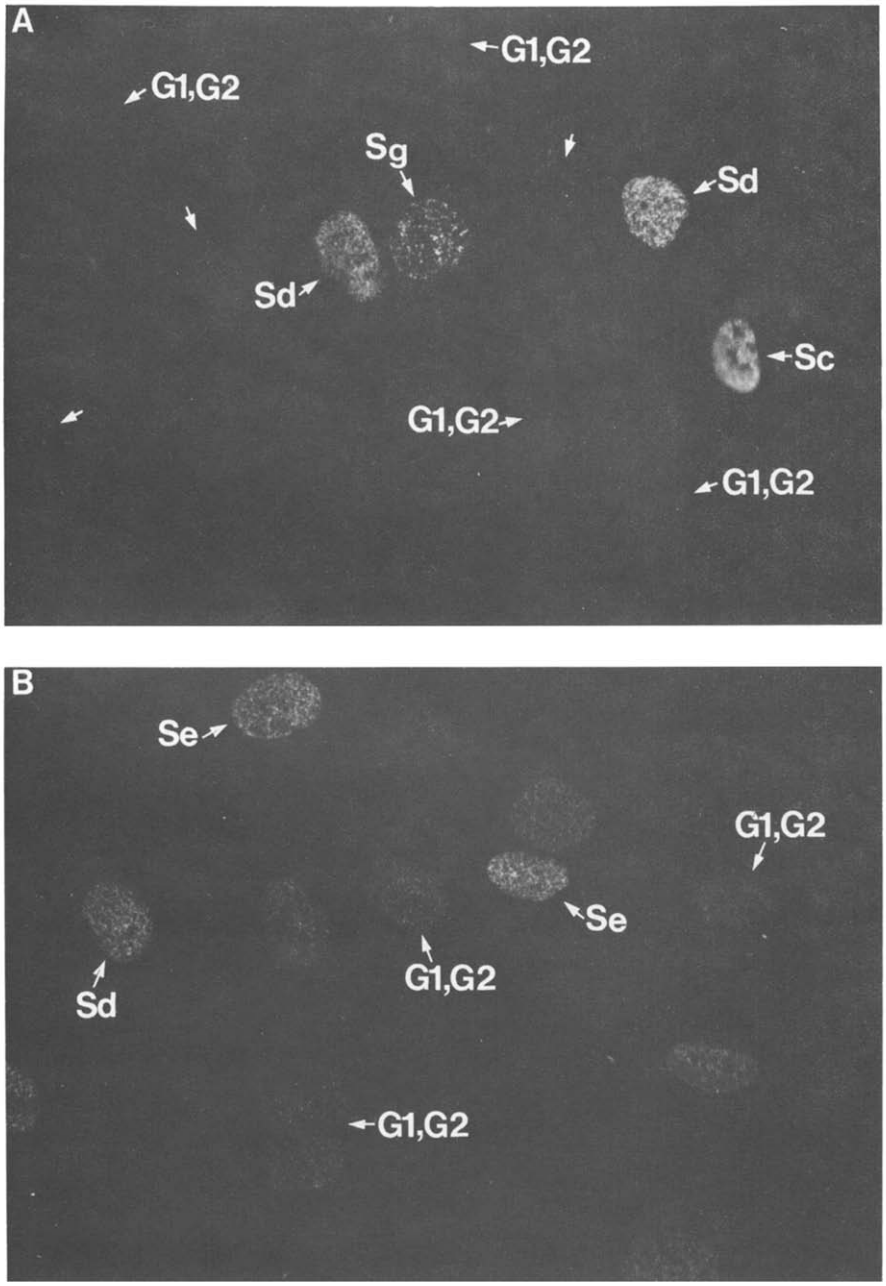

Fig.5. Cyclin/PCNA antigen staining (PCNA antibodies) in (A) control and (B) UV irradiated (30 J/m^2) normal human C5RO fibroblasts.

staining may be due to a redistribution of pre-existing cyclin/PCNA. Cycloheximide alone, on the other hand, had no effect on the patterns of cyclin/PCNA antigen distribution, although the staining intensity was weaker than that observed in untreated control cells (not shown).

4. DISCUSSION

The most striking effect of UV irradiation on cyclin/PCNA antigen staining was the substantial increase in nuclear fluorescence observed in non S-phase cells. These cells which are engaged in nucleotide excision DNA repair exhibited an almost continuous speckled staining throughout the nucleus. Similar results have been observed with other cultured cell types of human (C5RO fibroblasts, fig.5A and B; A431, SV40 transformed keratinocytes), mouse (3T3) and goat (goat synovial) origin.

The increase in cyclin/PCNA antigen staining observed in non S-phase cells was not abolished by doses of cycloheximide that inhibited protein synthesis, suggesting that this phenomenon may be due to a redistribution of pre-existing cyclin/PCNA. This is in line with the fact that G_1 AMA cells synthesize significant levels of this protein (fig.6A) as compared to S-phase cells (fig.6B, see also [14]). G_1 cyclin/PCNA however, is not recognized by polyclonal (autoantibodies) ([6,14] and references therein) or monoclonal antibodies [24,25]. The reason for the masking of the antigenic site(s) is at present unknown, although there is evidence suggesting that cyclin/PCNA antigenicity is linked somehow to the status of DNA replication [12,21]. From these observations it is therefore tempting to speculate that changes in cyclin/PCNA antigen staining observed in UV irradiated non S-phase cells may reflect changes in antigenicity brought about by the migration of this protein to the sites of DNA damage. Interestingly, UV irradiation (30 J/m^2) of *Xeroderma pigmentosum* XP25RO cells (complementation group A), which are almost completely deficient in DNA repair, resulted in an increased nuclear cyclin/PCNA antigen staining of non S-phase cells that was similar to that observed in normal human C5RO fibroblasts (not shown, but see fig.5B). These results are interpreted to suggest that not all steps involved in DNA repair are deficient in these cells. Cyclin/PCNA may still migrate to the site of damage (leading to changes in antigenicity) irrespective of whether DNA repair takes places or not. A comparable situation has been observed in synchronized G_1 cells treated with hydroxyurea [12,21]. These cells, which are blocked at the G_1/S transition border of the cell cycle, exhibit a strong nuclear granular staining with PCNA/cyclin antibodies in spite of the fact that they do not synthesize DNA. The possibility cannot be excluded however that the increase in nuclear fluorescence of non S-phase cells is unrelated to DNA repair synthesis. Functional studies are now underway as large amounts of this protein can be obtained by a purification protocol recently devised by Ogata et al. [17,26].

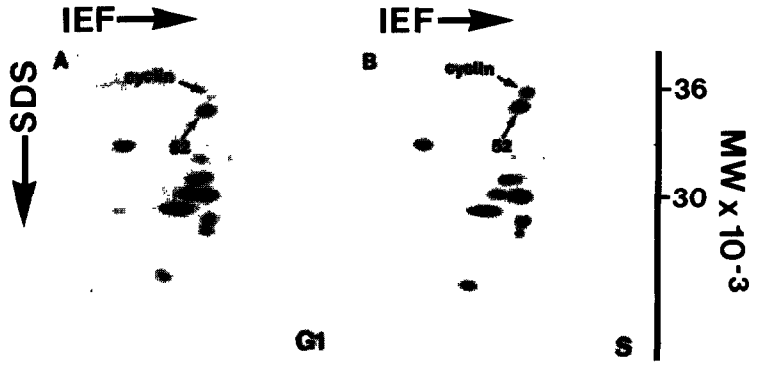

Fig.6. Cyclin synthesis in (A) G_1 and (B) late S-phase cells. Mitotic AMA cells obtained by mechanical detachment were labelled for (A) 2 h and (B) 13 h after plating mitotic cells.

ACKNOWLEDGEMENTS

We would like to thank S. Himmelstrup Jørgensen for typing the manuscript and O. Jensen and O. Sønderskov for photography. We would also like to thank Dr E. Tan for providing the PCNA antibodies specific for cyclin. The XP25RO cells were a kind gift from J. Hoeyandeers. This work was supported by grants from the Danish Cancer Foundation, the Danish Medical and Natural Science Research Councils, NOVO, Kong Christian den Tiendes Fond and Fonden af 17.12.1981.

REFERENCES

[1] Celis, J.E., Bravo, R., Larsen, P., Fey, S.J., Bellatin, J. and Celis, A. (1984) in: Two Dimensional Gel Electrophoresis of Proteins: Methods and Applications (Celis, J.E. and Bravo, R. eds) pp.307–362, Academic Press, New York.
[2] Bravo, R., Fey, S.J., Bellatin, J., Mose Larsen, P., Arrevalo, J. and Celis, J.E. (1981) Exp. Cell Res. 136, 311–319.
[3] Bravo, R. and Celis, J.E. (1982) Clin. Chem. 28, 766–781.
[4] Celis, J.E., Bravo, R., Mose Larsen, P. and Fey, S.J. (1984) Leukemia Res. 8, 143–157.
[5] Miyachi, K., Fritzler, M.J. and Tan, E.M. (1978) J. Immunol. 121, 2228–2234.
[6] Takasaki, Y., Deng, J.S. and Tan, E.M. (1981) J. Exp. Med. 159, 1899–1909.
[7] Mathews, M.B., Bernstein, R.M., Franza, B.R. and Garrels, J.I. (1984) Nature 309, 374–376.
[8] Celis, J.E. and Nielsen, S. (1986) Proc. Natl. Acad. Sci. USA, 83, 8187–8190.
[9] Nielsen, S., Celis, A., Petersen Ratz, G. and Celis, J.E. (1987) Leukemia, 1, in press.
[10] Bravo, R. and Celis, J.E. (1980) J. Cell Biol. 48, 795–802.
[11] Bravo, R. and Celis, J.E. (1985) FEBS Lett. 182, 435–440.
[12] Celis, J.E. and Celis, A. (1985) EMBO J. 4, 1187–1192.
[13] Madsen, P. and Celis, J.E. (1985) FEBS Lett. 193, 5–11.
[14] Celis, J.E., Madsen, P., Nielsen, S. and Celis, A. (1986) Leukemia Res. 10, 237–249.
[15] Nakamura, H., Morita, T. and Sato, C. (1986) Exp. Cell Res. 165, 291–297.
[16] Vishwanataha, J.K., Coughlin, S.A., Wesolowski-Owen, M. and Baril, E.F. (1986) J. Biol. Chem. 261, 6619–6628.
[17] Ogata, K., Celis, J.E. and Tan, E.M. (1987) Methods Enzymol., in press.
[18] Bravo, R., Fey, S.J., Small, J.V., Mose Larsen, P. and Celis, J.E. (1981) Cell 25, 195–202.
[19] Celis, J.E. and Bravo, R. (1981) Trends Biochem. Sci. 6, 197–201.
[20] Bravo, R., Small, J.V., Fey, S.J., Mose Larsen, P. and Celis, J.E. (1981) J. Mol. Biol. 152, 121–143.
[21] Bravo, R. and MacDonald-Bravo, H. (1985) EMBO J. 4, 655–661.
[22] Celis, J.E. and Celis, A. (1985) Proc. Natl. Acad. Sci. USA 82, 3262–3266.
[23] Bravo, R. (1986) Exp. Cell Res. 163, 287–293.
[24] Ogata, K., Kurki, P., Celis, J.E., Nakamura, R.M. and Tan, E. (1987) Exp. Cell Res., in press.
[25] Madsen, P., Ogata, K. and Celis, J.E., (1987) Leukemia 3, in press.
[26] Ogata, K., Ogata, Y., Nakamura, R. and Tan, E.M. (1985) J. Immunol. 135, 2623–2630.