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Progressin: a novel proliferation-sensitive and cell cycle-regulated human protein whose rate of synthesis increases at or near the G_1/S transition border of the cell cycle

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A novel proliferation-sensitive and cell cycle-specific basic protein, termed progressin ($M_r = 33\,000$), has been identified in proliferating human cells of epithelial, fibroblast and lymphoid origin. Progressin is synthesized almost exclusively during the S-phase of transformed human amnion cells (AMA). Increased synthesis of this protein is first detected late in G_1, at or near the G_1/S transition border, reaches a maximum in mid to late S-phase, and declines thereafter. Contrary to histones, progressin synthesis is not coupled to DNA replication. As expected for an S-phase-specific protein, no detectable synthesis of progressin was observed in non-proliferating human MRC-5 fibroblasts and epidermal basal keratinocytes. Elevated, but variable levels of this protein were observed in proliferating normal fibroblasts and transformed cells of fibroblast, epithelial and lymphoid origin. Taken together the above observations suggest that progressin may be a component of the common pathway leading to DNA replication and cell division.

Cell proliferation; Cell cycle; Initiation of DNA replication; Transformation; Cancer

1. INTRODUCTION

Understanding of the molecular mechanisms underlying malignant transformation and cancer will ultimately require unraveling of the pathways that control cell proliferation in normal cells. In this laboratory we have focused our research on the identification of proteins that are putative components of the common pathway leading to DNA replication and cell division in human cells ([1,2] and references therein). These proteins are expected (i) to be common to all cell types and (ii) to be synthesized in a cell cycle specific fashion: increased synthesis starting late in G_1, near the G_1/S transition border. So far, two proteins have been identified that exhibit these characteristics. These correspond to the DNA replication protein cyclin [1–5], also termed PCNA [6–8] or auxiliary protein of DNA polymerase δ [9–11], and the nuclear phosphoprotein dividin [12,13]. Here we describe a novel proliferation-sensitive and cell cycle-specific human basic protein that we have termed progressin (M_r = 33 000, fig.1). The role of progressin, cyclin (PCNA) and dividin in cell cycle progression is discussed.

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Abbreviations: NEPHGE, nonequilibrium pH gradient electrophoresis; PCNA, proliferating cell nuclear antigen; 2D, two dimensional

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2. MATERIALS AND METHODS

2.1. *Cells*

All the cells used in this study were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal calf serum and antibiotics (penicillin at 100 U/ml, steptomycin at 50 μg/ml). Human epidermal basal keratinocytes were prepared as described [14]. The SV40-transformed human keratinocytes (SVK14) [15] were kindly provided by E.B. Lane (Imperial Cancer Research Laboratories, London).

2.2. *Cell cycle studies*

Mitotic AMA cells were obtained by mechanical detachment essentially as described by Terasima and Tolmach [16]. Two 250-ml flasks containing 1–2 × 10^6 cells per flask were used. The homogeneity of the collected cells was assessed by phase-contrast microscopy. Cells for [^{35}S]methionine labeling were plated in microtiter wells (96-well plates, Nunc), while those for immunofluorescence were plated in round glass coverslips. Entrance of the cells into S-phase was determined by indirect immunofluorescence using PCNA antibodies specific for cyclin [6,17] (a kind gift of E. Tan).

2.3. *Quiescent cultures*

MRC-5 human fibroblasts were trypsinized and plated (microtiter wells or glass coverslips) in DMEM containing 10% (v/v) fetal bovine serum. One day later, the medium was changed to DMEM plus 0.5% serum. The cultures were then left for 4 days in the same medium. Cells were released by adding DMEM containing 10% (v/v) serum.

2.4. *[^{35}S]Methionine labeling*

Synchronous interphase cells grown in microtiter wells (96-well plates, Nunc) were labeled for 30 min with 1 mCi [^{35}S]methionine per ml as described [18,19]. At the end of the labeling period the medium was aspirated, and the cells were resuspended in 20 μl lysis buffer [20]. Mitotic cells (in suspension) were labeled for 30 min under similar conditions.

2.5. *Two-dimensional gel electrophoresis*

2D gel electrophoresis (NEPGHE) was carried out as described [21,22]. In short, the first dimension was performed in 130 mm × 1.2 mm, 4% (w/v) polyacrylamide gels containing 5% amholines (3% pH 7–9 (LKB); 2% pH 8–9.5 (LKB); 4.5 h at 400 V). Gels were processed for fluorography as described by Laskey and Mills [23].

2.6. *Indirect immunofluorescence*

Cells grown in round glass coverslips (12 mm in diameter) were washed three times with Hanks' saline solution and treated for 5 min at −20°C with methanol. After washing extensively with Hanks' saline solution, the coverslips were covered with 20 μl of anticyclin (PCNA) antibodies (1:200 dilution) and incubated for 60 min at 37°C in a humid environment. The coverslips were then washed several times with Hanks' saline solution and covered with 20 μl of rhodamine-conjugated goat antihuman IgG (diluted 1:150 in Hanks' saline dilution). After 60 min of incubation at 37°C in a humid environment, the coverslips were washed thoroughly with Hanks' saline solution and were mounted in Gelvatol. Observations were made on a Zeiss photomicroscope equipped with epifluorescence and phase contrast optics.

3. RESULTS

3.1. *Increased synthesis of progressin in proliferating human cells of fibroblast, epithelial and lymphoid origin*

Fig.2A and B shows the appropriate areas of NEPHGE 2D gels of [^{35}S]methionine-labeled proteins from slowly proliferating MRC-5 fibroblasts (p38, fig.2A) and their SV40 transformed counterparts (MRC-5 V2, fig.2B). Similar gels of non-proliferating human epidermal basal keratinocytes and SV40 transformed keratinocytes (K14) are shown in fig.2C and D, respectively. As seen in Fig.2A and C, low levels of synthesis of progressin were observed in the slowly proliferating fibroblasts (fig.2A), while little of this protein was detected in the non-proliferating basal cells (fig.2C). Low levels of synthesis of progressin have also been observed in normal human lymphocytes (not shown). Of the so far studied transformed human cell types, those that exhibit high levels of progressin synthesis include: W138 SV40, Hela (fig.2E), Detroit-98, Chang Liver, Fl-amnion,

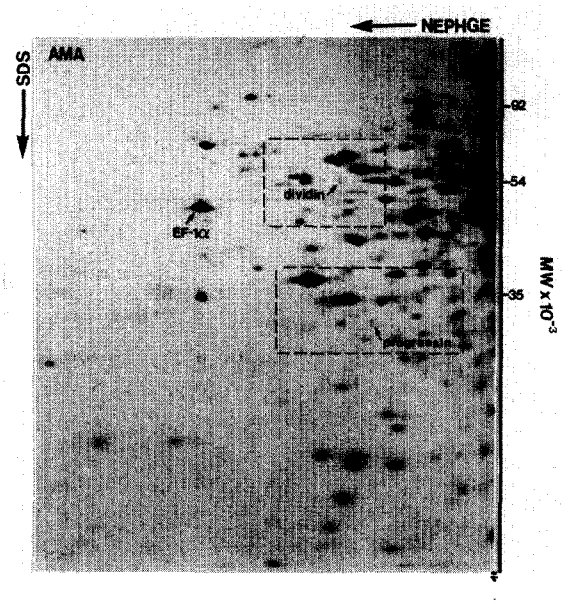

Fig.1. Position of progressin in 2D gels (NEPHGE) of [^{35}S]methionine-labeled proteins from AMA cells. The position of elongation factor EF-1α and dividin are indicated for reference.

A431 and Molt-4 (fig.2F). Polypeptides having the same coordinates (M_r and p*I*) as progressin have not been observed in proliferating mouse cells.

3.2. *Progressin in synthesized preferentially during the S-phase of the cell cycle of transformed human amnion cells (AMA)*

Mitotic AMA cells obtained by gently mechanical shake off as well as synchronized interphase cells were labeled for 30 min at 37°C with [^{35}S]methionine and the radioactive proteins were analyzed by NEPHGE 2D-gel electrophoresis [21,22]. Representative gels of labeled proteins from mitotic, G_1 (labeled 2, 5 and 9 h after plating mitotic cells), S (labeled 12 and 15 h after plating mitotic cells) and G_2 cells (labeled 20.5 h after plating mitotic cells) are shown in fig.3B. The duration of the cell cycle phases (sub-S-phases included, fig.3A; see [25]), patterns of cyclin (PCNA) antigen distribution (fig.3D) and levels of dividin (fig.3C) are also shown in fig.3. Increased synthesis of progressin is first detected late in G_1 near the G_1/S transition border, reaches a maximum in mid to late S-phase, and declines thereafter (fig.3B). Low levels of synthesis of progressin were detected in mitotic, G_1 and G_2 (fig.3B). Increased rate of synthesis of progressin has also been observed in growth-arrested MRC-5 fibroblasts (kept for 96 h in DMEM containing 0.5% serum) stimulated to enter S-phase by addi-

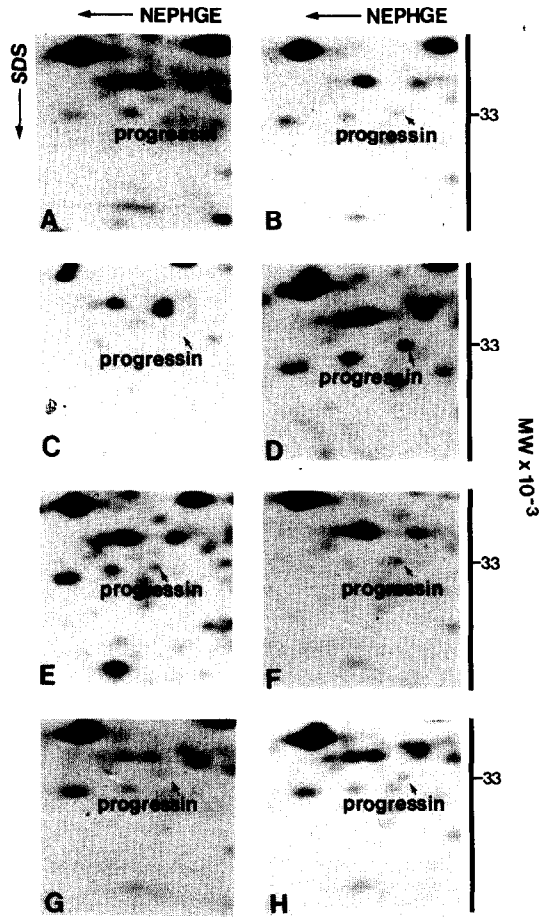

Fig.2. Synthesis of progressin in normal and transformed human cells. Cells were labeled for 2 h with [^{35}S]methionine as described in section 2. (A) Human MRC-5 fibroblasts (p38); (B) SV40 transformed MRC-5 (MRC-5 V2); (C) non-proliferating human epidermal basal keratinocytes (kept in DMEM with high Ca^{2+} concentration); (D) SV40 transformed keratinocytes (SV14); (E) HeLa; (F) Molt-4; (G) quiescent human MRC-5 fibroblasts; and (H) MRC-5 fibroblasts labeled 19 h after release. Only the appropriate region of the gels are shown.

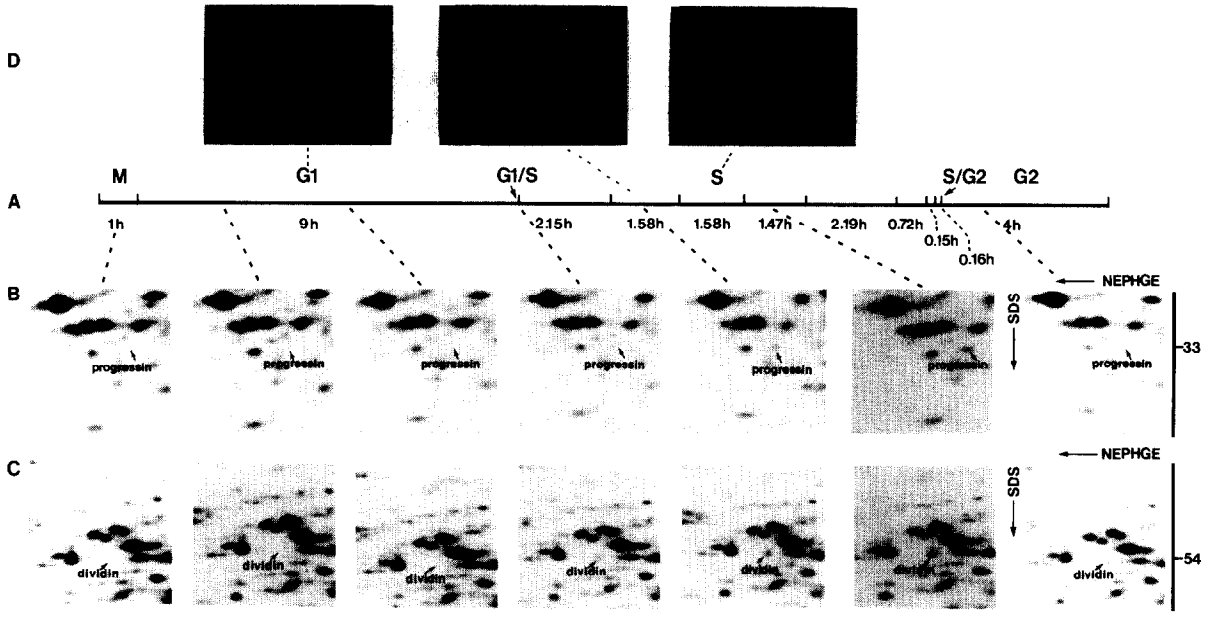

Fig.3. Synthesis of [^{35}S]methionine-labeled progressin throughout the cell cycle of AMA cells. (A) Duration of the cell cycle phases (duration of sub-S-phases is also included; see [25]). (B,C) synthesis of progressin (B) and dividin (C) at various stages of the cell cycle. Cells were labeled for 30 min as described in section 2. G_1 cells were labeled 2.5, and 9 h after plating mitotic cells. S-phase cells were labeled 12 and 15 h after plating, while G_2 cells were labeled 20.5 h later. (D) Patterns of cyclin (PCNA) antigen distribution as determined by indirect immunofluorescence of methanol-fixed cells. Areas of the gels shown in this figure are enclosed in fig.1.

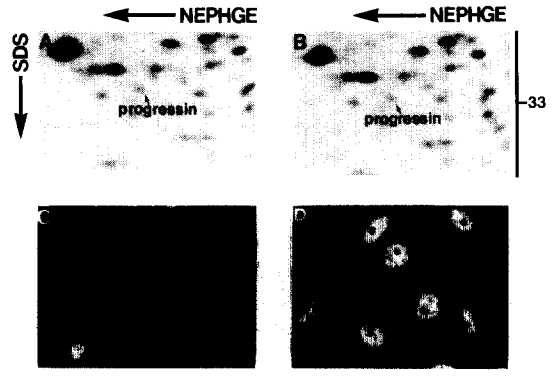

Fig.4. Progressin synthesis and cyclin (PCNA) antigen distribution in synchronized AMA cells treated with thymidine. G_1 AMA cells were treated with thymidine (2 mM final concentratgion; added 1 h after plating mitotic cells) and labeled with [^{35}S]methionine for 30 min, 14 h after its addition. (A) Control untreated cells; (B) thymidine-treated cells; (C) patterns of cyclin (PCNA) antigen distribution in control; and (D) patterns of cyclin (PCNA) antigen distribution in thymidine-treated cells.

tion of DMEM containing 10% serum (fig.2H). No detectable levels of progressin were observed in the growth-arrested cells (fig.2G).

3.3. *Progressin synthesis is not coupled to DNA replication*

To determine if progressin synthesis was dependent on DNA replication we added thymidine (2 mM final concentration) to synchronized G_1 cells (1 h after plating mitotic cells) and analyzed the [^{35}S]methionine-labeled proteins synthesized during a 30 min period, 14 h after its addition (S-phase, see also fig.3A). As shown in fig.4, similar levels of progressin were observed in the thymidine-treated cells (fig.4B) as compared to the untreated control cells (fig.4A). Indirect immunofluorescence analysis of methanol-fixed cells confirmed that the thymidine-treated cells were blocked at the G_1/S transition border of the cell cycle (fig.3D). Untreated cells on the other hand progressed into S-phase as judged by the late patterns of cyclin (PCNA) antigen distribution (fig.4C) [17,24,26].

4. DISCUSSION

Identification of proteins that may be components of the common pathway leading to DNA replication and cell division may be instrumental in understanding the mechanisms that control cell cycle progression and proliferation. So far, we have identified three proliferation-sensitive and cell cycle-specific proteins that are likely components of this pathway. In addition to progressin, these include the DNA replication protein cyclin (PCNA) [4-9,25,27-29] and the nuclear phosphoprotein dividin [12,13]. In contrast to histones, the synthesis of progressin, cyclin (PCNA) and dividin is independent of DNA replication ([26]; this article). In vivo replicative DNA synthesis on the other hand has not been observed in the absence of synthesis of these proteins.

What is the role of progressin, cyclin (PCNA) and dividin in the initiation of DNA replication and cell cycle progression in human cells? Our studies concerning distribution of cyclin (PCNA) and DNA replication in binucleated homokaryons produced by polyethylene glycol (PEG)-induced fusion of mitotic AMA cells have shed some light onto this question [25]. A small percentage of asynchronous S-phase homokaryons were observed in these fusions suggesting that (an) intranuclear event(s) was (were) required for cyclin (PCNA) distribution and DNA replication irrespective of the fact that these nuclei shared common cytoplasmic factors [25,30] (fig.5). The nature of this (these) intranuclear event(s) is at present unknown, although it may correspond to the formation of pre-replicative complexes. Here, we would like to propose that newly synthesized S-phase cyclin (PCNA) (and perhaps progressin and dividin) may be one of the factors that trigger initiation of DNA replication once pre-replicative complexes are formed.

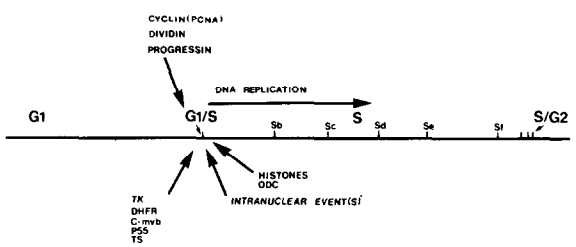

Fig.5. Some events at the G_1/S transition border of the cell cycle of human AMA cells. Increased synthesis of histones, ODC (ornithine decarboxylase), TK (thymidine kinase), DHFR (dihydrofolate reductase), c-myb, p55 and TS (thymidine synthetase), during S-phase has been observed in various cell types ([32] and references therein), but has not been investigated in AMA cells. The synthesis of histones and ODC is dependent on DNA replication while synthesis of cyclin (PCNA), dividin, progressin, TK and DHFR is unaffected by inhibitors of DNA replication ([26,32] and references therein; this article).

Taken together, our studies also imply that, most likely, cell cycle progression is not controlled by a single labile protein as suggested by Rossov et al. [31], but rather by a set of events (a few are indicated in fig.5; see [32]), some of which seem to be independent of each other [33].

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REFERENCES

[1] Celis, J.E., Bravo, R., Mose 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.) pp. 308-362, Academic Press, New York.
[2] Celis, J.E., Madsen, P., Celis, A., Nielsen, H.V. and Gesser, B. (1987) FEBS Lett. 220, 1-7.
[3] Bravo, R. and Celis, J.E. (1980) J. Cell Biol. 48, 795-802.
[4] Bravo, R., Fey, S.J., Bellatin, J., Mose Larsen, P., Arevalo, J. and Celis, J.E. (1981) Exp. Cell Res. 136, 311-319.
[5] Celis, J.E., Bravo, R., Mose Larsen, P. and Fey, S.J. (1984) Leuk. Res. 8, 143-157.
[6] Miyachi, K., Fritzler, M.J. and Tan, E.M. (1978) J. Immunol. 121, 2228-2234.

[7] Takasaki, Y., Deng, J.S. and Tan, E.M. (1981) J. Exp. Med. 154, 1899–1909.
[8] Mathews, M.B., Bernstein, R.M., Franza, R. and Garrels, J.I. (1984) Nature 309, 274–276.
[9] Tan, C.K., Castillo, C., So, A.G. and Downey, K.M. (1986) J. Biol. Chem. 261, 12310–12316.
[10] Bravo, R., Frank, R., Blundell, P.A. and Macdonald-Bravo, H. (1987) Nature 326, 515–517.
[11] Prelich, G., Tan, C.-K., Kostura, M., Mathews, M.B., So, A.G., Downey, K.M. and Stillman, B. (1987) Nature 326, 517–520.
[12] Celis, J.E. and Nielsen, S. (1986) Proc. Natl. Acad. Sci. USA 83, 8187–8190.
[13] Nielsen, S., Celis, A., Petersen Ratz, G. and Celis, J.E. (1987) Leukemia 1, 69–77.
[14] Celis, J.E., Fey, S.J., Mose Larsen, P. and Celis, A. (1984) Proc. Natl. Acad. Sci. USA 81, 3128–3132.
[15] Taylor-Papadimitriou, J., Purkis, P., Lane, E.B. and Chang, S.E. (1982) Cell Differ. 11, 169–180.
[16] Terasima, T. and Tolmach, L.J. (1963) Exp. Cell Res. 30, 344–362.
[17] Celis, J.E., Madsen, P., Nielsen, S. and Celis, A. (1986) Leuk. Res. 10, 237–249.
[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] O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021.
[21] O'Farrell, P.H., Goodmann, H.M. and O'Farrell, P. (1977) Cell 12, 1133–1142.
[22] Bravo, R., Small, J.V., Fey, S.J., Mose Larsen, P. and Celis, J.E. (1982) J. Mol. Biol. 152, 121–143.
[23] Laskey, R. and Mills, A.D. (1975) Eur. J. Biochem. 56, 335–341.
[24] Celis, J.E. and Celis, A. (1985) Proc. Natl. Acad. Sci. USA 82, 3262–3266.
[25] Celis, J.E. and Celis, A. (1985) EMBO J. 4, 1187–1192.
[26] Bravo, R. and Macdonald-Bravo, H. (1985) EMBO J. 4, 655–661.
[27] Madsen, P. and Celis, J.E. (1985) FEBS Lett. 193, 5–11.
[28] Nakamura, H., Takasaki, Y., Hirose, S.-I., Masaku, S. and Sajto, C. EMBO J., submitted.
[29] Prelich, G., Kostura, M., Marshak, D.R., Mathews, M.B. and Stillman, B. (1987) Nature 326, 471–475.
[30] Blow, J.J. and Watson, J.V. (1987) EMBO J. 6, 1997–2002.
[31] Rossov, P.W., Riddle, V.G.H. and Pardee, A.B. (1979) Proc. Natl. Acad. Sci. USA 76, 4446–4450.
[32] Denhardt, D.T., Edwards, D.R. and Parfett, C.L.J. (1986) Biochim. Biophys. Acta 865, 83–125.
[33] Celis, J.E., Madsen, P., Nielsen, S.O., Gesses, B., Nielsen, H.V., Petersen Ratz, G., Lauridsen, J. and Celis, A. Cancer Cells, in press.