

# Synthesis of the nuclear protein cyclin in growing, senescent and morphologically transformed human skin fibroblasts

Julio E. Celis and Rodrigo Bravo\*

*Division of Biostructural Chemistry, Department of Chemistry, Aarhus University, DK-8000, Denmark*

Received 4 November 1983

Quantitative two-dimensional gel electrophoretic analysis (IEF) of the nuclear polypeptide cyclin in normal human skin biopsies, growing and senescent fibroblasts and morphologically transformed skin fibroblasts (limited life span) has revealed a direct correlation between the levels of this protein and the proliferative state of the cells. These results strengthen the notion that cyclin may be a key component of the pathway(s) that control cell proliferation.

<i>Transformation sensitive protein</i>	<i>Cell proliferation</i>	<i>Limited life span</i>
<i>Two-dimensional gel electrophoresis</i>		

## 1. INTRODUCTION

We have described a human nuclear protein of  $M_r$  36000 (cyclin; IEF 49 in HeLa protein catalogue [1–3]) whose relative proportion is sensitive to changes in the rate of cell proliferation and to transformation [4–16]. The rate of synthesis of cyclin increases in S phase of HeLa cells [5], and its level may be linked to events related to DNA replication. To date, most of what is known about cyclin comes from studies of immortal and immortal and tumorigenic cells [4–16]. It is important, therefore, to determine the level of this protein in normal tissues as well as cultured cells (limited life span) derived from them. Here we present a comparative study of the levels of cyclin in normal human skin biopsies, growing and senescent skin fibroblasts and morphologically transformed skin fibroblasts (limited life span) that arose spontaneously amongst a population of senescent cells. The results show that there is very

little synthesis of cyclin in the skin tissue as compared to early passaged skin fibroblasts (passage 1 (p1)–p9; split ratio 1:3). From p9 to 22 (the cells died at p22) the cultured fibroblasts showed a decrease in the synthesis of cyclin that was accompanied by a concomitant decrease in the percentage of cells labelled with [ $^3\text{H}$ ]thymidine (thymidine labelling index). Synthesis of cyclin as well as an increase in the thymidine labelling index was observed in morphologically transformed fibroblasts. The synthesis of cyclin declined again as these cells ceased to proliferate and died. Taken together these results show a direct correlation between the levels of cyclin and the proliferative state of the cells and support the notion that this protein may be a key component of the pathway(s) that control cell proliferation [13].

## 2. MATERIALS AND METHODS

### 2.1. Cells

Primary fibroblasts were prepared from human skin biopsies as in [17] and were a gift from the Department of Human Genetics of this University. The fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and antibiotics

\* Present address: European Molecular Biology Laboratory, Postfach 10.2209, Meyerhofstrasse 1, 6900 Heidelberg 1, FRG

*Abbreviation:* IEF, isoelectric focussing

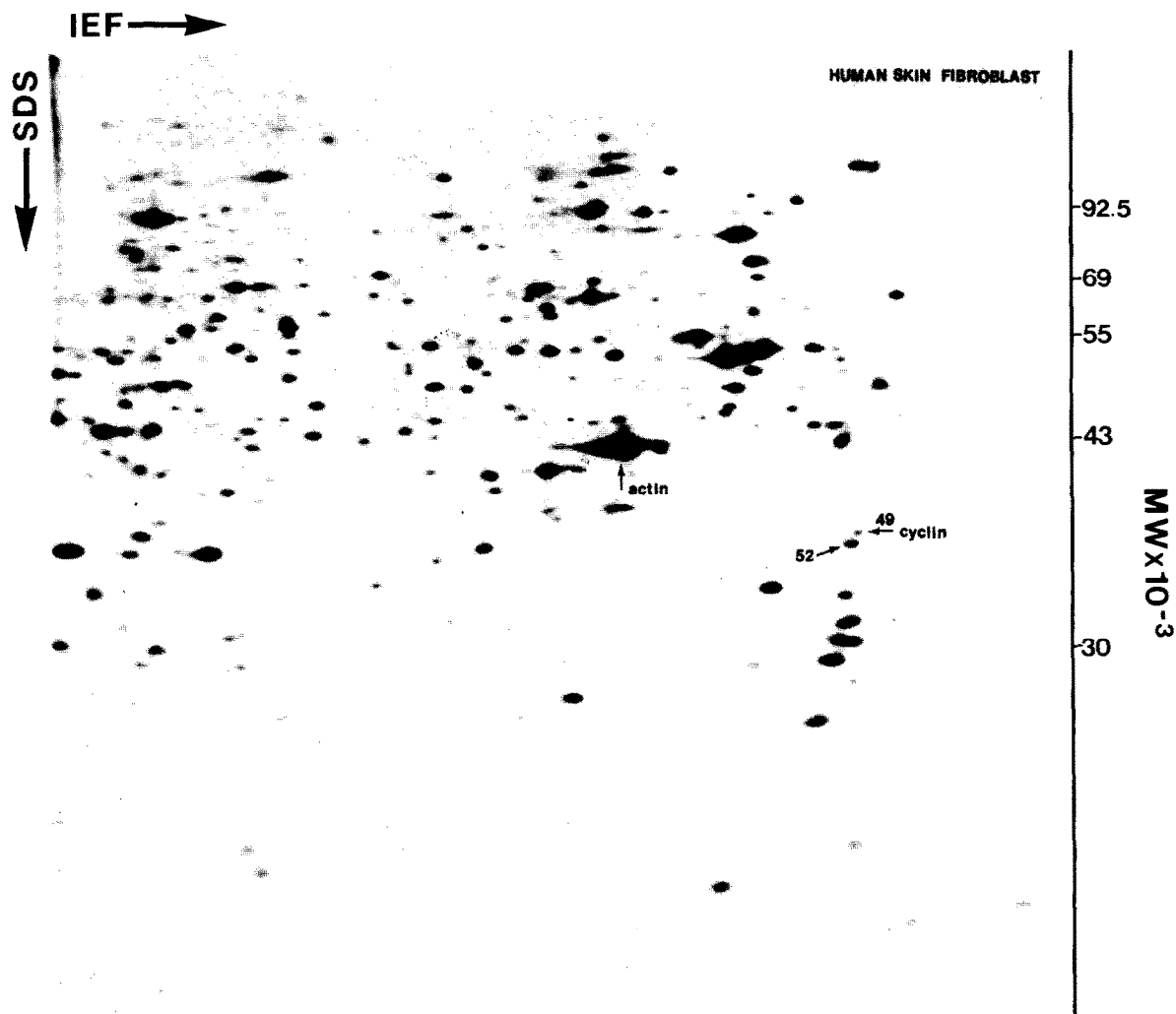


Fig.1. Two-dimensional gel electrophoresis (IEF) of [ $^{35}\text{S}$ ]methionine labelled proteins from cultured human skin fibroblasts labelled at p3. Cells were labelled for 16 h with [ $^{35}\text{S}$ ]methionine (1 mCi/ml) as in [18,19].

(penicillin, 100 units/ml; streptomycin, 50  $\mu\text{g}/\text{ml}$ ) and were passed at a split ratio of 1:3.

## 2.2. Labelling of cells with [ $^{35}\text{S}$ ]methionine

Cells were labelled for 16 h with [ $^{35}\text{S}$ ]methionine (1 mCi/ml) as in [18,19].

The procedures for two-dimensional gel electrophoresis (IEF) [20] have previously been described.

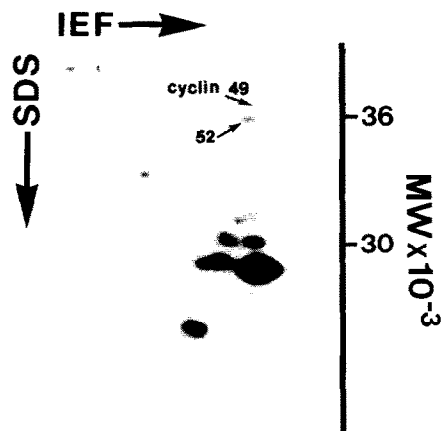


Fig.2. Cyclin synthesis in a human skin biopsy. A skin biopsy (punch biopsy) was labelled for 16 h with [ $^{35}\text{S}$ ]methionine (1 mCi/ml) as in [18,19].

## 3. RESULTS AND DISCUSSION

Fig.1 shows a two-dimensional gel electrophoretic pattern (IEF) [20] of [ $^{35}$ S]methionine-labelled proteins (16 h labelling) from human skin fibroblasts (p3) prepared from a skin biopsy. The position of the nuclear polypeptide cyclin (IEF 49) [6,21], the tropomyosin polypeptide IEF 52 [6] and total actin are indicated as reference. Fig.2 shows the appropriate region of an IEF gel of [ $^{35}$ S]methionine-labelled proteins from a human skin biopsy labelled for 16 h. Only very small amounts of cyclin could be observed in line with the fact that few cells are dividing in the tissue [22]. Similar levels of cyclin have been reported in human dermis and epidermis [14].

The levels of cyclin throughout the life span of the cultured skin fibroblasts were determined by quantitative two-dimensional gel electrophoretic analysis of [ $^{35}$ S]methionine-labelled proteins from cells labelled at each passage during their life span (p3–p22). The thymidine labelling index (percentage of cells labelled with [ $^3$ H]thymidine as determined by autoradiography) was also determined at each passage and some of the data are given in table 1. A drastic decrease in the labelling index was observed after passage 10 and this remained low until the cells died at p22. Fig.3 shows two examples of IEF gels (only the pertinent region is shown) of [ $^{35}$ S]methionine-labelled proteins from fibroblasts labelled at p8 (fig.3a) and 11 (fig.3b), respectively. The gel patterns of cultures labelled

Table 1

Relative proportion of cyclin (IEF 49) in growing, senescent and morphologically transformed human skin fibroblasts

Human skin fibroblast passage number	Thymidine labelling (%)	Cyclin relative proportion <sup>c</sup>	Tropomyosin IEF 52 relative proportion <sup>c</sup>
p3	30	0.120	0.130
p8	20	0.084	0.130
p11	4	0.048	0.276
p19	<0.1	<0.030	0.285
p28 <sup>a</sup>	6	0.086	0.156
p31 <sup>a</sup>	<0.5	<0.030	0.224

<sup>a</sup> The morphologically transformed fibroblasts appeared spontaneously in a culture of senescent fibroblasts that had reached p23. The levels of cyclin and the thymidine labelling index at p23 were the same as those given for p19

<sup>b</sup> Cells grown in coverslips were labelled for 30 min with 2  $\mu$ Ci/ml of [ $^3$ H]thymidine and processed for autoradiography according to standard procedures (1 week exposure). About 300 cells were counted in each case. Cells containing 10 or more grains per nucleus were considered as positive. The thymidine labelling index corresponds to the % of cells labelled with [ $^3$ H]thymidine

<sup>c</sup> Radioactive spots were cut out from the gels and the radioactivity determined in a scintillation counter. The radioactivity is expressed as % of total protein. The relative proportion of total actin remained constant throughout the life span of the fibroblasts

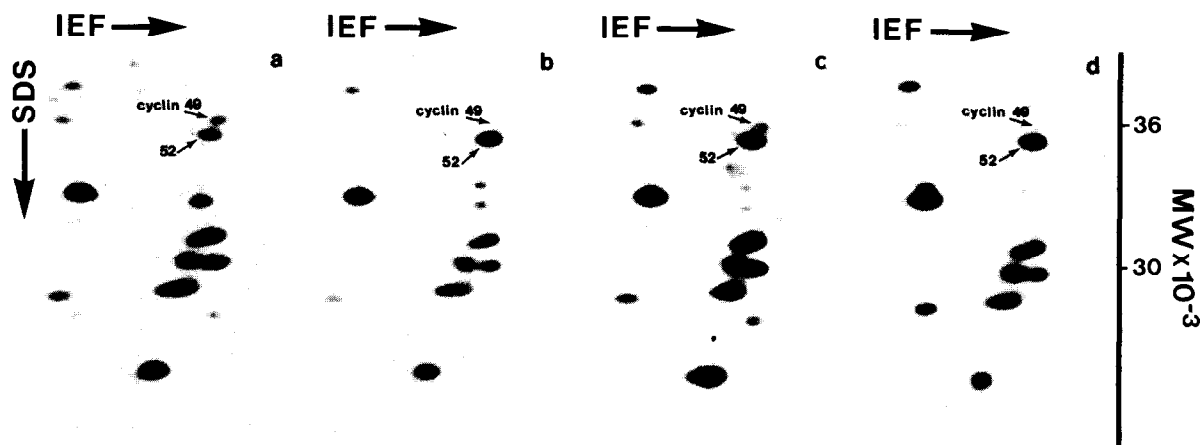


Fig.3. Synthesis of cyclin in growing, senescent and morphologically transformed fibroblasts. Cells at different passages were labelled with [ $^{35}$ S]methionine as in [18,19]. (a) p8, (b) p11, (c) p28 and (d) p31.

between p12 and 22 were similar to that of p11 except for a slight increase in the relative proportion of the tropomyosin polypeptide IEF 52 [6]. Quantitations of the levels of cyclin and tropomyosin IEF 52 at various passages are given in table 1. Clearly, a decrease in the synthesis of cyclin parallels a decrease in the number of cells that label with [ $^3\text{H}$ ]thymidine. Occasionally, cultures of low passaged fibroblasts synthesized very little cyclin (not shown), and this is most likely due to the fact that these cultures have reached confluency.

Further evidence suggesting that the synthesis of cyclin is directly linked to cell proliferation was obtained from studies of morphologically transformed skin fibroblasts (fig.4b) that arose spontaneously in one of the senescent cultures that reached p23 (fig.4a). The morphologically transformed cells were passed 8 times (split ratio 1:2) before they ceased to proliferate and died. Fig.3c,d shows IEF gels of [ $^{35}\text{S}$ ]methionine proteins from the morphologically transformed cells labelled at p28 and 31, respectively. The relative proportion of cyclin at both passages is given in table 1. The increase in cyclin was observed at p24 (not shown) and its level remained more or less constant until p29. The in-

crease in cyclin was accompanied by an increase in the thymidine labelling index (table 1).

Experiments are now underway to prepare antibodies against cyclin. These will be used for purifying the protein for functional studies and should be of value in assessing premalignant and neoplastic growth.

## ACKNOWLEDGEMENTS

We would like to thank our colleagues A. Celis, S.J. Fey and P.M. Larsen for discussions. R.B. was recipient of a fellowship from the Danish Medical Research Council. This work was supported by grants from Euratom, The Danish Cancer Foundation, the Danish Medical and Natural Science Research Councils, Carlsberg and NOVO.

## REFERENCES

- [1] Bravo, R., Bellatin, J. and Celis, J.E. (1981) *Cell Biol. Int. Rep.* 5, 93-96.
- [2] Bravo, R. and Celis, J.E. (1982) *Clin. Chem.* 28, 766-781.
- [3] Bravo, R. and Celis, J.E. (1983) in: *Two-dimensional Gel Electrophoresis of Proteins: Methods and Applications* (Celis, J.E. and Bravo, R. eds) Academic Press, New York, in press.
- [4] Bravo, R. and Celis, J.E. (1980) *Exp. Cell Res.* 127, 249-260.
- [5] Bravo, R. and Celis, J.E. (1980) *J. Cell Biol.* 48, 795-802.
- [6] Bravo, R., Fey, S.J., Bellatin, J., Mose Larsen, P., Arevalo, J. and Celis, J.E. (1981) *Exp. Cell Res.* 136, 311-319.
- [7] Bravo, R., Fey, S.J. and Celis, J.E. (1981) *Carcinogenesis* 2, 769-782.
- [8] Bravo, R. and Celis, J.E. (1982) *Clin. Chem.* 28, 949-954.
- [9] Bellatin, J., Bravo, R. and Celis, J.E. (1982) *Proc. Natl. Acad. Sci. USA* 79, 4367-4370.
- [10] Bravo, R., Fey, S.J., Bellatin, J., Mose Larsen, P. and Celis, J.E. (1982) in: *Embryonic Development, Part A* (Burger, M. ed) pp.235-248, Alan Liss, New York.
- [11] Bravo, R., Bellatin, J., Fey, S.J., Mose Larsen, P. and Celis, J.E. (1983) in: *Gene Expression in Normal and Transformed Cells* (Celis, J.E. and Bravo, R. eds) pp.263-290, Plenum, New York.

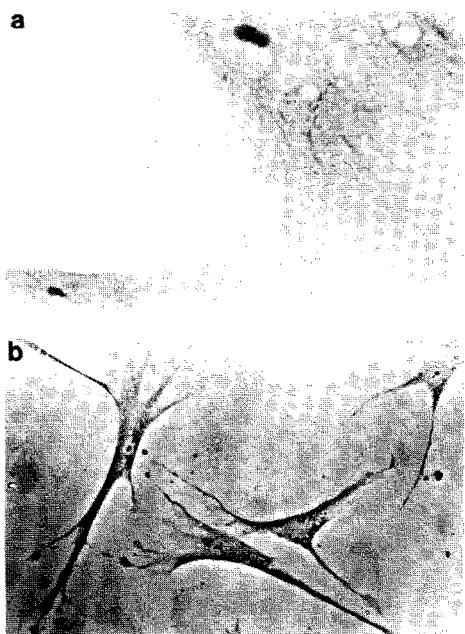


Fig.4. Morphology of (a) senescent and (b) morphologically transformed human skin fibroblasts. (a) Giemsa staining. (b) Phase contrast. (a,b)  $\times 320$ .

- [12] Forchhammer, J. and McDonald-Bravo, H. (1983) in: *Gene Expression in Normal and Transformed Cells* (Celis, J.E. and Bravo, R. eds) pp.291–314, Plenum, New York.
- [13] Celis, J.E., Bravo, R., Mose Larsen, P., Fey, S.J., Bellatin, J. and Celis, A. (1983) in: *Two-dimensional Gel Electrophoresis of Proteins: Methods and Applications* (Celis, J.E. and Bravo, R. eds) Academic Press, New York, in press.
- [14] Celis, J.E., Fey, S.J., Mose Larsen, P. and Celis, A. (1983) in: *The Cancer Cell* (Levine, A. et al. eds) Cold Spring Harbor Laboratory, in press.
- [15] Garrels, J. and Franza, R. (1983) in: *The Cancer Cell* (Levine, A. et al. eds) Cold Spring Harbor Laboratory, in press.
- [16] Bravo, R. (1983) in: *The Cancer Cell* (Levine, A. et al. eds) Cold Spring Harbor Laboratory, in press.
- [17] Martin, G.M. (1973) in: *Tissue Culture* (Kruse, P.F. and Patterson, M.K. eds) p.39, Academic Press, New York.
- [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] Bravo, R., Celis, A., Mosses, D. and Celis, J.E. (1981) *Cell Biol. Int. Rep.* 5, 479–489.
- [22] Montagna, W. and Billingham, R.E. (1964) *Advances in Biology of Skin*, vol.v, Wound Healing, Pergamon, Oxford.