FEBS Letters 358 (1995) 126–128 FEBS 15020

Chaos in DNA partition during the last mitoses of the proliferative life-span of human fibroblasts

A. Macieira-Coelho*

INSERM and University of Paris VI, 73 bis rue Maréchal Foch, 78000 Versailles, France

Received 23 November 1994

Abstract We have followed with autoradiography the course of DNA synthesis and with cytofluorometry the partition of DNA during mitosis, through the different phases of the life-span of human fibroblasts. Towards the end of the proliferative potential of the cell population, DNA synthesis was disturbed during the late part of the S period. The DNA content of metaphases and of anaphases had a normal Gaussian distribution during the whole proliferative life-span of the cell population. At the very end, however, when cells entered the post-mitotic phase, the distribution of DNA content deviated from the normal Gaussian probability, becoming significantly skewed.

Key words: Human fibroblast; Aging; Senescence; Terminal differentiation; Quantal mitosis

1. Introduction

Human fibroblasts have a finite division potential, a feature with implications inter alia for development and aging [1]. The mechanisms, though, determining this property of several mammalian somatic proliferative cells have not been elucidated. During serial replication the cell population evolves through different phases towards a final post-mitotic state [1]. It has been proposed that this is a process of terminal differentiation [2] and a consensus is building up favouring this view [3].

We have analyzed DNA synthesis and the partition of DNA between sister cells throughout the fibroblasts' life-span and found that the last mitoses are characterized by a chaotic behaviour in the distribution of DNA between daughter cells. It could correspond to the 'quantal mitosis' proposed by Holtzer [4].

2. Materials and methods

A human embryonic lung fibroblast strain was used, maintained as previously described [5].

The course of DNA synthesis was followed as described by Stanners and Still [6]. [^{3}H]Tdr at a concentration of 0.01 μCi/ml was added, duplicate samples were removed at hourly intervals, and were processed for autoradiography as reported previously [5]. Grains were counted over 100 labeled metaphases to determine the peak metaphase grain count [6].

For the measurement of DNA content, the cells were washed with phosphate-balanced salt solution (PBS), fixed with ethanol:acetone (1:1) for 30 min, dried and incubated for 1 h at 37°C in a buffer solution (0.1 M Tris, 0.1 M NaCl, pH 7.5), containing 30 units/ml of RNase, and then for 30 min in the same buffer containing 10 μg/ml of ethidium bromide. This solution was renewed after 15 min and the cells were mounted in the staining solution. The measurements were made with a Zeiss microscope fluorometer equipped with a BP exciter filter, a FT 580 beam splitter and a LP 590 barrier filter. A standard uranyl glass was used to control the stability of the fluorometer.

The slides were screened for mitoses, and the DNA content of metaphases and of each half of the anaphases was measured. Histograms were built with the distributions of the respective DNA contents found at different cell population doublings (PDLs). For each PDL, the cumulative percentage of cells with the same DNA content was plotted on probit paper; a normal Gaussian distribution plotted on probit paper gives a straight line. The DNA content of more than 4000 metaphases and anaphases was measured during the proliferative life-span of the cell population; 325 anaphases and 385 metaphases were screened in the last population doubling.

3. Results

Fig. 1 illustrates the different phases of the life-span of a population of human fibroblasts, which are characterized by distinct structural and metabolic features [7]. The proliferative period (phases I, II and III) is followed by a terminal phase (phase IV) where the cells perform the last mitoses of their division potential. Many events take place in a sudden fashion during phase IV [7], after which the cells enter a post-mitotic phase where they can survive for several months [1].

For an asynchronous population the method of Stanners and Till [6] gives the most accurate estimate of the duration of the S period. The time interval between the first appearance of labeled metaphases and the point when the number of grains over the metaphases reaches a plateau during continuous labeling, corresponds to the length of the S period (Fig. 2). In addition the method also gives information on the time–course of DNA synthesis. The results show that the time–course of DNA synthesis during the first part of the S period is identical in both cultures although it starts 1 h later in cells entering phase IV. The second part of the S period, however, takes 1 h to terminate in actively proliferating cultures, while in cells entering phase IV it takes 3 h.

Since DNA synthesis is semiconservative, the scatter of the distribution of the DNA contents between daughter cells should be the same as that of metaphases when that of the latter is halved. This was evaluated by plotting on probit paper the distributions of the DNA contents of phase II and III cells in these stages of mitosis (Fig. 3), in such a way that the scale of the abscissa for the metaphases is twice that of the anaphases. The values corresponding to the metaphases and to each half of the anaphases are linear and overlap, in agreement with the semiconservative synthesis of DNA. Thus the plot shows that the distribution of the DNA contents of cells in metaphase and anaphase follows a normal Gaussian probability. The same results were obtained at different PDLs during phases II and III of the fibroblast population.

However, when the measurements were made on cells at the very end of the proliferative life-span, the plot of DNA contents

*Corresponding author. Fax: (33) (1) 39 49 51 45.

SSDI 0014-5793(94)01407-8

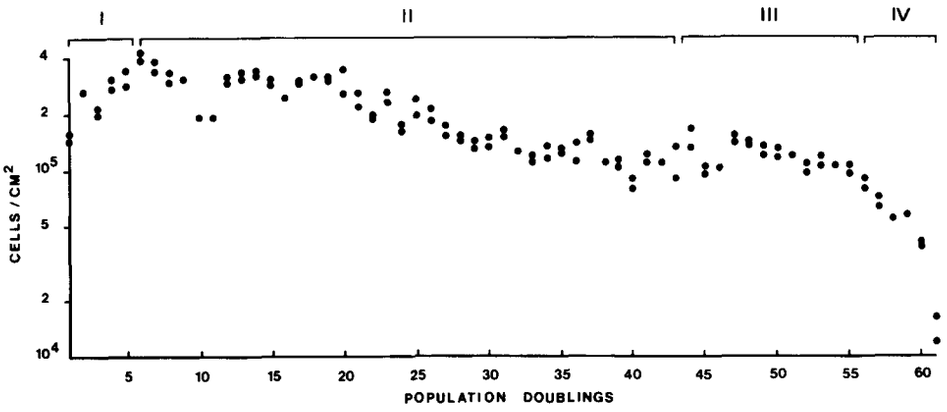

Fig. 1. Survival curve of a human embryonic lung fibroblast population expressed as the maximal cell densities reached after each population doubling. Each dot corresponds to a cell count of a separate culture. The different phases traversed by the cells during their life-span are indicated. The phases are characterized by the kinetics of proliferation and by metabolic events.

was not linear either for anaphases or metaphases (Fig. 4). Furthermore, the DNA contents of metaphases and of anaphases diverged at high DNA contents. The cells' DNA content also reached significantly higher figures during the last mitoses of the fibroblast population proliferative life-span, suggesting a deregulation not only of the partition of DNA but also of its synthesis.

4. Discussion

Previous results have shown that the evolution of the kinetics of proliferation of human embryonic lung fibroblasts is characterized by an accumulation of subtle changes and a final phase with abrupt events [8]. This evolution is accompanied in parallel by functional modifications that follow the same pattern [7].

One of the abrupt events consists of the destabilization of nucleosomes and the decondensation of heterochromatin, both phenomena probably being related [9]. There is also a disorganization of the 30 nm chromatin fibers [9] mainly at the nuclear periphery in their relationship with the lamina densa where DNA synthesis initiating sites are located. The disturbance in the time-course of DNA synthesis during the last part of the S period shown above in cells entering phase IV could be related to the chromatin modifications since heterochromatin is known to be synthesized at the end of the S period. It could be the prelude to the final chaotic mitoses.

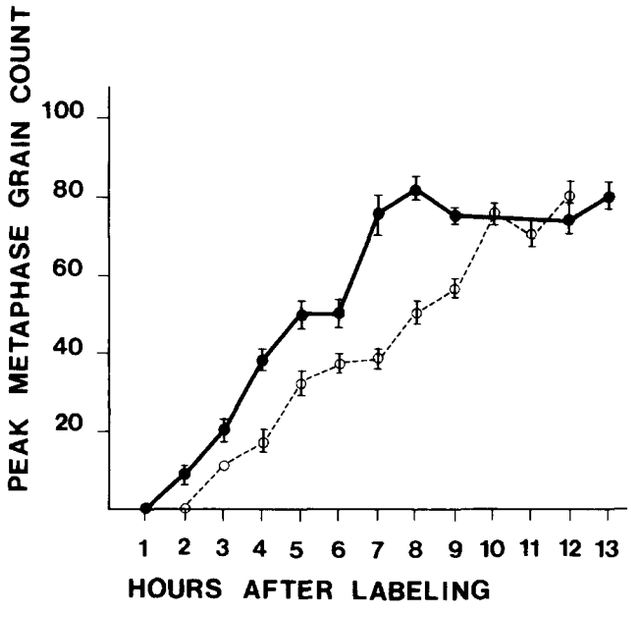

Fig. 2. Peak metaphase grain count found during continuous labeling of cells in phase II (—) and entering phase IV (- - - -). The standard errors are indicated.

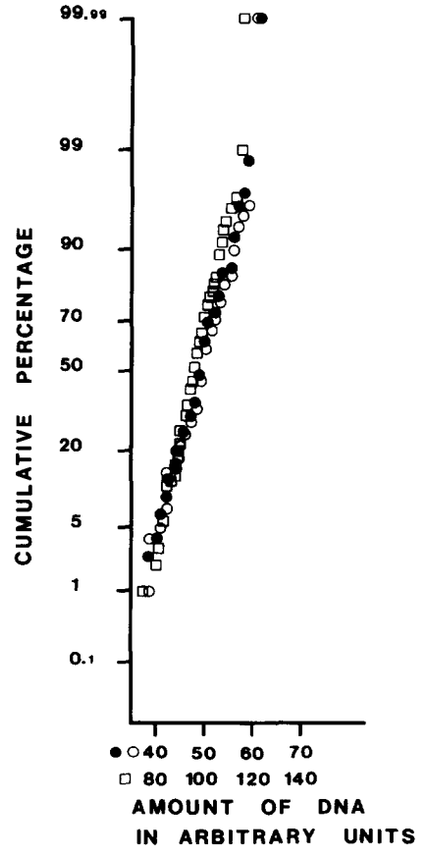

Fig. 3. Plot on probit paper of the distribution of the amount of DNA on metaphases (□) and on each half of anaphases (●, ○) of human embryonic lung fibroblasts at the 16th PDL.

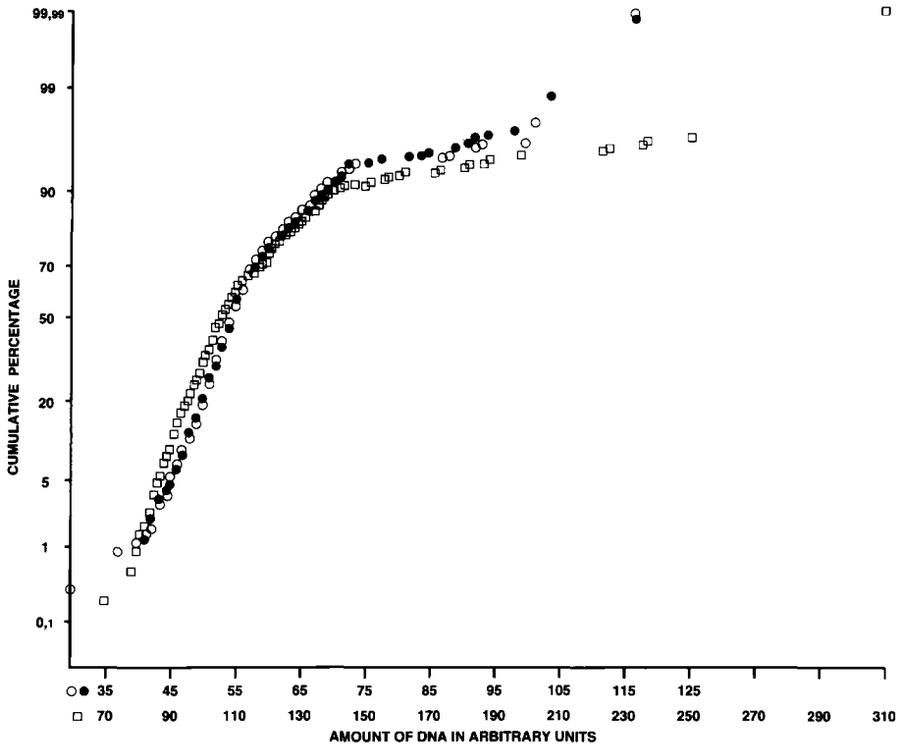

Fig. 4. Plot on probit paper of the distribution of the amount of DNA on metaphases (□) and on each half of anaphases (●, ○) of human embryonic lung fibroblasts during the last population doubling.

The deregulation of the synthesis and partition of DNA during the last divisions could also be related to other events, such as the loss of telomeres during replication leading to a final critical deletion [10]. Telomeres apparently function in the attachment of chromosome ends to the nuclear envelope [11] where DNA synthesis initiating sites are located.

Fibroblasts have an important role in homeostasis through the creation of a microenvironment and through their inductive properties on the differentiation of other cells [12–14]. Terminal fibroblasts can be seen in the normal human organisms, and their number seems to be increased in aging-related pathologies [9,15]. It has been proposed that the evolution of the fibroblast properties through proliferation is a programmed process of terminal differentiation [2], necessary to fulfill a role in homeostasis. Holtzer [4] hypothesized that differentiated cells become committed in the course of a last mitosis, the 'quantal mitosis'. The chaotic deregulation of the synthesis and partition of DNA between daughter cells, reported herein, could be the final commitment step of the fibroblast differentiation program.

Acknowledgements: This work was supported by contract no. 901003 from INSERM and by the Sandoz Foundation for Gerontology Research.

References

[1] Hayflick, L. (1965) Exp. Cell Res. 37, 614–622.
[2] Martin, G.M., Sprague, C., Norwood, T.H. and Pendergrass, W.R. (1974) Am. J. Path. 74, 137–151.
[3] Martin, G.M. (1993) J. Geront. 48, B171–B172.
[4] Holtzer, H. (1970) in: Cell Differentiation (Scheijde, A. and de Vellis, J. eds.) pp. 476–503, Van Nostrand, Reynhold, New York.
[5] Macieira-Coelho, A., Pontén, J. and Philipson, L. (1966) Exp. Cell Res. 43, 20–29.
[6] Stanners, C.P. and Till, J. (1960) Biochim. Biophys. Acta 37, 406–415.
[7] Macieira-Coelho, A. (1988) Biology of Normal Proliferating Cells in Vitro. Relevance for in Vivo Aging, p. 111, Karger, Basel.
[8] Macieira-Coelho, A. and Azzarone, B. (1982) Exp. Cell Res. 141, 325–332.
[9] Macieira-Coelho, A. (1991) Mutat. Res. 256, 81–104.
[10] Harley, C. (1991) Mutat. Res. 256, 271–282.
[11] Agard, D.A. and Sedat, J.W. (1983) Nature 302, 676–681.
[12] Auerbach, R. (1961) Dev. Biol. 2, 271–284.
[13] Kratochwill, K. (1969) Dev. Biol. 20, 46–71.
[14] Cunha, G.R., Donjacour, A.A., Cooke, P.S., Mee, S., Bigsby, R.M., Higgins, S.S. and Sugimura, Y. (1987) Endocr. Rev. 8, 338–362.
[15] Macieira-Coelho, A. (1994) Ann. NY Acad. Sci. 719, 108–128.