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SOME ASPECTS OF THE PATHOLOGY OF MITOSIS IN A CHINESE HAMSTER CULTURE DURING DISTURBANCES OF THE PROTEIN-SYNTHESIZING SYSTEM

I. A. Alov and L. S. Strochkova

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After disturbance of protein synthesis by puromycin and disturbance of transcription of chromosomal and ribosomal RNA by actinomycin D considerable changes were observed in the normal course of mitosis. An increase in the number of colchicine-like mitoses (c mitoses) sometimes accompanied, in particular, by segmentation of their cytoplasm with the formation of racemose structures, were observed. It is suggested that the development of c mitoses is associated not only with disturbances in the system of formation of the mitotic apparatus, but also with blocking of the synthesis of one of the chromosomal proteins that stabilize the spiralization of DNA strands. The other disturbance of division arising as a result of depression of metabolism, namely hollow metaphase, is associated not only with disturbances of the formation of the division spindle, but also with chromosomal changes. Selective depression of transcription of ribosomal RNA led to definite delay of anaphase and to coupling of the telomeric regions of the chromosomes, evidently on account of disturbance of the "protective membrane" of the chromosomes formed by RNA of the disintegrating nucleoli and RNA of the perichromatin granules.

KEY WORDS: *pathology of mitosis; RNA transcription; protein synthesis; cell culture.*

Pathological mitoses (PM), the mechanism of their origin, and also the applied importance of their investigation have repeatedly been examined in different types of cells [1]. In the study of the mitotic regime when protein synthesis is blocked and transcription of chromosomal and ribosomal RNA inhibited in culture, the writers discovered certain distinguishing features of the disturbance of mitosis. Knowing the character of the cytochemical changes causing them, it is possible to imagine the possible mechanisms with which these disturbances of normal cell division are connected.

EXPERIMENTAL METHODS

Observations were made on a synchronized culture of Chinese hamster fibroblast-like cells (line Bl1dii FaF-28 clone 237), in which different metabolic processes were inhibited during different periods of interphase. Protein synthesis was inhibited by puromycin (Serva, 10 μg/ml), transcription of total chromosomal RNA (cRNA) by actinomycin D (Reanal, 1 μg/ml), and synthesis of ribosomal RNA (rRNA) selectively by actinomycin D in a dose of 0.1 μg/ml. The incidence and character of PM were investigated during the 1st and 2nd waves of mitosis after synchronization of the cells by mitotic selection after preliminary treatment with colcemid.

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EXPERIMENTAL RESULTS

Disturbance of protein and RNA synthesis led to a considerable increase in the number of PM, which consisted of colchicine-like mitoses (c mitoses), scattering of the chromosomes in metaphase, delayed movement of the chromosomes, multiple bridges, hollow metaphases, etc.

The appearance of c mitoses was particularly interesting: A sharp increase in their number (sometimes up to 70-100% of all PM) was observed during the 1st and 2nd waves of proliferation after treatment with all doses of actinomycin D and, in particular, of puromycin. As a rule the c mitoses were in the form of ball or sphere metaphases with strongly hyperspiralized, swollen, and partly fused chromosomes (Fig. 1A). The c mitosis [1] is a multifaceted process, which arises after treatment with various plasmokinetic poisons (colchicine, colcemid, vinblastine, vincristine, etc.), inducing primarily disorganization of the cell division spindle. This disorganization is connected chiefly with a disturbance of equilibrium between the precursor pool and the intact microtubules toward depolymerization. Although nearly all investigators of this pathology of mitosis have observed simultaneous changes in the chromosomes also (hyperspiralization, delayed disjunction of kinetochores), they have not been subjected to closer investigations. The results of the present investigations with blocking of proteins and RNA synthesis, like the analogous observations made by other workers who studied the action of actinomycin D on dividing cells [2, 7, 10], suggest that the hyperspiralization and fusion of chromosomes typical of the c mitosis are connected with blocking of the synthesis of proteins essential for normal spiralization of DNA strands. The nature of these proteins (X proteins) is not yet known. One suggestion is that they are histone fractions (fraction F_1, for example), a change in the quantity of which [13] or their acetylation or phosphorylation [6, 12] is connected with condensation of the chromosomes in mitosis. The possibility likewise cannot be ruled out that the DNA-gyrase enzyme–substrate system which, as has been shown in the case of *E. coli* regulates the degree of spiralization of the circular chromosome [5], also participates in the phenomenon.

Whatever the case, our own observations, together with data of other workers, enable considerable additions to be made to modern hypotheses of the development of the c mitosis. The onset of this pathology is probably connected not only with disturbances in the system of formation of the mitotic apparatus, but also with blocking of the synthesis of one of the chromosomal proteins which stabilizes the spiralization of chromosomes.

When the mechanisms of development of the c mitosis are discussed, changes in the mitotic apparatus, chromosomes, and certain organelles are usually considered. This range of objects must probably be considerably enlarged. For instance, after the action of puromycin on the final stages of interphase, pictures of mass fragmentation of cells were frequently observed in c mitosis. The dividing cell lost its circular shape, it developed several constriction rings separating shortened chromosomes scattered throughout the cytoplasm. Individual viable fragments, forming "clusters" or chains (Fig. 1B), contained several strongly hyperspiralized chromosomes or no chromosomes whatsoever.

Similar pictures were observed previously during microfilming of human fibroblasts after prolonged treatment with low doses of colchicine [4], and also in synchronized Chinese hamster cells after the action of colchicine selectively on the G_2 period of interphase [3]. The connection, on the one hand, between the premature formation of multiple constriction rings in the cell, the system of microtubules, and the subplasmalemmal actin microfibrils and, on the other hand, the blocking of protein synthesis and also the action of colchicine, is not yet clear. Among the PM which were observed after blocking of the protein-synthesizing system (usually after inhibition of transcription of cRNA), the hollow metaphases (2-7% of all PM) were particularly interesting. This form of pathology was found both in the 1st (nearer) and in the 2nd (distant) mitoses; in the latter case about 3 times more often than in the first mitosis. After the addition of actinomycin D to the cells hollow metaphases appeared 5 times more often than after the addition of puromycin. The development of the typical hollow metaphase is usually attributed to displacement of the chromosomes to the periphery of the cell on account of swelling of the division spindle and with an irregular arrangement of the chromosomal microtubules formed at a distance from the central spindle [1]. However, yet another possibility can be added to these views. For instance, in the experiments described above, besides displacement of the chromosomes to the periphery of the cell in hollow metaphase, they appeared to be fused together by their centric and telomeric regions and they were located not only along the radii of the cell, but also parallel to the surface (Fig. 1C, D). This suggests that the formation of the hollow metaphase in this case is probably not

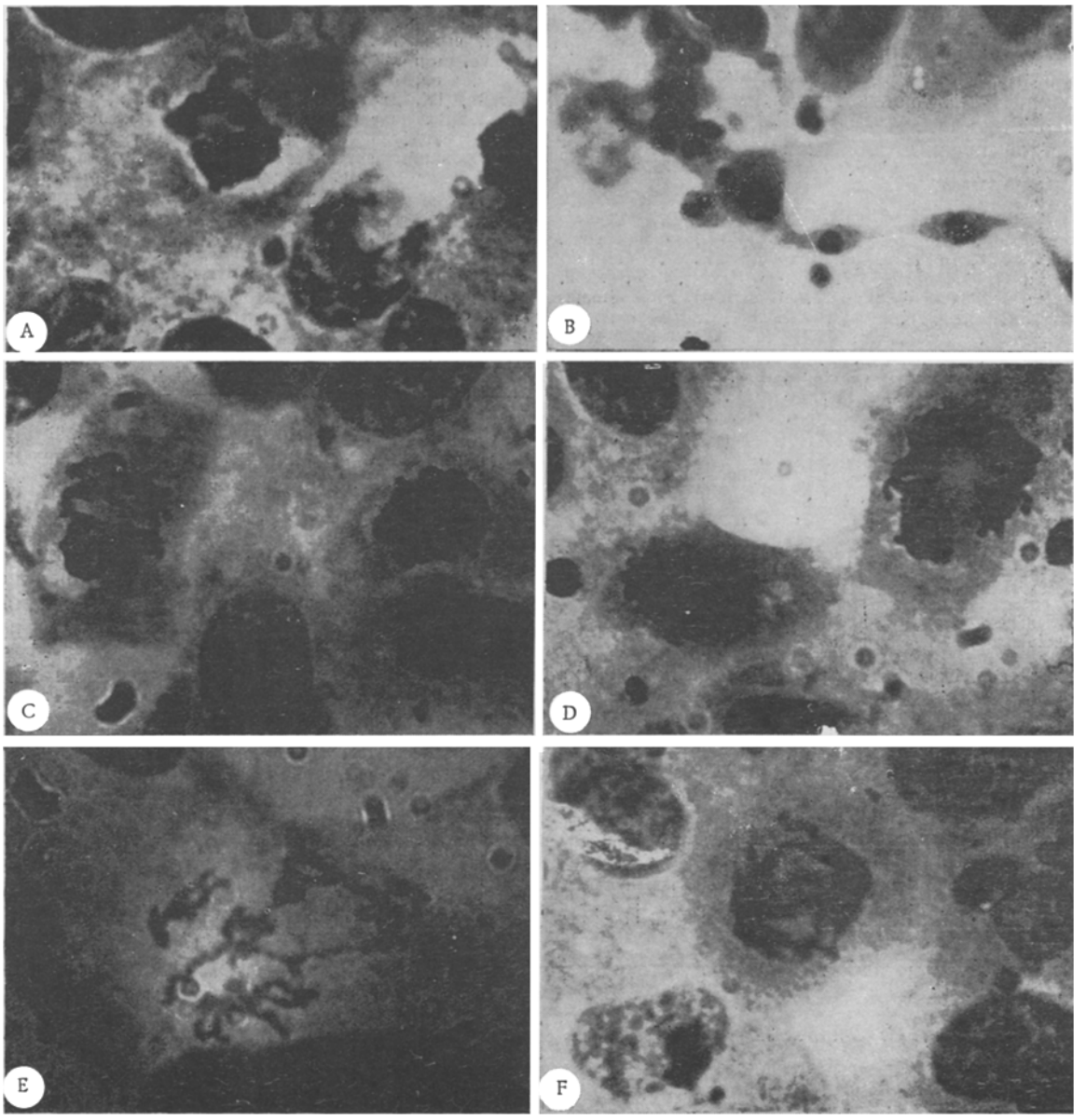

Fig. 1. Some forms of pathological mitosis in culture of Chinese hamster cells during inhibition of protein-synthesizing system. A) c Mitosis; B) fragmentation of cell during c mitosis; C, D) hollow metaphases; E) circular linking of chromosomes; F) multiple coupling of telomeric regions of chromosomes in anaphase. Carazzi's hematoxylin, 600×.

only connected with changes in the division spindle, but may also be chromosomal in nature. Another possibility is that this pathology of mitosis may be either the result of a disturbance of RNA transcription or the result of the direct action of actinomycin D on the DNA template. A distant prototype of such a hollow metaphase could perhaps be the formation of circular chromosomal structures observed following the action of actinomycin D (Fig. 1E), and which has also been described by other workers, for example when studying fragmentation and reunion of chromosomes in locust spermatozoa [8].

In conclusion, attention must be drawn to yet another feature distinguishing the course of the first mitosis after inhibition of rRNA synthesis in any period of interphase. Prolongation of anaphase ("anaphase delay") was observed under these circumstances, although under ordinary conditions it is extremely rare. Prolongation of anaphase as a rule was accompanied by chromatid or chromosomal bridges, when multiple coupling of the telomeric ends of the chromosomes was observed (Fig. 1F).

A similar phenomenon has also been described by other workers. Patnak and McGill [11], for instance, showed in several lines of mammalian cells that there is a marked increase in the number of abnormal anaphases after treatment with actinomycin D (linking of chromatids in the region of the nucleolar organizer), whereas after treatment of the cells with ethidium bromide, an inhibitor of mitochondrial RNA synthesis, anaphase movement of the cells also was delayed, for the chromosomes appeared to be bound by submicroscopic fibrils. During mitosis some of the material of the disintegrating nucleoli and the so-called perichromatin granules covering the surface of the chromosomes are distributed between the daughter cells as the chromosomes separate in anaphase. The absence of this material (as a result of inhibition of rRNA synthesis), and also suppression of the synthesis of perichromosomal RNA in the G_2 period [9] caused the chromosomes to loose their "protective membrane" of RNP, and this could facilitate their more intensive coupling in anaphase.

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