COMPARISON OF LATE INCORPORATION PATTERNS OF THYMIDINE-H^3 WITH DISTRIBUTION OF G-SEGMENTS IN EASILY IDENTIFIABLE CHROMOSOMES OF *Macaca mulatta*

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Replication of easily identifiable chromosomes Nos. 1, 2, 8, and 9 was studied in a peripheral blood lymphocyte culture of *Macaca mulatta*. Replication of the chromosomes studied was not the end of the S period. Late-replicating segments corresponded completely to darkly stained segments. The constancy of linear differentiation of homologous chromosomes in different types of somatic cells indicates that darkly stained segments consist of structural heterochromatin.

KEY WORDS: lymphocyte culture; thymidine-H^3; replication.

It is now generally accepted that genetically inactivated segments of separate chromosomes and even whole chromosomes replicate at the end of the S period [5]. It is considered that the phenomenon of late DNA replication has been established precisely for segments heterochromatinized in interphase, irrespective of whether the segments in question belonged to heterochromatic or euchromatic regions of the chromosomes [6]. Comparison of the differential staining patterns of chromosomes with their late replication patterns in different animals has yielded valuable information for the understanding of the functional organization of chromosomes.

The objects of the present investigation were: 1) to study late replication of easily identifiable chromosomes of *M. mulatta*; 2) to compare the late-labeling and longitudinal differentiation patterns of the chromosomes studied.

EXPERIMENTAL METHOD

Peripheral blood lymphocyte cultures from three sexually mature (2♀ and 1♂) monkeys (*M. mulatta*) obtained by the usual method [9] were used in the experiments. Considering that the mean duration of the G_2 period in lymphocyte cultures of *M. mulatta* is 5 h [4], thymidine-H^3 (2 μCi/ml) was added to the culture 5 h or, in some cases, 6 h before fixation. Colchicine was added 1 h before hypotonic treatment of the cells with 0.55% KCl solution. The procedure for obtaining the preparations and autoradiographs was as described previously [4]. Metaphase plates with a satisfactory scattering of the chromosomes and with clear labeling (not less than 150 silver grains per metaphase) were photographed before and after removal of the label. Interchromosomal asynchrony of DNA synthesis was analyzed by Slezinger's method [7]. The value of the index K was determined for visually identifiable chromosomes Nos. 1, 2, 8, and 9 in accordance with the writers' classification [3]. Altogether 17 labeled metaphase plates were used in the analysis. To study the longitudinal order of replication, chromosome No. 1 was divided into six, and chromosomes Nos. 2, 8, and 9 into five, equal segments. The number of silver grains above each segment was counted. The distribution of grains of label along the length of metacentric chromosomes Nos. 2 and 8 was analyzed, as other workers [1, 7] also did. Some chromosomal preparations were used for differential staining by the method described earlier [2].

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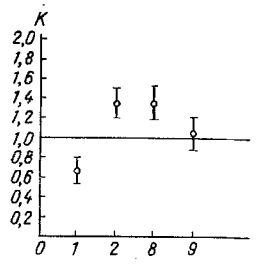

Fig. 1

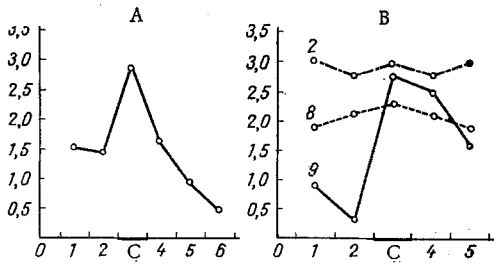

Fig. 2

Fig. 1. Distribution of K indices at end of S period. Abscissa, No. of chromosome; ordinate, mean values of K index.

Fig. 2. Intensity of labeling of chromosomal segments in leukocytes: A) chromosome No. 1; B) chromosomes Nos. 2, 8, and 9. Abscissa, segments of chromosomes (C—centromeresegment, 2) region of secondary constriction of chromosome No. 9); ordinate, mean number of grains of silver above segments of chromosomes.

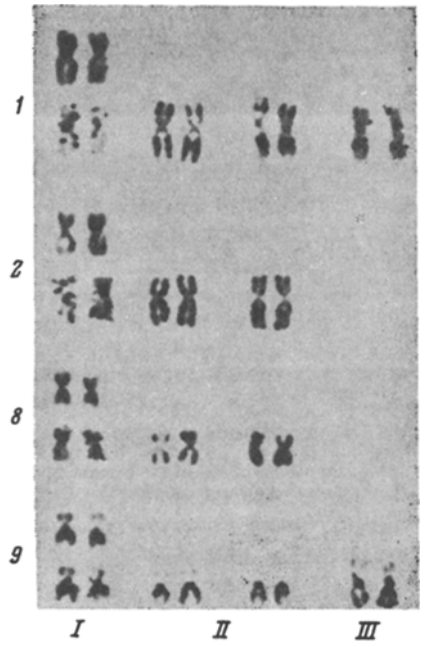

Fig. 3. Comparison of late-labeling patterns and differential staining patterns of chromosomes Nos. 1, 2, 8, and 9 (Romanovsky—Giemsa staining method, 630x): 1, 2, 8, and 9) No. of chromosomes; I) labeling patterns of chromosomes at end of S period; II) differential staining patterns; III) labeling patterns of chromosomes Nos. 1 and 9 before end of S period.

EXPERIMENTAL RESULTS

The results of analysis of interchromosomal asynchrony of replication of the chromosomes chosen for study are illustrated in Fig. 1. Clearly chromosomes Nos. 2 and 8 contained many more grains of label than was expected theoretically. In chromosome No. 1 at the end of the S period, on the other hand, the number of grains of label was significantly smaller than the theoretically expected number. Chromosome No. 9 (nucleolus-forming) contained label in a quantity corresponding to its relative length. Consequently, the chromosomes in peripheral blood lymphocyte cultures from M. mulatta complete replication highly asynchronously: chromosome No. 1 earlier, but chromosomes Nos. 2 and 8 later than chromosome No. 9.

The results of analysis of the longitudinal nonsynchronization of replication of the segments of chromosomes Nos. 1, 2, 8, and 9 are illustrated in Fig. 2. In chromosome No. 1, the centromere segment was most intensively labeled at the end of the S period. The mean number of grains of label in segments nearest to the centromere and also in the telomere of the short arm was reduced. The number of grains in the telomere of the long arm was sharply reduced. Unlike chromosome No. 1, in chromosomes Nos. 2 and 8 it was impossible to distinguish a single segment labeled definitely more intensively than the rest. In chromosome No. 9 the largest number of grains at the end of the S period was found in the region of the centromere and adjacent portion of the long arm. In the region of the secondary constriction (the organizer of the nucleolus) and in the short arm the concentration of label was minimal.

Comparison of the order of replication along the length of the chromosomes with the distribution of darkly stained segments showed (Fig. 3) that at the end of the S period in chromosome No. 1 the centromere segment is late-replicating and, correspondingly, is darkly stained. When the isotope was added 6 h before fixation of the cultures, i.e., before the end of the S period, the middle of the long arm was intensively labeled, in a region corresponding to the darkly stained segment of that arm. Chromosomes Nos. 2 and 8 were intensively labeled throughout their length. They were also darkly stained throughout their length. In chromosome No. 9, the centromere and adjacent area of the long arm were intensively labeled and, correspondingly, they were the darkly stained segments of that chromosome. Consequently, good correlation was observed between the patterns of late labeling and of differential staining: late-replicating segments of these

chromosomes corresponded exactly to the darkly stained segments. On the basis of late replication of the darkly stained segments, they can be classed as heterochromatinized regions. However, heterochromatinized regions may include both structural heterochromatin [8, 10] and temporarily heterochromatinized areas of euchromatin [6, 7]. To study this problem in more detail, the patterns of differentiation of homologues were compared in several species of monkeys with 42 chromosomes.

Observations showed that longitudinal differentiation of homologous chromosomes was identical in different types of cells (bone marrow, lymphocytes, epithelial cells of the kidneys) and in different species of macaques (M. mulatta, M. nemestrina, M. arctoides). Structural differentiation of most of the homonymous pairs of homologous chromosomes is also similar in M. mulatta and Papio hamadryas belonging to different genera of the Cercopithecidae family. The constancy of structural differentiation of homologous chromosomes of different types of somatic cells in the species of monkeys studied suggests that the darkly stained segments, distinguishable on differential staining by the Romanovsky–Giemsa method, evidently consists of structural heterochromatin. Autoradiography combined with differential staining revealed at least two types of structural heterochromatin in chromosome No. 1, terminating DNA synthesis at different stages of the end of the S period of the mitotic cycle. Results also showed that analysis of linear chromosomal replication can be studied by the more sophisticated method of counting silver grains above lightly and darkly stained segments. This approach may perhaps help to reveal late-replicating heterochromatinized segments of euchromatin.

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