Mammalian Cell Fusion

V. Replication Behaviour of Heterochromatin as Observed by Premature Chromosome Condensation

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Abstract. The behaviour of heterochromatin during premature chromosome condensation (PCC) was studied in a cell line of Microtus agrestis after fusion with mitotic HeLa cells. In the G₁- and G₂-PCC, the heterochromatic nature of the X-chromosomes was detectable by their intense staining. The pulverized appearance of the S-phase PCC was correlated with incorporation of ³H TdR into the DNA. Three types of S-PCC were observed. PCC with a pulverized appearance of: (a) only the autosomes (early S); (b) autosomes and X-chromosomes (mid S); and (c) only the X-chromosomes (late S). The behaviour of heterochromatin during replication, as observed by the PCC method, was no different from that of euchromatin. The data on the sequence of chromosome replication indicate that the centromeric regions of the X-chromosomes were the last segments to replicate. The completion of DNA synthesis in the X-chromosomes appears to be followed by progressive chromosome condensation during G₂ even before the actual initiation of prophase.

Introduction

Normally, the chromosomes of mammalian cells can only be visualized during a limited time span of their cell cycle, that is during mitosis or meiosis. However, Johnson and Rao (1970) have shown that it is possible to induce premature chromosome condensation (PCC) in interphase cells by fusing them with those in mitosis. The chromatin from G₁ cells condenses into very long single stranded chromosomes and the G₂ nuclei produced chromosomes with two chromatids but are relatively less condensed than metaphase chromosomes. The unevenly condensed chromosomes with “pulverized” appearance, obtained from the S phase cells, reflect the extent of DNA replication in progress. Since all these observations were made on euchromatin we were interested to study the behaviour of heterochromatin during PCC induction with particular reference to the following two points: (1) whether heteropyenosis can be demonstrated in the prematurely condensed G₁ or G₂ chromosomes, and (2) whether heterochromatin undergoes decondensation at the time of DNA replication.
A favorable object in this respect is the European field vole, *Microtus agrestis*; in which all of its constitutive heterochromatin is confined to the large sex chromosomes (Schmid *et al.*, 1965; Wolf *et al.*, 1965). In the male the entire long arm of the X-chromosome and the proximal quarter of the short arm and the whole Y-chromosome are composed of constitutive heterochromatin. In the female, the remaining portion of the short arm of one of the two X-chromosomes undergoes facultative heterochromatinization. This offers an opportunity to study constitutive and facultative heterochromatin in the same structure.

**Materials and Methods**

For this study a cell line, established from the ear biopsy of a female *Microtus agrestis* in 1967, grown in modified McCoy's 5A medium supplemented with 20% of fetal calf serum was used. HeLa cells, grown as monolayer in Eagles minimal essential medium supplemented with non-essential amino acids, sodium pyruvate, glutamine and 10% fetal calf serum, were partially synchronized by exposing them to 2.5 mM of thymidine for 20 hours. Four hours after the reversal of the excess thymidine block colcemid (0.05 mg/ml) was added to the dishes and they were incubated for another 11 hours. The floating and loosely attached mitotic cells were then harvested by gentle shaking. Fusion between interphase cells of *Microtus agrestis* and mitotic HeLa cells was achieved by means of UV-inactivated Sendai virus as described earlier (Rao and Johnson, 1972). In short, the virus-cell mixture was kept at 4°C for 15 min, then transferred into a waterbath maintained at 37°C for 10 min. The cell suspension was diluted with hypotonic 0.075 M potassium chloride and reincubated for another 10 min. After centrifugation, the cells were fixed in methanol/acetic acid (3:1) and the fixative was changed three times. The cells were then spread on wet, ice-cold slides, flame dried and stained with Giemsa. For autoradiography, the stain was removed by placing the slides in 70% ethanol for 6 hours and covered with liquid emulsion.

**Results**

The *Microtus* cell strain exhibited a high incidence (about 40%) of polyploidy including some octoploid cells. In about 10% of the metaphases scored, we found marker chromosomes, derived mainly from the sex chromosomes, which contained less than the normal amount of constitutive heterochromatin as could be demonstrated by the shortness of the C-band region.

For most of our studies on PCC, we checked normal diploid cells. However, for an estimation of the number of cells at different stages of the cell cycle, each PCC was scored and rescored after autoradiography: altogether 134 cells were analysed, 36% were in G1-, 52% in S- and 12% in G2-phase.