Chemical induction of quadruple and octuple chromosomes in Chinese hamster CHO-K1 cells and relationship between their three-dimensional structure and spatial distribution of BrdU-labeled chromatids

Kyomu Matsumoto, Toshihiro Ohta
Laboratory of Genetic Toxicology, Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira, Tokyo 187, Japan

Received: 31 January 1994 / in revised form: 18 April 1994 / Accepted: 25 April 1994

Abstract. Double endoreduplication of Chinese hamster CHO-K1 cells that exhibited quadruple chromosomes at metaphase was induced by a combination of rotenone and ammonium vanadate treatments. Analysis of sister chromatid differential staining patterns (using 5-bromo-2'-deoxyuridine) revealed that approximately 50% of the quadruple chromosomes did not keep the scheme of “outside replication” of DNA. Based on the ratio of the staining patterns observed, we suggest that the two diplochromosomes forming a quadruple chromosome are held together by a physical link connecting the two original chromatids. Metaphases with octuple chromosomes were also produced by the same treatment. Each chromosome constituting an octuple chromosome was longer and thinner than ordinary metaphase chromosomes. This suggests incomplete chromosome condensation at metaphase. The majority of octuple chromosomes showed the eight constituent chromosomes to be so enmeshed that a planar alignment could not be observed in air-dried preparations.

Introduction

An endoreduplication cycle consists of two successive DNA synthetic periods (ES₁ and ES₂) without an intervening mitosis, leading to the formation of diplochromosomes in the following mitotic metaphase (Schwarzacher and Schnedl 1965). When [³H]thymidine or 5-bromo-2'-deoxyuridine (BrdU) is given to endoreduplicating cells during the first DNA synthetic period (ES₁) and air-dried slides are prepared, the label is restricted to the two outer chromatids of a diplochromosome (Walen 1965; Schwarzacher and Schnedl 1966; Wolf and Perry 1974). This phenomenon could be due to the new DNA strands being synthesized on the outside of the old ones at each DNA synthetic period of endoreduplication, though the native state of DNA strands is a double helix (Walen 1965; Schwarzacher and Schnedl 1966). This characteristic mode of DNA replication in endoreduplication is called “outside replication” (Takanari 1985). Even if this is so, the two-dimensional scheme of outside replication could still be applicable to diplo- and quadruple chromosomes organized in a three-dimensional configuration. This is something that needs to be clarified for an understanding of the mechanism of outside replication.

Recently, we found that a natural insecticide, rotenone, affected cultured Chinese hamster cells at metaphase and led them to endoreduplicate (Matsumoto and Ohta 1992). An application of this finding made it possible to induce endoreduplicated cells with diplochromosomes at remarkably high frequency (about 80% of all the metaphases). In the present experiments, such an endoreduplicated cell population was treated with another endoreduplication inducer, ammonium vanadate (Owusu-Yaw et al. 1990), so that double and triple endoreduplicated cells with quadruple and octuple chromosomes could be induced. By analyzing the sister chromatid differential staining (SCD) patterns in many BrdU-labeled quadruple chromosomes, we attempted to define the relationship between the three-dimensional configuration of the chromosomes and outside replication of DNA.
Materials and methods

Cell line and chemicals. Chinese hamster CHO-K1 cells were grown in Ham's F12 medium (Nissui Pharmaceutical, Japan) supplemented with 10% fetal bovine serum (Bocknek Laboratories ICN), penicillin (50 U/ml), and streptomycin (50 μg/ml) in a humidified atmosphere with 5% CO₂ at 37°C. The doubling time of the cells was about 15 h.

Rotenone (Aldrich) and 3-(1-anilinoethylidene)-5-benzylpyrrolidine-2,4-dione (TN-16, Wako Pure Chemical, Japan) were dissolved in dimethyl sulfoxide. Ammonium vanadate (Wako) was dissolved in 1% ammonia solution. BrdU (Sigma) was dissolved in distilled water. The final concentration of these solvents in the medium was 0.5%.

Induction of quadruple and octuple chromosomes. The procedures for obtaining a cell population in which about 80% of cells have diplochromosomes were previously described (Matusmoto and Ohta 1992). In brief, Chinese hamster CHO-K1 cells were seeded in 100 mm dishes and grown to approximately 70% confluence. Then they were treated with 0.5 μg/ml of TN-16 for 1 h to arrest metaphase. The cells were quickly rinsed and permitted to recover in fresh medium for 20 min. Rotenone was added to the cells at a final concentration of 10 μg/ml. After 30 min, metaphase cells were selectively detached from the monolayer by gentle pipetting. The metaphase cell suspensions containing rotenone were incubated for a further 2.5 h. They were then collected by centrifugation, washed, and recultured on 35 mm dishes in fresh medium. After 24 h, the cells were treated with 25 μg/ml of ammonium vanadate for 6 h. Chromosome preparations were made 24 to 48 h after the end of ammonium vanadate treatment.

BrdU labeling. Double and triple endoreduplication involve, respectively, three and four successive DNA syntheses without intervening mitosis. In order to label these endoreduplicating cells during these three and four DNA synthetic periods, BrdU was added to cultures at a final concentration of 5 μM, beginning at 15 h (1 cell cycle) before the addition of TN-16. The induction of quadruple and octuple chromosomes described above was carried out in the presence of BrdU, except during rotenone treatment. Labeled chromosomes were stained by the tetrasodium EDTA-Giemsa method for SCD (Takayama and Tachibana 1980), i.e., the preparations were stained at 40°C for 7 min with 3% Giemsa (Merck) diluted in 2% tetrasodium EDTA solution. By this staining method, the chromatids bifilarly substituted with BrdU were darkly stained, while the unifilar chromatids were lightly stained.

Chromosome preparations. Chromosome preparations were made by the conventional air-drying method. Colchicine was added to the cultures at a concentration of 0.5 μg/ml 2 h prior to harvest. The cells were exposed to 0.075 M KCl, fixed in 3:1 methanol:acetic acid, and air-dried. The chromosome preparations were usually stained with 2% Giemsa diluted in 1/15 M phosphate buffer, pH 6.8.

Results

Appearance of quadruple and octuple chromosomes

The reason ammonium vanadate was used to induce the second endoreduplication was that this compound showed relatively high effectiveness with less cytotoxicity in CHO-K1 cells than several other known inducers, such as colchicine (Rizzoni and Palitti 1973; Takanari 1985), vincristine (Takanari et al. 1985), hydrazine (Speit et al. 1984), and 4-nitroquinoline 1-oxide (Sutou and Tokuyama 1974).

Metaphase cells with quadruple chromosomes first appeared 30 h following treatment with ammonium vanadate and reached a peak frequency of 3.3% after 36 h. Since the reported frequencies of double endoreduplicated cells induced by Colcemid (Herreros and Giannelii 1967), colchicine (Rizzoni and Palitti 1973; Takanari 1985), or 8-azaguanine treatment (Ronchi et al. 1965) were 0.1%–1.1%, the present treatments were very effective. In well-spread metaphases, the two sister diplochromosomes of a quadruple chromosome tended to lie a small distance apart, so that each quadruple chromosome was taken to be two diplochromosomes at first...