An improved method for staining kinetochores of human chromosomes

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An improved method, which exhibited simultaneously clearly kinetochores and the nucleolar organizer regions of human chromosomes by pretreating of human metaphase chromosomes with HCl and NaOH, followed by staining with silver nitrate and visualizing using ammoniacal silver, is described in the present communication. It has important role for analysis of kinetochore variation, mechanism of chromosomal non-disjunction as well as identification of functional active centromeres.

Keywords: Chromosomal segregation, Kinetochore, Nucleolar organizer regions, Silver nitrate staining

Kinetochore, located at the site of the primary constriction of the chromosome, a trilamellar plate composed of proteins, RNA, and DNA1 is essential for chromosomal segregation. This structure can themselves function as microtubule-organizing centers2. The centromeres with kinetochores on the metaphase chromosome represent the functional active centromeres, while centromeres without kinetochore lack this property. At present, kinetochores can be visualized by three methods, including high temperature treatment, silver staining and indirect immunofluorescence staining to chromosome preparation3-5. A stable, fine effect method for exhibiting clearly kinetochores of human metaphase chromosome is reported in the present communication.

Materials and Methods

Human gastric adenocarcinoma cell strain (BGC823) was obtained from the Department of Pathology, Chongqing Medical University. The cells were cultured at 37°C for 2 days using RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum. Then, they were treated for 4 hr with 0.2 μg/ml colcemid and removed from the tissue-culture flask with 0.1% trypsin solution; sample of this suspension were collected by centrifugalization. Chromosome preparation was made by standard chromosomal technique.

The air-dried preparation from 12 days old was pretreated for 30 min with 0.2 N HCl solution, then washed with distilled water and dried. The preparation was again treated for 90 sec with 0.1% NaOH solution, followed by rinsing the slide with distilled water. The slide by pretreating with HCl and NaOH was placed on culture capsule. Five drops of 50% AgNO3 solution (the AgNO3 solution was prepared fresh every time) were added to the slide. The slide was covered with vegetable parchment, and then treated at 65°C for 30 min in thermostat-controlled water bath, followed by rinsing the slide with distilled water. The next step consists of simultaneously applying four drops of each of 40% ammoniacal silver solution and 3% formalin solution to the slide, and then the slide was covered with cover slip and observed under microscope at room temperature. When kinetochores in centromeres showed deep black spots (the two small dark spots at each of centromeres) and chromosomal arm turn to a golden color, the cover slip was immediately removed with distilled water so that the reaction was stopped.

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was neutralized with 97 ml of 3.2% sodium acetate solution, then the solution is regulated to a pH of 5 with methanoic acid). The two solutions were prepared fresh every time.

**Results**

Two small dark spots, one on each sister chromatid, were very clearly exhibited on both sides of the each centromere of the metaphase chromosome, the remainder of the chromosome were yellow in colour (Fig. 1). The silver stainable materials in the centromeric region represent those specific proteins that form the “kinetochore filaments” which associate with microtubules of the spindle apparatus. Nucleolus-organizer regions (NORs) were also stained simultaneously in a small proportion of the acrocentrics (Fig. 1).

**Discussion**

Eiberg described a technique called Cd-band that stains two small dark spots on both sides of centromere. However, there was controversy as to whether it represented the centromere. Further the exhibition of Cd-band at centromere was very vague and difficult for relative analysis. Denton et al. introduced a technique called simultaneous silver staining for both NOR and kinetochores. In centromere region of human chromosome two small black dots, one on each chromatid, can be exhibited by this method. They believed that the small black dots represented those specific proteins, which formed the kinetochore. However, it is insufficient for distinguishing clearly kinetochores between two chromatids in some chromosomes. The discovery that individuals with CREST-syndrome scleroderma had antikinetochore antibodies in their peripheral blood has made it possible to visualize clearly kinetochores using indirect immunofluorescence staining. The sister kinetochore of each chromosome can clearly show intensive fluorescence with the immunofluorescence staining, but the specimen can not be preserved forever due to fluorescent decrescence, and the cost of the experiment is very expensive.

With respect to Denton’s method, the following modifications were made in the present study: (i) the preparation was pretreated with 0.2 N HCl solution in order to avoid “hair-brush” of chromosome and kept clean background of chromosome preparation; (ii) the concentration of NaOH solution used was increased (0.1% solution of NaOH) in order to remove preferably some protective covering of centromere, (iii) the concentration of AgNO₃ solution (50% solution of AgNO₃) was increased and reaction time was prolonged to react more fully. By the improvement method described in the present communication, the two sister kinetochores at centromere of metaphase chromosome can be exhibited very clearly and distinguished distinctly. The uniform effect of staining kinetochores was also observed on the metaphase chromosomes of mouse by this method. The repeatability and stability of this method is fine. It may apply to analysis of...
kinetochore variation, mechanism of chromosomal non-disjunction as well as identification of functional active centromeres.

References