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Whole-genome chromosome distribution during nuclear fragmentation of giant trophoblast cells of *Microtus rossiaemeridionalis* studied with the use of gonosomal chromatin arrangement

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Abstract

Gonosomal chromatin bodies (GCBs), i.e. blocks of condensed chromatin consisting of heterochromatized region of the sex chromosomes of the field vole *M. rossiaemeridionalis*, were used as a natural interphase chromosome marker in order to clarify the regularities of GCB rearrangement during nuclear fragmentation of secondary giant trophoblast cells (SGTCs) at the end of their differentiation. Cytophotometrical measurements of DNA content in the nuclei, nuclear fragments and simultaneously in the GCBs were made in the secondary giant SGTCs of field vole *M. rossiaemeridionalis*. In most cases 1 to 2 GCBs get into the nuclear fragments at different ploidy levels. In the nuclear fragments, GCB DNA content decreased mostly proportionally to DNA content in the whole fragments corresponding to 2c, 4c and 8c. The data obtained demonstrate a regular whole-genome chromosome distribution into nuclear fragments. A possible mechanism of nuclear fragmentation that largely ensures a balanced genome in nuclear fragments is discussed.

Keywords: X-chromosome; Heterochromatin; Endoreduplication; Trophoblast; Placenta; Rodents

1. Introduction

Differentiation of a number of mammalian tissues involves genome multiplication that usually is irreversible (Nagl, 1978; Brodsky and Uryvaeva, 1985). A special case is represented by giant trophoblast cells that undergo depolyploidization via breakdown of highly polyploid nuclei into fragments (Zybina et al., 1979; Zybina, 1986). DNA distribution into the fragments tends to be whole-genome (Zybina et al., 1979; Zybina, 1990). However the mechanism of nuclear dissociation into fragments and chromosome distribution is unclear. In addition, binucleate and multinucleate trophoblast cells are encountered in a wide range of mammalian placentae (Hoffman and Wooding, 1993). In this connection, chromosome distribution in secondary giant trophoblast cells (SGTCs) seems to be important, as exemplified in the field vole *Microtus rossiaemeridionalis*, whose cell nuclei contain large clumps of condensed gonosomal chromatin consisting mainly of sex chromosome heterochromatin (Mazurok et al., 2001; Zybina et al., 2003). Recently we showed that DNA content in the GCBs increases proportionally to the ploidy level of giant trophoblast nuclei in the course of polytenization (Zybina et al., 2003). In the case of nuclear fragmentation, GCBs may also represent a natural chromosome marker of interphase nucleus that helps to follow the distribution of defined chromosomes during giant cell depolyploidization. Such investigations help in verifying the possibility of balanced genome distribution being achieved in the newly formed fragments of the trophoblastic polykaryocytes.

Abbreviations: SGTC, secondary giant trophoblast cells; GCB, gonosomal chromatin body.

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2. Materials and methods

Placentae of the East European field vole were used in the work; this species was initially described as *Microtus subarvalis* (Meyer et al., 1972), but

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later, according to the nomenclature codex, was changed to *Microtus rossiaemeridionalis* (Malygin, 1983). Placentae were kindly supplied by ED Sholl. To obtain the placentae, females were caged with males, and the morning on which vaginal plugs were confirmed was designated by the day 1 post-coitum.

The placentae were fixed with a 3:1 mixture of ethanol and glacial acetic acid. The totals of 12 embryos from 6 females were studied: 2 females each at day 10, 12, and 14 of gestation. Permanent squash preparations were made of the main part of the material. For this purpose the fixed placentae were dehydrated in 96% ethanol, and incubated in 70% ethanol. Pieces (2–3 mm) of placentae were macerated for 20 min in 45% acetic acid, placed on object glasses, covered with coverslips, and slightly pressed. On freezing the slides on dry-ice (solid CO_2), the coverslips were removed and the slides air-dried. The slides were Feulgen-stained (hydrolysis in 5 N HCl for 30 min at room temperature).

A part of the fixed material was dehydrated in a graded series of ethanols and embedded in paraffin; from this material, 10 μm histological sections were cut and Feulgen-stained. The DNA content was measured using a ''Videotest'' image analyzer composed of a CPT 83 60 digital CCD-video camera (Chipper, USA) installed on an EC Bimam-13 microscope and of computer IBM PC. Input of the image and measurement of the integral optical density characterizing the DNA amount in the nucleus and separately in GCB were performed using Videotest-Morpho software (St. Petersburg). The possibility of measuring DNA content in hepatocyte nuclei with this image analyzing system had been previously shown (Stein et al., 1998). An objective of 40 × 0.65 and an interference filter at 550 nm were used. In each placenta, from 150 to 400 trophoblast cell nuclear fragments were analysed, and 50 nuclei of fetal erythrocyte nuclei as a control of the DNA content in diploid nuclei. In processing the results, the following parameters were analyzed: the amount of DNA per nucleus in arbitrary units (a.u.) and ploidy (c) as well as the DNA content (a.u.) in GCB (in individual bodies and as the total in nuclei with 2–3 GCB). From these data, the means and standard errors were calculated as well as the correlation coefficient (r) between various parameters.

3. Results

Secondary giant trophoblast cells were observed at different stages of placenta development till term, although in a part of them, nuclei dissociated into small fragments. This process began on day 10 of gestation (Figs. 1 and 2). By day 15–17, fragmentation extended to a significant part of SGTCs, whereas by term, rather many mononuclear giant trophoblast cells are retained. During fragmentation either gradual budding or separation of nuclear fragments into the cytoplasm or their isolation inside the initial giant nucleus was observed; in this case a cluster of nuclear fragments initially retained the shape of nucleus (Figs. 1 and 2). A peculiarity of the field vole SGTCs is dissociation of non-classic polytene chromosome bundles into a multitude of endochromosomes at the late stages of differentiation (beginning from day 12). In contrast, GCBs mostly do not disintegrate into sister chromonemes or endochromosomes, and are retained in a form of 1–2 large condensed chromatin bodies, their size being comparable to the size of polytene chromosome bundles (Zybina et al., 2003).

Cytophotometry of DNA content in the nuclear fragments showed that they corresponded to 2c, 4c, and 8c (Fig. 3). The results are in a good agreement with our data that indicated the tendency of the whole-genome DNA distribution into nuclear fragments (Zybina et al., 1979; Zybina, 1990; Zybina and Zybina, 1996).

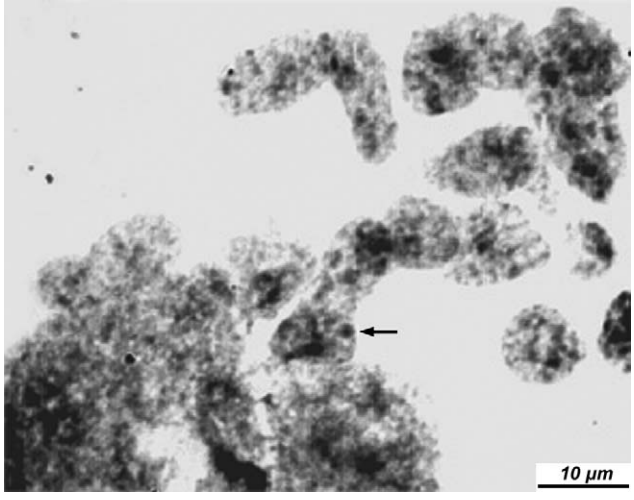

Fig. 1. Nuclear fragmentation in the secondary giant trophoblast cells of the *M. rossiameridionalis* into low-polyploid nuclear fragments on the day 10 of gestation. Note separation of nuclear fragments from the surface of the giant nucleus; one or few small gonosomal chromatin bodies are observed in the fragments (*arrow*). Feulgen-stained squash.

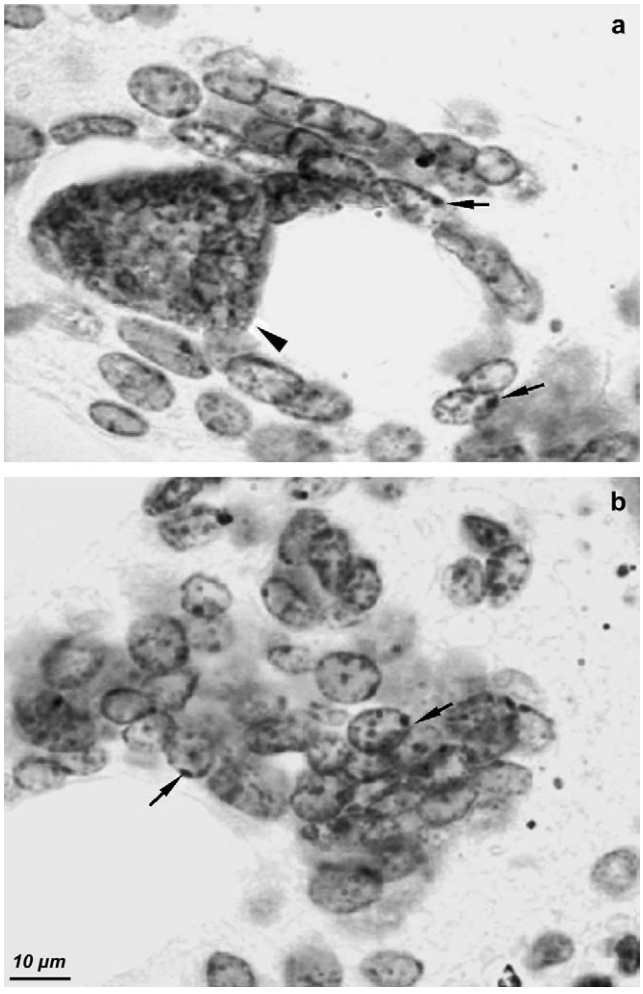

Fig. 2. Isolation of nuclear fragments inside the initial nucleus of the secondary giant trophoblast cells of *M. rossiameridionalis* on day 12 of gestation. a – part of the initial nucleus with progressively isolating and pinching off fragments (*arrowheads*); b – a compact cluster of nuclear fragments inside giant trophoblast cells; 1–2 gonosomal chromatin bodies are observed in the nuclear fragments (*arrow*). Feulgen-stained section.