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Cell Cycle Modification in Trophoblast Cell Populations in the Course of Placenta Formation

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1. Introduction

It is well established that the presence of the diploid chromosome set in cell nuclei of the majority of tissues is a characteristic of vertebrates. This peculiarity was supplemented by the rule of the relative constancy of DNA in nuclei with diploid chromosome set (Boivin et al., 1948). However, some cells contain doubled or multiplied chromosome set as compared to other cells of the organism or other individuals of the species. As distinct from the generative polyploidy in which polyploidy covers all cell types including gametes, somatic polyploidy extents to cells of several tissues (Brodsky, Uryvaeva, 1985).

At present there are numerous data that the cells of some tissues of animal and plants appear to be polyploid. The incidence of polyploidy in the tissues of animals and plants as well as levels of ploidy is described in detail in quite a few books and reviews (Geitler, 1953; Tschermak-Woess, 1971; Nagl, 1978, 1981, 1995; Brodsky, Uryvaeva, 1985; Raikov, 1982; Zybina, 1986; D'Amato, 1989; Zybina, Zybina, 1996; Edgar, Orr-Weaver, 2001; Ravid et al., 2002). A large body of data on incidence of polyploidy in animal and plant tissues enabled W. Nagl to make a conclusion that polyploidy is a widespread phenomenon characteristic of the overwhelming majority of eukaryotes (Nagl, 1978; 1981; 1995). According to Nagl, Vertebrata represent a specific taxon that differs from other eukaryotes in the extent of polyploidy among the differentiated cells. Thus, in insects and higher plants, nearly all normal, differentiated cells are polyploid. The majority of Mammalia and, most probably, other Vertebrates, are characterized by the diploid chromosome set in most cells including both proliferative and the tissue-specific cells. Polyploidy in Vertebrata is present in many cell types, i.e., hepatocytes, cardyomyocytes, megakaryocytes and some others (Brodsky, Uryvaeva, 1985; Hancock et al, 1993; Kudryavtsev et al, 1993; Nagl, 1995; Anatskaya et al, 2001). It suggests that propagation of vertebrates is, probably, due to an advantage of the mitotic cycle that involves a machinery of accurate reproduction of diploid cells over other cell reproduction cycles that result in polyploidization. It cannot be ruled out that the biological advantage of mitosis has made it possible the wide distribution of Vertebrata. Nevertheless, genome multiplication characteristic of wide range of highly differentiated cells in different taxons suggests an undoubtedly important biological significance of polyploidy in the lifespan of some specific cell types.

Most of suggestions consider polyploidy as a factor promoting some tissue-specific functions of some cell types (Nagl, 1978, 1985; Brodsky, Uryvaeva, 1985; Edgar, Orr-Weaver, 2001; Lee et al., 2009). In some mammalian tissues the number of polyploid cells increases under conditions of the physiological stress: in cardiomyocytes in heart disease (Rumyantsev, 1991); in the aorta vascular smooth muscle cells of hypertensive animals (Owens, Schwartz, 1982; Ravid et al., 2002; Hixon, Gualberto, 2003; Nagata et al., 2005; Gui et al., 2007); in the smooth muscle cells of myometrium during pregnancy (Heiden, James, 1975), in thyrocytes and cells of the adrenal gland with age (Auer et al., 1985; Ravid et al., 2002). Besides, an increase in proportion of polyploidy cells correlates with aging and cell senescence (Yang et al., 2007; McCrann et al., 2008; Celton-Morizur, Desdoutes, 2010). Switching to polyploidy, as a rule, results in loss of capability for cell divisions that is also a characteristic of the differentiated cells. An increase of cell size leads to a change of cell metabolic characteristics. Switch to endoreduplication cycle in which cell growth is gained without periodic reorganization of cytoskeleton and cell contacts, concomitant to the entrance into mitosis allows cell to continue cell growth and proliferation without prominent structural transformation. Such a way of cell reproduction may be important for the highly specialized cell types. Therefore, it worth considering different types of cell cycle modification as possible ways to gain different levels of genome multiplication characteristic of different cell types of animals and plants.

1.1 Abbreviations

AER – agranular endoplasmic reticulum, GER – granular endoplasmic reticulum, NE – nuclear membrane; AL – annulate lamellae; BB - Barr body; GCB – gonosomal chromatin body

2. General considerations on the somatic polyploidization mechanisms

The clearest notions on ways of somatic polyploidization were developed basing on the cell cycle concept (Howard, Pelc, 1953; Nagl, 1978; Brodsky, Uryvaeva, 1985; Edgar, Orr-Weaver, 2001; Lee et al., 2009).

2.1 Restitution (uncompleted) mitoses

Switching off the last step of mitosis – cytotomy – may be the first step to polyploidy: binucleate cell is formed, each nucleus containing diploid chromosome set. In the next cell cycle and mitosis two metaphase plates may unite forming the common plate. If the mitosis comes to the end, it results in two mononucleated cells with tetraploid chromosome set. Such cycles may repeat and lead to formation of mono- and binucleate cells of the higher ploidy level:

$$2c – (2c \times 2) – 4c – (4c \times 2) – 8c – (8c \times 2)$$

Such a ploidy level was proved by V.Ja. Brodsky basing on the dynamics of transition of mono- and binucleate cells of different ploidy using combination of cytophotometry and ^{3}H-thymidine labeling of DNA replication (Brodsky, Uryvaeva, 1985). Acytokinetic mitoses with subsequent polyploidizing mitosis is rather widespread way of polyploidization, for example, in liver, myocardium, retina pigment epithelium (Rumyantsev, 1982). Similar ways were described later by using time-lapse video images in the aortic vascular smooth

muscle cells, however, as one of different ways of polyploidy genome gaining (Nagata et al., 2005). An essential point, in our opinion, is "mitotic origin" of polyploid cells: rise of a polyploid nucleus requires a diploid cell to enter mitosis.

Block of mitosis may occur somewhat earlier – at the prophase, metaphase, and anaphase. The cells reaching these phases then undergo chromosome despiralization and return into interphase. As the chromosome do not move to the poles, the cells retain the doubled chromosome set and become polyploid. Such a cycle was called the restitution cycle (Nagl; 1978; 1995) or the incompleted polyploidizing mitosis (Uryvaeva, 1979). Prophase restitution was described in the root tip cells of onion *Allium cepa* and *A. carinatum* (Nagl, 1978). In case of prophase restitution, the cells enter prophase, then return to interphase via telophasic decondensation.

Metaphase restitution was found in the onion root tip cells in which mitosis was blocked by colchicines; spontaneous metaphase restitution was observed in the pea endosperm (Nagl, 1978). The restitution mitoses can be recognized by overcontraction of chromosomes, their enhanced stickiness and failure of ordered arrangement being due to misfunction of the spindle (Nagl, 1978).

Anaphase restitution was also observed in the onion root tip cells under the colchicine treatment, and spontaneously – in the suspensor of *Lathyrus latifolius* (Nagl, 1978). In this case of chromosome bridges, these are probably responsible for non-disjunction of the daughter nuclei. In the next G1-phase a cell with a dumbbell-like nucleus or a binucleate cell with tightly attached nuclei is formed. Metaphase and anaphase restitution were also described in cancer cells (Therman et al., 1986).

Uncompleted polyploidizing mitoses were also found in the decidual cells in the rat endometrium – both in the normal pregnancy and in the induced deciduomata (Zybina, 1986). Uncompleted mitosis with delays of its middle phases was found in the vertebrate tissue, in particular, in megakaryocytes and cancer cells (Brodsky, Uryvaeva, 1985; Nagl, 1995).

Spontaneous polyploidization in human tissues often includes different types of restitution mitoses that seem to result from disorder in many mitotic events; these events also including reverse cytokinesis. Thus, the hypertensive aortic vascular smooth muscle cells (VSMC) at the time-lapse video showed delayed mitosis as compared to the normal one (Nagata et al., 2005). In most cases, sister chromatids were normally segregated and the cleavage furrow was also normally formed, but cytokinesis progression was stopped. Thereafter, reversion of cytokinesis occurred, and ultimately two nuclei were reversed into a single binucleate cell. In some cases, the cleavage furrow was formed again, and finally the VSMC were divided into two daughter cells. In other, rare, cases (4 times per 100 time-lapse images), there occurred exit from mitosis without cell division that resulted in polyploid mononuclear cell formation. In these cases, the cleavage furrow was formed even before sister chromatid disjunction and/or segregation that resulted in chromosome separation into two parts, the parts being bound by a chromosome bridge. These mitoses resulted in polyploid cells with single irregular-shaped lobular nuclei (Nagata et al., 2005). At last, there occurred some mitoses with a shallow cleavage furrow and missegregation of sister chromatids; thereafter the furrow disappeared and mononuclear polyploid cell with round nucleus was formed. Immunolabelling of β- and γ-tubulin proved that the cell retained mitotic spindle and centrosomes that allow mitosis progression, Aurora B was also found to be normally expressed, but another chromosome passenger protein Survivin was downregulated. Lack

of Survivin expression was, probably, the reason for disturbance of the Aurora B/Survivin complex formation, Aurora B kinase dysfunction and failure of chromosome movement leading to polyploidization. Thus, failure of expression of one regulator of mitotic machinery may exert a pleiotropic effect on the proceeding and result of mitosis. Such a pleiotropic effect is the reason for various mitotic modifications and aberrations in cancer cells (Therman et al., 1986; Therman, Kuhn, 1989) and for numerous cases of spontaneous and induced mitotic restitution (Nagl, 1978).

Regulation of the tissue-specific restitution cycle is not quite clearly elucidated. At present, processes of transition to polyploidization similar to uncompleted mitoses are considered as overcoming of mitotic spindle assembly checkpoint (Cahill et al., 1998; Gualberto et al., 1998). The former delays the onset of anaphase and the exit from mitosis. The mitotic checkpoint accomplishes its function by controlling activity of the cell cycle regulatory machinery composed by the Mitosis Promoting Factor (MPF), the Anaphase Promoting Factor, and proteasome. MPF is a protein complex that consists of a cyclin-dependent kinase $p34^{cdc2}$, cyclin B, and associated proteins (Hixon, Gualberto, 2003). MPF induces formation of a mitotic spindle by phosphorylating components of the mitotic spindle including microtubule-based motor proteins. Cyclins B are known to be involved in the microtubule metabolism (Nurse, 1991; Ookata et al., 1995). Degradation of CyclinB/$p34^{cdc2}$ correlates with entrance into the normal ana- and telophase (Lehner, O'Farrel, 1990; Holloway et al., 1993). The Anaphase Promoting Complex (APC) and Proteasome control metaphase-anaphase transition and the exit from mitosis. At the onset of anaphase, the activity of separins contribute to release cohesins from chromosomes, allowing for segregation of bivalents to opposite poles. Separin activity is blocked by securins. At the karyokinesis the APC-complex promotes destruction of secutins and cyclin B leading to chromosome segregation and cytokinesis. Therefore, mutation in $p34^{cdc2}$ in the phosphorylation sites blocks mitosis progression (Compton et al., 1995).

Some data about regulation of transition to polyploidization cycles were obtained on the model of VSMC. The aortic VSMC of mice genetically prone to hypertension were treated with colcemid to estimate effect of the spindle checkpoint mechanism. While VSMC from normotensive animals were able to arrest the cell cycle progression in mitosis, VSMC isolated from hypertensive animals re-entered the cell cycle in the absence of chromosomal segregation and undervent polyploid rounds of DNA synthesis. While VSMC of normotensive rats accumulated Cyclin B (mitotic arrest) in response to a mitotic spindle inhibitor, unscheduled degradation of Cyclin B was observed in VSMC isolated from capacitance arteries of spontaneously hypertensive animals (Hixon, Gualberto, 2003). In this case, exit from mitosis may proceed in the absence of chromosome segregation and karyokinesis thereby leading to cell cycle re-entry and polyploidization.

2.2 Endocycles

More profound reduction of mitotic cycle, as compared to uncompleted mitosis, results in *endocycles*. This term covers all phenomena of genome multiplication that are accomplished without nuclear envelope disappearance (Nagl, 1978; 1981; 1995; Therman et al., 1983; 1986; Edgar, Orr-Weaver, 2001). The endocycles include *classic and angiospermal endomitosis*, and *endoreduplication or polyteny* (Geitler, 1953; Nagl, 1978; 1995; Kiknadze, Istomina, 1980; Zybina, Zybina, 1996; 2005).

2.2.1 Endomitosis

In the course of the classic endomitosis, chromosomes undergo successive modifications similar to that in mitosis, but without nuclear envelope disappearance, metaphase plate arrangement, and anaphase movement. In this case, there occur chromosome condensation, sister chromatid separation that is followed by their decondensation (Geitler, 1953; Nagl, 1978; 1995). Morphology of the endomitotic chromosomes is somewhat different from the mitotic ones by less sharp contours.

Classic endomitosis was described in invertebrate tissues: in a Heteropteran *Gerris lateralis* (Geitler, 1953); in the parietal cells of testicular follicle of grasshoppers (Kiknadze, Istomina, 1980), in the albumen gland and neurons of the snail *Succinea lauta* (Anisimov, 1997 a, b, c; Anisimov, Kirsanova, 2002; Anisimov, 2005). Recently, the latter case was studied in detail. In the transition from endointerphase to endoprophase and, then, to endometaphase, nuclear envelope was retained, autosomes did not reach the complete shortening and thickening like in mitosis; meantime, the spindle and chromosome movement were not observed. In endoanaphase there occurred splitting of chromosomes into chromatids without spindle formation. In the transition to endotelophase and endointerphase the split chromatids stayed to lie in parallel, meantime undergoing progressive decondensation, so in endointerphase, numerous paired chromocenters were seen in the nucleus. In DNA synthesis study using ^{3}H-thymidine, the cycle of endomitosis was revealed, and duration of phases of endointerphase – G1, S, and G2 – was determined (Anisimov, 1997a). Each phase of endomitotic cycle was characterized by specific chromosome structure and arrangement, and in G1-, S-, and G2-phases, numerous chromocenters were observed, the degree of their condensation being characteristic of each phase of the endointerphase.

Endomitosis was also observed in the trophoblast of placenta and hydatidiform mole (Sarto et al., 1982; Therman et al., 1983, 1986). In these cells there occurred pictures of endomitosis, the degree of chromosome condensation differing in different cells. In the ^{3}H-thymidine-labelled cells, paired endomitotic chromosomes were seen, their condensation was less pronounced than in the unlabelled cells. Therefore, at the endointerphase, a partial chromosome decondensation and replication occurred (Therman et al., 1986); different chromosome regions probably were decondensed at different intervals.

The transcriptional activity of endomitotic nuclei appeared to be sufficiently high (Anisimov, 1997c). The highest ^{3}H-uridine labelling in snail tissues was observed in G1-, S-, and G2-phase, i.e., during the whole endometaphase. In endointerphase and endoprophase the ^{3}H-uridine labeling decreased up to minimum, so that in endometaphase it accounts for 4% of the endointerphase level. In endotelophase a fast increase of the ^{3}H-uridine labeling was observed. We can conclude that transcriptional activity persists throughout the whole endomitotic cycle, but undergoes cyclic transformation, so that at the endometaphase it is minimal. Electron microscopic observation also demonstrated a certain degree of chromosome condensation at all endomitotic stages in cells of the albumen gland of the snail. The uncomplete condensation is accompanied by the radial looping of thin chromatin threads running from the endochromosome axis; perichromatin granules were seen at the loops. Such an organization of endochromosomes resembles lampbrush chromosomes found in diplotene oocytes in many vertebrates (Callan, 1986). The extent of looping also seems to undergo the condensation cycle: maximal number of loops was observed at endointerphase, minimal – at endometaphase (Anisimov, 1997b, c). Thus, classic endomitosis represents a variation of the cell cycle that retains cycle of replication,

chromosome condensation, and transcriptional activity; however, this cycle is "shortened", some events corresponding to mitosis are omitted.
It seems to be of interest that throughout the endomitotic cycle not only the nuclear envelope, but also nucleolus persists; the level of transcription activity of nucleolar organizer varies at different phases of the endomitotic cycle (Anisimova, Anisimov, 2002). The nucleolar activity also varies depending on the functional state of the cell, in particular, of the differentiation rate and direction (Anisimova, Anisimov, 2002).

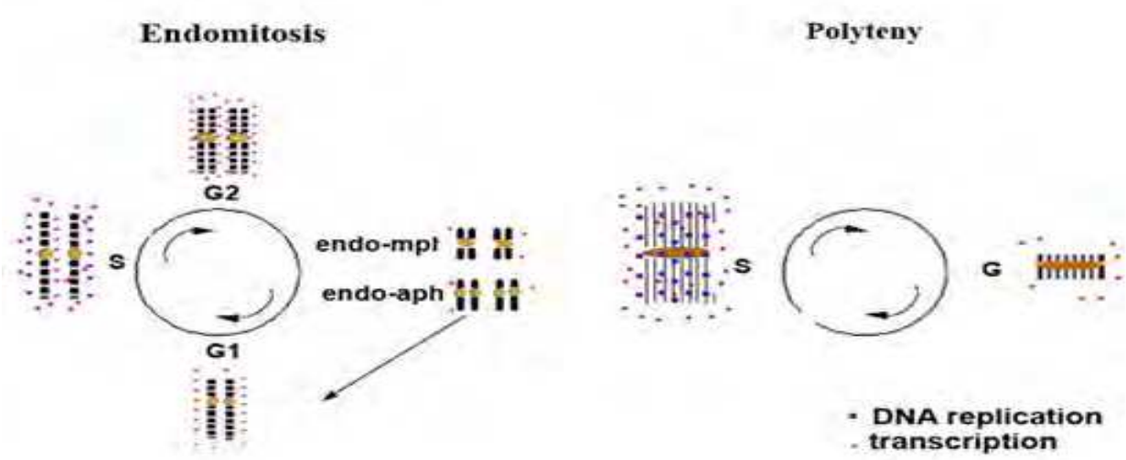

Fig. 1. Scheme of endocycles in endomitosis and polyteny. In the endomitotic cycle replication, chromosome condensation/decondensation and transcription are retained; replication is accomplished at the S-phase; transcription is active at the G1-, S, and G2-phases. In endometaphase (endo-mph) and endoanaphase (endo-aph) endochromosomes condense and transcription declines up to minimum; sister chromatid segregation takes place at the endoanaphase. Arrow indicates transformations of one pair of sister endochromosomes in the course of the endomitotic cycle. In polyteny chromosome replication is accomplished in S-phase; cyclic changes of chromosome condensation and transcription take place; chromatid segregation is not observed.

Apart from the classical or "insect-type" endomitosis, the so-called "angiospermal endomitosis" was observed in cells of many higher plants (Tschermak-Woess, 1971; Nagl, 1978). In this case the cyclic chromosome condensation appears to be much lower (rudimentary) than in the insect-type endomitosis. Therefore, it is rather difficult to detect different endomitotic phases as compared to the classic endomitosis.
It is to be specified that endomitotic pictures do not always reflect genome multiplication via endocycle progression. In cells of the testicular follicle of grasshoppers, all endomitotic stages were detected morphologically. Nevertheless, DNA synthesis in these cells was not detected at the stages when the phases of endomitosis can be seen (Kiknadze, Istomina, 1980). By contrast, these cells show intensive transcription by the ^{3}H-uridine labeling. Thus, in such a case, endomitotic chromosome appears to be a specific form of the polyploid nucleus with high transcription activity without genome multiplication (Kiknadze, Istomina, 1980). It can be explained, for example, by the specific differentiation of the testicular follicle cells, in which they lose the DNA replication cycle, but accomplish their tissue-specific function that implies high protein synthesis.

Interestingly, in this case, endometaphase chromosomes resemble the organization of lampbrush chromosomes. ^{3}H-uridine label was concentrated over the loops projected from the central part of endochromosomes (Kiknadze, Istomina, 1980). Such a peculiarity makes endomitotic chromosomes of grasshopper similar to other examples of the classic endomitosis.

To explain the difference between these two "endomitoses" – replicative and non-replicative one, it is of interest to mention that in the snail protein gland, DNA replication was not detected at the terminal stage of differentiation – despite the presence of typical endomitotic figures (Anisimov, 1997a). However, the level of ^{3}H-uridine labeling was decreased significantly as compared to the earlier, "replicative" stages in the lifespan of the cells.

To sum up, endomitosis represents a specific type of cell cycle, in which some degree of chromosome condensation and transcription is maintained throughout the whole endocycle (Fig.1). Therefore, endomitosis may be considered as a specific form of transcriptionally active nucleus (Brodsky, Uryvaeva, 1985). It is strengthened by the similarity of the endomitotic chromosomes with lampbrush chromosomes characteristic of the diplotene of the I meiotic division (Callan, 1986). Lampbrush organization and uncomplete condensation suggest that endomitosis is a way to gain highly specified cell types with high transcriptional activity of tissue-specific chromosome loci; this transcriptional activity may be important for the special function of the cells.

The classic endomitosis may be of doubtless interest due to its occurrence in malignant cells, first of all, in cervical cancer and complete hydatidiform moles (Therman et al., 1982; 1983; 1986).

2.2.2 Polyteny

The most shortened cycle that consists of merely two phases – presynthetic G and synthetic S – is *endoreduplication* that results in *polyteny* and *G2-block* (Pearson, 1974; Nagl, 1978, 1995; Brodsky, Uryvaeva, 1985; Zybina, 1986; Zybina, Zybina, 1996, 2005; Brodsky, Uryvaeva, 1985; Edgar Orr-Weaver, 2001).

According to V. Brodsky and I. Uryvaeva (1985), in G2-block and polyteny the cell cycle is devoid of the whole mitosis; therefore, the pre- and postmitotic phases are united. In this case the sister chromatids do not separate, each chromatid beginning a new round of replication in the new S-phase. As a result of a series of successive endoreduplication cycles, polytene chromosomes are formed.

Control of endoreduplication cycle is not elucidated completely. However, it is certainly associated with cessation of expression of cyclins A and B that are necessary for mitosis progression (Nagl, 1995; Edgar, Orr-Weaver, 2001). Nevertheless, the detailed study of expression of cyclins, cyclin-dependent kinases as well as other proteins regulating endocycle progression prove that endocycle does not imply the complete switching off the components of the mitotic machinery. The switching from mitotic cycle to endocycle itself appears to be impossible without expression of factors regulating transition through mitotic phases. In switching from mitosis to endocycles, cells in the Drosophila embryo downregulate the mitotic regulators Cdk1, Cyclin A, cyclins B and B3 as well as Cdc25/string (Sauer et al., 1995, Edgar, Orr-Weaver, 2001). Meantime, studies on Fzr/Cdh1, a regulator of APC, show that APC activity is required for switch from mitotic cycles to endocycles (Edgar, Orr-Weaver, 2001). A series of investigation proved that initiation of endocycles required expression of E cyclins (Knoblich et al., 1994; Lilly, Spradling, 1996; Su,

O'Farrell, 1998; Edgar, Orr-Weaver, 2001). Besides, it is oscillatory (i.e. cyclic) expression of cyclin E that is required for successive endocycles in Drosophila tissues, i.e.ovarian nurse cells, embryonic gut, and embryonic larval tissues (Lilly, Spradling, 1996; Su, O'Farrell, 1998; Edgar, Orr-Weaver, 2001). In the endopolyploid murine giant trophoblast cells the oscillation of cyclin E expression was not demonstrated; in this case, fluctuation of Cdk2 occurs due to the periodic activity of the S-phase inhibitor $p57^{kip2}$ (Hattori et al., 2000; Hu, Cross, 2010). Therefore, endocycle retains the cyclic expression of several components and regulators of the mitotic cell division machinery.

It seems to be of importance that the degree of polytene structure widely varies, and three main types may be distinguished: (1) classic polyteny with characteristical band structure, for example, in the salivary glands of Diptera (Beermann, 1972; Kiknadze et al., 1976; Zhimulev, 1992); (2) the cell with uncomplete expression of polytene features, for example, in the giant trophoblast cells (Zybina, Zybina, 1996, 2005), in the suspensor of higher plants (Nagl, 1978, 1981), in trophocytes of insect ovaries (Dej, Spradling, 1999), and in various endocycles of Invertebrata (Nagl, 1978); (3) the cells, in which the polytene features are not revealed, and polyteny may be suggested basing on the multifold DNA replication in the absence of mitosis (Brodsky, Uryvaeva, 1985).

At present it is considered that morphological and functional features of the classical polytene chromosomes are accounted for by the following factors. First, the homologous chromosomes are bound due to the somatic conjugation. Therefore, the cells with polytene chromosomes show the haploid chromosome number. Second, in the course of successive endocycles the daughter chromatids stay bound to the initial ones; as a result, accumulation of the bound chromatids leads to thickening of the chromosomes. Third, the chromosomes are characterized by the weakening of the cycle of spiralization-despiralization characteristic of mitotic cycle; the degree of spiralization corresponds to prophase or interphase. It is probably the reason of their significant length that exceeds many times the length of mitotic chromosomes. The tightness of chromatid attachment in the polytene chromosome, most probably, also correlates with the degree of their despiralization and depends on their degree of ploidy (Ashburner, 1972; Kiknadze, 1972, Nagl, 1978).

Non-classic polytene chromosomes are well-studied in the higher plants. They were discovered in the highly polyploid nuclei of the provisory cells important to embryo development: antipods, synergids, cells of suspensor and angiospermal haustorium (Geitler, 1953; Tschermak-Woess, 1971; Hasitschka, 1956, 1957; Hasitschka-Jenschke, 1957, 1959; Nagl, 1969, 1978). Somatic conjugation probably is not obligatory for these chromosomes. As a result, nuclei of suspensor of several plants contain diploid number of chromosomes, in rare cases – the haploid one (Geitler, 1953; Nagl, 1978; Tschermak-Woess, 1971, 1973). In general, polytene chromosomes are composed of loosely attached cable-like structures, their ends being split more or less (Nagl, 1981). Their disc pattern is less regular than in classical polytene chromosomes because of not very tight attachment of chromatids. In many cases, chromatid attachment is not observed at the full length of the chromosome.

The degree of chromatid attachment may widely vary at different lifespan steps and depends on many factors including physiological state of the cell. In particular, it depends on the stage of polytene chromosome formation, i.e., the number of replication rounds. At the initial endocycles, chromatid attachment is restricted by the pericentromeric heterochromatin blocks; in the euchromatic regions, chromatids are separated to a significant distance. Each subsequent endocycle makes polytenic structure more clear-cut, and the "well-developed" polytene chromosomes can be identified precisely, although their

separate loci are less distinguishable than in the classical polytene chromosomes (Nagl, 1978).

Besides, the degree of condensation depends on the stages of polytene nucleus cycle. In the highly endopolyploid cells of suspensor at the stage of replication chromonemes decondense and disjoin at a significance distance (Avanzi et al., 1970; Brady, Clutter, 1974). Disjunction of chromatids and their decondensation is observed in euchromatin and β-heterochromatin regions, whereas α-heterochromatin region stays condensed and retains chromatid attachment throughout the whole cycle of polytene nucleus (Brady, Clutter, 1974).

Transition to the endoreduplication (polytene) cycle that may be necessary in some specific cases. Thus, as pointed out by B. Edgar and T. Orr-Weaver, the endocycles, once initiated further mitotic divisions are "ill advised for mechanical reasons". Indeed, polyteny is present in the highly differentiated cells. Besides, polyteny allows cells to increase their mass and metabolic output. Another possible advantage of endoreduplication is that it allows combining fast growth of the tissue with its functioning. In this connection, a very important consideration is that transition to endocycles allows growth without periodic rearrangement of cytoskeletal elements or cell-cell contacts, as happens in mitoses, and it is less disruptive to highly structured tissues than in mitotic proliferation (Edgar, Orr-Weaver, 2001). All this seems to be appropriate to all kinds of endocycles. In this connection, polyteny appears to be the most advantageous to gain the highest possible ploidy levels (Nagl, 1978, 1981). By contrast, the lower ploidy levels are generally achieved via uncompleted mitoses, these cycles being more characteristic of mammalian tissues, i.e. in hepatocytes (Brodsky, Uryvaeva, 1985; Kudryavtsev et al., 1993; Celton-Morizur, 2010), megakaryocytes (Ravid et al., 2002); cardiac myocytes (Pfitzer, 1971; Rumyantsev, 1991; Anatskaya et al., 2001).

Meantime, it seems to be important that, in some cases genome multiplication implies entrance mitosis, in the other cases genome reproduction is achieved without the nuclear envelope disappearance. The biological significance of this difference, in our opinion, is demonstrated by the occurrence of different genome multiplication ways and their mutual transition in the trophoblast of mammalian placenta.

3. Cell cycle modification in trophoblast cell of mammalian placenta

The trophoblast cells of the rodent embryos represent an example that in the functionally different cell populations of the common origin have different ways of genome multiplication. The level of ploidy clearly correlates with the ability of cells to invade endometrium, to lyse and to phagocytose partly its tissues in the course of embryo implantation. Beginning from the onset of differentiation, the primary and secondary giant trophoblast cells undergo a series of endoreduplication cycle reaching very high ploidy level – 512c–1024c in rat and mouse (Zybina, Mosjan, 1967; Barlow, Sherman, 1972 ; Zybina, Zybina, 1996, 2005); 2048c and 16384c in the field vole (Zybina et al., 1975; Zybina et al., 2003; Zybina et al., 2009). The cells have the non-classical polytene chromosomes and can combine cell replication with the invasive and phagocytic activity (Zybina, Zybina, 2005).

3.1 Polytene nucleus cycle in the secondary giant trophoblast cells

Two phases – endointerphase (S) and endoprophase (G) – were determined in the polytene cycle of the secondary giant trophoblast cells in rat placenta by using ^{3}H-thymidine labeling (Zybina, Zybina, 1996, 2005). In endoprophase non-classic polytene chromosomes in a form

of bundles of quite condensed chromatids of chromomeric structure are observed (Fig. 2b). The bundles are attached to the nuclear envelope. At the endointerphase nucleus is filled by paired thin Feulgen-positive threads, the ″threads″ being scattered throughout the caryoplasm and bound to the nuclear envelope (Fig. 2a). The minimal chromosome condensation characteristic of the interphase nucleus is observed at the endointerphase; however, some chromosome regions are visualized at this phase. Thus, parallel chromosome threads with characteristic disc pattern can be observed in the site where chromosomes penetrate nucleolus (Fig. 2a). Later on, three phases of endoreduplication were discerned – G1, S, and G2 – based on the S-phase inhibitor $p57^{kip2}$ (Hattori et al., 2000).

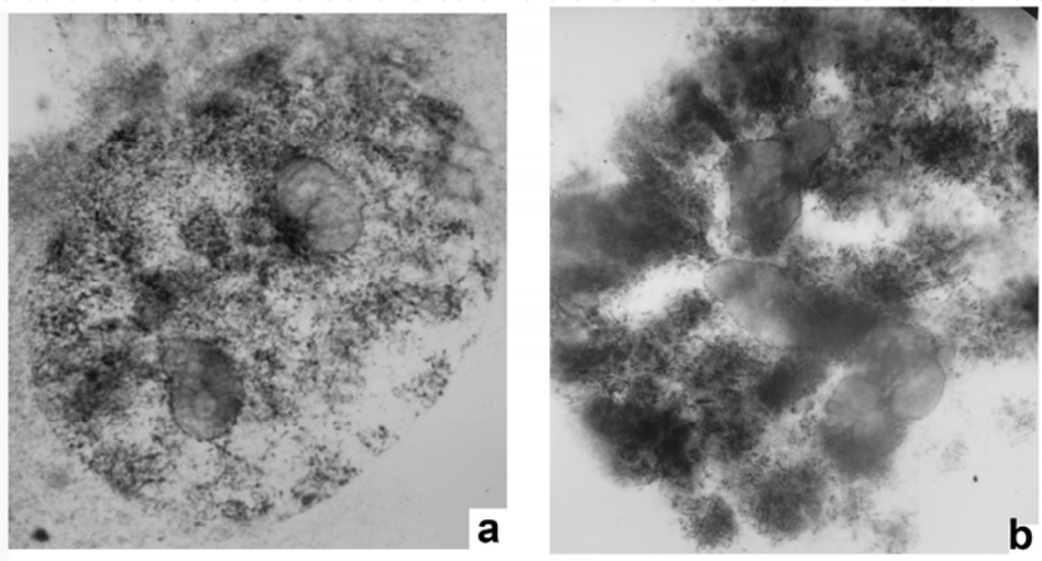

Fig. 2. Polytene chromosomes in the rat secondary giant trophoblast cell. A. endointerphase, b. endoprophase.

Two phases of polytene cycle are also observed in the mouse and rabbit giant trophoblast cells. In endoprophase some nuclei showed lampbrush-like structure (Fig. 3a, b): loops are running from the heterochromatic condenced region. At the phase contrast preparations, polytene chromosomes look like the long threads attached to the nuclear envelope (Fig. 3c).
DNA replication takes place at the endointerphase, as this was demonstrated by using both authoradiography (Zybina, 1986) and cytophotometry (Zybina et al., 1985). The level of ploidy of "meshwork" nuclei (Fig.2a) was 16c–256c, i.e., somewhat lower than in nuclei with chromosome bundles (Zybina, Zybina, 1985;1996). In particular, it may be accounted for by the presence of a number of nuclei, in which polytene chromosome bundles were not yet formed: the clear-cut bundles are observed beginning from 64c. In the group of nuclei of "polytene" type (Fig. 2b) the sharp peaks corresponding to ploidy classes 64c, 128c, 256c, and 512c are observed. By contrast, in the group of "meshwork" nuclei there are many intermediate values that correspond to DNA synthesis. The intermediate nuclei are also characterized by sharp peaks, but ploidy level is lower than in ″bundles″: the nuclei probably correspond to the process of decondensation of polytene chromosome bundles or the early endocycles when polytene structure is yet underdeveloped. The data confirm that endoreduplication cycle includes the cycle of polytene chromosome condensation, the maximum condensation level being in the endoprophase.

The rate of the polytene cycle proved to be rather high, it is especially high at the implantation period. Using DNA cytometry, it was demonstrated that at the 5-7 day of gestation in mouse some of the trophoblast cells undergo 3 cycles reaching 32c and 64 c. Thereafter, a decrease of polyploidisation is observed with subsequent constant rate of polyploidization: one endoreduplication cycle per day (Barlow, Sherman, 1972; Zybina, 1986); according to autoradiographic data, duration of S-phase in rat trophoblast cells is about 6 h, which is approximately equal to duration of S-phase in diploid cells. However, duration of G-phase upon polyplodidzation increases from 8.3 to 24.9 h for 12-15 days of gestation (Andreeva, 1964). Meantime, there also may be an increase of S-phase duration from 45-h to 6-7-h upon the endoreduplication progression (Zavarzin, 1967).
An increase of endoreduplication cycle duration was also detected in the case of classic polyteny of Drosophila (Rudkin, 1972). In the Drosophila salivary glands S-phase lengthens upon polyploidization from 2^{10}n to 2^{11}n by 20% (Gunderina et al, 1984). However, the highest ploidy levels are mainly achieved via endocycles.

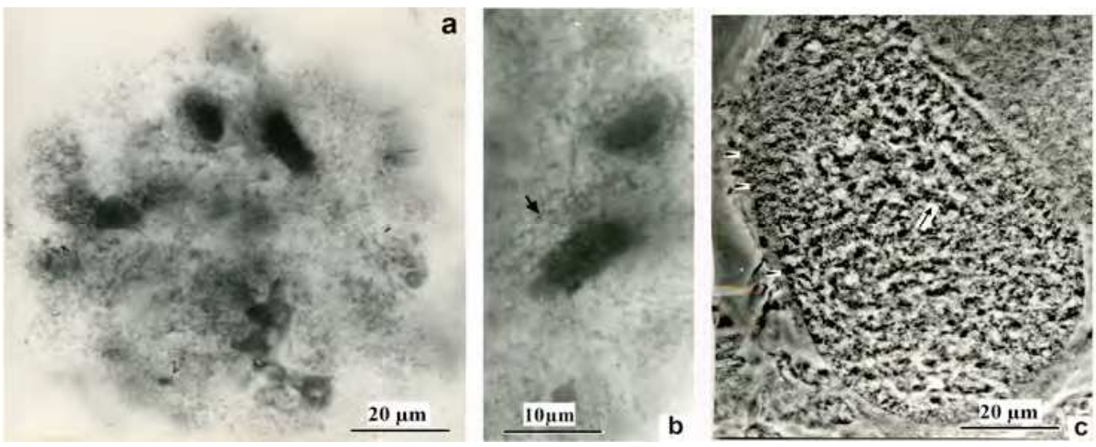

Fig. 3. Polytene chromosomes in the mouse (a, b) and rabbit (c) secondary giant trophoblast cells. (a) – non-classic polytene chromosome bundles; (b) – lampbrush chromosome-like structure: the loops (arrows) run out of the condensed part of the chromosome bundle; c – polytene chromosome bindles seen as parallel chromosome threads (arrow). a, b – acetoorcein staining, c – unstained preparation at phase contrast.

Both endointerphase or endoprophase are characterized by a certain level of transcriptional activity. However, in endoprophase the RNA synthesis is decreased (Zybina, 1963). Therefore, like in endomitosis, transcriptional activity is maintained, but oscillates in the course of the whole endoreduplication cycle. The nucleolus also persists throughout the cycle. According to the electron microscope study, the volume of the granular component that reflects the amount synthesis and accumulation of preribosome particle is higher at endointerphase; at this phase the number of fibrillar center that reflects the number of site of rRNA synthesis is also higher than at endoprophase (Zybina, 1986; Zybina, Zybina, 1996). In the highly differentiated giant trophoblast cells that exits from the cell cycle (the ^{3}H-thymidine labeling is not seen any longer), the polytene chromosome bundles become indiscernible, probably, due to weakening of the sister chromatid attachment. However, in this case, transcriptional activity does persist, and the transcriptionally active nucleolus may be observed (Zybina, 1986; Zybina, Zybina, 1996), although a decrease of granular

component and fibrillar center amount prove the attenuation of the preribosome synthesis and processing. In this case, however, two types of nuclei that differ in the degree of chromatin condensation also are observed; they are also different in the transcriptional characteristics of the nucleolus (Zybina, 1986; Zybina, Zybina, 1996). Therefore, in the absence of DNA replication the polytene cycle is maintained, it is reflected, at least, in the variation of the chromosome condensation and transcription activity.

Differentiation of the giant trophoblast cells that involves exit from the regular mitotic cycle and the onset of endoreduplication is under the developmental control. In the differentiation of giant trophoblast cells from the Rcho-1 choriocarcinoma cell line the downregulation of *Id1* and *Id2* occurs, being concomitant with upregulation of the basic helix-loop-helix factor *Hxt* and acquisition of an increased adhesiveness (McAuley et al., 1998) that triggers the onset of invasive capability of the giant trophoblast cells. Cell cycle modification is triggered by switched Cyclin D isophorm expression from D3 to D1. The initiation of the S-phase during endocycles appeared to involve synthesis of cyclins E and A, and termination of the S-phase during endocycles was associated with abrupt loss of cyclins E and A. Both cyclins were absent from the gap-phase during endocycles, which suggests that their degradation may be necessary to allow reinitiation of the endocycle. The arrest of the mitotic cycle at the onset of endoreduplication was associated with a failure to assemble cyclinB/p34^{cdk1} complexes during the first endocycle. In the subsequent endocycles, the cyclin B expression was suppressed. Therefore, at the first endocycle, its regulation retains some traits of mitotic cycle and then switches to the specific endoreduplicative regulation pattern. Interestingly, in the murine giant trophoblast cell population, expression of *cyclin B1* trascripts was found at the transcriptional level both in diploid and in polyploid cells, whereas the B1 protein was detected exclusively in the diploid cells. Therefore, downregulation of Cyclin B is the key event that switches off the mitotic machinery. Meantime, *Cyclin D1* transcripts were found only in giant trophoblast cells, even prior to that of *placental lactogen I* being early marker of giant trophoblast cell differentiation. This pattern of expression probably means that mural trophoblast differentiation and endoreduplication depend on the prolonged G1-phase. The authors state that D-type cyclins are required for endocycle maintenance, keeping in mind that antisence oligonucleotids to Cyclin D3 were found to block endoreduplication in murine megakaryocytes; besides, overexpression of cyclin D3 proved significant increase of endomitosis in the megakaryocytes (Zimmet et al., 1997). The zinc finger transcription factor Snail regulates the "G_2 decision point" whether the trophoblast cells go through mitosis or enter endocycle (Nakayama et al., 1998).

The polytene cycle of the giant trophoblast cells involves periodicity of S-phase during endoreduplication. During the mitotic cell cycle, biochemical events coincident with mitosis lead to the re-setting of the origins of replication (Elledge, 1996). This includes degradation of the protein Geminin that otherwise suppresses the firing of origins of replication (McGarry, Kirschner, 1998). Geminin mutant embryos die during preimplantation development and show ectopic endoreduplication in blastomeres (Gonzales et al., 2006; Hu, Cross, 2010). Another important mechanism to maintain periodicity during the endocycle involves cyclic expression of p57^{kip2}, a G1/S Cdk inhibitor (Hattori et al., 2000). During subsequent endocycles, the p57^{kip2} level fluctuates and this identifies two phases: the endo-G2 phase with the p57^{kip2} completion of S-phase and the endo-G1-phase with p57^{kip2} declining several hours before entry into S-phase (Hattori et al., 2000). It is hypothesized that periodic expression of p57^{kip2} protein promotes alternating S and "gap" phases in the endocycle (Hu, Cross, 2010), the "gap" probably corresponding to endoprophase.

3.2 Transition between different modes of genome multiplication

Junctional zone and labyrinth trophoblast in the rat and field vole placenta represents a cell population, in which a high proliferative activity is accompanied by a multidirectional differentiation. These cells do not reach a high ploidy level characteristical of the primary and secondary trophoblast cells, they achieve the ploidy level as high as 16c and 32c (Zybina, Zybina, 2000, 2005). These cell populations are characterized by different ways of genome multiplication in development of one cell line at different stages of ontogenesis. The incidence of this phenomenon among the multicellular organisms is not studied in detail. Nagl (1978) pointed out that the first step of polyploidization was achieved via restitution mitosis. In the rat proliferative trophoblast cell populations the acytokinetic and uncompleted mitosis are characteristic of the first step of polyploidization. The meta- and anaphase restitutions also were often found in these cells (Fig. 4).

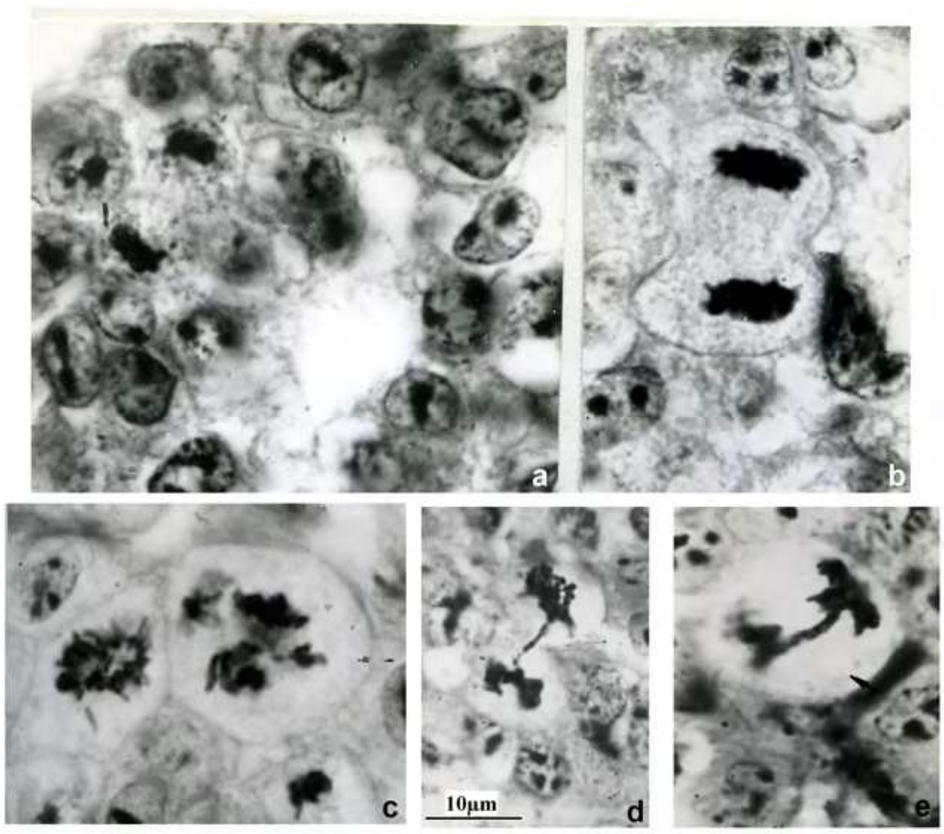

Fig. 4. Restitution mitoses in the junctional zone of the rat placenta. a, b -regular diploid (a) and polyploid (b) anaphases; c – a regular metaphase and metaphase with irregular chromosome alignment; d, e –restitutional anaphase with chromosome bridges.

Cytophotometrically among the mitotic figures of different ploidy (4c, 8c, and 16c), all mitotic stages were found – from prophase to telophase. Hence, mitosis in the junctional zone and labyrinth trophoblast can proceed up to octaploid level. Meantime, there was a noticeable predominance of earlier mitotic stages (pro- and metaphase) over the later ones (ana- and telophases) among the polyploid mitoses (Zybina et al., 2005). Among the polyploid mitotic figures, mostly restitution mitoses were present, very often anaphases

with multiple bridges were revealed, so the cells had no chance to segregate. Therefore, upon a rise of the ploidy level, the ability to undergo the complete mitosis falls down. Besides, it is to be emphasized that upon cessation of the mitotic activity their cycles of replication do not stop. The cells continue to uptake ^{3}H-thymidine and reach the ploidy level 32c, sometimes 64c and 128c via endoreduplication (Zybina et al., 2000, 2005).

Transition from the regular mitosis to the uncompleted one and then – to endocycle was described in several tissues of snail (Anisimov, Kirsanova, 2002). In the course of neuron development of the snail *Succinea lauta* in the dorsal bodies and procerebrums besides the usual mitoses there occurred metaphases resembling k-mitoses with chromosome disorientation in metaphase. The chromosome arrangement showed sparseness that made possible to count chromosome number. In anaphase there occurred chromosome lagging and bridges as well as lack of segregation to the poles. All this indicates progression of restitution mitoses. The cycle resulted in the cells 4c, 4c/2n, and 4c/4n, where c is ploidy determined by DNA content, and n – ploidy determined by the chromosome number counting. In the course of differentiation of neurons there occurred transition to the endomitosis cycle that resulted in nuclei 32c-64c and later – 512c and higher (Anisimov, Kirsanova, 2002). The data confirm the regularity found in the trophoblast cell whose initial steps of polyploidization (mainly up to 8c) are achieved by uncompleted (restitutional) mitoses, then switch to endocycles.

It is to be mentioned that the trophoblast cell population that starts their genome multiplication via uncompleted mitoses does not prove invasive properties. As soon as they move to the border with maternal tissues (decidua basalis) or penetrated endometrium, they lose their capability for mitosis and DNA replication and reside inside the semiallogenic tissue in a form of a polyploid cells (Zybina, Zybina, 2005). Arrest of mitosis and complete repression of DNA replication after a series of endoreduplication cycles makes hardly probable the renewal of mitotic activity in the deeply invading tertiary giant trophoblast cells, thereby preventing the possibility of their ectopic expanding in the maternal tissues during the normal pregnancy.

3.3 Genome multiplication in the trophoblast cells of carnivore and ruminants

Trophoblast in the hemochorial placenta of silver fox and mink is also highly invasive. Among the trophoblast cells of fox and mink, polyploid cells prevail, the level of ploidy being mainly 2c-64c, the cells with highly polyploid nuclei 128c and 256c being rare (Zybina et al., 1992, 2001).

The common ways of polyploidization appear to be the same as in the rodent placenta. The nuclei reach 4c-8c via restitution mitoses, whereas the higher ploidy levels are achieved via endoreduplication (Zybina et al., 2001, Zybina, Zybina, 2005).

Ruminants do not have highly invasive trophoblast cells: their placenta is epitheliochorial in which trophoblast cells mostly attach the uterine epithelium and do not penetrate uterine wall. Among the trophoblast cells there is a portion of polyploid cells mostly up to 8c (Klisch et al., 1999). The examples considered in this chapter confirm the regularities obtained in the rodent placenta. The invasive trophoblast cells in Carnivora that come into a close contact with the allogenic tissue reach high ploidy levels via endocycles, whereas the low-invasive trophoblast cells in ruminants reach 4c-8c and some higher via acytokinetic and other uncomplete mitoses.

3.4 Genome multiplication of the extravillous trophoblast cells in the human placenta alongside the invasive pathway

In the human placenta, polyploidization also takes place, though the cells do not reach as high ploidy level as other Mammalia. Meantime, like in other mammals, the highest ploidy level are characteristic of the invasive trophoblast cells.

In human placenta, the differentiation of the trophoblast cells that invade endometrium during pregnancy is accomplished in the cell columns at the tips of encoring villi (Kaufmann, Castellucci, 1997). The invasive pathway includes several steps (Zybina et al., 2002, Fig. 5a): (a) stem cells of cell column (CC) attached to the basal membrane at the apical part of the ancoring villi; they have high mitotic activity and express integrins $\alpha_6\beta_4$ (Kaufmann, Castellucci, 1997); (b) a proximal part of CC composed of a few compact layers of proliferative trophoblast cells, in which mitotic figures are present; (c) a distal part of CC composed of more loosely arranged EVT cells that contain larger nuclei and more abundant cytoplasm; they express integrins $\alpha_5\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_1$ (Kaufmann, Castellucci, 1997); (d) EVT

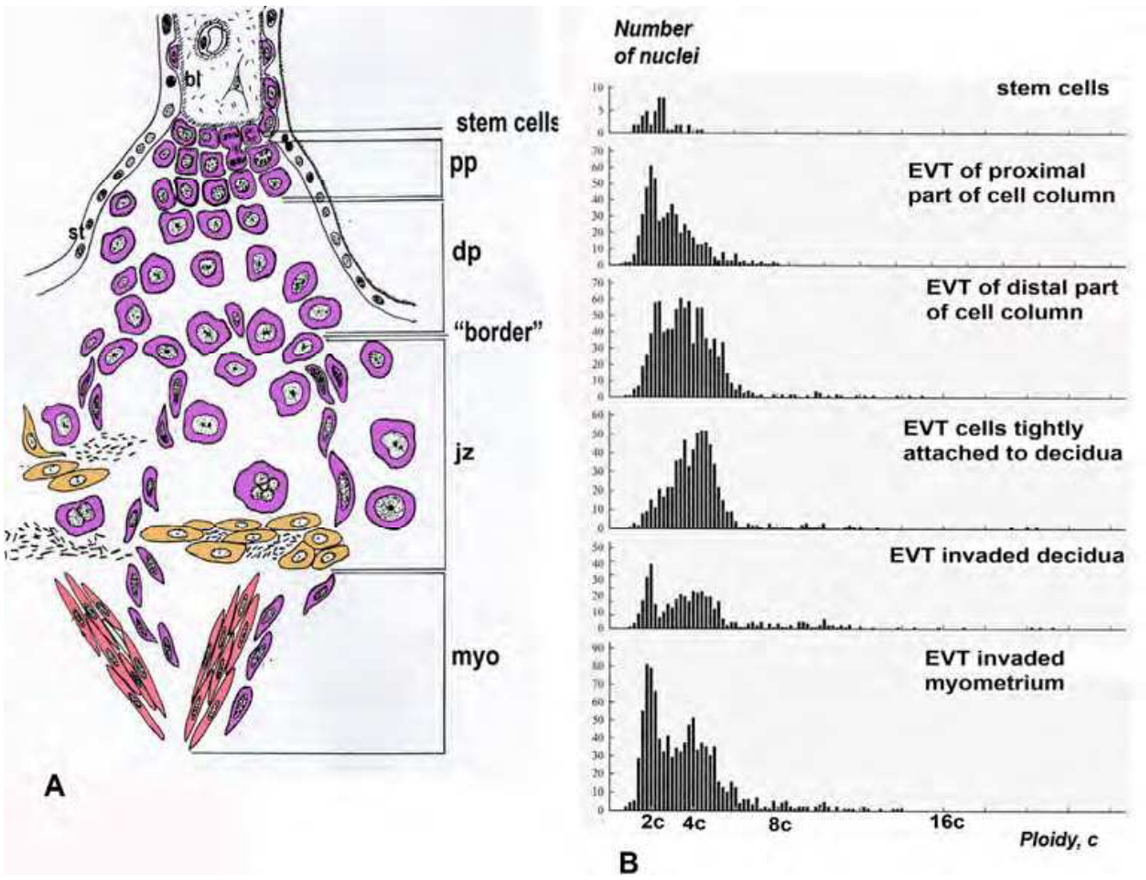

Fig. 5. Invasion of the extravillous trophoblast into the uterine wall in human placenta and its polyploidization. A - the trophoblast cells are proliferative in the zone of stem cells and proximal part (pp) of cell column, in the distal part (dp) they acquire invasive phenotype and migrate into junctional zone (jz), i.e. decidualized endometrium, a proportion of trophoblast cells reach myometrium (myo); B - polyploidization of the human extravillous trophoblast cells in the course of invasive pathway.

of the distal CC part, most closely attached to the basal plate; (e) invasive EVT cells determined by the cytokeratin-7 positivity within the materno-fetal junctional zone; (f) invasive, cytokeratin-7-positive EVT cells within myometrium.

The stem cells were found to be mainly diploid; a small amount of tetraploid cells (13.8 ± 2.3%) could be attributed to G_2-phase. The percentage of polyploid cells rose upon cell differentiation, so that in the distal part of CC the majority of EVT cells became polyploid, the portion of tetraploid cells (58 ± 1.5%) exceeded that of diploid ones (38 ± 1.5%); some amount of octaploid nuclei appeared. In the group of EVT attached to the basal plate, but not invading it, the percentage of tetraploid cells was the highest (74.7 ± 1.7%), and the octaploid cell group rose to 4.9 ± 0.8%. The EVT cells invading the decidualized endometrium also were predominantly polyploid: the fraction of octaploid nuclei increased to 9.7 ± 1.0% and some amount of 16c nuclei appeared (1.4 ± 1.0%), while the percentage of diploid cells rose slightly. This tendency did also take place in the group of invasive EVT cells within myometrium, although polyploid cells prevailed in the majority of the placentas studied (Fig. 5b). The portion of cells of different ploidy levels changed statistically significantly at every stage of differentiation of the EVT and invasive pathway ($P < 0.01$).

Besides, it is reasonable to note the individual variability of ploidy of invasive trophoblast cells, the higher ploidy level being established in the pregnancies with the greater amount of the extravillous trophoblast (Zybina et al., 2002). In the latter case, a higher level of pKi-67 expression was detected, which indicates their higher proliferative activity (data not shown).

Thus, in human placenta, like in other mammals, the trophoblast cells undergo polyploidization in the course of acquirement of invasive properties. However, the ploidy level is not as high as in rodent placenta.

As to mechanism of polyploidization, the uncompleted mitoses most probably do not play a significant role, despite the low ploidy level. The DNA content in the mitotic figures was 4c, i.e., the diploid cells, most probably, are only able to divide mitotically in the cell columns.

Simultaneously, in both the villous and, partly, the extravillous trophoblast cell populations of the human placenta, there occurs a high amount of cells containing a great number of chromocenters, some of which often resemble endomitotic chromosomes (Zybina et al., 2004). Rather numerous small nucleoli were observed to be attached to the chromocenters. The chromocenters/endochromosomes were found in the stem cells and in the proximal part of CC. Not infrequently, some large nuclei contained chromocenters that were markedly larger as compared with the neighbor cells. In the distal part of CC as well as in the EVT invaded endometrium and myometrium nuclei with a relatively uniform, network-like chromatin distribution were observed. The number of chromocenters in the EVT in the Feulgen and DAPI-stained sections was 15-30, the mode being 20-24 per nucleus, which is comparable to the haploid chromosome number in the human genome. The number of chromocenters / endochromosomes did not increase significantly with rise of the ploidy level. These findings indicate that polyplodization do not involve chromatid disjunction; moreover, the haploid number of endochromosomes suggests somatic conjugation characteristic of polyteny. Therefore, it is reasonable to suggest the first step of endoreduplication / polytenization in the extravillous trophoblast cells.

The intracellular localization of Ki-67 in EVT also provides some data on the ways of genome multiplication. In the stem cells and cells of the proximal part of CC, the Ki-67 immunostaining reminded the chromocenters revealed in Feulgen-, DAPI-, and hematoxylin-stained preparations. Besides, 2-5 small MIB1-positive nucleoli were often

revealed in the cells of this CC part. By contrast, in the EVT of the distal part of CC the pKI-67 was located mainly in 1-2 large nucleoli, rather than in chromocenters. Such difference in the intranuclear distribution of Ki-67 indicates changes in the cell cycle, when taking into account that different patterns of the Ki-67 localization characterize different stages of the cell cycle (Starborg et al., 1996; Bridger et al., 1998). Thus, in the early G1, pKi-67 is detected in numerous foci of centromeric and telomeric DNA localization scattered throughout the nucleus (Bridger et al., 1998). In the late G1, S, and G2-phase, pKi-67 is found inside the well-formed nucleolus (Verheijen et al., 1989; Kill, 1996), in mitosis this protein is associated with chromosomes (Bridger et al., 1998; Suurmejer, Boon, 1999). Transition to the predominant nucleolar localization of Ki-67 suggests that redistribution of the protein characteristic of the mitotic cycle does not take place. In combination with cycle progression indicated by Ki-67 immunopositivity and lack of mitoses, it is also a proof for transition to the shortened cell cycle – endoreduplication. However, the relatively low ploidy level suggests progression of a few endocycles, afterward the cells leave the cell cycle, and the invasive EVT loose their capability for replication.

Populatios of the non-invasive – villous – trophoblast also contain a number of octaploid cells (Wakuda, Yosida, 1992; Pötgens et al., 2001), although their frequency is not as high as in cells of invasive pathway.

Giant trophoblast cells undergoing endomitosis and non-classic polyteny were found in some cases of normal human pregnancy (at the early stages of embryonic development) as well as in some pathological cases. The giant cells with bundles of parallel chromatin threads are more specific for trophoblast malignant tumors (Sarto et al., 1982; Therman et al., 1983; Kuhn, Therman, 1988). Endomitotic chromosomes were revealed in the human hydatidiform moles (Therman et al.,1983). These chromosomes sometimes weree not detached from each other in the course of consecutive replication cycles and formed clusters of adjacent endochromosomes. Therefore, endocycles (endomitosis and endoreduplication) appear to be characteristic of human trophoblast cells. Meantime, the well-developed non-classic polyteny in the human trophoblast appears to be not numerous, as the cells, most probably, do not undergo many cycles of endopolyploidization.

Regulation of cell polyploidization of the human EVT is not understood completely, although some data suggest similarity and specificity as compared to the rodent trophoblast. Thus, "mitotic" cyclins A and B1 as well as Ki-67 and PCNA were immunolocalized in proximal and distal EVT as well as in the EVT that invaded the upper decidual segments (Korgun et al., 2006). Cell cycle inhibitors p27 and p57 were expressed in all extravillous trophoblast cells (EVT), but p21 was not. The authors state that the set of cell cycle regulators present in the cell column strongly suggests that cells in both proximal and distal part of CC do not acutely proliferate. However, they maintain this ability because they have not left cell cycle, i.e. they express Ki-67 and PCNA as well as mitotic cyclins. We are of opinion that p27 and p57 expression reflect attenuation of proliferative activity in the EVT trophoblast along the invasive pathway. Meantime, a strong expression of p57 may be a feature of the endoreduplication cycle, because p57, being the S-phase inhibitor, proves oscillatory expression in the course of mouse giant trophoblast cell endoreduplication (Hattori et al., 2000; Hu, Cross, 2010). Mitotic cyclin B1 (as judged from the photo, Korgun et al., 2006) also shows downregulation in the invasive pathway. The immunolabelled cells may be found in the CC, but not inside the decidualized endometrium. However, endomitotic polyploidization may involve cyclin B1/cdk2 activity, as this way of cell cycle involves chromatid segregation, in which p34/cdk2 may take part.

Cyclin D1 also was expressed in the human cytotrophoblast compartment, which suggests its cell cycle processes up to the third trimester of pregnancy (De Falco et al., 2004). In fact, Cyclin D expression may be associated with polyploidization.It is confirmed by the data obtained on megakaryocytes. Thus, Cyclin D3 upregulation in response to thrombopoetin in the megacaryocyte lineage and Cyclin D3 overexpression were found to increase the megacaryocyte ploidy (Zimmet et al., 1997). A decrease in the level of Cyclin D3 blocks polyploidization (Wang et al., 1995). In Drosophila, Cyclin/Cdk4 also increases ploidy in endocycling tissues (Datar et al., 2000); therefore, D cyclins appear to be critical for endocycle progression.

Cyclin E may be also involved in transition to endocycles in human extravillous trophoblast endocycle. Thus, Cyclin E was found to express in the EVT, the highest level was found in the CC. In the EVT of CC, expression of Cyclin E started more distal as compared to Ki-67 and was maintained (with less intensity) in the deeper layer of interstitial trophoblast (Bamberger et al., 2003). According to flow cytometry, there is a correlation between p27 and Cyclin E expression in the human cytotrophoblast of normal placenta and hydatidiform moles (Fukunaga, 2004). The role of Cyclin E is supported by the data of the knockout experiment on murine embryos (Parisi et al., 2003). Double knockout mice with targeted genes encoding Cyclins E1 and E2 showed a marked reduction of ploidy of the trophoblast cells. It suggest a specific role of Cyclin E in trophoblast cell endocycle entry, probably in the absence of mitoses.

Anyway, human trophoblast polyploidization needs further investigation.

4. Depolyploidization and genome segregation is a terminal step of the polytene nucleus cycle

4.1 Transition from the polytene nucleus to the polygenomic one

The lifespan of the giant trophoblast cells in most of rodent placentas finishes by disintegration of the polytene nucleus into low-polyploid nuclear fragments, so that the highly polyploid mononuclear cell becomes a polykaryocyte (Zybina, 1986, 1990; Zybina, Zybina, 1996; Zybina et al., 2005). It is to be emphasized that this process is accomplished at the stage of the complete cessation of DNA replication and attenuation of RNA synthesis. It is not quite clear how the redistribution of chromosomes is achieved. However, in some rodent giant trophoblast cells there occurs disintegration of the polytene chromosome bundles into numerous paired endochromosomes. It is the most strongly pronounced in the highly invasive supergiant trophoblast cells in the field vole placenta, which reach very high ploidy level 256c-16384c (Zybina et al., 2009). The degree of chromatid attachment in these cells is weakening, they detach one-by-one from the surface of the chromosome bundle. As a result, the supergiant nucleus appears to be filled with rather decondensed endochromosomes; at the later stage the endochromosomes undergo condensation and look like paired endomitotic chromosomes (Fig. 6). The stage may be considered as a maximal level of chromosome condensation in the polytene nucleus cycle; it cannot be ruled out that it is one of the stages of chromosome rearrangement before depolyploidization.

Polytene chromosome disintegration into endochromosomes was also described in the endocycle of Dipteran cell types, in which classic polyteny occurs at one of the stages of the cell lifespan. Thus, in the nurse cells of ovaries of *Calliphora erythrocephala* polytene chromosomes with the clear-cut disc pattern were seen in the early endocycles. At the later stages, loosening of the disc pattern occurred with simultaneous shortening of chromosomes

due to the spiralization of chromatid and their detachment from each other. As a result, the polytene chromosome broke down into endomitotic chromosomes (Wasserlauf et al., 2003; Ananina, Vedernikov, 2003, 2005). DNA hybridization *in situ* with chromosome 3 probe showed that endochromosomes did not spread throughout the nucleus, but form a chromosome territory formed by a multitude of homologous endochromosomes. This process seems to be analogous to the depolytenization in the giant trophoblast cells. In this case the chromosome structure and the activity cycling also take place. Thus, the nucleolar organizer activity varies during the polytene cycle and cell onthogenesis. In the course of polytene chromosome compactization, nucleolus falls into numerous small nucleoli, then they progressively decrease in number. At the stage of endomitotic chromosomes the nucleoli disappear completely (Wasserlauf et al., 2003). The stage functionally corresponds to mitosis. Thus, disaggregation of polytene chromosomes into endomitotic chromosomes occurs at the stage of attenuation of transcription.

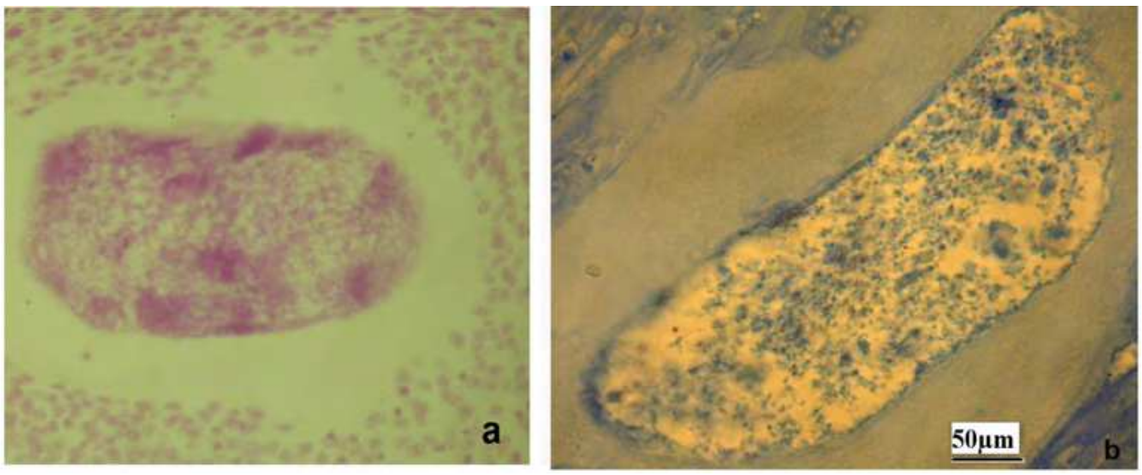

Fig. 6. Supergiant trophoblast cells of *Microtus rossiaemeridionalis*. a – a supergiant nucleus with bundles of non-classic polytene chromosomes; b – desaggregation of polytene chromosomes into endochromosomes.
Staining: a – Feulgen reaction, b – bromphenole blue.

4.2 Fragmentation of the giant trophoblast cells

Genome multiplication in the differentiation of a wide range of tissues in most cases proceeds irreversibly (Brodsky, Uryvaeva, 1985). One property intrinsic to many endocycles is that once they are initiated, further mitotic divisions are ill-advised for mechanical reasons (Edgar, Orr-Weaver, 2001). The mechanism of this phenomenon has not been yet clearly deciphered. These statements are proved, in particular by the examples that illustrate loss of the key components of the mitotic machinery in the cells undergoing endocycles. Thus, in the ovarian trophocytes of Drosophila (Mahowald et al., 1979), on reaching 8c when mitotic divisions decline, the centrioles migrated into oocyte through the intercellular bridges and finally lied adjacent to the oocyte nucleus. Thus, disconnection of chromosome, centrosome, and centriolar cycle and disaggregation of the centriolar complex may be a way of commitment to switching to the irreversible polytene cycle.
The exception is the mammalian trophoblast cell that undergo depolyploidization on reaching high levels of ploidy via a series of endoreduplication rounds (E. Zybina et al.,

1979, 1986). It should be emphasized that fragmentation of the highly endopolyploid nuclei begins after cessation of cycles of DNA replication (Zybina, 1986; Zybina, Zybina, 1996), i.e., at the second half of pregnancy. The non-classic polytene chromosomes are not revealed at this period any longer.

In the course of fragmentation, deep folds of nuclear endvelope (NE) dissect an initial nucleus into nuclei of smaller size (Fig. 7a). In many cases, NE emerges projections at one pole of the nucleus. Later on, small round nuclear fragments are detached from one side of the initial giant nucleus (Fig. 7b). In other cases, the whole nucleus turns out to be segregated into several dozens of small nuclei tightly attached to each other. Sometimes, the both processes take place in the same nucleus, i.e in case of binucleate giant cell; otherwise, initial nucleus first fall down into two giant nuclei, then they undergo fragmentation.

Distribution of chromosomes, most probably, proved to be genome-wise. The DNA cytometry demonstrated that the ploidy level of nuclear fragments corresponded to predominance of 1c, 2c, 4c, 8c (Zybina et al., 1975; Zybina, 1990; Zybina et al., 2005). The natural chromosome markers – nucleoli, Barr bodies (inactivated X-chromosomes) and condensed heterochromatin regions of polytene chromosomes – are distributed into nuclear fragments in accordance with ploidy their level (Zybina, 1986; Zybina, Zybina, 1996). The whole-genome chromosome distribution into the nuclear fragment is confirmed by the use of another natural chromosome marker of the interphase nucleus, i.e., gonosomal chromatin bodies (GCB, Zybina et al., 2003, 2005). Cytophotometrical measurement of DNA content in the nuclei, nuclear fragments, and simultaneously in the gonosomal chromatin bodies was made in the SGTCs of field vole *M. rossiaemeridionalis*. In most cases, 1-2 GCBs were found in the nuclear fragments of different ploidy levels. In the nuclear fragments GCB, the DNA content decreased mostly proportionally to the DNA content in the whole fragments corresponding to 2c, 4c and 8c.

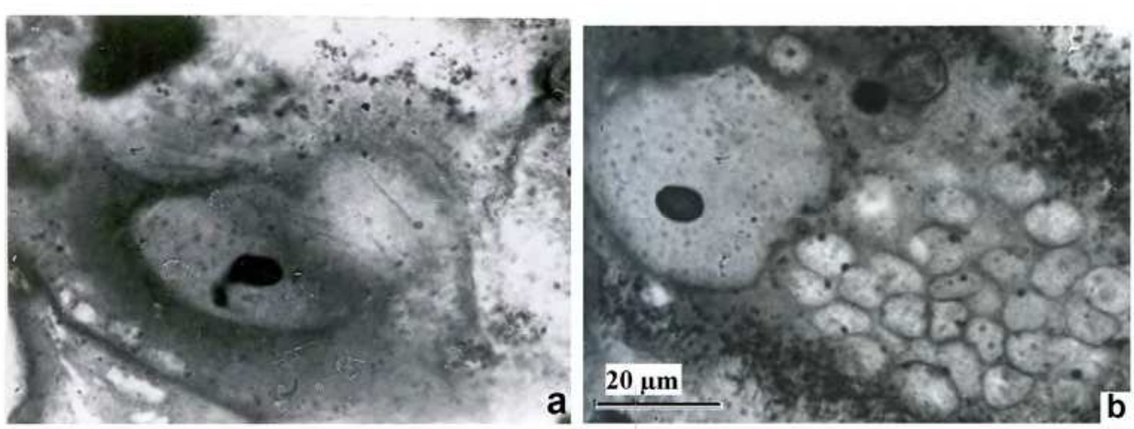

Fig. 7. Fragmentation of the secondary giant trophoblast cells in rat placenta. a - at the beginning of fragmentation the giant nucleus breaks down into two parts that undergo further fragmentation; b – the small nuclear fragments are budding off one side of the initial giant nucleus. Staining: Heidengein hematoxylin.

At the light microscope level it may be observed in rat SGTC that deep folds of the nuclear envelope (NE) dissect the nucleus, these folds separating single nuclei of smaller size (Fig. 7). In many cases the projections lie only at one pole of the nucleus (Fig. 7); in the same cases

their localization coincides with the nuclear zone, in which fragmentation begins and fragments start to detach from the initial nucleus (Fig. 7).

Observation of the ultrastructure of the initial giant nuclei during their subdivision into fragments proved NE and its derivatives to play an active role in this process (Zybina, Zybina, 2008). The very deep folds divide the nucleus into several lobes. Apart from NE, a vide range of other NE derivatives may provide a reserve material for the nuclear fragmentation: annulate lamellae (AL), membranous tubules packs of pore complexes, etc. AL represent outgrowth of the inner or outer membrane of nuclear envelope consisting of parallel membranes joined by numerous pore complexes (Kessel, 1992; Zybina, Zybina, 2008). The intranuclear ALs of SGTCs are observed mainly at the periphery of the nucleus, i.e., in the zone of the most extensive NE folding. The areas covered by the double smooth-surfaced membranes were also found in the superficial part of the karyoplasm in the zone of fragmentation.

It is worth mentioning that a significant portion of SGTC undergoing fragmentation does not show any signs of apoptosis; their cytoplasm is reach in ribosomes, the numerous mitochondria, AER channels and cisterns, as well as elements of the Golgi complex. Meantime, some SGTC undergoing fragmentation sometimes show some signs of degeneration (Fig. 8b).

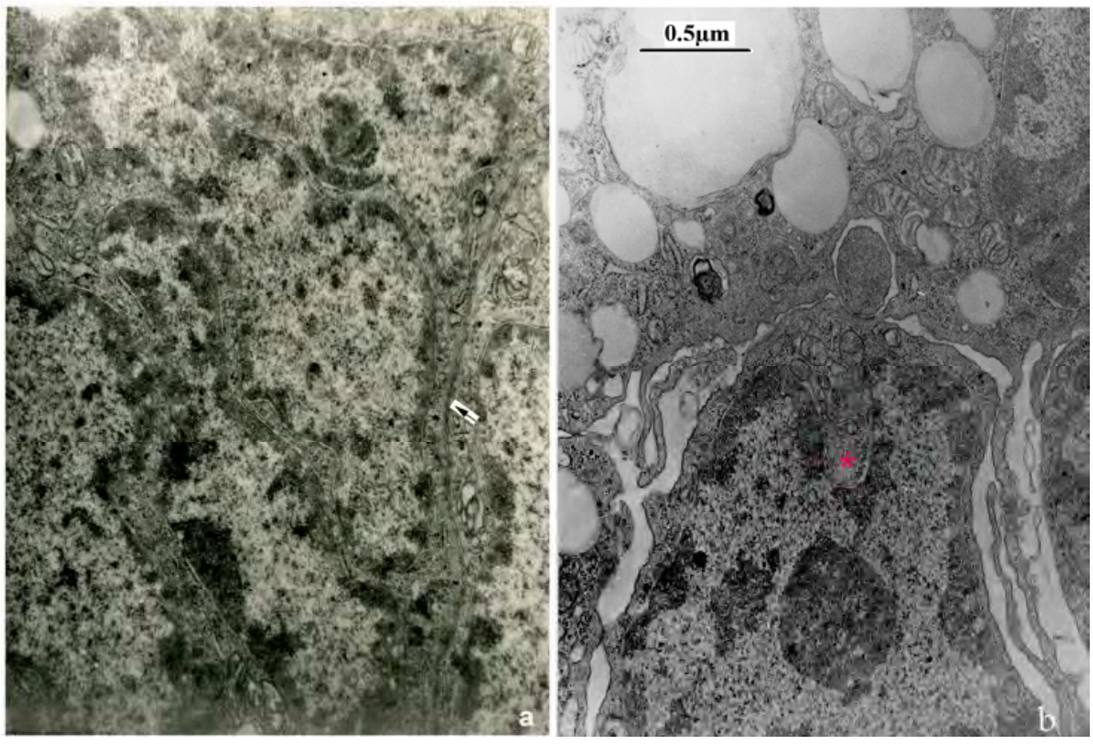

Fig. 8. Ultrastructure of the nuclear fragments. a – nuclear fragments surrounded by narrow cytoplasmic territories are separated by double membrane formed by agranular endoplasmic reticulum; b – cytoplasm shows signs of degeneration; a nuclear fragment shows a transcriptionally active nucleolus; it is isolated from the rest of the giant trophoblast cells by a wide channels of agranular endoplasmic reticulum; a bundle of intermediate filaments is seen inside the double membrane that delimits the nuclear fragment.

Each nuclear fragment is surrounded by the two-membrane NE (Fig.8a) containing numerous pore complexes. Progression of fragmentation is accompanied by the chromatin condensation and inactivation of the nucleolus. In the large fragments with the low-condensed chromatin the nucleolus consists mostly of the fibrillogranular component and the fibrillar centers surrounded by the dense fibrillar component, it indicating their transcriptional activity. The smallest nuclear fragments indicates, most probably, the final step of fragmentation: the chromatin is highly condensed and the nucleoli consist of electron dense fibrils that correspond to the most inactivated nucleolus (Zybina, Zybina, 2008). It indicates a decrease in attenuation of transcription that results, most probably, in apoptosis of the giant trophoblast cell.

Membranous structures were found to play another role in the transformation of giant trophoblast cells (Zybina, Zybina, 2008). At final steps of fragmentation, some nuclear fragments were observed to be surrounded by the cytoplasmic area delimited by the double membrane (Fig.8a, b). Upon compartmentalization of the cytoplasm, the narrow cisterns emerging from the outer membrane of NE pass to the system of the AER channels, which, in turn, participates in formation of the double membranes separating the cytoplasmic zones around individual nuclear fragments or their aggregates inside the trophoblast polykaryocytes (Fig. 8a). Interestingly, the desmosome-like structures appear in flat cisterns of AER that isolate the cytoplasmic area between two fragments (Fig. 8b, (Zybina, Zybina, 2008).). It suggests that the fragments behave, in some aspects, like individual cells. The cytoplamic territories that surround the separate nuclear fragments contain the complete set of viable cytoplasmic organelles — mitochondria, Golgi complex, GER and AER cisterns, numerous polysomes. Signs of degeneration in most cases were not observed in the cell compartments surrounding the nuclear fragments.

Sometimes, the nuclear fragments with the surrounding cell territory appear to be isolated from the rest of the cells, in which signs of degeneration are observed (Fig. 8b). These fragments look viable: they contain active nucleolus, many ribosomes and mitochondria in the narrow layer of the cytoplasm surrounding the nuclear fragment; in this case the cytoplasm territory is isolated from the rest of the cytoplasm by wide cisterns of the agranular endoplasmic reticulum.

A question arises, which structures play the role of the centriolar machinery in the course of fragmentation of the highly endopolyploid nucleus. Glasser (1984) put forward an idea that the centrosome-like bodies consisting of intermediate filaments may play this role. Indeed, at the stage of formation of the cytoplasmic zones around the nuclear fragment bundles, the 6–12-nm intermediate filaments (IF) are observed in the perinuclear zones of the cytoplasm (Zybina, Zybina, 2008).

The decrease of ploidy level is to involve the somatic reduction process, in which genome segregation is achieved in the absence of DNA replication. Non-numerous examples of somatic reduction are described in the literature. Thus, in ileum of the mosquito Culex, nuclei show signs of polyteny. In the course of metamorphosis, polyploid mitoses arise. The mitoses are followed by a series of reductional mitoses that proceed without intervening interphase and result in formation of octa- and tetraploid nuclei (Berger et al., 1938). Similar divisions are also described in ileum of the mosquito *Aedes aegypti* (Risler, 1959). The well-studied example of genome segregation is fragmentation of the highly polyploid nucleus in radiolarian (Raikov, 1982) that results in the breakdown of the primary endopolyploid nucleus into individual genomes.

The above-mentioned processes represent definite steps in differentiation of unicellular and invertebrates, they are less pronounced in vertebrates, by coinciding with completion of cell cycles. Fragmentation, i.e., somatic reduction of the trophoblast endopolyploid nuclei, also represent a definite, specifically terminal, step of the cell lifespan that coincides with cessation of cell cycles. These cells, most probably, are incapable of renewal of mitotic divisions. Therefore, the biological significance of the phenomenon is probably that cessation of functioning of provisory organ, i.e., placenta, makes impossible the renewal of proliferative activity of its cells, especially taking into account their characteristic feature of invasion of maternal tissues. Nevertheless, the possibility of isolation of diploid nuclear fragments with surrounding cell territories anticipates the probability of their transformation into full-value cells. Further studies might possibly throw light on significance of this phenomenon.

5. Reversibility of polyploidization in the lifespan of cells

The processes described above represent certain steps in differentiation of normal animal cells.

Nevertheless, some pathological processes, including carcinogenesis, imply a possibility of progression of polyploidization and depolyploidization cycles, the latter resulting in some cases in formation of the actively proliferating cell clones. Investigation of mechanisms of depolyploidization showed some traits similar to fragmentation of the highly endopolyploid nuclei in the mammalian trophoblast.

A reserve mechanism that allows overcoming the impossibility of cell division under conditions of disorder of the mitotic spindle microtubules was observed in the experiment of irradiation and treatment by a microtubule inhibitor SK&F on the p53 mutated Burkitt lymphoma cell lines (Erenpreisa et al., 2002). Electron microscopy showed large cells with the lobulating, segmenting, and budding nuclei that gave rise to many micronuclei and/or subnuclei. Study of ultrastructure revealed large, endopolyploid cells undergone significant changes, among them envelope-limited chromatin sheets (ELCS) and some other unusual membranous structures including AL and the so-called confronting cisterns (CC). The selective DNA cytometry in these nuclei and their segments showed the most frequently the 2N multiple DNA content. In irradiated cells, single ALs were seen in the close vicinity to ELCS, where they were observed to sequester the cytoplasm around the micronuclei, nuclear segments and buds (Erenpreisa, 2002). The process is similar to cytoplasm compartmentalization around the trophoblast cell nuclear fragments. However, unlike SK&F-treated cells, in trophoblast cells the compartmentalization of the cytoplasm is accomplished by cisterns of AER continuous with the outer nuclear membranes.

In search for a mechanism for genome reduction, acute radiation-induced endopolyploid tumor cells were demonstrated to exit from the mitotic cycle and altered DNA repair. It was found that the 10 Gy dose of photon irradiation (IR) to p53 function-deficient cell lines caused them to undergo extensive changes in the cell cycle progression, so that they transiently form endopolyploid giant cells. After G2-arrest for one to two days, the cells enter aberrant, often bridged mitoses and then either undergo mitotic death or, through "mitotic slippage" or bi-nucleation, become tetraploid. On days 5-6 post irradiation, this phase was switched to depolyploidization and bipolar and multipolar cell divisions occurred. It seems to be important that Aurora B-kinase was expressed in the newly-formed cells. From days 7-9 onwards death of most giant cells can be observed, and cell divisions

are seen in the endopolyploid tumor cells. The cells gave rise to small colonies of paradiploid cells (Illidge et al., 2000; Ivanov et al., 2003; Erenpreisa et al., 2005, 2008). It seems to be important that Aurora B-kinase was expressed during segregation of these newly-formed cells suggesting the mitotic mechanism to be involved in this process (Salmina et al., 2010). Interestingly, the β-galactosidase expression changed from positive in the giant cells to negative in the small proliferative cells resulted from reduction division (Puig et al., 2008; Wheatley, 2008). Therefore, restitutive divisions of large (endopolyploid) cells represent a possibility that the tumor can recover post-treatment and start up again (Whetley, 2006).

Upregulation of meiosis-specific genes, such as REC8, MOS, and SPO11, was demonstrated in these cells (Kalejs et al., 2006; Ianzini et al., 2009). Besides, signs of pluripotency and self-renewal stem cell genes NANOG, OCT4, and SOX2 in this polyploidy-dependent survival mechanism were found (Erenpreisa, Cragg, 2010). Therefore, polyploidization and depolyploidization cycles potentially facilitate survival and propagation of the tumor cell population.

Therefore, the recent findings proved that in some cases polyploidy may provide cells a reserve way to survive. The polyploid cells, being more resistant to mutagenic factors and mitotic poisons, can be viable for some time and, moreover, may give rise to a viable diploid progeny with high proliferative potential.

6. Conclusion

Somatic polyploidization characteristic of quite a few tissues in multicellular organisms is achieved by means of various modifications of cell cycle, i.e., uncompleted mitoses including restitution and acytokinetic mitoses followed by polyploidizing mitoses as well as endocycles implying endomitosis and polyteny. These cell cycle modifications are accounted for by reduction of different phases of mitosis or by its almost complete absence. Nevertheless, if in the absence of mitotic division cycle of DNA, replication is retained, it is accompanied by the cyclic changes of transcription activity and chromosome condensation. Progression of the modified cell cycles, like in the regular mitotic cycle, is regulated by the activity of the cyclin-kinase complexes. However, some specific difference in their expression is observed. In particular, in the course of transition of trophoblast cells from mitotic cycles to endocycles a change of cyclin D isophorms – from D3 to D1 – was found. Arrest of mitotic cycle in transition to endoreduplication cycle is accounted for by the lack of assembly of cyclin B/p34^{cdk1}-complex in the first endocycle, the subsequent endocycles not involving at all the cyclin B expression.

In the course of uncompleted mitoses the majority of mitotic machinery components and regulation are retained, which favors retaining their ability to undergo mitoses. Thus, Aurora B-kinase, in most cases, proved to retain the mitosis-specific time-table of relocation. By contrast, switching off the expression of several factors that ensure the sequence of mitosis progression (p34^{cdk1}, Survivin, etc.) may be accounted for by the lack of kinetochore binding to the spindle and other event that result in chromosome missegregation in ana- and telophase and polyploidization. Unlike the uncompleted mitoses, endocycles are more prone to lose their capability for renewal of mitotic activity.

In the mammalian placenta, various types of cell cycles are characterstic of the functionally different trophoblast cell populations. In this aspect the giant rodent trophoblast cells combine lysis and phagocytosis of allogenic maternal cells with their intensive growth and

polyploidization (mostly up to 128c-512c) via endoreduplication, i.e. without disappearance of the nuclear envelope. It allows ruling out contact of genome to the rests of phagocytosed cells that may be mutagenic to the trophoblast cells. By contrast, cells of the placenta junctional zone that partly share the traits of the trophoblast stem cells and undergo polyploidization via uncompleted mitoses do not contact to the allogenic maternal cells that probably favor their genome protection. This example illustrates the significance of different ways of genome multiplication for the tissue-specific functions.

In the rodent giant trophoblast cells, cessation of the DNA replication does not result in the complete cessation of the cyclic changes of transcription activity and chromosome condensation. It cannot be ruled out that the highest level of "cyclicity" is disintegration of polytene chromosomes onto the olygotene chromonemes and/or endochromosomes that may be a prerequisite of the whole-genome depolyploidization of the giant nuclei that result in the polykaryocyte formation. This event represents the terminal step of giant cell lifespan before their apoptosis. However, similar processes described in many pathological processes suggest retaining some reserve mechanisms that allow cells to survive under extreme conditions.

In the human placenta, highly invasive extravillous trophoblast cells also undergo several endocycles before the start of invasion of endometrium. Transition to endomitosis and endoreduplication before the complete cessation of DNA replication favors formation of cells more resistant under condition of their contact with allogenic maternal tissues.

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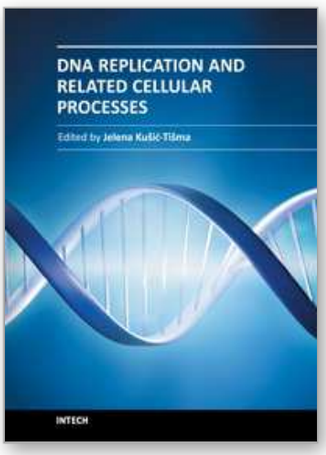

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Since the discovery of the DNA structure researchers have been highly interested in the molecular basis of genome inheritance. This book covers a wide range of aspects and issues related to the field of DNA replication. The association between genome replication, repair and recombination is also addressed, as well as summaries of recent work of the replication cycles of prokaryotic and eukaryotic viruses. The reader will gain an overview of our current understanding of DNA replication and related cellular processes, and useful resources for further reading.

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