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## HYPOTHESIS

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# Centriolar Mechanisms of Differentiation and Replicative Aging of Higher Animal Cells

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**Abstract**—The centrosome (centriole) and the cytoskeleton produced by it are structures, which probably determine differentiation, morphogenesis, and switching on the mechanism of replicative aging in all somatic cells of multicellular animals. The mechanism of such programming of the events seems to include cytoskeleton influences and small RNAs related to the centrosome. 1) If these functions are really related with centrioles, the multicellular organism's cells which: a) initially lack centrioles (e.g., higher plant cells and also zygote and early blastomeres of some animals) or cytoskeleton (e.g., embryonic stem cells); or b) generate centrioles *de novo* (e.g., zygote and early blastomeres of some animals), will be totipotent and lack replicative aging. Consequently, the absence (constant or temporary) of the structure determining the counting of divisions also means the absence of counting of differentiation processes. 2) Although a particular damage to centrioles or cytoskeleton (e.g., in tumor cells) fails to make the cells totipotent (because the morphogenetic status of these cells, as differentiated from that of totipotent ones, is not zero), but such a transformation can suppress the initiation of the aging mechanism induced by these structures and, thus, make such cells replicatively “immortal”.

**Key words:** cell aging, differentiation, apoptosis, centrosome, siRNA

Death is the fate of all living structures, and this also concerns cells. However, regular age-related changes leading to cell death because of old age occur only in multicellular animals (Metazoa).

In addition to the impossibility for dividing cells to produce offspring (replicative aging), the cell aging of nondividing (or virtually incapable of division) cells has another component manifested by a decrease in the cell “working capacity”. In the organism of multicellular animals, the aging of skin and the hemopoietic system is mainly associated with the first mechanism, whereas the second mechanism is the aging pathway of nerve and muscle cells. And aging of the organism in total is a *systemic phenomenon* mainly contributed by aging of nerve and muscle cells.

Somatic animal cells placed into nutrition medium undergo a strictly defined number of divisions (Hayflick's limit) [1, 2]. As noted in [3], “on reaching the final differentiation, somatic cells lose reproductive potential, and from this moment they are condemned to die”. This thesis was not refuted even under the boom on plasticity of hemopoietic stem cells (HSC) of bone marrow. During

the last two years, these cells were shown to be not totipotent and pluripotent but only multi- and unipotent [4] (in contrasting to data of the previous three-four years). Therefore, the involvement of these cells in regeneration is caused not by their own plasticity and trans-differentiation, but mainly due to their fusion with cells of the damaged organ [5, 6]. The potential of HSC is high but not unlimited, and they are incapable of self-differentiation [7]. Thus, the idea of Hayflick's limit retains validity for somatic cells.

What structures precisely determine the onset of Hayflick's limit? Today telomeric shortening of chromosomes seems to be the most likely candidate for the role of a replicative timer [8].

However, some difficulties described below prevent the replicative aging hypothesis from being considered as sufficient.

1. Blasco et al. [9] obtained mice genetically deprived of telomerase (the enzyme maintaining the length of telomeres). These mice were not only viable, but they could reproduce until the sixth generation, despite the telomere shortening with each successive generation. In other words, each next generation of the mice started their life at the length of telomeric ends of chro-

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mosomes equal to those at the death of the preceding generation, but nevertheless developed normally. If telomere shortening was the cause of aging of their cells, these mice would not be able to develop. Only the sixth generation animals displayed disorders associated with the ultimate shortening of telomeres. As the authors reasonably noted, the shortening of telomeres finally resulted in the termination of proliferation, but the "normal" senescence of the cells occurred before the limiting shortening of telomeres.

2. In mice with knocked-out telomerase gene [10], aging was not accelerated as judged by physiological and biochemical parameters, although the life span of these animals was decreased and the incidence of tumors increased.

3. In the majority of somatic cells of adult humans, the activity of telomerase is very low or immeasurable. But in somatic cells of mice, telomerase activity can also be determined postnatally [11]. Their telomeres are 5-10 times longer than human telomeres, and the constant expression of telomerase prevents the telomere shortening to the critical size [12]. Consequently, the shortening of telomeres cannot be responsible for the replicative aging of cells in mice.

4. It has been rather recently shown that expression of the telomerase catalytic subunit gene is insufficient for immortalization of human mammary gland normal fibroblasts and endothelial cells [13, 14]. Similar results were obtained when the gene of the mouse telomerase catalytic component (mTERT) was introduced into mouse embryonic fibroblasts. These cells had longer telomeres than in the control, but this failed to immortalize them [15].

5. The telomere hypothesis is also invalidated by data on changes in the length of telomeres in cloned animals. Thus, in Dolly and other cloned sheep, the telomeres were shorter than in normal age-matched sheep, and this seems to characterize the age of the "mother" sheep<sup>1</sup> [17]. Nevertheless, signs of premature senescence were not and still are not observed in Dolly and other cloned animals<sup>2</sup>.

These and other findings make doubtful the correctness of the telomere hypothesis. In particular, the cloning possibility itself contradicts the theory of the location of "senescence factors" in the nucleus, because it would be impossible to obtain a viable individual from

the nucleus of somatic cells of an adult animal if Hayflick's limit had been determined by the nuclear DNA [1]. Thus, just cytoplasmic factors and structures determine the onset of this limit, especially as normal calves have been already obtained [19] from *in vitro* "aged" somatic cells. This unambiguously indicates that the "senescence factors" are not concentrated in the nucleus.

## CYTOPLASMIC FACTORS AND CELL DEVELOPMENT

Cytoplasmic factors differentially activate genes, and this underlies differentiation [20]. In particular, involvement of the recipient's cytoplasm in reprogramming the nucleus was shown in transgenic mice with a fluorescent marker of the expression of gene *HSB 70.1*. Virtually the same can be said about the reprogramming of the nucleus on cloning other organisms [21].

Supporters of exclusively programming function of DNA in development usually argue by the unequivocal determination of all levels of organization by the genome via the primary structure of the encoded proteins and polypeptides. However, data on inheritance during cell reproduction (together with DNA) of the spatial organization of the cell, its locomotor system [22], and dynamical properties of the microtubular polymers [23] makes fruitless searches for the origin and initial cause (nucleus versus cytoplasm, egg versus hen). To decide these questions, one has to mentally come back to arising of the cellular forms of life [24]. At present, this question has no solution. Certainly, information about proteins is coded in the genome, but at the same time many of these proteins (e.g., signaling ones) in their turn control the genome, expressing some genes and suppressing others [25]. The spatial redistribution of macromolecules plays the most important role during ontogenesis, whereas genes, by definition, are unable of doing this [26]. According to [27], knowledge of molecular composition is insufficient to determine shape, and the organisms' morphology cannot explain the effects of their genes.

Up to now, DNA itself is not shown to have autonomous programs of changes in gene expression (except some particular cases). Structural rearrangements of the genome during development are not a result of the genetic inheritance but of epigenetic control, i.e., the previous morphofunctional architectonics of the cell [24]. Not all properties of the cell are coded in the genome. The genome only encodes molecules and lays into them the possibility of interactions. Later they behave as self-learning systems and became autonomous, though having feedbacks with the genome [28]. Of course, nobody denies that the genome does determine all levels of organization, but the spatial redistribution of macromolecules and time parameters of changes in the gene expression are

<sup>1</sup> This was not confirmed by experiments with cattle. The telomerase activity arose anew in the cloned embryos during early stages, and the length of telomeres recovered to normal [16].

<sup>2</sup> Dolly fell ill with arthritis, and, although this disease more often occurs in elderly sheep, she had no other signs of premature aging. The researchers had to lull her to sleep because of disease of upper respiratory pathways. However, she gave normal posterity several times [18].

functions of cytoplasmic factors. Consequently, the phenotypic diversity of cells is a function of epigenetic mechanisms [29, 30], which via repression/derepression of particular genes induce cell differentiation and senescence.

There are some reasons against considering the genome to be an integral and self-sufficient unit of the functional and hereditary information of the organism. If it were so, the totality of cells ingredients could be created completely *de novo* under conditions of the genome integrity and availability of the biosynthetic apparatus. But neither the nucleus nor the most important cell organelles can be created *de novo* in the absence of only fragments of these structures. These organelles become fragmented before cell division and then are assembled anew from these fragments [31]. In any case, we are still far from understanding how these structures depend on the genome [32, 33]. The cell is indeed a kind of “symbiosis” of the genome and cellular structures [32], and the full-value development of the organism needs not only genomic but also cytoplasmic information that is present in germ cells and absent in the genome and nucleus [28].

These facts have been known for a long time, and they are in favor of the *determining* role of extranuclear factors. This is especially evident during cell differentiation and division. Thus, it was shown by injection of nuclei of differentiated cells into the cytoplasm of other type cells that *the pattern of gene expression changes to correlate with the host's cytoplasm* [34]. Other researchers injected the nucleus isolated from kidney carcinoma into an enucleated oocyte, and this nucleus continued its normal development in the new environment resulting in a tadpole and then normal frog [35]. Note that there are cells that normally do not undergo nuclear influences but do not lose their differentiation, e.g., erythrocytes and the eye lens cells [36]. Cytoplasmic influences on the nucleus are also known to determine the rhythm of mitosis in embryonic cells [37]. It has also been directly shown that the cell cycle and partially regulation of the morphogenesis program are controlled on the level of cytoplasm [38].

Implantation of the adult cell nucleus into enucleated embryonic stem cells (ESC) resulted in stem cells of any desired type [7]. Thus, just cytoplasmic factors maintain the stem properties of ESC!

There are many discrepancies in the “telomeric theory of replicative aging”; however, just introduction of the human telomerase catalytic component gene (hTERT) makes human cells immortal [8]. If aging is a cytoplasmic phenomenon, how can these experiments be explained?

The following answer seems reasonable: the length of the telomeric DNA is maintained due not only to the interaction with telomerase and telomere-binding proteins, but also due to other still unknown factors regulating the generation of components of the telomere-pro-

ducing complex [39]. This can be shown by a number of examples.

a) In some cloned organisms (in particular, in cattle) telomerase activity appeared anew at the early embryo stage and the chromosome ends recovered to normal length [17, 40]. What factors were responsible for this? Telomerase expression in somatic cells is low, and this prevents the recovery of the telomere length after mitoses. By contrast, in the line of sex cells, the expression of the telomerase catalytic subunit gene is high [41, 42], but the oocytes used for the cloning were denucleated. Consequently, the normalization of the telomere length and/or *de novo* arising of the telomerase activity may be explained only by activation of the genome of the reconstructed cell under the influence of cytoplasmic factors of the oocyte.

b) Embryo maturation is associated with the shutdown of the gene (genes) encoding telomerase [42, 43]. But the differentiated activity of genes leading to the phenotypic diversity of cell types is provided by epigenetic mechanisms [29, 44]. Consequently, it is cytoplasmic factors that switch on (as well as switch off) the mechanisms which, being switched on, can immortalize the cell. Thus, the putative pathway of the cell depends on these extranuclear factors.

The ability of dividing cells to differentiate into a certain tissue (tissues), the intrinsic factor limiting/ensuring the possibility of gene repression and derepression determines the individual histological state of the cell, its morphogenetic status. Change in the morphogenetic status of the cell means an *irreversible* change in the spectrum of tissues that it can differentiate into. During embryogenesis, this process in most cases is directed to diminishing this spectrum (totipotency → ... → absence of potency), until the cells become highly specialized [45, 46]. The limitation of “potency” to produce generations of morphogenetically different cells continues until the appearance of a cell generation with a “final” morphogenetic status, and from this moment the cells are doomed to aging and death. (An exception is presented by the differentiation vector leading to the sex cell, for which the “final” morphogenetic status is specified by meiosis, and after the meiosis the cell reacquires the null morphogenetic status, as if “charges” anew.) Thus, for dividing cells the “final” morphogenetic status really means the termination of the life cycle. The “null” morphogenetic status means that the cell does not yet enter the pathway of differentiation. Consequently, such a cell is totipotent (in any case, still totipotent). Among the cells of multicellular animals, such cells are represented by zygote, first division blastomeres, embryonic germinative cells (EGC), ESC (many authors think EGC and ESC to be not totipotent but pluripotent), and partly oocytes (in the case of parthenogenesis) [47–50]. These cells have the “null” morphogenetic status. There are multicellular organisms with cells lacking the morpho-

genetic status (i.e., their status is virtually zero, although they display a certain morphogenetics of development), because their cells are totipotent (e.g., higher plants) [51, 52].

It should be noted the morphogenetic status of the progeny cannot change without division of the somatic cell. Programmed death is also related with changes in the morphogenetic status, and *this relation is realized via the counting of cell divisions*, starting from the “null” status. In general, it may be fancied as follows: exactly after a definite number of divisions, the cell (or more accurately, the cell descendant) has to differentiate into one or another histological unit. And this has to occur several times until after a definite number of divisions the cell exhausts the limit of divisions (Hayflick’s limit) and replicative aging begins—and this will be the “final” morphogenetic status. But really, not every division changes the status. Thus, division of ESC, parthenogenetic division of the oocyte, and even several divisions of the zygote should be considered unassociated with changes in the status while these cells retain totipotency. *Totipotency is the main characteristic of the “null” morphogenetic status of the cell*. Only the division, which changes the morphogenetic status, should be considered the starting point for counting “intracellular hours” eventually resulting in the “final” morphogenetic status. Division is the necessary but not sufficient condition for changes in the given status.

The close relation existing between division, differentiation, and programmed death makes one think these processes to be regulated by the same structure. No doubt, the structure carrying such information has to be capable of self-reproduction or at least be a “self-regulated autonomous organelle” (in terms of Alberts et al. [31]). Somatic cells of multicellular animals have a number of such organelles, including centrioles.

***The centrosome (centriole)<sup>3</sup> and cytoskeleton formed with involvement of the centrosome are likely to be structures which determine the histological distribution of all somatic cells and appearance of age-related changes. Just they determine and change the morphogenetic status of somatic cells of multicellular organisms<sup>4</sup>.***

## CENTRIOLAR/CYTOSKELETAL MECHANISMS OF CELL DIFFERENTIATION AND REPLICATION

Cytoskeletal structures are known to be the most suitable structures for coding the cytoplasm-stored information. The cytoskeleton, which is a kind of command processor, integrates and coordinates the cell metabolism [22]. Even the cleavage type [55] and joining up of cells into a tissue [56, 57] are determined by the cytoskeleton. Microtubular components of the cytoskeleton determine the direction of displacements of virtually all intracellular components [58, 59]. They are also (together with actin filaments) involved in cytokinesis, assemblage of the spindle, and mitotic movements [55, 60]. This concerns both somatic and sex cells. The egg cytomatrix is generally accepted to play a role in the anisotropic distribution of morphogenetic determinants and, thus, the primary determination of earlier development [61].

The cytoskeleton transmits both exogenous signals and endogenous influences into the cell nucleus [28, 48, 62]. Mechanisms of such influences are associated with the mechanical tension of the cytoskeleton and location of certain cytochemical components on the cytomatrix [55]. These effects control the synthetic and mitotic activities of the cells, their motive behavior, and morphogenesis [22]. This system in multicellular organisms functions independently of the simultaneous activity of the genome [24]. The cytoskeleton of the mother cell can transmit information about specific features of its organization directly to the daughter cell cytoskeleton [32].

Differentiation, proliferation, and maintaining of tissue architectonics are associated with both intercellular interactions and cell contacts with the extracellular matrix [63]. During these interactions, information is transmitted from the environment of the cell to its surface receptors and from them into the cell. *Ultimately changing activities of transcriptional factors, this process mediates morphogenesis and differentiation of the cell* [64]. Thus, an integral system has been proved to exist which consists of the extracellular matrix, plasma membrane, and cytoskeleton and is involved in spreading and transmis-

<sup>3</sup> In this paper, we do not concern differences between the centrosome and centriole. Although centrioles are present in the centrosome of the absolute majority of animal cells, this is not obligatory. Therefore, we identify them in this paper only technically (as it is also done in many other works) because of absence of reliable knowledge about functions of these structures [53].

<sup>4</sup> It should be noted that we do not set the equality sign between division halting and cell aging. When we speak that the cell dies on getting the “final” morphogenetic status, this does not mean that the cell has to ultimately die immediately after the halting of division. It is known that many nondividing or virtually nondividing cells (neurons, etc.) can successfully function without division even for 120 years. Thus, the replicative aging of dividing cells is fundamentally unlike the aging of nondividing (virtually nondividing) cells. Indeed, according to the literature, replicative aging is a component of the aging process. Moreover, the senile phenotype is the same whether it is induced replicatively or via premature aging [54]. Obviously, there are different reasons for aging of dividing and nondividing cells. The leading role in replicative aging belongs to centriolar mechanisms, whereas aging of nondividing cells is conditioned by many factors, first of all, oxidative stress (premature aging of all cells is also caused by free radicals). Possibly, just this is the reason for such a variety of cytogerontological concepts and a strong competition between free radical and “genetic” theories of aging.

sion of an external regulatory signal, *and this system functions as a trigger of gene expression* [65]. The close relation between the cytoskeleton and extracellular matrix is a pledge for every change in the structure of one component reflecting on the other's structure. It has long been known that changes in the cytoskeleton structure can modulate the configuration of chromatin and gene expression [66, 67].

This short review of the literature confirms the opinion of the majority of researchers that the above-presented cytoplasmic influences on the nucleus are virtually totally associated with the cytoskeleton and its constituents.

The cytoskeleton has been established to include three main types of filaments that form three systems: microtubules (of tubulin), microfilaments (of actin), and intermediary filaments. Production of varied structures from actin microfilaments is regulated by the system of microtubules, which determines regions of actin polymerization and location of microfilament bundles, i.e., microtubules determine the dynamic architecture of the cytoskeleton and the total cell [56, 68]. The organization of microtubules in animal cells is controlled by the centrosome (centrioles), which is an extragenomic carrier of information about the spatial position of microtubules [69, 70], and this forms the center of microtubule organization.

Of course, there are no direct indications that only centrioles are responsible for the morphogenetic status of the cell. It would be surprising if such findings existed not leading to adequate conclusions about functions of the centrioles. However, in the work [71] it is said that *changes in the centrosome structure can be an early marker of cell differentiation*. Moreover, based on the centrosome structure, the beginning of enterocyte differentiation can be dated even by the 14th day of embryogenesis, whereas [<sup>3</sup>H]thymidine autoradiography gives only the 16-17th day [71]. Enterocyte differentiation with their advancement from the crypt into villi is associated with termination of DNA synthesis and decrease in the synthesis of RNA and protein and also with involution of the centrosome. The authors [72] believe these findings to indicate cell differentiation. But involution of the centrosome in the villus enterocytes of mice was found to start when the levels of RNA and protein syntheses were still unchanged [72]. *Thus, the centrosome does change initially and the synthesis of nucleic acids changes only afterwards!*

The centrosome sets the consecutive order of the cell passing phases of the cell cycle [73] or, in any case, is involved in programming (the authors' term) events of the cell cycle at least two phases before their realization [74, 75] (but the nature of structures in the centrosome or around it responsible for this effect was not identified by the authors). However, on speaking about the effect of centrioles on the cell cycle, they had in mind the regulation only of one cycle but might extrapolate their data

onto all cell cycles without exception and, thus, approach our idea about the determination of Hayflick's cycle by centrioles. In principle, a factor regulating a single cell cycle can also regulate the remaining ones!

Microsurgical removal of the centrosome from the cell prevented the G<sub>2</sub>-phase of the cell cycle, i.e., the cell did not begin preparation for mitosis, although DNA synthesis in them was not inhibited [76, 77].

The centrosomes taken from the G<sub>1</sub>-phase-synchronized cells of different animal species and injected into the metaphase-synchronized oocytes of clawed frog could induce the division of the egg [78, 79]. *And if we remember that the culture age (i.e., also Hayflick's limit and aging) is determined by not the time but the number of cell divisions* (this is pointed by both supporters and opponents of Hayflick) [3, 80, 81] *and the cell division is directly related with the centrosomal structures, it is reasonably to conclude that the centrosome should be related to the initiation of cell aging, i.e., the mechanism of counting "cell divisions" seems to be concentrated in this organelle.*

The above-presented findings suggest that normally the centriolar cycle in animal cells is closely related with the cell cycle. But there are data indicating that the centrioles can be replicated independently of the nucleus [78, 82], i.e., the nuclear (chromosomal) cycle can be separated from the centriolar and centrosomal cycles. In this case, how can we speak about the determining influence of the centrosome on cell division?

First, normally, the replication of centrioles is related with events in the nucleus [76].

Second, the above-mentioned "separation" of these cycles is more closely associated with the independence of the centriole replication from nuclear influences. This thesis needs some explanations.

Nearly all cells of multicellular animals possess the centriole, but there are some exceptions, such as oocytes of mouse, sea urchin, drosophila, and some mollusks, spermatozooids of rodents, Balbiani cells of salivary glands of Diptera, erythrocytes of some species, polyploid follicular cells of drosophila [53], muscle fibers of mammals [83], and some others. Centriole-lacking cells are capable of division, e.g., initial cleavage divisions in mice, mitosis in cultured centriole-free cells of drosophila, the cleavage division in parthenogenesis in the Diptera *Sciara* [53]. But virtually all these examples are characterized by the same important feature (except sex cells): nearly all interphase centriole-lacking cells present the final form of differentiation (follicular cells, secretory cells of salivary glands of Diptera, etc.); thus, the life cycle of such cells can be terminated only by death, whereas virtually all cases of the centriole absence in the poles of the cell mitotic apparatus are meiotic divisions [84].

The presented and other data (for instance, cells with removed chromosomes can perform cytokinesis to the end and divide into two anucleate cells [85]) suggest that

among cellular structures those of the centrosoma play the most important role in division.

Consequently, the data in the literature about uncoupling of the two above-mentioned cycles essentially reflect the independence of the centriolar cycle from the nuclear one. But in no case this means that the nuclear cycle is similarly independent from the centriolar cycle! The data presented by us (some arguments will be given further) confirm this formulation of the problem.

There is also another reason for the fitness of the centriole for the role of the cytoplasmic determinant of genome expression. The self-reproducibility of the cytoplasm and whole cell structure is well known to be a common base for continuity of the morphofunctional organization on the cellular and subcellular levels [22]. The centrioles reproduce both *de novo* and on initiation with the pre-existent centriole [86], whereas on destruction of microtubular structures they never recover with involvement only of nuclear genes [87].

Centrioles were not once supposed to function as an intracellular detector of external signals [88], i.e., to be involved in transmitting information.

The absence of centrioles at the stage of preleptotenic condensation of chromosomes in mice [89] seems to evidence the relation between the "null" morphogenetic status and centrioles. We think that the "null" status is identical to the preleptotenic stage of the oogonium in these organisms. Thus, the intracellular "morphogenetic clock" is as if wound at this moment and set to the initial "null" state.

Experiments with cloning seem to be the most promising for detection of factors of cell differentiation.

To provide the successful development of the embryo obtained by introduction of the somatic cell nucleus into the denucleated oocyte (or at least, into the zygote), the nucleus needs a complete reprogramming, and this is determined by cytoplasmic factors of the denucleated oocyte [90]. But a reasonable question arises: why in this case do the cytoplasmic factors of the somatic cell donor of the nucleus not prevail over the oocytic factors and the reconstructed cell not continue the development as a differentiated cell? Note that many cloning techniques include electrofusion, but not a strict transfer of the nucleus from a somatic cell [18, 91-93]. As a result, the somatic cell cytoplasm mixes with the cytoplasm of the denucleated cell and is likely to influence the further fate of the reconstructed cell. Especially as we think the differentiation be related with the centrosome, and this structure can "be represented" by both the oocyte and somatic cell of the oocyte donor. And which of them will "dominate"? If we are right, the "predominance" of the oocyte's centrosome will promote the development of the cloned organism, whereas with the prevalence of the other centrosome the cell will continue the pathway of the nucleus donor cell. Because different animals have been successfully cloned, obviously, the first model is realized.

We have not found in the literature studies on the fate of centrosomes inside the cloned cell. Here we would finish analysis of the literature data, but our attention was attracted by studies on the fate of mitochondria in cloned organisms.

*Mitochondrial DNA (mtDNA) in Dolly and nine cloned sheep was shown to nearly exclusively originate from the recipient's denucleated oocytes*, with a small contribution from the corresponding somatic cells [94]. In such an embryo, mtDNA originated from the donor's cells partially or completely disappeared rather rapidly even during earlier stages of the embryogenesis [95]. Similar results were obtained for mtDNA in other cloned animals [91].

The "behavior" of centrosomes may be similar to that of mitochondria. With our hypothesis in mind, the predominance of centrosomes of the recipient's denucleated oocytes would be a good explanation why the cell obtained as a result of cloning takes the pathway of embryogenesis and fails to continue the line of the somatic cell used as donor of the nucleus!

It is a good time to look into the experiments of Hayflick (and some other researchers), which are insisted to confirm the relation between aging and nuclear factors [96, 97].

These experiments on fusion of young and old cells and later on the direct transfer of nuclei from old cells into young ones and vice versa have shown that the hybrid cells obtained from the cytoplasm of post-replicative cells and nuclei of dividing ones can perform the same number of doublings as the control replicatively young cultures. And the hybrids constructed from the cytoplasm of dividing cells and nuclei of post-replicative ones were unable to divide [96, 97]. Thus, the aging was controlled by the nucleus and not the cytoplasm.

But we believe that the results of these studies contradict the data of cloning. If the experiments of Hayflick really indicated the dominating role of the nucleus in determination of the cell fate, the transfer of somatic cell nuclei into the denucleated oocyte would produce a reconstructed cell unable of originating the whole organism but continuing the pathway of the differentiated cell of the nucleus donor. But this does not occur! Then why in the experiments of Hayflick did the nucleus determine the pathway of the cell?

Hayflick and other researchers removed the nuclei by treatment of the cells with cytochalasin B and centrifugation [96, 97]. This technique is still widely used [98, 99].

Because the centrosome is rather closely associated with the nucleus (the centrosome can be most easily removed together with the nucleus [98, 100]), could not the centrosome of the nucleus donor cell occur in the hybrid cell together with the nucleus?

When cells are treated with cytochalasin at relatively low accelerations (12,000g), only about 20% of the cyto-

plasts lack centrioles, and some of them contain the only centriole, and sometimes it is inactive [99]. Thus, on such treatment the major part of the centrioles goes into cytoplasts.

But Hayflick used considerably higher accelerations (25,000g) [97]. He used cytochalasin B, but at relatively high accelerations (without cytochalasin) centrioles remained in karyoplasts and did not go into cytoplasts [98, 101]. However, cytochalasin is used just to denucleate at lower accelerations [98]. Therefore, at high accelerations (both with and without cytochalasin), the centriole will more likely go into karyoplasts. Thus, under a certain experimental technique (as it particularly occurred in the studies of Hayflick), the centriole "cotraveled" with the nucleus, but the authors supporting the DNA-programmed mechanism of aging simply neglect the role of centrioles.

An important role of the centrosome in intracellular signal transmission and mitosis becomes more obvious. The role of the centrosome (similarly to the Golgi apparatus) as a signaling platform is supported by identification of transduction of various signaling molecules in these organelles (from members of the family Rho/Rac/Cdc42 to AKAP450) [102]. This also concerns proteinases and some other proteins. Thus, the protein CG-NAP is responsible for location of proteinase PKN on the centrosome, and this promotes the involvement of this complex in the regulation of interactions between the cytoskeleton components [103].

But at present, the problem of cell differentiation does not explain the following finding: in the majority of mammals, on fertilization of the oocyte the plasma membrane of the spermatozoon's tail fused with the oolemma. Although axial filaments and the centriole introduced by the spermatozoon are disintegrated, the structural components of the spermatozoon's tail in the oocyte's cytoplasm influence the further behavior of this region. Thus, the blastomere which received this region of the oocyte's cytoplasm is thought to enter the second division of cleavage more rapidly than the sister blastomere [104]. This mechanism seems to underlie the resulting diversity of germ cells with initially the same amount of the genetic material. The nuclear structures are unable to directly affect this process, because the unicellular embryo genome is transcriptionally inactive [105]. As these changes occur in the blastomeres, which have received the spermatozoon's tail, these findings support once more the appropriate role of the centriolar structures.

#### CENTRIOLAR/CYTOSKELETAL MECHANISMS OF APOPTOSIS

Many authors consider the initiation of cell aging as a program similar to apoptosis [106, 107] (although some researchers believe it to be a special type of cell death

[108, 109]). Therefore, we have attempted to find similarities between the centriolar influences and apoptosis.

During apoptosis, internal and external factors activate the corresponding genetic program and lead the cell to death [110]. Researchers mainly pay attention to the external factors inducing apoptosis, such as chemical and physical agents and also specific external stimuli, such as TNF, FAS, etc., triggering mechanisms of apoptosis [111, 112]. Consequently, in most cases apoptosis can be prevented by isolation of the cell from inducers of apoptosis, and the cell would be able to normally divide and develop. But the death because of old age cannot be prevented by such measures because it is a manifestation of the *exclusively* "intrinsic" program set in the cell.

Because factors determining cell differentiation also determine its aging, let us try to find out in apoptosis the mechanisms which could be related with centriolar influences.

Apoptosis is thought to have the following causes: DNA damages, binding to receptors of specific killer ligands, shortage of growth factors, destruction of the cytoskeleton, separation from the extracellular matrix, hypoxia, etc. [67, 113, 114]. Note that virtually all extracellular signals capable of triggering the mechanism of apoptosis have to realize the effect via the cytoskeleton.

Such phenomena as detachment of cells from the substrate and absence of signals from the integrin receptors as well as destruction of microtubules activate p53 [115] and, as a result, suppress proliferation and induce apoptosis [116]. And p53, in addition to the regulation of cytodifferentiation, genome stability, and passing over the cell cycle, also controls the cell architecture, adhesion, and migration [115], i.e., the cytoskeleton-associated functions (there are also data on the involvement of p53 in the control of centrosome replication [117]). It has been also proposed [118] that, under conditions of hypoxia and, possibly, other stress exposures, the stabilization and increase in the protein p53 content should result in its primary interaction with transcriptional corepressors inducing apoptosis. The process under consideration is realized via  $\alpha$ - and  $\beta$ -tubulins. In this publication, we were interested in data on the relation between p53 and tubulin structures (microtubules and cytoskeleton) and the possibility for inducing apoptosis of the cell. If we supplement these data with reports about cell differentiation under the influence of protein p53 [119], such a summarized exposure (apoptosis + differentiation) can lead to final differentiation and death. Note that aging is accompanied by loss of cells because of apoptosis [120].

#### CENTRIOLAR/CYTOSKELETAL MECHANISMS OF TUMOR TRANSFORMATION

The idea is now being more commonly accepted that tumor transformation of cells is, first of all, a result of epi-

genetic mechanisms. Immortalized and transformed cells were shown to be specified in aberrant hypermethylation of CpG-islets of DNA [121] along with concurrent demethylation of the genome [122]. Now numerous gene suppressors of tumor growth have been described, which are inactivated in various tumors through hypermethylation of CpG-islets located in their regulatory regions. These suppressors include the genes *Rb1*, *p53*, and many other [123, 124]. Methylation is in essence an epigenetic event [122]. Methylation of genes results in similar inactivation and similar phenotypic manifestation of the genes as the corresponding mutation [125, 126]. Even those scientists who believe the damage of functionally significant genes to be the initial cause of carcinogenesis admit that both genetic and epigenetic components contribute to arising of tumors, and the relative contribution of each of them to different neoplasms varies over wide limits [122, 125]. In many studies, the epigenetic influences are shown rather to precede malignization and not to be its consequence [121, 122, 127-131]. Moreover, carcinogens are known which have no mutagenic activity [132]. Therefore, carcinogenesis is proposed to have an epigenetic basis, and the genotoxicity of carcinogens may be their side effect. This hypothesis does not contradict thousands of reports about polymorphism of hundreds of genes in tumors. However, this polymorphism is usually manifested in mature tumors and increases with their progress, spontaneous or induced by nonmutagenic promoters, as a rule, in the absence of carcinogen; therefore, this does not elucidate the mechanism of the initiating effect of the carcinogen [133]. This hypothesis is supported by data on the role of functional inactivation of the p53 protein with proteins of HPV oncoviruses for accumulation of genetic anomalies [134]. Most recently, radiation-induced instability of the genome has been shown to be also based on the acquired stable change in cell functioning, which is inherited by epigenetic mechanisms, and this is associated with premature aging and tumor transformation [119].

As Sverdlov remarked [28], “inadequate ... integration of different signals leads to development ... of cancer”. We have noted above that the signal transmission into the cell is provided by the cytoskeleton and centrosomes.

It seems that tumor transformation induced by cytoplasmic factors is associated with repression/derepression of certain genes and gene networks. The expression of many genes changes after the start of cell aging and on immortalization [135, 136]. But now it is unknown which changes are primary or secondary [137]. The importance of nongenetic changes in the pathogenesis of cancer is proved by the existence of normal “phenocopies”, i.e., reversible rearrangements similar to transformation but not accompanied by changes in the genome. Such rearrangements are based on reorganization of the cytoskeleton [56]. But greater changes in the cytoskeleton

and/or centrosome are likely to result in malignization of the cells.

Certainly, we do not want to disprove that tumor transformation is associated with mutations in DNA. But tumor cells contain significantly more genes with changes in expression caused by epigenetic influences than genes with changes in structure, i.e., mutations [138].

Of course, we have mainly paid attention to the gene *p53* as the principle “curator of the genome”, but naturally, changes in expression of various other tumor suppressors under the influence of cytoplasmic factors also contribute to the induction of apoptosis.

According to the literature, p53 is one of leading factors determining aging. In particular, an irreversible termination of old cell proliferation is accompanied by p53-dependent transcriptional activation of the p53 target genes [139]. Contents of p33/ING1 protein and the corresponding mRNA are considerably increased in aging human fibroblasts, and this suggests the involvement of gene *ING1* in cell aging [140]. Functions of products of genes *ING1* and *p53* are similar [141]; therefore, they are suggested to belong to the same signaling pathway. The involvement of p53 in premature aging is shown even directly in Werner's syndrome and ataxia-telangiectasia [142, 143]. These findings are interesting because of reports on the location of p53 protein in the centrosome [76, 144].

In addition to tumor suppressors, the cell seems also to have other mechanisms of centriolar influences on apoptosis. We speak about protooncogenes. Their expression is known to be the main mechanism of tumor transformation of the cell, and they are also involved in initiation of apoptosis.

The leading Ras-oncogenes are associated with organization of the cytoskeleton, cytokinesis, and morphogenesis of the cell [145, 146], and hyperexpression of Ras along with normal expression of anti-oncogenes can result in apoptosis [147, 148]. A factor is released from the ends of microtubules that activates Rac [102, 149] and is also involved in realization of Ras effects. Injection of an oncogene (upon the normal functioning of tumor suppressors) leads to syndrome of early senescence of the cell, and it is suggested that programmed cell death because of aging could be a result of expression of Ras and some other oncogenes regulated by the centrosome. This mechanism of cell senescence seems to supplement the pathways associated with functioning of tumor suppressors.

Even in 1971, Harris reported on the certain role of centrioles in the inheritance of tumor properties of cells. Disorders in the cycle of centrosome doubling lead to instability of mammalian cells [150]. Similar phenomena, such as disorders in the division of centrosomes and aneuploidy, are observed in cultured cells under the influence of Aurora-A kinases also located in the centrosomes [23]. Rearrangements of chromosomes and aneuploidy are found in the majority of tumors.



It is unclear whether the amplification of centrosomes is sufficient for inducing transformation [151, 152], but anomalies of centrosomes and aneuploidy are detected in pre-invasion state carcinomas and, thus, can be early events in cell transformation [153-155]. The degree of centrosomal amplification seems to increase with progress of the tumor [156-159]. The protein CAS associated with microtubules in normal tissues is shown to be expressed in tumor cells [160].

All these findings indicate an extremely important role of centrosomes in cell malignization.

#### CENTRIOLAR/CYTOSKELETAL MECHANISMS OF STORAGE AND REPRODUCTION OF INFORMATION

The centrosome *has to possess* a mechanism providing for the memorizing, storage, and reproduction of information, because the determinativeness of cell aging suggests the presence of a mechanism permitting the cell to "count" the number of DNA doublings.

We think that two mechanisms, the cytoskeleton proper and RNA-dependent, can exist, which do not exclude one another.

**The cytoskeleton proper mechanism.** When the storage and reproduction of information are considered, the DNA molecule seems to be the best candidate for the role. As to centrioles, no DNA was detected in them. However, a kind of template reproduction of the conformation occurs on formation of the cytoskeleton during the growth of actin and tubulin filaments and also on reproduction of the nuclear membrane. The same mechanisms underlie epigenetic inheritance [161].

On the macromolecular level, the transmission of information without the involvement of DNA is most clearly associated with the prion phenomenon [162, 163]. It seems the most interesting that mutations by the *SUP35* gene encoding the termination factor (i.e., virtually the yeast prion) make the cells supersensitive to benomil, an agent specifically destroying microtubules of the cytoskeleton which produce the division spindle [164]. Inactivation of the *SUP35* homolog in *Drosophila melanogaster* results in a similar effect during meiosis [165]. Therefore, prions were suggested to be a kind of byproduct of the conformational copying the cytoskeleton elements in the cell [161].

Note a very interesting detail: the brain tissue of patients with Alzheimer's disease, senile dementia, and even of apparently healthy subjects display the same alterations as the brain tissue of humans who died from prion-caused diseases [166], and this confirms similarity of normal aging of the central nervous system and prion-caused diseases. Therefore, prions were supposed to be the main inducer of aging in higher animals [167]. We have mentioned prions for two reasons.

First, because prions are byproducts at the conformational copying of the cytoskeleton elements [161], it seems that the aging of even nonproliferating brain cells cannot occur without the influence of the cytoskeleton structures.

Second, PrP<sup>c</sup>, which is a normal cellular form of the prionic protein, seems to regulate circadian *rhythms in the cell* and in the total organism [168]. But our hypothesis is virtually based on assuming the central role of the centrioles/cytoskeleton in the regulation of all cell cycles without exception (consequently, also including Hayflick's limit).

These findings are unlikely to be accidental, and they indirectly support the reasonability of our approach.

**The RNA-dependent mechanism.** Two variants of the RNA-dependent mechanism are likely to exist which function concurrently (similar to the cytoskeleton proper mechanism and the RNA-dependent ones).

*Cytoskeleton/centrosome-mRNA.* The local limitation of protein synthesis through mRNA location is known to be crucial for creation of the effective asymmetric distribution of cytoplasmic factors, and it is detected in various eukaryotic cells [169, 170]. This process is important for establishing and retaining the polarity in both somatic and germinative cells and for asymmetric segregation of determinants during development [171].

A significant fraction of mRNA in animal cells is associated with the cytoskeleton [172, 173]. Although the functional meaning of this association remains unclear [174], all falls into place in the framework of our approach. Really, just the centrosome regulates development via the cytoskeleton, and this is proved by starting of mRNA translation *only after termination* of mRNA transfer inside the cell and location ("anchorage") in a definite region of the cytoplasm [175, 176]. Indeed, mRNAs are mainly transported along microtubules and partially actin filaments [172, 177, 178] and "anchored" to actin filaments [169, 172, 176], i.e., to the cytoskeleton structures. Microtubules are key participants in the location of mRNAs in some structures, including oocytes and embryos of *Xenopus* and *Drosophila* [170, 179]. In total, the active transport of mRNAs along the cytoskeleton filaments is the main mechanism of their location in the majority of cells [170, 179, 180]. The American geneticist Lewine suggests [175] the binding with the cytoskeleton be a functional property of mRNA. Location of mRNAs not only activates the translation and prevents degradation of mRNAs [181, 182], but leads to synthesis of a definite protein in the proper place that is extremely necessary during oogenesis and embryogenesis to create varied biochemical status in daughter cells [105]. Therefore, the role of the translational regulation is often considered decisive during embryogenesis. Note that mRNA, which is nonhomogeneously distributed in the cytoplasm, has long been believed to be the most popular candidate for

the role of morphogenetic determinant of development [24].

However, a direct involvement of the centrosome in location of RNA is also shown [171]. For the first time, concentration of cyclin B1 mRNA was found on the spindle and centrosomes in the dividing oocytes of *Xenopus* [183]. The RNA sorting with involvement of centrosomes was also described in embryos of the mollusk *Ilyanassa obsoleta* [184]. According to the authors, the inherent differences between the centrosomes are used by the located mRNAs and ensure the asymmetric segregation, and the absence of the centrosomal association affects the subsequent steps of location [171]. This seems to be the most distinct assumption of the central role of inner centrosomal structures in the location of mRNAs and the subsequent development of the embryo.

*Centrosome-small RNAs (siRNAs).* The inner cavity of somatic cell centrioles seems to contain molecules that are capable of determining the morphogenetic status of the cells (it should be noted that the inner region of the centriolar cylinder is poorly available for study [76]). Every mitotic division is associated with release of a certain number of such molecules into the cytoplasm, and their number in centrioles of the daughter cells is somewhat lower than in the mother cell centrioles. The number of molecules in the centrioles decreases with every mitotic division. The last molecule indicates the "final" morphogenetic status of the cell. Thus, the number of such molecules has to correspond to the number of putative mitotic divisions, starting from the cell with the "null" morphogenetic status and finishing by the descendant cell with the "final" morphogenetic status. These molecules are transcribed from the nuclear DNA after each mitosis (but a strictly definite number lower), and then their release in turn during mitosis determines the expression of the nuclear DNA genes (as if the nuclear DNA "lays" in the centrioles information about the sequence of switching on the DNA loci in the differentiated descendant cells). Such a "funny loading" mechanism is likely to be associated with the influence of the released molecules on expression of the corresponding genes. The released RNA molecules not only induce a successive decrease in expression of the genes related with the mechanism of their own "loading" but, possibly, also of other genes directly associated with provision of vital activity of the cell. Thus, we predict that genes should exist whose expression has to steadily decrease from mitosis to mitosis until Hayflick's limit is reached. In the absence of centrioles or structures transmitting the centriolar influences, in particular, the cytoskeleton, a dramatic transformation of these structures and, possibly, of the inner architectonics of centrioles (even on the background of cell division) means a derangement of the RNA-dependent mechanism of suppression of the gene expression (either because of impossibility of RNA to be located in the centrosome or leave the centrosome and

reach the appropriate cellular structures), and this, in turn, can prevent replicative aging.

We do not deny that the existence of such a mechanism is hypothetical. Nevertheless, some mechanisms must be responsible for realization of the centrosome functions. We consider the low-molecular-weight RNA as the best candidate for the role of carrier of information on the morphogenetic status. This is indirectly confirmed by the latest discoveries of new classes of small RNAs: interfering RNAs (siRNAs) and microRNAs (miRNAs) with regulatory activities. The RNA interference is responsible for a strictly selective inactivation of gene expression on the posttranslational level in the cells of various organisms [185-187] including mammals [188]. Gene expression is suppressed as a result of mRNA degradation [185, 187]. Here we have to remind of the above-presented data that mRNA "anchoring" on the cytoskeleton (that accelerates or slows down the mRNA degradation) is the crucial mechanism of the translational regulation of embryogenesis!

MicroRNAs have a widespread function of regulation of gene activity during development and cell differentiation in higher animals [189]. In particular, mechanisms are described of involvement of RNA interference genes in the regulation of time-associated expression of the genes required for development of *Caenorhabditis elegans* [190]. These molecules have even been directly shown to be involved in cell division [191-193]. Moreover, in 2002 the effect of siRNAs was found not to be limited by the transient switching off of genes on the level of RNA. In some organisms (only in plants), siRNAs can change the chromatin structure (i.e., act on the transcriptional level) and promote a long-term silencing of some and activating of other genes [194]. In addition to the transcriptional and post-transcriptional homology-dependent suppression of gene expression, the interaction between DNA/chromatin structure and RNA is manifested by many phenomena, e.g., the dose compensation in drosophila and inactivation of X-chromosome in mammals, when noncoding RNAs determine changes in the chromatin structure [195].

RNA interference seems to play a special role in maintaining the length of telomeres in *Drosophila melanogaster* [189]. However, contrastingly to the majority of eukaryotes, drosophila's telomeres are built on another principle; nevertheless, the involvement in maintaining the length of telomeres is also a function of RNA interference [196]. This convinced us that the relation centrosome/cytoskeleton → RNA interference → cell aging exists indeed.

There are contradictory but definite data on the presence of RNA inside the centrosome or in the centrosome-bound state [184, 197-199]. Therefore, due to their size (20-300 nucleotides) small RNAs seem to be ideal candidates for being located inside the centrosome.

These findings, as well as many others unambiguously indicate that the centriolar/cytoskeleton structures can

*in principle* contain the information that is capable of inducing programmed changes in the cell.

### CENTRIOLAR/CYTOSKELETAL MECHANISMS OF CELL REPLICATIVE AGING

It was recently proposed to discriminate the concepts of “cell life duration” and its “replicative potential”. The cell life duration is thought to be associated with senescence, whereas the replicative potential relates to proliferation, differentiation, and apoptosis [200]. Thus, the telomere hypothesis seems to be poorly related with the essence of aging, and it may be considered only as an explanation of the reason for the existence of Hayflick’s limit [3]. But we do affirm that all these mechanisms, in spite of different pathways of realization, are controlled from the same center.

We have already noted that during ontogenesis just the cytoplasmic factors switch on/off the telomerase activity, because the differential activities of genes are shown to be maintained by epigenetic mechanisms. According to our hypothesis, they can be related to the centrosome. What are the possible hypothetical mechanisms of the centrosomal control of telomere length and telomerase activity?

1. There are definite (and rather similar) relations between telomeres, telomerase, and nucleoli [201]. Thus, RNA of human telomerase contains the sequences responsible for transport into the nucleolus [202]. A syndrome is described when mutation in the protein diskernin (a normal nucleolar component) results in a sharp decrease in the telomerase activity, possibly, at the cost of enhanced interaction of diskernin with RNA of telomerase [203]. There are hypotheses that connect cell aging and the nucleolus: the nucleolus can be the place of accumulation of telomere-binding proteins [201, 204]. It is also known that factors directly influencing the assemblage of nucleoli are located in the centrosome during mitosis. In particular, damage to the centrosome in anaphase led to disorders in formation of the nucleoli after the cell had entered interphase. This function of the centrosome was not associated with the function of organization of microtubules [205].

2. Telomerase (or the *hTERT* gene) was found to be controlled by a number of cellular enzymes, such as proteins p53, myc, etc. Directly influencing the promoter of the gene *hTERT* (myc) or the protein regulators of this gene (p53), they can enhance or inhibit the synthesis/activity of telomerase [206]. These facts are interesting in addition to the above-presented data on location of protein p53 in the centrosome [76, 144] and association of myc with microtubules [207].

3. Recent studies have shown that telomeres can be controlled by the protein tankirase [208]. Tankirase itself is activated with MAP kinase [209]. The activity of MAP

kinase is regulated through the signaling pathway Ras-MAPK (mitogen-activated protein kinases) [208]. We have already mentioned that from the ends of microtubules a factor is released, which activates Rac proteins assigned to GTPases [102, 149] and is also involved in realization of Ras effects.

Consequently, both telomeres themselves and telomerase can be controlled via centriolar mechanisms!

### CONCLUSIONS FROM THE HYPOTHESIS AND EMPIRICAL FINDINGS

How can the determination by centrioles of the cell morphogenetic status be manifested?

*Differentiation of the majority of cells is accompanied by either the loss of certain features of the centrosome or their hypertrophy* [53]. At the terminal differentiation accompanied by an irreversible loss of proliferative potential of the cells, four different variants are realized [53]. In some cases, centrioles totally disappear (e.g., on myogenesis of skeletal muscles) [71, 72], in other cases the centriole becomes a basal body and produces a cilium (e.g., in nervous tissue) [72], in the third situation the centrosome is present but its certain components partially disconnect [53] (i.e., it is invalid), and only in some of the cells the centrosome functions normally and acts as the center of microtubule organization. But it seems to be also invalid in these cells because of inability of providing normal division. Thus, in all these cells the centrosome reaches the “final” status that caused an irreversible differentiation of the cells. Only cells with reversibly inactivated centrosome (e.g., hepatocytes of intact liver of adult animals) can return to the pathway of division.

According to our hypothesis, if triggering the differentiation and start of the programmed age-related changes are really associated with the centrioles, the cells of multicellular organisms which display either: a) the initial absence of centrioles or cytoskeleton, or b) *de novo* appearance of centrioles (centrioles may be considered as *de novo* produced until the cell enters the pathway of irreversible differentiation) will be totipotent and “immortal” (i.e., they will lack replicative aging). It is reasonable that the *absence (permanent or transitory) of a structure determining the account of divisions also means the absence of counting of irreversible differentiation processes. Therefore, such cells are totipotent and “immortal”*. This is the first conclusion from our hypothesis.

The loss of centrioles by some cells of a few representatives of higher animals during ontogenesis [71, 83, 210], as well as experimental removal of them, never make the cells immortal and totipotent. In the first case, this loss is *always* associated with the cell achieving the final stage of differentiation [71], whereas in the other case the removal of such an important organ makes the

cells incapable of dividing [211]. Consequently, these findings do not contradict our hypothesis.

The second conclusion is as follows: certain (naturally, not any) damage to centrioles and/or cytoskeleton has to result in the absence of replicative aging of the cell because it can suppress the switching on of the aging mechanism induced by these structures.

Let us compare these two conclusions from our concept with the facts.

1. It is known that centrioles are initially absent in the cells of higher plants [212, 213] and zygote and early blastomeres of some animals [69, 214]. The cytoskeleton is absent in embryonic stem cells of animals [48]. As a consequence, they are immortal (*in vivo* and/or *in vitro*) and totipotent (or at least pluripotent), and this supports our theory.

2. Centrioles appear *de novo* in the zygote and cells of early embryos<sup>5</sup> of some animals [69, 215, 216], and they are also totipotent and immortal.

3. Dramatic changes in the cytoskeletal structure are observed in cancerous and transformed cells [32, 63, 66, 217]. In particular, in these cells actin bundles are significantly reduced or absent, the length of the actin edge and the area of lamelloplasma of active microfilaments are decreased, and the endoplasmic plast of microfilaments is spoiled [218]. Although the structure of centrioles in transformed cells seems morphologically normal, the centrosome → cytoskeleton relation is disturbed because of changes in the cytoskeletal structure. Moreover, in the transformed cell the orientation of centrioles is affected [84], and a nonrandom orientation of the centrioles seems to be one of most important, although enigmatic, feature of the normal cell [219]. Although disorders in the normal organization of fibrillar material do not prevent mitosis, mitotic figures appear affected [53].

Cancerous and transformed cells are immortal [123], and this is also in the framework of our second conclusion. However, these cells are not totipotent, and this needs explanation. We have already noted that totipotency is related with the “null” morphogenetic status of the cells, and the status of cancerous and transformed cells naturally is not “null” because before the transformation they have already passed a certain number of differential mitoses (“transformation “freezes” the direction and level of differentiation of the precursor cell” [220]). Consequently, such immortal cells descending from somatic cells cannot be totipotent.

Obviously, the “behavior” of *all* immortal and/or totipotent *cells of multicellular organisms* that occur in nature completely fits our concept.

Does the simultaneous presence of centrioles and immortality in many unicellular organisms contradict our concept?

According to the literature, the centriole functions only as the basal body during early phylogenetic stages of eukaryotes [84]. Only after a certain evolution, the centriole can be involved not only in formation of flagella and cilia but in creation of the intracellular carcass [84]. This seems to be a starting point for arising the division counting and later results in appearance of replicative aging. An apparent “blossoming” of centrioles occurs only after appearance of multicellularity [221]. This is not contradicted by data on the aging of infusorian clones prevented from conjugation, because in the case of normal proliferation it “rejuvenated” the culture. Consequently, in some unicellular eukaryotes, centrioles are just beginning to acquire new functions and are yet unable to determine the cell morphogenetics; this function is peculiar only to multicellular organisms with irreversibly differentiated tissues. Thus, the simultaneous presence of both centrioles and “immortality” in various unicellular organisms does not contradict our theory.

The data presented in our paper allow us, using Harman’s term of cellular “molecular clock” for mitochondria, to believe that centrioles are the true molecular clock of the cell.

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