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Amoeba proteus and ploidy cycles: from simple model to complex issues*

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Summary

This short topical review/opinion paper is inspired by the recent study of *Amoeba proteus* cell cycle. These obligate agamic amoebae have a special type of cyclic polyploidy – an alternation of unproportional polyploidization and depolyploidization, with the latter provided by chromatin extrusion from the nucleus into the cytoplasm. Here we discuss possible significance and mechanisms of this phenomenon, reconsider similar strategies of life cycles in other unicellular eukaryotes, and debate provocatively the fundamental issues, which could be brought up during its study – from functions of meiotic genes to evolution of sexual process to survival strategy of cancer cells.

Key words: *Amoeba proteus*, atavistic theory of carcinogenesis, cell cycle, cyclic polyploidy, meiosis-specific genes, polyploid giant cancer cells

Amoeba proteus and cyclic polyploidy

In spite of extraordinary biodiversity of unicellular eukaryotes, there is hardly a better known organism than *Amoeba proteus* (Fig. 1). The majority of laymen and all biologists are familiar with it – as a curious (or not) theme in biology class, or a protozoological object and, moreover, a model organism for cell biology. A plethora of fundamental and applied studies carried out on this organism has been summarized in a number of fundamental reviews (Hirshfield, 1959; Jeon, 1973, 1995; Yudin, 1990; Anderson, 2017; etc.).

Amoeba proteus (Amoebozoa, Tubulinea, Euamoebida, Amoebidae)) is a free-living lobose amoeba that is traditionally regarded as an obligate agamic protist (Raikov, 1982; Yudin, 1990; Anderson, 2017). Nevertheless, the *Amoeba* species have a very special cell (=life) cycle (Fig. 2), during which a strategy of the so-called cyclic polyploidy, or ploidy cycle, is implemented (Demin et al., 2019).The latter implies an alternation of polyploidization and depolyploidization stages preceding reproduction in the life cycle (Kondrashov, 1994, 1997; Parfrey et al., 2008; Lahr et al., 2011). In *A. proteus* cell cycle the presynthetic phase G_1 is absent and an intensive DNA hyperreplication (stage of unproportional polyploidization) may occur starting from the interphase until the next mitosis (Ord, 1968; Makhlin, 1987, 1993). By the end of the cell cycle,

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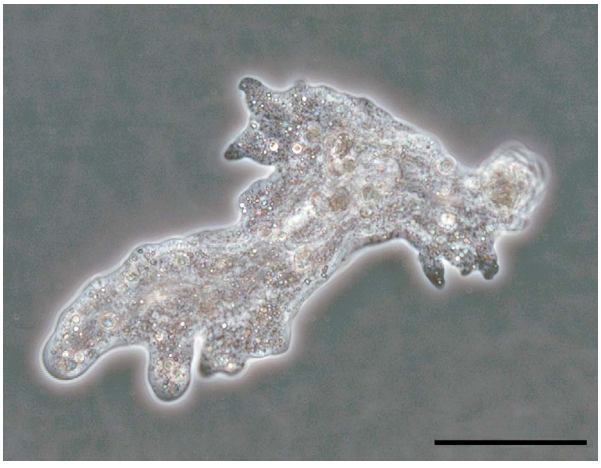

Fig. 1. *Amoeba proteus*, strain B, locomoting interphase cell (phase-contrast microscopy). Scale bar: 50 μm.

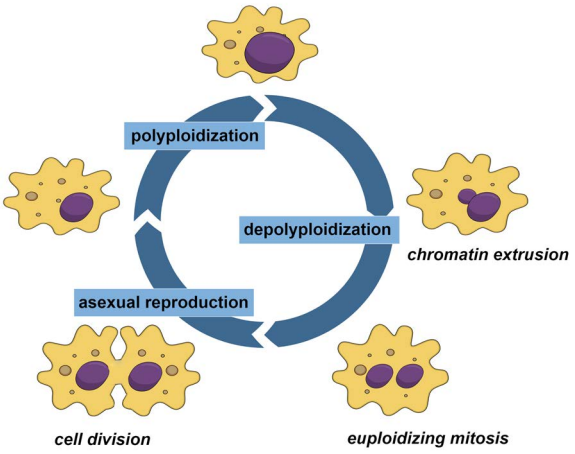

Fig. 2. Generalized scheme of the life cycle of *Amoeba* (created in the Mind the Graph platform: www.mindthegraph.com).

the amount of nuclear DNA may exceed the initial level by more than three times, according to previous fluorimetric measurements (Makhlin, 1987, 1993; Afon'kin, 1989). The depolyploidization occurs through the phenomenon of chromatin extrusion in the late interphase and in the prophase (Demin et al., 2019; Goodkov et al., 2019). The "excessive" nuclear DNA is eliminated from the amoeba nucleus by ejection into the cell cytoplasm. The depolyploidization, including prophase reduction of the chromosome number (Demin et al., 2019), reverts the *Amoeba* nucleus to the euploid state and therefore prepares the cells for equal separation of chromosomes in the daughter nuclei.

Cyclic polyploidy has also been described in some lower eukaryotes, not only agamic as *Amoeba*, but in sexuals as well (Parfrey et al., 2008; Parfrey and Katz, 2010). In Foraminifera (Rhizaria, Retaria), a single gamontic nucleus increases in size and in DNA content (polyploidization) and subsequently divides by mitosis into hundreds or thousands of nuclei for haploid gametes (depolyploidization) (Parfrey et al., 2008; Parfrey and Katz, 2010). Additionally, DNA degradation – the so-called Zerfall process – can occur at the depolyploidization stage. Some DNA and nucleoli are expelled from the nucleus and degrade before mitotic division (Goldstein, 1997; Parfrey et al., 2008). In other groups, depolyploidization is also carried out by multiple genomes segregations. In Phaeodarea (Rhizaria, Cercozoa, Thecofilosea) – the asexual marine protists with a complex silicone skeleton – a polyploid nucleus is formed by endoreplication, i.e. replication of the nuclear genome in the absence of endomitosis. Then, after multiple rounds of divisions, the genome sets are segregated into thousands of daughter nuclei for haploid spores (Raikov, 1982). In radiolaria from the group Polycystinea (Rhizaria, Retaria, Radiolaria), genome copies segregate into gametic nuclei, and residual nuclear material appears to degrade during gametogenesis before division (Parfrey and Katz, 2010). In the trophozoites of Entamoeba (Amoebozoa, Archamoebea, Entamoebidae), intensive polyploidization precedes cell division, while depolyploidization occurs before or during encystment (Lohia, 2003; Parfrey et al., 2008). And furthermore, ploidy cycles are described in the life history of cancer cells survived after irradiation and (or) chemotherapy (Erenpreisa and Cragg, 2013).

Thus, the cyclic polyploidy as a means of genome rearrangement had evolved several times in the eukaryotes (Parfrey and Katz, 2010). The mechanisms of cyclic polyploidy are not fully clear; besides, we are far from full understanding of its significance. Further on, we will discuss some topics associated with the study of this phenomenon and the *Amoeba* position in similar investigations.

Meiotic toolkit: the chicken or the egg?

At the first glance, being an agamic organism is extremely disadvantageous. Direct mutations, which inflict most damage on genes, occur much more frequently than reverse ones. The effective

disposing of deleterious mutations is a challenge of paramount importance in all organisms, both in sexuals and in agamic. But the latter, apparently, should use alternative mechanisms of recombination. Otherwise, the effect of the so-called Muller's ratchet (Muller, 1964) should occur. This mechanism implies the reduction of fitness in the course of an agamic organism's natural history due to the accumulation of deleterious mutations and the extreme rarity of reverse mutations. The existence of such a ratchet in microorganisms has been proved experimentally (Andersson and Hughes, 1997). In theory, this phenomenon should considerably shorten the existence time of a biological species. However, agamic organisms, and *Amoeba proteus* in particular, apparently find a way around this limitation. In this regard, the study of cyclic polyploidy is closely intertwined with the issue of functionality and evolution of meiosis-specific genes.

Intense explorations aimed at elucidation of the origin of sex with application of genomic and transcriptomic approaches have recently been launched (Ramesh et al., 2005; Stanley, 2005; Speijer et al., 2015; Tekle et al., 2017; Wood et al., 2017; Hofstatter et al., 2018; Hofstatter and Lahr, 2019; Morse, 2019). The search of orthologs of syngamy- and meiosis-associated genes was carried out in various lineages of unicellular eukaryotes. As a group including "traditional" agamic organisms, Amoebozoa is a primary focus of researchers' attention (Stanley, 2005; Ehrenkaufer et al., 2013; Tekle et al., 2017; Wood et al., 2017; Hofstatter et al., 2018). The orthologs of individual genes or their complete sets have been found in all major groups. In some of these protists, sexual or sexual-like processes were observed and well documented during *in vivo* studies of their life histories, and functions of these genes are beyond doubt (Tekle et al., 2017; Wood et al., 2017). Moreover, meiotic genes have been found even in some organisms known as "ancient asexuals" (Tekle et al., 2017; Hofstatter et al., 2018).

Among others, *Amoeba proteus* also possesses some of meiotic toolkit genes according to the results of recent analysis of transcriptomic data (Hofstatter et al., 2018). The expression of genes encoding proteins included in the cohesin and synaptonemal complexes, responsible for double strand breaks, recombination and gene conversion, occurs (Table 1). At the same time, HAP2 and GEX1 proteins implicated in plasmo- and karyogamy were not detected. And since no one has ever observed sexual process in *Amoeba* it is logically to assume that the so-called meiotic toolkit genes can be employed in amoeba's ploidy cycle.

Table 1. Meiotic toolkit proteins detected in *Amoeba proteus* based on transcriptomic data according to Hofstatter and Lahr (2019).

Functional group	Protein
Sister chromatid cohesion	SCM3, SMC1, RAD21, REC8
Introduction of double-strand breaks	SPO11, MRE11, RAD50
Homologous recombination	RAD51A, HOP2, MND1
ZMM complex	MER3, ZIP4, MSH4, MSH5
Crossover	MLH1, EXO1, MUS81, MMS4
Gene conversion	MSH6, MSH2, PMS2

Recently Maciver (2016) supposed that genes participating in the homologous recombination (DMC1, RAD50, RAD51) and SPO11 responsible for double-strand breaks are retained in the agamic unicellular eukaryotes due to their possible employment in the gene conversion. According to his hypothesis, the latter combined with polyploidy may be a good alternative to the sexual process and meiotic recombination. Consequently, the "cyclic polyploidy + gene conversion" strategy could be a way around the Muller's ratchet effect. In a series of successive generations, polyploidy reduces the likelihood of expression of harmful mutations, while gene conversion corrects them (Maciver, 2016). The cyclic polyploidy was also considered as a compensatory mechanism in agamic unicellular eukaryotes (Kondrashov, 1994, 1997; Parfrey et al., 2008; Lahr et al., 2011). Thus, the cyclic polyploidy in *Amoeba* cell cycle, apparently, performs the function of providing recombination for reducing the mutational load and maintaining the genetic diversity in the absence of meiosis. The functioning of "meiosis-specific" genes, in doing so, can underlie these processes.

The issue of multifunctionality of meiotic genes brings us inevitably to the thorny path of the discussion of the sexuality origin and evolution. The origin of the sexual process and hence the meiosis is a broadly debated issue (Maguire, 1992; Kondrashov, 1994; Bogdanov, 2003; Erenpreisa et al., 2005; Goodenough, 2014; Speijer et al. 2015; Hofstatter and Lahr, 2019). According to the discussed above data on the occurrence of syngamy-associated and meiotic genes, most authors are inclined to believe that agamic protists lost meiosis in the course of evolution (Speijer et al., 2015; Kang et al., 2017; Tekle et al., 2017; Hofstatter et al., 2018; Hofstatter and Lahr, 2019). The Last Eukaryotic Common

Ancestor (LECA) is even considered as possessing traits of sexual process that inherited "meiotic toolkit" from the archaeal DNA repair machinery (Speijer et al., 2015; Hofstatter and Lahr, 2019). In turn, the present asexuals, including those among Amoebozoa, have lost sex secondarily.

On the other hand, Maciver with co-authors (2019) recently demonstrated that meiotic genes are expressed in *Acanthamoeba* cells in the exponentially growing culture where no any traits of sexual behavior were observed. In this regard, up-regulation of HAP2 and GEX1 genes are of particular interest, although this point was not discussed in the paper. These results contribute to the concept of participation of meiotic molecular machine in the homologous recombination in polyploid nucleus in the absence of sex (Maciver et al., 2019). As we already assumed, events that occur in *Amoeba proteus* cell cycle could also be regulated by similar molecular mechanisms. Further research of gene activity in the consecutive cycle phases could elucidate the validity of this hypothesis (or disprove it).

It should be recalled here that in "classical asexuals" – bdelloid rotifers – some meiotic genes are expressed – SPO11, REC8, HOP1, MSH4, and MSH5 – but there are no any evidences of sexual reproduction in this metazoan group (Hofstatter and Lahr, 2019). The observed situation is associated with participation of these genes' products in the DNA repair system that is highly efficient and allows rotifers to demonstrate extreme survival (Hofstatter and Lahr, 2019).

Surprisingly, mammalian cancer cells can also evidence for the meiotic genes multifunctionality and, particularly, participation in ploidy cycles. In the next section we will dwell on the issue of ploidy cycles in the cancer cells and on why association of their study with unicellular eukaryotes is not so incredible. However, here we only mention briefly that polyploidization/depolyploidization processes occurs in some cases in tumor cells (Erenpreisa and Cragg, 2013; Salmina et al., 2019; Chen et al., 2019). A number of meiotic genes were shown to be activated in tumor cells during the depolyploidization phase. These are the genes whose products are part of the cohesin complex, involved in conventional meiosis in sister chromatid cohesion, recombination, and homologues chromosomes separation – REC8, SGO1, SGO2, DMC1, SPO11, SCYP1,2,3, STAG3, RAD51 (Erenpreisa et al., 2005; Kalejs et al., 2006; Ianzini et al., 2009; Erenpreisa and Cragg, 2013; Salmina et al., 2019). Besides, MOS/MAP-kinase pathway responsible for spindle arrest in meiosis was shown to be active in endomitotic nuclei (Erenpreisa and Cragg, 2013). In any case, it is not possible to talk about "canonic" meiosis in the tumor cells, although researchers assume that the special types of division emerged in their life history (Ianzini et al., 2009; Salmina et al., 2019). Alternatively, the meiotic genes up-regulation in such a scenario of the ploidy cycle could mean their possible participation in the implementation of the similar strategies in other organisms.

Thus, distribution of "meiosis-specific apparatus" and its multifunctionality may yield new insights about development of sexuality in eukaryotes. It cannot be completely denied that this molecular machinery could emerge as a mechanism of providing genetic diversity or DNA repair independent of sexual reproduction *sensu stricto*. Then, meiosis originated in different lineages and this set of genes obtained new distinct specialization. We believe that this issue is not yet closed, and thorough study of the meiotic genes' functions in eukaryotes from different groups should contribute to its elucidation.

Look back to go forward?

The strategy of ploidy cycles, apparently, emerged in different, phylogenetically distant groups of eukaryotes, and mammalian cells are no exception. These are cancer cells, whose "life history" is currently the subject of many studies. Erenpreisa and Cragg (2007) proposed a model describing how the cancer cells switch from the regular mitotic cycle that provides their reproduction to the ploidy cycle (Fig. 3). The latter includes polyploidization phase that leads to the formation of giant polyploid cells and depolyploidization, or reduction, stage producing new generation of tumor cells. The authors described this strategy as a "life cycle" comparable to that of unicellular eukaryotes (Erenpreisa and Cragg, 2007). Such a transition can be realized in the tumor cells as a result of various – physiological or pathological – stresses (Chen et al., 2019).

Polyploid giant cancer cells (PGCCs) are formed in response to starvation, changes in temperature or pH, radiation (Chen et al., 2019); they are detected in tissue in hypoxia or hypoxia-mimic condition (Zhang et al., 2014). The emergence of PGCCs is a consequence of genotoxic chemotherapy that arrests the regular mitotic division, causing the

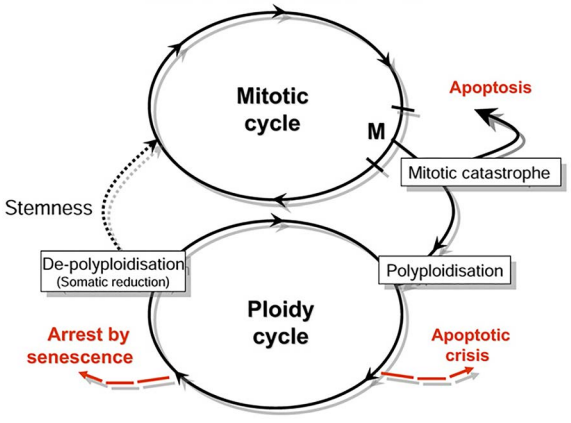

Fig. 3. Schematic model of a cancer cell life cycle that includes switch from the regular mitotic cycle to the ploidy cycle (with permission from Erenpreisa and Cragg, 2007).

mitotic catastrophe state, or induces DNA damage (Erenpreisa and Cragg, 2013; Torgovnick and Schumacher, 2015; Chen et al., 2019). In all these cases, PGCCs become a key to preservation of tumor in the conditions of the death of many cells. Some of them survive and reprogram to embryonal stem-like state providing the emergence of renewed population (Salmina et al., 2010; Erenpreisa and Cragg, 2013; Erenpreisa et al., 2018b; Salmina et al., 2019). A return to the mitotic cycle and recovery of the population (a new "clone") of tumor cells is achieved through depolyploidization (Erenpreisa et al., 2018b; Salmina et al., 2019). It is carried out either by reduction divisions of a special type, or by multipolar asymmetric or amitosis-like divisions (Salmina et al., 2019; Chen et al., 2019). In the latter cases, small cells with sub-nuclei separate, or even "bud", from the PGCC and produce a new generation of cancer cells, returning to the mitotic cycle (Rajaraman et al., 2006; Erenpreisa and Cragg 2013; Niu et al., 2016). The main benefit of such a strategy is providing genetic diversity of new population and its resistance to the therapy. In turn, renewed generation of cancer cells has new potential for spreading in the organism.

Here it should be noted, that in some scenarios separation of "micronuclei" containing damaged DNA is one of the important events of depolyploidization phase (Erenpreisa et al., 2012, 2018b). The nuclear lamina and peripheral heterochromatin are eliminated with nuclear envelope. As has been shown, the fate of eliminated material is degradation through the autophagy pathway (Yang et al., 2011; Ivanov et al., 2013; Erenpreisa et al., 2018b). The genetic mission of this phenomenon is to get rid of damaged "chromatin waste" and, therefore, to reduce mutational load. At the same time, the polyploidy provides sufficient number of variants for chromatin sorting (Erenpreisa et al., 2018b). Thus, despite the small number of survivors, the negative effects of genotoxic treatment can be mitigated and the cell population is capable of rescuing itself. Some patterns of the chromatin material elimination by budding, when separating "micronuclei" (Erenpreisa et al., 2012), notably resemble the "excessive" chromatin extrusion process, which we have observed in the *Amoeba* cells (Goodkov et al., 2019). Obviously, it does not make sense to talk about the phylogenetic relationship of protists and mammalian tumor cells. Nevertheless, these and other examples of the nuclear chromatin extrusion into the cytoplasm during the cell cycle indicate that this is an extremely ancient and universal phenomenon (Goodkov et al., 2019).

Moreover, the atavistic theory of carcinogenesis suggests that cancer cells switch to a "selfish" lifestyle like unicellular organisms (Davies and Lineweaver, 2011; Niculescu, 2016; Trigos et al., 2017, 2018; Erenpreisa et al., 2018a; Salmina et al., 2019). Analysis of RNAseq expression data for seven solid tumor types revealed that expression of genes with orthologs in bacteria and protists – "unicellular" genes – is elevated (Trigos et al., 2017). In contrast, the "multicellular" genes, including unique for placentals, are down-regulated. The coexpression between the genes responsible for the basic and evolutionarily early cellular processes and the genes, providing more complex cellular functions that are found only in the metazoan genomes, is disrupted in the tumor cells (Trigos et al., 2017, 2018; Erenpreisa et al., 2018a). In the abovementioned PGCCs, overexpression of "unicellular" genes occurs as well (Erenpreisa et al., 2018a). Besides, the genes involved in the stress response are also up-regulated. Moreover, the authors point out the phenotype changes in these cells – shape, cytoskeleton organization, locomotion pattern (Erenpreisa et al., 2018a). They start to look more like amoeboid protists, rather than cells in a tissue or organ. Noteworthy, somatic polyploidy also turned out to be associated with upregulation of genes related to stress response, stemness and unicellularity alongside the presence of some features of cancer cell profile (Vazquez-Martin et al., 2016).

Thus, the highly conserved, ancient programs of functioning of the cell as an individual organism are activated, while those associated with existence as a

part of tissue and organism are suppressed (Trigos et al., 2017, 2018; Erenpreisa et al., 2018a). The cancer cells seem to "look back", "remember" what it is to be an individual cell-organism. The autonomy is considered as a main hallmark of tumour cells (Hanahan and Weinberg, 2011). Consequently, use of analogous mechanisms that underlie passing of the life cycle could be assumed. Particularly, this implies that protists, including *Amoeba proteus*, might well merit consideration as a model for the study of the mechanisms of ploidy cycle in cancer cells. A more complete picture of distribution and features of such a strategy in different organisms will lead to a better understanding of its significance and evolution.

Conclusion

To sum up, we hope that this small discussion touched upon several important issues, which are currently in the focus of researchers' attention. And even such an "ordinary" organism like *Amoeba proteus* can be important, for instance, for study of sexual process origin and evolution, or can contribute to the understanding of cancer cells' biology. The analogous strategies of development, survival under pressure of the mutational load, or providing genetic diversity are apparently used across the eukaryotic tree. Therefore, every "building block" of knowledge of different organisms and consideration of all possible scenarios are necessary for obtaining the whole picture of evolution.

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