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ORIGINAL PAPER

Genome Re-duplication and Irregular Segregation Occur During the Cell Cycle of *Entamoeba histolytica*

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Abstract Heterogeneity of genome content is commonly observed in axenic cultures of *Entamoeba histolytica*. Cells with multiple nuclei and nuclei with heterogenous genome contents suggest that regulatory mechanisms that ensure alternation of DNA synthesis and mitosis are absent in this organism. Therefore, several endo-reduplicative cycles may occur without mitosis. The data also shows that unlike other endo-reduplicating organisms, *E.histolytica* does not undergo a precise number of endo-reduplicative cycles. We propose that irregular endo-reduplication and genome partitioning lead to heterogeneity in the genome content of *E.histolytica* trophozoites in their proliferative phase. The goal of future studies should be aimed at understanding the mechanisms that are involved in (a) accumulation of multiple genome contents in a single nucleus; (b) genome segregation in nuclei that contain multiple genome contents and (c) maintenance of genome fidelity in *E. histolytica*.

Keywords Genome reduplication · Regulation of cell division · Protist pathogen · *Entamoeba histolytica* · Mitotic events

Introduction

Accurate chromosome duplication, segregation and maintenance of genome content are critical for the survival of any organism. In this context, the protist pathogen, *Entamoeba histolytica* is an interesting case because it can tolerate variations in its genome content. *E. histolytica* infects the human host to cause amoebic dysentery and liver abscesses. Following a simple two-stage life cycle, it alternates between the dormant but infectious cyst and the proliferating trophozoite. Proliferation occurs anaerobically within the human intestine or in micro-aerophilic conditions at other sites

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of infection such as the liver or brain. Laboratory cultivation commonly uses micro-aerophilic conditions for propagating *E.histolytica* trophozoites.

Proliferating cells, in general, ensure the constancy of their genome by various mechanisms during each cycle. Checkpoint proteins do not permit progression of the cell cycle if all the conditions required for the preceding phase have not been faithfully completed. Initiation of DNA synthesis requires 'licensing' of DNA by specific protein complexes before new DNA strands are synthesized by polymerases. Similarly, other proteins ensure error free DNA synthesis, repair of damaged DNA and inhibition of DNA synthesis once genome duplication is complete. The cell ensures this through a large number of 'checks and controls' so that over-replication does not occur. Over-replication would pose critical problems during segregation, since each genome copy must be identical.

Therefore, for accurate chromosome duplication and segregation, DNA synthesis (S-phase) ends after 2 copies of the genome have been synthesized and segregated (mitosis) immediately thereafter.

Digressions from the above paradigm are observed during both S-phase and mitosis in *E. histolytica*. The average DNA content of an asynchronous cell population fluctuates between 1x-6x genome contents (Gangopadhyay et al. 1997a). Multiple genome contents maybe present in a single nucleus or distributed over multiple nuclei in a single cell (Das and Lohia 2002). This is achieved by re-duplication of the genome without segregation (Gangopdahyay et al. 1997a, Das and Lohia 2002). Unlike endo-reduplicating cells of Drosophila embryos, rodent trophoblasts and specific plant tissues (Grafi 1998), genome reduplication does not occur for a predicted number of cycles in *E. histolytica*.

Following DNA duplication, equal genome segregation typically occurs on a bipolar microtubular spindle, where two identical sets of chromosomes are aligned on the equator and segregated by moving towards the two poles to eventually form two identical daughter cells. Instead of bipolar mitotic spindles, a radial microtubular assembly is commonly seen in *E. histolytica* (Vayssie et al. 2004; Orozco et al. 1988; Dastidar et al. 2007), raising questions about the mode of chromosomal segregation in euploid or polyploid nuclei. Multi-nucleated cells represent uncoupling of cell division from mitosis. Heterogeneity is further aggravated in these multi-nucleated cells since daughter nuclei may not remain synchronous. These observations led to the hypothesis that polyploid nuclei or multi-nucleated cells result from alterations in the typical eukaryotic checkpoint control mechanisms (Banerjee et al. 2002; Lohia 2003). This review explores various mechanisms that explain the above hypothesis. These include: endo-reduplication, atypical microtubular assemblies at mitosis, genome partitioning and modifications in known processes that mediate cell cycle progression.

Endo-Reduplication versus the Mitotic Cycle

Many organisms exhibit endo-reduplication of their genome under special circumstances that disrupts the alternation of genome duplication and mitosis (Reviewed in Grafi 1998, Larkins et al. 2001). These include-differentiation of giant trophoblast cells in the rodent placenta, DNA replication of the polytene chromosomes, embryos of Drosophila and several plant tissues. In aberrant states, polyploidy has been observed in tumor cells an is a hallmark of many cancers. Often endo-reduplication is linked to terminal cells that do not need to divide any further. In several plant tissues, endo-reduplication is believed to be required for increased gene expression in order to maintain the high metabolic activity of the cells.

The net result of endo-reduplication is an increased number of genome copies that have not been segregated. While there are many hypotheses why mitotic cells switch to endocycles, the reasons vary in different cell types. Changes in CDK-cyclin activity, absence of B and A type cyclins, transcriptional and post-transcriptional modifications, changes in plant hormone levels etc are few examples of mechanistic changes between endo-reduplication and mitotic cycles. Apart from changes in the activity of mitotic cycle regulators, several unique genes have been identified in plant and Drosophila tissues that were required for endo-reduplicative cycles. These include *gnu, png, plu* (Renault et al. 2003) and other plant genes such as *ccs52* (Cebolla et al. 1999). Importantly, endo-reduplication is usually programmed to form a predicted number of exact copies of the genome either through multiple re-initiations of replication origins, or through repeated rounds of the S-phase with or without the gap phases (Grafi 1998).

In contrast, the mitotic cycle is precisely regulated to form only two copies of the genome followed by segregation into daughter cells. The regulation of a mitotic cell cycle has been extensively studied in many organisms using different mutants, which often display accumulation of multiple genome contents. In the fission yeast, a number of mutants were isolated that over-replicated their DNA without passing through mitosis. For example both over-expression of the $rum1^{+}$ (replication uncoupled mitosis) and s$rw1^{+}$(suppressor of r*ad1*w*ee1*) genes, inhibit onset of mitosis by negatively regulating p^{34}Cdc2/Cig2 complex (Moreno and Nurse 1994; Yamaguchi et al. 1997). Mutations that were identified as alleles of CDC2 and CDC13, the genes encoding the Cdc2 and cyclin B proteins showed similar effect. (Broek et al. 1991; Hayles et al. 1994). Mitotic CDK activity was also demonstrated to play a role beyond activation of mitosis. Suppression of DNA synthesis during meiosis in starfish oocytes required the activation of the mitotic CDK while reduction in mitotic CDK activity was required for endo-reduplication in several plant tissues, Drosophila salivary glands and megakaryocytes (reviewed in Grafi 1998).

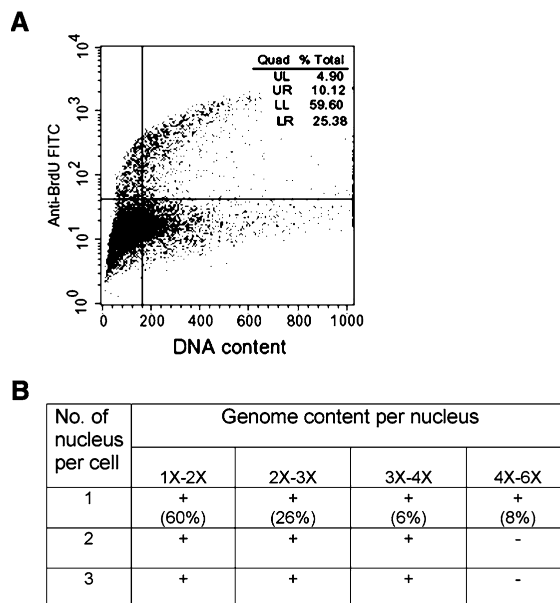
Loss of regulation of origin firing to once per cell cycle has been attributed to lack of phosphorylation of substrates by S-phase CDKs, degradation of proteins by ubiquitination and related checkpoint controls (Larkins et al. 2001). In addition to regulating entry into mitosis, oscillation of Anaphase promoting complex (APC) regulates pre-RC formation. Thus high APC activity in late mitosis/G1 allows pre-RC formation and low APC activity in S-phase/G2 prevents pre-RC formation and therefore prevents re-replication. Emi1 (*e*arly *m*itosis *i*nhibitor *1*), a negative regulator of APC, is believed to co-regulate pre-RC formation through its action on APC (Machida and Dutta 2007). Other genes that have been implicated in endo-reduplication are subunits of the DNA replication-licensing complex. Importantly, each redundant pathway that prevents re-replication is dependant on regulating components of the pre-replication complex (pre-RC) (Sivaprasad et al. 2007).

Unlike other endo-reduplicating or mitotic cells, *E.histolytica* lack a precise program to generate a fixed number of copies of the genome. Observations pertaining to this conclusion are given in the following section.

Genome Reduplication Occurs Commonly in *Entamoeba* trophozoites

Flow cytometric analysis from different studies demonstrated that discrete populations of cells with 1x and 2x genome contents were not identifiable in axenic cultures of *E. histolytica* (Dvorak et al. 1995, Gangopadhyay et al. 1997a, Das and Lohia

2002). Instead the cell population showed genome contents ranging from 1x to 6x or more. Estimation of genome content was based on fluorescence of DNA binding dye either by flow cytometry (whole cell genome content) or scanning cytometry (individual nuclear genome content). Increased heterogeneity was observed in late log phase culture, suggesting that depletion of nutrients maybe responsible for loss of cell cycle control (Gangopadhyay et al. 1997a, Vohra et al. 1998). However, later studies showed that genome reduplication occurred within 2 h after initiation of DNA synthesis in freshly cultured synchronized cells (Das and Lohia 2002). This important observation established that *E.histolytica* trophozoites intrinsically reduplicate their genome within an extended S-phase without mitosis. Additional factors like nutrient / growth factor depletion may exacerbate the accumulation of multinucleated cells by delaying cytokinesis. Importantly, uni-nucleated and multi-nucleated cells containing 1x-6x genome contents were found in axenic cultures of *E.histolytica* (Fig. 1).



No. of nucleus per cell	Genome content per nucleus			
	1X-2X	2X-3X	3X-4X	4X-6X
1	+ (60%)	+ (26%)	+ (6%)	+ (8%)
2	+	+	+	-
3	+	+	+	-

Fig. 1 Genome reduplication during the cell cycle of *E.histolytica.* (**A**) Dotplot shows incorporation of 5′-bromo-2′-deoxyuridine (BrdU) on the *y*-axis and total DNA content on the *x*-axis in *E. histolytica* trophozoites after 24 h growth (early log phase) in axenic culture. Genome content of whole cells was measured by Propidium iodide (PI) fluorescence. Lower left (LL) quadrant shows cells with 1x-2x genome content; lower right (LR) quadrant shows cells with greater than 2x genome contents; upper left (UL) quadrant shows DNA synthesis in euploid (1x-2x) cells and upper right (UR) quadrant shows DNA synthesis in cells with greater than 2x genome contents. Percentage of cells in each quadrant is shown in inset table. (**B**) Table shows percentage of uni-nucleated cells with 1x-6x genome contents. Similar distribution was observed in bi- and tri- nucleated cells (quantitative data not available). Genome content of individual nuclei was estimated by DAPI fluorescence in a scanning cytometer and data from 2000 nuclei was normalized (Adapted from Das and Lohia 2002). Average distribution (%) of genome content in uni-nucleated cells was calculated from more than three independent sets of experiments. A similar analysis for multi-nucleated cells has not been done

E. histolytica Genome Encodes Most of the Eukaryotic DNA Replication Licensing Factors but Lacks Crucial Regulators

Since endo-reduplication without mitosis was common in these cells, we asked if genes regulating initiation of DNA replication were encoded in the genome. Licensing of DNA replication is achieved by the formation of the pre-RC at the replication origin in eukaryotes. Formation of pre-RC requires the ordered assembly of the origin recognition complex (ORC), Cdc6, Cdt1 and the Mcm2-7 proteins. Loading of Mcm2-7 to replication origins requires the binding of the ORC to the replication origin (Stillman 1994) and recruitment of Cdc6 (Diffley 1996) and Cdt1 (Nishitani et al. 2000). The pre-RC is activated by protein kinases Cdc7 (Masai et al. 2002) and cyclin-dependant kinase (Dahmann et al. 1995) for initiation to occur. Mcm8 and Mcm9, two additional members of Mcm2-7 family have been recently found in metazoa, but not in yeast (Maiorano et al. 2006). Preliminary characterization of Mcm8 showed that it is not associated with Mcm2-7 complex and possibly functions after pre-RC formation (Maiorano et al. 2005). Other factors that regulate the transition from pre-RC to replication initiation are Mcm10 and Cdc45 (Merchant et al. 1997; Wohlschlegel et al. 2002; Gregan et al. 2003). Both these proteins also have a role in elongation (Bell and Dutta 2002). The GINS complex allows the helicase to interact with regulatory proteins in replisome progression complexes (RPCs) that are assembled during initiation and disassembled at the end of S phase (Gambus et al. 2006). Replication origin licensing is inactivated during S-phase but Mcm2-7 may function as a helicase that unwinds DNA ahead of the replication fork during S-phase (Labib et al. 2001). Once S-phase has begun, the formation of new pre-RC is kept in check by high CDK activity and by the activity of the protein geminin (Bell and Dutta 2002).

Data mining of known genome sequences is a useful tool that shows the presence or absence of a gene based on sequence homology. If present, the degree of sequence similarity reflects on the protein's ability to carry out homologus function. However, absence of sequence homologs does not rule out the possibility of functional homologs being encoded by heterologus genes. Data mining of the *E. histolytica* genome sequence (http://www.tigr.org/tdb/e2k1/eha1) revealed that, sequence homologs of most of the conserved components of the pre-replicative complex were encoded in the amoeba genome with few notable exceptions (Table 1; Mukherjee and Lohia, unpublished observations). Genes encoding the heterohexamer-origin recognition complex (ORC) required for loading Mcm proteins on replication origins were absent except for a single homolog that was similar to both Orc1 and Cdc6. It is possible that Eh Cdc6/Orc1 subunit alone may form a homo-hexamer to fulfill the functions of the ORC complex. A similar situation is seen in archea (Kelman and Kelman, 2003) where a single gene encoded a Cdc6/Orc1 homolog was hypothesized to function both as Cdc6 and a homo-hexamer of Orc1 instead of the ORC complex. Sequence analysis shows the absence of the Cdt1, the Cdc7-Dbf4 pair and the epsilon unit of DNA polymerase—a substrate of the GINS complex. Absence of DNA pol ε, is intriguing since all the components of the GINS complex were encoded in *E. histolytica*. While the catalytic domains of Mcm2-7 were conserved, truncations in the N-and C-terminal domains were observed in Mcm3-6. Additionally, the sequence of Eh Mcm2-7 proteins was found to be more divergent than the yeast and human homologs using standard bioinformatics analysis (Gangopadhyay et al. 1997b, Das et al. 2005). Biochemical analysis of the Mcm2-3-5 proteins showed constitutive association of these proteins with the chromatin fraction (Das et al.

Table 1 Comparison of components of the pre-Replication Complex in *E. histolytica* with yeast and humans

Homologs	*H. sapiens*	*S. cerevisae*	*E. histolytica*
MCM2-7	+	+	+
MCM8	+	–	+
MCM9	+	–	+
MCM10	+	+	–
ORC2-6	+	+	–
ORC1	+	+	ORC1/CDC6
CDC6	+	+	
CDC7	+	+	–
DBF4	+	+	–
CDT1	+	–	–
Geminin	+	–	–
CDC45	+	+	+
GINS	+	+	+
Polε	+	+	–

The table shows the presence or absence of known genes that have been identified as components of the pre-RC in human (*H. sapiens*), yeast (*S. cerevisae*) and *E. histolytica*. *E. histolytica* sequence homologs were identified by using yeast or human sequences in a BLAST search against the *E. histolytica* genome database

2005). Whether this association indicates constitutively 'replication-competent' DNA or simply the helicase function of Mcm proteins remains to be established. Strikingly, MCM8, 9, present mostly in higher organisms, were well conserved in *E. histolytica*. Therefore while a large number of proteins required for DNA replication licensing are encoded in the amoeba genome, absence of crucial regulators like Cdt1, geminin, Cdc7/Dbf4, Mcm10, DNA pol ε may lead to loss of replication licensing to 'once per cell cycle' thereby permitting endo-reduplication without mitosis in *E. histolytica*. During the DNA synthetic phase cells accumulated multiple genome contents (upto 6x in individual nuclei) (Das and Lohia 2002). It was demonstrated that DNA synthesis stopped after 5–6 h in synchronized cells (Gangopadhyay et al. 1997a) following which the average genome content was reduced to 1x-2x. However, a discrete G2 phase or synchronized mitotic phase was not observed. In order to analyze the mechanism of genome segregation, the microtubular assembly at mitosis was studied.

Alterations in the Microtubular Assembly May Lead to Erratic Genome Partitioning

Instead of typical microtubular spindles, a radial microtubular assembly has been observed during mitosis in *E. histolytica* (Orozco et al. 1988; Solis and Barrios 1991; Vayssie et al. 2004). It has been contemplated that differences in the C-terminal amino acid sequences of Eh $\alpha\beta$ tubulins from mammalian and other tubulins may alter physico-chemical properties of microtubular assembly in *E. histolytica* (Sanchez et al. 1994; Katiyar and Edlind 1996). Homology modeling of the tertiary structure of Eh $\alpha\beta$ tubulins showed that 16 amino acids at the C-terminal of Eh β tubulin formed an extension when compared to the crystal structure of mammalian $\alpha\beta$ tubulins (Roy and Lohia 2004). The extended C-terminal suggested a steric hindrance in tubulin polymerization and the formation of unstable microtubular assembly. A direct proof of this concept has yet to be demonstrated.

Bipolar spindle assemblies are nucleated at microtubule organizing centers (MTOCs). In spite of the presence of the components of microtubule organizing centers (MTOCs)-such as γ-tubulin in *E. histolytica* (Ray et al. 1997, Vayssie et al. 2004), bipolar spindle assembly could not be visualized in normally cycling cells. However, after incubation with taxol-a microtubule stabilizing drug, bipolar spindles were observed at low frequency (8–12%) (Dastidar et al. 2007). This suggests that the bipolar spindle assembly is extremely unstable and explains why it was not observed earlier. Whether the instability is due to changes in the tubulin monomers as suggested above or due to changes in proteins required to stabilize the bipolar spindles or both, is an important issue.

Kinesin motor proteins are required both for formation and stabilization of bipolar spindles during mitosis (Miki et al. 2005). Six kinesin genes have been identified in *E. histolytica* (Dastidar et al. 2007). Eh Klp5, a homolog of the BimC/Eg5 kinesin family was studied for its role in regulating microtubular assembly (Dastidar et al. 2007). Using a functional genomics approach it was demonstrated that increased expression of Eh Klp5 showed greater numbers of bipolar spindles than control cells (Fig. 2). Additionally, increased expression of Eh Klp5 led to decrease in the polyploid population compared to control cells. Therefore, euploidy in Eh Klp5 overexpressing transformants must be facilitated by increased stability or number of bipolar spindles that in turn facilitate equational chromosome segregation. It is not known if Eh Klp5 also plays a role in preventing endo-reduplication in *E.histolytica*. Other genes that have been identified to be involved in regulating the genome content are discussed below.

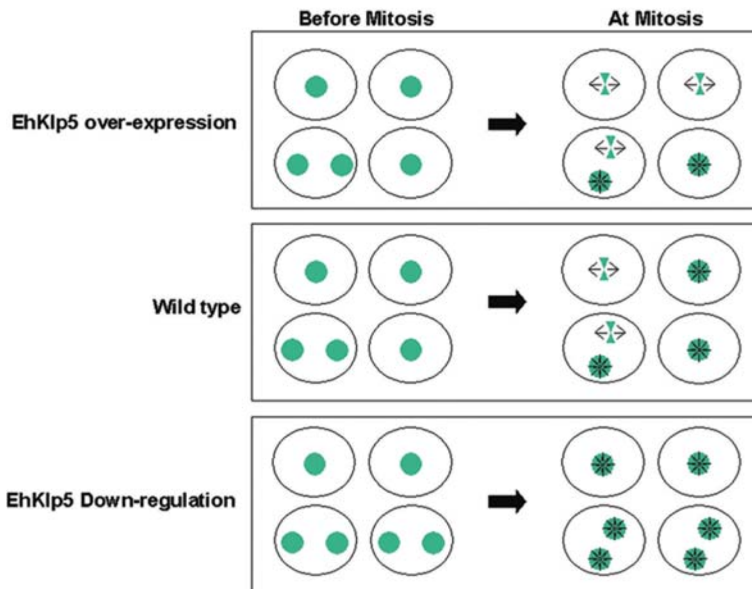


Fig. 2 Microtubular assembly is regulated by Eh Klp5 in *E. histolytica.* Cartoon shows distribution of radial (star-like) and bipolar spindles (spindle-like) of *E. histolytica* microtubules after taxol treatment in Eh Klp5 stable transformants, control cells and Eh Klp5 down regulated transformants. Nuclear DNA content is shown as closed green circles, which are distributed on radial or bipolar microtubular assemblies at mitosis. Eh Klp5 shows greater number of bipolar spindles compared to control (represented qualitatively here). Down regulated Eh Klp5 transformants do not show any bipolar spindles (Adapted from Dastidar et al. 2007)

Multi-Nucleated Cells in *E. histolytica* Mutants

Over-expression or constitutive activity of some genes have been shown to yield multi-nucleated cells suggesting that these proteins maybe involved in regulating cytokinesis and chromosome segregation. Several Ras superfamily small GTPases were identified in *E. histolytica*, which may regulate signal transduction pathways in this organism (Lohia and Samuleson 1993; Shen et al. 1994; Lohia and Samuelson 1996; Ghosh et al. 1997; Guillen et al. 1998). Over-expression of the constitutively active (G12V) mutants of Eh RacG, Eh RacA and the kinase domain of Eh PAK2 (substrate of Eh RacA) led to the formation of multi-nucleated cells (Guillen et al. 1998; Ganguly and Lohia, unpublished observation; Arias-Romero et al. 2006). Over-expression of Eh Meth transferase, a protein involved in methylation and silencing of gene expression at multiple targets, also leads to multinucleated cells (Fisher et al. 2006). However, in the absence of directed studies, it is difficult to assign a specific target of this protein that may inhibit cytokinesis or related events. In an interesting study, it was shown that *E.invadens* trophozoites required assistance from helper cells to carry out cytokinesis (Biron et al. 2001). This observation hints at subsets of cells within a population to have different functions and a level of complexity where *Entamoeba* cells may not be a vegetatively dividing simple system.

Bacterial toxins are known to regulate cell division by effectively paralyzing the Rho mediated actin polymersiation (Aktories and Barbieri 2005). Toxin A and B from *Clostridium difficile* are glucosylating enzymes which share similar substrate specificity for mammalian Rho proteins. Interestingly, it was observed that EhRho1, the homolog of HsRhoA is glucosylated at the similar residue (Thr37 in HsRhoA) by toxinB alone while toxinA could not modify any other Rho proteins from *E.histolytica* (Majumder et al. 2006). It was also observed that EhRho1 could not be modified by C3 toxin from *Clostridium botulinum*, though the predicted residue for modification is conserved in Eh Rho1 (Godbold and Mann 2000; Godbold et al. 2002). Since *E. histolytica* co-habits the human colon with bacteria that secrete rho-modifying toxins, it is conceivable that amoeba Rho proteins are modified to render them resistant to some of the bacterial toxins. Although the molecular changes leading to this resistance are yet unclear, these changes may well influence the role of Eh Rho1 to regulate cytokinesis in *E. histolytica*.

Regulation of DNA Synthesis During Encystation

Current methods do not allow encystation of *E.histolytica* trophozoites in axenic cultures, but the reptilian parasite *Entamoeba invadens* can successfully encyst under conditions of low salt and glucose in the growth medium. Prior to encystation, these cells undergo an additional cycle of DNA replication so that each cell contains 4x genome contents. Two nuclear divisions yielding 4 nuclei, each containing 1x genome content, follow this. Thus the cell ensures that 4 complete copies of the genome are contained in each cyst. While axenic cultures of *E.invadens* switch from vegetative growth to encystation when the glucose or NaCl concentrations are lowered, this effect has not been mimicked in *E.histolytica*. Changes in intracellular calcium were seen during encystation of *E.invadens* suggesting that calcium mediated pathways regulate differentiation from vegetative growth to encystation (Ganguly and Lohia 2001).

Irregular DNA Synthesis and Chromosome Segregation: Observations and Questions

The studies described above suggest that heterogeneity of genome content in *E. histolytica* is a combined effect of uncontrolled endo-reduplication without chromosome segregation (Das and Lohia 2002) and instability of the bipolar spindle assembly (Dastidar et al. 2007). Multiple MTOCs, variations in the tertiary structure of $\alpha\beta$ tubulin and a predominantly radial microtubular structure are indicative of differences in the regulation of equational segregation.

Genome analysis suggests that reduplication may occur because of the absence of proteins that function to couple genome duplication with genome partitioning. Data mining also revealed an absence of members of the Anaphase promoting complex,

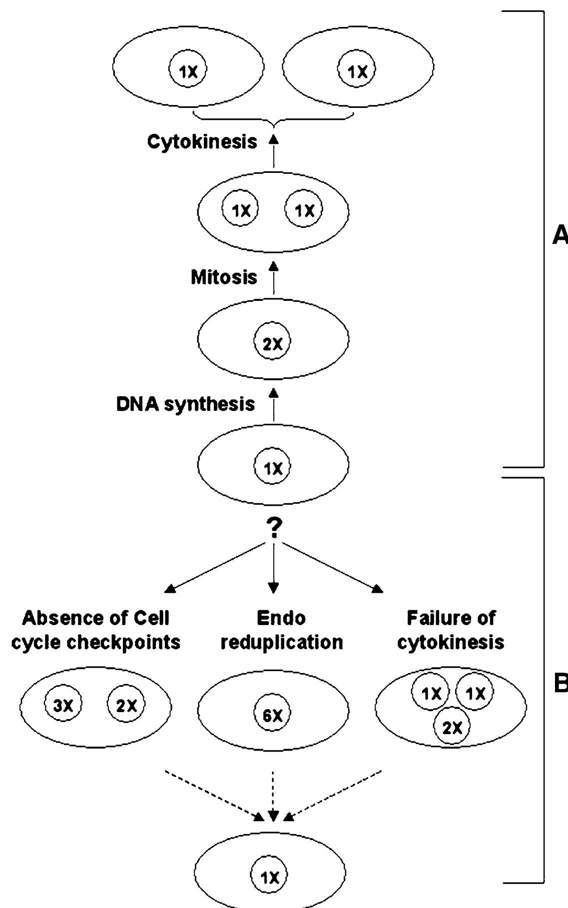


Fig. 3 Heterogeneity of genome content in *E. histolytica* trophozoites during vegetative growth. This cartoon is summarized from observations presented in Gangopadhyay et al. (1997a), Das and Lohia (2002) and Dastidar et al. (2007). Majority of cells in log phase of growth are euploid with single nucleus containing 1x-2x genome contents (**A**). A subset of the cell population is detected with heterogenous genome contents in a single nucleus. Another subset of multi-nucleated cells are also found (**B**). The different subsets may occur due to—(a) endo-reduplication or irregular mitotic events or absence of regulatory proteins that couple DNA duplication to mitosis [uni-nucleated cells with >2x genome contents] and (b) failure of cytokinesis coupled to mitosis [multi-nucleated cells]

kinetochore complex and other proteins required for ensuring alternation of genome duplication with chromosome segregation. Approximately, 9,938 genes were identified in the genome initially, of which almost 50% were unidentified ORF's or hypothetical proteins (Loftus et al. 2005). In spite of the absence of conserved sequence homologs, it is possible that functional homologs of checkpoint proteins are encoded in the amoeba genome. In addition, epigenetic mechanisms such as gene silencing or chromatin modification may influence genome content in *Entamoeba*. Replacing glucose with short chain fatty acids leads to restriction in the ploidy of *E. invadens* trophozoites (Byers and Eichinger 2005) due to their effect on chromatin modifying enzymes.

Based on all the available information, it appears that endo-reduplication is inherent to amoeba cells. At some point, it is likely that most of these cells return to euploidy or 1x genome content. However, the genome content of a significant number of cells fluctuates between 2x-6x in one or more nuclei (Fig. 3). How do these cells segregate multiple copies of their genome? Are there sequential mitotic events? Do radial microtubular assemblies segregate the genome by atypical methods?

Cells with either two bipolar spindles, or two different assemblies-one bipolar and another radial, have been seen in taxol arrested Eh Klp5 transformants. How does the cell regulate these multiple and diverse mitotic events? Is each nucleus an independent entity, without cross talk with other nuclei? What is the mechanism by which polyploid cells return to 1x genome content (Fig. 3)? Alternatively, polyploid cells could be terminal, while euploid cells continue to cycle.

Is the heterogeneity of genome content, essential for the survival of the organism under adverse conditions? If polyploidy does not lead to extinction, then does the heterogeneity have a bearing on the success of target tissue invasion? The advantages of accumulating multiple genome copies may ensure survival under hostile conditions. A logical possibility is that cells with multiple genome copies may precede encysting cells and we simply cannot monitor this process in vitro, for lack of suitable methods.

Conclusions

Proliferating *E. histolytica* cells undergo a combination of DNA endo-reduplication and irregular genome segregation. Both processes suggest absence of control mechanisms that regulate the typical eukaryotic cell cycle. As more information becomes available from functional genomics studies, it should be possible to gain a better understanding of gene networks involved in the cell division cycle of *E. histolytica*.

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