Eukaryotic checkpoints are absent in the cell division cycle of *Entamoeba histolytica*

SULAGNA BANERJEE, SUCHISMITA DAS and ANURADHA LOHIA*

Department of Biochemistry, Bose Institute, P1/12 CIT Scheme VIIM, Kolkata 700 054, India

*Corresponding author (Fax, 91-33-3343886; Email, anuradha@boseinst.ernet.in)

Fidelity in transmission of genetic characters is ensured by the faithful duplication of the genome, followed by equal segregation of the genetic material in the progeny. Thus, alternation of DNA duplication (S-phase) and chromosome segregation during the M-phase are hallmarks of most well studied eukaryotes. Several rounds of genome reduplication before chromosome segregation upsets this cycle and leads to polyploidy. Polyploidy is often witnessed in cells prior to differentiation, in embryonic cells or in diseases such as cancer. Studies on the protozoan parasite, *Entamoeba histolytica* suggest that in its proliferative phase, this organism may accumulate polyploid cells. It has also been shown that although this organism contains sequence homologs of genes which are known to control the cell cycle of most eukaryotes, these genes may be structurally altered and their equivalent function yet to be demonstrated in amoeba. The available information suggests that surveillance mechanisms or 'checkpoints' which are known to regulate the eukaryotic cell cycle may be absent or altered in *E. histolytica*.

1. Introduction

The decision for a cell to divide is a tightly regulated process that integrates signals from different sources. These signals arise mainly from the extracellular environment or intracellular checkpoint controls. Environmental signals help the cell to determine when the nutrients are available and when there are no overriding reasons to arrest cell division. Signals from internal checkpoints ensure completion of the preceeding steps before the next stage of division takes place (Hartwell and Weinart 1989). All these signals are integrated and processed by proteins involved in cell cycle control. In most eukaryotes, the entire genome must be faithfully duplicated before the cell can divide. Reduplication of the genome is prevented until cell division is completed. This control ensures that each daughter cell receives an identical copy of its genetic material. An exception to this rule is observed in cancer cells which occasionally become polyploid as a result of failure to restrain the S-phase, despite the failure to undergo complete mitosis (Pathak *et al* 1994).

Most of the information about the regulation of cell division in eukaryotes has been obtained from genetic and biochemical studies on the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Forsburg and Nurse 1991). It was shown that the proteins controlling the cell division cycle of these yeasts were well conserved in other eukaryotes (Nurse 1990) and the checkpoints controlling the cell cycle of yeast are similar in the eukaryotic kingdom. Based on this notion, several groups have compared the cell cycle of different eukaryotes to identify unique/similar sequences in homologs of cell cycle proteins and aberrations in the molecular

Abbreviations used: BrdU, 5′-bromo-2 deoxyuridine; MTOC, microtubule organizing centre.

regulation of cell cycle control, particularly in relation to disease.

Interesting case studies are pathogenic eukaryotes, which infect the human host. Analysis of the cells cycle of these organisms may offer novel targets and thereby, an important method of controlling disease. Pathogenic protozoan parasites, like *Plasmodium*, *Leishmania*, *Giardia* and *Entamoeba* are complex eukaryotes, which lead a parasitic existence. In doing so, they often use host proteins to their advantage, harbour prokaryotic genes (acquired as a result of lateral transfer) and also show loss of 'typical' eukaryotic characteristics, particularly as seen in *Entamoeba* (Clark *et al* 2000).

Do these cells then conform to the known parameters of the typical eukaryotic cell cycle? What are the checkpoints controlling the cell division of these organisms? Is the cell division cycle of these organisms driven by different extracellular signals? Are the intracellular effector proteins homologus to those in other eukaryotes? It is difficult to answer many of these questions since protozoan parasites would have to be studied *in vivo* within a complex vertebrate host to identify the different host signals and the parasite's response. It is quite likely that different genes would be activated if these organisms were to parasitise a vertebrate host, than if they were cultured under axenic conditions. Nonetheless, *in vitro* studies of axenically cultured *Entameoba* trophozoites, have demonstrated several unique features which suggest that the regulation of cell division in this parasite is intrinsically different from that observed in other single celled eukaryotes. Thus, these protozoa may proliferate by hitherto unidentified rules either in isolation or within their host environment.

2. Lack of genetic methods to study the cell cycle of *E. histolytica*

E. histolytica is axenically cultured – without any associated organisms – in a complex broth (Diamond *et al* 1978) in the laboratory. It is extremely difficult to use routine microbiological methods (such as obtaining single colonies) and virtually impossible to use genetic methods similar to those used in the study of yeasts to study *E. histolytica*. Thus understanding the progression of the cell division cycle in this organism has largely relied on the use of multi-parameter flow cytometry. Orthologues of eukaryotic cell cycle proteins have been cloned and characterized from this organism using degenerate oligonucleotide primers to conserved domains in the eukaryotic proteins, amplifying DNA fragments by polymerase chain reaction (PCR), cloning and sequencing these products to characterize these genes. Functional complementation of mutants to identify genes regulating

the progression of the cell cycle in *E. histolytica* was not possible since classical genetical and microbiological methods could not be used here.

Another problem in the study of cell division of *Entamoeba* has been the difficulty in obtaining synchronous cultures with a high mitotic index. Several methods have been used as an attempt to synchronize *Entamoeba* culture, such as hydroxyurea and removal of serum deprivation from the growth medium.

3. Identification of cell cycle phases of *E. histolytica*

Using fluorescent DNA dyes such as Propidium iodide on fixed *E. histolytica* trophozoites, Dvorak *et al* (1995), have analysed the different cell cycle phases of *E. histolytica* by flow cytometry. They have used mathematical modelling to display the G1, S, G2 phases of the *Entamoeba* cell cycle as a series of overlapping Gaussian curves, unlike other eukaryotes where discrete phases of the cell cycle can be identified (Dvorak *et al* 1995). Consistent with this, results from another laboratory demonstrated a unimodal DNA distribution ranging from 1n to 10n genome contents, in an asynchronous cell population (Gangopadhyay *et al* 1997a).

The two discrete fluorescent peaks for G1 and G2 cells commonly observed in the yeasts and other eukaryotes show that most of the cells in an asynchronous population contain either 1n or 2n genome contents, with an intermediate population in S-phase which contains $> 1n$ and $< 2n$ genome contents. But results obtained with *E. histolytica* trophozoites suggested that asynchronously growing cells may contain greater than 2n genome contents in a typical axenic culture. Thus, defined G1, S and G2 phases within 1n–2n genome contents could not be identified in these cells.

4. Growth arrest and cell synchronization

A couple of studies have shown that *E. histolytica* cells could be partially synchronized using hydroxyurea (Austin and Warren 1983) or 2% serum (Orozco *et al* 1988). Antimitotic drugs such as colchicine had no effect on *E. histolytica* even at concentrations greater than 200 μg/ml (Orozco *et al* 1988), suggesting changes in tubulin binding sites or impermeability to colchicine. However, complete removal of serum from the growth medium for 10–12 h led to growth arrest and inhibition of DNA synthesis (Gangopadhyay *et al* 1997a). Remarkably, the DNA content profile of serum starved cells suggested that inhibition of DNA synthesis arrests cells in whichever cell cycle phase they may be in. Thus, these cells are incapable of completing the cell division cycle if the

nutrients and growth factors provided by serum are removed from the culture medium. It was observed that re-addition of serum was required for re-entry of cells in the cell cycle (Gangopadhyay *et al* 1997a). Interestingly, there was a lag phase after re-addition of serum, during which all the cells with genome contents > 1n–2n up to 10n, possibly underwent cell division or completed their cycle and accumulated as an euploid population before DNA synthesis was initiated again (Gangopadhyay *et al* 1997a).

Thus, *E. histolytica* trophozoites appeared to accumulate greater than 2n genome contents in axenic culture, but serum starvation and re-addition could effectively synchronize these cells in the G1 phase prior to initiation of the S-phase. S-phase cells in this population were identified by adding the thymine analogue, 5′-bromo-2 deoxyuridine (BrdU) to mid-log phase cells (Gangopadhyay *et al* 1997a). BrdU incorporating cells or DNA synthesizing cells were identified by flow cytometry using fluorescin isothyacyanate (FITC) tagged anti-BrdU antibody. It was noted that the genome content of BrdU incorporating cells ranged from > 1n to 10n (Gangopadhyay *et al* 1997a). This further underscored the earlier observation and suggested that DNA reduplication could occur during one cell cycle of *E. histolytica* without cells having to undergo division.

5. Cultures show progressive deregulation of DNA synthesis and cell division in *E. histolytica* with time

Axenic cultures of *Entamoeba* are made up of a heterogenous population of cells with varying DNA contents. Two independent studies reported that polyploid cells are more prevalent in late log phase cultures (Vohra *et al* 1998; Gangopadhyay *et al* 1997a) whereas freshly subcultured cells are mostly euploid (Das and Lohia 2002). In a typical eukaryotic cell, DNA synthesis stops after one genome duplication and mitosis occurs (Stillman 1996). In contrast, *Entamoeba* cells show (i) DNA synthesis beyond 2n genome content, and (ii) progressive accumulation of polyploid cells in culture with an increase in time. This progressive deregulation of DNA synthesis from cell division may result partly from depletion of growth factors in the culture medium with time (Vohra *et al* 1998; Gangopadhyay *et al* 1997a). It is also possible that the amoeba cell cycle is programmed to produce multiple copies of the genome before cell division irrespective of external factors (Das and Lohia 2002).

To minimize the effect of depletion of growth factors from the culture media, cells were sub-cultured every 24 h in a fresh medium. When the DNA synthesis profile of these cells were studied by monitoring BrdU incorporation using flowcytometry, it was seen that the DNA content of the cells increased to almost 10n genome content in 90 min, suggesting genome reduplication within one cell division cycle. Furthermore, as the S-phase of *Entamoeba* lasts for 5–6 h (Gangopadhyay *et al* 1997a; Vohra *et al* 1998), it appears that genome reduplication occurs within the S-phase. Thus, unlike other eukaryotes, genome reduplication without cell division may be an inherent property of the *E. histolytica* cells.

6. Genome reduplication without nuclear division occurs in *E. histolytica*

The continued synthesis of DNA without alternation with cell division in *E. histolytica* may be loosely classified as endoreduplication. Unlike other eukaryotes in which mitosis is inhibited when they switch to endoreduplication before differentiation (Grafi 1998), *E. histolytica* trophozoites continue to proliferate as they undergo reduplication. Furthermore, the occurrence of multinucleated cells suggests that more than one nuclear division may occur before cell division. Experimental results obtained from quantitation of DNA content in each nucleus in uni-nucleated and multi-nucleated trophozoites showed that the DNA content of each nucleus varied from 1n to 10n on an average (Das and Lohia 2002). Sporadically, nuclei with greater than 10n genome contents could also be detected. Similar results were also observed with trophozoites of the reptilian parasite *Entamoeba invadens* [S Das and A Lohia, unpublished observations].

Although data from *E. histolytica* cysts is not available, by using cysts from axenically cultured *E. invadens*, Das and Lohia (unpublished observations) showed that each nucleus of a quadri-nucleate cyst contained equal amounts of DNA, which was approximately 25% of the DNA amount seen in trophozoites with 1n genome content. A case in point here is the lack of conclusive evidence for meiotic division during encystation of *Entamoeba* trophozoites. Whether the *Entamoeba* undergoes meiosis, homologus recombination and all other processes commensurate with sexual reproduction has long been debated. Indeed, old texts refer to 'amitosis' as the method of cell division in the *Entamoeba*. However, homology cloning and DNA sequencing have identified a large number of orthologues of eukaryotic genes which are known to control the mitotic cycle. It is possible that similar orthologues of key genes involved in meiosis may also be discovered once the genome sequencing of *E. histolytica* is complete.

The cell division cycle of the reptilian parasite *E. invadens*, is largely similar to that of *E. histolytica* (Ganguly and Lohia 2001). Interestingly, BrdU incorporation studies demonstrated that 4n genome contents were synthesized before encystations occurred in this

parasite. Additionally, it was shown that the concentration of free intracellular calcium ions changed remarkably during encystations (Ganguly and Lohia 2001). Thus analysis of the role of second messengers will be important in understanding the progression of the amoeba cell cycle as well.

7. Cell cycle genes of *E. histolytica*

One of the most important cell division regulators is p34^{cdc2}, a 34 kDa Ser/Thr kinase, which is the product of the cdc2 gene of *Schizosaccharomyces pombe* and of the *cdc28* gene of *S. cerevisiae* (Hindley and Phear 1984; Lorincz and Reed 1984). The p34^{cdc2} is activated at the 'start', the beginning of S-phase, and at mitosis and meiosis when replicated chromosomes are segregated into the daughter cells (M-phase). The p34^{cdc2} binds cyclins, proteins which vary in concentration during the cell cycle and which contain conserved sequences called the 'cyclin box' to form an active complex. The activity of p34^{cdc2}–cyclin complex is regulated by the phosphorylation of Ser, Thr, and Tyr residues of p34^{cdc2} (Ducommun *et al* 1991). Activation of mitotic cyclin-cdc2 kinase complex (Stern and Nurse 1996) is normally required for entry into mitosis. Inhibition of this mitotic CDK activity has been shown to cause cells to undergo multiple rounds of DNA replication (Stillman 1996). Cyclin E has been reported to play a role in regulating the initiation of S-phase in both mitotic and endocycles (Sauer *et al* 1995). The *Eh cdc2* gene was one of the first *Entamoeba* cell cycle genes to be cloned and characterized (Lohia and Samuelson 1993). It was also one of the first genes to be demonstrated to contain an intron. Prior to this discovery, it was largely believed that amoeba genes did not contain introns. Interestingly, the position of the intron was evolutionarily conserved with the first intron in the *S. pombe cdc2* gene (Lohia and Samuelson 1993). The *Eh cdc2* gene was seen to be different from other cdc2 homologs in the PSTAIRE motif and later identified to be similar to cdk4 (unpublished observations). Unfortunately, homologs for cyclins have not been identified yet. Although the amino acid sequence of the *Eh cdc2* gene is similar to other homolgs of the same gene, its role in regulating the amoeba cell cycle is not yet clear.

7.1 *MCM proteins in* Entamoeba

Several factors essential for the regulation of initiation of DNA replication have been identified in eukaryotic cells. These are hexameric origin recognition complex (ORC), *CDC6*, *CDC28* and six minichromosome maintenance proteins (Mcm2-7) from *S. cerevisiae* and their homologues from other eukaryotes. Mcm proteins are known to act as licensing factors in regulating initiation of DNA replication (Tye 1999), and their presence in *E. histolytica* (Gangopadhyay *et al* 1997b; Das and Lohia 2000) suggests that they may be involved in the regulation of DNA replication. Paradoxically, several rounds of DNA reduplication occur in *Entamoeba*, suggesting that these *MCM* homologs are either inactive or their function may be altered in *E. histolytica*.

7.2 *Tubulin proteins of* Entamoeba

In all eukaryotic cells studied so far the microtubule skeleton is required for mitosis and cytoplasmic organization. In higher eukaryotes the onset of mitosis is marked by the condensation of the chromosomes and formation of spindle fibers from the two poles of the cells, which gradually grow and extend throughout the cell. As mitosis progresses, the condensed chromosomes align themselves on the spindle fibers along the equator of the cell, prior to segregation into the daughter cells. These microtubules are made up of *a*- and *b*-tubulin subunits fused along their length. They nucleate from two bodies on either pole of a dividing cell, the microtubule organizing centre (MTOC). One of the key proteins in the MTOC is *g*-tubulin, which is actually involved in microtubule nucleation in higher eukaryotes (Joshi 1994).

Homologs of *a*-, *b*-, and *g*-tubulin have been identified in *Entamoeba* (Sanchez *et al* 1994; Katiyar and Edlind 1996; Ray *et al* 1997). Though the homologues for *a*- and *b*-tubulin genes have been identified in *Entamoeba*, microtubular spindle structure has not yet been seen. Electron microscopic analysis of cryofixed and cryosubstituted trophozoites of *E. histolytica* failed to demonstrate microtubules in both the cytoplasm and the nuclei of non-dividing amoeba (Clark *et al* 2000). It has been suggested that the differences in the amino acid sequences of *a*- and *b*-tubulin in amoeba – compared to other eukaryotic tubulins – is responsible for their inefficient polymerization and this is possibly the reason why no observable spindle structure is seen in these organisms (Katiyar and Edlind 1996). In the light of this information, the method of chromosome segregation in *Entamoeba* remains an elusive and intriguing question.

Immunofluorescent confocal microscopy shows Eh *g*-tubulin to be localized as multiple extra-nuclear bodies. Expression of *g*-tubulin was measured in the different phases of the cell cycle by flow cytometry. It was seen that the G1 cells showed a lower level of *g*-tubulin expression whereas maximal expression was obtained in the S/G2 phase of the cell cycle (Ray *et al* 1997). The increase in the *g*-tubulin content prior to the onset of mitosis indicates an increase in the number of MTOC in the cell thereby implying that a centrosome duplication cycle

exists in *Entamoeba* just as in higher eukaryotes where the centrosome division cycle precedes the nuclear division. However, the presence of centrosomes and MTOCs is arguable in the absence of visible microtubular structure. However, the three tubulin components involved in microtubule polymerization and centrosomal formation are expressed in amoeba. It remains to be demonstrated unequivocally whether they polymerize to form a microtubular spindle nucleated by the MTOC.

Anti-microtubule drugs as colchicines, usually used to study cell division in higher eukaryotes, do not have any marked effect on these cells (Orozco *et al* 1988). A recent study has shown that the antimicrotubule drug oryzalin inhibits the growth of *Entamoeba invadens* as well as *E. histolytica*, the former being more resistant to the drug than the latter, and that effective doses of oryzalin are higher for *Entamoeba* than for the other parasitic protozoa examined thus far. Pretreatment of trophozoites with oryzalin prevents encystation of trophozoites without the drug. Accumulation of trophozoites in the mitotic phase was observed after culture with oryzalin (Makioka *et al* 2000). This is of considerable significance in the study of the cell cycle in amoeba because oryzalin is a potent mitotic-phase inhibitor and may become a useful tool for studies on the relationship between the cell cycle and encystation of this parasite.

8. Cytokinesis in *Entamoeba*

One of the most important aspects of the cell division cycle is cytokinesis – the final process, which physically separates the two daughter cells with equal DNA content from each other. During cytokinesis, a contractile ring forms at the equator of the dividing cell. This ring is a contractile bundle of actin filaments that is similar to a stress fibre. In response to specific signals, different proteins are recruited which modulate changes in the actin cytoskeleton to bring about this cellular process. In *Entamoeba*, homologues of actin (Edman *et al* 1987), myosin (Arhets *et al* 1992), profilin (Binder *et al* 1995), p21 rho (Lohia and Samuelson 1993), ras and rap (Shen *et al* 1994), p21 rac (Lohia and Samuelson 1996) and Diaphanous (Ganguly and Lohia 2000) genes have been identified and fully characterized. Overexpression of a mutant RacG-V12 not only deregulated cell polarity but also caused a defect in cytokinesis (Guillen *et al* 1998). The Eh Diaphanous gene product has also been shown to be colocalized with polymerized actin at the site of cell division (Ganguly and Lohia 2000). While scanty information is available on the molecular factors and mechanisms which regulate cytokinesis, an interesting report showed helper cells or 'midwives' assisting trophozoites to divide (Biron *et al* 2001). It was suggested that extracellular

factors secreted by 'midwives' were required for the final separation of two daughter cells.

9. Conclusion

It may be seen the primitive protozoan *Entamoeba* is unique as far as its cell division cycle is concerned. The organism lacks the surveillance mechanisms which in higher eukaryotes function as checkpoints and regulate cell division. As a result, *Entamoeba* cells reduplicate their genome several times before cell division occurs. Several homologues of eukaryotic genes that are involved in the regulation of the cell division cycle have been identified and cloned. From the little that has been studied, it is apparent that the story of cell division in this parasite may well be very different from that of other eukaryotes. While there is hope for discovering a novel drug target, the real fun will be in understanding how the wily parasite proliferates inspite of the complex surveillance system of the vertebrate host, often using this very system to its advantage.

References

Arhets P, Rahim Z, Raymond-Denise A, Sansonetti P and Guillen N 1992 Identification of a myosin heavy chain gene from *Entamoeba histolytica*; *Arch. Med. Res.* **23** 41–43

Austin C J and Warren L G 1983 Induced division synchrony in *Entamoeba histolytica*: Effects of hydroxyurea and serum deprivation; *Am. J. Trop. Med. Hyg.* **32** 507–511

Binder M, Ortner S, Erben H, Scheiner O, Weidermann G, Valenta R and Duchene M 1995 The basic isoform of profilin in pathogenic *Entamoeba histolytica*. cDNA cloning, heterologus expression and actin binding properties; *Eur. J. Biochem.* **233** 976–981

Biron D, Libros P, Sagi D, Mirelman D and Moses E 2001 Midwives assist dividing amoebae; *Nature* (*London*) **410** 430

Clark C G, Espinosa-Cantellano M and Bhattacharya A 2000 *Entamoeba histolytica*: an overview of the biology of the organism; in *Amebiasis* (ed.) J I Ravdin (London: Imperial College Press) pp 1–45

Das S and Lohia A 2000 MCM proteins of *Entamoeba histolytica*; *Arch. Med. Res.* **31** 269–270

Das S and Lohia A 2002 Delinking of S-phase and cytokinesis in the protozoan parasite *Entamoeba histolytica*; *Cell Microbiol.* **4** 55–60

Diamond L S, Harlow D R and Cunnick C A 1978 New medium for axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*; *Trans. R. Soc. Trop. Med. Hyg.* **72** 431–432

Ducommun B, Brambilla P, Felix M A, Franza B R Jr, Karsenti E and Draetta G 1991 cdc2 phosphorylation is required for its interaction with cyclin; *EMBO J.* **10** 3311–3319

Dvorak J A, Kobayashi S, Alling D W and Hallahan C W 1995 Elucidation of the DNA synthetic cycle of *Entamoeba* spp. Using flow cytometry and mathematical modeling; *J. Euk. Microbiol.* **42** 610–616

Edman U, Meza I and Agabian N 1987 Genomic and cDNA actin sequences from a virulent strain of *Entamoeba histolytica*; *Proc. Natl. Acad. Sci. USA* **84** 3024–3028

Forsburg S L and Nurse P 1991 Cell cycle regulation in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*; *Annu. Rev. Cell. Biol.* **7** 227–256

Gangopadhayay S S, Ray S S, Kennady K, Pande G and Lohia A 1997a Heterogeneity of DNA content in axenically growing *Entamoeba histolytica* HM1: IMSS clone A; *Mol. Biochem. Parasitol.* **90** 9–20

Gangopadhayay S S, Ray S S, Sinha P and Lohia A 1997b Unusual genome organization in *Entamoeba histolytica* leads to two overlapping transcripts; *Mol. Biochem. Parasitol.* **89** 73–83

Ganguly A and Lohia A 2000 The diaphanous protein from *Entamoeba histolytica* controls cell motility and cytokinesis; *Arch. Med. Res.* **31** 137–139

Ganguly A and Lohia A 2001 Cell cycle of *Entamoeba invadens* during vegetative growth and differentiation; *Mol. Biochem. Parasitol.* **112** 279–287

Grafi G 1998 Cell cycle regulation of DNA replication: the endoreduplication perspective; *Exp. Cell Res.* **244** 372–378

Guillen N, Boquet P and Sansonetti P 1998 The small GTP binding protein RacG regulated uroid formation in the protozoan parasite *Entamoeba histolytica*; *J. Cell. Sci.* **111** 1729–1739

Hartwell L H and Weinert T A 1989 Checkpoints:controls that ensure the order of cell cycle events; *Science* **246** 629–634

Hindley J and Phear G A 1984 Sequence of cell division gene CDC2 from *Schizosacchharomyces pombe*; patterns of splicing and homology to protein kinases; *Gene* **31** 129–134

Joshi H C 1994 Microtubule organizing center and *g* tubulin; *Curr. Opin. Cell. Biol.* **6** 55–62

Katiyar S K and Edlind T 1996 *Entamoeba histolytica* encodes a highly divergent *b* tubulin; *J. Euk. Microbiol.* **43** 31–34

Lohia A and Samuelson J 1993 Cloning of Ehcdc2 gene from *Entamoeba histolytica* encoding a protein kinase p34 cdc2 homologue; *Gene* **127** 203–207

Lohia A and Samuelson J 1993 Molecular cloning of ras homologue (rho) from *Entamoeba histolytica*; *Mol. Biochem. Parasitol.* **58** 177–178

Lohia A and Samuelson J 1996 Heterogeneity of *Entamoeba histolytica* rac genes encoding p21 rac homologues; *Gene* **173** 205–208

Lorincz A T and Reed S I 1984 Primary structure homology between the product of yeast cell division control gene and CDC28 and vertebrate oncogenes; *Nature (London)* **307** 183–185

Makioka A, Kumagai M, Ohtomo H, Kobayashi S and Takeuchi T 2000 Effect of the antitubulin drug oryzalin on the encystation of *Entamoeba invadens*; *Parasitol. Res.* **86** 625–629

Nurse P 1990 Universal control mechanism regulating onset of M-phase; *Nature (London)* **344** 503–508

Orozco E, Solis F J, Dominguez J, Chavez B and Hernandez F 1988 *Entamoeba histolytica*: Cell cycle and nuclear division; *Exp. Parasitol.* **67** 85–95

Pathak S, Dave B J and Gagos S 1994 Chromosome alterations in cancer development and apoptosis; *In Vivo* **8** 843–850

Ray S S, Gangopadhayay S S, Pande G, Samuelson J and Lohia A 1997 Primary structure of *Entamoeba histolytica g* tubulin and localization of amoebic microtubule organizing center; *Mol. Biochem. Parasitol.* **90** 331–336

Sanchez M A, Peattie D A, Wirth D and Orozco E 1994 Cloning, genomic organization and transcription of the *Entamoeba histolytica a* tubulin gene; *Gene* **146** 239–244

Sauer K, Knoblich J A, Richardson H and Lehner C F 1995 Distinct modes of cyclin E/cdc2c kinase regulation and S-phase control in mitosis and endoreduplication cycles of *Drosophila* embryogenesis; *Genes Dev.* **9** 1327–1339

Shen P S, Lohia A and Samuelson J 1994 Molecular cloning of ras and rap genes from *Entamoeba histolytica*; *Mol. Biochem. Parasitol.* **64** 111–120

Stern B and Nurse P 1996 A quantitative model for the cdc2 control of S-phase and mitosis in fission yeast; *Trends Genet.* **12** 345–350

Stillman B 1996 Cell cycle control of DNA replication; *Science* **274** 1659–1664

Tye B 1999 MCM proteins in DNA replication; *Annu. Rev. Biochem.* **68** 649–686

Vohra H, Mahajan R C and Ganguly N K 1998 Role of serum in regulating the *Entamoeba histolytica* cell cycle: a flow cytometric analysis; *Parasitol. Res.* **84** 835–838