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Evolution of the Cell Cycle

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SUMMARY

Cell proliferation involves duplication of all cell constituents and their more-or-less equal segregation to daughter cells. It seems probable that the performance of primitive cell-like structures would have been dogged by poor duplication and segregation fidelity, and by parasitism. This favoured evolution of the genome and with it the distinction between 'genomic' components like chromosomes whose synthesis is periodic and most other 'functional' components whose synthesis is continuous. Eukaryotic cells evolved from bacterial ancestors whose fused genome was replicated from a single origin and whose means of segregating sister chromatids depended on fixing their identity at replication. Evolution of an endo- or cytoskeleton, initially as means of consuming other bacteria, eventually enabled evolution of the mitotic spindle and a new means of segregating sister chromatids whose replication could be initiated from multiple origins. In this primitive eukaryotic cell, S and M phases might have been triggered by activation of a single cyclin-dependent kinase whose destruction along with that of other proteins would have triggered anaphase. Mitotic non-disjunction would have greatly facilitated genomic expansion, now possible due to multiple origins, and thereby accelerated the tempo of evolution when permitted by environmental conditions.

1. INTRODUCTION

Sustained cell proliferation entails the duplication of all cell constituents and their segregation to two daughter cells in a manner sufficiently equal for both to inherit the capacity for repeating this process. Cell division is the basis of all life. Moreover the similar chemistry of living organisms suggests that we are all descended through an unbroken line of divisions from a single ancestral cell. Most cell constituents, for example ribosomes, are present in large numbers and are synthesised (with the exception of M phase) continuously thoughout the interval between divisions: this process is called cell growth. In contrast, cells contain only one or two copies of each chromosome and microtubule organizing centres (MTOCs), whose duplication and segregation (known as the chromosome cycle) involves greater care (i.e. fidelity) is template driven, and is periodic. How did this distinction between 'functional' and 'genomic' components arise and how do cells coordinate the periodic synthesis of the latter with the continuous synthesis of the former?

Coordination between cell growth and the chromosome cycle is largely achieved by a dependence of the chromosome cycle on cell growth but not vice versa; growth continues unabated for two or three cycles regardless of any progression through the chromosome cycle (Creanor & Mitchison 1984). However, both DNA replication and mitosis are usually dependent on growth of the cell to a critical size (Killander & Zetterberg 1965; Nurse 1975). By storing up growth beforehand, embryonic cells emancipate themselves temporarily from the need for ongoing growth. This paper is concerned with the evolution of the chromosome cycle, how and why it evolved.

2. PROPERTIES OF EUKARYOTIC CHROMOSOMES AND THEIR SEGREGATION APPARATUS

All eukaryotic cells have multiple linear chromomsomes which are duplicated by replication forks that originate from multiple sites within each chromosome. With few exceptions, origins can only fire once per cycle with the result that all chromosomal DNA sequences are duplicated only once: the genome is in this sense democratic. Each chromosomal duplicate (sister chromatid) is segregated to opposite poles of the cell through forces exerted by microtubules that connect unique structures on each chromatid, called kinetochores, to microtubule organizing centres (MTOCs).

MTOCs are also duplicated only once per cycle. Their segregation towards opposite poles of the cell usually preceeds that of sister chromatids and is due to the activity of motor proteins that travel along microtubules in one direction or another. Motors connecting microtubules emanating from opposite MTOCs cause their repulsion, whereas others that associate with cortical structures near the cell's exterior can attract MTOCs and thereby orient the 'mitotic spindle apparatus' (see figure 1 and the paper from Mitchison).

Segregation of sister chromatids to opposite poles is only possible because of their previous 'alignment' on the mitotic spindle (Koshland 1994). Each pair of

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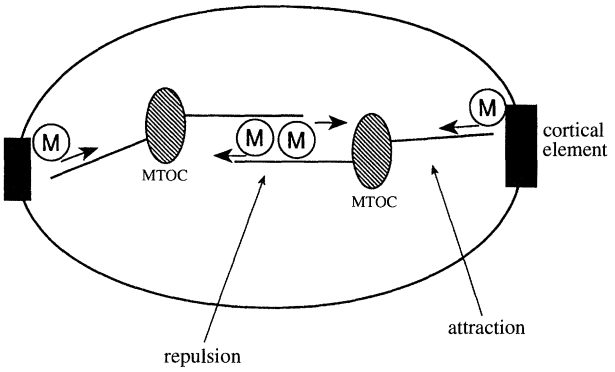

Figure 1. The mitotic apparatus without chromosomes. Spindles emanating from microtubule organizing centres (MTOC) have a defined polarity with the plus end pointing away from the MTOC. Repulsion between spindles or bundles of spindles of opposite polarity (which emanate from sister MTOCs) is thought to be brought about by motor proteins of the kinesin or dynein family and to cause repulsion of sister MTOCs.

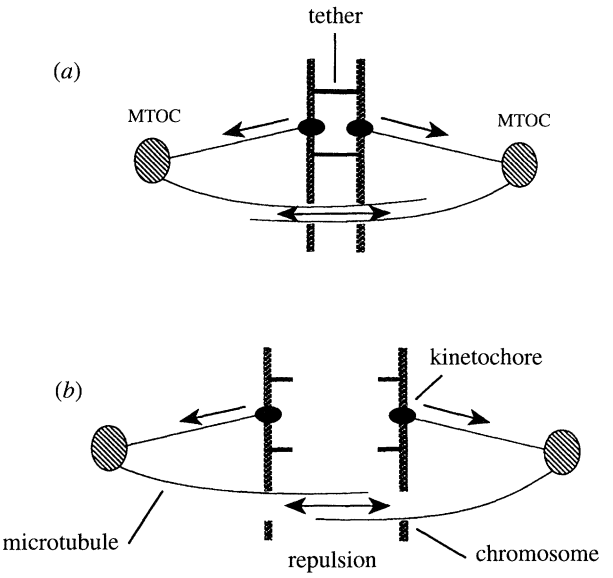

Figure 2. (*a*) Alignment of chromosomes on the spindle apparatus (metaphase) and (*b*) the disjunction/segregation of sister chromatids at anaphase. Two different sets of microtubules are required: those attached to kinetochores and those which run between sister MTOCs. The former are responsible for attracting sister chromatids to opposite poles (at anaphase A) and the latter for ensuring that sister MTOCs are not pulled together and for driving them further apart during anaphase B.

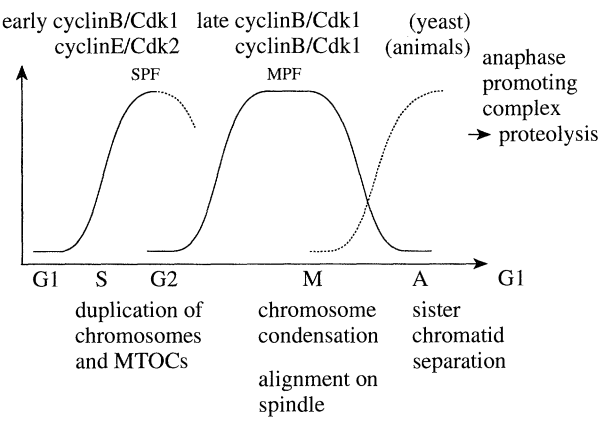

Figure 3. Important signals during the eukaryotic cell cycle. The cyclin/cdks that trigger S and M phases in yeast and animal cells are shown above.

sisters becomes attached to eachother during replication by tethers that are still ill-defined. Kinetochores are captured by microtubules emanating from an MTOC and attracted towards it by means of microtubule depolymerization or motor proteins. Sister kinetochores are prevented from attaching to the same pole either by geometrical constraints on the orientation of sister kinetochores or by controls that prevent segregation until all pairs of sister kinetochores are under tension, i.e. are being pulled to opposite poles (Murray 1995). In many, but not all, eukaryotes, pairs of sister chromatids align on a 'metaphase' plate that is midway between the poles. Tension between all pairs of sister chromatids (or simply their attachment to the mitotic spindle) is the stable endpoint of the alignment process and is thought to signal the next crucial step: severing of the tethers that connect sister chromatids, which allows their attraction to opposite MTOCs (anaphase A) and repulsion between MTOCs themselves and/or their attraction to cortical elements (anaphase B) (see figure 2).

It is vital that the duplication, alignment, and segregation of chromosomes and MTOCs occur in the correct order; alignment depends on previous duplication and segregation depends on previous alignment. A key feature of the chromosome cycle of eukaryotes that distinguishes it from the equivalent process in bacteria is the dependence of chromosome reduplication on their previous segregation at anaphase. We shall see later why this is so.

The last key feature of mitosis is chromosome condensation, which accompanies their alignment and segregation. If modest, as is the case in cells with small genomes, chromosome duplication is compatible with the alignment process and a single signal could trigger both processes (though, obviously, alignment must await duplication of sister kinetochores). Chromosome condensation is so modest in yeast (Guacci *et al.* 1994) that cells continue to transcribe chromatin throughout mitosis. Indeed in certain mutants it is possible to delay DNA replication until after the formation of a mitotic spindle (Pringle & Hartwell 1981). It is, however, essential that the signal for segregation (i.e. anaphase) be distinct from that which triggers duplication and alignment. The tethers that connect sisters must not be broken before alignment is complete. Eukaryotic cells monitor the state of chromosome and MTOC duplication and chromosome alignment on the spindle and prevent anaphase while replication forks still exist and while there exist pairs of sister chromatids that are not under tension on the mitotic spindle. Such controls are known as surveillance mechanisms or, more confusingly, as checkpoints (Hartwell & Weinert 1989).

If chromosome condensation is extreme, as in mammals, and thereby incompatible with duplication, then the latter must be triggered before condensation. Thus in most if not all eukaryotes, separate signals, known as S and M phase promoting factors, signal the onset of S and M phases respectively (see figure 3). This

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is an important point to grasp because the whole framework currently used for describing the eukaryotic cell cycle, including G1 and G2, the gaps between S and M phases, revolves around the distinction between S and M phases, which is minor compared to the distinction between these two processes and anaphase.

3. CYCLIN-DEPENDENT KINASES REGULATE KEY TRANSITIONS

A class of protein kinase whose activity depends on unstable regulatory subunits called cyclins have a key role in triggering S and M phases (Nurse 1990; Schwob *et al.* 1994). In yeast, a single cyclin-dependent kinase subunit (Cdk) complexed with B-type cyclins regulates chromosome duplication, condensation and alignment (Nasmyth 1993). In mammals, the Cdks that trigger S and M phases differ in both kinase and cyclin subunits (Sherr 1994): Cdk2 associated with cyclins E and A promotes S phase, whereas Cdk1 associated with cyclins A and B promotes M phase (see figure 3). Anaphase, in contrast, is triggered by a 20S particle with ubiquitin ligase activity called the Anaphase Promoting Complex (APC), which triggers proteolysis of cyclins and other proteins (Irniger *et al.* 1995; King *et al.* 1995). Proteolysis of the tethers that hold sisters together might be the key to chromosome segregation (Murray 1995), whereas cyclin destruction is vital for disassembly of the mitotic spindle and for setting up a new round of chromosome duplication (see below).

If cells use surveillance mechanisms to delay anaphase until chromosome duplication and alignment is complete, how do they ensure that the next round of duplication depends on the previous execution of anaphase? Initiation of DNA replication depends not only on S phase-promoting Cdks but also on the previous assembly of pre-replication complexes (pre-RCs) at future origins (Cocker *et al.* 1995; Diffley *et al.* 1994). S phase-promoting Cdks possibly trigger the transition of pre-RCs into replication forks (see figure 4). Because S and M phase-promoting Cdks inhibit the de novo assembly of pre-RCs (at least in yeast (Dahmann *et al.* 1995)), cells can only form these vital structures upon cyclin destruction at anaphase. Thus activation of the APC, when all chromosomes are correctly aligned on the mitotic spindle, triggers both chromosome segregation and formation of pre-RCs; the linkage between anaphase and re-replication is therefore built into the regulatory logic of the eukaryotic cell cycle (see figure 5).

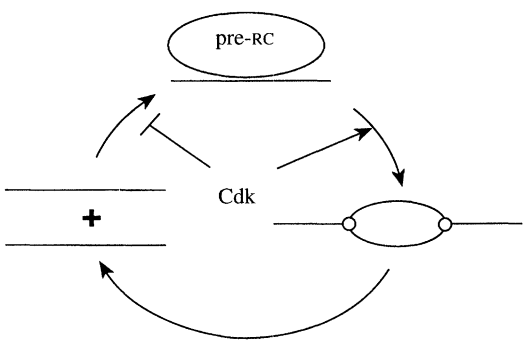

Figure 4. Initiation of DNA replication requires two steps: formation of a pre-replication complex (pre-RC) at future origins and the subsequent activation of an S phase promoting Cdk in late G1. In yeast, S and M phase promoting Cdks not only promote the initiation of DNA replication from pre-RCs but also inhibit the de novo assembly of pre-RCs at future origins. The dual function of Cdks ensures that a cycle of Cdk activation/destruction is necessary for a round of DNA replication, thereby ensuring that no origin can fire more than once per cell cycle.

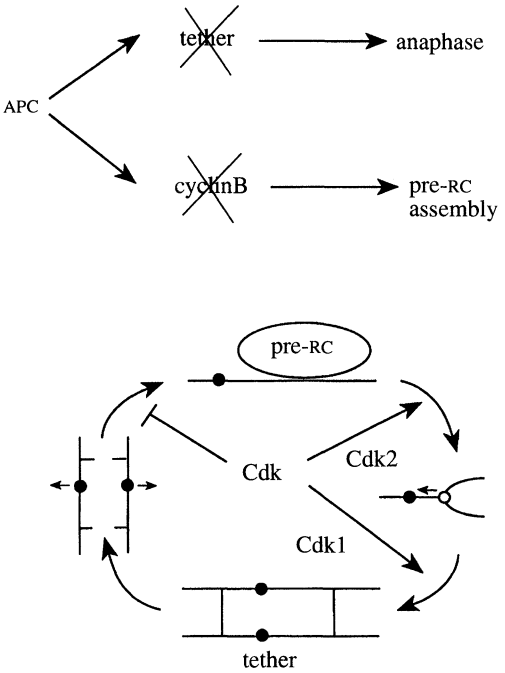

Figure 5. Linkage of re-replication with passage through anaphase. M phase Cdks are destroyed at the end of mitosis due to proteolysis of their cyclin B subunits, which depends on the activity of a ubiquitin protein ligase. The latter is a 20S particle which helps a ubiquitin conjugating enzyme to polyubiquitinate cyclin B via its destruction box. The 20S ubiquitin ligase is needed not only for ubiquitination of cyclin B but also for that of unknown proteins whose destruction is needed for sister chromatid separation. It is therefore known as the Anaphase Promoting Complex (APC). The putative tethers that hold sister chromatids together are possible targets for the APC. Thus, activation of the APC at metaphase triggers both anaphase and the destruction of Cdks. The latter is a precondition for the reassembly of pre-RCs. Rereplication cannot therefore preceed chromosome segregation.

4. EVOLUTION OF THE GENOME

Why does the eukaryotic cell cycle work in this manner and how did it evolve? My premise is that it is descended from a bacterial cell cycle. To appreciate how the eukaryotic cycle might have evolved from the bacterial one, we must first appreciate what sort of problems bacteria had already solved and what were and still are the limitations of their solutions.

We must start by confronting the one aspect of the cell cycle that I have so far avoided: how cells duplicate all those other cell constituents (i.e. how cells grow). Let us go back to a precellular world of free, functional, self replicating, RNA 'ribozymes' whose

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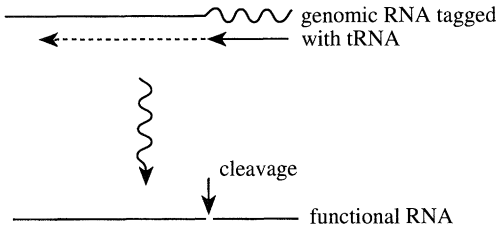

Figure 6. tRNAs may have initially distinguished genomic RNAs to be replicated from functional RNAs to be used.

replication is template driven. There is no distinction between growth and genome replication in this world. The concentration of each RNA (R) will be determined by the differential equation $dR/dt = (k1.R–K2.R)$; R at equilibrium is not 'determined' by the parameters affecting its synthesis or degradation but by competition between different RNAs for scarce resources and, as soon as we include interactions between RNAs, in some complicated manner on the concentration of cooperating and hindering RNAs. Life may have been short, brutal and unpredictable, but there will have been no problems with inheritance until sets of cooperating RNAs became associated with a structure, for example the inside out Obcell (Cavalier-Smith 1987).

Several problems would have beset the first precellular structures: (i) the system would have been very sensitive to parasitic RNAs; (ii) failure to inherit a crucial member of a cooperative RNA set would have been lethal; (iii) high fidelity replication would have been too time consuming and costly; (iv) copy number control would have been problematic to say the least (in effect, each RNA would have needed a copy number control system analogous to that of present-day bacterial episomes). Many of these problems were largely solved by a device to distinguish 'genomic' from 'functional' RNAs. It has been suggested that the marking of 'genomic' RNAs by tags that resemble modern day tRNAs was an early step in the evolution of ribosomal protein synthesis (Maizels & Weiner 1994). According to this hypothesis, only RNAs that carried tRNAs at their 5′ ends could be duplicated by a primitive replicase (see figure 6). Subsequent cleavage of the transcript at a position immediately 3' to the tag sequence would generate 'functional' RNAs no longer capable of replication. Such a device could have solved or at least ameliorated the segregation problem by having multicopy 'fused' genomic RNAs from which several 'functional' RNAs could be cleaved. Parasitic RNAs would have to join the genome to survive. Furthermore, if there were a system to control carefully the amount or copy number of the genomic RNA, then the concentration of functional RNAs could easily be determined by their half lives and rates of synthesis. Finally, by distinguishing duplication of the genome from the production of functional RNAs (before or after the genome's transition to DNA), the primitive cell or precellular structure (obcell?) would have been able to devote more time and energy to high fidelity replication of genomic RNAs without compromising the rate of production of functional RNAs.

The single copy fused DNA genome of modern bacteria is the logical end point of this line of inovation. Replacement of genomic RNA by more stable DNA would have further facilitated more stable and therefore larger genomes, whereas the evolution of a primitive segregation system that involved attachement of the genome to a cell or obcell wall would have facilitated a lowering of its copy number and thereby facilitated yet higher fidelity of replication with minimum cost. This transition from organisms in which enzyme synthesis was due to self-catalysis to one in which it was directed by a genome was clearly a key step in the evolution of cells. It is also one which has important implications for the nature of modern cell division cycles, where the duplication of functional and genomic components is regulated very differently.

5. THE CELL CYCLE OF THE CENANCESTOR

All living organisms share a large number of sophisticated biochemical networks involving hundreds of enzymes. It is therefore probable that the breakthrough organism, or 'cenancestor' from which all current living cells are descended would have needed a relatively sophistated genome not unlike that of modern bacteria. It is hard to imagine, as proposed by Woese, that it was a 'progenote' in which the relationship between genotype and phenoytype had not become fully established (Woese 1987).

There are three possible geneological relationships (*a*, *b*, and *c* of figure 7) between the cenancestor and the three existing domains: archebacteria, eubacteria and eukaryotes. Eukaryotes have many more features in common with archebacteria than with eubacteria, suggesting that they have a common ancestor not shared by eubacteria. But what is the relationship between this common ancestor with eubacteria and the cenancestor? An ingenuous method of 'rooting' the tree, using duplicated genes, suggests that pedigree *a* is the correct one, according to which eubacteria were the first group to branch off (Iwabe *et al.* 1989). This thesis is supported by two other considerations. It seems likely that the cenancestor obtained its energy by photosynthesis, a property that is confined to modern eubacteria or their organellar descendants. Photosynthesis need only have been lost once according to pedigree (*a*) but twice according to (*b*) and (*c*). More persuasive still, the earliest accepted fossils, which date to circa 3500 Ma BP, appear to have been formed from organisms that were remarkably similar to modern cyanobacteria (i.e. members of the eubacterial line);

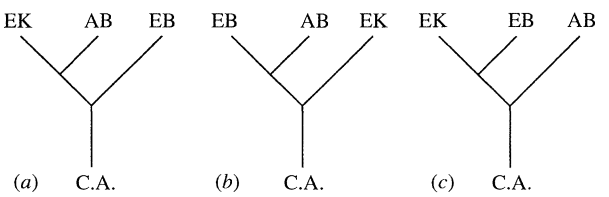

Figure 7. The three possible geneologies connecting archebacteria (AB), eubacteria (EB), and eukaryotes (EK) with their common ancestor (C.A.).

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that is, eubacteria evolved very early. The earliest eukaryotic fossils do not appear until about 2000 Ma BP (Knoll 1992). The implication is that we need to discover what sort of genome and chromosome cycle the common ancestor of eukaryotes and archea possessed. Did they resemble those of eukaryotes or those of archebacteria?

What little we know about the genome of archebacteria suggests that they look remarkably similar to that of eubacteria (Cohen *et al.* 1992). The halophilic archebacterium Haloferax volcanii has a genome predominantly composed of a single circular chromosome containing 3 000 000 b.p. of DNA (i.e somewhat smaller than that of *E. coli*). Its operon structure and gene order are remarkably similar to that of eubacteria. Whether it is replicated from a single origin is not known. Though it is early days for any hard conclusion, it would seem that the cell cycles and genomes of archebacteria may be much more similar to eubacteria than they are to eukaryotes. If true, this is a dramatic finding given the shared ancestry of eukaryotes and archea. The implication is that the eukaryotic cell cycle must have evolved from one that resembled that of modern day bacteria. Furthermore, there must have been dramatic changes in the ancestral eukaryotic line to produce genomes that are fragmented, linear, replicated from mulitple origins, and segregated by microtubule organising centres. To appreciate the significance of these changes and to start to speculate as to how they might have come about, we need to have a closer look at the bacterial chromosome cycle.

6. THE BACTERIAL CHROMOSOME CYCLE

Most, if not all, existing bacteria have genomes composed of a single circular double stranded DNA molecule. This arrangement simplifies the segregation problem to such an extent that mutations which affect chromosome partitioning are not even lethal; the consequence of non-disjunction is merely the loss of some cytoplasm (Niki *et al.* 1991). Another feature that distinguishes bacteria from eukaryotes is the initiation of chromosome duplication from a single bidirectional origin and its termination when the two forks meet at a unique site called Ter. This arrangement means that it takes a minimum of 40 mins to complete replication (Donachie 1993).

Bacterial genomes are invariably less than 5 000 000 b.p., which is about one third of the size of the smallest eukaryotic genomes, and two to three orders of magnitude smaller than that of humans. The small size of their genomes could well be a limitation imposed by the mechanisms that they use to duplicate and segregate their chromosomes. Their segregation apparatus may not be able to handle multiple chromosomes and the size of each chromosome is limited by having only a single replication origin. It is also possible, however, that their style of life never demanded larger genomes and hence more sophisticated mechanisms to duplicate and segregate them; that is, other aspects of their cell biology limited the evolution of bacteria that needed larger genomes.

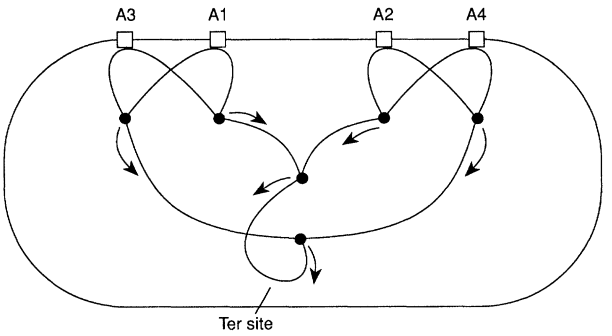

Figure 8. Re-initiation of DNA replication in bacteria can preceed termination of the previous round. Unique origin of replication at sites A1-A4. Origins may be associated with the cell wall, at least shortly after the initiation of replication. Termination at a unique Ter site is necessary to trigger partition. Arrows mark the movement of three sets of bidirectional replication forks; an old one approaching the Ter site and two new ones that have just moved away from the origins.

The use of only a single replication origin by bacteria is distinctly curious given the limitations that this imposes on the speed of chromosome duplication. Might it be a consequence of the mechanism by which they partition chromosomes? Unfortunately, this process is still poorly understood. One idea, proposed over 30 years ago, is that bacterial replication origins are attached to their rigid cell walls and that sister origins are segregated away from each other due to the geometry of cell wall growth (see figure 8). Recent data suggest, however, that the mechanism cannot be as simple as this. Bacterial chromosomes, or nucleoids, do not begin to show any signs of separation until DNA replication is complete (after which time sister nucleoids move apart very suddenly) which hints that a cytoskeletal apparatus might be responsible (Donachie 1993). Indeed one of the genes needed for partitioning, Muk B, encodes a protein with some similarity to motor proteins (Niki *et al.* 1991). Nevertheless, cell wall growth is also implicated because the distance by which chromosomes move apart is reduced in spherical mutants by an amount that is consistent with them being moved a fixed distance (1.2 μm) along the circumference of the cell. Lastly, there is some evidence that in polyploid cells, sister nucleoids like sister chromatids in eukaryotes, segregate away from each other soon after replication is complete (Donachie 1995). The picture that emerges is one in which sister chromatids are segregated via a cytoskeletal apparatus attached to sites in the cell wall, though it still unclear whether this attachment is via a unique locus equivalent to the eukaryotic kinetochore.

A key question concerns how bacteria determine which chromosomes in the cell are sisters that must be segregated away from eachother. Two sorts of mechanism are possible. The method used by eukaryotes is to hold sister chromatids together (even after their replication) and to use this property to attach them to opposite poles of the cell either by measuring the resistance to their being pulled apart by the mitotic apparatus or by sister kinetochores having a rigid geometry that forces their attachment to opposite

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poles. An alternative is to mark newly replicated DNAs as sisters at the time of their replication. One suspects that bacteria use the latter method and that they mark sisters at the time of initiation. If so, this would provide an explanation for why they use only a single replication origin. It would be difficult to coordinate the marking of sister chromatids at multiple origins.

Though they constrain genome size, the unique DNA replication origins of bacteria do not restrict their rate of proliferation because unlike eukaryotes reinitiation can preceed partition (see figure 8). Thus in rich media where cells proliferate rapidly, cells can contain four or even eight copies of sequences near the origin while they have only a single copy of sequences near the Ter site. The signal for partitioning sister nucleoids therefore stems from the Ter site, soon after termination (though only when the putative cell wall attachment sites have separated sufficiently). By allowing reinitiation before partitioning and thereby running several chromosome cycles in parallel, bacteria are able to proliferate with doubling times which are much shorter than the time it takes them to replicate their genomes. This method contrasts with the multiple origins used by eukaryotes, which achieves the same end but is probably incompatible with reinitiation before partitioning. With multiple origins, there would be no unique Ter site and it is hard to see how cells could link chromsome partitioning to the completion of DNA replication (as opposed to the absence of replication forks). This may therefore be yet another reason why bacteria use unique replication origins (though there is clearly a circularity to this logic).

7. EVOLUTION OF MITOSIS

Given the differences in strategies used by bacteria and eukaryotes to organize their chromosome cycles, it is all the more remarkable that the latter seem to have a bacterial ancestry. This is one reason why it has been proposed that the eukaryotic lineage might have deeper roots than suggested by the pedigree in figure 7, a view echoed in the paper by Doolittle in this symposium.

It is currently thought that a key transition in the evolution of the eukaryotic cell was the development of an endo- or cytoskeleton as an alternative method of protecting against osmotic shock to the rigid peptidoglycan cell wall or exoskeleton of bacteria (Cavalier-Smith 1987). This might have occured in an archebacterial lineage in which cells had already emancipated themselves from the constraints of a cell wall, for example by living in a high saline environment. Evolution of a cytoskeleton presumably enabled our ancestors to reemerge from such a narrow niche and, due to their lack of a cell wall, to develop phagocytosis, i.e. to survive by feeding on bacteria that were in plentiful supply. The ability to endocytose not only provided a new food supply but also facilitated the formation and acquisition of organelles. The nucleus of our eukaryotic ancestor might have evolved originally to protect the genome from enzymes used to digest that of its prey.

The 'invention' of microtubules will have been one of the key steps in the evolution of a radically new system of chromosome segregation. The chief merit of this cytoskeletal component is its stiffness. It seems likely that it would have initially evolved for some structural purpose, for example to protect cells against shearing forces, and later used to act as 'railway lines' for transporting material from one side of the cell to the other. Most but not all microtubules in existing eukaryotes are organised by MTOCs, whose ability to duplicate and segregate to opposite poles of the cell forms the basis for aligning sister chromatids on the mitotic spindle. It seems probable that the ability to duplicate and segregate MTOCs would have evolved as a means of ensuring their inheritance by each daughter cell; i.e. before their use in chromosome segregation. Though it is possible that bundles of microtubules could have played important roles in transporting material from one side of the cell to another without MTOCs, it is hard to envisage how this could have been useful for disjoining sister chromatids until the cells had developed a means by which opposite ends of the cell produced bundles with different polarity. The evolution of the MTOC and a system for controlling its duplication and segregation could therefore have been a critical step in the evolution of the mitotic spindle. It is still unclear how these organelles are duplicated once and only once during the cell cycle. Their segregation to opposite poles of the cell is less of a mystery (see figure 1).

Another key invention will have been sites to which the plus end of microtubules can attach and on which forces could be exerted either by localized microtubule depolymerization or by associated motor proteins. Such attachment sites might have originally evolved as a means of localizing sister MTOCs to specific poles of the cell (see figure 1), but the subsequent transfer of a protein domain capable of such activity to a site specific DNA binding protein could have created a primitive kinetochore. These then were the ingredients for a new system capable of specifying the identity of sister chromatids and of pulling them towards sister MTOCs. If sisters remain attached to each other after replication (by catenation or special tether proteins), it becomes possible to use measurement of tensile force to determine whether they have attached to opposite poles of the cell. Here then was a system whose robustness relies on exploration by trial and error (see Kirschner, this volume) rather than rigid structural attachments set up at the time of DNA replication. One of the great merits of the new system was its compatibility with multiple replication origins and, possibly even more important, with an almost unlimited number of chromosomes. It is inconceivable that a primitive mitotic system would have worked sufficiently well to be relied on and it would presumably have coexisted for some time (it may still do so) with the preexisting bacterial chromosome segregation apparatus. It has been suggested that microtubules still perform a merely supporting role in Dinoflagellates (Kubai 1975). Even if true, this subservience is probably secondary (Heath 1980), because even primordial eukaryotes, like Giardia,

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which branched off before the acquisition of mitochondria (Sogin *et al.* 1989), seem to use intranuclear spindles to segregate their chromosomes (Filice 1952; Adam 1991). The implication is that the 'mitotic' system for segregating chromsomes had already evolved in the common ancestor of all eukaryotes and was presumably one of the keys to their success.

8. CONSEQUENCES FOR GENOME REPLICATION

The mitotic system, once perfected, not only allowed genomes to escape the tyranny of unique replication origins but also permitted their fragmentation into multiple chromosomes. We have little or no idea how telomeres evolved. Circular chromsomes may avoid problems associated with replicating chromosome ends but cause problems when it comes to recombination. Their universal retention by bacteria presumably has something to do with their using termination as a means of signalling chromosome partitioning. The linearization of eukaryotic chromosomes might therefore have been an inevitable consequence of their using alternative methods to perform this task (see below). The evolution of mitosis therefore set the stage for expansion of the genome. It may even have accelerated the whole tempo of evolution in the eukaryotic lineage. Occasional chromosomal non disjunction at mitosis produces progeny in which whole chromosomes are duplicated instantly and if compatible with survival continue to be inherited. Such duplicates could have greatly accelerated the evolution of new enzymatic functions.

One of the disadvantages of the mitotic system is that it is incompatible with reinitiating rounds of replication before chromsome partition. There are at least two reasons for this (see figure 9). First, re-replication of kinetochores before disjunction of sisters at anaphase would lead to ambiguity as to which pair were to be segregated to opposite poles. Second, if tethers holding sister chromatids together are essential for alignment and their severence is the signal for anaphase, then it would be difficult if not impossible to sever selectively those tethers that hold 'aunts' together while retaining the tethers that attach the next generation of sisters for use during the subsequent mitosis. The development of a method to prevent re-replication before anaphase has been completed would therefore have been a crucial step. One of its consequences would have been a tighter control over ploidy than is seen in bacteria, which would have facilitated the subsequent evolution of haploid/diploid cycles and eventually meiosis. Whether the hypothesis I have proposed to explain this aspect of the eukaryotic cell cycle is correct (see figure 5) and if so how it evolved are clearly important questions for the future.

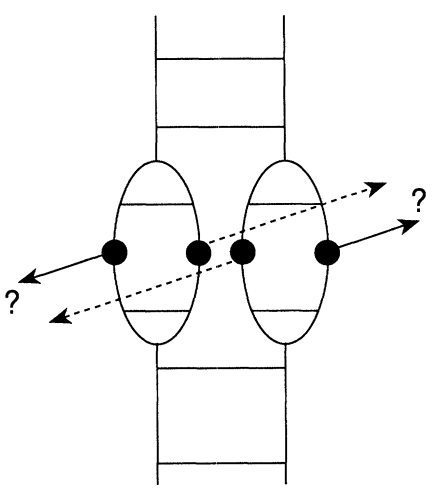

Figure 9. Two reasons why a second round of DNA replication must not preceed anaphase in eukaryotes. First, re-replication of kinetochore DNA would create ambiguities as to which pair of sister kinetochores should be pulled in opposite directions by microtubules emanating from sister MTOCs. Second, it would be difficult to distinguish new from old tethers.

The evolution of a mechanism that ensures that origins fire only once between successive anaphases would have been crucial not only for mitosis to function as an effective sister chromatid segregation system but also for the evolution of a system by which chromosomes can be reliably replicated from multiple origins. While there is only a single origin within a single chromosome genome, it is easy to ensure that each sequence in the genome is replicated equally; every initiation event leads to replication of the entire genome. The evolution of a system capable of segregating multiple chromosomes would have raised the problem of how to ensure that each origin (there might only have been one per chromosome initially) fired with the same frequency so that different parts of the genome were replicated equally. It is plausible, if not probable, that the same mechanism that prevents firing of origins more than once between successive anaphases (see figures 4 and 5) also solved the problem of selfish replication origins, and thereby facilitated the proliferation of origins within chromosomes. Having a single Cdk both promote replication from preRCs and inhibit de novo assembly of preRCs ensures that a cycle of Cdk activation/destruction is necessary for any origin to fire; origins cannot therefore fire more than once during the chromosome cycle. Thus the ability to use an unlimited number of replication origins, without which embryogenesis would be impossible, could have been an inherent property of the mitotic system of chromosome segregation, in which cyclins are only destroyed at anaphase along with the tethers between sister chromatids.

9. EVOLUTION OF CDKs

The cyclin dependent kinases (Cdks) implicated in regulating S and M phases in fungi and animal cells have also been detected in a wide variety of eukaryotic lineages, including plants, Paramecium (Tang *et al.* 1994), and Plasmodium (Ross-macdonald *et al.* 1994). It seems likely, though not yet proven, that the

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S	M phase	
CycD / Cdk4 CycE / Cdk2 CycA / Cdk2	CycB / Cdk1	animal cells
CycB1 / Cdk1 2 / 3 / 4 / 5 / 6 /	CycB1 / Cdk1 2 / 3 / 4 /	fungi (yeast)

Figure 10. Cyclin dependent kinases in yeast and animal cells. Anyone of six B-type Cdk1 kinases can trigger S phase in yeast. However, the Cyclin B5 (Clb5) and Cyclin B6 (Clb6) kinases probably perform this task in wild type cells for they are the first to appear. It seems that the S phase promoting cyclins from yeast and animal cells do not have a common ancestor not shared by mitotic cyclins; that is, cyclin B5 from yeast does not resemble cyclin E from animals more than it does the mitotic cyclins from both organisms.

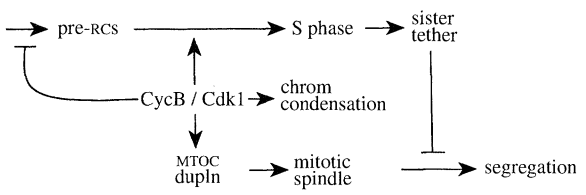
common ancestor of eukaryotes used these enzymes to regulate their chromosome cycles. Figure 10 compares modern cyclins and Cdks involved in controlling S and M phases in fungi and animal cells. B-type cyclins and Cdk1 are implicated in controlling mitosis in both kingdoms (Nurse 1990), but there is greater divergence between the cyclins and Cdk subunits that regulate S phase. Cdk1 and B-type cyclins also regulate S phase in ascomycetes (Schwob *et al.* 1994), whereas variants on the B-type theme, cyclins E and A, associate with Cdk2 to do so in animal cells (Knoblich *et al.* 1994). Responsibility for coordinating the cell cycle with growth is performed by highly diverged variants on this theme: cyclin D/Cdk4 in animals (Sherr 1994), and Cln/Cdk1 in yeast (Nasmyth 1993). The implication is that the common ancestor of these two kingdoms possessed B-type cyclins and a Cdk1-like kinase but not cyclins A, D, or E.

Given their likely bacterial ancestry and the limitations of the methods used by bacteria to duplicate and segregate chromosomes, it is safe to assume that primordial eukaryotes would also have had small genomes. It is therefore plausible that chromosome condensation need not have been an extreme feature of chromosome alignment during mitosis and that oscillations in the activity of a single cyclin dependent kinase, composed of a B-type cyclin and a Cdk related to Cdk1, could have driven the chromosome duplication/alignment cycles of our ancestral eukaryote. Because Cdks of this type regulate M phase in modern eukaryotes, it seems likely that the primordial Cdk evolved initially to regulate the duplication and segregation of MTOCs or microtubules themselves and may later have been coopted to coordinate chromosome duplication and segregation. Cdks have now been implicated in processes unrelated to the chromosome cycle, for instance in regulating transcription (Roy *et al.* 1994). Whether the primordial Cdk started life as a transcription factor or a regulator of microtubules is impossible to judge at present.

10. A PRIMORDIAL EUKARYOTIC CELL CYCLE

Figure 11 depicts what the cell cycle of the primordial eukaryote might have looked like. Activation of a Cdk resembling cyclin B/Cdk1 might have triggered DNA replication, formation of the mitotic spindle, and modest chromosome condensation, whereas destruction of cyclin B and the tethers that hold sister chromatids together would have triggered anaphase and the formation of pre-replication complexes needed for the next round of DNA replication. Thus the cell cycle might have been driven by an oscillation in a single Cdk. Proteolysis might always have been a fundamental feature of the eukaryotic cell cycle. There are at least two reasons for this. First, proteolyis is an ideal mechanism for imposing irreversibility, i.e. for ensuring that the cell cycle never operates in reverse. Second, proteolysis of proteins at the end of mitosis means that protein synthesis can be used to regulate Cdk activation in the next cycle, which might have helped link the chromosome cycle with cell growth (i.e. protein synthesis). In most cases, crucial cell cycle proteolytic events in eukaryotes involve ubiquitination; a process that is absent in prokaryotes and which might have evolved initially to distinguish host proteins from those of prey that had been phagocytosed.

The evolution, for whatever reason, of a system capable of segregating multiple chromosomes (i.e. mitosis) would have permitted and indeed facilitated genome expansion, initially via increasing chromsome numbers. Genome expansion might have been exacerbated or even partly driven by the presence of the nucleus, which by separating translation and



activation of CycB / Cdk1 → S phase, mitotic spindle, modest, chrom condensation

destruction of sister tether and CycB → anaphase and pre-RC formation

Figure 11. A putative regulatory network for the cell cycle of the primordial eukaryotic cell.

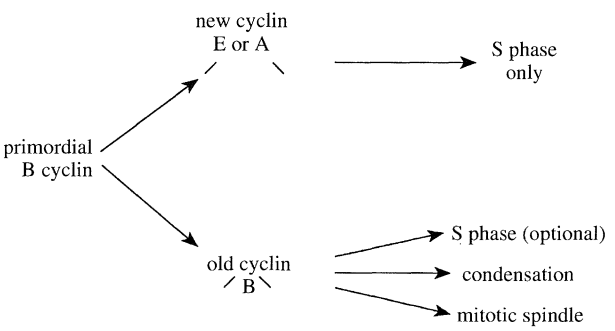


Figure 12. The origin of S and M phase specific cyclins via a gene duplication.

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transcription was very possibly responsible for the acquisition of most introns. However, the load of non-disjunctional events imposes an upper limit on the number of chromosomes and there will have been selection at some point for cells with larger chromosomes, which needed to be more highly condensed during mitosis to avoid their being caught by the cytokinetic apparatus. This would have necessitated a separation of S and M phases, because it seems unlikely that DNA replication can occur on highly condensed chromsomes.

It is easy to imagine that duplication of a primordial cyclin B gene could have led to the evolution of S phase specific cyclin variants (e.g. cyclins E and A) that had lost the ability to promote chromosome condensation and alignment on the mitotic spindle but had retained the ability to promote DNA replication. Their activation before that of the ancestral cyclin capable of promoting all three events might have been sufficient to generate the more familiar modern cell cycle with S phase preceeding M phase. Initially the ancestral cyclin may have retained the ability to promote replication, a property retained by mitotic B-type cyclins in yeast, but could have gradually lost this property. The B-type cyclins that still promote S phase in yeast (e.g. Clbs5 and 6) do not resemble cyclins E or A, suggesting that the cyclin gene duplication events that led to S and M phases becoming separated in time may have occured relatively recently (i.e. after the divergence of fungi and animals) and probably more than once. However, we clearly need to delve into the phylogeny of cyclins and Cdks further – particularly in primitive eukaryotes like Giardia – before we can be certain about this.

11. SURVEILLANCE MECHANISMS

Chromosome partition in bacteria is dependent on termination. Thus surveillance mechanisms, which ensure that late cell cycle events like partition do not precede earlier ones like replication termination, will have existed long before the evolution of eukaryotes. It is nevertheless interesting to consider what sort of mechanisms would have been essential for the primordial eukaryotic cell cycle. If we are correct in thinking that DNA replication and chromosome alignment were once triggered by the same Cdk, it is clear that cells must have had a means of preventing the onset of anaphase until DNA replication was completed and all sets of sister chromatids were aligned on the spindle apparatus. Current work in yeast implicates negative controls in both phenomena. It is thought that individual replication forks generate a signal that inhibits the onset of anaphase (Kelly *et al.* 1993; Piatti *et al.* 1995), presumably by inhibiting activation of the Anaphase Promoting Complex (Irniger *et al.* 1995) A similar inhibitory signal may be transmitted by the presence of a single pair of sister chromatids attached to only one pole (how the latter is measured is not clear but could be due to an absence of tension on the kinetochore) (Li & Murray 1991). Thus replication forks and incompletely attached chromosomes appear to veto anaphase in eukaryotic

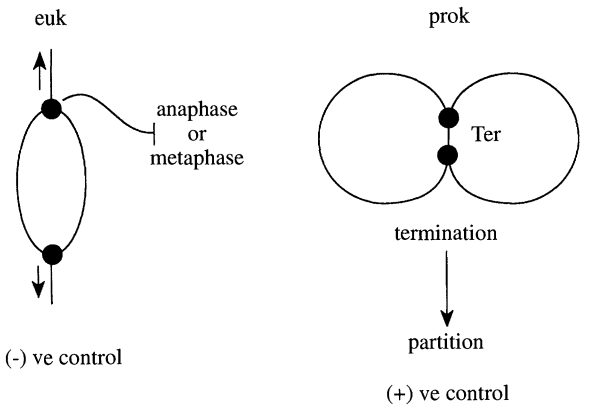

Figure 13. Surveillance mechanisms that monitor the state of DNA replication and prevent chromosome segregation until complete may operate in fundamentally different ways in prokaryotes and eukaryotes. The presence of replication forks vetoes both metaphase and anaphase in eukaryotes, whereas termination itself triggers partition in prokaryotes.

cells. In organisms with greater chromosome condensation, on-going replication forks need not only inhibit anaphase but also the onset of M phase. In this case, there is some evidence that the mitotic cyclin B/Cdk1 kinase is the target for inhibition.

It is worth pointing out that the evolution of a cell cycle in which re-replication cannot precede anaphase enabled eukaryotes to use a surveillance mechanism for linking chromosome duplication and segregation that sensed the presence of replication forks. This is not possible in bacteria, which need to reinitiate DNA replication before termination of the previous round and must therefore measure termination itself, which sends a positive signal to the partitioning apparatus (see figure 13). Indeed, the need to measure termination might be a reason for retention of circular genomes in bacteria.

12. CONCLUSION

I have tried to describe some of the constraints that may have shaped the evolution of the eukaryotic system for duplicating and partitioning genomes and present a scenario how this might have evolved. Given the primitive state of our understanding of the cell cycle in a few model organisms and the complete absence of any information on that of 'primitive' eukaryotes, it is unlikely that this scenario will stand the test of time; however it may prove a useful basis for future studies. If we are to understand more about our origins, however, we will need not only continued study of crown eukaryotes like yeast and man but also that of 'early' eukaryotic lineages amongst the protozoa. It is also clear that there is much to be learnt about chromosome segregation in bacteria, without which it will not be possible to appreciate the mechanisms used by eukaryotic cells.

It is currently fashionable to ascribe our history or evolution more to chance than necessity. Consideration of the cell cycle suggests that necessity may have played a vital role in the way we evolved. There seems not the remotest possibility that cells with bacterial cell cycles

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could ever have evolved into multicellular organisms of our complexity without first developing a chromosome segregation system whose performance matches current mitotic processes. Bacterial partitioning systems could never have supported sufficiently large genomes either to programme the developmental processes needed or to act a the raw material for their evolution. Nor is it likely that their replication system could ever have been compatible with the huge inbalance between growth and cell division that is required for embryonic development in most metazoa. The first bacterial fossils are ca 3500 Ma old and the earliest ones ressembling eukaryotic cells are between 1700 and 2000 Ma old (Knoll 1992). Complex multicellular 'crown' eukaryotes did not evolve much before 1200 Ma BP. It seems likely that the evolution of mitosis might have been an important (i.e. rate determining) step in the first transition, from bacteria to eukaryote, and that the evolution of meiosis might have played an important role in the the second transition, from microorganisms to metazoa and plants.

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