DNA replication in the fission yeast: robustness in the face of uncertainty

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Abstract

DNA replication, the process of duplication of a cell’s genetic content, must be carried out with great precision every time the cell divides, so that genetic information is preserved. Control mechanisms must ensure that every base of the genome is replicated within the allocated time (S-phase) and only once per cell cycle, thereby safeguarding genomic integrity. In eukaryotes, replication starts from many points along the chromosome, termed origins of replication, and then proceeds continuously bidirectionally until an opposing moving fork is encountered. In contrast to bacteria, where a specific site on the genome serves as an origin in every cell division, in most eukaryotes origin selection appears highly stochastic: many potential origins exist, of which only a subset is selected to fire in any given cell, giving rise to an apparently random distribution of initiation events across the genome. Origin states change throughout the cell cycle, through the ordered formation and modification of origin-associated multisubunit protein complexes. State transitions are governed by fluctuations of cyclin-dependent kinase (CDK) activity and guards in these transitions ensure system memory. We present here DNA replication dynamics, emphasizing recent data from the fission yeast Schizosaccharomyces pombe, and discuss how robustness may be ensured in spite of (or even assisted by) system randomness.

Keywords: fission yeast; Schizosaccharomyces pombe; DNA replication; origin selection; stochasticity; licensing; random completion problem; hybrid dynamics

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Introduction: uncertainty and robustness in DNA replication

Every time a cell divides, its entire genome must be replicated fully and only once during S-phase of the cell cycle, followed by chromosome segregation in mitosis, in order to ensure that the genetic material is transmitted to the next generation and genomic instabilities are avoided.

In bacterial and viral genomes, replication initiates from the same genomic site in every cell, termed the origin of replication [28]. Given the large genomes of eukaryotes, replication would fail to be completed in the observed time if it
started from a single point on every chromosome. Thus, eukaryotes initiate replication from hundreds of replication origins at every cell cycle [16]. These origins are able to initiate firing throughout the length of S phase. Surprisingly, accumulating evidence suggests that the location of active origins exhibits a high degree of uncertainty in most eukaryotic organisms [16]: although replication generally starts from defined regions, many potential origins exist, of which only a subset will be randomly selected to fire in any given cell in a population. In addition, the time at which a given origin will fire within S phase is not precisely determined.

DNA replication is therefore a complex process which involves considerable uncertainty. It must, however, be carried out with great precision for the genetic information to be maintained. The foregoing features introduce some possible problems to its successful completion:

- How are the positions of origins selected so that DNA replication is completed within the allocated S-phase duration?
- How does the system have memory and coordinate firing in such a way that every region is replicated and no region is rereplicated within the same cell cycle?

In this overview paper we outline some of the mechanisms that have been proposed to regulate this random yet highly robust process, concentrating on the fission yeast *Schizosaccharomyces pombe*. We survey recent findings which suggest that randomness plays an integral role in the DNA replication process. We also discuss the mechanisms that the cell uses to ensure that each part of the genome replicates once, and only once, in a normal cell cycle. Finally, we highlight potential problems associated with the random nature of the process and survey mechanisms that have been suggested to explain how the cell deals with these problems.

**Replication initiation sites are selected randomly**

Origin positioning is a crucial part of replication and various organisms have evolved different ways to define origins of replication. In bacteria, there is only a single non-redundant origin of replication. The single circular bacterial chromosome is entirely replicated by two replication forks starting from this origin [28]. Origin positioning is sequence-specific and origin activation is deterministic: the origin fires in every cell cycle with high fidelity. Early work from budding yeast offered support to the defined origin model, as *S. cerevisiae* origins were shown to be defined by specific sequences and fire with high efficiency [16,22]. However, eukaryotic origins in general appear less well defined and their firing pattern appears non-deterministic. Indeed, in early fly and frog embryos, replication is carried out without any noticeable sequence specificity [21,49], while in human cells, any sequence larger than 10 kb appears suitable to sustain replication initiation on a plasmid at random sites inside the sequence [29,30].

Most eukaryotic cells seem to follow an intermediate route between a fully deterministic and a fully random origin selection mechanism: replication does start from relatively specific regions along the genome; however, these regions are not selected in every cell cycle, thereby giving rise to a semi-random distribution of initiation events along the DNA. In addition, while certain origins tend to fire early and others late, the timing of firing of each origin is not fully determined, giving rise to a semi-random timing pattern of initiation. Recent work from fission yeast lends support to the notion that stochasticity is an integral part of the DNA replication process.

Plasmid maintenance assays have been applied to map origins in *S. pombe*. The average length of fragments capable of sustaining autonomous replicating sequence (ARS) activity was shown to be 500–1500 bp [9,14]. While two-dimensional gel electrophoresis suggested DNA replication starts at discrete genomic loci [14,17,58], mutational analysis in plasmids showed lack of a consensus sequence which could define an origin [13]. Origins were shown to be composed of multiple redundant elements, the sequential excision of which did not have an all-or-none effect, but led to a gradual decrease in origin function [9,24,42]. All identified origins were shown to reside preferentially in intergenic locations, be above average AT rich and contain a number of short stretches of highly asymmetric adenine and thymine residues, which contributed synergistically to origin activity. Characterized origins were shown to initiate replication
at their genomic location in only a fraction of cells in a population [14,17,25,48].

Following the completion of the S. pombe sequencing project [53], full genome analyses became feasible and have shed more light on the nature and function of S. pombe origins. Segurado et al. employed a bioinformatics analysis to find the coordinates of genomic locations of AT-rich sequences (AT content > 72% in a 0.5–1.0 kb window) which could serve as origin sites [47]. This approach led to the identification of 384 AT-rich islands with potential origin activity. AT-rich islands were shown to reside in intergenic regions and to be over-represented between divergent transcriptional units. Average origin density was one every 33 kb, with a higher density in centromeric, subtelomeric and mating-type loci (about four-fold higher than the genomic average). Twenty of the 384 AT-rich islands were tested for origin function at their genomic location by two-dimensional gel electrophoresis; 90% of the islands tested had origin activity, predicting a total of 345 origins associated with AT-rich islands across the fission yeast genome.

More insight into the process was given recently by Dai et al. [11], who showed that the properties of known fission yeast origins were similar to fission yeast intergenic regions in general. Of 26 intergenic regions tested on plasmids for origin function, four functioned as monomers and 10 functioned as dimers, leading to the suggestion that approximately half of fission yeast 4978 intergenic regions may have potential origin function. A lack of a consensus sequence was also noted, while AT content and intergenic size were suggested to constitute the major determinants of origin activity. Based on these findings, a hypothesis was formulated, suggesting that DNA replication is stochastic in nature and that, from the many potential origins across the fission yeast genome, only a few will fire in any given cell cycle.

Further evidence for the stochastic nature of origin selection was given by Patel et al. [43] Labelling of newly synthesized DNA with thymidine analogues in the presence of hydroxyurea (to arrest fork progression), as well as pulse-labelling of DNA in early and late S-phase, was used to identify replication intermediates. These intermediates were analysed at the single-cell level with DNA combing. In combination, fluorescence in situ hybridization (FISH) was used to map the replicating areas on specific genomic locations. After measurement of more than 1000 interbubble distances, the distribution of interorigin gaps was found to be exponential, clearly indicative of a random initiation pattern across the genome. The stochasticity of origin firing was further solidified through the analysis of origins at the ura4 and nmt1 regions. The firing probabilities of origins in these regions were in the approximate range 10–70%. In addition, this analysis showed no evidence for coordinated regulation of neighbouring origins, while labelling of DNA intermediates in two sequential S-phases showed that origin selection is random between successive cell cycles.

From these analyses, key characteristics of fission yeast origins have emerged (Figure 1A): localization in intergenic locations and preferential association with promoter regions; absence of a specific sequence-level definition; high AT content and AT asymmetry; correlation with large intergenic size. Why are origins located exclusively in intergenic regions? An obvious explanation is that ongoing transcription and origin specification are mutually exclusive; origin specification may not be possible in transcribed regions which are constantly scanned by the transcriptional machinery. In addition, elements in promoter regions which enhance transcriptional activity could have a concomitant positive effect on origin specification.

The most striking emerging feature of origin specification in fission yeast, however, is its highly probabilistic nature. Figure 1B shows the spatial stochasticity in origin selection. From a large pool of potential origins, a different collection is selected in every cell cycle, which leads to a semi-random distribution of selected origins. This distribution is different in every cell cycle of every cell. Figure 1C shows the temporal stochasticity in origins firing. It is known that origins do not fire concurrently at the G1–S transition but rather have the potential to fire throughout S-phase. While some origins tend to fire early and others late, there is a continuous distribution of firing events throughout S phase and it is highly unlikely that timing is deterministically defined in a precise time point, giving rise to temporal stochasticity.

The two aspects of stochasticity are in fact linked, as the probability of firing of a given origin will affect the average time within S at which it will fire, while an intrinsically late firing origin will tend to appear inefficient, as it will
Figure 1. (A) Origin characteristics. Fission yeast origins are located in intergenic regions and are characterized by high AT content and regions of AT asymmetry. The length of the intergenic region is also a determinant in origin specification. (B) Spatial stochasticity in origin selection. Many putative origins exist along the fission yeast genome which will fire in only a certain fraction of cells in a population (percentage efficiencies are shown above origin locations). Every cell in the population will therefore be characterized by a different subset of active origins. (C) Temporal stochasticity in origin selection. Origins fire throughout S phase and, while some tend on average to fire early and others late, there is uncertainty as to when each origin will fire in any given cell in the population.

have a high probability of being replicated passively before it fires. The early or late character of a given origin may be deterministically defined independently of its firing efficiency. It is, however, conceivable that the observed timing of firing represents a read-out of firing efficiency for at least some origins, with highly efficient origins tending by probability to replicate early in S phase, in contrast to inefficient origins appearing, on average, late. Kim and Huberman [25] analysed the replication timing of several fission yeast ARS elements, using two-dimensional gel electrophoresis and various synchronization methods, and found that origins tend to be replicated in a given order. In cells arrested in early S phase by hydroxyurea treatment, early-replicating ARS elements contained replication intermediates while late-replicating ones did not, whereas in cells lacking the Rad3 or Cds1 checkpoint kinases, almost all regions tested contained replication intermediates, suggesting active repression of late-replicating regions, a result which was further supported by subsequent full-genome analysis [15]. Yompakdee and Huberman [57] identified a 200 bp sequence containing GC-rich elements which could confer a late-firing character to an origin on a plasmid, and similar sequences were identified close to late-replicating regions in the genome. The above data suggest that origin timing patterns may be determined, at least for some origins. Patel et al. [43], on the other hand, found no evidence for exclusively late-firing origins in the fission yeast genome. Further experiments will be required to clarify whether deterministic or probabilistic firing timing patterns prevail for the majority of fission yeast origins.

Replication forks move continuously

Starting at origins, replication forks move bidirectionally along the chromosome in a continuous fashion. Forks are generally considered to be moving stably on DNA until they meet another fork moving in the opposite direction. In that case every part between the two origins from which the forks initiated is replicated. Many origins are
actually replicated by passing replication forks that originated from nearby origins, so a single fork traverses large parts of the chromosomes. Fork speed may vary considerably along the genome and may be dependent on primary sequence as well as higher order chromatin structure. Replication pause sites or fork barriers exist along the genome, such as in the ribosomal RNA repeats [45]. Such barriers may be required to ensure that head-on collisions between RNA and DNA polymerases are avoided. In order to safeguard the continuous movement of the replication forks, control mechanisms ensure that polymerases remain on the DNA and that the forks do not collapse even if replication is impeded. Ablation of certain control proteins results in polymerase fall off and can lead to severe chromosomal instabilities through recombination [1,3].

**Discrete memory ensures once per cell cycle replication**

Origins can be in two discrete states: pre-replicative, when they are ready to fire as soon as conditions permit; and post-replicative, when they cannot fire. The transition from the post-replicative state to the pre-replicative state takes place in M/G1 through the process of licensing. The transition from the pre-replicative state to the post-replicative state takes place during S phase, through origin activation or passive replication. The two transitions are temporally separated and involve the formation and modification of multi-subunit protein complexes on origin DNA. While origin characteristics are not conserved during evolution, the factors which bind to origins to define origin states function in very similar ways in all organisms, and our current understanding of the process stems from experiments in several model systems.

In this section we outline the process with which the cell ‘stores information’ in this discrete memory and the molecular mechanism behind this process, concentrating on data from fission yeast.

**Licensing: transition to the pre-replicative state**

Licensing occurs from late mitosis and through G1 phase and is a prerequisite for DNA replication [4,38]. It involves the sequential recruitment of licensing factors on origin sequences forming a multisubunit complex, termed the pre-replicative complex (PreRC, Figure 2). Only origins at the pre-replicative state possess the licence to replicate during the following S phase.

A central component believed to provide spatial regulation of origin positioning is the origin...
recognition complex (ORC). ORC is a six-subunit complex originally identified in budding yeast, based on its ability to bind in a sequence-specific manner to origins of replication [2]. Subsequent analyses permitted characterization of fission yeast ORCs and both in vivo and in vitro experiments revealed specific association of ORC subunits with origins of replication [7,27,34,41,51]. One of the fission yeast ORC subunits, Orc4, possesses multiple AT-hook motifs at its amino-terminal part which were shown to interact with the AT-rich S. pombe origins [8,32]. These data suggest that the ORCs may define which regions in the genome have the potential to act as origins of replication. The ORC is found on chromatin throughout the cell cycle, albeit differentially modified [34], and is therefore unlikely to be the major determinant of when licensing takes place. The temporal regulation of licensing appears to be mainly provided by two other licensing factors, Cdc18 and Cdt1.

Cdc18 was originally discovered through genetic screening [37]. It is tightly regulated during the cell cycle at both the transcriptional and the proteolytic level and is present only in late M and G1 phase, when it is recruited to origin DNA. Cdt1 (cdc10-dependent transcript 1) was initially identified as a target of the G1 transcription factor Cdc10 [19]. Further analysis showed that it is absolutely required for origin licensing in this organism [39]. It is expressed similarly to Cdc18, peaking close to the M–G1 transition.

Dependent on the presence of ORC, Cdc18 and Cdt1, the MCM complex is recruited onto chromatin, and this loading confers to DNA the licence to replicate. The six subunit MCM complex associates with chromatin in a cell cycle-specific manner and is believed to act as the replicative helicase during the following S phase [31].

Apart from regulating once per cell-cycle replication, licensing also appears important for blocking mitosis until replication has taken place. Cells depleted of Cdc18 or Cdt1 are not only unable to initiate DNA replication, but also do not sense the presence of unreplicated DNA and enter abortive mitosis, resulting in a ‘cut’ phenotype [19,23].

Activation and passive replication: transition to the post-replicative state

At the onset of S phase, several factors, pivotal amongst which is Cdc45, associate with the already formed pre-RC to assemble the pre-initiation complex (PreIC, Figure 2; reviewed in Refs 12,38). This complex is able to promote replisome formation at origin sites and facilitate DNA unwinding, thereby leading to initiation of replication. Several factors such as Sld3, Cut5/Rad4 (a Dpb11 homologue), Cdc23 (a homologue of MCM10) and the GINS complex, as well as the S phase cyclin-dependent kinase (S-CDK) and Cdc7-Dbf4 kinase (DDK, Hsk1-Dfp1 in fission yeast), are required for stable Cdc45 association with origins, activation of the PreIC complex and DNA replication initiation.

It is clear that origin specification and firing is a two-step process: licensing takes place at late M/early G1 while activation of licensed origins takes place at the G1–S transition. In order to ensure once-per-cell-cycle replication, licensing must be coupled to passage through mitosis, while activation of origins and initiation of replication must be coupled to the inactivation of de novo licensing.

What triggers the transitions and sets the guards?

Early research in fission yeast received a major boost from the observation that mutants undergoing over-replication could be isolated and studied, thereby allowing the identification of factors which are essential for once per cell cycle replication. Genetic screens to identify over-replicating mutant strains were carried out and identified two classes of mutants (Figure 3; reviewed in Ref. 35).

In the first class, ectopic activation of key licensing factors by overexpression of Cdc18 [40], Cdc18 together with Cdt1 [39,56], or mutations in Orc2 [52,55] results in repeated rounds of DNA replication in the absence of mitosis. Strikingly, another class of mutants had defects due to inactivation of the mitotic cyclin-dependent kinase (M-CDK, cdc2/cdc13 [10,18]), suggesting that the mitotic CDK has a dual role: to promote mitosis at the end of the cell cycle and to inhibit DNA replication until mitosis has been completed.

It is interesting to note that the two classes of mutants are believed to give rise to different types of genome over-replication (Figure 3): misregulation of licensing factors is believed to give rise to repeated firing of origins within the same S-phase (rereplication), while M-CDK inactivation gives rise to complete rounds of S phase without
Defects in system memory lead to over-replication. Two classes of system perturbations can lead to loss of controls ensuring once-per-cell-cycle replication. Ectopic expression of key licensing factors (Cdc18, the prototype of this category, is depicted) leads to refiring of origins within the same S-phase (rereplication). Defects in mitotic CDK activity lead to complete cycles of S phase in the absence of mitosis (endoreduplication). These two types of over-replication are reminiscent of physiologically occurring over-replication phenomena, such as chorion gene amplification (rereplication) or polyploidization (endoreduplication), and may be related to certain chromosomal abnormalities observed in cancer cells.

These experiments showed that accurate regulation of licensing factors, as well as CDK activity, is essential for the cell to remember which genomic regions have been replicated. We now know that this memory is achieved through the cross-talk between CDK and licensing factors. Continuous changes in CDK activity dictate which specific function should take place, while the discrete states of origins provide memory. In Figure 4, the so-called quantitative model of cell cycle regulation is illustrated [50]. Cell cycle events are regulated by the periodic fluctuations in the activity of CDKs, the master regulators of the cell cycle. There are two identified thresholds in CDK activity. Threshold 1 defines entry into S phase and threshold 2 defines entry into mitosis.

To avoid incomplete DNA replication, regulatory mechanisms have evolved which prevent CDK activity from reaching threshold 2 if any region of the genome remains unreplicated. Before replication, and while CDK activity is low, origins are present in the pre-replicative state. Only in this state can they support initiation of DNA replication when CDK activity passes threshold 1. When an origin fires, or when it is passively replicated by a passing replication fork from a nearby origin, it automatically switches to the post-replicative state and can no longer support initiation of replication. CDK activities over threshold 1 inhibit conversion of the post-replicative state to the pre-replicative state. Only when cell division is completed and CDK activity resets to zero can origins reacquire the pre-replicative state. With this simple mechanism, rereplication is inhibited.

### Origin sites, origin states and stochasticity

How does the cross-talk between putative origin sites along the genome and trans-acting factors imposing origin states give rise to the observed stochasticity in origin usage in fission yeast?

The fact that only a subset of putative origins fire in any given cycle suggests that there is a rate-limiting step in the process of origin licensing and/or activation:

1. At the level of licensing. Dai et al. [11] suggested that every intergenic region in the...
genome is a potential ORC binding site. Orc4 contains multiple AT-hook motifs at its N-terminus and it could bind with varying affinities to multiple asymmetric AT regions present in different numbers in every intergenic region along the genome. The number of potential Orc4 binding sites greatly exceeds the number of ORC molecules present in the cell and therefore ORC would associate with only a subset of potential origins in any given cell.

2. At the level of origin activation. A factor(s) required for origin activation may be limiting and therefore only a subset of licensed origins will initiate replication. Candidates for such a limiting factor include Cdc45, cyclin-dependent kinase activity and DDB kinase activity, and differences in efficiencies between origins could reflect the accessibility of licensed origins to these factors, e.g. due to chromatin structure.

It should be noted, however, that the observed efficiency of firing of an origin in a cell population is not only affected by the intrinsic propensity of this origin to fire in any given cell cycle but also by the probability that it is passively replicated from an adjacent origin. In an extreme case scenario, randomness may be solely generated by passive replication coupled to a degree of uncertainty in timing of origin firing, even if most potential origins are able to fire in every cell cycle (see Redundancy model, below).

The random completion problem

In contrast to deterministic origin selection, where origins fire in most cells and therefore inter-origin distances and S phase length are precisely defined, random origin selection may result in the occasional formation of large inter-origin gaps and a possible inability to replicate those gaps within the time allocated for S phase (the random completion problem). It should be noted, however, that DNA replication dynamics at the whole-genome level are the result of many parameters, such as replication speed at any given location, origin number and spacing, intrinsic origin efficiencies and S phase duration, which will influence S-phase completion. Accurate genome-wide analysis of DNA is therefore required to reveal the extent of the random completion problem for a given organism.

Several models could explain how the random completion problem may be overcome [16,20]. Properties of fission yeast replication render some of the models suitable, while others are inconsistent with recent research findings.

Origin redundancy model

One solution to the random completion problem would be a high redundancy of putative origins possessing high intrinsic firing efficiencies. In this model (Figure 5A), putative origins are present in high excess, spaced closely together, and can all fire within the length of S phase. A degree of stochasticity in timing of activation within S phase will result in early firing of some origins and passive replication of origins that have not yet fired, giving rise to a random distribution of active origins in any given cycle and a low observed firing efficiency for any given origin in a population of cells. However, since every non-replicated origin

![Figure 5. Possible solutions to the random completion problem (see text). Origin redundancy. (A) Defined spacing. (B) Redistribution. (C) 'G2' replication. Positions of putative origins are marked with vertical red lines. Unreplicated DNA is marked in blue, replicated DNA in green. In (B), inter-origin distances in individual cells in a population are marked. In (D), the maximum inter-origin region in each cell is marked. Regions unable to become fully replicated during what we define as S-phase are shown in red.](copyright)
can initiate replication and will do so if not passively replicated soon enough, the random completion problem will not ensue. This explanation is attractive for the early divisions of *Xenopus* embryos where transcription is not yet active, replication can initiate at any sequence and there is a large excess of licensing and initiation factors. It is, however, unclear whether it could explain timely replication in organisms where restrictions to origin positioning exist and factors are limiting, such as in fission yeast, where origins are located exclusively in intergenic regions, or in mammalian cells, where ongoing transcription and chromatin structure are unlikely to permit origins to be located anywhere along the genome.

**Defined spacing model**

The defined spacing model [5,21] postulates that though origins can be selected from a pool of putative origins, there is a defined spacing between origins that fire in any given cell, which ensures that large inter-origins gaps do not appear (Figure 5B). Using yeast, bacterial and viral plasmids, it has been observed that replication never starts from a second origin if their spacing is less than a specific length [6,36,46] while larger plasmids are able to initiate replication from multiple points in *Xenopus* extracts [33].

The mechanism that measures and defines inter-origin distances could rely on chromatin structure or lateral inhibition. Higher-order chromatin structure could induce spatial constraints to origin selection, while lateral inhibition is an active mechanism which could explain origin interference. Sequential addition of a factor on DNA which is recruited at origins, extends bidirectionally and covers DNA sequences flanking the origin could provide lateral inhibition, and MCM proteins have been proposed as such a factor [20,54].

This model, although attractive, nevertheless seems highly unlikely for fission yeast. Recent findings revealed an exponential distribution of inter-origin distances analysed at the single-cell level by DNA combing, which clearly contradicts the defined spacing model [43].

**Redistribution model**

The redistribution model posits an increase in firing efficiency of origins remaining unreplicated as S phase proceeds [33]. In contrast to the redundancy model postulating an excess of factors for initiation, the redistribution model assumes a rate-limiting factor, which enables only a specific number of potential origins to be selected for firing at any time (Figure 5C). This factor is not consumed by the firing process but is released and retains ability to activate further origins. As S phase proceeds and more origins convert to the post-replicative state, the pool of the available limiting factor is constantly increasing, thus facilitating activation of the remaining origins. Unreplicated regions can thus replicate fast in late S, through efficient firing of late origins induced by redistribution of the limiting factor. Note that for this model to hold true, the limiting factor must function at the level of origin activation, rather than origin licensing, since *de novo* licensing is believed to be globally inhibited as soon as S-phase starts. Cdc45 offers a likely candidate for such a rate-limiting factor.

**Replication in ‘G2’?**

A possible, although somewhat heretical, solution would be that S phase occasionally does run into what we term the ‘G2 phase’ (Figure 5D). S phase is defined based on our ability to detect ongoing DNA replication; however, a small amount of late replication in certain cells in a population could go undetected and proceed during what we define as the G2 phase. Given a functional S–M checkpoint to sense the presence of unreplicated DNA and arrest M phase entry until replication has been completed, variations in the length of S phase between individual cells in a population could be inconsequential. This solution appears particularly attractive for fission yeast, given its unusually long G2 phase. G2 is approximately four times longer than an average S phase, providing ample time for duplication of late-replicating regions of the genome corresponding to randomly arising large inter-origin gaps. In the very rare cases in which unreplicated parts still persist after G2 completion, the S–M checkpoint will ensure that mitosis is arrested until the remaining genomic regions are replicated.

Although this explanation appears unlikely for the early rapid division of amphibian and insect embryos, which lack a G2 phase and an efficient S–M checkpoint [26,44], it could hold true for several other organisms, provided that they have
a checkpoint to ensure complete genome replication before mitosis. In this scenario, the time required for DNA replication would show heterogeneity within a population, with no clear distinction between S and G2, the sum of S and G2 for a given cell being the total time required for DNA synthesis and cellular growth to take place in order to prepare the cell for the next mitosis.

**Randomness as a source of robustness**

Since randomness in origin selection can lead to problems, why have organisms not opted for a deterministic mode of origin specification? In fact it appears that a degree of randomness in initiation of replication is the rule for most organisms, rather than the exception. Despite making the process more unpredictable, randomness may also be a source of robustness. Randomness is intricately linked to redundancy: many potential origins exist, of which only a subset will be chosen in any given cycle. Redundancy is a clear source of robustness by providing system versatility and plasticity: in a changing environment, some origins may be unable to fire and having a large number of potential origins offers a clear benefit. In a deterministic model, inability of a given origin to fire would have an immediate effect on the overall process. In a redundant, random model, such a failure would be inconsequential. For example, if transcription through a given region and initiation of replication from that region are indeed mutually exclusive, higher eukaryotes must contain a large number of potential origins to accommodate different transcriptional programmes in different cell types.

**Perspectives**

We have described the process of DNA replication, highlighting the control mechanisms that ensure robustness in the face of randomness. However, several key questions require further investigation. Given random origin selection, is there a random completion problem in fission yeast, or is the number and spacing of origins sufficient to ensure replication within the allocated time? If a random completion problem exists, are there control mechanisms in place to avoid the consequences of prolonged S phase duration, or is there S-phase length heterogeneity and ‘G2’ replication? Are there intrinsically early firing and late firing origins in fission yeast, or is the tendency of certain origins to appear earlier than others a probabilistic event reflecting their firing efficiencies?

Recent genome-wide experiments based on microarrays provide hope for answering these questions. In addition, experiments at the single-cell level will be required to address open questions and the development of methodologies permitting full-genome single cell analyses will become increasingly needed. Given the complexity and probabilistic nature of the process, system behaviour will, however, be difficult to capture and accurately analyse at the whole-genome level. Analytical methods, modelling and *in silico* experiments will be needed to analyse the data and gain insight into potential mechanisms.

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