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review

Replication fork barriers: pausing for a break or stalling for time?

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Defects in chromosome replication can lead to translocations that are thought to result from recombination events at stalled DNA replication forks. The progression of forks is controlled by an essential DNA helicase, which unwinds the parental duplex and can stall on encountering tight protein–DNA complexes. Such pause sites are hotspots for recombination and it has been proposed that stalled replisomes disassemble, leading to fork collapse. However, in both prokaryotes and eukaryotes it now seems that paused forks are surprisingly stable, so that DNA synthesis can resume without recombination if the barrier protein is removed. Recombination at stalled forks might require other events that occur after pausing, or might be dependent on features of the surrounding DNA sequence. These findings have important implications for our understanding of the regulation of genome stability in eukaryotic cells, in which pausing of forks is mediated by specific proteins that are associated with the replicative helicase.

Keywords: checkpoint; DNA replication fork barriers; mini-chromosome maintenance helicase; recombination; replisome

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Introduction

DNA replication forks are established from a single origin in the circular chromosome of *Escherichia coli* and at multiple origins on each linear chromosome in eukaryotic cells. An assembly of proteins termed the replisome is associated with each fork, and comprises the replicative helicase together with DNA polymerases, a primase to initiate each new DNA fragment and other accessory factors. Progression of a fork normally continues until it encounters another fork coming in the opposite direction, at which point termination occurs and the replisome is disassembled.

In the presence of certain kinds of alkylation on the DNA template, or if the production of nucleotides is inhibited, the replicative helicase progresses much more slowly, so that the fork is said to have 'stalled' (Katou *et al*, 2003; Tercero & Diffley, 2001). Forks can also stall in response to agents that inhibit polymerases but that allow the helicase to continue unwinding the parental duplex (Pacek *et al*, 2006; Walter & Newport, 2000). Eukaryotic cells activate checkpoint kinases under such conditions, in response to altered features of the fork, including the exposure of more single-stranded DNA (ssDNA; Branzei & Foiani, 2005; Li & Zou, 2005; McGowan & Russell, 2004). Checkpoint kinases have an essential role in such situations because they prevent an irreversible collapse of the stalled forks, which would otherwise be fatal for the cell (Lopes *et al*, 2001; Tercero & Diffley, 2001).

Forks also pause during the normal process of chromosome replication at 'replication fork barriers' (RFBs), where particular proteins bind tightly to DNA. In the *E. coli* genome, a range of pause sites have been identified. Terminator (Ter) sequences are located on the opposite side of the circular chromosome to the origin and these represent programmed pause sites that are bound by a protein called Tus. The Tus–Ter complex forms a polar barrier that blocks progression of the replicative helicase when a fork arrives in the non-permissive direction (Hill & Marians, 1990). The stalled fork is resolved when a fork arrives from the opposite direction and triggers termination (Fig 1A). Other RFBs in *E. coli* represent accidental pause sites, which are usually less efficient. For example, forks might pause on encountering the Lac or Tet repressors bound to their cognate operators, but efficient stalling requires tandem arrays of many such complexes (Payne *et al*, 2006; Possoz *et al*, 2006). Finally, a bacterial fork moves more slowly when it encounters the ribosomal RNA (rRNA) transcriptional machinery moving in the opposite direction (French, 1992) and pauses for longer periods at sites where the transcription machinery has stalled in DNA sequences that are rich in GC repeats (Krasilnikova *et al*, 1998).

RFBs also exist in eukaryotic cells and can be programmed or accidental, polar or bi-directional. The best examples of programmed RFBs are the polar barriers that ensure that the rDNA is largely replicated in the same direction as transcription (Brewer & Fangman, 1988; Krings & Bastia, 2005; Linskens & Huberman, 1988; Sanchez-Gorostiaga *et al*, 2004) and the replication termination sequence 1 (*RTS1*) barrier, which causes unidirectional replication of the fission yeast mating-type locus (Dalgaard & Klar, 2001). Eukaryotic forks also encounter a range of weaker accidental RFBs, such as the polar barriers that form at transfer RNA (tRNA) genes (Deshpande & Newlon, 1996), and the bi-directional barriers that are present at centromeres (Greenfeder & Newlon, 1992). Stalling is not normally observed when eukaryotic forks encounter the RNA

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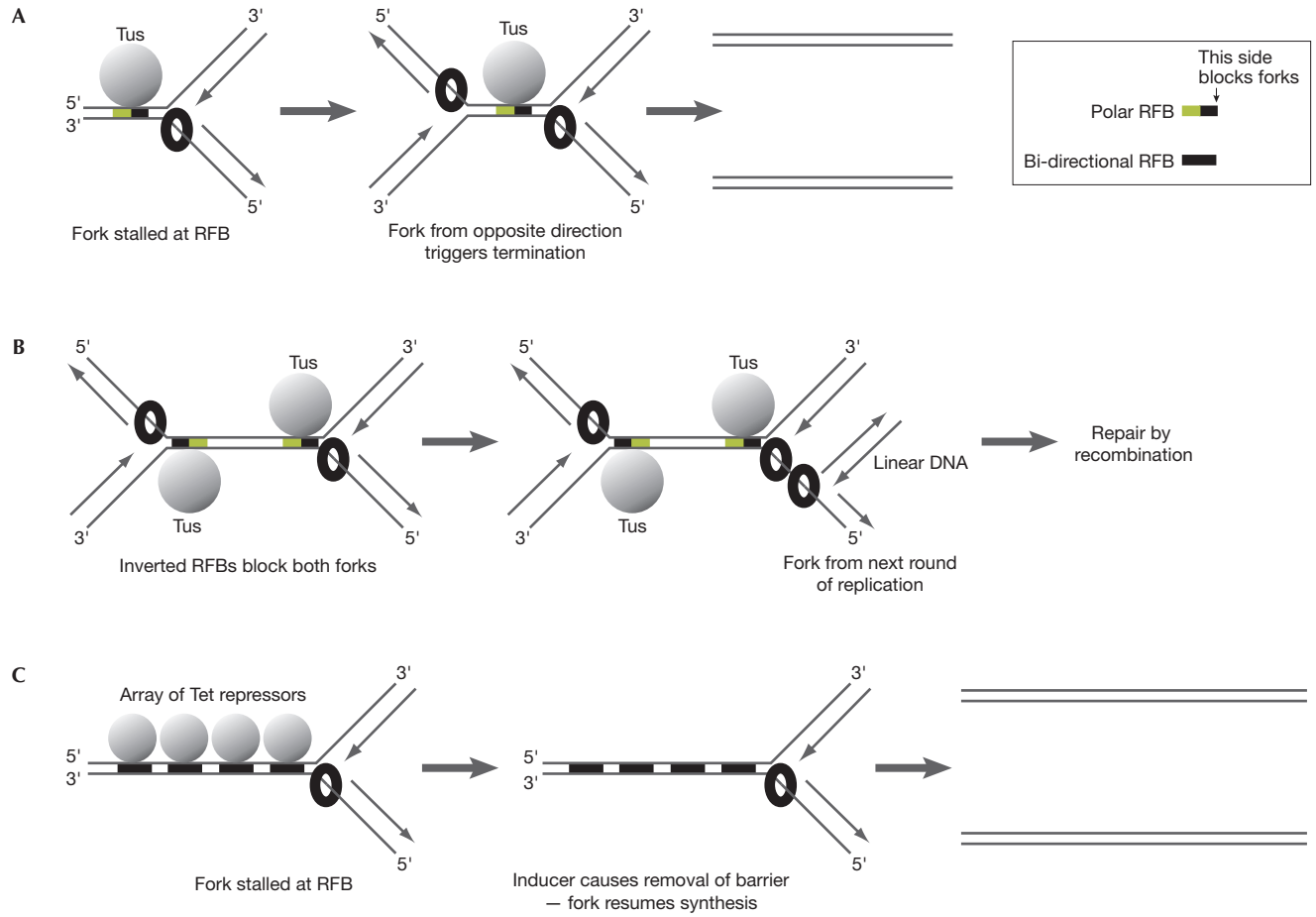

Fig 1 | Pausing of forks at replication fork barriers in *Escherichia coli*. (**A**) A fork normally pauses at a Tus protein–terminator sequence (Tus–Ter) barrier until another fork arrives from the opposite direction and induces termination. (**B**) Artificial inversion of Tus–Ter barriers blocks forks in both directions. Arrival of a new fork from the next round of replication produces linear DNA that might drive repair by recombination. (**C**) Forks stall at large arrays of Tet operators bound by the repressor protein, but removal of the barrier by an inducer (anhydrotetracycline) allows the rapid resumption of DNA synthesis in the absence of recombination. RFB, replication fork barrier.

polymerase II (Pol II) machinery moving in the opposing direction through linear chromosomes (Brewer & Fangman, 1988; Ivessa *et al*, 2003), but can be seen when such clashes occur in circular plasmids, suggesting that an additional torsional contribution is required (Prado & Aguilera, 2005). As in *E. coli*, eukaryotic forks also pause on encountering sites where Pol II stalls in GC-rich DNA, perhaps owing to the encounter with defective messenger ribonucleoprotein complexes (Wellinger *et al*, 2006). Finally, forks also pause transiently at other protein–DNA complexes, such as pre-replicative complexes at origins of replication and protein complexes at, or near, telomeres (Makovets *et al*, 2004; Miller *et al*, 2006; Wang *et al*, 2001).

In both prokaryotes and eukaryotes, RFB sites are often associated with an increased frequency of recombination, leading to the theory that paused forks stimulate recombination directly through the rapid disassembly of the stalled replisome and the collapse of the fork (see, for example, Defossez *et al*, 1999; Admire *et al*, 2006; Kobayashi *et al*, 1998; Lambert *et al*, 2005). When forks pause at RFBs they do not seem to elicit or require a checkpoint response (Calzada *et al*, 2005; Lambert *et al*, 2005), probably owing to the lack of substantial amounts of ssDNA (Gruber *et al*, 2000; Lucchini & Sogo, 1994). This lack of stabilization by checkpoint kinases has been taken as a potential explanation for the collapse of paused forks that can lead to recombination (Admire *et al*, 2006; Lambert *et al*, 2005).

Nevertheless, there is a growing body of evidence from both prokaryotes and eukaryotes to support an alternative view. It seems that paused forks and their associated replisomes are surprisingly stable, so that the act of pausing *per se* is unlikely to induce collapse of the fork. Furthermore, there is evidence, in several cases, to indicate that hotspots of recombination are dependent on other factors, in addition to the pausing of a DNA replication fork. The literature concerning these issues is both fascinating and highly complex, and forms the topic of this review.

Recombination at paused forks in *E. coli*

Several studies have shown that the pausing of forks at Tus–Ter complexes can stimulate recombination at these sites. A Ter sequence acts as a deletion hotspot when placed in a plasmid (Bierne *et al*, 1991), and the λ prophage excises at a higher

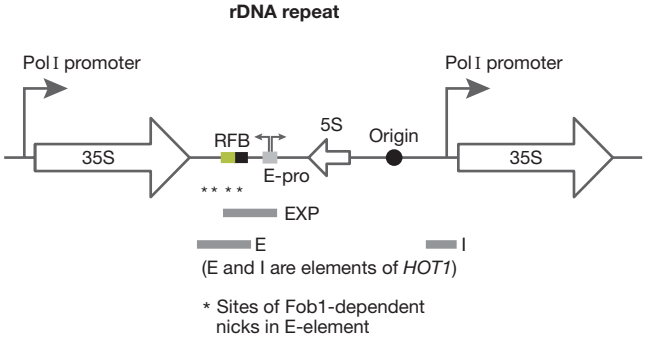

Fig 2 | The ribosomal DNA locus in budding yeast. The replication fork barrier (RFB) ensures that the 35S gene is replicated in the same direction as RNA polymerase I (Pol I) transcription. Maintenance of the copy number of the rDNA repeats is dependent on the EXP sequence (expansion of rDNA repeats) that comprises the RFB and the bi-directional Pol II promoter E-pro (expressing two non-coding mRNAs). The enhancer of rDNA transcription (E) element (containing the RFB and associated 5' sequences) and the initiation of rDNA transcription (I) element (containing the Pol I promoter) of HOT1 stimulate recombination when placed at other chromosomal loci. Fob1, fork blocking 1; rDNA, ribosomal DNA.

frequency at the terminus than at other sites in the *E. coli* chromosome (Louarn *et al*, 1991). Furthermore, a screen for 'hot' DNAs that stimulate recombination identified Ter sequences (Nishitani *et al*, 1993) and such hot activity requires the Tus protein, suggesting a link to paused forks (Horiuchi *et al*, 1994).

Insertion of additional Ter sequences into the *E. coli* chromosome—so as to create a region that forks cannot enter from either direction—produces strains that grow slowly and are dependent on the RecA protein, which mediates strand invasion during recombination (Horiuchi & Fujimura, 1995; Sharma & Hill, 1995). Such strains accumulate linear DNA in the presence of Tus and have increased rates of recombination adjacent to the pause sites, indicating that the stalled forks lead to the formation of double-strand breaks in DNA (DSBs; Bierne *et al*, 1997; Michel *et al*, 1997). However, DSBs are not produced by the collapse of the fork that initially pauses at the Tus–Ter complex; instead, they result from the arrival of a new fork in the subsequent round of chromosome replication (Fig 1B). Replication of the daughter strand from the first round then produces a linear molecule that stimulates recombination (Bidnenko *et al*, 2002; Sharma & Hill, 1995).

The presence of the *Lac* promoter in an *E. coli* plasmid also stimulates recombination in a manner that is apparently linked to the replication of the plasmid (Vilette *et al*, 1992). Insertion of a tandem array of 34 *Lac* operators into the *E. coli* chromosome makes the resultant strain dependent on RecA for viability when the Lac repressor is expressed (Payne *et al*, 2006). Furthermore, a large array of 240 Tet operator sites blocks growth altogether in the presence of the Tet repressor protein (Possoz *et al*, 2006). Importantly, however, two-dimensional DNA gels show that a paused DNA replication fork can resume DNA synthesis within 5 min of the addition of inducer to remove the Tet repressor protein from the array (Possoz *et al*, 2006). This is true even in the absence of RecA, indicating that restart in this case might simply involve the direct resumption of DNA synthesis by the stalled replisome (Fig 1C).

Clashes between forks and the transcriptional machinery can also promote the formation of deletions in *E. coli* plasmids (Vilette *et al*, 1992), and many genes in the chromosome are orientated so as to avoid such clashes with DNA replication forks (Brewer, 1988). Nevertheless, this seems to correlate more with the essential nature of the genes involved than with the level of expression, indicating that such preferential orientation represents a kind of 'insurance policy' to protect essential genes, whereas cells are still able, in most cases, to deal with clashes between replication forks and active RNA polymerase (Rocha & Danchin, 2003).

Together, these data indicate that the stalling of forks at protein–DNA barriers is, indeed, associated with increased recombination in *E. coli*. Paused forks do not necessarily collapse, however, and DSBs are only produced in an indirect manner by the subsequent round of DNA replication. Paused bacterial replisomes have not been examined directly; however, it seems likely that they persist at RFBs, as DNA synthesis resumes rapidly after removal of a barrier protein.

Pausing of forks and recombination in budding yeast

The link between replication and recombination in eukaryotic cells has been studied most intensively with regard to the rDNA locus in budding yeast (Fig 2). The rDNA array comprises several hundred repeats of a 9-kb unit that contains the genes encoding the rRNAs. Recombination in the rDNA is regulated in a highly complex manner, as recombination between repeats is required to maintain the copy number of the array, but must also be restrained to prevent the excision of extra-chromosomal circles (ERCs). These comprise one or more rDNA repeats and are thought to contribute to ageing (Defossez *et al*, 1999).

The first insight into the mechanisms of rDNA recombination came from a screen for hot DNAs that can stimulate recombination when placed in a plasmid. This led to the identification of HOT1, which is a fragment derived from the rDNA repeats (Keil & Roeder, 1984). The HOT1 sequence was subsequently found to comprise two key elements. The first component is the promoter for RNA Pol I (I element), which transcribes the rRNA genes; HOT1 only stimulates recombination when Pol I transcribes into the recombining sequences (Huang & Keil, 1995; Voelkel-Meiman *et al*, 1987). The second component functions as an enhancer of Pol I transcription in the HOT1 fragment (E element; Voelkel-Meiman *et al*, 1987), although it is not required for Pol I transcription in the rDNA (Burkhalter & Sogo, 2004; Wai *et al*, 2001). This enhancer element was found to contain a polar RFB that ensures unidirectional replication throughout the rDNA repeats (Brewer & Fangman, 1988; Linskens & Huberman, 1988). A screen for factors required for HOT1 activity identified the fork blocking 1 (Fob1) protein, which is required for forks to pause at the RFB (Kobayashi & Horiuchi, 1996), and binds directly to the RFB sequence and surrounding sites (Huang & Moazed, 2003; Kobayashi, 2003; Mohanty & Bastia, 2004).

These findings initially suggested that pausing of forks at the RFB might drive recombination in the rDNA in collaboration with Pol I transcription. Consistently, subsequent studies showed that Pol I is required to maintain the high copy number of the rDNA repeats (Brewer *et al*, 1992; Kobayashi *et al*, 1998), similar to the Rad52 (Radiation sensitive 52) protein, which is required for almost all recombination events in budding yeast (Gangloff *et al*, 1996). Fob1 also has a crucial role in the regulation of rDNA copy number;

however, the situation is complex, as Fob1 is required for both the decrease of copy number that occurs after the loss of Pol I and for the increase after the subsequent re-introduction of the enzyme (Kobayashi *et al*, 1998). The formation of monomeric ERCs is reduced in the absence of Fob1 and the lifespan is increased, which is consistent with Fob1 having a crucial role in regulating recombination within the rDNA (Defossez *et al*, 1999).

The link between the RFB and recombination is enigmatic, however, as the insertion of *HOT1* outside the rDNA promotes recombination in a manner that is dependent on Fob1 and Pol I, even when the direction of replication is such that forks do not pause at the RFB (Ward *et al*, 2000). This indicates that Fob1 contributes to *HOT1* recombination independently of its role in pausing forks at the RFB. In addition, although *HOT1* recombination normally requires Fob1, this is not the case after hyperactivation of Pol I transcription in a strain with a reduced number of rDNA repeats (Serizawa *et al*, 2004).

Within the endogenous rDNA, the maintenance of copy number requires an element called expansion of rDNA repeats (EXP), which comprises not only the RFB sequence, but also the adjacent 400 bp (Fig 2). The 400 bp region is not required for RFB function, but instead contains a non-coding bidirectional promoter, E-Pro, which can stimulate the dissociation of cohesin from the surrounding region (Kobayashi & Ganley, 2005).

The Sir2 deacetylase represses transcription by E-Pro within the EXP element (Kobayashi & Ganley, 2005) and limits unequal sister-chromatid recombination within rDNA (Kobayashi *et al*, 2004). In the absence of Sir2, a plasmid containing the Fob1–RFB integrates into the rDNA with high frequency, but only if the RFB is oriented such that forks pause at the RFB (Benguria *et al*, 2003). However, recombination under such conditions requires the full EXP element, and so pausing of forks at the RFB is only part of the mechanism.

In addition to mediating fork pausing at the RFB, it is now clear that Fob1 has other functions that could explain its role in *HOT1* recombination and the maintenance of rDNA copy number. An elegant study showed that Fob1 is required for the formation of nicks at specific locations within the rDNA enhancer (Burkhalter & Sogo, 2004). These nicks are present even during G1 phase and occur at sites that are distinct from the RFB but are important for *HOT1* activity. The formation of such nicks was subsequently found to require topoisomerase I, in addition to Fob1, and an *in vivo* footprint at the corresponding sites was found to be dependent on Fob1 (Di Felice *et al*, 2005).

Together, these studies show that recombination at both *HOT1* and the endogenous rDNA is stimulated by a multitude of factors and activities. Stalling of forks at the RFB might be one important element; however, it is clear that a paused fork is not sufficient to stimulate recombination between the rDNA repeats and that other events are also required. These are dependent on both surrounding DNA sequence elements and the activity of other proteins.

Sites where forks pause on encountering stalled Pol II complexes are also hotspots for recombination (Prado & Aguilera, 2005; Wellinger *et al*, 2006). Recombination at such sites requires transcription to occur during S phase, and both pausing and recombination are stimulated in the absence of the Rrm3 (ribosomal DNA recombination mutation 3) helicase (Prado & Aguilera, 2005; Wellinger *et al*, 2006), which aids the passage of forks past protein–DNA barriers (Ivessa *et al*, 2003). This is consistent with a mechanistic link between the stalling of forks and the stimulation of recombination. However, plasmids with active pause sites of this kind can be maintained even in cells lacking either the RecA homologue Rad51 or the Rad52 protein, and the presence of replication intermediates in two-dimensional DNA gels is not altered by the absence of these proteins, indicating that the paused forks often recover without recombination (Prado & Aguilera, 2005).

Recombination after fork pausing in fission yeast

The pausing of DNA replication forks has a crucial role in a specific recombination reaction that mediates the switching of mating type in fission yeast (Dalgaard & Klar, 1999, 2000). A genomic imprint is formed at the recipient *mat1* locus on chromosome 2 and promotes recombination with the donor loci. Formation of the imprint requires that *mat1* is replicated by a fork moving towards the centromere; this is achieved by the *RTS1* replication fork barrier that is located before *mat1* on the centromere proximal side, and that blocks forks from this direction (Dalgaard & Klar, 2001). *RTS1* therefore contributes indirectly to the subsequent recombination reaction of mating-type switching, by determining the direction of replication of the mating-type region.

Two studies have shown, however, that transposition of *RTS1* to other loci on chromosome 3 stimulates recombination at these sites (Fig 3A). In the first study, insertion of either one or two copies of *RTS1* at the *ura4* locus produced a notable phenotype, in which optimal viability of cells became dependent on Rad22—the fission yeast homologue of Rad52—and the frequency of chromosomal translocations was greatly increased (Lambert *et al*, 2005). A similar phenotype was observed in cells in which endogenous RTS1 was deleted, so that *RTS1–ura4* on chromosome 3 was the only copy of the RTS1 sequence (Lambert *et al*, 2005). The Rad22 protein could be detected by chromatin immunoprecipitation (ChIP) at the *RTS1–ura4* locus, in contrast to the endogenous *RTS1* locus on chromosome 2 (Lambert *et al*, 2005).

In a second study, transposition of *RTS1* to the *ade6* locus on chromosome 3 stimulated recombination at adjacent sequences, but did not make cells dependent on Rad22 for optimal viability (Ahn *et al*, 2005).

These studies confirm the link between paused replication forks and increased recombination at adjacent loci; however, they also indicate that the consequences of a DNA replication fork stalling at a particular protein–DNA barrier might be influenced by the chromosomal context surrounding the RFB.

Preservation of the paused replisome at RFBs

To understand the molecular events that result from pausing of replication forks at RFBs, it is necessary to study the kinetics of pausing at specific loci during a single round of chromosome replication (Fig 3B).

One such approach in eukaryotic cells was the creation of a budding yeast strain with two opposed Fob1–RFBs between two highly active and early origins of replication on chromosome 3 (Calzada *et al*, 2005). Synchronous cell cultures were used to show that forks arrive at each barrier with similar kinetics, pause for an extended period and then pass the barrier before completing replication of the intervening region. ChIP experiments showed that replisome components can be detected at each RFB throughout the period during which pausing occurs. Pausing and recovery are independent of checkpoint kinases and Rad52, and might simply involve the resumption of synthesis by the stalled fork after removal

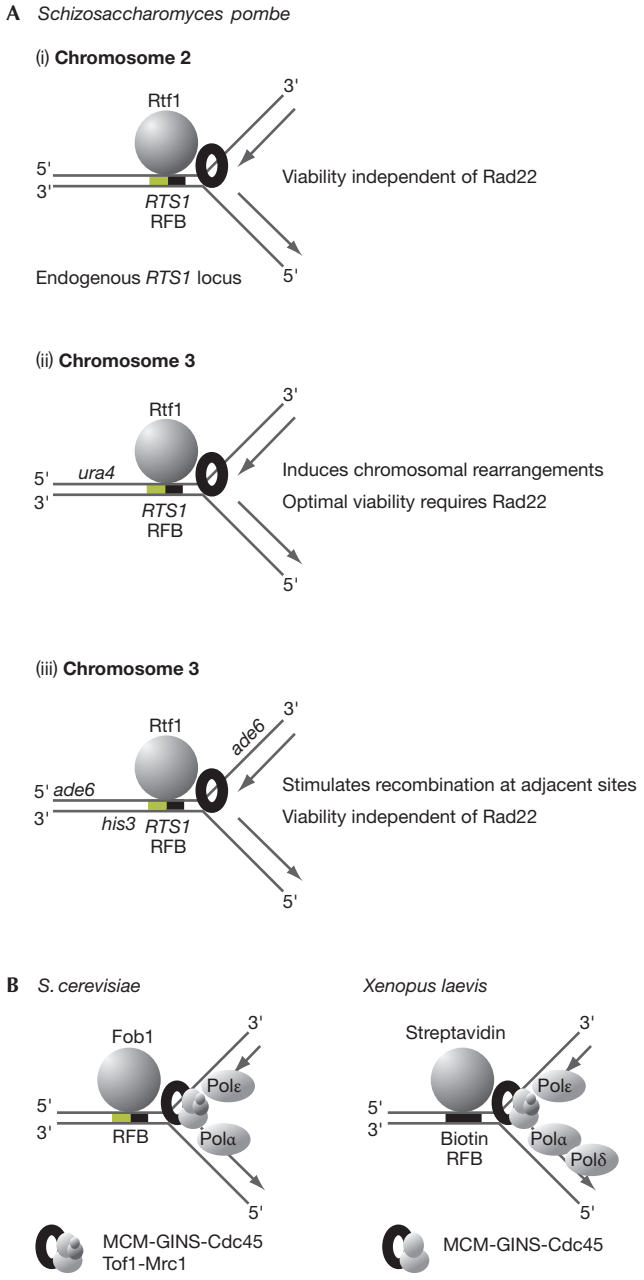

Fig 3 | Pausing of forks at eukaryotic replication fork barriers. (**A**) Pausing of forks at the replication termination sequence 1 (RTS1) barrier in *Schizosaccharomyces pombe* can have different outcomes depending on the chromosomal context (for (ii) see Lambert *et al*, 2005; for (iii) see Ahn *et al*, 2005). (**B**) Chromatin immunoprecipitation studies have shown that replisome components persist at paused forks in *Saccharomyces cerevisiae* (see Calzada *et al*, 2005) and *Xenopus laevis* (see Pacek *et al*, 2006). Fob1, fork blocking 1; Pol, DNA polymerase; Rtf1, replication termination factor 1. The MCM (mini-chromosome maintenance) helicase is required for the progression of eukaryotic DNA replication forks, together with the Cdc45 protein and the four-protein GINS complex (Sld5–Psf1–Psf2–Psf3). In budding yeast, MCM–GINS–Cdc45 also interact with the regulatory proteins Tof1 and Mrc1.

of the barrier. Consistently, the Rrm3 helicase specifically accumulates at both barrier sites during the period of pausing (Calzada *et al*, 2005)—note that a recent study also detected Rrm3 at normal replication forks (Azvolinsky *et al*, 2006).

In another study using extracts of *Xenopus* eggs, an 'accidental' RFB was created at a specific site on a plasmid, in the form of a biotin–streptavidin complex (Pacek *et al*, 2006). ChIP experiments showed that replisome components accumulated at the RFB during the period of pausing, as seen at the budding yeast Fob1–RFB.

Together, these experiments indicate that paused eukaryotic replisomes do not simply disassemble on pausing, but instead can remain stable for extended periods. Therefore, although collapse and recombination represent one possible outcome of stalling, it seems likely that eukaryotic forks can often simply resume synthesis once the barrier has been removed, just as stalled forks in the *E. coli* genome are able to recover after barrier removal at large Tet arrays (Possoz *et al*, 2006).

Stalling of DNA replication forks at fragile sites

Specific sites in eukaryotic chromosomes are known to be particularly sensitive to breakage when chromosome replication is perturbed, resulting in the formation of chromosomal translocations (Richards, 2001).

In mammalian cells, common fragile sites can be visualized by treating cells with the polymerase inhibitor aphidicolin and then subsequently examining metaphase spreads. Reduced expression of the checkpoint kinase ATM and Rad3-related (ATR) increases the frequency of rearrangements at fragile sites, even in the absence of aphidicolin (Casper *et al*, 2002). A related phenomenon might exist in budding yeast, in which absence of the Mec1 (Mitosis entry checkpoint 1) kinase—which is functionally similar to ATR—causes increased breakage of chromosomes at particular loci that correspond to 'replication slow zones' (Cha & Kleckner, 2002).

Two screens for fragile sites in budding yeast showed that they are usually associated with sequences that contain both Ty transposons and tRNAs (Admire *et al*, 2006; Dunham *et al*, 2002). Fragile sites have also been detected in budding yeast by reducing the expression of DNA polymerase-α. Such fragile sites are associated with the inverted repeat sequences of Ty elements (Lemoine *et al*, 2005). A model has been proposed in which the breakage of fragile sites requires two factors: slowing of replication and the presence of sequence elements, such as inverted repeats, which can form potentially recombinogenic secondary structures when unwound (Lemoine *et al*, 2005). Impairing polymerase-α function should increase the amount of ssDNA on the lagging-strand template, thereby favouring secondary-structure formation when the fragile site is unwound.

tRNAs act as polar RFBs; therefore, the association of yeast fragile sites with Ty elements and tRNAs raises the possibility that the stalling of forks at tRNA RFBs might initiate the formation of fragile sites (Admire *et al*, 2006). It seems likely that nearby sequence elements, such as the long terminal repeats of the Ty elements, will also contribute to fragile-site formation. Perhaps the role of checkpoint kinases in protecting cells against fragile sites might relate to the stabilization of DNA secondary structures that are formed when such elements are unwound, particularly as checkpoint kinases are not required for pausing or recovery of forks *per se* at the budding yeast Fob1–RFB (Calzada *et al*, 2005), or at the fission yeast *RTS1–ura4* RFB (Lambert *et al*, 2005).

Mechanisms of fork pausing at RFBs

Pausing of replication forks at accidental barriers, such as Tet repressor/operator arrays in *E. coli*, might simply result from the physical clash between the replisome and the protein–DNA complex at the RFB. At the Tus–Ter barrier, however, pausing occurs by a more sophisticated mechanism. One possibility is that specific interactions between Tus and the replicative helicase dnaB might contribute to pausing (Mulugu *et al*, 2001). However, a recent study has identified a different mechanism in which unwinding of the RFB from the non-permissive side springs a 'molecular mousetrap', which allows a conserved base in the displaced strand to 'flip-out' so that it can be bound tightly by Tus (Mulcair *et al*, 2006).

Eukaryotic cells seem to have evolved a different strategy to deal with the pausing of forks at protein–DNA barriers. Pausing at the fission yeast mating-type locus, at the rDNA of both budding and fission yeast, and also at a budding yeast tRNA has been found to require two specific proteins: Swi1/3 (identified through mutations that inhibit switching of mating type) in fission yeast and Tof1/Csm3 (Topoisomerase I interacting factor 1/Chromosome segregation in meiosis 3) in budding yeast (Calzada *et al*, 2005; Dalgaard & Klar, 2000; Krings & Bastia, 2004; Mohanty *et al*, 2006; Tourriere *et al*, 2005). These two factors bind each other and associate with the replicative mini-chromosome maintenance (MCM) helicase at DNA replication forks (Gambus *et al*, 2006; Katou *et al*, 2003; Lee *et al*, 2004; Mayer *et al*, 2004; Noguchi *et al*, 2004).

It therefore seems that pausing of eukaryotic forks is—at least in these particular cases—an active process mediated by the replisome itself. This could allow eukaryotic cells to do other important things at paused replication forks, such as establishing the imprint at the fission yeast mating-type locus. It is interesting to note that two studies have shown that recombination rates are increased in fission yeast cells lacking Swi1/3 (Ahn *et al*, 2005; Sommariva *et al*, 2005). Furthermore, both fission yeast and budding yeast cells lacking Swi1/Tof1 often have subnuclear foci of recombination proteins (Noguchi *et al*, 2004; Tourriere *et al*, 2005). This indicates that the ability of eukaryotic DNA replication forks to pause their progression at RFBs might help to preserve genome stability. Perhaps proteins such as Swi1/Tof1 assist the fork in pausing stably at tight protein–DNA barriers until helicases, such as Rrm3, unwind the RFB and displace the barrier protein, so that the replicative helicase can proceed and allow the fork to resume synthesis.

Conclusions

It is clear that much remains to be learnt about the mechanisms by which eukaryotic forks pause at protein–DNA barriers, and about the molecular events that follow the stalling of forks at such RFBs. It will be fascinating to discover whether 'active' pausing by Tof1/Swi1 is a general feature of all eukaryotic RFBs, and if this mechanism helps to preserve genome stability or has evolved for other reasons. It is clear that pause sites can be hotspots for recombination, although this seems to be linked to other factors besides the act of pausing itself. Perhaps there is competition at protein–DNA barriers between the inherent stability of a replisome that arrests its own progression through Tof1/Swi1, and the possibility that collapse and recombination might occur stochastically or be promoted by other features at fragile sites. If this is the case, although eukaryotic cells often hold forks, they do not necessarily have a knife in the other hand.

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