“Chromosome kissing” and modulation of replication termination

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Previously, inter-chromosomal interactions called “chromosome kissing” have been reported to control tissue-specific transcription and cell fate determination. Using the fission yeast as a model system we have shown that physiologically programmed replication termination is also modulated by chromosome kissing. The published report reviewed here shows that a myb-like replication terminator protein Reb1 of S. pombe and its cognate binding sites (Ter) are involved in chromosome kissing that promotes a cooperative mechanism of replication termination. We also suggest that at least one other replication terminator protein namely Sap1, which is also an origin binding protein, is likely to be involved in a similar mechanism of control not only of fork arrest but also of replication initiation and in possible ori-Ter interaction. We discuss the roles of chromatin remodeling and other proteins in this novel mechanism of replication control.

“Chromosome kissing” refers to direct physical interactions between pairs of chromosomes that often control a DNA transaction.1 Chromosome kissing can be driven by an oligomeric DNA binding protein that simultaneously binds to sites on two non-homologous chromosomes that form a kissing pair. Alternatively, each site could bind to a different protein but these proteins would interact with each other directly or via a third protein with an affinity that is strong enough to promote site-site interaction. Historically, it was Herman Muller who first commented on the observation that the tendency of the somatic chromosomes of Drosophila to associate closely with their respective homologs might cause regulation of a gene located on one chromosome by its allele located on the homolog.2 This trans-activation phenomenon was further studied by E.B. Lewis at the bithorax locus, and he named it as transvection.3 Transvection or kissing between homologous chromosomes at the bithorax locus is brought about by the oligomeric form of the protein called Zeste4 that promotes physical interaction between two enhancer elements. A few other examples of chromosome kissing are described here. Random inactivation of one of a pair X chromosome in human females requires a transient kissing interaction at the X inactivation center.5 Furthermore, tissue specific transcription and cell fate determination by chromosome kissing is provided by interaction between the chromosome X and XI in naïve T helper cells. Upon receiving signal to differentiate into Th1 or Th2 cells, the kissing interaction is abolished. In Th1 cells and chromosome XI moves into heterochromatic regions and gets transcriptionally repressed. In Th2 cells the chromosome XI remains active whereas chromosome X (10) becomes heterochromatic and is rendered inactive.6

Previously, it was not known whether chromosome kissing also controlled DNA replication. Recently, we have reported that chromosome kissing modulates site-specific termination of DNA replication in Schizosaccharomyces pombe.7 DNA replication can be divided into three steps: initiation, ongoing replication and termination. The biochemistry of replication initiation and ongoing replication have been reviewed in references 8 and 9. Replication termination can occur at random sites where two fork coming from the
opposite direction in a chromosome meet each other and topoisomerase II catalyzes the process of separation of the daughter molecules at the random terminus. A second category of replication termination is physiologically programmed and occurs at sequence-specific replication termini (Ter) that bind to cognate terminator proteins. The Ter complex arrests forks in a polar mode, i.e., a fork approaching the sites from one direction is arrested whereas that approaching from the opposite direction passes through unimpeded or the second fork meets the 1st fork that is already arrested at the Ter sites. Programmed replication termination is of special interest because of its connections with other DNA transactions such as transcription, recombination etc.

At least three terminator proteins have been identified to date in fission yeast namely Rtf1, Reb1, and Sap1. Rtf1 binds to a site called RTS1 that is located 5' to the mating type switch locus Mat1. This site prevents a fork traveling from left to right from passing through the switching locus because such a passage would erase a strand-specific imprint that determines which of the descendent cells will undergo mating type switch. The imprint is either a site specific nick or a piece of RNA left-over from lagging strand replication at the switch site. The imprint becomes a ds break by another round of replication in the next cell cycle and provides an insertion site for either Mat2 or Mat3 that replace Mat1. Three closely spaced Ter sites are present in each of the intergenic spacer of rDNA clusters that are present at either ends of chromosome III. These are called Ter1, Ter2 and Ter3 (Fig. 1A). Ter1 binds to the multifunctional protein Sap1 that also binds to the ARS sites and to the SAS1 site that promotes efficient mating type switching. Only the Sap1-Ter1 complex promotes polar fork arrest. We have also discovered the presence of Ter1 like sequences outside the rDNA (unpublished).

Ter2 and Ter3 bind to the dimeric myb-like protein called Reb1. The protein has an N-terminal dimerization domain and C-terminal, twin myb motifs that are parts of the DNA binding domain (Fig. 1B). Reb1-Ter2/Ter3 complexes not only cause polar arrest of replication forks approaching from one direction but also of rRNA synthesis catalyzed by RNA polymerase I approaching the complex from the opposite direction (Fig. 1A). Reb1-binding Ter sites are also located at other regions of the chromosomes I, II and III, outside the rDNA. Many of these sites are located in the enhancer regions of several genes that are transcribed by RNA polymerase II. Reb1 binding to these sites not only promotes polar fork arrest but also activates transcription from the corresponding promoters. The truncated monomeric Reb1 binds to a consensus binding site with almost the same affinity as the WT protein and promotes fork arrest.

Hitherto, most of our knowledge about the mechanism of polar fork arrest has been derived from the study of replication termination in prokaryotic systems. We and others have shown that the terminator protein of E. coli called Tus, and that of B. subtilis called RTP bind to the cognate Ter sites and arrest the respective replicative helicases in a polar mode. The process not only involves terminator protein-Ter interaction but also the interaction between the arresting and the arrested proteins. The mechanism of polar fork arrest by terminator protein has been reviewed in references 26 and 27. The Ter3-Reb1 complex does not arrest forks in vivo when present in the cell milieu of S. cerevisiae—an observation that supports the interpretation that binding of Reb1 to Ter is necessary but not sufficient to cause polar fork arrest and that the mechanism appears to be replisome-specific. The protein binds to the consensus sequence GAG TAA TGG TAA that causes a G to T at the 7th position resulting in the sequence GAG TAA TGG TAA TGC ACC A causes a ~10-fold reduction in protein binding and abolition of fork arrest.

Using two in vitro methods, we have shown that the dimeric but not the truncated monomeric form of Reb1 can bring together two WT Ter site present in cis in a plasmid and separated by 1.3 kb of DNA regardless of whether the sites are positioned in a parallel or an antiparallel orientation with respect to each other. We have reported further that that a WT site can not only loop DNA by interacting with the M7 mutant site by enhancing cooperativity at a distance but can also promote fork arrest in vivo at the mutant site (the solo M7 site is nonfunctional) as an observation that supports the interpretation that binding of Reb1 to Ter is necessary but not sufficient to cause polar fork arrest and that the mechanism appears to be replisome-specific.

The pathway has been derived from the study of replication termination in prokaryotic systems. We and others have shown that the terminator protein of E. coli called Tus, and that of B. subtilis called RTP bind to the cognate Ter sites and arrest the respective replicative helicases in a polar mode. The process not only involves terminator protein-Ter interaction but also the interaction between the arresting and the arrested proteins. The mechanism of polar fork arrest by terminator protein has been reviewed in references 26 and 27. The Ter3-Reb1 complex does not arrest forks in vivo when present in the cell milieu of S. cerevisiae—an observation that supports the interpretation that binding of Reb1 to Ter is necessary but not sufficient to cause polar fork arrest and that the mechanism appears to be replisome-specific. The protein binds to the consensus sequence GAG TAA TGG TAA TGC ACC A causes a ~10-fold reduction in protein binding and abolition of fork arrest.
the mutant sites is necessary but not sufficient for fork arrest; the two sites have to contact each other in an antiparallel orientation to cause fork arrest at M7. These experiments unequivocally demonstrated that Reb1 can loop DNA and the looping process enhances replication fork arrest by a cooperative mechanism.7

Can Reb1 promote a trans-interaction between a M7 Ter site located on one chromosome with a WT Ter site located on a different chromosome? We addressed this question by mutating the WT Ter located at the coordinate 344314 on chromosome II to the M7 form and performed circular chromosome conformation capture as shown in Figure 2 to look for interacting sequences. The cells were treated with HCHO to cross-link proteins to DNA, the chromatin digested with a 4 bp cutter, diluted to prevent intermolecular contacts of one complex with another, ligated, the cross link reversed and the captured sequence (shown in red) amplified by polymerase chain reaction (PCR) with a pair of primers. The reaction products were fractionated by agarose gel electrophoresis, the DNA bands eluted and sequenced. Consistently, the M7 Ter located in chromosome II captured a major Ter sequence located at 4689236 and a minor one at 4257637, both located in chromosome I. Although not all of the Ter sites on the chromosomes have been mapped, it seems that the capture is non-random. Consistent with this suggestion, the ~400 Ter sites that bind to Reb1 and located in the 2 rDNA clusters located at either ends of chromosome III were not captured by this method (unpublished). In control experiments, as expected, Ter-Ter kissing interactions were abolished in reb1-deleted cells and also in those cells expressing only the monomeric form of Reb1.7

Does the chromosome kissing described above modulate replication termination? We addressed this question by mutating multiple residues in the Ter (the major Ter site on chromosome I captured by 4C) that caused the site to become nonfunctional (i.e., incapable of binding to Reb1). We then asked the question as to whether the mutated TerM was capable of promoting fork arrest at TerM and the answer was in the negative. The results provided compelling evidence that kissing interactions between the Ter located on chromosome II with the Ter located on chromosome I controlled replication termination.7

Why has nature evolved such a cooperative inter-chromosomal interaction mechanism for controlling replication termination? Several possibilities come to mind. Since Reb1 is not only a replication terminator protein but also activates transcription catalyzed by RNA polymerase II at certain promoters, the kissing mechanism perhaps ensures coordinated control of transcription. Concomitant replication termination at the sites is probably necessary because it prevents interference of transcription by replication and vice versa.28 Such interference is likely to cause unscheduled fork stalling that might lead to fork regression and chromosome breaks resulting in genome instability. An inspection of the direction of transcription and replication about some of the Ter sites shows that replication and transcription appear to be co-directional. Recent work supports the conclusion that replication-transcription interference can occur not only when replication forks approach a transcription unit from the opposite direction but also when the processes are co-directional.29 The mechanism could also modulate cell cycle-directed gene expression. For example, transcription of Ste9 seems to be controlled by Ter344314, and under nitrogen starvation Ste9 is induced and promotes G1 arrest that is a prerequisite for sporulation (Pablo Hernandez personal communication). Extensive chromosome kissing that involves Ter344314 could help stronger Reb1 binding and help catalyze a burst of synthesis of Ste9 needed for sporulation. Interruption or reduction of kissing at other stages of the cell cycle and under favorable nutritional conditions would reduce kissing interactions and consequently the level of Ste9 when it is no longer needed. In summary, the kissing mechanism could be a convenient way to control temporally and spatially replication fork arrest and the physiological process that it promotes.

Figure 2. A diagrammatic representation of the 4C technique used to determine chromosome kissing at the Ter sites located in chromosome 1 and chromosome 2 of fission yeast.
It has been shown that chromosomes at interphase are not randomly located but tend to occupy chromosome territories. It has been further suggested that a chromatin loop emanating from one territory contacts a loop from another to promote kissing-dependent DNA transactions. Are such contacts random and guided by stochastic movements or are these vectorial and involve a molecular motor such as myosin and an ATP hydrolysis? There are a few reports of chromatin movement in interphase catalyzed by myosin-dependent ATP hydrolysis. However, further work is needed to determine unequivocally whether such ATP-dependent locomotion is needed for chromosome kissing.

If chromosome kissing is facilitated by contacts between chromatin loops, how is such a loop formed and/or stabilized? Silent mating type loci of S. cerevisiae appear to be located in a chromosomal loop that could be formed by interaction between barrier elements that form the stem of the loop. Mutations in the cohesins Smcl and Smc3 cause silencing to spread beyond the loop domain. This observation would suggest that cohesins might be involved in the organization of interphase chromatin into looped domains. It is therefore possible that cohesins could be indirectly involved in Ter-Ter interaction via chromosome kissing by generating chromatin loops. Work is in progress to investigate this possibility. Another cohesion-like protein namely condensin (cohesin type of tripartite rings) is known to be involved in fork arrest in S. cerevisiae. It might be useful to investigate whether condensing might be performing a similar function in fission yeast.

The Ter sites are probably masked by nucleosomes that would inhibit interaction with the cognate terminator proteins. Therefore, nucleosome removal or displacement from Ter sites by chromatin remodeling proteins is likely to be necessary for replication termination. In fact our preliminary data show that chromatin remodeling proteins are required for efficient fork arrest at Ter sites. Furthermore, 2D gel analysis of fork arrest at Ter sites show that certain remodeling proteins promote whereas others inhibit fork arrest. Further work will be needed to elucidate the interplay of terminator protein, chromatin remodeling proteins, possibly a motor protein, possibly cohesion and other yet to be identified components that drive chromosome kissing.

Does chromosome kissing also regulate initiation of replication? Recent work on the conformation of interphase chromosomes of S. cerevisiae suggests that early replicating ARS elements seem to interact with each other. Our preliminary data suggest that in S. pombe ARS and Ter sites interact with each other in a Rebl-independent mode. We suspect that Sap1 is likely to be involved in promoting this interaction. These observations suggest that chromosome kissing may be involved in regulation of replication by providing a reversible sequestering mechanism that places origins and termini in replication factories. Experiments to test this hypothesis are in progress. On the basis of the observations discussed in this perspective it appears that control of major DNA transactions such as replication, transcription etc., are 3-dimensional and are promoted by specific protein-mediated inter and intra-chromosomal interactions. These topics invite further investigations in a variety of different systems.

Acknowledgements

This work was supported by the grants GM 049264 and GM049264-17S1 to D.B. The references are in regulation of replication by providing a reversible sequestering mechanism that places origins and termini in replication factories. Experiments to test this hypothesis are in progress. On the basis of the observations discussed in this perspective it appears that control of major DNA transactions such as replication, transcription etc., are 3-dimensional and are promoted by specific protein-mediated inter and intra-chromosomal interactions. These topics invite further investigations in a variety of different systems.

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