Current Biology, Vol. 14, R915–R917, November 9, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.10.012

DNA Replication: Stalling a Fork for Imprinting and Switching

Dispatch

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Mating-type switching in fission yeast has long been known to be directed by a DNA 'imprint'. This imprint has now been firmly characterized as a protected site-specific and strand-specific nick. New work also links the widely conserved Swi1–Swi3 complex to the protection of stalled replication forks in general.

This story concerns two yeast species which reach similar ends by very different means. The fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* can switch mating type by reprogramming their genomic DNA. The general rules of this interconversion are relatively simple: two alternative cell states — complementary cell types — are encoded by the mating-type 'cassettes', which reside at three distinct loci in the genome (Figure 1). One of these loci can be actively expressed, whereas the others merely serve as silent backup copies for the interconversion process. The exchange reaction itself is accomplished by homologous recombination, guided by repetitive sequence elements which are identical for either end of all three loci. To initiate this recombination, a damage-like event is inflicted upon the DNA at a boundary of the active mating-type locus. To resolve this damage, recombinational repair takes over, using the reciprocal silent cassette as a template for bypass replication, switching back to the active locus at the opposite end.

This is how far the similarity between the two yeasts extends: all the molecular tools to program the initial damage are different in the two species, reflecting the independent evolution of the underlying mechanisms. In budding yeast, the recombination is initiated by a staggered double-strand break, created by the tightly controlled HO endonuclease at a specific ~20 base pair recognition sequence. This cut is usually carried out in late G1 phase of the cell cycle, to be fully repaired before the beginning of S phase replication. Notably, this double-strand cut is lethal if no information elsewhere in the genome is available for appropriate repair. The HO endonuclease, together with the corresponding recognition site, have been developed as a versatile research tool, allowing selected target sites to be cleaved and repaired *in vivo* at the discretion of the experimenter.

In fission yeast, on the other hand, the mechanism that causes the initial switch-related damage — here often referred to as an 'imprint' — is less invasive and not fully understood. Even the biochemical structure has remained somewhat controversial, and a stretch of 500–600 base pairs is required to specify the imprint, made in the middle of the sequence. Most remarkably, the imprint is not lethal in the absence of other homologous sequences in the genome. While the imprint is generated during S phase of one cycle, its repair is delayed until S phase of the following cycle, when it causes cassette switching in one of the two daughter DNA molecules.

This integration of the fission yeast mating-type switch into the local organization of DNA synthesis is corroborated by recent progress of several research groups. New work of Kaykov and Arcangioli [1], reported in this issue of *Current Biology*, has firmly established the molecular structure of the damage-like initial imprint as a single-strand break. Complementary work of Vengrova and Dalgaard [2] has characterized a replication pause site, which is critical for the imprinting reaction. Moreover, Russell's group [3] has added general significance to key components of the *S. pombe* switching system by linking them to the replisome complex of ubiquitous replication forks.

In fission yeast, the two mating types are termed P, for plus, and M, for minus; in budding yeast, they are called **a** and α. The functional mating type is determined by the P or M state of the *mat1* locus. The silent backup cassettes for the switching reaction reside at *mat2-P* and *mat3-M*, respectively, some distance from *mat1*. As illustrated in Figure 2, imprinting and switching at *mat1* require that the relevant replication fork approaches from the right-hand side; replication from the left is barred by the strong RTS1 terminator, some 700 base pairs to the left of the *mat1* cassette [4]. The flanking homology boxes are termed H1 to the right, where the imprint is set to start the switching reaction, and H2 to the left, where resolution occurs later on.

Within the *mat1-M* cassette, a directional pausing site MPS1 has been detected and mapped 340 base pairs to the left of the imprint at H1 [2]. Transient

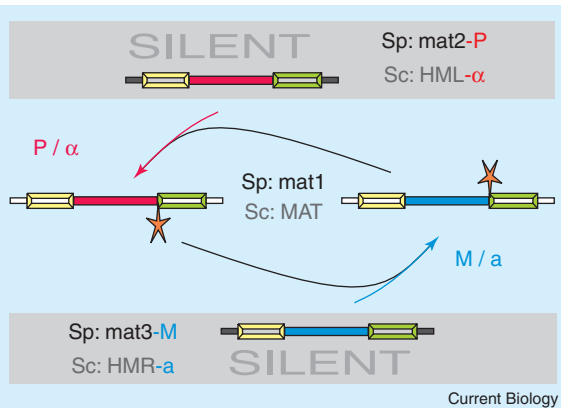

Figure 1. Cassette model of mating-type switching in the fission yeast *Schizosaccharomyces pombe* (Sp) and budding yeast, *Saccharomyces cerevisiae* (Sc).

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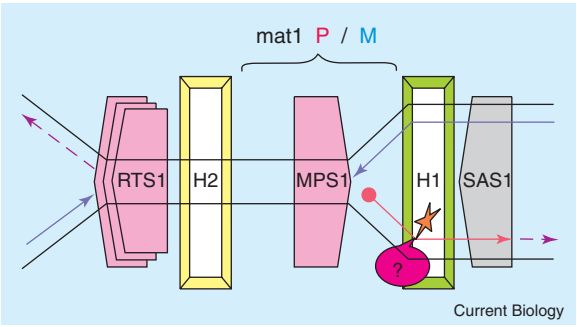

Figure 2. Organization of the *S. pombe mat1* cassette at the imprinting stage during S phase, one cell cycle before mating-type switching.
The potentially interfering replication from the left is terminated at RTS1. Homology boxes H1 and H2 flank the M/P-specific inner range of the cassette. Interactions between the external boundary SAS1 and the internal replication pause site MPS1 are necessary for placing a protected nick in the middle of a longer than usual Okazaki fragment, presumably by a structure-dependent endonuclease (?).

cessation of the replication fork at this pause site, after passing the site of the imprint from the right-hand side, is critical for setting the imprint at H1. A similar pause site in the *mat1-P* cassette remains to be characterized. The overall topology of replication at *mat1* indicates that the imprinting event occurs on the newly synthesized lagging strand, perhaps during maturation of a critical Okazaki fragment. An external boundary is defined by the SAS1 binding site for the essential Sap1 protein of still unknown function [5].

Kaykov and Arcangioli [1] have now resolved the molecular structure of the imprint as a site-specific and strand-specific nick, which lacks a phosphate group on either side. This conclusion was based on a series of reconstitution experiments, characterizing the conditions that allow the exact regeneration of the parental DNA sequence from the imprint fragments nicked *in vivo*. The original sequence could be perfectly restored by phosporylation and ligation — not only for wild-type, but also when six Ts around the imprinting site were replaced by a CTGCAG *Pst*I restriction site. Significantly, these data are incompatible with the alternative interpretation of Vengrova and Dalgaard [2], assuming 1–2 interspersed ribonucleotides in the DNA chain at the site of the imprint. It is remarkable that the artificial *Pst*I restriction target at the imprinting site had little effect on the efficiency of single-strand DNA cleavage, which still occurred at the equivalent position. This means that the imprint is positioned more by overall structure than by local sequence of the target DNA. The earlier notion of a double-strand break at this site has been refuted as a preparation artefact [6,7].

As the strains used in these studies lacked silent donor cassettes, imprinting was uncoupled from mating-type switching, a tremendous experimental advantage. This provides us with a powerful model to study in detail what happens when a replication fork encounters damage at a known position. Beyond this general relevance, the structure and behaviour of this peculiar DNA discontinuity raise interesting and important questions at various levels, concerning fission yeast in particular.

How does single-strand DNA cleavage regularly arise during DNA replication in the first place? Which sequence elements are required to specify the site and efficiency of the imprint? How is it protected for an entire cell cycle, without being annihilated by the cell's ubiquitous repair mechanisms? How does it lead up to directed mating-type switching in the subsequent S phase, only affecting one of the daughter molecules? How is it actually repaired in the template strand, each time replication manages to pass the imprint, whether or not there is mating-type switching on the other strand? Not all the answers are available yet, but a range of circumstantial observations bear on these issues. These will undoubtedly be followed up in the specialist literature.

Useful functional cues of general importance relate to three proteins involved in the imprinting reaction: Swi1, Swi3 and Swi7. Mutations in the corresponding genes reduce the level of imprinting and, in turn, the frequency of *mat1-P*/*mat1-M* interconversion. The only known *swi7* allele changes DNA polymerase α by a single amino acid, without abolishing the catalytic activity of this essential enzyme [8]. This implicates the polymerase α–primase complex in setting the imprint. Swi1 and Swi3, on the other hand, have only recently been shown to participate in the stabilization of stalled replication forks in general [3,9] and, more specifically, in transiently halting replication at the MPS1 pause site inside the *mat1-M* cassette ([10] and A. Klar, personal communication).

Moreover, a prominent Okazaki fragment has been detected in the critical area (Figure 2) [2]. This fragment spans more than 540 nucleotides, compared to the average of 100–150 nucleotides for eukaryotes in general. It is uniquely primed close to the MPS1 pause site and, in a wild-type strain, it remains unprocessed at the 5′ end, considerably longer than in a *swi3* mutant. Evidence for other Okazaki fragments in the vicinity of the imprinting site itself was not obtained in this experiment. This indicates that the imprinting nick has to be introduced in the middle of the extraordinarily long fragment, likely by some structure-specific endonuclease. At any rate, the site-specific nicking in *S. pombe* appears more complex than the direct cutting of both strands by the HO endonuclease in *S. cerevisiae*.

What then is known about the consequences of the imprint for replication in the succeeding cell cycle? When the leading strand of the next replication fork is halted at the nick, the fork is transiently retracted as a 'chickenfoot' structure, as observed experimentally [2,11]. This allows the 3′ overhang to invade the homologous H1 sequence of a silent cassette, where bypass synthesis results in mating-type interconversion [12]; the transient retraction of the replisome also allows the nick to be closed by gap repair, whereafter the replication fork can progress beyond the site of the previous imprint.

The removal of the original imprint in every cell cycle has long been postulated [13]. In the course of an effective switching event, the leading strand will

only reenter the *mat1* cassette in the H2 area for resolution. The long, pause-related Okazaki fragment of the lagging strand is in turn marked by a new imprint in the lower branch of DNA, representing the unswitched daughter cell which usually is able to switch one of its own daughter cells during the following cell division. In fully viable cell lines, however, where there is no silent cassette available for switching, the closing of the nick in front of the chickenfoot structure allows the stalled replication fork to be fully reconstituted within the original *mat1* cassette, which now resembles the unimprinted DNA of Figure 2.

New work in a related field by Russell's group [3] has given wider significance to key components of the *S. pombe* switching system. Here, the Swi3 protein was isolated anew as a specific binding partner for Swi1. The Swi1–Swi3 complex tightly follows the movement of replication forks, probably as ancillary proteins associated with the replisome. While this newly discovered complex has no vital role in replication as such, it becomes critical if replication forks are arrested by single-strand damage in the DNA template. If one or both proteins are missing, mutual coordination is lost for leading-strand and lagging-strand synthesis, resulting in the accumulation of single-stranded gaps and, in turn, the destabilization of replication forks.

Given this novel function, the protein has been dubbed a fork protection complex (FPC). As orthologs occur from other fungi to insects, mice and man, this complex is widely conserved and may have retained similar protective functions. In particular, Tof1 and Src3 in *S. cerevisiae* — homologs of *S. pombe* Swi1 and Swi3, respectively — have been implicated in replication pausing and efficient sister chromatid cohesion [14,15].

References

1. Kaykov, A., and Arcangioli, B. (2004). A programmed strand-specific and modified nick in *S. pombe* constitutes a novel type of chromosomal imprint. Curr. Biol. *14*, this issue.
2. Vengrova, S., and Dalgaard, J.Z. (2004). RNase-sensitive DNA modification(s) initiates *S. pombe* mating-type switching. Genes Dev. *18*, 794-804.
3. Noguchi, E., Noguchi, C., McDonald, W.H., Yates III, J.R., and Russell, P. (2004). Swi1 and Swi3 are components of a replication fork protection complex in fission yeast. Mol. Cell. Biol. *24*, 8342-8355.
4. Dalgaard, J.Z., and Klar, A.J. (2001). A DNA replication-arrest site RTS1 regulates imprinting by determining the direction of replication at *mat1* in *S. pombe*. Genes Dev. *15*, 2060-2068.
5. Arcangioli, B., and Klar, A.J. (1991). A novel switch-activating site (SAS1) and its cognate binding factor (SAP1) required for efficient *mat1* switching in *Schizosaccharomyces pombe*. EMBO J. *10*, 3025-3032.
6. Arcangioli, B. (1998). A site- and strand-specific DNA break confers asymmetric switching potential in fission yeast. EMBO J. *17*, 4503-4510.
7. Dalgaard, J.Z., and Klar, A.J. (1999). Orientation of DNA replication establishes mating-type switching pattern in *S. pombe*. Nature *400*, 181-184.
8. Singh, J., and Klar, A.J. (1993). DNA polymerase-alpha is essential for mating-type switching in fission yeast. Nature. *361*, 271-273.
9. Noguchi, E., Noguchi, C., Du, L.L., and Russell, P. (2003). Swi1 prevents replication fork collapse and controls checkpoint kinase Cds1. Mol. Cell. Biol. *23*, 7861-7874.
10. Dalgaard, J.Z., and Klar, A.J. (2000). *swi1* and *swi3* perform imprinting, pausing, and termination of DNA replication in *S. pombe*. Cell *102*, 745-751.
11. Kaykov, A., Holmes, A.M., and Arcangioli, B. (2004). Formation, maintenance and consequences of the imprint at the mating-type locus in fission yeast. EMBO J. *23*, 930-938.
12. Arcangioli B. (2000). Fate of i DNA strands during mating-type switching in fission yeast. EMBO Rep. *1*, 145-150.
13. Klar, A.J., Bonaduce, M.J., and Cafferkey, R. (1991). The mechanism of fission yeast mating type interconversion: seal/replicate/cleave model of replication across the double-stranded break site at *mat1*. Genetics *127*, 489-496.
14. Katou, Y., Kanoh, Y., Bando, M., Noguchi, H., Tanaka, H., Ashikari, T., Sugimoto, K., and Shirahige, K. (2003). S-phase checkpoint proteins Tof1 and Mrc1 form a stable replication-pausing complex. Nature *424*, 1078-1083.
15. Mayer, M.L., Pot, I., Chang, M., Xu, H., Aneliunas, V., Kwok, T., Newitt, R., Aebersold, R., Boone, C., Brown, G.W., and Hieter, P. (2004). Identification of protein complexes required for efficient sister chromatid cohesion. Mol. Biol. Cell *15*, 1736-1745.