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Commentary

Relationship between transcription and initiation of meiotic recombination: Toward chromatin accessibility

*Alain Nicolas**

Institut Curie, Section de Recherche, UMR144 Centre National de la Recherche Scientifique, 26 rue d'Ulm, 75248 Paris Cedex 05, France

Since the high level of meiotic recombination at the *ARG4* locus of the budding yeast *Saccharomyces cerevisiae* was found to be controlled by a cis-acting initiator located within its 5′ noncoding region (1), the question of the relationship between transcription and meiotic recombination control was raised. Are promoters and sites at which meiotic recombination initiates functionally related? Transcription by RNA polymerase I and II appears to stimulate mitotic recombination in *S. cerevisiae* (2, 3), but how is meiotic recombination affected by local gene expression? The paper by Kon *et al.* (4), in conjunction with other recent contributions concerning the M26 hot spot of meiotic recombination in the fission yeast *Schizosaccharomyces pombe*, provides compelling new insights and prompts additional questions. This commentary/mini-review will focus on the control of the initiation of meiotic recombination in these yeasts.

Meiosis, a eukaryotic innovation, allows diploid cells to generate haploid gametes. Homologous recombination, which occurs during the prophase of the meiosis I division, has two essential functions. First, the overall high rate of recombination (100- to 1,000-fold above that in vegetative cells) contributes to the increased genetic diversity of the gametes. Second, crossing-over between two nonsister chromatids creates a physical link between homologs and ensures their proper disjunction during meiosis I division (5–7). It is well established that defects in meiotic recombination lead to abnormal chromosome segregation, which can result in inviable progeny. It follows that understanding the initiation and the maturation of recombination intermediates is an important issue. Recombination frequencies are not distributed at random, and chromosomal regions that undergo unusually high levels of recombination are termed "hot spots." Our current understanding of the mechanisms and of the control of meiotic recombination owes much to the study of these hot spots (8).

The M26 Hot Spot of *S. pombe*. The meiotic recombination hot spot *ade6-M26* (M26) has been well characterized (9, 10). M26 was one of 394 *ADE6* mutations isolated by H. Gutz (11). M26 is unique: in pairwise *ade6* heteroallellic crosses, it manifests a higher level of recombination, up to 15-fold, compared with nearby mutations. The patient dissection of this "marker effect" has been very fruitful. Significantly, when homozygous, M26 increases the frequency of gene conversion frequencies of nearby markers, suggesting that M26 creates a site that promotes recombination in its vicinity (11–13). The cloning and sequencing of *ade6* mutations established that M26 is a G-T substitution, which creates a nonsense mutation near the 5′ end of the coding region. In contrast, the *ade6-M375* mutation (M375), also a G to T change located in the preceding codon (12, 14), does not exhibit hot-spot activity (11). The M26 effect was further found to depend on the fortuitous creation of a specific DNA sequence motif, the heptamer 5′-ATGACGT-3′ (the M26 mutation is underlined) (15). Mutation of any of these seven nucleotides results in the loss of hot-spot activity. The heterodimeric protein Mts1/Mts2 (*M-t*wenty-*s*ix binding proteins) later was identified as a factor that specifically binds to this motif (16). Additional experiments demonstrated a perfect correlation between the sequence requirement of the heptamer for hot-spot activity *in vivo*, and Mts1/Mts2 binding activity *in vitro* (16). The conclusive evidence that this heterodimeric protein is essential for high level of recombination at M26 now is provided by the identification of the MTS1 and MTS2 genes and the finding that their disruption abolishes hot-spot activity *in vivo* (4). These authors also report that Mts1 and Mts2 are identical to the recently described transcription factors Atf1(Gad7) and Pcr1, respectively (17–19). These proteins are implicated in several biochemical pathways, but their mode of action is not yet elucidated. These observations, in conjunction with the previous finding that a 510-bp deletion covering the presumed ADE6 promoter also inactivates M26 activity (20), directly speak to the question of the relationship between recombination and the control of transcription.

Hot Spots in *S. cerevisiae*. In budding yeast, native hot spots at several loci and some artificial constructs have served as paradigms for understanding how meiotic recombination is initiated (8). At the *ARG4* and *HIS4* loci, several lines of evidence indicate that cis-acting sequences, located upstream of the coding regions, control hot-spot activity and are the initiation sites. First, these regions sustain the highest levels of conversion (21, 22). Second, deletions that remove part or all of these intergenic sequences reduce the frequencies of conversion of adjacent markers and abolish conversion gradients (1, 21–23). Third, meiotically induced DNA double-strand breaks (DSBs) can be detected here (24, 25). Analysis of numerous strains bearing deletions, substitutions, or insertions in these regions has demonstrated a clear correlation between hot-spot activity and the extent of formation of DSBs, providing strong evidence for their role in the initiation of recombination (23–26). The determination of the location of DSBs in several chromosomal regions showed that most DSBs are located within intergenic regions upstream of ORFs (23–30). It is then possible that one promoter-containing region controls recombination in two adjacent, but functionally unrelated, genes, regardless of their orientations (21, 22, 29).

Elements that control in cis or trans the transcription of *ARG4* and *HIS4* have been assessed for their influence on recombination (or DSB) levels. At *ARG4*, small deletions that remove the mRNA start site, the TATA element, and the Gcn4 transcription factor binding site have no effect on hot-spot activity (31). Deletion of a poly(dA·dT)$_{14}$ tract located near the TATA element confers a 3-fold reduction in recombination, although deletion of the gene coding for the DATIN protein that binds to poly(dA·dT) DNA has no effect (31). Notably, the poly(dA·dT) deletion does not decrease gene conversion to the

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*To whom reprint requests should be addressed. e-mail: anicolas@curie.fr.

basal level seen with larger deletions, suggesting that there are other cis-acting elements that are not yet identified.

Gcn4, Rap1, Bas1, and Bas2 are four proteins that bind in the *HIS4* 5′ region and activate transcription. Experiments in which their binding sites have been deleted or in which the genes encoding these proteins have been disrupted show that Bas1p, Bas2p, and Rap1p are required for hot-spot activity (32–34). Interestingly, the replacement of the normal upstream region with two Rap1 binding sites stimulates gene conversion, although no stimulation can be observed in strains with a wild-type and a mutant Rap1 binding site (34). Nicely, DSBs do not form at *HIS4*, in strains in which the Rap1 binding site is mutated or which lack the Bas1 and/or Bas2 proteins (25). These findings provide strong evidence for a relation between recombination and transcription signals. What is the nature of this link?

Toward Chromatin Accessibility. The steady-state level of *ARG4* and *HIS4* mRNA has been determined in diploids with promoter mutations or which lack transcriptional activators (31, 35). No consistent correlation with gene conversion frequencies was observed (31–34, 36). Similarly, the level of ADE6 mRNA has been measured in strains with or without the M26 site (36) or in mts1 and mts2 mutants (4). The levels were found equivalent in all cases. Therefore, in both yeasts, it appears that hot-spot activity is not correlated with transcriptional strength.

The yeast hot spots described above differ in two important ways. First, the known cis-acting elements are intergenic in *S. cerevisiae* but intragenic in *S. pombe*, at least in the case of M26. Second, the *S. cerevisiae* hot spots appear to have no sequence specificity, as determined by the mapping of DSB sites (25, 37–39), whereas M26 activity relies on a discrete nucleotide sequence. More puzzling, in *S. cerevisiae*, hot spots that are strong DSB sites can be created by introduction of foreign DNA without the apparent creation of a promoter (40–41). A way to resolve the difference between natural and artificial hot spots and between the two yeasts is to consider the influence of chromatin structure. What do we know about DNA accessibility in recombinationally active regions?

In the budding yeast, all natural and artificially created DSB sites examined so far are located in regions of chromatin that are hypersensitive to DNase I and micrococcal nuclease (MNase), suggesting that DSBs occur at sites where the DNA is particularly accessible (27, 42–44). In these studies, strains carrying alterations of cis-acting sequences or mutations in the transcriptional factors were examined. Variations of DSBs and recombination frequencies generally were found to parallel the modifications in chromatin structure. These results underscore the primacy of DNA accessibility for hot-spot activity.

Hypersensitivity to nuclease increases specifically at hot spots during early meiotic prophase, before the appearance of DSBs (42–44). Two current, but not mutually exclusive, interpretations of these changes have been proposed. One holds that chromatin structure undergoes, in early meiosis, a regulated modification that is necessary to provide a proper substrate for the DSB nuclease, recently identified as the product of the *SPO11* gene (45, 46). Alternatively, the change of accessibility can be a consequence of the assembly of a preinitiating recombination complex at the future DSB site, reminiscent of the molecular strategy used in the initiation of replication.

Studies of the chromatin structure of the *ADE6* locus of *S. pombe* confirm and extend the results from *S. cerevisiae* and reveal striking similarities (47). The MNase-digestion patterns of wild-type vegetative chromatin is consistent with the presence of two hypersensitive sites in the presumed *ADE6* promoter region (position −210 and −80) and nucleosomes in the coding region. In contrast, the chromatin of the M26 strain is different. There is a novel hypersensitive site at the position of M26 (+170), and the regular spacing of nucleosomes is destroyed. In the wild-type strain, MNase hypersensitivity at promoter sites increases moderately during meiosis, as observed in *S. cerevisiae*. Intriguingly, in M26 strains, both the M26 site itself and the distant −80 site exhibit a marked increase in hypersensitivity in meiosis. Transcription factors can alter chromatin structure by binding to their target sequences, so it is possible that the interaction of Mts1/Mts2 with the M26 site affects the binding of nucleosomes further away, and thereby alters their nucleosome positioning (47). This is consistent with the additional observation that local remodeling of chromatin in M26 strains requires the heptamer motif (47) and depends on the presence of the Mts1/Mts2 heterodimer (4).

Possible Models. Because M26 stimulates meiotic recombination above the wild-type level and the binding of the Mts1/Mts2 heterodimer strictly correlates with hot-spot activity, it seems likely that M26 affects an early, rate-limiting step in the initiation of recombination. Does this mutation create a novel initiation site or does it behave as an enhancer that stimulates an endogeneous site (9, 45, 48)? If meiotic chromatin hypersensitivity is a definitive feature of initiation sites, as in *S. cerevisiae*, a simple hypothesis is that in wild-type strains, recombination initiates in the presumed *ADE6* promoter, and in M26 strains recombination initiates both in the promoter and in the vicinity of M26. Further, the binding of transcription factors in meiosis (specifically Mts1/Mts2 at M26 but perhaps others at other promoter regions) may be sufficient to drive a change in chromatin that directly or indirectly attracts the initiating nuclease. Bearing the *S. cerevisiae* data in mind, a plausible model is that the M26 −Mts1/Mts2 DNA-protein complex mimics a promoter structure, as was postulated to explain the rare cases in *S. cerevisiae* where DSBs do not occur in intergenic promoter-containing regions (28).

Alternatively, M26 may stimulate initiation only at the promoter region, via a direct or indirect interaction between the M26-Mts1/Mts2 complex and initiator elements at the promoter. This "two cis-acting elements" hypothesis is consistent with observations that M26 is not active in strains with the presumed *ade6-delXB* promoter deletion, which covers the −80 hypersensitive site (20). It also could explain why M26 heptamers, created at several sites downstream in the *ADE6* coding region, all behave as hot spots, and in an orientation-independent manner (49). M26 has many of the features that have been attributed to enhancers. In this light, the apparent mechanistic differences between the hot-spot activity in the two yeasts may be accounted for by considering that an initiator site and its enhancer are separated in the case of M26 but are closely linked in *S. cerevisiae*.

Conclusion and Perspectives. To distinguish between the models proposed for the mode of action of M26, it seems critical to detect the lesion initiating meiotic recombination in fission yeast and to compare M26 and wild-type strains. The Rec12 protein of *S. pombe* is homologous to Spo11 in *S. cerevisiae*, and the similarity of mutant phenotypes suggests that in *S. pombe* meiotic recombination also might be initiated by DSBs (45, 46, 50). This possibility remains to be confirmed.

Another question is whether or not M26 sites are distributed throughout the genome and whether they contribute to the general enhancement of meiotic recombination in *S. pombe* (and in other organisms if evolutionarily conserved), as proposed by Wahls and Smith (16). Kon *et al.* (4) mention unpublished observations that "intergenic recombination in *mts* mutants is reduced as much as 50% in four intervals tested so far . . ." They estimate that there are 600 M26 sites in the diploid *S. pombe* genome and calculate that if each site is, on average, as recombinogenic as ade6-M26, the predicted M26 sites could account for approximately half of meiotic homologous recombination. Although, data are already available to show that chromosomal context can affect the ade6-M26 recombinogenicity (51, 52), this is an interesting path of

research, because it is relevant for understanding the general control of meiotic recombination in *S. pombe*, and it also may tell us how the recombination processes might have evolved. Proteins involved in the formation and the repair of meiotic DSBs have been evolutionarily conserved across taxa (53). Whether different organisms, although highly divergent for genome organization, have conserved a common mode of controlling the accessibility of initiation sites remains to be answered. Perhaps in certain eukaryotes, recombination enhancers have been placed in coding regions as well as in noncoding 5′, 3′, and intronic regions, as has been found for transcriptional enhancers?

In conclusion, one might appreciate that both yeasts in their own ways have contributed to progress in the field. La Fontaine in ''Le Lièvre et la Tortue'' (''The Tortoise and the Hare,'' Les Fables, 1668), told us ''It is no use to run fast, one should just start on time.'' *S. cerevisiae* and *S. pombe* left the departure gate many years ago: neither can be considered fast or slow. The payoff will be our understanding of how meiotic recombination is stimulated in the many innovative contexts that nature has devised to reassort the genetic information. Today, our current knowledge of meiotic recombination hot spots in the two yeasts strongly suggests that there is a mechanistic relationship between meiotic recombination and transcription, one that moves toward the issue of chromatin accessibility. The variety of promoters and ways to modulate DNA accessibility in chromatin may explain why meiotic DSB frequencies vary by at least two orders of magnitude within the *S. cerevisiae* genome (8, 28). The observation that meiotic DSBs along the *S. cerevisiae* chromosomes are not distributed randomly but are clustered in large chromosomal domains (28, 30) reflects another level of regulation. Is chromatin structure at the finish line once again?

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