

Hierarchical regulation of recombination

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Meiotic recombination is regulated at multiple levels such as at DNA sequence, chromatin structure, recombination proteins, and intracellular signal levels. Initial processes of meiotic recombination occur within accessible chromatin regions, which are often formed by binding of transcription factors to the target DNA sequences. Signal transduction pathways play important regulatory roles in chromatin opening at some recombination hot spots. To introduce DNA breaks, recombination initiating-proteins are then recruited to such accessible chromosomal sites. Elucidation of the hierarchy of these regulatory elements would provide important insights into the principles and constraints of the dynamic property of genomes.

Since the discovery of genetic maps, meiotic recombination has been assumed to occur randomly along the chromosome. However, recent findings have revealed that meiotic recombination is controlled by highly organized regulatory systems.

Level 1: DNA sequences and high DNA accessibility in chromatin

The basic elements of recombination regulation are DNA sequences and DNA accessibility in chromatin. Meiotic recombination is triggered by transient DNA double-strand breaks (DSBs) at defined chromosomal sites. In chromosome III of the budding yeast *Saccharomyces cerevisiae*, nearly 90% of the DSB sites are located in transcription promoter regions¹⁾ that exhibit high DNA accessibility.^{2,3)} Alteration of cis-acting sequences for transcription influences both local DNA accessibility and efficiency of DSB formation.³⁾

Collaboration between transcription and recombination is also observed in the fission yeast *Schizosaccharomyces pombe*. The *ade6-M26* is a single transversion mutation that creates a meiosis-specific recombination hot spot (Fig. 1). Hot spot activity strictly requires binding of the heterodimeric protein Atf1-Pcr1 to cAMP-responsive element (CRE)-like DNA sequences around the *M26* mutation [5'-N-T-G-A-C-G-T-(C, A)-3', *M26* mutation underlined].⁴⁾ In the wild-type *ade6* locus, nucleosomes are aligned at defined positions with regular spacing (nucleosome phasing). However, in *ade6-M26*, chromatin around the *M26* mutation meiotically remodels to form open configuration (Fig. 1).⁵⁾ Such chromatin remodeling can occur in natural CRE-like sequences (e.g., in the catalase *ctt1* promoter region). In addition, it requires the specific binding of Atf1-Pcr1 to CRE-like sequences, which shows a good correlation with the *M26* hot spot activity.

Level 2: Intracellular signals regulate chromatin opening

The second level of recombination regulation is established by intracellular signal transduction pathways. Atf1-Pcr1 is a CREB/ATF family transcription factor important for

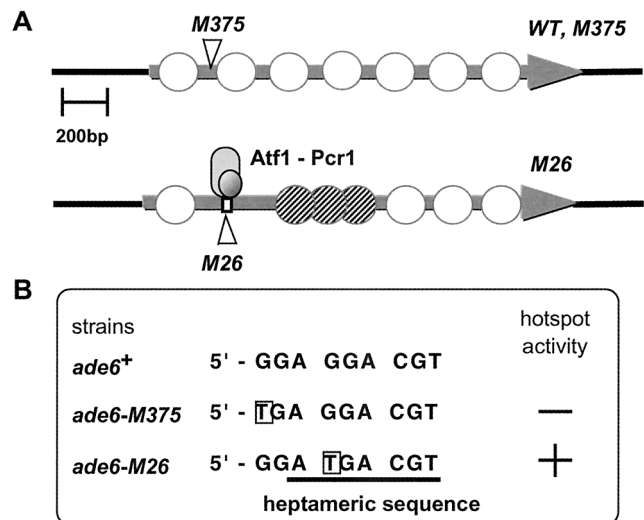


Fig. 1. Chromatin remodeling in *ade6-M26* recombination hot spot. The diagrams indicate the *ade6* open reading frame (horizontal arrow), position of CRE-like heptanucleotide sequence (open box), positions of the *M375* and *M26* mutations (triangles). Open and shaded circles represent phased and randomly positioned nucleosomes, respectively. Shaded ovals indicate Atf1-Pcr1 heterodimeric protein. B) Differences between the DNA sequences of *ade6*⁺, *ade6-M375* (negative control), and *ade6-M26* (recombination hot spot) alleles. T in open box indicates the G-to-T transversion. The underlined sequence indicates a heptameric DNA site required for hot spot activity.

S. pombe sexual differentiation and is regulated counteractively by stress-activated MAP kinase (SAPK) and cAMP-dependent kinase (PKA) cascades (Fig. 2). In the vegetative growth stage, the activated PKA pathway suppresses functions of Atf1-Pcr1. Various cellular stresses such as starvation inactivate the PKA pathway but activate the SAPK cascade that in turn phosphorylates and activates Atf1. We found that the SAPK cascade and the PKA pathway play positive and negative regulatory roles in the CRE-mediated chromatin remodeling, respectively (Fig. 2).

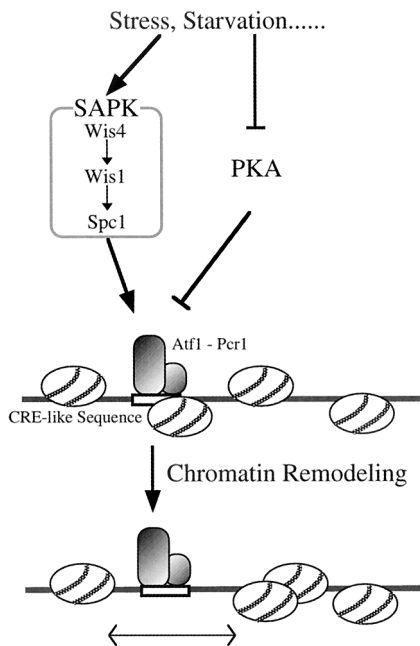


Fig. 2. Regulation of the CRE-related chromatin remodeling by SAPK cascade and PKA pathway. Under vegetative growth condition, the PKA pathway negatively regulates the function of Atf1-Pcr1 for chromatin remodeling. Nitrogen starvation results in the reduction of PKA activity, and the SAPK cascade is activated. The activated SAPK facilitates local chromatin remodeling mediated by Atf1-Pcr1. Possibly, phosphorylated Atf1-Pcr1 can bind to CRE more strongly or recruit the chromatin remodeling machinery to CRE sites more efficiently than unphosphorylated one.

Level 3: Loading of recombination proteins

The third level of recombination regulation can be observed

during loading of the recombination machinery onto the accessible chromosomal sites. Mre11 and Spo11 proteins are particularly important in this aspect. Meiotic chromatin in *S. cerevisiae* meiotic recombination hot spots exhibits higher sensitivity to micrococcal nuclease than that in mitosis (chromatin transition). The C-terminal domain of Mre11, which is essential for DSB formation, is involved in chromatin transition⁶⁾ possibly through its direct interaction with DNA. Accessible chromosomal sites with such Mre11-dependent chromatin transition are probably preferential targets of the DSB nuclease Spo11. Recent analysis revealed that these steps occur as facilitated by premeiotic DNA replication.⁷⁾

Hierarchy of recombination regulatory elements

It is very likely that recombination regulatory elements influence each other. The aim of the ongoing project is to understand the hierarchic architecture of these regulatory elements and to investigate key molecules that link these elements together. The expected data would give us important information on the principles and constraints of genome dynamics.

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