Genome **Biology**

## RESEARCH HIGHLIGHT

# Mapping meiotic breaks: Spo11 oligonucleotides precisely mark the spots

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### Abstract

Initiation sites for meiotic recombination have now been precisely mapped across the budding yeast genome using a widely applicable deep-sequencing approach.

## Meiosis and the roles of recombination

The consecutive cell divisions of meiosis precisely halve the cellular chromosome complement to produce haploid gamete cells. Prior to first division, replicated paternal and maternal chromosomes (homologs) become intimately associated before segregating to opposite poles of the cell. During the second division, sister-chromatids separate much as they do during mitosis.

The template-directed DNA repair process called homologous recombination has fundamental roles in the pairing and accurate segregation of homologs during the first meiotic division. Multiple recombination events are initiated along each chromosome and the ensuing homologous pairing and strand-exchange reactions align the homolog pairs and facilitate their intimate synapsis. Following synapsis, a fraction of the recombination events are completed with an associated exchange of chromosome arms. These crossovers physically tether the homologs and allow them to attain a stable bipolar connection to the spindle apparatus, thereby facilitating accurate homolog segregation. Without a crossover, paternal and maternal homologs are at risk of missegregation. The aneuploid gametes that result from homolog missegregation lead to gene dosage imbalances in the zygote that cause pregnancy miscarriage and chromosomal diseases such as Down syndrome. Meiotic recombination also has a less immediate, although no

less critical, role in determining patterns of inheritance and shaping the genome over evolutionary time.
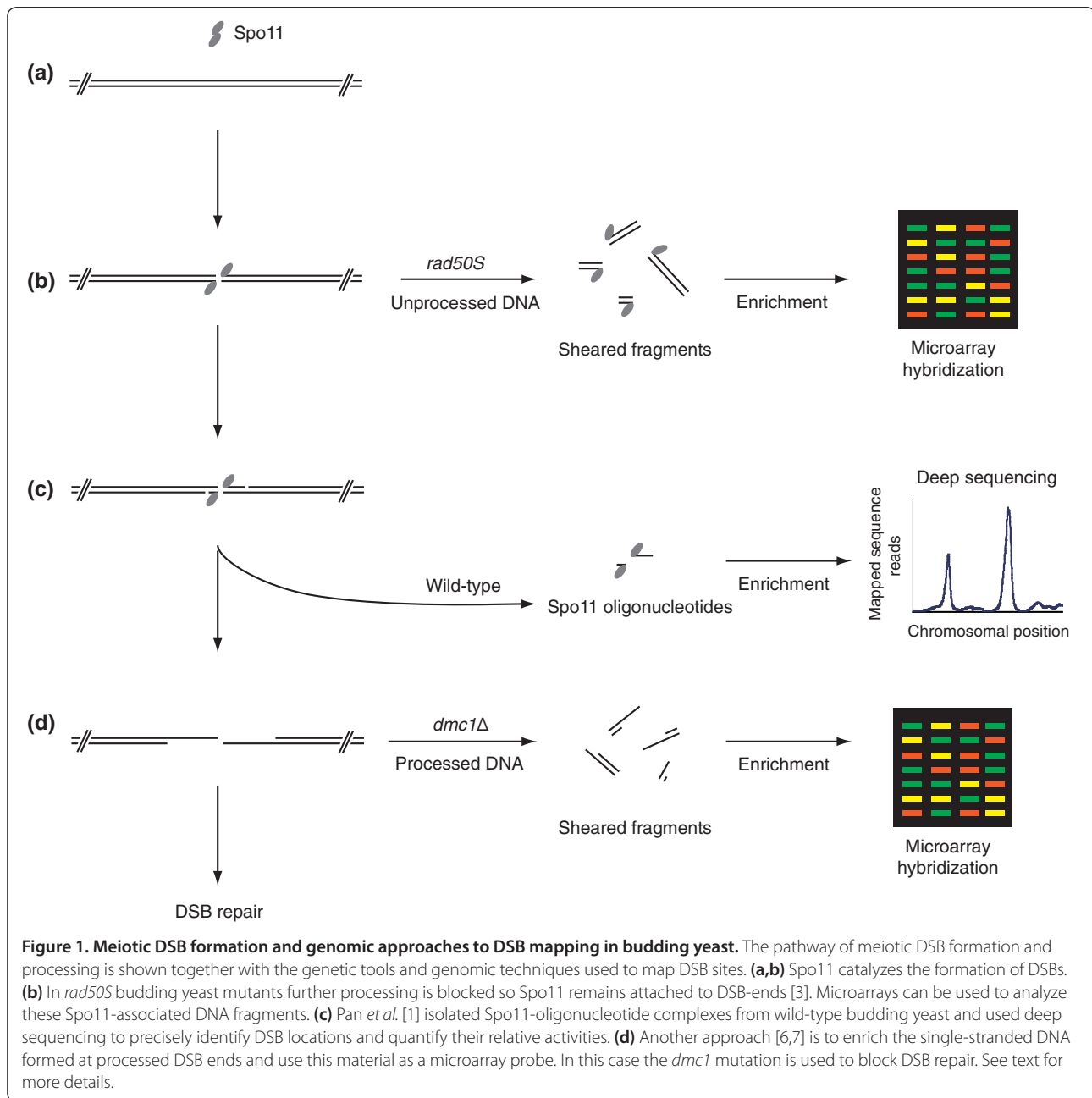
## Hotspots of meiotic recombination

It has long been known that rates of meiotic recombination vary widely across genomes. Most notably, at a fine scale, genetic maps are punctuated by discrete 'hotspots' with extended intervening regions of greatly suppressed recombination. Recombination hotspots have fundamental consequences for genome evolution, patterns of inheritance and genetic studies: hotspots create intervals of high linkage disequilibrium (LD) flanked by sharp transitions where LD breaks down; this limits population diversity and drives the archetypal block structure of haplotypes. Thus, the precise identification of recombination hotspots is key for describing their impact on LD and for understanding the molecular mechanisms that underlie their distribution and activity. A new genomic approach to precisely map sites where recombination is initiated moves us towards these goals [1].

## Programmed DNA double-strand breaks initiate meiotic recombination

Recombination hotspots define hotspots for the initiation of recombination. Meiotic recombination is initiated by DNA double-strand breaks (DSBs), which are catalyzed by a highly conserved enzyme called Spo11. To break DNA, the active-site tyrosines of a dimer of Spo11 catalyze dual nucleophilic attacks on the backbones of the complementary DNA strands (Figure 1a). These transesterification reactions result in covalent Spo11-DNA complexes, with Spo11 being attached to the 5' termini of both broken ends (Figure 1b). Subsequently, DSB ends undergo extensive nucleolytic resection of the 5'-strands during which Spo11 is liberated to yield complexes of Spo11 attached to short oligonucleotides (under 40 nucleotides) [2]. The long 3' single-stranded tails then assemble into nucleoprotein filaments comprising RecA-like proteins, Dmc1 and Rad51, which catalyze homologous pairing and DNA strand-exchange reactions.

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**Figure 1. Meiotic DSB formation and genomic approaches to DSB mapping in budding yeast.** The pathway of meiotic DSB formation and processing is shown together with the genetic tools and genomic techniques used to map DSB sites. **(a,b)** Spo11 catalyzes the formation of DSBs. **(b)** In *rad50S* budding yeast mutants further processing is blocked so Spo11 remains attached to DSB-ends [3]. Microarrays can be used to analyze these Spo11-associated DNA fragments. **(c)** Pan *et al.* [1] isolated Spo11-oligonucleotide complexes from wild-type budding yeast and used deep sequencing to precisely identify DSB locations and quantify their relative activities. **(d)** Another approach [6,7] is to enrich the single-stranded DNA formed at processed DSB ends and use this material as a microarray probe. In this case the *dmc1* mutation is used to block DSB repair. See text for more details.

## Genome-wide mapping of DSB hotspots in budding yeast

The first studies to map DSB sites on a large scale exploited the *rad50S* mutation, which blocks processing, leaving Spo11 attached to DSB ends (Figure 1b). This reagent allowed detection of accumulated DSB fragments by gel electrophoresis and Southern analysis, leading to a map of DSB hotspots along chromosome 3 of budding yeast [3]. With the advent of microarray technology, DSBs accumulated in *rad50S*-like mutants could be mapped genome-wide [4,5]. For these experiments, DNA probes were prepared from Spo11-DNA complexes that

had been enriched by binding to glass fiber filters or by immunoprecipitating Spo11.

It was subsequently discovered that a subset of meiotic DSBs detected in wild-type cells fails to form in *rad50S* and related mutants. Although intriguing, this fact meant that DSB maps from *rad50S*-like mutants were inaccurate. To solve this problem, two groups simultaneously developed a strategy to enrich the single-stranded DNA formed at processed DNA ends, which was then used to prepare microarray probes [6,7]. This approach used the *dmc1* mutation, which blocks DSB repair after processing to produce single-stranded tails (Figure 1d). The resulting

DSB maps were more biologically meaningful and provided considerable insight (discussed below), but have limited spatial and quantitative resolution because of microarray design, the dynamic range of hybridization signals, and the relatively large sizes of DSB-associated DNAs used as probes.

In 2005, the group of Scott Keeney confirmed the existence of Spo11-oligonucleotide complexes and showed that they are relatively stable and readily isolated from wild-type cells by immunoprecipitation of Spo11 [2]. It was immediately obvious that the sequences of Spo11-oligonucleotides can precisely identify the sites of meiotic DSBs. These handy 'address tags' have now been used to produce perhaps the ultimate map of DSB sites in the budding yeast genome [1] (Figure 1c). By deep sequencing Spo11-oligonucleotides, Pan *et al.* [1] were able to quantitatively map DSBs across the genome at nucleotide resolution and with exquisite sensitivity.

## The budding yeast DSB map

In many ways, the Spo11-oligonucleotide DSB map [1] corroborates features inferred from other genomic and non-genomic analyses of DSB locations [3,4,6,7]. However, the precise spatial and quantitative resolution of this map greatly extends our understanding of the features that underlie hotspot location and activity. For budding yeast, the meiotic DSB map is dictated by the fundamental organization of the genome, because the vast majority (98.2%) of Spo11-oligonucleotide sequences map to non-repetitive elements, primarily gene promoters where nucleosomes are relatively depleted and DNA is accessible to Spo11.

With 3,604 hotspots identified as clusters of Spo11-oligonucleotides, it seems that a majority of gene promoters have the potential to function as DSB hotspots. In addition, when analyzed over windows of several kilobases, DSB density positively correlates with GC content [1]. This probably reflects the location of DSBs in chromatin loops; loops are relatively GC-rich whereas the loop bases associated with the homolog axes are relatively AT-rich [8].

## Treasure your exceptions

Exceptions to the general patterns described above provide a more detailed picture of the meiotic DSB landscape, the factors that shape it and potential biological implications [1]. First, 1.8% of DSBs occur in repetitive elements, including telomeres, rDNA, tRNAs and retrotransposons. Although DSBs are relatively suppressed in these elements, this class of breaks creates a significant risk of non-allelic recombination, which may cause chromosomal rearrangements, defective homolog pairing and chromosome missegregation.

Second, the sensitivity of deep sequencing reveals that the designation of any given site as a DSB hotspot is rather arbitrary. The ranked activities of DSB hotspots follow a striking continuum over a 410-fold range with no clear cut-off [1]. Moreover, more than 10% of DSBs are not located in clear hotspots.

Third, although DSBs are generally suppressed around all centromeres, this effect varies for different chromosomes [1]. Given that centromere-proximal crossovers are associated with chromosome missegregation [9], the authors [1] suggest that the distinct centromere-proximal DSB profiles of different chromosomes may differentially predispose them to segregation errors.

Fourth, location of DSB hotspots to gene promoters is not the rule because 4.8% of Spo11-oligo sequences map to non-promoter locations [1]. Also, the majority of hotspots show clustering of Spo11 oligonucleotides (indicating cleavage sites) within a region of 189 bp on average. However, around 10% of hotspots are at least 500 bp wide and some show overlap with open reading frame sequences.

## A hierarchy of factors governing DSB sites

Pan *et al.* [1] also used deep sequencing to generate high-resolution maps of meiotic nucleosome positions. Comparison with the Spo11-oligonucleotide map showed that the widths of nucleosome-depleted regions positively correlate with the widths, and to some extent the activities, of DSB hotspots. Strikingly, DSBs form almost exclusively in non-nucleosomal DNA. However, a simple relationship between nucleosome occupancy and DSB activity is not observed: low nucleosome occupancy *per se* does not predict the locations or intensities of DSB hotspots. Thus, although absence of stable nucleosomes seems to be a prerequisite for Spo11 cleavage, it is not sufficient. Other factors, such as sub-chromosomal chromatin structure and location within a chromatin loop, seem to be dominant factors in hotspot activity. This inference is consistent with the studies of Lichten and colleagues [10], who showed that the activity of a defined DSB hotspot varied depending on its chromosomal location.

## Mechanistic insights

From the precise locations of the 2.2 million Spo11-oligonucleotide sequences, Pan *et al.* [1] showed that local DNA composition also influences Spo11 cleavage sites. As expected from previous studies, Spo11 does not have a specific recognition or cleavage site. However, sequence biases were detected: the 10 to 12 bp surrounding the cleavage site and predicted to be bound directly by Spo11 is relatively AT rich, predicting relatively narrow and deep helix grooves facing the bound Spo11 dimer. Cleavage favors sites immediately 3'

of a C and disfavors G in the same position. Also, within a 32-bp core surrounding the Spo11 cleavage sites, a twofold rotational symmetry for complementary dinucleotide composition can be discerned, suggesting separate contributions of the flanking 'half sites' to Spo11 binding and/or cleavage. In addition, cleavage sites are negatively correlated with positioned nucleosomes. Similarly, Spo11 is generally occluded from cleaving sites where transcription factors are bound, even though the binding sites of several different transcription factors positively correlate with hotspot sites.

## Limitations and prospects

Although Spo11-oligonucleotide DSB maps [1] have much greater accuracy than those produced by other genomic DSB mapping approaches, there may still be some caveats to their interpretation. For example, Spo11-oligonucleotides appear to turn over as DSBs are repaired [1]. Thus, the relative activities of DSB hotspots may be exaggerated depending on how rapidly DSBs at a given site are repaired. This artifact may be compounded by the fact that DSBs form at different times throughout the genome.

In addition, the probabilistic nature of DSB formation means that hotspot maps can tell us little about DSB regulation at the level of individual cells. In particular, understanding critical processes that control the number, distribution and timing of DSBs will require further development of techniques to detect DSBs in single cells.

The Spo11-oligonucleotide DSB mapping technique [1] should be broadly applicable to any species from which sufficient amounts of meiotic material can be obtained. This raises the exciting prospect of being able to contrast DSB maps across phyla and understand the impact of DSB hotspots on patterns of inheritance and genome evolution in individual species.

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