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SI CHROMATIN AND DEVELOPMENT

Meiotic recombination hotspots – a comparative view

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SUMMARY

During meiosis homologous chromosomes pair and undergo reciprocal genetic exchange, termed crossover. Meiotic recombination has a profound effect on patterns of genetic variation and is an important tool during crop breeding. Crossovers initiate from programmed DNA double-stranded breaks that are processed to form single-stranded DNA, which can invade a homologous chromosome. Strand invasion events mature into double Holliday junctions that can be resolved as crossovers. Extensive variation in the frequency of meiotic recombination occurs along chromosomes and is typically focused in narrow hotspots, observed both at the level of DNA breaks and final crossovers. We review methodologies to profile hotspots at different steps of the meiotic recombination pathway that have been used in different eukaryote species. We then discuss what these studies have revealed concerning specification of hotspot locations and activity and the contributions of both genetic and epigenetic factors. Understanding hotspots is important for interpreting patterns of genetic variation in populations and how eukaryotic genomes evolve. In addition, manipulation of hotspots will allow us to accelerate crop breeding, where meiotic recombination distributions can be limiting.

Keywords: meiosis, recombination, hotspots, chromatin, DNA motifs.

INTRODUCTION

Meiosis is widely conserved in sexually reproducing eukaryotes, during which a single round of DNA replication is coupled with two rounds of chromosome segregation (Villeneuve and Hillers, 2001). Prior to the first meiotic division homologous chromosomes pair and undergo programmed recombination, which can result in reciprocal genetic exchange, termed crossover (Hunt Morgan, 1916). In addition to generating new combinations of sequence diversity, an obligate crossover is required to physically connect homologs and ensure balanced chromosome segregation at meiosis I (Page and Hawley, 2003). The frequency of meiotic recombination is highly non-random along chromosomes, and typically occurs within narrow regions termed hotspots (Lichten and Goldman, 1995; Kauppi *et al.*, 2004; de Massy, 2013; Lam and Keeney, 2014; Mercier *et al.*, 2014). In addition, large (megabase) chromosomal regions can be suppressed for recombination, for example the heterochromatic regions around centromeres (Copenhaver *et al.*, 1999; The Tomato Genome Consortium, 2012; Mayer *et al.*, 2012; Choulet *et al.*, 2014). Despite such regions showing low recombination rates in crop genomes, they frequently contain variation in agriculturally important genes, for example those controlling disease resistance or stress tolerance. Therefore, greater understanding of the control of recombination distributions has the potential to accelerate crop breeding and allow use of the available variation more effectively.

Meiotic recombination is initiated by DNA double-stranded breaks (DSBs) generated by the SPO11 endonuclease (Bergerat *et al.*, 1997; Keeney *et al.*, 1997) (Figure 1a–c). DSB ends then undergo 5′ to 3′ resection to generate 3′-single-stranded DNA (ssDNA) that is bound by the DMC1 and RAD51 recombinases (Figure 1d,e) (Sun *et al.*, 1991; Bishop *et al.*, 1992; Shinohara *et al.*, 1992). The resulting nucleoprotein filament then performs strand invasion of a homologous chromosome (Kurzbauer *et al.*, 2012; Da Ines *et al.*, 2013). These inter-homolog strand invasion events are further processed to form a double Holliday junction (dHJ) that can be resolved as a crossover (Figure 1f–h) (Szostak *et al.*, 1983; Schwacha and Kleckner, 1995). A larger number of DSBs initially form then mature into final crossover molecules. For example in Arabidopsis approximately 200 DSB foci are observed, based on RAD51 and DMC1 foci, whereas only approximately 10 crossovers

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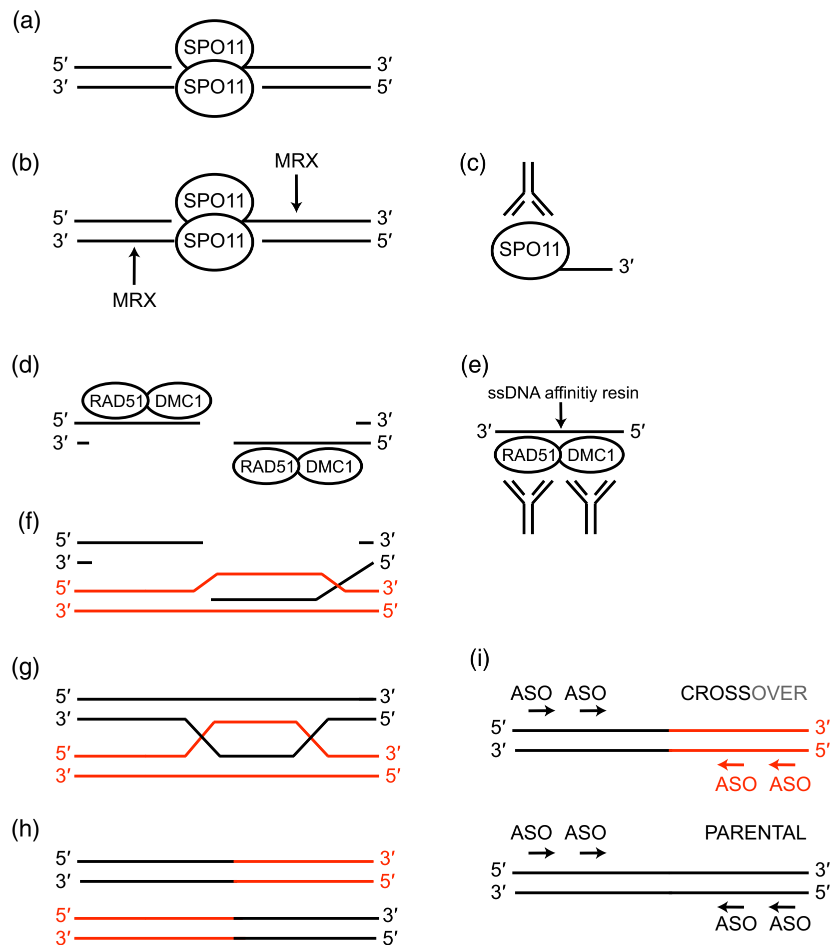

Figure 1. Methodologies to profile meiotic recombination hotspots.
(a) Meiotic recombination is initiated by DNA double-stranded breaks (DSBs) generated by SPO11 endonuclease dimers.
(b) SPO11 molecules become covalently bound to DSB target site 5′ ends. Upstream and downstream cleavage by the MRX complex releases SPO11-oligonucleotide complexes.
(c) SPO11 can be immunopurified and the bound oligonucleotides analysed to generate maps of meiotic DSBs.
(d) Following DSB formation resection occurs to generate single-stranded DNA (ssDNA) that is bound by the RAD51 and DMC1 recombinases.
(e) RAD51 or DMC1 can be immunopurified and bound ssDNA used to analyse DSB sites. Alternatively, the ssDNA can be directly purified using an affinity resin.
(f) Resected ssDNA invades a homologous chromosome (red) to form a displacement loop.
(g) DNA synthesis and ligation generate a double Holliday junction.
(h) Double Holliday junctions can be resolved as crossover molecules.
(i) Crossover hotspots can be analysed using pollen-typing with allele-specific oligonucleotides (ASOs) that anneal to polymorphic sites between the homologs. This situation allows recombinant (crossover) and non-recombinant (parental) molecules to be specifically amplified.

are detected per meiosis (Copenhaver *et al.*, 1998; Chelysheva *et al.*, 2010; Giraut *et al.*, 2011; Ferdous *et al.*, 2012; Salomé *et al.*, 2012). Most DSBs are thought to enter the strand invasion pathway, but are then resolved as non-crossovers via synthesis-dependent strand annealing (SDSA), without exchange of flanking genetic markers (Allers and Lichten, 2001; McMahill *et al.*, 2007). As this mode of repair involves template-driven DNA synthesis post-invasion, it can result in gene conversion over heterozygous sites, which can be detected as 3:1 inheritance between the meiotic products (Sun *et al.*, 2012; Wijnker *et al.*, 2013).

The majority (approximately 85%) of Arabidopsis crossovers are interfering, meaning they are distributed further apart than expected if they were independent, and are generated by the ZMM pathway (*MSH4*, *MSH5*, *MER3*, *HEI10*, *ZIP4*, *SHOC1*, *PTD*) (Higgins *et al.*, 2004, 2008; Chen *et al.*, 2005; Mercier *et al.*, 2005 Wijeratne *et al.*, 2006; Macaisne *et al.*, 2008; Chelysheva *et al.*, 2012). The ZMM pathway is best characterized in budding yeast in which it acts to stabilize inter-homolog strand invasion intermediates and promotes formation of dHJs and their resolution as crossovers (Lynn *et al.*, 2007). The remaining non-ZMM crossovers in Arabidopsis are generated by a non-interfering pathway, that includes *MUS81* (Berchowitz *et al.*, 2007; Higgins, Buckling, *et al.*, 2008). FANCM, MHF1 and MHF2 are required for non-crossover formation in Arabidopsis, and in their absence DSBs that would normally become non-crossovers are repaired as non-interfering crossovers, leading to an approximately 3-fold increase in total crossover frequency (Crismani *et al.*, 2012; Knoll *et al.*, 2012; Girard *et al.*, 2014). These non-interfering crossovers are assumed to be generated via the *MUS81* pathway, though a formal test of this model is precluded due to *fancm mus81* lethality (Crismani *et al.*, 2012). More recently TOPOISOMERASE3α and the RECQ4A and RECQ4B helicases have been identified as additional anti-crossover factors in Arabidopsis (Séguéla-Arnaud *et al.*, 2015). As a consequence of these recombination pathways one or a small number of crossovers form per chromosome, although the positions of crossovers observed between independent meiosis are highly variable.

Measurement of DSBs and crossovers in multiple species has demonstrated concentration in hotspots, which

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are typically 1–10 kb in width and show significantly higher recombination activity than surrounding regions (Lichten and Goldman, 1995; Kauppi *et al*., 2004; de Massy, 2013; Lam and Keeney, 2014; Mercier *et al*., 2014). In this review we examine the methodologies used in different eukaryotic species to profile meiotic break and crossover hotspots (Table 1). We then discuss what these studies have revealed concerning the genetic and epigenetic factors that contribute to variation in recombination frequency along eukaryotic chromosomes and the formation of hotspots.

DETECTING AND MEASURING HOTSPOTS

DSB hotspots – mapping breaks and ssDNA

Meiotic recombination initiates from DNA DSBS, which can be directly measured to reveal hotspots. A major advantage to the study of meiosis in budding yeast *Saccharomyces cerevisiae* is the ability to synchronize large numbers of cells to enter meiosis simultaneously, which facilitates biochemical analysis of meiotic recombination. Due to the effect of DSBs on DNA molecule size, gel electrophoresis of staged meiotic DNA can be used to analyse DSBs in yeast (Nicolas *et al*., 1989; Baudat and Nicolas, 1997; Garcia *et al*., 2015). This is a versatile method that allows DSB variation to be measured on scales from whole chromosomes to single nucleotides, according to electrophoresis conditions and probe choice (Nicolas *et al*., 1989; Baudat and Nicolas, 1997; Garcia *et al*., 2015). The detection of DSBs can be increased by using DNA processing mutants such as *rad50S* and *sae2D*, which are deficient in endonucleolytic release of SPO11 from DSBs and accumulate unrepaired breaks (Buhler *et al*., 2007). However, it is important to note that mapping DSBs in *rad50s* and *sae2D* mutants may lead to biased detection of early forming DSBs over late DSBs, which account for a substantial fraction of breaks in *S. cerevisiae* (Buhler *et al*., 2007; Joshi *et al*., 2015). Although the application of gel electrophoresis assays to larger genomes is challenging, a modified Southern blotting method using terminal transferase and nested PCR was able to detect DSBs at the *H2-Ea* hotspot in mouse testicular germ cells (Qin *et al*., 2004).

A sensitive method to detect meiotic DSBs relies on the catalytic mechanism of the SPO11 endonuclease (Neale *et al*., 2005). SPO11 is related to topoisomerases and becomes covalently bound to 5′-target sites at a catalytic tyrosine residue (Bergerat *et al*., 1997; Keeney *et al*., 1997; Neale *et al*., 2005). Subsequent upstream or downstream cleavage by the MRX nuclease complex liberates SPO11 bound to oligonucleotides approximately 20–100 nt in length (Figure 1b) (Neale *et al*., 2005; Garcia *et al*., 2011; Lange *et al*., 2011; Pan *et al*., 2011). Immunoprecipitation of SPO11 and purification of the bound oligonucleotides can be used to analyse DSB target sites, using end-labelling and gel electrophoresis, or generation of sequencing libraries (Figure 1c) (Neale *et al*., 2005; Lange *et al*., 2011; Pan *et al*., 2011). These approaches have been performed successfully in budding yeast, fission yeast and mice (Lange *et al*., 2011; Pan *et al*., 2011; Fowler *et al*., 2014). This technique has also been repeated in mouse *atm* kinase signaling mutants, which accumulate higher DSB levels (Lange *et al*., 2011). In budding yeast Spo11 functions with a group of accessory proteins, including Rec114, Mer2 and Mei4 (Panizza *et al*., 2011). These proteins have been analysed by chromatin immunoprecipitation (ChIP) and shown to associate with cohesin-rich regions (Panizza *et al*., 2011), which may reflect a mechanism to tether DSBs formed on chromatin loops to repair sites at the meiotic chromosome axis (Kleckner *et al*., 2004). Alternatively, the locus where breaks will form is first tethered to the axis, where SPO11 is present, and then a DSB forms (Kleckner *et al*., 2004; Sommermeyer *et al*., 2013).

Following DSB resection, meiotic ssDNA can be directly purified and analysed using an affinity resin, which can also be combined with recombination/processing mutants that accumulate unrepaired breaks (Figure 1e) (Buhler *et al*., 2007, 2009). As ssDNA is bound by the DMC1 and RAD51 recombinases, ChIP of these factors has been used to generate high-resolution recombination maps in yeast, mouse and humans, using an approach termed ssDNA-sequencing (SSDS) (Figure 1e) (Smagulova *et al*., 2011; Brick *et al*., 2012; Khil *et al*., 2012; He *et al*., 2013; Pratto *et al*., 2014). Similar methods have also been developed in

Table 1 Methodologies used to detect meiotic recombination hotspots throughout eukaryotes

Recombination step	Technique	Fungi	Plants	Animals
DNA double strand breaks	Gel electrophoresis & Southern blotting	o		o
DNA double strand breaks	RPA & DMC1 ChIP-seq (SSDS)	o	o	o
DNA double strand breaks	ssDNA purification	o		
DNA double strand breaks	SPO11 oligonucleotide sequencing	o		o
ZMM pathway	Zip3 chromatin immunoprecipitation	o		
Crossovers/non-crossovers	Tetrad analysis	o	o	o
Crossovers/non-crossovers	Classical genetic mapping	o	o	o
Crossovers/non-crossovers	Gamete/spore genome sequencing	o	o	o
Crossovers/non-crossovers	Sperm-typing/pollen-typing		o	o
Crossovers/non-crossovers	LD-based analyses	o	o	o

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maize (He *et al.*, 2013). It will be important to apply these or related methods in plants to fully understand genomic distributions of meiotic DSBs. A variety of Arabidopsis meiotic mutants are available that may facilitate the study of DSBs, including those altering break processing (*sae2/com1*) (Uanschou *et al.*, 2007), DNA damage kinase signaling (*atm*, *atr*) (Garcia *et al.*, 2003; Roitinger *et al.*, 2015) and chromatin modification (*arp6*, *met1*, *ddm1*) (Colomé-Tatché *et al.*, 2012; Melamed-Bessudo and Levy, 2012; Mirouze *et al.*, 2012; Yelina *et al.*, 2012; Choi *et al.*, 2013).

Crossover hotspots – direct mapping

A classical method to detect crossovers is via analysis of co-inheritance of linked heterozygous markers through meiosis (Hunt Morgan, 1916), in which change of linkage phase between markers indicates the occurrence of a crossover. Meiotic tetrad analysis is a powerful genetic technique, where all four daughter cells from a single meiosis are analysed (Lichten, 2014). In addition to distinguishing between two, three and four strand crossover events, tetrad analysis also allows measurement of gene conversion events via detection of 3:1 inheritance between sister gametes (Nicolas *et al.*, 1989; Berchowitz and Copenhaver, 2008; Martini *et al.*, 2011; Sun *et al.*, 2012). Recently tetrad analysis has been extended to both mice and plants (Berchowitz and Copenhaver, 2008; Cole *et al.*, 2014). In Arabidopsis the *quartet1* (*qrt1*) mutant is altered in pollen wall biogenesis, such that the four products of male meiosis remain physically attached (Francis *et al.*, 2006). Combination of *qrt1* with linked heterozygous transgenes expressing different colours of fluorescent protein provides an elegant visual method to score crossovers (Francis *et al.*, 2007; Berchowitz and Copenhaver, 2008), and an equivalent approach has been developed in budding yeast (Thacker *et al.*, 2011). Alternatively, *qrt1* can be complemented and single pollen grains analysed using flow cytometry to increase measurement throughput (Yelina *et al.*, 2012, 2013). Single *qrt1* tetrad pollinations can also be used to isolate siblings related through a common meiosis, which are then sequenced to provide insight into genome-wide patterns of crossover and gene conversion (Wijnker *et al.*, 2013; Qi *et al.*, 2014). Equally, large numbers of crossover events can be readily mapped using genotyping or sequencing of F_2 or backcross populations (Salomé *et al.*, 2012; Rowan *et al.*, 2015). New methods that use multiple annealing- and looping-based amplification cycles (MALBEC) have allowed sequencing and identification of crossovers in a single human sperm or oocyte (Lu *et al.*, 2012; Hou *et al.*, 2013). This outcome has also been achieved in maize via sequencing of DNA from isolated single microspores (the male meiotic products) (Li *et al.*, 2015). However, a major limitation to all of these approaches for the study of hotspots is the paucity of crossovers per meiosis. For example, known plant hotspots have genetic distances between approximately 0.1–0.5 cM (Table 2) (Yelina *et al.*, 2012; Choi *et al.*, 2013; Drouaud *et al.*, 2013). Therefore, to analyse 100s of crossovers at a given hotspot it is necessary to screen 100 000s of meioses, which is generally not possible when generating populations of plants.

To isolate large numbers of crossovers at individual hotspots a general approach is to use allele-specific amplification from post-meiotic gamete DNA, termed sperm typing or pollen typing (Tiemann-Boege *et al.*, 2006; Baudat and de Massy, 2007; Cole *et al.*, 2010; Berg *et al.*, 2011; Drouaud and Mézard, 2011; Yelina *et al.*, 2012; Choi *et al.*, 2013; Drouaud *et al.*, 2013). These approaches rely on the generation of individuals that are heterozygous over a hotspot region of interest. Collection of gametes and isolation of DNA means that samples consist of a mixture of non-recombinant (parental) and crossover molecules that are distinguishable by patterns of polymorphisms. Allele-spe-

Table 2 Plant meiotic recombination hotspots

Species	Hotspot	Interval (base-pairs)	cM	cM/Mb	Location	Chromosome cM/Mb	Reference
Arabidopsis thaliana	*3b*	5746	0.11	20.01	Intergenic	4.77	Choi *et al.* (2013)
Arabidopsis thaliana	*3a*	5825	0.21	36.22	Gene 5′ and 3′ end	4.77	Choi *et al.* (2013); Yelina *et al.* (2012)
Arabidopsis thaliana	*14a*	7283	0.55	75.52	Gene 5′ end & intergenic	4.8	Drouaud *et al.* (2013)
Arabidopsis thaliana	*130x*	12 488	0.53	42.44	Intergenic	4.8	Drouaud *et al.* (2013)
Zea mays	*a1*	1900	0.08	40.00	*a1*	2.1	Brown and Sundaresan (1991)
Zea mays	*a1*	710	0.04	59.00	Gene promoter	0.5–1.5	Yao and Schnable (2005)
Zea mays	*a1*	500	0.005	9.10	Gene 5′ end	0.5–1.5	Yao and Schnable (2005)
Zea mays	*Yz1*	1900	0.06	32.00	Gene 5′ end	0.5–1.5	Yao and Schnable (2005)
Zea mays	*Yz1*	540	0.03	48.00	Gene 3′ end	0.5–1.5	Yao and Schnable (2005)
Zea mays	*B*	620	0.03	52.00	Gene 5′ end	nd	Patterson *et al.* (1995)
Zea mays	*bronze*	1056	0.051	48.30	Gene 5′ end	nd	Fu *et al.* (2002)
Zea mays	*bronze*	793	0.033	41.61	Gene 5′ end	nd	Fu *et al.* (2002)
Triticum aestivum	*HGA3*	23 000	0.20	8.82	Gene 5′ end	0.85	Saintenac *et al.* (2009)

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cific primers are designed to anneal to polymorphisms flanking a hotspot and used in different configurations to amplify either parental or crossover molecules (Figure 1i) (Baudat and de Massy, 2009; Kauppi *et al.*, 2009; Cole and Jasin, 2011; Drouaud and Mézard, 2011). Typically, the products of male meiosis (pollen or sperm) are collected due to the ease of isolating the required large numbers of cells (Kauppi *et al.*, 2009; Drouaud and Mézard, 2011), although analysis of female gametes is also possible (de Boer *et al.*, 2013). This analysis is important as sex-specific differences in meiotic recombination are widespread, yet poorly understood (Lenormand and Dutheil, 2005; Giraut *et al.*, 2011; Campbell *et al.*, 2015). Estimating recombination rate within the amplified region is possible by diluting the gamete template DNA in order to titrate parental and crossover molecules. It is possible to then sequence or genotype amplification products from single crossover molecules to identify internal crossover positions to the resolution of individual polymorphisms (Tiemann-Boege *et al.*, 2006; Baudat and de Massy, 2007; Cole *et al.*, 2010; Berg *et al.*, 2011; Drouaud and Mézard, 2011; Yelina *et al.*, 2012; Choi *et al.*, 2013; Drouaud *et al.*, 2013). These methods allow the fine-scale characterization of crossover patterns within single hotspots. A further method to map crossover recombination is via ChIP of associated factors, for example Zip3 within the ZMM pathway has been analysed in budding yeast (Serrentino *et al.*, 2013). In some cases early and late cytogenetic foci are observed with distinct properties (e.g. RNF212), and so it is important to consider whether ChIP approaches will profile different foci classes equally (Reynolds *et al.*, 2013; Qiao *et al.*, 2014).

Crossover hotspots – historical mapping

The signature of crossover can also be detected via analysis of natural genetic polymorphisms, due to the effect recombination has on non-random associations between mutations (Auton and McVean, 2012). Specifically, crossovers cause decay of linkage disequilibrium (LD) between linked polymorphisms, which can be analysed using coalescent theory (Charlesworth and Charlesworth, 2010; Auton and McVean, 2012). For example, consider two linked, independently arising mutations *a* and *b.* The only way an *a*–*b* haplotype can occur is through recurrent mutation, or via recombination joining *a* and *b* onto the same chromosome (Hudson and Kaplan, 1985; Charlesworth and Charlesworth, 2010; Auton and McVean, 2012). Packages such as LDhat and SequenceLDhot use this principle to analyse SNP patterns and estimate the population-scaled recombination rate $4N_er$ (where r is the per generation recombination rate and N_e is the effective population size) (Fearnhead, 2006; Auton and McVean, 2007). These approaches are powerful as they sample the very large numbers of meioses occurring in the history of the individuals compared. However, there are a number of limitations to these methods, including that SNPs are influenced by population genetic forces in addition to recombination, including selection, drift and migration (Charlesworth and Charlesworth, 2010; Auton and McVean, 2012). Further caveats associated with these approaches include the potential error introduced by structural variation between individuals, for example insertions, deletions, inversions and translocations. Mis-calling of SNP positions relative to a given reference sequence may under- or over-estimate actual physical distances between variants and thus cause erroneous recombination rate estimates. This may be a particular problem in repetitive regions, where accurately identifying SNPs from short read sequencing data can be problematic. Therefore, it is important to combine historical and experimental mapping of crossover recombination.

GENETIC AND EPIGENETIC CONTROL OF HOTSPOTS

Gene-associated recombination hotspots in plants and fungi

Recombination mapping techniques have revealed much concerning hotspot locations and their control in plant, animal and fungal species (Lichten and Goldman, 1995; Kauppi *et al.*, 2004; de Massy, 2013; Lam and Keeney, 2014; Mercier *et al.*, 2014). Despite the widespread occurrence of hotspots throughout eukaryotes major differences in the relative importance of genetic and epigenetic control exist. In Arabidopsis a combination of historical and experimental mapping of crossovers has revealed concentration of recombination at gene promoters and terminators (Figure 2a) (Yelina *et al.*, 2012; Choi *et al.*, 2013; Drouaud *et al.*, 2013). The historical crossover signal in this species is highest immediately downstream of gene transcriptional start sites (TSSs), overlapping the highly positioned +1 nucleosome (Figure 2a) (Choi *et al.*, 2013). Significant but lower peaks are also observed in proximity to gene transcriptional termination sites (TTSs), also within gene open reading frames (Figure 2a) (Choi *et al.*, 2013). Historical crossover hotspots at the start and end of genes were also observed in *Mimulus guttatus* (Hellsten *et al.*, 2013). Direct mapping of crossovers has shown gene-associated hotspots in maize (*Bronze*, *a1*) and wheat (*HGA1*, *HGA3*, *SOS1*) (Table 2) (Dooner, 1986; Xu *et al.*, 1995; Fu *et al.*, 2001; Saintenac *et al.*, 2009; Li *et al.*, 2015), and is concordant with the high recombination rates in gene-rich euchromatin and low rates in repeat-rich heterochromatin observed in multiple plant genomes (Gore *et al.*, 2009; Liu *et al.*, 2009; Wei *et al.*, 2009; The Tomato Genome Consortium, 2012; Mayer *et al.*, 2012; Choulet *et al.*, 2014; Li *et al.*, 2015; Rodgers-Melnick *et al.*, 2015).

Productive and accurate gene transcription by RNA polymerase II is known to rely on both specific DNA sequences and chromatin states at gene promoters (Venters and Pugh, 2009; Deal and Henikoff, 2011). For example, the +1

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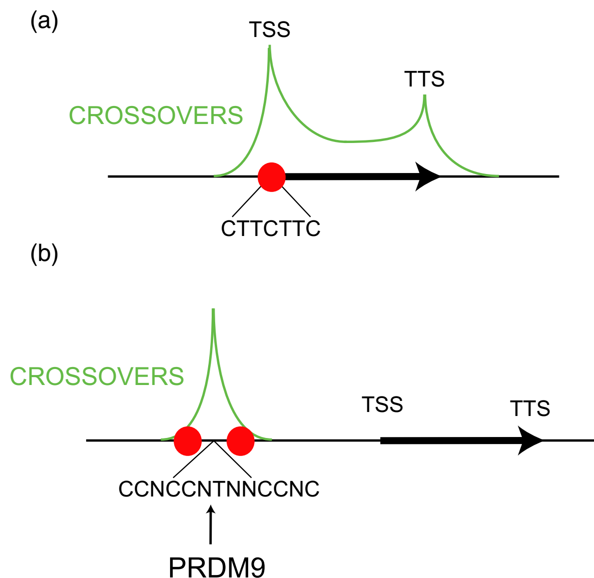

Figure 2. Plant versus human crossover hotspots.
(a) Crossover frequency (green) is represented at plant gene-associated hotspots. Crossovers are highest in proximity to gene transcriptional start (TSSs) and termination (TTSs) sites. The gene open reading frame is shown by the black arrow. Recombination hotspots at the gene promoter (TSS) are also associated with +1 nucleosomes (red circle) that contain H2A.Z and are modified with H3K4^{me3} and CTT-repeat DNA sequence motifs (Choi *et al.*, 2013; Wijnker *et al.*, 2013).
(b) In humans crossover hotspots occur at intergenic CCN-like motifs (Myers *et al.*, 2008), which are bound by PRDM9 zinc finger domains. PRDM9 also contains a histone methyltransferase SET domain, which catalyses H3K4^{me3} (red circles) and causes high recombination rates.

nucleosome shows the H3K4^{me3} modification and the presence of the histone variant H2A.Z, both of which are important for regulation of transcription (Zhang *et al.*, 2009; Coleman-Derr and Zilberman, 2012). The deposition of H2A.Z has also been shown to be required for normal levels of meiotic recombination at multiple physical scales, via analysis of the *arp6* mutation (Choi *et al.*, 2013). ARP6 is a component of the SWR1 chromatin remodeling complex that deposits H2A.Z into chromatin (Choi *et al.*, 2005). RAD51 and DMC1 foci were also significantly reduced in *arp6*, which indicates that DSB frequency may be lower, in addition to final crossover numbers (Choi *et al.*, 2013). It is also important to note that *arp6* plays an important role in the transcription of meiotic genes, including *DMC1*, which may contribute to mutant phenotypes (Qin *et al.*, 2014).

The presence of hotspots at plant gene promoters is similar to the occurrence of DSB hotspots at gene promoters in budding yeast (Nicolas *et al.*, 1989; Wu and Lichten, 1994; Lichten and Goldman, 1995; Berchowitz *et al.*, 2009; Pan *et al.*, 2011). In budding yeast nucleosome occupancy is a major determinant of recombination and high levels of DSBs are observed in nucleosome-free regions upstream of TSSs (Nicolas *et al.*, 1989; Wu and Lichten, 1994; Lichten and Goldman, 1995; Berchowitz *et al.*, 2009; Pan *et al.*, 2011). The H3K4^{me3} chromatin modification plays an important role in promoting hotspot recombination in budding yeast (Borde *et al.*, 2009; Tischfield and Keeney, 2012). Mutations to the *SET1* histone H3K4 methyltransferase cause severely reduced DSB frequency at the majority of hotspots, although a smaller number showed increased break frequency (Borde *et al.*, 2009). The Spp1 subunit of the COMPASS complex was found to interact simultaneously with both H3K4^{me3} and a component of the meiotic axis (Mer2), revealing how epigenetic marks can function to promote tethering of DSBs to axis-associated repair sites (Acquaviva *et al.*, 2013; Sommermeyer *et al.*, 2013). As H3K4^{me3} is also enriched at plant gene promoter hotspots it will important to test the extent to which this mechanism is conserved. Importantly, hotspot distributions can be evolutionarily diverse even between more closely related species. For example, mapping Spo11-oligos in *Schizosaccharomyces pombe* has revealed distinct patterns relative to budding yeast (Cromie *et al.*, 2007; Hyppa and Smith, 2010; Fowler *et al.*, 2014). Specifically, DSB hotspots are broader, observed in intergenic regions and show a weaker correlation with nucleosome occupancy (Cromie *et al.*, 2007; Hyppa and Smith, 2010; Fowler *et al.*, 2014). Interestingly, fission yeast hotspots also show a bias for inter-sister repair, leading to the phenomenon of crossover invariance (Young *et al.*, 2002; Hyppa and Smith, 2010). Crossover invariance is defined by the observations that in fission yeast large variations in DSB frequency are observed per physical distance, yet crossover frequency is not observed to show matching variation (Hyppa and Smith, 2010). Together this demonstrates the importance of studying hotspots and meiotic recombination in multiple species.

Although chromatin plays a major role in directing recombination at plant hotspots an involvement for specific DNA sequence motifs has also been observed (Choi *et al.*, 2013; Wijnker *et al.*, 2013). An association was detected between crossover hotspots and poly dA:dT sequences located upstream of gene TSS sites, which are known to disfavor nucleosome occupancy (Iyer and Struhl, 1995; Segal and Widom, 2009). One possibility is that A-rich sequences cause a greater degree of nucleosome exclusion that facilitates accessibility of the recombination machinery, including during DSB formation by SPO11 (Nicolas *et al.*, 1989; Wu and Lichten, 1994; Lichten and Goldman, 1995; Berchowitz *et al.*, 2009; Pan *et al.*, 2011). In addition to A-rich sequences, a class of CTT-repeat sequence motifs was detected as hotspot-enriched and located immediately downstream of TSSs, overlapping the +1 H2A.Z-containing nucleosome (Figure 2a) (Choi *et al.*, 2013; Wijnker *et al.*, 2013). These degenerate motifs are of unknown function but it is possible that they also contribute to organization of promoter chromatin in plants with consequences for meiotic recombination. Alternatively the CTT motifs may be specifically recognized by a component

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of the recombination machinery, in a manner similar to PRDM9 in animals (Jeffreys *et al*., 2001; Tiemann-Boege *et al*., 2006; Baudat and de Massy, 2007; Berg *et al*., 2010; Cole *et al*., 2010).

PRDM9-dependent hotspots in animals

Extensive direct mapping of crossovers using sperm typing approaches in humans and mice has revealed the presence of punctate hotspots (Jeffreys *et al*., 2001; Tiemann-Boege *et al*., 2006; Baudat and de Massy, 2007; Berg *et al*., 2010; Cole *et al*., 2010). This finding is supported by LD-based analysis that revealed thousands of hotspots throughout the human genome (Crawford *et al*., 2004; McVean *et al*., 2004; Myers *et al*., 2005; Khil and Camerini-Otero, 2010). Analysis of human hotspots identified a family of degenerate C-rich DNA sequence motifs associated with hotspots (Myers *et al*., 2008). Subsequently this motif family was found to reflect binding sites for the PRDM9 protein, which contains both zinc fingers and a SET histone methyltransferase domain (Figure 2b) (Baudat *et al*., 2010; Myers *et al*., 2010; Parvanov *et al*., 2010; Grey *et al*., 2011). PRDM9 has been shown to direct meiosis-specific $H3K4^{me3}$ that reorganizes nucleosomes around target motifs and drives DSB formation (Figure 2b) (Mihola *et al*., 2009; Brick *et al*., 2012; Baker *et al*., 2014; Pratto *et al*., 2014). Intriguingly, *PRDM9* is an extremely fast-evolving protein, which is reflected in distinctive hotspot locations between human populations and mammalian species (Oliver *et al*., 2009; Myers *et al*., 2010; Hinch *et al*., 2011; Auton *et al*., 2012). The action of PRDM9 tends to place hotspots in intergenic regions located away from genes (Figure 2b). PRDM9 has mutated to non-functionality in some species, including the canine lineage, where it is associated with reversion of recombination to promoters (Oliver *et al*., 2009; Auton *et al*., 2013). This has also been directly demonstrated in *prdm9* mutant mice, where hotspots were found to revert to promoter locations via SSDS mapping (Brick *et al*., 2012). Clear orthologs of PRDM9 have so far not been identified in plants or fungi. Together these results are consistent with gene promoters representing an ancestral mode of hotspot designation and PRDM9 representing a derived mechanism within animals. These findings again clearly demonstrate the importance of studying meiotic recombination hotspots in multiple eukaryotic lineages.

FUTURE DIRECTIONS

The occurrence of meiotic recombination hotspots is widespread within eukaryotes, but surprisingly the mechanisms that specify their locations and activity are varied. It will be important to study these phenomena in further diverse species to assess evolutionary changes in the relative roles of genetic and epigenetic information in hotspot control. Further work is required to compare hotspots measured early (e.g. DSBs) and late (e.g. crossovers) in the meiotic recombination pathway to understand their inter-relationships, particularly in relation to homeostatic mechanisms such as crossover interference. Equally, how different levels of meiotic chromosome organization interact to control hotspots and broad scale rates of recombination will be important, including interactions between chromatin and the meiotic axis/synaptonemal complex. The biological significance of hotspots will be important to further explore. For example, are specific types of genes enriched for hotspots and does recombination play an adaptive role at such genes? Finally, a mechanistic understanding of hotspots will allow us to manipulate this process within plant genomes and facilitate crop breeding and improvement.

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