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Regulating double-stranded DNA break repair towards crossover or non-crossover during mammalian meiosis

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Abstract

During meiosis the programmed induction of DNA double-stranded breaks (DSB) leads to crossover (CO) and non-crossover products (NCO). One key role of CO is to connect homologs before metaphase I and thus to ensure the proper reductional segregation. This role implies an accurate regulation of CO frequency with the establishment of at least one CO per chromosome arm. Current major challenges are to understand how CO and NCO formation are regulated and what is the role of NCO. We present here the current knowledge about CO and NCO and their regulation in mammals. CO density varies widely along chromosomes and their distribution is not random as they are subject to positive interference. As documented in the mouse and human, a significant excess of DSB are generated relative to the number of CO. In fact, evidence has been obtained for the formation of NCO products, for regulation of the choice of DSB repair towards CO or NCO and for a CO specific pathway. We discuss the roles of Msh4, Msh5 and Sycp1 which affect DSB repair and probably not only the CO pathway. We suggest that, in mammals, the regulation of NCO differs from that described in *Saccharomyces cerevisiae*.

Introduction

In most sexually reproducing eukaryotes, at the beginning of the first meiotic prophase, a unique set of events is programmed: the induction of DNA double-stranded breaks (DSB) at various locations in the genome of each meiotic cell. These DSB are generated by the catalytic activity of Spo11 in a reaction, in part, similar to that described for type II DNA topoisomerases (Keeney 2001). The repair of these DSB by recombination with a chromatid from the homologous chromosome generates two types of recombinant molecules, reciprocal exchanges or crossover (CO) and gene conversion without crossover (NCO). CO plays an essential mechanical role for the reductional division at meiosis I by connecting homologs and thus allowing their proper orientation at metaphase I (Petronczki *et al*. 2003). Thus, at least one CO per chromosomal arm is required for the proper reductional division.

Studies in *Saccharomyces cerevisiae* have shown that CO and NCO are the products of two distinct DSB repair pathways (Allers & Lichten 2001, Hunter & Kleckner 2001) (Figure 1): after DSB formation and ends processing, a putative intermediate common to both CO and NCO pathways, and resulting from the strand exchange reaction of one DSB end, is formed. NCO products are thought to be generated by a mechanism called synthesis-dependent strand annealing (SDSA) involving the extension of the 3′ invading end by a DNA polymerase, followed by its displacement and re-annealing onto the other end of the broken chromatid. The initiating chromatid thus contains heteroduplex DNA, which can lead to gene

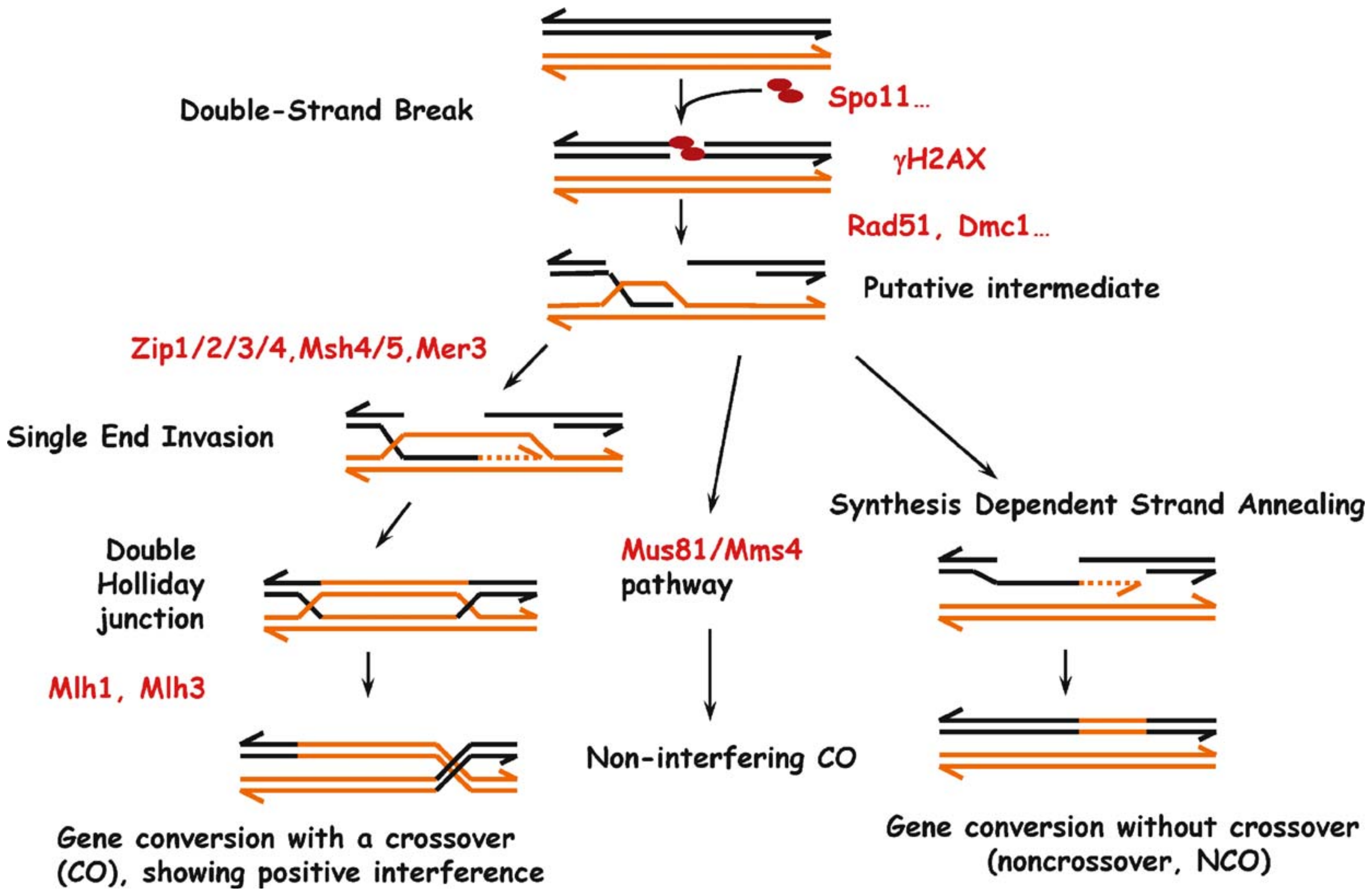

Figure 1. The DSBR model based on studies in *S. cerevisiae*.

conversion without crossover upon mismatch repair. Mutants specifically altering this pathway have not been identified so far. It can be considered as a default pathway and its function is discussed below. Along the CO pathway, a single-end invasion intermediate (SEI) has been identified and is converted into a double Holliday junction by the annealing of the free DSB end to the displaced strand. This double Holliday junction is resolved such as to generate an exchange of flanking markers, and upon mismatch repair of heteroduplex DNA, a CO product with gene conversion is formed. Several functions have been shown to be specifically required for the CO pathway. These are called the ZMM functions, that include *ZIP1*, *ZIP2*, *ZIP3*, *ZIP4*, *MSH4*, *MSH5* and *MER3* genes (ZMM) (Borner *et al.* 2004). These functions seem to act at the time or soon after DSB formation at the leptotene/zygotene stage of meiotic prophase and one of their roles is to antagonize the anti-crossover activity of the Sgs1 helicase (Jessop *et al.* 2006). Strains carrying mutations in one of these genes have a reduction of CO whereas NCO are, in general, not affected. In addition, formation of CO in this pathway also requires Mlh1 and Mlh3 proteins, thought to act at a late step in the recombination reaction (Hunter & Borts 1997, Wang *et al.* 1999, Alpi *et al.* 2003, Argueso *et al.* 2004). A unique manifestation of CO regulation is positive interference which leads to the non-random distribution of CO that are more widely spaced than expected (Jones 1984). In ZMM mutants the remaining CO do not show interference as measured genetically, and most depend on Mus81/Mms4 (De Los Santos *et al.* 2003). Interestingly, in *S. pombe*, where CO do not show interference, CO are completely dependent on Mus81/Eme1 (the *S. pombe* homolog of Mus81/Mms4) and go through a single Holliday junction intermediate (Hollingsworth & Brill 2004, Cromie *et al.* 2006).

One current major issue is to understand how the decision for CO/NCO is made (Borner *et al.* 2004, Bishop & Zickler 2004) and how the proper number and position of CO are achieved in each meiotic cell. Understanding this obviously requires an understanding of the whole pathway of DSB repair and to detect and analyze not only CO but also those DSB repair events not leading to CO in order to answer the following questions: When is a DSB event committed to be repaired as a CO? How is the non-random spacing of CO regulated? Why are there recombination events in excess of the number of CO and what are their roles?

Here, we present the current information about meiotic DSB repair mechanisms and regulation in mammals related to these issues of CO control. Most of the data presented takes advantage of recent studies in humans and mice. We first outline the evidence for DSB formation and repair and describe the data showing the excess of DSB repair events as compared to CO. We then summarize the large amount of information about CO distribution and frequencies as well as the limited information about NCO and the evidence for NCO/CO control. We then discuss the results obtained from the analysis of mice carrying mutations in ZMM orthologs (Msh4, Msh5 and Sycp1) and suggest interpretations for their roles along the DSB repair pathway.

Conservation of the DSB repair pathway

Initiation by Spo11-generated DSB

The Spo11 protein is widely conserved and the current evidence for initiation of meiotic recombination by DSB in mammals comes from the observation that a high level of H2AX phosphorylation is detected at the leptotene stage of meiotic prophase. In mice this phosphorylation has been shown to be Spo11-dependent (Mahadevaiah *et al.* 2001). In addition, the detection of Spo11-oligo molecules from wild-type mouse testis strongly supports the notion that Spo11 is catalytically active in meiosis (Neale *et al.* 2005). These Spo11-oligo covalent molecules, where Spo11 is bound to the 5′ end of a short oligonucleotide (15–30 bp long), are thought to result from the processing of Spo11–DNA cleavage complexes that have been released by an unknown endonuclease activity. DNA breaks have also been visualized in mouse testis sections by assays allowing the detection of ends with 3′ overhangs (Zenvirth *et al.* 2003), and at a mouse CO hotspot by the use of terminal transferase (Qin *et al.* 2004). One property of initiation by DSB and repair as depicted in Figure 1 is that the initiating chromosome is the recipient of information. This prediction of the model has indeed been confirmed at several CO hotspots in human and mouse (Jeffreys & Neumann 2002, Yauk *et al.* 2003, Jeffreys & Neumann 2005: Baudat and de Massy, *PLoS Genetics*, in press).

Given that the phosphorylation of H2AX at leptotene spreads over large overlapping chromatin domains the total number and distribution of DSB could not be determined.

DSB repair events: an excess compared to CO

Given the model outlined in Figure 1, one predicted intermediate common between CO and NCO is a strand exchange product. The two major strand exchange proteins acting in meiosis, Rad51 and Dmc1 (Shinohara 2004), have been analyzed by immunocytochemistry. In mouse and human spermatocytes and oocytes, Rad51 and Dmc1 co-localize and form multiple foci at leptotene and zygotene (Ashley *et al*. 1995, Plug *et al*. 1996, Barlow *et al*. 1997, Moens *et al*. 1997). Rad51 foci are detected in early leptotene and appear to be mostly located on the chromosome axis. The number of foci decreases as synapsis proceeds from zygotene to early pachytene and goes down to an undetectable level at the middle of pachytene (Moens *et al*. 2002, Oliver-Bonet *et al*. 2005). Thus, the number of Rad51/Dmc1 foci peaks at leptotene to about 200–400 in mice and humans, with differences between sexes (Table 1). These values are a minimum estimate of the total number of DSB repair events, which exact value is therefore unknown. In any case the number of these foci by far exceeds the number of CO (Table 1 and see below, 'Properties of CO products'), raising the questions of why are so many DSB generated and how are their repair regulated? The decrease of Rad51/Dmc1 foci from leptotene to the middle of pachytene is thought to reflect the progression of the

Table 1. Quantitative data on meiotic recombination events in humans and mice (male and female)

	Mus musculus			Human		
	Female	Male	Reference	Female	Male	Reference
Rad51 foci	250–350	230–270	Plug *et al*. 1996	350–400		Lenzi *et al*. 2004
	420–370 (394)		Kolas 2005		91–262 (152)	Barlow *et al*. 1997
		250–300	Tarsounas 1999		104 ± 28	Oliver-Bonet *et al*. 2005
		300	Moens *et al*. 2002			
		400	de Vries *et al*. 2005			
Mlh1 foci	23–33 (28)		Kolas 2005	48–102 (70)		Tease 2002
	19–34 (26.1)		Lynn *et al*. 2005	10–107 (60)		Lenzi *et al*. 2004
	26.6 ± 3.2		Koehler *et al*. 2006		41–59 (50.9)[c]	Barlow *et al*. 1998
	24.1 ± 2.6[b]		Koehler *et al*. 2006		49.1 ± 4.8	Lynn 2002
		22.7	Anderson *et al*. 1999		50	Oliver-Bonet *et al*. 2005
		23	Moens *et al*. 2002		32–63 (47.8)	Codina-Pascual *et al*. 2006
		22.9[c]	Froenicke 2002			
		21.5–24.3[c]	Koehler *et al*. 2002			
Chiasmata		22.6–23.9	ref. in Anderson *et al*. 1999		49.6–53.7	ref. in Vallente *et al*. 2006
	24.1		Woods *et al*. 1999			
Genetic map (cM)	1385[b]		Dietrich *et al*. 1996	3799–4600 (4400)	2590–2813 (2700)	ref. in Vallente *et al*. 2006[d]
	1398[b]		Rhodes 1998			
	1817	1386	Shifman *et al*. 2006			
Rad51 foci / CO (genetic map)[a]	394/36 = 11	250/28 = 9		370/88 = 4	152/54 = 3	

[a]50 cM correspond to an average of one CO per meiocyte.
[b]*M. musculus* × *M. spretus* interspecific cross.
[c]Autosomal Mlh1 foci only have been counted. One focus has to be added for taking into account the obligatory CO on the pseudo-autosomal region of the XY pair.
[d]for additional data on human genetic maps, see also Lynn *et al*. 2004.

repair events with the elimination of Rad51/Dmc1 once the strand exchange reactions are complete. Several other proteins have been immunolocalized and found to mark recombination foci. These have been reviewed (Cohen & Pollard 2001, Vallente *et al*. 2006) and will not be described here except for those whose analysis provides insight into the understanding of CO/NCO pathways and CO regulation (see below, 'The CO/NCO decision, the ZMM proteins in mammals').

Properties of CO products

Frequency and distribution

CO can be measured over the whole genome by cytological, genetic or population analysis. The total number of chiasma is around 23 in mice and 52 in humans, with differences between sexes (see below, 'Variations of CO activity'; Table 1). CO can also be monitored by the immunolocalization of the Mlh1 protein, whose foci have been shown to correspond to sites of chiasma (Marcon & Moens 2003). In principle, a perfect correlation between the numbers of Mlh1 foci and CO is not necessarily expected given the potential existence of a Mlh1-independent CO pathway, as shown in *S. cerevisiae* for instance. In fact, in mice, Mlh1 is required for the formation of most, but not all, CO given the 10–20-fold reduction of CO frequencies in Mlh1 −/− mice, which have therefore a residual level of CO activity corresponding to 5–10% of wild-type (Woods *et al.* 1999, Guillon *et al*. 2005). We note a slight excess of CO as measured genetically as compared to Mlh1 foci in wild-type mice, which could be due to technical bias or to the presence of a small proportion of Mlh1-independent CO in wild-type mice (Table 1). These cytological analyses also show that at least one CO is formed per chromosome arm both in humans and mice, apart from short heterochromatic arms from acrocentric chromosomes. This regulation of CO frequency is a manifestation of the rule of the obligatory CO. Interestingly, mouse strains carrying Robertsonian translocations have two CO per chromosome, one per euchromatic arm (Dumas & Britton-Davidian 2002). The rule of the obligatory CO is also observed between the X and Y, where one CO is always observed in the pseudoautosomal region. A second level of CO regulation is shown by the measures of distances between chiasmata or Mlh1 foci, or by genetic distances. These show that, both in mouse and human, CO are not randomly distributed and are more evenly spaced than expected if they occurred independently, a phenomenon defined as positive interference (Lawrie *et al.* 1995, Laurie & Hulten 1985, Anderson *et al.* 1999, Broman & Weber 2000, Broman *et al*. 2002).

The recent genetic maps determined by pedigree or based on population analysis provided a genome-wide high-resolution map of CO distribution both in the human and the mouse genomes (McVean *et al.* 2004, Myers *et al*. 2005, Shifman *et al*. 2006). These approaches confirmed at large scale previously established properties of CO distribution and led to additional conclusions: CO are not randomly distributed, but occur in multiple specific regions of the genome called CO hotspots (de Massy 2003, Kauppi *et al*. 2004). A hotspot is a region 1–2 kb wide where CO are clustered, as a result of localized initiation events (see below). The average spacing between hotspots is 50–100 kb and hotspot activities vary over three to four orders of magnitude, from 0.9 to 0.0005 cM as determined in the human genome. In the human genome the number of hotspots is estimated around 25 000–50 000 (Myers *et al.* 2005). The variations of hotspot density and activity along chromosomes result in domains with high (jungles) or low (desert) recombination activity. Most sub-telomeric regions are recombination jungles in male meiosis whereas centromeric regions are recombination deserts. However, the determinants of CO variation along chromosomes are not known, even though some factors correlated with CO density are beginning to be analyzed (Buard & de Massy 2007, TIG in revision).

In addition, molecular analysis of recombinant products has been performed in specific regions of the human and mouse genomes by allele-specific PCR that have allowed not only measures of CO frequencies, but also the determination of the distribution of exchange points at several CO hotspots. In all cases, 17 human hotspots (Holloway *et al.* 2006) and two mouse hotspots (Guillon & de Massy 2002, Yauk *et al.* 2003) exchange points follow a bell-shaped curved with a peak of density in a narrow interval (on the order of a few hundred bp) predicted to correspond to the region of initiation (Figure 2). This distribution of exchange points is coherent with the DSB repair model where

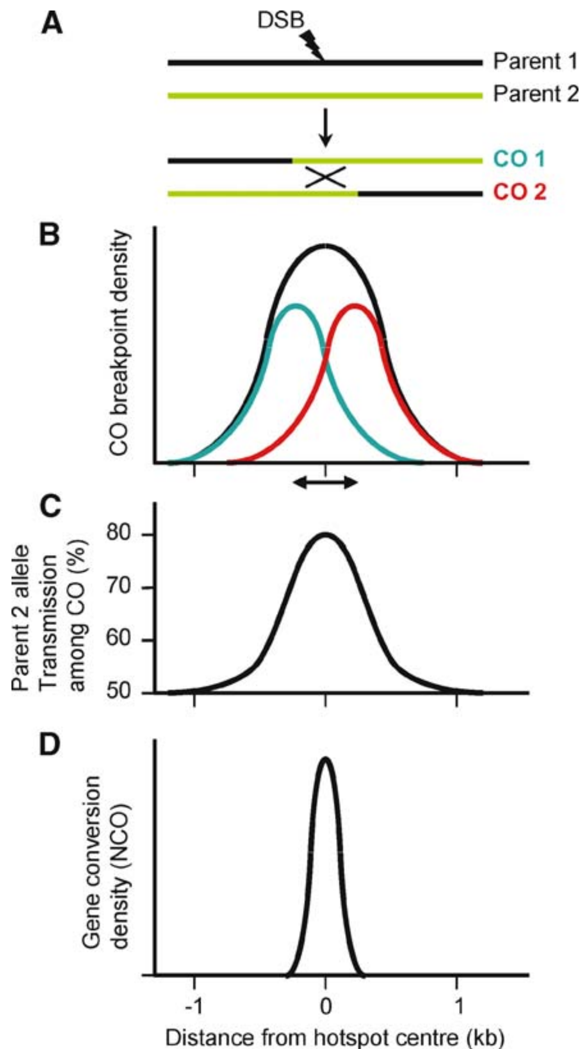

Figure 2. Theoretical distributions of CO breakpoints and gene conversions resulting from NCO events at a hotspot. **A**: Schematic representation of the chromatids involved in one CO event at a hotspot. The Spo11-generated DSB occurs only on Parent 1 molecule. CO1 and CO2 represent both exchange products generated by one reciprocal CO event. **B**: Distribution of the CO breakpoints along the hotspot. DSB are highly localized at the hotspot centre. In blue, CO1 breakpoints. In red, CO2 breakpoint; these two curves overlap due to the spreading of DSB sites. In black, CO1 + CO2 exchange points. The double arrow represents the average length of gene conversion tracts associated with CO. **C**: Percentage of transmission of Parent 2 alleles (from the non-initiating chromosome) into CO products. **D**: Density of gene conversion resulting from NCO events.

intermediates extend to a variable distance from initiation (Figure 1). The analysis of the distribution of CO exchange points can also provide information on the initiation activity of each homolog, and on the average size of gene conversion tracts involved in the CO events. Indeed, when initiation occurs on one chromatid, because mismatch repair is directed and leads to a conversion bias towards the unbroken chromatid, the exchange points of each CO product are distributed asymmetrically on either side of the region of initiation. In a population of CO products a differential initiation activity on each homolog results in a transmission distortion for markers located in the region of initiation and correlated with a non-overlapping distribution of the two types of reciprocal products (Figure 2). When a strong distortion is observed, the distance between these two distributions provides an estimate of the average gene conversion tract. Several such cases have been observed in humans and mice, and have shown that conversion tract lengths associated with CO are around 500 bp (Jeffreys & Neumann 2002, Baudat and de Massy, *PLoS Genetics*, in press). An important output from direct molecular analysis is that they have allowed a real-time analysis of CO formation and showed at one CO hotspot in the mouse genome (*Psmb9*) that CO products are formed at the end of the pachytene stage in male meiosis (Guillon & de Massy 2002). Therefore, about 8 days separate DSB from CO formation in male meiosis.

Variations in CO activity

The overall number and distribution of CO are not identical for all individuals in a given species; on the contrary, they display both DNA sequence-dependent and -independent variations. The differences between genders also illustrate the occurrence of DNA sequence-independent variations: in humans and to a lesser extent in mouse, overall CO rates are significantly higher in females than in males (Table 1), with, however, higher male-specific densities of CO in the centromere-distal sub-telomeric regions (Broman *et al*. 1998, Kong *et al*. 2002, Shifman *et al*. 2006). The number and distribution of MLH1 foci in XY female mice are similar to those observed in normal XX females rather than those observed in normal XY males, demonstrating that the biological sex rather than the genotype (i.e. the XY chromosome pair) determines the gender-specific pattern of meiotic recombination in this organism (Lynn *et al*. 2005).

The genetic background is also a major determinant of the variations in the number and distribution of CO. Indeed, the comparison between several inbred mouse lines revealed small but reproducible differences in the overall number of MLH1 foci (Koehler *et al*. 2002). In crosses between different lines, such as those used for genetic linkage analyses, the pattern of recombination might be affected by additional factors, including the level of polymorphism and a possible genetic interaction between the genomes of both parents. For example, the female genetic maps generated from interspecific *Mus m. domesticus* × *M. spretus* backcrosses are not longer than the sex-averaged maps generated from *M. musculus* intraspecific intercrosses, despite the fact that female genetic maps are usually longer than their male counterparts (Dietrich *et al*. 1996 and Table 1). A recent comparison between both parent strains (C57BL/6 and SPRET) and their F1 hybrid confirmed that the number of MLH1 foci is reduced significantly in the hybrid (Koehler *et al*. 2006). In humans, differences among individuals are well documented, suggesting that genetic factors affect the overall CO rate (Broman *et al*. 1998, Kong *et al*. 2002, Lynn *et al*. 2004, Codina-Pascual *et al*. 2006). Besides these large-scale differences in CO number, the local distribution of CO can also depend on the DNA sequence. This is illustrated by the fact that several CO hotspots identified in the mouse MHC are specific for one or a few haplotypes (Shiroishi *et al*. 1995). Similarly, at several human CO hotspots, the CO rate was found to vary widely from one individual to another (Neumann & Jeffreys 2006). In some cases, these variations were found to correlate with specific sequence polymorphisms located close to the center of the hotspot, suggesting that these variations of CO activity reflect variations in the rate of DSB formation (Jeffreys & Neumann 2005).

Properties of NCO products

The large excess in the number of Rad51/Dmc1 foci over CO in mice and humans suggests that NCO outnumber CO in mammals (see above; Table 1). However, given that NCO events lead to a highly localized modification of the genotype, their detection requires specific tools and their description and analysis are still poorly documented in mammalian genomes. Most of the data comes from molecular analysis at a few specific CO hotspots in the human and mouse genomes. These studies have shown that most CO hotspots are also NCO hotspots, indicating that the same initiating regions can lead to both CO and NCO products (Guillon & de Massy 2002, Jeffreys & May 2004).

The relative ratio of CO to NCO was found to vary considerably from one hotspot to another, as shown by the comparison of several hotspots in the human genome. The measured values went from 1:2.7 to 4:1, and even to more than 12:1 at one hotspot where no NCO was detected (Holloway *et al*. 2006). It should be kept in mind that most NCO rates are probably under-evaluated in proportions which are difficult to estimate, given that their detection depends on the presence of polymorphic markers. Nevertheless, it is interesting to compare these values for individual hotspots with the predictions made at the scale of the whole genome, based on cytological analysis as described above (Table 1). Given that a significant fraction of NCO may remain undetected, these ratios indicate that NCO indeed represent a substantial proportion of the meiotic DSB repair events in mammals.

Several aspects of NCO formation have been compared with CO. NCO are clustered at the center of CO hotspots, with a sharp decrease in gene conversion frequencies on both sides, indicating that both CO and NCO are the products of initiation events taking place in the same region (Jeffreys & May 2004, Guillon *et al*. 2005). NCO products are formed with a kinetics similar to those of CO, from middle to late pachynema (Guillon *et al*. 2005).

However, detailed mapping analyses have shown an amazing and unexpected difference between CO and NCO: the size of the conversion tract. Whereas the size of gene conversion tracts among CO products was found to be around 500 bp (see above), among NCO the conversion tracts are considerably shorter, with a mean length estimated between 50 and 300 bp (Jeffreys & May 2004, Guillon *et al*. 2005). This is consistent with the fact that NCO and CO are outcomes of different DSB repair pathways, involving different recombination intermediates. In fact, Mlh1 and Mlh3 were found to be required for CO but not NCO formation (Guillon *et al*. 2005; Svetlanov, Baudat, Cohen & de Massy, personal communication).

The CO/NCO decision, the ZMM proteins in mammals

Four mammalian homologs of the yeast ZMM proteins have been identified: Sycp1 (Sage *et al.* 1995, Meuwissen *et al.* 1997, de Vries *et al.* 2005), Msh4 (Paquis-Flucklinger *et al.* 1997, Kneitz *et al.* 2000), Msh5 (Bocher *et al.* 1999, Her *et al.* 1999, Edelmann *et al.* 1999) and Mer3 (also called HFM1, Entrez IDs: 330149 and 164045 for mouse and human, respectively). The Mer3 gene and protein have not been analyzed. Msh4 and Msh5 have been detected in testes and ovaries only, and do not seem to be expressed at significant levels in other tissues. Protein–protein interaction studies of mouse and human proteins expressed in cultured cells indicate that Msh4 and Msh5 dimerize via their carboxy-termini (reviewed in Kolas & Cohen 2004). Msh4 interacts with Rad51 and Dmc1 (Neyton *et al.* 2004) and with Mlh1 (Santucci-Darmanin *et al.* 2000). Immunolocalization shows that Msh4 and 5 co-localize and form foci in zygotene until late pachytene (Lenzi *et al.* 2004). The number of mouse Msh4 foci goes from 142 to 47 at mid-pachytene (Kneitz *et al.* 2000) where it co-localizes with Mlh1 (Santucci-Darmanin *et al.* 2000). Therefore, unlike *S. cerevisiae*, in mammals the number of Msh4 foci exceeds the number of CO. Msh4 foci are evenly distributed, show a low level of interference and their distribution is clearly different from that of Mlh1, which shows a strong level of interference (de Boer & Heyting 2006). Mutant Msh4 or Msh5 mice show a strong synapsis defect, but have high levels of Rad51 foci (tested in Mhs4−/− mice only), possibly indicating a defect in the processing of recombination intermediates and a failure to establish or maintain stable homologous interactions (de Vries *et al.* 1999, Kneitz *et al.* 2000). While mutant spermatocytes undergo apoptosis at the end of zygotene/beginning of pachytene, oocytes proceed until diakinesis, but are completely eliminated at 1 week of age. Although these phenotypes indicate an early role for Msh4 and 5 in the process of DSB repair, these proteins have been proposed to also play a role late in recombination, based on their co-localization with Mlh1 and on *in-vitro* assays (Snowden *et al.* 2004). The observation of a major synapsis defect in Msh4 or 5 mutants and of the number and distribution of Msh4/5 foci suggest that, differently from *S. cerevisiae*, the role of these proteins is not restricted to the formation of CO regulated by interference. We propose that, in mammals, Msh4 could be involved in both the formation of CO and NCO by acting before the separation of NCO/CO pathways. In mammals, NCO are therefore predicted to be subject to a specific constraint as revealed by the low level of interference between Msh4 foci, whereas no interference could be detected genetically between NCO in *S. cerevisiae* (Malkova *et al.* 2004). The action of Msh4 both in mammals and yeast could in fact be functionally similar and linked to interference control but operating at different times, and therefore at different stages, along the DSB repair pathway in these organisms (de Boer & Heyting 2006, Fig. 3).

Sycp1 is a meiosis-specific protein homologous to *S. cerevisiae* Zip1 based on weak sequence homology, and is a transverse filament protein, part of the central element of the synaptonemal complex (Schmekel *et al.* 1996). Sycp1 mutant male mice show defects in prophase progression and are defective in synaptonemal complex formation. However, pachytene spermatocytes have their homologs aligned at distance, suggesting that homologous interactions have been established but not converted into tight associations (de Vries *et al.* 2005). In Sycp1−/− mice, Rad51 and Msh4 form foci as in wild-type mice, showing that Sycp1 is not required for the low level of interference observed on Msh4 foci at late zygotene. However, these foci persist longer than in wild-type mice, suggesting a defect during the process of DSB repair. Indeed, γ-H2AX foci also persist longer and Mlh1/3 foci do not form. Whether DSB are repaired toward NCO remains to be determined. Among the rare spermatocytes that reach metaphase I, most chromosomes form univalents suggesting a CO defect. (Whether Sycp1 is required for interference between Mlh1 foci in wild-type mice cannot be determined.) In yeast the Sycp1 homolog, Zip1, is not required for the establishment of interference between Zip2 foci which largely co-localize with Msh4 (Novak *et al.* 2001). This could be coherent with the normal level of interference between Msh4 foci in Sycp1−/− mice. Another property of the ZMM proteins in *S. cerevisiae* is that they define the SIC (Synapsis Initiation Complex). Several arguments have led to the proposition that SIC are located at the same sites where crossovers will form (Henderson & Keeney 2005). In humans, analysis of synapsis initiation has indeed suggested a

limited number of sites preferentially located near sub-telomeric regions in correlation with CO density (Brown *et al.* 2005).

Unexplored DSB repair events

We have mentioned above that the number of meiotic DSB far exceeds the final number of interhomolog CO (see Table 1). Although recent data show that a significant fraction of DSB are repaired leading to NCO between homologs (see above), alternative, unexplored possibilities have to be considered. Although meiotic recombination events are channeled towards homologous interactions, the fraction that possibly goes through interactions between sister chromatids is unknown. This control is in part mediated by meiotic specific cohesins. Indeed, a mutant defective for a meiosis-specific cohesin (Rec8) displays synapsis associating sister chromatids rather than synapsis between homologous chromosomes (Xu *et al.* 2005).

In addition, DSB repair can occur by intra-chromatid interactions. Meiotic intra-allelic rearrangements have been observed at unstable minisatellites (Buard & Vergnaud 1994, Jeffreys *et al.* 1994). The localization of these tandem-repeat DNA sequences in CO hotspots strongly suggests that these rearrangements are a product of the repair of meiotic DSB (Jeffreys *et al.* 1998, Buard *et al.* 2000). Though rearranged products resulting from intra- or inter-chromatids cannot be distinguished from each other, some of them might result from intra-chromatid events. Since the inter-homolog CO are the sole recombination products to have been counted exhaustively at the scale of the whole genome so far, the respective proportions of the other types of products (inter-homolog NCO, inter-sister recombination and intra-chromatid recombination) remain unknown.

An alternative DSB repair pathway to homologous recombination is non-homologous end joining (NHEJ), which results in the ligation of the ends on both sides of the DSB. This pathway is a major one for repairing DNA DSB in somatic cells, especially during the G1 phase of the cell cycle. During meiosis, KU70, a component essential to the NHEJ, is absent from the spermatocyte nuclei during the leptotene and zygotene stages of prophase, when meiotic DSB are generated and processed. Moreover, at the later pachytene stage, KU70 reappears but is mostly concentrated in the sex body, which corresponds to the chromatin domain of the non-pseudoautosomal regions of the X and Y chromosomes (Goedecke *et al.* 1999). These observations suggest that the NHEJ is indeed prevented during the course of meiotic recombination, at least in the autosomes.

During male meiosis the sex chromosomes do not have a homolog, with the exception of the short pseudoautosomal region. DSB are likely to be generated on the X and Y since RAD51 foci form on their axes (except for the heterochromatic region of the Y). However, these foci accumulate and persist until late pachytene, and are different from the autosomal foci that disappear progressively during the progression into the pachytene stage (see above). It is therefore possible that the DSB generated on the X and Y chromosomes are repaired by one of the

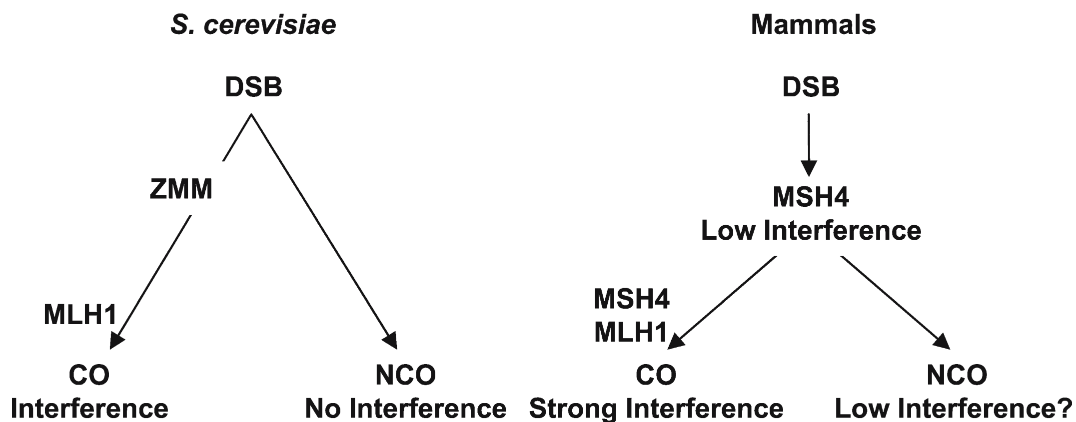

Figure 3. Regulations of NCO/CO decision in *S. cerevisiae* and mammals.

alternative pathways mentioned above. The persistence of the X/Y RAD51 foci through the pachytene stage suggests that this alternative repair might necessitate the relaxation of some constraints (for instance, against inter-sister recombination) that would be imposed at earlier stages to favor interhomolog recombination interactions.

Conclusions

The current data suggest that a large number of DSB are induced at the beginning of meiosis and lead to several hundred DSB repair events in mammals. These repair events are highly regulated to result in the formation of at least one CO per chromosome arm. These CO show a high level of interference and depend on Mlh1/3 proteins, which co-localize with CO sites. Whether another CO pathway is active is not known, but the Mus81 protein clearly does not play an essential role during meiosis given the normal fertility of Mus81 mutant mice (McPherson *et al*. 2004, Dendouga *et al*. 2005). Several observations show that the repair of DSB towards CO or NCO is indeed regulated in mammals. First, the distribution of Msh4 foci, which mark most if not all DSB repair events, significantly differs from that of Mlh1, suggesting that all DSB sites do not have the same probability to become a CO. Second, when measured at the molecular level at various CO hotspots, variable ratios CO/NCO were found. Specific functions are thus predicted to be required for the CO/NCO decision, and how this regulation works remains to be understood. Interestingly, Mlh1 and 3 are specifically required for the CO pathway, not for NCO (Guillon *et al*. 2005; Svetlanov, Baudat, Cohen & de Massy personal communication). However, unlike in *S. cerevisiae*, Msh4/5 functions do not seem to be exclusively involved in CO formation given the localization of the proteins and the mutant phenotypes. The lack of requirement for Msh4/5 for NCO in yeast might be related to the fact that the NCO pathway is a default pathway. Instead, in mice, Msh4/5 might also participate in NCO regulation in order to ensure the proper frequency and distribution of these events (Figure 3). This raises the question of what is the function of NCO events, which can be envisioned in at least two ways: (1) Could the process of NCO formation play a role in promoting homologous pairing, or contributing to bring homologous chromosome axis together? (2) Could the outcome of NCO confer an evolutionary advantage such as removal of DNA elements from the genome? The answers seem to differ between organisms, and it is possible that the NCO play a more critical role with respect to pairing in mammals and thus require specific functions such as Msh4/5 and possibly Sycp1. The combination of various approaches and comparisons between organisms, already extremely fruitful, is expected to continue to provide a better understanding of the regulation of DSB repair and CO control.

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