Recombination: a frank view of exchanges and vice versa

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The study of double-strand chromosome break repair by homologous and nonhomologous recombination is a growth industry. In the past year, there have been important advances both in understanding the connection between recombination and DNA replication and in linking recombination with origins of human cancer. At the same time, a combination of biochemical, genetic, molecular biological, and cytological approaches have provided a clearer vision of the specific functions of a variety of recombination proteins.

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Abbreviations

ATM	ataxia telangiectasia mutation
DSB	double-strand break
HR	homologous recombination
NHEJ	nonhomologous end-joining
Sir	silent information regulator

Introduction

Much progress in our understanding of recombination has been associated with the impressive and rapid development of specific recombination assays in vertebrate cells that permit a direct comparison between yeast and vertebrates. Site-specific rare-cutting endonucleases, such as HO and I-SceI, are making it possible to create double-strand breaks (DSBs) in chromosomes, producing results that are quite different from those obtained on the basis of the transfection of 'naked' DNA into cells. One important realization is that homologous recombination (HR) and nonhomologous end-joining (NHEJ) compete with each other and take place at comparable frequencies. Although budding yeast favors HR over NHEJ and mouse cells prefer NHEJ, the differences are much less than an order of magnitude [1••,2•]. The idea that the ratio of HR to NHEJ is developmentally has recently received support from a study of Ku DNA end-binding proteins during meiosis in mouse cells [3•]. Ku proteins participate in NHEJ. Goedecke *et al.* [3•] found that the level of Ku proteins decreases during mouse cell meiosis, so that presumably HR becomes favored over NHEJ.

HR proteins

HR in *Saccharomyces* requires the RecA-homologous strand exchange proteins Rad51p (and the two Rad51-related proteins Rad55p and Rad57p), Rad52p, Rad54p and Rad59p. Rad50p, Mre11p and Xrs2p are also important. All these proteins have vertebrate homologues [4,5]. Investigation of the functions of these proteins has produced much new information and has provided further evidence that these proteins are important in preventing cancer. The roles of these recombination proteins are discussed below.

Rad51p

In vertebrates, the absence of Rad51p is lethal. When it is depleted from chicken DT40 cells, it causes an accumulation of chromosome breaks. Takeda and colleagues [6•], using chicken DT40 cells, created a mutation in Rad51p that prevents ATP hydrolysis but allows ATP binding; this mutated Rad51p rescues lethality in Rad51p null cells. However, recombination is surprisingly robust in this Rad51p mutant, showing that the essential functions of Rad51p are independent of ATP. By depleting Rad51p, or eliminating Rad54p (another recombination protein), Takeda and colleagues have also shown that sister chromatids in mammalian cells undergo exchange through HR [7]. In addition to Rad51p, vertebrates have five Rad51 homologues (Rad51B, Rad51C, Rad51D, XRCC1 and XRCC2) whereas yeast has only two. It now seems that all of them play important roles in recombination, although none of them is essential for cell viability. For example, Jasin and colleagues [8,9] have shown that XRCC2 and XRCC3 deletions reduce I-SceI-induced recombination in mouse cells.

Rad54p

Rad54p facilitates Rad51p's strand exchange activity *in vitro* [10]. The importance of Rad54 homologues in HR has been demonstrated in fruit flies [11], chicken DT40 cells [7] and mice [12]; although, unlike Rad51p, Rad54p is not essential. In addition, *rad54*$^{-/-}$ yeast cells are not as severely defective in recombination as *rad51*$^{-/-}$ mutants [13]. In fact, from yeast to humans, there are only two Rad54-like proteins: Rad54p and Rad54Bp. In yeast, they apparently participate in different pathways, with Rad54p playing the key role in sister-chromatid recombination [14], and Rad54Bp (known in yeast as Tid1p or Rdh54p) being more important for inter-chromosomal transactions. (How cells know which recombination machinery to use, depending on the homologous partner chosen, is an enduring mystery). However, much of the increased interest in Rad54p and Rad54Bp has come from the demonstration that these genes are often mutated in primary cancers [15,16]. Loss of heterozygosity near other human recombination genes has also been noted, suggesting that when a mutant allele is homozygous, cells have an elevated probability of developing cancer [17].

BRCA1p and BRCA2p

The connection between HR and cancer has been strengthened by the demonstration that a mutation in the breast cancer gene *BRCA1* reduces recombination in mouse cells [18••]. BRCA1p interacts with Rad51p, as well as with the Mre11–Rad50 complex [19], which has been implicated both in HR and in NHEJ. Whether another

breast cancer-associated protein, BRCA2p, which also complexes with Rad51p, is involved directly in recombination should be known soon.

Rad52p

In yeast, *RAD51* does not appear to be as essential as it is in vertebrates. A number of HR events can occur without *RAD51*, including the maintenance of telomeres in the absence of telomerase [20•]. In contrast, *RAD52*, which encodes a strand annealing protein, seems to be the most essential recombination gene. Interestingly, yeast appear to have an alternative HR pathway for telomere maintenance that uses Rad50p, Mre11p and Xrs2p [20•]. Both pathways require *RAD52*. Perhaps this second pathway could be investigated using an allele of *RAD52* that is only defective in recombination in the absence of *RAD51* [21]. There is another Rad52-like protein, Rad59p, that also appears to be part of a *RAD52*-dependent, *RAD51*-independent, pathway [21]. Like Rad52p, Rad59p may be involved in strand annealing.

Studies of *RAD52* in higher eukaryotes pose the question of whether it has evolved a different role than in budding yeast. In fission yeast, which is evolutionarily remote from budding yeast, it now seems that there are two Rad52 homologues (both homologous to yeast Rad52 and not to yeast Rad59). A double mutant of these homologous is inviable, possibly because of failures to repair DNA damage arising during replication, but perhaps for other reasons [22]. In vertebrates, cells with a Rad52 knockout are not seriously compromised. Perhaps there are additional Rad52 homologues still to be found. In any case, *in vitro*, human and yeast Rad52p stimulates Rad51p-mediated strand exchange and these two proteins clearly interact [23]. In addition, Rad52p appears to bind selectively to DNA ends [24]. Curiously, in both *Caenorhabditis* and *Drosophila* the apparently complete genome sequence provides no evidence of a Rad52 homologue.

Mre11

Yeast Mre11, Rad50 and Xrs2 proteins participate in a remarkably diverse set of functions, affecting HR, NHEJ telomere maintenance, the induction of meiotic DSBs and checkpoint regulation of the G_2/M DNA damage response (reviewed in [5]). Surprisingly, a *mre11* deletion specifically reduces recombination in the G_2 phase of the cell cycle, between both sister chromatids and homologous chromosomes, much more profoundly than inter-homologue HR in G_1 [25]. This seems to be another example of different repair pathways using distinct protein complexes.

Connecting homologous recombination to replication

The lethality of vertebrate cells lacking Rad51p seems to be caused by the accumulation of chromosome breaks that are believed to arise during the normal process of DNA replication. It is now becoming evident that one major role of recombination is to re-establish replication at broken replication forks. This idea is strongly supported by recent work in Michel's laboratory on bacteria that describes how recombination proteins participate in the re-initiation of DNA replication [26]. At the same time, it is becoming evident that recombination events themselves involve nearly all of the components of normal DNA replication [27•]. This result adds to a growing body of evidence that the mechanism of gene conversion repair of DSBs may not follow the mechanism outlined by Szostak *et al.* [28], but instead takes place through one of several versions of synthesis-dependent strand annealing (SDSA) [5,29]. These ideas have found further experimental support from several studies showing that recombination of bacteriophage in *Escherichia coli* involves extensive DNA synthesis, consistent with a 'copy choice' or 'break-induced replication' mechanism [30,31], which also appears to be important in eukaryotes (reviewed in [32]).

Homologous recombination during meiosis

All eukaryotes examined so far have a second *RAD51*-like gene, *DMC1*, that is expressed only in meiotic cells. Knocking out *dmc1* impairs or prevents meiosis. Whether Dmc1p acts in the same 'recombinosome' as Rad51p is an important issue that remains to be addressed. In *Saccharomyces*, these proteins are sometimes colocalized in foci that are visible in meiotic prophase. A recent electron microscopic study argues that they are present in the same complexes of recombination proteins in mouse meiosis as well [33]. Genetic evidence suggests, however, that the two proteins do not perform all the same tasks. Loss of Dmc1p eliminates both inter-homologue and inter-sister chromatid recombination intermediates, whereas the loss of Rad51p reduces and delays the appearance of these intermediates [34]. Structural studies show that the two RecA-like proteins form different polymeric structures on DNA *in vitro*. Yeast and human Rad51p make an extended helical filament — which was first described for bacterial RecA protein (reviewed in [4]) — whereas human Dmc1p forms eight-membered rings [35]. It is not known whether they form different structures when both are present at the same time. There is also strong evidence that Rad51p and Dmc1p interact preferentially with different homologues of Rad54p [36].

HR during meiosis is quite different from mitotic recombination in many respects. In *Saccharomyces*, meiotic recombination is initiated by creating 5′-ended DSBs by a meiosis-specific topoisomerase II-like protein, Spo11p. So far, meiotic DSBs have only been found in *Saccharomyces*, but homologues of Spo11p have been found in *Schizosaccharomyces*, *Caenorhabditis* and *Drosophila*, and recently, in mice [37•,38•]. We await knockouts of the mouse *SPO11* gene to see whether it will prevent meiotic recombination as observed in other organisms.

Another special feature of meiosis is the high level of crossing over associated with DSB-induced recombination. One pair of proteins involved in crossover regulation during

meiosis is Msh4p and Msh5p, homologues of the Msh2–Msh3–Msh6 mismatch repair proteins. Neither Msh4p nor Msh5p affects mismatch repair *per se*; rather, they appear to be involved in the resolution of Holliday junction-containing recombination intermediates. Whether Msh4p and Msh5p are capable of binding to Holliday junctions has not yet been shown. Ironically, Msh2p and Msh6p have been reported to bind to these structures [39], despite the fact that *msh2* deletions do not seem to affect mitotic or meiotic crossing over [5,40].

In mice and *Caenorhabditis*, the absence of the Msh4p and Msh5p homologues is far more severe. In Msh4p/Msh5p-deficient worms, crossing over appears to be completely abolished, although noncrossover events may still take place [41•]. In mice, homozygous deletion of *msh5* completely destroys meiosis [42•,43•]. Thus, as with deletions of *rad51*, *rad50* or *mre11*, the absence of Msh5p in mice has a much more severe phenotypes than in yeast.

Recent analysis of cohesin proteins involved inholding sister chromatids together has interjected another level of complexity into the way meiotic recombination occurs. The Rec8 proteins of *Saccharomyces* and *Schizosaccharomyces* are components of a meiosis-specific cohesin complex that appears to establish associations between sister chromatids. In *Schizosaccharomyces pombe*, the absence of Rec8p causes a marked reduction in recombination, especially in the centromere-proximal regions [44–46]. In *S. cerevisiae*, the defect is even more pronounced, apparently blocking the completion of repair of DSBs between both homologous and sister chromosomes [47•]. These results suggest that cohesin function is required for establishing the chromosomal context necessary for interhomolog recombination, as well as maintaining a proper relationship between sister chromatids.

Nonhomologous end-joining

Some striking advances have been made in the analysis of NHEJ. The Ku70 and Ku80 proteins, as well as DNA ligase IV and its associated XRCC4 protein, are required for end-joining in organisms ranging from yeast to humans (reviewed in [5,48]). As mentioned above, *Saccharomyces* uses the NHEJ pathway more than previously suspected, but only to ligate short complementary ends; yeast are not very effective at joining incompatible ends. Mammals use other microhomologies further from the end to produce joining, but this activity is inefficient in yeast. In addition, yeast lacks the Ku-associated protein kinase catalytic subunit, DNA-PKcs, whereas in mammals NHEJ is strongly influenced by this protein.

Until recently, the only 'programmed' role for the NHEJ system appeared to be in V(D)J rearrangements in the immune system, but two reports have now shown that the absence of DNA ligase IV has profound effects on the maturation of murine brain cells [49••,50••]. As with the immune system, the absence of Ku and DNA-PKcs has less pronounced effects on brain cells than the loss of DNA ligase IV.

In budding yeast, NHEJ also depends strongly on Mre11p, Rad50p and Xrs2p. These three proteins form a complex with DNA-unwinding and nuclease activity. Mre11 and Rad50 homologues are found in vertebrates, and homozygous knockouts are lethal to the cell. A protein of similar size, but with little homology to Xrs2, has also been found, and mutations in this subunit are associated with Nijmegan breakage syndrome. This syndrome is characterized by chromosome instability and cancer-prone phenotypes reminiscent of mutations of the checkpoint regulator, ATM (ataxia telangiectasia mutation). Recently, another human ATM-like disease has been linked to Mre11 [51•]. But it is still not clear whether the defect in these cases is due to the role of Mre11 in homologous or nonhomologous recombination or in the sensing of DNA damage. In fact, the loss of the Mre11 homologue in fission yeast does not affect telomere length or NHEJ, although cells are radiation sensitive [52]. In DT40 cells, the absence of Mre11 affects homologous recombination, but does not affect end-joining [53]. The different requirements for the Mre11 complex in budding and fission yeasts illustrate the necessity of having more than one model system for characterizing the mechanisms of NHEJ. The number of apparently different NHEJ pathways being identified continues to proliferate. For example, in the absence of Mre11p, budding yeast cells exhibit gross chromosomal rearrangements with junctions that lack the microhomology seen in other cases [54•].

There has been a flurry of interest in the role of Sir (silent information regulator) proteins in NHEJ. These proteins are involved in the creation of regions of heterochromatin at telomeres; therefore, the idea that broken ends might become heterochromatic to retard degradation and facilitate NHEJ was attractive. However it is now clear that the effect of knocking out *SIR* genes is largely caused by the unsilencing of cryptic mating-type genes in *Saccharomyces* [2•,55]; cells expressing both mating types have increased HR and decreased NHEJ, although the mating-type-regulated genes that are responsible have not yet been identified.

Checkpoint regulation of the repair of DSBs

DNA damage signals an arrest in cell cycle progression, ostensibly to allow cells more time to repair a DSB, but it is evident that there is more going on than simply providing a longer period of grace prior to mitosis. In budding yeast, DNA damage provokes a dramatic rearrangement of the nucleus. Even a single DSB causes the delocalization of Ku and Sir proteins from telomeres [56•–58•]. This reorganization is dependent on a functional checkpoint system and seems to occur predominantly during S phase. How this occurs is not known. The loss of Ku proteins from telomeres correlates with the arrival of Ku at the site of a DSB (detected by chromatin immunoprecipitation), but it is not at all clear that Ku's participation in NHEJ depends on its release from telomeres.

The absence of the checkpoint genes also causes a change in the way homologous sequences are recruited to repair

DNA damage during both meiosis and mitosis. During meiosis, *rad17*$^{-/-}$ and *rad24*$^{-/-}$ strains exhibit an increased use of ectopically located homologous sequences and sister chromatids in reciprocal recombination [59,60]. There also seems to be a loss of the normal inhibition of sister-chromatid recombination. During mitosis, *rad9*$^{-/-}$ cells show an increase in the formation of translocations by HR [61]. We still need to determine whether sequences actively released from a nuclear matrix search out partners more promiscuously, or whether a premature entry into mitosis leaves some ends with no alternative.

Finally, a connection has been made between the checkpoint gene *ATM* in mammalian cells and recombination. Takeda and colleagues have now knocked out both copies of the *ATM* gene in their DT40 cell line and shown that the radiosensitivity of these cells is due to a deficiency in homologous recombination rather than NHEJ [62•]. Biochemical support for this idea comes from two papers showing that mammalian Rad51p is phosphorylated by c-Abl in an ATM-dependent manner after ionizing radiation [63,64]. Curiously, these authors find that the modified Rad51p is less efficient in *in vitro* strand exchange assays and at forming complexes with Rad52p. This finding is contrary to our expectations and to what is observed *in vivo* in DT40 cells. The question that remains is why should Rad51p become less active at the time when there is DNA damage to repair.

Conclusions and prospects

The interactions and functions of many recombination proteins are being characterized, and considerable progress has been made in learning about the multiple repair pathways in which these proteins participate. From my point of view, one of the most exciting and satisfying developments over the last year has been the rapid emergence of vertebrate model systems to examine HR mechanisms in detail, and the demonstration of great similarity in the way similar events proceed in budding yeast. I notice a growing interest in the importance of recombination, both from cancer researchers and from students of DNA replication. Further progress awaits us in this millennium.

Update

Several papers of interest have appeared recently. The universality of Spo11 control of meiosis is suggested by the finding of two homologues in *Arabidopsis*, which are expressed not only in reproductive cells but to a lesser extent in somatic tissue [65•]. A third report of Spo11 in mouse and human has also appeared [66•].

Control of homologous recombination in mammalian cells has also been investigated by overexpressing UBL1p, a Rad51p- and Rad52p-interacting, ubiquitin-like, protein [67]. Li *et al*. [67] report that UBL1p overexpression down-regulates DSB-induced homologous recombination and makes cells more sensitive to ionizing radiation. In budding yeast, further evidence of a Rad51p-independent pathway requiring Rad59p has been presented [68].

The role of recombination in the expansion of trinucleotide CAG repeats has been studied during yeast meiosis by two groups. Both groups find that these repeats have a higher rate of instability in meiosis than in growing cells [69,70•]. Jankowski *et al*. [70•] make the important observation that the CAG region becomes a prominent site for Spo11p-mediated DSBs and that both large expansions and contractions are frequently found during recombination between two different-sized CAG-containing regions at the same site on homologous chromosomes. A related finding is that CAG repeats show expansions during HO endonuclease-induced mitotic recombination, whereas replication of these sequences only produces contractions [71]. Moreover, an apparent block in repair-associated DNA replication is suppressed by over-expressing the Mre11p-Rad50p-Xrs2p complex.

During nonhomologous end-joining, it now appears that the budding yeast homologue of XRCC4, Lif1p, binds to DNA ends and targets DNA ligase IV to these sites [72]. Further evidence of the importance of this pathway in mammalian cells is provided by Karajawala *et al*. [73], who show that primary dermal fibroblasts of mice exhibit increased rates of chromosome breakage when either Ku86 or Lig4 cells are heterozygous and especially when cells are homozygous null for Ku86

A potentially important finding is that Swi2/Snf2 proteins (whose yeast family members include both the UV-repair Rad16p and the recombination proteins Rad54p and Tdi1p) all contain a domain that specifically recognizes a single- to double-strand transition in DNA structures [74•]. Such structures would be expected to form in many steps of DNA replication, repair and recombination. Interestingly, Rad51p also appears to prefer double-stranded substrates with a single-stranded DNA end [75,76].

Finally, a recent paper has appeared that provides strong evidence of a programmed use of a replication-induced DSB to promote replication repair during the switching of mating-type genes in *S. pombe*. Dalgaard and Klar [77] showed that the production of a DSB by replication across an 'imprinted' site in the mating-type locus is related to the direction of the replication fork traversing the mating-type locus. Arcangioli and de Lahondes [78] identify recombination intermediates reflecting strand invasion and new DNA synthesis that arise from this process.

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