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The search for the right partner: Homologous pairing and DNA strand exchange proteins in eukaryotes

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Abstract. Finding the right partner is a central problem in homologous recombination. Common to all models for general recombination is a homologous pairing and DNA strand exchange step. In prokaryotes this process has mainly been studied with the RecA protein of *Escherichia coli.* Two approaches have been used to find homologous pairing and DNA strand exchange proteins in eukaryotes. A biochemical approach has resulted in numerous proteins from various organisms. Almost all of these proteins are biochemically fundamentally different from RecA. The in vivo role of these proteins is largely not understood. A molecular-genetical approach has identified structural homologs to the *E. coli* RecA protein in the yeast *Saccharomyces cerevisiae* and subsequently in other organisms including other fungi, mammals, birds, and plants. The biochemistry of the eukaryotic RecA homologs is largely unsolved. For the fungal RecA homologs (*S. cerevisiae RAD51, RAD55, RAD57, DMC1; Schizosaccharomyces pombe rad51; Neurospora crassa mei3*) a role in homologous recombination and recombinational repair is evident. Besides recombination, homologous pairing proteins might be involved in other cellular processes like chromosome pairing or gene inactivation.

Key words. Homologous pairing; hybrid DNA; recombination; strand exchange; RecA.

Introduction

A major problem in homologous recombination and generally in life is finding the right partner. In prokaryotes, the central role of the *Escherichia coli* RecA protein in the homology search, homologous pairing, and strand exchange phase of recombination is documented in this issue (Kowalczykowski; Stasiak and Egelman) and has been excellently reviewed elsewhere[51,71,72,94]. Similar proteins must exist in eukaryotes to perform the homolog search during meiotic prophase (Loidl, in this issue) and to provide the enzymatic functions for homologous pairing and DNA strand exchange in recombination and recombinational repair. This review will describe the two approaches that have been taken to identify homologous pairing and DNA strand exchange proteins from eukaryotes. First, the molecular-genetical approach to identify eukaryotic sequence homologs of RecA and second, the biochemical approach to directly purify activities that catalyze RecA type reactions.

Recognition of homology in recombination is almost surely achieved by direct DNA-DNA interactions. The initial probing of homology might involve novel DNA structures, like triple helices, which must be designed for rapid scanning (reviewed in 82). Once homology has been established the current models for general recombination[56,90] foresee a step in which hybrid DNA (also called heteroduplex DNA) is formed. If the hybrid DNA leads to pairing of DNA segments which are genetically marked (mutant and wild type allele), the heteroduplex region will contain mispaired bases. The repair of such mispairs can lead to conversion, the non-reciprocal type of recombination, or restoration of the original situation, whereas failure to repair leads to post-meiotic segregation (see fig. 1; Nicolas and Petes, Fox et al., in this issue). In *E. coli* RecA performs the homology search, the homologous pairing and strand exchange steps during recombination[51,71,72,94]. It is unclear at the moment whether this will also be the case in eukaryotes or whether the different functions of RecA have been segregated into different polypeptides.

Mechanisms of hybrid DNA formation in *Escherichia coli*

In *E. coli* several pathways of recombination have been identified genetically (reviewed in 81). Most of the homologous recombination in this organism is dependent on the RecA protein. The ability of the RecA protein to form hybrid DNA in vitro strongly supports the central role of this intermediate. However, *recA* independent recombination occurs in *E. coli* as well. Hybrid DNA is also an intermediate in these pathways (80, reviewed in 81). The mechanism by which RecA protein forms hybrid DNA is probably the most studied aspect of homologous recombination (see Stasiak and Egelman, Kowalczykowski, in this issue). Briefly, RecA forms helical RecA:DNA complexes with single-stranded or partially double-stranded DNA molecules. These complexes can selectively recognize and bind homologous double-stranded DNA molecules. It is believed that within the enclosure of the RecA filament the two aligned molecules can exchange their strands[33]. RecA independent recombination includes the *RecE* pathway of plasmid recombination and phage λ recombination[81].

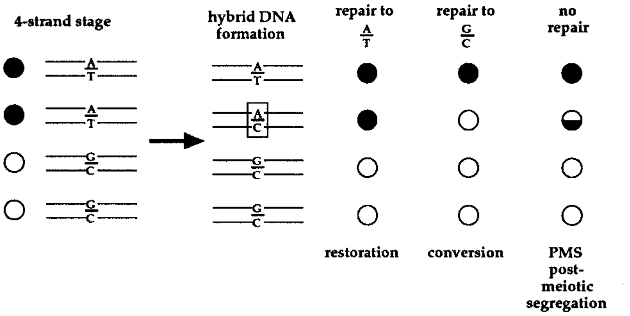

Figure 1. Post-meiotic segregation (PMS): Evidence for the hybrid DNA intermediate in homologous recombination in fungi. Hybrid DNA formation at one genetically marked site is followed. After pre-meiotic S-phase (4-strand stage) two chromosomes carry a hypothetical marker for black colony color (top lanes; A:T base pair), whereas the other two chromosomes carry a mutation in this hypothetical gene (bottom lanes; G:C base pair) leading to white colony color. When asymmetric hybrid DNA is formed at this site, an A:C mispair is formed. This mispair may enjoy three different fates. First, it may be repaired to an A:T base pair, leading to restoration which is undetectable here. Second, it may be repaired to a G:C base pair, leading to conversion (3:1 or 6:2 segregation). Third, it may be left unrepaired, leading to a mixed meiotic product whose genotype will segregate in the first ensuing mitotic division. Therefore, this event is called post-meiotic segregation (PMS). In the yeast *S. cerevisiae* this may lead to a colony with sectors of different color.

Their mechanism of hybrid DNA formation is fundamentally different from RecA. Both pathways, *RecE* and phage λ, employ 5′ to 3′ exonucleases to produce single-stranded tails and a second protein for the formation of the hybrid DNA[28,81]. The mechanism of action is much less understood than that of RecA, but it is apparent that binding of ATP is not required.

Hybrid DNA during recombination in eukaryotes

The phenomenon of postmeiotic segregation (PMS) illustrated in figure 1 is genetic evidence for the hybrid DNA intermediate during recombination in fungi (reviewed in 70). In addition, physical methods detected hybrid DNA during meiotic prophase of *S. cerevisiae*[24,63]. Genetic reasoning similar to the one shown in figure 1 suggests that hybrid DNA is formed during intrachromosomal gene conversion in mammalian cells[7] and in cells infected by the Shope fibroma virus[20]. In the Shope fibroma virus infected cells hybrid DNA was also detected physically[20] and a strand exchange activity catalyzing the formation of hybrid DNA in vitro has been partially purified[95]. Thus, it appears that hybrid DNA is a central recombination intermediate in eukaryotes as well.

RecA homologs in eukaryotes

Sequence comparisons identified several fungal and plant genes as eukaryotic RecA homologs and allowed the targeted isolation of further RecA homologs from vertebrates. The schematic alignment shown in figure 2 reveals the varying degrees of conservation and relatedness between the eukaryotic RecA homologs. Phylogenetic analysis by Ogawa and coworkers[67,77] suggested the existence of two sub-families within the eukaryotic RecA homologs: the Rad51-like (Rad51 of human, mouse, chicken, *S. cerevisiae*, *Schizosaccharomyces pombe*, and Mei3 of *Neurospora crassa*) and the Dmc1-like genes (*S. cerevisiae* Dmc1 and *Lilium longiflorum* LIM15). Within these sub-families sequences not only share the homology to RecA identified as Domain II or 'homologous core' but also significant homology in the more amino-terminal domain I and in the carboxy-terminal region which are unrelated to the RecA sequence (fig. 2). Rad55 and Rad57 from *S. cerevisiae* cannot be included in either sub-family as they lack significant homologies to either the Rad51 or Dmc1 sequences outside the homologous core (fig. 2). Less conservation is evident in the *Ustilago maydis* Rec2 protein (see fig. 2) which shows significant sequence homology to RecA only around the motifs A and B of the ATP binding site in domain II. Another plant homolog, AtRecAhom from *Arabidopsis thaliana*, shows high conservation to RecA throughout the entire sequence.

The homologous core is probably involved in functions common to the RecA homologs. This region shows the best conservation on the primary sequence level and has been shown in RecA to be responsible for functions of DNA repair and recombination[65]. The crystal structure of the RecA protein determined that the major central region which almost exactly coincides with the homologous core of the protein is likely to be responsible for ATP binding and DNA binding[84,85]. Three dimensional modeling on the basis of the RecA crystal structure revealed significant structural conservation between RecA, T4 uvsX and Dmc1, Rad51, and Rad57 (ref. 86). This is confirmed by the low resolution structural analysis of the protein:DNA filaments formed by RecA, Rad51, and T4 uvsX which are highly similar (66, reviewed in 16). Particularly noteworthy is the case of the T4 uvsX protein which shows very weak homology with RecA yet behaves very similarly to RecA in in vitro reactions and is required for recombination in T4 in vivo.

Rad51 sub-family

Members of the Rad51 sub-family of eukaryotic RecA homologs have now been found in two yeast species[1,4,62,76,77], in a filamentous fungus[12], in avians[5], and in two mammalian species[60,77,96]. Therefore, it is likely that this gene has been conserved throughout the evolution of eukaryotes. The proteins differ in size due to NH_2-terminal extensions of different length. The vertebrate proteins are smaller ($M_r \sim 37{,}000$; refs 5, 60, 77, 96) than the yeast proteins (*S. pombe* M_r 39,800; refs 62, 77; *S. cerevisiae* M_r 43,000; refs 1, 4, 76). For the *N.*

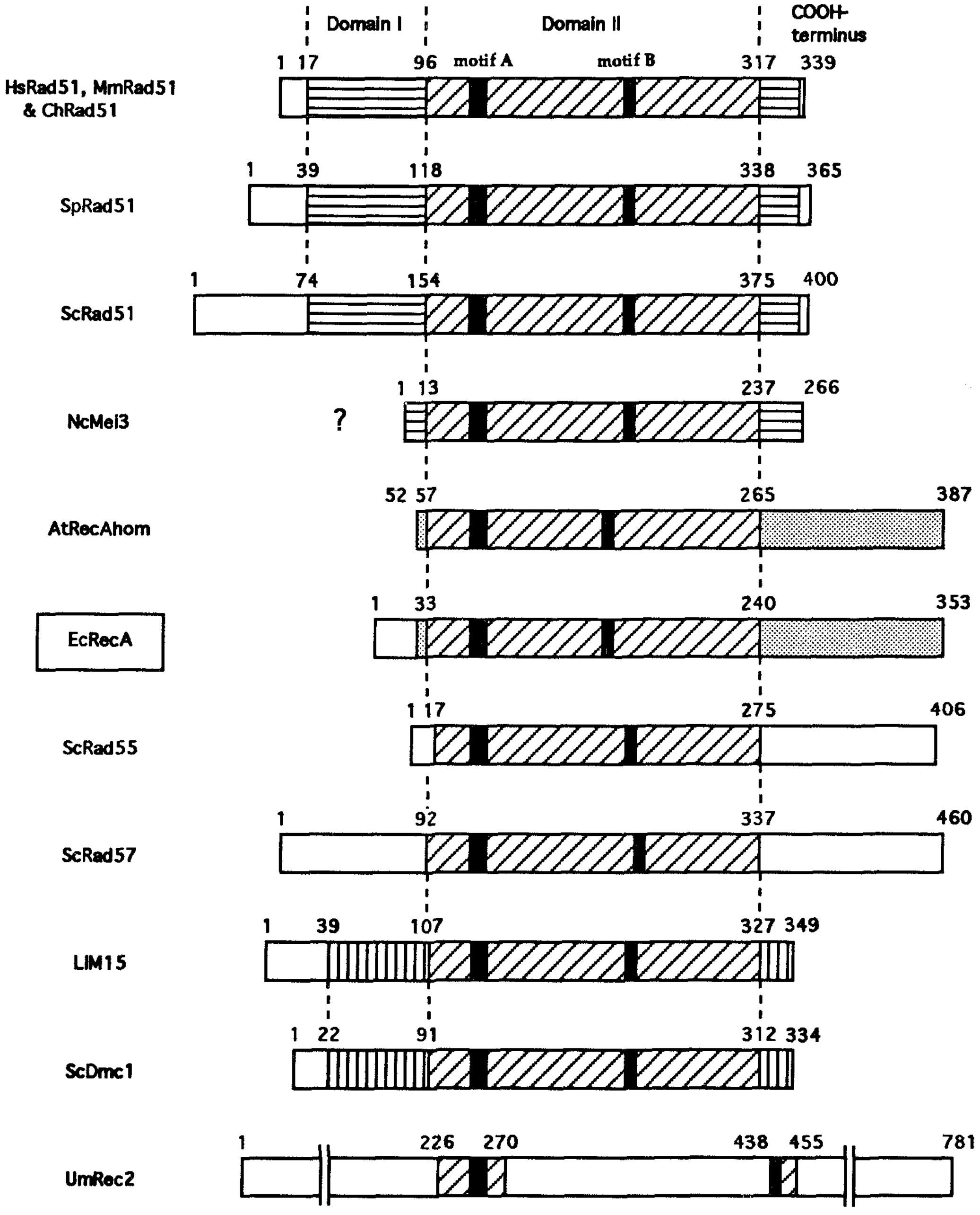

Figure 2. Relation of the eukaryotic RecA homologs to the *E. coli* RecA protein. Diagonal bars (▨) represent Domain II, ('homologous core'), horizontal bars (▤) the region of homology in the Rad51 sub-family, vertical bars (▥) the region of homology in the Dmc1 sub-family, and dots (▦) the homology between *E. coli* RecA and the *A. thaliania* RecA homolog (AtRecAhom). Open bars indicate sequences without apparent homology, and black bars indicate the position of motif A and B for the ATP binding consensus sequence[93]. The sequence representations are almost drawn to scale. The human (HsRad51), mouse (MmRad51), and *S. pombe* (SpRad51) sequences are from ref. 77, the chicken sequence (ChRad51) from ref. 5, the *S. cerevisiae* sequence (ScRad51) from refs 1, 4, 76, the *N. crassa* Mei3 sequence (NcMei3) from ref. 12, the *A. thaliana* sequence (AtRecAhom) from ref. 9, the *S. cerevisiae* Rad55 sequence (ScRad55) from ref. 53, the *S. cerevisiae* Rad57 sequence (ScRad57) from ref. 40, the alignment for the *L. longiflorum* sequence (LIM15) was taken from ref. 77, the *S. cerevisiae* Dmc1 sequence (ScDmc1) from ref. 6, and the *U. maydis* sequence (UmRec2) from W. Holloman (pers. comm.). For NcMei3 the ? denotes the observation that sequences upstream of the proposed initiator codon exhibit significant homology to the Rad51 sub-family suggesting that the real start of the *mei3* open reading frame has not been identified yet (R. Rothstein, pers. comm.). For AtRecAhom only the mature peptide is shown as the first 51 amino acids are cleaved as the chloroplast transit peptide[9]. This figure was expanded based on alignments in figure 7 of Ogawa et al.[67] for the Rad51 and Dmc1 sub-families. Numbers denote amino acid residues.

crassa mei3 gene product the predicted M_r is 29,000 (12, see discussion in legend to fig. 2). Most knowledge about the in vivo function of these proteins comes from genetic analysis in fungi. Mutations in *RAD51* or *mei3* lead to extreme sensitivity to X-rays and the X-ray mimetic drug methyl-methansulfonate in *S. cerevisiae* (reviewed in 22), *S. pombe*[62,67], and *N. crassa* (12 and refs therein). A single double-strand break will kill an *S.*

cerevisiae cell without Rad51 function showing a clear deficiency in double-strand break repair (reviewed in 22, 70). Besides a role in recombinational repair, Rad51 plays a role in general recombination in mitosis (refs 61, 67, 73, 76; reviewed in 22, 70) and meiosis. The *N. crassa mei3* mutation leads to meiotic arrest at pachytene (discussed in 12). In wild type recombination is induced a 100–1000 fold during meiosis and is believed to be important for the correct segregation of the chromosomes[70]. The meiotic recombination phenotype of *S. cerevisiae rad51* cells is complex, but certainly less drastic than in *E. coli* cells lacking RecA. Analysis of the few viable spores made suggested normal intra- and intergenic recombination[61] pointing to other recombination pathways or redundant functions in the same pathway. Other assays designed to analyze the entire population of cells measured a large reduction in intragenic recombination at one locus[61] and essentially no difference to wild type at two other loci[76]. However, meiotic recombination between direct repeats was found to be reduced 20 fold[76]. Candidates for redundant functions are the other RecA homologs of *S. cerevisiae*. No results have been reported for genetic interaction of *RAD51* with *RAD55* or *RAD57*. However, *RAD51* seems to interact genetically with *DMC1* as meiotic induction of intragenic recombination is virtually abolished in the double mutant (A. Shinohara, pers. comm.) whereas the single mutants show comparably little difference to wild type[6,76]. Physical analysis of the DNA during meiotic prophase established that the meiosis specific double-strand breaks, believed to be an early intermediate in meiotic recombination (review in 47), are formed in *rad51* cells[76]. It is unclear whether physical crossover products are made to lesser extent or whether the formation is simply delayed[76]. Unfortunately, no genetic information is available about meiotic intergenic recombination (crossover) in the bulk of the cells. In addition, meiotic development is abnormal in both yeasts lacking Rad51 including induced lethality under meiotic conditions[76], reduced sporulation and reduced viability of the meiotic products[61,67,76]. It is unclear whether these phenotypes are consequences of a primary meiotic recombination defect or point to an additional role of Rad51 during meiosis.

The expression patterns of the mammalian[60,77] and avian[5] Rad51 genes with rather specific expression in meiotic tissues and lymphoid organs suggest a role in meiotic recombination and possibly in recombination during antibody formation and class switching. (See also Bezzubova and Buerstedde in this issue.) The genes might be used in complementation studies in yeast. Partial complementation of the *rad51-1* mutation in *S. cerevisiae* by the mouse *RAD51* gene was reported[60] but the *rad51*Δ mutation is not complemented (cited in 67). The reported interaction of Rad51 with other proteins (see below) might complicate the interspecies complementation.

The ScRad51 protein has been purified from *E. coli* and *S. cerevisiae*. The binding of Rad51 protein to both single-stranded and double-stranded DNA is enhanced by the high energy cofactor ATP. However, only single-stranded DNA supports the ATPase activity of Rad51 which has a four-fold lower turnover than *E. coli* RecA protein[67,76]. Structural studies of the Rad51:double-stranded DNA complex revealed a filament structure surprisingly similar to that of the active RecA:DNA filament[66] suggesting that it might represent a universal structure in biology (reviewed in 16). Interestingly, Rad51 is unable to form filaments on single-stranded DNA whereas RecA can readily do so[16]. This is surprising as only single-stranded DNA supports the ATPase activity of ScRad51 which for RecA is dependent on filament formation (see Stasiak and Egelman, in this issue). Rad51 protein does not (yet?) catalyze the annealing of homologous single-stranded DNA or the formation of joint molecules in the strand exchange reaction shown in figure 3. This might be related to the present difference in ATPase activity and filament formation between RecA and ScRad51. Rad51:Rad51 protein interactions which might be relevant in the filament could be reconstructed using the dual hybrid system[18] for protein:protein interaction (D. Weaver and C. Bendixen, pers. comm.). The results of the structural studies[66] strongly supports the conclusions about the homology between RecA and its eukaryotic homologs (see below; fig. 2). They show that the conservation of the invariant residues in an overall modest conservation is sufficient to maintain the critical active filament structure. It is unclear at present why Rad51 protein does

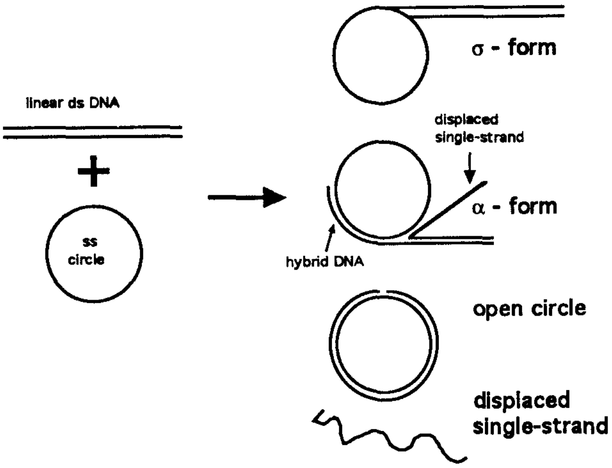

Figure 3. Assay for in vitro hybrid DNA formation: the strand exchange reaction. Linear double-stranded DNA and circular single-stranded DNA, usually linearized replicative form and viral DNA from filamentous phages, respectively, are the substrates. Different structures of joint molecules are shown on the right and can be distinguished by electron microscopic analysis. Most informative are α-forms where the region of hybrid DNA and the displaced single-stranded region can be clearly demonstrated.

not perform the in vitro recombination assays like RecA. Since the protein has now been purified from the cognate host, it seems unlikely that the protein has been purified in an inactive form. It has been suggested that other proteins of the recombinational repair group interact with Rad51 protein[67]. In fact, purified Rad51 protein is retained on an affinity matrix containing Rad52 protein[76]. *RAD52* is another member of the recombinational repair group (reviewed in 22). Mutations in *RAD52* lead to the severest recombination defects in *S. cerevisiae* (reviewed in 70). The structural interaction between Rad51 and Rad52 protein has also been shown by the dual hybrid system defining a Rad51 interacting domain at the carboxy-terminus of Rad52 (ref. 57). Additional genetic arguments from experiments with the *Kluyveromyces lactis RAD52* gene in *S. cerevisiae* strongly suggest a DNA repair/recombination complex including Rad51, Rad52, and possibly other proteins[57].

Dmc1 sub-family

Both proteins in this group, *S. cerevisiae* Dmc1 (predicted M_r 36,600; ref. 6) and *L. longiflorum* LIM15 (cited in 49), are specifically expressed in meiosis which might be the reason for their relatedness. As expected from the presence of similar proteins in this organism (Rad51, Rad55, Rad57) the recombination defects in *S. cerevisiae* lacking Dmc1 are only modest in comparison to *E. coli recA* cells[6]. Genetic interaction with the other *recA* homologs, *RAD51*, *RAD55*, and *RAD57*, remains largely to be tested (see above). Unlike *rad51* cells which undergo meiosis and spore formation to some extent[76], cells lacking Dmc1 arrest in meiotic prophase[6]. This suggests that Dmc1 is not merely a meiotic reinforcement for the Rad51 function. Formation of physical crossover was strongly depressed[6] but it is not clear whether this is due to the arrest or due to the loss of the enzymatic function involved in crossover formation. Dmc1 protein localizes in multiple discrete complexes with the meiotic chromatin before formation of the synaptonemal complex (D. Bishop, pers. comm.) suggesting a direct role in meiotic recombination.

S. cerevisiae Rad55 and Rad57

The *S. cerevisiae* Rad55 and Rad57 proteins (predicted M_r 52,200 and M_r 46,253, respectively) show significant sequence conservation[53,40] to the homologous core of RecA (domain II in fig. 2). For Rad57 the structural significance of this primary homology was confirmed by model building[86]. Both proteins show no significant additional homology to the Rad51 or Dmc1 subfamilies or to each other. The homology in domain II is equally high to both, Dmc1 and Rad51. Therefore, Rad55 and Rad57 cannot be included in either subfamily. *RAD55* and *RAD57* are members of the *RAD52* epistasis group in which mutations lead to X-ray sensitivity and defects in mitotic recombination and meiosis (reviewed in 22). Surprisingly, deletion of *RAD55* or *RAD57* confers cold-sensitive X-ray damage repair suggesting that the proteins might be stabilizing a larger recombinational repair complex[40,52], possibly the one suggested to involve Rad51 and Rad52 (see above). Since the *rad55 rad57* double mutant is indistinguishable from the single mutants[52], Rad55 and Rad57 cannot be simply redundant functions but probably act in the same structure or complex.

Ustilago maydis Rec2

Mutations in the *REC2* gene cause multiple defects in homologous recombination, extreme sensitivity to X-rays, and defects in meiosis[21,32]. This supports the functional significance of the, in comparison, modest sequence relationship of Rec2 to *E. coli* RecA. The *REC2* gene has a predicted product of 781 amino acids with a molecular mass of 84.4 kDa (W. Holloman, pers. comm.). It is unclear at the moment how *REC2* is related to the rec1 activity[48] of *U. maydis* that promotes homologous pairing and strand exchange in vitro (see below).

Arabidopsis thaliana AtRecAhom

This M_r 41,900 plant homolog (predicted mature peptide) shows by far the highest degree of sequence conservation, 52.6% overall identity and 61.8% identity in domain II to *E. coli* RecA[9]. As the protein product is localized in chloroplasts, this nuclear gene is likely to derive from the bacteria giving rise to plant chloroplasts. This gene is apparently also present in pea[9] and might be responsible for the ATP-dependent strand transfer activity observed in pea chloroplast extracts[10]. Anti-RecA antibodies employed for cloning of AtRecAhom reveal a 39 kDa protein in pea chloroplasts[11] which is potentially the gene product. A different potential *A. thaliana* homolog of *E. coli* RecA has been cloned by direct complementation of the repair and recombination defects of *E. coli recA* cells[68]. The sequence of this gene, DT100, suggests again chloroplast localization and reveals only very limited homology with RecA in motif A of the ATP binding site[68].

DNA strand exchange and homologous pairing proteins identified by biochemical criteria

In the pioneering work with *E. coli* RecA a number of in vitro assays for homologous pairing and DNA strand exchange have been developed (reviewed in 51, 71, 72, 94; see also fig. 3). Generally two classes of proteins can be distinguished: ATP-dependent homologous pairing activities which at least in some aspects conform to the RecA paradigm (*U. maydis* rec1) and ATP-independent activities (all others) which catalyze hybrid DNA formation differently. Biochemically these proteins are more similar to enzymes involved in RecA independent recombination of *E. coli* (see above). Only purified proteins will be discussed as reports of activities in extracts and partially purified activities are too numerous to be included. The biochemical properties of some of these proteins have been reviewed previously[17,29].

ATP-dependent homologous pairing proteins

The first homologous pairing protein to be purified from eukaryotes was an activity called rec1 from *U. maydis*[48]. The rec1 activity which is likely to reside in a 70 kDa polypeptide (ref. 48; Kmiec and Holloman, manuscript submitted) is not encoded by the *REC1* gene of *U. maydis* which encodes a M_r 57,000 polypeptide (W. Holloman, pers. comm.; 31). rec1 is the only purified protein from eukaryotes that conforms to the RecA model at least in some respects (reviewed in 17) by showing single-stranded DNA dependent ATPase activity[48]. While the renaturation of homologous single-stranded DNA and DNA strand exchange activity (fig. 3) of rec1 are dependent on ATP as with RecA, the uptake of linear single-strand into supercoiled duplex shows an ATP independent component unlike RecA[48]. rec1 is also distinguished from RecA by the requirement of sub-stoichiometric amounts of protein, probably less than 1 monomer per 200 nucleotides (48; discussed in 17). The relationship of the rec1 activity to the *REC2* gene of *U. maydis* which has certain homologies to *E. coli* RecA (see above) is unclear at the moment.

ATP-independent homologous pairing proteins

Partial purification of the first mammalian homologous pairing protein revealed the unexpected characteristic that formation of joint molecules was catalyzed independent of ATP binding or hydrolysis[34]. While RecA can form joint molecules independent of ATP hydrolysis, ATP binding is necessary to activate the protein for homologous pairing (reviewed in 51). ATP hydrolysis of RecA allows the protein to move through heterologies and to turn over (Stasiak and Egelman in this issue; 51). In the strand exchange reaction which has been most widely used to purify these activities various types of joint molecules (σ-forms, α-forms, open circles; see fig. 3) are formed. They can be easily separated from the substrates by gel electrophoresis and the structure of the joint molecules can be visualized by electron microscopy. Previous discussions of assays[25] suggested a combination of gel electrophoresis and electron microscopy as sufficient for the demonstration of true strand transfer. The argument centers on the demonstration of α-forms where the displaced strand can be visualized (see fig. 3) which has been generally taken as evidence for strand exchange. Recent experiments have shown that under non-standard reaction conditions joint molecule formation and strand exchange can be achieved spontaneously without added protein[69,79]. Reduction of the substrate size from more natural DNA like full size replicative form of filamentous phages (5.4–7.2 kbp) to smaller DNAs[41,55] like short oligonucleotides may lead to serious artifacts[42] because small double-stranded DNA fragments (<400 bp) are prone to denaturation during ethanol precipitation and sample drying[88]. Thus a simple reannealing reaction will mimic complete strand exchange. Even more disturbing are recent observations (Käslin and Heyer, submitted for publication) under standard reaction conditions and M13 size substrates. It was shown that *S. pombe* fatty acid synthase, an enzyme obviously unrelated to DNA metabolism, can catalyze the formation of joint molecules (including α-forms) provided an exonuclease was present before or during the reaction. Since most of the eukaryotic enzymes discussed below have an associated exonuclease activity and act independently of a high energy cofactor, demonstration of their involvement in homologous recombination cannot rest on their biochemical characteristics but must include more direct in vivo evidence.

Human HPP-1

HPP-1 (homologous pairing protein 1) has been purified to apparent homogeneity from a human leukemic T-lymphoblast cell line as a M_r 120,000 polypeptide[58]. The protein catalyzes the strand exchange reaction (fig. 3) in a 3′ to 5′ polarity with respect to the displaced strand which is opposite to that of RecA[58]. This directionality might be dictated by the 3′ to 5′ exonuclease activity present in purified HPP-1[58] but it is unclear whether HPP-1 itself is a nuclease. HPP-1 is required in stoichiometric amounts (1 monomer per 25 bp) and acts non-catalytically[58]. While an earlier fraction in the purification was dependent on ATP[19], the purified HPP-1 protein catalyzed the reaction independent of ATP binding or hydrolysis[58]. However, photoaffinity labeling has shown that HPP-1 binds specifically ATP and addition of exogenous ATP or of the non-hydrolyzable analog $\beta\gamma$-CH_2-ATP interfered with activity[58]. A putative turn-over factor might utilize the HPP-1 bound ATP as the less pure fraction catalyzes strand exchange faster and with only catalytic amounts of protein[19]. HPP-1 may be associated with and is stimulated by stoichiometric amounts of the human trimeric single-stranded DNA binding protein RP-A but not by other non-cognate single-stranded DNA binding proteins[59].

Drosophila melanogaster Rrp1

Rrp1 (recombination repair protein 1) has been purified from *D. melanogaster* embryos using a version of the assay shown in figure 3[55]. It catalyzes the formation of joint molecules at sub-stoichiometric amounts of only 1 monomer per 400 nt single-stranded DNA without requirement for a high energy cofactor[54]. The 670 amino acid protein (M_r 75,000) shows significant sequence homology in its carboxy-terminal 252 amino acids to a class of DNA repair enzymes known as apurinic/apyrimidinic (AP) endonuclease like *E. coli* exonuclease III[74]. It was shown that Rrp1 contains intrinsic 3′ to 5′ exonuclease activity and AP endonuclease activity besides strand exchange and DNA renaturation activity[75]. Compared to exonuclease III the exonuclease activity of Rrp1 is attenuated by about 10 fold but the AP endonuclease activity is qualitatively and quantitatively similar to exonuclease III[64]. Rrp1 is likely to act as an AP endonuclease in vivo as it complements *E. coli xth nfo* mutants which define structural genes for AP endonucleases in protection against oxidative and alkylation damage to the DNA[26]. It is unclear whether the protein plays a role in recombination besides DNA repair as mutants in the gene are unfortunately not available.

Herpes simplex virus 1 ICP8

The M_r 128,000 protein ICP8 of Herpes simplex virus 1, a delayed early protein, is essential for viral replication and is viewed as the viral single-stranded DNA binding protein. Purified ICP8 forms compact filaments on single-stranded DNA but not on double-stranded DNA at a stoichiometry of 1 ICP8 monomer per 10 nt of single-stranded DNA. These ICP:single-stranded DNA complexes can promote transfer of one strand from a duplex DNA molecule to the circle dependent on a divalent cation but independent of a high energy cofactor (reaction shown in fig. 3). The reaction exhibits an unexpected sensitivity to the length of the duplex substrate which needed to be less than full length M13 and favored duplex DNA with resected ends. No formation of a paranemic joint between supercoiled duplex circle and circular single-stranded DNA was observed. Although trace amounts of nuclease contamination were observed, several controls seem to suggest that this contaminant was not responsible for the activity[8]. Herpes simplex is an attractive model as genetic analysis is possible. Besides its known essential role in replication ICP8 is needed for viral gene expression and nuclear localization of the replication complex[23].

S. cerevisiae SEP1, DPA, STPα

S. cerevisiae has been a focus for the isolation of homologous pairing and strand exchange proteins. Three different homologous pairing activities have been purified from *S. cerevisiae* and they are encoded by different genes (reviewed in 17, 29).

The first protein purified from *S. cerevisiae* that effectively catalyzed the strand exchange reaction shown in figure 3 is SEP1, for strand exchange protein 1[50]. It is the most extensively studied homologous pairing protein from eukaryotes and a test case to elucidate the biochemical action of the ATP-independent homologous pairing proteins and their relationship to genetic recombination. SEP1 was initially purified as a proteolytic fragment of M_r 132,000 (ref. 50) but the relevant in vivo form is a M_r 175,000 polypeptide[14,37], here called p175^{SEP1}, whose properties will be reviewed. This full length form can be overexpressed from the cloned gene[37] which has a coding potential for 175 kDa protein[15,92]. p175^{SEP1} is present with high abundance throughout all stages of the life cycle in mitotic and meiotic cells (W.-D. Heyer and R. Kolodner, unpubl. results). Surprisingly, the majority of SEP1 is localized not in the nucleus but in the cytoplasm (A. Johnson and R. Kolodner, pers. comm.) and a complementing SEP1-lacZ fusion has been localized to the nuclear periphery in *S. cerevisiae*[44]. p175^{SEP1} catalyzes efficiently the strand exchange reaction (fig. 3) at a stoichiometry of 1 monomer per 100 nt single-stranded DNA independent of a high energy cofactor[38]. In addition, p175^{SEP1} exhibits exonuclease activity on single-stranded and double-stranded DNA[37-39] and RNA[83], RNAseH activity[83], and is capable of aggregating DNA[38] as well as renaturing complementary single-stranded DNA (E. Käslin and W.-D. Heyer, unpubl. results). Single-stranded DNA is clearly preferred over double-stranded DNA as substrate for the exonuclease. However, it is difficult to judge whether RNA or DNA is the preferred substrate for lack of directly comparable substrates and due to sequence specific pausing of the enzyme[37-39,83]. The 5′ to 3′ double-stranded DNA exonuclease activity is essential for strand exchange and dictates the polarity in this reaction (5′ to 3′ relative to the displaced strand)[37]. Unlike RecA[51,71,72,94] p175^{SEP1} itself has no intrinsic polarity for strand exchange[37,39]. Thus, SEP1 catalyzes hybrid DNA formation fundamentally differently from RecA for it does not require ATP binding or hydrolysis, involves an exonuclease step, and does not exhibit an intrinsic polarity in the reaction. The mode of action might be comparable in some respects to the *recA* independent pathways (phage λ and *E. coli* RecE), described above. The gene encoding SEP1 has been cloned and sequenced five times in four unrelated approaches and is also known as *KEM1*, *RAR5*, *DST2*, and *XRN1* (reviewed in 29, 43). Mutations in the gene lead to pleiotropic consequences which have been interpreted as defects in RNA metabolism (for a recent paper see 35), in the cytosceleton (see ref. 45), and in homologous recombination[15,92]. During mitosis *sep1* mutants show little to no phenotype in intra- or intergenic recombination[15,45,92]. SEP1 is not required for an in vitro reaction catalyzed by cell free extract from mitotic cells between two linear double-stranded DNAs where a 5′ to 3′ exonuclease and a pairing function is required[36]. The mutants are defective in meiosis[3,15,45,92] with a delay during meiotic prophase[3] and specific arrest at pachytene during meiotic prophase (ref. 3; R. Kolodner, pers. comm.). Therefore, genetic analysis of meiotic recombination can only be indirect as the cells do not undergo meiotic cell divisions. These indirect measurements of intragenic meiotic recombination have resulted in diverse observations ranging from reduction[15] over no difference[3] to enhancement[92] in the mutant which may be due to differences in strains, test systems or *SEP1* mutations used. Data in two different test systems for intergenic recombination during meiosis showed a significant reduction (4–20 fold) in the mutant (ref. 3; R. Kolodner, pers. comm.). The meiotic recombination phenotypes could be a side effect of the delay/arrest phenotype in meiotic prophase or might be interpreted as a direct involvement in one of several pathways in meiotic recombination. The pachytene arrest itself cannot be taken as an argument for an involvement in meiotic DNA metabolism as also mutants in genes unrelated to DNA metabolism like *cdc28*, a cell cycle control gene, arrest at pachytene[78]. Further genetic studies including double mutant analysis will have to shed more light on the role of SEP1 in meiotic recombination.

The second strand exchange activity purified from *S. cerevisiae* mitotic cells, DPA for DNA pairing activity, is a M_r 120,000 protein[27]. DPA acts in stoichiometric amounts to renature DNA and requires resected double-stranded DNA substrates in order to catalyze the three strand reaction (fig. 3). The joint molecules formed are limited to 3–5 kb of hybrid DNA while no open circular DNA is produced[27]. Like SEP1[37] DPA lacks intrinsic polarity in strand exchange while the overall polarity in the reaction is dictated by the polarity of the exonuclease used to resect the double-stranded DNA ends[27]. DPA is identical to the translation elongation factor EF-3 (K. McEntee, pers. comm.), an essential translation component in *S. cerevisiae* (reviewed in 30). A role in recombination for DPA is therefore highly unlikely.

The third homologous pairing protein from *S. cerevisiae*, STPα for strand transfer protein α, has been purified as a 38 kDa protein from meiotic cells using the assay shown in figure 3[87]. It acts in sub-stoichiometric amounts in the presence of high and stoichiometric amounts of single-stranded DNA binding proteins independent of any high energy cofactor[87]. The STPα activity shows a >15 fold increase during meiotic prophase[87] due to an unknown circumstance since the STPα protein level does not exhibit a concomitant increase[13]. Recombination in cells lacking STPα is normal for intrachromosomal deletion formation, mitotic intragenic recombination, and meiotic intergenic recombination. Only meiotic intragenic recombination between two *his1* heteroalleles was found to be reduced[13]. The significance of this reduction is difficult to understand as the mutants show no decrease in spore viability, a common trait for mutations affecting meiotic recombination (reviewed in 70). The gene encoding STPα[13] is identical to the transcription elongation factor SII (TFIIS) of *S. cerevisiae* (discussed in 46). Given the phenotypes of cells lacking STPα and the well established biochemistry of TFIIS a role in recombination seems highly unlikely for STPα. The biochemical activity, strand exchange, used to purify this protein might be relevant for its action during transcription in liberating the transcript from the DNA substrate.

S. pombe HPP, p140^{exo2}

Besides *S. cerevisiae* the fission yeast *S. pombe* is uniquely suited to study the in vivo function of purified proteins by reverse genetics. Using a version of the assay shown in figure 3 with a mixture of shorter double-stranded DNA substrates, Arai et al.[2] purified from mitotic *S. pombe* cells an activity requiring three proteins called homologous pairing-promoting protein (HPP). The activity is dependent on a divalent cation but independent of a high energy cofactor for the formation of joint molecules. Three proteins are required for maximal activity at high stoichiometry and at an optimal ratio of about 1:2:1 of a 30 kDa, 65 kDa, and a 100 kDa protein, respectively. The 65 kDa polypeptide alone is capable of low level homologous pairing which is enhanced 4–5 fold by the other proteins. Although DNAse activity has not been reported for the purified fractions, a minor contamination has not been directly excluded. The exact structure of the joint molecules is unclear in the absence of electron microscopy.

Another homologous pairing and strand exchange activity has been purified from mitotic *S. pombe* cells using the assay shown in figure 3 (Käslin and Heyer, submitted for publication). This protein, termed p140^{exo2}, is identical to exo2 of *S. pombe* an exonuclease previously purified from meiotic cells[89]. The protein is homologous to SEP1 from *S. cerevisiae* (P. Szankasi and G. R. Smith, pers. comm.) and exhibits nearly identical nuclease activities (ref. 89; Käslin and Heyer, submitted for publication). p140^{exo2} catalyzes the formation of joint molecules similar to SEP1 independent of ATP with a requirement of stoichiometric amounts of protein (one monomer p140^{exo2} per 40 nt single-stranded DNA). Electron microscopic analysis of reaction products demonstrated the existence of α-form molecules (shown in fig. 3) indicative of strand exchange. Preliminary results with strains lacking p140^{exo2} indicate that they are able to sporulate with some loss of viability (P. Szankasi and G. R. Smith, pers. comm.).

Are the non-RecA-type proteins involved in homologous recombination?

The two approaches for isolation of proteins that are involved in hybrid DNA formation in eukaryotes have not merged pointing to the same proteins. *U. maydis REC2* is a possible exception. While an involvement in recombination and recombinational repair is evident at least for the RecA homologs from the yeasts, the role of the biochemically identified strand exchange proteins in these processes is much less evident at present. In the two cases (*S. cerevisiae* DPA, STPα) where the relevant in vivo function has been elucidated it is unrelated to recombination. The existence of multiple recombination pathways has been established in *E. coli*[81] and in *S. cerevisiae*[70]. Therefore, the interpretation of results from single mutants is not straightforward in light of possible multiple pathways and redundant functions. Yet only genetic data can support a role of the biochemically identified proteins in homologous recombination, since the assay used for their purification cannot provide certainty on their in vivo role.

Biological role of homologous pairing proteins

One might speculate about the involvement of homologous pairing proteins in other cellular processes which might use DNA pairing in their biological function. Pre-meiotic chromosome pairing culminating in the formation of the synaptonemal complex and its relation to meiotic recombination is discussed by Loidl (in this issue). It is unclear whether the same functions are used for chromosome pairing and meiotic recombination. Gene inactivation triggered by recognition between DNA repeats almost surely involves direct DNA:DNA pairing necessitating homologous pairing proteins as discussed by Rossignol and Faugeron (in this issue). Other trans-sensing effects are evident in diptera which show somatic pairing of chromosomes. They result in changes of gene expression in transvection and possibly related phenomena in mammals[91].

Homologous pairing proteins are obviously essential for genetic recombination and recombinational repair. Structural RecA homologs (see fig. 2) have now been isolated from all groups of eukaryotes and can be assumed to be universal. In yeasts the RecA homologs are clearly involved in genetic recombination although many details remain unsolved. The functional biochemistry of these proteins is largely not understood and rests heavily on the structural homology to *E. coli* RecA. One might expect differentiation of the functions that are all integrated in RecA (homology search, homologous alignment, strand exchange) into several proteins in eukaryotes. Differential expression of some RecA homologs is already evident from *S. cerevisiae* Dmc1 and Lily LIM15. While eukaryotes have clearly retained RecA like functions for homologous recombination, recombinational repair, and maybe other cellular processes, it is unclear at the moment whether the ATP-independent eukaryotic strand exchange proteins are involved in these processes. Understanding these potentially central recombination proteins will greatly advance our knowledge about recombination and recombinational repair in eukaryotes. This will lead to a more informed use of these processes in gene manipulation.

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