Biochimie (1991) 73, 163–176
© Société française de biochimie et biologie moléculaire / Elsevier, Paris

An overview of homologous pairing and DNA strand exchange proteins

AK Eggleston, SC Kowalczykowski*

Department of Cell, Molecular, and Structural Biology, Northwestern University Medical School, 303 East Chicago Ave, Chicago, IL 60611 USA

(Received 2 January 1991; accepted 8 February 1991)

Summary — Processes fundamental to all models of genetic recombination include the homologous pairing and subsequent exchange of DNA strands. Biochemical analysis of these events has been conducted primarily on the recA protein of *Escherichia coli*, although proteins which can promote such reactions have been purified from many sources, both prokaryotic and eukaryotic. The activities of these homologous pairing and DNA strand exchange proteins are either ATP-dependent, as predicted based on the recA protein paradigm, or, more unexpectedly, ATP-independent. This review examines the reactions promoted by both classes of proteins and highlights their similarities and differences. The mechanistic implications of the apparent existence of 2 classes of strand exchange protein are discussed.

genetic recombination / DNA strand exchange / homologous pairing / recA protein

Introduction

The homologous pairing and exchange of DNA strands is a central step in all general recombination pathways, yet the specific mechanism by which homology is recognized, DNA is paired, and strands are exchanged remained obscure until the purification of the *Escherichia coli* recA protein [1–3]. This protein promotes both the renaturation of complementary ssDNA and the exchange of DNA strands between homologous ssDNA and dsDNA molecules. Although both *in vitro* reactions reflect different aspects of models for genetic recombination, the distinctiveness of the strand exchange reaction has made it the focus of recombination studies. The DNA strand exchange reaction promoted by recA protein involves multiple steps, including coating of ssDNA by recA protein, homologous alignment and nascent exchange of DNA strands, and polar extension of the heteroduplex DNA joint [4]. Since recA protein is a DNA-dependent ATPase, the exchange of DNA strands is normally accompanied by the hydrolysis of ATP [1–3]. As might be expected for a complex reaction, DNA strand exchange between ssDNA and dsDNA substrates is stimulated by an auxiliary factor, the *E coli* ssDNA binding protein (SSB protein) [5, 6].

Given the importance of the recA protein-promoted DNA strand exchange reaction, homologous pairing activities were initially sought and purified from organisms as diverse as bacteriophage T4 [7–9], *Proteus mirabilis* [10], *Bacillus subtilis* [11], and *Ustilago maydis* [12]. Although differing in some respects, they behaved much like the *E coli* recA protein, particularly in their requirements for either ATP or dATP as an obligatory cofactor and for stoichiometric amounts of protein. Recently, however, strand exchange (or strand transfer) proteins that deviate from the recA protein model have been isolated from mammalian [13, 14], *Drosophila melanogaster* [15–17], and *Saccharomyces cerevisiae* [18–21] cells. Their most striking properties are that they promote DNA strand exchange without the need for a nucleoside triphosphate cofactor and can function at nearly catalytic concentrations. These apparent contraventions of the recA protein paradigm raise interesting questions regarding the molecular details of the DNA strand exchange mechanism. By comparing and contrasting the properties of and the reactions promoted by these proteins, further insight into the roles of nucleotide cofactors and accessory protein compo-

---

*Correspondence and reprints
Abbreviations: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; bp, base pair; kb, kilobase pair; nt, nucleotide; kDa, kilodaltons; ATPγS, adenosine 5'-*O*-(3-thio-triphosphate); AMP-PNP, adenylyl β,γ-imidophosphate

nents may be gained. This knowledge is likely to lead to a more holistic appreciation of the biochemical steps which comprise the cellular recombination process. Consequently, this article will briefly review properties of the protein-dependent homologous pairing and DNA strand exchange reactions catalyzed by recA and other ATP-utilizing proteins, then the similarities and differences of those reactions promoted by proteins which have no requirement for an ATP cofactor will be examined. To facilitate this comparison, the relevant properties of the proteins discussed are summarized in table I.

ATP-dependent homologous pairing proteins

Escherichia coli *recA protein*

The *E coli* recA protein (M_r 37 842 Da) has several characteristic biochemical activities *in vitro*: 1), ss- and dsDNA binding; 2), ss- and dsDNA-dependent NTP hydrolysis; 3), ssDNA renaturation and aggregation; 4), joint molecule formation and DNA strand exchange; and 5), lexA and lambdoid phage repressor protein cleavage [1–3]. It effects the exchange of DNA strands by a sequential multi-step process [4]. Initially, recA protein binds tightly and cooperatively to ssDNA in a step termed presynapsis. ATP or the essentially non-hydrolyzable ATP analogue, ATPγS, enhances the stability of this interaction [22] and is required for the formation of a complex that is active in DNA pairing [5, 23]. This complex saturates at a stoichiometry of 1 recA protein monomer/3-4 nt of ssDNA and hydrolyzes ATP at a rate of ≈ 25–30 ATP molecules/min/recA protein monomer [24–26]. The stability and functionality of the presynaptic complex are sensitive to the presence of ADP and are disrupted when the ratio of ADP to ATP exceeds 1:≈ 2-3 [26–28].

Table I. Properties of known homologous pairing proteins.

Protein	Organism	M_r [a]	Interaction with ATP: bind	hydr	req	stoich [b]	mode [c]	pairing end bias [d]	joint extension [e]	renat [f]	co/aggreg [g]	accessory factors [h]	stoich [i]	assay [j]
ATP-Dependent Proteins:														
recA	E. coli	38	Y	Y	Y	3	S	none [k]	5' to 3'	Y	Y/Y	SSB	3	fb,jm,ns,se,em
recA	P. mirabilis	38	Y	Y	Y	1.5	S	ND	ND	ND	ND	ND		fb,jm,se
recE	B. subtilis	42	Y [l]	Y	Y	ND	S	ND	ND	ND	ND	SSB	ND	jm,se
uvsX	Phage T4	44	Y	Y	Y	3-5	S	5'	5' to 3'	Y	Y/ND	G32P	8	fb,jm,em
												uvsY	3-5	
HPP	U. maydis	110	Y	Y	Y	2000 [m]	C	3'	3' to 5'	Y	ND	RPA-like	2000	fb,jm,ns
ATP-Independent Proteins:														
Protein	Organism	M_r	Interaction with ATP: bind	hydr	req	stoich	mode	pairing end bias	joint extension	renat	co/aggreg	accessory factors	stoich	assay
STP	D. melanogaster	105	N	N	N	400	C	ND	ND	ND	Y/Y	ND		jm,se,em
SEP	Human B-cells	ND	ND	N	N	ND	ND	3'	ND	ND	ND	ND		jm,em
HPP-1	Human T-cells	120	Y [n]	N	N	50 [o]	S	3'	3' to 5'	ND	ND	RPA, others	ND	jm,se,em
SEP1	S. cerevisiae	170	N	N	N	12	S	none	5' to 3'	Y	ND	RPA1 subunit	12	jm,em
												SF1	725	
DPA	S. cerevisiae	120	ND	N	N	ND	S	none	none	Y	ND	ND		jm,em
STPa	S. cerevisiae	38	ND	N	N	ND	ND	ND	ND	Y	N/ND	ySSBs	2000	jm,em
STPb	S. cerevisiae	180	ND	N	N	ND	ND	ND	ND	ND	ND	ySS8s	2000	jm,em

ND/ not determined. [a]kDa. [b]Optimal stoichiometry of homologous pairing protein (nt ssDNA/protein monomer) in the absence of any other protein. [c]Mode of action (Stoichiometric or Catalytic) in the absence of any other protein. [d]Preferred end (5' or 3') of displacement of the non-complementary strand during initial pairing. [e]Polarity of extension of the heteroduplex joint relative to the displaced non-complementary strand. [f]Protein-mediated renaturation of complementary ssDNA. [g]Ability to either coaggregate ssDNA and dsDNA or to aggregate ssDNA. [h]Accessory factor(s) which stimulate the pairing protein. [i]Optimal stoichiometry of homologous pairing protein (nt ssDNA/protein monomer) in the presence of the respective stimulatory factor. [j]Assay used to determine activities: se = gel assay with production of form II molecules; em = electron microscopy with visualization of the displaced strand; ns = nuclease sensitivity assay; jm = gel assay with production of joint molecule intermediates; fb = filter binding assay with retention of joint molecules. [k]Results depend on the particular substrates and reaction conditions used (see [27, 38, 35, 59, 100]). Some of this disparity may be attributable to the differences in experimental technique; the former two references employed filter-binding assays and electron microscopy, whereas the latter three references used agarose gel assays. [l]Although ATP is bound by this protein, only dATP will bind to and activate nucleotide hydrolysis and DNA strand exchange. [m]Value based on the estimate of the proportion of HPP in the partially purified fraction. [n]Based on uv crosslinking by the ATP analogue, 8-azido ATP. [o]Expressed as nt dsDNA/protein monomer.

In the next phase of DNA strand exchange, synapsis of the DNA substrates, the extended helical ssDNA-recA protein filament is an active participant in pairing with another DNA molecule to form a nucleoprotein complex containing 2 DNA molecules. If the second DNA molecule is single-stranded and complementary, recA protein promotes renaturation of the strands, but this process requires that the ssDNA be no more than 10–15% saturated with recA protein and that SSB protein be absent [5, 29, 30]. If the second DNA molecule is double-stranded and complementary, recA protein promotes the formation of homologously paired DNA [23, 31]. These homologously paired joint molecules are detected experimentally either by a nitrocellulose filter assay [23, 31] or by an agarose gel assay [32]. In the former assay, the retention of labeled dsDNA on the filter is dependent on its pairing with homologous ssDNA as only ssDNA is retained under the conditions used, while in the gel assay, joint molecules are detected as an intermediate species with lower mobility than the parental DNA substrates due to the complexation of the DNA molecules by recA protein. With topologically constrained substrates (*ie* neither participant contains either a free end or a region of homologous ssDNA), only paranemic joints whose strands are not interwound can be formed; these molecules are unstable when deproteinized [33, 34]. In the absence of topological barriers to the interwinding of strands, however, plectonemic joint molecules, which are stable in the absence of protein, are produced. Joint molecule formation between circular ssDNA and linear dsDNA displays essentially no preference for pairing at either dsDNA end, but a distinct preference for pairing at the dsDNA end containing the 3' complementary strand is exhibited in the presence of ADP [27]. In contrast, pairing between linear ssDNA and supercoiled DNA demonstrates a 5- to 10-fold preference for complementarity at the 3' end of the ssDNA in the absence of ssDNA binding protein (SSB protein), and a 50-fold preference in its presence [35]. In addition to these homology-dependent reactions, recA protein can both aggregate non-homologous ssDNA and coaggregate non-homologous ss- and dsDNA [36].

Following formation of the joint molecule, the nascent heteroduplex DNA joint is extended. This phase requires the continual hydrolysis of ATP and proceeds at a rate of ≈ 10 bp/s [32], ultimately resulting in heteroduplex DNA containing 1 strand from each of the parental molecules. Thus, in the most typical strand exchange reaction between circular ssDNA and linear dsDNA, the products are a nicked circular (form II) molecule and a linear ssDNA molecule. For recA protein, heteroduplex DNA extension is polar, proceeding in a 5' to 3' direction relative to the displaced (non-complementary) strand of the duplex molecule [37–39]. The polarity of recA protein-dependent reactions almost certainly stems from the polar (5' to 3') polymerization of the protein on ssDNA [40].

Although the formation of a plectonemic joint molecule may imply that the exchange of DNA strands has occurred concurrent with displacement of the non-complementary strand of the duplex DNA, recent results which suggest the formation of joint molecules containing putative triple-stranded DNA structures potentially refute this simplistic view [41–43]. The physical exchange of DNA strands can be readily demonstrated in 3 ways: 1), by the formation of an S1 nuclease- or P1 nuclease-sensitive strand displaced from the labeled parental dsDNA [32]; 2), by the appearance of form II molecules in the agarose gel assay using circular ssDNA and linear dsDNA substrates [32]; or 3), by the visualization of a displaced single-strand using electron microscopy [37]. Consequently, the detection of joint molecules by means of either the nitrocellulose or the gel assay permits definition of a homologous pairing protein (provided that contaminating nuclease, helix-destabilizing, and helicase activities are absent), whereas classification as a DNA strand exchange protein requires positive demonstration of strand displacement by at least 1 of these 3 experimental criteria.

The SSB protein of *E coli* (M_r 18.9 kDa) affects nearly all of the recA protein-dependent processes to some extent, although the type and magnitude of the effect are dependent on both the reaction studied and the experimental conditions. Thus, SSB protein inhibits recA protein-promoted DNA renaturation [30]; has little effect on the 4-stranded DNA exchange reaction between gapped and linear duplex DNA molecules [44]; and generally stimulates ssDNA-dependent ATPase activity [25, 45, 46], joint molecule formation [5, 6], and DNA strand exchange [32]. Inhibition of renaturation results from disruption of recA protein-dependent aggregation of ssDNA, a likely reaction intermediate [36]. The lack of an effect on the 4-stranded reaction presumably reflects the relatively low concentration of ssDNA present [44]. The basis of the stimulatory effect of SSB protein is quite complex [1–3, 47]. Enhancement of joint molecule formation occurs only at suboptimal concentrations of recA protein, relative to the ssDNA concentration, and results from an alleviation of the inhibitory effects of excess ssDNA; stimulation is maximal at ≈ 1 SSB protein monomer/20 nt, with higher concentrations of SSB protein being inhibitory [5, 6]. In contrast, stimulation of complete DNA strand exchange (*ie* that which results in the production of form II DNA molecules) by SSB protein occurs at saturating recA protein concentrations [32]. This stimulation has, at least, a presynaptic component because it coincides with stimulation of the recA protein ssDNA-dependent ATPase activity [25, 46] due to the melting of regions of secondary structure

within the ssDNA which are otherwise inaccessible to recA protein [25, 48, 49]. Under conditions favorable for DNA strand exchange, recA protein subsequently displaces the bound SSB protein to form a saturated nucleoprotein filament [40, 49, 50]. This saturated complex is more stable than the discontinuous complex formed in the absence of SSB protein [50], and its formation can be transiently elicited by preincubation of the presynaptic complexes at low (1 mM) Mg^{2+} concentrations in the absence of SSB protein [25, 48–51]. The different roles of SSB protein in joint molecule formation and extensive DNA strand exchange presumably reflect the need for longer stretches of contiguously bound recA protein in the latter process than for the smaller stretches in the former process. These effects of SSB protein are nonspecific because other helix-destabilizing proteins from *E coli*, bacteriophage T4, and yeast are also able to stimulate joint molecule formation [6, 52], ssDNA-dependent ATPase activity [25], and DNA strand exchange [52–54]. In addition to SSB protein, the recBCD enzyme of *E coli*, a DNA helicase, stimulates recA protein-dependent heteroduplex DNA formation between pairs of DNA substrates which are normally unsuitable for use by recA protein alone [55–57]; the helicase activity of recBCD enzyme serves an essential role as an initiator of these reactions.

To address the question of how the homologous pairing and exchange of DNA strands can be accomplished by proteins which are either ATP-dependent or ATP-independent, the role of ATP hydrolysis in these reactions must be understood; this has been accomplished by examination of the effect of ATP binding and hydrolysis on the DNA binding properties of recA protein. The binding of ATP by recA protein induces a high-affinity DNA binding state that represents the 'active' form of the protein which is proficient in both DNA strand exchange and repressor cleavage (see [3]). The resultant high DNA binding affinity state enables recA protein to both displace SSB protein and underwind duplex DNA. When the bound ATP is hydrolyzed, this allosteric change is reversed, the affinity of the resultant ADP-recA protein-DNA complex is reduced, and recA protein can potentially dissociate from the ssDNA. Thus, at least one role of ATP in the recA protein-mediated strand exchange reaction is as a cycling factor whose binding and hydrolysis modulate the affinity of recA protein for ssDNA.

The specific mechanism by which the free energy derived from the ATP hydrolysis cycle is transduced into a force which drives heteroduplex DNA formation remains a topic of discussion (see [3]). Although it was tacitly assumed that ATP hydrolysis was necessary for DNA heteroduplex formation, the discovery that substantial heteroduplex formation occurs in the absence of ATP hydrolysis undermines this restriction. RecA protein which is locked in the high affinity conformation by the binding of ATPγS is competent in the formation of plectonemically joined molecules containing an average of 2.4–3.4 kb of heteroduplex DNA [58, 59]. It has been proposed that, with ATPγS bound to recA protein, the complex of ssDNA–recA protein–dsDNA is arrested in a transition state due to the inability of recA protein to turn over, and that disruption of the protein filament results in the release of the exchanged DNA strands. Thus, the energy of ATP hydrolysis is not utilized, *per se*, in the exchange of DNA strands. Instead, only the induction of the functionally active, high-affinity DNA binding mode is required for the steps preceding and including joint molecule formation. This suggests that ATP hydrolysis may serve solely in the release of product through the polar dissociation of recA protein so that the protein can be utilized in another round of recombination. Both the formation of heteroduplex DNA exceeding ≈ 3 kb in length and the polarity of exchange apparently require ATP hydrolysis, although the mechanistic basis for this requirement remains unclear [3]. The implication of this interpretation with regard to the ATP-independent proteins is that ATP hydrolysis in fact is not necessary for homologous pairing and DNA strand exchange, although since these proteins may not necessarily recycle effectively or demonstrate directionality, they may lack some intrinsic properties which characterize the ATP-dependent protein-mediated reaction.

Proteus mirabilis *recA protein*

The existence of recA-like proteins throughout the bacterial kingdom was confirmed by the isolation of genes from many bacterial species which complement the UV-sensitivity of *E coli* strains containing recA mutations. DNA sequencing of these genes revealed considerable sequence identity to the *E coli* recA protein, confirming the genetic complementation studies and demonstrating considerable evolutionary conservation [60]. Of these prokaryotic recA protein analogues, only the proteins from *P mirabilis* and *B subtilis* have been extensively purified and characterized.

The amino acid sequence of the *P mirabilis* recA protein (M_r 38, 176 Da) is 73% identical to that of *E coli* [61]. Consequently, it is not surprising that the *P mirabilis* protein has ssDNA-dependent ATPase [10], DNA strand exchange [10], and lexA repressor cleavage activities [62]. The protein promotes the formation of D-loop molecules between ssDNA and supercoiled DNA and catalyzes reciprocal strand exchange between gapped and linear dsDNA substrates. In contrast to the *E coli* protein, the *P mirabilis* recA protein does not effect the complete exchange of DNA strands between circular ssDNA and linear

dsDNA when *E coli* SSB protein is present; only intermediate joint molecules are detected. Consistent with this observed inhibition of DNA strand exchange, SSB protein also substantially reduces the ATPase activity of *P mirabilis* recA protein, which suggests that this protein, unlike that from *E coli*, can only partially resist displacement by SSB protein from ssDNA.

Bacillus subtilis *recE protein*

Although evolutionarily distant from *E coli*, *B subtilis* encodes a 42-kDa polypeptide, the recE protein, which shares several properties with recA protein, such as ssDNA binding, nucleotide hydrolysis, and lexA protein cleavage [11]. These common activities are reflected in the conservation of nucleotide sequences of the 2 genes; the *B subtilis recE* gene has 60% identity with the *E coli recA* gene [63]. The most notable feature of this protein is that, despite its homology to recA protein, it is unable to hydrolyze ATP; instead, dATP is stringently required for the functioning of the recE protein. The dATPase activity is ssDNA-dependent, has a turnover number ≈ 65% of that of *E coli* recA protein, and has a similar apparent K_m. ATP acts as an inhibitor of both the dATPase and the DNA strand exchange activities; it can, however, support a low level of lexA protein cleavage. In the presence of both dATP and *E coli* SSB protein, recE protein promotes proficient DNA strand exchange as measured by the agarose gel assay, with ≈ 60% conversion to form II product molecules compared to nearly 100% for the *E coli* recA protein.

Bacteriophage T4 uvsX protein

In the genetically well-defined recombination system encoded by the bacteriophage T4, DNA strand exchange is mediated by the uvsX protein. UvsX protein resembles recA protein in that it has a similar M_r (43 760 Da), binds stoichiometrically (1 monomer/3–5 nt) and cooperatively to both ss- and dsDNA, has ssDNA-dependent (d)ATPase and DNA renaturation activities, and promotes both joint molecule formation and strand displacement [7–9, 64]. Its amino acid sequence, however, is the most divergent of the prokaryotic DNA strand exchange proteins known, sharing only 23% sequence identity and an additional 15% conservation with the *E coli* recA protein [65]. The ATPase activity of uvsX protein is unusual in that it generates both ADP and AMP; whether this unique property is significant mechanistically is unknown. The uvsX protein-ssDNA filament is stabilized in the presence of ATP or ATPγS [7], suggesting that a nucleoside triphosphate functions as an allosteric effector in this system also. Although synapsis is relatively inefficient in the absence of accessory factors (see below), both paranemic and plectonemic joint molecules are formed with roughly equal efficiency [66]. Concentrations of uvsX protein which exceed saturation of the ssDNA display reduced rates of joint molecule formation as a result of protein binding to the dsDNA [66]. The turnover of ATP by uvsX protein is ≈ 8-fold greater than that of recA protein (240 ADP and 145 AMP molecules generated/min/monomer) [8], and the rate of branch migration is somewhat higher (15 bp/s) [67, 68]. Thus, models proposed for uvsX protein function envision the uvsX protein-ssDNA filament as being more dynamic than the recA protein-ssDNA filament, with subunit turnover within the filament occurring more frequently as a consequence of ATP hydrolysis (*eg* as in treadmilling models) [68, 69].

T4 phage encodes a 33.5-kDa helix-destabilizing protein analogous to SSB protein, termed gene 32 protein (G32P), which binds tightly and cooperatively to ssDNA. Excess G32P inhibits both the ATPase and the joint molecule formation activities of uvsX protein, but, as in the *E coli* model, stoichiometric amounts of G32P (1 monomer/8–12 nt) allow nearly 100% joint molecule formation by subsaturating concentrations of uvsX protein (1 monomer/8 nt ssDNA) [7, 70, 71]. Limiting concentrations of G32P increase both the rate and the extent of formation of joint molecules that are almost exclusively plectonemic, yet the rate of ATP hydrolysis is decreased by > 50% [70]. Substitution of SSB protein for G32P results in slower (by at least 5-fold) joint molecule formation at subsaturating concentrations of uvsX protein and in ≈ 25% of the molecules remaining paranemically joined [70]. Although uvsX protein coaggregates non-homologous ss- and dsDNA, coaggregates are not detected under conditions which are optimal for joint molecule formation or in the presence of G32P [70]. Analysis of DNA strand exchange between circular ssDNA and linear dsDNA is complicated by the formation of homology-dependent DNA networks. Strand exchange, as measured by network formation, is stimulated 5- to 10-fold by G32P [68]. SSB protein can stimulate the production of both form II molecules and homology-dependent DNA networks by uvsX protein, although the stimulation is 15-fold less than for G32P [8]. In the presence of G32P, the polarity of pairing and strand displacement is 5' to 3' relative to the displaced strand, as inferred from gel assays employing either linear dsDNA blocked with heterologous sequences at the ends [7] or completely homologous dsDNA [68], respectively. DNA strand exchange requires continuous ATP hydrolysis, since the addition of ATPγS inhibits an ongoing reaction, but only after a transient acceleration [8, 68]. Although the *E coli* SSB protein has an important presynaptic role, no equivalent func-

tion for G32P is postulated; a proposal for the role of G32P in DNA strand exchange lies in stabilization of the displaced strand, either directly or indirectly [71].

In addition to G32P, bacteriophage T4 encodes 2 proteins that stimulate the uvsX protein specifically (*ie* they do not affect recA protein activity). The uvsY protein (M_r 16 kDa) binds cooperatively to both ss- and dsDNA [72], and it stimulates strand exchange ≈ 3-fold by interacting with uvsX protein at molar ratios (uvsY:uvsX) ranging from 1:1 [73], 1:3 [69], or 1:10 [72]. UvsY protein increases the rate of ATP hydrolysis by uvsX protein 2- to 3-fold under sub-optimal conditions (*eg* in the presence of either excess G32P or high salt) [69, 72, 73]. By increasing the apparent affinity of uvsX protein for ssDNA [73], uvsY protein allows uvsX protein to resist displacement from ssDNA by G32P [72]. Thus, similar to the *E coli* system, enhancement of ssDNA binding affinity, particularly relative to the cognate DNA binding protein, is an important determinant of DNA strand exchange ability. In addition to uvsY protein, the dda protein, a helicase involved in T4 replication, also stimulates (> 4-fold) the rate of branch migration by unwinding the dsDNA ahead of the uvsX protein-ssDNA filament branch point [67]. This effect is not observed using another T4 phage-encoded helicase, the 41 protein, implying interaction specificity.

Ustilago maydis *homologous pairing protein (HPP)*

Based upon the prokaryotic systems, several generalizations might be made. A strand exchange protein would bind ssDNA tightly, cooperatively, and stoichiometrically, resulting in the formation of a saturated helical filament. The binding of ATP, and possibly the interaction with another protein, would be required for optimal activity. Finally, ATP hydrolysis would be required to permit recycling of the protein. In addition, the protein might also have DNA renaturation activity. These properties provided the basis for the isolation of eukaryotic strand exchange proteins by biochemical criteria.

The first eukaryotic pairing protein to be purified was from the fungus, *U maydis*. Although the activity was found to be lacking in extracts from *rec1* mutants, resulting in its preliminary designation as the rec1 protein [12, 74], recent data indicate that the 70-kDa protein believed to possess the strand transfer activity is not the responsible factor. Instead, the *rec1* gene encodes a 58-kDa protein [75]. It is now believed that the pairing activity might reside in a 110–120 kDa protein which was present in the initial preparations; this protein has been designated homologous pairing protein (HPP) (Holloman, personal communication). The 70-kDa protein may be an ancillary DNA binding protein.

In some respects, HPP conforms to the recA protein model, having both ssDNA-dependent ATPase and ssDNA renaturation activities [12] in addition to DNA strand exchange activity [76]. Its ATPase specific activity (230 ADP generated/min), like that of uvsX protein, is ≈ 10-fold greater than that of recA protein, but joint molecule formation between ssDNA fragments and supercoiled DNA by HPP has a significant (40%) ATP-independent component [12], suggesting that nucleotide hydrolysis may not be obligatorily linked to joint molecule formation. Nevertheless, it appears that ATP hydrolysis is required for both heteroduplex extension and plectonemic molecule formation; joint molecules formed in the presence of the non-hydrolyzable ATP analogue AMP-PNP are unstable when deproteinized and hence are presumably paranemic [76]. Unlike either recA or uvsX protein, HPP is active at substoichiometric concentrations (1 monomer/200 nt ssDNA). This value, and the ATPase specific activity, were based on activity residing in the 70-kDa protein; since it is now believed that a different polypeptide in the preparation is the pairing protein, the actual values need to be adjusted by as much as 10-fold to account for the lower amount of active polypeptide. Unlike either recA or uvsX protein, HPP does not appear either to bind ssDNA cooperatively or to form filaments, and it can mediate the pairing of 2 supercoiled molecules in the presence of topoisomerase [77]. Continual ATP hydrolysis is required for extension of the heteroduplex joint, which forms preferentially at the dsDNA end containing the 5' complementary end (3' displaced end). Similarly, strand displacement proceeds with a polarity (3' to 5' relative to the displaced strand) opposite that of both recA and uvsX proteins [76].

The initial formation of paranemic joint molecules by HPP results in dsDNA unwinding; as with the *E coli* recA protein, these paranemic joints are unstable in the presence of ADP, presumably due to protein dissociation. The formation of plectonemic joint molecules results in the generation of left-handed DNA that is recognized by anti-Z-DNA antibodies, implying that Z-DNA is present at the joint [78]. HPP binds Z-DNA with an affinity that is reported to be from 2- to 6-fold [79] to 20- to 75-fold [80] greater than for B-DNA. Subsequent studies on recA protein have revealed that it also has an apparently higher affinity (2- to 6-fold) for Z-DNA than for B-DNA [79], but this enhancement is kinetic in nature; Z-DNA promotes faster nucleation than B-DNA does, but at equilibrium, B-DNA is preferentially bound [81]. This suggests that recombination proteins which bind Z-DNA might be involved in the initiation of homologous pairing, a proposal which is supported by the observation that joint molecule formation by HPP between 2 duplex molecules containing Z-DNA inserts initiates within this region [82]. No stimulatory

or accessory factor has been identified for HPP, and the mechanism of its action at such a low protein to DNA ratio remains obscure.

ATP-independent homologous pairing proteins

The earliest reports of the partial purification of putative homologous pairing activities from other eukaryotic cells – human [83–87], mouse [88, 89], lily [88, 90], and yeast [91] – did not appear to contradict the mechanistic conception of strand exchange as revealed by work on recA protein; most notably, ATP hydrolysis appeared to be required in all cases. Recently, however, studies with purified proteins from human, *D melanogaster*, and *S cerevisiae* cells have appeared. The most intriguing aspects of these proteins are that they appear to conduct DNA strand exchange in the absence of any exogenous nucleotide cofactor and that they can function at substoichiometric or even near catalytic concentrations. Consequently, the observation that recA protein can promote the exchange of DNA strands in the absence of ATP hydrolysis may be universally relevant, even if the eukaryotic proteins function by a mechanistically distinct pathway.

Human B-lymphoblast cell strand transferase (SEP)

The first DNA strand exchange protein to be partially purified from mammalian cells exhibited an unanticipated trait [13, 92]. Like the recA protein, this protein fraction can both renature complementary ssDNA and form joint molecules between circular ss- and linear dsDNA; it does not, however, require a nucleoside triphosphate for DNA strand exchange. In fact, the addition of non-hydrolyzable analogues such as ATPγS and AMP-PNP inhibits joint molecule formation by up to 50%. The joint molecules formed by this protein are plectonemic but contain limited lengths (200–300 bp) of heteroduplex DNA. Joint molecule formation occurs at the end of the linear dsDNA possessing the 3' end of the non-complementary strand. The reaction displays a concentration dependence on ssDNA, but since the protein fraction was impure and the activities could not be ascribed to any one polypeptide, determination of the stoichiometry of the reaction is unfeasible. Although minor nuclease activity was detected in the preparation, several controls seem to suggest that this contaminant was not responsible for the activity observed.

Human T-lymphoblast cell pairing protein (HPP-1)

A DNA strand transferase from human T cells (M_r 120 kDa), designated HPP-1 for human pairing protein 1, has been extensively purified and characterized [14, 93]. Interestingly, while ATP is neither required for activity nor hydrolyzed by HPP-1, the protein specifically binds the photoaffinity analogue, 8-azido ATP, in a site distinct from its DNA binding site. The rate of joint molecule formation between circular ssDNA and linear dsDNA promoted by HPP-1 is comparable to that of recA protein, but only ≈ 6% of the input DNA is converted into complete heteroduplex products (*ie* form II molecules). The rate of DNA strand exchange by HPP-1 (2 nt/s) is ≈ 5-fold less than that of recA protein, but this slower rate is more typical of other eukaryotic proteins. Pairing requires a 5' complementary strand in the dsDNA (*ie* a 3' displaced strand) and proceeds 3' to 5' relative to the displaced strand. The question of how DNA strand exchange has polarity without the input of free energy (*eg* through ATP hydrolysis) appears problematic, but since the DNA substrates themselves are asymmetric, directional exchange may result from thermal branch migration in joint molecules blocked at one end of the DNA [94]; also, the presence of a trace 3' to 5' exonuclease activity may contribute to the observed bias. The optimal rate of strand exchange is reported to occur at a stoichiometry of 1 monomer/50 nt dsDNA, but the reaction is dependent on both ssDNA and dsDNA concentration, suggesting that binding of the protein to dsDNA ends may also be important [93]. Even though HPP-1 appears to bind to ssDNA cooperatively, it does not form extensive filaments on ssDNA. Both the rate and the extent of the reaction are dependent on the concentration of protein, implying that the protein can participate in only one round of DNA strand exchange and is unable to turn over [14].

A 500-kDa recombination complex which is active for DNA strand exchange can be isolated at earlier stages of purification; it consists of HPP-1 and several other activities [93]. A significant difference between the properties of this complex and those of purified HPP-1 is that the reaction promoted by the complex fraction is both ATP-dependent and catalytic. These results suggest that HPP-1 is normally associated with accessory factors, at least one of which utilizes ATP (possibly that bound to HPP-1). The presumptive ATP-dependent factor may assist in HPP-1 turnover, since the rate of strand exchange in crude extracts is 10-fold faster. Nevertheless, the ability of the highly purified HPP-1 to produce form II molecules indicates that turnover is dispensable for heteroduplex extension and DNA strand exchange.

Drosophila melanogaster *strand transfer protein (STP)*

Two laboratories have isolated ATP-independent strand transfer proteins from *D melanogaster* embryo nuclear extracts [15–17]. The similarity in their pro-

perties indicates that they are identical [17]. This 105-kDa protein carries out both ss- and dsDNA aggregation and ssDNA renaturation in addition to DNA strand exchange; thus, it appears to have some of the essential nucleic acid interaction properties of a recA protein analogue. The partially purified fraction demonstrates a pairing bias for displacement of the 3' non-complementary strand. The maximum extent of DNA heteroduplex formation is ≈ 600 bp [15], and joint molecule formation requires as little as 13 bp of homology [41]. The DNA strand transfer reaction displays a sigmoidal dependence on protein concentration, possibly suggesting that it binds cooperatively to ssDNA. The reaction appears to be catalytic since the rate but not the extent of the strand transfer reaction is affected by the protein concentration. This rate is > 8-fold more rapid than that mediated by recA protein alone and, unlike the recA protein-promoted reaction, is not stimulated by SSB protein.

In contrast to the partially purified fraction, the purified strand transferase efficiently promotes the formation of a species which co-migrates with form II DNA, using circular ssDNA and linear dsDNA substrates [17]. This result is all the more surprising given that optimal activity is observed at only 1 monomer/400 nt ssDNA. The optimum suggests the need either for the formation of a catalytically active multimer on the ssDNA or for interaction between proteins on different DNA molecules. The latter alternative is favored by data which correlate the properties of DNA strand exchange with those of ssDNA aggregation. Although it elutes from a gel filtration column with a M_r of 500 kDa, this purified protein, if it is truly catalytic, does not appear to require a stoichiometric accessory factor (although it co-purifies with a 3'-exonuclease), as seen with uvsX and the eukaryotic proteins which can function at substoichiometric concentrations (see below). In this respect, it is more similar to the *U maydis* HPP, except that the *U maydis* reaction is largely ATP-dependent.

It has been reported that the human B-cell strand transferase, a different partially purified human cell strand transferase, and the *D melanogaster* strand transferase are unable to unwind a 20 bp duplex region on a circular ssDNA molecule [95]. This result suggests that the search for homology by these proteins may proceed by a different mechanism than that proposed for recA protein and uvsX protein, which are able to displace similar fragments (not exceeding 30 bp in length).

Saccharomyces cerevisiae *strand exchange proteins (SEP1/STPβ, DPA, and STPα)*

Three apparently different DNA strand exchange proteins have been identified in the yeast, *S cerevisiae*. The first protein described, and the most extensively characterized activity, is the mitotic protein, SEP1 (strand exchange protein 1) [18]. Independently, a similar and antigenically related protein designated STPβ (for strand transfer protein β) has also been described [21]. Although the molecular mass of the 2 proteins differs (SEP1 is 132-kDa; STPβ is 180-kDa), SEP1 is presumably a proteolytic product. SEP1 promotes homologous DNA strand exchange and the renaturation of ssDNA in an ATP-independent manner; in fact, for an unknown reason, the addition of ATP inhibits the reaction 2- to 3-fold. Like recA protein, SEP1 is required in stoichiometric amounts (1 monomer/12-14 nt) in the absence of a stimulatory factor, and its binding to ssDNA is cooperative. The average length of heteroduplex DNA formed is 4.1 kb, which is (perhaps coincidentally) similar to the amount of heteroduplex DNA formed by recA protein in the absence of turnover (*ie* in the presence of ATPγS). SEP1 displays no end-bias in joint molecule formation, and branch migration is reported to proceed 5' to 3' relative to the displaced strand (based on electron microscopic studies which detect α structures using 3' end complementary dsDNA *versus* the occurrence of σ structures using 5' end complementary dsDNA). These results distinguish SEP1 from the other known eukaryotic strand transfer proteins, which display both an end-bias in pairing and a polarity of branch migration which is opposite that of both recA and uvsX protein. As is the case for recA protein, a substoichiometric concentration of SEP1 (1 monomer/100 nt) is optimal for ssDNA renaturation [96].

SEP1 binds both ss- and dsDNA, with the relative binding affinity for ssDNA being higher than that for dsDNA [96]. Binding to ssDNA is not detectably cooperative, which leaves unexplained the sigmoid dependence on SEP1 concentration in the DNA strand exchange reaction. SEP1–ssDNA complexes are stable to 200 mM NaCl, but both ssDNA renaturation and DNA strand exchange are inhibited well below this salt concentration. Therefore, a step succeeding complex formation must be responsible for the salt sensitivity of the reaction. Since SEP1 binds to dsDNA, the decrease in activity observed with greater than stoichiometric amounts of SEP1 may be analogous to the inhibition seen with excess uvsX protein.

Two different stimulatory factors for SEP1 and a number of stimulatory factors for STPβ have been identified. One SEP1 stimulatory factor is a yeast ssDNA binding protein (ySSB protein), which binds ssDNA cooperatively and with high affinity [54]. At subsaturating concentrations of SEP1, there is a temporal lag in the formation of joint molecules which can be relieved by the addition of ySSB protein, resulting in an 18-fold increase in the initial rate. The inclusion of this ySSB does not influence the

sigmoidicity of the SEP1 titration, but it does decrease (by ≈ 2-fold) the amount of SEP1 required for optimal levels of strand exchange in the presence of stoichiometric amounts of ySSB protein to 1 monomer/18 nt. As with the uvsX protein-G32P reaction, enhancement of strand exchange by ySSB protein is negligible at saturating concentrations of SEP1. Stimulation may result from a specific protein–protein interaction because *E coli* SSB protein cannot substitute, although the ySSB protein does stimulate recA protein. The gene encoding the 34-kDa ySSB protein has been cloned, and based on its homology to the large subunit of a cellular protein (RP-A) involved in SV40 replication, it has been designated *RPA1* [97]. The *RPA1* gene is essential for viability, and it encodes a 70-kDa polypeptide. Thus, the purified y-SSB is a proteolytic fragment of the RPA1 protein comprising approximately the central portion of the polypeptide and containing the Zn^{2+} finger DNA binding domain. Likewise, a variety of DNA binding proteins stimulate joint molecule formation by STPβ. A 50-fold stimulation is observed at optimal concentrations, and up to 1.5 kb of heteroduplex DNA are formed. Only 2–3 molecules of STPβ per linear dsDNA molecule, however, are required when optimal concentrations of these ySSBs are present [21].

A second SEP1 stimulatory factor, SF1, is a 33-kDa protein which dramatically alters the amount of SEP1 required for DNA strand exchange [98]. Using an optimal amount of SF1 (1 monomer/20 nt), the extent of joint molecule formation at a suboptimal SEP1 concentration (1 monomer/5800 nt) is equivalent to that obtained under the optimal unstimulated conditions (*ie* at an SEP1:nt ratio of 1:12). This represents an enhancement of > 400-fold; at even higher concentrations of SEP1, with an optimum at 1:725, the rate of strand exchange is increased 3- to 4-fold, and the distribution of intermediates is altered–joint molecules are converted to large DNA networks. SF1 is similar to other ssDNA binding proteins in that it is required in stoichiometric amounts; it stimulates DNA strand exchange by its cognate protein to a great extent; and it has ssDNA renaturation activity [98, 99]. By virtue of its ability to alleviate the amount of SEP1 required to essentially catalytic amounts, however, SF1 appears to function in actively promoting DNA strand exchange, rather than in assisting the formation or maintenance of a presynaptic filament composed primarily of the strand exchange protein.

Although SF1 can aggregate both ssDNA and dsDNA in the absence of SEP1, this property is not the basis of its stimulatory effect since it is effective under conditions which reduce its aggregation activity. Instead, SF1 likely plays a direct role in DNA strand exchange. Assuming that SEP1 and SF1 have distinct roles in the reaction, 2 possibilities exist. First, SF1 could be involved in bringing the substrates together and aligning them, while SEP1 catalyzes branch migration itself. Alternatively, SEP1 could participate in the initiation of synapsis, after which strand exchange and branch migration could be promoted by SF1-coated ssDNA. The second explanation is considered to be more likely because it does not require turnover of SEP1. In this scenario, SF1 inhibits unproductive (or promotes productive) interactions of SEP1 with ssDNA so that SEP1 can act nearly catalytically (at 1–2 monomers/dsDNA molecule).

The second strand exchange activity to be purified from mitotic yeast cells, termed DNA pairing activity (DPA) [20], has properties so similar to those of SEP1 that it may, at 120 kDa, be a further proteolytic product of the full-length SEP1. Although the stoichiometry of its binding to ssDNA in DNA strand exchange has not been reported, this protein renatures ssDNA at a ratio of 1 monomer/50 nt. Assuming that renaturation by this protein also requires subsaturating amounts of protein, DPA appears to act stoichiometrically. One unusual property of DPA is its requirement for dsDNA substrates with either 5' or 3' tails of 4-50 nt in order to initiate strand transfer and branch migration, which appears to proceed with no preferred polarity. The joint molecules formed by DPA are limited to 3–5 kb of heteroduplex DNA. Thus, pairing appears to initiate by renaturation and to extend by thermal branch migration. Since both the rate and the extent of DNA renaturation (and, by extension, joint molecule formation) are dependent on the protein concentration, DPA may also lack the intrinsic capability to turn over.

The third yeast DNA strand exchange protein has been designated STPα (strand transfer protein α). STPα is a 38-kDa strand exchange protein isolated from meiotic cells [19]. It catalyzes DNA strand exchange and DNA renaturation in a manner very reminiscent of that described for STPβ. For example, both STPα and STPβ act catalytically in the presence of a 26-kDa ySSB protein; at a ratio of 1 ySSB protein monomer/6-8 nt, only 2–3 molecules of either STPα or STPβ per dsDNA molecule are required for maximal joint molecule formation. Both proteins are sensitive to the concentration of ySSB proteins or other stimulatory agents, such as histone H1 and spermidine, with optimal stimulation being observed in each case at a concentration which aggregates 50% of the DNA. These proteins differ in 2 respects, however: STPβ is more sensitive to ionic conditions, and STPα catalyzes the formation of complete strand exchange products (form II DNA molecules) more efficiently. These proteins are distinct since STPβ protein and activity are still detected in an STPα disruption strain. Thus, all 3 strand transfer activities isolated from yeast (SEP1/STPβ, DPA, and STPα) have similar though distinct properties. The reported differences in

stoichiometry and properties of the mitotic proteins may be due to variations in the specific activities of the different preparations and to the effects of proteolysis.

Conclusion

Strand transfer proteins having some similarities to the *E coli* recA protein have been isolated from many organisms. These proteins bind cooperatively to ssDNA (and sometimes dsDNA), promote both DNA aggregation and DNA renaturation, and form joint molecules with extensive regions of heteroduplex DNA. Despite these broad similarities, proteins which promote these reactions can be classified into two types based on their cofactor and stoichiometric requirements. Such a classification scheme divides the proteins along roughly organismal lines, with the need for a nucleotide cofactor and stoichiometric amounts of protein being primarily prokaryotic requirements. Since many of the eukaryotic proteins execute proficient DNA strand exchange in the absence of a hydrolyzable ligand such as ATP, they must employ a different mode of regulating their binding to and dissociation from DNA. Two possibilities may be considered: 1), that ssDNA binding affinity and protein turnover are regulated by an interaction with other protein(s), in which an ATP-dependent factor might be involved; or 2), that no catalytic turnover of the proteins exists, and the proteins are arrested in a tight binding mode. The possibility that these catalytic factors may use a nucleotide cofactor other than ATP is highlighted by the requirement for dATP by the *B subtilis* recE protein. The second significant difference between the prokaryotic and the eukaryotic proteins is the ability of most eukaryotic proteins to promote DNA strand exchange when present at nearly catalytic concentrations. Under these conditions, the DNA strand exchange protein typically requires the inclusion of accessory proteins which somewhat resemble their prokaryotic counterparts, SSB protein and G32P; prokaryotic stimulatory factors, however, do not drastically reduce the amount of prokaryotic DNA strand exchange protein required. As exemplified by *D melanogaster* STP, even this requirement

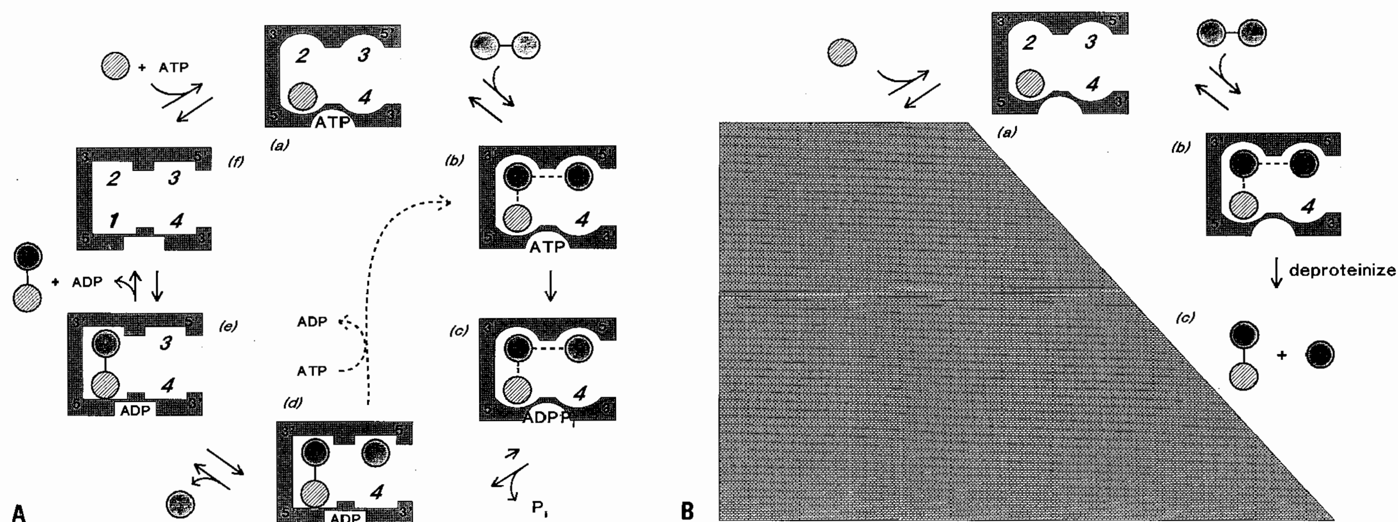

Fig 1. A. DNA strand exchange coupled to ATP binding and hydrolysis. In (f), a recA protein monomer containing 4 potential sites for the binding of individual DNA strands is pictured. Upon binding an ATP molecule, the conformation of the protein is changed, and a ssDNA molecule occupies the highest affinity site (a). A dsDNA molecule is subsequently bound to the second and third highest affinity sites (b) in such a manner that a transition state is achieved in which hydrogen bonding between the dsDNA strands is strained, and bond formation between the ssDNA and its complement within the dsDNA is favored. After the ATP molecule is hydrolyzed, reversing the allosteric change (c), the initial bonding is disrupted and a new bond is formed (d). The displaced strand, in a low affinity site, is released (e), followed by release of the heteroduplex DNA and ADP (f). As ATP hydrolysis by recA protein is processive, more than one ATP molecule may be hydrolyzed per bp formed (dashed arrow). **B.** Strand exchange promoted in the absence of an ATP hydrolytic cycle. In (a), the strand exchange protein, which exists in a high ssDNA affinity conformation, binds a ssDNA molecule in its highest affinity site. Non-equivalent binding of the dsDNA molecule places stress on the hydrogen bonding between the strands and favors pairing of the complementary strand with the ssDNA (b). Interaction with another protein to permit turnover or physical disruption of the protein results in release of the heteroduplex DNA molecule and displaced ssDNA (c).

for an accessory factor may be dispensable. This distinction, in turn, calls into question the obligation of a DNA strand exchange protein to form an extensive nucleoprotein filament.

Given these similarities and differences among the DNA strand exchange proteins, it is fair to ask whether the archetypal DNA strand exchange protein, the *E coli* recA protein, is a good model for the mechanism of DNA strand exchange promoted by these other proteins. Although an unequivocal answer to this question is not yet available, two limiting possibilities exist. The first is that ATP-independent homologous pairing and DNA strand exchange occur by a completely different and yet to be discovered (and discussed) mechanism. The second is that the mechanism for DNA strand exchange by recA protein is, in fact, applicable but that the ATP-dependent steps are obviously absent. Figure 1A presents a model of DNA strand exchange promoted by recA protein [3, 26], which may be applicable to all of the ATP-dependent proteins. A salient feature of this model is that ATP hydrolysis is required only for protein dissociation and directionality but is not required for DNA strand exchange. Figure 1B illustrates the same model but with the ATP hydrolysis-dependent steps blocked out to demonstrate those steps which may be catalyzed by the ATP-independent proteins. In this case, we assume that the ATP-independent strand exchange protein behaves effectively like the ATP-bound form of recA protein but does so without nucleotide binding; hence it cannot turn over. This simple model shows that this hypothetical strand transfer protein can bind both DNA molecules and promote stabilization of a transition state-like complex in which strand exchange has (nearly) occurred; removal of the protein permits detection of joint molecules. Apparent catalytic action can occur if protein-dependent DNA strand exchange, as depicted in figure 1, occurs only at one end of the linear dsDNA (perhaps due to stereospecific constraints), and due to this bound protein, thermal (*ie* protein-independent) branch migration extends the nascent heteroduplex joint in an apparently polar manner [94]. Alternatively, an auxiliary factor could promote the dissociation of the stand exchange protein from the product heteroduplex DNA, permitting subsequent catalytic action. Elucidation of the actual mechanism pathway remains a tantalizing subject for further inquiry.

Acknowledgments

The work in this laboratory has been supported by NIH research grants AI-18987 and GM-41347 (to SCK) and by NIH NRSA training grant GM-08061 (to AKE). The authors thank D Dixon, B Rehrauer, and S Lauder of this laboratory for their helpful suggestions concerning the manuscript.

References

1 Cox MM, Lehman IR (1987) Enzymes of general recombination. *Annu Rev Biochem* 56, 229–262

2 Radding CM (1989) Helical recA nucleoprotein filaments mediate homologous pairing and strand exchange. *Biochim Biophys Acta* 1008, 131–145

3 Kowalczykowski SC (1991) Biochemistry of genetic recombination: energetics and mechanism of DNA strand exchange. *Annu Rev Biophys Biophys Chem* 20, 539–575

4 Radding CM, Flory J, Wu A, Kahn R, DasGupta C, Gonda D, Bianchi M, Tsang SS (1983) Three phases in homologous pairing: Polymerization of recA protein on single-stranded DNA, synapsis, and polar strand exchange. *Cold Spring Harbor Symp Quant Biol* 47, 821–828

5 McEntee K, Weinstock GM, Lehman IR (1980) RecA protein-catalyzed strand assimilation: stimulation by *Escherichia coli* single-stranded DNA-binding protein. *Proc Natl Acad Sci USA* 77, 857–861

6 Shibata T, DasGupta C, Cunningham RP, Radding CM (1980) Homologous pairing in genetic recombination: formation of D-loops by combined action of recA protein and a helix-destabilizing protein. *Proc Natl Acad Sci USA* 77, 2606–2610

7 Yonesaki T, Minagawa T (1985) T4 phage gene uvsX product catalyzes homologous DNA pairing. *EMBO J* 4, 3321–3328

8 Formosa T, Alberts BM (1986) Purification and characterization of the T4 bacteriophage uvsX protein. *J Biol Chem* 261, 6107–6118

9 Hinton DM, Nossal NG (1986) Cloning of the bacteriophage T4 *uvsX* gene and purification and characterization of the T4 uvsX recombination protein. *J Biol Chem* 261, 5663–5673

10 West SC, Countryman JK, Howard-Flanders P (1983) Purification and properties of the recA protein of *Proteus mirabilis*: comparison with *Escherichia coli* recA protein; Specificity of interaction with single strand binding protein. *J Biol Chem* 258, 4648–4654

11 Lovett CM, Roberts JW (1985) Purification of a recA protein analogue from *Bacillus subtilis*. *J Biol Chem* 260, 3305–3313

12 Kmeic E, Holloman WK (1982) Homologous pairing of DNA molecules promoted by a protein from *Ustilago*. *Cell* 29, 367–374

13 Hsieh P, Meyn MS, Camerini-Otero RD (1986) Partial purification and characterization of a recombinase from human cells. *Cell* 44, 885–894

14 Moore SP, Fishel R (1990) Purification and characterization of a protein from human cells which promotes homologous pairing of DNA. *J Biol Chem* 265, 11108–1117

15 Eisen A, Camerini-Otero RD (1988) A recombinase from *Drosophila melanogaster* embryos. *Proc Natl Acad Sci USA* 85, 7481–7485

16 McCarthy JG, Sander M, Lowenhaupt K, Rich A (1988) Sensitive homologous recombination strand-transfer assay: Partial purification of a *Drosophila melanogaster* enzyme and detection of sequence effects on the strand transfer activity of recA protein. *Proc Natl Acad Sci USA* 85, 5854–5858

17 Lowenhaupt K, Sander M, Hauser C, Rich A (1989) *Drosophila melanogaster* strand transferase. *J Biol Chem* 264, 20568–20575

18 Kolodner R, Evans DH, Morrison PT (1987) Purification and characterization of an activity from *Saccharomyces cerevisiae* that catalyzes homologous pairing and strand transfer. *Proc Natl Acad Sci USA* 84, 5560–5564

19 Sugino A, Nitiss J, Resnick MA (1988) ATP-independent DNA strand transfer catalyzed by protein(s) from meiotic cells of the yeast *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 85, 3683–3687

20 Halbrook J, McEntee K (1989) Purification and characterization of a DNA-pairing and strand transfer activity from mitotic *Saccharomyces cerevisiae*. *J Biol Chem* 264, 21403–21412

21 Dykstra CC, Hamatake RK, Sugino A (1990) DNA strand transfer protein β from yeast mitotic cells differs from strand transfer protein α from meiotic cells. *J Biol Chem* 265, 10968–10973

22 Menetski JP, Kowalczykowski SC (1985) Interaction of recA protein with single-stranded DNA. Quantitative aspects of binding affinity modulation by nucleotide cofactors. *J Mol Biol* 181, 281–295

23 Shibata T, DasGupta C, Cunningham RP, Radding CM (1979) Purified *E coli* recA protein catalyzes homologous pairing of superhelical DNA and single-stranded fragments. *Proc Natl Acad Sci USA* 76, 1638–1642

24 Brenner SL, Mitchell RS, Morrical SW, Neuendorf SK, Schutte BC, Cox MM (1987) RecA protein-promoted ATP hydrolysis occurs throughout *recA* nucleoprotein filaments. *J Biol Chem* 262, 4011–4016

25 Kowalczykowski SC, Krupp RA (1987) Effects of the *Escherichia coli* SSB protein on the single-stranded DNA-dependent ATPase activity of *Escherichia coli* recA protein: Evidence that SSB protein facilitates the binding of *recA* protein to regions of secondary structure within single-stranded DNA. *J Mol Biol* 193, 97–113

26 Menetski JP, Varghese A, Kowalczykowski SC (1988) Properties of the high-affinity single-stranded DNA binding state of the *Escherichia coli* recA protein. *Biochemistry* 27, 1205–1212

27 Wu AM, Kahn R, DasGupta C, Radding CM (1982) Formation of nascent heteroduplex structures by recA protein and DNA. *Cell* 30, 37–44

28 Cox MM, Soltis DA, Lehman IR, DeBrosse C, Benkovic SJ (1983) ADP-mediated dissociation of stable complexes of recA protein and single-stranded DNA. *J Biol Chem* 258, 2586–2592

29 Weinstock GM, McEntee K, Lehman IR (1979) ATP-dependent renaturation of DNA catalyzed by the recA protein of *Escherichia coli*. *Proc Natl Acad Sci USA* 76, 126–130

30 McEntee K (1985) Kinetics of DNA renaturation catalyzed by the recA protein of *Escherichia coli*. *Biochemistry* 24, 4345–4351

31 McEntee K, Weinstock GM, Lehman IR (1979) Initiation of general recombination catalyzed *in vitro* by the recA protein of *Escherichia coli*. *Proc Natl Acad Sci USA* 76, 2615–2619

32 Cox MM, Lehman IR (1981) RecA protein of *Escherichia coli* promotes branch migration, a kinetically distinct phase of DNA strand exchange. *Proc Natl Acad Sci USA* 78, 3433–3437

33 Bianchi M, DasGupta C, Radding CM (1983) Synapsis and the formation of paranemic joints by *E coli* recA protein. *Cell* 34, 931–939

34 Riddles PW, Lehman IR (1985) The formation of paranemic and plectonemic joints between DNA molecules by the recA and single-stranded DNA-binding proteins of *Escherichia coli*. *J Biol Chem* 260, 165–169

35 Konforti BB, Davis RW (1990) The preference for a 3' homologous end is intrinsic to recA-promoted strand exchange. *J Biol Chem* 265, 6916–6920

36 Tsang SS, Chow SA, Radding CM (1985) Networks of DNA and recA protein are intermediates in homologous pairing. *Biochemistry* 24, 3226–3232

37 Cox MM, Lehman IR (1981) Directionality and polarity in recA protein-promoted branch migration. *Proc Natl Acad Sci USA* 78, 6018–6022

38 Kahn R, Cunningham RP, DasGupta C, Radding CM (1981) Polarity of heteroduplex formation promoted by *Escherichia* recA protein. *Proc Natl Acad Sci USA* 4786–4790

39 West SC, Cassuto E, Howard-Flanders P (1981) Heteroduplex formation by recA protein: polarity of strand exchanges. *Proc Natl Acad Sci USA* 78, 6149–6153

40 Thresher RJ, Christiansen G, Griffith JD (1988) Assembly of presynaptic filaments. Factors affecting the assembly of recA protein onto single-stranded DNA. *J Mol Biol* 201, 101–113

41 Hsieh P, Camerini-Otero CS, Camerini-Otero RD (1990) Pairing of homologous DNA sequences by proteins: evidence for three-stranded DNA. *Genes Dev* 4, 1951–1963

42 Rao BJ, Jwang B, Radding CM (1990) A three-stranded DNA complex remains after strand exchange mediated by recA protein. *In: Molecular Mechanisms in DNA Replication and Recombination* (Richardson CC, Lehman IR, eds) Alan R Liss, NY, 387–398

43 Rao BJ, Jwang B, Radding CM (1990) RecA protein reinitiates strand exchange on isolated protein-free DNA intermediates: an ADP-resistant process. *J Mol Biol* 213, 789–809

44 West SC, Cassuto E, Howard-Flanders P (1982) Role of SSB protein in recA promoted branch migration reactions. *Mol Gen Genet* 186, 333–338

45 Lavery PE, Kowalczykowski SC (1988) Biochemical basis of the temperature-inducible constitutive protease activity of the recA441 protein of *Escherichia coli*. *J Mol Biol* 203, 861–874

46 Morrical SW, Lee J, Cox MM (1986) Continuous association of *Escherichia coli* single-stranded DNA binding protein with stable complexes of recA protein and single-stranded DNA. *Biochemistry* 25, 1482–1494

47 Kowalczykowski SC (1987) Mechanistic aspects of the DNA strand exchange activity of *E coli* recA protein. *Trends Biochem Sci* 12, 141–145

48 Muniyappa K, Shaner SL, Tsang SS, Radding CM (1984) Mechanism of the concerted action of recA protein and helix destabilizing proteins in homologous recombination. *Proc Natl Acad Sci USA* 81, 2757–2761

49 Tsang SS, Muniyappa K, Azhderian E, Gonda DK, Radding CM, Flory J, Chase JW (1985) Intermediates in homologous pairing promoted by recA protein: isolation and characterization of active presynaptic complexes. *J Mol Biol* 185, 295–309

50 Kowalczykowski SC, Clow JC, Somani R, Varghese A (1987) Effects of the *Escherichia coli* SSB protein on the binding of *Escherichia coli* recA protein to single-stranded DNA: demonstration of competitive binding and the lack of a specific protein-protein interaction. *J Mol Biol* 193, 81–95

51 Morrical SW, Cox MM (1990) Stabilization of *recA* protein-ssDNA complexes by the single-stranded DNA binding protein of *Escherichia coli*. *Biochemistry* 29, 837–843

52 Egner C, Azhderian E, Tsang SS, Radding CM, Chase JW (1987) Effects of various single-stranded DNA-binding proteins on reactions promoted by recA protein. *J Bacteriol* 169, 3422–3428

53 Chow SA, Rao BJ, Radding CM (1988) Reversibility of strand invasion promoted by recA protein and its inhibition by *Escherichia coli* single-stranded DNA-binding protein or phage T4 gene 32 protein. *J Biol Chem* 263, 200–209

54 Heyer WD, Kolodner RD (1989) Purification and characterization of a protein from *Saccharomyces cerevisiae* that binds tightly to single-stranded DNA and stimulates a cognate strand exchange protein. *Biochemistry* 28, 2856–2862

55 Roman LJ, Kowalczykowski SC (1989) Formation of heteroduplex DNA promoted by the combined activities of *Escherichia coli* recA and recBCD proteins. *J Biol Chem* 264, 18340–18348

56 Kowalczykowski SC, Roman LJ (1990) Reconstitution of homologous pairing activity dependent upon the combined activities of purified *E coli* recA, recBCD, and SSB proteins. *In: Molecular Mechanisms in DNA Replication and Recombination: UCLA Symposia on Molecular and Cellular Biology, New Series* (Richardson CC, Lehman IR, eds) Alan R Liss Inc, NY, 357–373

57 Roman LJ, Dixon DA, Kowalczykowski SC (1991) RecBCD-dependent joint molecule formation promoted by the *Escherichia coli* recA and SSB proteins. *Proc Natl Acad Sci USA* 88 (in press)

58 Menetski JP, Bear DG, Kowalczykowski SC (1990) Stable DNA heteroduplex formation catalyzed by the *Escherichia coli* recA protein in the absence of ATP hydrolysis. *Proc Natl Acad Sci USA* 87, 21–25

59 Rosselli W, Stasiak A (1990) Energetics of recA-mediated recombination reactions: polar exchange of DNA strands is driven by recA-DNA binding energy. *J Mol Biol* 216, 335–352

60 Miller RV, Kokjohn TA (1990) General microbiology of *recA*: environmental and evolutionary significance. *Annu Rev Microbiol* 44, 365–394

61 Akaboshi E, Yip ML, Howard Flanders P (1989) Nucleotide sequence of the *recA* gene of *Proteus mirabilis*. *Nucleic Acids Res* 17, 4390

62 West SC, Little JW (1984) *P mirabilis* recA protein catalyses cleavage of *E coli* lexA protein and the lambda repressor *in vitro*. *Mol Gen Genet* 194, 111–113

63 Stranathan MC, Bayles KW, Yasbin RE (1990) The nucleotide sequence of the *recE* + gene of *Bacillus subtilis*. *Nucleic Acids Res* 18, 4249

64 Griffith J, Formosa T (1985) The uvsX protein of bacteriophage T4 arranges single-stranded and double-stranded DNA into similar helical nucleoprotein filaments. *J Biol Chem* 260, 4484–4491

65 Fujisawa H, Yonesaki T, Minagawa T (1985) Sequence of the T4 recombination gene, *uvsX*, and its comparison with that of the *recA* gene of *Escherichia coli*. *Nucleic Acids Res* 13, 7473–7481

66 Harris LD, Griffith J (1987) Visualization of the homologous pairing of DNA catalyzed by the bacteriophage T4 uvsX protein. *J Biol Chem* 262, 9285–9292

67 Kodadek T, Alberts BM (1987) Stimulation of protein-directed strand exchange by a DNA helicase. *Nature (Lond)* 326, 312–314

68 Kodadek T, Wong ML, Alberts BM (1988) The mechanism of homologous DNA strand exchange catalyzed by the bacteriophage T4 uvsX and gene 32 proteins. *J Biol Chem* 263, 9427–9436

69 Harris LD, Griffith JD (1989) UvsX protein of bacteriophage T4 is an accessory protein for *in vitro* catalysis of strand exchange. *J Mol Biol* 206, 19–27

70 Harris LD, Griffith JD (1988) Formation of D-loops by the uvsX protein of T4 bacteriophage: a comparison of the reaction catalyzed in the presence or absence of gene 32 protein. *Biochemistry* 27, 6954–6959

71 Kodadek T (1990) The role of the bacteriophage T4 gene 32 protein in homologous pairing. *J Biol Chem* 265, 20966–20969

72 Yonesaki T, Minagawa T (1989) Synergistic action of three recombination gene products of bacteriophage T4, uvsX, uvsY, and gene 32 proteins. *J Biol Chem* 264, 7814–7820

73 Kodadek T, Gan DC, Stemke Hale K (1989) The phage T4 uvsY recombination protein stabilizes presynaptic filaments. *J Biol Chem* 264, 16451–16457

74 Holliday R, Taylor SY, Kmiec EB, Holloman WK (1984) Biochemical characterization of *rec1* mutants and the genetic control of recombination in *Ustilago maydis*. *Cold Spring Harbor Symp Quant Biol* 49, 669–673

75 Tsukuda T, Bauchwitz R, Hollomanb WK (1989) Isolation of the *REC1* gene controlling recombination in *Ustilago maydis*. *Gene* 85, 335–341

76 Kmiec EB, Holloman WK (1983) Heteroduplex formation and polarity during strand transfer promoted by *Ustilago* rec1 protein. *Cell* 33, 857–864

77 Kmiec EB, Kroeger PE, Brougham MJ, Holloman WK (1983) Topological linkage of circular DNA molecules promoted by *Ustilago* rec1 protein and topoisomerase. *Cell* 34, 919–929

78 Kmiec EB, Holloman WK (1984) Synapsis promoted by *Ustilago* rec1 protein. *Cell* 36, 593–598

79 Blaho JA, Wells RD (1987) Left-handed Z-DNA binding by the recA protein of *Escherichia coli*. *J Biol Chem* 262, 6082–6088

80 Kmiec EB, Angelides KJ, Holloman WK (1985) Left-handed DNA and the synaptic pairing reaction promoted by *Ustilago* rec1 protein. *Cell* 40, 139–145

81 Kim JI, Heuser J, Cox MM (1989) Enhanced *recA* protein binding to Z DNA represents a kinetic perturbation of a general duplex DNA binding pathway. *J Biol Chem* 264, 21848–21856

82 Kmiec EB, Holloman WK (1986) Homologous pairing of DNA molecules by *Ustilago* rec1 protein is promoted by sequences of Z-DNA. *Cell* 44, 545–554

83 Ganea D, Moore P, Chekuri L, Kucherlapati R (1987) Characterization of an ATP-dependent DNA strand transferase from human cells. *Mol Cell Biol* 7, 3124–3130

84 Cassuto E, Lightfoot LA, Howard Flanders P (1987) Partial purification of an activity from human cells that promotes homologous pairing and the formation of heteroduplex DNA in the presence of ATP. *Mol Gen Genet* 208, 10–14

85 Fishel RA, Detmer K, Rich A (1988) Identification of homologous pairing and strand-exchange activity from a human tumor cell line based on Z-DNA affinity chromatography. *Proc Natl Acad Sci USA* 85, 36–40

86 Kenne K, Ljungquist S (1984) A DNA-recombinogenic activity in human cells. *Nucleic Acids Res* 12, 3057–3068

87 Kucherlapati RS, Spencer J, Moore PD (1985) Homologous recombination catalyzed by human cell extracts. *Mol Cell Biol* 5, 714–720

88 Hotta Y, Tabata S, Bouchard RA, Pinon R, Stern H (1986) General recombination mechanisms in extracts of meiotic cells. *Chromosoma* 93, 140–151

89 Kenne K, Ljungquist S (1987) RecA-like activity in mammalian cell extracts of different origin. *Mutat Res* 184, 229–236

90 Hotta Y, Stern H (1978) DNA unwinding protein from meiotic cells of *Lilium*. *Biochemistry* 17, 1872–1880

91 Angulo JF, Schwencke J, Moreau PL, Moustacchi E, Devoret R (1985) A yeast protein analogous to *Escherichia coli* recA protein whose cellular level is enhanced after uv irradiation. *Mol Gen Genet* 201, 20–24

92 Hsieh P, Meyn S, Camerini-Otero RD (1987) Partial purification and characterization of a recombinase from human cells. *In: DNA Replication and Recombination* (McMacken R, Kelly TJ, eds) Alan R Liss Inc, NY, 671–680

93 Moore SP, Rich A, Fishel R (1989) The human recombination strand exchange process. *Genome* 31, 45–52

94 Fishel R, Derbyshire M, Dowjat K, Harris CJ, Moore SP (1990) Studies on homologous pairing and strand exchange in human cells. *In: Molecular Mechanisms in DNA Replication and Recombination* (Richardson CR, Lehman IR, eds) Alan R Liss Inc, NY, 343–356

95 Hsieh P, Camerini-Otero RD (1989) Formation of joint DNA molecules by two eukaryotic strand exchange proteins does not require melting of a DNA duplex. *J Biol Chem* 264, 5089–5097

96 Heyer WD, Evans DH, Kolodner RD (1988) Renaturation of DNA by a *Saccharomyces cerevisiae* protein that catalyzes homologous pairing and strand exchange. *J Biol Chem* 263, 15189–15195

97 Heyer WD, Rao MRS, Erdile LF, Kelly TJ, Kolodner RD (1990) An essential *Saccharomyces cerevisiae* single-stranded DNA binding protein is homologous to the large subunit of human RP-A. *EMBO J* 9, 2321–2329

98 Norris D, Kolodner R (1990) Purification of a strand exchange stimulatory factor from *Saccharomyces cerevisiae*. *Biochemistry* 29, 7903–7911

99 Norris D, Kolodner R (1990) Interaction of a *Saccharomyces cerevisiae* strand exchange stimulatory factor with DNA. *Biochemistry* 29, 7911–7917

100 Konforti BB, Davis RW (1991) DNA substrate requirements for stable joint molecule formation by the RecA and SSB proteins of *Escherichia coli*. *J Biol Chem* (in press)