An overview of homologous pairing and DNA strand exchange proteins

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Summary — Processes fundamental to all models of genetic recombination involve 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 remains 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 contradictions 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 these proteins, further insight into the roles of nucleotide cofactors and accessory protein compo-

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Abbreviations: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; bp, base pair; kb, kilobase pair; n, nucleoside; kDa, kilodaltons; ATPγS, adenosine 5′-O-(3-thio-triphosphate); AMP-PNP, adenylyl 5′-imidophosphate
nts 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, and 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 1.

ATP-dependent homologous pairing proteins

E. coli recA protein

The E. coli recA protein (M, 37 842 Da) has several characteristic biochemical activities in vitro: 1, ssDNA 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, less A and homologous 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 pre-synapsis. ATP or the essentially non-hydrolyzable ATP analogue, ATP7S, 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/homocDNA protein monomer [24–26]. The stability and functionality of the pre-synaptic 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 1. Properties of known homologous pairing proteins.

ATP-dependent Proteins:

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gramnegative</th>
<th>Interaction with ATP</th>
<th>RecA protein binding</th>
<th>RecA protein cleavage</th>
<th>ATP-dependent NTP hydrolysis</th>
<th>RecA protein renaturation</th>
<th>Joint molecule formation</th>
<th>DNA strand exchange</th>
<th>RecA protein cleavage</th>
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Kd not determined, d4A. Optimal stoichiometry of homologous pairing protein (nt ssDNA/protein monomer) in the absence of any other protein. aMode of action (Stoichiometric or Catalytic) in the absence of any other protein. bPreferred end (5' or 3') of displacement of the non-complementary strand during initial pairing. cPotency of extension of the heteroduplex joint relative to the displaced non-complementary strand. dProtein-mediated renaturation of complementary ssDNA. eAbility of other aggregate ssDNA or dsDNA to aggregate ssDNA. fAccessory factors which stimulate the pairing reaction. gOptimal stoichiometry of homologous pairing protein (nt ssDNA/protein monomer) in the presence of the respective catalytic factor. hAssay used to determine activities: ge = gel assay with production of form II molecules; em = electron microscopy with visualization of the displaced strand; ms = macromolecular sensitivity assay; jg = gel assay with production of joint molecule intermediates; fb = filter binding assay with retention of joint molecules. iResults 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. Although ATP is bound by this protein, only ADP will bind to and activate nucleotide hydrolysis and DNA strand exchange. jValue based on the estimate of the proportion of HPP in the partially purified fraction. kBased on uv crosslinking of the ATP analogue, 8-azido ATP. lExpressed as 1000 dsDNA/protein monomer.
Homologous pairing proteins

In the next phase of DNA strand exchange, synap-
sis 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 mole-
cules. 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 homo-
logously paired joint molecules are detected experi-
mentally 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 depend-
ent 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 (or neither participant contains
either a free end or a region of homologous ssDNA),
only paracentric joints whose strands do not inter-
wind can be found; these molecules are unstable
when deproteinized [33, 34]. In the absence of topolo-
getic barriers to the interwinding of strands, however,
plectonemic joint molecules, which are stable in the
absence of protein, are produced. Joint molecule for-
mation is observed in linear and circular ssDNA.
Displaying essentially no preference for pairing at either
dsDNA end, but a distinct preference for pairing at the
circular DNA end containing the noncomplementary strand
is exhibited in the presence of ADP [27]. In contrast,
pairing between linear ssDNA and supercoiled DNA
occurs approximately at a 5- to 10-fold preference for comple-
mentarity at the 3' end of the ssDNA in the absence of ssDNA binding protein and a 100-fold preference for comple-
mentarity at the 3' end of the ssDNA in the presence of ssDNA binding protein and a 50-fold preference in its presence [35]. In addition to these homology-dependent reactions, recA protein can bind
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
protein synthesis. The strand exchange reaction
ultimately resulting in heteroduplex DNA containing 1 strand from each of the parental molecules. Thus, in the most typi-
cal 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 displa-
ced (non-complementary) strand of the duplex mol-
ecule [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 mol-
cule may imply that the exchange of DNA strands has
occurred only 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
observed using 3 ways: 1), by the formation of an S1
nuclease- or PI nuclease-sensitive strand displaced
from the labeled parental dsDNA [32, 2), by the ap-
pearance of form II molecules in the agarose gel
assay using circular ssDNA and linear dsDNA
substrates [32]; or 3), by the visualization of a dis-
placed 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-detesti-
lizing, and helicase activities are absent), whereas
classification as a DNA strand exchange protein
requires positive demonstration of strand displace-
ment by at least 1 of these 3 experimental criteria.
The SSB protein of E. coli (M, 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 in-
hibits recA protein-promoted DNA renaturation [30];
hinders the effect of recA protein on DNA strand
exchange action between gapped and linear duplex DNA
molecules [44]; and generally stimulates ssDNA-depend-
t DNA gyrase activity [25, 45]. In addition, SSB protein has an effect on the formation of heterogeneous ssDNA,
a likely reaction intermediate [36]. The lack of an effect on the conversion of ssDNA to semiconservative DNA,
the relatively low concentration of ssDNA present [44].
The basis of the stimulatory effect of SSB protein is
clearly not known [1, 5-7]. Formation of joint mol-
cule formation occurs only at suboptimal concentra-
tions of recA protein, relative to the ssDNA concen-
tration, and results from an alleviation of the inhibit-
ory effects of excess ssDNA; stimulation is maximal at
[1] SSB protein with higher concentrations of ssDNA 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 [48, 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 pretreatment of the presynaptic complexes at low (1 mM) MgCl₂ 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 continuously bound recA protein in the later process than for the shorter stretches in the former process. These effects of SSb protein are non-specific because other helix-stabilizing proteins from E. coli, bacteriophage T4, and yeast are also able to stimulate joint molecule formation [6, 52], ssDNA-dependent ATPase activity [27], and DNA strand exchange [52–54]. In addition to SSb protein, the recBCD enzyme of E. coli, a DNA helicase, stimulates recA protein-ssDNA-dependent heteroduplex DNA formation between parts 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 processes 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 of recA protein to both double-stranded SSb protein and underwind duplex DNA. When the bound ATP is hydrolyzed, this allosteric charge 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 for the formation of plecokonircularly 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-δDNA 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 and the genes were studied 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, 58, 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 ExoA 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 δDNA 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

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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 recE 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 Kₐ. 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 of 23-base-pair nucleic acid 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 M₉ (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 activity, 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 Asp; 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 synthesis is relatively inefficient in the absence of accessory factors (see below), both parasitic and plasmidic 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 bps) [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 ~ 8-fold the filament occurring more frequently as a consequence of ATP hydrolysis (e.g. as in tearing-out models) [68, 69].

T4 phage encodes a 33.5-kDa helix-stabilizing protein analogous to SSB protein, termed gene 32 protein (G32P), which binds tightly and cooperatively to ssDNA. Excess G32P inhibits both the ATPasase 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 subverting 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 plasmic, yet the rate of ATP hydrolysis is decreased by > 50% [70]. Substitution of SSB protein for G32P results in slower formation of joint molecules under conditions which are optimal for joint molecule formation or in the presence of G32P [70]. Analysis of DNA strand exchange between circular SSODNA and linear ssDNA is complicated by the formation of homology-depend- ent 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 strand exchange resembling network formation 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 reactions require con- tinuous ATP hydrolysis, since the addition of ATPγS inhibits an ongoing reaction, but only after a transient acceleration [8, 66]. 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 (i.e. they do not affect RecA protein activity). The uvsY protein (M, 16,600) binds cooperatively to both ss- and dsDNA [72], and it stimulates strand exchange = 3-fold by interacting with uvsX protein at molar ratios (uvsX/uvsY) 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 suboptimal conditions (or in the presence of either excoc 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 displace- ment 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 uvsX protein, the dda protein, a helicase involved in T4 replication, also stimulates (3 > 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 ei 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 sta- tionarily, resulting in the formation of a satu- rated helical filament. The binding of ATP, and possi- bly 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, uvsX 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 designator as the rec1 protein [12, 34], 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) (Hollozsik, 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 (300 ADP generated/min, i.e. that of uvsX protein, is 10-fold greater than that of recA protein, but joint nucleotide formation between ssDNA frag- ments 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 hete- roduplex extension and electrophoretic molecule forma- tion; joint molecules formed in the presence of the non-hydrolyzable ATP analogue AMP-PNP are un- stable when denatured and hence are presumably paramecian [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 read- ing 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 heterodu- plex joint, which forms preferentially at the dsDNA end including tail (3 displaced end). Similarly, strand displacement proceeds with a polarity (5' to 3') relative to the displaced strand; oppo- site that of both recA and uvsX proteins [76]. The initial formation of paramecian joint molecules by HPP results in dsDNA unwinding; as with the E.coli recA protein, these paramecian joints are unstable in the presence of ADP, presumably due to protein inactivation. The formation of phage lambda- derived 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 (2-6-fold) for Z-DNA than for B-DNA [79], but this enhancement is kinetically in nature; Z- DNA promotes faster nucleation than B-DNA does, but the equilibrium constant, B-DNA, is in favor of this complex [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. 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 uncharacterized activity [92, 93]. Like the recA protein, this protein fraction can both reneat complementarily 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 electropositive 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 [94] was 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 picoaffinity 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 5% of the input DNA is converted to complete heteroduplex products (ie form II molecules). The rate of DNA strand exchange by HPP-1 (2 nM) is 5-fold less than that of recA protein, but this slower rate is more typical of other eukaryotic proteins. Pairing requires a 5' complementarstrand 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 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 only one of the many copies of ATP (possibly that bound to HPP-1) is essential for strand exchange in crude extracts (10-fold faster). Nevertheless, the ability of the highly purified HPP-1 to promote thermal strand exchange 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-
kdalpha protein carries out both ss- and dsDNA aggre-
gation 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 concen-
tration, 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 the more surprising
given that optimal activity is observed at only 1
monomer/600 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 alter-
native is favored by data which correlate the proper-
ties of DNA strand exchange with those of ssDNA
aggregation, which are seen from gel filtration with
a 550 kDa, purified protein, if it is truly catalytic, does not appear to require a sto-
ichometric accessory factor (although it co-purifies with a 3’-exonuclease), as seen with uV-X
and the eukaryotic recombination function at substoichio-
metric concentrations (see below). In this respect, it is
more similar to the U mu/dis HPP, except that the
U mu/dis 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 melagaster strand trans-
ferase are unable to unwind a 20 bp duplex
region in 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 function, and an ATP-dependent
protein, which are able to displace similar fragments (not
exceeding 30 bp in length).

Saccharomyces cerevisiae strand exchange proteins
(SEP1/STPβ, DPA, and STPα)
The first protein described, and the most exten-
sively characterized activity, is the nontoxic 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 protostylid 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 to 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 (is 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 struc-
tures using 5’ end complementary dsDNA versus the
occurrence of α structures using 5’ end complementar-
ty dsDNA). These results distinguish SEP1 from
the other known eukaryotic strand transfer proteins,
which display both an end-bias in pairing and a polar-
ity of branch migration which is opposite of that of
both recA and avaX protein. As is the case for recA
protein, a substoichiometric concentration of SEP1
(1 monomer/100 nt) is optimal for ssDNA renatu-
ration.

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 sigmoidal
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 analog-
ous to the inhibition seen with excess avaX protein.

Two different stimulatory factors for SEP1-ssDNA
binding protein (ySSB protein), which binds
ssDNA cooperatively and with high affinity [54]. At
subsaturation 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 SEPI titration, but it does decrease (by ~2-fold) the amount of SEPI required for optimal levels of strand exchange in the presence of stoichio-
metric amounts of ySSB protein to 1 monomer/18 nt. As with the avEX protein-G32P reaction, enhancement of strand exchange by ySSB protein is negligible at saturating concentrations of SEPI. 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 RPAn [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 Zn2+ finger DNA binding domain. Likewise, a variety of DNA binding proteins stimulate joint molecule formation by STP3. A 50-fold stimulation is observed at optimal concen-
trations, and up to 1.5 kb of heteroduplex DNA is formed. Only 2–3 molecules of STP5 per linear dsDNA molecule, however, are required when opti-
mal concentrations of these ySSBs are present [21].

A second SEPI stimulatory factor, SF1, is a 33-kDa protein which dramatically alters the amount of SEPI 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 SEPI concentration (1 monomer) is equivalent to that obtained under the optimal unstimulated condi-
tions (ie at an SEPI:nt ratio of 1:1.2). This represents an ~12-fold stimulation over even higher concentra-
tions of SEPI, with an optimum at 1:75:2, 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 DNA 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 is dependent on the rest of the system. By virtue of its ability to alleviate the amount of SEPI 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 temperature-sensitive intermediate composed primarily of the strand exchange protein.

Although SF1 can aggregate both ssDNA and dsDNA to form stable-association of SF1, this property is not the basis of its stimulatory effect since it is effective under conditions which reduce its aggregative activ-
ity. Instead, SF1 likely plays a direct role in DNA strand exchange. Assuming that SEPI and SF1 have distinct roles in the reaction, 2 possibilities exist. First, SF1 could be involved in bringing the substrates to-
gether and aligning them, while SEPI catalyzes branch migration (see below). Alternatively, SEPI could participate in the initiation of synopsis, after which strand exchange and branch migration could be promoted by SF1-coated ssDNA. The second expla-
nation is considered to be more likely because it does not require turnover of SEPI. In this scenario, SF1 inhibits unproductive (or promotes productive) inter-
actions of SEPI with ssDNA so that SEPI can act nearly catalytically (at 1–2 monomers/dsDNA mol-
ecule).

The second strand exchange activity to be purified from mitotic yeast cells, termed DNA pairing activity (DPA) [20], has properties similar to those of SEPI that it may, at 120 kDa, be a further proteolytic product of the full-length SEPI. Although the stoi-
chiometry 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 sub saturating amounts of protein, DPA appears to act stoichiometri-
cally. One unusual property of DPA is its requirement for dsDNA substrates with either 3' or 5' tails of 4-50 nt in order to initiate strand transfer and branch migra-
tion, which appears to proceed with no preferred polar-
ity. 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 (like SF1) also lack the intrinsic ca-
bility to turn over.

The third yeast DNA strand exchange protein has been designated STP4s (strand transfer protein α). STP4s is a 38-kDa strand exchange protein isolated from mitotic cells [15]. It catalyzes DNA strand exchange and DNA renaturation in a manner very reminiscent of that described for STP5. For example, both STP4s and STP5 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 STP4s or STP5 per DNA molecule are required for maxi-
mal joint molecule formation. Both proteins are sensi-
tive to the concentration of ySSB proteins or other stimulatory agents, such as histone H1 and speri-
dine, with optimal stimulation being observed in each case at a concentration which aggregates 50% of the DNA. These proteins differ in 2 respects, however; STP5 is more sensitive to ionic conditions, and STP4s catalyzes the formation of recombinant strand exchange products (form II DNA molecules) more efficiently. These proteins are distinct since STP5 protein and activity are still detected in STP4s disruption strain. Thus, all 3 strand transfer activities isolated from yeast (SEPI, STP5, DPA, and STP4s) 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 protein 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 recF 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 for accessory proteins is absent.

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 binding 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 exensive
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 [13, 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 the 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 a protein-dependent DNA strand exchange, as depicted in figure 1, occurs only at one end of the linear DNA (perhaps due to tissue-specific constraints), and due to this bound protein, thermal (ie protein-independent) branch migration extends the nascent heteroduplexes 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.

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