

# Stoichiometry, Base Orientation, and Nuclease Accessibility of RecA·DNA Complexes Seen by Polarized Light in Flow-oriented Solution

IMPLICATIONS FOR THE MECHANISM OF GENETIC RECOMBINATION\*

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**By using flow linear dichroism, in combination with nuclease digestion and two spectroscopically distinguishable DNAs, we demonstrate the existence of two internal and one external DNA-binding sites in the RecA fiber. A number of different complexes between RecA and single- and double-stranded DNAs are characterized with respect to stoichiometry, location, and base orientation of each of the associated DNAs. Based on these results, we discuss important steps of the mechanism of general genetic recombination.**

The molecular mechanism of gene exchange has attracted great attention since it might in a paradigmatic way promote understanding of a number of fundamental biological processes. Despite intense study, though, the ways recombination proteins interact with and transfer DNA, and even the stoichiometries of the various adducts that are formed, have remained largely unknown. A primary limitation is the inapplicability of most biochemical and biophysical methods for studying non-specific protein-nucleic acid interactions *in situ*. We have found that flow linear dichroism (LD),<sup>1</sup> measured with phase-modulation technique, provides unique possibilities for probing such systems, in that it may provide information about both hydrodynamic (global) and optical (local) structural properties (1). In this way we have managed to characterize several distinct RecA·DNA complexes, both as to stoichiometry and average organization of crucial chromophoric groups; we here also report the discovery of a selective digestion of DNA in one of the complexes when subjected to micrococcal nuclease. The emerging picture of the RecA·DNA system is used to discuss the mechanistic role of RecA in the general recombination.

The RecA protein in *Escherichia coli* promotes strand exchange between homologous DNA in the general recombination process, post replicative repair, and the induction of the SOS system by activating the cleavage of the LexA repressor (for reviews, see Refs. 2-8). *In vitro*, RecA forms extended fibers that bind DNA in presence of ATP, or its non-hydro-

lyzable analog ATP $\gamma$ S, as a cofactor (9). The fiber may accommodate up to three different strands of single-stranded (ss)DNA or one double-stranded (ds)DNA and one ssDNA (10). The RecA protein recognizes homologous sequences in the dsDNA and ssDNA molecule or in two dsDNAs if one of the strands is nicked, and can perform strand exchange in such regions (11, 12). In order to understand the molecular basis of these reactions, physical studies on RecA-DNA interactions are being performed. Using flow linear dichroism and fluorescence spectroscopy, we have earlier demonstrated that RecA can simultaneously coordinate up to three molecules of ssDNA and also one each of an ssDNA and a dsDNA molecule (10). In this work we use the same physical techniques to study model systems composed of RecA + poly(d $\epsilon$ A) + unmodified synthetic homopolymers + unmodified DNA. These systems are specially designed to allow spectral discrimination between different DNA molecules simultaneously coordinated by a single RecA fiber. By exposing the complexes to nuclease, we also demonstrate, *in situ*, the selective degradation of one of the DNA molecules in a ternary RecA·DNA complex.

## MATERIALS AND METHODS

The RecA protein was prepared as described previously (10) except that the conventional DEAE-cellulose chromatography was replaced by high performance DEAE-5PW (Tosoh) chromatography in order to improve the purification. The concentration in subunits was determined spectroscopically by using  $\epsilon_{280} = 2.17 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  (13). Micrococcal nuclease (Pharmacia LKB Biotechnology Inc., 27-0584, lot PM58431) was a kind gift from Pharmacia and free from protease (no degradation of RecA after 30 min of incubation with the nuclease was observed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis). The specific nuclease activity was given by the manufacturer.

As dsDNA calf thymus DNA (Sigma, type I, lot 66F-9645) was used. Agarose gel electrophoresis showed that this DNA was mainly constituted of fragments larger than 20 kilobase pairs. Unmodified ssDNA was obtained by heat denaturation of the dsDNA. Poly(dA) (lot PG717836) and poly(dT) (lot OG 627834) were obtained from Pharmacia. The average length of these polynucleotides was given by the manufacturer and was about 300 bases. The absorption coefficient used for the determination of concentration was  $6.5 \times 10^{-3} \text{ M}^{-1} \text{ cm}^{-1}$  (in nucleotides) at 260 nm for dsDNA,  $8.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$  at 257 nm for poly(dA) and  $8.52 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$  at 264 nm for poly(dT). These polynucleotides were freshly dissolved just before use. Poly(d $\epsilon$ A) was prepared from poly(dA) (Pharmacia, lot 00417836) as described previously (14). The average length was estimated from sedimentation experiments to about 250 bases, and its concentration was determined by using  $\epsilon_{257} = 3.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$  (15).

ATP $\gamma$ S was obtained from Boehringer Mannheim (lot 11614821-78). The amount of ATP $\gamma$ S degraded to ADP was, according to the supplier, less than 20% at the time of the experiments. Since the binding affinity of ADP to RecA is two orders of magnitude lower

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<sup>1</sup> The abbreviations used are: LD, linear dichroism; ATP $\gamma$ S, adenosine-5'-O-(3-thiotriphosphate); dsDNA, double-stranded DNA; ssDNA, single-stranded DNA;  $\epsilon$ A, etheno derivative of adenine.

than that of ATP $\gamma$ S, this amount of ADP was considered not to influence the experiments. Experiments using "older" lots of ATP $\gamma$ S, that contained more of ADP, revealed no differences in the LD signals.

All experiments were performed at room temperature (22 °C) in a buffer containing 20 mM sodium phosphate, pH 6.6, 50 mM NaCl, 4 mM MgCl<sub>2</sub>, and 1 mM dithiothreitol. Complexes were formed by adding the components in the order RecA (1–4  $\mu$ M), polynucleotide, and ATP $\gamma$ S (40  $\mu$ M), and the mixture was incubated for 15 min. The order of addition did not affect the linear dichroism signal. When multiple polynucleotides were added, the mixture was further incubated for 30 min after each addition of polynucleotide. Longer incubation times (up to 3 h) did not modify the LD signals.

Linear dichroism was measured on a Jasco J-500 spectropolarimeter. The samples were oriented in a Couette cell (1) using shear gradients of 600–1500 s<sup>-1</sup>. The shapes of the LD spectra were independent of shear gradient between 150 and 1500 s<sup>-1</sup>. Recording of each spectrum took about 30 min and no alterations of the LD signal were observed during the recording. The cell length was 1 mm and the temperature 22 °C. All LD data are normalized to 1  $\mu$ M RecA concentration and 1-cm light path. Isotropic fluorescence was measured on an Aminco SPF-500 Spectrofluorimeter as described earlier (10). To ease the identification of the different complexes, we have indicated the order of component addition in the name.

## RESULTS

**RecA Interaction with Single-stranded DNA**—We shall first deal with the flow LD and fluorescence results for RecA upon complexing increasing amounts of poly(dεA) and unmodified ssDNA in presence of ATP $\gamma$ S. The complexes formed in the presence of ATP $\gamma$ S are kinetically very stable and their formation can be considered practically irreversible (16). It may thus be possible to "trap" complexes in intermediate stages of strand exchange. The use of an etheno-modified polynucleotide allows fluorescence detection of its association and also spectroscopic discrimination between the different DNAs in the RecA complexes. The LD probes any deviation from isotropic distribution: it is nearly zero for free single-stranded DNA due to high flexibility and to a nearly random organization of the light-absorbing purine and pyrimidine bases. By contrast, ssDNA exhibits a substantial LD magnitude in the presence of RecA showing that the DNA bases are now far from randomly distributed. From knowledge of how the light-absorbing transition dipoles are directed in the DNA bases and in the aromatic amino acid residues one may extract information about how these chromophores are organized in the complexes.

From the titration results in Fig. 1 the formation of several distinct RecA·ssDNA complexes may be inferred. Both LD and fluorescence intensity increase in magnitude up to a stoichiometry of approximately 3 bases/RecA monomer, corresponding to coordination of a first ssDNA molecule in the RecA fiber. This point is characterized by a maximum in LD at 285 nm and a change of slope in the fluorescence titration curve (10). Between the ratios of three and six nucleotides/RecA, the negative LD at 260 and 310 nm and the fluorescence intensity, now with an altered slope, increase in magnitude, whereas the positive LD at 285 nm decreases slightly. This reflects the association of a second molecule of ssDNA to the RecA-ssDNA fiber (10). Upon further addition of ssDNA (now unmodified ssDNA in order to distinguish it from the poly(dεA)), its coordination to the RecA complex is revealed by an increase toward positive values of the LD signal at 260 nm.

Fig. 2 displays the LD spectra of the three different DNA·RecA complexes. The LD profiles corresponding to the RecA complexes containing one and two ssDNA molecules are very similar, suggesting similar binding geometries for the first two strands. The negative LD features around 240, 260, and 315 nm, corresponding to (in-plane) absorption bands in the eth-

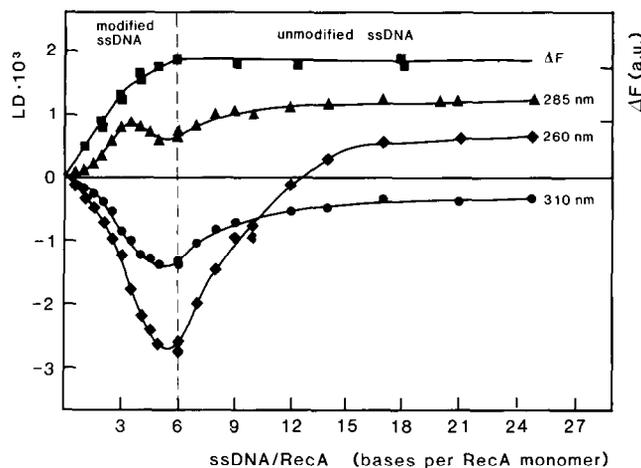


FIG. 1. LD signal at 260 nm (●), 285 nm (▲), and at 310 nm (○) representing ssDNA, tryptophans in RecA, and poly(dεA), respectively, and the fluorescence intensity of poly(dεA) (■) as a function of added poly(dεA) and unmodified ssDNA to RecA. Flow gradient 1200 s<sup>-1</sup>.

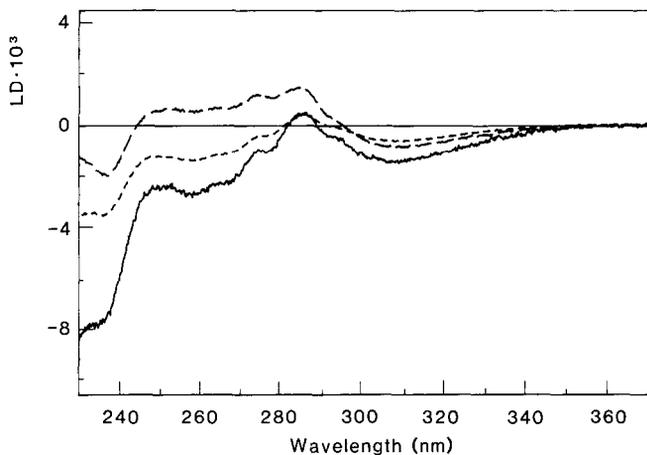


FIG. 2. LD spectra of the complexes RecA·poly(dεA) (---), RecA·[poly(dεA)]<sub>2</sub> (—) and RecA·[poly(dεA)]<sub>2</sub>·ssDNA (- · -). The stoichiometries are 3 bases of each poly(dεA) strand/RecA and saturating amounts of ssDNA (≈15 bases).

eno-modified adenine bases, demonstrates a preferentially perpendicular<sup>2</sup> base orientation in the first two ssDNAs (10, 17, 18). Around 285 nm a positive LD feature with a structure that can be identified with protein absorption (10, 17) indicates that the aromatic indole planes of the tryptophan residues in the RecA protein have a preferentially parallel orientation relative to the fiber axis.

The LD spectrum of the third complex differs dramatically from those of the other two. Around 260 nm the LD is now positive, meaning that the average base orientation in this complex is preferentially parallel to the fiber axis. Since the LD around 310 nm, where poly(dεA) but not normal ssDNA absorbs, remains negative the positive LD around 260 nm can be attributed to the extra contribution from the third ssDNA molecule. In contrast to the first two ssDNAs, the bases in the third ssDNA are thus oriented preferentially parallel to the fiber. In conclusion, the mode of association of the third

<sup>2</sup> The LD signal changes sign at an effective angle of 55° between the light-absorbing transition dipole and the local fiber axis (1). The term preferentially perpendicular (parallel) base orientation is used to denote an average base inclination of more (less) than 55° relative to the fiber axis.

ssDNA molecule to the RecA fiber differs from that of the other two.

Fig. 3a shows the time behavior of the LD signal at 260 nm after addition of micrococcal nuclease to the RecA complex containing three ssDNA molecules. The LD signal decreases rapidly but reaches an essentially constant level after approximately 10 min. The LD spectrum after the digestion, shown in Fig. 3b, is within experimental error identical to the spectrum of the complex containing two ssDNAs, *i.e.* the spectrum recorded before addition of the unmodified ssDNA. We may thus conclude that the last added ssDNA molecule is not protected from nuclease digestion, whereas the first two ssDNAs are.

**Simultaneous Interaction of RecA with Single- and Double-stranded DNA**—Fig. 4 shows LD spectra of the poly(dεA)·RecA·dsDNA and dsDNA·RecA·poly(dεA) complexes, which differ only with respect to the order of addition of the DNAs, before and after nuclease digestion. The stoichiometry in these complexes is 3 base pairs of dsDNA + 3 bases of poly(dεA)/RecA monomer, which is known to fully saturate the RecA fiber (10). In both of the complexes, the bases in both the dsDNA molecule and the poly(dεA) strand appear to be preferentially perpendicular to the fiber axis as evidenced from the large negative LD signal at 260 nm (dsDNA) and

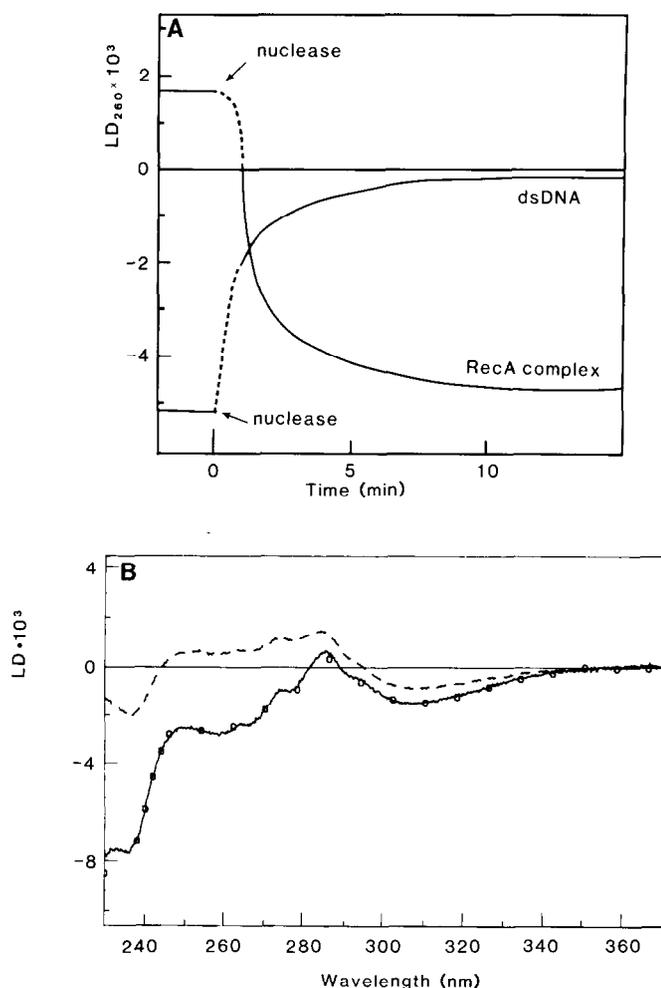


FIG. 3. *a*, time dependence of the LD signal at 260 nm of the RecA·[poly(dεA)]<sub>2</sub>·ssDNA after addition of micrococcal nuclease (in 1 mM CaCl<sub>2</sub>). For comparison, the corresponding time dependence of the digestion of free dsDNA is shown. *b*, LD spectra of the RecA·[poly(dεA)]<sub>2</sub>·ssDNA complex before (—○) and after (---○) addition of nuclease. The circles denote the spectrum of RecA·[poly(dεA)]<sub>2</sub> (○) reproduced from Fig. 2 for comparison.

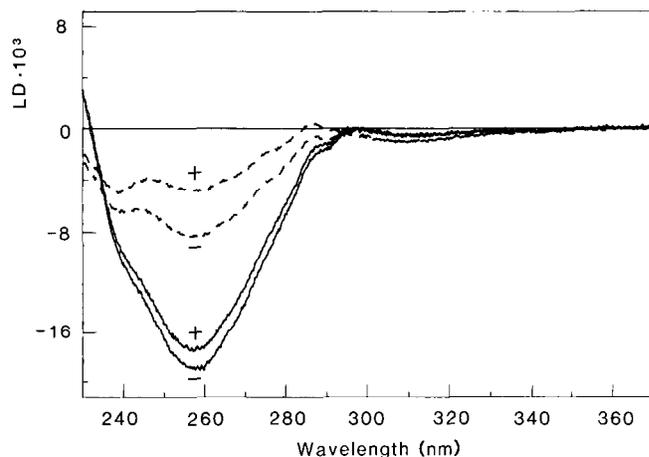


FIG. 4. LD spectra of RecA·dsDNA·poly(dεA) (—) and RecA·poly(dεA)·dsDNA (---) complexes before (—) and after (+) addition of nuclease. The stoichiometries are 3 bases of poly(dεA) and 3 base pairs of dsDNA/RecA monomer. Gradient 1200 s<sup>-1</sup>.

from the negative LD features at 240 and 315 nm (poly(dεA)). Further, in both complexes the characteristic positive LD feature from the RecA proteins is observed around 290 nm. The two complexes differ, however, with respect to the relative amplitudes of the dsDNA and poly(dεA) features. The pronounced negative LD features at 240 and 315 nm in the complex where poly(dεA) is added first show that the dεA bases are significantly more perpendicular in this complex than in the complex where dsDNA has entered first. Indeed, the similar magnitudes of the LD bands at 240 and 260 nm are consistent with the bases in the poly(dεA) being essentially coplanar to the bases in the dsDNA. We also note that the overall LD magnitude for the poly(dεA)·RecA·dsDNA complex is significantly lower than for the dsDNA·RecA·poly(dεA) complex and also lower than for the RecA complex with only dsDNA (10). The overall LD magnitude reflects the flow orientation properties of the complex (1, 10), so this is a clear indication of a lower persistence length. Since the persistence length, everything else being unchanged, increases with increasing mass, this must reflect a decreased rigidity of the dsDNA·RecA entity in the poly(dεA)·RecA·dsDNA complex, which, for example would be expected if the dsDNA is strand-separated.

Both DNAs in the dsDNA·RecA·poly(dεA) complex are protected from nuclease digestion as seen from the essentially unchanged features of the LD spectrum recorded after nuclease addition. The small decrease in the overall LD magnitude caused by the nuclease treatment is most likely owing to a lower degree of complex orientation as a result of spurious cuts at sites where the DNAs are locally exposed due to incomplete RecA binding. For the poly(dεA)·RecA·dsDNA, a more substantial decrease in LD magnitude is observed, suggesting only a partial protection. The digestion alters slightly the shape of the LD spectrum: the LD at 260 nm (dominated by dsDNA) decreases more in intensity than the LD at 240 nm (dominated by poly(dεA)), indicating that the dsDNA is the less protected DNA species. The reason why most of the dsDNA LD signal remains after the digestion might be due to that only a part of the dsDNA molecule is exposed to the nuclease, possibly only one strand.

We conclude that RecA forms two different complexes with one each of ssDNA and dsDNA depending on order of addition. The complexes have the same stoichiometries but differ in relative base orientation and rigidity. The ssDNA·RecA·dsDNA complex has a coplanar arrangement of the bases of

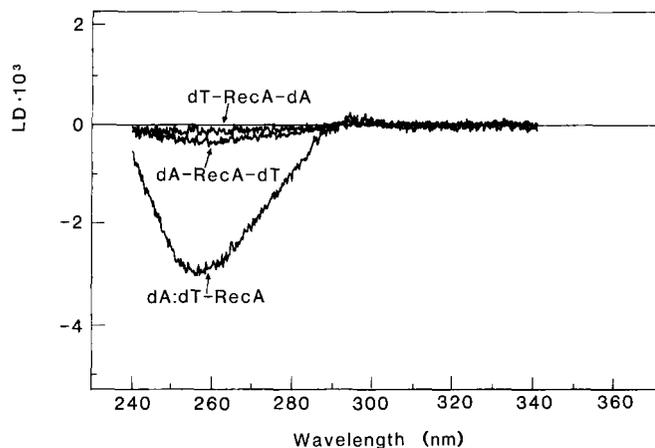


FIG. 5. LD spectra of poly(dA)·RecA·poly(dT), poly(dT)·RecA·poly(dA), and [poly(dA)·poly(dT)]·RecA complexes. Gradient  $600 \text{ s}^{-1}$ .

the ssDNA and the dsDNA strands, and the dsDNA molecule has an impaired rigidity, possibly being strand-separated. In the dsDNA·RecA·ssDNA complex, the bases of the ssDNA and dsDNA strands are not coplanar, and the dsDNA molecule is expected to have a more regular double helical structure. Both complexes are stable in absence of ATP hydrolysis.

**Base Pairing in Binary Complexes**—Fig. 5 compares the LD spectra of the poly(dA)·RecA·poly(dT) and poly(dT)·RecA·poly(dA) complexes with that of the [poly(dA)·poly(dT)]·RecA (the first two complexes were formed with the two ssDNAs bound one each in the two internal sites, whereas in the last complex the two ssDNAs were allowed to anneal, forming a dsDNA molecule, prior to the addition of RecA). The LD amplitudes of the complexes with two ssDNAs are an order of magnitude lower than the LD of the poly(dA)·poly(dT)·RecA. This demonstrates that the two strands when added separately, although being complementary, do not become regularly base paired with each other. This could indicate that ATP hydrolysis is associated with base pairing. However, recently Menetski *et al.* (19) have reported the formation of joint molecules of plectonemic nature in the presence of ATP $\gamma$ S, ruling out this function of ATP hydrolysis in the recombination reaction. As we shall see, a more plausible explanation is that annealing does not occur between strands bound in the two internal sites, but may occur between one strand in the internal and one in the external site.

#### DISCUSSION

**Ternary RecA·ssDNA Complexes**—As demonstrated by the results in Figs. 1 and 2, RecA can form a ternary complex with two molecules of poly(dεA) and one molecule of unmodified ssDNA. As suggested from the positive LD at 260 nm arising mainly from the last added unmodified ssDNA, the base orientation of this ssDNA molecule must be preferentially parallel to the fiber axis. The base orientation of the two other ssDNA molecules remains preferentially perpendicular as evident from the negative LD at 315 nm, and we conclude that in this complex the base orientation in the last added DNA is different from the average base orientation of the first two. This conclusion, though, may not be general for all RecA complexes with three ssDNAs, since the physical properties of ternary RecA·ssDNA complexes apparently depend on the chemical nature of the complexed ssDNAs. For example, etheno-modified DNA does not appear to bind at all in the third, low affinity binding site, when the two internal sites are occupied with ssDNAs (10). The complex with all three DNA molecules being unmodified ssDNA does not

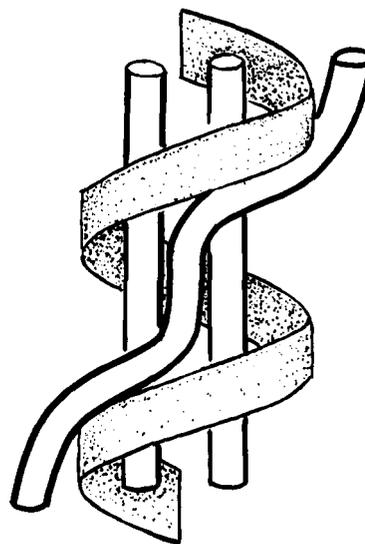


FIG. 6. Schematic structure of the RecA spiral with three associated strands of ssDNA (rods) as suggested from the spectroscopic results. Two initially bound strands are accommodated within the RecA spiral. A third strand is associated externally. Its bound section is tentatively placed parallel to the two interior strands and a nonquantitative binding is depicted to indicate the weak association.

orient in flow (10), and the physical properties of complexes formed with various homopolymers depend on the base composition of the ssDNAs.<sup>3</sup>

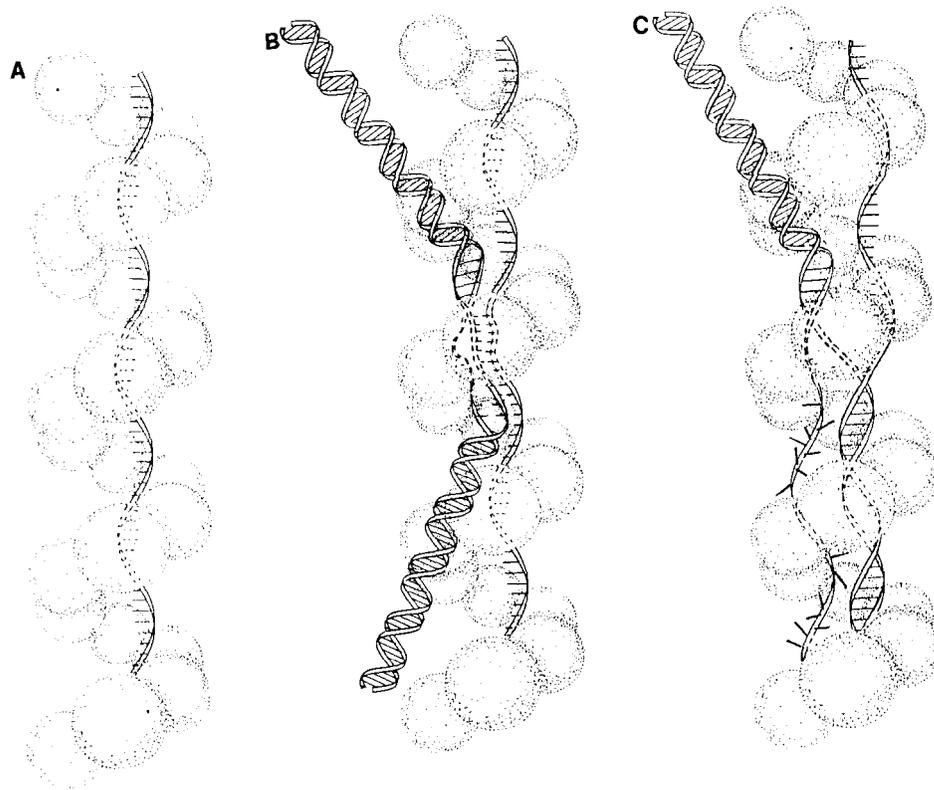
Nuclease treatment of the RecA·poly(dεA)·ssDNA ternary complex gives a product with the same LD as has the RecA complex with only two ssDNAs (Fig. 3b), clearly showing that only the last added ssDNA is degraded by the nuclease and that the other two ssDNAs are protected. Corresponding nuclease treatment of a complex with three molecules of unmodified ssDNA also reveals a selective degradation (results not shown), though because of the identical chemical nature of all ssDNAs, the molecule that has been degraded could not be identified here. Although there is obviously a complicated structural dependence on the chemical nature of the ssDNAs involved in ternary complexes with RecA, it appears a general conclusion that only the last added ssDNA is accessible to the nuclease.

A plausible explanation of these observations is that the first two added ssDNA molecules are accommodated inside the RecA fiber and effectively shielded from nuclease digestion, whereas the last added third ssDNA is exposed on the outside. Such an arrangement may be related to the inhibition of the RecA cleavage activity of the LexA and the  $\lambda$  phage repressors observed at ssDNA/RecA ratios above 6 (20–22), *i.e.* when DNA becomes externally bound. One may speculate that the RecA cleavage site is located at a position on the exterior of the fiber where the third, externally bound ssDNA may provide steric interference.

A schematic picture of the three-stranded complex emerging from the spectroscopic results is drawn in Fig. 6. The RecA proteins are assembled in a spiral, as suggested by electron microscopy (23, 24). Two ssDNA molecules are positioned in the center of the fiber and a third ssDNA is located on the outside. Only partial association of the third ssDNA is depicted, suggesting a nonquantitative interaction as indicated by the very high saturation level observed (Fig. 1) and its possible involvement in coaggregation.

**Ternary RecA·dsDNA·ssDNA Complexes**—As seen in Fig.

<sup>3</sup> M. Kubista, M. Takahashi, and B. Nordén, manuscript submitted.



**FIG. 7. Model for the RecA-mediated strand exchange reaction.** *a*, Presynapsis. RecA binds to the ssDNA forming a helical fiber with a stoichiometry of 3 bases/recA monomer. The DNA bases in the complex are perpendicularly oriented. *b*, Synapsis. dsDNA binds to the presynaptic fiber with one strand in each site. The initial contacts are in noncomplementary regions and only a small part of the dsDNA is incorporated. The dsDNA can diffuse through the binding site, like a thread through the eye of a needle. *c*, Strand transfer. When complementary regions in the ssDNA and the dsDNA are encountered at a DNA end, base pairing occurs and the two strands intertwine.

4, RecA forms two different ternary complexes with one each of ssDNA and dsDNA. The observation that the physical properties are different for the two complexes suggests that the DNA-binding sites in RecA are different, a finding that is consistent with the apparent saturation of the first binding site, prior to the onset of additional DNA binding (Fig. 1, and Ref. 12). Note though, that the binding sites may originally be equivalent, but coordination of one type of DNA may cause allosteric changes that affect the properties of the other sites.

The finding that the two ternary complexes ssDNA·RecA·dsDNA and dsDNA·RecA·ssDNA have different physical properties has interesting implications as to their function in the strand exchange reaction. RecA binds with much higher affinity to ssDNA than to dsDNA, and with access to both DNAs it will bind to the ssDNA. We suggest that this binding involves the "primary" internal site. We note that in the ternary complex with ssDNA in the primary site, the ssDNA bases are roughly as perpendicular to the RecA fiber as the dsDNA bases are, as judged from the comparable amplitudes of the LD at 240 and 260 nm (*cf.* Fig. 5). An arrangement with the bases perpendicularly oriented in all three DNA strands can be imagined as most economical in a machinery designed for recognition of homologous sequences and reformation of base pairs. Honigberg *et al.* (25) have shown that RecA·ssDNA complexes formed in the presence of ATP $\gamma$ S bind homologous dsDNA much more efficiently than non-homologous dsDNA which has been recently verified by Müller *et al.* (26). Our LD observations, demonstrating a base alignment suitable for base pairing interactions, allow us to propose that the observed difference in stability between homologous and non-homologous ssDNA·RecA·dsDNA complexes is caused by base-base interaction between the ssDNA and the dsDNA. This difference in stability can provide a mechanism for identifying homologous sequences since homologous ssDNA·RecA·dsDNA complexes are expected to be energetically favored. We note that the base-base interactions between ssDNA and dsDNA in the ternary ssDNA·

RecA·dsDNA complex occur in absence of ATP hydrolysis, indicating that energy is not required for the recognition of homologous sequences in the recombination reaction. This conclusion is supported by the recent report of Menetski *et al.* (19) of plectomenic joints formation between homologous DNA in the presence of ATP $\gamma$ S.

Müller *et al.* (26) report that the stability of dsDNA·RecA·ssDNA complexes is independent of DNA homology, in agreement with our observations that the DNA bases in this complex are not suitably aligned for base-base interactions between the ssDNA and the dsDNA. This complex is thus unlikely to be involved in the process of identifying homologous sequences in the recombination reaction, but may possibly be a later intermediate.

*Implications on the Mechanism for RecA-mediated Strand Exchange*—The spectroscopic data presented above indicate 1) the existence of three different DNA "binding sites," two internal and one external, in the RecA fiber. 2) A practically constant stoichiometry of about three bases (or base pairs) per RecA monomer of each quantitatively bound DNA molecule. 3) In presence of cofactor, a preferentially perpendicular base orientation for the first two bound DNA molecules. 4) Two distinguishable RecA complexes with one each of a dsDNA and an ssDNA molecule. 5) No noticeable spontaneous base pairing in absence of ATP hydrolysis between two complementary ssDNAs bound in the two internal binding sites.

The finding of three binding sites may provide a first step toward understanding the mechanism of RecA-mediated strand exchange. We note that RecA can simultaneously coordinate three strands of ssDNA (three ssDNAs or one dsDNA and one ssDNA), but apparently not four strands (complexes with two dsDNAs or one dsDNA and two ssDNAs or with four ssDNAs have not been reported). One could thus suspect that the RecA-mediated strand exchange occurs between two dsDNAs of which one has been strand separated prior to RecA binding. This hypothesis is strongly supported

by recent footprinting experiments by Chow *et al.* (27–28) which reveal similar protection patterns in symmetric and asymmetric strand exchange. The crucial step to generate single-stranded DNA substrates for the recombination could be related to the activity of the RecBCD protein which is known to separate the strands of dsDNA (29) and play a ubiquitous role in the principal pathway (RecBCD pathway) of recombination (4–6).

Our findings and conclusions may add a number of hints as to the molecular basis of the strand exchange reaction. We adopt the proposal by Smith *et al.* (6, 30, 31), that a RecBCD strand separating step precedes the actual recombination, providing RecA with ssDNA substrate. At physiological ATP concentrations (3–5 mM), the RecA is saturated with ATP and the ssDNA bases are preferentially perpendicularly oriented (10, 35) as tentatively illustrated in Fig. 7a. The ssDNA is bound in the primary internal site with the RecA proteins wound around in a spiral. The filament has a diameter of approximately 100 Å with about six RecA units/turn (23) and the stoichiometry is 3 bases of ssDNA/RecA monomer (10, 32).

The ssDNA·RecA·ATP complex now coordinates a dsDNA molecule, forming a ssDNA·RecA·dsDNA ternary complex. There are two free binding sites available, and we suggest that the dsDNA becomes bound with one strand in each of the two binding sites (Fig. 7b). Such binding requires an almost complete strand separation, which has been evidenced by electron microscopic observations (33). It is also consistent with the relatively small LD signals of the ssDNA·RecA·dsDNA complex and could account for the partial protection of the dsDNA from nuclease digestion (Fig. 4).

In the ssDNA·RecA·dsDNA ternary complex, the bases in all three strands are coplanar, and base-base interactions are anticipated between the ssDNA and the appropriate strand of the dsDNA molecule. Since two complementary ssDNA molecules bound in the two internal sites are unable to anneal (Fig. 5), we suggest that the ssDNA molecule initially coordinated by RecA and bound in the high affinity internal site, is in contact with the strand of the dsDNA that is bound in the external site; the other dsDNA strand being bound in the second internal site. This conclusion is supported by the footprinting experiments of Chow *et al.* (28) showing that the transferred strand in the strand exchange reaction is the only strand not being protected from nuclease digestion, and by the observation that RecA-stimulated DNA renaturation appears to occur between one strand bound in one of the internal sites and one in the external site.<sup>3</sup>

The proposed arrangement of ssDNA and dsDNA in the ssDNA·RecA·dsDNA complex may explain the ability of the recombination system to find complementary sequences, without the requirement of energy input by ATP hydrolysis (19). For statistical reasons, the initial contacts between the ssDNA·RecA fiber and the dsDNA will be in non-complementary regions. These complexes are rather weak (26) and only a small part of the dsDNA is likely to be incorporated. The remaining free part can be anticipated to bridge several fibers, making up the large three-dimensional networks observed *in vitro* (34). In non-complementary regions, the dsDNA is only loosely bound by the ssDNA·RecA complexes and may diffuse like a thread through the eye of a needle. When a homologous region is encountered, favorable base-base interactions largely increase the interaction energy and the dsDNA becomes locked. Since the dsDNA is strand separated, the ssDNA in complementary regions and in absence of topological constraints, *i.e.* at a DNA end, may intertwine

with the complementary dsDNA strand (Fig. 7c), explaining the stability of plectomenic joints in absence of RecA protein.

So far energy dissipation by ATP hydrolysis has not been required, raising the question which the energy consuming step is? As suggested by Menetski *et al.* (19), it may simply be the dissociation of the complex, which possibly provides directionality. The dsDNA·RecA·ssDNA complex formed in presence of ATP $\gamma$ S (Fig. 4) is a likely candidate for the final complex prior to dissociation (*top half* of Fig. 7c). In this complex the ssDNA, which should be the non-transferred strand of the dsDNA, is not coplanar with the bases of the dsDNA molecule, and the larger overall LD signal indicates a more rigid dsDNA structure, suggesting that the two dsDNA strands are interwound. Dissociation of this complex, possibly induced by ATP hydrolysis (19) gives only a single product, which is the hetero duplex.

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