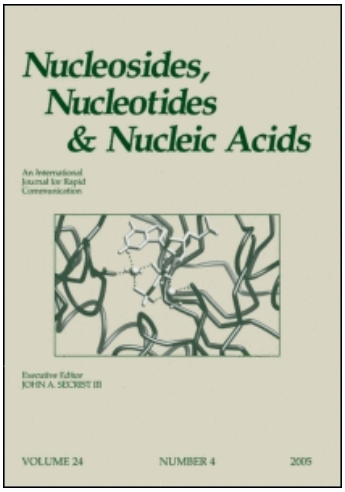

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Properties of RecA Complexes with Homopolymeric DNA Strands Depend on Sequence Complementarity. Implications for the Mechanism of Strand Exchange

Mikael Kubista[a]; Bengt Nordén[b]; Masayuki Takahashi[c]
[a] department of Biochemistry & Biophysics, Molecular Biotechnology group, Gothenburg, SWEDEN [b] department of Physical Chemistry, Chalmers University of Technology, Gothenburg, SWEDEN [c] Groupe d'Etude Mutagénèse et Cancérogénèse, CNRS, Institut Curie, Université Paris Sad, Orsay, Cedex, FRANCE

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PROPERTIES OF RECA COMPLEXES WITH HOMOPOLYMERIC DNA STRANDS DEPEND ON SEQUENCE COMPLEMENTARITY. IMPLICATIONS FOR THE MECHANISM OF STRAND EXCHANGE

Mikael Kubista,[1] Bengt Nordén,[2] and Masayuki Takahashi[3]

[1]Molecular Biotechnology group, department of Biochemistry & Biophysics and [2]department of Physical Chemistry, Chalmers University of Technology, S-412 96 Gothenburg, SWEDEN, and [3]Groupe d'Etude Mutagénèse et Cancérogénèse, CNRS, Institut Curie, Université Paris Sud, F-91405 Orsay, Cedex, FRANCE.

ABSTRACT

We have characterised complexes between RecA and single-stranded homopolynucleotides by linear dichroism spectroscopy and small angle neutron scattering to investigate base pairing possibilities among DNA strands bound in a RecA filament. We find that in the presence of the non-hydrolysable cofactor ATPγS, and very likely also in the presence of ATP, a RecA fiber has three distinct DNA binding sites, each of which can bind one strand of DNA at a stoichiometry of three nucleotides per RecA monomer. The structural and hydrodynamic properties of the complexes are found to depend on the number of strands bound and on sequence complementarity among the strands. For example, RecA-[homopolymer]$_3$-ATPγS complexes aggregate when either of the strands bound in sites I and II is complementary to the strand bound in site III.

Dedicated to the memory of Dr. Roland K. Robins

We have also studied the RecA catalysed annealing of complementary homopolymers and find it to be most efficient when two strands of one homopolymer are bound per RecA filament prior to the addition of the complementary homopolymer. These results suggest that a DNA strand bound in site III can base-pair with either of the strands in sites I and II, whereas the latter strands are unable to base-pair with each other.

INTRODUCTION

Strand exchange between homologous DNA molecules is an important process in post replicative repair. In Escherichia coli the RecA protein is a central component of this reaction and the protein also regulates the synthesis and activities of a series of proteins involved in DNA repair and mutagenesis.[1] To understand these functions the interaction of RecA with DNA has been extensively studied.[2,3] From electron microscopic observations it was inferred that RecA binds DNA in a cooperative manner by forming a helical structure around it,[4,5] and from linear dichroism studies it was early speculated that RecA can simultaneously bind three molecules of single-stranded (ss) DNA.[6,7]

To investigate the base pairing possibilities among the bound strands in a RecA filament we have in this work studied a large number of RecA complexes with single-stranded homopolymers by linear dichroism (LD) and small angle neutron scattering (SANS). The flow linear dichroism technique is very useful for obtaining structural information about filamentous DNA-protein complexes,[8] and is particularly suitable to study RecA-DNA interactions.[9] The complexes are macroscopically oriented by shear in a streaming solution and the absorption of two orthogonal forms of plane polarised light is measured as function of wavelength. In this way it is possible to determine the orientations of vital chromophoric groups, such as the DNA bases and aromatic amino acids, and, in combination with small angle neutron scattering anisotropy,[10,11] changes in the hydrodynamic properties of the fibres. For example, the build up of well orienting DNA-containing RecA filaments is reflected by an overall increase in LD

magnitude, whereas interactions between these filaments, resulting in netted structures with poor orientation properties, results in a substantial drop in LD magnitude.

MATERIALS AND METHODS

Chemicals

RecA was purified as described elsewhere,[9] except that the conventional DEAE cellulose (DE52 Whatman) chromatography step was replaced by high performance liquid chromatography using a DEAE 5 PW (Tosoh) column. The RecA, eluted with about 0.35 M NaCl at pH 8.0, was free from detectable contamination of DNase. Single stranded homopolymers (Pharmacia) were used without further purification. Their concentrations were determined spectroscopically using $\varepsilon_{257} = 8.6 \cdot 10^3$ $M^{-1}cm^{-1}$ for poly(dA), $\varepsilon_{264} = 8.52 \cdot 10^3$ $M^{-1}cm^{-1}$ for poly(dT) and $\varepsilon_{274} = 7.4 \cdot 10^3$ $M^{-1}cm^{-1}$ for poly(dC). All experiments were performed with poly(dA) and poly(dT). Poly(dC) was used sparingly owing to the much shorter lengths of commercially available poly(dC) homopolymers which makes them less suitable for LD and anisotropic SANS studies (most batches of poly(dC) contained too short polymers to give measurable signals). Poly(dG) could not be used owing to the tendency of this homopolymer to form multistranded structures.

Linear dichroism studies.

LD was measured as described previously using a shear gradient of 600 s^{-1} and a RecA concentration of 3-12 μM.[9] ATPγS (Boehringer-Mannheim) concentration was kept below 20 μM to avoid perturbation of the spectroscopic signals. This concentration was sufficient to saturate RecA under our condition. Most experiments were carried out at 22° C in a buffer containing 50 mM NaCl, 4 mM $MgCl_2$, 1 mM dithiotreitol, 0.1 mM EDTA and 20 mM potassium phosphate pH=6.6. In some experiments 20 mM Tris buffer pH 7.5 was used, but no significant differences in recorded spectra were observed. Titrations were

performed either by successive additions of homopolymer to a RecA solution, i.e., a regular titration, or by preparing fresh samples with the indicated mixing ratio. These samples were prepared by incubating RecA with the first aliquot of homopolymer, thereafter with the second and finally with the third aliquot. Each incubation was for 1 hours at 20°C. RecA catalysed annealing of homopolymers was performed analogously using 0.2-3 μM of RecA and replacing ATPγS with 300 μM ATP.

Small angle neutron scattering

SANS was measured at the neutron facility at Risö as described previously.[10] RecA concentration was 50 μM and the concentration of ATPγS was about 100 μM. Shear gradient was 900 s^{-1}. Owing to the long measuring times required for SANS (8-12 hours) the experiments were performed at 8° C. Scattering intensities were collected every hour, compared to confirm sample integrity, and averaged to give the final pattern.

RESULTS

Linear dichroism titrations in the presence of ATPγS

Figure 1 shows LD titrations of RecA with poly(dA) and poly(dT). With both homopolymers the LD magnitude increases until a ratio of three nucleotides per RecA monomer is reached, reflecting the formation of a RecA-$[ssDNA]_1$-ATPγS complex.[9] With further addition of polynucleotide the LD magnitude decreases and essentially vanishes at a ratio of roughly six nucleotides per RecA monomer, which reflects the formation of a RecA-$[ssDNA]_2$-ATPγS complex.[9] These stoichiometries are the same as previously observed in titrations with heat denatured random sequence DNA and with the chemically modified εDNA.[6,9] With further increase in binding ratio the LD signal recovers and grows in magnitude until a plateau is reached at a ratio of about nine nucleotides per RecA monomer. The LD signal is unaffected by further addition of DNA suggesting

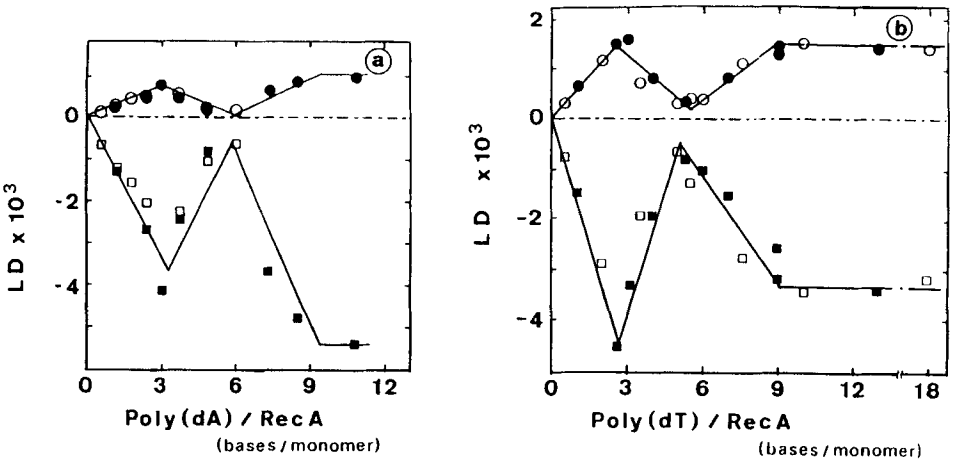

Figure 1. Flow linear dichroism signal as a function of nucleotide/RecA ratio for **A:** RecA-poly(dA)-ATPγS and **B:** RecA-poly(dT)-ATPγS (b) complexes. LD signal at 285 nm (○ , •) reflects tryptophan orientation and at 260 nm (□ , ■) reflects DNA base orientation. Open symbols refer to successive additions of homopolymer to a RecA solution, i.e., a regular titration, whereas closed symbols are freshly prepared samples with the indicated mixing ratio. The data are normalised to 1 μM RecA concentration.

that the plateau corresponds to complete saturation of the RecA filaments with the synthetic homopolymers.

Titrations with one homopolymer up to a ratio of three nucleotides per RecA followed by a second homopolymer up to a total ratio of six nucleotides per RecA all behaved similarly: LD magnitude increased up to three nucleotides per RecA and thereafter decreased becoming negligible at six nucleotides per RecA When a third homopolymer was added LD remained negligible if its sequence was complementary to any of those bound previously, but redeveloped with other sequences (Table I).

Linear dichroism spectra of complexes in the presence of ATPγS

LD spectra of RecA-[poly(dT)]$_1$-ATPγS, RecA-[poly(dT)]$_2$-ATPγS and RecA-[poly(dT)]$_3$-ATPγS, and for corresponding complexes with poly(dA), are shown

Table I. Sequence dependent orientation of RecA-[ssDNA]$_3$-ATPγS complexes evidenced from macroscopic orientability in shear flow.

Site I[a]	Bp[b]	Site III[a]	Bp[b]	Site II[a]	Orientation
A		A		A	YES
A	=	T	=	A	NO
A		A	=	T	NO
A	=	T		T	NO
A	=	T		C	NO
T	=	A		A	NO
T[c]		**C**		**A[c]**	**YES**
T		T	=	A	NO
T	=	A	=	T	NO
T		C		T	YES
T		T		T	YES
T	=	A		C	NO
T		T		C	YES
C		T	=	A	NO
C		T		C	YES
C		C		T	YES
C		T		T	YES
εA		ssDNA		εA	YES[d]
ssDNA	=	ssDNA	=	ssDNA	NO[e]

[a]A, T, C represent poly(dA), poly(dT) and poly(dC), respectively ssDNA is heat denatured random sequence DNA and εA is poly(dA) treated with chloroacetaldehyde which converts the adenine residues to their etheno derivatives. The sites are numbered in the order they bind ssDNA. [b]'=' indicates sequence complementarity. [c]Strands in sites I and II are complementary. [d]Reference 12. [e]Reference 9

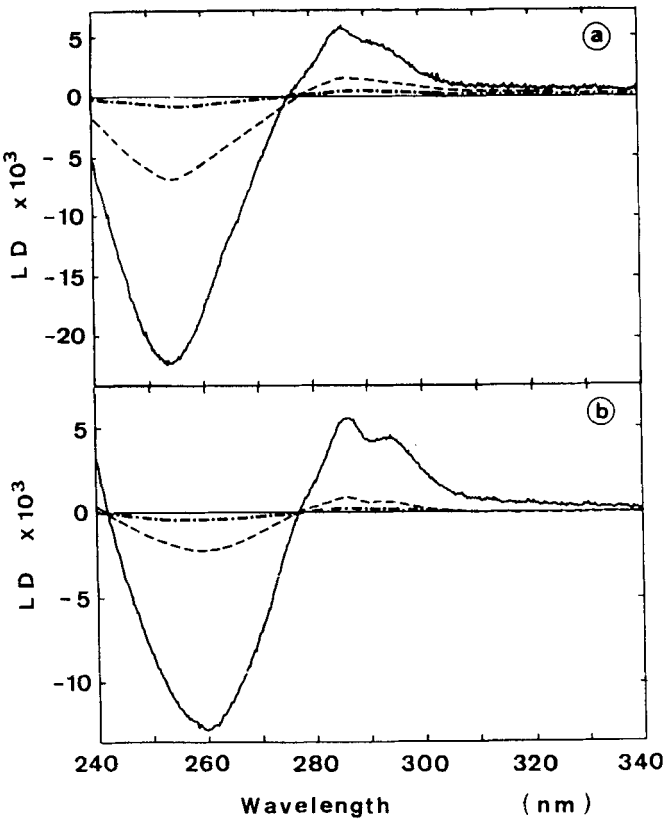

Figure 2. Linear dichroism spectra of RecA complexes with **A:** poly(dA) and **B:** poly(dT) in the presence of ATPγS at nucleotide:RecA ratios of 3:1 (————), 6:1 (- · - · -) and 9:1 (- - - -). The concentration of homopolymers is 12 μM in bases and the RecA concentration varies.

in figure 2. The spectra are recorded at the same homopolymer concentrations and their magnitudes differ therefore from the intensities normalised to constant RecA concentration shown in figure 1. All spectra display negative LD at the absorption of the DNA bases (260 nm) and positive LD at the absorption of the tryptophan residues in RecA (285 nm). These features indicate a more parallel orientation of the tryptophan residues and a more perpendicular orientation of the DNA bases relative to the fiber axis. In qualitative terms these features are

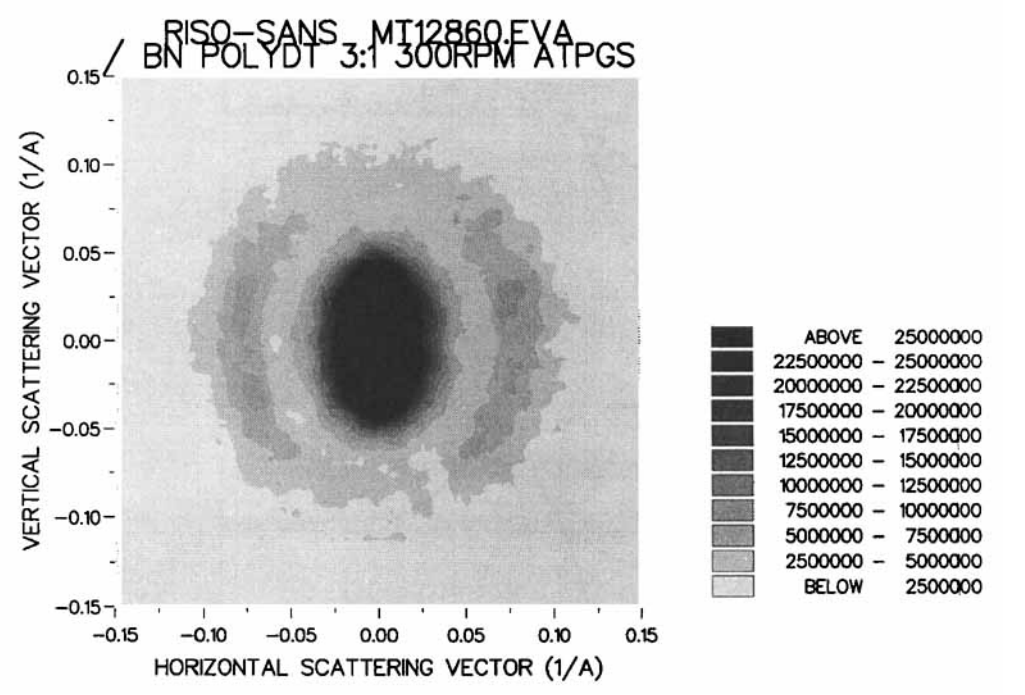

Figure 3. Small angle neutron scattering patterns reflecting the orientation of the complexes RecA-[poly(dT)]$_1$-ATPγS (3:1), RecA-[poly(dT)]$_2$-ATPγS (6:1) and RecA-[poly(dT)]$_3$-ATPγS (9:1). RecA concentration is 50 μM and the shear gradient 900 s^{-1}.

common to all three complexes and they are similar to those reported previously for complexes between RecA and heat denatured random sequence DNA.[9]

Small angle neutron scattering of complexes in the presence of ATPγS

Figure 3 shows anisotropic small angle neutron scattering (SANS) patterns for RecA-poly(dT)-ATPγS complexes oriented in shear flow. From the anisotropy of the central domain in the scattering pattern, which reflects the degree of fiber orientation,[10,12] it is clear that the RecA-[poly(dT)]$_2$-ATPγS complex has negligible orientation compared to that of the RecA-[poly(dT)]$_1$-ATPγS and RecA-[poly(dT)]$_3$-ATPγS complexes.

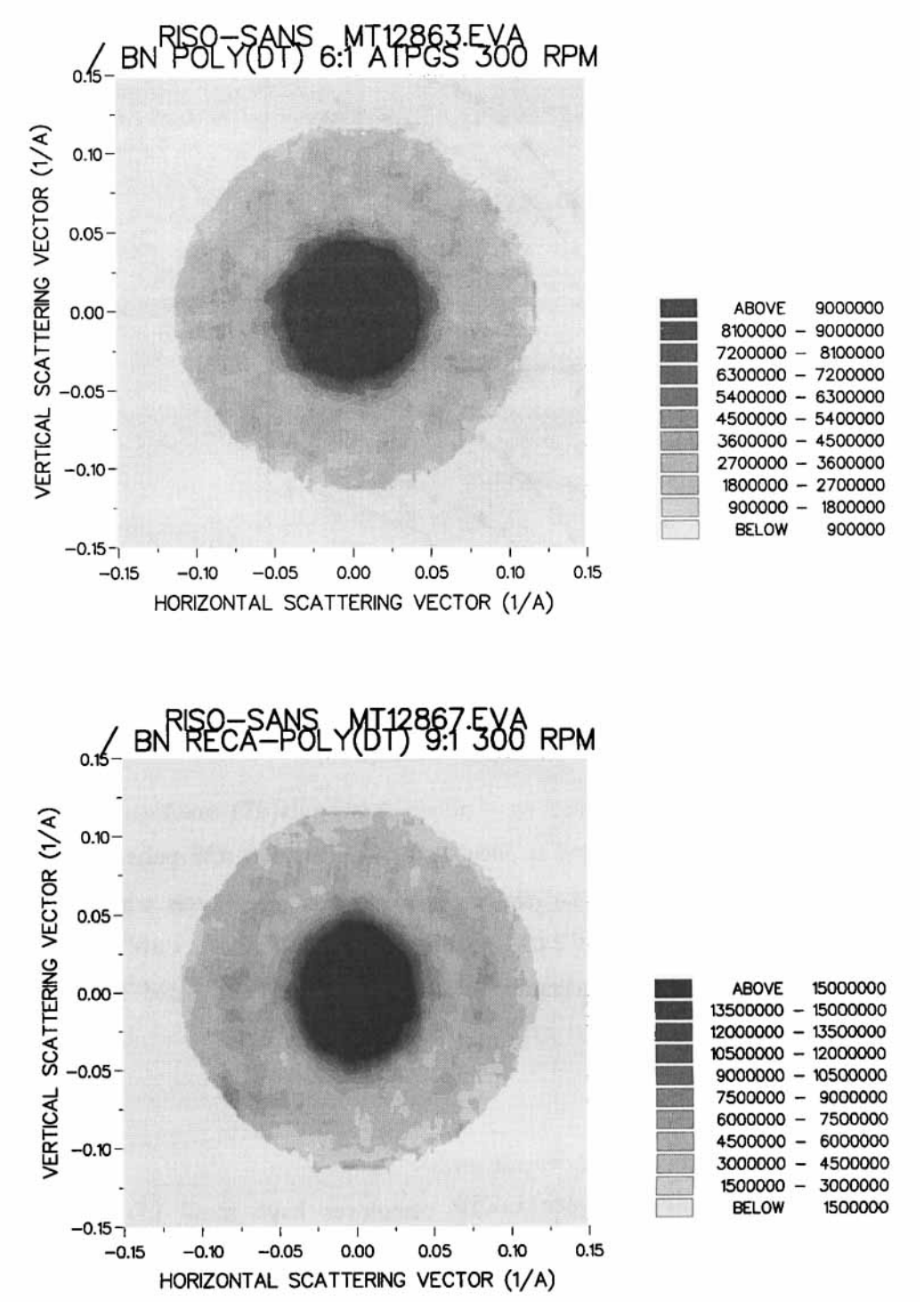

Figure 3. Continued

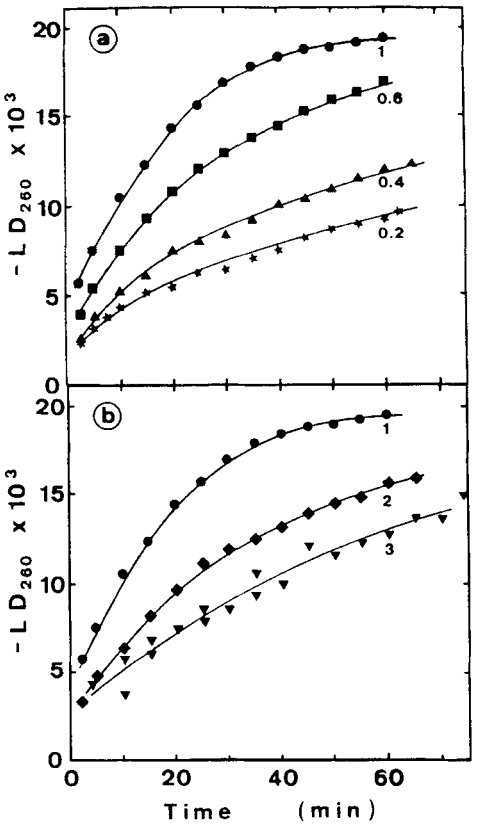

Figure 4. RecA catalysed annealing of poly(dA) and poly(dT) monitored by LD. 6 μM of poly(dA) was added to incubated mixtures of 6 μM poly(dT) and varying amounts of RecA. The rate of duplex formation increases with RecA concentration up to 1 μM **A:** 0.2 μM (*), 0.4 μM (▲), 0.6 μM (■), 1 μM (•), and decreases at higher RecA concentrations **B:** 1 μM (•), 2 μM (♦), 3 μM (▼). ATP concentration was 300 μM.

RecA catalysed annealing of homopolymers

Under our conditions all ssDNA-ATP complexes have small LD signals compared to free dsDNA. LD can thus be used to monitor the formation of poly(dA):poly(dT) duplexes *in situ*. Figure 4 shows the build up of the LD signal from annealed poly(dA):poly(dT) duplexes after the addition of poly(dA) to

preincubated mixtures of RecA, poly(dT) and ATP at various nucleotide/RecA ratios. As judged from the initial slopes and limiting values of the LD signals, the rate and fidelity of annealing is maximum when poly(dA) is added to a preincubated mixture of 6 μM poly(dT) and 1 μM RecA, which corresponds to a stoichiometry of six nucleotides bound per RecA monomer.

DISCUSSION

Binding of single stranded DNA to RecA

All titrations of RecA with single-stranded DNAs behave similarly up to an overall stoichiometry of six nucleotides per RecA monomer: LD magnitude increases until a ratio of three nucleotides per RecA monomer and thereafter it decreases until a ratio of six nucleotides per RecA monomer (see for example figure 1). These ratios have earlier been shown to correspond to saturation of the first and second binding site, respectively, of the RecA filaments with single-stranded DNA.[6,9] When more single-stranded DNA is added the LD behaviour depends on the chemical nature of the strands and whether there is sequence complementarity. In the titrations shown in figure 1, the LD magnitude starts to increase again reaching a plateau at nine nucleotides per RecA. The plateau suggests saturation of the third DNA binding site, meaning that each binding site of the RecA filament coordinates one DNA strand at a stoichiometry of three nucleotides per RecA monomer. We note this is the same stoichiometry as observed for the ternary RecA-ssDNA-dsDNA-ATPγS and RecA-dsDNA-ssDNA-ATPγS complexes that also contain three DNA strands.[9]

Titration of RecA with heat denatured random sequence DNA behaves differently. Here the LD magnitude *decreases* further above the ratio of six nucleotides per RecA monomer and vanishes at a mixing ratio of about 10-12.[9] In titrations where poly(dεA) is added up to a ratio of six nucleotides per RecA, followed by addition of heat denatured random sequence DNA, the LD signal *increases*, becoming even positive in sign, and does not saturate until a mixing ratio of about 15 nucleotides per RecA monomer.[13] Since binding stoichiometries

of different RecA-[ssDNA]$_3$-ATPγS complexes are likely to be very similar, if not identical, the observed variations in the saturation of the LD signal most likely reflects different affinities for the third strand in the complexes. The affinity of the third site thus appears to be largest for a short homopolymer when the first two sites are occupied by other short homopolymers. The other extreme are the chemically modified ssDNAs, εDNA and poly(dεA), which do not bind in the third site at all.[6,9] Presently we have no explanation for these differences in binding affinity of the third site.

RecA complexes with two DNA strands form sequence independent networks

The LD titration in figure 1 reveals that among complexes formed with poly(dT) the overall LD magnitude is lowest for the RecA-[poly(dT)]$_2$-ATPγS complex formed at an intermediate stoichiometry. This lowering of LD magnitude is observed at all wavelengths (figure 2) and hence corresponds to an impaired overall orientation of the RecA-[poly(dT)]$_2$-ATPγS filaments. Several factors influence the orientation of fibres in shear flow. Stiff fibres orient better than flexible ones, long polymers orient better than short ones, and globular structures, isometric aggregates and networks generally show poor orientation.[8] Although there may be some effects on flexibility it is hard to believe that the inclusion of a second DNA strand should make the RecA fiber much more flexible and inclusion of a third strand should stiffen it again. RecA-DNA complexes have a strong tendency for end-to-end association, as observed in electron microscopy,[14,15] but there are no indications that complexes with two DNA strands should be shorter than those with either one or three strands (unpublished results). The remaining alternative is that the RecA-[homopolymer]$_2$-ATPγS filaments form macroscopically isotropic structures, such as networks, that do not orient in gradient flow.

The decrease in orientation upon binding a second DNA strand is not a property unique for the homopolymers but is also observed with heat denatured random sequence DNA.[9] Nor is it related to sequence complementarity, since the

orientation vanishes when two non-complementary homopolymers are bound per RecA filament (figure 1) as when two complementary homopolymers are bound[13]. Since each RecA filament has on average two ssDNA strands bound, we believe that some of these strands may be shared between fibres forming bridges, thus tying the fibres into networks (figure 5 left). The networks are probably more compact when the fibres contain short DNA molecules as indicated by the almost negligible orientation of complexes with short homopolymers (figure 1) compared to the still significant orientation of complexes formed with long DNA single-strands[9]. These DNA-sequence independent networks may be related to the coaggregates that form between RecA, ssDNA and dsDNA in the early stages of RecA catalysed strand exchange.[16]

RecA complexes with three DNA strands form sequence dependent networks

The addition of a third poly(dT) strand to a RecA-[poly(dT)]$_2$-ATPγS complex, or addition of a third poly(dA) strand to a RecA-[poly(dA)]$_2$-ATPγS complex, leads to a redispension of the networks as judged from the reappearance of LD and SANS anisotropy (figures 1-3). This behaviour is in sharp contrast to titrations with, for example, heat denatured random sequence DNA, where addition of a third strand results in an even lower LD signal and thus apparently induces an even more aggregated structure.[9] Among the complexes between RecA and ssDNAs studied so far, we find a clear correlation between the formation of non-orientable structures and sequence complementarity (table I). Numbering the DNA-binding sites in the RecA fibre according to the order they bind single-stranded DNA, we find that when either of the strands bound in sites I and II is complementary to the strand in site III the LD disappears, demonstrating that these combinations give poor orientation. When all three strands are mutually non-complementary, or when only the strands in sites I and II are complementary (bold symbols in table I), the complexes orient readily. Thus, in contrast to RecA-[ssDNA]$_2$-ATPγS complexes, the orientation of RecA-

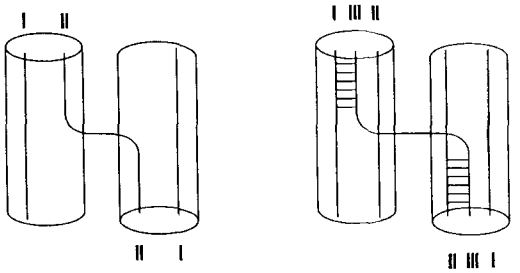

Figure 5. Schematic drawings of bridged RecA fibres illustrating possible mechanisms behind network formation. **Left:** sequence independent networks of RecA-$[ssDNA]_2$-ATPγS complexes caused by strands in site II bridging between fibres. **Right:** sequence dependent networks of RecA-$[ssDNA]_3$-ATPγS complexes caused by strands in site III, when firmly bound through base-pairing interactions with either of the strands in sites I and II, bridging between fibres.

$[ssDNA]_3$-ATPγS complexes depends on sequences complementarity among the bound strands.

The observed pattern in the sequence dependence (table I) indicates that aggregation takes place when the strand in site III is sequence complementary to either of the strands in sites I and II. This strand is much weaker bound in the RecA-$[ssDNA]_3$-ATPγS complex than the strands in sites I and II, as demonstrated earlier by its sensitivity to nucleases.[13] For it to create stable networks by bridging fibres may therefore require the additional binding strength provided by base-pairing interactions with the more firmly bound strands in sites I and II. Since sequence complementarity with one of these strands is sufficient to induce aggregation, the strand in site III should be able to base-pair with both these strands (figure 5, right).

We note that sequence complementarity between strands in sites I and II is not sufficient to cause aggregation (bold symbols in table I). Strands in these sites have earlier been shown unable to base-pair with each other when bound in the presence of ATPγS,[13] so the lack of effect of sequence complementarity between

them is not surprising. These strands are, however, tightly bound,[9,13] and should be able to hold a network together irrespectively of base-pairing interactions, as in fact they do in the sequence independent networks of RecA-$[ssDNA]_2$-ATPγS complexes discussed above. Since these networks disappear when a non-complementary third strand is added (figures 1-3), the binding of the third strand must prevent the sequence independent bridging between fibres containing two DNA strands. The mechanism behind it is presently unknown.

RecA catalysed annealing involves the strand bound in site III

All complexes discussed so far were formed in the presence of the ATP analogue ATPγS, which is virtually non-hydrolysable. Its presence results in a very tight binding of RecA protein to DNA and one cannot exclude the possibility that the modified cofactor induces structures that are different from those formed with ATP and which may be without biological significance. Zlotnik et al. recently identified the formation of RecA-$[ssDNA]_1$-ATP and RecA-$[ssDNA]_2$-ATP complexes,[17] and we have identified the ternary complex RecA-ssDNA-dsDNA-ATP.[18] Further, Lauder & Kowalczykowski have reported the existence of RecA-ssDNA-ATP complexes with 3:1 and 6:1 nucleotides per RecA ratios.[19] Clearly, the first two RecA-ssDNA complexes form both with ATP and ATPγS as cofactors, but so far there has been no evidence for a RecA-$[ssDNA]_3$-ATP complex.

In an attempt to obtain evidence for such a complex we studied the RecA catalysed annealing of poly(dA) and poly(dT) in the presence of ATP. By choosing homopolymers as substrates we could incubate RecA at an arbitrary ratio with either homopolymer before initiating annealing by the addition of the complementary one. In this way we avoided competing spontaneous annealing and, since the substrates are completely complementary, we also eliminated the search for homology step and could directly study the effect of stoichiometry.

Our results suggest that the rate and fidelity of annealing is highest when the stoichiometry of the preincubated RecA-poly(dT)-ATP complex is about six

nucleotides per RecA monomer (figure 4). At this stoichiometry two poly(dT) molecules are bound per RecA filament, occupying the first two binding sites, and the poly(dA) has to bind in site III. Annealing should thus take place between the poly(dA) strand in site III and one of the poly(dT) strands bound in sites I and II. Since the two strands in a DNA duplex are antiparallel the strand in site III should bind with opposite polarity relative to at least one, possibly both, of the strands in sites I and II.

When poly(dA) is added to a preincubated RecA-poly(dT)-ATP complex at a stoichiometry of three nucleotides per RecA monomer, i.e., when poly(dT) is bound exclusively in site I and poly(dA) can bind in site II, annealing is less efficient. The kinetic curves in figure 4 are compared at the same poly(dT) concentrations, so the observed higher rate at the 6:1 ratio relative to the rate at the 3:1 ratio means that the (average) ability of the poly(dT) strands in sites I and II to pair with the poly(dA) strand in site III is higher than the ability of the poly(dT) strand in site I to pair with the poly(dA) strand in site II. This finding is consistent with the previously observed inability of complementary strands bound in sites I and II to form a duplex in the presence of ATPγS.[13] An arrangement of three DNA strands bound in a RecA fibre that is consistent with our observations is schematically shown in figure 6. The barrier between the bases in sites I and II is symbolic, indicating their reduced ability to base-pair. It might be a real physical barrier, such as provided by a protein domain, but may also represent a topological constraint such as if the two strands are bound with the same polarity.

Binding of double stranded DNA to RecA

Our results on the interaction of RecA with homopolymers may explain some earlier observations on the interaction of RecA with duplex DNA and on the formation of ternary complexes between RecA and one each of a single-stranded and duplex DNA. Since the three RecA binding sites accommodate one DNA strand each, a DNA duplex should occupy two sites, one by each strand. Image analysis of electron micrographs has revealed that one of the strands in a RecA-

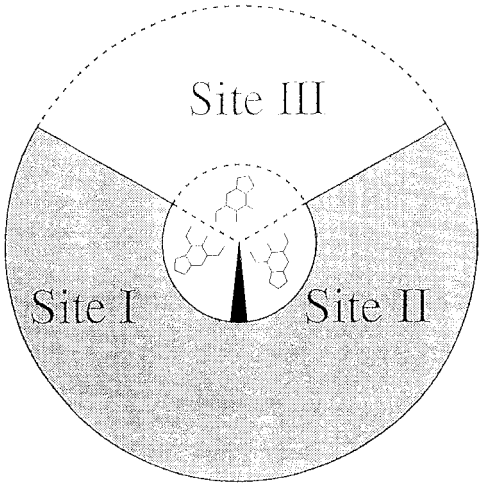

Figure 6. Cross section of a RecA fiber showing its three DNA binding sites. The sites are numbered in the order they are engaged in binding ssDNA. Site III is tentatively placed at the groove of the RecA fiber (open sector) as suggested from nuclease accessibility experiments.[13] The dark wedge symbolises the inability of strands in site I and II to base-pair with each other.

[dsDNA]-ATPγS complex follows the same path as the single-stranded DNA in a RecA-$[ssDNA]_1$-ATPγS complex.[20,21] One strand of the DNA duplex in the RecA-[dsDNA]-ATPγS complex should thus be bound in site I of the RecA filament. Where the other dsDNA strand is bound can be deduced as follows. The chemically modified single-stranded DNAs, εDNA and poly(dεA), bind only in sites I and II of the RecA filament; their affinity for site III is negligible.[6,9] Since these DNAs are bound by a RecA-[dsDNA]-ATPγS complex,[13] the available site cannot be III. Site I was concluded above to be occupied by one of the dsDNA strands, so the available binding site must be II. Consequently, the occupied sites in a RecA-[dsDNA]-ATPγS complex should be I and III, site II being available for binding ssDNA.

This is, however, not the only way to accommodate a DNA duplex in a RecA filament. The RecA-$[ssDNA]_1$-ATPγS complex binds dsDNA forming a

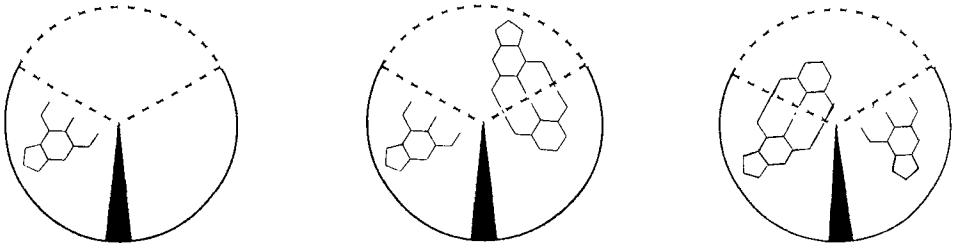

Figure 7. Possible scenario of RecA catalysed strand exchange. **Left:** RecA polymerises onto a DNA single strand. **Middle:** A dsDNA molecule enters the filament and becomes bound with its strands in sites II and III. The strands are in bound in register, each with a stoichiometry of three nucleotides per RecA monomer, and all bases are coplanar. **Right:** When complementary regions meet in the search for homology process the strand in site III flips over and base-pairs with the strand in site I.

ternary RecA-[ssDNA]-[dsDNA]-ATPγS complex.[9] In this complex site I is occupied by the ssDNA so the duplex must be bound in sites II and III. The strands in the two ternary RecA-[ssDNA]-[dsDNA]-ATPγS and RecA-[dsDNA]-[ssDNA]-ATPγS complexes are thus bound differently, which may explain their quite different physical properties.[13,22]

The winding of DNA strands in the RecA filament

In the RecA-[dsDNA]-ATPγS complex the RecA proteins form a spiral with about 6.17 RecA units per turn.[21,23] The DNA is unwound from 10.5 to about 18.6 base-pairs per turn,[24] and the stoichiometry is three base-pairs per RecA monomer.[9] Since 3×6.17=18.51, the dsDNA strands should have the same winding as the RecA proteins themselves. The same winding is expected also for coordinated single-strands, which at least in site I follow the same path as one of the dsDNA strands.[20,21] Hence, the DNA binding sites in the RecA filament should follow the protein spiral allowing each of the bound strands to make

identical contacts with every protein unit. A consequence of this arrangement is that RecA bound DNA strands should be coaxial.

A possible mechanism for strand exchange based on the three site concept

A possible mechanism for the strand exchange reaction based on the three binding sites per RecA filament and their base-pairing abilities is schematically shown in figure 7. Single stranded DNA, in vivo possibly generated by RecBCD protein,[25,26] is bound by RecA in site I (figure 7, left). dsDNA enters the filament and its strands become bound in sites II and III. The three strands are bound in register and their bases are coplanar.[13] The initial contact is, for statistical reasons, in a non-complementary region and the dsDNA strands remain paired (figure 7, middle). By either lateral diffusive motion or by repeated dissociation and association complementary regions between the ssDNA in site I and the dsDNA strand in site III find each other. When this occurs the strand in site III flips over and pairs with the strand in site I forming a new DNA duplex (figure 7, right).

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