596–600 Nucleic Acids Research, 1999, Vol. 27, No. 2

Conserved sequence preference in DNA binding among recombination proteins: an effect of ssDNA secondary structure

E. Biet, J.-S. Sun[1] and M. Dutreix*

Institut Curie, Section de Recherche UMR144-CNRS, 26 rue d'Ulm, F-75248-Paris Cedex 05, France and [1]Laboratoire de Biophysique, Muséum National d'Histoire Naturelle, INSERM U201, CNRS URA481, 43 rue Cuvier, F-75231 Paris Cedex 05, France

Received August 31, 1998; Revised November 13, 1998; Accepted November 25, 1998

ABSTRACT

Repetitive sequences have been proposed to be recombinogenic elements in eukaryotic chromosomes. We tested whether dinucleotide repeats sequences are preferential sites for recombination because of their high affinity for recombination enzymes. We compared the kinetics of the binding of the scRad51, hsRad51 and ecRecA proteins to oligonucleotides with repeats of dinucleotides GT, CA, CT, GA, GC or AT. Since secondary structures in single-stranded DNA (ssDNA) act as a barrier to complete binding we measured whether these oligonucleotides are able to form stable secondary structures. We show that the preferential binding of recombination proteins is conserved among the three proteins and is influenced mainly by secondary structures in ssDNA.

INTRODUCTION

Homologous recombination is a shared property of all living cells, and can occur at any location in the genome. The observation that some regions of prokaryotic and eukaryotic genomes are hotspots of recombinational activity led us to examine whether recombinogenicity is inherent to the DNA itself: specific sequences, with a high affinity for recombination enzymes, might be preferential sites of recombination. Microsatellite sequences in which a short sequence is tandemly repeated (1) have been proposed to be recombinogenic elements in eukaryotic chromosomes (2,3). The results presented in this work show that some dinucleotide repeat sequences are indeed preferentially bound by eukaryotic and prokaryotic recombination proteins.

Biochemical studies show that the product of the *Escherichia coli* recA gene, *E.coli* RecA (ecRecA) protein, has a central role in recombination. *In vivo*, it catalyses homologous pairing and strand exchange reactions between two DNA molecules (reviewed in 4–7). Proteins homologous to RecA have been found in other prokaryotes as well as in eukaryotes [yeast (8); chicken (9); mouse (10)]. Structural RecA analogues such as scRad51 (11) and Dmc1 (12) in *Saccharomyces cerevisiae*, spRad51 (rhp51) in *Schizosaccharomyces pombe* (13) and Mei-3 in *Neurospora crassa* (14) are particularly interesting, since mutations in the genes encoding these proteins confer significant defects in DNA recombination and repair as observed for bacterial recA mutations (15). Further, the inhibition of hsRad51 gene expression by antisense oligonucleotides reduces homologous recombination in human cells (16).

In addition to its catalytic function in genetic recombination, ecRecA provides structural framework for homologous pairing (17). In the presence of ATP, ecRecA binds DNA [either single-stranded DNA (ssDNA) or gapped duplex] and, with it forms a right-handed helical nucleoprotein filament (18,19). The *S.cerevisiae*, *Homo sapiens* and *Xenopus laevis* Rad51 proteins have recently been purified and also found to form filamentous complexes with DNA and to catalyse homologous recombination. Image reconstruction from electron micrographs of human and yeast Rad51 proteins polymerised on double-stranded DNA (dsDNA) indicates that the three-dimensional structures of the nucleoprotein filaments thus formed are similar to that of the equivalent RecA protein–DNA complex (20–22). These filaments have a characteristic striated appearance and they stretch DNA to a similar extent: the nucleoprotein filaments have a helical pitch of ~95 Å, and DNA is extended by ~50% with respect to the naked B-form duplex. Here, we compare the kinetics of binding of the bacterial, yeast and human recombination proteins with short ssDNA with the use of plasmon resonance biosensors to follow the reactions in real time.

MATERIALS AND METHODS

Oligonucleotides and protein

All the oligonucleotides are 39 nt long and are biotynylated (-b-) at the 5′-end. They were purchased from Genset and purified by ion exchange chromatography on HPLC. Oligonucleotides CA, CT, GT, CA, GA, AT and GC contain 19 repeats of the given dinucleotide and an additional biotinylated nucleotide. The MIX sequence 5′-b-GCTAAGTAACATGGAGCAGGTCGCGGATTTCGACACAAT is a 50% GC sequence derived from the phage M13. Oligonucleotides GT7 and GT7C sequences are shown in Figure 3. RecA protein (M_r 37 800) was purified as described (23). *Escherichia coli* ssDNA binding (Ssb) protein (M_r 18 900) was purchased from USB. *Saccharomyces cerevisiae* scRad51 protein (M_r 43 000) was a gift of S. Kowalczykowski (24). Human hsRad51 (M_r 37 000) was a gift of S. West (25).

*To whom correspondence should be addressed. Tel: +33 1 42 34 64 27; Fax: +33 1 42 34 64 38; Email: marie.dutreix@curie.fr

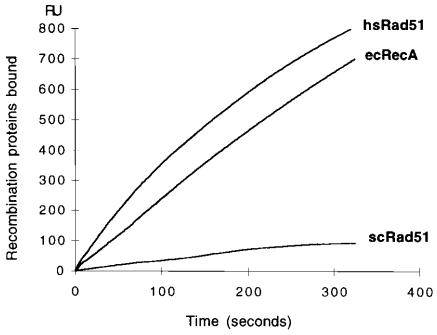

Figure 1. Kinetics of binding of hsRad51, ecRecA and scRad51 proteins to the MIX oligonucleotide. Kinetics were measured as described in Materials and Methods.

Protein binding determination

The binding analysis was accomplished with a BIAcore 2000 (BIAcore AB, Uppsala, Sweden). The fluidic system consists of four detection surfaces located in separate flow cells that are accessible either individually or serially in a multichannel mode. Oligonucleotides are immobilised on the detection surfaces and proteins are injected into a continuous buffer flow [Buffer A: 10 mM Tris–HCl (pH 7.2), 50 mM NaCl and 5 mM $MgCl_2$] that passes over the surfaces. For the binding measurement the flow rate was 10 µl/min and the temperature was 37°C. Proteins in Buffer A were injected at time zero and 0.5 mM ATP-γ-S was added, when recombination proteins were used. Surfaces were regenerated between each binding experiment by injection of 5 µl 0.05% SDS. The SPR response, expressed in resonance units (RU), depends on the refractive index in close proximity to the surface and therefore is directly correlated to the concentration of molecules in the surface layer (1 RU = 1 pg/mm^2). Proteins and oligonucleotides do not differ considerably with respect to their SPR response (26). Equivalent amounts of each biotin-labelled oligonucleotide (420 RU ± 10 corresponding to a oligonucleotide density of 1.2 pM/mm^2) were captured on sensor surfaces preimmobilised with streptavidin either supplied by the company (sensor chip SA, BIAcore) or prepared in our laboratory (27) (sensor chip CM5, BIAcore). One flow cell without bound oligonucleotide was used as a reference to correct for bulk refractive index contributions which are related to differences in the composition of injected samples.

DNA thermal denaturation by UV spectrophotometer

The thermal stability of double helices was measured with a UVIKON 940 Spectrophotometer using quartz cuvettes of 1 cm optical pathlength. The spectrophotometer was linked to an IBM-AT computer for data collection and analysis. The temperature of the cell holder was controlled with a circulating liquid (80% water/20% ethylene glycol) in a Haake P2 water bath. The temperature of the water bath was lowered from 80 to 0°C and then raised to 80°C at a rate of 0.15°C/min with a Haake PG 20 thermoprogrammer. The absorbance at 260 nm was recorded every 7 min. The sample temperature was measured with a Teflon-coated temperature probe immersed directly in a control cuvette. All oligonucleotide samples were prepared in buffer of 20 mM sodium cacodylate, pH 7.2, with 50 mM NaCl and 5 mM $MgCl_2$. The melting temperatures (T_m) were evaluated as the maximum of the first derivative of the melting profiles. Based on multiple experiments, the uncertainty in T_m was estimated at ±1°C.

RESULTS

Real time measurement of the DNA binding activities of ecRecA, scRad51 and hsRad51 proteins

In order to measure the kinetics of binding of proteins to oligonucleotides, we developed a new technique based on the use of the surface plasmon resonance biosensor (BIAcore). Using this technique, we compared the binding of three recombination proteins: ecRecA (*E.coli*), scRad51 (*S.cerevisiae*) and hsRad51 (*H.sapiens*), to ssDNA. Binding reactions were performed under identical experimental conditions for the three proteins and in the presence of ATP-γ-S and $MgCl_2$. The kinetics of binding were first measured with the MIX oligonucleotide, a sequence derived from the genome of the bacteriophage M13 which is devoid of obvious secondary structure (Fig. 1). The hsRad51 and ecRecA proteins bind with similar kinetics to ssDNA, and do not reach steady-state by 300 s after injection. The binding kinetics slowed down as filament filled-up and steady-state was not reached even when injection time was extended to 1000 s or when the concentration of protein was increased 5-fold (data not shown). The slow down of the kinetics when DNA is partially covered reflects the limited access of the protein to short naked region of the ssDNA. The study was done by comparing the initial association rate (Table 1). At that concentration and during this short period, the rate of binding to the MIX oligonucleotide is linear, probably because co-operative binding is negligible. The initial rates of association of ecRecA and hsRad51 proteins to the MIX oligonucleotide are 11×10^{-5} and 6.1×10^{-5} [fmol of monomers]/s, respectively. The binding of scRad51 is slow, with a initial association rate 10-fold lower than that of ecRecA and hsRad51. Moreover, the rate of scRad51 binding reaches a steady-state after 300 s of injection, which corresponds to fewer than one protein monomer per 10 oligonucleotides. These results are in agreement with the observation that scRad51 does not bind efficiently to long ssDNA of random sequence content (20,21,24).

Table 1. Initial binding rates of hsRad51, scRad51 and ecRecA recombination proteins

	Initial binding rate (10 -5 fmoles / sec.)		
DNA	hsRad51	scRad51	ecRecA
CT	45.9	62.7	463.8*
GT	56.7	80.0	502.3*
CA	51.0	21.2	303.8*
MIX	11.0	0.9	6.1

The initial rates of binding were estimated from the first 30 s of the kinetics shown in Figure 2 using the computer program Kaleida-Graph to calculate functions. The values were accepted when they corresponded to a linear function with >0.99 accuracy. Where binding kinetics were not linear, the estimation was done for the first 10 s of the reaction (*). Values, in fmol of monomers/s, were calculated using the molecular weight of proteins and the correlation of SPR response (1 RU = 1 pg/mm^2) indicated in Materials and Methods.

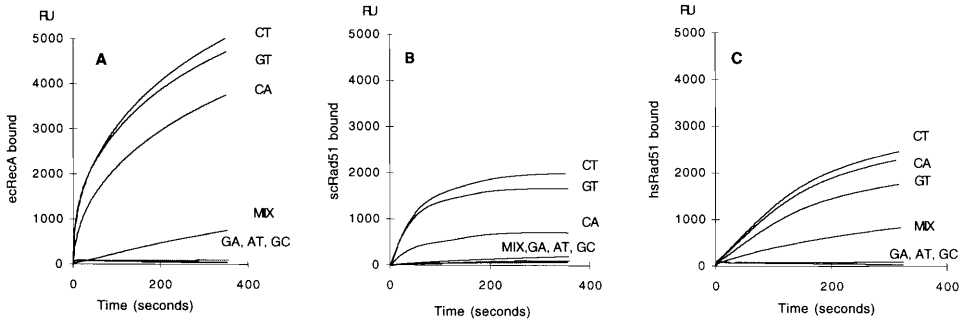

Figure 2. Kinetics of binding of recombination proteins to the oligonucleotides. Binding kinetics of ecRecA (**A**), scRad51 (**B**) and hsRad51 (**C**) proteins were measured as described in Materials and Methods. The CT, GT, CA, GA, AT and GC oligonucleotides consist of repeats of these dinucleotides.

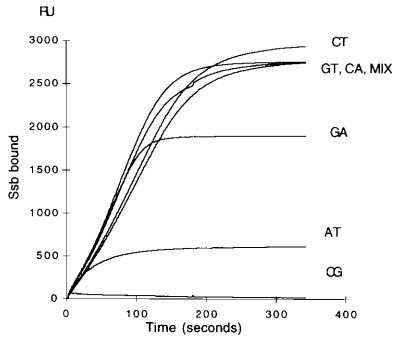

Figure 3. Kinetics of binding of Ssb protein to the different oligonucleotides.

Binding of hsRad51, scRad51 and RecA to dinucleotide repeat sequences

In a previous study we showed that ecRecA binds preferentially to GT and CA dinucleotide repeats (27). Here, we compared the kinetics of ssDNA binding of recombination proteins with all six possible dinucleotide repeat sequences (CA, GT, CT, AT, GC, GA). Different combinations of three oligonucleotides were bound to the sensorship surface. Although the initial rate of binding is strongly affected by the sequence of the oligonucleotide (Table 1), the general shape of the binding curves is specific for each recombination protein and is not influenced by the sequence (Fig. 2). For example, the rate of binding of scRad51 to CT and GT oligonucleotides, which is 15–20-fold greater than binding to the MIX sequence, reaches steady-state within 200 s, at a level which corresponds to only two monomers per oligonucleotide. Increasing the concentration of protein injected does not significantly improve the extent of binding (data not shown).The binding of ecRecA and hsRad51 do not reach saturation on any of the tested sequences, even after 350 s of injection.

All the three proteins share a common set of affinities for the different repeated sequences. The experiments were repeated several times on the same sequences with similar results. The sequences can be separated into three different classes according to the binding affinities: high affinity for the GT, CT and CA repeats, intermediate affinity for the MIX sequence and low affinity for the GA, GC and AT repeats.

Binding of Ssb protein to dinucleotide repeat sequences

Single-strand binding proteins such as *E.coli* Ssb and *S.cerevisiae* Rpa facilitate the binding of recombination proteins to ssDNA (21,22,28). These proteins destabilise unstable secondary structures, but do not bind to dsDNA. We measured the binding of Ssb protein to the different oligonucleotides to determine whether it prefers the same sequences than RecA protein. Indeed, the polyGT, polyCT, polyCA and MIX sequences are efficiently bound by Ssb. At steady-state every oligonucleotide is bound by four Ssb monomers (Fig. 3). These results are consistent with a model proposing that Ssb tetramers bind a region of 30–36 nt long and form nucleosome-like structures (29,30).

The AT and GC oligonucleotides can potentially fold on themselves and form perfect hairpins and are therefore poorly bound by Ssb. However, 25% of the AT oligomers were bound by Ssb protein indicating that secondary structures formed on this sequence can probably be removed by the protein. Interestingly, sequences containing GA dinucleotides which cannot form perfect hairpins are not entirely bound by Ssb. This result is in agreement with our finding that this sequence is bound as poorly by recombination proteins as polyCG and polyAT sequences. The order of binding affinity of Ssb protein to the different sequences was similar to the order of binding affinity of recombination protein: high affinity for GT, CT, CA and MIX sequences and lower affinities for GA, AT and GC repeats. These data indicate that the poor binding of recombination proteins to AT, CG and GA oligonucleotides may be due to the presence of structures in the ssDNA.

Determination of secondary structures formed at 37°C

To determine how the absence of secondary structure in oligonucleotides contributes to the preferential binding of recombination proteins, we estimated the extent of its formation in these different sequences. DNA thermal denaturation experiments were carried out under conditions similar to these of the binding assays, in order to assess whether specific repetitive sequences are involved in self-associated structures. Almost all oligomers, except those with the CT and CA repeats, show distinct curves with T_m ranging from 30 to >90°C, which is indicative of the presence of significant amounts of self-associated structures at temperatures near and below the melting point (Table 2). Therefore, the fraction of free (non-self-structured) single-stranded oligomers was estimated at 37°C according to an all-or-none (two states) model. Except for the CT and CA oligonucleotides, others oligomers exhibited at 37°C various proportions of self-associated structures (Table 2). The proportion of free ssDNA was then compared with the binding efficiency of the various proteins and found to correlate with their affinity for ssDNA.

The GA, AT and GC oligonucleotides, whose sequences are >75% structured, are not bound by recombination proteins. In contrast, the CT and CA oligonucleotides are almost devoid of secondary structure and are efficiently covered by recombination proteins. The GT oligonucleotide is ~30% self-associated, and indeed, the binding of hsRad51 to this sequence is reduced compared with the binding to CA and CT repeats. However, ecRecA and scRad51 proteins bind to polyGT sequence as efficiently as they bind to polyCT and polyCA sequences as if they were insensitive to its secondary structure. The low binding affinity of these two proteins for the MIX sequence, however, indicates that their binding is influenced by ssDNA structures.

Table 2. Determination of the melting temperature of the various oligonucleotides

Oligonucleotide	Tm (°C) (±1°C)	% of free single strand at 37 °C (±5%)
CT20	N.T.	100
CA20	< 20	95
GT20	30	70
MIX	35	55
GA20	48	25
AT20	55	15
GC20	> 90	0

The T_m were evaluated as described in Materials and Methods. The amount of free ssDNA at 37°C was calculated from the melting profiles assuming a two-state model. No change in the absorbance was detected for the CT oligonucleotide (N.T.).

Introduction of an inverted repeated sequence close to the dinucleotide repeat tract suppresses preferential binding to polyGT

The kinetics of binding of ecRecA and scRad51 proteins to GT dinucleotide repeats suggest that these proteins bind preferentially to this sequence. To test if this preferential binding is independent from the secondary structure on the ssDNA, we compared the binding of those recombination proteins to a 40 base oligonucleotide of random sequence with or without an insert of seven GT repeats. Although the MIX and GT7 oligonucleotides have the same T_m (40 ± 1°C), scRad51 and ecRecA bind preferentially to the sequence containing the GT repeats (Fig. 4). We created a perfect five bases inverted repeat sequence in the flanking regions by the addition of a single base. This change in sequence has only a moderate effect on T_m (44 ± 1°C) but induces the formation of a secondary structure that confers a specific DnaseI sensitivity to the oligonucleotide (Fig. 5). The single base addition completely suppressed the preferential binding of the two proteins to the GT repeats (Fig. 4). The binding of Ssb protein was similar for the three oligonucleotides tested (data not shown). These results confirm that both GT content and secondary structures influence the affinity of recombination proteins for ssDNA.

DISCUSSION

Although the protein-mediated process of DNA strand exchange was studied *in vitro* using various recombination proteins, many features of the *in vivo* reaction remain unknown (21,22,31). We have examined here the very first step of the reaction, the formation with ssDNA of a nucleoprotein filament. This step seems to be a prerequisite for the joining of two homologous DNA molecules. It has recently been shown that the ecRecA and scRad51 proteins had a preference for GT-rich sequences (32,33) but it is not clear whether this bias is an intrinsic feature of these proteins, or is shared by other ssDNA binding proteins. Using the Biacore to measure the kinetics of binding in real time, we found that the human hsRad51 also exhibits a preference for these sequences. We have compared the kinetics of binding to different simple sequences and we have studied the properties of ssDNA that control this preferential binding.

In this study we monitor the binding of recombination proteins to oligonucleotides that are immobilised on the surface of a biosensor chip. It is unlikely that immobilisation modifies the sequence preference of binding. Actually, preference for GT-rich

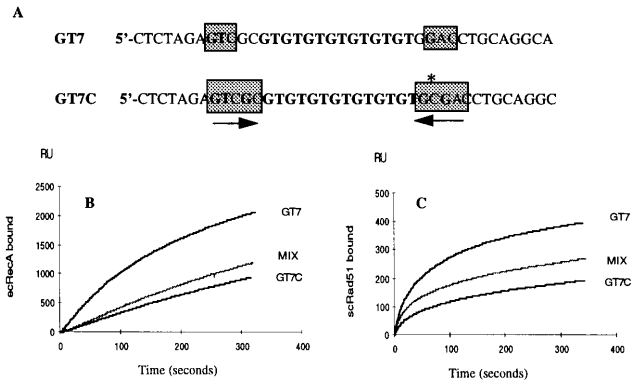

Figure 4. Kinetics of binding of ecRecA and scRad51 proteins to MIX, GT7 and GT7C oligonucleotides. Sequences of the oligonucleotides are indicated (**A**), with inverted repeated sequence (grey box) and the nucleotide change (*) between GT7 and GT7C. Binding kinetics of ecRecA (**B**) and scRad51 (**C**) were measured as described in Materials and Methods. Initial binding rates (10^{-5} fmol/s) were calculated as described in Table 1: MIX (6.1), GT7 (18.1) and GT7C (4.8) for ecRecA protein and MIX (0.9), GT7 (1.7) and GT7C (0.5) for scRad51 protein.

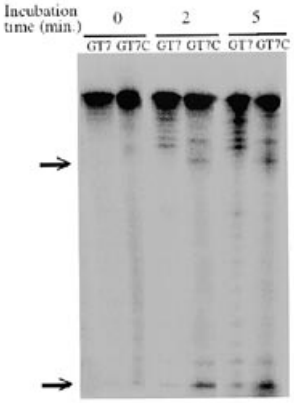

Figure 5. DNaseI sensitivity of GT7 and GT7C oligonucleotides. The 3′-end labelled GT7 and GT7C oligonucleotides were incubated with 1 U of DNaseI in 15 µl buffer (50 mM Tris pH 7.5, 10 mM $MnCl_2$, 50 µg/ml BSA) for 0, 2 and 5 min and analysed on a 12% acrylamide denaturing gel. Arrows indicate preferential sites of DNaseI cleavage introduced by the base addition in GT7C (located 10 and 28 nt from the 3′-end of the oligonucleotide).

sequences is observed on immobilised DNA (this work) and on oligonucleotides in solution (32,33). Moreover, recombination *in vivo* is initiated at single-stranded gaps or single-stranded ends of duplex DNA, the ssDNA produced must be under topological constraints as it is for DNA immobilised on a dextran surface.

The binding of hsRad51 and scRad51 is linear during the first 30 s, indicating that in our experimental conditions co-operative binding is not significant during this period. However, the binding of ecRecA to polyGT, polyCA and polyCT sequences is very fast and non-linear, even during the first few seconds. This difference suggests that cooperativity is more important for the ecRecA protein than for the eukaryotic Rad51 proteins.

The binding of scRad51on preferred sequences (GT, CT and CA) is very efficient at early times, but rapidly reaches steady-state. The reason for this inhibition of filament formation is not clear. Increasing the protein concentration in the injected solution does not increase the extent of binding, as might be expected if the protein were inactive. Electron microscopic observations indicate that scRad51 protein forms non-contiguous nucleoprotein filaments with long ssDNA (21). Filaments with a highly regular, right-handed helical appearance can be observed when scRad51 protein is incubated in the presence of Rpa protein. It has been proposed that secondary structure in ssDNA imposes a barrier to nucleoprotein filament continuity, which Rpa or Ssb can overcome through its ability to destabilise duplex DNA (21). Our observation that binding is also impaired on polyCT and polyCA sequences, which do not form secondary structure, indicates that Rpa might play another stimulatory role in the formation of the presynaptic complex via changes in DNA structure.

The binding preference order is (CT, GT, CA) > MIX > (GA, AT, CG) for all the proteins tested. The influence of secondary structure in ssDNA was confirmed by determining the T_m of the different sequences. The binding of Ssb and RecA proteins to sequences that are highly self-associated (polyAT and polyGC) is inhibited. Surprisingly, the binding of RecA and in lower extent Ssb is impaired on polyGA. Though we have no idea of the kind of structure involved, the melting study indicates that this sequence is actually partially structured. The only sequence whose binding does not follow perfectly the T_m classification was the GT dinucleotide repeats. According to the measured T_m the binding affinity of recombination proteins to this sequence is expected to be intermediate between that for the polyCA and the MIX sequences. This result was observed with hsRad51 protein but not with the two other proteins. A preference for GT-rich sequences by ecRecA and scRad51 proteins has been demonstrated by *in vitro* selection (32,33). We observe that GT dinucleotide repeats are indeed preferentially bound by ecRecA and scRad51. This preference indicates either a high affinity of the monomers for the GTG and TGT trinucleotides and/or that the secondary structures detected by T_m measurement, which are present in 30% of the molecules, stimulate ecRecA and scRad51 binding. However, the loss of GT effect when secondary structures are introduced near short GT stretches demonstrates that even in a GT-rich context, secondary structure plays a major role in determining protein binding affinity. In studies of the sequences flanking the recombination hot spot χ in *E.coli*, Tracy *et al.* observed that the χ sites are embedded within GGT rich islands (34). They found that, within these regions, these bases are over-represented. Such sequences are unlikely to form secondary structures and thus could shield χ sites from the effects of structured DNA.

ACKNOWLEDGEMENTS

We are grateful to Peter Bauman and Robert Tracy for providing Rad51 proteins and to Annick Boulet and Sophie Loeillet for technical assistance. We thank Kathleen Smith for critical review of the manuscript. This work was supported by grants from the Centre National de la Recherche Scientifique (UMR144), the Institut Curie, the Ligue Nationale Contre le Cancer and the Association pour la Recherche sur le Cancer (1252).

REFERENCES

1 Debrauwere,H., Gendrel,C.-G., Lechat,S. and Dutreix,M. (1998) *Biochimie*, **79**, 577–586.
2 Treco,D. and Arnheim,N. (1986) *Mol. Cell. Biol.*, **6**, 3934–3947.
3 Wahls,W.P., Wallace,L.J. and Moore,P.D. (1990) *Cell*, **60**, 95–103.
4 Cox,M.M. and Lehman,I.R. (1987) *Annu. Rev. Biochem.*, **56**, 229–262.
5 Kowalczykowski,S.C. and Eggleston,A.K. (1994) *Annu. Rev. Biochem.*, **63**, 991–1043.
6 Radding,C.M. (1991) *J. Biol. Chem.*, **266**, 5355–5358.
7 West,S.C. (1992) *Annu. Rev. Biochem.*, **61**, 603–640.
8 Shinohara,A., Ogawa,H., Matsuda,Y., Ushio,N., Ikeo,K. and Ogawa,T. (1993) *Nature Genet.*, **4**, 239–243.
9 Bezzubova,O., Shinohara,A., Mueller,R.G., Ogawa,H. and Buerstedde,J.M. (1993) *Nucleic Acids Res.*, **21**, 1577–1580.
10 Morita,T., Yoshimura,Y., Yamamoto,A., Murata,K., Mori,M., Yamamoto,H. and Matsushiro,A. (1993) *Proc. Natl Acad. Sci. USA*, **90**, 6577–6580.
11 Aboussekhra,A., Chanet,R., Adjiri,A. and Fabre,F. (1992) *Mol. Cell. Biol*, **12**, 3224–3234.
12 Bishop,D., Park,D., Xu,L. and Kleckner,N. (1992) *Cell*, **69**, 439–456.
13 Muris,D.F.R., Vreeken,K., Carr,A.M., Broughton,B.C., Lehman,A.R., Lohman,P.H.M. and Pastink,A. (1993) *Nucleic Acids Res.*, **21**, 4886–4991.
14 Cheng,R., Baker,T.I., Cords,C.E. and Radloff,R.J. (1993) *Mutat. Res.*, **294**, 223–234.
15 Clark,A.J. and Margulies,A.D. (1965) *Proc. Natl Acad. Sci. USA*, **53**, 451–459.
16 Xia,S.J., Shammas,M.A. and Reis,R.J. (1997) *Mol. Cell. Biol.*, **17**, 7151–7158.
17 Howard-Flanders,P., West,S.C. and Stasiak,A. (1984) *Nature*, **309**, 215–219.
18 West,S.C., Cassuto,E., Mursalim,J. and Howard-Flanders,P. (1980) *Proc. Natl Acad. Sci. USA*, **77**, 2569.
19 Stasiak,A., Stasiak,A.Z. and Koller,T. (1984) *Cold Spring Harbor Symp. Quant. Biol.*, **49**, 561–570.
20 Ogawa,T., Yu,X., Shinohara,A. and Egelman,E.H. (1993) *Science*, **259**, 1896–1899.
21 Sung,P. and Robberson,D.L. (1995) *Cell*, 453–461.
22 Benson,F.E., Stasiak,A. and West,S.C. (1994) *EMBO J.*, **13**, 5764–5771.
23 Shibata,T., Cunningham,R.P. and Radding,C.M. (1981) *J. Biol. Chem.*, **25**, 7557–7564.
24 Sugiyama,T., Zaitseva,E.M. and Kowalczykowski,S.C. (1997) *J. Biol. Chem.*, **272**, 7940–7945.
25 Bauman,P., Benson,F.E. and West,S.C. (1996) *Cell*, **87**, 757–766.
26 Watts,H.J., Yeung,D. and Parkes,H. (1995) *Anal. Chem.*, **67**, 4283–4289.
27 Dutreix,M. (1997) *J. Mol. Biol.*, **273**, 105–113.
28 Kowalczykowski,S.C. and Krupp,R.A. (1987) *J. Mol. Biol.*, **193**, 97–113.
29 Sigal,N., Delius,H., Kornberg,T., Gefter,M.L. and Alberts,B. (1972) *Proc. Natl Acad. Sci. USA*, **69**, 3537–3541.
30 Krauss,G., Sindermann,H., Shomburg,U. and Maass,G. (1981) *Biochemistry*, **20**, 5346–5352.
31 Stasiak,A., Egelman,A.H. and Howard-Flanders,P. (1988) *J. Mol. Biol.*, **202**, 659.
32 Tracy,R.B. and Kowalczykowski,S.C. (1996) *Genes Dev.*, **10**, 1890–1903.
33 Tracy,R.B., Baumohl,J.K. and Kowalczykowski,S.C. (1997) *Genes Dev.*, **11**, 3423–3431.
34 Tracy,R.B., Chedin,F. and Kowalczykowski,S.C. (1997) *Cell*, **90**, 205–206.