The β recombinase, in the presence of a chromatin-associated protein such as Hbsu, catalyzes DNA resolution or DNA inversion on supercoiled substrates containing two directly or inversely oriented six sites. Hbsu stabilizes the formation of the recombination complex (Alonso, J. C., Weise, F., and Rojo, F. (1995) J. Biol. Chem. 270, 2938–2945). In this study we show that resolution by β recombinase strictly requires supercoiled DNA, but inversion does not. On a substrate with two inversely oriented six sites, β recombinase catalyzed both resolution and inversion if the DNA was supercoiled but only inversion if the substrate was relaxed or linear. Hbsu was critical for the formation of synaptic complexes; its concentration relative to that of the supercoiled DNA substrate determined whether resolution or inversion products were preferentially formed. The results suggest that the β recombinase forms unproductive short-lived synaptic complexes between two juxtaposed inversely oriented six sites; the presence of 3 to 13 Hbsu dimers per supercoiled DNA molecule would stabilize a synaptic complex with a relative geometry of the six sites allowing β recombinase preferentially to achieve resolution. Supercoiling probably helps to overcome an energetic barrier, since resolution does not occur in relaxed DNA. The presence of >30 Hbsu dimers per DNA molecule probably favors the formation of a recombination complex with a different geometry since the reaction is directed preferentially toward DNA inversion.

Site-specific recombinases of the Tn3/yö family can be divided into three major subfamilies: DNA resolvases, DNA invertases, and DNA resolvase-invertases (reviewed in Refs. 1–3). The members of the three subfamilies normally require supercoiled DNA substrates containing two recombination sites (reviewed in Refs. 4–6). While DNA resolvases and DNA invertases are highly specialized in catalyzing resolution or inversion, respectively, members of the resolvase-invertase subfamily do not have this bias and catalyze both kinds of reactions efficiently (6, 7). The major difference between the enzymes of these three subfamilies lies in the DNA site required for recombination. The res site of DNA resolvases contains a crossover site (subsite I) and two essential accessory sites (subsites II and III) to which the recombinase binds (reviewed in Refs. 1 and 2). In the case of DNA invertases, the crossover site is analogous to the subsite I of DNA resolvases. The accessory site (sis or enhancer), which is not targeted by the recombinase, is located at some distance from the recombination site and is targeted by the sequence-specific DNA-binding and DNA-bending protein FIS (reviewed in Refs. 2, 8, and 9). The recombination site (six site) of resolvase-invertases includes a crossover site (subsite I) and an essential accessory site (subsite II). The accessory site(s) to which a sequence-independent chromatin-associated protein binds is not well defined (3, 11). The β recombinase is a well characterized member of the latter subfamily.

Both in vitro and in vivo, the activity of the β recombinase depends upon the presence of a chromatin-associated protein such as Bacillus subtilis Hbsu, the Escherichia coli HU, or eukaryotic HMG1, both when the protein mediates deletions between two directly oriented six sites, or inversions between inversely oriented six sites (7, 10–12). We have shown that, for DNA resolution, the role of the chromatin-associated protein is to facilitate the formation of the recombination complex (11). Our current model holds that the chromatin-associated protein works by recognizing and stabilizing a DNA structure at the six site. Our previous work had indicated that resolution occurred when the two six sites for the β recombinase are directly oriented and two or more Hbsu dimers per DNA molecule are present. The inversion reaction, which is less defined, takes place in the presence of 40 Hbsu dimers per DNA molecule and requires a substrate with two inversely oriented six sites (7, 12). With the aim of characterizing in detail the inversion reaction, we have studied the effect of the β recombinase on substrates containing two inversely oriented six sites. We have found that when such a substrate is supercoiled, both DNA inversion and DNA resolution can occur. The Hbsu concentration could determine the final direction of the reaction on a supercoiled DNA substrate. Relaxation of the DNA substrate totally inhibited the resolution activity of the β recombinase but did not affect its ability to catalyze inversion reactions. DNA inversion was observed even on linear DNA substrates. Therefore, the combination of the β recombinase six site and Hbsu allows an unprecedented flexibility of the DNA substrate, making it competent for the assembly of productive recombination complexes of different geometries.

MATERIALS AND METHODS

Bacterial Strains and Plasmids—The Escherichia coli strain XL-1 Blue (13) was used as host for DNA manipulations. Plasmids pCB8 and pCB12 (6) were used as substrates for site-specific recombination.

Proteins and Reagents—The β protein was overexpressed and purified as described previously (6, 14). Purified Hbsu protein was a gift from Prof. U. Heinemann (Max-Delbrück-Centrum für Molekulare Genetik, Ihnestrasse 73, D-14195 Berlin, Germany).
Medizin, Berlin). Since both wild-type $\beta$ (14) and Hbsu (6) proteins are dimers in solution, their concentration is expressed as mol of protein dimers.

**Preparation of Plasmids with Different Superhelical Densities**—Plasmids pCB8 and pCB12 were relaxed with topoisomerase I in the presence of ethidium bromide concentrations ranging from 0 to 20 $\mu$m, essentially as described (15). The mean superhelical densities of the samples obtained were calculated, as described (16), from the linking number difference between the center of the toposiomer distribution of a given sample and the center of the toposiomer distribution corresponding to relaxed DNA. The amount of DNA is expressed as mol of plasmid molecules.

**In Vitro Assays for Site-specific Recombination**—Plasmids pCB8, which contains two directly oriented six sites for the recombinase separated by about 2.3 kb, and pCB12, in which the two six sites are inversely oriented and separated by about 1.4 kb, were used as substrates for site-specific recombination. Reaction mixtures contained, in a total volume of 25 $\mu$l, 10 nM substrate plasmid, in 10 mM bis-Tris propane-HCl, pH 6.9, 10 mM MgCl$_2$, 10 mM NaCl, 1 mM DTT and proteins Hbsu and $\beta$ recombinase at the concentrations indicated in the Fig. legends. The $\beta$ protein-catalyzed resolution was saturated after 30 min of incubation, whereas saturation of the inversion reaction was achieved after 3 h. Hence, all reactions were incubated 3 h at 30 °C. To visualize the reaction products by agarose gel electrophoresis, the DNA was digested with the KpnI and EcoRV endonucleases. When working at higher ionic strength, reactions contained 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM MgCl$_2$, and the Hbsu and $\beta$ recombinase concentrations indicated in the Fig. legends (6).

**Electron Microscopy**—A sample containing supercoiled or linear DNA (10 nM) was incubated for 30 min at 30 °C, in the absence or presence of $\beta$ protein (500 nM) and/or Hbsu (800 nM), in a solution containing 10 mM triethanolamine-HCl, pH 7.5, 10 mM MgCl$_2$, 1 mM DTT in a total volume of 25 $\mu$l. Glutaraldehyde was then added to a final concentration of 0.1%, and allowed to react for 2 h at 30 °C. Samples were diluted with a buffer containing 10 mM triethanolamine-HCl, pH 7.5, 2 mM MgCl$_2$, to a final concentration of 2 mg/ml of DNA. Samples were directly adsorbed to freshly cleaved mica, stained with 2% uranyl acetate for 2 min and washed in double distilled water. After floating the samples in water, these were deposited onto copper grids covered with toulol. After being rotary shadowed with platinum at 10$^{-5}$ torr, a carbon film was deposited. Finally, replicas were obtained as described previously (17). Electron micrographs were taken at 80 kV, routinely at a magnification of $\times$50,000. Contour length measurements were carried out on photographic prints using a Summagraphic-type digitizer tablet.

**RESULTS**

### $\beta$ Recombinase Can Catalyze Both DNA Resolution and DNA Inversion on Supercoiled DNA Substrates with Two Inversely Oriented six Sites

Previously, we have demonstrated that $\beta$ recombinase, in the presence of Hbsu at concentrations corresponding to about 40 Hbsu dimers per DNA molecule, catalyzes a DNA inversion reaction on a supercoiled DNA substrate (plasmid pCB12) with two inversely oriented six sites (6, 7, 18). In this study we found that both DNA resolution and DNA inversion can occur on supercoiled DNA substrates with two inversely oriented six sites and that the Hbsu concentration present in the reaction mixture influenced whether resolution or inversion products were formed preferentially. In the presence of 10 nM supercoiled DNA substrate containing two six sites in an inverse orientation (plasmid pCB12) and an excess of $\beta$ recombinase (670 nM), the addition of 3–13 Hbsu dimers (30–130 nM) per DNA molecule (1 Hbsu dimer per 1600 to 370 base pairs) led to the appearance of recombination products corresponding preferentially to a resolution process rather than to the expected inversion reaction. Nevertheless, at higher Hbsu concentrations (>150 nM, corresponding to about 15–200 Hbsu dimers per DNA molecule), the amount of resolution products decreased while inversion products became predominant (Fig. 1, A and B). About 42 and 30% of the total DNA substrate was converted to resolution and inversion products, respectively, in 3 h at 30 °C.

An additional unexpected DNA fragment of about 2.6 kb, apparently associated to the resolution process, was also detected, although its nature is at present unknown. The amount of $\beta$ recombinase present from 30 nM to 2 $\mu$m did not seem to bias the reaction in either direction (resolution or inversion) (data not shown).

The addition of 2 $\mu$m Hbsu (200 Hbsu dimers per DNA molecule, or 1 dimer per 25 nucleotides) neither nicked nor unwound pCB12 DNA (Fig. 1A, lanes 1 and 2, and data not shown), ruling out the possibility that the decrease in resolution efficiency at high Hbsu concentrations could be due to nicking or unwinding of the substrate DNA (see below). It is likely, therefore, that the effect of $\beta$ recombinase on supercoiled DNA substrates containing two inversely oriented six sites is different from that of the enzymes of the other subfamilies (see the Introduction) that are very selective for the orientation of the recombination sites and generate only one product from a particular substrate; DNA invertases catalyze only inversions and DNA resolvases are highly specialized in resolution.

### The Role of Hbsu in $\beta$-Mediated DNA Inversion Is to Facilitate the Assembly of a Synaptic Complex

When the substrate of $\beta$ recombinase is a supercoiled plasmid containing two directly oriented six sites (pCB8 plasmid DNA), the role of Hbsu is to facilitate the joining of distant recombination sites to form a recombination complex (11). To investigate whether Hbsu has the same function in the inversion reaction, recombination complexes formed with plasmid pCB12 (10 nM) under conditions which favor DNA inversion (an excess of Hbsu) were analyzed by electron microscopy after fixation of the protein-DNA complexes with glutaraldehyde. When the DNA was incubated separately with $\beta$ recombinase or Hbsu, neither recombination complexes nor protein bound to DNA were observed (Fig. 2, A–C). Furthermore, the addition of 50 dimers of $\beta$ recombinase (500 nM), or 80 dimers of the Hbsu (800 nM) protein per DNA molecule, did not seem to affect the level of supercoiling or to relax pCB12 DNA (Fig. 2, A–C). When both Hbsu and $\beta$ recombinase were present in the reaction mixture, about 30 to 40% of the DNA molecules showed a dot-like complex that held together two DNA segments of the molecule, dividing the plasmid into two discrete domains. From the length of the DNA segments in each domain, we infer that the dot-like structures is located at the position expected for a recombination complex (Fig. 2, D–F). The proportion of DNA molecules containing a dot-like structure correlates with the amount of recombinant products observed by gel electrophoresis.

Although these assays do not allow us to discriminate whether the complexes observed correspond to resolution or inversion reactions, they indicate that Hbsu is essential for the joining of two distant inversely oriented six sites into a recombination complex, as was previously shown to be the case when the two six sites are in direct orientation (11).

### The Supercoiling Density of the DNA Substrate Dramatically Affects the Resolution Reaction but Not the Inversion Reaction

Supercoiling of the substrate DNA is essential for site-specific recombinases of the Tn3/γ6 family (reviewed in Refs. 1 and 2). In the case of $\beta$ recombinase, resolution with a plasmid containing two directly oriented six sites (plasmid pCB8) occurs efficiently if the molecule is supercoiled but is undetectable if the substrate is relaxed (7). To determine whether differences exist in the supercoiling requirements of the inversion and resolution reactions, we relaxed plasmid pCB12 with topoisomerase I and tested whether the relaxed DNA is a substrate for the inversion or resolution reactions. Fig. 3 shows that the relaxed pCB12 did not support the resolution reaction but was

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1 The abbreviations used are: kb, kilobase; DTT, dithiothreital; bp, base pair; six, site of crossing over.
as good a substrate for the inversion reaction as the supercoiled form.

To analyze this aspect in more detail, we prepared plasmid populations with different supercoiling densities from plasmids pCB8 and pCB12 and scored for DNA recombination. Under standard conditions (10 nM substrate DNA, 500 nM β protein, 300 nM Hbsu), resolution with pCB8 was efficient only for plasmids having superhelical densities between –0.03 and –0.07; below and above these values, the reaction efficiency decreased sharply (Fig. 4). Resolution was the only recombination product observed with pCB8 DNA (Ref. 7; data not shown). Plasmid pCB12 showed that the resolution reaction between two inversely oriented six sites has a DNA supercoiling requirement similar to that of a substrate with two directly oriented six sites. Nevertheless, efficient inversion was observed at all supercoiling densities tested (Fig. 4). It seems, therefore, that β recombinase does not strictly require DNA supercoiling for the inversion reaction.

Recombination was not detected between two negatively supercoiled or linear plasmid substrates, each containing a single six site (intermolecular recombination; data not shown).

β Recombinase Catalyzes Inversion on a Linear DNA with Inversely Oriented six Sites—Since, as shown above, the β recombinase efficiently catalyzed DNA inversion on a relaxed substrate containing two inversely oriented six sites, we asked whether this reaction could also take place when the substrate was in linear form. Plasmid pCB12 was linearized with KpnI and used as substrate for recombination. As revealed in Fig. 5, under standard conditions inversion products were readily observed. The reaction efficiency was similar, or even higher, than that obtained with a supercoiled pCB12 DNA. DNA recombination between two inversely oriented six sites on linear DNA strictly required the presence of the accessory protein Hbsu (data not shown).

To investigate the recombination products formed with linear pCB12 substrates, the protein-DNA complexes were fixed with glutaraldehyde and analyzed by electron microscopy. When the linear DNA was incubated separately with either β recombinase (500 nM) or Hbsu (800 nM), neither recombination complexes nor protein dots sitting on DNA were observed (data not shown). In the presence of both β (500 nM) and Hbsu (800 nM), about 30 to 40% of total DNA molecules were complexed.
substrates. pCB12 DNA (10 nM) was relaxed by incubation with topoisomerase I (lanes 1 and 4). Relaxed (R) or supercoiled (SC) DNA was incubated in the absence (lanes 1–3) or presence (lanes 4 and 5) of 250 nM Hbsu and 500 nM β protein. The products were analyzed by digestion with endonucleases KpnI and EcoRV. The lane labeled M corresponds to a DNA size marker. The DNA fragments corresponding to inversion or resolution products are indicated.

FIG. 3. Resolution and inversion reactions on relaxed DNA substrates. pCB12 DNA (10 nM) was relaxed by incubation with topoisomerase I (lanes 1 and 4). Relaxed (R) or supercoiled (SC) DNA was incubated in the absence (lanes 1–3) or presence (lanes 4 and 5) of 250 nM Hbsu and 500 nM β protein. The products were analyzed by digestion with endonucleases KpnI and EcoRV. The lane labeled M corresponds to a DNA size marker. The DNA fragments corresponding to inversion or resolution products are indicated.

FIG. 4. Effect of the DNA substrate supercoiling density on the resolution and inversion reactions. Recombination reactions were carried out with plasmid preparations having different supercoiled densities (see "Materials and Methods"). Plasmids pCB8 (two directly oriented six sites) and pCB12 (two inversely oriented six sites) were used. Reactions contained 10 nM substrate DNA, 300 nM Hbsu, and 500 nM β protein. Samples were digested with endonucleases KpnI and EcoRV, and the fragments generated were resolved by agarose-gel electrophoresis. The amounts of the DNA fragments corresponding to inverted or resolved molecules were quantitated by laser-scanning densitometry. The graph shows the reaction efficiency plotted versus the superhelical density of the plasmid preparation (circles correspond to resolution on pCB8, squares to resolution on plasmid pCB12, and triangles to inversion on pCB12). Reaction efficiency was calculated relative to the maximum recombination value observed in each case; similar amounts of unreacted material were present in all cases.

FIG. 5. A linear DNA with inversely oriented six sites is a substrate for β recombinase. Plasmid pCB12 was linearized with endonuclease KpnI and used as substrate for recombination in the absence (−) or presence (+) of proteins Hbsu and β recombinase, as indicated. Samples were subsequently digested with endonuclease EcoRV, and the fragments generated were resolved by agarose-gel electrophoresis. The DNA fragments corresponding to nonrecombinant plasmid (3.4 and 1.4, marked as nr), or to inversion products (4.1 and 0.8, marked as inv), are shown. Hbsu was present at 500 nM, pCB12 at 10 nM, and the β recombinase at 248, 496, 992, or 1984 nM. The lane labeled M corresponds to a DNA size marker.

cules. The length of the long arm was about 2821 ± 216 bp, that of the loop was 1,889 ± 197 bp, while the short arm was 167 bp ± 67 in length; the length measured for the protein-free plasmid was 4,984 ± 55 bp. It is likely, therefore, that the dot-like structures represent a synaptic complex formed by the joining of the two distant inversely oriented six sites present in the linear molecule.

In three cases (3.3%), the protein dot was bound to one DNA strand, whereas in the remaining 20% of the cases (n = 18), two or more individual DNA molecules bound to a protein dot were observed (data not shown). These complexes, which are probably preparation artifacts, were not further analyzed.

From these results, we conclude that the role of Hbsu with linear substrates is to facilitate the formation of a recombination complex, as it does with supercoiled plasmids (see Fig. 2).

DISCUSSION

β Recombinase Does Not Show a Directionality Bias on a Supercoiled Substrate with Two Inversely Oriented six Sites—The control of the reaction directionality of DNA resolvases and DNA invertases results from the formation of higher order protein-DNA complexes which control the DNA geometry within the synaptic complex. This discrimination is further enhanced by the free energy associated with DNA supercoiling (reviewed in Refs. 1 and 2). Our results show that β recombinase can catalyze both DNA resolution and DNA inversion on negatively supercoiled DNA substrates containing two inversely oriented six sites, and that the type of reaction (resolution or inversion) is determined to a significant extent by the amount of Hbsu available relative to DNA substrate. The effect of Hbsu in the directionality of the reaction is not the consequence of a nicking of the DNA by contaminant nucleases since addition of an excess of Hbsu did not nick the DNA. Previously, it has been shown that 10–13 dimers of HU (E. coli counterpart...
leading to resolution and the other one to inversion products. If this hypothesis is correct, whether β recombinase will catalyze resolution or inversion on these substrates will depend on the DNA geometry within the synaptic complex rather than on DNA site orientation discrimination. It should be taken into account that this model would hold only when the substrate has two inversely oriented six sites since, when the two six sites are directly oriented, only DNA resolution occurs, independently of the Hbsu concentration (6, 7).

Analysis of recombination complexes by electron microscopy showed that, both in the inversion and resolution reactions, the role of Hbsu is to stabilize the formation of the recombination complex (Ref. 11; this work). Since β protein catalyzes DNA inversion between two inversely oriented sites on linear DNA, we can rule out that the role of Hbsu is solely to increase the supercoiling of the DNA substrate. The stabilization of the recombination complex requires overcoming an energy barrier to stabilize a DNA conformation that allows the proper alignment of the two inversely oriented six sites in a productive recombination complex which, after strand exchange, generates recombination products. The results reported show that the presence of 3 to 13 Hbsu protein dimers per supercoiled DNA molecule helps to stabilize preferentially a complex with a geometry leading to resolution products. When 15–200 Hbsu dimers per supercoiled molecule are present, a recombination complex with a different geometry is stabilized which, after strand exchange, yields inversion products.

We have previously shown that a mammalian HMG1 protein, which shares neither sequence nor structural homology with Hbsu, or even plastid HlpA, can stimulate β-mediated recombination (11, 20). These chromatin-associated proteins bind preferentially to DNA sequences showing bent or altered conformations (21–23). It is likely, therefore, that the way through which the chromatin-associated protein participates in the reaction is by stabilizing one or more bent DNA conformations that arise upon the assembly of a recombination complex. This complex should be stable enough to allow the recombination molecules to assemble and undergo the conformational changes necessary to adopt a catalytically active state. A role for Hbsu in binding bent or altered DNA structures would allow a high flexibility for its positioning in the recombination complex: it may bind to more than one position, provided that the DNA can be distorted, and that the protein can physically fit into the complex. We believe that it is this characteristic which confers less selectivity to the β recombinase with respect to the final direction of the recombination reaction (resolution or inversion) when the two β protein six sites are in an inverted orientation.

**Formation of Synapsis Depends on Conformational Effects**—As discussed above, the β recombinase requires negative supercoiling of the DNA substrate for the resolution reaction but not for the inversion reaction. Productive recombination complexes were formed even on linear DNA molecules containing inversely oriented six sites. This behavior is different from that of the DNA resolvases or DNA invertases. The analysis of the role of DNA supercoiling on the recombination reactions catalyzed by DNA resolvases and DNA invertases has led to the conclusion that the critical mechanistic assistance provided by supercoiling leading to the formation of a synapsis depends on conformational effects while the transition to postsynapsis and strand exchange relies on torsional stress; the latter process requires a higher superhelical density than synapsis (24, 25). DNA invertases require an accessory protein, FIS, to mediate inversion and reaction selectivity (26). FIS-independent mutant derivatives of the Gin invertase do not require supercoiled DNA and are even active on linear DNA substrates, albeit with a low efficiency. Those mutations lie in the dimer interface and...
may render a constitutively active recombinase (27, 28). FIS-independent Gin mutants have also lost the selectivity for inversion. Furthermore, the requirement for supercoiling by DNA resolvases can be circumvented by using special “permissive” reaction conditions (5, 29). The β recombinase resembles in many respects the FIS-independent Gin invertase mutants or DNA resolvases assayed under special “permissive” reaction conditions (5, 28, 29). An essential difference between the β and Gin systems is that FIS binds to a specific site providing a directionality to the reaction, while Hbsu could in principle bind to any region of the DNA in the recombination complex that has an appropriately bent structure. This characteristic is most likely what allows the β protein to form recombination complexes of different geometries. It is worth mentioning that, like other DNA resolvases of this family (24, 25), the β protein can form synaptic complexes with a relaxed DNA substrate containing two directly oriented six sites, as judged by electron microscopy (data not shown), although these complexes are not productive (Figs. 3 and 4).

Finally, our results may shed some light on how the recombination sites find each other to form the recombination complex. Several models have been proposed to account for this process. Recombination sites could initially juxtapose either by random collision (30, 31) or by slithering in a plectonemically coiled. Several models have been proposed to account for this process. Recombination sites could initially juxtapose either by random collision (30, 31) or by slithering in a plectonemically coiled (30, 31) or by slithering in a plectonemically coiled (30, 31). When the substrate DNA is circular, it is proposed that a productive interwrapping of the sites and cognate proteins can only be achieved when the recombination sites exist in the substrate DNA molecule in a particular geometry that would impose a “topological filter” on the reaction (31). When the sites are not in the appropriate orientation, the energetic cost of distorting the DNA to achieve productive synapsis is probably too high and recombination is precluded (25, 30, 31). We suggest that the β recombinase, in the presence of Hbsu, probably forms the recombination complex by random collision because inversion occurs efficiently on linear substrates. In linear DNA, encountering of the recombination sites by slithering would only be successful when the DNA is moving in one direction relative to the first target site for the recombinase since the slithering in the opposite direction would reach the end of the molecule before the second target is found. This would considerably diminish recombination efficiency, and the β protein seems to be equally efficient with linear and supercoiled molecules. In a supercoiled substrate stabilization of a distorted DNA by Hbsu, binding could overcome the energetic cost of reorienting the inappropriate orientation of the inverted six sites to achieve productive synapsis; in the presence of an excess of Hbsu, the constraints on the DNA impose a barrier for such a reorientation and the juxtaposed, properly oriented sites form a synaptic complex.

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REFERENCES

1. Grindley, N. D. F. (1994) in Nucleic Acids and Molecular Biology (Eckstein, F., and Lilley, D. M. J., eds) vol 8, pp. 236–267, Springer-Verlag, Berlin
2. Stark, W. M., and Boocock, M. R. (1995) in Mobile Genetic Elements (Sherratt, R. J., ed.) pp. 101–129, Oxford University Press
3. Alonso, J. C., Ayura, S., Canosa, L., Weise, F., and Rojo, F. (1996) FEMS Microbiol. Lett. 142, 1–10
4. Kanaar, R., van de Putte, P., and Cazzaire, N. R. (1989) Cell 58, 147–159
5. Stark, W. M., Sherrat, D. J., and Boocock, M. R. (1989) Cell 58, 779–790
6. Rojo, F., and Alonso, J. C. (1994) J. Mol. Biol. 238, 159–172
7. Alonso, J. C., Weise, F., and Rojo, F. (1995) J. Biol. Chem. 270, 2938–2945
8. Johnson, R. C. (1991) Curr. Opin. Genet. Dev. 1, 404–411
9. van de Putte, P., and Gooosen, N. (1992) Trends Genet. 8, 457–462
10. Canosa, L., Rojo, F., and Alonso, J. C. (1996) Nucleic Acids Res. 24, 2712–2717
11. Alonso, J. C., Gutierrez, C., and Rojo, F. (1995) Mol. Microbiol. 18, 471–478
12. Canosa, L., Ayura, S., Rojo, F., and Alonso, J. C. (1997) Mol. Gen. Genet. 255, 467–476
13. Sambrook, J., Maniatis, T., and Fritsch, E. F. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
14. Rojo, F., Weise, F., and Alonso, J. C. (1993) FEBS Lett. 328, 169–173
15. Meiklejohn, A. L., and Gralla, J. D. (1989) J. Mol. Biol. 207, 661–673
16. Wang, J. C., Peck, J. L., and Becherer, K. (1982) Cold Spring Harbor Symp. Quant. Biol. 47, 85–91
17. Sogo, J. M., Stasiak, A., de Bernardin, W., Losa, R., and Koller, T. (1987) in Electron Microscopy in Molecular Biology, A Practical Approach (Somerville, J., and Scheer, U., eds) pp. 61–78, IRL Press, Oxford
18. Rojo, F., and Alonso, J. C. (1995) Nucleic Acids Res. 23, 3181–3188
19. Drlica, R. (1992) Mol. Microbiol. 6, 425–433
20. Grasser, K. D., Ritt, C., Krieg, M. E., Fernandez, S., Alonso, J. C., and Grimm, R. (1997) Eur. J. Biochem. 249, 76–76
21. Pontiggia, A., Negri, A., Beltrame, M., and Bianchi, M. E. (1993) Mol. Microbiol. 7, 343–350
22. Bianchi, M. E. (1994) Mol Microbiol. 14, 1–5
23. Bonnefoy, E., Takahashi, M., and Yaniv, J. R. (1994) J. Mol. Biol. 242, 116–129
24. Benjamin, K. R., Abola, A. P., Kanaar, R., and Cazzaire, N. R. (1996) J. Mol. Biol. 256, 50–63
25. Watson, M. A., Boocock, M. R., and Stark, W. M. (1996) J. Mol. Biol. 257, 317–329
26. Johnson, R. C., Glasgow, A. C., and Simon, M. I. (1987) Nature 329, 531–539
27. Klippel, A., Cloppenborg, K., and Kahmann, R. (1988) EMBO J. 7, 3983–3989
28. Klippel, A., Kanaar, R., Kahmann, R., and Cazzaire, N. R. (1993) EMBO J. 12, 1047–1057
29. Droge, P., and Cazzaire, N. R. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6062–6066
30. Craigie, R., and Mizzuuchi, K. (1986) Cell 45, 795–800
31. Boocock, M. R., Brown, J. L., and Sherratt, D. J. (1986) Biochem. Soc. Trans. 14, 214–216
32. Benjamin, K. R., and Cazzaire, N. R. (1986) Proc. R. A. Welch Found. Conf. Chem. Res. 29, 107–126