Direct Recombination Proteins

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The RAD54 and RAD51 genes are involved in genetic recombination and double-strand break repair in the yeast Saccharomyces cerevisiae. The Rad51 protein is thought to be a yeast analogue of the Escherichia coli recA gene product and catalyzes strand exchange between homologous single- and double-stranded DNAs in vitro. RAD54 exhibits homologies to several known ATPases and is a member of the SWI2/MOT1 family. We show here that the Rad54 protein interacts with the Rad51 protein in vivo and in vitro and that the NH2-terminal 115 residues of the Rad54 protein are necessary for this interaction. Combined with previously reported results, these data imply that the Rad54 protein is part of a multiprotein yeast recombination complex.

The RAD52 epistasis group includes genes involved in homologous recombination in the yeast Saccharomyces cerevisiae. Mutations in these genes result in phenotypes that include an inability to repair double-stranded breaks, as well as defects in mitotic and meiotic recombination (1–4). The Rad51, Rad55, and Rad57 proteins show considerable homology to the Escherichia coli RecA protein, the paradigmatic prokaryotic strand transferase. Indeed, Rad51 protein has been shown to mediate strand exchange in vitro between homologous single- and double-stranded DNAs in the presence of replication protein A (RPA),1 the yeast single-stranded DNA-binding protein (5).

A number of results suggest that the Rad51 protein functions as part of a multiprotein complex in vivo. For example, the Rad51 and Rad52 proteins have been shown to bind one another both in vivo (6) and in vitro (7), and there is genetic evidence that Rfa1 is associated with the putative recombination complex (8, 9). Furthermore, experiments from the Berg laboratory have suggested that the Rad54 protein acts as a member of the SWI2/MOT1 family. We show here that the Rad54 protein interacts with the Rad51 protein in vivo and in vitro and that the NH2-terminal 115 residues of the Rad54 protein are necessary for this interaction. Combined with previously reported results, these data imply that the Rad54 protein is part of a multiprotein yeast recombination complex.

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colonies were picked and tested for 
firmed the identity of the purified protein. Approximately 10 mg of
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resuspended in 200 ml of cold extraction buffer (20 mM HEPES, pH 7.5, 1 mM EDTA, 0.5% benzamidine, 0.5% Triton X-100, and 34 g/ml DNase I. Cells were lysed by incubation of a 5-fold molar excess of the protein with the TEV protease at 30°C for 1 h. The reaction was terminated by the addition of protein/liter of liquid culture was obtained. The metal-binding tag was cleaved from His6Rad51 protein to give GAMG-Rad51 by incubation of a 5-fold molar excess of the protein with the TEV protease for 5 min as described (17).

Purification of His6Rad51 Protein from Yeast—S. cerevisiae reg1-501 cells containing pKHYesHis651 were grown on SC (1.7 g of yeast nitrogen base (Life Technologies, Inc.), 5 g of ammonium sulfate, and 20 g of Bactoagar (Life Technologies, Inc.) per liter) Ura-Leu drop-out plates at 30°C using glucose as the carbon source. A single colony was picked and grown in SC ura-leu drop-out media overnight. The culture was diluted 1:500 in SC ura-leu drop-out media and grown until the OD600 was between 0.8 and 1.0. The cells were induced by the addition of galactose to 2%. The cell pellet was resuspended in 1 volume of CS buffer supplemented with 65 mM sodium phosphate, 10 mM Mops Cl–, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 25 mM MgCl2, 2 mM MgCl2, and 1 mM pepstatin A. The suspension was lysed by French Press (SLM Aminco). Cell debris was removed by centrifugation in a Beckman JA-17 rotor run an 14,000 rpm for 1 h. The protein was then purified using the procedure described above for His6Rad51 protein expressed in E. coli, except that a DE-52 column was substituted for the hydroxylapatite column. The DNA-cellulose pool was loaded in a buffer containing 100 mM NaCl and, after thorough washing, eluted with a buffer containing 400 mM NaCl. Approximately 150 μg of purified His6Rad51 protein per liter of liquid culture was obtained.

Purification of Rad54ΔBamHI—The RAD54 coding sequence was amplified by PCR and cloned into pKM260 to give pKHHis6RAD54. Expression of Rad54 protein in E. coli using the AR plasmid was unsuccessful. To produce a derivative of Rad54 protein, pKHHis6RAD54 was cleaved with BamHI, and after removal of the internal fragment, ligated to give pKHHis6RAD54Δ(BamHI). Detection of the BamHI fragment deletes the region of RAD54 encoding residues 115–750, but retains the reading frame. The protein was then purified by affinity chromatography using a Bio-Rad Prep Cell. NH2-terminal sequencing confirmed the identity of the polypeptide. The concentrations of each protein was determined by absorbance at 280 nm in denaturing solutions as described (18).

Far-Western Blot Analysis—The protocol employed was slightly modified from that employed by Horuchi et al. (19). Proteins were electrophoresed through a denaturing polyacrylamide gel and subsequently stained with Coomassie Blue or transferred to polyvinylidene difluoride membrane (Millipore). The membranes were then incubated in 8% urea containing 1% 2-mercaptoethanol, 1% 2-mercaptoethanol, 1% mercaptoethanol, and 1% mercaptoethanol. Detection of the proteins was achieved through stepwise dilutions of the urea concentration in FW buffer (20 mM Tris-HCl, pH 7.5, 60 mM NaCl, 0.1 mM EDTA, 10 mM MgCl2, 5% (w/v) glycerol, 0.02% Nonidet P-40) until the final urea concentration was below 10 mM. The membranes were blocked in FW buffer containing 5% non-fat dry milk, and then incubated in FW buffer (containing 2% non-fat milk) with TEV-cleaved His6Rad51 protein (purified from yeast) (2 mg/ml) or, as a control, in the same buffer lacking the Rad51 protein. The bands which retained the Rad51 protein were detected by probing the membrane with mouse polyclonal antibodies raised against TEV protease-cleaved His6Rad51 protein (1 h at room temperature) followed by a goat anti-mouse antibody conjugated to horseradish peroxidase (1 h) and then chemiluminescence reagent (DuPont) (1 min at room temperature). The membrane was washed with FW buffer between each incubation.

Preparation of the Rad51 Protein Affinity Column—Affi-Gel 10 beads (Bio-Rad) were washed with 5 volumes of double-distilled H2O, then twice with 5 volumes of FIX buffer (100 mM potassium phosphate, pH 6.5, 10% (w/v) glycerol). His6Rad51 protein was dialyzed into the same buffer. The beads were mixed with the protein and agitated gently on a rotatory table at 4°C. Typically, 5 ml of a 6 mg/ml protein solution was mixed with 5 ml of packed beads. The progress of the reaction was checked periodically by monitoring the A260 of the supernatant. When this had decreased 15%, the reaction was terminated by the addition of a large excess of ethanolamine, pH 7.4. The same method was employed to couple bovine serum albumin (BSA) to Affi-Gel 10. The beads were washed thoroughly with Bind buffer (see below) and stored at –20°C.

Protein Affinity Chromatography—Columns were constructed from 1 ml syringes plugged with glass wool. The Rad51 protein beads (0.9 mg of protein/packed ml) were added to the column to give a packed volume of 0.3 ml. A 0.3-ml BSA control column (6 mg of protein/packed ml) was also constructed. The columns were then washed extensively with

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Bind$_{2000}$ buffer (Bind buffer: 40 mM Tris-HCl, pH 7.4, 1 mM Na$_2$EDTA, 1 mM dithiothreitol, 2 mM MgCl$_2$, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamide hydrochloride, supplemented with NaCl as indicated in the subscript in millimolar), then equilibrated with Bind$_{sb}$ buffer.

Lysates from cells expressing S10 epitope-tagged derivatives of the Rad51, Rad54, and Rpa1 proteins were made according to the procedure of Melcher and Johnston (20). The lysates were cleared by centrifugation at 13,000 rpm in a benchtop microcentrifuge and passage through a 0.44-micron filter (Millipore), mixed together (ratio of lysates containing S10-Rad54, S10-RPA, S10-Rad51 = 10:3:1) in Bind$_{sb}$ buffer, and applied simultaneously to the Rad51 and BSA columns at a flow rate of 1 column volume/h. Each column was then washed with 10 column volumes of Bind$_{sb}$ buffer, then eluted with 1 column volume of Bind$_{50}$ buffer. The beads were then removed from the column and boiled in SDS-containing gel loading buffer to release bound proteins not eluted by the 500 mM salt wash. Each fraction was made 50 µg/ml in lysozyme, then the total protein was precipitated by the addition of sodium deoxycholate to 0.5 mg/ml and trichloroacetic acid to 20%, followed by incubation on ice for 1 h and centrifugation at 13,000 rpm for 30 min in a benchtop microcentrifuge at 4 °C. The precipitate was washed with acetic and diethyl ether then boiled in sample loading buffer for 3 min and applied to a 7.5% denaturing polyacrylamide gel. The proteins were analyzed by Western blot using monoclonal antibodies that recognize the S10 epitope (Novagen).

Complementation of X-ray Sensitivity—Cells were irradiated on solid selection media (galactose as the carbon source) with X-rays from a 60Co source at a dose rate of 115 rads. After 3–4 days growth, cell numbers were counted and compared to the number of cells growing on plates that had not been irradiated.

RESULTS

The Rad51 Protein Binds the Rad51 Protein in Vivo—We employed the yeast two-hybrid system (14, 21) to probe for protein-protein interactions between some of the known RAD genes. Plasmids were constructed in which the Gal4 reporter gene was fused to the AD and the Rad51 protein to the DBD a much lower level of β-Gal activity was observed. A similar result was obtained for the well characterized Rad51-Rad52 interaction. These observations suggest that fusion of the Rad51 protein to the DBD interferes with its interactions with other recombinational proteins, although apparently not with self-association.

No physical interaction between the RPA1 gene product, the yeast single-stranded DNA-binding protein, and any of the Rad proteins tested was indicated by the two-hybrid data, although Rpa strongly stimulates Rad51-mediated strand exchange in vitro (5). However, this negative result should be interpreted with caution. It might be that stoichiometric amounts of the other two components of heterotrimeric Rpa (22) are required for a stable interaction, or that fusion of Rpa1 protein to the GAL4 DBD interferes with its binding to other factors.

The Rad51 Protein-binding Domain Maps to the NH$_2$ Terminus of the Rad54 Protein—Many eukaryotic proteins contain independent functional domains. To investigate whether Rad51 protein binding activity could be mapped to a particular region of the Rad54 protein, fusion constructs were made in which the GAL4 DBD was linked to various fragments of Rad54 (Fig. 1). They were then employed in two-hybrid experiments along with the AD-Rad51 fusion. None of the Rad54 fragment fusions were themselves able to stimulate lacZ transcription. Rad54(A) (residues 1–327) and Rad51 protein provided a strong positive signal, while fragments lacking the NH$_2$-terminal region did not. These data show that residues in the NH$_2$-terminal region of Rad54 protein are important in binding Rad51 protein while the COOH terminus is not essential for this interaction. However, we cannot exclude the possibility the COOH-terminal region of Rad54 protein plays a secondary role in Rad54-Rad51 interactions, since we were unable to demonstrate that these fusions were stably expressed. Quantitation of the in vitro expression levels of these various constructs was attempted by Western blotting using antibodies raised against a fragment of Rad54 protein (see below), but no signal was detected in any case, including that of the full-length protein. It may be that the fusions were expressed at very low levels, that they were proteolytically unstable upon cell lysis, or that fusion with the Gal4 DBD interfered with antibody binding.

The Rad51 Proteins Binds to the NH$_2$-terminal 115 Residues of Rad54 Protein in Vivo—While the two-hybrid assay is useful as an indicator of protein-protein interactions, it is important to check results obtained in these experiments by an independent method. Furthermore, it is conceivable that GAL4 activity could be reconstituted by an indirect Rad51-Rad54 protein interaction bridged one or more endogenous factors. Therefore, in vitro experiments were employed to probe Rad54-Rad51 protein-protein interactions.

To facilitate these studies, large quantities of a Rad51 protein derivative containing a 6-histidine tag and a specific protease (TEV protease) cleavage site (17) was expressed in both yeast and E. coli and purified. The fusion protein contains 15 non-native residues between the NH$_2$-terminal methionine and the Rad51 amino acids (Fig. 2). To ensure that these amino acids did not interfere with the function of the Rad51 protein, we expressed this fusion (henceforth called the His6Rad51 protein) in a rad51 strain while the COOH terminus is not essential for this strain. As shown in Fig. 3, this was the case, demonstrating that the His6Rad51 protein was active in vivo. The presence of the 6-histidine tag facilitates purification and allows large quantities of the protein to be obtained easily (see Fig. 2 and “Materials and Methods”). The rate of DNA-dependent ATP hydrolysis catalyzed by His6Rad51 protein was measured (data not shown).
shown) and found to be essentially identical to that reported for native Rad51 protein (5, 23), again arguing that the NH2-terminal fusion does not compromise the activity of the protein. His6Rad51 protein was cleaved efficiently by purified TEV protease (17) in vitro, resulting in a polypeptide lacking the metal-binding tag and containing only four non-native residues, GAMG (Fig. 2).

Unfortunately, many attempts to express large quantities of Rad54 protein in E. coli, S. cerevisiae, and Pichia were unsuccessful.2 We therefore turned to the expression of fragments of Rad54 protein. Two polypeptides were expressed, the 327-residue NH2-terminal fragment, Rad54(A), and Rad54ΔBamHI, a fusion containing a large deletion of the central region of the protein. The corresponding gene was constructed by eliminating the internal BamHI fragment in the RAD54 gene and religating (this retains the reading frame). This derivative lacks the ATPase homology regions, but retains the NH2-terminal 115 residues and the COOH-terminal 182 amino acids.

Expression of each Rad54 protein fragment as 6-histidine fusions in E. coli BL21DE3 cells under the control of a T7 promoter resulted in the production of large amounts of protein, most of which was insoluble. Each was purified under denaturing conditions, the Rad54ΔBamHI protein to near homogeneity and Rad54(A) protein to approximately 90% purity (see Fig. 4).

Finally, mouse polyclonal antibodies were raised against the TEV protease-cleaved His6Rad51 protein and His6Rad54ΔBamHI to facilitate their detection in crude lysates (see below).

With these tools in hand, a Far-Western blotting experiment (19) was employed to look for direct binding between the Rad51 protein and the Rad54 protein fragments. The results are shown in Fig. 4. Part A shows a Coomassie Blue-stained denaturing gel of the purified Rad54 fragments (lanes 1 and 3) and the crude lysates from which the purified proteins were derived (lanes 2 and 4). A control lysate lacking any Rad54-derived proteins is shown in lane 5. This gel was blotted onto nitrocellulose and the proteins renatured. The blots were then probed with either TEV-cleaved His6Rad51 protein (Fig. 4B) or with buffer as a control (data not shown). Mouse anti-Rad51 antibody, followed by goat anti-mouse antibodies conjugated with

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2 H. Jiang, K. Stemke-Hale, and T. Kodadek, unpublished observations.
horseradish peroxidase were then used to probe the blot to detect the position of the bands that bound Rad51 protein in the first step. As shown in lanes 2 and 4 of Fig. 4B, Rad51 protein bound to both the purified Rad54(A) and Rad54ΔBamHI polypeptides, respectively. More importantly, the Rad51 protein also recognized these Rad54 fragments selectively in the lysate lanes. While some E. coli proteins in these lanes “light up” (see also lane 5), they are also observed in the blot where the Rad51 protein probing step was omitted (data not shown). The background bands are therefore due to a low level of cross-reactivity of some bacterial proteins with the polyclonal anti-Rad51 antibody and/or the secondary antibody. These data strongly suggest that only the NH₂-terminal 115 amino acids of Rad54 are absolutely required for binding Rad51 protein in vitro.

Detection of Rad54-Rad51 Binding by Protein Affinity Chromatography—Since the Far-Western experiment described above employed fragments of the Rad54 protein which had been denatured and renatured prior to exposure to Rad51, it remained an important goal to demonstrate in vitro interactions between the intact proteins. Protein affinity chromatography using His6Rad51 protein immobilized on Affi-Gel beads was employed for this purpose. Since native Rad54 protein is present at exceedingly low levels in yeast (50 molecules/cell or less) and is difficult to detect with the polyclonal antibodies in hand, a vector was constructed that expressed an epitope-tagged derivative of the protein. This species was clearly detectable by Western blotting using a commercially available monoclonal antibody directed against the S10 epitope. Several other plasmids expressing S10-tagged derivatives of yeast recombination proteins were also constructed, including Rpa1 and Rad51. S10-Rad51 and S10-Rad54 proteins were shown to complement the x-ray sensitivity of yeast strains lacking the wild-type proteins, although at high doses, cells expressing S10-Rad54 protein were somewhat more sensitive than wild-type yeast (Fig. 3).

Lysates from yeast expressing S10-tagged Rpa1, Rad51, and Rad54 proteins were mixed to provide approximately equal amounts of the three proteins and the mixture was passed over the Rad51 protein column as well as a BSA control column. After extensive rinsing, the columns were eluted with a buffer containing 500 mM sodium chloride. Finally, the column matrix was then boiled in SDS-containing buffer to release bound proteins not eluted by the high salt wash. Each fraction was concentrated then electrophoresed through a denaturing polyacrylamide gel. The fate of each S10-tagged protein was assessed by Western blotting.

As shown in Fig. 5, significant quantities of the S10-tagged Rad54 protein bound to the Rad51 column, but not the BSA control column. After extensive rinsing, the columns were eluted with a buffer containing 500 mM sodium chloride. Finally, the column matrix was then boiled in SDS-containing buffer to release bound proteins not eluted by the high salt wash. Each fraction was concentrated then electrophoresed through a denaturing polyacrylamide gel. The fate of each S10-tagged protein was assessed by Western blotting.

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3 H. Jiang and T. Kodadek, unpublished results.
However, Rad54 protein is not required when the DNA is part of a relatively inaccessible chromatin structure (34). In mitotic recombination, it has shown that the Rad54 protein is required for an early step in histone-occluded substrate. Indeed, Haber and colleagues (34) have raised the exciting possibility that Rad54 may be involved in protein-DNA complexes in an ATP-dependent fashion. This binding protein-associated factor that disrupts TATA-binding (32, 33). MOT1 protein has been shown to be a TATA-binding (32, 33). MOT1 protein has been shown to be a TATA-binding (32, 33). MOT1 protein has been shown to be a TATA-binding (32, 33). MOT1 protein has been shown to be a TATA-binding (32, 33).

In vivo, Rad54 protein is also an integral part of this putative genetic recombination. Several genetic and biochemical techniques, including the two-hybrid system, protein affinity chromatography, and Far-Western blotting, have been employed to demonstrate a direct interaction between the yeast Rad51 and Rad54 recombination proteins. There is growing evidence that histone recombination in yeast is catalyzed by a multiprotein complex containing several RAD gene products, including Rad51, Rad52, Rad55, Rad57, and probably many other factors as well. Given the results reported here, and the fact that the RAD51 and RAD54 genes have been shown to be epistatic (24), it seems very likely that the Rad54 protein is also an integral part of this putative “recombinosome.”

The function of the Rad54 protein in recombination is unknown and the protein yet to be purified and characterized biochemically. It is homologous to a number of ATPases and appears to be a member of a family that includes Swi2/Snf2 (25–28) and MOT1 (29–31). ATPases involved in transcriptional regulation. The Swi-Snf complex is thought to remodel chromatin structure and may influence transcription factor binding (32, 33). MOT1 protein has been shown to be a TATA-binding protein-associated factor that disrupts TATA-binding protein-DNA complexes in an ATP-dependent fashion. This raises the exciting possibility that Rad54 may be involved in allowing the recombination machinery to access the DNA in a histone-occluded substrate. Indeed, Haber and colleagues (34) have shown that the Rad54 protein is required for an early step in mitotic recombination in vivo when one of the DNA partners is part of a relatively inaccessible chromatin structure (34). However, Rad54 protein is not required when the DNA is carried on a plasmid and is therefore part of a more accessible chromatin structure.

Alternatively, it has been suggested that Rad54 protein might be a DNA helicase. Given the central roles of DNA helicases in E. coli (35–41) and bacteriophage T4 (42–45) strand exchange, a DNA helicase is anticipated to be involved in yeast recombination as well. Obviously, elucidation of Rad54 protein’s function will have to await its purification and biochemical characterization.

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REFERENCES

1. Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J., and Stahl, F. W. (1983) Cell 33, 25–35
2. Fishman, L. J., Rubin, N., and Haber, J. E. (1992) Mol. Cell. Biol. 12, 1292–1303
3. Rubin, N. and Haber, J. E. (1988) Mol. Cell. Biol. 8, 3918–3928
4. Shinozawa, A., and Ogawa, T. (1995) Trends Biochem. Sci. 20, 387–391
5. Sung, P. (1994) Science 265, 1241–1243
6. Milne, G. T., and Weaver, D. T. (1993) Genes Dev. 7, 1755–1765
7. Shinozawa, A., Ogawa, H., and Ogawa, T. (1992) Cell 69, 477–477
8. Firmanen, A., Elias-Armann, M., and Berg, P. (1995) Mol. Cell. Biol. 15, 1629–1631
9. Smith, J., and Rothstein, R. (1995) Mol. Cell. Biol. 15, 1632–1641
10. Hays, S. L., Firmanen, A. A., and Berg, P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6925–6929
11. Johnson, R. D., and Symington, L. S. (1995) Mol. Cell. Biol. 15, 4843–4850
12. Moore, P. D., Simon, J. R., Wallace, L. J., and Chow, T. Y. (1993) Curr. Genet. 23, 1–8
13. Emery, H. S., Schild, D., Kellogg, D. E., and Mortimer, R. K. (1991) Gene (Amst.) 104, 103–106
14. Durrfure, T., Becherer, K., Chen, P.-L., Yeh, S.-H., Yang, Y., Kilbuen, A. E., Lee, W.-H., and Elledge, S. J. (1993) Genes Dev. 7, 555–569
15. Alani, E., Subbiah, S., and Kleckner, N. (1989) Genetics 122, 47–57
16. Guarente, L. (1983) Methods Enzymol. 101, 181–191
17. Parks, T. D., Leiber, K. K., Howard, E. D., Johnston, S. A., and Dougherty, W. G. (1994) Anal. Biochem. 218, 413–417
18. Edelhoch, H. (1967) Biochemistry 6, 1948–1954
19. Horihachi, J., Silverman, N., Marcus, G., and Guarente, L. (1995) Mol. Cell. Biol. 15, 1203–1209
20. Melcher, K., and Johnston, S. (1995) Mol. Cell. Biol. 15, 2839–2848
21. Fields, S., and Song, O.-K. (1989) Nature 340, 245–246
22. Heyer, W. D., Rao, M. R., Erdile, L. F., Kelly, T. J., and Kolodner, R. D. (1990) EMBO J. 9, 2321–2329
23. Ogawa, T., Shinozawa, A., Nabetani, A., Ikeya, T., Y., Yang, E. H., and Ogawa, H. (1995) Cold Spring Harbor Symp. Quant. Biol. 58, 567–576
24. Battay, A. J., and Symington, L. S. (1995) Genetics 139, 45–56
25. Peterson, C. L., and Herskovitz, I. (1992) Cell 68, 573–583
26. Peterson, C. L., Dingwall, A., and Scott, M. P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2905–2908
27. Laurent, B. C., and Carlson, M. (1992) Genes Dev. 6, 1707–1715
28. Laurent, B., Yang, X., and Carlson, M. (1992) Mol. Cell. Biol. 12, 1839–1902
29. Auble, D. T., Hansen, K. E., Mueller, G. C. F., Lane, W. S., Thorner, J., and Habib, S. (1994) Genes Dev. 8, 1920–1934
30. Davis, J. L., Kunisawa, R., and Thorner, J. (1992) Mol. Cell. Biol. 12, 1879–1892
31. Pom, D., Campbell, A. M., Bai, Y., and Weil, P. A. (1994) J. Biol. Chem. 269, 23135–23140
32. Imbalzano, A. N., Kwon, H., Green, M. R., and Kingston, R. E. (1994) Nature 370, 481–485
33. Kwon, H., Imbalzano, A. N., Khaviri, A., Kingston, R. E., and Green, M. R. (1994) Nature 370, 477–481
34. Sugawara, N., Ivanov, E. L., Fisher-Lobell, Ray, B. L., Wu, X., and Haber, J. E. (1995) Nature 374, 84–88
35. Iwasaki, H., Takahagi, M., Nakata, A., and Shinagawa, H. (1995) Genes Dev. 6, 2214–2220
36. Lloyd, R. G., and Sharples, G. J. (1993) Nucleic Acids Res. 21, 1719–1725
37. Muller, B., Tseneva, I. R., and West, S. C. (1993) J. Biol. Chem. 268, 17185–17189
38. Parsons, C. A., and West, S. C. (1993) J. Biol. Chem. 268, 573–583
39. Tseneva, I. R., Muller, B., and West, S. C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1315–1319
40. Tseneva, I. R., Muller, B., and West, S. C. (1992) Cell 69, 1171–1180
41. Morel, P., Hejna, J. A., Ehrlich, S. D., and Cassuto, E. (1993) Nucleic Acids Res. 21, 3205–3209
42. Kodadek, T., and Alberts, B. M. (1987) Nature 332, 312–314
43. Kodadek, T. (1991) J. Biol. Chem. 266, 9712–9718
44. Salinas, F., and Kodadek, T. (1994) Biochem. Biophys. Res. Commun. 205, 1004–1009
45. Salinas, F., and Kodadek, T. (1995) Cell 82, 111–119