DNA-Dependent Adenosinetriphosphatase A Is the Eukaryotic Analogue of the Bacteriophage T4 Gene 44 Protein: Immunological Identity of DNA Replication-Associated ATPases†

Larry D. Mesner,‡ William M. Sutherland,§ and Joel W. Hockensmith*,‡

Departments of Biochemistry and Anatomy and Cell Biology and Molecular Biology Institute, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908

Received September 5, 1991; Revised Manuscript Received October 9, 1991

ABSTRACT: We report the construction of three stable murine hybridomas that secrete monoclonal antibodies which recognize calf thymus DNA-dependent adenosinetriphosphatase A. All three of the antibodies react specifically with calf thymus ATPase A and the gene 44 protein from the bacteriophage T4 DNA-dependent ATPase. Each of the three anti-ATPase A antibodies appears to recognize a different epitope and none of the antibodies inhibit DNA-dependent ATP hydrolysis by ATPase A. Furthermore, one of the antibodies has been shown to react with two different preparations of HeLa cell DNA-dependent ATPases and a yeast DNA-dependent ATPase, all of which have been implicated in the enzymology of DNA replication. These findings provide strong evidence for the role of ATPase A in DNA replication. These observations lead us to conclude that, apart from the nucleotide binding sites, there are at least three epitopes common to both the bacteriophage and eukaryotic DNA-dependent ATPases that we have examined and that the different preparations of the eukaryotic ATPases contain the same DNA-dependent ATPase.

Monoclonal antibodies have been employed for exhaustive studies of F_1 ATPase structure–function relationships, providing much insight into the protein–protein interactions that occur in complex, multiple protein adenosinetriphosphatase enzymatic systems (Aggeler et al., 1990). Such studies of DNA-independent ATPases have made significant progress toward an understanding of the regulation of ATP hydrolysis in these systems. Like the DNA-independent ATPases, DNA-dependent ATPases may play a role in multiprotein complexes but our understanding of DNA-dependent ATPases remains relatively limited. Moreover, the progress of DNA-dependent ATPase studies has differed vastly in prokaryotic and eukaryotic systems. The role of many prokaryotic DNA-dependent ATPases has been established for both DNA replication and recombination using a variety of genetic and enzymatic approaches (Kornberg, 1980, 1982). However, enzymes catalyzing DNA-dependent ATP hydrolysis in eukaryotes have been more unyielding with respect to identification, purification, genetics, and role in nucleotide metabolism. While a few eukaryotic DNA-dependent ATPases have been highly purified based on their ability to hydrolyze ATP, some of these lack assignment of a definitive role in nucleic acid metabolism (Hockensmith et al., 1986; Thomas et al., 1988). The reports of highly purified eukaryotic DNA-dependent ATPases with defined roles include enzymes such as DNA helicases and polymerase-stimulatory factors (Poll & Benbow, 1988; Seki et al., 1987; Thommes & Hubscher, 1990b; Vishwanatha & Baril, 1990; Harosh et al., 1989). Other eukaryotic DNA-dependent ATPases, such as those in the CF IIB, Activator 1, and RF-C complexes, have had a role specified but the ATPase polypeptide has not been characterized in the absence of the other proteins composing the complex (Weinberg et al., 1990; Lee et al., 1991; Tsurimoto & Stillman, 1991). With the exception of the yeast RAD3 protein and viral gene products such as the T-antigen from simian virus 40 or the helicase–primase from herpesvirus, genetic studies have been essentially nonexistent for these enzymes (Sung et al., 1987; Crute et al., 1989; Tegtmeyer, 1972).

Recognizing the importance of understanding DNA-dependent ATP hydrolysis and its role in DNA replication, we have developed a number of monoclonal antibodies against calf thymus DNA-dependent ATPase A. These antibodies demonstrate that ATPase A has antigenic determinants that are common to the bacteriophage T4 gene 44 protein, which is a DNA-dependent ATPase involved in DNA replication (Nossal & Peterlin, 1979; Sinha et al., 1980). We also establish that HeLa cell and yeast protein fractions that demonstrate ATP-dependent stimulation of DNA replication contain a similar, if not the same, DNA-dependent ATPase. Consequently, we conclude that these data support the supposition that ATPase A plays a role in DNA replication.

Materials and Methods

All the reagents used were analytical grade. Modifications of a previously published protocol (Hockensmith et al., 1986) for the isolation of calf thymus ATPase A are shown in Table I. Bacteriophage T4 gene 44/62 and 45 proteins were a gift from Dr. Peter von Hippel (Jarvis et al., 1989a). The HeLa cell fractions known as Activator 1 and the glycerol gradient fractions of Activator 1 were kindly prepared and assayed for replicative stimulatory activity by Dr. Jerard Hurwitz and his colleague Dr. Ann Kwong (Lee et al., 1991). A HeLa cell DNA polymerase α replication complex containing DNA-dependent ATPase activity was a gift from Dr. Earl Baril (Malkas et al., 1990). *Saccharomyces cerevisiae* RF-C complex was a gift from Dr. Peter Burgers (Yoder & Burgers, 1991). Anti-DNA-dependent ATPase A monoclonal antibo-

† This research was supported in part by U.S. Public Health Service (USPHS) Research Grant GM-43569 (J.W.H.), by American Cancer Society Institutional Research Grant IN 149B (J.W.H.), and by March of Dimes Basil O'Connor Starter Scholar Research Award 5-619 (J. W.H.). L.D.M. was a predoctoral trainee supported by USPHS Institutional Research Service Award GM-08136.

‡ Department of Biochemistry and Molecular Biology Institute.
§ Department of Anatomy and Cell Biology.

0006-2960/91/0430-11490$02.50/0

Table I: Purification of Multiple Forms of Calf Thymus DNA-Dependent ATPase A

fraction	protein (mg)		act. (units)		sp. act. (units/mg)		yield (%)		purification (x-fold)	
	68 kDa	83 kDa	68 kDa	83 kDa	68 kDa	83 kDa	63 kDa	83 kDa	68 kDa	83 kDa
I crude	546000[a]		(182)[b]		(0.0003)		(100)		1	
II PEG_{8000} pptn	19500		(182)		(0.0093)		(100)		31	
III denatured DNA–cellulose[c]	665		182		0.274		100		913	
IV phosphocellulose[d]	48.1		64.0		1.33		35		4430	
V denatured DNA–cellulose[e]	8.10	6.81	16.5	43.2	2.04	6.34	9.1	24	6800	21100
VI ATP–agarose	0.42	1.15	5.70	17.9	13.6	15.6	3.1	9.8	45300	52000
VII hydroxylapatite[f]	0.08	0.26	3.20	6.4	42.6	24.8	1.8	3.5	142000	82700

[a] The purification shown was begun with 9100 g of fetal calf thymus glands (Hockensmith et al., 1986). [b] Fraction III is the first fraction with which it is possible to identify DNA-dependent ATPase activity. The values in parentheses have been estimated by assuming 100% recovery of the activity during the initial steps of this purification. [c] One-quarter of fraction II was loaded and washed with 150 mM NaCl in buffer A and the column (8.2 × 32 cm) was eluted with 650 mM NaCl. [d] The four fraction IIIs were combined and loaded onto a 4.6 × 19 cm column. [e] Fraction IV was loaded, and the column (2.5 × 42 cm) was washed with 90 mM K_2SO_4 in buffer S and eluted with a 1.2-L linear gradient from 90 to 300 mM K_2SO_4 in buffer S. The 68- and 83-kDa polypeptides elute at 125 and 240 mM K_2SO_4, respectively. [f] Fraction VI was loaded onto a hydroxylapatite column (1.5 × 4 cm), which was washed with buffer S and eluted with a 150-mL linear gradient of 0–530 mM $(NH_4)_2SO_4$. Both the 68- and 83-kDa species elute at 270 mM $(NH_4)_2SO_4$.

dies were developed and characterized as IgG_1s (Chapman et al., 1984). Monoclonal antibodies used for analytical purposes were isolated from ascites fluid (Reik et al., 1987) and purified to homogeneity on protein G–Sepharose CL4B (Pharmacia-LKB) according to the manufacturer's protocol.

Gel electrophoresis in the presence of SDS[1] and subsequent silver staining of proteins was performed according to published procedures (Dreyfuss et al., 1984; Morrissey, 1981). Biotrace NT (Gelman, 0.45 μm pore size) nitrocellulose membranes were used in dot blot and electrophoretic transfer procedures. Western blotting was performed by a published procedure (Towbin et al., 1979) and probed with 5 μg/mL monoclonal antibody. Antigen–antibody complexes were detected with horseradish peroxidase conjugated F_c-specific goat anti-mouse IgG (Jackson ImmunoResearch Laboratories) and the colorimetric substrate diaminobenzidine (tetrahydrochloride salt).

Immunoprecipitations were performed with antibodies coupled to Affi-Gel HZ (Bio-Rad) according to the manufacturer's protocol. The antibody–bead complex was incubated with a fraction containing DNA-dependent ATPase activity for 2 h at 4 °C, precipitated by centrifugation, rinsed with buffer, and subsequently assayed for ATPase activity. ATPase assays have been previously described (Hockensmith et al., 1986).

Protein concentration was determined using a published procedure with bovine serum albumin as a standard (Bradford, 1976).

Results

We have previously reported the purification of calf thymus DNA-dependent ATPase A, which prefers a primer–template junction DNA effector for ATP hydrolysis (Hockensmith et al., 1986). We have slightly modified the original purification protocol to yield two different DNA-dependent ATPase polypeptides with estimated molecular masses of 68 and 83 kDa (Table I). Apart from the molecular weight and the immunogenic disparities described below, the two species of ATPase A are functionally indistinguishable with respect to γ-phosphohydrolysis and DNA effector preference. These preparations of DNA-dependent ATPases were used to develop monoclonal antibodies (Chapman et al., 1984). Three monoclonal antibodies (4B10, 6E12, and 2D1) were identified by their response to antigens in an ELISA where the protein is presumed to be in the native state. Each antibody reacted similarly to the 83-kDa DNA-dependent ATPase A (fraction VII). However, each of the antibodies appears to recognize a unique epitope, which allows immunogenic definition of the similarities and differences between the two ATPase A polypeptides.

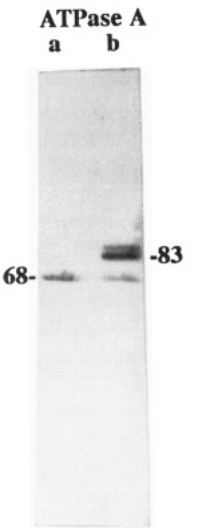

FIGURE 1: Western blot analysis of two preparations of DNA-dependent ATPase A. The 68-kDa fraction VI (a) and the 83-kDa fraction VII (b) were loaded onto an SDS–9% polyacrylamide gel (80 ng). Following electrophoresis and electrotransfer to Biotrace NT, the immobilized protein was probed with monoclonal antibody 4B10.

In an ELISA the 4B10 and 2D1 antibodies reacted with the 68-kDa fraction VII (>1.8 A_{414nm} units), but the 6E12 antibody did not yield any detectable response. Therefore, one of the antigenic determinants must be removed or altered by the removal of part of the 83-kDa polypeptide, resulting in loss of 6E12 binding. While all three of the antibodies reacted with at least one form of the native ATPase polypeptide, Figure 1 shows that the 4B10 antibody also reacted with the denatured polypeptides of both the 68- and 83-kDa species of the ATPase. Figure 1 also shows that the increased sensitivity afforded by the 4B10 antibody allowed detection of both the 68-kDa species and an 85-kDa species, which are present in some 83-kDa preparations. Furthermore, immunoprecipitations of fraction III revealed not only the 68- and 83-kDa polypeptides but also 100-, 105-, 150-, and 160-kDa polypeptides.[2] Thus, the results are suggestive of proteolytic

[1] Abbreviations: ELISA, enzyme-linked immunosorbent assay; SDS, sodium dodecyl sulfate.

[2] L. D. Mesner and J. W. Hockensmith, manuscript in preparation.

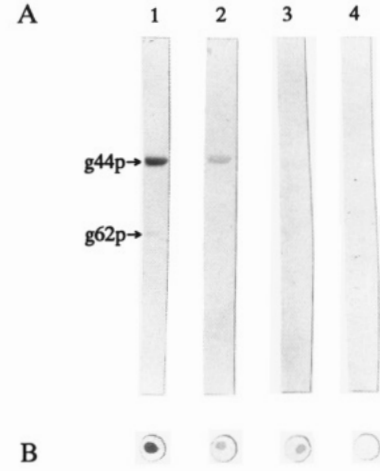

FIGURE 2: Western blot (A) and dot blot (B) analysis of the bacteriophage T4 gene 44 and 62 proteins. SDS–polyacrylamide gels (15%) were loaded with 0.58 μg (lane 1), 1.16 μg (lane 2), or 2.9 μg (lanes 3 and 4) of the gene 44/62 protein complex. Each dot blot (B) had 0.9 μg of gene 44/62 protein complex loaded. Immobilized protein was probed with monoclonal antibody 4B10 (lane 1), 2D1 (lane 2), 6E12 (lane 3), or no antibody (lane 4).

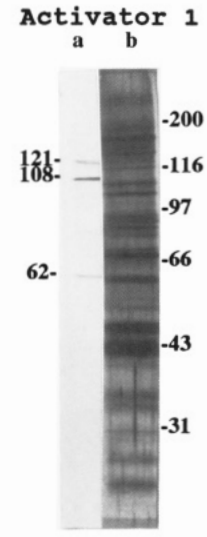

FIGURE 3: Western blot (a) and silver-stained gel (b) analysis of HeLa cell Activator 1 complex. SDS–polyacrylamide gels (10%) were loaded with 2.3 μg of Activator 1 fraction. The immobilized protein was probed with monoclonal antibody 4B10.

degradation. Similarly to the 4B10 antibody, the 2D1 antibody binds to both the 68- and 83-kDa denatured species but the reactivity is considerably weaker than for 4B10. The antibody 6E12 also reacted rather weakly with the denatured enzyme but recognized only the 83-kDa peptide and not the 68-kDa polypeptide in a manner analogous to that for the native polypeptides. These data support the conclusion that the three antibodies recognize at least two different epitopes available on the 83-kDa species.

Neither the 6E12 nor the 2D1 antibody reacted well with denatured ATPase A, suggesting that secondary and tertiary structure is important for recognition of these epitopes in the ATPase A polypeptide. Alternatively, the 4B10 antibody reacts very strongly with both the native and denatured ATPase A.[3] We have previously reported that ATPase A is a highly stable enzyme; however, we find that higher molecular weight species of ATPase A are relatively unstable and can be inactivated by relatively brief (overnight) storage at 4 °C (Hockensmith et al., 1986).[2] The 4B10 antibody continues to react with the ATPase A peptides even after they have been inactivated, while the other two antibodies fail to recognize them. In addition, the 4B10 antibody reacted with the expressed β-galactosidase fusion product of a partial clone from a fetal calf thymus λgt11 cDNA library, while neither of the other antibodies is useful for detection of that clone. Consequently, we conclude that the 4B10 antibody is recognizing epitopes different than those recognized by either 6E12 or 2D1 and suggest that the 4B10 epitope is directly related to a primary amino acid sequence.

While each of these three antibodies recognizes different epitopes, it should be noted that each antibody can be used to immunoprecipitate ATPase A in a manner that yields an antibody–enzyme complex that is competent for DNA-dependent ATP hydrolysis. Consequently, the three epitopes recognized by these antibodies must be available in the active conformation of the enzyme and none of the epitopes are likely to coincide with either the DNA-binding domain or the ATP-binding domain.

[3] The relative degree of responsiveness for the 4B10, 6E12, and 2D1 antibodies is identical for both the calf thymus ATPase A and the bacteriophage T4 gene 44 protein. Consequently, Figure 2 is appropriate for comparison of the individual antibody responses.

Our working hypothesis has been that ATPase A is the eukaryotic analogue of the bacteriophage T4 gene 44/62 proteins. During T4 DNA replication, the gene 44/62 proteins interact with three other proteins including a stimulatory protein (gene 45 protein), a DNA polymerase (gene 43 protein) and a single-stranded DNA binding protein (gene 32 protein) (Nossal & Peterlin, 1979; Sinha et al., 1980). Western blot and dot blot analysis of homogeneous preparations of the gene 44/62 proteins revealed that the gene 44 protein reacted with our antibodies (Figure 2) but that the gene 62 protein does not contain antigenic determinants recognized by our antibodies under these conditions. The gene 44 protein reacted with all three of the antibodies, suggesting that all three of the epitopes or structural features recognized in ATPase A are also present in the gene 44 protein. Additionally, we have previously established that the DNA primer–template effector specificity for both the gene 44 protein and ATPase A are virtually identical (Hockensmith et al., 1986; Jarvis et al., 1989b). We thus suggest that it is highly probable that ATPase A is also similar to the gene 44 protein in its DNA replication properties. We believe that this is the first direct physical evidence of a relationship between a prokaryotic and eukaryotic DNA-dependent ATPase and that this supports our earlier hypothesis (Hockensmith et al., 1986).

Recognizing that there are a number of reported eukaryotic DNA-dependent ATPases with a number of different functions, we have specifically sought preparations of these enzymes that might be related to ATPase A. An SDS–polyacrylamide gel and Western blot of a fraction from HeLa cells known as Activator 1 is shown in Figure 3 (pre-glycerol gradient fraction) (Lee et al., 1991). The Activator 1 fraction stimulates DNA replication and has been suggested to be analogous to the bacteriophage T4 gene 44/62 proteins (Weinberg et al., 1990; Lee et al., 1991; Tsurimoto & Stillman, 1991). The Activator 1 fraction has been suggested to comprise 145-, 40-, 38-, 37-, and 36.5-kDa proteins based on purification by glycerol gradient sedimentation. Figure 3 demonstrates that 4B10 recognized three polypeptides (121, 108, and 62 kDa) other than those previously detected by silver staining of this preparation. The lack of correspondence between the reported polypeptides and those observed by Western blotting is further substantiated by our finding that highly purified preparations of ATPase A have a specific activity for ATP hydrolysis ap-

proximately 3 orders of magnitude higher than Activator 1 and RF-C preparations (Lee et al., 1991; Tsurimoto & Stillman, 1990). The differences in the specific activity lead us to suggest that the polypeptide responsible for DNA-dependent ATP hydrolysis in the Activator 1 preparations is less than one-thousandth of the total protein and hence is not visible by means of silver staining. Furthermore, the presence of the high molecular weight immunogenic species (121 kDa) coincides with the peak of ATPase and ATP-dependent DNA replicative stimulatory activity as revealed by Western blots of fractions from a glycerol gradient of Activator 1 (Lee et al., 1991). These immunogenic reactions lead us to suggest that the peptides responsible for DNA-dependent ATP hydrolysis in the Activator 1 fraction are a minor component not detected on the silver-stained SDS–polyacrylamide gels.

Activator 1 complex is reputed to be equivalent to a complex called RF-C (Thommes & Hubscher, 1990). Recently, RF-C has been purified from yeast and has been shown to contain six proteins of 130, 86, 41, 40, 37, and 27 kDa (Yoder & Burgers, 1991). Monoclonal antibody 4B10 reacted with a polypeptide that corresponds to the highest molecular weight species in this yeast complex, providing evidence that activator 1, RF-C, and DNA-dependent ATPase A contain at least one similar antigen. Our data lead us to conclude that four disparate systems, bacteriophage T4, bovine calf thymus, HeLa, and yeast, all contain antigenically related proteins, which have been shown in the T4 and calf thymus systems to be DNA-dependent ATPases that recognize primer–template junctions as effectors for ATP hydrolysis. Hence, we infer that both the Activator 1 complex and RF-C complex contain DNA-dependent ATPase A.

We have also obtained a 21S enzyme complex from HeLa cells that stimulates DNA polymerase α-primase activity and contains an associated DNA-dependent ATPase activity (Malkas et al., 1990). Western blot analysis of this fraction with the 4B10 antibody identified two distinct species, 168 and 143 kDa, of protein as having a common epitope. Both species were found during all the steps of purification from the postmicrosomal supernatant through chromatography on Q-Sepharose.

Discussion

We first proposed that a calf thymus DNA-dependent ATPase was the eukaryotic analogue of the bacteriophage T4 gene 44/62 proteins on the basis of functional hydrolysis of ATP in a DNA-dependent manner (Hockensmith et al., 1986). Unlike any other class of DNA-dependent ATPases, both calf thymus DNA-dependent ATPase A and the T4 gene 44/62 and 45 proteins exhibit preferential use of primer–template junctions as effectors for ATP hydrolysis (Hockensmith et al., 1986; Jarvis et al., 1989b). On the basis of this observation and the gene 44/62 proteins' role in the elongation phase of DNA synthesis (Nossal & Peterlin, 1979; Sinha et al., 1980), we suggested that DNA-dependent ATPase A could be involved in DNA synthesis (Hockensmith et al., 1986). Further purification of ATPase A has allowed us to develop a series of monoclonal antibodies against this enzyme. We have used these antibodies to explore the possibility that ATPase A is a homologue of the gene 44 protein, which is the ATP-hydrolyzing subunit of the gene 44/62 protein complex (Rush et al., 1989). Western and dot blot analyses using these antibodies demonstrate that there are distinct epitopes common to both the bacteriophage T4 gene 44 protein and the calf thymus ATPase A. Considering that both the gene 44 protein and ATPase A catalyze DNA-dependent ATP hydrolysis (Hockensmith et al., 1986; Rush et al., 1989) and consequently must both have ATP- and DNA-binding sites which coordinate specific discrimination among DNA effectors and considering that these two polypeptides also have at least three common epitopes, we suggest that the gross homology of the two enzymes must be very high.

Recent reports have also identified eukaryotic DNA-dependent ATPases that have a functional similarity to the bacteriophage T4 gene 44/62 proteins, but structural similarities have not been reported (Weinberg et al., 1990; Lee et al., 1991; Tsurimoto & Stillman, 1990, 1991). These ATPases are reported to stimulate various types of DNA replication in a manner analogous to that of the gene 44/62 proteins. We have shown that these enzyme fractions contain polypeptides that are antigenically related to ATPase A and to each other, thereby confirming speculation that these enzymes are similar, if not the very same enzyme (Weinberg et al., 1990; Lee et al., 1991; Tsurimoto & Stillman, 1990, 1991). From a quantitative point of view, ATPase A and the other eukaryotic DNA-dependent ATPases exhibit a large disparity in specific activities for DNA-dependent ATP hydrolysis. Coupling this observation with the Western blot and silver-stained gel analyses leads us to suggest that the ATPase is a minor protein component of these stimulatory fractions. Furthermore, our Western blot analyses identify polypeptides of differing molecular weights from each of these preparations. This observation coupled with our purification of the two ATPase species, 68 and 83 kDa, leads us to postulate that these multiple species may be the result of proteolysis similar to that reported previously in eukaryotic DNA replication proteins (Sauer & Lehman, 1982). Thus, differences in the preparations of RF-C and Activator 1 may be directly related to the presence of differing molecular weight species of DNA-dependent ATPase. Furthermore, our antibodies do not react with any polypeptides in the size range of the 40-kDa polypeptide species from RF-C that has been identified as cross-linking to ATP (Weinberg et al., 1990; Tsurimoto & Stillman, 1991). Consequently, we suggest that either the 40-kDa species is a protein such as phosphoglycerate kinase (Jindal & Vishwanatha, 1990) and is unrelated to ATPase A, or the 40-kDa species is a proteolytic degradation product which has lost the appropriate epitopes for recognition by our antibodies. Regardless of how the different polypeptides are generated, the three anti-ATPase A monoclonal antibodies demonstrate multiple interrelated peptides in each of the eukaryotic DNA-dependent ATPase preparations.

Although we have demonstrated that ATPase A is a component of the Activator 1 and RF-C complexes, we have neither established the role of this enzyme in DNA replication nor excluded the possibility that other proteins in the complex may play an important role in the replication stimulatory activity. We are currently pursuing additional studies which define an improved purification of this enzyme along with establishment of its functional activities and characteristics.

Acknowledgments

We thank W. Dennis Rinehart for technical support during these endeavors. We are particularly grateful for the generosity of Drs. Baril, Burgers, Hurwitz, and von Hippel and their colleagues.

References

Aggeler, R., Mendel-Hartvig, J., & Capaldi, R. A. (1990) *Biochemistry 29*, 10387–10395.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Chapman, M. D., Sutherland, W. M., & Platts-Mills, T. A. E. (1984) *J. Immunol. 133*, 2488–2495.

Crute, J. J., Tsurumi, T., Zhu, L., Weller, S. K., Olivo, P. D., Challberg, M. D., Mocarski, E. S., & Lehman, I. R. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 2186–2189.

Dreyfuss, G., Adam, S. A., & Cho, Y. D. (1984) *Mol. Cell. Biol. 4*, 415–423.

Harosh, I., Naumovski, L., & Friedberg, E. C. (1989) *J. Biol. Chem. 264*, 20532–20539.

Hockensmith, J. W., Wahl, A. F., Kowalski, S., & Bambara, R. A. (1986) *Biochemistry 25*, 7812–7821.

Jarvis, T. C., Paul, L. S., & von Hippel, P. H. (1989a) *J. Biol. Chem. 264*, 12709–12716.

Jarvis, T. C., Paul, L. S., Hockensmith, J. W., & von Hippel, P. H. (1989b) *J. Biol. Chem. 264*, 12717–12729.

Jindal, H. K., & Vishwanatha, J. K. (1990) *J. Biol. Chem. 265*, 6540–6543.

Kornberg, A. (1980) *DNA Replication*, W. H. Freeman, San Francisco, CA.

Kornberg, A. (1982) Supplement to *DNA Replication*, W. H. Freeman, San Francisco, CA.

Lee, S.-H., Kwong, A. D., Pan, Z.-Q., & Hurwitz, J. (1991) *J. Biol. Chem. 266*, 594–602.

Malkas, L. H., Hickey, R. J., Li, C., Pedersen, N., & Baril, E. F. (1990) *Biochemistry 29*, 6362–6374.

Morrissey, J. H. (1981) *Anal. Biochem. 117*, 307–310.

Nossal, N. G., & Peterlin, B. M. (1979) *J. Biol. Chem. 254*, 6032–6037.

Poll, E. H., & Benbow, R. M. (1988) *Biochemistry 27*, 8701–8706.

Reik, L. M., Maines, S. L., Ryan, D. E., Levin, W., Bandiera, S., & Thompson, P. E. (1987) *J. Immunol. Methods 100*, 123–130.

Rush, J., Lin, T.-C., Quinones, M., Spicer, E. K., Douglas, I., Williams, K. R., & Konigsberg, W. H. (1989) *J. Biol. Chem. 264*, 10943–10953.

Sauer, B., & Lehman, I. R. (1982) *J. Biol. Chem. 257*, 12394–12398.

Seki, M., Enomoto, T., Hanaoka, F., & Yamada, M. (1987) *Biochemistry 26*, 2924–2928.

Sinha, N. K., Morris, C. F., & Alberts, B. M. (1980) *J. Biol. Chem. 255*, 4290–4303.

Sung, P., Prakash, L., Weber, S., & Prakash, S. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6045–6049.

Tegtmeyer, P. (1972) *J. Virol. 10*, 591–598.

Thomas, D. C., Rein, D. C., & Meyer, R. R. (1988) *Nucleic Acids Res. 16*, 6447–6464.

Thommes, P., & Hubscher, U. (1990a) *Eur. J. Biochem. 194*, 699–712.

Thommes, P., & Hubscher, U. (1990b) *J. Biol. Chem. 265*, 14347–14354.

Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.

Tsurimoto, T., & Stillman, B. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 1023–1027.

Tsurimoto, T., & Stillman, B. (1991) *J. Biol. Chem. 266*, 1950–1960.

Vishwanatha, J. K., & Baril, E. F. (1990) *Biochemistry 29*, 8753–8759.

Weinberg, D. H., Collins, K. L., Simancek, P., Russo, A., Wold, M. S., Virshup, D. M., & Kelly, T. J. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 8692–8696.

Yoder, B. L., & Burgers, P. M. J. (1991) *J. Biol. Chem.* (in press).