Ku autoantigen is the regulatory component of a template-associated protein kinase that phosphorylates RNA polymerase II

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ABSTRACT The carboxyl-terminal domain of RNA polymerase II contains a tandemly repeated heptapeptide sequence. Previous work has shown that this sequence is phosphorylated at multiple sites by a template-associated protein kinase, in a reaction that is closely associated with the initiation of RNA synthesis. We have purified this kinase to apparent homogeneity from human (HeLa) cells. The purified kinase phosphorylates native RNA polymerase II only in the presence of DNA and the general transcription factors TFIID (TBP), TFIIB, and TFIIF. Two kinase components are required for full activity: a catalytic component and a DNA-binding regulatory component. The regulatory component has been identified as Ku autoantigen, based on the molecular weights of its component polypeptides, its DNA-binding properties, and its reactivity with anti-Ku monoclonal antibodies. The Ku autoantigen recruits the catalytic component of the kinase to the template. Ku autoantigen has been previously proposed to interact with DNA by a characteristic bind-and-slide mechanism. This mode of interaction may provide a mechanism for targeting the kinase to the transcription complex and other DNA-bound substrates.

Eukaryotic messenger RNA biosynthesis requires RNA polymerase II (RNAP II). In the presence of transcription factors, RNAP II binds to promoter sites on the DNA template to form a transcription complex. It then initiates transcription, moving along the template and copying the nucleotide sequence into RNA. RNAP II is phosphorylated at about the same time as RNA synthesis begins (1–3), suggesting that phosphorylation may facilitate the transition from the promoter-bound to the elongating form of the enzyme.

The principal sites of RNAP II phosphorylation are in the carboxyl-terminal domain (CTD) of the largest subunit (4, 5). The CTD is composed of repeats of the heptapeptide (Ser-Pro-Thr-Ser-Pro-Ser-Tyr) (6, 7). Phosphorylation occurs cooperatively at multiple serine and threonine residues and induces a conformational change, as evidenced by altered immunoreactivity and sedimentation rate (8, 9). The CTD is essential in yeast, *Drosophila*, and cultured murine cells (10–13). Mutations in the CTD impair the response of RNAP II to certain transcriptional activator proteins (14–16).

Arias and Dynan (17) have developed a system based on an immobilized template that allows the isolation of preinitiation transcription complexes. A protein kinase present in these preparations phosphorylates endogenous, promoter-bound RNAP II (3). The kinase can be eluted from the complexes with high concentrations of KCl. The eluted kinase efficiently phosphorylates a model substrate containing the CTD joined to the DNA-binding domain of the yeast protein GAL4 (18). The reaction is dependent on DNA and on the presence of the DNA-binding domain in the substrate, suggesting that DNA

binding, in itself, may provide a signal for phosphorylation. Physically and enzymatically, the template-associated CTD kinase resembles a previously identified DNA-dependent protein kinase (DNA-PK) (19–21).

Although both partially purified DNA-PK preparations and the template-associated kinase contain a major 350-kDa polypeptide, this species and the kinase activity do not always comigrate during chromatography (21). This observation suggests that enzyme activity may require more than one component. We report here that the template-associated CTD kinase can be resolved into two essential components. One is the 350-kDa DNA-PK polypeptide, and the other is Ku autoantigen, a moderately abundant chromatin protein originally identified by reaction with antibodies from patients with systemic lupus erythematosus and other autoimmune diseases (22–24). Ku antigen has previously been shown to be a substrate for DNA-PK (21). Several lines of evidence have suggested that Ku protein might be involved in transcriptional regulation (25–27). Our present findings confirm that Ku affects the transcriptional apparatus and suggest that it does so by activating a protein kinase that phosphorylates transcriptionally poised RNAP II.

MATERIALS AND METHODS

Phosphorylation Assays. Reaction mixtures contained, in a final volume of 50 μ l, 25 mM Tris Cl (pH 7.9), 6.25 mM MgCl₂, 0.5 mM EDTA, 2.5% (vol/vol) glycerol, 0.5 mM dithiothreitol, 12.5 μ M [γ -³²P]ATP (NEN, specific activity 30–40 Ci/mmol; 1 Ci = 37 GBq), *EcoRI*-*Bgl* I AdUAS DNA fragment (18) at 20 ng/ml, GAL4-CTD (18) at 2 μ g/ml, and 1–4 μ l of CTD kinase fractions. Reaction mixtures were incubated at 30°C for 60 min. Radiolabeled products were analyzed by SDS/6% PAGE and visualized by autoradiography or Molecular Dynamics (Sunnyvale, CA) PhosphorImager analysis.

Protein Purification. A detailed description of the kinase purification will be published elsewhere (A.D., L. Y. Stein, B. Calore, and W.S.D., unpublished data). The kinase was purified from the HeLa cell nuclear extract (17) in a buffer containing 50 mM Tris Cl (pH 7.9); 1 mM EDTA; 5% glycerol; 0.02% Tween 20; 1 mM dithiothreitol; pepstatin A, leupeptin, and soybean trypsin inhibitor, each at 1 μ g/ml; and phenylmethylsulfonyl fluoride at 10 μ g/ml, to which KCl or (NH₄)₂SO₄ was added at the various chromatographic steps. The two components of the kinase cofractionated through the first three steps, which included sequential binding and elution with KCl from phosphocellulose (active peak at 0.3 M), DEAE-Sephacel (active peak at 0.2 M), and heparin-agarose (active peak at 0.3 M). The kinase was then

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Abbreviations: RNAP II, RNA polymerase II; CTD, carboxyl-terminal domain; DNA-PK, DNA-dependent protein kinase.

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loaded on a phenyl-Superose Pharmacia FPLC column. Kinase component B flowed through in 0.75 M $(\text{NH}_4)_2\text{SO}_4$ and was further purified by Superdex 200 gel filtration and Mono S (0.1–0.4 M KCl gradient) and, where indicated, Mono Q (0.1–0.4 M KCl gradient) ion-exchange chromatography. Kinase component A was eluted from phenyl-Superose at 0.25 M $(\text{NH}_4)_2\text{SO}_4$ and was further purified in the same way as component B.

Native RNAP II was purified from calf thymus by using 8WG16 monoclonal antibodies (28). General transcription factors TFIID (TBP), TFIIB, and TFIIF were expressed in *Escherichia coli* and purified as described (29–31). The general factors were a gift of the D. Reinberg lab. Ku protein was prepared by phosphocellulose and double-stranded DNA-Sepharose chromatography and anion-exchange HPLC (27).

Electrophoretic Mobility-Shift Assays. Binding reaction mixtures contained, in a final volume of 20 μl : 50 mM Tris Cl (pH 7.9), 50 mM KCl, 4 mM MgCl_2 , 0.5 mM EDTA, 5% glycerol, 0.5 mM dithiothreitol, 1.0–2.0 ng of ^{32}P -labeled 262-base-pair *EcoRI*–*Bgl*I fragment from pDNAdMLP [containing the adenovirus 2 major late promoter (32)], and kinase components as indicated in the figure legend. Reaction mixtures were incubated for 20 min at room temperature and analyzed by electrophoresis in a nondenaturing 4% polyacrylamide gel in Tris/borate/EDTA buffer. Protein–DNA complexes were visualized by autoradiography.

Antibodies, Immunoblotting, and Immunodepletion. Anti-Ku monoclonal antibodies N3H10 (IgG2b) and N9C1 (IgG1) are directed against the 68-kDa and 83-kDa subunits of Ku, respectively (27). Control monoclonal antibody 2F8 (IgG2b) is directed against *E. coli* RNAP σ subunit.

For immunoblotting, purified component B was fractionated by SDS/10% PAGE, transferred to nitrocellulose, and probed with ascites fluid diluted 1:700 in 3% nonfat dry milk/0.2% Tween 20. Immune complexes were visualized with an ECL Western blot detection kit (Amersham).

For immunodepletion, N3H10 antibody bound to protein A-Sepharose was incubated for 2 hr at room temperature with kinase (a fraction containing both components A and B, prepared by successive DEAE-Sepharose, heparin-agarose, and Superdex 200 chromatography of HeLa cell nuclear

extract). The beads were pelleted by centrifugation, and kinase activity remaining in the supernatant was assayed.

RESULTS

Purification of the Template-Associated CTD Kinase. We discovered, in the course of extensive fractionation of HeLa cell extracts, that the template-associated CTD kinase activity resolved into two essential components. Each was purified to apparent homogeneity based on its ability to complement the other for activity. Component A, which was purified by six sequential chromatographic steps, contained a single polypeptide of ≈ 350 kDa, as analyzed by SDS/PAGE (Fig. 1A), that precisely comigrated with activity in the final two chromatographic steps. Monoclonal antibody against a similar-size polypeptide inhibits the activity of DNA-PK (20), consistent with this polypeptide being a kinase component. Component A evidently contains the kinase active site. In agreement with previous observations with DNA-PK, we have found that it is capable of autophosphorylation, albeit at a low level (A.D., L. Y. Stein, B. Calore, and W.S.D., unpublished data).

Kinase component B was purified by seven sequential chromatographic steps. SDS/PAGE analysis showed only two polypeptides, 68 kDa and 83 kDa, present in apparent 1:1 stoichiometry (Fig. 1A). These precisely comigrated with activity in the final three chromatographic steps. Kinase activity required both components (Fig. 1B).

Phosphorylation of Native RNAP II. Because the template-associated CTD kinase was purified based on its ability to phosphorylate a GAL4-CTD model substrate, it was important to establish whether the purified enzyme was also able to phosphorylate the CTD of native RNAP II (Fig. 2). Efficient phosphorylation occurred in a reaction requiring seven purified macromolecular components: DNA, nonphosphorylated RNAP IIA, recombinant transcription factors TFIID (TBP), TFIIB, and TFIIF, and both components of the kinase. The majority of the radiolabeled large subunit of RNAP II was converted to the lower-mobility RNAP IIO form, indicative of phosphorylation at multiple sites (4, 9). The requirement for general transcription factors is of particular interest. TFIID, TFIIB, and TFIIF are known to be involved in recruiting RNAP II to DNA (33, 34). It is likely

FIG. 1. Purified template-associated kinase components A and B. (A) Analysis of purified kinase components by SDS/PAGE with silver staining. Proteins are as indicated above each lane: A, kinase component A (10 ng of Mono S activity peak fraction); Mr, molecular weight markers (sizes indicated in kilodaltons at left); B, kinase component B [20 ng of Mono S (MS) or 20 ng of Mono Q (MQ) activity peak fractions as indicated]. Arrows indicate the polypeptides associated with kinase activity. (B) GAL4-CTD phosphorylation. Reaction mixtures contained purified kinase component A or component B or both, as indicated. Arrow at right denotes multiply phosphorylated GAL4-CTD₀ reaction product.

FIG. 2. Phosphorylation of RNAP II by purified template-associated kinase. The complete reaction mixture (lane 5) contained purified kinase components A (0.5 ng) and B (1.0 ng), 100 ng of immunoaffinity-purified RNAP IIA, recombinant transcription factors TFIID (rIID, 70 ng), TFIIB (rIIB, 80 ng), and TFIIF (rIIF, 40 ng of RAP30 and 10 ng of RAP74), and a 262-base-pair DNA fragment from pAdUAS, containing the adenovirus major late promoter. Other reactions had individual components omitted as indicated. All reactions contained 12.5 μ M [γ - 32 P]ATP (30 Ci/mmol) in a final volume of 50 μ l. Arrows at right denote positions of fully phosphorylated RNAP II marker (lane M) and singly phosphorylated RNAP IIA marker (not shown). Below the autoradiogram, bars show relative amounts of radiolabeled RNAP II produced in each reaction, as determined by PhosphorImager analysis.

that these factors enhance the phosphorylation by positioning RNAP II on DNA, where it is more efficiently recognized as a substrate by the template-associated kinase.

Kinase Component B Recruits Component A to DNA. To determine whether either of the kinase components had DNA binding activity, we performed electrophoretic mobility-shift assays. The experiments used an adenovirus major late promoter-containing DNA fragment similar to that in Fig. 2. At low concentration, kinase component B generated a discrete protein-DNA complex (Fig. 3). Kinase component A did not bind DNA on its own but shifted the component B-DNA complex to a new position. At higher concentration, component B generated additional discrete protein-DNA complexes, perhaps containing two, three, or four molecules of Ku protein. Component A shifted these complexes to new positions. Therefore we conclude that component B binds DNA directly, and component A binds to DNA only in the presence of component B.

Kinase Component B Is Ku Autoantigen. In its polypeptide composition and DNA-binding properties, kinase component B resembles the Ku autoantigen (22-24). The cloned subunits of Ku have predicted molecular weights of 69,851 and 82,713 (25, 35, 36), in agreement with the observed molecular weights of the component B polypeptides. Previously, Ku has been shown to interact nonspecifically with DNA ends and, in a proposed bind-and-slide mechanism, produce a ladder of multimeric forms similar to that in Fig. 3 (37-41).

Kinase component B was immunoblotted and probed with a mixture of monoclonal antibodies against Ku protein. Both

FIG. 3. Electrophoretic mobility-shift assay. Binding reaction mixtures (see Materials and Methods) contained adenovirus 2 major late promoter DNA probe, purified kinase component A (2 ng), and purified kinase component B [0.4 ng (1X) or 1.0 ng (2.5X), as indicated]. Protein-DNA complexes were analyzed by electrophoresis in a nondenaturing 4% polyacrylamide gel and visualized by autoradiography. Bands marked B denote complexes between kinase component B and DNA. Bands marked AB denote supershifted complexes that appear only in the presence of both kinase components A and B.

subunits reacted, whereas neither reacted with a control antibody (Fig. 4A). To further confirm that Ku protein is identical to component B, we tested Ku protein prepared by an independent method. Ku protein prepared by this method has previously been subjected to partial amino acid sequencing and shown to contain peptides identical to those predicted from cDNA clones (27). This preparation of authentic Ku protein was capable of substituting for component B in a phosphorylation assay (Fig. 4B). Finally, we tested the ability of monoclonal antibody against Ku protein to selectively deplete kinase activity from solution. Activity was depleted in reactions containing anti-Ku antibody, but not in control reactions (Fig. 4C). Taken together, these data indicate that kinase component B is Ku protein.

DISCUSSION

We have shown that the template-associated CTD kinase is a two-component system. One component, which is identical to the Ku autoantigen, recruits the other to DNA, forming a complex that efficiently phosphorylates DNA-bound substrates. Ku is required for phosphorylation of both the GAL4-CTD model substrate and the CTD of native RNAP II. Other experiments have shown that Ku protein is also required for phosphorylation of the heat shock protein HSP90 (A.D., L. Y. Stein, B. Calore, and W.S.D., unpublished data), which has been widely used as a substrate for DNA-PK (20, 21). Several lines of evidence support the identification of component B as Ku protein. These include the sizes of the polypeptides of component B, their reactivity with anti-Ku monoclonal antibodies, and the ability of authentic Ku protein to substitute for component B in a phosphorylation assay. In addition, purified component B gave a pattern of DNA binding similar to that previously observed for Ku protein (37-41). Also, the previous observation that DNA ends are required for *in vitro* activation of DNA-PK (20) strikingly parallels the requirement for DNA ends for Ku protein to come on and off DNA *in vitro*.

FIG. 4. Kinase component B is Ku antigen. (A) Immunoblot. Nuclear extract (20 μ g) and purified component B (20 ng) were fractionated by SDS/10% PAGE, transferred to nitrocellulose, and probed with a mixture of anti-Ku monoclonal antibodies (MAB) directed against the 68-kDa and 83-kDa subunits of Ku, or with a control monoclonal antibody. Immune complexes were visualized by using a chemiluminescence detection kit. Positions of prestained electrophoretic markers are indicated at left with molecular masses in kilodaltons. (B) Substitution of Ku protein for kinase component B. Phosphorylation reaction mixtures contained GAL4-CTD substrate, a GAL site-containing DNA fragment, purified kinase component A (0.5 ng), and purified kinase component B (1 ng) or purified Ku protein (25 ng). Arrow indicates multiply phosphorylated GAL4-CTD₀ reaction product. (C) Depletion of kinase activity by use of anti-Ku monoclonal antibody. Purified kinase fraction was incubated with anti-Ku monoclonal antibodies attached to protein A-Sepharose. Kinase activity was assayed with a GAL4-CTD substrate. Upper and lower arrows denote multiply phosphorylated GAL4-CTD₀ and singly phosphorylated GAL4-CTD_a reaction products, respectively.

Although Ku is a relatively abundant nuclear protein (400,000 per mammalian cell), its biochemical function has not been previously established. A number of prior observations have suggested, however, that it has a role in transcription. (In previous literature, Ku has also been referred to as PSE I, TREF1 and TREF2, and NFIV.) The small subunit has clusters of acidic amino acids that resemble acidic transcriptional activating domains (25). Purified Ku protein stimulates transcription of the U1 promoter (26) and a U1-transferrin hybrid promoter (27) in nuclear extracts immunodepleted with anti-Ku antibody. Recent work demonstrated Ku activation of the cytomegalovirus immediate early and adenovirus major late promoters at high template concentration (42). In contrast to conventional activators, however, attempts to show that specific DNA sequences are required for transcriptional activation by Ku have been unsuccessful.

Previous studies have shown that Ku protein interacts with DNA in a highly distinctive manner. It binds nonspecifically to ends of DNA fragments and slides to internal sequences (37–41). The sliding is processive; once loaded onto a fragment of DNA, there is very little exchange of Ku protein to other fragments unless DNA ends are present (39). The distribution of Ku at internal sites is nonrandom. It has some intrinsic affinity for sequences that occur in certain promoters (27, 38, 43). Ku also binds cooperatively with several transcription factors (38), at least one of which, Oct-1, is a substrate for DNA-PK (21).

The special properties of Ku protein might be important in the regulation of CTD phosphorylation and transcription. The ability to slide along DNA makes Ku ideally suited as a DNA-bound carrier for moving the kinase catalytic component along the template to the transcription complex. Transcription factors could influence this process in several ways. For example, some DNA-bound proteins might block the movement of Ku protein along the DNA, interfering with CTD phosphorylation. Others, particularly those with an affinity for Ku protein, might increase the local concentration of kinase in the vicinity of the promoter, enhancing CTD phosphorylation.

Despite much circumstantial evidence that CTD phosphorylation occurs during transcription, it has been difficult to

demonstrate its precise role. One of the problems is the multiplicity of CTD kinases in the cell. A number of reports describe kinases that phosphorylate the CTD in solution (1, 44–48), and at least one of these kinases influences the phosphorylation state of the CTD *in vivo* (49). In addition, recent reports describe at least one other CTD kinase that copurifies with a basal transcription factor (48, 50–52). This factor is a multi-polypeptide complex and has essential functions other than as a kinase. It has different enzymatic characteristics than the kinase we have purified, and it appears to have no subunits in common with that kinase. Preliminary phospho amino acid analysis of the phosphorylated CTD indicates that our kinase phosphorylates the CTD at different sites—i.e., serine and threonine residues equally rather than primarily serine (refs. 51 and 52; A.D., L. Y. Stein, B. Calore, and W.S.D., unpublished data). We expect that the elucidation of the polypeptide composition of different mammalian template-associated CTD kinases, together with the availability of immunologic reagents against the kinases and their accessory proteins, will allow a more detailed assessment of their involvement in the RNAP II initiation mechanism.

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1. Payne, J. M., Laybourn, P. J. & Dahmus, M. E. (1989) *J. Biol. Chem.* **264**, 19621–19629.
2. Laybourn, P. J. & Dahmus, M. E. (1990) *J. Biol. Chem.* **265**, 13165–13173.
3. Arias, J., Peterson, S. R. & Dynan, W. S. (1991) *J. Biol. Chem.* **266**, 8055–8061.
4. Cadena, D. L. & Dahmus, M. E. (1987) *J. Biol. Chem.* **262**, 12468–12474.
5. Zhang, J. & Corden, J. L. (1991) *J. Biol. Chem.* **266**, 2290–2296.
6. Corden, J. L., Cadena, D. L., Ahearn, J. M., Jr., & Dahmus, M. E. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7934–7938.
7. Allison, L. A., Moyle, M., Shales, M. & Ingles, C. J. (1985) *Cell* **42**, 599–610.

8. Laybourn, P. J. & Dahmus, M. E. (1989) *J. Biol. Chem.* **264**, 6693–6698.
9. Zhang, J. & Corden, J. L. (1991) *J. Biol. Chem.* **266**, 2297–2302.
10. Allison, L. A., Wong, J. K. C., Fitzpatrick, V. D., Moyle, M. & Ingles, C. J. (1988) *Mol. Cell. Biol.* **8**, 321–329.
11. Bartolomei, M. S., Halden, N. F., Cullen, C. R. & Corden, J. L. (1988) *Mol. Cell. Biol.* **8**, 330–339.
12. Zehring, W. A., Lee, J. M., Weeks, J. R., Jokerst, R. S. & Greenleaf, A. L. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3698–3702.
13. Nonet, M. L. & Young, R. A. (1989) *Genetics* **123**, 715–724.
14. Allison, L. A. & Ingles, C. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2794–2798.
15. Scafe, C., Chao, D., Lopes, J., Hirsch, J. P., Henry, S. & Young, R. A. (1990) *Nature (London)* **347**, 491–494.
16. Liao, S. M., Taylor, I. C., Kingston, R. E. & Young, R. A. (1991) *Genes Dev.* **5**, 2431–2440.
17. Arias, J. A. & Dynan, W. S. (1989) *J. Biol. Chem.* **264**, 3223–3229.
18. Peterson, S. R., Dvir, A., Anderson, C. W. & Dynan, W. S. (1992) *Genes Dev.* **6**, 426–438.
19. Walker, A. I., Hunt, T., Jackson, R. J. & Anderson, C. W. (1985) *EMBO J.* **4**, 139–145.
20. Carter, T., Vancurova, I., Sun, I., Lou, W. & DeLeon, S. (1990) *Mol. Cell. Biol.* **10**, 6460–6471.
21. Lees-Miller, S. P., Chen, Y.-R. & Anderson, C. W. (1990) *Mol. Cell. Biol.* **10**, 6472–6481.
22. Mimori, T., Skizuki, M., Yamagata, H., Inada, S., Yoshida, S. & Homma, M. (1981) *J. Clin. Invest.* **68**, 611–620.
23. Reeves, W. H. (1985) *J. Exp. Med.* **161**, 18–39.
24. Mimori, T., Hardin, J. A. & Steitz, J. A. (1986) *J. Biol. Chem.* **261**, 2274–2278.
25. Reeves, W. & Stoege, Z. M. (1989) *J. Biol. Chem.* **264**, 5047–5052.
26. Gunderson, S. I., Knuth, M. W. & Burgess, R. R. (1990) *Genes Dev.* **4**, 2048–2060.
27. Knuth, M. W., Gunderson, S. I., Thompson, N. E., Strasheim, L. A. & Burgess, R. R. (1990) *J. Biol. Chem.* **265**, 17911–17920.
28. Thompson, N. E., Steinberg, H., Aronson, D. B. & Burgess, R. R. (1989) *J. Biol. Chem.* **264**, 11511–11520.
29. Maldonado, E., Ha, I., Cortes, P., Weis, L. & Reinberg, D. (1990) *Mol. Cell. Biol.* **10**, 6335–6347.
30. Ha, I., Lane, W. S. & Reinberg, D. (1991) *Nature (London)* **352**, 689–695.
31. Finkelstein, A., Kostrub, C. F., Li, J., Chavez, D. P., Wang, B. Q., Fang, S. M., Greenblatt, J. & Burton, Z. F. (1992) *Nature (London)* **355**, 464–467.
32. Conaway, R. C. & Conaway, J. W. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7356–7360.
33. Conaway, R. C., Garrett, K. P., Hanley, J. P. & Conaway, J. W. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6205–6209.
34. Flores, O., Lu, H., Killeen, M., Greenblatt, J., Burton, Z. F. & Reinberg, D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9999–10003.
35. Yaneva, M., Wen, J., Ayala, A. & Cook, R. (1989) *J. Biol. Chem.* **264**, 13407–13411.
36. Mimori, T., Oshsone, Y., Hama, N., Suwa, A., Arizuki, M., Homma, M., Griffith, A. J. & Hardin, J. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1777–1781.
37. Mimori, T. & Hardin, J. A. (1986) *J. Biol. Chem.* **261**, 10375–10379.
38. de Vries, E., van Driel, W., Bergsma, W. G., Arnberg, A. C. & van der Vliet, P. C. (1989) *J. Mol. Biol.* **200**, 65–78.
39. Paillard, S. & Strauss, F. (1991) *Nucleic Acids Res.* **19**, 5619–5624.
40. Griffith, A. J., Blier, P. R., Mimori, T. & Hardin, J. A. (1992) *J. Biol. Chem.* **267**, 331–338.
41. Zhang, W.-W. & Yaneva, M. (1992) *Biochem. Biophys. Res. Commun.* **186**, 574–579.
42. Knuth, M. W. (1991) Ph.D. thesis (Univ. of Wisconsin, Madison).
43. Roberts, M. R., Miskimins, W. K. & Ruddle, F. H. (1989) *Cell Regul.* **1**, 151–164.
44. Cisek, L. J. & Corden, J. L. (1989) *Nature (London)* **339**, 679–684.
45. Guilfoyle, T. J. (1989) *Plant Cell* **1**, 827–836.
46. Lee, J. M. & Greenleaf, A. L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3624–3628.
47. Stevens, A. & Maupin, M. K. (1989) *Biochem. Biophys. Res. Commun.* **159**, 508–515.
48. Feaver, W. J., Gileadi, O., Li, Y. & Kornberg, R. D. (1991) *Cell* **67**, 1223–1230.
49. Lee, J. M. & Greenleaf, A. L. (1991) *Gene Expression* **1**, 149–167.
50. Gileadi, O., Feaver, W. J. & Kornberg, R. D. (1992) *Science* **257**, 1389–1392.
51. Lu, H., Zawel, L., Fisher, L., Egly, J.-M. & Reinberg, D. (1992) *Nature (London)* **358**, 641–645.
52. Serizawa, H., Conaway, R. C. & Conaway, J. W. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7476–7480.