Interaction of *Xenopus* TFIIC with a 5S RNA gene

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ABSTRACT

Using fractionated *Xenopus* transcription factors we have identified and characterized a unique protein-DNA complex formed between TFIIA, TFIIC and a 5S RNA gene. The formation of this complex was blocked by specific competitor DNAs and by the inactivation of TFIIC using two different methods. In addition, TFIIC activity was retained when the complexes were affinity purified using a reversibly immobilized DNA template. The TFIIA(A+C)-5S RNA gene complex has a distinct electrophoretic mobility on band-shift gels and a unique DNase I footprint. The characteristic feature of the DNase I footprint is a TFIIC-dependent extension of the TFIIA footprint an additional 25 bp toward the 5' end of the gene. This indicates a direct interaction between *Xenopus* TFIIC and the template DNA.

INTRODUCTION

Transcription factor IIC (TFIIC) plays a central role in the transcription of certain genes by RNA polymerase III (1-3). The TFIIC-dependent group of class III genes contains their principle promoter elements within the coding regions and includes the tRNA and 5S RNA genes. The current view is that TFIIC (together with TFIIA for 5S RNA genes) functions to position TFIIB just upstream of the genes, and that once bound TFIIB acts to engage RNA polymerase III in multiple rounds of transcription. TFIIB, which contains TBP (the TATA-box binding protein), is required for the transcription of all class III genes. Class III genes with promoter elements located entirely outside the transcribed region, e.g. the U6 RNA gene, use an alternative assembly pathway for TFIIB involving a TATA-box, and do not require TFIIC for transcription. TFIIC is specifically required for the transcription of the group of class III genes with internal promoters and its function as an essential assembly factor for TFIIB places TFIIC in a position to regulate the expression of these genes.

The structure of TFIIC from *Saccharomyces cerevisiae* and its interaction with DNA templates have been extensively characterized. Highly purified yeast TFIIC contains six tightly associated polypeptides. Four of these polypeptides have been mapped to specific positions along the DNA templates for both tRNA and 5S RNA genes. In addition, the genes for three of the yeast TFIIC

subunits have been cloned. Human TFIIC appears to be different. Human TFIIC sub-fractionates into a DNA binding component, TFIIC2, and a second essential component (4,5). cDNA clones corresponding to the largest subunit of human and rat TFIIC2 have been isolated and although they are nearly identical to each other, they show no similarity to the primary amino acid sequences of the cloned yeast TFIIC subunits (6,7). *Xenopus* TFIIC, although not highly purified, has been reported to have significantly different properties from the yeast or human counterparts. Several reports show that *Xenopus* TFIIC stabilizes the TFIIA-5S RNA gene complex, but conclude that addition of TFIIC does not alter the band-shift mobility or the DNase I footprint of the complex (8-11). Another report identifies three band-shift complexes with *Xenopus* TFIIC (12). However, in all cases the DNase I footprint of a TFIIA-5S RNA gene complex was unchanged by the addition of TFIIC.

Using a new method, called a bead-shift, we recently identified TFIIC-dependent protein-DNA complexes formed on a 5S RNA gene during incubation in a *Xenopus* oocyte extract (13). Using this method, the protein-DNA complexes formed in a relatively large volume (~1 ml) can be analyzed on a single lane of a band-shift gel. The increased sensitivity of this method, relative to a conventional band-shift, makes possible the study of individual complexes present in only small amounts. In the work reported here, we have used fractionated *Xenopus* transcription factors to extend and confirm our earlier observations on the interaction of TFIIC with a 5S RNA gene.

MATERIALS AND METHODS

DNA probes

The plasmid ps (14) containing the somatic 5S RNA gene from *Xenopus laevis* was used in the experiments reported here. DNA probes were radioactively labeled at the *Ngo*MI site, biotinylated at the *A*/III site, and released from the beads by digestion with *Eco*RI (13). The released *Eco*RI-*Ngo*MI fragment containing the 5S RNA gene was 420 bp in length.

Xenopus oocyte extracts and transcription factors

Extracts from *Xenopus* oocytes were prepared by the method of Glikin *et al.* (15) using the following extract buffer: 30 mM Na-HEPES (pH 7.6), 70 mM KCl, 1 mM DTT, 50 μ M EDTA, 50 μ M EGTA, 10 μ M ZnSO₄, 10% glycerol. For some extracts, 100

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mM potassium glutamate replaced 70 mM KCl and 2 mM MgCl₂ was also included. Chromatography on phosphocellulose (16) was used to fractionate the oocyte extract into TFIIB (350 mM KCl step) and TFIIC (700 mM KCl step). The TFIIC step fraction was then dialyzed, concentrated on a small DE52-cellulose column, dialyzed into extract buffer with 2 mM MgCl₂, and stored at a final protein concentration of about 250 µg/ml. TFIIA was purified from immature *Xenopus* ovaries as 7S particles using gel filtration on Sephacryl S-400, flow through on octyl-phenyl Sepharose, and salt gradient elution from DE52-cellulose. The calpain containing calcium inactivation factor, CIF, was obtained by salt gradient phosphocellulose chromatography of an oocyte extract as described (16).

Bead-shift isolation of protein–DNA complexes

Bead-shift isolation of protein–DNA complexes was carried out in buffer T2 as described by Peck *et al.* (13) with minor modifications. Competitor DNA was included at final concentrations ranging from 2 to 25 µg/mg total protein when whole oocyte extracts were used and from 25 to 625 µg/mg total protein when fractionated factors were used. The competitor DNA consisted of a mixture of 15% plasmid DNA, 40% poly(dA-dC)·poly(dG-dT), 15% poly(dA-dT)·poly(dA-dT), 15% poly(dG-dC)·poly(dG-dC) and 15% poly(dI-dC)·poly(dI-dC). Plasmids containing cloned genes replaced vector plasmid when specific competitors were used. A typical 150 µl reaction for the formation of protein–DNA complexes included 28 µg of TFIIC, 0.2 µg of TFIIA (added as 7S particles), 3.5 µg of competitor DNA, and 20 µl of streptavidin coated paramagnetic beads with 70 ng (35 fmol) of immobilized DNA probe. Where indicated, 28 µg of CIF and/or 1 mM Ca²⁺ were included in the reactions. Complex formation took place for 2–3 h before washing away unbound material and proceeding with the bead-shift method as described (13). Protein–DNA complexes were separated by electrophoresis for 5 h at 80 V in 1% agarose gels using 90 mM Tris-borate (pH 8.3) and 50 µM EDTA as the buffer.

DNase I footprinting

The mixture of affinity purified and soluble protein–DNA complexes released from the paramagnetic beads by *EcoRI* was digested with DNase I at a final concentration of 5 µg/ml for 1 min. DNase digestion was stopped by the addition of EDTA to a final concentration of 10 mM and the complexes were separated on a 1% agarose gel. The DNA in each complex was electroeluted and equal amounts were re-run on denaturing sequencing gels. Chemical sequencing ladders were used to determine the locations of the DNase I cleavages.

In vitro transcription

TFIIC was assayed using *in vitro* transcription in a *Xenopus* oocyte extract lacking TFIIC activity (13,16). Inactivation of TFIIC in whole oocyte extracts was accomplished by a 15 min incubation at 42°C. Control reactions used the same amount of template DNA with normal or heat-treated extracts.

RESULTS

Using the bead-shift method we observed the formation of a specific protein–DNA complex between a 5S RNA gene and the *Xenopus* transcription factors TFIIA and TFIIC. Figure 1 shows

Figure 1. Specific complex of TFIIA and TFIIC with a 5S RNA gene. The bead-shift method (13) was used to isolate complexes formed on a *Xenopus* somatic 5S RNA gene during incubation with *Xenopus* TFIIA and TFIIC. An autoradiograph of a 1% agarose band-shift gel is shown. Lanes f–h show the effect of adding increasing amounts of a specific competitor DNA, plasmid ps, during complex formation. In the reactions for lanes c–e, non-specific vector DNA replaced the specific competitor. In addition to plasmid DNA, all reactions contained synthetic non-specific competitor DNA during complex formation. The total amount of DNA in each reaction was 0.7 µg, lanes c and f; 3.5 µg, lanes d and g; 17.5 µg, lanes e and h. Control reactions were: free DNA, lane i; DNA with TFIIA, lane b; DNA with oocyte extract, lane a.

the electrophoretic mobility of this complex (lanes c–e) relative to the complex with TFIIA alone (lane b) and relative to several complexes formed on this DNA probe in a whole oocyte extract (lane a, complexes 1–6). As indicated in the figure, we refer to this complex as an A+C complex. The formation of the A+C complex was sensitive to competition with unlabeled plasmid DNA containing a 5S RNA gene as shown in Figure 1, lanes f–h. In this case, the unlabeled competitor DNA was identical to the labeled probe DNA and contained a cloned *Xenopus* somatic 5S RNA gene. The A+C complex was also sensitive to competition by unlabeled plasmid DNA containing a cloned *Xenopus* 5S RNA pseudogene (data not shown). Since the DNA sequences surrounding the coding regions of the cloned somatic and 5S RNA pseudogenes are completely different, the fact that both cloned genes were effective competitors for A+C complex formation implies that the A+C complex is specific for the 5S RNA coding region.

Specific protein–DNA interactions within the A+C complex were next analyzed by DNase I footprinting. The DNase I footprint of the A+C complex on the transcribed strand is shown in Figure 2, lane c, and can be compared with the TFIIA footprint shown in lane b. The A+C footprint contains a large central protected region starting near +20 and extending to the 3' boundary of a TFIIA footprint at +95. This central protected region includes all of the TFIIA binding site and includes an additional 25 bp on the 5' side of the TFIIA site. This extension of the TFIIA protected region toward the 5' end of the gene is the most distinctive feature of the A+C footprint. In addition, alterations in the cleavage pattern were observed over a region

Figure 2. DNase I footprint of the specific complex of TFIIIA and TFIIIC with a 5S RNA gene. The bead-shift method (13) was used to affinity purify and concentrate protein-DNA complexes formed on a 5S RNA gene during incubation with *Xenopus* TFIIIA and TFIIIC (see Fig. 1, lane d). The mixture of soluble complexes was briefly digested with DNase I and then separated on a 1% agarose gel. The DNA probe in each of the complexes was eluted from the gel and re-run on a denaturing sequencing gel. Footprints on the transcribed strand are shown. Lanes a and b correspond to bands 1 and 2 of Figure 1, i.e. free probe and probe complexed with TFIIIA. Lane c corresponds to the complex labeled A+C in Figure 1. The position of the 5S RNA gene is indicated. Note that a TFIIIA footprint is observed in lane a. Some TFIIIA complexes were probably disrupted by the EDTA added to stop the DNase I digestion and therefore the probe ran at the position of free DNA on the band-shift gel.

from +1 to +20. The general features of the DNase I footprint of the A+C complex on the non-transcribed strand were similar to those seen on the transcribed strand (data not shown).

Figure 3. CIF with Ca^{2+} blocks formation of the A+C complex on a 5S RNA gene. Prior to the addition of 5S RNA genes, transcription factors TFIIIA (A) and TFIIIC (C) were incubated for 45 min with CIF and/or Ca^{2+} , as indicated. For lane g, the factors and 5S RNA genes were incubated for 2 h before Ca^{2+} addition. Protein-DNA complexes were then assayed using the bead-shift method. An autoradiograph of a band-shift gel is shown. Control reactions were: free DNA, lane a; DNA with TFIIIA, lane b; DNA with oocyte extract, lane h.

We previously examined the DNase I footprints of protein-DNA complexes formed on a somatic 5S RNA gene incubated in a whole oocyte extract (13). This earlier work suggested that TFIIIC was required for the formation of two complexes (Fig. 1, lane a, bands 5 and 6). Significantly, the DNase I footprint of the A+C complex was the same as the footprints of bands 5 and 6 over the coding region of the gene. In particular, all three complexes share the large central protected region which includes the TFIIIA site and the distinctive 25 bp extension toward the 5' end of the gene. In addition, both bands 5 and 6 showed an additional protected region from +115 to +160 resulting from a protein now named EP3. Some preparations of TFIIIC contained small amounts of EP3 and when these TFIIIC preparations were used, additional complexes containing EP3 and corresponding to bands 4 and 5 (Fig. 3, lane c) were observed. Adding increasing amounts of a double stranded oligonucleotide with a sequence corresponding to the EP3 binding site caused a decrease in the amount of band 5 and a corresponding increase in the amount of the A+C complex (data not shown). Except for the EP3 footprint, which was missing from the A+C complex, the DNase I footprints of the A+C complex and band 5 were found to be indistinguishable.

To demonstrate that TFIIIC activity was required for the formation of the A+C complex we inactivated TFIIIC using two different methods and found that in both cases the A+C complex was not observed. First, *Xenopus* TFIIIC was inactivated by a mild heat treatment, 15 min at 42°C (16,17). The A+C complex was not observed when heat-treated TFIIIC was used, but was observed under identical conditions with non-heat-treated TFIIIC (data not shown). Secondly, *Xenopus* TFIIIC was inactivated with a combination of CIF (calcium inactivation factor) and Ca^{2+} . We have previously shown that TFIIIC is a specific target for proteolytic digestion by the Ca^{2+} dependent calpain found in CIF (16). Figure 3 (lanes c-e) shows that when either CIF alone or Ca^{2+} alone was added to the reaction, the A+C complex was

observed. Lane f shows that when a combination of CIF and Ca^{2+} was added to the reaction, the A+C complex was not observed. The results of these experiments support the conclusion that TFIIC activity is required for the formation of the A+C complex.

We also found that TFIIC activity was retained with the 5S RNA gene probe following affinity purification and enzymatic release of the protein–DNA complexes formed during incubation with *Xenopus* TFIIA and TFIIC. Using the bead-shift method we prepared a sample containing affinity-purified A+C complexes which had been released from the beads. One aliquot of these complexes was analyzed on a band-shift gel (Fig. 4A) and another aliquot was assayed for TFIIC using *in vitro* transcription in an extract lacking TFIIC activity. Significant levels of 5S RNAs were produced and thus showed that the complexes retained TFIIC activity (Fig 4B, lane f). In addition, competition with an EP3 specific oligonucleotide had no effect on the amount of TFIIC activity retained. Using the bead-shift method, only a very small fraction of the protein present during the initial complex formation is retained in the final preparation of soluble affinity-purified complexes. The combination of extensively washing the complexes with non-specific competitor DNA and the gentle enzymatic release of the complexes from the beads argues that non-specific retention and subsequent release of TFIIC activity would be very unlikely. Thus this experiment supports the view that the A+C complex contains TFIIC activity.

DISCUSSION

The significant finding of the work presented in this report is the identification of a specific protein–DNA complex formed between a 5S RNA gene and the *Xenopus* transcription factors TFIIA and TFIIC. These results have defined two key properties of this complex. First, we have established the electrophoretic mobility of this complex on a band-shift gel relative to other complexes formed on 5S RNA genes. Secondly, we have determined the DNase I footprint of the specific A+C complex.

The conclusion that the A+C complex which we have identified (Fig. 1, lanes c–e) actually contains TFIIC is supported by the following results. The A+C complex was formed using TFIIA and fractionated TFIIC in the absence of TFIIB. The A+C complex was specific for the 5S RNA coding region since complex formation was sensitive to competition with unlabeled DNA containing different types of 5S RNA genes with divergent flanking sequences. TFIIC activity was required for the formation of the A+C complex since inactivation of TFIIC by two different methods resulted in the concomitant loss of the A+C complex. TFIIC was likely to reside in the A+C complex since TFIIC activity was retained with the affinity purified complexes isolated using the bead-shift method.

In a previous report (13), several complexes were observed to form on a 5S RNA gene incubated in a whole oocyte extract (Fig. 1, lane a, bands 1–6). Four of these complexes (bands 3–6) contained EP3, a protein which binds to the 3' flanking sequences of the *Xenopus* somatic 5S RNA gene. The results also suggested that two complexes (bands 5–6) contain TFIIC. However, a complex containing TFIIA and TFIIC, without EP3, was not observed. The results reported here confirm our earlier notions regarding the factors present in these complexes and identify a new complex containing only TFIIA and TFIIC.

The most distinctive feature of the DNase I footprint of the A+C complex is an extension of the TFIIA footprint an additional 25

Figure 4. TFIIC activity in affinity purified complexes. (A) Band-shift gel of purified complexes. (B) TFIIC activity of purified complexes. Complexes were formed between *Xenopus* TFIIA and TFIIC and a 5S RNA gene and purified using the bead-shift method (see Fig. 1). Aliquots of the purified complexes were analyzed on a band-shift gel, shown in (A), and tested for TFIIC activity, shown in (B). Complexes were formed with TFIIA or with TFIIA and TFIIC as indicated. (In addition to non-specific competitor DNA, complex formation with TFIIA and TFIIC included 25 $\mu\text{g}/\text{mg}$ protein of a double stranded oligonucleotide containing the EP3 binding site.) The TFIIC activity of the purified complexes was assayed using *in vitro* transcription with buffer (lanes c, e and g) or with heat-treated oocyte extract lacking TFIIC activity (lanes b, d and f). An autoradiograph of the denaturing gel used to separate the transcription products is shown in (B). The positions of the labeled DNA templates and the 5S RNA transcripts are indicated.

bp toward the 5' end of the gene. The region between +20 and +45 is not protected from DNase I cleavage when TFIIA alone is bound to the gene, but is protected in the A+C complex. Thus the A+C complex contains a large central protected region starting near +20 and extending to +95. This large central protected region was also observed in the footprints of bands 5 and 6 which were previously suggested to contain TFIIC (13). Taking all of the known properties of these three complexes (A+C, bands 5 and 6) into account, we conclude that all three contain TFIIC. It is important to note that this conclusion is largely based on the relationship of the complexes to TFIIC activity as described above and not solely on competition with other DNA molecules.

The relationship between EP3, the protein which binds to the 3' flanking sequences of the *Xenopus* somatic 5S RNA gene, and the formation of a transcription complex on a 5S RNA gene has been clarified by our results. Some preparations of TFIIC contained small amounts of EP3 and additional complexes were observed (Fig. 3, lane c). These additional complexes could be competed away using an oligonucleotide containing the EP3 binding site with no loss in the TFIIC activity of the affinity purified complexes. We conclude that EP3 is not required for TFIIC activity and suggest that EP3 is not an essential transcription factor. However, in view

of other recent work from our laboratory demonstrating that EP3 acts as a transcriptional enhancer for class III genes, it is possible that EP3 interacts with TFIIC.

The results presented here are significantly different from previous work in other laboratories to characterize a complex between the *Xenopus* transcription factors TFIIA and TFIIC and a 5S RNA gene. Our results show that TFIIA and TFIIC form a complex on a 5S RNA gene with a distinct gel mobility and a unique DNase I footprint. These results are in contrast to several previous studies which found that although TFIIC stabilized the TFIIA-5S RNA gene complex, both the gel mobility and the DNase I footprint in the presence of TFIIC remained identical to that of the TFIIA-5S RNA gene complex (8-11). These studies used conventional band-shift and DNase I footprinting methods. We used the bead-shift method (13) which is considerably more sensitive than a standard band-shift assay and allows the DNase I footprint of each complex to be individually analyzed. The A+C complex which we have identified may not have been observed in these earlier studies because the amounts of this complex were below the levels of detection for the assays used.

A subsequent study by Keller *et al.* (12) reported the identification of three specific band-shift complexes containing TFIIC. Remarkably, the DNase I footprints of these complexes were identical to that of the TFIIA-5S RNA gene complex, i.e. no TFIIC specific footprint was observed. As noted by the authors, the lack of a TFIIC specific footprint could be explained by a protein-protein interaction between TFIIC and TFIIA. The absence of a TFIIC specific footprint in these studies suggests that TFIIC does not interact directly with the DNA. Our results are clearly different. We have identified a single complex containing TFIIC with a distinctive footprint which indicates a direct interaction with the DNA. Perhaps the band-shift complexes observed by Keller *et al.* contain proteins which are either unrelated to TFIIC or which contain only a subset of the polypeptides which make up TFIIC.

TFIIC is the limiting factor for transcription of class III genes in *Xenopus* oocyte extracts (16). We have estimated the maximum amount of TFIIC present in a whole oocyte extract using single round transcription and by reducing the concentration of template DNA to a level where the transcription signal starts to drop. Both approaches are in rough agreement with an estimate for the number of A+C complexes observed using the bead-shift method. A typical *in vitro* transcription reaction using 20 μ l of extract (~100 μ g protein) contains <1 fmol of TFIIC. This estimate of the maximum amount of TFIIC activity in the oocyte extract is at least 1000-fold less than the amount of the band-shift complex reported by Keller *et al.* [see Fig. 1 of (12)]. Thus the A+C complex which we have examined is both quantitatively and qualitatively different from that observed by Keller *et al.*

The distinctive features of the DNase I footprint of the A+C complex which we have identified are significantly different from the published footprints of *Xenopus* transcription complexes on 5S RNA genes (8,11,18-21). In particular, the region diagnostic for TFIIC, between +20 and +45, was protected from DNase I cleavage in the A+C complex (Fig. 2, lane c), but was not protected in the published transcription complex footprints [e.g. see Fig. 3 of (19)]. This suggests that the previously observed complexes did not contain *Xenopus* TFIIC. Yeast TFIIC is a component of active yeast 5S RNA gene transcription complexes formed *in vitro*. However, it may be removed from established transcription complexes without destroying transcriptional activity (22). If

Xenopus TFIIC functions in the same way, then active *Xenopus* transcription complexes need not retain *Xenopus* TFIIC. Alternatively, it is possible that TFIIC was a component of the transcriptionally active complexes in the earlier experiments, but went undetected because the bulk of the templates were transcriptionally inactive and did not contain TFIIC, but were responsible for the footprint patterns.

The general features of the DNase I footprint of the A+C complex which we have identified are similar to the footprints of TFIIC(A+C)-5S RNA gene complexes obtained using human and yeast transcription factors. The region diagnostic for *Xenopus* TFIIC, between +20 and +45, was protected from DNase I cleavage in both the *Xenopus* A+C complex (Fig. 2, lane c) and in a similar complex formed using human TFIIC [see Fig. 8, lane 6 of (23)]. Yeast TFIIC also extends the yeast TFIIA footprint in a manner analogous to the 5' extension of the *Xenopus* TFIIA footprint by *Xenopus* TFIIC although the details are somewhat different (22,24). Some preparations of yeast TFIIC generate both a 3' and a 5' extension of the yeast TFIIA footprint, while other preparations generate only a 3' extension and lack the 5' extension. However, photo-crosslinking of yeast TFIIC subunits to a 5S RNA gene clearly positions TFIIC subunits in the region just upstream of the TFIIA binding site when TFIIB or a subfraction of TFIIB is also present (25). Therefore, the interaction of TFIIC with DNA sequences just upstream of the TFIIA binding site is a consistent feature of all systems analyzed to date.

Although the essential promoter elements for transcription of a 5S RNA gene have been localized to a region largely corresponding to the TFIIA binding site, several studies have shown that DNA sequences 5' to this internal control region influence the level of transcription (11,26). In particular, linker-scanning mutations in DNA sequences just upstream of the TFIIA binding site were shown to reduce the transcriptional efficiency of *Xenopus* 5S RNA genes because of reduced abilities to compete for limiting amounts of TFIIC (10). These results support the conclusion that TFIIC interacts with the DNA sequences upstream of the TFIIA binding site in the region defined by the DNase I footprint of the A+C complex.

Using fractionated *Xenopus* TFIIC we have identified a specific TFIIC(A+C)-5S RNA gene complex and found that this complex is the same as previously observed in oocyte extracts, except for the addition of EP3. The bead-shift method has enabled us to identify some key properties of this complex even though only rather impure preparations of transcription factors were used. Future studies using more highly purified transcription factors should provide even more critical insights into the assembly, structure and regulation of class III transcription complexes.

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REFERENCES

- 1 Geiduschek, E. P. and Tocchini-Valentini, G. P. (1988) *Annu. Rev. Biochem.* **57**, 873-914.
- 2 Gabrielsen, O. S. and Sentenac, A. (1991) *Trends. Biochem. Sci.* **16**, 412-416.

- 3 Geiduschek, E. P. and Kassavetis, G. A. (1992) In McKnight, S. L. and Yamamoto, K. R. (eds), *Transcriptional Regulation*. Cold Spring Harbor Laboratory, Cold Spring Harbor, Vol. I, pp. 247–280.
- 4 Yoshinaga, S. K., Boulanger, P. A. and Berk, A. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3585–3589.
- 5 Chiang, C. M., Ge, H., Wang, Z., Hoffmann, A. and Roeder, R. G. (1993) *EMBO J.* **12**, 2749–2762.
- 6 L'Etoile, N. D., Fahnestock, M. L., Shen, Y., Aebersold, R. and Berk, A. J. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 1652–1656.
- 7 Lagna, G., Kovelman, R., Sukegawa, J. and Roeder, R. G. (1994) *Mol. Cell. Biol.* **14**, 3053–3064.
- 8 Wolffe, A. P. (1988) *EMBO J.* **7**, 1071–1079.
- 9 Hayes, J., Tullius, T. D. and Wolffe, A. P. (1989) *J. Biol. Chem.* **264**, 6009–6012.
- 10 Keller, H. J., You, Q. M., Romaniuk, P. J. and Gottesfeld, J. M. (1990) *Mol. Cell. Biol.* **10**, 5166–5176.
- 11 Wolffe, A. P. and Morse, R. H. (1990) *J. Biol. Chem.* **265**, 4592–4599.
- 12 Keller, H. J., Romaniuk, P. J. and Gottesfeld, J. M. (1992) *J. Biol. Chem.* **267**, 18190–18198.
- 13 Peck, L. J., Bartilson, M. and DeRisi, J. L. (1994) *Nucleic Acids Res.* **22**, 443–449.
- 14 Seidel, C. W. and Peck, L. J. (1992) *J. Mol. Biol.* **227**, 1009–1018.
- 15 Glikin, G. C., Ruberti, I. and Worcel, A. (1984) *Cell* **37**, 33–41.
- 16 Sturges, M. R. and Peck, L. J. (1994) *J. Biol. Chem.* **269**, 5712–5719.
- 17 Kovelman, R. and Roeder, R. G. (1992) *J. Biol. Chem.* **267**, 24446–24456.
- 18 Wolffe, A. P. and Brown, D. D. (1986) *Cell* **47**, 217–227.
- 19 Wolffe, A. P., Jordan, E. and Brown, D. D. (1986) *Cell* **44**, 381–389.
- 20 Engelke, D. R. and Gottesfeld, J. M. (1990) *Nucleic Acids Res.* **18**, 6031–6037.
- 21 Chipev, C. C. and Wolffe, A. P. (1992) *Mol. Cell. Biol.* **12**, 45–55.
- 22 Kassavetis, G. A., Braun, B. R., Nguyen, L. H. and Geiduschek, E. P. (1990) *Cell* **60**, 235–245.
- 23 Schneider, H. R., Waldschmidt, R., Jahn, D. and Seifart, K. H. (1989) *Nucleic Acids Res.* **17**, 5003–5016.
- 24 Braun, B. R., Riggs, D. L., Kassavetis, G. A. and Geiduschek, E. P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2530–2534.
- 25 Braun, B. R., Bartholomew, B., Kassavetis, G. A. and Geiduschek, E. P. (1992) *J. Mol. Biol.* **228**, 1063–1077.
- 26 Reynolds, W. F. and Azer, K. (1988) *Nucleic Acids Res.* **16**, 3391–3403.