0014-4754/93/100831-05$1.50 + 0.20

Reviews

Transcription factor IIIA (TFIIIA): An update

B. S. Shastry

Eye Research Institute, Oakland University, Rochester (Michigan 48309, USA)
Received 6 November 1992; accepted 25 June 1993

Abstract. *Xenopus* transcription factor, termed TFIIIA, is the first eukaryotic transcription factor purified to homogeneity and one of the most extensively characterized polymerase III gene factors at the levels both of the protein and its gene. It is an abundant protein in oocytes and is specifically required for the 5S RNA gene transcription. It promotes the formation of a stable transcription complex by first binding to the internal control region of the 5S RNA gene through its zinc finger motifs. It contains two structural domains and associates with 5S RNA to form 7S ribonucleoprotein particles in oocytes. Its expression is developmentally controlled at the level of transcription and translation. It participates in the assembly of active chromatin templates and, at least in part, is responsible for the differential expression of two kinds of 5S RNA genes in *Xenopus*.
Key words. Transcription; factor; polymerase III; chromatin; gene; DNA-protein.

In recent years, it has become increasingly clear that specific transcription of eukaryotic genes involves the presence of several discrete DNA sequences called regulatory elements and an ordered interaction of multiple factors with the target DNA and with RNA polymerases. The regulatory sequences are the target binding sites for the sequence-specific DNA binding factors. Over the past several years, a major effort has been undertaken to identify the control elements of several tissue and cell-type specific and inducible genes, and to isolate and characterize the sequence-specific DNA binding proteins. Although the exact role played by the various factors in the specific transcription is still not understood, protein:protein interactions, post-translational modification (e.g., phosphorylation, glycosylation and dimerization) and the cellular concentration of factors appear to be involved in modulating the activities of individual factors[1–5]. Now the major challenge is to understand how recognition of these factors by target sequences on the DNA brings about the regulated expression of specific genes.

Among eukaryotic genes, the 5S RNA genes from *Xenopus* have become the model system for the study of the developmental regulation of eukaryotic genes[6,7]. Their organization and regulatory sequences have been well characterized. They also require multiple factors along with polymerase III for specific transcription in vitro. Among these factors, one factor, designated TFIIIA, is specifically required for the 5S RNA gene transcription. This is the first eukaryotic transcription factor to be isolated and purified to homogeneity. It is unique in its structure and function, and has been the subject of intense study during the last 10 years. The purpose of this article is to update our current knowledge of this novel protein. Since the first comprehensive review on TFIIIA[8], progress has been made in understanding the interaction of the zinc fingers, the structure of the internal control region, and the identification of the DNA sequences that are necessary for the developmental expression of the TFIIIA gene. Major emphasis is given to these areas. Because of space limitations, the number of references is restricted. More comprehensive lists can be found in reviews on TFIIIA[8], polymerase III gene transcription[6] and the developmental expression of 5S RNA genes[7].

Purification and properties

TFIIIA was originally purified to homogeneity from *Xenopus* oocytes by using conventional chromatographic procedures, which was very tedious and time consuming. Simpler and more efficient methods, such as glycerol gradient centrifugation followed by DEAE cellulose chromatography[9], and a recombinant method in which the protein is expressed in *E. coli*[10], are now available for obtaining a highly purified factor of a yield of 2–3 mg protein. The factor obtained by these methods is functionally indistinguishable from that obtained by the original procedure. Another abundant protein of 42 kDa has been partially purified, and reported to replace TFIIIA[11] for somatic 5S RNA genes and repress the transcription of oocyte 5S RNA genes, but this work is highly controversial and has not been replicated by others[12].

Xenopus TFIIIA in its native state contains a single polypeptide of 38.5 kDa and is a highly asymmetric molecule. The corresponding protein from yeast[13] is slightly larger (50 kDa), and that from HeLa cells[14] is smaller (35 kDa). Although these proteins differ in their molecular mass they are functionally identical (specifi-

cally required for the 5S RNA gene transcription), and the HeLa protein can replace the frog factor in a transcription assay. A wide variety of properties such as binding to 5S RNA in addition to 5S DNA, DNA dependent ATPase activity, and DNA reassociation have been described for *Xenopus* TFIIIA[8]. However, it is not known whether the corresponding proteins from yeast and HeLa cells also possess these properties. Moreover, all three proteins seem to exhibit high affinity binding to the internal control region (ICR) of the homologous 5S RNA genes. This is the most remarkable feature of TFIIIA. Additionally, the *Xenopus* factor was shown to interact with the corresponding intragenic region of the mammalian 5S RNA genes[15,16]. Interestingly, frog TFIIIA may also be involved in the unidirectional transport of the 5S RNA from nucleus to cytoplasm[17] in the form of 7S RNPs. In this respect TFIIIA is very similar to the ribosomal protein L5 which can also bind to the 5S RNA and mobilize the 5S RNPs both into and out of the nucleus. In fact, it has been suggested[6,18], based on their striking similarity in 5S RNA interaction, that TFIIIA may be an evolutionary descendent of the ribosomal protein.

Structure and functions

By a variety of studies[6–8] it has been demonstrated that frog TFIIIA has many structural characteristics similar to those of prokaryotic repressors and activators. For instance, it possesses one structural domain which can bind to the DNA but cannot direct the transcription (fig., panel A). On the other hand, the second domain of 10,000 Da at the C-terminus does not bind the DNA but is important in activating the gene for transcription. This may imply that the basic mechanisms of gene activation in prokaryotic and eukaryotic cells may be similar. Interestingly, yeast TFIIIA also exhibits a similar structure of domains which are required for the promotion of transcription[19,20]. In addition, *Xenopus* TFIIIA has another interesting property: it can make a complex with DNA in the absence of any other factors. This complex, however, does not appear to be stable during repeated rounds of transcription by either RNA polymerase III or bacteriophage Sp6 or T7 RNA polymerase[21]. On the other hand, yeast TFIIIA requires the presence of TFIIIC to interact with the 5S RNA gene[22]. The availability of TFIIIA cDNA from both frog[6–8] and yeast[19] systems has further enhanced our understanding of the structure and function of this protein. The deduced amino acid sequence of the corresponding cDNAs from *Xenopus* and yeast revealed that both proteins contain 9 zinc finger motifs, although considerable divergence in the amino acid sequence of the corresponding fingers between the two systems has been reported. Amino acid sequence comparison of TFIIIA

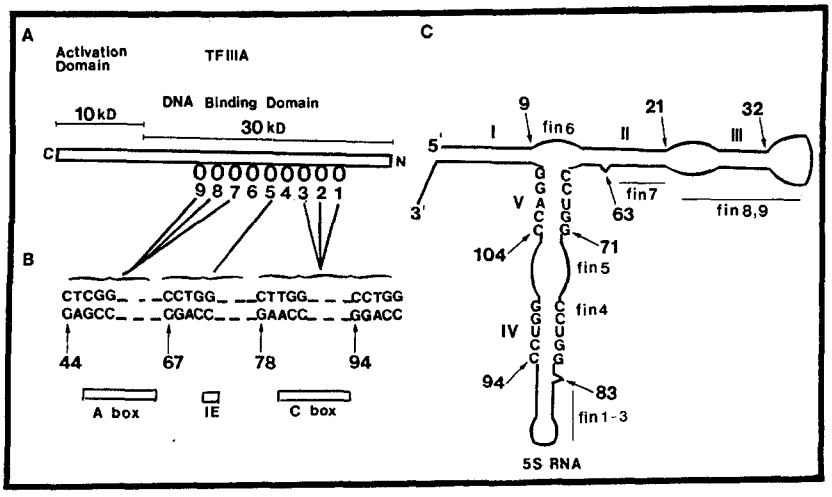

Figure. A schematic representation of the structural features of TFIIIA, ICR and 5S RNA. Panel A: TFIIIA is represented by an open rectangular box. The structures and the numbers 1–9 beneath the rectangle show nine imperfect tandemly repeated zinc fingers. The N-terminal (N in the figure) 30 kDa proteolytic fragment (residues 1-276) which contains the nine zinc finger motifs is the DNA binding domain. The C-terminal (C in the figure) 10 kDa fragment does not bind DNA but is required for the activation[6–8]. This C-terminal domain may also be a protein:protein interaction site. Panel B: The sequence shown is the common 5 base pair repeating element present in the ICR and 5S RNA. Since the ruthenium(II) probe specifically recognizes the 'A' conformation (RNA conformation) and cleaves at the border of these pentanucleotide regions[8,31] these elements, along with linker regions, are proposed to possess the necessary structural features[31] in the ICR to accommodate the zinc fingers of TFIIIA. The individual finger binding sites are shown in the figure. Fingers 4 and 6 each bind across the minor groove spanning the A box, intermediate element (IE) and C box regions[32–35]. Panel C: The secondary structure of *Xenopus* 5S RNA proposed by Garrett et al.[57]. There are two repeating units of pentanucleotide in helices IV and V which are reported to be providing necessary structure for the TFIIIA binding. Since these helices are also present in the ICR they are thought to be common structural features recognized by TFIIIA in 5S DNA and 5S RNA. The proposed binding sites for the zinc fingers are indicated as shown by others[39]. fin = zinc fingers. The figure is not drawn to scale.

from *Bufo americanus*, *Rana pipiens* and *Xenopus* reveals more variation (40%) than in other DNA binding proteins[23]. The yeast protein also differs in the sequence of the amino and carboxyl terminal domains[19,24]. However, it remains to be seen whether a similar structure is also present in the HeLa TFIIIA. The detailed structural feature of zinc fingers of *Xenopus* TFIIIA has been described before[6–8].

A variety of studies, including studies on truncated *Xenopus* TFIIIA[25,26], have shown that the first three fingers are essential for the sequence specific DNA binding. Interestingly, sequence-specific interaction was not observed with polypeptides containing fingers 1 and 2, 3 and 4 of TFIIIA. Furthermore, in vitro experiments on mutagenesis of zinc finger domains, and the conserved tetra peptide (TGEK) in the N-terminal region, indicate that zinc fingers 1 and 2, a terminal positive charge in the tetra peptide, the bend structure, and cooperativity among zinc fingers are the major determinants of TFIIIA interaction with DNA[27]. Additionally, reconstitution experiments using a synthetic 26 amino acid peptide[28] containing the seven most highly conserved amino acids of TFIIIA suggests that these conserved amino acids are minimally needed for the metal binding and to produce a correctly folded structure. By using a set of mutant forms of *Xenopus* TFIIIA[29] containing single amino acid substitutions in one of the nine zinc fingers, it has been demonstrated that the three C-terminal zinc fingers (fingers 7–9) are involved in higher-order interactions (protein:protein interaction). Without the C-terminal end the TFIIIA can still bind to DNA but there is no transcription, so this region can be regarded as an activation domain.

One of the unique functions of *Xenopus* TFIIIA is that it recognizes two kinds of nucleic acid molecules, DNA and RNA. Since these molecules have different structures, an extensive effort has been made to understand the common structural requirements of these molecules for TFIIIA binding. These have been discussed in detail previously[6–8]. In addition to the protein modification studies, photo cross-linking of the TFIIIA-DNA complex with 5′-azido-deoxy uridine 5′-triphosphate[30] has shown that two T residues at positions +84 and +88 are in contact with protein in the coding and non-coding strands respectively. Further analysis by V8 protease digestion of the complex suggested that the contact regions with +84 are zinc finger 2 and the linker region of fingers 2 and 3. These results are consistent with the previous observations[25,26]. Additionally, the use of metal probes (ruthenium and rhodium), which recognize DNA purely on the basis of shape selection[31] indicated that the structure of the internal control region is not uniform and contains distinct segments or variations which appear to be encoded in the DNA sequence (fig., panel B). There are short segments of 'A'-like conformation and distinct openings of the major groove at the junction between 'A'- and 'B'-type segments within the ICR region. These types of structural features (a nonuniform distribution of 'A' and 'B'-type elements) of ICR may accommodate the multiple finger domain of TFIIIA. Furthermore, the missing nucleotide experiment supports the notion that TFIIIA wraps around the DNA in the major groove of the helix[32]. This is again in accordance with the finding that the openings in the major groove closely correspond to the individual finger binding sites of TFIIIA. Based on the crystal structure of the DNA: protein complex and other experiments involving deletion mutants, an alignment of zinc fingers of TFIIIA with its DNA binding site is proposed[33–35]. From this, it appears that fingers 1–3 bind to the C box region, fingers 7–9 are assigned to the A box, finger 5 binds to the intermediate element, and fingers 4 and 6 each bind across the minor groove spanning the A box, intermediate element and C box regions. Since the CD spectrum of the 5S gene ICR does not change with TFIIIA[6–8], these structural changes in the ICR are believed not to be induced by TFIIIA but to be inherent in the DNA sequence. Although several experiments suggest that there are certain sequences in the ICR which can assume the 'A'-type conformation, there are no simple explanations at present for how TFIIIA can bind to both RNA and DNA.

In addition to the DNA binding and transcriptional activity, *Xenopus* TFIIIA has another, different biological function. It binds to the 5S RNA to form 7S ribonucleoprotein particles. The purpose of this interaction appears to be to store and stabilize the 5S RNA in oocytes until it is required for ribosome assembly. A variety of studies on the structural requirement of 5S RNA for the interaction with TFIIIA have been described in detail[8]. Recent reports using chemical nuclease protection experiments indicate that nucleotides +73 to +77 and +99 to +102 are essential contact sites for TFIIIA[36]. The zinc fingers 8 and 9 of TFIIIA may be involved in the interaction with the distal portion of the helix V (fig., panel C) in the 5S RNA[37]. A comparative analysis of DNA and RNA binding properties using a set of TFIIIA derivatives with one or several fingers deleted suggests that fingers 3 and 6 have special properties for DNA and RNA binding respectively[38]. In a study involving a series of truncated proteins it has been shown that fingers 4–7 and 1–3 are required for specific high affinity RNA and DNA binding, respectively[39]. The C-terminal domains of TFIIIA do not bind the 5S RNA similar[1] to 5S DNA, and the N-terminal zinc fingers are also not involved in the recognition of helix V of 5S RNA. Studies involving mutant 5S RNA containing substitutions or deletions in all three helical stems indicate that the secondary structure of all three helical stems plays a critical role in TFIIIA binding[40]. It would be interesting to determine

whether the corresponding TFIIIAs from HeLa and yeast systems also bind RNA and exhibit similar structural requirements. Although a 43 kDa protein with a structure similar to those of TFIIIA has been isolated from *Xenopus* oocytes[41] its function other than storage of 5S RNA is not understood. This protein binds exclusively to the 5S RNA but not to the DNA, and exhibits binding regions similar to those observed for TFIIIA on 5S RNA, but with different protection patterns[42]. In addition, the N-terminal fingers in the 43 kDa protein are needed for the 5S RNA binding[43] but the C-terminus of TFIIIA shows the highest affinity in 5S RNA binding.

TFIIIA has been shown to be involved in the assembly of the active chromatin template[6–8]. Chromatin assembled with the purified histones in the presence of TFIIIA is found to be actively transcribed. Moreover, when the 5S DNA was assembled with a tetramer of histones H_3 and H_4, TFIIIA could recognize its binding site as with the naked DNA. However, the DNA complexed with an octamer of histones (H_3, H_4, H_{2A} and H_{2B}) appears to be refractory to TFIIIA interaction[44]. When the N-terminal tails of the core histones are acetylated or removed, TFIIIA binding to the histone DNA complex is restored[45]. This suggests that TFIIIA does not recognize its binding site in the nucleosome. Although a slightly different observation was made with yeast[46] the above experiments are consistent with the idea that TFIIIA is at least one of the components that must be present at the time of nucleosome assembly in order to form a transcriptionally active template[8].

TFIIIA gene

To better understand the structure and functions of TFIIIA, a cDNA clone from yeast and cDNA and genomic clones from frog have been isolated and characterized[8,19]. Blot hybridization data suggested that both yeast and frog contain a single copy gene for TFIIIA[19,24]. The pattern of developmental expression of the TFIIIA gene indicates that it is maximally expressed in young oocytes, but during later stages of oogenesis its level decreases several fold. Northern blot analyses show longer and more heterogeneous RNAs in kidney cells and swimming tadpoles[7,8]. Thus it appears that TFIIIA gene expression is developmentally controlled at the transcriptional level. Similarly, the available data on the cellular level of frog TFIIIA, together with microinjection experiments using mRNA and different concentrations of purified TFIIIA either into unfertilized eggs or coenocytic embryos[6–8], suggest that high concentrations of TFIIIA may be responsible in part for the activation of oocyte-type genes, so that TFIIIA thus fulfils a transcriptional role. However, the potential influence of other proteins and the chromatin structure cannot be ruled out. For instance, TFIIIA binding to the ICR, and TFIIIA dependent transcription of the 5S RNA gene, are reported to be suppressed by an increase in the biosynthesis of thionein which may be due to the depletion of zinc atoms[47]. Therefore, by restricting the intracellular concentration of metal ions, it is possible to modulate the activity of metal-dependent proteins like TFIIIA, and this may be another cellular mechanism to control the gene activity. Alternatively, this may also imply that zinc metal may be a physiological regulator of TFIIIA activity, as suggested by others for several metalloproteins[48,49].

The promoter elements required for the expression of the frog TFIIIA gene have been identified and discussed in detail[8]. In addition to the more general kind of polymerase II promoters, it contains several elements in the upstream region which function both as negative and positive regulatory sequences[50]. More interestingly, within the coding region, it contains two stretches of nucleotides which are highly homologous with the tRNA 'B' box promoter element, and another region which shows homology to the 5′- and 3′-border of the TFIIIA binding site of the 5S RNA gene. Recently[51] it has been shown that the TFIIIA gene can specifically bind TFIIIC over the 'B' box sequences and can form a stable complex with TFIIIC in a tRNA gene transcription competition assay. Although the biological significance of these data awaits further experiments, they may imply that the TFIIIA gene could regulate the expression of other class III genes by sequestering TFIIIC activity. Although several protein factors[8,52,53] including TFIIIA, TFIIIC, B3 and an adenovirus major late transcription factor have been shown to be specifically reacting with several different regions of the TFIIIA gene, it is not evident at present whether any of these factors have roles in cell-type or in vitro expression of the TFIIIA gene.

Concluding remarks

In this article an attempt has been made to summarize some of the latest developments and our current knowledge of a gene specific transcription factor very widely known as TFIIIA. Since its discovery in 1980, remarkable progress has been made in understanding the structure and function of this interesting protein. With its unusual properties of sequence-specific DNA and RNA binding it plays an important role in the transcriptional activation of the 5S RNA gene. Similarly to the ribosomal protein L5, it may also participate in the unidirectional transport of 5S RNA from nucleus to cytoplasm. However, it is not known whether TFIIIA has functions other than those mentioned above. What role TFIIIA plays in the overall process of gene activation, and the structural and functional significance of its zinc fingers in carrying out its transcriptional role, remain to be elucidated. The ribosomal protein L5 without zinc

fingers also binds to 5S RNA, and the 43 kDa protein isolated from the frog oocyte – although it contains 9 zinc finger motifs similar to TFIIIA – binds to 5S RNA but not DNA. Therefore it is tempting to speculate that the zinc fingers in TFIIIA do not only contribute to the unique folding property of the protein but play an important role in specific gene transcription. Although metal-dependent folding of a single zinc finger[54], zinc-induced bending of the internal control region of the 5S RNA gene[55], and the reconstitution of an EDTA-treated TFIIIA with several divalent metal ions[56] have been reported, it remains to be seen in an apo-TFIIIA whether substitution of either iron, copper, Co and Ni metal for zinc will alter the TFIIIA activity, and whether such a substituted protein will be able to carry out its transcriptional role. Although the nature of TFIIIA binding and the structural requirements of the binding domain of TFIIIA, 5S DNA and 5S RNA are fairly well understood, much less is known about the structural and functional consequences of protein:protein interactions and the surfaces of the proteins that facilitate this interaction. In the next few years, many of these intriguing questions are likely to be answered. Such studies will undoubtedly enrich our understanding of the molecular mechanisms involved in the cell/tissue specific expression of eukaryotic genes.

Acknowledgements. I am grateful to Dr. Barry Winkler of Oakland University for his valuable corrections, comments and suggestions. Due to limited space for references, I regret that many original papers could only be cited through reviews.

1 Johnson, P. F., and McKnight, S. L., A. Rev. Biochem. *58* (1989) 799–839.
2 Sawadogo, M., and Sentenac, A., A. Rev. Biochem. *59* (1990) 711–754.
3 Gabrielsen, O. S., and Sentenac, A., Trends Biochem. Sci. *16* (1991) 412–416.
4 Sollner-Webb, B., and Mongey, E. B., Trends Biochem. Sci. *16* (1991) 58–62.
5 Latchman, D. S., Biochem. J. *270* (1990) 281–289.
6 Geiduschek, E. P., and Tocchini-Valentini, G. P., A. Rev. Biochem. *57* (1988) 873–914.
7 Wolffe, A. P., and Brown, D. D., Science *241* (1988) 1626–1632.
8 Shastry, B. S., Prog. Biophys. molec. Biol. *56* (1991) 135–144.
9 Smith, D. R., Jackson, I. J., and Brown, D. D., Cell *37* (1984) 645–652.
10 Rio, S. D., and Setzer, D. R., Nucl. Acids Res. *19* (1991) 6197–6203.
11 Blanco, J., Millstein, L., Razik, M. A., Dilworth, S., Cote, C., and Gottesfeld, J., Genes & Dev. *3* (1989) 1602–1612.
12 Brown, D. D., Genes & Dev. *5* (1991) 1737–1738.
13 Wang, C. K., and Weil, P. A., J. biol. Chem. *264* (1989) 1092–1099.
14 Seifarts, K. H., Wang, L., Waldschmidt, R., Jahn, D., and Wingender, E., J. biol. Chem. *264* (1989) 1702–1709.
15 Emerson, B. M., and Roeder, R. G., J. biol. Chem. *259* (1984) 7926–7935.
16 Shastry, B. S., and Greenstein, D., Gene *107* (1991) 269–278.
17 Allison, L. A., Romaniuk, P. J., and Bakken, A. H., Devl Biol. *144* (1991) 129–144.
18 Enver, T., Nature *317* (1985) 385–386.
19 Archambault, J., Milne, C. A., Schappert, K. T., Baum, B., Friesen, J. D., and Segal, J., J. biol. Chem. *267* (1992) 3282–3288.
20 Milne, C. A., and Segall, J., J. biol. Chem. *268* (1993) 11364–11371.
21 Campbell, F. E., and Setzer, D. R., Molec. cell Biol. *11* (1991) 3978–3986.
22 Struksnes, K., Forus, A., Gabrielsen, O. S., and Øyen, T. B., Nucl. Acids Res. *19* (1991) 565–571.
23 Gaskins, C. J., Smith, J. F., Ogilvie, M. K., and Hanas, J. S., Gene *120* (1992) 197–206.
24 Woychik, N. A., and Young, R. A., Proc. natl Acad. Sci. USA *89* (1992) 3999–4003.
25 Christensen, J. H., Hansen, P. K., Lillelund, O., and Thogersen, H. C., FEBS Lett. *281* (1991) 181–184.
26 Liao, X., Clemens, K. R., Tennant, L., Wright, P. E., and Gottesfeld, J. M., J. molec. Biol. *223* (1992) 857–871.
27 Smith, J. F., Hawkins, J., Leonard, R. E., and Hanas, J. S., Nucl. Acids Res. *19* (1991) 6871–6876.
28 Michael, S. F., Kilfoil, V. J., Schmidt, M. H., Amann, B. T., and Berg, J. M., Proc. natl Acad. Sci. USA *89* (1992) 4796–4800.
29 Rio, S. D., and Setzer, D. R., Proc. natl Acad. Sci. USA *90* (1993) 168–172.
30 Lee, D. K., Evans, R. K., Blanco, J., Gottesfeld, J., and John, J. D., J. biol. Chem. *266* (1991) 16478–16484.
31 Huber, P. W., Morii, T., Mei, H-Y., and Barton, J. K., Proc. natl Acad. Sci. USA *88* (1991) 10801–10805.
32 Hayes, J. J., and Tullins, T. D., J. molec. Biol. *227* (1992) 407–417.
33 Fairall, L., and Rhodes, D., Nucl. Acids Res. *20* (1992) 4727–4731.
34 Hayes, J. J., and Clemens, K. R., Biochem. *31* (1992) 11600–11605.
35 Clemens, K. R., Liao, X., Wolf, V., Wright, P. E., and Gottesfeld, J. M., Proc. natl Acad. Sci. USA *89* (1992) 10822–10826.
36 Darsillo, P., and Huber, P. W., J. biol. Chem. *266* (1991) 21075–21082.
37 Sands, M. S., and Bogenhagen, D. F., Nucl. Acids Res. *19* (1991) 1791–1796.
38 Theunissen, O., Rudt, F., Guddat, U., Mentzel, H., and Pieler, T., Cell *71* (1992) 679–690.
39 Clemens, K. R., Wolf, V., McBryant, S. J., Zhang, P., Liao, X., Wright, P. E., and Gottesfeld, J. M., Science *260* (1993) 530–533.
40 Bogenhagen, D. F., and Sands, M. S., Nucl. Acids Res. *20* (1992) 2639–2645.
41 Joho, K. E., Darby, M. K., Crawford, E. T., and Brown, D. D., Cell *61* (1990) 293–300.
42 Sands, M. S., and Bogenhagen, D. F., Nucl. Acids Res. *19* (1991) 1797–1803.
43 Darby, M. K., and Joho, K. E., Molec. cell. Biol. *12* (1992) 3155–3164.
44 Hayes, J. F., and Wolffe, A. P., Proc. natl Acad. Sci. USA *89* (1992) 1229–1233.
45 Lee, D. Y., Hayes, J. J., Pruss, D., and Wolffe, A. P., Cell *72* (1993) 73–84.
46 Felts, S. J., Weill, P. A., and Chalkley, R., Molec. cell Biol. *10* (1990) 2390–2401.
47 Zeng, J., Vallee, B. L., and Kagi, J. H. R., Proc. natl Acad. Sci. USA *88* (1991) 9984–9988.
48 Zhang, Z-Y., Reardon, I. M., Hui, J. O., O'Connell, K. L., Poorman, R. A., Tomasselli, A. G., and Heinrikson, R. L., Biochem. *30* (1991) 8717–8721.
49 Karlstrom, A. R., and Levin, R. L., Proc. natl Acad. Sci. USA *88* (1981) 5552–5556.
50 Pfaff, S. L., Hall, R. K., Hart, G. C., and Taylor, W. L., Dev. Biol. *145* (1991) 241–254.
51 Shastry, B. S., and Zhang, Z. G., BBRC *175* (1991) 1036–1041.
52 Tafuri, S. R., and Wolffe, A. P., Proc. natl Acad. Sci. USA *87* (1990) 9028–9032.
53 Pfaff, S. L., and Taylor, W. L., Dev. Biol. *151* (1992) 306–316.
54 Frankel, A. D., Berg, J. M., and Pabo, C. O., Proc. natl Acad. Sci. USA *84* (1987) 4841–4845.
55 Nickol, J., and Rau, D. C., J. molec. Biol. *228* (1992) 1115–1123.
56 Hanas, J. S., Hazuda, D. J., Bogenhagen, D. F., Wu, F. Y.-H. and Wu, C.-W., J. biol. Chem. *258* (1983) 14120–14125.
57 Garrett, R. A., Douthwaite, S., and Noller, H. F., Trends Biochem. Sci. *6* (1981) 137–139.