Volume 16 Number 13 1988 Nucleic Acids Research

Heavy metal ions in transcription factors from HeLa cells: Sp1, but not octamer transcription factor requires zinc for DNA binding and for activator function

Gunnar Westin and Walter Schaffner

Institut für Molekularbiologie II der Universität Zürich, Hönggerberg, CH-8093 Zürich, Switzerland

Received April 28, 1988; Accepted June 8, 1988

ABSTRACT

Zinc is an important cofactor for many enzymes involved in nucleic acid metabolism such as DNA and RNA polymerases, reverse transcriptase and tRNA synthetases. We have developed an inducible in vitro transcription system using metal-depleted nuclear extracts to reveal the presence and functional relevance of heavy metal ions in transcription factors. Using protein-DNA binding assays (band shift and DNAase I footprint) we show that Sp1, a promoter-specific vertebrate transcription factor that binds to the "GC box" (5'-$^{GG}_{TA}$GGCG$^{GGGC}_{TAAT}$), is reversibly inactivated by metal-depletion. Zinc is required for specific DNA binding in vitro and is also essential for Sp1 factor-directed transcription. In contrast, another factor from HeLa cells, the so-called octamer transcription factor (OTF) that binds to the sequence 5'-ATGCAAATNA, is not affected by metal-depletion and thus seems not to be a zinc metalloprotein.

INTRODUCTION

Zinc plays a major role as cofactor in many enzymes. More than 200 zinc metalloenzymes have been identified belonging to different enzyme classes including oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Also, many Zn metalloenzymes are involved in nucleic acid metabolism such as DNA and RNA polymerases, reverse transcriptase and tRNA synthetases (1, and references therein). The transcription factors TFIIIA of Xenopus and GAL4 of Saccharomyces cerevisiae contain zinc ions essential for DNA binding and activity (2,3). Structural studies of TFIIIA led to the discovery of a novel protein motif used for DNA (and RNA) binding, the so-called zinc finger (4,5). The finger structure, which is postulated to mediate sequence-specific binding of the factor to DNA, is maintained by a zinc ion which binds to cysteine and histidine residues. Thus, in this case zinc may play a structural role rather than a catalytic one. Multiple zinc finger-like structures have been found in several eukaryotic RNA polymerase II transcription factors (5,6) and it has been directly demonstrated by mutational analyses that two zinc finger structures are essential for the function of the yeast protein ADR1, a positive regulator of transcription (7).

The mammalian transcription factor Sp1 has been characterized extensively by R. Tjian and his colleagues. It binds to, and activates, transcription from many viral and cellular promoters (8,9). Two polypeptides of 105 and 95 kilodaltons have been correlated with Sp1 activity (10). Recently, a partial Sp1 cDNA has been isolated and the C-terminal 168 amino acid residues, which contain three zinc finger motifs, were shown to retain DNA binding activity. It was also found that purified Sp1 requires zinc for DNA binding (11).

A conserved "decanucleotide" motif 5'-ATGCAAATNA, most often referred to as "octamer" motif, is present in the tissue-specific promoters of the IgH and IgL variable genes and also in the immunoglobulin heavy chain enhancer (12-14). Mutagenesis experiments, in our and in other laboratories, demonstrated a crucial role for the octamer motif in cell type-specific transcription (15,16). The explanation of cell type specificity is complicated by the fact that at least two factors can bind to the octamer sequence, referred to as OTF-1 and OTF-2 (or NF-A1 and NF-A2, respectively). OTF-1 (100 kD, 17) is ubiquitously present in all cell lines tested so far, while OTF-2 (60 kD, 18) is typical for B cells. The octamer binding factor of HeLa cells described by other laboratories (19-21) is probably identical to OTF-1, though this remains to be demonstrated. The lymphoid-specific activity of immunoglobulin genes is most likely mediated by OTF-2, while the ubiquitously present OTF-1 protein contributes to the activity of many other genes, for example those encoding histone H2B (22, and references therein) or various U snRNAs (23,24, and references therein) whose promoter/enhancer region contains an octamer motif. In addition, OTFs seems to be involved in DNA replication: An octamer motif is present in the replication origin of adenoviruses and a protein with the properties of OTF stimulates viral DNA replication (25,26).

In this paper we show, by using simple and straightforward experimental procedures, that transcription factor Sp1 requires zinc for specific DNA binding and that zinc is essential for Sp1-directed transcription. In contrast, the octamer transcription factor from HeLa cells is not affected by metal-depletion and thus seems not to be a zinc metalloprotein.

MATERIALS AND METHODS

Preparation of nuclear extracts

HeLa cell nuclear extracts were prepared as previously described (27,28) with the following modifications for the metal-depleted extract used in the band shift assay. 50μl of extract was diluted to 400μl in a buffer consisting of 20mM HEPES-KOH pH7.9, 20%

glycerol, 50mM NaCl, 0.5mM DTT, 0.5mM PMSF and 4mM EDTA (final concentration). After 20 min incubation on ice, the extract was dialyzed against the dilution buffer (omitting the EDTA). The metal-depleted extracts used for the DNAase I protection assay and for the in vitro transcription reactions were differently prepared. Before $(NH_4)_2SO_4$ precipitation of a newly prepared extract, EDTA was added to 20mM and the extract was stirred for 20 min on ice. Prior to dialysis, EDTA was again added, to 12 mM, and the extract was dialyzed against 20mM HEPES-KOH pH7.9, 20% glycerol, 50mM NaCl, 0.5mM DTT and 0.5mM PMSF.

Band shift and DNAase I protection assay

In the band shift assay (29,30) binding reactions were carried out by incubating 2-4 fmol end-labelled DNA with 2-3 μg of nuclear protein and 0.5 μg poly(dI-dC) in a buffer containing 15mM HEPES pH7.9, 15% glycerol, 2% polyvinylalcohol, 36mM NaCl, 0.4mM DTT, 0.4mM PMSF and $ZnSO_4$ (when indicated). After 20 min at 30 ^{0}C, the reaction mixture was chilled on ice, loaded on a 4% polyacrylamide (29:1) gel in 0.25xTBE buffer and electrophoresed at 10 V/cm at 4 °C. For competition experiments, 2.5 pmol of oligonucleotide was added to the reaction mixture prior to the addition of the extract. The same binding conditions were used in the DNAase I protection assay (31), except that 5 fmol of a 5'end-labelled DNA fragment from the plasmid SvPr (labelled at a EcoRI site upstream of the SV40 early promoter) was incubated with 20-30 μg nuclear protein and with 1 μg poly(dI-dC). After incubation with the extract, reactions were mixed with $MgCl_2$ to 0.8mM. 0.5 μg DNAase I (no extract) or 2 μg DNAase I (with extract) was then added and allowed to digest for 90 sec on ice, before the DNAase I was inactivated by the addition of 0.36mM NaCl, 0.12% SDS, 12mM EDTA (final concentrations), followed by phenol/chloroform/isoamylalcohol (25:24:1) extraction and ethanol precipitation. The end-labelled DNA was analyzed in a 10% polyacrylamide (19:1) 7.5M urea gel.

Construction of DNA templates

The SV40 early promoter template (SvPr) was made by ligating a blunt-ended NcoI-XbaI fragment from the pET-1 plasmid (32) containing the SV40 early promoter (positions 38 to 99 in the SV40 genome (8) as determined by DNA sequencing) into a blunt-ended HindIII site in front of the rabbit ß-globin gene TATA box. This plasmid was derived from a 5'external deletion mutant of the ß-globin gene with endpoint at -37, that was linked to the HindIII site in pBR322 via HindIII linkers (33). The SvPr plasmid was subsequently recloned by ligating the EcoRI-BamHI fragment from SvPr with the BamHI-XbaI fragment from the ß-globin gene in plasmid pß1E (15) to EcoRI-XbaI digested pUC18 vector plasmid. The OTF-template and HSV IE-3 Sp1-template were

constructed in the following way. An oligonucleotide, with SacI and SalI protruding ends, comprising 25 bp of mouse IgH promoter (gene 104.2) sequences with an octamer factor binding site (34), was inserted in front of the ß-globin gene TATA box in the OVEC plasmid (28, T.Gerster, unpublished). The 32 bp HSV IE-3 Sp1 oligonucleotide (the sequence is shown in Figure 1a) was cloned into the same vector but by using a blunt-ended SalI site in front of the ß-globin gene TATA box.

In vitro transcription

In vitro transcription reactions and S1 nuclease mapping were as previously described (28) except that 3 µl of metal depleted extract, 0.2µg test gene and 0.2µg OVEC-REF (which has a deletion of 28 bp around the transcription initiation site ,28) and $ZnSO_4$ (when indicated) were used.

RESULTS

Transcription factor Sp1 requires zinc for specific DNA binding

In order to detect the Sp1 factor and the octamer transcription factor (OTF), we have used the band shift assay technique (29,30). In this assay, an end-labelled fragment is incubated with a nuclear extract and is then electrophoresed through a native polyacrylamide gel. Binding studies were done using HeLa cell nuclear extracts. A well-characterized Sp1 binding site was used, namely a 32 bp oligonucleotide containing a strong binding site present in the promoter of the Herpes simplex virus immediate-early 3 (HSV IE-3) gene (35). As shown in Figure 1a, the herpesvirus fragment gives rise to a major retarded complex and complex formation is prevented by competition with an excess of the unlabelled HSV IE-3 Sp1 oligonucleotide (lanes 1-3). In addition, several faint faster migrating complexes are seen which were not further analyzed. The results indicate that the Sp1 transcription factor can be detected in the bandshift assay using crude nuclear extracts.

To test if metal ions play a role in Sp1 binding we performed bandshift experiments with the metal chelator EDTA present in the binding reactions. By increasing the EDTA concentration Sp1 binding was gradually lost. Since it was not possible to restore binding in the presence of EDTA by addition of excess metal ions (not shown) further experiments were carried out in which the extract was first treated with EDTA and EDTA-metal complexes were then removed by extensive dialysis. As shown in Figure 1a, the binding of Sp1 to the site from the HSV IE-3 gene is completely abolished in such a metal-depleted extract (lane 4). Interestingly however, complex formation is restored upon addition of increasing amounts of zinc, with a maximal effect at 400µM zinc (lanes

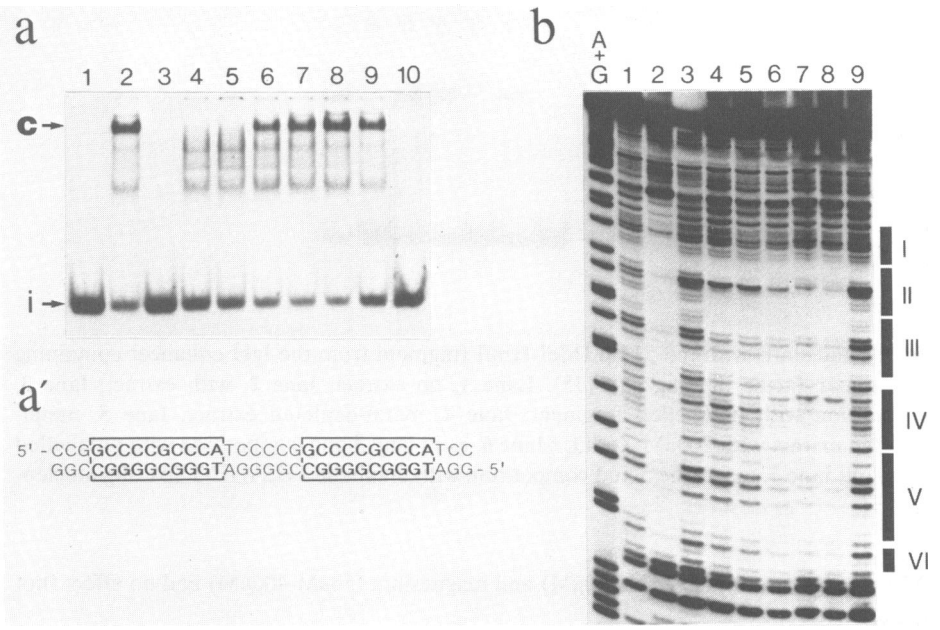

Fig.1. Band shift analyses using untreated or metal-depleted HeLa cell nuclear extracts. **(a)** Band shifts with a 32 bp Sp1 binding oligonucleotide (a'). The oligonucleotide contains two tandem copies of a 16 bp segment from the HSV IE-3 gene promoter. The Sp1 consensus binding sites (10) are boxed. Untreated HeLa cell nuclear extract was used in **lanes 1-3** and metal-depleted extract in **lanes 4-10. Lane 1**, no extract; **lane 2**, with extract; **lane 3**, competition with unlabelled oligonucleotide; **lane 4**, metal-depleted extract; **lane 5**, addition to 50μM $ZnSO_4$; **lane 6**, 100μM $ZnSO_4$; **lane 7**, 200μM $ZnSO_4$; **lane 8**, 400μM $ZnSO_4$; **lane 9**, 500μM $ZnSO_4$; **lane 10**, 400μM $ZnSO_4$ and competition with unlabelled oligonucleotide. i, input fragment; c, major complex.
(b) DNAase I footprint analyses on the SV40 early promoter (coding early strand). Untreated **(lanes 1-3)** or metal-depleted **(lanes 4-9)** Hela cell nuclear extracts were used. **Lane 1**, no extract; **lane 2**, with extract; **lane 3**, competition with unlabelled HSV IE-3 Sp1 oligonucleotide; **lane 4**, metal-depleted extract; **lane 5**, addition to 50μM $ZnSO_4$; **lane 6**, 100μM $ZnSO_4$; **lane 7**, 200μM $ZnSO_4$; **lane 8**, 400 μM $ZnSO_4$; **lane 9**, 400μM $ZnSO_4$ and competition with unlabelled HSV IE-3 Sp1 oligonucleotide. A+G, sequence ladder of the probe. The Sp1 binding sites I-VI are indicated by black bars (five bp are missing from site VI in the SvPr plasmid).

5-9). These results demonstrate that binding of Sp1 to its DNA recognition sequences is abolished by metal depletion and that binding is restored by addition of zinc. As expected, the complex formed by addition of zinc is also competed by unlabelled HSV IE-3 Sp1 oligonucleotide (lane 10). Addition of other metal ions such as cadmium

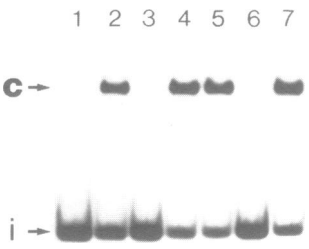

Fig.2 Band shifts with the 51 bp DdeI-HinfI fragment from the IgH enhancer containing an octamer factor binding site (15). **Lane 1**, no extract; **lane 2**, with extract; **lane 3**, competition with unlabelled fragment; **lane 4**, metal-depleted extract; **lane 5**, metal-depleted extract with 200μM $ZnSO_4$; **lane 6**, as in lane 5 and competition with unlabelled fragment; **lane 7**, as in lane 5 and competition with unlabelled HSV IE-3 Sp1 oligonucleotide.

(0.5μM-800μM), copper (1μM-200μM) and magnesium (50μM-400μM) had no effect (not shown).

In addition to the band shift experiments, we also performed DNAase I footprint analyses of the SV40 early promoter region, which contains multiple Sp1 binding sites (8,9). The strong protection of the tandemly organized Sp1 binding sites, evident in lane 2 of Figure 1b, is eliminated by depletion of zinc and other metals from the extract (lane 4). Addition of increasing concentrations of zinc almost completely restores the footprint pattern (lanes 5-8). Furthermore, competition with excess unlabelled Sp1 binding site from herpesvirus reveals the identity of the binding factor as Sp1 (lanes 3 and 9).

Binding of octamer transcription factor (OTF) is not sensitive to metal-depletion

In order to test if the octamer transcription factor requires heavy metals for binding, we used the 51 bp DdeI-HinfI fragment of the immunoglobulin heavy chain gene enhancer which contains a "decanucleotide", "octamer" consensus binding site 5'-ATGCAAATNA (15,36). The results presented in Figure 2 show that binding of the ubiquitous octamer transcription factor present in HeLa cells is not affected by depletion of metal ions or addition of zinc.

Binding of Sp1, but not OTF, is sensitive to the metal chelator 1,10-phenanthroline

Since Sp1 needs zinc to bind to its recognition sequences we expected the metal chelator 1,10-phenanthroline to inhibit the binding. This compound is an efficient chelator of Zn, Mn, Cu and Fe ions, and has been widely used to detect zinc in metallo-proteins such as methionyl-tRNA synthetase of Escherichia coli (1). As expected, binding of Sp1, but

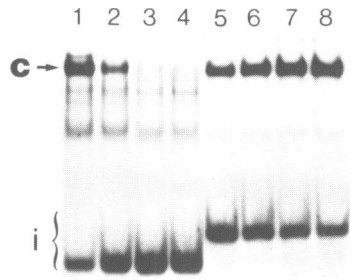

Fig.3 Band shifts using a HeLa cell extract with increasing amounts of 1,10-phenanthroline in the binding reaction. The following probes were used: **Lanes 1-4**, 32 bp HSV IE-3 Sp1 oligonucleotide; **lanes 5-8**, 51 bp IgH enhancer fragment. **Lanes 1** and **5**, with extract only; **lanes 2** and **6**, addition to 90μM 1,10-phenanthroline; **lanes 3** and **7**, 450μM; **lanes 4** and **8**, 900μM.

not OTF, is very sensitive to 1,10-phenanthroline. At 90μM phenanthroline only a small amount of the Sp1 complex is formed and at 450μM complex formation is abolished. (Figure 3).

Sp1 requires zinc also for activator function

As demonstrated above, transcription factor Sp1 requires zinc to bind to its recognition sequences in vitro. To address the functional relevance of this finding we have performed in vitro transcription reactions using the metal-depleted extract. Rabbit ß-globin gene templates with the following Sp1-binding sites, inserted in front of the TATA box, were tested: the simian virus 40 (SV40) early promoter and the HSV IE-3 Sp1 oligonucleotide (see Figure 1a). A template with a promoter containing an octamer motif upstream of the ß-globin gene TATA box (OTF-template) that is efficiently transcribed in HeLa cell extracts (M.M. Müller and W. Schaffner, unpublished results) and the vector plasmid were also included in the analyses. The reference plasmid OVEC-REF (28), which contains a 28 bp deletion around the transcription initiation site, was included as an internal standard in each reaction. Figure 4 shows S1-nuclease mapping of RNA synthesized from circular templates in the metal-depleted HeLa cell nuclear extract. Transcription from the vector plasmid, which has no promoter elements other than the TATA box, or from the OTF template is not severely impeded and is only marginally increased when zinc ions are added to the reactions (Figures 4a, 4b, lanes 1-4). Our results show that it is possible to largely deplete a nuclear extract of heavy metals and still retain transcriptional activity from the OTF template. This is in agreement with the results obtained in the binding studies presented in Figures 2 and 3 and indicates that the HeLa cell octamer

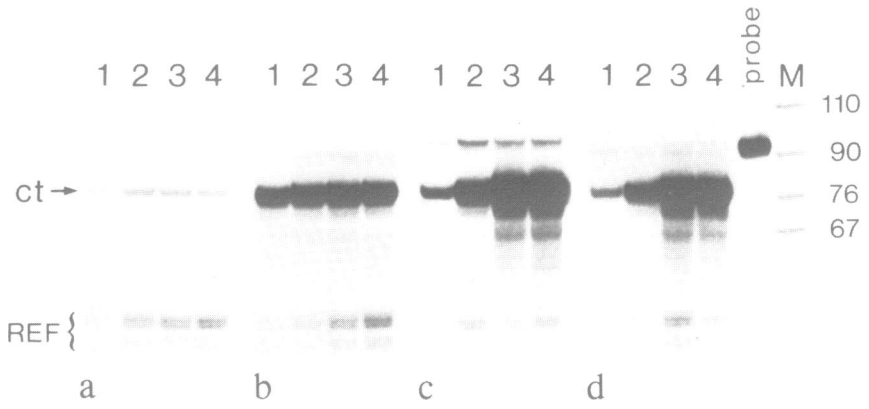

Fig.4. In vitro transcription in a metal-depleted HeLa cell nuclear extract. Quantitative S1 nuclease mapping of RNA synthesized from circular templates. The following templates were used. **(a)** OVEC plasmid, no elements inserted in front of TATA box; **(b)** octamer transcription factor (OTF)-template; **(c)** SV40 early promoter (SvPr); **(d)** HSV IE-3 Sp1-template. **Lane 1**, extract only; **lane 2**, addition to 400μM $ZnSO_4$; **lane 3**, 800μM $ZnSO_4$; **lane 4**, 1600μM $ZnSO_4$. probe, a 93 nt S1 probe extending between positions -18 and +75 on the non-coding strand of the ß-globin gene; M, HpaII-digested pBR322 marker DNA; ct, correctly initiated transcripts; REF, reference gene transcripts.

transcription factor does not need zinc for activity or, less likely, that zinc is not released from OTF by EDTA. The SV40 early promoter and the Sp1 binding sites of the HSV IE-3 gene promoter stimulate transcription, to some extent, in the depleted extract (Figures 4c, 4d, lane 1). However, addition of increasing amounts of zinc to the reaction greatly stimulates transcription from the two templates, to a level well above that of the OTF template (Figures 4c, 4d, lanes 2-4). The zinc concentration required for maximal transcription is quite high but is similar to that required for binding of Sp1 to the SV40 early promoter (Figure 1b) when the different experimental conditions, such as the amount of extract, reaction volume, and additional EDTA in the transcription buffer are taken into account. Transcription from the reference gene (OVEC-REF), which contains the SV40 enhancer in front of the globin gene TATA box, is also stimulated to some extent by zinc. This latter observation has not been further analyzed. Taken together, the results show that zinc is essential for Sp1 factor-directed transcription.

DISCUSSION

We have developed an inducible in vitro transcription assay to reveal the presence and functional relevance of heavy metal ions in transcription factors. Our data show that Sp1

requires zinc for binding and for transcriptional activity. The results are in agreement with the recently published findings of R. Tjian and collegues that Sp1 contains three zinc finger motifs and that purified Sp1 requires zinc for DNA binding (11). However, in their case binding of Sp1 was found to be insensitive to the metal chelator 1,10-phenanthroline. The difference to our data may be explained by the fact that an excess of magnesium ions over metal chelator was used in their binding reaction (11).

The HeLa cell octamer transcription factor was found to be insensitive to metal-depletion, both in the binding assay and in the in vitro transcription assay. This indicates that OTF does not need zinc for activity or, less likely, that zinc is not released from the protein by treatment with EDTA or 1,10-phenanthroline. Thus, we predict that HeLa cell octamer transcription factor does not contain zinc finger-like structures important for specific DNA binding. In agreement with this expectation Sp1, but not OTF, must be renatured in presence of zinc ions after SDS polyacrylamide gel electrophoresis in order to regain specific DNA binding (G. Westin and M.M. Müller, unpublished). RNA polymerases are known to contain zinc ions (1) but in a very tightly bound form since the activaty of this enzyme is hardly, if at all, affected by the EDTA/dialysis treatment.

Multiple zinc finger-like structures have been found in several RNA polymerase II transcription factors (5,6) and similar structures have also been found in developmental proteins, such as Krüppel, Hunchback and the protein encoded in the sex-determining region of human Y chromosomes (37-39). Indeed, multigene families encoding zinc finger-like structures have been cloned and these genes are likely to play a regulatory role at the transcriptional level (40,41). We think that the simple procedures described in this work should be helpful in determining the role of heavy metal ions in proteins with putative metal-binding domains.

ACKNOWLEDGEMENTS

We thank Dr. Gotthold Schaffner for synthesizing the oligonucleotides used for our studies and Fritz Ochsenbein for expert graphical work. We also thank Drs. Hugh Pelham and Deborah Maguire for critical reading of the manuscript and valuable comments. This work was supported by the Kanton of Zürich, by the Swiss National Science Foundation and by an EMBO long-term fellowship to G.W.

REFERENCES

1. Posorske, L. H., Cohn, M., Yanagisawa, N. and Auld, D. S. (1979) Bioch. Biophys. Acta, 576, 128-133, and references therein.

2. Hanas, J.S., Hazuda, D.J., Bogenhagen, D.F., Wu, F.Y.-H. and Wu, C.-W. (1983) J.Biol.Chem., 258, 14120-14125.
3. Johnston, M. (1987) Nature, 328, 353-355.
4. Miller, J., McLachlan, A.D. and Klug, A. (1985) EMBO J., 4, 1609-1614.
5. Klug, A. and Rhodes, D. (1987) Trends Biochem., 12, 464-469.
6. Berg, J.M. (1986) Science, 232, 485-487.
7. Blumberg, H., Eisen, A., Sledziewski, A., Bader, D. and Young, E.T. (1987) Nature, 328, 443-445.
8. Dynan, W.S. and Tjian, R. (1983) Cell, 35, 79-87.
9. Kadonaga, J.T., Jones, K.A. and Tjian, R. (1986) Trends Biochem., 11, 20-23.
10. Briggs, M.R., Kadonaga, J.T., Bell, S.P. and Tjian, R. (1986) Science, 234, 47-52.
11. Kadonaga, J.T., Carner, K.R., Masiarz, F.R. and Tjian, R. (1987) Cell, 51, 1079-1090.
12. Falkner, F.G., Neumann, E. and Zachau, H.G. (1984) Hoppe-Seyler's Z.Physiol.Chem., 365, 1331-1343.
13. Parslow, T.G., Blair, D.L., Murphy, W.J. and Granner, D.K. (1984) Proc.Natl.Acad. Sci. USA, 81, 2650-2654.
14. Falkner, F.G., Mocikat, R. and Zachau, H.G. (1986) Nucleic Acids Res., 14, 8819-8827
15. Gerster, T., Matthias, P., Thali, M., Jiricny, J. and Schaffner,W. (1987) EMBO J., 6, 1323-1330.
16. Wirth, T., Staudt, L. and Baltimore, D. (1987) Nature, 329, 174-133.
17. Fletcher, C., Heintz, N. and Roeder, R.G. (1987) Cell, 51, 773-781.
18. Scheidereit, C., Heguy, A. and Roeder, R.G. (1987) Cell, 51, 783-793.
19. Bohmann, D., Keller, W., Dale, T., Schöler, H.R., Tebb, G. and Mattaj, I.W. (1987) Nature 325, 268-272.
20. Rosales, R., Vigneron, M., Macchi, M., Davidson, I., Xiao, J.H. and Chambon, P. (1987) EMBO J., 6, 3015-3025.
21. Sturm, R., Baumruker, T., Franza, B.R. and Herr, W. (1987) Genes and Dev., 1, 1147-1160.
22. Barberis, A., Superti-Furga, G. and Busslinger, M. (1987) Cell, 50, 347-359.
23. Carbon, P.,Murgo, S., Ebel,J.P., Krol, A., Tebb, G. and Mattaj, I.W. (1987) Cell, 51, 71-79.
24. Janson, L., Bark, C. and Pettersson, U. (1987) Nucleic Acids Res., 15, 4997-5016.
25. Pruijn, G.J.M., van Driel, W. and van der Vliet, P.C. (1986) Nature, 322, 656-659.
26. Pruijn, G.J.M., van Driel, W., van Miltenburg, R.T. and van der Vliet, P.C. (1987) EMBO J., 6, 3771-3778.
27. Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) Nucleic Acids Res., 11, 1475-1489.
28. Westin, G., Gerster, T., Müller, M.M., Schaffner, G. and Schaffner, W. (1987) Nucleic Acids Res., 15, 6787-6798.
29. Fried, M. and Crothers, D.M. (1981) Nucleic Acids Res., 9, 6505-6525.
30. Garner, M.M. and Rezvin, A. (1981) Nucleic Acids Res., 9, 3047-3060.
31. Galas, D. and Schmitz, A. (1981) Nucleic Acids Res., 5, 3157-3170.
32. Weber, F., de Villiers, J. and Schaffner, W. (1984) Cell, 36, 983-992.
33. Dierks, P., van Ooyen, A., Cochran, M.D., Dobkin, C., Reiser, J. and Weissmann, C. (1983) Cell, 32, 695-706.
34. Ballard, D.W. and Bothwell, A. (1986) Proc. natl. Acad. Sci. USA, 83, 9626-9630.
35. Jones, K.A. and Tjian, R. (1985) Nature, 317, 179-182.

36. Staudt, L.M., Singh, H., Sen, R., Wirth, T., Sharp, P.A. and Baltimore, D. (1986) Nature, 323, 640-643 .
37. Rosenberg, U.B., Schröder, C., Preiss, A., Kienlin, A., Côté, S., Riede, I. and Jäckle, H. (1986) Nature, 319, 336-339.
38. Tautz, D., Lehmann, R., Schnürch, H., Schuh, R., Seifert, E., Kienlin, A., Jones, K. and Jäckle, H. (1987) Nature, 327, 383-389.
39. Page, D.C., Mosher, R., Simpson, E.M., Fischer, E.M.C., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A. and Brown, L.G. (1987) Cell, 51, 1091-1104.
40. Chowdhury, K., Deutsch, U. and Gruss, P. (1987) Cell, 48, 771-778.
41. Chavrier, P., Lemaire, P., Revelant, O., Bravo, R. and Charnay, P. (1988) Mol. Cell. Biol., 8, 1319-1326.