Identification of a Conserved Oxidation-sensitive Cysteine Residue in the NFI Family of DNA-binding Proteins*

(Smarrjit Bandyopadhyay and Richard M. Gronostajski†)

From the Department of Cancer Biology, Cleveland Clinic Foundation Research Institute, Cleveland, Ohio 44195 and Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106

The nuclear factor I (NFI) family of site-specific DNA-binding proteins plays a role in both transcription and adenovirus DNA replication. The DNA binding domain of NFI family members contains 4 cysteine residues (Cys-2, Cys-3, Cys-4, and Cys-5) that are conserved in all NFI proteins. Mutation of the Cys-2, Cys-4, and Cys-5 residues in the human NFI-C protein to several other amino acids abolished DNA binding, while 8 of 10 mutations of the Cys-3 residue had little or no effect on binding. Wild-type NFI-C was inactivated by N-ethylmaleimide, while the active Cys-3 mutant proteins were resistant to N-ethylmaleimide. Treatment of wild-type NFI in vitro with the oxidizing agent diamide also inactivated DNA binding, and subsequent reduction with dithiothreitol restored binding activity. The active Cys-3 mutant NFI proteins were resistant to diamide-inactivation, indicating that the Cys-3 residue is required for modulation of DNA-binding by oxidation state. These studies indicate that oxidative-inactivation can play an important role in the modifying NFI-DNA-protein interactions. The presence of this nonessential Cys-3 residue in all known NFI proteins raises the possibility that it may function in a manner similar to redox-sensitive cysteine residues found in other site-specific DNA-binding proteins.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Cleveland Clinic Foundation, Dept. of Cancer Biology NN-1, 9500 Euclid Ave., Cleveland, OH 44195. Tel.: 216-445-6629; Fax: 216-445-6269.

*The abbreviations used are: NFI, nuclear factor I; NEM, N-ethylmaleimide; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); DTT, dithiothreitol; GMIS, gel mobility shift; PAGE, polyacrylamide gel electrophoresis.

The nuclear factor I (NFI) family of site-specific DNA-binding proteins plays a role in both transcription and adenovirus DNA replication. The DNA binding domain of NFI family members contains 4 cysteine residues (Cys-2, Cys-3, Cys-4, and Cys-5) that are conserved in all NFI proteins. Mutation of the Cys-2, Cys-4, and Cys-5 residues in the human NFI-C protein to several other amino acids abolished DNA binding, while 8 of 10 mutations of the Cys-3 residue had little or no effect on binding. Wild-type NFI-C was inactivated by N-ethylmaleimide in vitro, while the active Cys-3 mutant proteins were resistant to N-ethylmaleimide. Treatment of wild-type NFI in vitro with the oxidizing agent diamide also inactivated DNA binding, and subsequent reduction with dithiothreitol restored binding activity. The active Cys-3 mutant NFI proteins were resistant to diamide-inactivation, indicating that the Cys-3 residue is required for modulation of DNA-binding by oxidation state. These studies indicate that oxidative-inactivation can play an important role in the modifying NFI-DNA-protein interactions. The presence of this nonessential Cys-3 residue in all known NFI proteins raises the possibility that it may function in a manner similar to redox-sensitive cysteine residues found in other site-specific DNA-binding proteins.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Cleveland Clinic Foundation, Dept. of Cancer Biology NN-1, 9500 Euclid Ave., Cleveland, OH 44195. Tel.: 216-445-6629; Fax: 216-445-6269.

*The abbreviations used are: NFI, nuclear factor I; NEM, N-ethylmaleimide; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); DTT, dithiothreitol; GMIS, gel mobility shift; PAGE, polyacrylamide gel electrophoresis.

 Plasmaid DNA and Mutagenesis—The pET220 vector encoding the NH2-terminal 220 amino acids of the human NFI-C protein (hNFI-C220) expressed from a T7 promoter was described previously (18). The cloned human NFI-C cDNA was a gift of Dr. R. Tjian. This 220-residue DNA binding domain of hNFI-C/CTFl protein was shown previously to be sufficient for DNA binding activity and stimulation of adenovirus replication but lacks the COOH-terminal proline-rich domain impart-
Oxidative-sensitivity DNA Binding by NFI

Our previous studies indicated that the DNA binding activity of NFI was inactivated by the sulfhydryl alkylating agent NEM and that Ser substitutions at the Cys-2, Cys-3, Cys-4, and Cys-5 positions of hNFI-C220 inactivated DNA binding while Ser substitution at Cys-1 had no effect on binding (15, 33). However, we recently observed that cells expressing the C3S mutant NFI protein had a somewhat slower growth rate in liquid cultures and a smaller colony size at 37°C when compared to those expressing the wild-type protein. In addition, we determined that 5–10-fold more active NFI protein was present in soluble extracts of E. coli when isopropyl 1-thio-β-D-galactopyranoside induction was performed at 17°C rather than the 37°C temperature used earlier (15). These results suggested that low temperature induction of NFI protein might reduce any potential toxicity of the wild-type and mutant NFI proteins in E. coli. In the present study we have used this low temperature induction system to examine the effects of multiple mutations at the 4 conserved Cys residues present in the hNFI-C220 protein. Our initial aim was to determine whether any other amino acids could substitute for the conserved Cys residues of hNFI-C220 with retention of DNA binding activity. Several amino acid substitutions were made at each conserved Cys position by site-directed mutagenesis, and the DNA binding activity of NFI in extracts containing wild-type and mutant NFI proteins was measured using a gel mobility shift assay (6, 15). As summarized in Fig. 1, extracts containing recombinant hNFI-C220 with substitutions at the Cys-2 (Ser, Trp, Phe, Gly, Leu, Val, Ile, His), Cys-4 (Ser, Trp, Arg, Phe, Gly, Leu, Val, His, Ala, Asp, Tyr, Met), and Cys-5 (Ser, Trp, Gly, Leu, His, Ala, Tyr, Thr, Ile, Lys, Pro) positions were devoid of DNA binding activity (binding <0.1% of wild-type NFI-containing extracts). However, DNA binding activity was detected in 8 of 11 extracts expressing wild-type or mutant NFI proteins generated a single specific protein-DNA complex with the FIB-2.6 nucleotide (Fig. 2A, lane 4, denoted by an arrow at the left) and showed no binding of the mutant oligonucleotide FIB-2.6C2 (Fig. 2A, lane 2). The level of DNA binding activity was similar in extracts containing either wild-type NFI (Fig. 2A, lane 4) or mutant NFI proteins with serine, leucine, valine, threonine, isoleucine, alanine, or histidine substitutions at the Cys-3 position (Fig. 2A, lanes 5, 7, 8, 9, 10, and 11, respectively). However, substitution of the Cys-3 position with glycine yielded extracts with reduced DNA binding activity (Fig. 2A, lane 6).

DNA binding activity was abolished by arginine or tryptophan

\[\text{R. M. Gronostajski and S. Bandyopadhyay, unpublished results.}\]
FIG. 1. Effects of mutation of cysteine residues of hNFI-C220. Schematic diagram showing the non-conserved cysteine 1 residue (Cys-1) and the four conserved cysteine residues 2, 3, 4, and 5 (Cys-2 to Cys-5) present in the DNA binding domain of hNFI-C220. Mutations were generated at each of these positions as described under "Materials and Methods," and substitutions that were shown to be active and inactive for DNA binding as assessed by gel mobility shift assays are shown above and below the sequence line, respectively. The designations Cys-1 to Cys-5 correspond to absolute residue positions Cys-79 (Cys1), Cys-95 (Cys2), Cys-111 (Cys3), Cys-145 (Cys4), and Cys-154 (Cys5) of the hNFI-CTF1 protein (4) (NCBI sequence identification no. 30266).

substitution at Cys-3 (Fig. 2A, lanes 13 and 14, respectively). As seen previously, any substitution at the Cys-2, Cys-4, and Cys-5 positions of NFI abolished DNA binding activity (Fig. 2A, lanes 15–17, and data not shown).

To ensure that similar quantities of wild-type or mutant proteins were used to assess DNA binding activity, immunodetection of NFI-C protein was performed after SDS-PAGE analysis of bacterial extracts. Equivalent quantities of NFI-C were present in extracts containing either wild-type (Fig. 2B, lane 2, NFI band denoted by the arrow at the right) or mutant NFI-C proteins (Fig. 2B, lanes 3–12); including extracts containing the inactive arginine (Fig. 2B, lane 11) and tryptophan (Fig. 2B, lane 12) mutants. Similar levels of immunoreactive NFI protein were also present in extracts containing the inactive Cys-2, Cys-4, and Cys-5 mutant proteins (not shown). Titration experiments indicated that less than a 3-fold difference in NFI protein levels could be readily detected by this immunoblot assay (not shown). Control extracts from cells containing the parent vector lacking an NFI coding region contained only low levels of a number of nonspecific stained bands (Fig. 2B, lane 1).

We had previously shown that the DNA binding activity of NFI was inactivated by incubation with the sulphydryl modifying agents NEM and DTNB (15). The finding that the conserved Cys-3 residue of NFI-C could be substituted with a variety of different amino acids with complete retention of DNA binding activity, led us to test the NEM sensitivity of the active Cys-3 mutants. While the wild-type NFI protein was completely inactivated by NEM (Fig. 3, lane 2 versus 1), all the active Cys-3 mutants were resistant to NEM-inactivation (Fig. 3, lanes 3–18). The active Cys-3 mutants were also resistant to inactivation by the sulphydryl modifying agent DTNB (not shown). These data indicate that the cysteine residue present at the Cys-3 position is the only readily accessible alkylation-sensitive sulphydryl residue involved in NFI-DNA interaction.

Since sensitivity to NEM is a common feature of a number of proteins whose DNA binding activity is affected by oxidation (22, 24, 27, 28), we examined the sensitivity of the DNA binding activity of wild-type and mutant NFI proteins to oxidation by the chemical oxidizing agent diamide (22, 41). Treatment of wild-type NFI with 1 mM diamide abolished DNA binding activity (Fig. 4A, lane 2 versus lane 1, NFI-DNA complex denoted by the arrow marked A at the left). Although inactivation of NFI by NEM is irreversible (data not shown), the inactivation by diamide was reversed by subsequent addition of a 10-fold molar excess of DTT (Fig. 4A, lane 3). Inactivation by diamide and reactivation by excess DTT was also seen with a mutant
with both wild-type NFI and mutant NFI proteins where the Cys-3 position was replaced by an alanine, leucine, or valine residue (Fig. 5, lanes 8, 12, 14, and 16, respectively). As seen with wild-type NFI, no cross-linking was detected when the C2 oligonucleotide was incubated with the mutant proteins (Fig. 5, lanes 11, 13, and 15) or in the absence of UV irradiation (Fig. 5, lane 9, and data not shown). Thus, NFI proteins with alanine, leucine, or valine substitutions at the Cys-3 position form UV-induced cross-links with DNA with an efficiency similar to that of wild-type hNFI-C220.

**DISCUSSION**

These findings indicate that of the 4 cysteine residues in hNFI-C which are completely conserved between known NFI family members (15), the Cys-2, Cys-4, and Cys-5 residues are intolerant of amino acid substitutions (Figs. 1 and 2), while the Cys-3 position may play a role in reox regulation of NFI DNA binding activity (Fig. 4). The complete intolerance of the Cys-2, Cys-4, and Cys-5 residues to substitution indicate an essential role for these residues in some aspect of NFI-DNA interaction. Although our lack of information on the structure of the NFI DNA binding domain prevents us from determining the precise role of these essential residues, our findings indicate a number of possibilities. Since the relatively conservative substitutions of serine or alanine at these positions completely abolished DNA binding activity (Fig. 1), it appears that even minor changes in the charge or size of the residues at these positions disrupt NFI function. This finding, in combination with our observation that all of the active Cys-3 substitution mutants are resistant to inactivation by NEM and diamide (Figs. 3 and 4), suggests that the Cys-2, Cys-4, and Cys-5 residues may be inaccessible to modification with NEM or diamide and thus may be buried in the core of the NFI protein. It is possible, for example, that two of these residues may participate in an intramolecular disulfide bond in NFI that is required for DNA binding activity, although the known resistance of the DNA binding activity of NFI to the presence 50–100 mM DTT (not shown) would indicate that such a disulfide bond would have to be unusually resistant to reduction. Another possibility is that these residues operate together for some essential function in NFI, such as the coordination of a metal ion needed for DNA binding. However, no such essential metal ion has been demonstrated for NFI-DNA interaction (11, 15), and DNA binding by NFI is reduced by less than 50% in the absence of MgCl₂ and in the presence of 10 mM EDTA. Since NFI has been shown to interact with DNA as a dimer (8–10), it will be important to determine which potential step in NFI function (i.e. initial subunit folding, subunit dimerization, or dimer-DNA interaction) is affected by mutation of the Cys-2, Cys-4, and Cys-5 residues.

The ability of the majority of NFI Cys-3 substitution mutants to bind DNA indicates that this cysteine residue is unlikely to be directly involved in NFI-DNA interactions. However, Arg or Trp substitution at the Cys-3 position of NFI abolished DNA binding activity (Figs. 1 and 2), and these two residues produce the largest side-chain substitutions generated in this study. In addition, the sensitivity of wild-type NFI to inactivation by NEM (Fig. 3), diamide (Fig. 4), and DTNB (15), and the resistance of Cys-3 substitution mutants to such inactivation indicates that the addition of large chemical adducts at the Cys-3 position can abolish DNA binding by NFI. Given these results, it is perhaps surprising that DNA binding by NFI partially protects the protein from inactivation by diamide (Fig. 4B), NEM or DTNB (15). It may be that the Cys-3 residue is located close to the DNA in the NFI-DNA complex but is not directly involved in protein-DNA interactions. This appears to be true

---

38. Bandopadhyay and R. Gronostajski, unpublished data.
for the oxidation-sensitive cysteine residues detected in the c-Fos and c-Jun proteins, which are located near the basic DNA binding domains of these proteins but do not appear to directly contact DNA (22). Alternatively, DNA binding by NFI may induce a conformational change in the protein that reduces the accessibility of the Cys-3 residue to modification even though the Cys-3 position is quite distant from the actually DNA binding interface. Another possibility is that the Cys-3 residue in wild-type NFI does indeed interact directly with DNA, but that mutation of the residue has little or no effect on DNA binding affinity. Although such an effect was seen in the bovine papilloma virus type 1 E2 protein, where conversion of a cysteine residue that only a small effect on DNA binding affinity (27). Further analysis of the structure and function of the DNA binding domain of NFI will be required to distinguish between these possibilities. The DNA binding activity of the active C3S mutant protein was somewhat surprising, since we had previously failed to detect binding activity in a C3S mutant of hNFI-C220 (15). We attribute the former inability to detect activity in extracts of this mutant to two potential causes. First, the C3S mutation appears to modestly increase the toxicity of the NFI gene product in E. coli as assessed by a reduced growth rate in liquid cultures and a smaller colony size following transformation of plasmids containing this mutation. In addition, the induction conditions used previously (37 °C for 3 h) yield levels of active NFI that are 5-10-fold lower than those obtained using the conditions described here (17 °C for 16 h). Induction of NFI expression at 17 °C appears to significantly reduce the toxicity seen at 37 °C, and we have determined that both the new C3 mutants described above and the former C3S mutant protein possess levels of DNA binding activity comparable to that of wild-type NFI. We are currently determining whether mutations at the Cys-3 position affect the temperature sensitivity, pH dependence, or other parameters of the DNA binding activity of hNFI-C.

The ability of NFI proteins containing alanine, isoleucine, and valine substitutions at the Cys-3 position to cross-link to DNA after UV irradiation (Fig. 5) demonstrates a second property of NFI that is unaffected by mutagenesis at this position. Although the precise residue(s) of NFI that cross-link to DNA have not been determined, previous studies with other DNA-binding proteins indicate that a variety of amino acids can participate in such cross-linking reactions. While a specific cysteine residue of the bacteriophage fd gene 5 protein cross-links to single-stranded DNA (Cys-33) (42) after UV irradiation, specific phenylalanine residues on the bacteriophage T4 gene 32 protein (Phe-183) (44), the E. coli SSB protein (Phe-60) (45), and the adenovirus DNA-binding protein (Phe-418) (46) cross-link to DNA under similar conditions (47, 48). While UV cross-links have not been identified between alanine residues of proteins and specific bases of native DNA, an alanine residue of GCN4 (Ala-238) cross-links to 5-bromouridine-substituted DNA when a 5-bromouridine base replaces a thymine base at position +3 of a GCN4 binding site (49). In addition, although no studies have been reported which examine the relative ability of different natural amino acids at the same position of a protein to cross-link to DNA after UV irradiation, it is likely that the efficiency of cross-linking is related to both the proximity of an amino acid residue to DNA and its chemical reactivity (47, 48). Thus, the equivalent degree of cross-linking seen after UV irradiation of wild-type and mutant NFI-DNA complexes suggests that the Cys-3 position is not the site of UV cross-linking, but is not conclusive. However, the similar efficiency of both DNA binding ability, as measured in the gel mobility shift assay (Fig. 2), and UV cross-linking (Fig. 5) of the
Fig. 5. UV Cross-linking assay of wild-type and Cys-3 mutants of NFI-C220. Extracts (20 μg) of control cells (Control), or cells expressing wild-type NFI (WT) or Cys-3 mutant NFI-C220 proteins (C3A, C3I, and C3V) were incubated in reactions containing 100 fmol of [\(^{32}P\)]PPIB-2.6 (2.6) or [\(^{32}P\)]PPIB-2.6C2 (C2) DNA as indicated, UV irradiated (+) or not irradiated (−), and analyzed by SDS-PAGE. Lane 1, control extract, 2.6 DNA, −UV; Lane 2, control extract, 2.6 DNA, +UV; Lane 3, control extract, 2.6 DNA, −UV; Lane 4, control extract, 2.6 DNA, +UV; Lane 5, wild-type NFI, C2 DNA, −UV; Lane 6, wild-type NFI, C2 DNA, +UV; Lane 7, wild-type NFI, 2.6 DNA, −UV; Lane 8, wild-type NFI, 2.6 DNA, +UV; Lane 9, C3A NFI, C2 DNA, −UV; Lane 10, C3A NFI, C2 DNA, +UV; Lane 11, C3I NFI, 2.6 DNA, −UV; Lane 12, C3A NFI, 2.6 DNA, +UV; Lane 13, C3I NFI, C2 DNA, −UV; Lane 14, C3I NFI, 2.6 DNA, +UV; Lane 15, C3V NFI, C2 DNA, +UV; Lane 16, C3V NFI, 2.6 DNA, +UV.

Fig. 6. Sequences surrounding oxidation-sensitive cysteine residues in NFI-C and other redox-sensitive DNA-binding proteins. The protein sequences surrounding the known oxidation-sensitive cysteine residues of the NFI-C (this report), Myb (26), NFκB/Rel (24, 53), BPV-1 E2 (27), c-Fos (22), and c-Jun (22) proteins are shown with the sensitive cysteine residue in the center. Basic protein residues (arginine, R, and lysine, L) are denoted by + and acidic residues (aspartate, D, and glutamate, E) are denoted by − above the sequence, respectively.

Various Cys-3 mutants and wild type NFI proteins, indicates that a large change in the overall structure of the DNA-protein complex due to these mutations is unlikely. It will be necessary to map the specific amino acid residue(s) involved in NFI-DNA cross-linking to more fully interpret these data.

The finding that all of the active Cys-3 mutants of NFI are resistant to inactivation by both NEM (Fig. 2) or diamide oxidation (Fig. 4) suggests that NFI may be subject to redox regulation in vitro and in vivo. Redox regulation of DNA binding activity was first demonstrated with the OxyR protein of E. coli, where oxidation of the protein changes its footprint on DNA and is required for transcriptional activation by OxyR on specific promoter sites (50, 51). Subsequent studies in eukaryotes have demonstrated that the DNA binding activities of a number of site-specific DNA-binding proteins, including c-Fos and c-Jun (22, 23, 52), NFκB/Rel (24, 25), BPV-1 E2 (27), USF (28), and Myb (26), are inactivated by oxidation of specific cysteine residues in vitro. These proteins contain a wide variety of different DNA binding structural domains, which include the basic leucine zipper domain (c-Fos and c-Jun), the helix-loophelix domain (USF), an α-helix, dimeric β-barrel domain (BPV/E2) (43), and two as yet uncharacterized DNA binding domains (Myb and NFκB/Rel). For each of these DNA-binding proteins, a specific cysteine residue or residues is sensitive to oxidation by diamide or other oxidizing and alkylating agents in vitro, and mutation of these sensitive residues generates proteins that are resistant to oxidative inactivation or NEM (22, 24, 26–28, 53). These properties, sensitivity to oxidation/alkylation in vitro and subsequent resistance to inactivation following mutation, are similar to those described here for NFI. However, there are numerous intracellular proteins that share similar properties for which there is no evidence of redox regulation. Thus, additional studies on oxidative inactivation and the characteristics of oxidation-resistant mutants of NFI in vivo are essential to determine whether the transcriptional activation properties of NFI family members are modified by intracellular redox state.

Although there appear to be common features in the oxidative inactivation of several DNA-binding proteins, there are also distinct differences in the apparent mechanism of inactivation between the different proteins. Inter- and intramolecular disulfide bonds have been shown to form during oxidative inactivation of USF (28) and NFκB (24) and have been proposed to interfere with DNA binding by these proteins. However, inter- or intramolecular disulfide bonds have not been detected in the oxidative inactivation of c-Fos/c-Jun heterodimers or the BPV-1 E2 protein, and several groups have proposed that inactivation of these proteins may be mediated by oxidation of sulfenic acid residues (–SOH) (22, 27, 52, 54). Reversible oxidation of a specific cysteine residue to sulfenic acid has also been proposed for the reversible modification of the OxyR protein in E. coli (50). Although frequently unstable, stabilized sulfenic acid residues have now been detected in a number of flavoproteins and oxidized enzymes and represent a reversible oxidation state of cysteine residues (see Ref. 54 for a review). While the mechanism for stabilization of sulfenic acid residues on proteins is unknown, one model that has been proposed is that such oxidized residues may be stabilized by a highly basic local environment surrounding the oxidation-sensitive cysteine residues of a number of proteins (Fig. 6) (22, 27, 50, 54). It is of interest that the oxidation sensitive Cys-3 residues of hNFI-C is located within such a potentially highly basic environment (Fig. 6, hNFI-C). In addition, although the mechanism of oxidative inactivation of NFI is currently unknown, we have failed to detect any evidence by SDS-PAGE for inter- or intramolecular disulfide bond formation during oxidative inactivation of wild-type NFI. Also, the faster mobility of NFI-DNA complexes after treatment with diamide is more consistent with a small change in the charge of the complex than with covalent dimer formation. Similar increases in the mobility of NFI-DNA complexes were seen previously after treatment of the complexes with DTNB (15). Since mutation of the conserved Cys-2, Cys-4, and Cys-5 residues inactivates DNA binding, it will be difficult to determine by mutagenesis whether any of these residues participate in inter- or intramolecular disulfide linkages during oxidative inactivation of NFI. Thus, further biochemical analysis of the mechanism for oxidative inactivation of NFI in vitro is needed to resolve this issue.

In addition to studies on oxidative inactivation in vitro, there is growing evidence that redox regulation may play an important role in gene expression and cellular physiology in vivo.
example, changing the oxidation sensitive cysteine residue of the c-Fos protein to a serine residue both activates its DNA-binding activity in vitro and increases its oncogenic potential in vivo (55). Similarly, the oxidation-sensitive cysteine residue in the c-Jun protein is converted to a serine residue in the oncogenic v-Jun protein (22). Also, overexpression of human thioredoxin, a protein expected to increase the intracellular reducing environment, or treatment of cells with anti-oxidants can modulate transcriptional activation by NF-{kappa}B.

18. Lovering, R., Hanson, I. M., Borden, K. L., Martin, S., O'Reilly, N. J., Evan, G. I., Rahman, D., Pappin, D. J., Towseal, J., and Freeman, P. S. (1995). Proc. Natl. Acad. Sci. U. S. A. 92, 7171-7175.

19. Jackson, S. P., and Tjian, R. (1988) Cell 55, 125-133.

20. Jackson, S. P., and Tjian, R. (1988) Proc. Natl. Acad. Sci. U. S. A. 86, 2113-2117.

21. Jackson, S. P., MacDonald, J. J., Lee-Miller, S., and Tjian, R. (1990) Cell 63, 155-165.

22. Abate, C., Pael, L., Kauwer, J. J., and Curran, T. (1990) Science 240, 1157-1161.

23. McBride, A. A., Klauser, R. D., and Howley, P. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7351-7355.

24. Pulugurta, P., Kato, H., and Rodger, R. G. (1992) J. Biol. Chem. 267, 24569-24577.

25. Huang, R. P., and Adamson, E. D. (1993) DNA Cell Biol. 12, 265-273.

26. Staal, P. F., Roederer, M., Herzenberg, L. A., and Herzenberg, L. A. (1990). Proc. Natl. Acad. Sci. U. S. A. 97, 9544-9547.

27. Kalbac, T., Kinter, A., Poll, G., Anderson, M. E., Meister, A., and Fauci, A. S. (1991). Proc. Natl. Acad. Sci. U. S. A. 88, 955-958.

28. Schreck, R., Rieber, P., and Baeuerle, P. A. (1991) EMBO J. 10, 2247-2254.

29. Novak, A., Goyal, N., and Gronostajski, R. M. (1991) J. Biol. Chem. 266, 26302-26308.

30. Deng, W. P., and Nickoloff, J. A. (1992) Anal. Biochem. 200, 81-88.

31. Reithardt-Olson, J. F., Bowis, J. J., Breyer, R. M., Hu, J. C., Knight, K. L., Lim, W. A., Musing, M. C., Farnell, D. A., Shoemaker, K. R., and Sauer, R. T. (1995) Methods Enzymol. 228, 545-568.

32. Tabor, S., and Richardson, C. C. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4767-4771.

33. Le Gros, S. F., and Gruning-Leitch, F. (1990) Eur. J. Biochem. 187, 307-314.

34. Bradford, M. (1976) Anal. Biochem. 72, 248-254.

35. Gronostajski, R. M. (1986) Nucleic Acids Res. 14, 9117-9123.

36. Laemmli, U. K. (1970) Nature 227, 680-685.

37. Koosower, N. S., and Kossower, E. M. (1987) Methods Enzymol. 143, 264-270.

38. Paradiso, P., and Konigsweg, W. (1982) J. Biol. Chem. 257, 1463-1467.

39. Hwang, Y. R., Goodman, S. R., Laimins, L. A., and Sigler, P. B. (1990) Nature 349, 505-512.

40. Stanov, Y., Williams, K. R., and Konigsweg, W. H. (1988) Proteins 4, 1-6.

41. Merrill, B. W., Williams, K. R., Chase, J. W., and Konigsweg, W. H. (1984) J. Biol. Chem. 259, 10850-10856.

42. Cheglov, V., and Klessig, D. F. (1992) J. Biol. Chem. 267, 17782-17781.

43. Williams, K. R., and Konigsweg, W. H. (1991) Methods Enzymol. 200, 516-529.

44. Hoekstra, J. W., Kubasek, W. L., Vorachek, W. E., Evtimov, E. M., and van Hulp, P. H. (1991) Methods Enzymol. 200, 231-236.

45. Blessing, K. E., Elbright, Y. W., and Elbright, R. H. (1992) Nature 359, 650-652.

46. Storz, G., Tartaglia, L. A., and Ames, B. N. (1990) Science 248, 189-194.

47. Tartaglia, L. A., Gimeno, C. J., Storz, G., and Ames, B. N. (1992) J. Biol. Chem. 267, 2039-2045.

48. Xanthoudakis, S., Miso, G., Wang, F., Pan, Y. C., and Curran, T. (1992) EMBO J. 11, 3323-3335.

49. Kumar, S., Rabson, A. B., and Gelinas, C. (1992) Mol. Cell. Biol. 12, 3094-3106.

50. Chibnall, A., Miller, H., Parsonage, D., and Ross, R. F. (1992) FASEB J. 7, 1483-1490.

51. Okuno, H., Akaahori, A., Sato, H., Xanthoudakis, S., and Tjian, R. (1993) Oncogene 8, 665-673.

52. Murer, M., Eppendel, T., and Hurzh, J. H., and Novak, A., Goyal, N., and Gronostajski, R. M. (1992) Mol. Cell. Biol. 10, 1041-1048.

53. Kruse, U., Qiao, P., and Sippel, A. E. (1991) Nucleic Acids Res. 19, 6641.

54. de Vries, E., van Driel, V., and van Hael, S. J. L., and van der Vliet, P. C. (1997) EMBO J. 6, 161-168.

55. Gounari, F., De Francesco, E., Schmitt, J., van der Vliet, P. C., Curtiss, R., and Stunnenberg, H. (1990) EMBO J. 9, 559-566.

56. Mermod, N., O'Neill, E. A., Kelly, T. J., and Tjian, R. (1989) Cell 58, 741-753.

57. Meisterernst, M., Gander, I., Rogge, L., and Winnacker, E. L. (1988) Nucleic Acids Res. 16, 4419-4435.

58. Gronostajski, R. M., Adhya, S., Nagata, K., Guggenheimer, R. A., and Hurwit, J. S. (1985) Mol. Cell. Biol. 6, 964-971.

59. Gronostajski, R. M. (1987) Nucleic Acids Res. 15, 5545-5559.

60. Meisterernst, M., Rogge, L., Foerchler, R., Karaghiosoff, M., and Winnacker, E. L. (1989) Biochemistry 28, 8191-8200.

61. Novak, A., Goyal, N., and Gronostajski, R. M. (1992) J. Biol. Chem. 267, 2286-2290.

62. Pubs, C. O., and Sauer, R. T. (1999) Annu. Rev. Biochem. 61, 1053-1095.

63. Struhl, K. (1989) Trends Biochem. Sci. 14, 137-140.

64. Lovering, R., Hanson, I. M., Borden, K. L., Martin, S., O'Reilly, N. J., Evan, G. I., Rahman, D., Pappin, D. J., Towseal, J., and Freeman, P. S. (1995). Proc. Natl. Acad. Sci. U. S. A. 92, 7171-7175.

65. Jackson, S. P., and Tjian, R. (1988) Cell 55, 125-133.

66. Jackson, S. P., and Tjian, R. (1988) Proc. Natl. Acad. Sci. U. S. A. 86, 1781-1785.

67. McBride, A. A., Klauser, R. D., and Howley, P. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7351-7355.