The Journal of Biological Chemistry

© 1994 by The American Society for Biochemistry and Molecular Biology, Inc.

Vol. 269, No. 11, Issue of March 18, pp. S176-S181, 1994

Printed in U.S.A.

The Nuclear Localization Signal of NGFI-A Is Located within the Zinc Finger DNA Binding Domain*

(California Matheny, Mark L. Day†, and Jeffrey Milbrandt§)

From the Division of Laboratory Medicine, Departments of Pathology and Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

NGFI-A is an immediate-early gene that encodes a transcription factor whose DNA binding domain is composed of three C2H2 zinc fingers. To identify its nuclear localization signal (NLS), wild type NGFI-A and various mutants were transfected into COS cells and their cellular location assayed by indirect immunofluorescence. Although wild type NGFI-A was located exclusively within the nucleus, deletions lacking the highly basic zinc finger region were not efficiently translocated to the nucleus. However, DNA binding per se is not required for nuclear localization, as an NGFI-A mutant (A Y339G), which does not bind DNA, is still faithfully directed to the nucleus. To determine the minimal region(s) of NGFI-A sufficient to direct nuclear localization, the cellular location of various NGFI-A/β-galactosidase fusion proteins was examined. Fusion proteins containing all three zinc fingers were found in the nucleus, but those containing only two zinc fingers were predominantly cytoplasmic. When the zinc finger structure was altered by mutating a zinc-chelating cysteine residue in any one of the three zinc fingers, the resulting domain was no longer capable of directing β-galactosidase to the nucleus. Furthermore, the mutation of an arginine residue in the third zinc finger of NGFI-A, a position which is occupied by a leucine residue in most C2H2 zinc fingers, abolished nuclear localization, but had no effect on DNA binding. These studies suggest that NGFI-A contains a novel NLS which is dependent on the overall structure of the DNA binding domain and not solely upon its highly basic nature.

NGFI-A (also called Egr-1 (1), zif268 (2), Krox-24 (3)) is a transcription factor encoded by an immediate-early gene that was first isolated by virtue of its induction by nerve growth factor in PC12 cells (a rat pheochromocytoma cell line) (4). It is a member of a family of immediate-early genes, including Krox-20 (5), NGFI-C (6), and Egr-3 (7), which contain very similar C2H2-type zinc fingers (8) and whose cognate proteins all bind to the nucleotide sequence 5'-CGG/G/TGGGCC (6, 7, 9). Additionally, the zinc fingers comprising the DNA binding domain of NGFI-A are similar to those present in the Wilms’ tumor gene product (WT1) (10–12), which also recognizes the GCG/G/TGGGCC element (13, 14) and the transcription factor Sp1 (15).

The transport of large proteins into the nucleus is an active process that requires the presence of a suitable nuclear localization signal (NLS) (16). Nuclear import begins with an association between the NLS and proteins responsible for transportation to the nuclear pore complex (for reviews see Refs. 17 and 18). This is followed by the translocation of the protein into the nucleus via an ATP-dependent process (19). Many nuclear localization signals have been described; although not identical in sequence, they generally fall into two categories. The first is typified by that of SV40 T antigen, in which a stretch of basic amino acids (PKKKRRK) contains all the components necessary for nuclear transport. Furthermore, these residues are also sufficient to promote nuclear accumulation of a cytoplasmic reporter protein (20–22). This type of NLS is present in a variety of proteins, including the oncoproteins v-Rel, c-Myb, and c-Myc, several viral proteins, and the tumor suppressor p53 (23). The second type of NLS is a bipartite signal usually composed of 2 basic amino acids and a spacer region of approximately 10 amino acids, followed by another cluster of basic amino acids (24). A bipartite NLS was first identified in nucleoplasm, but has also been found in a number of nuclear receptors as well (25).

Nuclear entry is now recognized as an important mode of regulating transcription factor function (26). The transcription factor NFκB is sequestered in the cytoplasm by binding to an inhibitory protein, IκB. Upon phosphorylation of IκB, the complex dissociates and NFκB is translocated to the nucleus where it regulates expression of a variety of genes (27). The yeast transcription factor SWI5 is phosphorylated on specific serine residues which results in its sequestration in the cytoplasm; upon dephosphorylation it enters the nucleus and becomes transcriptionally active (28). In other studies, the phosphorylation of sites in or near the NLS has been shown to regulate the rate of transport into the nucleus (29). Additionally, many nuclear receptors contain an NLS that directs them to the nucleus upon binding to their hormone ligand (30–34).

Because nuclear localization may also provide a mechanism for regulating the transcriptional activity of NGFI-A, we have identified the nature of the NGFI-A NLS. Two approaches were utilized: a subtractive method in which the cellular locations of NGFI-A deletion mutants were assayed by indirect immunofluorescence to identify residues required for nuclear localization and an additive approach in which regions of NGFI-A were linked to a cytoplasmic reporter protein (β-galactosidase) to identify regions sufficient to promote nuclear entry. Through these mutational analyses, we have shown that the zinc fingers of the DNA binding domain contain the NLS of NGFI-A and that this domain is sufficient to target β-galactosidase to the nucleus. Additional mutational analyses indicated that the secondary structure of the zinc finger must be maintained for efficient nuclear localization; however, DNA binding per se is not a necessity for nuclear localization. The DNA binding do-

* 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.

† Present address: Division of Urologic Surgery, Washington University School of Medicine, St. Louis, MO 63110.

§ To whom correspondence should be addressed. Fax: 314-362-8756.

The abbreviations used are: NLS, nuclear localization signal; PCR, polymerase chain reaction; PBS, phosphate-buffered saline.

8176
main of NGFI-A is very similar to that present in other members of this gene family (NGFI-C, Krox-20, and Egr-3), suggesting that this region also serves to target these proteins to the nucleus.

**EXPERIMENTAL PROCEDURES**

**Antibodies and DNA Constructs**—The anti-NGFI-A antisera (A310) has been described previously (35). The anti-β-galactosidase monoclonal antibody was obtained from J. Sanes (Washington University, St. Louis, MO). The pCMV mammalian expression plasmid was obtained from M. Roth (University of Texas, Southwestern). The β-galactosidase (placF) and SV40 T antigen β-galactosidase (placF-CAT) plasmids have been described previously (36).

Internal deletion mutants of NGFI-A (4) were constructed by cleaving the cDNA clone in pBSKS (Stratagene) with restriction enzymes and inserting appropriate length BamHI linkers to maintain the correct reading frame. Deletion mutants were designated using an A as followed by the numbers of the residues which were deleted. Carboxyl-terminal truncation mutants were constructed by cleaving with a unique restriction enzyme and inserting an oligonucleotide with termination codons in all three reading frames. All mutants were transferred into the pCMV expression vector. The NGFI-A mutant A379GD has been described previously (38).

The NGFI-A fusion constructs were produced by linking NGFI-A PCR fragments to the β-galactosidase gene at the NcoI site of placF. The identity of all constructs was confirmed by sequencing, and they were designated according to the amino acids of NGFI-A that were linked to β-galactosidase. The fragment encoding the fusion protein was then transferred to pCMV using the Kpn1 and HindIII sites. The mutant containing zinc fingers one and three linked to β-galactosidase (NGFI-A 331-365,394-433 β-galactosidase) and all of the point mutations linked to β-galactosidase were cloned similarly using PCR products bearing mutations derived from a genetic selection in yeast (37) as a template for PCR synthesis. For simplicity, the β-galactosidase fusion proteins containing mutations within the NGFI-A zinc fingers (amino acids 328-433) have been named AZF β-galactosidase with the location and identity of the residue (native to mutated) included in parentheses (i.e., AZF (R412G) β-galactosidase).

**Cell Culture and Transfections**—COS-7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Cells were plated 24 h before transfection at a density of 20,000-30,000 cells/cm² chamber slide. The cells were transfected by calcium phosphate precipitation using 0.77 μg of plasmid DNA/chamber. All DNA constructs were transfected a minimum of four times using at least two different plasmid preparations.

**Immunohistochemistry**—COS-7 cells were transfected with various expression constructs; 48 h later they were washed twice with cold phosphate-buffered saline (PBS) and fixed in a solution of 3.7% formaldehyde in PBS for 10 min at 25°C. Cells were then washed three times in PBS, incubated for 15 min in blocking buffer (0.5% Triton X-100), washed with 0.5% Triton X-100, and incubated 1 h with primary antiserum diluted in buffer B for 2 h at 25°C. The anti-NGFI-A and anti-β-galactosidase antibodies were used at dilutions of 1:500 and 1:50, respectively. The cells were then washed with 0.5% Triton X-100, incubated with secondary antibody (fluorescein isothiocyanate-conjugated goat anti-rabbit or antimouse IgG) at a dilution of 1:150 for 90 min at 25°C, washed with 0.5% Triton X-100, three times for 5 min each, and mounted in a solution of 50% glycerol in 0.5% Triton X-100. Slides were examined in a blinded fashion (i.e., without knowledge of what plasmid was transfected). Fifty or more cells were scored using the following scale: nuclear > cytoplasmic/nuclear = cytoplasmic/cytoplasmic = nuclear > cytoplasmic. SV40 T antigen β-galactosidase and β-galactosidase alone were used as positive and negative controls, respectively. From these analyses an average score was determined for each DNA construct.

**RESULTS**

**Determining the NLS of NGFI-A by Deletional Analysis**—Cell fractionation studies had previously demonstrated that wild type 84-kDa NGFI-A is located exclusively within the nucleus (35). To determine which regions of NGFI-A are required for its nuclear localization, we engineered deletions within NGFI-A and evaluated the ability of the resulting mutant proteins to enter the nucleus in transiently transfected COS cells by indirect immunofluorescence. Most of the mutant proteins were found exclusively or predominantly in the nucleus. However, a mutant which lacks the zinc fingers and carboxyl terminus (Δ322-536) remained in the cytoplasm (Fig. 1). A deletion (Δ322-420) that lacks only the zinc fingers gave a similar result, indicating that the NLS is located within the highly basic region of the DNA binding domain. An NGFI-A mutant (A379GD) that does not bind to the NGFI-A recognition site (37) was also examined. This mutant was translocated to the nucleus as efficiently as wild type NGFI-A (Fig. 1), indicating that sequence-specific DNA binding per se is not required for nuclear localization. To further define the region within the zinc fingers necessary for nuclear localization, constructs lacking individual zinc fingers were tested. NGFI-A deletion mutants that contain any two zinc fingers were translocated to the nucleus (Fig. 1); however, removal of the third zinc finger and the basic region immediately carboxyl-terminal to it partially disrupted nuclear localization, suggesting that this region contributes more to the NLS than the first or second zinc fingers.

**The Structure of the Zinc Fingers Is Required for Full Nuclear Localization of the Heterologous Cytoplasmic Protein, β-Galactosidase**—Deletional analysis showed that the zinc finger region was necessary for nuclear localization and suggested that the NLS may be located within the third zinc finger. However, because the elimination of this finger did not completely abolish nuclear localization, a further examination of the sequences necessary for nuclear localization was pursued. Full-length and portions of NGFI-A were linked to a heterologous cytoplasmic protein, β-galactosidase, and the subcellular localization of these fusion proteins was examined along with controls for nuclear (SV40 T antigen β-galactosidase) and cytoplasmic (β-galactosidase alone) localization (Fig. 2). Full-length NGFI-A targeted β-galactosidase to the nucleus as efficiently as the NLS from SV40 T antigen. Since deleting the NGFI-A zinc fingers disrupted nuclear localization, a chimeric protein that contained all three NGFI-A zinc fingers fused to β-galactosidase (NGFI-A 331-433 β-galactosidase (AZF β-gal)) was tested and shown to target β-galactosidase to the nucleus as efficiently as full-length NGFI-A. To determine if the third zinc finger and the adjacent basic region of NGFI-A are sufficient for nuclear localization, these regions were linked to the β-galactosidase protein, and the subcellular localization of resulting chimeric proteins was assessed (Fig. 2, NGFI-A 389-436 β-gal). The cytoplasmic staining of these transfected cells closely resembled that obtained with β-galactosidase alone, indicating that residues within the third zinc finger and adjacent basic region alone are not sufficient to direct nuclear translocation (Fig. 2). Using additional mutants, we demonstrated that proteins containing only two of the three zinc fingers of NGFI-A were also inefficiently targeted to the nucleus, although proteins containing the second and third zinc fingers (NGFI-A 367-433 β-galactosidase) demonstrated greater nuclear staining than those containing the first and third zinc fingers (NGFI-A 331-365,394-433 β-galactosidase) (Fig. 2). From this analysis, it is clear that all three zinc fingers of NGFI-A are necessary and sufficient to promote maximal targeting of β-galactosidase to the nucleus.

**The Structure of the Zinc Fingers Is Required for Full Nuclear Localization**—The structure of the C_{9H_{2}} zinc finger is critically dependent upon the zinc chelating cysteine and histidine residues (8, 39). If any of these residues are mutated in NGFI-A, it is no longer capable of binding to its DNA recognition site (37). Furthermore, the mutation of these residues would be predicted to radically alter the structure of the zinc finger (8). Three constructs were made in which a cysteine residue was substituted within each zinc finger was independently mutated within the context of the entire DNA binding domain.
Fig. 1. Determination of the nuclear localization signal of NGFI-A. A schematic representation of the NGFI-A proteins. COS-7 cells were transfected with wild type NGFI-A or the indicated deletion mutants, cultured for 2 days, and the cellular location of the NGFI-A protein was assayed by indirect immunofluorescence using NGFI-A-specific antisera. The zinc fingers are shown in black. The asterisk denotes the location of the mutation Y339G in the non-DNA binding NGFI-A mutant. Cell staining was scored as described in the methods: N, represents nuclear staining; N>C, nuclear greater than cytoplasmic staining; N=C, nuclear equal to cytoplasmic staining; C>N, cytoplasmic greater than nuclear staining. B, representative immunofluorescent images are shown for each deletion mutant.

resulting domains were deficient in their ability to target β-galactosidase to the nucleus; however, nuclear targeting was not completely abolished, as occurred when an entire zinc finger was deleted (Fig. 2). For example, the mutant AZF (C346F) β-galactosidase (Fig. 3) was equally distributed between the nucleus and the cytoplasm, in contrast to the predominately cytoplasmic location of the mutant which lacks the first zinc finger (NGFI-A 367–433 β-gal) (Fig. 2). Since the basic nature of the amino acid sequence remains intact in these mutations, and DNA binding per se is not required for nuclear localization.
Nuclear Localization Signal of NGFI-A

FIG. 2. NGFI-A zinc fingers target β-galactosidase to the nucleus. A, schematic representation of the NGFI-A β-galactosidase fusion proteins. The indicated regions of NGFI-A were linked to β-galactosidase, transfected into COS-7 cells, and the cellular localization determined by indirect immunofluorescence using anti β-galactosidase antisera. The unmodified β-galactosidase construct was used as a control for a cytoplasmic protein. The NLS of SV40 T antigen (T&+) was linked to β-galactosidase and used as a control for a nuclear protein. B, representative immunofluorescent images are shown for each deletion mutant.

(Fig. 1), these results suggest that the NGFI-A NLS is not solely dependent on the basic nature of the DNA binding domain, but on its secondary structure as well.

An Arginine at Residue 412 Is Critical for the Nuclear Localization of NGFI-A—Since deleting the third zinc finger of NGFI-A partially disrupted nuclear localization, but deletion of either the first or second zinc finger had no effect, the sequence of zinc finger three was closely examined. One significant difference was noted; in most Cys2His2 zinc fingers, a conserved leucine is present three amino acids before the first zinc-chelating histidine, whereas NGFI-A zinc fingers one and two do contain a leucine residue at this position it is occupied by an arginine in the third zinc finger of NGFI-A and by either arginine or lysine in the third zinc finger NGFI-C, Egr-3, and Krox-20.

Previously, an in vivo mutational analysis using genetic selection in yeast was used to define the important residues of the NGFI-A zinc fingers (37). Most of these mutations impaired DNA binding; however, several mutants were recovered with normal DNA binding activity, including one in which arginine 412 was replaced by glycine. This suggested that arginine 412 is functionally significant and that the function of these zinc fingers is more diverse than recognized previously (37). Because of the unique nature of this residue and the phenotype of this mutant in yeast, we tested whether it played a role in NGFI-A nuclear localization. The zinc fingers of NGFI-A with a mutation at amino acid 412 (AZF (R412G) β-galactosidase) were linked to β-galactosidase, and the resultant protein was assayed for nuclear localization. The cellular location of this mutant is indistinguishable from the native β-galactosidase protein (Fig. 3), demonstrating that this mutation totally abolished the ability of the NGFI-A zinc fingers to target β-galactosidase to the nucleus.

DISCUSSION

These mutational studies demonstrate that the NLS of NGFI-A is contained within its zinc finger DNA binding domain. All three zinc fingers were required to target the heterologous cytoplasmic protein β-galactosidase to the nucleus. The inability to further delimit the NLS suggests that a new type of global domain NLS exists in NGFI-A.

To characterize the NLS, two complementary strategies were used: 1) a subtractive approach which identifies a polypeptide segment which contains information required for nuclear localization and 2) an additive approach which identifies a polypeptide segment that is sufficient for nuclear localization. The advantage of the subtractive approach is that the NLS is identified within its normal context, an important consideration as the efficacy of the NLS can be influenced by its position. For example, when the SV40 T antigen NLS was inserted into different locations within the cytoplasmic protein pyruvate kinase (PK), one insertion was unable to direct PK to the nucleus because it was buried in a hydrophobic domain (40). This is not
The zinc finger structure is required for nuclear targeting and a conserved arginine at residue 412 is critical for NGFI-A nuclear localization. NGFI-A zinc fingers containing a cysteine mutation in zinc finger one, two, or three and the mutation of arginine 412 to glycine were all linked to β-galactosidase. Cellular localization was determined as above and compared with the intact NGFI-A zinc fingers β-galactosidase chimeric protein. Representative immunofluorescent images are shown. The AZF β-galactosidase chimera was located in the nucleus. The staining of AZF (C346F) β-galactosidase, AZF (C374W) β-galactosidase, and AZF (C402S) β-galactosidase was determined to be nuclear equal to cytoplasmic. The β-galactosidase control protein and AZF (R412G) β-galactosidase were located primarily in the cytoplasm.

AZF (C346F) β-gal  AZF (C374W) β-gal  AZF (C402S) β-gal

a significant concern in our study because all of the test sequences were introduced into the same site at the amino terminus of β-galactosidase, where masking does not occur as evidenced by the nuclear localization of the SV40 T antigen β-galactosidase positive control. However, subtractive studies address only what is necessary for nuclear localization and cannot define the boundaries of the functional sequence as can be achieved by additive studies linking regions of the nuclear protein to a heterologous cytoplasmic protein. The additive approach may be a more stringent assay, as targeting a large (450 kDa) tetrameric protein to the nucleus may be more difficult than targeting native NGFI-A (84 kDa) to this location.

The only clear commonality between the various NLSs is the predominance of basic amino acids in DNA binding domains and nuclear targeting signals may reflect the fact that DNA binding domains were the archetypal targeting signals (24), a possibility that is strengthened by our results with NGFI-A. In general, DNA binding is not required for nuclear localization; indeed, when the same region of the protein contains both DNA binding and nuclear localization functions, they are often separable. For instance, the deletion of the NLS of poly(ADP-ribose) polymerase, which is located immediately carboxy-terminal to the zinc fingers, does not affect DNA binding. The same is true for Gal4 where DNA binding alone does not necessarily imply that the protein will be translocated to the nucleus (45).

Despite the large number of NLSs that have been identified, no consensus sequence has emerged at the amino acid level. The only clear commonality between the various NLSs is the predominance of basic amino acids. Two types of NLSs have been characterized previously, those containing a single basic cluster and those with a bipartite domain. Of the proteins containing a bipartite NLS, manipulating the spacer region between the two basic clusters has little effect, providing evidence that the primary structure is not critical for nuclear localization (25, 46). This independence from spacer length could be explained if the two basic domains are in close proximity in the tertiary structure so that they can function as a cohesive nuclear localization unit (24, 46). Support for this hypothesis has been obtained from the x-ray crystal structure of the glu-
corticoid DNA binding domain and NLS (24), where computer modeling indicates that the spacer region can be looped out to juxtapose the two basic clusters of the NLS (47). The crystal structure of NGFI-A indicates that the three zinc fingers are arranged in a C-shaped structure which fits into the major groove of the DNA (39). In this arrangement the basic amino acids of the zinc fingers are brought into close proximity and could represent a new global domain type of NLS. Alternatively, the many basic clusters contained within the zinc fingers of NGFI-A could reflect many weak NLSs, all of which are required for full nuclear localization.

Between amino acids 328 and 433, which includes the zinc fingers and the adjacent basic regions, NGFI-A contains 26 basic amino acids (Fig. 4). This region is highly conserved among Krox-20, NGFI-C, and Egr-3 (80% identity, 96% similarity). Based on this extreme conservation, the NLSs of the other NGFI-A family members are also likely to be present within the zinc fingers and of the global domain type. In support of this hypothesis, we have found that deleting the zinc fingers of NGFI-C eliminated the ability of this protein to localize to the nucleus (data not shown) and studies of Krox-20 have shown that regions outside of the zinc fingers can be deleted without affecting nuclear localization (48).

In summary, the zinc fingers of NGFI-A appear to function as a global domain NLS, a contention that is supported by the requirement for all three zinc fingers in translocating β-galactosidase to the nucleus. An arginine at position 412 is also required for nuclear localization of NGFI-A, a position occupied by leucine in most C$_2$H$_2$ zinc fingers, but by a basic residue in the third zinc finger in members of this gene family. Based on the functional analysis presented here and the extreme conservation of the zinc fingers between NGFI-A and its family members, the NLS of the other members of the NGFI-A family may also be located within the DNA binding domain.

REFERENCES

1. Sukhatme, V. P., Cao, X., Chang, L. C., Tsai-Morris, C. H., Stamenkovich, D., Ferrara, P. C. P., Cohen, D. B., Edwards, S. A., Shows, T. B., Curran, T., Le Beau, M., and Adelson, E. D. (1988) Cell 53, 37–42.
2. Christy, B. A., Lau, L. F., and Nathans, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7587–7591.
3. Lemaire, P., Revelat, O., Bravo, R., and Charnay, P. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 4691–4696.
4. Milbrandt, J. (1987) Science 238, 797–799.
5. Chevrier, F., Janssen-Timmus, U., Mattei, M.-G., Zerial, M., Bravo, R., and Charnay, P. (1989) Mol. Cell. Biol. 9, 787–797.
6. Crosby, S. D., Puett, J. J., Simburger, K. S., Fahrner, T. J., and Milbrandt, J. (1991) Mol. Cell. Biol. 11, 3853–3861.
7. Patwardhan, S., Gashler, A., Siegel, M. G., Chang, L. C., Joseph, L. J., Shows, T. B., Le Beau, M. M., and Sukhatme, V. P. (1991) Oncogene 6, 917–928.
8. Berg, J. M. (1990) Annu. Rev. Biochem. 19, 405–421.
9. Christy, B., and Nathans, D. (1989) Proc. Natl. Acad. Sci. U. S. A. 66, 8737–8741.
10. Schneider, C., King, R. M., and Philpason, J. (1988) Cell 54, 787–793.
11. Schreiber, V., Molinete, M., Boeuf, H., de Murcia, G., and Menissier-de Murcia, J. (1992) Biochim. Biophys. Acta 1071, 83–101.
12. Pullen, A. M., Choi, Y., Kappler, J., and Marrack, P. (1992) Eur. J. Immunol. 22, 159–165.
13. Silver, P., and Christy, B. A., Lau, L. F., and Nathans, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6335–6340.
14. Issaeva, T. B., Le Beau, M. M., and Sukhatme, V. P. (1991) Oncogene 6, 917–928.
15. Marshall, C. (1991) Science 250, 1259–1262.
16. Nigg, E. A., Baeuerle, P. A., and Laemmli, U. K. (1991) Cell 66, 15–22.
17. García-Bustos, J., Bertet, J., and Domínguez, M. (1982) Cell 35, 374–380.
18. Moor, M. E., and Blobel, G. (1982) Cell 30, 939–960.
19. Kalderon, D., Richardson, W. D., Markham, A. T., and Smith, A. E. (1984) Nature 311, 33–38.
20. Goldfarb, D. S., Gariepy, J., Schoolin, R., and Kornberg, R. (1986) Nature 322, 641–644.
21. Lampson, R. F., and Butel, J. S. (1986) Cell 47, 809–813.
22. Goldfarb, D. S., Gariepy, J., Schoolin, R., and Kornberg, R. (1986) Nature 322, 641–644.
23. Park, S. H., and Parker, A. Z. (1988) J. Biol. Chem. 263, 18019–18022.
24. Dingwall, C., and Laskey, R. A. (1991) Trends. Biochem. Sci. 16, 478–481.
25. Robbins, J., Dilworth, S. M., Laskey, R. A., and Dingwall, C. (1991) Cell 64, 615–623.
26. Hunter, T., and Karin, M. (1992) Cell 70, 276–377.
27. Ghoosh, S., and Baltimore, D. (1990) Nature 344, 678–682.
28. Moll, T., Tebb, G., Surana, U., Robitsh, H., and Nasmyth, K. (1991) Cell 66, 743–758.
29. Rihs, H. P., Janz, D. A., Fan, H., and Peter, K. (1991) EMBO J. 10, 633–639.
30. Picard, D., and Yamamoto, K. R. (1987) EMBO J. 6, 3335–3340.
31. Picard, D., and Yamamoto, K. R. (1987) Cell Regul. 1, 291–299.
32. Jenster, G., van der Kortum, J. A., Praplan, J., and Brinkmann, A. O. (1992) J. Biol. Chem. 267, 3718–3724.
33. Giuochon-Mantel, A., Loefelt, H., Leesh, P., Christain-Maître, S., Perrot-Appel, M., and Milgrom, E. (1992) J. Steroid Biochem. Mol. Biol. 41, 671–675.
34. Giuochon-Mantel, A., Leesh, P., Christain-Maître, S., Loofelt, H., Perrot-Appel, M., and Milgrom, E. (1992) J. Steroid Biochem. Mol. Biol. 41, 671–675.
35. Giuochon-Mantel, A., Leesh, P., Christain-Maître, S., Loofelt, H., Perrot-Appel, M., and Milgrom, E. (1992) J. Steroid Biochem. Mol. Biol. 41, 671–675.
36. Chen, C., and Okayama, H. (1988) Mol. Cell. Biol. 18, 741–749.
37. Pavletich, N. P. (1992) Science 252, 809–817.
38. Blackman, M. A., Kappler, J. W., and Marrack, P. (1991) Adv. Exp. Med. Biol. 299, 159–165.
39. Kappler, J. W., Herman, A., Clements, J., and Marrack, P. (1992) J. Exp. Med. 175, 387–396.
40. Ylikoski, T., Boocock, M. T., Berry, M., Gronemeyer, H., and Chambon, P. (1992) EMBO J. 11, 3681–3694.
41. Hamy, F., and Belbeque, N. (1988) Biochem. Biophys. Res. Commun. 182, 289–293.
42. Schreiber, V., Molinete, M., Boeuf, H., de Murcia, G., and Menissier-de Murcia, J. (1992) EMBO J. 11, 3263–3269.
43. Pullen, A. M., Choi, Y., Kuehn, E., Kappler, J., and Marrack, P. (1992) J. Exp. Med. 175, 41–47.
44. Zhao, L. J., and Padmanabhan, R. (1991) New Biol. 3, 1074–1088.
45. Hard, T., Kellenbach, E., Boeclens, R., Mäler, H. A., Dahlman, K., Freedman, L. P., Carstads-Duke, J. T., Vranenrego, K. R., Gustafsson, J., and Rapp, K. (1990) Nature 349, 157–160.
46. Vesque, C., and Charnay, P. (1992) Nucleic Acids Res. 20, 2485–2492.