Role of Sp1 in cAMP-dependent Transcriptional Regulation of the Bovine CYP11A Gene

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The pituitary peptide hormone ACTH regulates transcription of the cholesterol side chain cleavage cytochrome P450 (CYP11A) gene via cAMP and activation of cAMP-dependent protein kinase. A G-rich sequence element conferring cAMP-dependent regulation has been found to reside within region −118 to −100 of the bovine CYP11A promoter. Previous studies have suggested that it binds a protein antigenically related to the transcription factor Sp1. We now report that the −118/−100 element binds both Sp1 and Sp3, members of the Sp family of transcription factors. We have made use of Drosophila SL2 cells, which lack endogenous Sp factors, to dissect the possible functional roles of Sp1, Sp3, and Sp4. All factors stimulated the activity of cotransfected reporter constructs in which the promoter of the bovine CYP11A gene regulates luciferase expression. Sp3 did not repress Sp1-dependent activation, as has previously been shown for other G-rich promoters. Mutation of the −118/−100 element of CYP11A abolished Sp1-mediated activation of a CYP11A reporter gene in SL2 cells as well as cAMP responsiveness in human H295R cells. Furthermore, cotransfection of SL2 cells with the catalytic subunit of cAMP-dependent protein kinase together with Sp1 and a CYP11A reporter construct enhanced Sp1-dependent activation of the reporter 4.2-fold, demonstrating that Sp1 confers cAMP responsiveness in these cells. Thus, we show that introduction of Sp1 alone in an Sp-negative cell such as SL2 is sufficient to achieve the cAMP-dependent regulation observed using the −118/−100 element of CYP11A in adrenocortical cells.

The pituitary peptide hormone ACTH regulates steroidogenesis, via cAMP and the activation of cAMP-dependent protein kinase (PKA), by two temporally distinct processes (reviewed in Refs. 1 and 2). An acute response involves the rapid mobilization of cholesterol from intracellular stores to the inner mitochondrial membrane, where the first step of steroid hormone synthesis is catalyzed by the cholesterol side chain cleavage enzyme (P450scc). By a more chronic and slower process, ACTH release from the pituitary stimulates transcription of the cytochrome P450 steroid hydroxylase genes, leading to optimal steroidogenic capacity such that steroid hormones can be generated on demand via the acute response. Analyses of the promoter regions of the genes encoding the steroid hydroxylases have led to identification of elements that can confer cAMP responsiveness to reporter genes. Interestingly, these promoter elements usually lack sequence similarity to the classical cAMP-responsive element, and furthermore, the factors interacting with these atypical cAMP-responsive sequences are not related to classical cAMP-responsive element-binding proteins (reviewed in Ref. 1).

Analyses of the regulatory regions of the human, bovine, rat, and mouse CYP11A genes, which encode P450scc, have revealed species-specific variations in both the location and composition of cAMP-responsive elements (3–8). Deletion analysis of the bovine CYP11A gene through transfections in the mouse adrenocortical tumor cell line Y1 identified a CAMP-responsive sequence between −183 and −83 base pairs upstream of the start site of transcription (3). Subsequently, the functional CAMP-responsive sequence was found to reside within region −118 to −100, which is a G-rich sequence that is highly conserved in this position when comparing CYP11A from different species (9). This element is similar to binding sites for the transcription factor Sp1, and a previous study has shown that the −118/−100 sequence binds a protein that is recognized by antibodies to Sp1. Mutations within the site that interfere with the binding of the Sp1-related protein in vitro also block cAMP induction mediated by the element in vivo (10). In addition, a sequence between −70 and −50 was shown to bind a protein antigenically related to Sp1 and to support cAMP-dependent transcription (10). However, the Sp1-related factor could account only for part of the −118/−100 and −70/−50 binding activities present in Y1 extracts, and the ability of Sp1 to activate transcription was not tested directly.

Sp1 is not the only protein binding to and acting through so-called GC boxes and related motifs present in many promoters. Recently, cDNAs encoding Sp1-related proteins have been isolated, indicating the existence of an Sp1 multigene family (11, 12). Three Sp1-related proteins called Sp2, Sp3, and Sp4 have been characterized. They contain zinc-finger motifs as well as glutamine- and serine/threonine-rich stretches similar to those of Sp1. The DNA-binding domains of Sp1, Sp3, and Sp4 are highly conserved, and they recognize GC boxes with very similar specificities and affinities. Sp2, on the other hand, appears to have a DNA binding specificity that is very different from those of the other family members. The high degree of structural conservation among Sp1, Sp3, and Sp4 is, however,
not mirrored by a similar functional conservation. Several studies have indicated that whereas Sp1 and Sp4 act as transcriptional activators, Sp3 has bifunctional activities and acts both as an activator and a repressor of transcription (13–17). Furthermore, in contrast to Sp1, Sp4 is not able to act synergistically through adjacent binding sites (14). These studies suggest that the different members of the Sp family of transcription factors may have unique rather than redundant functions. In light of this, we wanted to explore the possibility of using Drosophila SL2 cells, which lack endogenous Sp factors, to test directly the function of individual Sp factors on the CYP11A gene and to study the influence of activation of the cAMP-dependent signal transduction pathway. We also set out to determine whether the G-rich –118/–100 element of the CYP11A gene binds factors in addition to the suggested Sp1-related factor and, if so, could these be other members of the Sp family.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The Scc-wt-luc plasmid contains the region between –186 and +12 of the bovine CYP11A gene fused to the firefly luciferase gene in the pGL3 vector (Promega, Madison WI). In Scc-mut-luc positions –107 and –108 have been changed to Cs (18). Dr. Evan R. Simpson (Monash Medical Center, Clayton, Australia) generously provided the Scc-luc reporter plasmids. The BCAT2 plasmid contains two Sp1-binding sites from the human T-cell lymphotropic virus promoter fused to the E1B TATA box in front of the CAT reporter gene (19). A schematic outline of all reporter gene constructs is presented in Fig. 1A. The Sp1, Sp3, and Sp4 expression plasmids have been described previously (14); the expression plasmid containing the catalytic subunit of PKA was a generous gift from Dr. Stanley McKnight (University of Washington, Seattle, WA).

**Cell Culture**—H295R cells were obtained from Dr. William R. Rainey (University of Texas Southwestern Medical Center, Dallas, TX). H295R cells were cultured in a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium (Life Technologies, Inc.) supplemented with insulin (6.25 μg/ml), transferrin (6.25 μg/ml), selenium (6.25 ng/ml), linoleic acid (5.35 μg/ml), sodium selenite (6.25 μg/ml), and 10% fetal bovine serum. Both SL2 and H295R cells were cultured at 37 °C in 5% CO2.

**Preparation of Nuclear Extracts and Electrophoretic Mobility Shift Assays**—Nuclear extracts were prepared according to Andrews and Faller (21). Electrophoretic mobility shift assays (EMSA) were performed with double-stranded synthetic oligonucleotides that were labeled with [γ-32P]ATP (Amersham Pharmacia Biotech) and T4 polynucleotide kinase (New England Biolabs, Inc.). EMSA conditions were as described (10), and antibodies in supershift experiments were added to the nuclear extracts at 4 °C for 30 min prior to use in EMSAs. The antibodies against Sp1, Sp3, and Sp4 have been described previously (13), as have antibodies against AS1 (22), and antibodies against Egr-1 (antibody 189) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

**RESULTS**

**Identification of Proteins That Bind the –118/–100 Element in Adrenocortical Cells**—EMSA was performed using nuclear extracts prepared from the human and mouse adrenocortical tumor cell lines H295R and Y1, respectively. When the –118/–100 element (indicated in **boldface** in Fig. 1B) was radioactively labeled and incubated with Y1 or H295R extracts, three major complexes were observed (Figs. 2 and 3, indicated by **arrows** and **arrowheads**, respectively). All three complexes were specific in nature as judged by the ability of an excess of unlabeled –118/–100 element to abolish the appearance of the complexes (Fig. 2, second through fourth lanes). Also, an excess of an oligonucleotide encompassing an Sp1-binding site (23) abolished the formation of all three complexes (Fig. 2, *fifth through seventh lanes*). Thus, these results provide further evidence that members of the Sp family of transcription factors participate in binding to the –118/–100 element. However, the –118/–100 element appears to be a weak Sp1-binding site since the unlabeled oligonucleotide encompassing the Sp1 consensus sequence was more effective in competing for binding than the unlabeled –118/–100 element.

To determine which of the complexes may contain Sp factors, antibodies specific for Sp1, Sp3, and Sp4 were then added to the extracts. As shown in Fig. 3, anti-Sp1 antibodies significantly reduced or abolished the complex with the lowest mobility (complex I), whereas anti-Sp3 antibodies abolished the formation of a complex with middle mobility (complex II) and reduced formation of complex I. The addition of anti-Sp4 antibodies had no effect on the formation of either complex. Significant amounts of the fastest migrating complex (complex III) were resistant to the addition of antibodies to Sp1, Sp3, or Sp4, either when added individually or in combination with the nuclear extracts (Fig. 3). Similar results were obtained when an oligonucleotide encompassing the Sp1-binding site was employed as a probe (Fig. 3). These results suggest that both Sp1 and Sp3 are present in adrenocortical nuclear extracts and bind to the –118/–100 element of the bovine CYP11A gene and that additional factors also recognize the element. Further-
**FIG. 1.** Schematic diagram and sequence of the CYP11A proximal promoter. **A,** schematic diagram of the reporter gene constructs used. Scc-wt-luc contains CYP11A sequences between −186 and +12 in front of the luciferase reporter gene. BCAT2 contains two Sp1-binding sites from the human T-cell lymphotrophic virus promoter fused to the E1B TATA box in front of the CAT reporter gene. **B,** sequence of the bovine CYP11A proximal promoter. The G-rich Sp1-binding sites are boxed; the SF-1 site (47) and the consensus binding site for ASP (22) are enclosed; and the TATA box is underlined. The −118/−100 element used in EMSA is in boldface, and the asterisks indicate the positions that were changed to C in the mutant construct Scc-mut-luc.

**FIG. 2.** EMSA profiles with the −118/−100 element and nuclear extracts from Y1 cells. 32P-Labeled double-stranded oligonucleotides corresponding to the −118/−100 element of the bovine CYP11A gene were incubated with 8 μg of nuclear extract protein from Y1 cells. Competition analysis was carried out by the addition of increasing concentrations (25, 50, and 100 ng) of unlabeled oligonucleotides encompassing the −118/−100 element or an Sp1-binding site to the reactions. Protein-DNA complexes were resolved by native 4% polyacrylamide gel electrophoresis and autoradiography of the fixed and dried gels. The arrows indicate specific complexes I–III.

**FIG. 3.** EMSA profiles with the −118/−100 element and nuclear extracts from H295R and Y1 cells. 32P-Labeled double-stranded oligonucleotides corresponding to the −118/−100 element of the bovine CYP11A gene or the Sp1-binding site were incubated with 8 μg of nuclear extract (n.e) protein from H295R or Y1 cells. Protein-DNA complexes were resolved on native 4% polyacrylamide gels. The arrowheads indicate specific complexes I—III. Anti-Sp1, anti-Sp3, and anti-Sp4 antibodies (1 μl) were added to the indicated reactions by means of a 30-min preincubation with the nuclear extract at 4 °C. as, antiserum.
more, the results suggest that the levels of Sp4 in Y1 or H295R nuclear extracts are very low since the addition of anti-Sp4 antibodies was without effect on complexes formed when the −118/−100 element or the Sp1-binding site was employed as a probe.

We also performed an EMSA in which antibodies against the transcription factors Egr-1 and ASP were employed. The addition of antibodies against Egr-1 had no effect on the formation of the fastest migrating complex (complex III) in EMSA (data not shown). Egr-1 has previously been shown to bind Sp1-binding sites, but these results indicate that Egr-1 is not part of the complexes formed between the −118/−100 element and adrenocortical nuclear extracts. Similarly, antibodies to ASP, a protein that participates in the cAMP-dependent regulation of the CYP21 gene and that binds GC-rich sequences (22), had no effect (data not shown).

Functional Roles of Sp Factors in Control of the Bovine CYP11A Gene—Transient transfections of H295R cells were performed with a reporter construct in which sequences between −186 and +12 of the bovine CYP11A gene have been fused to the luciferase gene (Scc-wt-luc) (Fig. 1A). The results show that the bovine promoter is active also in this human adrenocortical cell line (Fig. 4), as has been previously shown to be the case in mouse Y1 cells (3). Furthermore, the promoter activity was stimulated by forskolin treatment of the cells.

To determine the effects of individual Sp factors on CYP11A promoter activity, we next investigated the activities of Scc-wt-luc in Drosophila SL2 cells. These insect cells provide a useful model since there are no endogenous Sp factors present. As shown in Fig. 5, Scc-wt-luc was stimulated in a dose-dependent manner by cotransfection of a plasmid (pPacSp1) containing the Sp1 cDNA under the control of the Drosophila melanogaster actin 5C promoter (Fig. 5). A 9-fold stimulation was obtained when 1 µg of Sp1 expression plasmid was cotransfected with Scc-wt-luc. Cotransfection with pPacSp3 or pPacSp4 also resulted in stimulation of luciferase activity, 3- and 3.5-fold, respectively (Fig. 5).

To determine if the effects of cotransfection of Sp1, Sp3, and Sp4 on luciferase activity are mediated by the −118/−100 element, we next employed the Scc-mut-luc construct, in which this element has been mutated (Fig. 1B), in cotransfection experiments. Mutation of the element has previously been shown to interfere with protein binding in EMSA (10, 18), and the mutation did indeed abolish Sp1- and Sp4-mediated effects on luciferase activity in SL2 cells and reduced the effects of Sp3 (Fig. 5). Thus, these results establish that Sp1, Sp3, and Sp4 can act as positive regulators of the bovine CYP11A gene and suggest that these effects are mediated through the GA-rich element localized between −118 and −100.

Sp3 has previously been demonstrated to repress Sp1-dependent activation of several genes, and therefore, the effect of Sp3 on Sp1-mediated activation of a CYP11A reporter construct was explored in cotransfection experiments. As shown in Fig. 6, there was no effect of Sp3 on Sp1-mediated activation of the Scc-wt-luc construct in SL2 cells, whereas Sp3 repressed Sp1-mediated activation of the BCAT2 construct by 40%.

Regulation of Sp1 Levels in Adrenocortical Cells—To test the possibility that Sp1 levels in adrenocortical cells are regulated by cAMP, we performed Western blot analysis of extracts from H295R and Y1 cells that had been treated with forskolin for various lengths of time. As shown in Fig. 7, forskolin did not have an appreciable effect on Sp1 levels at any of the time points investigated. EMSA experiments were performed with the −118/−100 element and nuclear extracts from Y1 and H295R cells maintained in the presence or absence of forskolin for 6 h. The results of these experiments indicate that forskolin treatment does not alter the binding of protein factors to the −118/−100 element (data not shown).
Sp Factors in Control of CYP11A

The cAMP-dependent regulation of the CYP11A promoter thus appears to be mediated, at least in part, through the −118/−100 element (Fig. 4) (18) and since Sp1 acts via this element (Fig. 5), we wanted to determine if the Sp1-dependent activation of the CYP11A gene is modulated by PKA. For this purpose, SL2 cells were transiently cotransfected with Scc-wt-luc and BCAT2 together with pPacSp1 (0.5 μg), pPacSp3 (1 μg), pPacSp4 (1 μg), or the empty vector pPac (1 μg), as indicated. Cells were harvested 48 h after transfection, and luciferase and CAT activities were determined. Results are expressed as a percentage of reporter gene activity obtained in extracts from cells cotransfected with Scc-wt-luc or BCAT2 together with pPacSp1 and pPac. Values represent the average of at least two independent experiments.

DISCUSSION

Previous studies have shown the −118/−100 sequence of the bovine CYP11A gene to be involved in cAMP-dependent transcription in Y1 (9) and bovine ovarian luteal (24) cells. It has also been shown to bind a protein antigenically related to Sp1. However, Sp1 is not the only transcription factor that can bind to and act via GC boxes and related motifs. In this study, we have performed supershift EMSA employing antibodies against several other GC box-binding proteins to determine what factors could bind the −118/−100 element. We demonstrate herein that this element, in addition to binding Sp1, also binds Sp3, another member of the Sp family of transcription factors, and that it binds additional factors, which remain to be identified.

We also made use of Drosophila SL2 cells, which lack endogenous Sp factors, to dissect the possible functional roles of Sp1, Sp3, and Sp4. Intriguingly, all factors were shown to stimulate the activity of a cotransfected reporter construct driven by the bovine CYP11A promoter. Sp3 can act both as an activator of transactivation (25–28) and as a repressor of Sp1-mediated transcription (15–17, 28); however, in the case of the CYP11A promoter, Sp3 did not repress the Sp1-dependent activation. In contrast, Sp3 could repress Sp1-mediated activation of the BCAT2 reporter construct in SL2 cells (Fig. 6), suggesting that the effect of Sp3 is dependent on the promoter context in which the Sp1 site is localized. The BCAT2 construct harbors two Sp1 sites, and this has previously been shown to be important for the negative effect of Sp3 (27). Constructs containing a single Sp1 site, such as the CYP11A reporter, were less prone to be repressed by Sp3. In fact, Sp3 often activated transcription of such constructs (27). The results obtained with CYP11A thus further illustrate the complexity of Sp1 sites and their context dependence.

The −118/−100 element is required for basal and cAMP-mediated transcription of transfected CYP11A reporter genes in several cell types, including murine adrenocortical Y1 cells (9, 10) and bovine luteal cells (18, 24). In this study, we dem-
onstrate that this is also the case for human adrenocortical H295R cells, in which mutation of the −118/−100 element abolished cAMP-mediated stimulation of the Scc-luc construct. To establish that the effects of Sp1 factors on CYP11A-driven luciferase activity is mediated through the −118/−100 element, the same reporter gene construct mutated in the −118/−100 element was employed in transfection experiments in SL2 cells. Our results establish a positive role for Sp1, Sp3, and Sp4 in the regulation of the bovine CYP11A gene via this element since the mutant construct was unresponsive. Evidence suggests that, in addition to the element at −118 to −100, a region between −70 and −50 binds factors antigenically related to Sp1 and may contribute to cAMP-dependent regulation of CYP11A (3, 10). However, the results presented in this study suggest that this element is of less importance for Sp1-mediated activation since the construct mutated in the −118/−100 element, but with an intact Sp1 site at −70 to −50, was unresponsive.

Sp1 has traditionally been characterized as a constitutive transcription factor and has been considered to be a regulator of basal promoter activity. However, it is becoming increasingly clear that Sp1 binding and transactivation can be modulated by a variety of stimuli, and Sp1 has been suggested to mediate the effects of various growth factors and hormones (29–31). This suggests that Sp1 may have a critical role also in directing tissue-specific (32), developmental (33), and hormonal regulation of gene expression. Sp1 sites have been mapped to reside within cAMP-responsive regions in several genes, including the adrenodoxin gene (34), the human surfactant protein 2A gene (35), the rhesus growth hormone variant gene (36), the human urokinase gene (37), and the rat skg gene (38). The close association between the Sp1 site at −118 to −100 in the bovine CYP11A gene and cAMP responsiveness prompted us to investigate the effect of cAMP on Sp1-mediated activation of the Scc-luc construct in SL2 cells. Our results indicate that forskolin or 8-CPT-cAMP treatment of the cells, as well as cotransfection of the PKA catalytic subunit, enhanced the effect of Sp1 on Scc reporter gene activity. The effect of forskolin or 8-CPT-cAMP was only observed in cells transfected with Sp1, linking this transcription factor to the response. Interestingly, a recent report (39) demonstrated that cotransfection of insect SF9 cells with Sp1 and the PKA catalytic subunit enhances Sp1-dependent activation of a reporter gene under the control of the SV40 promoter. Combined, these results suggest that Sp1 may be a bona fide nuclear target of the PKA-dependent signal transduction pathway.

The mechanism by which Sp1 confers cAMP responsiveness to the CYP11A promoter is not yet defined. Tissue-specific and/or hormonal regulation of Sp1 expression is a possible mechanism by which Sp1 activity could be modulated. However, the levels of Sp1 protein in adrenocortical cells (Fig. 7) or ovarian granulosa cells (38) are not altered by forskolin treatment as determined by immunoblot analysis. This suggests that other mechanisms, such as post-translational modifications of Sp1 or regulated interactions of Sp1 with other factors, are more likely to be involved. Sp1 has been shown to be a phosphoprotein, but the effect of phosphorylation on Sp1 activity is complex. Phosphorylation by a DNA-dependent kinase appears not to alter the activity (40), whereas casein kinase II-mediated phosphorylation results in a decreased DNA binding activity (41). Of particular interest is the finding that PKA phosphorylation of Sp1 in vitro increases its DNA binding activity (39). However, when the −118/−100 element was employed as a probe in EMSA, the same gel shifts were obtained with nuclear extracts from untreated or forskolin-treated adrenocortical cells, indicating that forskolin does not affect the DNA binding activity of Sp1 in this cell type for this particular site (data not shown). Thus, although Sp1 can serve as a substrate for PKA phosphorylation in vitro, it has not yet been demonstrated that this occurs in vivo in adrenocortical or other steroidogenic cells.

It is recognized that Sp1 can interact with several other transcription factors, including CCAAT/enhancer-binding protein β (42), p53 (43), the chicken ovalbumin upstream promoter transcription factor (44), and the estrogen receptor (31), and thereby activate transcription. Increasing evidence is emerging that Sp1 enhances cooperative interactions among multiple transcription factors to juxtapose the transcriptional regulatory domains of the proteins with the transcription initiation complex (45, 46). Whether similar cooperative interactions of Sp1 with other transcription factors underlie the cAMP responsiveness of CYP11A remains to be determined.

Finally, the sequence between −70 and −50 also predicts a binding site for steroidogenic factor 1 (SF-1), a nuclear orphan receptor required for normal development of adrenals and gonads and a positive regulator of all steroid hydroxylase genes studied so far (reviewed in Ref. 47). In most cases, the SF-1-binding sites are localized within cAMP-responsive regions, and SF-1 has been proposed to mediate the response in certain cases (48, 49). In fact, it has recently been demonstrated that both Sp1 and SF-1 are necessary to achieve cAMP-dependent regulation of the CYP11A gene in Y1 cells as well as in primary bovine luteal cells (18). Furthermore, a two-hybrid assay revealed protein–protein interactions between Sp1 and SF-1, although no physical interaction could be demonstrated by co-immunoprecipitation assays (18). This suggests that the interaction between the two factors could be through interaction with a coactivator. Recently, we have shown that mutation within the activation function-2 domain of SF-1 transforms the transcription factor into a dominant-negative mutant with respect to cAMP-dependent activation of the bovine CYP17 gene (50). Also, others have shown that SF-1 can interact with steroid receptor coactivator 1 (51). It could therefore be envisioned that cAMP might stimulate the interaction of coactivators, e.g. steroid receptor coactivator 1, not only with SF-1, but possibly also with Sp1. In conclusion, our results establish Sp1 as a cAMP-responsive transcription factor in the context of the bovine CYP11A promoter and suggest the interesting possibility that cAMP may influence the interaction of Sp1 with cofactors shared with other transcription factors involved in CYP11A regulation.

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REFERENCES
1. Waterman, M. R. (1994) J. Biol. Chem. 269, 27783–27786
2. Miller, W. L. (1988) Endocr. Rev. 9, 295–318
3. Ahlgren, R., Simpson, E. R., Waterman, M. R., and Lund, J. (1990) J. Biol. Chem. 265, 3313–3319
4. Chung, B. C., Hu, M. C., Lai, C. C., and Lin, C. H. (1989) Biochem. Biophys. Res. Commun. 160, 276–281
5. Moore, C. C., Hum, D. W., and Miller, W. L. (1992) Mol. Endocrinol. 6, 2045–2058
6. Morohashi, K., Zanger, U. M., Honda, S., Hara, M., Waterman, M. R., and Omura, T. (1993) Mol. Endocrinol. 7, 1196–1204
7. Ooki, R. B., Parker, L. E., Gibson, J. L., and Richards, J. S. (1990) J. Biol. Chem. 265, 22922–22931
8. Rice, D. A., Kirkman, M. S., Aitken, L. D., Mouw, A. R., Schimmer, B. P., and Waterman, M. R. (1992) J. Biol. Chem. 267, 22392–22401
9. Murakami, K., Waterman, M. R., Simpson, E. E., and Zanger, U. M. (1992) Mol. Endocrinol. 6, 1682–1690
10. Venepally, P., and Waterman, M. R. (1995) J. Biol. Chem. 270, 25402–25410
11. Kingsley, C., and Winoto, A. (1992) Mol. Cell. Biol. 12, 4251–4261
12. Hagen, G., Muller, S., Beato, M., and Suske, G. (1992) Nucleic Acids Res. 20, 5519–5525
13. Hagen, G., Muller, S., Beato, M., and Suske, G. (1994) EMBO J. 13, 3843–3851
14. Hagen, G., Dennig, J., Preuss, A., Beato, M., and Suske, G. (1995) J. Biol. Chem. 270, 24989–24994
15. Majello, B., De Luca, P., Hagen, G., Suske, G., and Lania, L. (1994) Nucleic Acids Res. 22, 4914–4921
16. Majello, B., De Luca, P., Saske, G., and Lania, L. (1995) Oncogene 10, 1841–1848
17. Denz, J., Beato, M., and Suske, G. (1996) EMBO J. 15, 5659–5667
18. Liu, Z., and Simpson, E. R. (1997) Mol. Endocrinol. 11, 127–137
19. Pascal, E., and Tjian, R. (1991) Genes Dev. 5, 1646–1656
20. Lund, J., Ahlgren, R., Wu, D. H., Kagimoto, M., Simpson, E. R., and Waterman, M. R. (1990) J. Biol. Chem. 265, 3304–3312
21. Andrews, N. C., and Faller, D. V. (1991) Nucleic Acids Res. 19, 101–110
22. Kagawa, N., and Waterman, M. R. (1992) J. Biol. Chem. 267, 25213–25219
23. Dynan, W. S., Saffer, J. D., Lee, W. S., and Tjian, R. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4915–4919
24. Begeot, M., Shetty, U., Kilgore, M., Waterman, M., and Simpson, E. (1993) J. Biol. Chem. 268, 17317–17325
25. Prowse, D. M., Bolgian, L., Molnar, A., and Dotto, G. P. (1997) J. Biol. Chem. 272, 1308–1314
26. Nielsen, S. J., Praestegaard, M., Jørgensen, H. F., and Clark, B. F. (1998) Biochem. J. 333, 511–517
27. Majello, B., De Luca, P., and Lania, L. (1997) J. Biol. Chem. 272, 4921–4926
28. Chen, S. J., Arlitt, C. M., Jimenez, S. A., and Varga, J. (1998) Gene 215, 101–110
29. Jensen, D. E., Rich, C. B., Terpstra, A. J., Farmer, S. R., and Foster, J. A. (1995) J. Biol. Chem. 270, 6555–6563
30. Merchant, J. L., Shirotani, A., Mortensen, E. R., Shumaker, D. K., and Abraczinskas, D. R. (1995) J. Biol. Chem. 270, 6314–6319
31. Porter, W., Saville, B., Hovik, D., and Safe, S. (1997) Mol. Endocrinol. 11, 1569–1580
32. Zhang, D. E., Hetherington, C. J., Tan, S., Dziennis, S. E., Gonzalez, D. A., Chen, H. M., and Tenen, D. G. (1994) J. Biol. Chem. 269, 11425–11434
33. Marin, M., Karis, A., Visser, P., Grosveld, F., and Philipsen, S. (1997) Cell 89, 619–628
34. Chang, C. Y., Huang, C., Guo, I. C., Tsai, H. M., Wu, D. A., and Chung, B. C. (1992) Mol. Endocrinol. 6, 1362–1370
35. Young, P. P., and Mendelson, C. R. (1997) Mol. Endocrinol. 11, 1082–1093
36. Schanke, J. T., Durning, M., Johnson, K. J., Bennett, L. K., and Golos, T. G. (1998) Mol. Endocrinol. 12, 405–417
37. Grimaldi, P., Geremia, R., Albanesi, C., and Rossi, P. (1996) Mol. Cell. Endocrinol. 117, 167–173
38. Alliston, T. N., Majyar, A. C., Base, P., Firestone, G. L., and Richards, J. S. (1997) Mol. Endocrinol. 11, 1934–1949
39. Rohlf, C., Ahmad, S., Borellini, P., Lei, J., and Glazer, R. I. (1997) J. Biol. Chem. 272, 21137–21141
40. Jackson, S. A., Barry, D. A., Leggett, R. W., and Mueller, C. R. (1997) J. Biol. Chem. 272, 13488–13495
41. Lee, Y. H., Yano, M., Liu, S. Y., Matsunaga, E., Johnson, P. F., and Gonzalez, P. J. (1994) Mol. Cell. Biol. 14, 1383–1394
42. Borellini, F., and Glazer, R. I. (1993) J. Biol. Chem. 268, 7923–7928
43. Rohr, O., Aunis, D., and Schaeffer, E. (1997) J. Biol. Chem. 272, 31149–31155
44. Bessereau, J. L., Mendelzon, D., LePoupon, C., Fiszman, M., Changeux, J. P., and Piette, J. (1993) EMBO J. 12, 443–449
45. Dittmer, J., Degonne, A., Gitlin, S. D., Ghysdael, J., and Brady, J. N. (1994) J. Biol. Chem. 269, 21428–21434
46. Bakke, M., and Lund, J. (1995) Mol. Endocrinol. 9, 327–339
47. Clemens, J. W., Lala, D. S., Parker, K. L., and Richards, J. S. (1994) Endocrinology 134, 1499–1508
48. Jacob, A. L., and Lund, J. (1998) J. Biol. Chem. 273, 13391–13394
49. Crawford, P. A., Polish, J. A., Ganz, G., and Sadovsky, Y. (1997) Mol. Endocrinol. 11, 1628–1635