Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 1440–1444, February 1994
Biochemistry

Two orphan receptors binding to a common site are involved in the regulation of the oxytocin gene in the bovine ovary

(promoter/transcription factors SF-1 and COUP-TF/luteinization)

UWE WEHRENBERG, RICHARD IVELL, MARTINA JANSEN, STEPHANIE VON GOEDECKE, AND NORBERT WALTHER*

Institute for Hormone and Fertility Research, University of Hamburg, Grandweg 64, D-22529 Hamburg, Germany

Communicated by Elwood V. Jensen, October 11, 1993

ABSTRACT **The peptide hormone oxytocin is highly expressed in the hypothalamus within only a small number of magnocellular neurons. However, it is also expressed in a much larger number of cells in the bovine corpus luteum at high levels in an estrous cycle-dependent manner. By using nuclear extracts from this tissue for *in vitro* binding studies, two protein complexes have been shown to bind to a common site in the bovine oxytocin promoter. One of these proteins has been identified as the bovine homologue of the chicken ovalbumin upstream promoter transcription factor (COUP-TF). The second protein is here characterized as the bovine homologue of a tissue-specific transcription factor, steroidogenic factor 1 (SF-1). The relative expression of these two factors during luteal development correlates with the level of luteal oxytocin gene expression, with SF-1 being the factor binding to the promoter of the oxytocin gene when this promoter is activated. Cotransfection experiments using the murine testicular cell line TM4 show that SF-1 can stimulate the expression of a transfected oxytocin gene, suggesting that SF-1 may be involved in up-regulation of the oxytocin gene *in vivo*, possibly by transducing a stimulatory signal to the RNA polymerase.**

The gene for the hypothalamic peptide hormone oxytocin forms part of a more complex gene locus which also includes the homologous vasopressin gene (1–3). Because of the paucity of the hypothalamic neurons which express the oxytocin gene, and the lack of any oxytocin-expressing cell lines, very little is known about the mechanisms by which the oxytocin gene is regulated.

The oxytocin gene is also expressed in some peripheral tissues (4). In particular, it is highly up-regulated in the large cells of the early bovine corpus luteum, which are derived by luteinization of preovulatory granulosa cells (5), and in the bovine testis (6). A transgenic study using a bovine oxytocin gene construct comprising only the gene and 600 bp of the 5′ noncoding sequence indicated consistent expression in the Sertoli cells of the transgenic mouse, evidently using the same transcription start site as in the hypothalamus or as in the bovine ovary and testis (6). These observations suggest that, unlike in the hypothalamus, the bovine oxytocin gene can be tissue-specifically expressed in the gonads by a minimal functional promoter contained within 600 bp of the transcription start site.

Computer comparison of the 5′ noncoding region of the oxytocin gene among a number of species revealed a conserved promoter region of about 200 bp (7). *In vitro* DNA–nuclear protein binding studies have identified a highly conserved DNA element within this 200-bp promoter region at ≈160 nucleotides upstream from the transcriptional initiation site (8). This element shows good homology with a direct repeat of the sequence motif AGGTCA, which is known to constitute a part of the binding site for a diverse group of nuclear hormone receptors. In the rat and human oxytocin promoter this binding site in addition contains a good homology with the classic palindromic estrogen-responsive element, such that in a heterologous transfection system the rat and human but not the bovine oxytocin gene promoter can be stimulated by estradiol (9, 10). However, estrogen receptor expression could not be detected in the oxytocinergic cells of the rat hypothalamus (11) suggesting that direct estrogen-dependent activation is not the mechanism regulating oxytocin gene expression *in vivo*.

Taking advantage of the relatively high activity of the oxytocin gene in the bovine ovary, we have used a combination of DNA–nuclear protein binding studies and transient-transfection assays, to identify the transcription factors binding to this promoter *in vivo*. Two factors have been identified, COUP-TF (chicken ovalbumin upstream promoter transcription factor) and SF-1 (steroidogenic factor 1), which bind to the conserved element at position −160. Although both these factors belong to the superfamily of nuclear hormone receptors, they have no known ligands and, thus, belong to the subgroup of orphan receptors. We show here that these two factors both interact with a common binding site in the oxytocin promoter and that SF-1 is involved in regulation of oxytocin gene transcription.

MATERIALS AND METHODS

Extraction of Nuclear Proteins. Nuclear proteins from frozen bovine tissues were extracted as described (8). Bovine granulosa cells were prepared from preovulatory follicles and maintained as primary cultures for 48 hr (12, 13). For extraction of nuclear proteins from granulosa cells or tissue culture cells, a rapid micropreparation technique (14) was used. Nuclear proteins were divided into aliquots, frozen in liquid nitrogen, and stored at −80°C.

Gel Retardation Experiments. Gel retardation experiments were performed (8) with a fragment from the bovine oxytocin promoter (−185 to −121) as probe. The following double-stranded oligonucleotides were used as specific competitors: CTATCAGTGACCTTGGATGCA [optimized binding site for the bovine homologue of SF-1 (15), designated SF-1] and AGTTTGACCTTTGACACCATA [chicken ovalbumin promoter COUP-TF binding site, −89 to −69 (16), designated COUP]. For immunological characterization of proteins, specific antisera against SF-1 and COUP-TF (generous gifts of K. Parker, Duke University Medical Center, Durham, NC, and M.-J. Tsai, Baylor College of Medicine, Houston) were added at a dilution of 1:5 to the binding reaction mixture.

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

Abbreviations: SF-1, steroidogenic factor 1; COUP-TF, chicken ovalbumin upstream promoter transcription factor.

*To whom reprint requests should be addressed.

Methylation Interference Footprinting Analysis. Methylation interference footprinting analysis was performed as described (8). The partially methylated DNA was cleaved at the positions of G and A residues by treatment with 0.1 M NaOH at 90°C (16).

Establishment of a Bovine Granulosa-Derived Cell Line. Bovine granulosa cells were prepared from preovulatory follicles (12, 13) and the primary cultures were supplemented with insulin (10 μg/ml) and 2% horse serum. For immortalization the cells were cotransfected with a subgenomic fragment of simian virus 40 containing the complete early region (generous gift of W. Deppert, Heinrich-Pette-Institut, Hamburg, Germany) and the neomycin-resistance vector pSV2neo (17) by using the Lipofectin reagent (GIBCO). After 17 days of selection with the antibiotic G418 (GIBCO), a single immortalized neomycin-resistant clone was isolated and grown into the I1 cell line. Supernatants of I1 cells were tested at regular intervals for production of steroids by radioimmunoassays (13) and found to produce progesterone, but not estradiol. Oxytocin gene expression could not be detected in I1 cells by RNase protection assay.

Transient Transfection of Cell Lines. TM4 cells were obtained from the American Type Culture Collection. For transient transfection, I1 cells (described above) or TM4 cells were plated into Petri dishes and transfected by calcium phosphate precipitation (18). Expression vectors for transcription factors SF-1 (19) and COUP-TF (20) were generous gifts of K. Parker and M.-J. Tsai, respectively. As reporter plasmid, pBOPLUC-185, containing the bovine oxytocin promoter (−185 to +17), or pT109LUC, containing the herpes simplex virus thymidine kinase promoter (−109 to +52), both inserted into the pXP2 luciferase vector (21), were used; the β-galactosidase expression vector pCH110 (Pharmacia) was employed as a control for transfection efficiency. After 24 hr cells were lysed, and luciferase activities (luciferase assay system, Promega), and β-galactosidase activities (Galacto-Light System, Tropix, Bedford, MA) were determined.

RESULTS

Characterization of Specific DNA-Binding Proteins from Bovine Granulosa Cells and Corpus Luteum. When nuclear proteins are prepared from bovine corpora lutea and analyzed by gel retardation assays using a DNA fragment from the promoter region of the bovine oxytocin gene (−185 to −121), two major protein–DNA complexes, A and B, are formed. A 20-bp oligonucleotide encompassing the conserved DNA element at −160 competes with the probe to block formation of the labeled complexes (8). These complexes are identified in different experiments using different nuclear extracts by their reproducible electrophoretic mobility and the specificity of their binding to oligonucleotide competitors. A 21-bp oligonucleotide containing a binding site for SF-1 specifically blocked formation of complex A, whereas formation of complex B was specifically blocked by a 21-bp oligonucleotide containing a COUP-TF binding site. Nuclear extracts from granulosa cells isolated from preovulatory follicles form complex A exclusively (Fig. 1). Whereas complex A was predominant in the early corpus luteum, there was increased formation of complex B in the mid to late cycle and in pregnancy. The prevalence of complex A correlates well with maximal *in vivo* expression of the oxytocin gene in the bovine corpus luteum, with highest levels of oxytocin mRNA being present immediately following ovulation (5). The gene is evidently switched off after day 5, with an exponential decline in mRNA thereafter. This down-regulation correlates well with the increase of complex B, which includes the bovine homologue of COUP-TF (22). The gene for bovine COUP-TF is activated during luteal differentiation, thus explaining the increase in complex B content by *de novo* synthesis of COUP-TF (22).

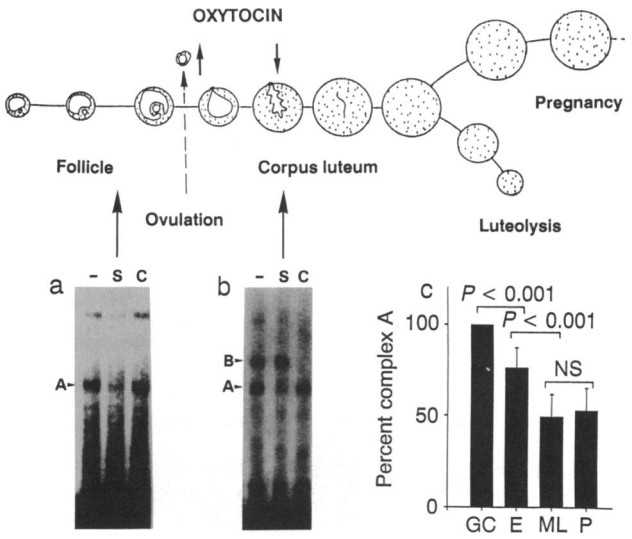

FIG. 1. Time course of prevalence of complex A during luteal differentiation. At the top, the time course of luteal differentiation and the phase of active oxytocin transcription in the early corpus luteum are shown. (*a* and *b*) Two gel retardation experiments using nuclear extracts from preovulatory granulosa cells (*a*) and from corpora lutea from the middle to late cycle (*b*) are illustrated. The retarded complexes A and B were identified by their relative electrophoretic mobility, together with specific competition with oligonucleotides containing binding sites for SF-1 (lanes S) or COUP-TF (lanes C). (*c*) Summary of the results of a larger number of such experiments using nuclear extracts from granulosa cells (GC), or corpora lutea from the early cycle (E), the middle to late cycle (ML), or pregnancy (P). The autoradiographic intensities of the retarded complexes A and B were estimated by computer-assisted quantitation with the Java system (Jandel, Erkrath, Germany) and expressed as percent complex A in relation to the sum of complexes A and B. The results are given as means and standard deviations from four to eight independent experiments and were compared by using Student's *t* test. NS, not significant.

Gel retardation experiments using various concentrations of oligonucleotide competitors (Fig. 2) showed that a 30-fold excess of the SF-1 binding site completely blocked formation of complex A, whereas a 3000-fold excess was necessary to block formation of complex B. Competition with a 30-fold excess of the COUP-TF binding site led to complete inhibition of formation of complex B, whereas only at a 3000-fold excess was complex A effectively eliminated. Additional bands appeared with varying intensities in gel retardation experiments using different preparations of luteal nuclear extracts. Formation of the low-mobility complex was inhibited as well by the SF-1 as by the COUP-TF binding site, suggesting that both factors were constituents of this complex, whereas formation of the two high-mobility complexes was inhibited with some degree of specificity only by the SF-1 binding site.

To confirm the characterization of the proteins present in the luteal complexes, the nuclear extracts were incubated with specific antisera against either SF-1 or COUP-TF (Fig. 3). The SF-1-specific antiserum recognized complex A, the low-mobility complex, and the upper one of the high-mobility complexes, leading to total disappearance of these bands in the gel retardation assay. The COUP-TF-specific antiserum recognized complex B and the low-mobility complex, resulting also in the disappearance of these bands in the gel retardation assay. This result differs somewhat from the "supershift" effect of this antiserum described by Wang *et al.* (23). This difference may be due to a lower affinity of the COUP-TF-containing complex for the oxytocin gene pro-

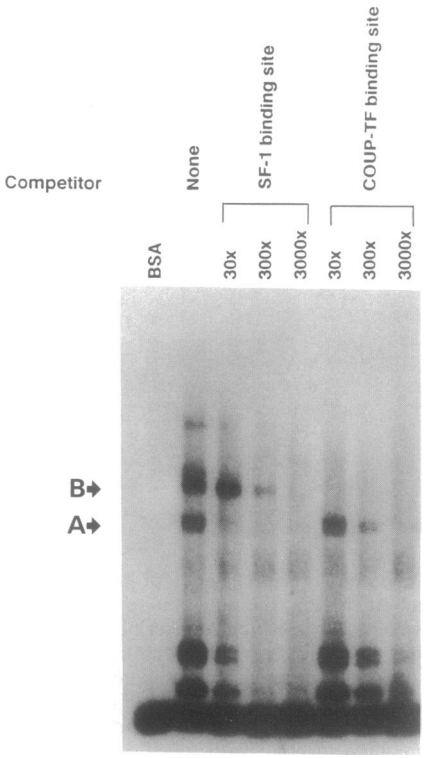

FIG. 2. Interaction of proteins from complexes A and B with DNA binding sites for SF-1 or COUP-TF. Nuclear protein (1 μg) from middle- to late-phase corpora lutea was subjected to gel retardation analysis. For competition studies of binding to the oxytocin promoter, unlabeled SF-1 or COUP oligonucleotide was added to the reaction in the molar excess indicated above the lanes. Bovine serum albumin (BSA, 1 μg) was used as control.

moter, addition of the antibody thus exacerbating complex instability. Using the same antiserum, Rice *et al.* (24) also observed disappearance of COUP-TF complexes. No cross-reaction of the two antisera with their noncognate complexes was observed. Addition of control antisera failed to show any effect on complex formation.

To analyze the interactions of the luteal nuclear proteins with the bovine oxytocin promoter in detail, methylation interference footprinting was employed (Fig. 4). A nuclear extract from day 5 corpora lutea exhibiting a prominent complex A band and relatively large amounts of the low-mobility complex (termed here complex C) was compared with a nuclear extract from day 11 corpora lutea showing complex A and complex B bands of comparable intensity. All protein–DNA interactions map to the promoter region from −151 to −163 encompassed by the oligonucleotide competitor used to define this site (8). Complex A shows strong interaction in the region from −156 to −163 and weak interaction at position −154 in the coding strand. The resulting footprint reveals the complex A binding site as TCAAGGTTAT, a sequence showing high similarity with the binding sites for the bovine (15), mouse (25), and rat (26) homologues of SF-1. This binding site contains the complete distal nuclear hormone receptor half-site (AGGTTA) and shows considerable overlap with the proximal half-site. The sequence of the binding site indicates that SF-1 binds as a monomer to the oxytocin promoter. In contrast, the complex B footprint covers the whole region from −151 to −163, containing both nuclear hormone receptor half-sites, indicating that COUP-TF binds to this sequence as a homodimer or possibly as a heterodimer with another factor such as retinoid X

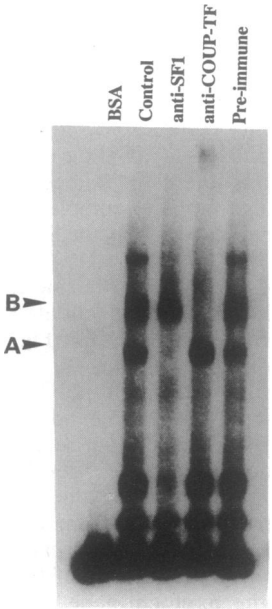

FIG. 3. Characterization of proteins from complexes A and B with antisera specific for SF-1 or COUP-TF. Nuclear protein (1 μg) from middle- to late-phase corpora lutea was subjected to gel retardation analysis. For immunological characterization of the proteins, 2 μl of antiserum specific for SF-1 or COUP-TF or 2 μl of preimmune serum were added to the binding reaction mixtures as indicated. Reaction mixtures with 1 μg of nuclear extract without antiserum added (Control) and 1 μg of bovine serum albumin (BSA) were used as controls.

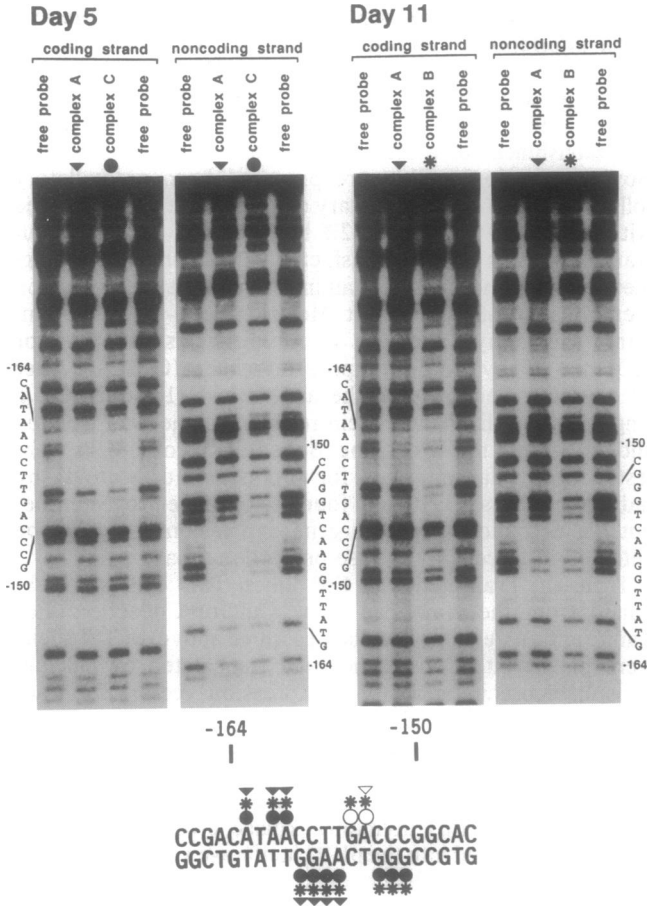

FIG. 4. Methylation interference footprinting of the binding sites for the luteal nuclear proteins. The retarded bands from 10 parallel gel retardation experiments employing nuclear extracts from 5- or 11-day corpora lutea were used for analysis of the protein–DNA interactions present in complex A, complex B, and the low-mobility complex C. The type of complex analyzed is indicated above the lanes. The sequence of the footprint region is shown beside the autoradiograms. The results of the analysis are summarized at the bottom. Positions of interference with protein binding are indicated by triangles (complex A), asterisks (complex B), and circles (complex C). Filled symbols represent positions of strong interference with protein binding, whereas open symbols indicate weaker interference.

receptor (27). The footprint of the low-mobility complex (complex C) covers the same range as the complex B footprint, but the interactions at positions −154 and −155 in the coding strand are substantially weaker. This result can be explained by assuming that SF-1 and COUP-TF bind simultaneously to this site, with SF-1 occupying the distal half-site and COUP-TF the proximal half-site, but, being unable to form a heterodimer, they hinder each other from optimal binding. The footprints of the two high-mobility complexes (data not shown) show high similarity to the footprint of complex A, suggesting that these proteins are derived from SF-1, possibly by proteolytic degradation. Similar complexes have been observed in nuclear extracts from other tissues expressing SF-1 (25). Taken together, these results identify the overlapping binding sites for the orphan receptors SF-1 and COUP-TF in the bovine oxytocin promoter. Both factors can bind alternatively to this site; simultaneous binding apparently leads to a steric hindrance, suggesting that the observed low-mobility complex may not represent a functional heterodimer.

SF-1 and COUP-TF Are Necessary for the Formation of Complexes A and B. The *in vitro* protein–DNA binding studies described above show that the transcription factors SF-1 and COUP-TF are expressed in the bovine corpus luteum in a cycle-dependent fashion and can bind to a common element in the bovine oxytocin gene promoter. To show that heterologous SF-1 and COUP-TF can substitute for the endogenous bovine proteins in the formation of complexes A and B, transfection experiments were performed with the bovine granulosa-derived cell line I1 (Fig. 5). Nuclear proteins were prepared from I1 cells transfected with expression vectors encoding human COUP-TF or murine SF-1 and analyzed by gel retardation experiments. Nuclear proteins from untransfected I1 cells did not form complex A or B (Fig. 5, lanes 1–3), consistent with the inability of these cells to express the endogenous oxytocin gene. Only after transfection with the COUP-TF (Fig. 5, lanes 4–6) or SF-1 (lanes 7–9) expression vector, respectively, was complex B or A evident. The specificity of these complexes was verified by competition with the oligonucleotides representing the respective binding sites for COUP-TF or SF-1. Cotransfection with both expression vectors (Fig. 5, lanes 10–12) resulted in the formation of both complexes. Formation of the low-mobility complex appearing in the cotransfections with SF-1 and COUP-TF was only partially blocked by the SF-1 binding site in this case. A high-mobility complex with characteristics similar to the complexes found with the luteal nuclear extracts appeared in all transfections with SF-1, confirming that the DNA-binding protein in this complex was derived from SF-1. This experiment clearly shows that the heterologous transcription factors SF-1 and COUP-TF can substitute for endogenously expressed bovine proteins in the formation of the specific protein–DNA complexes A and B. Thus the bovine homologues of SF-1 and COUP-TF can be assumed to be the constituents of these luteal protein–DNA complexes.

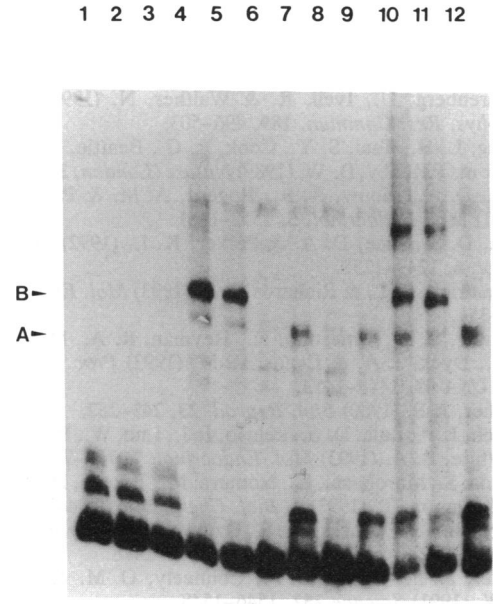

FIG. 5. Analysis of nuclear proteins from I1 cells transfected with expression vectors for SF-1 and COUP-TF. I1 cells were transfected with control plasmid (lanes 1–3), 10 μg of COUP-TF expression vector (lanes 4–6), 10 μg of SF-1 expression vector (lanes 7–9), or 10 μg each of both expression vectors (lanes 10–12). Competitor oligonucleotides were added to the reaction mixtures for lanes 2, 5, 8, and 11 (SF-1 oligonucleotide) and for lanes 3, 6, 9, and 12 (COUP oligonucleotide).

Influence of SF-1 on Oxytocin Gene Expression in TM4 Cells. The peripheral expression of the bovine oxytocin gene is not restricted to the female gonad but is also appreciable within the Sertoli cells in the testis of the bull and of transgenic mice carrying the bovine oxytocin gene with only 600 bp of the promoter region (6). As the I1 cell line established from bovine granulosa cells apparently had shut off the expression of the endogenous oxytocin gene during tissue culture (data not shown) the mouse testicular cell line TM4, supposedly of Sertoli cell origin (28), was chosen to investigate the regulation of oxytocin gene expression by transient-transfection studies.

Nuclear protein extracts of TM4 cells showed that these were able to form complex B and thus contained COUP-TF (data not shown). Transfection of these cells with a construct comprising the bovine oxytocin gene promoter coupled to the luciferase reporter gene led to a measurable basal expression (Fig. 6). Cotransfection with the SF-1 expression vector caused a 2-fold increase in luciferase activity, comparable to the transcriptional effect of murine or bovine SF-1 reported by Lynch *et al.* (29) and Honda *et al.* (30). Control experiments using a herpes simplex virus thymidine kinase promoter–luciferase reporter construct (21) resulted in no increase in the basal transcription by cotransfection of SF-1. These results show that the observed increase in transcription is dependent on the specific binding of SF-1 to the bovine oxytocin promoter. This factor appears to be necessary for the up-regulation of oxytocin gene transcription in the bovine corpus luteum.

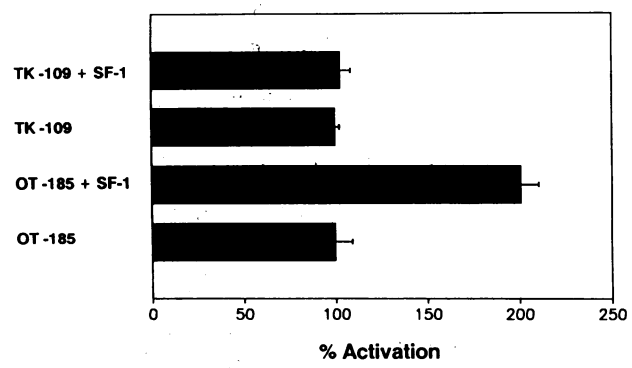

FIG. 6. Effect of SF-1 on oxytocin promoter activity in transfected TM4 cells. TM4 cells were transfected with 10 μg of pT109LUC (TK-109) or pBOPLUC-185 (OT-185) together with 10 μg of SF-1 expression vector or 10 μg of control plasmid. The promoter activity in the absence of cotransfected SF-1 was set to 100%. Results are given as means and standard deviations from three independent experiments.

DISCUSSION

As no cell lines expressing oxytocin exist to date, and the gene locus involved in hypothalamic expression appears to be very complex, it was decided to access the oxytocin gene and its regulation by working with preovulatory granulosa cells and luteal cells of the ruminant ovary, the only cell type that in primary culture appears to express high levels of oxytocin mRNA endogenously under controlled conditions.

The results present a consistent image of two orphan receptors, SF-1 and COUP-TF, binding to a highly conserved transcription factor binding site in the oxytocin promoter region. These two factors are differentially expressed *in vivo*, with the expression of SF-1 being associated with active transcription of the bovine oxytocin gene. The bovine homologue of SF-1 is implicated in the activation of several of the steroidogenic enzymes in the adrenal, ovary, and testis and appears to mediate the response induced by cAMP-generating agents such as gonadotropins or forskolin (19, 24, 26). However, the observation that it is also present in unstimulated primary cultures of granulosa cells, before the oxytocin gene is up-regulated and before progesterone production by these cells is increased (data not shown), as well as the presence of SF-1 in the adrenal gland (30) and in Leydig cells (19), where the oxytocin gene is not highly up-regulated, suggests that its presence alone is not sufficient to induce the massive up-regulation of the oxytocin gene *in vivo*. This correlates with the recent finding that granulosa cells from early preantral follicles that do not have aromatase activity already contain a protein presumed to be SF-1, before the aromatase promoter that binds this protein is activated (29). COUP-TF is able to activate transcription for some genes *in vitro* (16). However, COUP-TF homodimers or heterodimers with several hormone receptors may bind to hormone response elements leading to an inhibition of hormone-induced transcription (20, 27, 31). The increase in COUP-TF expression during luteal differentiation correlates with the down-regulation of oxytocin transcription. However, the dramatic up- and down-regulation of oxytocin transcription cannot be explained exclusively by the binding of SF-1 or COUP-TF. Interaction of SF-1 and/or COUP-TF with other regulatory proteins, or specific modification of the orphan receptors, is needed for the high up-regulation of oxytocin transcription in the bovine corpus luteum. Like SF-1 (see above), COUP-TF may be influenced by cAMP agonists (32). It has been suggested that phosphorylation may be a general mechanism for regulating the activity of orphan receptors (33, 34). SF-1 might function as a permissive factor enabling the RNA polymerase to transcribe the oxytocin gene, by transducing an activating signal from an enhancer-like element located further away from the transcription start site, while the occupation of the oxytocin promoter binding site with COUP-TF might interfere with this activation.

The DNA element identified in this study is one which in heterologous systems using the rat or human promoters can also respond to binding of the estrogen receptor (9, 10), the thyroid hormone receptor (35), or the retinoic acid receptor (36). However, there is no positive evidence that this element is bound by these receptors *in vivo*. Nevertheless, it is possible that in different tissues or even in different species, the same element may answer differently to binding of several dissimilar but related transcription factors. It will therefore be important to extend the studies presented here to an analysis of other species and tissues.

We thank Drs. K. Parker and M.-J. Tsai for kindly providing the expression vectors and antisera for SF-1 and COUP-TF, respectively, and Dr. W. Deppert for the kind gift of the simian virus 40 plasmid. The excellent technical assistance of B. Bartlick and D. Kann is gratefully acknowledged. We are grateful to Prof. F. Leidenberger for his generous support and encouragement and to the Deutsche Forschungsgemeinschaft for financial support (Grant Ho 388/6-3/10). Aspects of this study form part of a doctoral thesis by U.W.

1. Sausville, E., Carney, D. & Battey, J. (1985) *J. Biol. Chem.* **260,** 10236–10241.
2. Hara, Y., Battey, J. & Gainer, H. (1990) *Mol. Brain Res.* **8,** 319–324.
3. Schmitz, E., Mohr, E. & Richter, D. (1991) *DNA Cell Biol.* **10,** 81–91.
4. Ivell, R. (1987) in *Neuropeptides and Their Peptidases*, ed. Turner, A. J. (Horwood, Chichester, U.K.), pp. 31–64.
5. Ivell, R., Brackett, K. H., Fields, M. J. & Richter, D. (1985) *FEBS Lett.* **190,** 263–267.
6. Ang, H. L., Ivell, R., Walther, N., Nicholson, H., Ungefroren, H., Millar, M., Carter, D. & Murphy, D. (1994) *J. Endocrinol.*, in press.
7. Ivell, R., Hunt, N., Abend, N., Brackmann, B., Nollmeyer, D., Lamsa, J. C. & McCracken, J. A. (1990) *Reprod. Fertil. Dev.* **2,** 703–711.
8. Walther, N., Wehrenberg, U., Brackmann, B. & Ivell, R. (1991) *J. Neuroendocrinol.* **3,** 539–549.
9. Burbach, J. P. H., Adan, R. A. H., van Tol, H. H. M., Verbeeck, M. A. E., Axelson, J. F., Leeuwen, F. W. & Beekman, J. M. (1990) *J. Neuroendocrinol.* **2,** 633–639.
10. Richard, S. & Zingg, H. H. (1990) *J. Biol. Chem.* **265,** 6098–6103.
11. Axelson, J. F. & van Leeuwen, F. W. (1990) *J. Neuroendocrinol.* **2,** 209–216.
12. Luck, M. R. & Jungclas, B. (1987) *J. Endocrinol.* **114,** 423–430.
13. Holtorf, A. P., Furuya, K., Ivell, R. & McArdle, C. A. (1989) *Endocrinology* **125,** 2612–2620.
14. Andrews, N. C. & Faller, D. V. (1991) *Nucleic Acids Res.* **19,** 2499.
15. Morohashi, K. I., Honda, S. I., Inomata, Y., Handa, H. & Omura, T. (1992) *J. Biol. Chem.* **267,** 17913–17919.
16. Tsai, S. Y., Sagami, I., Wang, H., Tsai, M. J. & O'Malley, B. W. (1987) *Cell* **50,** 701–709.
17. Southern, P. & Berg, P. (1982) in *Eukaryotic Viral Vectors*, ed. Gluzman, Y. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 41–45.
18. Schöler, H. R. & Gruss, P. (1984) *Cell* **36,** 403–413.
19. Ikeda, Y., Lala, D. S., Luo, X., Kim, E., Moisan, M. P. & Parker, K. L. (1993) *Mol. Endocrinol.* **7,** 852–860.
20. Cooney, A. J., Tsai, S. Y., O'Malley, B. W. & Tsai, M. J. (1992) *Mol. Cell. Biol.* **12,** 4153–4163.
21. Nordeen, S. K. (1988) *BioTechniques* **6,** 454–457.
22. Wehrenberg, U., Ivell, R. & Walther, N. (1992) *Biochem. Biophys. Res. Commun.* **189,** 496–503.
23. Wang, L. H., Tsai, S. Y., Cook, R. G., Beattie, W. G., Tsai, M. J. & O'Malley, B. W. (1989) *Nature (London)* **340,** 163–166.
24. Rice, D. A., Mouw, A. R., Bogerd, A. M. & Parker, K. L. (1991) *Mol. Endocrinol.* **5,** 1552–1561.
25. Lala, D. S., Rice, D. A. & Parker, K. L. (1992) *Mol. Endocrinol.* **6,** 1249–1258.
26. Fitzpatrick, S. L. & Richards, J. S. (1993) *Mol. Endocrinol.* **7,** 341–354.
27. Kliewer, S. A., Umesono, K., Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A. & Evans, R. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1448–1452.
28. Mather, J. P. (1980) *Biol. Reprod.* **23,** 243–252.
29. Lynch, J. P., Lala, D. S., Peluso, J. J., Luo, W., Parker, K. L. & White, B. A. (1993) *Mol. Endocrinol.* **7,** 776–786.
30. Honda, S., Morohashi, K., Nomura, M., Takeya, H., Kitajima, M. & Omura, T. (1993) *J. Biol. Chem.* **268,** 7494–7502.
31. Berrodin, T. J., Marks, M. S., Ozato, K., Linney, E. & Lazar, M. A. (1992) *Mol. Endocrinol.* **6,** 1468–1478.
32. Power, R. F., Lydon, J. P., Conneely, O. M. & O'Malley, B. W. (1991) *Science* **252,** 1546–1548.
33. O'Malley, B. W. & Tsai, M.-J. (1992) *Biol. Reprod.* **46,** 163–167.
34. O'Malley, B. W. & Conneely, O. M. (1992) *Mol. Endocrinol.* **6,** 1359–1361.
35. Adan, R. A. H., Cox, J. J., van Kats, J. P. & Burbach, J. P. H. (1992) *J. Biol. Chem.* **267,** 3771–3777.
36. Richard, S. & Zingg, H. H. (1991) *J. Biol. Chem.* **266,** 21428–21433.