Molecular Cloning, Structure, and Expression of Mouse Estrogen-responsive Finger Protein Efp

CO-LOCALIZATION WITH ESTROGEN RECEPTOR mRNA IN TARGET ORGANS*

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We have previously identified a human estrogen-responsive gene, efp (estrogen-responsive finger protein), which encodes a putative transcription regulator (Inoue, S., Orimo, A., Hosoi, T., Kondo, S., Toyoshima, H., Kondo, T., Ikegami, A., Ouchi, Y., Orimo, H., and Muramatsu, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11117–11121). Here, we report isolation of mouse Efp cDNA and its structure containing three cysteine-rich domains (RING finger and B1 and B2 boxes), a coiled-coil domain, and a C-terminal domain. High levels of Efp mRNA were detected in uterus, ovary, and placenta by RNase protection assay. By in situ hybridization histochemistry the transcripts of efp were also detected in uterus, mammary gland, ovary, and brain, and the co-localization of Efp and estrogen receptor mRNA was particularly demonstrated in these female organs. Moreover, the level of Efp mRNA in uterus and brain, which are known as target organs for estrogen, was up-regulated in vivo by 17β-estradiol. Furthermore, both the Efp and estrogen receptor mRNA were stained in the brain vesicles of 11.5-day embryos by whole mount in situ hybridization. These findings raise the possibility that efp is an estrogen-responsive gene that mediates estrogen action in various target organs.

Estrogen plays important roles in the reproductive system as a sex steroid hormone. It is involved in the growth and development of female organs such as the uterus and mammary gland. Estrogen receptor (ER) acts as an estrogen-dependent transcription factor recognizing and binding to specific estrogen-responsive elements (ERE) in the enhancer region of target genes and regulating their transcription directly. Thus, estrogen exerts its action on target organs by regulating target gene products (1, 2). ER has also been identified in the central nervous system (3, 4), in the skeletal system (5, 6), and in the cardiovascular system (7, 8), implying some important roles for estrogen and estrogen responsive genes in a number of nonreproductive organs. From the clinical point of view, estrogen replacement therapy is effective to protect postmenopausal women from osteoporosis (9) and coronary heart disease (10). Furthermore, estrogen plays critical roles in carcinogenesis and growth of breast cancers (11).

In contrast to the wide variety of estrogen action on different organs, tissues, and cells, estrogen-responsive genes that are known so far are relatively few and include vitellogenin (12), prolactin (13), pS2 (14), uteroglobin (15), ovalbumin (16), progesterone receptor (17), and lactoferin (18). More genes that mediate estrogen action in a number of organs should be present. To identify estrogen-responsive genes, we have developed a method designated “genomic binding site cloning” used for isolation of ERE-containing fragments from human genomic DNA (19). Using one of those fragments, we have cloned a novel estrogen-responsive gene, efp (for estrogen-responsive finger protein) (20). The predicted human Efp protein had a RING finger motif present in a new family of apparent nuclear proteins including transcription regulators (21, 22). Human efp contained a consensus ERE sequence at the 3′ region that could act as a downstream estrogen-dependent enhancer, and it was up-regulated by estrogen in ER-positive cells derived from mammary gland.

Here, we have identified the mouse homologue of human efp. The predicted mouse Efp showed a high degree of conservation with human Efp. Interesting differential conservation of different domains of the protein was also noted. Mouse Efp mRNA was detected in reproductive organs and in the central nervous system by in situ hybridization histochemistry. Northern blot analysis indicated that the level of Efp mRNA was up-regulated within 2 h in uterus and brain by 17β-estradiol. Moreover, the co-localized expression of Efp mRNA with ER mRNA in female reproductive organs and in brain vesicles of embryos was demonstrated.

EXPERIMENTAL PROCEDURES

Screening of Mouse cDNA Libraries and DNA Sequence Analysis—A λZAPII (Stratagene) cDNA library prepared from poly(A)+ RNA of mouse placenta was screened as described previously (20). 600,000 plaques were screened by hybridization with the 32P-labeled 2.4-kilobase EcoRI fragment of human Efp cDNA (20). Sequence analysis was performed by the dideoxy method according to the manufacturer’s instructions (Sequenase, U.S. Biochemical Corp.).

Southern Blot Analysis—Mouse, human, and rat genomic DNAs were prepared as described (23). Purified DNA was digested overnight with restriction enzymes separated in 0.7% agarose gel. A transferred membrane was hybridized with a 32P-labeled 522-bp EcoRI fragment encompassing a part of the cysteine-rich region of mouse Efp cDNA overnight as described previously (19). Each total RNA was extracted as described previously (23) for the RNase protection assay. 17β-Estradiol (500 μg/kg) mixed with olive oil was injected subcutaneously into 3-week female ICR mice. After the injection of 17β-estradiol, uterus and brain were collected at the in-
cated hours. RNA was extracted, and poly(A)^+ RNA was prepared for Northern blot analysis.

RNA Probes—For in situ hybridization, antisense and sense RNA probes labeled with digoxigenin-UTP (Boehringer Mannheim) were produced in vitro from linearized plasmid bearing a 522-bp EcoRI fragment of mouse Efp cDNA or a 2.1-kilobase EcoRI fragment of mouse ER cDNA (24). The length of the 522-bp EcoRI fragment was reduced to 100–200 bp by alkaline hydrolysis (25). Probes were used for hybridization at 1 μg/ml. For the RNase protection assay, antisense RNA probes were generated with [32P]UTP (Amersham Corp.) in vitro according to the manufacturer’s protocol (Nippongene, Tokyo) from linearized plasmid bearing a 522-bp EcoRI fragment of mouse Efp cDNA or an 180-bp Xho-I-PstI fragment of mouse ER cDNA (24). For internal control, antisense RNA probes were generated with [32P]UTP in vitro from linearized plasmid bearing a 114-bp EcoRI fragment of mouse glyceraldehyde-3-phosphate dehydrogenase cDNA or a 250-bp fragment of β-actin cDNA supplied with the kit.

RNase Protection Assay—RNase protection assays were performed using the Ambion ribonuclease protection assay RPA Kit (Ambion, Texas). Each 15 μg of total RNA derived from mouse tissues was hybridized with Efp and β-actin RNA probes or ER and glyceraldehyde-3-phosphate dehydrogenase RNA probes. Each 15 μg of total RNA derived from the heads of mouse embryos was hybridized with Efp and β-actin RNA probes.

Northern Blot Analysis—For each sample, 3 μg of poly(A)^+ RNA from uterus and 10 μg of poly(A)^+ RNA from brain were separated in 1% agarose. Northern blot analysis was performed as described previously (20). The 32P-labeled 522-bp EcoRI fragment of mouse Efp cDNA, β-actin cDNA fragment (Nippongene, Tokyo), or glyceraldehyde-3-phosphate dehydrogenase cDNA fragment was used as the probe. Autoradiography was carried out at ~80 °C with an intensifying screen for 3 days in Efp probe and for 1 day in β-actin cDNA probe. This experiment was performed three times with consistent results.

Nuclear Extract Preparation—Nuclear extracts were prepared as described by Gorski et al. (26) with several modifications. Mouse placenta was minced finely with scissors, homogenized in the indicated buffer (26), loaded on top of the sucrose cushion containing 2M sucrose and centrifuged at 23,000 rpm for 60 min. The nuclear pellet was dissolved in the indicated buffer, quantitated by measuring absorbance, and frozen in liquid nitrogen until use.

Antibody Preparation and Western Blot Analysis—Partial mouse Efp cDNA (amino acids 303–457) generated by polymerase chain reaction was ligated in the Xho-I-PstI fragment of mouse ER cDNA (24). In-frame fusions were constructed. The fusion protein expressed in E. coli was eluted from a Sepharose 4B column (Pharmacia) by competition with reduced glutathione (27). Rabbit polyclonal anti-Efp antiserum was generated by subcutaneous injection of the fusion protein emulsified in complete Freund’s adjuvant. Western blot analysis was as described previously (20). The membrane was probed with the anti-mouse Efp antibody (1:10,000) and then anti-rabbit IgG (F) (F) conjugated with alkaline phosphatase (1:7500). In Situ Hybridization—Tissues of 5-week ICR mice were collected and fixed. In situ hybridization was performed essentially as described previously (28). Slides were hybridized with mouse Efp or ER RNA probe overnight at 50 °C and reacted with anti-digoxigenin-alkaline phosphatase (Boehringer Mannheim) (1:700) diluted in blocking reagent. Then color reactions were performed with nitro blue tetrazolium and X-phosphate (Boehringer Mannheim) overnight.

Whole Mount in Situ Hybridization—Whole mount in situ hybridization was performed as described by Wilkinson (29). 11.5-day embryos were removed from the uteruses of pregnant ICR mice, fixed in 4% paraformaldehyde in phosphate-buffered saline at 4 °C for 12 h, and hybridized with the digoxigenin-labeled mouse Efp and ER RNA probes.

RESULTS

Isolation of Mouse Efp—Seven clones were isolated by the screening the mouse placenta cDNA library (see “Experimental Procedures”). All the clones were found to be isolated from the same RNA by restriction mapping and partial sequencing. The clone n3 that had the longest insert was sequenced full-length for both strands and was found to have the longest open reading frame (634 amino acids) that showed the same domain organization and a high degree of sequence homology to the human Efp (Fig. 1A). An isoform cDNA lacking 99 bp was also identified, which might be derived from an alternative splicing (Fig. 1A).

Structure of Mouse Efp—Mouse Efp cDNA (n3) encodes a protein of calculated relative molecular mass (Mr) of 71,768, containing the RING finger, B1 box, B2 box, coiled-coil, and C-terminal domains (Fig. 1A). The RING finger and B1 and B2 boxes form the cysteine-rich region of the Efp. An alignment between the predicted mouse and human Efp proteins shows a high degree of homology in the cysteine-rich sequence domain (89%) including RING finger (83%), B1 box (92%), B2 box (93%), and C-terminal (88%) domains at the amino acid level (Fig. 1B). The coiled-coil domain (67%) and the spacing region between coiled-coil and C-terminal domains show a lower degree of homology. The efp Gene in the Mouse Genome—A single band of 3.5 kilobases was detected from EcoRI-digested mouse genomic DNA by cross-species genomic Southern blot analysis (Fig. 1C). The mouse Efp cDNA probe strongly hybridized with human as well as rat DNA.

The Expression of Mouse Efp and Its Estrogen Responsiveness—The RNase protection assay showed that relatively abundant Efp mRNA was present in uterus, ovary, and placenta, whereas it was detected at a medium level in mammary gland and at lower levels in brain and liver (Fig. 2A). In other organs such as spleen, kidney, heart, lung, and thymus, the level of Efp mRNA was also relatively low (data not shown). On the other hand, the ER mRNA was detected at high amounts in uterus and ovary (Fig. 2B). Western blot analysis showed the existence of Efp in placenta (Fig. 2C). By immunoblotting with an anti-mouse Efp antibody a specific band was detected in the nuclear extract derived from mouse placenta (Fig. 2C). The size of the band agreed well with the predicted Mr.

In situ hybridization histochemical studies detected transcripts of efp in mouse uterus, mammary gland, ovary, and brain. In uterus, Efp mRNA was localized predominantly over endometrium (Fig. 3A). The highest stain density was found in columnar epithelial cells of uterus, and lower levels were found in stromal cells of the lamina propria (Fig. 3A) and in smooth muscle cells of the myometrium (data not shown). No staining was seen with the sense Efp RNA probe (Fig. 3B). The hybridization signal with antisense ER RNA probe showed the co-localization of Efp and ER mRNA in endometrium (Fig. 3, A and C). Both Efp and ER mRNA were detected in luminal epithelial cells in mammary gland (Fig. 3, D and F), while no staining was found with sense Efp RNA probe (Fig. 3E). In mouse ovary, only granulosa cells but not thecal cells were well stained by antisense Efp RNA probe (Fig. 3G). Antisense ER RNA probe stained the granulosa cells well and also stained a part of the thecal cells (Fig. 3, G and I). No staining was detected with sense Efp RNA probe here too (Fig. 3H). In mouse brain, Efp mRNA was shown widely distributed. The Efp mRNA-containing neurons were found with greater cell densities on the sections of cerebral cortex (Fig. 4A) and hypothalamus including the ventromedial hypothalamic nucleus (Fig. 4, B and D), while no staining was detected with sense Efp RNA probe (Fig. 4C).

To examine estrogen responsiveness of the mouse efp gene in vivo, the effect of estrogen administration on the amount of Efp mRNA was studied in uterus and brain. By Northern blot analysis, a 6.0-kilobase transcript of mouse efp was detected in these organs, and its amount was increased by subcutaneous injection of 17β-estradiol (500 μg/kg) to 2.5 times in uterus and 2.0 times in brain as early as 2 h, using β-actin mRNA as an internal standard and then returned to normal level by 4–6 h (Fig. 5).

The Expression of Efp mRNA in Mouse Embryos—The expression of Efp mRNA in the heads of embryos was also
studied by RNase protection assay. The Efp mRNA was detected from 10.5 days to 18.5 days, the level being highest at 16.5 days (Fig. 6). By whole mount in situ hybridization, Efp as well as ER mRNA staining was found predominantly in brain vesicles of 11.5-day mouse embryo (Fig. 7, A and C). The Efp mRNA staining was distributed over the regions corresponding to telencephalon, mesencephalon, and metencephalon, but it was much less over the region corresponding to spinal cord (Fig. 7A). No staining was detected with sense Efp RNA probe (Fig. 7B). The staining of ER mRNA was also present in the regions corresponding to telencephalon and mesencephalon, whereas it was rather low in metencephalon and spinal cord (Fig. 7C). In Fig. 7, D and F, paraffin-embedded in situ hybridization also showed that both the Efp and ER mRNA were expressed in telencephalon, while no staining was detected with sense Efp RNA probe (Fig. 7E).
FIG. 2. The tissue distributions of efp and ER transcripts. A, RNase protection assay with Efp and β-actin RNA probe. Lane 1, M is the HapII-digested pBR 322 as size marker (calibration in bp); lane 2, undigested Efp RNA probe; lane 3, undigested β-actin RNA probe; lanes 4–9, placenta, uterus, ovary, mammary gland, brain, and liver RNA, respectively. 15 μg of total RNA was used in each assay. Full-length protected fragments for each probe are indicated. As an internal control, β-actin RNA probe was included in the Efp assay. The Efp mRNA was shown to be expressed in uterus, ovary, and placenta at relatively high levels followed by mammary gland and liver. The Efp mRNA in brain was detected as a faint band. B, RNase protection assay with ER and glyceraldehyde-3-phosphate dehydrogenase RNA probe. Lane 1, M is the HapII-digested pBR 322 as size marker (calibration in bp); lane 2, undigested ER RNA probe; lane 3, undigested glyceraldehyde-3-phosphate dehydrogenase RNA probe; lane 4, yeast total RNA control; lanes 5–10, placenta, uterus, ovary, mammary gland, brain, and liver RNA, respectively. 15 μg of total RNA was used in each assay. Full-length protected fragments for each probe are indicated. As an internal control, glyceraldehyde-3-phosphate dehydrogenase RNA probe was included in the ER assay. The ER mRNA was shown to be expressed in uterus and ovary at high levels. C, Western blot analysis shows the existence of Efp. Nuclear extract prepared from mouse placenta was separated on a 10% SDS-polyacrylamide gel and electroblotted to polyvinylidene difluoride membrane (Millipore Corp.). Western blot analysis with anti-mouse Efp antibody (1:10,000) detects a 70-kDa native protein with the M, predicted from Efp cDNA. Molecular masses are given in kilodaltons.

FIG. 3. In situ hybridization histochemistry of Efp mRNA in reproductive organs. Photographs A–C represent a set of serial uterus sections. D–F represent a set of serial mammary gland sections. G–I represent a set of serial ovary sections. (A, D, and G) are hybridized with the Efp antisense probe. (B, E, and H) are hybridized with the Efp sense probe for the negative control. (C, F, and I) are hybridized with the ER antisense probe. To facilitate orientation, the epithelium (e) and the stroma (s) of uterus and granulosa cells (g) and thecal cells (t) of ovary are indicated. The scale bar indicates 1 μm (A–C) and 100 μm (D–I). The co-localization of Efp and ER mRNA in female organs is shown.
Isolation of Mouse Efp and Comparison with Other Proteins in the RING Finger Family—

In this work, the mouse homologue of the human Efp has been cloned and characterized. Human efp was isolated as an estrogen-responsive gene by genomic binding site cloning using a recombinant ER protein (20). The predicted mouse Efp shows a high degree of conservation with human Efp over the cysteine-rich sequence and C-terminal domain, with a higher divergence at their spacing region and coiled-coil domain. Cross-species genomic Southern blot analysis using mouse EfpcDNA probes suggest that mouse efp exists as a single copy in mouse genome. Indeed, this gene was mapped to a single locus in the mouse chromosome 11C region (30). Strong cross-hybridizing bands in human and rat DNA suggest that efp is highly conserved in mammalian species. The cysteine-rich regions at the amino terminus of Efp fit the consensus of this new family of zinc finger motifs called the RING finger, B1 box, and B2 box (21, 22).

The seven RING finger containing proteins (i.e. the human PML or Myl protein (31–34), the mouse T18 protein (35), the mouse Rpt-1 regulatory protein (36), the human Rfp protein and the related Ret fusion protein (37), the human 52-kDa SS-A/Ro autoantigen (38, 39), XNF7 from Xenopus (40), and PWA33 from the newt Pleurodeles waltl (41) are known to have B box domains so far. All of these B box-containing proteins have a coiled-coil domain present immediately carboxyl-terminal to the B boxes (34, 40). Amino acid comparison of proteins with the putative C-terminal domain shows the
existence of conserved residues (38, 42). Excepting the PML, T18, and Rpt-1, the RING-B box-containing subfamily has a C-terminal domain, which is highly conserved, though nothing is known about its function.

A number of proteins having the RING finger motif are involved in regulating gene expression. For example, Rpt-1 is a down-regulator of the interleukin-2 receptor and human immunodeficiency virus type 1 genes (36). The Rfp is proposed to be a transcription regulator in spermatogenesis (37). XNF-7 is a putative transcription regulator expressed maternally in Xenopus laevis (40). PWA33 is associated with the nascent transcripts on the lampbrush chromosome loops and likely to be a regulatory protein during early development (41). These data suggest that Efp may also be a transcription regulator, although rigorous proof awaits more experimentation.

Several RING finger-containing proteins are implicated in cell transformation. For example, PML produces a fusion protein with the retinoic acid receptor α in acute promyelocytic leukemia (31–34). T18 is a transforming mouse fusion protein with the B-raf proto-oncogene (35). Human Rfp fused with the ret proto-oncogene acquires transforming activity (37). Mouse bmi-1 cooperates with the myc oncogene in lymphoma development (43, 44). Freemont et al. (45, 46) report that two oncogenes, c-cbl (47) associated with lymphoma and mdm-2 (48), which forms a complex with p53 protein and inhibits its trans-activation, also contain a RING finger motif. Recently, the BRCA 1 gene that was identified as a tumor-suppressor gene for the early-onset breast cancer and ovarian tumor by linkage analysis has been cloned (49). Interestingly, the BRCA 1 gene also had the RING finger motif and was localized in the chromosome 17q21.3 locus close to 17q23.1, where human Efp was localized (30). Efp is a member of the RING-B box-containing subfamily that includes PML and T18, which raises an interesting possibility that the Efp may be involved in cell transformation or make a fusion protein related to oncogenesis.

The Expression of the efp Gene—The Efp mRNA was found to be expressed at a relatively high level in reproductive organs in which ER mRNA was highly expressed. In other organs, the expression of Efp mRNA was relatively low. The high staining of Efp and ER mRNA in mouse uterus examined by in situ hybridization histochemistry was co-localized in epithelial cells of the endometrium. This is in agreement with the previous immunohistochemistry (50–52) and in situ hybridization histochemistry (53) data that showed a high staining of ER mRNA in epithelial cells of the uterus. In human mammary gland, it was reported that ER mRNA was expressed in luminal epithe-
Efp mRNA Co-localized with Estrogen Receptor mRNA

In the mouse, Efp mRNA was up-regulated by estrogen within 2 h in uterus as well as in brain in vivo. Human efp was also transcriptionally regulated by estrogen at a short response time (within 2 h) in ER-positive cells derived from mammary gland (20). Mouse efp and human efp contain ERE sequences in the 3′-untranslated region. An ERE in mouse efp is an imperfect palindromic sequence (AGGGCAGGGTGACCT) (Fig. 1A), but it is known that an imperfect palindromic ERE sequence can actually function (e.g., in the cases of pS2 (14) and prolactin (13)). This imperfect palindromic ERE of mouse efp might act as a downstream estrogen-dependent enhancer just like the ERE of human efp. Further analysis is required to establish this point.

In embryonic tissues, Efp mRNA was detected in the head during the gestation by RNase protection assay and also detected throughout the developing brain vesicles by in situ hybridization histochemistry. ER mRNA was also detected in the heads of 10.5- and 12.5-day embryos by the reverse transcriptase-polymerase chain reaction method and in the heads of 14.5-18.5-day embryos by RNase protection assay (data not shown), although the level of ER mRNA in 10.5- and 12.5-day embryos was shown to be rather low. The expression of Efp and ER mRNA during embryogenesis may be related to the regulation of brain development in terms of sexual dimorphism, etc.

In a mouse ER gene targeting model (57, 58), the female heterozygote was found to be infertile, having a hypoplastic uterus, hyperemic cystic ovary, and decreased skeletal mineralization, etc. During embryogenesis may be related to the regulation of brain development in terms of sexual dimorphism, etc.

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39. Itoh, K., Itoh, Y., and Frank, M. B. (1991) J. Clin. Invest. 87, 177–186
40. Reddy, B. A., Kloc, M., and Etkin, L. D. (1991) Dev. Biol. 148, 107–116
41. Bellini, M., Lacroix, J.-C., and Gall, J. G. (1993) EMBO J. 12, 107–114
42. Jack, L. J., and Mather, I. H. (1990) J. Biol. Chem. 265, 14481–14486
43. Haupt, Y., Alexander, W. S., Barri, G., Klinken, S. P., and Adams, J. M. (1991) Cell 65, 753–763
44. van Lohuizen, M., Verbeek, S., Scheijen, B., Wientjens, E., van der Gulden, H., and Berns, A. (1991) Cell 65, 737–752
45. Lovering, R., Hanson, I. M., Borden, K. L. B., Martin, S., O’Reilly, N. J., Evan, G. I., Rahman, D., Pappin, D. J. C., Trowsdale, J., and Freemont, P. S. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 2112–2116
46. Bodi, M. N., Freemont, P. S., and Borden, K. L. B. (1993) Trends Biochem. Sci. 19, 198–199
47. Blake, T. J., Shapiro, M., Morse, H. C., II, and Langdon, W. Y. (1991) Oncogene 6, 653–657
48. Momand, J., Zambratto, G. P., Olson, D. C., George, D., and Levine, A. J. (1992) Cell 69, 1237–1245
49. Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L. M., Ding, W., Bell, R., Rosenthal, J., Hussey, C., Tran, T., McClure, M., Frye, C., Hattier, T., Phelps, B., Haugen-Strano, A., Katcher, H., Yakumo, K., Gholami, Z., Shaffer, D., Stone, S., Bayer, S., Wray, C., Bogden, R., Dayananth, P., Ward, J., Tonin, P., Narod, S., Bristow, P. K., Norris, F. H., Helvering, L., Morrison, P., Roetek, P., Lai, M., Barrett, J. C., Lewis, C., Neuhausen, S., Cannon-Albright, L., Goldgar, D., Wiseman, R., Kamb, A., and Skolnick, M. H. (1994) Science 7, 66–71
50. Yamashita, S., Newbold, R. R., McLachlan, J. A., and Korach, K. S. (1989) Endocrinology 125, 2888–2896
51. Zaino, R. J., Clarke, C. L., Fel, P. D., and Satyaswaroop, P. G. (1989) Endocrinology 125, 2728–2734
52. Iwai, T., Fujii, S., Nambu, Y., Nonogaki, H., Konishi, I., Mori, T., and Okamura, H. (1991) Endocrinology 129, 1840–1848
53. Koji, T., and Brenner, R. M. (1993) Endocrinology 132, 382–392
54. Petersen, O. W., Hoyer, P. E., and Deurs, B. V. (1987) Cancer Res. 47, 5748–5751
55. Agthoven, T. V., Timmermans, M., Foekens, J. A., Dorssers, L. C. J., and Henzen-Loomans, S. C. (1994) Am. J. Pathol. 144, 1238–1246
56. Billiar, R. B., Loukides, J. A., and Miller, M. M. (1992) J. Clin. Endocrinol. Metab. 75, 1159–1165
57. Lubahn, D. B., Moyer, J. S., Golding, T. S., Couse, J. F., Korach, K. S., and Smithies, O. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 11162–11166
58. Korach, K. S. (1994) Science 266, 1524–1527
59. Smith, E. P., Boyd, J. F., Frank, G. R., Takahashi, H., Cohen, R. M., Specker, B., Williams, T. C., Lubahn, D. B., and Korach, K. S. (1994) N. Engl. J. Med. 20, 1056–1061