Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 1686–1691, February 1999
Neurobiology

The 70-kDa heat shock cognate protein (Hsc73) gene is enhanced by ovarian hormones in the ventromedial hypothalamus

CHRISTOPHER J. KREBS*, ERICH D. JARVIS†, AND DONALD W. PFAFF*‡

Laboratories of *Neurobiology and Behavior and †Animal Behavior, The Rockefeller University, 1230 York Avenue, Box 275, New York, NY 10021

Contributed by Donald W. Pfaff, December 10, 1998

ABSTRACT **Estrogen (E) and progesterone (P) orchestrate many cellular responses involved in female reproductive physiology, including reproductive behaviors. E- and P-binding neurons important for lordosis behavior have been located within the ventromedial hypothalamus (VMH), and several hormone-responsive genes have been observed there as well. In attempts to identify additional E- and P-responsive genes in the VMH that may contribute to sexual behaviors, we used the differential display mRNA screening technique. One of the genes identified encodes the 73-kDa heat shock cognate protein (*Hsc73*). Quantitative *in situ* hybridization analysis of brains from naturally cycling female rats revealed a significant increase in *Hsc73* mRNA in the VMH and arcuate nucleus of animals during proestrus compared with those at diestrus-1. To confirm that these increases were steroid hormone dependent, we compared vehicle-treated ovariectomized females with ovariectomized females treated with estradiol benzoate and P. Northern analysis and *in situ* hybridizations showed that the *Hsc73* gene is enhanced by E and P in the pituitary and subregions of the VMH. Incidentally, by examining the primary amino acid sequence of rat, human, and chicken progesterone receptors, we noticed that putative Hsc73 binding sites are conserved across species with similar sites existing in the androgen and glucocorticoid receptors. Together these findings suggest a possible mechanism through which E could influence the activities of progesterone, androgen, and glucocorticoid receptors, by enhancing the expression of Hsc73 in cells where these proteins colocalize.**

Estrogen (E) and progesterone (P) are responsible for a constellation of cellular events involved in female reproductive physiology. In the brain, in particular, the binding of E and P to receptors in neurons located within the ventromedial hypothalamus (VMH) has been implicated in the facilitation of reproductive behaviors (1). In intact female rats, these behaviors are observed on the afternoon of proestrus, a stage in the estrous cycle in which an increase in estradiol is followed by a surge of P (2). Removal of the ovaries abolishes these behaviors, and they can be reinstated by treating the animals with exogenous hormone. The treatment of ovariectomized (ovx) rats in this way has allowed reproductive behaviors and other centrally mediated aspects of female reproductive physiology to be studied in a controlled and predictable manner.

One way that steroid hormones mediate a physiological response is by activating nuclear receptors to alter gene expression in target cells (3). The finding that female sexual behavior in rats is diminished by E receptor (ER) and P receptor (PR) antagonists (4, 5) as well as inhibitors of transcription (6) and protein synthesis (7) suggests that E and P and their receptors also alter gene expression in the VMH. In fact, E has been shown to induce several genes in the VMH linked to female sexual behavior (for a review see ref. 1); however, the full range of genes responsive to P in this brain region has been difficult to identify. Therefore, we have applied the differential display–PCR (DD-PCR) technique (8) to hormone-treated ovx female rats, in an attempt to identify additional E and P target genes in the vicinity of the VMH and to clarify the molecular pathways leading to female sexual behavior.

One of the genes we have identified with DD-PCR is the 70-kDa heat shock protein, *Hsc73*. Northern blotting and quantitative *in situ* hybridization studies confirm the steroid-dependent modulation of *Hsc73* expression in the brains and pituitaries of both gonadectomized female rats treated with hormones, as well as intact rats with natural estrous cycles. It has been known for some time that the steroid hormone receptors interact with members of the heat shock family of proteins, and that this interaction is required for the proper functioning of the receptors within the cell (9). The interfaces of the heat shock proteins with the receptors, however, are poorly defined. Here we have expanded on the work of Terlecky *et al.* (10) and observed several putative Hsc73 binding sites in the amino acid sequences of PRs, androgen receptors (ARs), and glucocorticoid receptors (GRs), but not ERs. Together, these findings suggest intriguing mechanisms for the influence of E on the functioning of PR, and perhaps AR and GR, in cells simultaneously targeted by these hormones.

MATERIALS AND METHODS

Animals and Treatments. Intact and ovx adult female Sprague–Dawley rats were obtained from the Charles River Breeding Laboratories and maintained on a 12-h light, 12-h dark schedule (lights on at 1000 h) with food and water supplied ad libitum. The ovx rats were allowed to rest for 10 days postsurgery before any experimentation was performed. Hormone treatments of ovx rats were as follows: injections with estradiol benzoate (EB) (s.c.; 12.5 μg/rat) were followed 24 h later with injections of P (i.p., to guarantee a fast route; 500 μg/rat) (EB + P). Other groups of rats received either EB followed 24 h later with sesame oil vehicle (EB + vehicle) or vehicle only at both time points. Three hours after the final injection, all animals were CO_2-asphyxiated and sacrificed. The estrous cycles of the intact rats were monitored by cytological examination of vaginal smears taken between 0900 and 1100 h. The animals were sacrificed after three consecutive 4-day cycles to ensure consistent normal reproductive physiology. To ensure that potential maximum gene expression differences were seen, animals with contrasting serum hormone levels were compared. One group of rats was sacrificed on the afternoon (1400 h) of proestrus and the other on the afternoon of diestrus-1 (1400 h). The brain, pituitary,

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.

PNAS is available online at www.pnas.org.

Abbreviations: DD-PCR, differential display PCR; E, estrogen; P, progesterone; EB, estradiol benzoate; VMH, ventromedial hypothalamus; ER, E receptor; PR, P receptor; AR, androgen receptor; GR, glucocorticoid receptor; ovx, ovariectomized.

‡To whom reprint requests should be addressed. e-mail: krebsc@rockvax.rockefeller.edu.

uterus, liver, and heart from each animal were dissected and either snap-frozen in liquid nitrogen for RNA isolation or embedded in freezing media (TissueTek, Miles) for *in situ* hybridization.

RNA Isolation and Analysis. To obtain RNA for DD-PCR, the VMH was dissected from a 2-mm coronal section taken from the rostral edge of the optic chiasm. Anterior and lateral tissue was removed by cutting a trapezoid, around the third ventricle, inscribed by the fornix and the optic tracts. Total RNA from all tissues described above then was isolated by the guanidinium isothiocyanate method (11). To eliminate potential DNA contamination, RNA was treated with RNase-free DNase I (GenHunter, Nashville, TN). Northern blots were prepared by size-fractionating 10 μg or 20 μg total RNA on 1.2% agarose gels containing 6.5% formaldehyde, transferred to GeneScreen Plus Nylon (NEN/DuPont) membranes, and hybridized to random primed-^{32}P-labeled (Boehringer Mannheim) probe specific to the 3′ end of the *Hsc73* gene. The blots were hybridized according to the manufacturer's instructions (NEN/DuPont) and washed at 65°C with a final stringency of 0.5× standard saline citrate/1% SDS. The hybridization signal was quantified by PhosphorImager (Molecular Dynamics) analysis. Before transferring the RNA to nylon membranes, the ethidium bromide-stained gels were photographed over UV light with Poloroid 665 positive/negative film. To correct for variations in the loading of RNA, the 18S rRNA bands visible in the negative image of the gels were quantified by densitometry (NIH Image). The PhosphorImager values then were divided by the densitometry values to normalize the Hsc73 signal. The results are expressed in arbitrary PhosphorImager units and presented as mean values obtained from three or more animals.

DD-PCR. The DD technique was performed essentially as described by Liang and Pardee (8). Briefly, total RNA (100 ng) isolated from the VMH of the hormone-treated and control animals described above was reverse-transcribed in a 20-μl reaction by using an anchored oligo(dT) primer (8) and Superscript-RT (Life Technologies, Grand Island, NY). One microliter of the reverse transcription reaction was used in PCR with arbitrary 10 mers and anchored oligo(dT) primers in the presence of [α-^{33}P]dATP according to the methods of Liang and Pardee (8, 12). The radioactive products were size-fractionated on 6% denaturing polyacrylamide gels and visualized by autoradiography. The resulting autoradiographs were examined to locate cDNA bands that satisfy two criteria: first, they must exhibit a differential intensity between treatment groups, relative to the majority that displayed a uniform intensity between groups; and second, they must be present in each individual within the group. Adhering to these criteria ensured that the cDNAs chosen for further analysis were altered by the treatment of the animals and not caused by individual variation. Satisfactory bands then were cut from the dried polyacrylamide gels, reamplified by PCR (8), and cloned into the plasmid vector pCR-Script SK(+) (Stratagene) according to the manufacturer's instructions. The resulting clones were sequenced by the Protein/DNA Technology Center at Rockefeller University and confirmed to be differentially expressed by the slot blotting procedure of Liu and Raghothama (13). Clone identity was determined by performing BLAST searches against the GenBank database.

***In Situ* Hybridization.** The *in situ* hybridization methodology was essentially as described by Mello *et al.* (14). Slides containing brain tissue were hybridized and washed at 60°C, and then treated with RNase (5 μg/ml, Boehringer Mannheim) to eliminate nonspecific binding. The final wash was performed at room temperature at a stringency of 0.1× standard saline phosphate/EDTA (0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA). After determining the signal by PhosphorImager analysis, the slides were dipped in photographic emulsion (NBT-2, Kodak) and exposed for 3 weeks in light protected boxes at 4°C. Anatomically matched coronal brain sections were quantified by counting the number of silver grains per cell (15–35 cells/rat) by using the Bioquant image analysis system (R & M Biometrics, Nashville). To ensure accurate measurements, three adjacent sections per animal were analyzed, and the mean value was used for comparisons between groups.

Statistical Analysis. All results are presented as mean values ± SEM. Student's *t* tests were used to compare the mean differences between groups. Statistical significance was accepted at $P < 0.05$ (one-tailed).

RESULTS

***Hsc73* mRNA Is Responsive to Ovarian Steroid Hormones in the Female Rat VMH.** To identify P-sensitive genes that may be involved in the facilitation of female rat sexual behaviors, DD-PCR was performed on VMH RNA from ovx rats given EB + P and EB + vehicle (*see Materials and Methods*). Following the criteria described in *Materials and Methods*, 25 differentially displayed bands were revealed, some showing increases, others decreases. One of the bands, which showed a slight increase in intensity because of the administration of P after E priming (Fig. 1*A*) was isolated from the gel, reamplified by PCR, and cloned into pCR-Script SK(+), and its enhanced appearance was confirmed by differential slot blotting (13) (Fig. 1*B*). Sequencing of this cloned DNA band revealed that it has 100% identity to the 3′ portion of the gene encoding the rat heat shock cognate 73-kDa protein (*Hsc73*) (Fig. 1*C*). Thus, expression of the *Hsc73* gene in the VMH of an E-primed rat is slightly enhanced by P.

***Hsc73* mRNA Levels in the VMH Correlate with Changes in Endogenous Ovarian Steroid Hormone Levels.** To determine whether *Hsc73* expression is ovarian steroid hormone dependent in an endogenous endocrine context, we next used quantitative *in situ* hybridization analysis to examine *Hsc73* expression in the brains of diestrus-1 and proestrus rats. *Hsc73* mRNA was ≈40% higher throughout the VMH of rats in proestrus than in rats in diestrus-1 (Fig. 2*B*). In contrast, there was no significant difference in *Hsc73* expression in the neocortex in the same plane as the VMH of these animals (Fig. 2 *A* and *B*). In an attempt to more precisely map *Hsc73* expression around the third ventricle of the hypothalamus, we quantified the *in situ* hybridization results from the dorsomedial, central, and ventrolateral regions of the VMH and arcuate nucleus. *Hsc73* mRNA levels in all four subdivisions were significantly higher at proestrus compared with diestrus-1 (Fig. 2*B*).

***Hsc73* mRNA Levels in Pituitary and Liver Correlate with Changes in Endogenous Ovarian Steroid Hormone Levels.** To determine whether *Hsc73* expression is altered in other ovarian steroid hormone target tissues during the estrous cycle, we performed Northern blots on total RNA isolated from several peripheral organs (pituitary, uterus, liver, and heart) obtained from rats sacrificed during proestrus and diestrus-1 (Fig. 3*A*). Those organs were selected because of their involvement in reproduction or because they contain ERs and PRs (16). Only the pituitary and liver showed statistically significant enhancements (52% and 40%, respectively) in *Hsc73* message levels at proestrus compared with diestrus-1 (Fig. 3*B*). Although there was substantial variability in the measurements, we observed a similar trend with uterine RNA (Fig. 3*B*). In contrast, no difference was observed in *Hsc73* message levels in heart (Fig. 3*B*).

E, But Not P, Enhances *Hsc73* mRNA Levels in the Pituitary. Because the largest and most consistent difference in *Hsc73* message level between proestrus and diestrus-1 was observed in pituitary, we used this tissue to begin determining how E and P might influence *Hsc73* mRNA. For these studies, total RNA was isolated from pituitaries of ovx rats given vehicle, EB + vehicle, or EB + P. Northern blot results showed

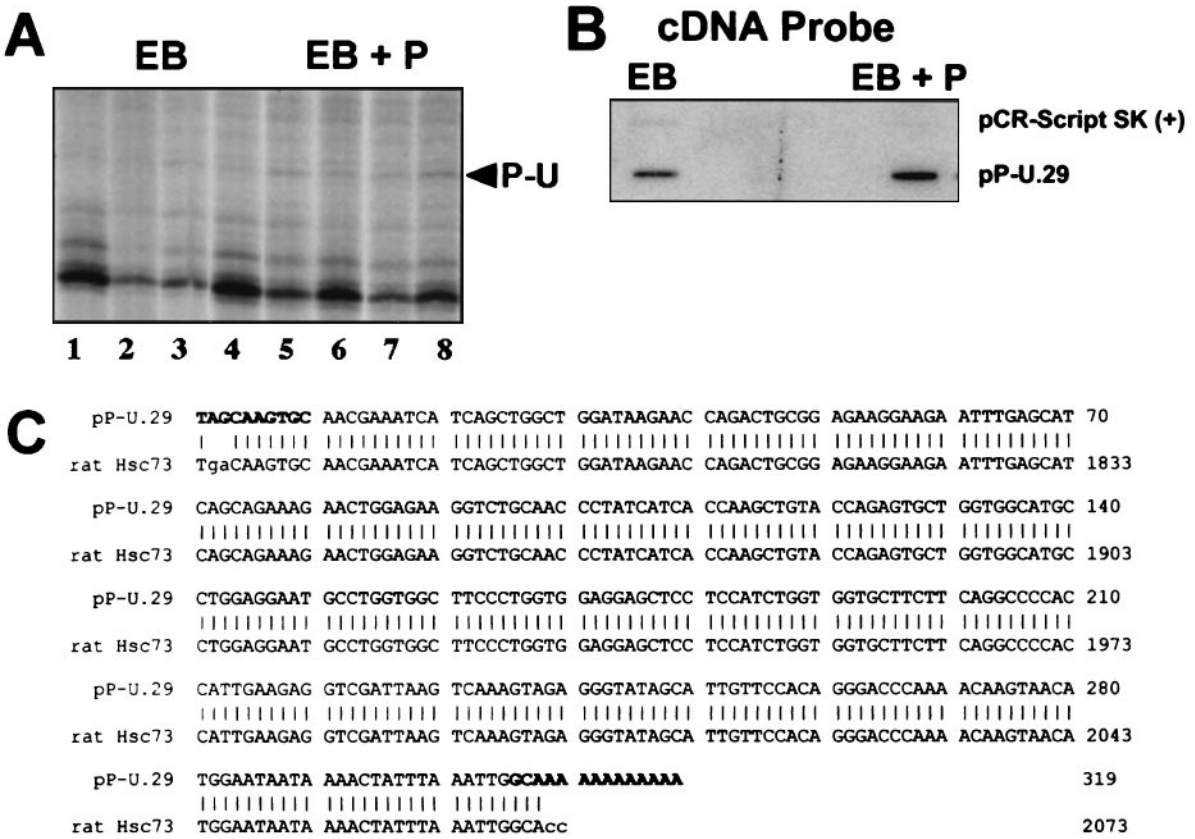

FIG. 1. DD-PCR with VMH RNA from EB + vehicle and EB + P-treated ovx rats. (*A*) DD-PCR products. Total RNA from the VMH of four individual EB + vehicle-treated ovx rats was compared with that from four individual EB + P-treated ovx rats by using the DD-PCR technique of Liang and Pardee (8). The band, P-U, displaying a higher intensity in every member of the EB + P group (lanes 5–8) was cut from the acrylamide gel, reamplified by PCR, and cloned into the pCR-Script SK(+) plasmid vector. (*B*) To confirm the DD-PCR result, the cloned DNA, pP-U.29, was slot-blotted onto nylon membranes in duplicate and hybridized to ^{32}P-labeled cDNA probes synthesized from poly(A)$^+$ RNA isolated from the VMH of EB and EB + P-treated ovx rats according to the procedures of Liu and Raghothama (13). Plasmid pCR-Script SK(+) was blotted in adjacent wells and served as a negative control. (*C*) Plasmid pP-U.29 was sequenced, and this information was used to perform a BLAST search against the GenBank database. The alignment of pP-U.29 with the most homologous GenBank entry, rat Hsc73 (GenBank accession no. M11942), is shown. Vertical lines and uppercase letters indicate sequence identity. The numbers correspond to those reported by Sorger and Pelham (15). Bold text indicates the location of the DD-PCR primer annealing sites.

that *Hsc73* message is about 20% higher after a 27-h treatment with EB only (EB + vehicle) compared with vehicle-treated animals. Also, a 3-h treatment with P after a 24-h treatment with EB (EB + P) had no additional influence on the *Hsc73* message level (Fig. 4). Because the 20% elevation in *Hsc73* message observed 27 h after the EB injection seemed rather modest, we analyzed the pituitaries of ovx rats just 6 h after an injection of EB and observed 44% more *Hsc73* message in those animals than in vehicle-treated controls (Fig. 4). Thus, the *Hsc73* mRNA increase in the pituitary seen during proestrus compared with diestrus-1 (Fig. 3) appears to be caused by the actions of E, with no further augmentation by the subsequent administration of P.

E Enhances *Hsc73* mRNA in the Central and Ventrolateral VMH, Whereas the Addition of P Elevates *Hsc73* mRNA in the Central VMH and Arcuate Nucleus. Finally, to examine the effects of E and P on *Hsc73* expression in the brain, we used quantitative *in situ* hybridization analysis on ovx animals treated with hormones as described above. The *Hsc73* message throughout the entire VMH was ≈30% higher in the hormone-treated animals than in the vehicle-treated control group ($P < 0.05$). A more detailed quantitative analysis of these results indicated that the ventrolateral and central regions of the VMH are responsive to EB (68% and 31%, respectively), whereas the dorsomedial portion, having fewer E-binding neurons (17), remained unaffected (Fig. 5*B*). In addition, after the subsequent P treatment, the *Hsc73* message was increased by 22% and 20% in the arcuate nucleus and central portion of the VMH, respectively. In contrast, the neocortex was unaffected by the hormone treatments.

Putative Hsc73 Recognition Sites in the Carboxy-Terminal Region of the PR. During these investigations, by comparing known amino acid sequences, we have built on the initial observation of Terlecky *et al.* (10) to note an additional putative Hsc73 recognition site in the extreme C-terminal regions of rat, human, and chicken PR (Fig. 6). All sites are conserved across species. Related sites appear in similar locations within the AR and GR (Fig. 6). No similar sites were seen in the entire amino acid sequences of either the α or β forms of ER. Although the binding of Hsc73 to these sites remains unproven, the possibility leads to intriguing predictions (*see Discussion*) about the way in which E could indirectly influence the functioning of PR, as well as AR and GR, by stimulating the expression of Hsc73 in target cells.

DISCUSSION

ERs and PRs are members of a large family of related proteins, the steroid hormone nuclear receptor superfamily (for a review see ref. 3). These receptors are associated with a variety of accessory proteins, many belonging to the heat shock family (e.g., Hsp90, Hsp70/Hsc73, Hsp23, etc.) (9). Upon ligand binding, the accessory proteins dissociate and the receptor, activated in the nucleus, alters the expression of target genes

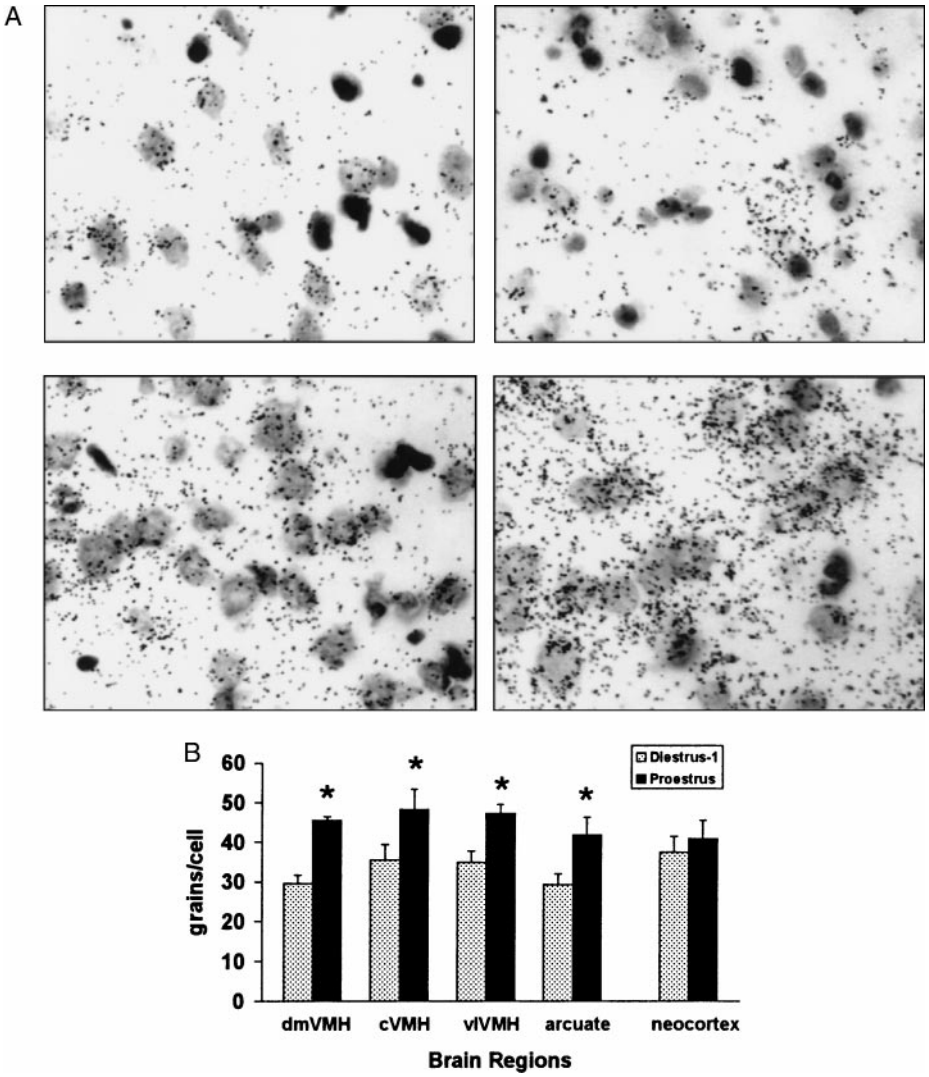

FIG. 2. *In situ* hybridization analysis of Hsc73 in the brains of rats at diestrus-1 and proestrus. (*A*) High-power magnification (×30) of representative *in situ* hybridization results from neocortex (*Upper*) and ventrolateral VMH (*Lower*) of intact rats at diestrus-1 (*Left*) and proestrus (*Right*). The brains from intact rats at diestrus-1 and proestrus were dissected, embedded in freezing media, and cut into 10-μm sections. The sections were hybridized to an antisense ^{35}S-labeled riboprobe, specific to the 3′ end of the *Hsc73* gene (Fig. 1*C*), transcribed *in vitro* from plasmid pP-U.29. (*B*) Quantitative analysis of results exemplified in *A* was performed on sections from each animal by dividing the number of silver grains by the number of cells within the visual field at ×40 magnification. Each bar represents the mean ± SEM of three or more animals. * indicates a significant difference ($P < 0.05$) in the level of *Hsc73* mRNA, measured in grains per cell, between animals at proestrus and diestrus-1.

(3). Another class of accessory proteins, transcriptional coactivators (e.g., P300 and SRC-1) (19, 20) and corepressors (e.g., NCoR) (21), modify the transcriptional properties of receptors once the receptors are activated in the nucleus. Although much is being learned about how accessory proteins and steroid hormone receptors interact, little is known about how they might influence the gene expression of one another. Here we demonstrate that the expression of the *Hsc73* gene in hormone target tissues is influenced by E and P (Figs. 1, 4, and 5) and that this influence likely results from a transcriptional mechanism because Hsc73 is only hormone responsive in cell groups with substantial amounts of ER and PR, such as the pituitary (16) and ventrolateral VMH (22–24), but not neocortex or heart (Figs. 4 and 5).

Hsc73 is one of several heat shock proteins with molecular masses of approximately 70 kDa. The binding of 70-kDa heat shock proteins to steroid hormone receptors has been reported by a number of investigators (for a review see ref. 9), yet the roles of these proteins in the receptor complex remain in question. Heat shock proteins, in general, function as molecular chaperones, ensuring proper protein folding, the facilitation of protein–protein interactions, and the transport of protein complexes across membranes (25). In this latter capacity, 70-kDa members of the heat shock family have been identified in the movement of proteins into the nucleus (26), mitochondria (27), endoplasmic reticulum (28), and lysosome (29). Hsc73, specifically, has been observed to facilitate the transport of proteins into the lysosome after the withdrawal of serum from cells in culture (29).

The Hsc73 recognition site for lysosomal transport, frequently found in the C termini of target proteins, has been loosely defined as four alternating charged and hydrophobic amino acids before or after a glutamine (10, 18) (Fig. 6). Terlecky *et al.* (10) have observed two putative Hsc73 recognition sites in the C-terminal region of the primary amino acid sequence of PR. Here we note an additional site within 22 aa of the C terminus of PR and further note that all of these sites are evolutionarily conserved from birds to mammals, not only residue for residue, but in their relative locations within the proteins as well. We also noticed additional putative Hsc73 recognition sites within the C termini of AR and GR (Fig. 6). Together, these findings suggest that the induction of Hsc73 by

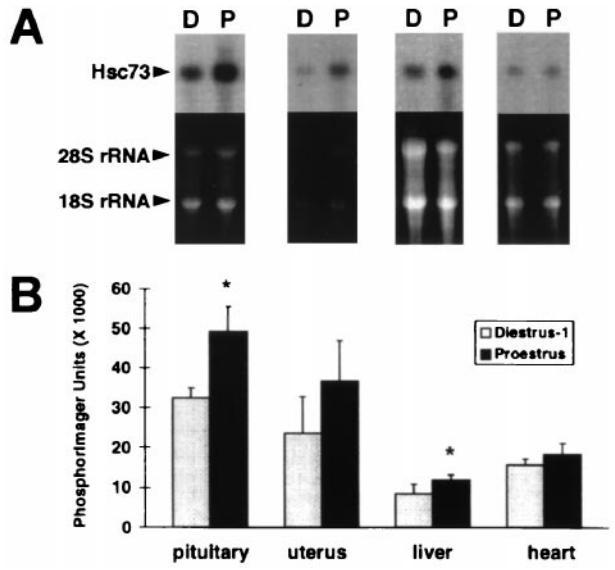

FIG. 3. Northern analysis of *Hsc73* mRNA in several peripheral tissues of rats at proestrus and diestrus-1. (*A*) Total RNA from pituitary, uterus, liver, and heart was subjected to Northern analysis with a *Hsc73* cDNA probe prepared from plasmid pP-U.29. *Hsc73* hybridization signals from representative animals at diestrus-1 and proestrus are shown above the corresponding ethidium bromide-stained gels. The intensely staining 28S and 18S rRNA bands are indicated. (*B*) To quantitate the Northern results, the intensity of the *Hsc73* hybridization signal, determined by PhosphorImager analysis, was normalized to the amount of RNA loaded in the corresponding lane of the gel. Each bar represents the mean ± SEM. ∗ indicates a significant difference ($P < 0.05$) between animals at proestrus ($n = 3$) and diestrus-1 ($n = 4$). The magnitude of enhancement of *Hsc73* mRNA at proestrus relative to diestrus-1 observed in pituitary, uterus, and liver was 52%, 55%, and 40%, respectively.

E acting through ER could influence the activities of PR, AR, and GR by expediting their movements within cells where these receptors colocalize.

Hsc73 has additional functions in the cell besides its role as a molecular chaperone. For instance, some of the properties assigned to Hsc73 include (*i*) an ATPase activity for the uncoating of clathrin-coated vesicles during endocytosis and vesicle recycling (30), and (*ii*) association with cell cycle regulatory proteins, retinoblastoma (31), p53 (32), and Bcl-2 (33).

Hormone target cells, like most cells of the endocrine system, experience elevated vesicle transport and secretion in

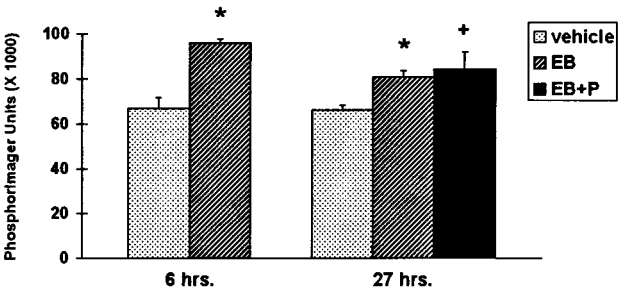

FIG. 4. Effect of E and P on pituitary *Hsc73* mRNA levels. Total RNA from individual animals in each treatment group was subject to Northern analysis. The *x* axis indicates the time of sacrifice after a single injection of hormone or vehicle. For the EB + P group, P was injected 24 h after EB, and the animals were sacrificed 3 h later for a total treatment time of 27 h. Quantitative analysis was performed as described in Fig. 3*B*. Each bar represents the mean ± SEM. ∗ indicates a significant difference ($P < 0.05$) between vehicle- and EB-treated animals. + indicates a difference ($P < 0.10$) between vehicle- and EB + P-treated animals.

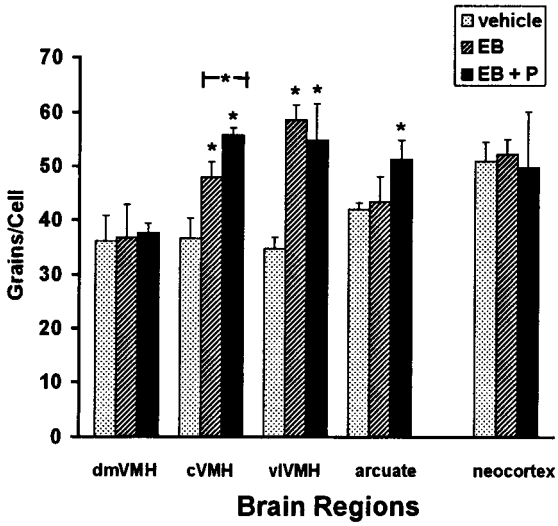

FIG. 5. *In situ* hybridization analysis of Hsc73 in the brains of ovx rats treated with EB and P. The brains from ovx rats treated with sesame oil vehicle, EB and EB + P (*see Materials and Methods*) were dissected and sectioned as in Fig. 2. *In situ* hybridization and quantitative analysis also were performed as in Fig. 2. Each bar represents the mean ± SEM of two animals per group. ∗ indicates a significant difference ($P < 0.05$) between vehicle- and hormone-treated animals. ∗ above the cVMH bars indicates a significant difference ($P < 0.05$) between animals treated with EB only and those that received EB + P.

response to an appropriate endocrine signal. Because Hsc73 is involved in the cellular management of vesicles, through the disassembly of clathrin cages (30), it is logical that an increase in Hsc73 synthesis is required to meet the demands the ovarian steroid hormones place on their target cells for secretion. Thus, Hsc73 could influence sexual behavior, as well as other aspects of reproductive physiology, because of its role in vesicle transport in hypothalamic neurons and gonadotrophs in the pituitary.

Hsc73 interacts with cell-cycle regulatory proteins. With the demonstration here that Hsc73 expression also is induced by E

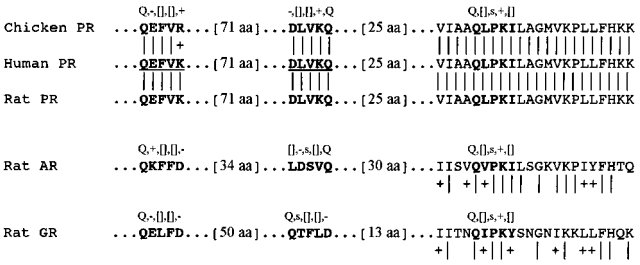

FIG. 6. Putative Hsc73 binding sites in the C termini of related nuclear receptors. Terlecky *et al.* (10) and Chiang *et al.* (18) described a consensus Hsc73 recognition site as glutamine before or after a combination of four charged and hydrophobic amino acid residues. Terlecky *et al.* (10) noticed two related sites in the C-terminal region of human PR (underlined). A third similar site, QLPKI, also exists in PR within the most C-terminal 22 aa. Chicken, human, and rat PR amino acid sequences are aligned to show the conservation of the Hsc73 binding sites across species. The numbers in brackets indicate the distance, in animo acid residues, between sites. Similar putative Hsc73 binding sites are indicted in rat ARs and GRs. All putative Hsc73 recognition sites are in bold. The vertical lines below the single-letter amino acid designation indicates identity with the rat PR sequence. + indicates similar, but nonidentical, amino acids. Symbols that define the consensus are as follows: Q, glutamine; +, positively charged amino acids; −, negatively charged amino acids; [ ], hydrophobic amino acids; s, small amino acids. The GenBank accession numbers for these sequences are L16922, M15716, M37518, M20133, and M14053.

and may interact with PR (Fig. 6), it is possible that Hsc73 contributes to the mitogenic properties of E and P in breast, uterine, and ovarian tissues (16). For example, Hsc73 expression changes during the remodeling of mouse breast tissue, which accompanies gestation, lactation, and postweaning (34). Hsc73 expression correlates very closely with circulating P levels during these processes and has been suggested to function within the PR-mediated pathways that govern breast tissue remodeling (34). In addition, Hsc73, but not Hsp70, has been identified in complexes with retinoblastoma protein, Rb110, in ovarian carcinoma (31) and the tumor suppressor protein, p53, in glioblastoma (35) and ovarian carcinoma (36) derived cell lines. Also, the Bcl-2 associated protein BAG-1 interacts with both the C-terminal region of steroid hormone receptors and Hsc73 (33) and may facilitate prostate cancer by potentiating the activity of AR (37). Taken together, these links between Hsc73 and cell cycle regulatory molecules, and the connection between ovarian steroid hormones and Hsc73 shown here, warrant further investigation into how Hsc73 influences cellular proliferation, and the mechanisms by which the *Hsc73* gene is regulated by steroid hormone receptors. To that end, we have noticed two putative E response element half-sites, a P response element half-site and an AP-1 site within the first intron and the genomic sequence 5′ to the transcriptional start site of the rat *Hsc73* gene (15). Although the transcriptional regulatory properties of these sites await further study, they suggest that E and P, acting through their nuclear receptors, directly enhance the expression of Hsc73.

We thank Drs L. M. Kow and S. Ogawa for their technical expertise and Dr. M. Bagchi for his helpful comments. This work was supported by National Institutes of Health Grants HD-08389 (C.J.K.) and HD05751 (D.W.P.).

1. Pfaff, D. W., Schwartz-Giblin, S., McCarthy, M. M. & Kow, L.-M. (1994) in *The Physiology of Reproduction,* eds. Knobil, E., Neill, J. D., Greenwald, G. S., Markert, C. L. & Pfaff, D. W. (Raven, New York), Vol. 2, pp. 107–220.
2. Powers, J. B. (1970) *Physiol. Behav.* **5,** 831–835.
3. Tsai, M.-J. & O'Malley, B. W. (1994) *Annu. Rev. Biochem.* **63,** 451–486.
4. Brown, T. J., Moore, M. J. & Blaustein, J. D. (1987) *Endocrinology* **121,** 298–304.
5. Etgen, A. M. (1987) *Horm. Behav.* **21,** 528–535.
6. Yahr, P. & Ulibarri, C. (1986) *Brain Res.* **387,** 153–165.
7. Glaser, J. H. & Barfield, R. J. (1984) *Neuroendocrinology* **38,** 337–343.
8. Liang, P. & Pardee, A. B. (1992) *Science* **257,** 967–971.
9. Pratt, W. B. & Toft, D. O. (1997) *Endocr. Rev.* **18,** 306–360.
10. Terlecky, S. R., Chiang, H.-L., Olson, T. S. & Dice, J. F. (1992) *J. Biol. Chem.* **267,** 9202–9209.
11. Ausubel, F. J., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1989) *Current Protocols in Molecular Biology* (Wiley, New York), 2nd Ed.
12. Liang, P. & Pardee, A. B. (1995) *Science* **267,** 1186–1187.
13. Liu, C. & Raghothama, K. G. (1996) *BioTechniques* **20,** 576–580.
14. Mello, C. V., Jarvis, E. D., Denisenko, N. & Rivas, M. (1997) in *Methods in Molecular Biology, Vol. 85: Differential Display Methods and Protocols*, eds. Liang, P. & Pardee, A. B. (Humana, Totowa, NJ), pp. 205–217.
15. Sorger, P. K. & Pelham, H. R. B. (1987) *EMBO J.* **6,** 993–998.
16. Clark, J. H. & Markaverich, B. M. (1988) in *The Physiology of Reproduction,* eds. Knobil, E., Neill, J. D., Ewing, L. L., Greenwald, G. S., Markert, C. L. & Pfaff, D. W. (Raven, New York), Vol. 1, pp. 675–724.
17. Pfaff, D. & Keiner, M. (1973) *J. Comp. Neurol.* **151,** 121–158.
18. Chiang, H.-L. & Dice, J. F. (1988) *J. Biol. Chem.* **263,** 6797–6805.
19. Hanstein, B., Eckner, R., Direnzo, J., Halachmi, S., Liu, H., Searcy, B., Kurokawa, R. & Brown, M. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 11540–11545.
20. Oñate, S. A., Tsai, S. Y., Tsai, M. J. & O'Malley, B. W. (1995) *Science* **270,** 1354–1357.
21. Hörlein, A. J., Näär, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamel, Y., Söderström, M., Glass, C. K. & Rosenfeld, M. G. (1995) *Nature (London)* **377,** 397–404.
22. Parsons, B., Rainbow, T. C., Pfaff, D. W. & McEwen, B. S. (1981) *Nature (London)* **292,** 58–59.
23. Romano, G. J., Krust, A. & Pfaff, D. W. (1989) *Mol. Endocrinol.* **3,** 1295–1300.
24. Blaustein, J. D. & Turcotte, J. C. (1989) *Neuroendocrinology* **49,** 454–461.
25. Morimoto, R. I., Tissieres, A. & Georgopolous, C. (1994) in *The Biology of Heat Shock Proteins and Molecular Chaperones,* eds. Morimoto, R. I., Tissieres, A. & Georgopolous, C. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 1–30.
26. Shi, Y. & Thomas, J. O. (1992) *Mol. Cell. Biol.* **12,** 2186–2192.
27. Murakami, H., Pain, D. & Blobel, G. (1988) *J. Cell Biol.* **107,** 2051–2057.
28. Chirico, W. J., Waters, M. G. & Blobel, G. (1988) *Nature (London)* **332,** 805–810.
29. Chiang, H.-L., Terlecky, S. R., Plant, C. P. & Dice, J. F. (1989) *Science* **246,** 382–385.
30. Chappell, T. G., Welch, W. J., Schlossman, D. M., Palter, K. B., Schlesinger, M. J. & Rothman, J. E. (1986) *Cell* **45,** 3–13.
31. Nihei, T., Takahashi, S., Sagae, S., Sato, N. & Kikuchi, K. (1993) *Cancer Res.* **53,** 1702–1705.
32. Hainaut, P. & Milner, J. (1992) *EMBO J.* **11,** 3513–3520.
33. Takayama, S., Bimston, D. N., Matsuzawa, S., Freeman, B. C., Aime-Sempe, C., Xie, Z., Morimoto, R. J. & Reed, J. C. (1997) *EMBO J.* **16,** 4887–4896.
34. Soulier, S., Vilotte, J.-L., L'Huillier, P. J. & Mercier, J.-C. (1996) *Gene* **172,** 285–289.
35. Matsumoto, H., Shimura, M., Omatsu, T., Okaichi, K., Majima, H. & Ohnishi, T. (1994) *Cancer Lett.* **87,** 39–46.
36. Nihei, T., Sato, N., Takahashi, S., Ishikawa, M., Sagae, S., Kudo, R., Kikuchi, K. & Inoue, A. (1993) *Cancer Lett.* **73,** 181–189.
37. Froesch, B. A., Takayama, S. & Reed, J. C. (1998) *J. Biol. Chem.* **273,** 11660–11666.