The induction by estrogen of rat α_{2u} -globulin gene expression in mouse L-cells

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ABSTRACT

Expression of the rat α_{2u} -globulin gene family is regulated in the adult male liver by a number of hormones, including growth hormone, thyroid hormone and several steroids. Upon injection into ovariectomized females, estrogens first induce α_{2u} -globulin expression and then suppress this gene after several days of hormone administration. To study this phenomenon, we developed a mouse L-cell line that expressed the human estrogen receptor. High levels of rat α_{2u} -globulin transcript were induced in stable transfectants of this line carrying a cloned α_{2u} -globulin gene, following exposure to 17β -estradiol. Since this induction was inhibited by cycloheximide, the response to estrogen, as to other steroids, appears to be secondary.

Using genes with variously deleted 5'-upstream regions, sequences responsible for this induction were located between -730 bp and -223 bp relative to the start of transcription. Examination of the DNA in this region revealed that an estrogen receptor element was located at -590 bp in an area that is highly conserved in most known α_{2u} -globulin genes. Administration of both dexamethasone and estrogen produced a synergistic effect in this system. The induction of α_{2u} -globulin RNA by estrogen in L-cells may re-capitulate the initial response to estrogen *in vivo*, and therefore represents a good model system to seek the identity of the other factors required to effect full induction. *Journal of Molecular Endocrinology* (1998) **20**, 141–150

INTRODUCTION

The α_{2u} -globulin gene family in rats consists of about 30 members (Kurtz 1981a, Dolan *et al.* 1982) which are expressed primarily in adult male rat liver (Roy & Neuhaus 1966). The expression is under multi-hormonal control (Roy *et al.* 1983) and androgens, glucocorticoids, thyroid hormone and growth hormone act as inducers. The effect of estrogen on the transcription of α_{2u} -globulin genes is more complex. Some of the early *in vivo* studies were conducted with female rats that were ovariectomized to establish hormonally deficient tissues. Following an initial exposure to estrogenic hormones, an increase in the hepatic concentration of α_{2u} -globulin was observed, which could be blocked with cycloheximide. Induction of α_{2u} -globulin gene expression by estrogen is just as effective as with the inductive hormone, androgen,

but the proteins are gradually lost after continued daily treatment (Roy 1977).

Daily treatment of mature male rats with estrogenic hormones likewise brings about a large decrease in the hepatic synthesis of α_{2u} -globulin, a 50% decrease in four days and complete suppression of α_{2u} -globulin synthesis in eight days (Roy *et al.* 1975). The continued treatment of males with estrogen eventually produces a two-week-long quiescent period, during which the α_{2u} -globulin gene does not respond to androgen. Although estrogen can decrease the hepatic level of androgen receptor (Kurtz *et al.* 1976), the suppression of androgen's ability to induce α_{2u} -globulin gene transcription by estrogen does not appear to be via this mechanism (Kurtz & Feigelson 1978, Roy 1979).

The effects of estrogen are probably mediated through its sex-specific modulation of the growth

hormone regime. In males the release of growth hormone is periodic whereas in females the fluctuations are dampened out (Saunders *et al.* 1976, Tannenbaum & Martin 1976). Estrogen treatment of male rats has been shown to reduce the pulsatile profile of growth hormone release, making it more similar to that observed in females (Mode *et al.* 1982). The continuous infusion of growth hormone into male rats causes a decrease in hepatic α_{2u} -globulin synthesis (Norstedt & Palmiter 1984, Husman *et al.* 1985) similar to the effect of estrogen. In contrast, periodic injection of growth hormone into female mice causes induction of the major urinary proteins (MUPs), the murine homologues of the α_{2u} -globulins (Al-Shawi *et al.* 1992). These experiments with estrogen suggest that an early inductive phase probably occurs in females during development but that it is replaced with a growth hormone-dependent inhibitory phase that represses α_{2u} -globulin expression.

The regulation of gene expression by estrogen-activated receptor has been studied in many systems. In most cases, the estrogen receptor binds to the estrogen response element (ERE) in the promoter region of the regulated gene. The consensus sequence of the ERE is a palindromic sequence with a half site of GGTCA separated by a 3 bp spacer. Sequences of TCAGGTCA or TTAGTTCA are also functional as estrogen receptor binding sites (Mangelsdorf *et al.* 1995). Upon binding to the promoter region, the estrogen receptor interacts with the transcription initiation complex to alter the transcription of the gene. The precise mechanism underlying this interaction is not clear. The requirement of protein synthesis in some cases implies that factors other than the estrogen receptor and the transcription initiation complex are involved in this process. Several nuclear factors that can interact with the estrogen receptor have been cloned and identified. One of these factors, ERAP160, exhibited binding specificity to estrogen-activated receptor (Halachmi *et al.* 1994), implicating a direct involvement in the estrogen-regulated gene transcription. Nuclear factor RIP140 was also found to interact specifically with the activation domain of the estrogen receptor in an estrogen-dependent manner (Cavaillès *et al.* 1995), which suggested that this nuclear factor is also a potential co-activator of estrogen-regulated transcription.

Although the regulation of rat α_{2u} -globulin genes by estrogen *in vivo* has been evident for over two decades, there is little known about how this occurs at the molecular level. That either the inductive or repressive phase is receptor-mediated is suggested by *in vitro* studies of α_{2u} -globulin gene clone RAO01, which showed that a fragment from

– 606 bp to – 575 bp upstream of the transcription start site has significant binding affinity for the estrogen receptor. A potential estrogen receptor binding site (ERE), in which a 7 bp spacer separates the two half sites, was noted in this fragment (Van Dijck & Verhoeven 1992). The requirement for protein synthesis during induction suggests that additional *trans*-acting proteins are also needed for full induction. However, to determine the functional significance of the ERE and the identity of any *trans*-acting factors will require the development of an appropriate *in vitro* model system. In this study, we describe a mouse L-cell line that expresses the human estrogen receptor. An α_{2u} -globulin gene, clone 91, which was previously shown to be inducible by dexamethasone in mouse L-cells (Kurtz 1981b), is inducible by estrogen in this cell line. Similar to the initial induction by estrogen *in vivo*, this induction also requires the synthesis of other proteins. We have delimited a region in the gene's 5'-upstream region that includes the ERE and is necessary for full induction.

MATERIALS AND METHODS

Plasmid constructs

The construction of plasmids 91RX4, 91RX2, 91RX3 and 91 Da, which contain 6.3 kb, 3.2 kb, 2.7 kb and 226 bp of α_{2u} -globulin 5'-upstream sequence respectively, the entire coding region and 400 bp of 3'-sequence is described in Wang (1996). Plasmid 91R91, which we have determined contains 762 bp of upstream sequence (Wang 1996), was constructed by Addison & Kurtz (1986) who originally described it as having 500 bp of upstream sequence.

Transfection of mouse L-cells

Two plasmids, pΔHER/91023 which carries the human estrogen receptor cDNA under the control of an SV40 promoter (Bradshaw *et al.* 1988), and p220.2 which contains a hygromycin resistant gene (Yates *et al.* 1985), were used to establish receptor-positive stable transfectants of mouse *tk*[–] *aprt*[–] L-cells (Wigler *et al.* 1979). The cells were transfected with 15 µg pΔHER/91023, 1 µg p220.2 and 15 µg mouse L-cell carrier DNA as described in Addison and Kurtz (1986). After two weeks of selection in the presence of 400 µg/ml hygromycin B, single colonies were isolated and further cultivated as cell lines. The presence of the human estrogen receptor gene was confirmed by Southern blotting and receptor was visualized in the nuclei by specific antibody reaction. One of these estrogen

receptor positive cell lines, E2, was then transfected with the five constructs containing the α_{2u} -globulin gene clone 91 described above. These transfections were carried out as described by Addison and Kurtz (1986). Cloned lines were examined for the presence of α_{2u} -globulin sequences by Southern blotting and the presence of α_{2u} -globulin RNA in these cell lines was confirmed by RNA S1 protection assays (see below).

Another receptor positive cell line, E3a, was established in a slightly different way by transfecting an L-cell line which already carried the 91R91 construct with p Δ HER/91023 as described above. Both Southern blotting and receptor localization confirmed the presence of an expressed estrogen receptor gene in this line.

DNA extraction and Southern hybridization

Genomic DNA from tissue culture cells was extracted according to Strauss (1994). For Southern analysis, 20 μ g DNA from each cell line was digested with restriction enzymes (BRL), fractionated on a 0.8% agarose gel and transferred onto a nylon membrane (Zeta-probe, Bio-Rad Laboratories) according to the manufacturer's instructions. The probe used in Southern blots to detect p Δ HER/91023 in transfected cells was the plasmid's 2.1 kb EcoRI fragment that comprises the entire estrogen receptor cDNA labeled with [α - 32 P]dATP. The probe used to detect α_{2u} -globulin sequences in transfected cells was the 4.2 kb HindIII fragment from 91R91 that includes 762 bp of upstream sequence and virtually the entire transcribed region.

Induction of α_{2u} -globulin RNA with hormones

Cells containing α_{2u} -globulin constructs were fed with Dulbecco's Modified Essential Medium (DMEM)+10% calf serum medium and split 1:8 the day before induction. Two plates of cells were used in each sample group. In control groups, cells were fed with DMEM+10% calf serum. In the induced groups, cells were fed with DMEM+10% calf serum+hormone. In inductions with estrogen, 2.75×10^{-5} M 17β -estradiol were used. In inductions with dexamethasone, 1×10^{-6} M dexamethasone was used. The medium in all cell cultures was replaced once every two days and the cells were harvested on the ninth day of induction for RNA isolation.

To analyse the dosage dependency of estrogen induction, one group of control cells was fed with DMEM+10% calf serum, and five induction groups were fed with DMEM+10% calf serum plus 0.55×10^{-5} M, 1.1×10^{-5} M, 1.65×10^{-5} M,

2.2×10^{-5} M and 2.75×10^{-5} M 17β -estradiol respectively. Cells were fed once every two days and were harvested on the ninth day of induction. The kinetics of estrogen induction were examined by feeding E3a cells with fresh medium containing 2.75×10^{-5} M 17β -estradiol on the 0, 2nd, 4th, 6th, 8th, and 10th day. Two plates of cells were harvested on the 1st, 3rd, 5th, 7th, 9th, and 11th day of induction. Cells in two control plates were fed every two days with unsupplemented fresh medium and harvested on the 7th day. RNA was isolated from these cells and the quantity of α_{2u} -globulin RNA was determined by S1 protection.

RNA isolation and S1 protection assay

The isolation of total RNA from cells and liver tissue and the quantitation of α_{2u} -globulin RNA by S1 protection were carried out as described in Wang (1996).

Reverse transcription (RT)-PCR

Total RNA (1 μ g) in a volume of 10 μ l was heated at 90 °C for 2 min and chilled on ice. Reverse transcription, the subsequent amplification by PCR and diagnostic restriction digestion of the products were carried out as previously described (Wang *et al.* 1997).

RESULTS

Tissue specificity of clone 91 expression

We have been able to distinguish three classes of transcript for the α_{2u} -globulin family that are tissue-specific in their distribution (Wang *et al.* 1997). To assign the specific member of the gene family used in our studies, clone 91 (Kurtz 1981b), to one of these classes, we subjected amplified cDNA from L-cells carrying plasmid 91R91 to diagnostic restriction analysis. As a comparison, RNA from an adult male liver was subjected to the same analysis. The data in Fig. 1 show that clone 91 belongs to class N whose amplified cDNA is not cut by any of the three enzymes used in this analysis (Wang *et al.* 1997). The liver sample also contains transcripts from this same class N, as may be judged from the failure of the triple digest to cleave all of the uncut 312 bp product (Fig. 1). In addition, the liver sample exhibits transcripts from the other two classes of genes in the α_{2u} -globulin family that we have been able to differentiate: class A whose cDNA product is cleaved by both ApaLI and SstI but not VspI, and class V which is cut only by VspI.

FIGURE 1. Assignment of clone 91 to subset N of the α_{2u} -globulin gene family. RT-PCR was performed with 1 μ g total RNA from mouse liver tissue or L-cells carrying plasmid 91R91 that had been induced by exposure to 1×10^{-6} M dexamethasone. The amplified cDNA was digested with restriction enzymes, fractionated by electrophoresis on a 2% agarose gel and the radioactive products were visualized by autoradiography. Samples were loaded as follows: M: molecular weight markers, C: undigested RT-PCR product, expected size=312 bp, A: ApaLI, S: SstI, V: VspI, T: triple digest.

α_{2u} -globulin RNA is induced by estrogen

Four independent cell lines, E2-R8, E2-R9, E2-R10 and E2-R11, were derived from the transfection of E2 with 91R91, which carried the coding region, 762 bp of 5'-sequence and 400 bp of 3'-sequence of clone 91. All were shown to contain α_{2u} -globulin sequences by Southern blotting, and α_{2u} -globulin RNA could be induced in each by dexamethasone (data not shown). In these cell lines, and E3a as well, estrogen induction of the transcription of α_{2u} -globulin RNA was observed (Fig. 2). The S1 protection assay showed that the α_{2u} -globulin RNA in these cell lines increased 5- to 15-fold following nine days of estrogen treatment. The 40 base fragment evident in all lanes is the product of an unknown RNA crossing the transcription start site. It has been detected previously (Addison & Kurtz 1986) and may correspond to the transcript initiated 77 bp upstream of the one that gives rise to the 32 base protected fragment (Winderickx *et al.* 1987).

The dependence of α_{2u} -globulin RNA induction on estrogen concentration was determined for cell line E3a (Fig. 3). The fold-induction increases as the concentration of 17β -estradiol rises until it reaches about 2.0×10^{-5} M, at which point α_{2u} -globulin RNA levels plateau.

The kinetics of estrogen-induced α_{2u} -globulin transcription in E3a cells (Fig. 4) showed a lag period of 2 to 3 days, which is similar to that observed during dexamethasone induction of the α_{2u} -globulin gene in L-cells (Addison & Kurtz

1986). Following the lag period, the α_{2u} -globulin RNA increases until the 9th day of treatment, when the level of α_{2u} -globulin RNA reaches a plateau. The presence of a lag period suggests that the induction by estrogen may be a secondary response that requires the synthesis of other transcription factor(s). A requirement for protein synthesis has been confirmed by quantitating the amount of the α_{2u} -globulin RNA induced by estrogen in E3a cells in the presence of cycloheximide (Fig. 5). The administration of 0.05 μ g/ml cycloheximide reduced the induction of α_{2u} -globulin transcription 5-fold to the basal level seen in uninduced cells.

Synergistic effects of estrogen and glucocorticoid on α_{2u} -globulin gene transcription

In vivo studies have shown that continued daily treatment with estrogen for eight days suppresses the expression of α_{2u} -globulin gene in male rat livers. To investigate the effect of estrogen on the dexamethasone-induced transcription of the α_{2u} -globulin gene in mouse L-cells, 17β -estradiol was added at the same time as dexamethasone. In two independent cell lines, E2-R8 and E2-R11, in which the transcription of the α_{2u} -globulin gene was inducible by either 17β -estradiol or dexamethasone, the treatment with both 17β -estradiol and dexamethasone for nine days produced a much higher

FIGURE 2. Induction of α_{2u} -globulin RNA by estrogen in mouse L-cells. Five independent cell lines, containing 91R91 (see text), were fed once every two days with (+) or without (–) 2.75×10^{-5} M 17β -estradiol. Total RNA was isolated from cells on the 9th day of induction and 25 μ g total RNA from each sample was used in the S1 protection assay. The 32 base fragment is the part of the oligonucleotide KF40 protected by α_{2u} -globulin mRNA from S1 digestion. This band was excised from the gel to quantitate α_{2u} -globulin mRNA by scintillation counting. The 40 base fragment that appears on the autoradiogram corresponds to the complete protection of KF40, the oligonucleotide used in the S1 protection assay, by an unknown RNA crossing the transcription initiation site of the α_{2u} -globulin gene (see text).

level of α_{2u} -globulin gene transcription than that produced by treatment with either of the hormones alone (Fig. 6). In fact, the level of induction by both hormones is higher than the sum of the levels of induction produced by these two hormones individually, indicating a synergistic effect was produced.

Delimiting the 5'-upstream sequences necessary for the estrogen-induced transcription of α_{2u} -globulin RNA

To examine the effect of 5'-upstream sequences on the hormonal induction of α_{2u} -globulin RNA accumulation, plasmids which contained different lengths of 5'-upstream sequences from clone 91 (see Materials and Methods) were transfected into E2 cells. Three to five independent cell lines from the transfectants of each construct, in which the α_{2u} -globulin gene was found to be inducible by dexamethasone, were established. The effects of 17β -estradiol, dexamethasone or both hormones on α_{2u} -globulin RNA levels are summarized in Fig. 7.

FIGURE 3. Dose-response of estrogen-induced α_{2u} -globulin RNA. E3a cells were grown in medium supplemented with 17β -estradiol at the concentrations shown for nine days. Total RNA was isolated and the amount of α_{2u} -globulin-specific transcript was quantitated by the S1 protection assay.

The average level of induction by estrogen in cells transfected with 91R91, which contains 762 bp of 5'-upstream sequence, was significantly higher

FIGURE 4. Kinetics of α_{2u} -globulin induction by estrogen. E3a cells were fed with medium supplemented with 2.75×10^{-5} M 17 β -estradiol. At the indicated times total RNA was isolated and the α_{2u} -globulin mRNA was quantitated by the S1 protection assay.

than in any of the other transfectants (8-fold). The removal of sequences between -762 bp and -220 bp substantially reduced the inducibility of α_{2u} -globulin RNA by either hormone. The addition of sequences between -762 bp and -2.7 kb likewise reduced the inducibility by estrogen but had little effect on the glucocorticoid response. The synergistic effect upon induction caused by estrogen and dexamethasone together was only apparent for the construct (91R91) that exhibited significant induction by estrogen alone. When the induction of the α_{2u} -globulin gene by estrogen was low, the induction by both hormones was more or less additive rather than synergistic.

DISCUSSION

Estrogen is capable of both inducing and repressing α_{2u} -globulin expression. Induction is seen upon first injection of the hormone into ovariectomized female rats. Repression is seen upon continued hormone administration to such females or upon its injection into male rats. In this paper we set out to develop an *in vitro* model to study the inductive effects of the hormone upon α_{2u} -globulin RNA accumulation. Stable mouse L-cell lines that expressed the human estrogen receptor were transfected with plasmids carrying derivatives of clone 91, a member of the α_{2u} -globulin gene family whose response to glucocorticoids has been analyzed in some detail (Kurtz 1981b, Addison & Kurtz 1986, 1989, Hess *et al.* 1990). The data of Fig. 1 show that clone 91 belongs

FIGURE 5. Inhibition of estrogen-induced transcription of α_{2u} -globulin by cycloheximide. Cultures of E3a cells were fed with medium containing 2.75×10^{-5} M 17 β -estradiol and the indicated concentrations of cycloheximide once every two days. After six days of incubation, cells were assayed for the α_{2u} -globulin RNA by S1 protection.

to the class of α_{2u} -globulin genes designated as N (Wang *et al.* 1997). The tissues in which these genes are predominantly expressed are those of the liver and the preputial gland (Wang *et al.* 1997). Gene expression in the former is clearly under multi-hormonal control which is consistent with the responsiveness of clone 91 to both estrogen (this study) and dexamethasone (Kurtz 1981b). Clone 91 cannot be expressed in the salivary glands where only class A genes are active (Wang *et al.* 1997).

Transfected cells carrying both the estrogen receptor and a clone 91 plasmid with 762 bp of 5'-upstream sequence, demonstrated estrogen inducibility of α_{2u} -globulin. The dose-response curve (Fig. 3) indicates that full induction is achieved at a concentration near 2×10^{-5} M, which is 4- to 30-fold higher than the physiological concentration range of serum estrogen detected in the rat estrous cycle (Nequin *et al.* 1979). We have no ready explanation for this fact other than to note that comparable levels of dexamethasone are required for maximal induction of α_{2u} -globulin in these same cells (Addison & Kurtz 1986). Furthermore, the response to estrogen exhibited an absolute requirement for the estrogen receptor, since no α_{2u} -globulin induction could be detected in L-cells transfected with just clone 91 (data not shown).

Induction of gene 91 by both estrogens and glucocorticoids requires the region between -226 bp and -762 bp to achieve maximal levels of RNA accumulation. This region includes a glucocorticoid response element (GRE) at position

FIGURE 6. Synergistic effect of estrogen and glucocorticoid on the transcription of α_{2u} -globulin, clone 91. Two independent cell lines, E2-R8 and E2-R11, which are E2 cells transfected with 91R91, were treated with hormones as indicated above for nine days. Total RNA was isolated and the α_{2u} -globulin RNA was quantitated by S1 protection. (A) S1 protection assay of α_{2u} -globulin gene transcripts in cells treated with hormones. (B) Level of induction relative to the control (from A). Con, no hormones; Dex, dexamethasone (1×10^{-6} M); Es, 17 β -estradiol (2.75×10^{-5} M).

– 513 bp that we have argued is a functional component of the *cis*-acting regulatory region of clone 91 (Wang 1996). Analysis of the DNA sequence of this region (Wang 1996) reveals the presence of just one sequence, 5'-GGTCAttctctgTGACT-3' centered at – 590 bp, with similarity to an estrogen receptor binding site (ERE). The DNA sequence of the – 606 bp to – 575 bp region in clone 91, which includes the putative ERE, is identical to that in clone RAO01, which showed binding affinity to the estrogen

receptor *in vitro* (Van Dijck & Verhoeven 1992). This site differs from the canonical ERE, 5'-GGTCAnnnTGACC-3', in its 7 bp spacing of the two half-sites. However, a genetic screen for elements that could function as EREs in yeast and mammalian cells revealed considerable diversity in the number, orientation and spacing of half sites in EREs (Dana *et al.* 1994). Furthermore, a cluster of four very widely spaced half-sites acts as an ERE in the well characterized ovalbumin regulatory region (Kato *et al.* 1992). Thus, the two half sites at

FIGURE 7. Effect of hormones on the induction of α_{2u} -globulin RNA from clone 91 constructs carrying various 5'-upstream sequences. The average levels of induction in independent cell lines carrying 91 Da (226 bp), 91R91 (762 bp), 91RX3 (2.7 kb), 91RX2 (3.2 kb) and 91RX4 (6.3 kb) are shown. Five lines were assayed for each construct except 91 Da and 91RX4 for which three and six lines respectively were examined.

– 590 bp of gene 91 may act synergistically to form a functional ERE.

Like the initial inductive response seen in castrated females, the effect of estrogen on transfected L-cells was inhibited by cycloheximide which implies that newly synthesized transacting factors, whose identity remains unknown, are involved. We hypothesize that the ERE at – 590 bp is necessary for estrogen-induced expression of the α_{2u} -globulin gene but that a hormone-receptor complex bound to this site participates with other transcription factors to effect full induction. Two proteins that are involved in the regulation of gene expression by estrogen have recently been cloned and characterized. Nuclear protein RIP140 was found to interact specifically with the activation domain of estrogen receptor *in vitro* in an estrogen-dependent manner (Cavaillès *et al.* 1995). This protein was also found to interact with the estrogen receptor in intact cells and modulates its function in the presence of estrogen, suggesting that RIP140 mediates the activation of transcription by

the estrogen receptor. ERAP160, an estrogen receptor-associated protein, has also been shown to interact with the receptor in a hormone-dependent manner and the ability of estrogen receptor to activate transcription was correlated with its ability to bind to this protein (Halachmi *et al.* 1994). It is not clear at this moment what factors are involved in the estrogen-induced transcription of the α_{2u} -globulin gene, but the mouse L-cell system provides us with an opportunity to determine their identity and to understand the nature of their interaction with the receptor and the basal transcription complex.

In cell lines transfected with plasmids that contain more than 762 bp of 5'-upstream sequence, the induction of the α_{2u} -globulin gene by estrogen, but not dexamethasone, is lower than that in cell lines transfected with just 762 bp of 5'-upstream sequence (Fig. 7). This suggests that silencing elements specific to the estrogen response lie within the region between – 2.7 kb and – 762 bp but a more detailed analysis of this region will be required to confirm their identity.

A pronounced synergistic effect of estrogen and dexamethasone on the α_{2u} -globulin gene transcription was observed in cells transfected with 91R91 (Fig. 6). The synergism seems dependent on the inducibility of this gene by estrogen as indicated in Fig. 7. Synergistic effects have been observed with glucocorticoid receptors that bind to multiple GREs (Schmid *et al.* 1989), or estrogen receptors that bind to multiple EREs (Martinez & Wahli 1989). These effects were attributed to co-operative binding of the receptors to their response elements. The synergistic effect that we have observed between these two hormones has not been reported before. Co-operative binding of the two types of receptor could result in a synergistic response but since the induction of α_{2u} -globulin by either hormone requires other transcription factors, another possibility is that recruiting of these transcription factors by both receptors synergizes transcription. Earlier work on the induction of gene 91 by glucocorticoids identified a non-GRE sequence at about – 130 bp that was essential for the glucocorticoid response (Addison & Kurtz 1986). The same linker scanning mutation, LS-137/-125, that abolished the glucocorticoid response of gene 91, abolishes estrogen induction of the gene (data not shown). This suggests that the receptors for the two steroid hormones may interact with α_{2u} NF1, the nuclear protein identified by Addison & Kurtz (1989) that binds at – 130 bp.

That the ERE sequence in clone 91 is active *in vivo* is suggested by the inductive effect of estrogen seen following the initial injection of the

hormone into hormonally depleted female rats. The suppressive effects of continued estrogen administration to females or males that are observed *in vivo* were not seen in the mouse L-cell system. Since the addition of estrogen did not suppress the dexamethasone-induced transcription, even in cells transfected with 6.3 kb of 5'-upstream sequences (Fig. 7), it is unlikely that the absence of suppression is due to missing 5'-upstream sequences. The failure of estrogen to suppress α_{2u} -globulin transcription in L-cells may reflect the hormone's apparent ability to modify the growth hormone release profile from the periodic pattern seen in males to the less pulsatile female pattern (Mode *et al.* 1982). It is now clear that the sexually dimorphic distribution of the major urinary proteins in rodents (low to absent in females, high in males), is a result of the different modes of growth hormone release in the two sexes (Norstedt & Palminter 1984, Husman *et al.* 1985, Al-Shawi *et al.* 1992). The *cis*-acting elements that mediate the response of the major urinary protein genes in mice to intermittent growth hormone have recently been mapped to the promoter proximal region and the first intron (Johnson *et al.* 1995). These authors propose that a c-Fos/c-Jun complex, bound to the AP1 site within the intron, interacts with transcription factors bound to the promoter proximal region. Down-regulation of the complex would occur in the female-like growth hormone environment and up-regulation, leading to increased MUP transcription, would occur under a male-like growth hormone regime.

The recent discovery that two sexually dimorphic traits, body weight and MUP expression, depend upon STAT5b (Udy *et al.* 1997), could also implicate this protein in the complex multihormonal regulatory system that controls the major urinary proteins. We have noted elsewhere (Wang & Hodgetts, unpublished observations) that two STAT5 binding sites are located within the upstream region that we have shown is required for the induction of α_{2u} -globulin by either dexamethasone or estrogen. Although the details have yet to be worked out, it is apparent that one or more components of the growth hormone signaling pathway is missing in L-cells, which appear to possess an intracellular environment similar to that set up by pulsatile growth hormone. Thus dexamethasone, which in the absence of intermittent growth hormone cannot induce α_{2u} -globulin expression *in vivo* (Lynch *et al.* 1982, Kulkarni *et al.* 1985), causes induction in L-cells. The creation of cells expressing the growth hormone receptor in addition to the estrogen receptor would be a useful next step in the development of an easily

manipulated *in vitro* culture system within which the study of the multihormonal dimension of α_{2u} -globulin regulation could be undertaken.

ACKNOWLEDGEMENTS

The Medical Pathology Laboratory at the Cross Cancer Institute, Edmonton, Alberta performed the estrogen receptor localization experiments. We are grateful to Drs M-J Tsai and Bill Sugden for their gifts of plasmids. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada to R B H and W A.

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RECEIVED 6 June 1997