Testosterone and dihydrotestosterone regulate AUF1 isoforms in a tissue-specific fashion in the mouse

LOWELL G. SHEFLIN1 AND STEPHEN W. SPAULDING2
Veterans Affairs Western New York Health Care System, and the Departments of 1,2Medicine and
2Physiology and Biophysics, State University of New York at Buffalo, Buffalo, New York 14215

Testosterone and dihydrotestosterone regulate AUF1 isoforms in a tissue-specific fashion in the mouse. Am. J. Physiol. Endocrinol. Metab. 278: E50–E57, 2000.—The sex difference in the metabolism of certain mRNAs in the murine submaxillary gland (SMG) prompted us to determine whether androgens regulate the expression of any of the four isoforms of AUF1, proteins that bind differentially to AU-rich RNA. We found that cytosol from female SMGs contains two major isoforms (p45 and p40), whereas cytosol from male SMGs contains a prominent p37 and a weaker p42. Injecting female mice with testosterone decreases p45 levels by 81% after 7 days \( (P < 0.05, n = 4) \), whereas p42 and p37 increase 74 and 449% at 7 days \( (P < 0.05, n = 4, \) for both). Orchietomy, conversely, decreases p37 levels in the male SMG by 91% \( (P < 0.006) \) while increasing p45 5-fold and p40 2.5-fold \( (P < 0.05, n = 5 \) for both). Both male and female kidney cytosol contains a prominent p37 and a faint band of ~42 kDa, but neither shows a significant change when circulating androgen levels are altered. Dihydrotestosterone (DHT) changes the pattern of AUF1 isoforms in female SMG cytosol more rapidly than does testosterone. Nuclear extracts from female SMG contain predominantly p45, and DHT decreases its level slightly \( (35\%, P < 0.05 \) at 24 h). Polysomal extracts from female SMG contain p45 and p42, and DHT increases p45 levels 58% \( (P < 0.02, n = 6) \) at 24 h. In certain nonreproductive tissues, androgens may differentially regulate AUF1 isoform levels to modulate the metabolism of AU-rich mRNAs posttranscriptionally.

AUF1/heterologous nuclear ribonucleoprotein D; androgens; sexual dimorphism; submaxillary gland

ANDROGENS CAN REGULATE GENE EXPRESSION at transcriptional or posttranscriptional levels, but how “anabolic” actions occur selectively in nonreproductive tissues remains unclear. For example, despite the fact that no androgen response element has been identified within 7 kb of the genome upstream of the epidermal growth factor (EGF) gene \( (8) \), manipulating circulating androgen levels dramatically alters the expression of EGF mRNA in the submaxillary gland (SMG) of mice \( (21–23) \). Consistent with an action at the posttranscriptional level, androgens produce several changes in EGF transcripts, including changes in the length of the poly-A tails \( (18, 21–23) \). We have previously demonstrated that several androgen-dependent proteins in SMG cytosol bind to a highly conserved 23-bp adenosine-uridine \( (AU) \)-rich sequence that overlaps the terminal polyadenylation signal in the 3′ untranslated region \( (3′UTR) \) of EGF mRNA \( (22, 23) \). Similar AU-rich elements are found in the 3′UTR of many genes whose transcripts display rapid turnover \( (20) \). Several families of heterologous nuclear ribonucleoproteins \( (hnRNPs) \) can bind to such AU-rich sequences, and some of these proteins can influence the subcellular location, metabolism, and/or polyadenylation of such mRNAs \( (6, 7, 13, 14, 27, 28) \). One highly conserved family of proteins, called AUF1/hnRNPD, binds to AU-rich elements found in 3′UTR of several growth factors, cytokines, and protooncogenes to regulate their rate of decay \( (6, 7, 11, 13, 14, 27, 28) \). Both the human and the murine AUF1/hnRNPD genes encode four isoforms \( (p45, p42, p40, \) and p37) which are generated by alternative splicing events \( (6, 27) \) and which have different affinities for AU-rich mRNA \( (11, 27) \). We therefore employed an antibody to AUF1 to probe immunoblots of proteins prepared from SMGs of male and female mice to determine whether circulating levels of androgen influence AUF1 isoforms in a tissue-specific manner.

METHODS

Animal studies. Experiments were conducted in accordance with National Institutes of Health guidelines for animal treatment and housing. Litters of young adult (10- and 12-wk-old) female BALB/c mice were divided into groups that contained representatives of each litter. The mice were injected with testosterone propionate \( [200 \mu g \) in sesame oil-ethanol \( (21)] \) subcutaneously every other day for up to 7 days, or with dihydrotestosterone \( [DHT; \) \( 200 \mu g \) suspended in vehicle: \( 0.9\% \) NaCl, \( 0.4\% \) polysorbate 80, \( 0.5\% \) carboxymethyl cellulose, and 0.9% ethanol \( (17) \)] subcutaneously \( (21) \). The mice were injected with testosterone propionate \( [200 \mu g \) in sesame oil-ethanol \( (21)] \) subcutaneously every other day for up to 7 days, or with dihydrotestosterone \( [DHT; \) \( 200 \mu g \) suspended in vehicle: \( 0.9\% \) NaCl, 0.4% polysorbate 80, 0.5% carboxymethyl cellulose, and 0.9% ethanol \( (17) \)] subcutaneously. Controls received vehicle alone. Three, 5, and 7 days after the testosterone injections, and 6, 24, and 48 h after the DHT injections, the animals were euthanized by a lethal intraperitoneal injection of pentobarbital. The SMGs and kidneys were removed and either processed immediately \( (11, 27) \) or snap-frozen in liquid \( N_2 \) and processed at a later date. The tissue samples were homogenized in 1 ml of freshly prepared iced \( \text{buffer} \) A \( [20 \text{mM HEPES, pH} 7.4, \) containing 50 mM K acetate, \( 5 \mu M \) Mg acetate, protease inhibitors \( (5 \mu g/ml \) phenylmethylsulfonyl fluoride, \( 10 \mu M \) soybean trypsin inhibitor, \( 10 \mu M \) leupeptin), ribonuclease inhibitor \( (1 U/\mu l \), Promega), and 1 mM dithiothreitol \( (21) \))

Preparation of cytosol and nuclear and polysomal extracts. Cytosol was prepared by centrifuging 1 ml of homogenized tissues at 800 g for 10 min at 4°C. The 800-g supernatants...
were layered on 4-ml cushions of 30% sucrose in iced buffer A and then centrifuged at 100,000 g for 2 h at 4°C, and the supernatants were saved as cytosol and frozen at −70°C (21). For the DHT experiments we also prepared polysomal and nuclear extracts by the following procedures. The 100,000-g pellet was resuspended in buffer A containing sodium chloride (0.3 M final concentration), incubated on ice for 1 h, and then centrifuged at 10,000 g for 15 min at 4°C. The resultant supernatant (polysomal extract) was frozen at −70°C. The 800-g pellets were resuspended in buffer A containing sodium chloride (0.3 M final concentration) and centrifuged at 12,000 g for 10 min at 4°C, and the resultant supernatant (nuclear extract) was frozen at −70°C. Protein concentrations were estimated by optical density (OD280 nm).

Detection of immunoreactive AUF1. Proteins in the different fractions were dissolved in 10% glycerol, 5% mercaptoethanol, 2% SDS, and 62.5 mM Tris·HCl (pH 6.8), heated to 90°C for 2 min, and then briefly centrifuged to remove insoluble material. The samples were run on 1% SDS-12.5% polyacrylamide gels, and then the gels and polyvinylidene fluoride membranes (Immobilon 0.45 μM, Millipore) were equilibrated in iced 25 mM Tris and 192 mM glycine, pH 8.3, containing 20% methanol. The proteins in the gels were transferred by use of a Bio-Rad Mini Trans-blot apparatus and a stepwise transfer protocol (50, 150, and 300 mA for 15, 15, and 60 min, respectively) (22, 23). After transfer, the membranes were stained with Ponceau red to confirm that the proteins in each lane had been transferred homogeneously and to establish the mobility of standard molecular mass markers. The membranes were then blocked with 3% nonfat dry milk in Tris-buffered saline and incubated with a rabbit polyclonal antibody to human AUF1 [Dr. Gary Brewer, no. 1871; 1:5,000 dilution for 1 h at 4°C (28)]. The distribution of the primary antibody was determined by incubation with a goat anti-rabbit antiserum conjugated with alkaline phosphatase (Amersham, Arlington Heights, IL; 1:10,000 dilution) and detected by chemifluorescence (Vistra ECF, Amersham). The immunoblots were scanned with a Storm fluorescence imaging system, and immunospecific bands were quantified using ImageQuant software, version 4.2 (Molecular Dynamics, Sunnyvale, CA). Molecular masses were estimated with Promega mid-range and Bio-Rad prestained high-range protein standards. Statistical analyses were performed by ANOVA (with StatView, SE 1 Graphics, version 1.03, Abacus Concepts, Calabasas, CA).

RESULTS

Tissue-specific androgen-dependent responses of AUF1 isoforms. Representative samples of kidney and SMG cytosol from two controls and two orchiectomized males were compared by Western blot analysis using antibodies raised against human AUF1. AUF1 is a heterogeneous family of 11 members with molecular masses ranging from 45 kDa to 37 kDa. Figure 1A shows the detection of the AUF1 isoforms in kidney and SMG cytosol of two control males and two males 2 wk after bilateral orchiectomy. Female SMG cytosol from two controls and two animals treated with testosterone for 7 days was also analyzed. The data show that the AUF1 isoforms were downregulated in the orchiectomized males but upregulated in the treated females.

Figure 1B shows the quantification of total AUF1 immunoreactivity in different tissues in response to changes in circulating androgen levels, 2 wk after orchiectomy of males or after 7 days of testosterone treatment of females (means ± SE, n = 4). *P < 0.05.

Figure 1C shows the Western blot analysis of female kidney before and after treatment with dihydrotestosterone (DHT) for 48 h compared with female SMG, before and after DHT, and male SMG, before and 2 wk after orchiectomy.
and of SMG cytosol from two controls and two testosterone-treated females are shown in Fig. 1 to demonstrate the relative tissue distribution and abundance of the immunoreactive AUF1 bands, which run in the 35- to 45-kDa region. Bands with apparently higher molecular masses are occasionally also detected. Cytosol from control male kidney and SMG (pair-matched tissue from the same animals) each contain a prominent 37-kDa (p37) and a minor 42-kDa (p42) immunoreactive AUF1 band. In striking contrast to the male tissues, cytosol from control female SMG generally contains two different and more prominent immunoreactive AUF1 bands, of 45 kDa (p45) and 40 kDa (p40). Orchiectomy of male mice markedly changes the AUF1 isoform pattern in the SMG cytosol, causing it to resemble control female SMG. Orchiectomy also increases the total AUF1 immunoreactivity in the male SMG by 2.5-fold (Fig. 1, A and B; P, 0.05, n = 5). In the kidney cytosol, however, orchiectomy does not significantly alter either the AUF1 isoform pattern or the total level of AUF1 immunoreactivity in female SMG in response to testosterone, as the mirror image of the 2.5-fold increase after orchiectomy in the male SMG (Fig. 1B; P < 0.05, n = 5). Thus manipulating circulating androgen levels caused consistent changes in both sexes: the pattern of AUF1 isoforms and also the total level of immunoreactive AUF1 change toward the male pattern when females are injected with testosterone, whereas they change toward the female pattern when males are orchiectomized.

Further support for the tissue-specific nature of the androgen-dependent AUF1 responses was obtained when we assessed the level of AUF1 isoforms in kidney cytosol from female mice injected with DHT. The AUF1 pattern in control female kidney cytosol resembled the pattern in the male, and it did not change after 48 h of DHT (Fig. 1C). Thus these AUF1 isoforms are unresponsive to in vivo changes in androgen levels in both male and female kidney cytosol.

The sizes of these immunoreactive AUF1 bands in SMG cytosol correspond to the sizes of the AUF1 isoforms predicted by cDNA analyses from human and murine tissues (6, 13, 28). A previous report indicated that the relative abundance of certain AUF1 isoforms differed between tissues from male mice (28), but this is the first demonstration that all four isoforms can be present in a single murine tissue and that the circulat-
ing level of androgen modulates their relative abundance. Because the four isoforms of AUF1 result from alternative splicing of the primary AUF1 gene transcript (6, 27, 28), the androgen-dependent changes in isoform expression could reflect tissue-specific differences in mRNA splicing.

Quantitative analysis of the effect of testosterone injections on specific AUF1 isoforms present in the cytosol of female SMG. We analyzed the time course of the change in the distribution and levels of AUF1 isoforms after the injection of female mice with 200 µg of testosterone QOD, and we quantified the individual AUF1 isoforms present at 0, 3, 5, and 7 days (Fig. 2). As shown above, the cytosol from control female SMGs routinely displayed the prominent p45 and p40 AUF1 isoforms (Fig. 2, A and B). Treatment with testosterone for 3 days decreased the mean level of immunoreactivity in p45 by 44% (P < 0.05, n = 4). The p45 continued to decrease progressively for up to 7 days of treatment, when its level fell by 81% (P < 0.05, n = 4) (Fig. 2, A and B). The decrease in p40 did not reach statistical significance, but immunoreactivity in the neighboring p42 and p37 increased 70 and 450%, respectively, after 1 wk of testosterone (P < 0.05, n = 4; Fig. 2, A and B). Note that there is inherent variability among the responses of animals to testosterone, and that in some individuals all four AUF1 isoforms can be detected (e.g., the last lane of Fig. 2A).

Quantitative analysis of the effect of orchiectomy on specific AUF1 isoforms present in cytosol from male SMG. As we reported, 2 wk after orchiectomy the AUF1 isoform pattern changed dramatically in the male SMG. The major isoform present in the control cytosol (p37) decreased by 91% (P < 0.006, n = 5), whereas the mean level of immunoreactivity in p45 increased 5.1-fold (P < 0.05, n = 5) and that in p40 increased 2.5-fold (P < 0.05, n = 5; Fig. 3B). The decrease in p42, which is flanked by p45 and p40, did not reach statistical significance (n = 5). Thus orchiectomy changes both the pattern and the amount of AUF1 in SMG cytosol in the opposite direction from the effect of injecting female mice with testosterone, in which p45 and p40 decrease while p37 becomes predominant.

Comparative analysis of the effect of DHT on AUF1 levels and isoforms in the cytosol and nuclear and polysomal fractions from female SMG. Because the murine SMG contains 5α-reductase, which can convert testosterone to DHT (12), and because female mice can aromatize testosterone to estrogen, we also assessed the effect of DHT on AUF1 isoforms in female mice.
In addition, because AUF1 isoforms have been detected in nuclear and polysomal fractions as well as cytosol, we expanded our investigation of androgen-dependent changes in AUF1 isoforms to include all three compartments.

DHT changed the distribution of AUF1 isoforms in the cytosol of female SMG more rapidly than did testosterone (Fig. 4, A and B), although the pattern of change in the isoforms closely resembled the change in response to testosterone. DHT decreased immunoreactivity in the predominant p45 and p40 isoforms by 73 and 30%, respectively (Fig. 4B; P < 0.05, n = 6) at 24 h, whereas both p42 and p37 increased at 24 h (by 115% and 11-fold, respectively, P < 0.05; n = 6 for both) (Fig. 4B). In general, the AUF1 responses to DHT resembled those observed after testosterone but occurred earlier.

Fig. 4. Time course of changes in AUF1 isoforms in cytosol and nuclear and polysomal extracts from female SMG after injection of DHT. SMG cytosol and nuclear and polysomal extracts (25 µg/lane) from female mice (controls and animals treated with DHT 6, 24, or 48 h). A: immunoblot shows effect of DHT treatment on AUF1 isoforms in cytosol from 3 different animals at each time point. Arrows indicate 45, 42, 40, and 37 kDa. B: quantification of responses (means ± SE; n = 6) of p45, p42, p40, and p37 in relative fluorescence units in cytosol of female SMG relative to DHT treatment (h). *P < 0.05. C: immunoblot shows effect of DHT on AUF1 isoforms present in nuclear extracts from 3 different animals at each time point. Arrows indicate 45, 42, 40, and 37 kDa. D: quantification of responses (means ± SE; n = 6) of major AUF1 isoform, p45 (in relative fluorescence units), in nuclear extracts from female SMG relative to DHT treatment (h). *P < 0.05. E: immunoblot shows effect of DHT on AUF1 isoforms in polysomal extracts from 3 different animals at each time point. Arrows indicate 45 and 42 kDa. F: quantification of responses of major AUF1 isoforms, p42 and p45 (in relative fluorescence units), in polysomal extracts of female SMG (means ± SE; n = 6). *P < 0.05.
which may reflect its action being more direct or its greater potency because of its slower dissociation from androgen receptors.

A previous immunoblotting study has shown that p45 predominates in the nucleus of various human and murine cells (28). Consistent with this, we found p45 to be the major isoform present in nuclear extracts from the SMG of both females and males. DHT reduced the level of p45 in female SMG nuclei by 35% at both 24 and 48 h (P < 0.05, n = 6 for both groups; Fig. 4, C and D). The size of the decrease in p45 levels in the nucleus is substantially less than the 85% decrease in cytoplasmic p45 in response to DHT at 24 h. The nuclear compartment contained relatively little immunoreactive p37, p40, or p42, and DHT caused no significant change in any of these minor AUF1 isoforms (n = 6). Because the pattern of change in the minor nuclear isoforms parallels the pattern observed in cytosol, it could simply reflect the contamination of nuclear fractions with small amounts of cytosol. In light of the changes produced by DHT in the cytosol, a shift from nuclear stores does not appear to be the explanation for the major increase in the level of the p37 that occurs in SMG cytosol.

Polysomes from human and hamster cell lines have been shown to contain AUF1 (6, 19, 28), so we also examined the distribution of AUF1 isoforms in extracts of polysomal fractions from the SMG of control and DHT-treated female mice (Fig. 4, E and F). Polysomal extracts from control female SMGs contained only p45 and p42. DHT did not change the level of p42 significantly but did increase immunoreactivity in p45 after 24 h (Fig. 4F; 58%, P < 0.02, n = 6). This DHT-induced increase in polysomal p45 is strikingly different from the decrease in p45 that DHT produced in both cytosol and in nuclear extracts. Although the fractionation method employed does not permit us to quantify total intracellular AUF1 levels or track shifts in the subcellular distribution of AUF1 isoforms, the increase in polysomal p45 could reflect a redistribution of p45 from stores in the cytosol and/or the nucleus in response to DHT (Table 1).

### Table 1. Summary of relative levels of AUF1 isoforms in female SMG before and after DHT treatment

<table>
<thead>
<tr>
<th>Subcellular Location</th>
<th>AUF1/hnRNP D Isoform</th>
<th>Cytoplasm</th>
<th>Polysomal fraction</th>
<th>Nuclear fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p45</td>
<td>High (Decreased 73%*)</td>
<td>High (Increased 58%)</td>
<td>High (Decreased 35%)</td>
</tr>
<tr>
<td></td>
<td>p42</td>
<td>Low (Increased 115%)</td>
<td>Medium (NC)</td>
<td>Low (NC)</td>
</tr>
<tr>
<td></td>
<td>p40</td>
<td>High (Decreased 30%)</td>
<td>ND (NC)</td>
<td>Low (NC)</td>
</tr>
<tr>
<td></td>
<td>p37</td>
<td>Low (Increased &gt;450%)</td>
<td>ND (NC)</td>
<td>Low (NC)</td>
</tr>
</tbody>
</table>

*Percent change after 48 h of DHT; NC, no significant change; ND, not detectable.

DISCUSSION

The pronounced sexual dimorphism in AUF1 isoforms in SMG cytosol, but not kidney cytosol, suggests that tissue-specific regulation of AUF1 isoforms is involved in the well-known sex difference in the metabolism of certain mRNAs in the mouse (8, 18, 21–23). The p45 and p40 isoforms predominate in female SMG cytosol, whereas the predominant form in male SMG cytosol is p37. Orchiectomy converts the male pattern to that found in the female, whereas injecting female mice with testosterone or DHT converts the female pattern to that found in male SMG cytosol. Thus circulating androgen levels differentially regulate the expression of AUF1 isoforms, supporting the hypothesis that changes in AUF1 mediate some of the effects of androgen on mRNA metabolism in this nonreproductive organ.

Four different protein isoforms of AUF1/hnRNP D are predicted on the basis of alternative splicing of the gene’s transcripts in human and murine tissue (6, 7, 13, 27, 28). The smallest AUF1 isoform, p37, is encoded by a transcript lacking both exons 2 and 7. The p40 isoform contains exon 2, the p42 isoform contains exon 7, and the p45 isoform contains both exons 2 and 7 (6, 27, 28). The p45 isoform of both human and murine AUF1 genes is highly conserved (96.9% amino acid identity between the two species). Our study is the first to demonstrate that all four murine isoforms can occur in one tissue, depending on the circulating level of androgen.

In contrast to the SMG, the AUF1 isoforms in kidney cytosol from both male and female mice did not show androgen responsiveness, despite the fact that a number of genes in the murine kidney are androgen responsive (23). One possible explanation for the tissue specificity of the response could be that androgen-dependent exon splicing of AUF1 gene transcripts occurs in the SMG but not in the kidney. Tissue-specific androgen-dependent regulation of mRNA splicing has been demonstrated for dopamine D2 receptor mRNA in the rat hypothalamus (10) and for PEM1 in the rat epididymis (15), so there are precedents for our suggesting such splicing of AUF1 transcripts. Because EGF mRNA and peptide levels also correlate directly with changes in endogenous androgen levels in the SMG but not in the kidney (21, 23), the regulation of specific AUF1 isoforms may play a role in the tissue-specific androgen-dependent regulation of EGF expression.

Another possible explanation for the tissue-specific changes in AUF1 isoforms could be that circulating androgen levels affect protease activities in the SMG (9, 26) that are not androgen responsive in the kidney. It is possible that there are differences in the proteolytic susceptibility of certain isoforms due to alternate splicing of AUF1 mRNA, inasmuch as the pair of isoforms present in control males (p37 and p42) lacks the sequence encoded by exon 2 that is present in the pair predominant in control females (p40 and p45).

The circulating level of testosterone or its tissue-specific conversion to DHT could also regulate covalent
modifications of specific AUF1 isoforms. Such modifications could selectively target certain isoforms for proteolytic degradation and alter their subcellular localization or their binding affinities for other proteins or for nucleic acids (3, 24, 25). In fact, recent evidence in HeLa cells indicates that the ubiquitination and subsequent proteosomal degradation of p37 are correlated with the rapid decay of AU-rich mRNAs (28). Subjecting these cells to heat shock or inhibiting ubiquitin-mediated proteosomal degradation specifically increased p37 levels 10- to 20-fold, whereas the other isoforms were only slightly increased, indicating that the p37 isoform is preferentially ubiquitinated and normally is rapidly degraded via the proteasome. Thus the increase in p37 we observed in female SMG cytosol after we had injected androgens, as well as the decrease in p37 after the orchietomy of males, could reflect changes in a covalent modification of p37.

The AUF1 isoforms p45 and p40 contain exon 2 and have a lower affinity for AU-rich RNA than do p42 and p37, which lack this exon (11, 27). Thus, no matter whether the androgen-dependent changes observed in AUF1 isoforms are produced by a shift in transcript splicing, by a difference in the susceptibility of the products to proteolytic digestion, or by a change in their posttranslational modification, the end result is that animals with higher circulating androgen levels selectively express those AUF1 isoforms that have higher affinity for AU-rich RNA. Furthermore, because recombinant p37 can form multimers in vitro (4), changes in the relative abundance of this isoform could influence cooperative binding to RNA (4) or to other proteins.

Although AUF1 has been widely recognized for its role as a cytoplasmic protein involved in modulating the stability of AU-rich mRNAs (1, 2, 28), there is evidence that the p45 isoform is enriched in the nuclei of a variety of human tissue culture cells (6, 28). Recently, an AUF1 isoform corresponding to 45 kDa was shown to be part of the B-cell-specific DNA binding protein LR1, a nuclear transcriptional activator/recombination factor (5). Thus this isoform of AUF1 appears to play a role in gene transcription in the nucleus, in addition to its role in regulating the degradation of AU-rich labile RNA transcripts in the cytosol (1, 2, 5, 16, 20). Our studies also show p45 to be the predominant nuclear isoform in female and male SMGs.

Circulating androgen levels appear to change the abundance of p45 in the nucleus slightly but do not appear to influence the abundance of the minor isoforms. The nuclear level of AUF1 appears to be regulated by active gene transcription, as indicated by studies on WI38 cells in which actinomycin D increased cytoplasmic and decreased nuclear AUF1 immunoreactivity (28). Because we also find p45 to be the predominant AUF1 isoform in the nucleus of SMG, and because this isoform remains relatively abundant in the nucleus after DHT, whereas DHT causes p45 to disappear from female cytosol, androgen treatment does not appear to induce a major shift in AUF1 to the cytosol from the nucleus and is consistent with a separate role and regulation of the nuclear p45 isoform.

Our finding, that DHT increases p45 in female polynuclear extracts while decreasing the abundance of this isoform in both the cytoplasmic and nuclear compartments, could indicate that DHT causes p45 to leave one or both of the latter subcellular compartments and bind to polysomes, and/or that DHT increases the translation of mRNA encoding p45 that is already present in polysomes. Because the different isoforms of AUF1 have different affinities for AU-rich RNA, the tissue-specific changes in individual isoforms could be involved in differential posttranscriptional regulation of many AU-rich gene transcripts and hence could be involved in anabolic actions of androgens in nonreproductive tissues, such as the SMG.

We thank Dr. G. Brewer for providing the polyclonal antibody to AUF1 (no. 1871), Dr. M. Ettinger for comments on the manuscript, B. Keegan for assisting in the preparation of figures and data analysis, and Wan Zhang for technical assistance.

This work was supported by funds from the Medical Research Service, Department of Veterans Affairs.

Address for reprint requests and other correspondence: S. Spaulding, Western NY Healthcare System, 3495 Bailey Ave. (151), Buffalo, NY 14127 (E-mail: medspaul@acsu.buffalo.edu).

Received 10 May 1999; accepted in final form 30 August 1999.

REFERENCES


12. Katsukawa, H., Y. Ninomiya, and M. Funakoshi. Effects of repeated androgen treatments on metabolism and nuclear bind-


