Effect of Estrogen on the Expression of mRNAs of Different Actin Isoforms in Immature Rat Uterus

CLONING OF α-SMOOTH MUSCLE ACTIN MESSAGE*

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Cytoplasmic β- and γ-actin mRNAs as well as smooth muscle actin mRNAs have been shown to be transiently increased in rat uterus after treatment with the steroid hormone estradiol. A clone isolated as an estradiol-induced message from a λ-gt10 cDNA library prepared from the mRNA of estrogen-stimulated immature rat uterus was identified as α-smooth muscle actin. A single-stranded RNA probe composed mainly of the 3'-untranslated region of this clone, as well as DNA probes derived from the 3'-untranslated regions of other actin genes, were used to study the induction kinetics of different actin isoforms in rat uterus after being stimulated by estradiol. The β- and γ-cytoskeletal actins showed an induction peak at 4 h after estradiol administration with 1.4- and 1.8-fold increases, respectively. The smooth muscle actin was maximally increased 2.1-fold at 8-12 h. Messages of α-skeletal and α-cardiac actins were neither expressed nor induced by estradiol in this tissue. The different induction kinetics of the cytoplasmic and smooth muscle actins suggest that they are regulated by different mechanisms and possibly in different cell types of the uterus.

Actins, major structural proteins of eucaryotic cells, are products of a multigene family. Amino acid sequence analysis has revealed that at least six actin isoforms exist in mammals (1). These include two cytoplasmic actins, i.e. β- and γ-cytoskeletal actins, two striated muscle actins, i.e. α-skeletal and α-cardiac actins, as well as two smooth muscle actins, i.e. vascular and intestinal type (or α- and γ-) smooth muscle actins. The cytoskeletal actins are coexpressed in all nonmuscle cells (1, 2). The two striated muscle actins are coexpressed in skeletal muscle and heart (3, 4), although the relative amounts of these two isoforms differ significantly in differentiating and mature adult cells (4, 5). The α- and γ-smooth muscle actins are similarly coexpressed in tissues of smooth muscle despite significant differences with respect to the relative quantities of the two isoforms (2). It was thus postulated that actins are coexpressed in pairs in all mammalian cells (3).

Several actin genes have been isolated and structurally characterized. The striated muscle isoforms have been examined in detail (6-11). Their expression has been shown to be cell-type specific during differentiation in amphibian embryos (12). Recently, transfection experiments have been used to study their tissue-specific expression and developmental regulation during myogenesis. The results indicate that cis-acting elements in the 5'-flanking region (13-17), and trans-acting factors (18) are involved in the modulation of muscle actin gene expression. Experiments with transgenic mice (19) and human x mouse heterokaryons (20) resulted in the same conclusion. The structures of several cytoplasmic actin genes have also been determined (21-24). Examination of the structure of a human α-smooth muscle actin gene was reported by Kakunaga et al. (25), and recently, Schwartz and co-workers (26) presented the complete structure and sequence of a chicken α-smooth muscle actin gene. However, there has been no report of the isolation and structural analysis of γ-smooth muscle actin genes, and no studies on the regulated expression of the smooth muscle actins have been reported.

Amino acid and nucleotide sequences have revealed that the genes for all actin isoforms share similar coding regions. The differences reside mainly in the N-terminal region of the polypeptide, ranging from 4 to 25 amino acid exchanges within the total of 374-375 residues (1). The four muscle actins share a closer relationship with each other than with the nonmuscle actins. Thus far, each actin mRNA isoform has been shown to possess a unique 3'-transcribed but untranslated region (3'-UTR). This region contains both conserved and nonconserved segments among different species (27), with the exception of α-smooth muscle actin which does not show any sequence conservation in 3'-UTR between chicken and human (28). Probes made from the 3'-UTR region are thus isotype- and even species-specific (18, 28, 29) and are useful for selective hybridization to the message of a specific actin isotype. Such message-specific probes have been prepared for all actin isoforms except γ-smooth muscle actin.

It was previously reported that bovine uterus contains four types of actin proteins, β- and γ-nonmuscle actins as well as α- and γ-smooth muscle actins (1). Using probes prepared from both coding regions and 3'-UTRs of different actin genes, we have observed that both muscle and nonmuscle actin genes are expressed in rat uterus, and their mRNA levels increase after stimulation by estradiol. This appears to be the first report that actin gene expression is affected by a steroid hormone. One of the first estrogen-regulated proteins described in the rat uterus (IF, induced protein) (30) has been shown to be composed of a major component, creatine kinase, and a minor component, enolase (31). This induced protein complex was recently shown to be associated with actin in rat uterus (32). The authors suggest that these estrogen-induced enzymes may be associated with cytoskeleton as an energy

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1 The abbreviations used are: 3'-UTR, 3'-untranslated region; ER, estrogen receptor; Pipes, 1,4-piperazinediethanesulfonic acid.
source to support the hormonal stimulation of some cytoskeletal functions. In this report we present the identification of a cDNA clone representing the message of α-smooth muscle actin from a library constructed in λ-gt10. The cDNA was prepared from mRNA of estrogen-treated immature rat uteruses. Using this cDNA clone genomic clones can be isolated, and the structural and regulatory features of this smooth muscle actin gene can be studied.

**EXPERIMENTAL PROCEDURES**

Materials—The cloned human α-skeletal actin (pHMCA-3’-UT), human α-skeletal actin (pHMCA-3’-UT), human α-fibriloblast β-actin (pHFβ-3’-UT), and human fibroblast γ-actin (pHγF-3’-UT) were gifts from Dr. Larry Kedes (Stanford University). The clone of human β-actin-coding region was a gift from Dr. Robeno Weinmann (Wistar Institute). pV-fgr, containing a human ß-actin-coding region, was from Cell Center, University of Pennsylvania. The 700-base pair Smal-fragment of pV-fgr containing the actin sequence (33) was purified by low melting point agarose gel electrophoresis and Blutip-d chromatography (Schleicher & Schuell). pGem-1 and rabbit reticulocyte lysate were purchased from Promega Biotec. [35S]Methionine was purchased from Du Pont-New England Nuclear. All restriction enzymes were purchased from New England BioLabs.

Poly(A) mRNA Preparation—Uteri were isolated from control or estrogen-treated immature (21-24 day) female Sprague-Dawley rats. Estrogen treatment was performed by subcutaneous injection of 1 μg of estradiol/day in 0.1 ml of saline/ethanol (90:10). Poly(A) mRNA was isolated by homogenizing the tissue in Tissue-nodium dodecyl sulfate solution (34), followed by oligo(dT)-cellulose chromatography (35). The mRNA samples used for kinetic experiments were further purified through a second oligo(dT)-cellulose column.

Northern Blot Hybridization—RNA samples were denatured at 60°C in 50% formamide and 2.2 M formaldehyde and fractionated by 1% agarose-formaldehyde gel electrophoresis (36). The RNA was then transferred to Nylo paper (GeneScreen transfer membrane, Du Pont-New England Nuclear Research Products) by the procedure of Thomas (37). After overnight transfer in 10 × SSC, blots were baked at 80°C in a vacuum oven for 1 h. Each blot was prehybridized at the condition described by Wahl et al. (38) and hybridized to 1.0-1.5 × 10^6 cpm/mL probe (1-3 × 10^8 cpm/μg) or a single-stranded RNA probe synthesized by in vitro transcription (1 × 10^8 cpm/μg). After hybridization the DNA probe-hybridized blots were washed in 2 × SSC, 0.1% SDS at room temperature for 3 × 10 min and at 65°C for 3 × 45 min. The riboprobe-hybridized blots were washed in 0.1% SDS at room temperature for 3 × 10 min, followed by 60°C for 3 × 45 min. In several experiments further washes under more stringent conditions, i.e. 1 mM EDTA at 65°C, were applied. The blots were then exposed to x-ray film in the presence of a Du Pont Lightning Plus intensifying screen at -70°C. The autoradiograms of the kinetic results were scanned by a densitometer (LKB Laboratories, Inc.).

Cloning and Subcloning—Clone λ-10C was picked from a cDNA library constructed in λ-gt10 as an estrogen-induced message. The library was constructed according to procedures suggested by T. Huynh.2 DNA strand cDNA was prepared (39, 40) from uterine mRNA isolated from 24-day-old female rats (41) treated for 3 days with estradiol. The cDNA insert of clone λ-10C is 1400 base pairs long and was subcloned into plasmid pGem-1 (Promega Biotec). This plasmid contains two RNA polymerase (SP6 and T7) promoters in opposite orientations, permitting RNA synthesis from either strand of DNA.

In Vitro Transcription—RNA probes were synthesized by the procedure described by Promega Biotec. pGem-10C (pGem-1 containing the cDNA insert of clone λ-10C) linearized by PvuII was used as template to synthesize the complete transcript of 10C. The transcript synthesized from the SP6 promoter of pGem-10C is complementary to the cellular mRNA. BglII and HaelII cut pGem-10C were treated with Klenow fragment of DNA polymerase I to fill in the 3’ protruding ends. These two DNA, and BstN-I cut pGem-10C were used as templates by SP6 RNA polymerase to synthesize shortened transcripts from the 3’ end of the cDNA insert. The reaction mixture contained 40 mM Tris-HCl, pH 7.5, 6 mM MgCl₂, 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 20 units of RNasin, 0.5 mM each of ATP, GTP, and UTP, 12 μM CTP, 0.2-1.0 μg of linearized plasmid DNA template, 50-60 units of SP6 RT, 10 units of R. amylaceus DNA polymerase, and 15 units of SP6 RNA polymerase in a total volume of 20 μl. After incubation at 37°C for 60 min, the DNA template was digested by addition of 1 unit of RQI Nase and further incubation at 37°C for 15 min. The solution was extracted twice with phenol/chloroform (with one back extraction of H₂O), once with chloroform, and the RNA transcript was recovered by sodium acetate-EDTA precipitation. The yield was 4-5 μg of RNA/μg of full length plasmid DNA. The specific activity was 0.4-1.0 × 10⁶ cpm of each used) using a mini-gel apparatus. After electrophoresis at 250 V for 30 min, the gel was wrapped in Saran Wrap and exposed to x-ray film at room temperature for 10 min.

Hybrid Selection of mRNA and in Vitro Translation—The procedures are described by Ricchiardi et al. (42, 43). 10 μg of Poly(A) mRNA from the T7 promoter was incubated in dry ice-ethanol, thawed by finger, and dotted onto nitrocellulose paper. After air drying at room temperature for 1 h, the dot was baked at 80°C in a vacuum oven for 1 h. The dot, cut into 6 pieces, was incubated in 500 μl of H₂O at 100°C for 1 min. Water was removed, 100 μl of hybridization buffer (50% formamide, 8.7 M NaCl, 10 mM Pipes, pH 6.4, and 4 mM EDTA), pH 7.2, was then added and 150 μg of mRNA from estrogen-treated rat uteri was dissolved in 100 μl of hybridization buffer and heated at 70°C for 10 min. The prehybridization solution was then removed from the DNA paper, and the hot mRNA solution was added. Hybridization was allowed to proceed at 37°C overnight. The hybridized paper was then washed with 100 μl of 1× SSC, 0.5% SDS at room temperature 3 times, 0.1× SSC, 0.1% SDS at 60°C 5 times, 0.1× SSC at room temperature twice, 2 mM EDTA, pH 7.2, at 0°C twice, and finally ice-cold H₂O twice. The selected mRNA was then eluted from 300 μl of boiling H₂O containing 3 μg of SNAP (1:100) DNA. The solution with the paper was immediately snap frozen, thawed by finger, and the RNA solution removed to a clean tube. mRNA was recovered by precipitation.

Half of the hybrid-selected mRNA was used for in vitro translation using rabbit reticulocyte lysate and [35S]methionine by the protocol described by Promega Biotec. 15,000 trichloroacetic acid-precipitable counts was applied to a 10% SDS-polyacrylamide gel using Laemmli electrophoresis conditions (44). After running overnight, the gel was fixed in 10% acetic acid, 30% methanol for 1 h and treated with En'chance (Du Pont-New England Nuclear) for 1 h followed by H₂O washes for 30 min. The gel was then dried and exposed to x-ray film with a Du Pont Lightning Plus intensifying screen at -70°C overnight.

Quantitation of Poly(A) mRNA by Poly(U) Hybridization—Poly(A) mRNA samples from the time-course experiment were quantitated by a poly(U)-hybridization method (45, 46). 0.3 μg of each RNA sample was mixed with 0.22 μg of [3H]poly(U) (0.16 μCi/μg) in 1.5 ml of 2 × SSC. The solution was heated at 90°C for 3 min and then incubated at room temperature for 60 min. The hybrids were treated with RNase A at 40 μg/ml at room temperature for 20 min. 100 μg of yeast tRNA, 75 μg of 2’-3’ uridylic acid and 5 ml of 10% cold trichloroacetic acid were then added, and the mixture was put onto ice for 2 h. The precipitate was collected on glass fiber discs by filtration, dried, and counted in a liquid scintillation counter.

Sequenceing—The 5’ end of the cDNA insert of pGem-10C was directly sequenced using the GeneSeq K/RT system from Promega Biotec. The transcript synthesized from the SP6 promoter was complementary to mRNA. Therefore, the sequence of the 5’ end of the message was obtained by synthesis from the T7 promoter. The plasmid DNA was denatured in 0.2 M NaOH and 0.2 mM EDTA (47) at room temperature for 5 min and precipitated in ethanol-sodium acetate-70% cold ethanol, dried pellets were dissolved, and 3 μl of this solution and 4 μl of Klenow reverse transcriptase reaction buffer and allowed to anneal with T7 promoter primer at 37°C for 30-60 min. The sequence was then determined by enzymatic DNA synthesis in the presence of dideoxynucleotide chain terminators (48) using [α-32P]dATP as the label. Both Klenow and Sequenase were used to confirm the sequence. The final concentration of cold dATP was adjusted to 1 μM for both enzymatic reactions.

2 T. Huynh, personal communication.
Expression of Different Actin mRNAs in Rat Uterus and their Induction by Estrogen—Although the polypeptide length of all actin isomers are almost identical (with the maximum difference of only 1 amino acid residue (1)), the lengths of the transcripts of the nonmuscle and muscle pairs differ significantly. The cytoskeletal actin (β- and γ-) transcripts are 2.2 kilobases long and the α-cardiac and α-skeletal actin transcripts comigrate at 1.6 kilobases, and the smooth muscle actins are coded by 1.3-kilobase long transcripts (12, 50). The striated muscle and the smooth muscle pairs are usually not separable on 1% agarose gels. In order to examine the possible effect of estrogen on the expression of actin genes in the immature rat uterus, probes made from the coding regions of three different actins were used to hybridize to Northern blots made from mRNAs of both control uteri and uteri isolated from rats treated for 2 days with estradiol. The results are shown in Fig. 1. All three probes hybridized to two broad bands flanking the position of 18S rRNA under our standard hybridization and washing condition. The probe of human α-skeletal muscle actin hybridized more strongly to the lower band than to the upper band (Fig. 1A), while the probes of human cytoskeletal β-actin and human cytoskeletal and γ-actin hybridized more strongly to the upper band than to the lower band (Fig. 1, B and C). This is due to the greater thermal stability of the homologous hybridization than the heterologous hybridization (27, 50). More interestingly, both bands were more abundant in the hormone-treated sample than the control sample when any coding region probe was used.

To characterize specifically which type of actin is expressed and induced in rat uterus, probes made from the messagesspecific 3'-UTR of four different actins were used. Fig. 2 shows that probes of 3'-UTR derived from α-skeletal and α-cardiac actins did not, as expected, hybridize to any message under our detection condition while each of the probes prepared from the 3'-UTR of β- and γ-cytoskeletal actin probes hybridized to a specific band above the 18S rRNA position, and both were more abundant in the hormone-treated sample than the control sample. The lower band observed in Fig. 1 shows that probes of 3'-UTR derived from α-skeletal and α-cardiac actins did not, as expected, hybridize to any message under our detection condition while each of the probes prepared from the 3'-UTR of β- and γ-cytoskeletal actin probes hybridized to a specific band above the 18S rRNA position, and both were more abundant in the hormone-treated sample than the control sample. The lower band observed in Fig. 1 when using a coding region probe is thus assigned to smooth muscle actin. It is known that smooth muscle actin is much more similar to skeletal muscle actin than the cytoplasmic isomers at the protein level (1). The stronger hybridization to the lower band shown in Fig. 1A is thus due to better sequence homology between the skeletal and smooth muscle actins. A

![Fig. 1. Northern blot analysis of actin gene expression and induction by estrogen in immature rat uterus using coding region mRNAs of different actin isomers as probes. Poly(A) mRNAs from control (c) and 2-day-estradiol-treated (e) rat uteri were isolated. Northern blots containing 2 μg of mRNA in each sample were prepared as described under "Experimental Procedures." Probe used for each blot was: A, pHMx A-px (human α-skeletal actin); B, human β-cytoskeletal actin; C, SmaI fragment of pV-fgr (human γ-cytoskeletal actin). The marker position of 18S rRNA is indicated.](image)

![Fig. 2. Expression and induction by estrogen of different actin isotypes in rat uterus. Northern blots were prepared as in Fig. 1. Nick-translated probes (approximately 5 × 10⁶ cpm/μg) were pHMCA-3'UT (A), pHMCA-3'UT (B), pHFP A-3'UT (C), and pHFP A-3'UT (D). Blots A and B were exposed for 4 days. Blots C and D were exposed for 2 days.](image)

![Fig. 3. Hybridization pattern of pGem-10C on Northern blots. A, nick-translated pGem-10C DNA was used as probe. B, 32P-labeled full length RNA transcript synthesized from PavII-linearized pGem-10C template was used as probe. Northern blots were prepared as in Fig. 1.](image)
mologous to clone 10C was hybrid-selected from estrogen-treated rat uterus and translated in vitro using a rabbit reticulocyte lysate system. The synthesized proteins were then analyzed on a 10% SDS-polyacrylamide gel. Fig. 4 shows that the major protein translated from the selected mRNA comigrated with authentic actin protein.

**Characterization of Clone 10C as α-Smooth Muscle Actin**

To identify the type of actin that 10C codes for, we attempted to prepare a probe composed mainly of the 3'-UTR of 10C. Three enzymes, BstN-I, HaeII, and BglII, were used to restrict the 10C cDNA insert at different distances from its 3' terminus. 32P-labeled RNA transcripts were synthesized from these restricted DNA templates and analyzed by 1% agarose-formaldehyde gel electrophoresis. Fig. 5A shows that, as expected from their DNA restriction patterns, BstN-I generated a transcript of 400 bases, BglII restricted the transcript to 800 bases, and the transcript of HaeII-cut pGem-10C was 1200 bases long. These RNAs were then hybridized to Northern blots prepared from uterine mRNA. BglII- and HaeII-cut 10C still hybridized to two bands with similar intensity as shown in Fig. 5B, lanes 1 and 2. However, BstN-I cut 10C transcript hybridized preferentially to the lower bands (lanes 3 and 4), and after washing the blot under very stringent conditions (1 mM EDTA at 65 °C) only hybridization to the lower band was retained (lanes 5 and 6). Therefore, this probe shows greatest sequence homology to the RNA in the lower band, which corresponds to smooth muscle actin mRNA. Clone 10C was thus concluded to represent a message for smooth muscle actin.

To characterize the specific type (α- or γ-) of smooth muscle actin that clone 10C codes for, we performed partial sequencing of the cDNA insert. Since the only amino acid sequence difference between α- and γ-smooth muscle actins resides at residues 4 and 5 (1), the 5' end sequence of the message that clone 10C codes for will distinguish its specific type. The 5' end of the cDNA insert of pGem-10C was directly sequenced from the T7 promoter of the plasmid by both Klenow and reverse transcriptase reactions primed with T7 promoter primer. We found that our 10C cDNA was nearly full length copy of the cognate mRNA. Its 5' end nucleotide sequence is shown in Fig. 6A. Comparison of this sequence and the corresponding amino acid sequence with the known amino acid sequences of different iso types of actins (1) is shown in Fig. 6B. The nucleotide sequence at this region of clone 10C clearly indicates that it codes for α-smooth muscle actin.

**Induction Kinetics of β-Cytoskeletal, γ-Cytoskeletal, and Smooth Muscle Actins by Estrogen in Rat Uterus**—Poly(A)-mRNAs of rat uteri at different hours after estradiol administration were isolated and purified two times by oligo (dT)-cellulose column chromatography. The poly(A)-mRNA concentration was measured by poly(U) hybridization. Fig. 7A shows that the quantity of 3H-poly(U) that hybridized to equal amounts of poly(A) mRNA (measured by A260) was relatively constant among the six samples. Furthermore, a probe isolated from the same library which showed relatively little change after a single injection of hormone was used to hybridize to the Northern blot, and the hybridization to
but reduced hyperplasia in response to estrogen administration, whereas the myometrium and stroma undergo marked hypertrophy between the first hours of hormone administration, new mRNA synthesis begins, followed by major increases in rRNA and protein between 8 and 16 h, histone and DNA synthesis at about 18 h, and a wave of cell division at 24 h (51, 52). These responses are directed to the overall growth of the uterus in preparation for pregnancy.

These events have generally been assayed in preparations from whole uterus, in spite of the fact that the tissue is composed of the following three differentiated cell types: epithelium, stroma, and myometrium. Histological and autoradiographic studies have shown that estrogen induces cell proliferation in the luminal and glandular epithelium, whereas the myometrium and stroma undergo marked hypertrophy but reduced hyperplasia in response to estrogen administration (53–57). Viable cells from each of the regions of the immature rat uterus have been separated and analyzed biochemically for their differential responses to estrogen (58). The three cell types contain estrogen receptor (ER), and estradiol administration caused a significant increase in the

FIG. 6. Identification of clone 10C as α-smooth muscle actin.
A. 5′ end nucleotide sequence and the corresponding amino acid sequence of pGem-10C. Quoted sequence is EcoRI cloning site.  B, comparison of the N-terminal residues of clone 10C with the known sequences of different actin isotypes. The residues that distinguish this clone as α-smooth muscle actin are underlined.

different time samples was relatively constant (Fig. 7B). This blot thus appeared to be appropriate to study the induction kinetics of different acts by estrogen in uterus. 32P-labeled RNA, synthesized by in vitro transcription using BstN-I cut pGem-10C as template, and nick-translated DNA of 3′-UTR of β- and γ-cytoskeletal acts were used as probes. The autoradiographic results are shown in Fig. 7, C–E. Smooth muscle actin was maximally induced between 8 and 12 h, while both β- and γ-cytoskeletal acts showed an induction peak at 4 h. The densitometric results are graphed in Fig. 8 (only the lower band in Fig. 7C was scanned). The message for smooth muscle actin increased 2.1-fold, while β- and γ-cytoskeletal actin mRNAs were increased 1.4- and 1.8-fold, respectively.

DISCUSSION

The response of the uterus to estrogen stimulation has been defined broadly in terms of the synthesis of the major classes of macromolecules and other biochemical events. Within the first hours of hormone administration, new mRNA synthesis begins, followed by major increases in rRNA and protein between 8 and 16 h, histone and DNA synthesis at about 18 h, and a wave of cell division at 24 h (51, 52). These responses are directed to the overall growth of the uterus in preparation for pregnancy.

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FIG. 7. Hybridization of message-specific actin probes to uterine poly(A) mRNA isolated at different times after administration of 1 µg of estradiol. 1.5 µg of each mRNA sample was used to prepare the time-course Northern blot. A, trichloroacetic acid precipitable counts of 3H-poly(U) that hybridized to 0.3 lg of each RNA sample (measured by A260). B, hybridization with a probe made from clone 1a which showed relatively little change after a single injection of estradiol. C, single-stranded RNA transcript synthesized from BstN-I cut pGem-10C template was used as probe for smooth muscle actin. Stringent wash in 1 mM EDTA at 65 °C was applied after the regular wash. The induction patterns of the lower band were the same before and after stringent wash. D, 3′-UTR of β-cytoskeletal actin (pPHβA-3′UT) was nick-translated and used as probe. E, 3′-UTR of γ-cytoskeletal actin (pPHγA-3′UT) was nick-translated and used as probe.

ER content of each. Although myometrium contained 85% of the total uterine ER, its ER concentration was lowest. Epithelial cells had the highest ER concentration and showed the greatest increase of ER concentration after estrogen administration. The stroma and myometrium produce the major skeletal elements of the uterus. The stromal cells are associated with collagen fibrils which increase in amount after estrogen administration. The myometrium is composed of bundles of circular and longitudinal muscle cells.

It has been reported that bovine uterus contains equal amounts of α- and γ-smooth muscle acts as well as β- and γ-cytoskeletal acts (1). The smooth muscle actin isoforms are clearly required for the contraction and support roles that the uterus plays during development of the embryo. The role of cytoskeletal acts in cells is less clear, but is probably involved in intracellular movement, organelle organization,
cytokinesis, and the transmission of signals between the plasma membrane and the cell interior (59). Although actins are major components of all of the uterine cell types, the effect of estrogen on their expression has not previously been examined.

We have isolated a cDNA clone from a rat uterine cDNA library and characterized it as a message of α-smooth muscle actin using Northern blot analyses, in vitro translation, and DNA sequencing. Using this clone, and probes for other actin isoforms, we also show that the levels of messages for different actins are increased with different kinetics in the rat uterus in response to estradiol. The cytoplasmic β- and γ-isoactins were induced maximally at 4 h after hormone injection, while the induction peak for α-smooth muscle actin was at 8-12 h. The γ-smooth muscle actin may share similar kinetics with its α-isomer, although a specific γ-smooth muscle actin probe was neither present nor induced by the hormone.

Estradiol may stimulate the expression of these actin genes by direct or indirect means. Estradiol is a mitogen in the immature rat uterus (57, 60, 61) and was shown to cause a 55-fold increase in the mitotic index of epithelium and a 6-fold increase in the stroma and myometrium (57). Thus, the hormone may initiate a sequence of reactions in the proliferation pathway of which actin gene expression may be one reaction. After initiation, subsequent reactions may or may not require direct interaction with the hormone.

Studies in other systems indicate that proliferating cells, including skeletal and smooth muscle myoblasts (62, 63), express mainly the nonmuscle β- and γ-cytoskeletal isoactins. Only nonreplicating, differentiated cells fully express the appropriate muscle actin isoforms. The induction of β- and γ-cytoskeletal actin mRNAs that we see may occur predominantly in the epithelial cells, which shows the most extensive cell division in response to estradiol. In the myometrium, which contains mainly smooth muscle cells and experiences hypertrophy but relatively little hyperplasia as a result of treatment with hormone, expression of smooth muscle actin mRNAs may be increased directly or indirectly by the hormone. It is possible that estradiol turns on the synthesis of factors which then act to increase the mRNA level of actins. Whether this increase results from enhanced genomic transcription or from altered message stability remains to be determined.

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