P3-434

Estrogen Up-Regulates Estrogen Receptor-a (ESR1) Gene Expression Posttranscriptionally by Inducing A + U-Rich Binding Factor 1 to Bind & Stabilize ESR1 mRNA

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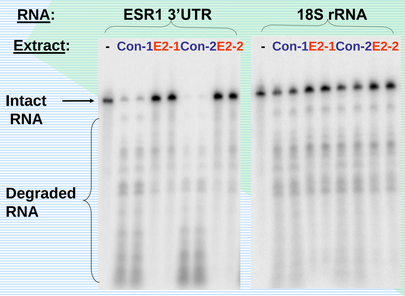
Introduction

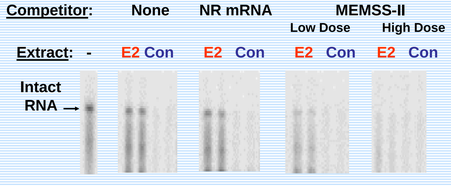
Hormones regulate gene expression at transcriptional and post-transcriptional levels*. Estrogen receptor alpha (ESR1) mRNA levels are primarily regulated posttranscriptionally by estrogen in the sheep uterus. Estrogen increases the concentrations of ESR1 mRNA five-fold in 24 h by stabilizing the mRNA. In previous work, we defined two regions (Minimal Estradiol-Modulated Stability Sequences or "MEMSS") in the 3'UTR that conferred E2-enhanced stability to heterologous mRNAs. Here, we use those RNA sequences to identify the E2-induced proteins that bind them and stabilize ESR1 mRNA to up-regulate its concentrations.

*Reviewed in Ing, 2005, *Biol. Reprod.*, 72:1290-1296

Stability Assay

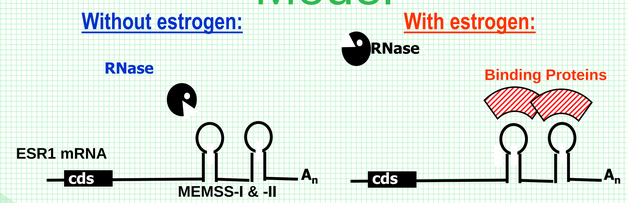


Estradiol (E2) treatment stabilizes ESR1 mRNA specifically. Four ovariectomized ewes were hysterectomized 24 h after injection with 50 ug E2 ("E2") or vehicle ("Con"). Uterine extracts from the ewes were incubated with radiolabeled ESR1 3' UTR and 18S rRNA in duplicate reactions prior to electrophoresis on polyacrylamide gels. ESR1 RNA was more stable in extracts from E2-treated ewes, while 18S was unaffected by treatment.



Minimal E2-Modulated Stability Sequence (MEMSS) of ESR1 mRNA competitively inhibits enhanced ESR1 mRNA stability. Unlabeled MEMSS RNA was added to the in vitro stability reactions with radiolabeled *ESR1* 3' UTR and uterine extracts from E2-treated or Control ewes in duplicate reactions. MEMSS-II RNA inhibited the enhanced stability of *ESR1* mRNA in extracts from the E2-treated ewe in a dose-dependent manner, while addition of Non-Regulated ("NR") RNA had no effect.

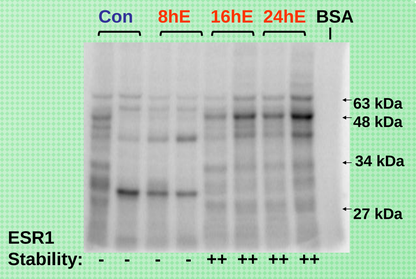
Model



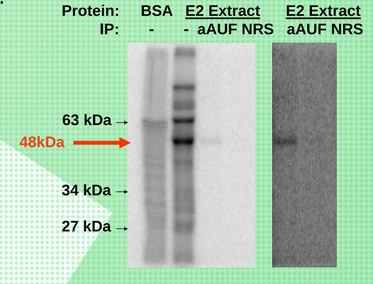
Model for E2 induction of a protein that binds and stabilizes ESR1 mRNA. A cartoon of ESR1 mRNA is shown with the coding sequence (cds) 5' to the 4375 base long 3'UTR. Within the latter, two 82 base Minimal E2-Modulated Stability Sequences (MEMSS) –I and –II carry stem loop structures*. Our **hypothesis** is that E2 induces a protein (hatched box) to bind the MEMSS and thereby stabilize *ESR1* mRNA against degradation by ribonuclease (RNase).

*Mitchell and Ing, 2003, *Mol Endocrinology* 17:562-574

MEMSS Binding Proteins

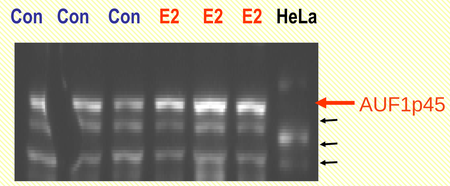


E2 treatment increases binding of specific proteins to MEMSS RNA. Extract proteins from two control ewes ("Con") and two ewes treated with E2 at 8, 16 and 24 h previously ("8hE", "16hE" and "24hE") were labeled by UV-crosslinking to radiolabeled MEMSS-II. After RNAse digestion, the radiolabeled proteins were analyzed by 12.5% SDS-PAGE. Bovine serum albumin (BSA) was a negative control protein.

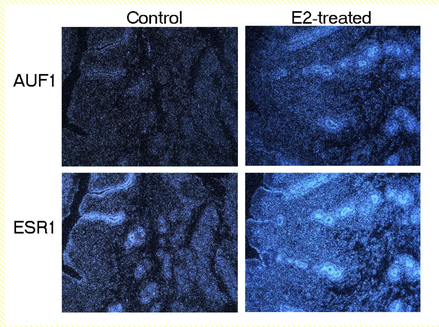


Immunoprecipitation of AUF1 UV-crosslinked to MEMSS RNA. Uterine extract proteins from an E2-treated ewe were labeled by UV-crosslinking to radioactive MEMSS-II RNA. Immunoprecipitation ("IP") with antiserum raised to AUF1 ("aAUF") showed that AUF1p45 bound MEMSS (red arrow) in normal and enhanced contrast panels (left and right, respectively). Negative controls were performed with nonimmune rabbit serum ("NRS").

E2 Induces AUF1



***Western blot shows E2 treatment increases concentrations of the AUF1p45 protein* (red arrowhead).** Uterine extract samples were from three control and three ewes treated with E2 24 h previously. HeLa cell nuclear extract is a positive control. The other 3 forms of AUF1 (p42, p40 and p37) are indicated with black arrows.



In situ hybridization demonstrates that E2 coordinately up-regulates AUF1 and ESR1 mRNA concentrations. Uterine cross-sections from control and E2-treated ewes were hybridized with antisense cRNA probes for AUF1 and ESR1 mRNAs. Darkfield views of the bright silver grains indicate that E2 treatment increases concentrations of AUF1 and ESR1 mRNAs in several endometrial cell types, especially glandular epithelium. The uterine lumen is at upper left.

Summary

1. E2 strongly up-regulates ESR1 mRNA concentrations by stabilizing the messenger RNA.
2. Used as a competitor, the regulatory region "MEMSS" RNA inhibits the enhanced stability of ESR1 mRNA.
3. E2 induces MEMSS binding proteins, concurrent with enhancing the stability of ESR1 mRNA.
4. The major E2-induced MEMSS binding protein is AUF1p45, which is known to stabilize other mRNAs.
5. E2 induces the expression of AUF1 and ESR1 genes in the same uterine cells.