TISSUE-SPECIFIC STIMULATION OF RNA SYNTHESIS BY TRANSFORMED ESTRADIOL-RECEPTOR COMPLEX*

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SUMMARY: Exposure to the estradiol-receptor complex of uterine cytosol increases the RNA synthetic capacity of purified uterine nuclei but not of liver or kidney nuclei. This stimulation is effected only by complex in which the binding unit of the receptor has undergone estrogen-induced conversion from the native (4S) to the transformed (5S) form. It is suggested that an important function of estrogenic hormone is to promote transformation of the receptor protein to an active form which can enter the nucleus and stimulate RNA synthesis.

INTRODUCTION: It is now established that estrogen-dependent tissues contain characteristic estradiol-binding macromolecules, commonly called estrogen receptors, with which the hormone interacts by a stepwise mechanism of at least three clearly defined stages**. On entering uterine cells, estradiol associates with the 4S binding unit of an 8S extranuclear receptor protein, activating it to undergo a temperature-dependent transformation to a 5S form, as indicated by its sedimentation in salt-containing sucrose gradients. This conversion is accompanied by migration of the transformed hormone-receptor complex to the nucleus where it associates with an acceptor site in the uterine chromatin and in some way induces an acceleration of biosynthetic processes.

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*A preliminary report of these findings was given at the 62nd meeting of the American Society of Biological Chemists, June, 1971 (1).

**This sequential interaction pathway, originally called a two-step mechanism but now better described as three-step, is discussed in more detail in references 2 and 3.

Numerous studies (4-6) have demonstrated that increased RNA synthesis is an early uterine response to estrogen and that production of messenger RNA for a specific soluble protein appears to be an especially early event (7). Although uterine nuclei isolated from estrogen-treated rats show enhanced ability to incorporate labeled precursors into RNA (8-11), attempts to stimulate uterine nuclei by direct treatment with estradiol proved unsuccessful. In 1969, Raynaud-Jammet and Baulieu (12) made the important observation that nuclei isolated from heifer endometrium, while not affected by estradiol or cytosol alone, show an increased ability to incorporate radioactive nucleotide into RNA after incubation with a mixture of estradiol and uterine cytosol containing the hormone in the form of the estradiol-receptor complex. Subsequent studies (13) indicated that the RNA polymerase activity of heifer endometrium nuclei or of the enzyme prepared from these nuclei could be enhanced by adding a mixture of estradiol and various uterine fractions directly to the polymerase assay system.

This paper presents evidence that susceptibility of RNA synthesis to stimulation by the estrogen-receptor complex is a specific characteristic of nuclei from hormone-dependent tissues, such as uterus, and that only the transformed (5S) form of the estradiol binding unit is able to produce this activating effect. These results support the previous suggestion (2) that an important function of the estrogenic hormone is to promote the conversion of the receptor protein to an active form which can enter the nucleus and there participate in biosynthetic processes.

EXPERIMENTAL PROCEDURES: Calf endometrium and uterus, kidney and liver from immature rats were homogenized at 2°C in 3 to 6 volumes 2.2 M sucrose -1 mM $MgCl_2$ using a Polytron PT-35 or a loose glass homogenizer with 10 sec homogenization periods, each followed by 50 sec cooling.

Nuclei and cytosol were separated by the method of Chauveau et al. (14). The nuclei were resuspended in the appropriately treated cytosol and incubated under the described conditions, after which they were separated again by centrifugation and resuspended for polymerase assay in 0.32 M sucrose-3 mM $MgCl_2$ in 10 mM Tris, pH 7.4. The integrity of the nuclear preparations was checked by light microscopy after crystal violet staining, and the DNA content was determined colorimetrically (15). For estimation of bound and soluble RNA polymerase activities, incubation with or without hormone was carried out in the whole 2.2 M sucrose homogenate, after which the nuclei were separated, suspended in 0.32 M sucrose-1 mM $MgCl_2$ in 20 mM Tris, pH 7.5, and the bound and soluble polymerase activities separated as described by Liao, et al. (16).

For RNA polymerase assay, 100 μl of nuclear suspension (100-150 μg DNA) and 300 μl mixture A (containing in μ moles: Tris, 100; $MgCl_2$, 5; NaF, 30; KCl, 75; mercaptoethanol, 10; ATP, 0.5; at pH 8.0) were equilibrated for 2 min at 37°C. The reaction was started by adding 100 μl mixture B (containing ATP, CTP, GTP and phosphoenolpyruvate, 0.5 μmole each, plus 10 μg pyruvate kinase and 5 μCi tritiated UTP (Schwarz), spec. act. 200 μCi/μmole). After 10 min at 37°C, the tubes were chilled, 5 ml of 10% TCA containing 0.2 N $Na_4P_2O_7$ was added and the precipitate collected on an HA millipore filter and washed with cold 0.5 N PCA-0.1 N pyrophosphate followed by 95% ethanol. After air drying, the filters were combusted in a Packard Model 300 oxidizer and the tritiated water counted by liquid scintillation. Results are reported as mean values of 7 replicate assays from which were subtracted blank determinations from similar assays containing 0.12 M EDTA. Assay of the soluble enzyme was carried out similarly except that 100 μl (200 μg) calf thymus DNA in 10 mM Tris, pH 8.0, was added as template; in this case, blanks contain-

ing no added DNA were used. The incorporation of UTP radioactivity into RNA was found to require all four nucleotide triphosphates and to be inhibited by actinomycin D. Similar results were obtained when the labeled precursor was CTP or GTP.

The 5S nuclear complex was obtained by extracting crude calf uterine nuclei with 0.4 M KCl after they had been incubated 1 hr at 25°C with uterine cytosol containing 10 nM estradiol. The extract was concentrated with a Diaflo XM-50 membrane and the residue diluted with 2.2 M sucrose to a final KCl concentration of 35 mM. The integrity of the 4S and 5S estradiol-receptor complexes was checked by sedimentation in sucrose gradients containing 0.4 M KCl.

RESULTS AND DISCUSSION: After incubation at 25°C for 30 min with uterine cytosol in 2.2 M sucrose, purified nuclei from immature rat uteri show a much lower ability to incorporate labeled nucleotide into RNA then do kidney or liver nuclei (Figure 1). When 10 nM estradiol was also present during incubation, RNA synthesis in uterine nuclei was increased 2.7 fold, whereas

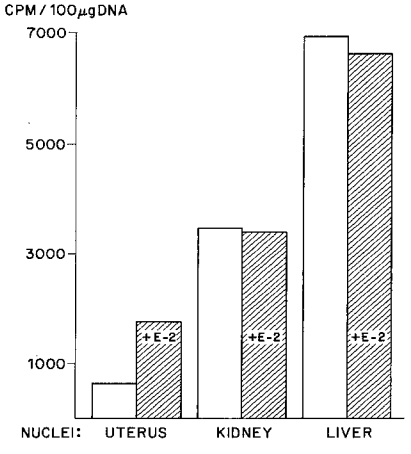

Figure 1. RNA synthesis in isolated nuclei from various rat tissues after incubation with rat uterine cytosol for 30 min at 25°C in the presence and absence of 10 nM estradiol.

there was no enhancement of the already high synthetic capacity in kidney or liver nuclei after incubation with hormone, either in uterine cytosol or in their own cytosols. Nuclei from the hormone-dependent tissue appear to possess a deficiency in RNA synthetic capacity which can be alleviated by treatment with the estradiol-receptor complex of uterine cytosol.

Stimulation of uterine nuclei requires hormone-induced conversion of the receptor protein binding unit from the 4S to the 5S form (Table I). Nuclei from calf endometrium are activated by incubation with estradiol and

Table I. RNA SYNTHESIS IN CALF ENDOMETRIUM NUCLEI

Expt.	Nuclei incubated with:	Incubation Temp., °C	Subsequent UMP-H^3 into RNA (% of control)
1 a	Cytosol + estradiol	25	220*
b	Cytosol + estradiol	0	95
c	Cytosol + estradiol (pretransformed at 25°)	25	240*
d	Cytosol + estradiol (pretransformed at 25°)	0	270*
2 a	Cytosol + estradiol	25	170*
b	Cytosol + estrone	25	105
c	Sucrose + estradiol	25	90
3 a	Cytosol + estradiol	25	195*
b	Nuclear extract	25	235*
c	Nuclear extract	0	175*

Asterisks indicate experiments with receptor in 5S form.

Incubations were carried out in 2.2 M sucrose for 30 min (expts. 1 and 2) or 45 min (expt. 3). In expt. 3, 35 mM KCl was also present. Estradiol and estrone were 10 nM. In expts. 1c and 1d, receptor transformation was effected by incubating the estradiol-cytosol mixture at 25°C for 30 min before the nuclei were added. Control values were obtained with cytosol without steroid, except in expts. 3b and 3c where heat-inactivated (50°C, 15 min) nuclear extract was used for the control.

endometrium cytosol at 25°C, where receptor transformation takes place readily, but not at 0°C, where it does not. However, if the estradiol-cytosol mixture is first warmed to 25°C to effect transformation of the receptor, the resulting 5S complex can stimulate nuclei on incubation at either 0°C or 25°C. Estrone, which is known to form the 4S complex but is unable to induce its conversion to the 5S form (2, 3), does not cause nuclear stimulation under conditions where estradiol is effective. The 5S estradiol-receptor complex, extracted by 0.4 M KCl from calf uterine nuclei previously incubated with estradiol and uterine cytosol, is fully active in stimulating fresh nuclei.

The magnesium-dependent RNA polymerase activity of mammalian nuclei prepared in 2.2 M sucrose can be separated into two fractions: bound enzyme, firmly associated with chromatin, and soluble enzyme, which is extracted from the nuclei by 0.32 M sucrose (16). It was found that both types of RNA polymerase activity were enhanced after incubation of endometrial nuclei with the estradiol-cytosol mixture (Figure 2). This still preliminary observation

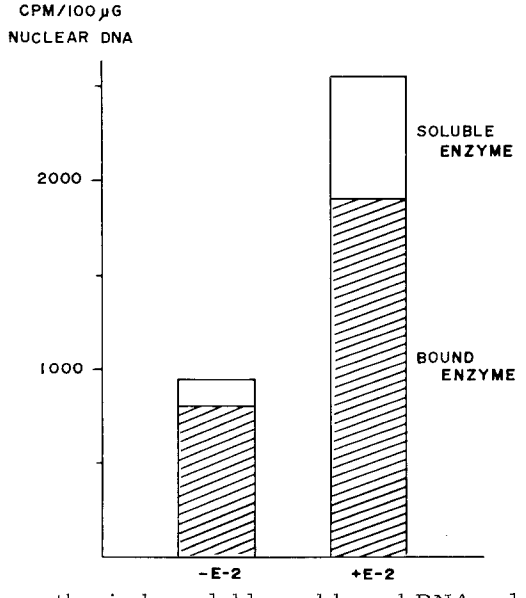

Figure 2. RNA synthesis by soluble and bound RNA polymerase of calf endometrium nuclei after incubation of homogenate for 35 min at 25°C in the presence and absence of 10 nM estradiol. Calf thymus DNA used for template with soluble enzyme.

suggests that at least part of the stimulation of RNA synthesis in uterine nuclei by the transformed estrogen-receptor complex involves an effect other than on chromatin template activity.

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