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TRANSLOCATION OF 4S AND 5S FORMS OF ESTROGEN-RECEPTORS INTO RAT LIVER NUCLEI IN VITRO

W. Powell-Jones[1], S. N. Nayfeh[2] and G. W. Lucier[1]

[1]National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

[2]Department of Biochemistry and Hospital Laboratories
School of Medicine
University of North Carolina
Chapel Hill, NC 27514

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Summary

We report the temperature-dependent translocation of cytoplasmic estrogen-receptor complexes into purified rat liver nuclei in vitro. In contrast to uteri cytosol which translocates only 5S forms of the uterine cytoplasmic receptor into nuclei, liver nuclei contain both 4S and 5S complexes following incubation with [^{3}H]-labeled liver cytosolic fractions at 29°C. Data suggests that the liver 4S nuclear form may represent activated receptor. Thus a shift in the sedimentation coefficient from 4S to 5S may not be obligatory to estrogen receptor activation and subsequent nuclear retention of the complex in this tissue.

INTRODUCTION: The response of target cells to estrogen is believed to be dependent upon binding of the hormone to a specific cytoplasmic receptor (1, 2). Nuclear translocation of the hormone-receptor complex then occurs followed by binding to chromatin resulting in gene activation and increased RNA biosynthesis (3, 4). The presence of estrogen-binding proteins in mammalian liver supernatants, possessing characteristics assigned to specific receptor proteins (5), has recently been established in studies from several laboratories (6-9). Extensive studies performed on the uterus (10) have demonstrated that association of estradiol with specific cytoplasmic receptor induces a temperature-dependent activation of the receptor with conversion from the 4S to the 5S state, (activation being defined as a rise in affinity for nuclear chromatin). The molecular basis for the changes in sedimentation coefficient have been examined in recent studies (11). It has been proposed

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that only the 5S form has high affinity for target tissue chromatin and the ability to bind to purified uterine nuclei in vitro (4). However, some uncertainty surrounds the translocation process (12, 13), and recent evidence for in vivo nuclear binding of estradiol-receptor complexes in liver (14) prompted our investigation of the translocation process in this organ.

EXPERIMENTAL

Preparation of cytosols: All procedures were performed at 4°C. Tissues, isolated from intact, adult (60-65 day old) female Sprague-Dawley rats, were washed with 0.25 M sucrose, 1 mM dithiothreitol in 10 mM Tris, pH 7.4 (buffer A), and minced finely using scissors. Liver was homogenized in two volumes of buffer A with four strokes of a Potter-Elvehjem homogenizer. Homogenates of minced uteri were prepared in 5 volumes of buffer A with two ten-second bursts from a Polytron PCU-2. Cytosol was prepared by centrifugation of crude homogenates at $100,000g_{av}$ for 1 hr. Protein was quantitated by the method of Lowry et al (15).

Preparation of nuclei: Minced liver and uterine tissues were homogenized, as described above, in ten volumes of 0.5 M sucrose 5 mM $MgCl_2$ in 10 mM Tris, pH 7.4 (buffer B). Homogenates were filtered and then centrifuged at $800g_{av}$ for 15 min. The resulting crude nuclear pellet was washed once in buffer B and resuspended in 2.2 M sucrose containing 1 mM $MgCl_2$. Nuclei were then purified by centrifugation through this medium at $27,000g_{av}$ for 1 hr. Integrity of nuclei was checked using electron microscopy. Nuclei were shown to be intact and free from cytoplasmic contamination. DNA was quantitated by the method of Burton (16).

Determination of specific nuclear binding: Liver (10-15 mg/ml protein) and uterine (3-7 mg/ml protein) cytosols were incubated (4°C for 2 hr.) with 4 nM [^{3}H] estradiol (115 Ci/mmol, New England Nuclear Corp.). In some cases, a 100-fold excess of unlabeled diethylstilbestrol was present in liver cytosolic incubations. Purified nuclei were resuspended in 2 ml of buffer A (0.7-1.0 mg/ml liver DNA; 0.2-0.4 mg/ml uterine DNA) and incubated, under conditions specified in the legend to the figures, with 5 ml of the labeled cytosol. Following incubations, nuclei were pelleted by centrifugation at $800g_{av}$ for 15 min and washed twice in buffer A. In order to minimize cytoplasmic contamination in some experiments, nuclei were repurified by centrifugation through 2.2 M sucrose containing 1 mM $MgCl_2$ and then washed once in buffer A containing 0.2% (v/v) triton-X-100. Following a final wash in buffer A alone the resulting nuclear pellets were resuspended in 0.4 M KCl, 1 mM dithiothreitol in 10 mM Tris, pH 7.4 (600 µl), and incubated on ice for 30 min. The resulting viscous extract was centrifuged at $800g_{av}$ for 20 min and aliquots (400 µl) of the supernatant were layered over 5-20% sucrose-gradients (4 ml) prepared in 1 mM dithiothreitol and 0.4 M KCl in 10 mM Tris, pH 7.4. Gradients were centrifuged at $199,000g_{av}$ for 18h at 4°C in the SW 56 Titanium rotor, fractionated and each fraction (100 µl) assessed for bound radioactivity. Fractions containing peak levels of radioactivity were extracted with benzene and the extracts chromatographed on thin-layer plates using a developing solvent of benzene:ether (1:1).

RESULTS AND DISCUSSION: Sedimentation analysis of the salt-extract of purified liver nuclei, previously incubated (29°C) with cytosol pre-labeled at 4°C,

yields two peaks of radioactivity corresponding to [^{3}H] steroid-macromolecular complexes sedimenting in 4S and 5S regions of the gradients (Fig. 1). Similar profiles were obtained from salt extracts of purified nuclei following incubation (29°C) of liver minces in Eagles basal medium containing [^{3}H] estradiol (data not shown). Neither peak is detected following incubation (29°C) of nuclei with [^{3}H] estradiol in buffer alone (Fig. 1), demonstrating that the formation of nuclear complexes requires the presence of cytoplasmic estrogen-binding components. When incubations of nuclei and labeled cytosol are performed at 4°C, considerably less nuclear transfer of 4S and 5S complexes is observed (Fig. 1), reflecting the temperature dependency of the translocation process. However, the translocation of cytosolic hormone-receptor complexes into nuclei at 4°C is markedly enhanced if the cytosol is prelabeled at 29°C prior to incubation with purified nuclei (Fig. 2). Thus warming of the native cytosolic receptor, even in the absence of nuclei, produces activation resulting in an increased nuclear affinity of both 4S and 5S complexes. Two possibilities are suggested by these results; either activation is not necessarily accompanied by a shift in the sedimentation coefficient to 5S, or the 5S form is converted back to an activated 4S form which is also capable of translocation into nuclei.

Since translocation of both 4S and 5S contrasts to the behavior of estrogen receptors in rat uterus (4), it was considered that the nuclear 4S form might reflect binding by an estradiol metabolite. However, following benzene extraction and thin-layer chromatography of both 4S and 5S fractions of sucrose-gradients, radioactivity was detected only in zones corresponding to estradiol (data not shown). Moreover, radioactivity is not detectable in the water fraction following benzene extraction indicating that polar estradiol metabolites are not present in 0.4 M KCl extracts of nuclei.

The possibility that 4S complexes arise as a consequence of cytoplasmic contamination of nuclei was also considered. Following incubations (29°C) of pre-labeled cytosol with purified nuclei, the nuclei were repurified by

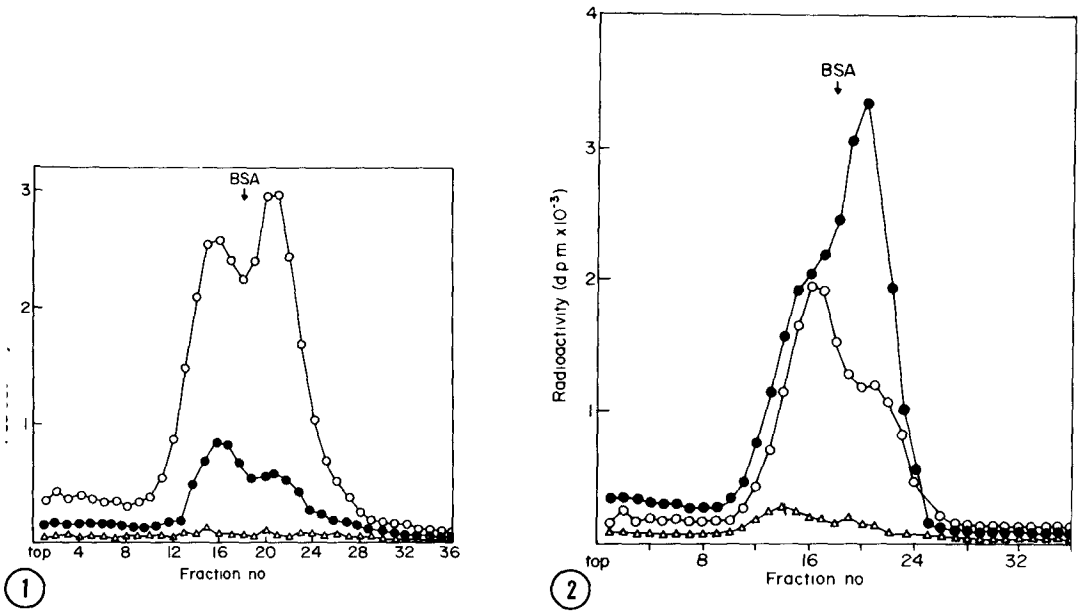
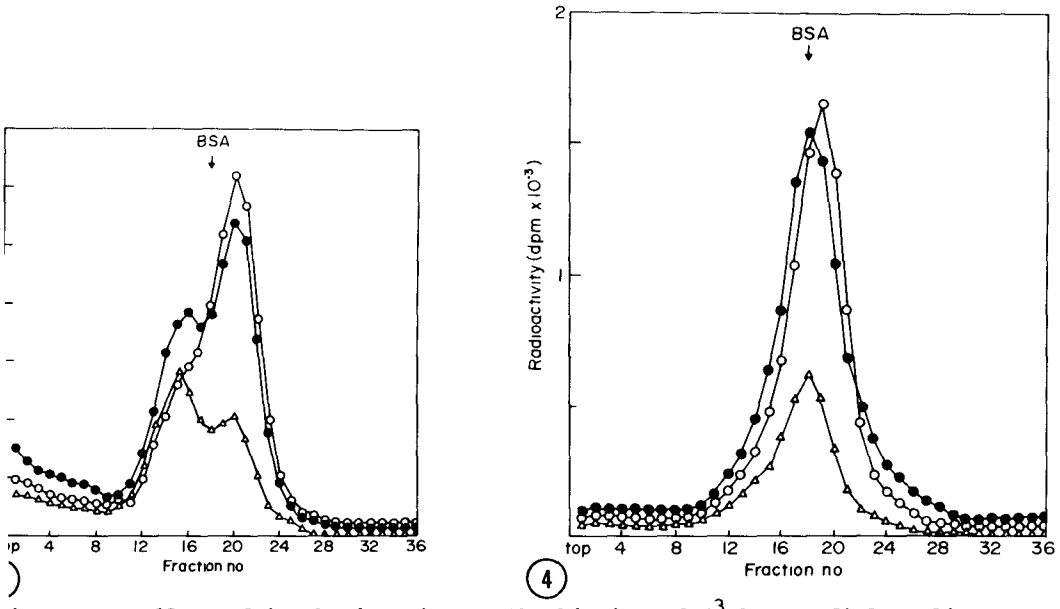


Figure 1. Sedimentation analysis of the binding of [^{3}H] estradiol by liver nuclei. Purified nuclei were incubated (40 min. at 29°C) with liver cytosol pre-incubated (2 h at 4°C) with either [^{3}H] estradiol (4 nM) alone (○) or together with 100-fold excess of diethylstilbestrol (△). Parallel incubations of purified nuclei with cytosol preincubated (2 h at 4°C) with [^{3}H] estradiol were performed at 4°C (●). Buffer containing [^{3}H] estradiol was incubated (40 min. at 29°C) with purified liver nuclei (△). Salt extracts of nuclei were analysed on 5-20% sucrose gradients as described in the Experimental section.

Figure 2. Temperature dependency of the uptake of hormone-receptor complexes by liver nuclei. Samples of liver cytosol pre-incubated (2 h at 4°C) with [^{3}H] estradiol (4 nM) were then incubated (40 min) with purified liver nuclei at 4°C (△) or 29°C (●). Parallel incubations were undertaken in which liver cytosol was pre-incubated (2 h at 4°C) with [^{3}H] estradiol and then warmed (30 min., 29°C) prior to incubation with nuclei at 4°C (○). Salt extracts of nuclei were analysed on 5-20% sucrose gradients as described in the Experimental section.

sedimentation through heavy sucrose and the pellets washed in buffer A containing 0.2% (v/v) triton-x-100 in order to remove the outer nuclear membrane. Sedimentation profiles of salt-extracts of these nuclei yielded similar profiles of 4S and 5S forms and equal amounts of binding to those presented in Fig. 1 (data not shown).

Data presented in Fig. 3 illustrate changes observed in sedimentation profiles when liver nuclei are incubated (29°C) for varying lengths of time with cytosol prelabeled at 4°C. The 4S complex is predominant at 15 min.,



igure 3. Effect of incubation time on the binding of [^{3}H] estradiol by liver uclei. Purified liver nuclei were incubated (29°C) with liver cytosol preinubated (2 h, 4°C) with [^{3}H] estradiol (4 nM) for 15 min. (△), 30 min. (●) or 0 min. (○). Salt extracts of nuclei were analysed on 5-20% sucrose gradients s described in the Experimental section.

igure 4. Effect of incubation time on the binding of [^{3}H] estradiol by uterine uclei. Purified uterine nuclei were incubated (29°C) with [^{3}H] estradiol (4 nM) or 7 min. (△), 15 min. (●) or 30 min. (○). Salt extracts of nuclei were analysed n 5-20% sucrose gradients as described in the Experimental section.

eaching maximal level at 30 min. and remaining relatively constant thereafter. he 5S complex, however, is minimal at 15 min. but increases rapidly up to 30 in. at which time the 5S peak is almost double that of the 4S peak. Since the atio of the 5S to 4S peak increases with time it is unlikely that a 5S to 3 intranuclear conversion is occurring. However, the possibility remains hat the 4S form is converted to the 5S form in liver nuclei. In contrast to iver, incubation (29°C) of uterine nuclei with [^{3}H] estradiol-labeled uterine ytosolic fractions for various periods of time results in the presence of ily 5S nuclear hormone-receptor complexes (Fig. 4).

Results from cross-over translocation studies (Table 1) demonstrate that ily the 5S component is found in liver nuclei following incubations with erine cytosol. This finding supports the concept that 5S to 4S conversion

Table 1

Cross-over translocation studies of liver and uterine estradiol-receptor complexes[1]

Incubation mixtures	Conformation of complexes extracted from nuclei
liver nuclei and liver cytosol	4S, 5S
liver nuclei and uterine cytosol	5S
uterine nuclei and uterine cytosol	5S
uterine nuclei and liver cytosol	4S, 5S

[1]Purified nuclei were incubated (40 min., 29°C) with cytosol preincubated (2 h, 4°C) with [^{3}H] estradiol (4nm). Salt extracts of nuclei were analysed on 5-20% sucrose gradients as described in the Experimental section.

is not occurring subsequent to the translocation step. Moreover, translocation of liver 4S complexes into uterine nuclei provides further evidence that the 4S form represents an activated estrogen-receptor complex.

In conclusion, these results demonstrate that in liver the cytosolic 5S complex is not the only species of estrogen receptor translocated into the nucleus. This suggests that qualitative differences may exist between liver and uterus in the transfer of estradiol from cytosolic to nuclear binding sites although some previous reports have shown both 4S and 5S complexes in immature rat uterine nuclei (17, 18). It is of interest to note here that no change from 4S to 5S forms can be detected in the sedimentation behavior of glucorticoid (19), progesterone (20) nor androgen-receptor complexes (21) during the nuclear translocation process. Although we have demonstrated the presence of the 4S form of the estrogen receptor in liver nuclei following incubation of [^{3}H] estradiol with whole cells, neither the mechanism of the formation of the 4S nuclear receptor nor the physiological significance of multiple forms of estrogen receptors in the mechanism of action of the hormone are fully understood.

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