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RECEPTOR TRANSFORMATION, THE KEY TO ESTROGEN ACTION

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SUMMARY

The transformation of the native (4S) uterine cytosol–receptor complex to an active (5S) form is an estrogen-requiring, temperature-dependent process, the rate of which is affected by the pH and ionic strength of the cytosol. Unlike its 4S precursor, the transformed complex is retained by uterine nuclei and chromatin but by non-target tissue chromatin as well. The specificity of the *in vitro* nuclear uptake of transformed receptor complex appears to reside in the biochemical response; transformed receptor effects *in vitro* the same target tissue specific, quantitative stimulation and qualitative change in nuclear RNA synthesis as that observed in the target tissue after administration of the hormone *in vivo*.

It is now generally recognized that estrogen-dependent tissues contain characteristic estrogen-binding macromolecules, commonly referred to as estrogen receptors, which are intimately involved in the target tissue interaction with the hormone [1, 2]. The interaction of estradiol with uterine cells appears to proceed by initial association of the hormone with the 4S binding unit of the extranuclear, estrogen–receptor protein followed by a temperature-dependent translocation of the resulting complex to the nucleus. During this process the receptor protein undergoes a change, called receptor transformation, which was first recognized by an increase in the sedimentation rate of the complex to 5S [3, 4], but which also includes the acquisition of an ability to bind to uterine nuclei [5] and to stimulate the activity of magnesium dependent nuclear RNA polymerase [6].

EXPERIMENTAL

Calf endometrium cytosol was prepared from a 20% homogenate in 0·32 M sucrose, 10 mM Tris, pH 7·4, by centrifugation for 1 h at 78,000 *g*. For receptor transformation studies cytosol aliquots were warmed at 25°C for the indicated times, either prior to or after addition of [6,7-^{3}H]-estradiol (S.A. 57 Ci/mmol) at 3–5 nM final concentrations. The effect of KCl on receptor transformation was studied using the above cytosol, made 150 mM in KCl by addition of 3 M KCl. The pH of cytosol aliquots was adjusted by titrating with dilute NaOH and compensating for cytosol dilution by addition of additional buffer as required.

For comparison of the binding of transformed and native receptor complexes with nuclei or chromatin, nuclei were isolated from 2·2 M sucrose homogenates [7], and chromatin was prepared from the purified nuclei by the method of Spelsberg and Hnilica [8]. For binding with purified nuclei, ^{3}H-estradiol (5·6 nM final concentration) was preincubated with endometrium cytosol (from a 20% homogenate in 0·32 M sucrose, 10 mM Tris, pH 7·4) for 45 min at either 0°C or 25°C prior to addition of 2 ml portions to equal portions of nuclei (2 mg DNA) and incubation for 60 min at either 0°C or 25°C. After incubation, the nuclei were separated by centrifugation at 1000 *g* resuspended twice in the cytosol buffer and collected by centrifugation. The nuclei were then extracted by gentle homogenization in 2 ml 0·4 M KCl, 10 mM Tris, pH 8·5, and after 30 min incubation in the cold, separated by centrifugation from the resulting extract. For binding with chromatin, the procedure was similar except that cytosol in 10 mM Tris buffer was used, the pre-incubation was with 10 nM ^{3}H-estradiol for 60 min and the chromatin was washed with 150 mM NaCl, 10 mM Tris buffer before extraction with 0·4 M KCl as above.

For study of specificity and saturability of receptor uptake by endometrium or thymus chromatins, each chromatin (35 μg DNA), prepared as described above, was incubated for 60 min at 25°C with various quantities of endometrium cytosol containing 5 nM ^{3}H-estradiol in a total volume of 865 μl of 10 mM Tris buffer containing 150 mM NaCl. The chromatins were collected by centrifugation, washed 4 times with 5 ml of 150 mM NaCl in 10 mM Tris buffer, pH 7·4, and resuspended in fluor for scintillation counting.

For sedimentation analyses 200 μl portions of cytosols (diluted with 1·5 vol. of 10 mM Tris buffer, pH 7·4) or nuclear extracts were layered on linear 5–20% sucrose gradients prepared in 10 mM Tris, 400 mM KCl, 1 mM EDTA, pH 7·4 buffer. Centrifugation was for 16 h at 308,000 *g* after which 100 μl portions were collected by displacement from the bottom of the tube and radioactivity determined by scintillation counting at 25–35% efficiency.

For study of the effect of partially purified nuclear receptor on magnesium-dependent nuclear RNA poly-

merase activity, increasing amounts of calf uterus nuclear–receptor complex, purified by salt precipitation and gel filtration on Sephadex G200 [9] were desalted by dialysis and incubated for 5 min at 30°C with purified endometrium or thymus nuclei suspended in 10 mM Tris buffer, pH 7·4 containing 0·32 M sucrose and 3 mM $MgCl_2$. Aliquot portions of each incubation mixture were assayed in replicate for RNA polymerase activity as previously described [6].

For the nearest neighbor nucleotide frequency study of the RNA, a 20% homogenate of calf endometrium in 2·2 M sucrose containing 10 mM $NgCl_2$ was incubated for 60 min at 25°C in the presence or absence of 10 nM estradiol. The nuclei were isolated by centrifugation at 50,000 *g* and taken up in 0·32 M sucrose for synthesis of RNA using α-^{32}P labeled GTP or UTP as precursor in the magnesium dependent RNA polymerase assay system previously described [6]. The determination of base linkages in the product after hydrolysis was performed by paper electrophoresis as described by Liao *et al.*[10].

RESULTS AND DISCUSSION

The estrogen-requiring, temperature-dependent receptor transformation with calf uterine cytosol is shown in Fig. 1. In the presence of estradiol (Fig. 1a) but not in its absence (Fig. 1b), warming the cytosol at 25°C results in a decrease in the amount of 4S complex, moving slower than the BPA marker, with a concomitant increase in the transformed, or 5S complex, moving faster than BPA. Under the conditions used for the experiment shown in Fig. 1 (cytosol of a 20% homogenate in hypotonic sucrose buffer at pH 7·4) the receptor transformation is not complete in one hour at 25°C, but a number of factors have been found to increase the rate of 5S complex formation.

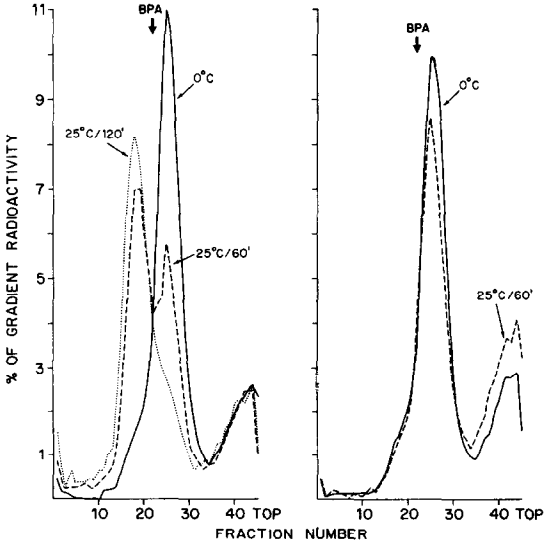

Fig. 1. Transformation of native (4S) cytosol receptor complex to 5S form. Calf endometrium cytosol, in the presence (left) or absence (right) of 4 nM ^3H-estradiol, was incubated at 0° or 25°C for the indicated times. After incubation, 4 nM ^3H-estradiol was added to the cytosols not containing it (right), and aliquot portions of each mixture were subjected to sedimentation analysis in the presence of 0·4 M KCl. BPA indicates the location of the bovine plasma albumin marker (4·6S). Migration is from right to left.

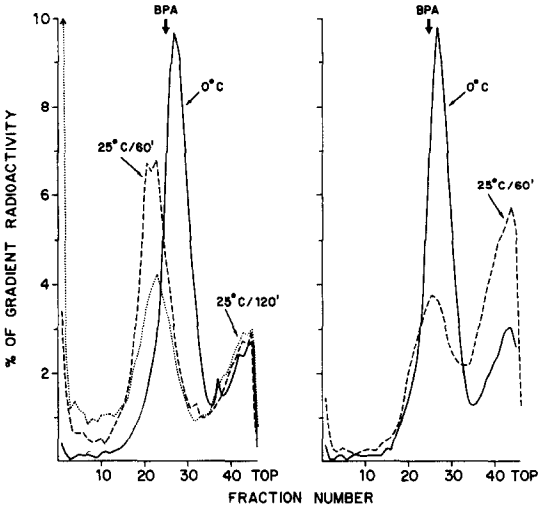

Fig. 2. Receptor transformation in endometrium cytosol in the presence of 150 mM KCl. Aliquots of cytosol to which KCl at a final concentration of 150 mM had been added were treated as described in Fig. 1.

When the salt concentration of the cytosol is increased to a physiologic concentration, 0·15 M in KCl (Fig. 2), the receptor transformation is complete in less than one hour at 25°C while no appreciable 5S formation is evident in the absence of the hormone. The pH of the cytosol has a dramatic influence on the rate of receptor transformation (Fig. 3), increasing with an increase in pH from pH 7·0, where no significant 5S is seen, to pH 7·8 where the reaction has proceeded about halfway to completion, to pH 8·5 where most of the 4S has been converted to the 5S form in the same time period. Although it has been reported that under certain conditions receptor transformation appears to be a bimolecular process [11], in hypotonic Tris–sucrose buffer more 5S is formed in a diluted cytosol than in the undiluted cytosol in the same time period (Fig. 4). It is possible that with hypotonic salt concentrations association of the 4S binding unit to 8S or higher macromolecular forms may retard the receptor transformation and result in more complex kinetics.

As previously reported [5], receptor transformation results in an estrogen-receptor complex with increased affinity for purified endometrium nuclei (Fig. 5—left). Similar differences in affinity of the transformed and untransformed receptor complexes toward endometrium chromatin are also found (Fig. 5—right). When incubated in the cold with nuclei or chromatin, the estradiol–cytosol mixture, preincubated at 25°C to effect receptor transformation, C, shows greater uptake of estradiol extractible as 5S complex than does the untransformed complex, A. When the incubation is conducted at 25°C, considerable amounts of com-

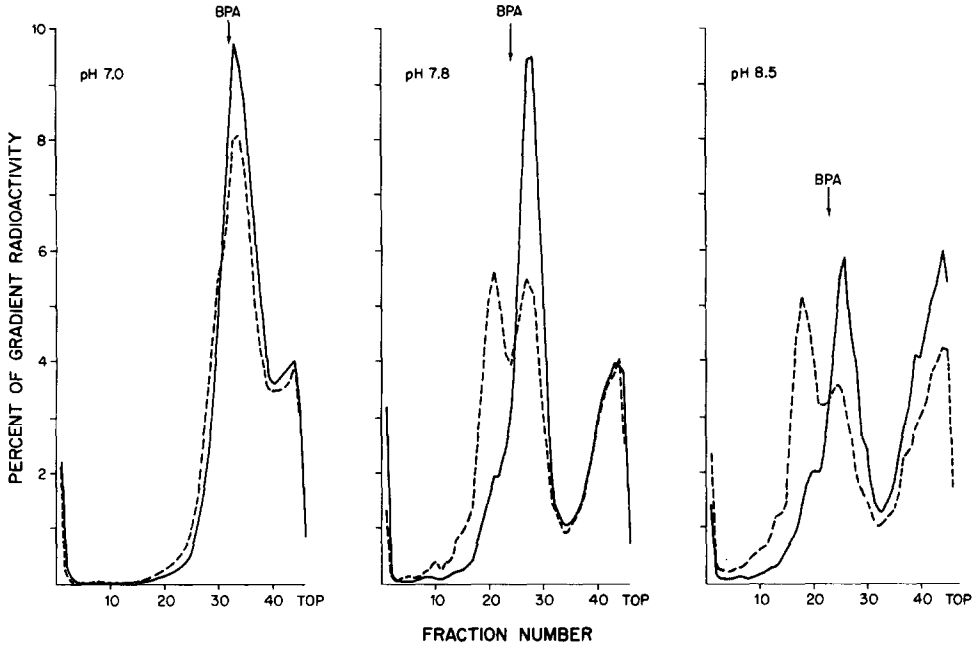

Fig. 3. Effect of pH on receptor transformation. Aliquots of endometrium cytosol were titrated to pH 7·0 (left), pH 7·8 (middle) or pH 8·5 (right). ^{3}H-estradiol was added (2·5 nM final concentration), and the mixtures incubated for 15 min in an ice bath (——) or at 25°C (– – – –), cooled and subjected to sedimentation analysis in the presence of 0·4 M KCl.

plex are taken up whether the cytosol pre-incubation mixture consisted of transformed, D, or untransformed complex, B, since receptor transformation occurs at the incubation temperature.

While it is clear that receptor transformation is a prerequisite for the incorporation of any appreciable quantity of receptor complex by uterine nuclei or chromatin, this uptake does not appear to be specific for target tissue nuclei or chromatin (Fig. 6). When chromatin prepared from purified calf endometrium and thymus nuclei and containing equivalent amounts of DNA was incubated with increasing concentrations of endometrial cytosol estrogen–receptor complex at 25°C, the receptor uptake by the target

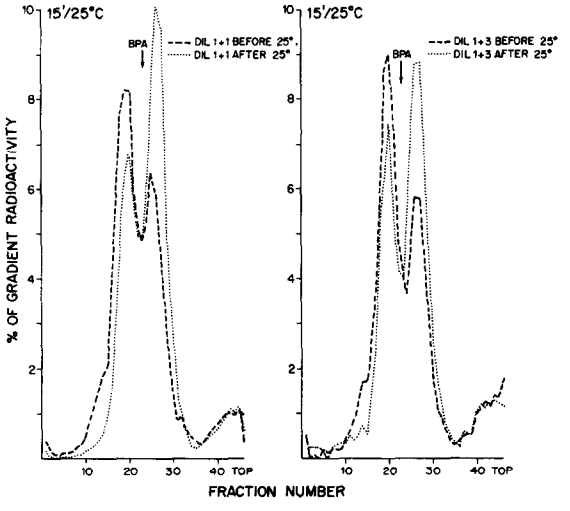

Fig. 4. Effect of cytosol dilution on receptor transformation in low ionic strength medium. Endometrium cytosol from a 20% homogenate in 0·32 M sucrose in 10 mM Tris buffer, pH 7·4, was made 4 nM in tritiated estradiol, and aliquots diluted 1 + 1 (left) or 1 + 3 (right) with homogenization buffer either prior to (– – – –) or after (·····) a 15 minute incubation at 25°C. Aliquot portions of each mixture were then analyzed on sucrose gradients in the presence of 0·4 M KCl.

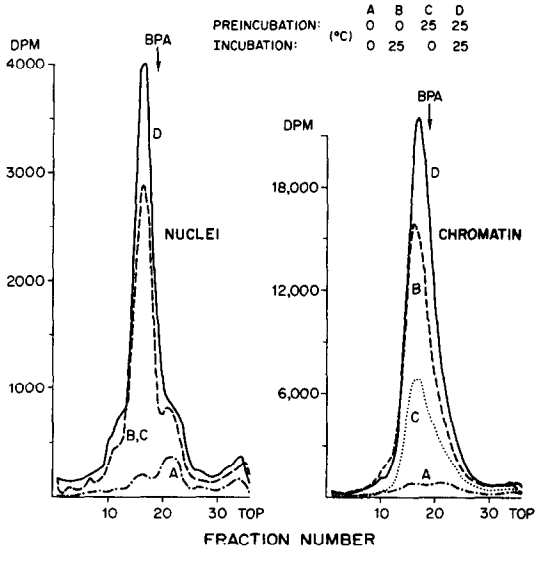

Fig. 5. Effect of receptor transformation on the association of receptor–complex with purified endometrium nuclei or chromatin. Aliquots of endometrium cytosol, pre-incubated with ^{3}H-estradiol in the cold (A and B) or at 25°C to effect receptor transformation (C and D), were incubated with endometrium nuclei (left) or chromatin (right) as described in Experimental Section. The nuclei and chromatin preparations were washed with buffer, and nuclear extracts, prepared in 0·4 M KCl, were subjected to sedimentation analysis in the presence of 0·4 M KCl.

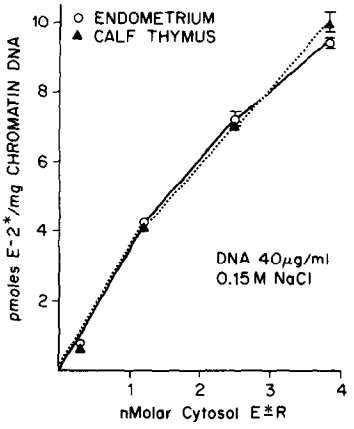

Fig. 6. Association of transformed receptor complex with endometrium and thymus chromatin. Various concentrations of endometrial cytosol–receptor complex were incubated for 60 min at 25°C with endometrium (——) or thymus (·····) chromatin (40 μg DNA/ml) at a NaCl concentration of 150 mM. After extensive washing, the retained radioactivity of the chromatin preparations was determined.

and non-target tissue chromatins was essentially the same. Furthermore, under the receptor transforming conditions used there was no marked tendency for saturation of chromatin sites. There is considerable disagreement in the literature as to the tissue specificity or saturability of the association of estrogen–receptor complex with nuclei and chromatin; some authors find tissue specificity [12] and saturability [13] while others do not [14–16]. Since it is possible that a lack of target tissue specificity for transformed receptor may exist *in vitro*, it is important to study possible biochemical effects resulting from the association of receptor complex with nuclei or chromatin.

One biochemical process that is clearly affected by estrogen is RNA synthesis. As has been previously

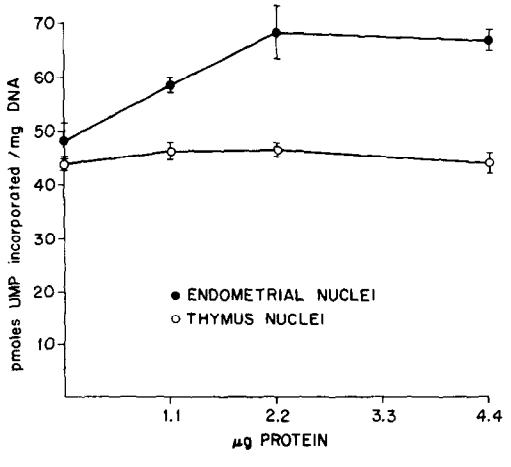

Fig. 7. Effect of different amounts of partially purified nuclear estradiol–receptor complex on nuclear RNA polymerase activity. Purified endometrium (solid circles) and thymus (open circles) nuclei were incubated for 5 min at 30°C with various amounts of partially purified nuclear complex from calf uterus, and aliquots of each mixture analyzed for magnesium-dependent RNA polymerase activity using ^{3}H-UTP as precursor.

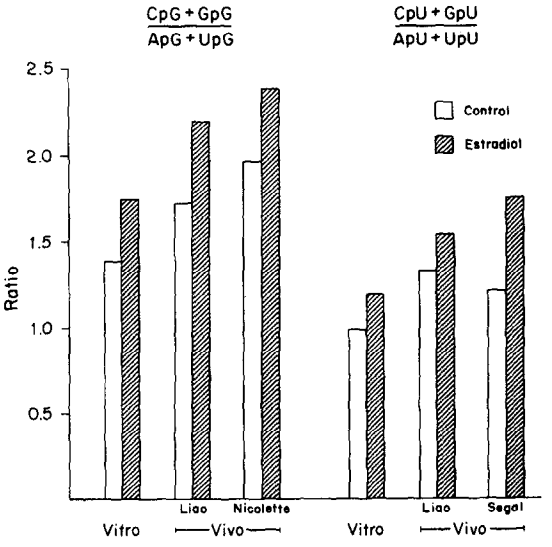

Fig. 8. Effect of estradiol *in vitro* on nearest neighbor nucleotide frequency (NNF) of RNA synthesized by isolated endometrium nuclei. The NNF of the RNA synthesized by calf endometrium nuclei after incubation with estradiol–receptor complex *in vitro* (as described in the Experimental Section) is compared with that reported by Barton and Liao[19], Nicolette and Babler[20] and Trachewsky and Segal[21] for rat uterine nuclei after administration of estradiol *in vivo*.

reported [5, 6, 17, 18], incubation of estradiol and uterine cytosol with uterine nuclei at 25°C effects a stimulation of the magnesium-dependent nuclear RNA polymerase activity. This *in vitro* response to the hormone requires the transformed receptor complex and furthermore is specific for target tissue nuclei. In addition, as seen in Fig. 7, partially purified transformed receptor complex isolated from uterine nuclei retains its ability to effect a stimulation in nuclear RNA synthesis of endometrial nuclei but has no such effect with the non-target, thymus nuclei.

Finally, as has been reported by Liao[19], Nicolette[20] and Segal[21], administration of estradiol *in vivo* causes a change in the qualitative nature of the RNA synthesized in isolated uterine nuclei. The nature of this change, indicated by determining the nearest neighbor frequency spectrum, corresponds to an increase in the cytidine and guanosine linkages in the RNA. A similar change in the qualitative nature of the RNA synthesized is observed after incubation of uterine nuclei with cytosol receptor complex *in vitro* (Fig. 8). Therefore, despite the apparent lack of tissue specificity of the association of receptor complex with nuclei *in vitro*, the biochemical effect of transformed receptor complex on RNA synthesis in nuclei after such *in vitro* incubations is a target tissue specific response which, both quantitatively and qualitatively, reproduces the effect of the hormone *in vivo*.

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