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Interaction of Steroid Hormones with the Nucleus

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I. Introduction

SINCE the early observations by Mueller (72) of the estrogen-enhanced incorporation of labeled precursors into uterine RNA and by Clever and Karlson (15) of the induction by ecdysone of chromosomal puffs in insect salivary glands, it has been well established that steroid hormones regulate biochemical reactions in target cell nuclei. There is much evidence to indicate that a principal action is on the production of RNA, apparently by enhancing the template function of target cell chromatin and possibly by other effects as well. Consistent with an influence on nuclear processes are the observations with all classes of steroid hormones that administration of physiological amounts of tritiated steroid to a hormone-deprived animal leads to an accumulation of radioactive hormone in target cell nuclei.

A major advance in the understanding of steroid hormone action came with the demonstration that target cells contain characteristic hormone-binding components or "receptors" and that it is a steroid-receptor complex rather than the steroid itself that exerts a regulatory effect in the cell nucleus. The concept of a two-step translocation mechanism, in which an activated hormone-receptor complex of cytoplasmic origin modulates RNA synthesis in the nucleus (Fig. 1), was originally developed for the interaction of estrogenic hormones with the rat uterus; it was later shown to provide a general model for the interaction of all classes of steroid hormones with their respective target tissues.

The overall pattern of the interaction of estrogenic hormones with the rat uterus is based largely on information derived from experiments that involved tritiated estrogens of high specific radioactivity to follow their reaction with receptor. The principal stages (and concepts) in the development of this knowledge were the following:

1) Uptake and retention of the hormone by reproductive tissues in vivo (receptor).

2) Uterotrophic action without metabolic alteration of estradiol.

3) Inhibition of specific binding by estrogen antagonists; correlation of binding inhibition with growth inhibition.

4) Uptake and retention of tritiated estrogens by uterine tissue in vitro, sensitive to the same binding inhibitors effective in vivo.

5) Two intracellular sites of hormone localization; temperature dependence of nuclear binding.

6) Identification and differentiation of nuclear and cytoplasmic estradiol-receptor complexes; direct formation of cytosol complex in vitro.

7) Extranuclear origin of nuclear complex (receptor translocation).

8) Hormone-induced conversion of cytosol complex to nuclear form (receptor activation).

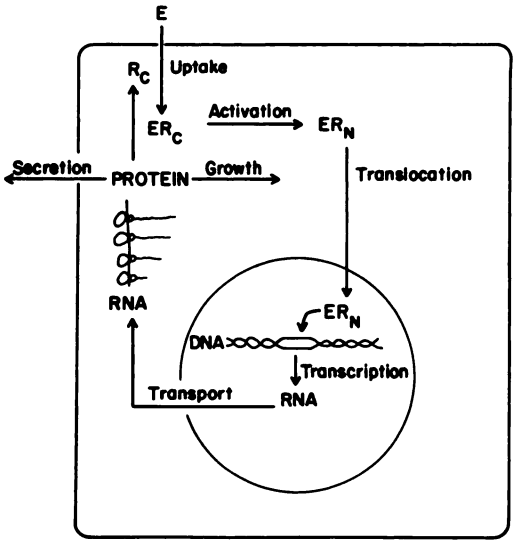

FIG. 1. Schematic representation of the estrogen interaction pathway and biochemical responses in target cells. The hormone (E) enters the cell and binds to an extranuclear receptor protein (Rc), inducing its conversion to an activated form (Rn) that is translocated to the nucleus where the hormone-receptor complex enhances the production of preribosomal and messenger RNAs involved in the synthesis of functional and/or secreted proteins.

9) Binding of activated estrogen-receptor complex by nuclei and chromatin (acceptor).

10) Tissue-specific influence of activated estrogen-receptor complex on transcription in isolated target cell nuclei.

In this paper, the experimental evidence on which the foregoing concepts are based is summarized, and more recent investigations into the nature of receptor activation, translocation, and nuclear binding, as well as the relation of these phenomena to RNA synthesis and growth induction, are described. Finally, mention will be made of the recent preparation of specific antibodies to purified estrogen receptor protein and their potential use as a new approach to unresolved questions of receptor structure, localization, and function. For the most part, the discussion will be limited generally to the estrogenic hormones, for which the interaction pathway was first elucidated, with brief reference to specific characteristics observed for other types of steroids. Detailed information concerning receptor interactions of other steroid hormones can be found in more extensive review articles and monographs (23, 39–41, 56, 60, 66, 81, 82, 88, 91, 122).

II. Hormone Binding in Target Tissues

That the female reproductive tissues, such as uterus, vagina, and anterior pituitary, contain characteristic estrogen-binding components, now called estrogen receptors or estrophilin, was first indicated by their striking ability to take up and retain tritiated hexestrol (20) and estradiol (45, 104) after the administration of physiological doses of these substances to immature animals. It is now recognized that most, if not all, mammalian tissues contain small amounts of estrogen receptor and that the unique characteristic of the hormone-dependent tissues is the magnitude of their estrophilin content (50). Estradiol was found to combine reversibly with the receptor and initiate growth of the immature rat uterus without itself undergoing chemical change (46), suggesting that the action of the hormone involves its influence on macromolecules rather than participation in reactions of steroid metabolism as had once been assumed. When excised uterine tissue is exposed to dilute solutions of tritiated estradiol at physiological temperature in vitro, an interaction of hormone with receptor takes place that shows all the characteristics of that observed in vivo (43, 106, 111), including sensitivity to inhibitors and formation of the same estradiol-receptor complexes that are found in vivo (51).

The specific uptake and retention of estradiol by target tissues, both in vivo (38, 93, 105) and in vitro (43, 47), are inhibited by a class of estrogen antagonists that are themselves very weak estrogens, but which prevent the uterotrophic action of the natural hormone. These substances, which include clomiphene, nafoxidine, Parke Davis

CI-628, and tamoxifen, provide a useful means for distinguishing specific binding of hormone to receptor from the nonspecific binding that estradiol shows with all tissues or with various macromolecules in broken cell systems (47). The correlation observed between the reduction in hormone incorporation and the inhibition of uterine growth when different amounts of nafoxidine are administered along with estradiol to the immature rat first provided evidence that binding of hormone to receptor actually is involved in its biological action (38). In contrast, actinomycin-D and puromycin, substances that prevent the growth response to estradiol (71, 116), show no inhibition of the characteristic uptake and retention of hormone (38). This suggests that the binding of estradiol to receptor is an early step in the uterotrophic process, initiating a sequence of biochemical events that can be blocked at later stages by these inhibitors of RNA and protein synthesis.

III. Estrogen-Receptor Interaction in Target Cells

A. Intracellular Localization of Bound Hormone

When uterine homogenates from estradiol-treated rats are subjected to differential centrifugation, the incorporated steroid appears in two cellular fractions (37, 43, 54, 76, 109, 118). Most of the hormone (70%–80%) is found in the nuclei (19, 43), with a smaller amount present in the high-speed supernatant or cytosol fraction. The predominance of nuclear binding, controversial in earlier reports, was confirmed by autoradiographic studies, (44, 107, 108) with a dry-mount procedure that minimizes steroid translocation during tissue processing. Similar nuclear localization of hormone is seen when excised uteri are exposed to estradiol at physiological temperature in vitro. The ratio between nuclear and extranuclear steroid remains remarkably constant over a wide variation in the amount of hormone administered, either in vivo (43, 118) or in vitro (19). In contrast to the nuclear localization seen at physiological temperature, after exposure of excised uteri to estradiol at 2°C, the incorporated steroid is mostly extranuclear, shifting to the nucleus as the tissues are warmed to physiological temperature (26, 51) and indicating that nuclear incorporation of estradiol is much more temperature dependent than is cytoplasmic binding.

B. Estrogen-Receptor Complexes

The estradiol taken up by rat uterus is associated with a different form of the receptor in the cytosol than in the nucleus. The application by Toft and Gorski (112) of ultracentrifugation in sucrose density gradients for characterizing the estrogen-receptor complexes of uterine cytosol was an important advance, for it provided a means for distinguishing between different modifications of the receptor. By this technique the radioactive hormone in the cytosol is found to sediment as a discrete band with a coefficient close to 8 S (Fig. 2a). In salt concentrations greater than 0.2 M, the 8 S complex is reversibly dissociated into subunits (18, 59) that sediment at about 4 S, just behind bovine plasma albumin (Fig. 2b). The estradiol bound in the nucleus can be solubilized, unaccompanied by DNA, by extraction with 0.3 M or 0.4 M KCl (52, 85) to yield an estradiol-receptor complex that, in the presence of salt, sediments at about 5 S, slightly faster than bovine plasma albumin (50, 52). As shown in Figure 2b, the nuclear complex is readily distinguished from the cytosol complex by careful ultracentrifugation in salt-containing sucrose gradients; this difference in sedimentation rates provided the first criterion for recognizing the important phenomenon of receptor activation (49).

The 8 S estradiol-receptor complex, or its 4 S subunit, forms directly in the cold when estradiol is added to the cytosol fraction of uteri not previously exposed to hormone (51, 52, 113). Thus, the receptor content of

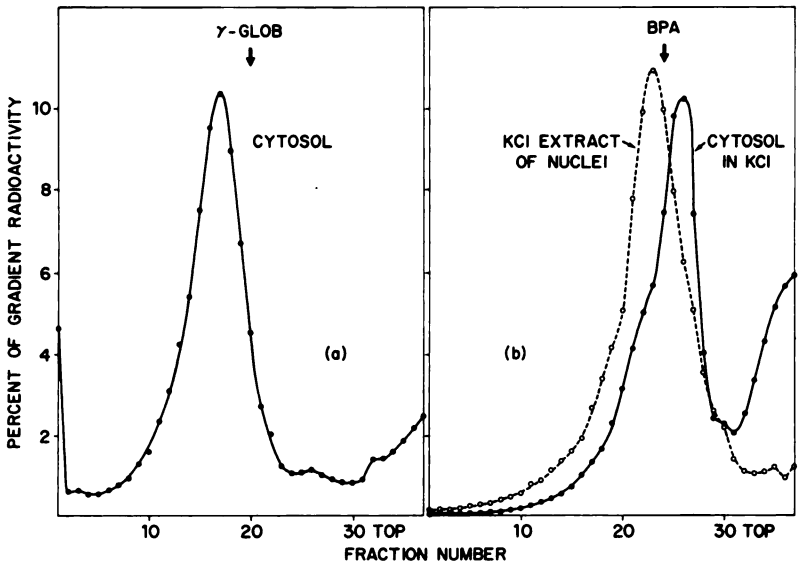

FIG. 2. Sedimentation patterns of radioactive estradiol-receptor complexes of rat uterine cytosol and nuclear extract (400 mM KCl) from uteri of immature rats excised 1 h after the subcutaneous injection of 100 ng (20.8 μCi) tritiated estradiol in saline. To saturate its receptor capacity the cytosol fraction was made 5 nM with additional tritiated estradiol. γ-GLOB and BPA indicate positions of bovine immunoglobulin (7.0 S) and bovine plasma albumin (4.6 S) markers. Gradients are: (a) 10% to 30% sucrose without added salt; (b) 5% to 20% sucrose containing 400 mM KCl. (From E. V. Jensen and E. R. DeSombre [41].)

the cytosol is estimated easily by adding sufficient tritiated estradiol to saturate the binding sites and determining the radioactivity present in the 8 S sedimentation peak. This interaction of estradiol with cytosol receptor is prevented by the presence of antiestrogens, such as nafoxidine or CI-628. Although noncovalent, the binding of estradiol to receptor proteins of uterine tissue is remarkably strong; association constant values from 10^9 to 10^{12} M^{-1} have been reported for the cytosol complex (56), with the nuclear form of the receptor showing even greater affinity (117). This tight binding appears to result from a very slow rate of dissociation (114); once formed, the complex does not readily lose estradiol in the cold except by receptor decomposition (117). From the sensitivity of their complexes to proteases but not to nucleases, the estrogen-binding substances of both cytosol (112) and nucleus (49) appear to be mainly protein in nature.

C. Receptor Translocation

A major advance in the understanding of the interaction of steroid hormones with target cells came with the recognition that the estradiol-receptor complex of the uterine nucleus is derived from the cytosol complex by a temperature-dependent process in which association with the hormone causes the extranuclear receptor to accumulate in the nucleus (Fig. 1). It was later shown that the temperature-dependent aspect of this phenomenon is the hormone-induced conversion of the native estrophilin to an active form that can bind in the nucleus. Although this two-step, receptor translocation mechanism is not proved with absolute certainty, it is supported by many different types of experimental evidence (Table 1).

A relation between the two intracellular sites of estrogen localization was first indicated by observations that a given dose of

TABLE 1
Evidence for Translocation Mechanism

1. Equal inhibition by nafoxidine of nuclear and extranuclear binding	In vivo
2. Unsaturable uptake; saturable retention	In vivo
3. Temperature-dependent intracellular redistribution	In vitro
4. Cytosol dependence of nuclear complex formation	In vitro
5. Hormone-induced depletion of cytosol receptor	In vivo and in vitro
6. Enhanced nuclear binding of activated receptor	In vitro
7. Tissue-specific effect of activated complex on RNA synthesis	In vitro
8. Immunochemical similarity of nuclear and cytosol receptors	In vivo and in vitro

nafoxidine in vivo inhibits cytosol and nuclear binding of estradiol by the rat uterus to the same degree (43), and that more radioactivity is bound to uterine nuclei when they are incubated with tritiated estradiol in uterine cytosol than in buffer alone (9). When it was found that there is a difference in saturability between the initial uptake of estradiol by the rat uterus in vivo and its longer term retention by the nucleus (43), and that the 8 S extranuclear complex can be produced in surprisingly large amounts by adding the hormone directly to uterine cytosol (52, 113), it was suggested as early as 1966 that the extranuclear 8 S protein, present in considerable reserve, might serve as an "uptake" receptor, bringing the hormone to the nucleus where it is retained in limited amount by a nuclear receptor (42). In the following year it was proposed independently from two laboratories (26, 51) that the nuclear receptor actually is an altered form of the cytosol receptor that has been translocated to the nucleus. At that time, this hypothesis was based on three principal experimental observations: 1) No 5 S nuclear estradiol-receptor complex is formed by treatment of immature rat uterine nuclei or nuclear extracts with estradiol alone, but incubation of nuclei with hormone in the presence of receptor-containing cytosol gives rise to extractable 5 S complex (51, 99); 2) when excised rat uteri are exposed to estradiol at 2°C, cell fractionation as well as autoradiographic experiments demonstrate that most of the hormone is present as extranuclear 8 S complex, shifting to nuclear 5 S complex if the tissues are then warmed to 37°C (26, 51); and 3) exposure to estradiol either in vivo (50, 51, 95) or in vitro (26, 99) causes a depletion of the receptor content of the uterine cytosol, consistent with its movement to the nucleus. Subsequent experimentation, discussed in the following sections, has provided additional evidence that supports the concept of the two-step receptor translocation mechanism (Table 1).

D. Receptor Activation

When it was recognized that the 8 S extranuclear receptor is composed of 4 S subunits, it became evident that the alteration of estrophilin that accompanies its hormone-induced migration to the nucleus is reflected by an increase in sedimentation rate of the hormone-binding unit from 4 S to 5 S (50). Originally this transformation was believed to involve nuclear factors, but later it was found that conversion of the 4 S cytosol complex to the nuclear form is effected simply by warming uterine cytosol to 25°C to 37°C in the presence of hormone (29, 36, 49). The 5 S estradiol-receptor complex thus produced, like that extracted from uterine nuclei, has two properties not shown by the native form; it can bind to isolated nuclei (14, 48a), chromatin (68), or DNA (70), and, as described below, it can enhance the RNA polymerase activity of isolated nuclei from hormone-dependent tissues and tumors. Because of these new properties acquired, the hormone-induced, temperature-dependent transformation of the native receptor to the biochemically

functional nuclear form is known as receptor activation.

That hormone-induced receptor activation can occur in the absence of nuclei does not preclude the possibility that, in the living cell, this process takes place preferentially in the nucleus (61a, 100), especially since the nuclear membrane might be expected to be more permeable to the smaller 4 S form of the receptor than to the larger 5 S form. There is evidence that DNA may increase the rate of the activation process (120), while, in the case of glucocorticoid receptors, it has been demonstrated that the cytosol contains a dissociable factor inhibitory to receptor activation (4a). In certain instances estrophilin is found in the cell nucleus uncomplexed with hormone. In contrast to results with uterus, nuclei of rat pituitary tumor (101) and MCF-7 human breast cancer cell lines (124), as well as chick (69, 83) and toad (83) liver, have been found to contain substantial amounts of unoccupied estrogen receptor even before exposure of the tissue to hormone, but whether this estrophilin is in the native or activated form has not been established. These findings suggest that native receptor may be distributed throughout the target cell, even though accumulation in the nucleus may depend on its conversion to the activated form that binds to chromatin, displacing an equilibrium between nuclear and extranuclear distribution. As discussed in a later section, more definite information about the intracellular localization of estrophilin before exposure to hormone could be provided by techniques for identifying the receptor that do not depend on labeled hormone as a marker.

Although the molecular details of receptor activation are not completely understood, the transformation of the native 4 S estradiol-receptor complex to the nuclear 5 S form appears to involve more than simply a conformational change. The activated complex has a higher molecular weight than the native form (62, 79, 120) and the conversion reaction follows second order kinetics, indicative of dimerization (63, 78). Contrary to some earlier observations, it was demonstrated recently that the activated form of estrophilin binds estradiol more tightly than the native form so that interaction with the hormone drives the equilibrium to the higher affinity dimeric state (117). How hormone-induced dimerization endows the activated receptor with the ability to bind to chromatin, as well as to DNA and other polyanions, is not completely clear.

E. Generality of Intracellular Interaction Mechanism

Investigations from many laboratories (60, 66, 81, 88, 92) have established that the interaction of all types of steroid hormones with their respective target cells takes place by a two-step, translocation mechanism, similar to that originally elucidated for the estrogens. In all cases accumulation of the extranuclear steroid-receptor complex in the nucleus is accompanied by a temperature-dependent, hormone-induced conversion of the receptor to a form that shows enhanced binding to chromatin. However, only in the case of estrogens is receptor activation characterized by an increase in sedimentation rate. Activation of the dihydrotestosterone-receptor complex of rat prostate causes a decrease in sedimentation rate from 3.8 S to 3.0 S (60); similar decreases have been reported for the progesterone receptor complexes of hamster (12) as well as guinea-pig and rabbit (94) uterus, while no difference was observed in the sedimentation rates of native and activated progesterone-receptor complex of chick oviduct (10). Whether activated receptors for these other classes of steroid hormones form dimers that are dissociated under the conditions of sedimentation analysis or whether dimerization is merely incidental to the acquisition of chromatin-binding properties by the estrogen receptor remains to be elucidated.

F. Nature of the Nuclear Interaction

Although it is clear that hormonal regulation in target cell nuclei is effected by steroid receptor complexes translocated from the cytoplasm, detailed understanding is lacking as to the precise nature of the nuclear "acceptor" sites with which the activated receptor complexes interact and the way in which this interaction modulates biochemical events, in particular RNA synthesis. In the case of estrogens, early studies indicated that radioactive estradiol, incorporated into rat or calf uterine nuclei after exposure of the tissue to hormone in vivo (55, 110) or in vitro (67), is associated with chromatin; however, the precise location of this binding is still uncertain. The conclusion from nuclear fractionation experiments with heifer endometrium (4) and with chick liver (5a) that estrogen is bound in the nucleolus is at variance with autoradiographic studies that show no indication of hormone localization in rat uterine nucleoli (108).

The concept of a limited number of acceptor sites involved in the regulation of a few specific genes is not supported by experimental observations. Although the capacity of rat uterine nuclei to retain estradiol in vivo shows saturability as the dose of administered hormone exceeds what is considered to be physiological (1, 43), the number of estradiol-receptor complexes translocated by a physiological amount of hormone is still rather high, having been variously estimated as 6,000 (2), 10,000 (73), and 14,000 (49) per cell nucleus. Yet in investigations of early responses to estradiol, such as the synthesis of induced protein in immature rat uteri (19) or of mRNA for ovalbumin and conalbumin in chick oviduct (73, 84), or the appearance of initiation sites for RNA synthesis on oviduct chromatin (52a), linear correlation between the concentration of nuclear receptors and biological response has been observed up to several thousand receptors per nucleus. It has been suggested (121, 122) that many of the translocated hormone-receptor complexes may be bound with low affinity to nonspecific sites in the nucleus, thus masking the interaction with a limited number of true acceptors actually involved in gene regulation. Treatment of rat uterine nuclei with micrococcal nuclease has provided evidence supporting the concept that estradiol-receptor complexes may be bound to two types of sites in the chromatin, one associated with nu bodies and the other with a region of the chromatin that is digested by the nuclease (98a). Observation of a lag of 3 h between the time that estradiol-receptor complex is bound in the nucleus and ovalbumin mRNA begins to accumulate has led to the interesting suggestion that there may be a rate-limiting transfer of receptor from initial, nonproductive chromatin binding sites to productive sites (84). However, comparable lag periods are not seen in other systems, such as mRNA for induced protein in rat uterus (17, 119), and the possibility that essentially all the receptor complexes translocated by physiological amounts of hormone participate equally in hormonal regulation cannot be ruled out on the basis of present knowledge. Experiments relating uterine growth to estrogen receptor concentration in the nucleus show clearly that full uterotrophic response requires the continued presence of substantial amounts of estrogen-receptor complex in the nucleus for a prolonged period of time (2, 25).

In attempts to detect a nuclear acceptor substance through its receptor-binding properties, it has been found that, under certain conditions, the estradiol-receptor complex can be extracted from rat uterine nuclei in combination with ribonucleoprotein (61), suggesting a role of this substance in nuclear binding and possibly in biological action. The activated but not the native form of the estradiol-receptor complex of bovine uterus is reported to bind strongly to nucleohistone (36). In other studies, a basic nonhistone nuclear protein has been

isolated from calf uterine nuclei, which, when attached covalently to Sepharose, shows a strong ability to bind estrogen-receptor complexes (86). Apparently this putative acceptor substance is not a specific component of target cell nuclei for it is found in varying amounts in all calf tissues tested (87). Because the activated or nuclear form of the steroid hormone-receptor complexes shows a strong tendency to bind to DNA as well as to chromatin, many investigators believe that DNA itself may participate in the nuclear binding of receptors in the nucleus. Supporting this concept are observations that careful exposure of uterine nuclei from estrogen-treated mice with deoxyribonuclease releases the bound complex (33), while pretreatment of nuclei with DNase destroys their ability to bind complex on subsequent incubation with hormone and uterine cytosol (74). Moreover, experiments with hormone-insensitive variants of glucocorticoid-sensitive lymphoma cells suggest that the decreased ability of the insensitive cells to accumulate hormone in their nuclei can be correlated with a lower affinity of the isolated DNA for the hormone-receptor complex (123). However, the limited availability of exposed DNA in the living cell and the lack of tissue specificity associated with DNA binding make it unlikely that DNA alone serves as the biochemically functional acceptor substance.

Attempts to study specific binding of receptor to chromatin in broken cell systems have yielded controversial results, probably because of artifacts resulting from the interference with receptor binding by nonreceptor cytoplasmic proteins (11). Although there have been some reports of saturable and selective binding of activated estradiol-receptor complexes to target cell nuclei or chromatin (49, 103), most studies have failed to confirm this tissue specificity (11, 14, 36, 41, 48, 74a). Doubt as to the biological relevance of chromatin binding studies in vitro is raised by experiments in which preincubation of whole rat uteri with estradiol in concentrations sufficient to produce maximal nuclear binding was found to have no effect on the subsequent ability of the isolated nuclei to bind estradiol-receptor complex in vitro (34). Similar results were observed for glucocorticoid receptor binding in nuclei from hepatoma cells pretreated with dexamethasone (34), supporting the concept that receptor binding in isolated nuclei may not be the same phenomenon as that which takes place in vivo.

In the case of androgen (65) and progesterone (103) receptors, chromatin from target cell nuclei is reported to bind more hormone-receptor complex than does chromatin from nontarget tissues. The most extensive studies have been carried out with the chick oviduct, where the selective binding of progesterone-receptor complex was shown to depend on the AP_3 subfraction of the nonhistone proteins of oviduct chromatin (102). On the basis of observations that the progesterone receptor of chick oviduct can be separated into two hormone-binding subunits, an A chain that binds nonspecifically to DNA but not to chromatin and a B chain that shows specific affinity for oviduct chromatin (96), O'Malley et al (82) have proposed a modified version of the two-step mechanism in which there is binding of the translocated progesterone-receptor complex both with DNA and with nonhistone proteins, with the latter association, involving the B component of the receptor, responsible for tissue specificity. This dual interaction is postulated to enhance template activity of the chromatin by making initiation sites available for the synthesis of mRNA for avidin (80) and other oviduct proteins. Extension of this model to the action of estrogens and other types of steroid hormones awaits experimental verification, although evidence has been presented for a role of nonhistone proteins in the estrogenic activation of the ovalbumin gene in chick oviduct chromatin (115).

Although the enhanced affinity for chromatin of the activated receptor has usually

been considered responsible for the accumulation of steroid-receptor complex in the nucleus, there are certain experimental observations that are difficult to reconcile with this simple explanation. It has been found that on exposure to varying amounts of estradiol, either in vivo (43, 118) or in vitro (19), the ratio of nuclear to extranuclear binding remains constant over a wide range of concentrations. If, as indicated by the saturability of nuclear retention in rat target cells by hyperphysiological doses of estradiol in vivo (2, 43), the number of nuclear acceptor sites that avidly bind activated estrogen-receptor complex is limited, one would expect the ratio of extranuclear to nuclear hormone to increase at higher hormone doses where most of the acceptors become occupied. In view of these considerations, it has been proposed (19) that translocation of extranuclear estrogen-receptor complex to the nucleus may not result from nuclear binding of the activated complex but rather from a "cytoplasmic exclusion" process, similar to that postulated to explain intracellular movement of solutes in oocytes (35), in which an excluded solute is distributed in accordance with the volume of the intracellular solution to which it has free access. Obviously more experimental information, perhaps obtained by using novel approaches, is required before the details of the interaction of hormone-receptor complexes in target cell nuclei can be elucidated.

IV. Estrogen-Receptor Complexes and RNA Synthesis

For all classes of steroid hormones, an early response is the enhancement of RNA synthesis in target cells. In the case of the primary stimulation of a hormone-deprived tissue, such as the immature rat uterus, incorporation of labeled precursors into all types of RNA is accelerated after the administration of estrogen (7, 24, 30, 57, 64, 116), although some early observations of extremely rapid stimulation probably reflect increased transport of nucleotide precursors rather than actual enhancement of synthesis itself (6, 28a, 79a). An especially rapid effect is on the production or processing of high molecular weight RNA (51a, 56a, 57, 58, 64) and on the synthesis of mRNA for a characteristic "induced protein" (17, 119), the synthesis of which is an early response to estrogenic stimulation (53, 77). In the secondary stimulation of a previously developed tissue, the effect of estrogen is predominantly to enhance the production of mRNAs required in the synthesis of specific proteins for export, such as ovalbumin and conalbumin in the estrogen-pretreated chick oviduct and vitellogenin in the stimulated frog liver. A detailed account of the many experiments concerning the molecular biology of the effect of steroid hormones on transcription is beyond the scope of this paper. We consider here the evidence for the participation of steroid-receptor complexes in the tissue-specific stimulation of RNA synthesis and the importance of receptor activation in this phenomenon.

Although the complete action of hormone-receptor complexes in target cell nuclei may involve various aspects of RNA synthesis and processing, a particularly striking effect is seen on RNA polymerase systems. It was first shown that the administration of testosterone to castrated rats leads to an increase in the ability of their isolated prostatic nuclei to incorporate labeled precursors into RNA (31), while similar enhancement of RNA polymerase activity was observed in uterine nuclei from rats injected with estradiol (22, 30). The template function of uterine chromatin from estrogen-treated rats (5, 110) or rabbits (13), or of oviduct chromatin from estrogen-treated chicks (98), is increased over that of corresponding chromatins from untreated animals. After estradiol injection, both nucleolar (I) and nucleoplasmic (II) RNA polymerases are stimulated in rat (21, 32) or rabbit (8) uterine nuclei but with different time patterns. Polymerase II activity shows an increase at 15 to 30 minutes

and then subsides to be followed by a second rise after 2 to 3 h, whereas polymerase I activity, as well as template function of the uterine chromatin, shows a prolonged enhancement, first detectable at about 1 h. The transient early increase in polymerase II activity is not in itself sufficient for hormonal response, because a single injection of estriol, which induces the transient stimulation of polymerase II but not the prolonged effect on both polymerase I and polymerase II, does not promote appreciable uterine growth (32). There is evidence, however, that the enhancement of polymerase I is somehow dependent on the initial stimulation and action of polymerase II, inasmuch as the increase in polymerase I is blocked by α-amanitin (8, 90), a substance that selectively inhibits the action of polymerase II. Moreover, the stimulation of polymerase I, but not polymerase II, is inhibited by the administration of cycloheximide (8), which suggests that protein synthesis is a prerequisite for the estrogen-induced enhancement of polymerase I activity, a conclusion that also has been reached on the basis of different experimental findings (75).

The participation of hormone-receptor complexes in the enhancement of RNA synthesis in target cell nuclei is substantiated by their direct effect on the RNA polymerase activity of isolated nuclei or on the template function of target cell chromatin. The RNA polymerase I activity of uterine nuclei, while not affected by estradiol itself, is doubled after the nuclei are incubated with estradiol in the presence of uterine cytosol containing the receptor (89). Only the activated or nuclear form of the estrogen-receptor complex is effective in stimulating RNA synthesis in isolated uterine nuclei, and the effect is specific for nuclei of hormone-dependent tissues (48). Nuclei of hormone-dependent rat mammary tumors resemble uterine nuclei in the sensitivity of their RNA polymerase systems to enhancement by estrogen-receptor complex in vitro, whereas nuclei from autonomous tumors are not susceptible to such stimulation (3). With either bacterial or endometrial RNA polymerase enzyme, the template function of chromatin isolated from target, but not from nontarget, cells is significantly increased after exposure to estradiol-receptor complex in vitro (48). This tissue-specific effect of estrogen-receptor complex on polymerase I activity is difficult to reconcile with the above-mentioned evidence suggesting a need for polymerase II stimulation and protein synthesis in the estrogenic enhancement of uterine polymerase I in vivo. Moreover, stimulation of endogenous RNA polymerase I activity has been observed by the direct addition of estradiol-receptor complex to chromatin from chick liver nucleoli (5a).

In case of androgenic hormones, incubation of prostatic nuclei with the dihydrotestosterone-receptor complex of prostatic cytosol leads to the enhancement of both polymerase I and polymerase II activities, with the largest effect seen on the polymerase I system, which also is stimulated in isolated nucleoli by exposure to the androgen-receptor complex (16). Highly purified progesterone-receptor complex from chick oviduct enhances the template function of chick oviduct chromatin, but not of liver or erythrocyte chromatin or of chick DNA, when the number of initiation sites is determined with a bacterial polymerase enzyme (97).

From the foregoing observations it appears that hormone-dependent tissues and tumors have a characteristic limitation in the activity of their RNA synthesizing or processing systems, probably involving, at least in part, a restriction on chromatin template function, which can be alleviated by an activated hormone-receptor complex of extranuclear origin. As mentioned earlier, full stimulation of uterine growth appears to require the continued presence of estrogen-receptor complexes in the nucleus for a period of several hours (2, 25). Thus it appears that the uterotrophic effect of estrogen involves a process requiring the ongoing participation of substantial numbers of hormone-receptor complexes rather than

the triggering of an initial event at a few specific gene sites.

V. Immunochemical Approaches to Receptor Studies

As mentioned above, essentially all our information about the occurrence, properties, and interactions of steroid hormone receptors has been obtained from experiments that involve the radioactive hormone as a marker for the receptor to which it binds. The recent preparation of antibodies to purified estrophilin (27, 28) provides for the first time a means to detect the receptor in the absence of bound steroid.

Immunoglobulin from the serum of rabbits or goats, immunized with highly purified preparations of the nuclear form of the estradiol-receptor complex of calf uterus, reacts with estradiol-receptor complexes to yield nonprecipitating products without release of the bound hormone. Thus, in addition to using conventional techniques of immunochemistry, one can conveniently study the interaction of antibody with estrophilin by examining its effect on the sedimentation properties of the receptor or on its elution in gel filtration, with the radioactive steroid as a marker. The antibody reacts with both nuclear and extranuclear estrophilin, not only of calf uterus from which the immunogen was obtained, but also from estrogen-dependent tissues of all species so far tested, including rat, mouse, guinea-pig, rabbit, sheep, and monkey uterus; monkey and hen oviduct; rat mammary, endometrial and pituitary tumors; and human breast cancer.

Nuclear and extranuclear forms of estrophilin differ in their reaction pattern with the antibody. The purified calf nuclear estradiol-receptor complex reacts with the rabbit antibody to form a single, more rapidly sedimenting product (11–12 S), crude nuclear complexes from various species yield this entity in addition to an apparently smaller (8 S) product, whereas extranuclear estradiol-receptor complexes show only the slower peak, which sediments at 7.5 S in salt-containing sucrose gradients. After hormone-induced activation, the cytosol receptor complex resembles the nuclear one in showing both the 8 S and 11-12 S peaks in the presence of antibody.

The fact that antibodies raised against the nuclear form of estrophilin crossreact with extranuclear as well as nuclear estrogen receptors from many tissues provides additional evidence for the concept that the nuclear receptor is derived from the translocation of the cytosol receptor (Table 1). The greater increase in sedimentation rate observed with activated or nuclear estrophilin suggests that the nuclear receptor can bind more antibody units per molecule than the extranuclear form, consistent with the proposal that receptor activation may involve dimerization of native estrophilin units.

In contrast to their reactivity with estrogen-receptor complexes from various sources, antibodies to estrophilin do not crossreact with either androgen-receptor complexes of rat prostate or with progesterone-receptor complexes from rabbit uterus, rat endometrial tumor or chick oviduct. Thus there is immunochemical similarity among estrophilins from different tissues of a wide variety of species, but receptors for different classes of sex hormones appear to be immunologically distinct. These crossreacting estrophilin-specific antibodies offer promise as useful reagents for the purification and assay of estrophilin through immunochemical techniques and for providing insight into many of the unresolved questions of receptor synthesis and activation, as well as intracellular localization before receptor distribution is perturbed by the entry of hormone.

VI. Summary

A principal action of steroid hormones takes place in the nucleus, mediated through the reversible interaction of a steroid-receptor complex with target cell chromatin. As illustrated for the case of estrogens, reaction of steroid hormones with

their responsive cells takes place by a two-step process in which the hormone binds to an extranuclear receptor protein, inducing its conversion to an activated form with a strong affinity for chromatin. Either before or after hormone-induced activation, the steroid-receptor complex is translocated to the nucleus, where it binds in the chromatin and in some way enhances the synthesis and/or processing of RNA. Present knowledge concerning steroid-receptor interaction in hormone-dependent tissues has been derived from experiments with the radioactive steroid as a marker for the receptor. The recent preparation of specific antibodies to the estrogen receptor protein promises a new approach to unresolved questions of receptor structure, localization, and function.

REFERENCES

1. ANDERSON, J. N., PECK, E. J., JR., AND CLARK, J. H.: Nuclear receptor-estrogen complex: Relationship between concentration and early uterotropic responses. Endocrinology **92**: 1488–1495, 1973.
2. ANDERSON, J. N., PECK, E. J., JR., AND CLARK, J. H.: Estrogen-induced uterine responses and growth: Relationship to receptor estrogen binding by uterine nuclei. Endocrinology **96**: 160–167, 1975.
3. ARBOGAST, L. Y., AND DESOMBRE, E. R.: Estrogen-dependent *in vitro* stimulation of RNA synthesis in hormone-dependent mammary tumors of the rat. J. Nat. Cancer Inst. **54**: 483–485, 1975.
4. ARNAUD, M., BEZIAT, Y., GUILLEUX, J. C., AND MOUSSERON-CANET, M.: Les RNA polymérases dans l'endomètre utérin de génisse. Stimulation de la RNA polymérase nucléolaire par le complexe cytoplasmique 5 S-oestradiol. C. R. Hebd. Séances Acad. Sci. Paris. **272**: 635–638, 1971.

4a. BAILLY, A., SALLAS, N., AND MILGROM, E.: A low molecular weight inhibitor of steroid receptor activation. J. Biol. Chem. **252**: 858–863, 1977.

5. BARKER, K. L., AND WARREN, J. C.: Template capacity of uterine chromatin: control by estradiol. Proc. Nat. Acad. Sci. U.S.A. **56**: 1298–1302, 1966.

5a. BIERI-BONNIOT, F., JOSS, U., AND DIERKS-VENTLING, C.: Stimulation of RNA polymerase I activity by 17β-estradiol-receptor complex on chick liver nucleolar chromatin. Fed. Eur. Biochem. Soc. Lett. **81**: 91–96, 1977.

6. BILLING, R. J., BARBIROLI, B., AND SMELLIE, R. M. S.: The mode of action of oestradiol. I. The transport of RNA precursors into the uterus. Biochim. Biophys. Acta **190**: 52–59, 1969.
7. BILLING, R. J., BARBIROLI, B., AND SMELLIE, R. M. S.: Changes in the patterns of synthesis of ribonucleic acid species in immature rat uterus in response to oestradiol-17β. Biochem. J. **112**: 563–569, 1969.
8. BORTHWICK, N. M., AND SMELLIE, R. M. S.: The effects of oestradiol-17β on the ribonucleic acid polymerases of immature rabbit uterus. Biochem. J. **147**: 91–101, 1975.
9. BRECHER, P. I., VIGERSKY, R., WOTIZ, H. S., AND WOTIZ, H. H.: An *in vitro* system for the binding of estradiol to rat uterine nuclei. Steroids **10**: 635–650, 1967.
10. BULLER, R. E., TOFT, D. O., SCHRADER, W. T., AND O'MALLEY, B. W.: Progesterone-binding components of chick oviduct. VIII. Receptor activation and hormone-dependent binding to purified nuclei. J. Biol. Chem. **250**: 801–808, 1975.
11. CHAMNESS, G. C., JENNINGS, A. W., AND MCGUIRE, W. L.: Oestrogen receptor binding is not restricted to target nuclei. Nature (London) **241**: 458–460, 1973.
12. CHEN, T. J., AND LEAVITT, W. W.: Nuclear progesterone receptor in hamster uterus: Measurement by tritiated progesterone exchange during the estrus cycle. Endocrinology **104**: 1588–1597, 1979.
13. CHURCH, R. B., AND MCCARTHY, B. J.: Unstable nuclear RNA synthesis following estrogen stimulation. Biochim. Biophys. Acta **199**: 103–114, 1970.
14. CLARK, J. H., AND GORSKI, J.: Estrogen receptors. An evaluation of cytoplasmic-nuclear interactions in a cell-free system and a method for assay. Biochim. Biophys. Acta **192**: 508–515, 1969.
15. CLEVER, U., AND KARLSON, P.: Induktion von Puff-veränderungen in den Speicheldrüsenchromosomen von *Chironomus tentans* durch Ecdyson. Exp. Cell. Res. **20**: 623–626, 1960.
16. DAVIES, P., AND GRIFFITHS, K.: Further studies on the stimulation of prostatic ribonucleic acid polymerase by 5α-dihydrotestosterone-receptor complexes. J. Endocrinol. **62**: 385–400, 1974.
17. DEANGELO, A. B., AND GORSKI, J.: Role of RNA synthesis in the estrogen induction of a specific uterine protein. Proc. Nat. Acad. Sci. U.S.A. **66**: 693–700, 1970.
18. ERDOS, T.: Properties of a uterine oestradiol receptor. Biochem. Biophys. Res. Commun. **37**: 338–343, 1968.
19. GANNON, F., KATZENELLENBOGEN, B., STANCEL, G., AND GORSKI, J.: Estrogen-receptor movement to the nucleus: Discussion of a cytoplasmic exclusion hypothesis. *In* The Molecular Biology of Hormone Action, ed. by J. Papaconstantinou, pp. 137–149, Academic Press, New York, 1976.
20. GLASCOCK, R. F., AND HOEKSTRA, W. G.: Selective accumulation of tritium-labelled hexoestrol by the reproductive organs of immature female goats and sheep. Biochem. J. **72**: 673–682, 1959.
21. GLASSER, S. R., CHYTIL, F., AND SPELSBERG, T. C.: Early effects of oestradiol-17β on the chromatin and activity of the deoxyribonucleic acid-dependent ribonucleic acid polymerases (I and II) of the rat uterus. Biochem. J. **130**: 947–957, 1972.
22. GORSKI, J.: Early estrogen effects on the activity of uterine ribonucleic acid polymerase. J. Biol. Chem. **239**: 889–892, 1964.
23. GORSKI, J., AND GANNON, F.: Current models of steroid hormone action: A critique. Annu. Rev. Physiol. **38**: 425–450, 1976.
24. GORSKI, J., AND NICOLETTE, J. A.: Early estrogen effects on newly synthesized RNA and phospholipid in subcellular fractions of rat uteri. Arch. Biochem. Biophys. **103**: 418–423, 1963.
25. GORSKI, J., AND RAKER, B.: Estrogen action in the uterus: The requisite for sustained estrogen binding in the nucleus. Gynecol. Oncol. **2**: 249–258, 1974.
26. GORSKI, J., TOFT, D., SHYAMALA, S., SMITH, D., AND NOTIDES, A.: Hormone receptors. Studies on the interaction of estrogens with the uterus. Recent Progr. Hormone Res. **24**: 45–80, 1968.
27. GREENE, G. L., CLOSS, L. E., DESOMBRE, E. R., AND JENSEN, E. V.: Antibodies to estrophilin: Comparison of rabbit and goat antisera. J. Steroid Biochem. **11**: 333–341, 1979.
28. GREENE, G. L., CLOSS, L. E., FLEMING, H., DESOMBRE, E. R., AND JENSEN, E. V.: Antibodies to estrogen receptor: Immunochemical similarity of estrophilin

from various mammalian species. Proc. Nat. Acad. Sci. U.S.A. 74: 3681–3685, 1977.

28a. GREENMAN, D. L.: Uridine uptake by rat uterus. Influence of injection route. Endocrinology 87: 716–722, 1970.

29. GSCHWENDT, M., AND HAMILTON, T. H.: The transformation of the cytoplasmic oestradiol-receptor complex into the nuclear complex in a uterine cell-free system. Biochem. J. 128: 611–616, 1972.

30. HAMILTON, T. H., WIDNELL, C. C., AND TATA, J. R.: Synthesis of ribonucleic acid during early estrogen action. J. Biol. Chem. 243: 408–417, 1968.

31. HANCOCK, R. L., ZELIS, R. F., SHAW, M., AND WILLIAMS-ASHMAN, H. G.: Incorporation of ribonucleoside triphosphates into ribonucleic acid by nuclei of the prostate gland. Biochim. Biophys. Acta 55: 257–260, 1962.

32. HARDIN, J. W., CLARK, J. H., GLASSER, S. R., AND PECK, E. J., JR.: RNA polymerase activity and uterine growth: Differential stimulation by estradiol, estriol and nafoxidine. Biochemistry 15: 1370–1374, 1976.

33. HARRIS, G. S.: Nature of oestrogen-specific binding sites in the nuclei of mouse uteri. Nature (London) New Biol. 231: 246–248, 1971.

34. HIGGINS, S. J., ROUSSEAU, G. G., BAXTER, J. D., AND TOMKINS, G. M.: Nuclear binding of steroid receptors: Comparison in intact cells and cell-free systems. Proc. Nat. Acad. Sci. U.S.A. 70: 3415–3418, 1973.

35. HOROWITZ, S. B., AND MOORE, L. C.: The nuclear permeability, intracellular distribution, and diffusion of inulin in the amphibian oocyte. J. Cell Biol. 60: 405–415, 1974.

36. JACKSON, V., AND CHALKLEY, R.: The cytoplasmic estradiol receptors of bovine uterus: Their occurrence, interconversion, and binding properties. J. Biol. Chem. 249: 1627–1636, 1974.

37. JENSEN, E. V.: Metabolic fate of sex hormones in target tissues with regard to tissue specificity. Proc. 2nd Int. Congr. Endocrinol., London, 1964, pp. 420–433. Excerpta Medica Foundation, Amsterdam, 1965.

38. JENSEN, E. V.: Mechanism of estrogen action in relation to carcinogenesis. Can. Cancer Conf. 6: 143–165, 1965.

39. JENSEN, E. V.: Receptor proteins: Past, present and future. Res. Steroids 7: 1–36, 1977.

40. JENSEN, E. V., AND DESOMBRE, E. R.: Mechanism of action of the female sex hormones. Annu. Rev. Biochem. 41: 203–230, 1972.

41. JENSEN, E. V., AND DESOMBRE, E. R.: Estrogen-receptor interaction. Science 182: 126–134, 1973.

42. JENSEN, E. V., DESOMBRE, E. R., HURST, D. J., KAWASHIMA, T., AND JUNGBLUT, P. W.: Estrogen-receptor interactions in target tissues. Arch. Anat. Micros. Morphol. Exp. 56:suppl, 547–569, 1967.

43. JENSEN, E. V., DESOMBRE, E. R., AND JUNGBLUT, P. W.: Interaction of estrogens with receptor sites *in vivo* and *in vitro*. Proc. 2nd Int. Congr. Hormonal Steroids, 1966, pp. 492–500. Excerpta Medica Foundation, Amsterdam, 1967.

44. JENSEN, E. V., DESOMBRE, E. R., JUNGBLUT, P. W., STUMPF, W. E., AND ROTH, L. J.: Biochemical and autoradiographic studies of ^{3}H-estradiol localization. *In* Autoradiography of Diffusible Substances, ed. by L. J. Roth and W. E. Stumpf, pp. 81–97, Academic Press, New York, 1969.

45. JENSEN, E. V., AND JACOBSON, H. I.: Fate of steroid estrogens in target tissues. *In* Biological Activities of Steroids in Relation to Cancer, ed. by G. Pincus and E. P. Vollmer, pp. 161–178. Academic Press, New York, 1960.

46. JENSEN, E. V., AND JACOBSON, H. I.: Basic guides to the mechanism of estrogen action. Recent Progr. Hormone Res. 18: 387–414, 1962.

47. JENSEN, E. V., JACOBSON, H. I., SMITH, S., JUNGBLUT, P. W., AND DESOMBRE, E. R.: The use of estrogen antagonists in hormone receptor studies. Gynecol. Invest. 3: 108–122, 1972.

48. JENSEN, E. V., MOHLA, S., GORELL, T. A., AND DESOMBRE, E. R.: The role of estrophilin in estrogen action. Vitamins Hormones 32: 89–127, 1974.

48a. JENSEN, E. V., MOHLA, S., GORELL, T., TANAKA, S., AND DESOMBRE, E. R.: Estrophile to nucleophile in two easy steps. J. Steroid Biochem. 3: 445–458, 1972.

49. JENSEN, E. V., NUMATA, M., BRECHER, P. I., AND DESOMBRE, E. R.: Hormone-receptor interaction as a guide to biochemical mechanism. Biochem. Soc. Symp. 32: 133–159, 1971.

50. JENSEN, E. V., NUMATA, M., SMITH, S., SUZUKI, T., BRECHER, P. I., AND DESOMBRE, E. R.: Estrogen-receptor interaction in target tissues. Develop. Biol. suppl. 3: 151–171, 1969.

51. JENSEN, E. V., SUZUKI, T., KAWASHIMA, T., STUMPF, W. E., JUNGBLUT, P. W., AND DESOMBRE, E. R.: A two-step mechanism for the interaction of estradiol with the rat uterus. Proc. Nat. Acad. Sci. U.S.A. 58: 632–638, 1968.

51a. JOEL, P. B., AND HAGERMAN, D. D.: Extraction of RNA from rat uterus. Biochim. Biophys. Acta 195: 328–339, 1969.

52. JUNGBLUT, P. W., HÄTZEL, I., DESOMBRE, E. R., AND JENSEN, E. V.: Über Hormone-"Receptoren;" die oestrogenbindenen Prinzipien der Erfolgsorgane. Colloq. Ges. Physiol. Chem. 18: 58–86, 1967.

52a. KALIMI, M., TSAI, S. Y., TSAI, M.-J., CLARK, J. H., AND O'MALLEY, B. W.: Effect of estrogen on gene expression in the chick oviduct. Correlation between nuclear-bound estrogen receptor and chromatin initiation sites for transcription. J. Biol. Chem. 251: 516–523, 1976.

53. KATZENELLENBOGEN, B., AND GORSKI, J.: Estrogen action *in vitro*: Induction of the synthesis of a specific uterine protein. J. Biol. Chem. 247: 1299–1305, 1972.

54. KING, R. J. B., AND GORDON, J.: The localization of [6, 7-^{3}H] oestradiol in rat uterus. J. Endocrinol. 34: 431–437, 1966.

55. KING, R. J. B., GORDON, J., COWAN, D. M., AND INMAN, D. R.: The intracellular localization of [6, 7-^{3}H] oestradiol-17β in dimethylbenzanthracene-induced rat mammary adenocarcinoma and other tissues. J. Endocrinol. 36: 139–150, 1966.

56. KING, R. J. B., AND MAINWARING, W. I. P.: Steroid-Cell Interactions. University Park Press, Baltimore, 1974.

56a. KNECHT, D. A., AND LUCK, D. N.: Synthesis and processing of ribosomal RNA by the uterus of the ovariectomised adult rat during early oestrogen action. Nature (London) 266: 563–564, 1977.

57. KNOWLER, J. T., AND SMELLIE, R. M. S.: The synthesis of ribonucleic acid in immature rat uterus responding to oestradiol-17β. Biochem. J. 125: 605–614, 1971.

58. KNOWLER, J. T., AND SMELLIE, R. M. S.: The oestrogen-stimulated synthesis of heterogenous nuclear ribonucleic acid in the uterus of immature rats. Biochem. J. 131: 689–697, 1973.

59. KORENMAN, S. G., AND RAO, B. R.: Reversible disaggregation of the cytosol estrogen binding protein of uterine cytosol. Proc. Nat. Acad. Sci. U.S.A. 61: 1028–1033, 1968.

60. LIAO, S.: Cellular receptors and mechanism of action of steroid hormones. Int. Rev. Cytol. 41: 87–172, 1975.

61. LIAO, S., LIANG, T., AND TYMOCZKO, J.: Ribonucleoprotein binding of steroid-"receptor" complexes. Nature (London) New Biol. 241: 211–213, 1973.

61a. LINKIE, D. M., AND SITTERI, P. K.: A re-examination of the interaction of estradiol with target cell receptors. J. Steroid Biochem. 9: 1071–1078, 1978.

62. LITTLE, M., SZENDRO, P. I., AND JUNGBLUT, P. W.: Hormone-mediated dimerization of microsomal estradiol receptor. Hoppe-Seyler's Z. Physiol. Chem. 354: 1599–1610, 1973.

63. LITTLE, M., SZENDRO, P., TERAN, C., HUGHES, A., AND JUNGBLUT, P. W.: Biosynthesis and transformation of microsomal and cytosol estradiol receptors. J. Steroid Biochem. 6: 493–500, 1975.
64. LUCK, D. N., AND HAMILTON, T. H.: Early estrogen action: Stimulation of the metabolism of high molecular weight and ribosomal RNAs. Proc. Nat. Acad. Sci. U.S.A. 69: 157–161, 1972.
65. MAINWARING, W. I. P., AND PETERKEN, B. M.: A reconstituted cell-free system for the specific transfer of steroid-receptor complexes into nuclear chromatin isolated from rat ventral prostate gland. Biochem. J. 125: 285–295, 1971.
66. MARVER, D., GOODMAN, D., AND EDELMAN, I. S.: Relationships between renal cytoplasmic and nuclear aldosterone-receptors. Kidney Int. 1: 210–223, 1972.
67. MAURER, H. R., AND CHALKLEY, R.: Some properties of a nuclear binding site of estradiol. J. Mol. Biol. 27: 431–441, 1967.
68. MCGUIRE, W. L., HUFF, K., AND CHAMNESS, G. C.: Temperature-dependent binding of estrogen receptor to chromatin. Biochemistry 11: 4562–4565, 1972.
69. MESTER, J., AND BAULIEU, E. E.: Nuclear estrogen receptor of chick liver. Biochim. Biophys. Acta 261: 236–244, 1972.
70. MILGROM, E., ATGER, M., AND BAULIEU, E. E.: Acidophilic activation of steroid hormone receptors. Biochemistry 12: 5196–5205, 1973.
71. MUELLER, G. C., GORSKI, J., AND AIZAWA, Y.: The role of protein synthesis in early estrogen action. Proc. Nat. Acad. Sci. U.S.A. 47: 164–169, 1961.
72. MUELLER, G. C., HERRANEN, A. M., AND JERVELL, K. J.: Studies on the mechanism of action of estrogens. Recent Progr. Hormone Res. 14: 95–129, 1958.
73. MULVIHILL, E. R., AND PALMITER, R. D.: Relationship of nuclear estrogen receptor levels to induction of ovalbumin and conalbumin mRNA in chick oviduct. J. Biol. Chem. 252: 2060–2068, 1977.
74. MUSLINER, T. A., AND CHADER, G. J.: A role for DNA in the formation of nuclear estradiol-receptor complex in a cell-free system. Biochem. Biophys. Res. Commun. 45: 996–1003, 1971.
74a. MUSLINER, T. A., CHADER, G. J., AND VILLEE, C. A.: Studies on estradiol receptors of the rat uterus. Nuclear uptake *in vitro*. Biochemistry 9: 4448–4453, 1970.
75. NICOLETTE, J. A., LEMAHIEU, M. A., AND MUELLER, G. C.: A role of estrogens in the regulation of RNA polymerase in surviving rat uterus. Biochim. Biophys. Acta 166: 403–409, 1968.
76. NOTEBOOM, W. D., AND GORSKI, J.: Stereospecific binding of estrogens in rat uterus. Arch. Biochem. Biophys. 111: 559–568, 1965.
77. NOTIDES, A., AND GORSKI, J.: Estrogen-induced synthesis of a specific uterine protein. Proc. Nat. Acad. Sci. U.S.A. 56: 230–235, 1966.
78. NOTIDES, A. C., HAMILTON, D. E., AND AUER, H. E.: A kinetic analysis of the estrogen receptor transformation. J. Biol. Chem. 250: 3945–3950, 1975.
79. NOTIDES, A. C., AND NIELSEN, S.: The molecular mechanism of the *in vitro* 4S to 5S transformation of the uterine estrogen receptor. J. Biol. Chem. 249: 1866–1873, 1974.
79a. OLIVER, J. M.: The effects of oestradiol on nucleoside transport in rat uterus. Biochem. J. 121: 83–88, 1971.
80. O'MALLEY, B. W., AND MCGUIRE, W. L.: Progesterone-induced synthesis of a new species of nuclear RNA. Endocrinology 84: 63–68, 1969.
81. O'MALLEY, B. W., AND MEANS, A. R.: Female steroid hormones and target cell nuclei. Science 183: 610–620, 1974.
82. O'MALLEY, B. W., SCHWARTZ, R. J., AND SCHRADER, W. T.: A review of regulation of gene expression by steroid hormone receptors. J. Steroid Biochem. 7: 1151–1159, 1976.
83. OZON, R., AND BELLE, R.: Récepteurs de l'oestradiol-17β dans le foie de poule et de l'amphibien *Discoglossus pictus*. Biochim. Biophys. Acta 297: 155–163, 1973.
84. PALMITER, R. D., MOORE, P. B., AND MULVIHILL, E. R.: A significant lag in the induction of ovalbumin messenger RNA by steroid hormones: a receptor translocation hypothesis. Cell 8: 557–572, 1976.
85. PUCA, G. A., AND BRESCIANI, F.: Receptor molecule for oestrogens from rat uterus. Nature (London) 218: 967–969, 1968.
86. PUCA, G. A., NOLA, E., HIBNER, U., CICALA, G., AND SICA, V.: Interaction of the estradiol receptor from calf uterus with its nuclear acceptor sites. J. Biol. Chem. 250: 6452–6459, 1975.
87. PUCA, G. A., SICA, V., AND NOLA, E.: Indentification of a high affinity nuclear acceptor site for estrogen receptor of calf uterus. Proc. Nat. Acad. Sci. U.S.A. 71: 979–983, 1974.
88. RASPÉ, G. (ed).: Schering Workshop on Steroid Hormone Receptors. Advances in the Biosciences, vol. 7. Pergamon-Vieweg, Braunschweig, 1971.
89. RAYNAUD-JAMMET, C., AND BAULIEU, E. E.: Action de l'oestradiol *in vitro*: Augmentation de la biosynthèse d'acide ribonucléique dans les noyaux utérine. C. R. Hebd. Séances Acad. Sci. Paris 268: 3211–3214, 1969.
90. RAYNAUD-JAMMET, C., CATELLI, M. G., AND BAULIEU, E. E.: Inhibition by α-amanitin of the oestradiol-induced increase in α-amanitin insensitive RNA polymerase in immature rat uterus. Fed. Eur. Biochem. Soc. Lett 22: 93–96, 1972.
91. ROUSSEAU, G. G.: Interaction of steroids with hepatoma cells: Molecular mechanisms of glucocorticoid hormone action. J. Steroid Biochem. 6: 75–89, 1975.
92. ROUSSEAU, G. G., BAXTER, J. D., HIGGINS, S. J., AND TOMKINS, G. M.: Steroid-induced nuclear binding of glucocorticoid receptors in intact hepatoma cells. J. Mol. Biol. 79: 539–554, 1973.
93. ROY, S., MAHESH, V. B., AND GREENBLATT, R. B.: Inhibition of uptake of radioactive estradiol by the uterus and pituitary gland of immature rats. Acta Endocrinol. 47: 669–675, 1964.
94. SAFFRAN, J., LOESER, B. K., BOHNETT, S. A., AND FABER, L. E.: Binding of progesterone receptor by nuclear preparations of rabbit and guinea pig uterus. J. Biol. Chem. 251: 5607–5613, 1976.
95. SARFF, M., AND GORSKI, J.: Control of estrogen binding protein concentration under basal conditions and after estrogen administration. Biochemistry 10: 2557–2563, 1971.
96. SCHRADER, W. T., TOFT, D. O., AND O'MALLEY, B. W.: Progesterone-binding protein of chick oviduct. VI. Interaction of purified progesterone-receptor components with nuclear constituents. J. Biol. Chem. 247: 2401–2407, 1972.
97. SCHWARTZ, R. J., KUHN, R. W., BULLER, R. E., SCHRADER, W. T., AND O'MALLEY, B. W.: Progesterone-binding components of chick oviduct. *In vitro* effects of purified hormone-receptor complexes on the initiation of RNA synthesis in chromatin. J. Biol. Chem. 251: 5166–5177, 1976.
98. SCHWARTZ, R. J., TSAI, M. J., TSAI, S. Y., AND O'MALLEY, B. W.: Effect of estrogen on gene expression in the chick oviduct. V. Changes in the number of RNA polymerase binding and initiation sites in chromatin. J. Biol. Chem. 250: 5175–5182, 1975.
98a. SENIOR, M. B., AND FRANKEL, F. R.: Evidence for two kinds of chromatin binding sites for the estradiol-receptor complex. Cell 14: 857–863, 1978.
99. SHYAMALA, G., AND GORSKI, J.: Estrogen receptors in the rat uterus. Studies on the interaction of cytosol and nuclear binding sites. J. Biol. Chem. 244: 1097–1103, 1969.
100. SIITERI, P. K., SCHWARZ, B. E., MORIYAMA, I., ASHBY, R., LINKIE, D., AND MACDONALD, P. C.: Estrogen

binding in the rat and human. *In* Receptors for Reproductive Hormones, ed. by B. W. O'Malley and A. R. Means, pp. 97–112, Plenum Press, New York, 1973.

101. SONNENSCHEIN, C., SOTO, A. M., COLOFIORE, J., AND FAROOKHI, R.: Estrogen target cells; establishment of a cell line derived from rat pituitary tumor MtT/F_4. Exp. Cell Res. **101**: 15–22, 1976.
102. SPELSBERG, T. C., STEGGLES, A. W., CHYTIL, F., AND O'MALLEY, B. W.: Progesterone-binding components of chick oviduct. V. Exchange of progesterone-binding capacity from target to nontarget tissue chromatins. J. Biol. Chem. **247**: 1368–1374, 1972.
103. STEGGLES, A. W., SPELSBERG, T. C., GLASSER, S. R., AND O'MALLEY, B. W.: Soluble complexes between steroid hormones and target-tissue receptors bind specifically to target-tissue chromatin. Proc. Nat. Acad. Sci. U.S.A. **68**: 1479–1482, 1971.
104. STONE, G. M.: The uptake of tritiated oestrogens by various organs of the ovariectomized mouse following subcutaneous administration. J. Endocrinol. **27**: 281–288, 1963.
105. STONE, G. M.: The effect of estrogen antagonists on the uptake of tritiated estradiol by the uterus and vagina of the ovariectomized mouse. J. Endocrinol. **29**: 127–136, 1964.
106. STONE, G. M., AND BAGGETT, B.: The *in vitro* uptake of tritiated estradiol and estrone by the uterus and vagina of the ovariectomized mouse. Steroids **5**: 809–826, 1965.
107. STUMPF, W. E.: Subcellular distribution of ^{3}H-estradiol in rat uterus by quantitative autoradiography: A comparison between ^{3}H-estradiol and ^{3}H-norethynodrel. Endocrinology **83**: 777–782, 1968.
108. STUMPF, W. E.: Nuclear concentration of ^{3}H-estradiol in target tissues. Dry-mount autoradiography of vagina, oviduct, ovary, testis, mammary tumor, liver and adrenal. Endocrinology **85**: 31–37, 1969.
109. TALWAR, G. P., SEGAL, S. J., EVANS, A., AND DAVIDSON, O. W.: The binding of estradiol in the uterus: a mechanism for derepression of RNA synthesis. Proc. Nat. Acad. Sci. U.S.A. **52**: 1059–1066, 1964.
110. TENG, C. S., AND HAMILTON, T. H.: The role of chromatin in estrogen action in the uterus. I. The control of template capacity and chemical composition and the binding of H^3-estradiol-17β. Proc. Nat. Acad. Sci. U.S.A. **60**: 1410–1417, 1968.
111. TERENIUS, L.: Specific uptake of oestrogens by the mouse uterus *in vitro*. Acta Endocrinol. **53**: 611–618, 1966.
112. TOFT, D., AND GORSKI, J.: A receptor molecule for estrogens: Isolation from the rat uterus and preliminary characterization. Proc. Nat. Acad. Sci. U.S.A. **55**: 1574–1581, 1966.
113. TOFT, D., SHYAMALA, G., AND GORSKI, J.: A receptor molecule for estrogens. Studies using a cell-free system. Proc. Nat. Acad. Sci. U.S.A. **57**: 1740–1743, 1967.
114. TRUONG, H., AND BAULIEU, E. E.: Interaction of uterus cytosol receptor with estradiol. Equilibrium and kinetic studies. Biochim. Biophys. Acta **237**: 167–172, 1971.
115. TSAI, S. Y., TSAI, M.-J., HARRIS, S. E., AND O'MALLEY, B. W.: Effects of estrogen on gene expression in the chick oviduct. Control of ovalbumin gene expression by nonhistone proteins. J. Biol. Chem. **251**: 6475–6478, 1976.
116. UI, H., AND MUELLER, G. C.: The role of RNA synthesis in early estrogen action. Proc. Nat. Acad. Sci. U.S.A. **50**: 256–260, 1963.
117. WEICHMAN, B. M., AND NOTIDES, A. C.: Estradiol-binding kinetics of the activated and non-activated estrogen receptor. J. Biol. Chem. **252**: 8856–8862, 1977.
118. WILLIAMS, D., AND GORSKI, J.: Kinetic and equilibrium analysis of estradiol in uterus: A model of binding site distribution in uterine cells. Proc. Nat. Acad. Sci. U.S.A. **69**: 3464–3468, 1972.
119. WIRA, C. R., AND BAULIEU, E. E.: Réponse ribonucléique précoce de l'utérus à l'oestradiol, *in vivo* et *in vitro*. C. R. Hebd. Séances Acad. Sci. Paris. **274**: 73–76, 1972.
120. YAMAMOTO, K. R., AND ALBERTS, B. M.: *In vitro* conversion of estradiol-receptor protein to its nuclear form: Dependence on hormone and DNA. Proc. Nat. Acad. Sci. U.S.A. **69**: 2105–2109, 1972.
121. YAMAMOTO, K. R., AND ALBERTS, B. M.: The interaction of estradiol-receptor protein with the genome: An argument for the existence of undetected specific sites. Cell **4**: 301–310, 1975.
122. YAMAMOTO, K. R., AND ALBERTS, B. M.: Steroid receptors: Elements for modulation of eukaryotic transcription. Annu. Rev. Biochem. **45**: 721–746, 1976.
123. YAMAMOTO, K. R., STAMPFER, M. R., AND TOMKINS, G. M.: Receptors from glucocorticoid-sensitive lymphoma cells and two classes of insensitive clones: Physical and DNA-binding properties. Proc. Nat. Acad. Sci. U.S.A. **71**: 3901–3905, 1974.
124. ZAVA, D. T., AND MCGUIRE, W. L.: Estrogen receptor. Unoccupied sites in nuclei of a breast tumor cell line. J. Biol. Chem. **252**: 3703–3708, 1977.