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The nature and development of steroid hormone receptors*

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Key words. Estrogen; nuclear; cytoplasmic; receptor; steroid; hormone action; receptor development.

Models of steroid hormone action

Our current conceptualization of the primary steps in steroid hormone (estrogens, androgens, progestins and corticoids) action is shown in figure 1[10]. We now believe that the steroids, which are generally hydrophobic molecules, diffuse through the outer cell membrane and cytoplasm to the nucleus[47]. In the nucleus the steroids bind to their respective receptors which are assumed to be bound with low affinity to some nuclear component[35]. This nuclear component could be DNA, nuclear matrix or some chromatin protein (acceptor) but this has not been proven conclusively. As a result of the steroid-receptor interaction, the receptor's conformation changes dramatically and its affinity for nuclear components becomes much higher[11, 13, 51]. This change in the association of receptor with nuclear components is correlated with, and may lead to the rapid (within minutes) changes in gene expression observed in the target tissues[1, 25, 29]. This general model may apply to all the steroid hormones, vitamin D metabolites and thyroxine, but has not been clearly demonstrated for all such hormones.

The model described above has undergone considerable revision in the last few years. Studies in the 1960s had shown that unoccupied steroid receptors were readily solubilized in cytosolic tissue extracts prepared by conventional homogenization procedures[41]. In contrast, the occupied (steroid bound) receptors were not readily solubilized and were bound in high proportion to nuclear fractions[39]. This led to the proposition of the translocation or two-step model of steroid hormone action[12, 16]. In the translocation model the receptor is initially a cytoplasmic protein. Upon binding steroid the receptor undergoes conformational changes which result in the steroid-receptor complex translocating to the nucleus where it binds to its site or sites of action. Early auto-

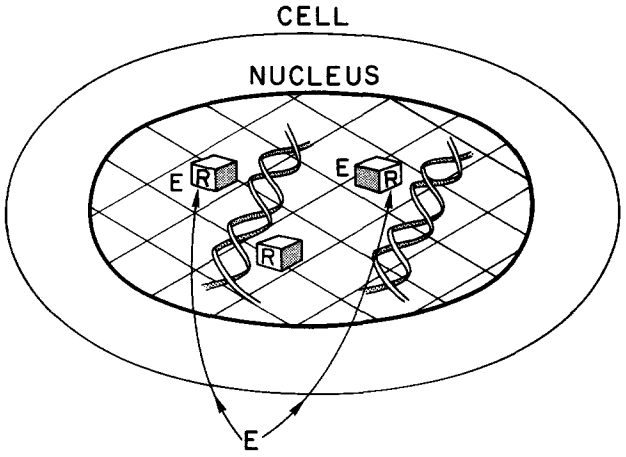

Figure 1. 'New' model of estrogen receptor. R, receptor; E, estrogen; #, nuclear matrix or scaffold; $\mathcal{S}$, DNA[10].

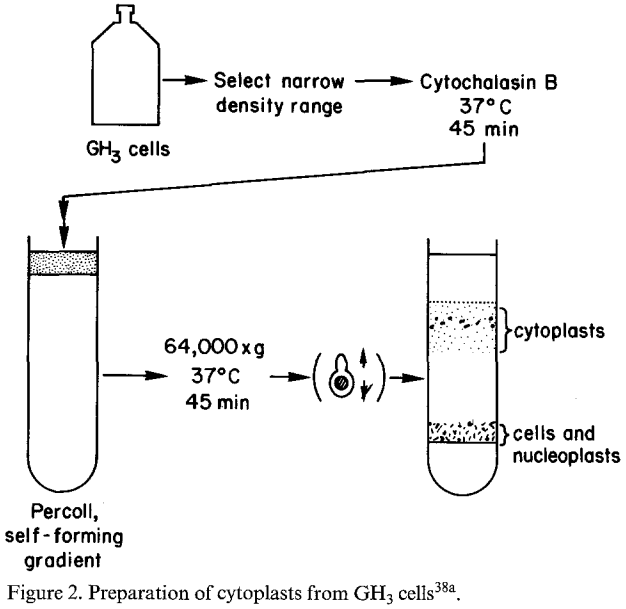

Figure 2. Preparation of cytoplasts from GH_3 cells[38a].

radiography studies were interpreted as supporting the translocation model[16].

While this was the consensus model, not all data were in agreement. Thyroid hormone receptors appeared to be unique as they were only detected in the nuclear compartment of target cells or tissues[30, 36]. The receptor for the vitamin D metabolite, 1,25-dihydroxy cholecalciferol, was reported to be both cytosolic and nuclear depending on homogenization conditions[45]. Sheridan and associates[38] raised some interesting questions about this model of steroid receptor localization after carrying out autoradiographic studies of estrogen receptor localization. They observed much higher nuclear content of unoccupied estrogen receptor and concluded that receptors exist in an equilibrium between nuclear and cytoplasmic compartments. These autoradiographic studies were complicated, as they required studying uptake of labeled estrogen into intact cells at low temperatures.

In the 1980s immunocytochemistry of the estrogen receptor became possible with the purification of receptor and subsequent preparation of monoclonal antibodies. Initially, such studies supported the translocation model[17, 26], but more recent work indicated all the receptor, with or without ligand, was present in the nucleus[21].

At the same time in our laboratory we were attempting to re-examine this problem using a newer method, cytochalasin induced enucleation[46]. In this method, illustrated in figure 2, cytochalasin-treated cells are centrifuged through Percoll gradients which allow the denser nuclei of the cell to centrifuge at a faster rate than the cytoplasm. The nucleoplast (nucleus with a rim of cytoplasm and cell membrane) is thus pulled away from the cytoplast (cytoplasm surrounded by an intact membrane). As shown in figure 3, estrogen receptors are found in only small concentrations in the cytoplasts. Unoccupied receptors are present in the nucleoplasts and the amount of cytoplasm remaining in the nucleoplast is not correlated with receptor concentration (fig. 4). Lactic dehydrogenase, a typical cytoplasmic enzyme, is found in the cytoplasts. We have concluded that the steroid receptors are nuclear proteins that without ligand are bound to nuclear components with low affinity which permits their extraction in low ionic strength homogenization media[10, 20]. We believe that all the steroid receptors are likely to have this characteristic. In support of this concept, recent autoradiographic studies have suggested that the unoccupied progesterone receptor is nuclear[9]. Furthermore, Welshons et al.[48] have recently demonstrated, using the enucleation technique, that the glucocorticoid receptor is also nuclear.

An important question that remains to be answered is what keeps the receptor in the nucleus. We believe that the receptor is bound to nuclear components. The basis for this is the observation that estrogen receptors immobilized by binding to hydroxylapatite behave like recep-

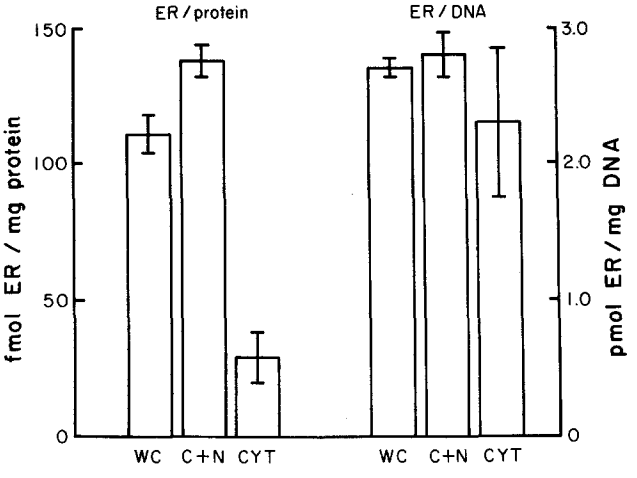

Figure 3. Intracellular distribution of estrogen receptor (ER). GH_3 cells were enucleated as described in the text. Estrogen receptor, protein and DNA were measured in untreated density-selected whole cells (WC), in the cell+nucleoplast fraction (C+N) and in the cytoplast fraction (Cyt). In intact cells or cytoplasts, estrogen receptor was measured as specific uptake of ^{3}H-estradiol (151 Ci mmol^{-1}, New England Nuclear) 2 nM in culture medium+DNase (Worthington DPFF 50 μg ml^{-1}) after 30 min at 37 °C, with or without 200 nM nonradioactive estradiol. Protein was measured with Coomassie blue staining[25] using the Bio-Rad microassay with bovine α-globulin (Sigma) as the standard. DNA was measured using diphenylamine[26] or the fluorescent dye Hoechst 33258[27]. The figure shows the results of three separate experiments (error bar is the standard error) in which cytoplasts contained 4%, 2.5% and 0.25% contaminating whole cells[46].

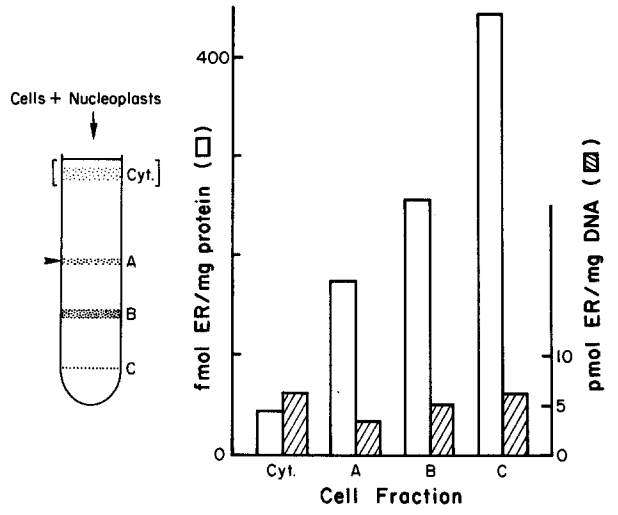

Figure 4. After enucleation, the cytoplasts were saved separately while the cell+nucleoplast layer was further fractionated on a density step gradient, with Percoll (Pharmacia) at 1.04, 1.05, 1.07, 1.08 and 1.10 g ml^{-1}. After 20 min at 400 g, cells were collected at interfaces between 1.05 to 1.07 g ml^{-1} (A), 1.07 to 1.08 g ml^{-1} (B), and 1.08 to 1.10 g ml^{-1} (C). The arrowhead at A indicates where the density-selected whole cells (1.058–1.063 g ml^{-1}) would have been found before enucleation, and the position that the cytoplasts would have occupied is indicated in brackets. Estrogen receptor, DNA and protein were measured in each fraction. Of the cells+nucleoplasts that were recovered after fractionation by density, 15% were recovered at A (which includes the pre-enucleation density), 76% were recovered at B and 9% were recovered at C[46].

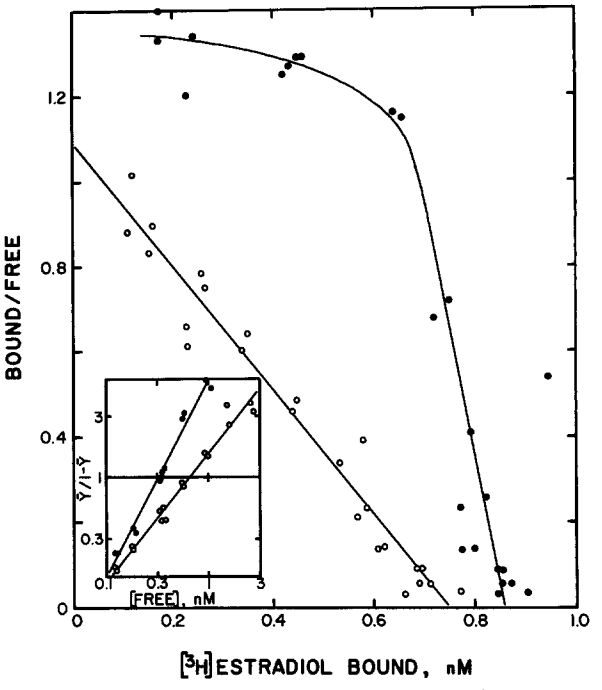

Figure 5. Scatchard plot of equilibrium binding at 0 °C of [^{3}H]-estradiol to native and monomeric estrogen receptors. Inset: Hill plots. (●), Binding to receptor in solution (receptor concentration = 0.85 nM, $S_{0.5}$ = 0.32 nM, n_H = 1.54); (○), Binding to monomeric receptor generated by treatment with 0.4 M KCl and adsorption onto hydroxylapatite (receptor concentration = 0.73 nM, $S_{0.5}$ = 0.64 nM, n_H = 1.01)[35].

tor in an intact cell[35]. Estrogen dissociation kinetics, which have been shown to discriminate between transformed and nontransformed receptors, were similar when receptor was immobilized on hydroxylapatite or when freely soluble[35]. On the other hand, the soluble receptor had estrogen binding characteristics that demonstrated positive cooperativity as first shown by Notides et al.[28] whereas the immobilized receptor did not[35] (fig. 5). Binding of estrogen in whole cells is not cooperative[49] and thus is similar to the immobilized receptor (fig. 6). In studies of amphibian oocyte nuclei, Feldherr and Pomerantz[8] have concluded that most nuclear proteins are bound to nuclear components although a number of nuclear proteins are readily solubilized. These and other data have led us to propose that the nuclear localization of unoccupied steroid receptor is due to low affinity binding to nuclear components.

Identification of the sites in the nucleus that estrogen receptors bind is difficult. Immunocytochemical studies indicate the receptor is dispersed widely in the nucleus[9]. There is no localization in the nucleolus although less appears in the denser heterochromatin than in less dense enchromatin. The ability to solubilize receptor in low ionic strength buffers suggests a weak association with the nuclear components.

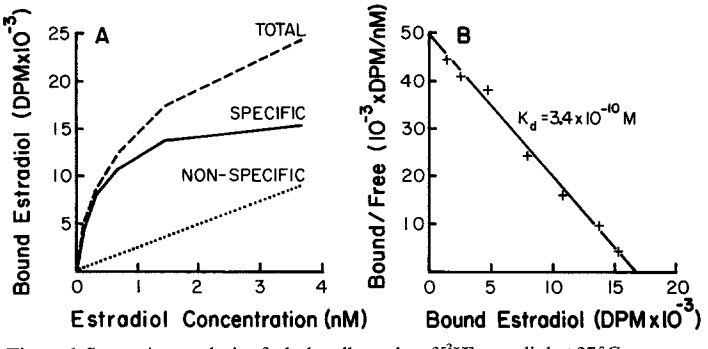

Figure 6. Saturation analysis of whole cell uptake of [^{3}H] estradiol at 37 °C by GH_3 cells[38a].

Nuclear binding sites

When the unoccupied estrogen receptor binds to an estrogen, a dramatic change occurs in surface properties of the complex indicating a conformational change in the protein[13]. When this interaction occurs at physiological temperatures, further changes are observed including a marked increase in binding to all polyanions, including DNA, RNA, proteins, etc. DNA binding by steroid receptor has been a popular topic for a number of years[51]. Most of such studies have concentrated on the fact that estrogen-receptor complexes bind tightly to DNA but it should be noted they also bind to a number of other substances as well[11]. More recently it has been observed that preferential binding to specific DNA sequences can be detected. Mulvihill et al.[27] were the first to show that a sex steroid receptor (progesterone receptor) bound to a specific sequence of DNA. They reported binding to sequences found upstream of several oviduct genes believed to be regulated by progesterone.

These reports have been followed in greater detail by studies by Yamamoto and associates[31] and others[18] on glucocorticoid receptor binding to mouse mammary tu-

mor virus (MMTV) and metallothionine genes. Specific DNA sequences scattered throughout the viral genome have been shown to have a somewhat higher affinity for the receptor than does the bulk of cellular DNA[31]. A site in the long terminal repeat of the MMTV has been identified as both a receptor binding site and a sequence essential for glucocorticoid activity on transfected MMTV genes[2]. This same sequence appears to have the characteristics of an 'enhancer' sequence[2]. Enhancers are genomic elements which increase the rates of transcription of nearby genes perhaps by increasing RNA polymerase loading[43].

Receptor binding to such sequences is only about 10-fold higher in affinity than binding to other DNA. This is in contrast to the three orders of magnitude higher binding that the lac repressor has for the lac operator compared to nonspecific DNA[24]. It is also in contrast to the recent report from Felsenfeld's group[7] reporting a protein specific for a globin gene expression had ~ 10,000 times higher affinity for its specific versus nonspecific sequence. Theoretical discussions of this problem have been presented by Lin and Riggs[24], Travers[42], Ptashne[32] and Von Hippel[44]. The sequence specificity of steroid receptor-DNA binding indicates some sequence homology but surprising numbers of mismatches are permitted[18]. Still, there is a good correspondence between DNA binding and the requirement of the sequence for steroid response. In all such studies purified, naked DNA is being studied. It is well known that in the intact nucleus, DNA is intimately associated with chromatin proteins, both histone and nonhistone proteins. Furthermore, the chromatin may be associated with a fibrillar network called the nuclear matrix or scaffold[15]. While still controversial, the nuclear matrix has been defined as nuclear proteins, insoluble under specific conditions, which may function as sites for replication and transcription[33]. Steroid receptors have been found to remain associated with nuclear matrix after extracting other nuclear components including over 90% of the DNA. Steroid-receptor complexes also bind to nuclear matrix in cell-free experiments[15]. However, if the small amount of residual DNA associated with the nuclear matrix contains operator regions or origins of replication, then DNA binding of the steroid receptor could still be involved in the nuclear matrix.

Chromatin 'acceptor' proteins are also a popular potential site of receptor interaction with chromatin. Ruh and Spelsberg[34] have characterized such proteins from the chick oviduct and others[4] report similar acceptors in rat uteri. A variety of other nuclear components have been identified as receptor binding entities at one time or another[11,23]. No clear cut evidence is available at this time that clearly defines a specific role for any such nuclear component. This is an active area of research and elucidation of this aspect of steroid hormone action is likely in the near future.

Development of steroid receptors: Ontogeny of estrogen receptors

Regulation of steroid receptor concentration is obviously critical for a cell to be able to respond to a particular steroid hormone. The female sex steroid receptors provide an interesting system for analysis because of their interrelationships and because they are not needed at all stages of life. Estrogen receptors in two rodent tissues, the uterus and pituitary, are present at very low concentrations at birth[3,40]. Figures 7 and 8 show the ontogeny of estrogen receptor development in these two tissues. The mouse pituitary has little or no receptor, but measurable levels of receptor are present in the rat uterus[3]. It should be noted that these concentrations are based on whole tissue studies and may reflect either low concentrations throughout the whole cell population or nonrandom dis-

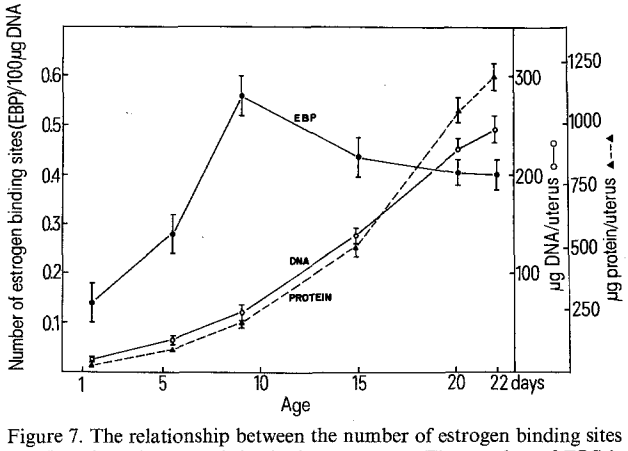

Figure 7. The relationship between the number of estrogen binding sites (EBS) and uterine growth in the immature rat. The number of EBS is expressed as the number of picomoles of estrogen bound. Points on the graph represent the mean ± SEM of 3–4 pooled samples[3].

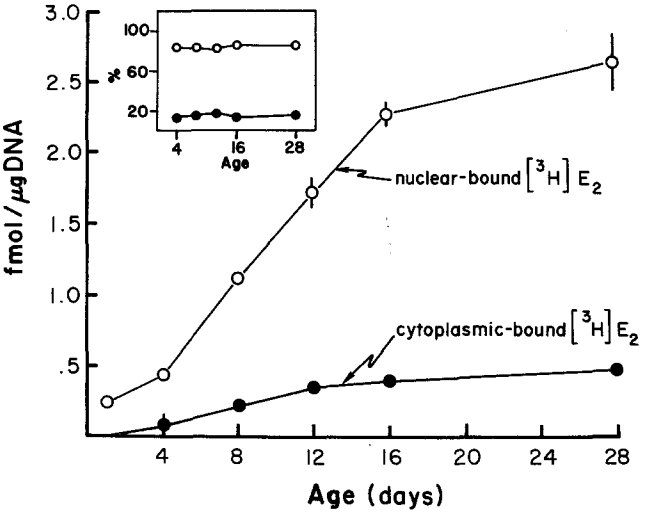

Figure 8. Estrogen binding in pituitaries of postnatal mice. Pituitaries were isolated from mice at the ages indicated. Half of the glands from each age group were incubated with 10 nM [^{3}H]E_2 (17β-estradiol), and the other half were incubated with 10 nM [^{3}H]E_2 plus 5 µM DES (diethylstilbestrol) for 1 h at 37 °C. Cytoplasmic and nuclear bound radioactivities were determined. Bound radioactivity measured in the presence of excess unlabeled competitor was subtracted from the total to estimate specific binding. Each point represents the mean ± SEM of triplicate assays of pooled material. Inset: Receptors measured in the nuclear and soluble fractions were summed, and the ratio found in each fraction was plotted as a function of age[40].

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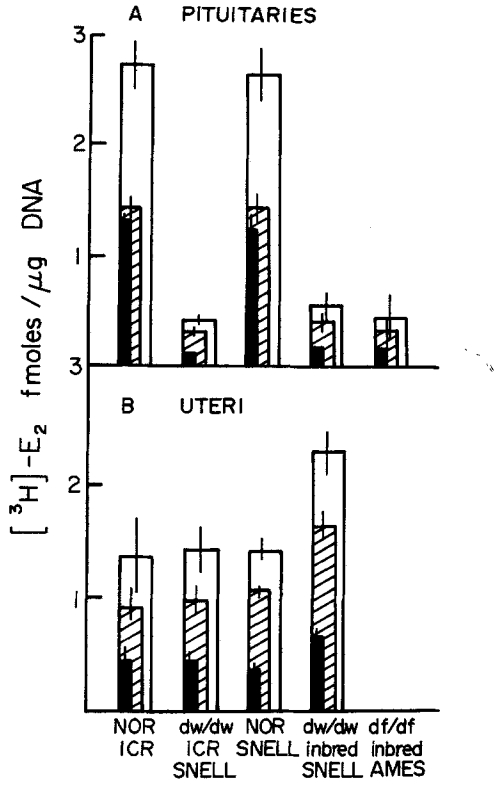

Figure 9. Estradiol receptor levels in pituitaries and uteri of normal and dwarf mice. Nuclear bound and soluble receptor levels were quantitated. Organs from normal and dwarf mice of the corresponding genetic background were assayed in the same experiment. Open bars = total specific binding; hatched bars = nuclear specific binding; closed bars = low-speed supernatant specific binding. A, Pituitary estrogen receptor. Each histogram represents the mean ± SEM of 3 assays (normals and Ames dwarfs) or 6 assays (Snell dwarfs). Pituitaries from normal mice were assayed individually whereas 3 pituitaries from dwarfs were pooled for each hot and hot and cold assay. B, Uterine estrogen receptor. Each histogram represents the mean ± SEM of 3 assays (normals) or 8 assays (Snell dwarfs). In several experiments, uterine receptor values ranging from 1.5 to 4.2 fmoles/g DNA have been measured. These differences are probably due to cyclic variations[37].

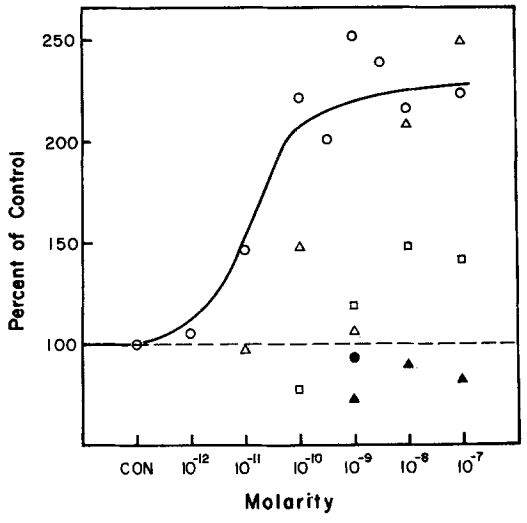

Figure 10. Induction of 130-K protein by various hormones. Cells were cultured for 3 days in media containing various concentrations of 17β-estradiol (○), 16α-estradiol (%), testosterone (□), progesterone (▲) and dexamethasone (●). CON, Control[19].

tribution limited to certain cell types. An example of the latter case would be the small number of lactotrophs present in the pituitary at birth. The lactotrophs are thought to be a principal target cell for estrogens.

Estrogen receptor concentrations rise until ~ 10–15 days of age in both pituitary and uterus (figs 7 and 8)[3,40]. After that time, the concentration of receptors remains relatively constant. The table shows that ovariectomy at 1 day after birth has no effect on estrogen receptor ontogeny in rat uteri. Similarly, there is no difference in uterine receptor concentration (fig. 9) in the Ames dwarf mouse whose pituitary is only $^1/_{10}$ of normal size and lacks growth hormone, prolactin and thyroid stimulating hormone[37]. It is apparent that the most obvious endocrine regulators do not play a role in regulation of the developmental acquisition of estrogen receptors. A report from Danforth et al.[5] has implicated melatonin in changes of estrogen receptor activity in hamster uteri suggesting this regulator should be examined for its role during receptor development. The importance of receptor development is illustrated by the observation that estrogen receptors in mammary carcinoma are related to the responsiveness of such tumors to endocrine therapy[50]. At the present time we are unaware of any additional data that present any convincing evidence as to how the estrogen receptors are controlled in these tumors.

The progestin receptor is an example of a receptor whose control is largely dependent on another hormone, the estrogens[22]. Progesterone receptor concentration in the uterus is relatively low in rats that have low estrogen levels, such as immature or ovariectomized females, and is dramatically increased by administration of estrogen[6].

A comparison of 8- to 10-day-old rat uteri taken from intact and ovariectomized rats. N.S., No significant difference between intact and ovariectomized group. Values represent the mean ± the SEM

Measurement	Intact	Ovariectomized
EBS[a]/uterus (pm)	0.33 ± 0.03	0.35 ± 0.04 N.S.
EBS/100 µg of DNA (pm)	0.53 ± 0.04	0.51 ± 0.05 N.S.
DNA/uterus (µg)	62.0 ± 5.6	60.30 ± 7.5 N.S.
Protein/uterus (µg)	202.0 ± 21.5	195.0 ± 19.6 N.S.
Wet wt (mg)	10.2 ± 0.5	9.9 ± 0.6 N.S.

[a] EBS, estrogen binding sites.

This effect of estrogen is directly on the uterine cells, as can be demonstrated in cell cultures of uterine cells (fig. 10)[19]. It also has been demonstrated in cultures of transformed mammary cells like the MCF-7 cell line[14]. While the details of how estrogen stimulates the production of progesterone receptor await the availability of molecular biological probes, the time course of the induction strongly suggests that a transcriptional site of action is involved. This would be consistent with other estrogen responses.

The state of knowledge concerning the developmental regulation of estrogen versus progesterone receptors are in marked contrast. In the case of the estrogen receptor

the major questions are biological, concerning what signals are involved. On the other hand, studies of the progesterone receptor will probably concentrate on the details of transcriptional regulation of its gene and the role of estrogen receptors. Scientific inquiry at all levels of organization, organismic, cellular and molecular, will be required for progress in elucidating the control of steroid receptor development.

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Receptor ontogeny and hormonal imprinting

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Key words. Hormonal imprinting; receptors; ontogenetic development; receptor adaptation; hormonal overlap.

Continuous interaction with the environment is a basic feature of cell life. Precise recognition of the environment is a prerequisite for cell function and survival. The environment of the unicellular organism is the external world. Transition from unicellular to multicellular forms of life involves dramatic alterations in the recognition system since, while the environment of the multicellular organism is still the external world, that of the single cell may be, depending on its localization, either the external or the internal milieu[15,16]. The at least partial 'internalization' of the environment at the level of multicellularity results in specialization of the recognition system (for the needs of the community). While the developing nervous system acquires the ability to receive scores of different signals and to control the entire (multicellular) organism, the primordial – chemical – system of signal recognition acquires different functions which become integrated into the complexity of the functions of the organism.

Differentiation of 'self' from 'non-self' (foreign) seems to exist at all levels of phylogenesis, because even unicellular organisms combine and form colonies exclusively with their own kind, and either escape or devour the organisms recognized as 'foreign'. From this basic mechanism develops at the multicellular level the marker – receptor recognition system, whose functions are to furnish morphogenesis and to maintain homeostasis of the immune system. Thus the immune system of multicellular organisms screens both the external and the internal environment for chemical (molecular) signals emitted by 'self' and 'foreign' cells. The second complex of the chemical recognition systems is represented by the pheromone system, which controls the relations between individuals, and is oriented exclusively towards the external environment. Last but not least, the endocrine system of multicellular organisms evolves from the primitive chemical recognition system of the unicellular organism. The endocrine system coordinates cellular functions initially in the absence of, but later in collaboration with, the nervous system. Since the endocrine system operates inside the multicellular organism, the recognitive function of the cells controlled by it is oriented towards the internal environment.

Analysis of the recognition system of multicellular organisms from the point of view of environmental relations has revealed that essentially four sub-systems operate in that system, of which one interacts exclusively with the external environment (pheromone system), two can process both external and internal signals (nervous system and immune system), and one responds exclusively to internal signals (recognition part of endocrine system). The operation of the sub-systems is genetically encoded, within the limits set by the needs and potentialities of the species. However, it appears that only the sub-system which responds exclusively to external signals (pheromone system) is encoded in every detail. In each condition in which recognition of the internal environment, i.e. recognition of the individually varying 'self' is decisive,