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A. Receptor proteins: past, present and future, ELWOOD V. JENSEN, Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois 60637, U.S.A.

The concept that biologically active substances elicit response by interacting with cellular receptor sites has long been a tenet of pharmacology, although the receptor substances themselves have, for the most part, been hypothetical entities. Recognition of specific receptors for steroid hormones dates from the late 1950's when the synthesis of estrogenic hormones labeled with carrier-free tritium made possible the determination of tissue distribution and intracellular localization of physiologic amounts of administered hormone. The striking ability of reproductive tissues to concentrate radioactive estrogens, without covalent binding or metabolic transformation of the steroid itself, indicated the presence in target tissues of an active receptor system. Within a few years, tritiated steroid hormones of high specific activity became commercially available; with the concurrent development of simplified, automated techniques for the measurement of tritium in biological materials, the early 1960's afforded an unusually favorable opportunity for experimentation in a new field. During the ensuing decade, a growing number of investigators made extensive contributions to an understanding of hormone–receptor interaction in target tissues. Aided by rapid developments in molecular biology, advances in the elucidation of early biochemical responses to hormonal stimulation paralleled those in the area of receptors, and more recently these two lines of investigation have begun to converge.

For the most part, the earlier studies establishing the main features of steroid–receptor interaction were carried out with estrogens, largely because these hormones were the first to become available in suitably labeled form. Their selective concentration by target tissues is especially striking; once formed, their complexes with receptor are not readily dissociable in the cold. Early investigations with mineralocorticoids and glucocorticoids, however, had the advantage that in each case a biological response to the hormone could be elicited in an *in vitro* system, a feature that was not realized for estrogens until relatively recently. Inception of receptor studies with androgens and progestins came somewhat later, following the important discoveries that in the classical target tissues for androgen it is not testosterone but dihydrotestosterone derived from it that binds to receptor, whereas the presence of significant levels of progesterone receptor in target tissues requires prestimulation with estrogen. Consideration of vitamin D as a steroid hormone that interacts in analogous fashion with specific receptors in target cells is a more recent development, depending on the demonstration that 1,25-dihydroxycholecalciferol rather than calciferol itself is the proximal antirachitic agent.

Like geologic time, receptor studies of the past may be divided into successive eras. After a Paleozoic age, in which the incorporation of labeled steroid hormones by whole tissues, first *in vivo* and later *in vitro*, demonstrated selective accumulation of unmetabolized hormone by receptor substances in target cells, there followed a Mesozoic period concerned with intracellular localization of the hormone, recognition that receptors are proteins,

and development of methods for solubilization of nuclear hormone–receptor complexes and for measurement of the capacity and affinity of hormone binding sites. During these studies, the use of binding inhibitors proved valuable, both in establishing the physiologic significance of hormone–receptor interaction and in distinguishing specific binding to the receptor from artifacts of nonspecific binding can occur *in vitro* with broken cell systems.

With the introduction of sucrose gradient ultracentrifugation for characterizing as well as measuring radioactive hormone–receptor complexes, the receptor field moved into its Cenozoic stage. During this period, the details of the intracellular interaction pathway were elucidated, leading to the concept of a two-step mechanism in which the nuclear hormone–receptor complex is derived from the translocation of an initial extranuclear complex. This was shown to be a temperature-dependent process involving the hormone–induced transformation of the receptor protein from its native state to an activated form possessing strong affinity for chromatin. This pathway, based on the fate of labeled exogenous hormones, is supported by the recent demonstration, using a nuclear exchange technique, that endogenous estrogen likewise undergoes translocation from an extranuclear to a nuclear form.

Using salt-containing sucrose gradients to permit recognition of the steroid-binding receptor units in an unaggregated condition, it was shown for both estrogen and androgen receptors that conversion of the complex from the native to the active form is accompanied by a change in sedimentation properties, a phenomenon that for estrogens appears to involve either a dimerization or the acquisition of a substantial molecular fragment. The importance of hormone-induced receptor transformation in biologic action was demonstrated by observations that only the transformed form of the estrogen–receptor complex can stimulate RNS polymerase in isolated uterine nuclei and that temperature-induced activation of the glucocorticoid–receptor complex is required for induction of specific enzymes in hepatoma cells or of glucose transport in thymus cells.

After an early demonstration of selective uptake of tritiated estrogens by hormone-dependent human breast cancers *in vivo* and by experimental rat mammary tumors either *in vivo* or *in vitro*, determination of estrogen receptors in excised specimens of human breast cancer has assumed clinical importance in predicting response of the patient to endocrine ablation. Results from many laboratories support the conclusion that patients whose tumors contain modest or negligible amounts of estrogen receptor have essentially no chance of benefit from adrenalectomy, hypophysectomy or other hormonal manipulation, whereas most but not all patients whose cancers contain substantial receptor levels will respond objectively to endocrine therapy.

Based on the knowledge accumulated from past investigations, several areas of receptor research are receiving attention in various laboratories at the present time. Especially important are attempts to bring together the results of receptor studies with those of biochemical investigations of early cellular responses. The nature of the acceptor site, with which the transformed receptor

associates in the nucleus and the precise biochemical mechanism by which this interaction enhances or modulates RNA synthesis are fundamental questions that need elucidation. For both estrogens and androgens, exposure of nuclei isolated from respective target cells to hormone—receptor complexes appears to elicit some but not all the effects on transcriptive behavior that are produced by hormone administration *in vivo*, thus affording a system for investigating genome-complex interaction in regard to effects both on template function and polymerase enzyme activity. The relation between responses of nucleolar and nucleoplasmic polymerases is being studied.

Considerable progress is being made toward the isolation of purified receptor proteins in quantities sufficient to permit determination of structure, molecular properties, composition and, eventually, aminoacid sequence. For such experiments, cell-free systems in which biologic responses can be detected and measured provide a valuable adjunct to steroid binding in evaluating the significance of purified receptor preparations. Demonstration that the progesterone receptor contains two steroid—binding components that may perform different functions in the nucleus indicates the complexity of the problem.

There are investigations of the molecular details of the hormone-induced transformation or activation of receptor proteins that appears to be required for binding to chromatin, as well as of the mechanism by which certain antagonists prevent response to the hormone, even though these substances also bind to the receptor and cause its translocation to the nucleus. Of considerable importance are studies of the control of the biosynthesis and cellular levels of receptor proteins and the question of why some breast cancers with high estrogen receptor levels still are not hormone-dependent. There are interesting indications that tumors of a truly hormone-dependent type may be characterized by the presence of receptors for more than one class of hormone. Genetic studies of alterations in mutant cells of one or more components of the glucocorticoid receptor system are bringing new insight into control of receptor function. Recent experiments have suggested that the extranuclear androgen—receptor complex may have a rapid effect on initiation factors for protein synthesis that does not involve its interaction with the genome.

What direction future studies of steroid hormone receptors will take is difficult to predict, and much will depend on the results of current lines of investigation. One might anticipate that receptor proteins for all classes of steroid hormones will be isolated in pure form, permitting complete elucidation of their composition and structure and of the differences between their native and activated forms. Specific antibodies prepared against the pure receptor proteins will permit determination of the immunochemical similarity of receptors from different target tissues or from different species. Such antibodies also should afford the possibility of immunochemical methods for the efficient isolation and purification of receptor proteins, as well as provide a relatively simple radioimmunoassay procedure for measuring the receptor content of tissues and tumors. Methods probably will be found for radiolabeling of the receptor protein during its biosynthesis so that its fate in the target cell nucleus can be ascertained independently from that of the hormone.

Among major unresolved problems that are certain to receive continued attention are the molecular basis of steroid-induced receptor transformation and the biochemical mechanism by which the transformed complex modulates RNA synthesis. Pertinent to the latter question is the nature of the modification in the target-cell genome during differentiation that results in its need for hormone—receptor complex, as well as changes during those neoplastic transformations of hormone-dependent tissues that result in loss of hormone dependency.

Information will be sought, perhaps from electron microscopic autoradiography, about the precise intranuclear localization of the hormone under physiologic conditions to furnish clues to the nature of the nuclear acceptor site and the relation between nuclear binding *in vivo* and in isolated nuclei. To complement knowledge about how the steroid moves into the nucleus, experiments will be designed to determine how the hormone and the receptor leave the nucleus after having served their function.

Despite the many gaps remaining in our knowledge, the past decade has seen remarkable progress in understanding the interaction of steroid hormones with target cells. Receptor studies have made a significant contribution to present concepts. Aided by developments in the general molecular biology of eukaryotic cells, the prognosis is excellent for the eventual elucidation of the detailed biochemical mechanism of steroid hormone action.

B. **A monomer-dimer equilibrium model for estrogen—receptor activation**, ANGELO C. NOTIDES, Department of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, U.S.A.

Cellular distribution analysis of the estrogen receptor of uterine tissues has shown that in the presence of estradiol and at $25-37°$, but not at $0°$, the receptor relocates from the cytoplasmic into the nuclear fraction [1—3]. Upon subsequent gradient centrifugation analysis of the receptor in buffer containing 0·4 M KCl, Jensen *et al.* [4] have demonstrated that the receptor found in the cytoplasm sediments as a 4S estradiol-binding protein (EBP), while the species from the nucleus sediments as a 5S EBP. Analysis of receptor activation was facilitated by the observation that an *in vitro* 4S to 5S EBP transformation occurred when the cytosol was incubated at $25-37°$ in the presence of estradiol [5].

Molecular properties of the estrogen—receptor. Our measurements of the molecular radii (by gel chromatography) and the sedimentation coefficients indicate that the 4S to 5S EBP transformation is the result of a bimolecular association reaction between the 4S EBP and a second macromolecule. The 4S EBP in the presence of 0·4 M KCl at pH 7·4 has a sedimentation coefficient of $4·2 \pm 0·04S$, a molecular Stokes radius of $44 \pm 0·4$ Å, and an apparent molecular weight of $7-8 \times 10^4$. The 5S $(5·5 \pm 0·02S)$ EBP, whether isolated from uterine nuclei or produced in the absence of nuclei by incubation of the cytosol-[^{3}H]-estradiol at $28°$ for 30 min, has an apparent molecular weight of $13-14 \times 10^4$ and a molecular Stokes radius of $58·5 \pm 0·5$Å. These data indicate that the 4S to 5S receptor transformation is a macromolecular association process resulting in an approximate doubling of the molecular weight of the 4S EBP, and not a result of a change in density or conformation of 4S EBP *per se* [6, 7].

Further support for a bimolecular reaction mechanism comes from a kinetic analysis of the *in vitro* 4S to 5S EBP transformation. Two alternative models are consistent with these experimental data: (a) a dimerization of two 4S EBP's and (b) an association of 4S EBP with a second, dissimilar monomeric unit that must be present at an approximately equal concentration. The second-order rate constant at $28°$ in the presence of 0·4 M KCl is 2×10^7 M^{-1} min^{-1} and is independent of the initial 4S EBP concentration, suggesting that the 4S EBP is dissociated into monomeric units.

In the absence of KCl or in the presence of 0·1 M KCl, the apparent second-order rate constant of 5S EBP