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 ± 0·04S, a molecular Stokes radius of 44 ± 0·4 Å, and an apparent molecular weight of $7–8 \times 10^4$. The 5S (5·5 ± 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 ± 0·5Å. 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 $2 \times 10^7$ $M^{-1}$ $min^{-1}$ 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

formation increases with decreasing 4S EBP concentration. The 4S EBP is weakly associated with other macromolecules or inhibitors in buffers of low ionic strength. Nevertheless, dilution of the cytosol with buffer of low ionic strength favors dissociation of the 4S EBP-macromolecular complex and thereby increases the fraction of the 4S EBP available for the activated dimer formation. The rate at which the 4S EBP forms the active dimer is very temperature dependent; the high energy of activation ($\sim$20 kcal mol$^{-1}$) suggests marked conformational changes are necessary [8, 9].

*The monomer-dimer equilibrium model for receptor activation by steroidal hormones.* We favour the following hypothesis for estrogen receptor activation, which may be a useful model for other steroidal hormones. The 4S EBP (monomer) with no or limited biological activity, by an estradiol- and temperature-mediated conformational change, dimerizes to form the 5S EBP. Even higher polymers may occur *in vivo*. This receptor model is analogous to that for a number of enzymes whose activity is modulated by a ligand- and, frequently but not always, a temperature-mediated association-dissociation process [10–12]. This molecular model is different from the site-site interaction of an allosteric protein that has been suggested by some investigators for the steroid hormone receptors. As a consequence of being a protein whose activity is regulated by a monomer-dimer equilibrium, the biological activity of the receptor is dependent upon the equilibrium constant between the monomer and dimer in the (a) absence or (b) presence of the steroid hormone, (c) the concentration of monomeric units in the cell, as well as (d) the concentration within any cellular compartment (e.g., cytoplasmic vs nuclear).

The activated state of the estrogen receptor, the dimer (5S EBP), is not dissociated during sucrose gradient centrifugation in buffers containing 0·4 M KCl at pH 7·4. Nevertheless, other steroidal hormone receptors may be readily dissociated as a consequence of any number of physiochemical forces that would drive their monomer-dimer equilibrium toward the monomeric state. These dissociating forces would include: (a) the equilibrium constant, (b) weak bonding forces between monomeric units, (c) high ionic strength buffers (0·4 M KCl), (d) lowered temperature (0–4°C), (e) receptor dilution, and (f) duration of centrifugation. Obviously, methods other than centrifugation may be necessary to examine the monomer-dimer equilibrium hypothesis for some steroidal hormone receptors.

Although the 5S EBP is invariably associated with estrogen–receptor activation, the possibility that dimerization is coincidental and unnecessary has not been demonstrated. Additional studies supporting a dimer model of estrogen–receptor structure have been reported by Jungblut and his associates [13] and Yamamoto and Alberts [14]. (This research was supported by National Institutes of Health Grant HD06707.)

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1. **Complementation analysis of steroid hormone action,** U. GEHRING, K. R. YAMAMOTO and G. M. TOMKINS, Biochemistry Department, University of California Medical Center, San Francisco, California 94143 and Institut für Biologische Chemie der Universität, D 69 Heidelberg, Germany

Wild type S49 mouse lymphoma cells are growth arrested and killed by physiological doses of glucocorticoids and hormone resistant variants can be isolated. The cause of steroid resistance has been traced to defects in the intracellular steroid receptor molecule and 3 types of receptor alterations have been identified among a variety of resistant clones: $r^-$ (receptor negative, no steroid binding), $nt^-$ (nuclear transfer negative), and $nt^-$ (increased nuclear transfer). Wild type (*wt*) cells have been hybridized with examples of each class of variant and the resulting hybrids are steroid sensitive although to a lesser extent than the *wt* X *wt* hybrids. This can be attributed to the lower concentration of wt receptor in these hybrids. Hybrids formed between various types of resistant cells are steroid unresponsive. No evidence of hybrid receptor molecules has been obtained. These results suggest that the steroid and nuclear binding domains of the receptor molecule are within the same complementation group.

2. **Mechanism of cytosol and nuclear $^3$H-estradiol binding in fetal kidney of guinea-pig,** C. SUMIDA and J. R. PASQUALINI, Foundation for Hormone Research, 26 Blvd. Brune, 75014 Paris, France

In previous studies the presence of specific estradiol ($E_2$) receptors in the fetal kidney of guinea-pig (40–55 days of gestation) was demonstrated: *in vivo*, after subcutaneous, *in situ*, administration of $7 \times 10^{-10}$ mol of [$^3$H]-estradiol (0·19 μg) per fetus or *in vitro*, after incubation of [$^3$H]-estradiol ($4 \times 10^{-8}$M) with kidney cell suspensions. Cytosol fraction is obtained after centrifugation at 250,000 *g* and the nuclear extracts are obtained by successive extractions with: (a) 0·1M Tris–HCl-0·0015M EDTA (Ext. a); (b) 0·3M NaCl-0·01M Tris–HCl (Ext. b) and (c) 1M NaCl-0·01M Tris–HCl (Ext. c).

In the cytosol fraction the $K_d$(4°C) for the [$^3$H]-estradiol macromolecule complex is $2{\cdot}5 \times 10^{-10}$M. [$^3$H]-Estradiol macromolecule complexes of the cytosol, obtained after incubation of this fraction with [$^3$H]-estradiol ($4 \times 10^{-8}$M), when incubated with purified nuclei showed that the formation of [$^3$H]-estradiol nuclear complexes was 2–3 times greater at 37°C than at 4°C. Purified nuclei incubated with [$^3$H]-estradiol can also form specific complexes in all the nuclear fractions. In another series of experiments, the incubation of purified nuclei with the cytosol fraction showed no increase in nuclear [$^3$H]-estradiol specific binding. Incubations of the 1M NaCl nuclear extracts (Ext. c) which contained 90–95% of the nuclear DNA showed "direct