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 7×10^{-10} mol of [^{3}H]-estradiol (0·19 μg) per fetus or *in vitro*, after incubation of [^{3}H]-estradiol (4×10^{-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 (4×10^{-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