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PROGRESS IN THE STUDY OF RECEPTORS INVOLVED IN STEROIDOGENESIS AND STEROID HORMONE ACTION

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INTRODUCTION

The use of modern tools in molecular biology and immunology has markedly enhanced our understanding of both the mechanisms of action of steroid hormones and of the regulatory processes of steroidogenesis. However, studies in both fields have not proceeded at the same pace: steroid hormone receptors were cloned in 1986–1988 and their general structural features are currently well understood. Their specific functional properties are now being studied using mainly *in vitro* mutagenesis methods. On the other hand, among the various hormonal receptors involved in steroidogenesis, only the LH receptor has recently been cloned and monoclonal antibodies obtained. Studies of ACTH, LHRH and CRH receptors still lag behind.

STRUCTURE AND FUNCTION OF THE PROGESTERONE RECEPTOR

Rabbit [1] and human [2] progesterone receptors have been cloned and sequenced. Over 100 monoclonal antibodies have been prepared against both receptors [3–5] and the epitopes recognized by several of these mapped with a rapid, new technique [3]. These antibodies have been used for immunocytochemical studies in animal [6, 7] and human tissues and specially for the evaluation of breast cancers using frozen [8] or paraffin embedded biopsies [9].

The gene for rabbit progesterone receptor has been isolated, the site of transcription determined and the promoter region characterized [10]. The human progesterone receptor has been localized to chromosome 11q23 [11]. *In vitro* mutagenesis has been used to study the mechanism of action of the antiprogesterone RU 486. The experimental data suggest that antagonist–receptor complexes bind to hormone responsive elements but that the interaction does not elicit transcriptional regulation [12].

Previous immunocytochemical studies at both the light and electronmicroscopic level have shown the progesterone receptor to be intranuclear in the presence or the absence of ligand [6, 7]. Deletion mutants have shown that this nuclear localization is due to the presence of two karyophylic signals; one constitutive and present in the hinge region of the receptor (between the DNA and steroid binding regions), the other hormone (or antihormone) dependent and present in the second finger region.

When cells are transfected with both a cytoplasmic form receptor (with both karyophylic signals deleted) and a nuclear form, hormone administration leads to the formation of hetero-oligomers and the transport of the "cytoplasmic" monomers into the nucleus. Both hormone and antagonist (RU 486) provoke oligomerization. The interaction between monomers takes place through the C-terminal part of the receptor [13].

STRUCTURE AND FUNCTION OF THE LH/HCG RECEPTOR

Immunoaffinity chromatography has been used to purify HCG–receptor complexes. An antiHCG monoclonal antibody (shown not to inhibit hormone binding to receptors) was used to prepare an immunomatrix. Porcine testes were the starting material which allowed the

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purification of hundreds of μg of receptor (sp. act. $\sim$1.5 nmol/mg protein). A mouse was immunized and its spleen used to prepare hybridomas of which 20 secreted antiLH–receptor antibodies [14].

The monoclonal antibodies have been used to screen a λgt library constructed from DNAs complementary to porcine testicular messenger RNAs. Clones were isolated and sequenced and shown to encode a 696 amino acid protein with 7 transmembrane domains and a long extracellular domain [15].

Expression in COS-7 cells has confirmed that this clone corresponds to a HCG binding protein, activating adenylate cyclase under the effect of the hormone. Cloning and sequencing has also shown the presence of variant messenger species, probably arising by alternate splicing, in which the transmembrane region was lacking. Two of these variants also lacked the intracellular domain, whereas the third variant retained this region. Further analysis will be necessary to establish if these variants may be secreted out of the cells. Recently, these variant proteins have been shown to bind the hormone [16], showing that this function is actually due in these receptors to the extracellular domain contrary to other G-protein linked receptors in which the ligand interacts with the transmembrane region.

Northern blot experiments showed the presence of similar species in the testes and ovaries with a major band of 4700 nucleotides and minor species of 6700, 5800, 4000, 2600 and 1400 nucleotides.

Cross hybridization has also allowed the cloning, sequencing and expression of the human TSH receptor [17] and of the human LH receptor [18]. The latter was localized by *in situ* hybridization to chromosome 2p21 [19] thus at quite a distance from the TSH receptor present on chromosome 14q31 [20].

Monoclonal antibodies have also allowed immunochemical studies of the LH receptor [14]. Immunoblot experiments showed a major species of $\sim$85,000 kDa corresponding probably to the major messenger species. Minor species of 45–48 kDa probably corresponding to the variant messenger RNA forms were observed. A $\sim$68 kDa species of unknown origin was also observed. The latter did not bind hormone. A 1:1 stoechiometry was observed between hormone and receptor. Immunocytochemical studies of LH receptor have been performed in ovaries and testes [21].

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