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Steroid Receptor Methods (Methods in Molecular Biology, Vol. 176)

Edited by Benjamin A. Lieberman,

Steroid hormones represent a powerful system for the study of the fundamental molecular mechanisms of gene regulation. Research in this field is also crucial for elucidating steroid receptor functions in endocrine disorders and some cancers.

In this volume of *Methods in Molecular Biology*, the reader will find a collection of novel protocols using the latest advances in yeast two-hybrid screening, fluorescence and immunoaffinity techniques, for isolating proteins that interact with steroid receptors. Other techniques enabling the analysis of histone acetylation and chromatin structure at steroid-regulated promoters are also described.

The identification of the steroid-regulated genes is also crucial for understanding the molecular and cellular processes controlled by steroid hormones. Described here are sensitive PCR-based differential display techniques for the discovery of gene expressions that change in response to hormone activation and in certain tumors. For elucidating receptor-functions in the context of a whole organism, there is also a description of receptor knockout technology.

Steroid receptor expression and localization are affected in several endocrine disorders and cancers, making the examination of their tissue distribution important. However, steroid

receptors are expressed at low levels in many target tissues and cells; this raises the necessity of developing highly sensitive methods. In this book, modern techniques based on the administration of [^{99m}Tc] technetium-labelled steroids or on the non-radioactive labelling of receptors with a photoactive ligand are proposed. Although the book lacks the immunochemistry techniques that would provide important information about the intratissue localization of steroid receptors, the reader will find details of a highly sensitive mRNA *in situ* hybridization technique.

Steroid hormones are being used in the treatment of several endocrine disorders and cancers, therefore, research in this field requires the development of new ligands, either agonists or antagonists. Combinatorial chemistry is described for the synthesis of ligands and a wide selection of both *in vivo* and *in vitro* screening approaches is presented to assess receptor binding and to increase the transcriptional properties of the ligands. Here, the traditional eukaryotic cell transfection assay is described, which uses hormone-inducible reporters and receptor expression vector constructs for the identification of potential ligands for steroid receptors. The yeast two-hybrid system has also been developed for identifying receptor subtype and function-selective retinoids.

Receptors have complex structures and are expressed at low levels, hence the book outlines the necessity of efficient overexpression and purification protocols to produce high amounts of receptors in a biologically active form with functional ligand-binding, DNA-binding and transcriptional activity. Large amounts are also necessary for crystallographic studies of the conformational changes induced by agonists and antagonists. The greatest difficulty is in obtaining proteins in a soluble form and this book provides protocols for the efficient production

and purification of a variety of steroid receptors.

Finally, because of the recent explosive increase in DNA sequences from genome sequencing projects, the question remains of how to identify novel members of the steroid receptor superfamily. Therefore, screening strategies are proposed, as well as bioinformatics, to analyze and interpret the biological data. The reader will also find detailed information about diverse computational tools that are currently available on the Internet.

In conclusion, this volume provides an excellent collection of top-quality chapters covering the latest techniques available in the field of steroid receptor biology. However, one point that could have improved the book, given the complexity of the topic, would be a chapter describing methods for studying the post-transductional modifications (i.e. phosphorylation) that modulate the biophysical and transcriptional properties of steroid receptors. There are also the inevitable oversights and redundancies inherent in any multi-author work, especially concerning the techniques of purification. Nevertheless, this book will serve as a useful reference guide.

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