

Molecular Biology of Steroid and Nuclear Hormone Receptors. Leonard P. Freedman (ed). Birkhauser, Boston. 1998. 319 pp. Price: SFr. 178.

This volume is the first in the series edited by Michael Karin and is entitled appropriately as 'Progress in gene expression'. This volume brings together in one place the diverse research information on steroid hormone receptors. The book is novel in many ways. All the contributors belong to the new generation of endocrinologists if one may say so. Molecular biology is their strength and in fact they have ushered in 'reverse endocrinology' whereby a cloned gene codes for a 'receptor' in search of a ligand and a physiological role. The gene product is analysed structurally and functionally by techniques of molecular biology and the physiological role established. This is a powerful and direct tool. With a foreword by Keith Yamamoto and a series preface by Michael Karin, the book provides a feast of new information, all generated in the nineties, and which has challenged the established paradigms in hormone action.

Mechanism of hormone action in the broadest sense has held the attention of many biochemists and endocrinologists for over five decades. This field of research activity has bagged several Nobel prizes. The basic paradigms of hormone action were clear by the seventies. Metabolic hormones were supposed to have receptors on the target cell membrane needing second messengers and signal transduction pathways while developmental hormones had nuclear receptors which act as transcription factors in regulating the expression of target genes. However, in the light of published research work in the last decade, a change in these basic paradigms is warranted. Molecular structures of hormone receptors have been revealed. Use of recombinant DNA techniques for cloning of receptors pointed to the presence of 'orphan nuclear receptors' without apparent activator ligands. Steroid receptors were also shown to be located on the cell membrane in some cases. The number of proteins associated with the conventional steroid receptor either initially or during its action has increased considerably and hence complicated the discussion of signal transduction mechanisms. Cross talk

among the post-receptor cellular events of different hormones has been noticed. Metabolic effects and developmental effects on gene expression have been integrated. The intranuclear molecular events in the process of DNA binding and activation of genes have been described in great detail. This book brings out all this with a series of lucidly-written chapters by modern authorities in the field.

The sub-cellular location of receptors to steroid hormones has been a matter of misunderstanding for over two decades now. Initially the opinion was that these are cytoplasmic and upon binding to the hormone they are transported into the nucleus. The two-step mechanism of action of steroid hormone proposed by Jensen, Gorski and others essentially was a paradigm for two decades. Green later asserted that there are only nuclear receptors. Subsequently, unliganded receptors were found both in the nucleus and in the cytoplasm demanding a rethinking of the subject. That the confusion arose because of lack of appreciation of the kinetically controlled bi-directional traffic of steroid receptors between nucleus and cytoplasm has been clearly explained by De Franco (chapter 2).

The interaction of steroid receptors with heat-shock proteins has been analysed by genetic, biochemical and pharmacological experimental approaches. The role of HSP 90 appears to be to keep the receptors inactive in the absence of hormone. Picard has succinctly brought this out in chapter 1.

Identification of an intra-cellular receptor has become complicated. The consensus till now was that steroid receptors have 5-6 domains of which the most important, from the functional point of view were the domain C (called the DNA binding domain, DBD) and domain E (called the ligand binding domain, LBD). The discovery of orphan receptors with no known ligand-binding property and of proteins like DAX-1 and SHP without DBD have brought confusion. Majority believe that there are two sub-families of intracellular receptors (IR). One family comprises the conventional steroid receptors and the second comprise the other nuclear receptors (orphan or otherwise). Estrogen receptors belong to both the classes. Simons Jr lucidly describes the structural details of LBD and the struc-

ture-function relationship information on LBD in chapter 3.

Chapter 4 by Rastinejud gives a detailed analysis of the DBD including the core region shared by all nuclear receptors and the unsolved problem of response element recognition by the activated receptors.

One of the puzzles in the field of mechanism of action of developmental hormones is the precise role of the agonist ligand. In chapter 5, Cheskis and Freedman discuss the recent data which suggest that the ligand (agonist, partial antagonist, etc.) could influence the dimerization of nuclear receptors. That dimerization of receptors is essential *in vivo* to make it bind to DNA was known earlier. Further, the dimerization was shown to be homo as well as hetero. This meant for example, that 9-*cis* retinoic acid-activated receptor (RAR) can form homodimers or heterodimer with another retinoic acid-activated receptor, RAR. The latter, of course, can be activated by all-*trans* retinoic acid and 9-*cis* retinoic acid. The consequences of homo or hetero dimerizations are however entirely opposite.

Elegant studies by Bert O'Malley, Anthony Means, P. Chambon and a host of others over three decades since the sixties have given us the basic information about steroid hormone action in different model systems like the laying hen oviduct, liver, etc. Regulation of synthesis of specific target gene products like ovalbumin, vitellogenin, etc. have been investigated. The most important finding from all these studies was the fact that steroid hormone action involves essentially transcriptional regulation of specific gene expression. This is not to belittle the important finding that came out of these studies that eukaryotes have split genes. Progress in our basic understanding of regulation of eukaryotic transcription obviously was necessary to solve steroid hormone action. When it was discovered that steroid hormone receptors were basically *trans*-activating factors just like transcription factors, the two fields of research work - spatial and temporal regulation of gene expression by transcription factors and the mechanism of steroid hormone action mutually influenced each other. One of the major goals, hence, of study of molecular biology of hormone action is the precise understanding of the regulation of assembly and function of transcription initiation complex by the specific

sequences in DNA. This is so because of the fact that regulation of gene expression of developmental hormones appears to be essentially targeted to regulation of this initiation complex formation and early elongation events. To be more precise, what is the interaction between hormone receptors and the factors including basal transcription factors involved in transcription initiation? The DBD of nuclear receptors does not have gene-activating function. This activation function is located at two domains – one near the N-terminus (AF-1) and the other hormone dependent AF-2 located within the C-terminus domain. These two activation domains have been identified in all steroid hormone receptors. Bagchi in chapter 6 describes the recent work explaining the facilitation, by the ligand-bound receptors, of the formation of pre-initiation complex comprising the basal transcription factors at the promoter site. This new information depended essentially upon the results of *in vitro* protein-protein interaction studies. The limitation of such studies, where due to use of excess of such proteins, non-specific interactions could occur has been explained by Bagchi. The role of co-activators in bridging the *trans*-activating factor and basal transcription factor is very lucidly explained. Silencing of gene expression by unliganded receptors, especially the oncogene-related products (e.g. v-erb A protein), and its role in oncogenesis is also explained here.

An enigma in hormone action is that concerning the mechanism behind termination of hormone signal on one hand and deceleration of cellular processes by growth and developmental hormones on the other. Glucocorticoid hormone-induced apoptosis of T-lymphocytes, feedback inhibition of gonadotropin synthesis and secretion by gonadal steroids are examples of the negative actions of hormones. Of course, even these effects are mediated by nuclear receptors as cell lines lacking nuclear receptors do not respond to the inhibitory actions of steroid hormones. In many cases, nuclear receptors and other transcription factors mutually inhibit each other's actions even though there are no corresponding *cis*-acting elements. That post-receptor events could be the site of such cross-inhibitory actions has been discussed by Herrlich and Gottlicher in chapter 7.

An additional complexity in the understanding of regulation of gene expression

in higher organisms is the organization of chromatin itself. The state of compaction of chromatin influenced by histone-DNA interaction can differ under various natural, physiological and artificial experimental conditions (aging for example). This has been shown to influence gene expression qualitatively and quantitatively. The mouse mammary tumour virus long terminal repeat (MMTV-LTR) can serve as a good model because it can be introduced along with a reporter gene into cells *in vitro* and it assumes a characteristic 6 nucleosome structure. It carries binding sites for a number of *trans*-acting factors including glucocorticoid receptors. These sites have been mapped with regard to particular nucleosomes. The molecular details of altered LTR structure as a consequence of hormone action has been described well by Watson and Archer (chapter 8). Creation of nuclease-hypersensitive site within the proximal portion of LTR appears to be a consistent feature of action of hormones like glucocorticoids, progesterone, estrogen and androgen. In essence, receptor-mediated chromatin remodelling including nucleosome displacement may become a new paradigm of developmental hormone action.

Post-translational modification of proteins, of which over 200 types are known, modulate protein functions in many cases. Of these the most well studied is phosphorylation. Steroid hormone receptors are phosphoproteins. This opens up the possibilities of cross-talk between signal transduction pathways involving protein kinases and phosphoprotein phosphatases and steroid hormone action. Phosphorylation affects many aspects of these receptors including nuclear localization, protein-protein interaction, intra-cellular traffic, DNA binding, etc. The transactivating function of glucocorticoid receptor is restricted to certain stages of the cell cycle only. Interestingly, phosphorylation of receptors also appears to be restricted to the target cells. Both mitogen activated protein kinases (MAP-kinases) and cyclin dependent kinases (CDK) appear to be involved in targeting these receptors. The details of sites of phosphorylation on these receptors and diverse consequences of such differential phosphorylation is discussed by Garabedian *et al.* in chapter 9. Ligand independent activation of receptor action might involve phosphorylation. Necessity for detailed

studies on the steady-state phosphorylation status *in vivo* of these receptors and its precise correlation to function is pointed out in this chapter. One is reminded of Philip Cohen's classic studies on the regulation of phosphorylase kinase in the seventies.

Nuclear receptors are evolutionarily related as the major motifs permitting functions like dimerization, transcriptional activation and target gene discrimination have been conserved. In addition to the orphan nuclear receptors, some receptors like NGF-1B, thyroid hormone receptor (TR), steroidogenic factor (SF1) bind DNA as monomers. A third α -helix formed by the C-terminal extension region of DBD can permit this. As mice deficient in SF-1 do not have developed adrenals and gonads, it is important to understand the action of SF-1 and other such receptors binding DNA as monomers. Recent data has brought to focus the importance of these receptors in tissue differentiation during development. Lazar and Harding, in an extremely lucid article bring out the current knowledge on these receptors (chapter 10).

The discovery, in 1988, of the new class of orphan nuclear receptors whose ligands were initially unknown and are now being revealed opened a new chapter in the history of developmental hormone action. One of the embarrassing problems in this area, as clearly brought out in this book (chapter 11 by Forman) is that the ligands identified as agonist, in some cases fail to bind to the putative receptors with high affinity! This leaves the possibility that a metabolite could be the *in vivo* ligand. A number of diet-derived ligands are natural activators of these orphan receptors. However whether these ligands occur *in vivo* in the tissues is not clear. In other cases, the target genes are not known. These facts have been brought out in this chapter.

This book is an extremely useful volume for post-graduate teachers and for researchers in the area of regulation of gene expression. The information given here represents the modern era of molecular endocrinology.

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