How do steroid hormones function to induce
the transcription of specific genes?

Review

Andrew C. B. CATO

Institut für Physiologische Chemie,
Philipps Universität, Deutschhausstr. 1-2,
D-3550 Marburg, West Germany

Introduction - Jensen's Model

The discovery in 1962 by Jensen and Jacobson (1) that radio-
actively labelled estrogenic compounds rapidly accumulated in certain
target-cell nuclei was followed by a demonstration that specific
high-affinity hormone-binding components existed in these cells (2).
In the uterus, these components were identified as protein molecules
located in the cytosol (2). These experimental findings heralded the
advent of a two-step hypothesis of a cytoplasmic-nuclear translocation
event proposed by Jensen et al. (3) which is generally used to explain
the regulation of gene expression by steroid hormones. According to
this model, steroid hormones interact with the high-affinity cyto-
plasmic proteins (receptors) causing a conformational change in the
receptors such that the receptor-hormone complexes are rapidly
translocated into the nucleus where they are thought to interact with
a variety of nuclear components and in particular with the genome
itself to induce or modulate the expression of a few specified genes.
Unfortunately, there is no direct experimental support for this model,
but many attempts have been made in the past 15 years to ask a
number of indirect questions that would verify certain aspects of this
model. Some of these questions are as follows:
1) Are there DNA sequences or some peculiar DNA structure around
inducible genes that render them sensitive to hormone induction?
2) Are there specific sequences around inducible genes that show
high-affinity to hormone/receptor complexes? and if so,
3) Does this interaction lead to transcriptional activation?

For a long time the vast size of the eukaryotic genome has
effectively prevented any direct study of particular inducible genes.
Fortunately, research in the past decade or so has provided a powerful
armoury of restriction enzymes to cut the genome into pieces,
bacterial vectors to clone these pieces, and other paraphernalia of
genetic engineering to isolate and study the structure of a number of
hormone-inducible genes (4-8). In most cases the individual base-
sequence of these genes and flanking regions have been meticulously
determined (9-11). The other bottle-neck in the study of the
mechanism of action of steroid hormones has been the multiple forms
exhibited by the receptor during preparation (12-16). It has therefore
been possible only recently to design a number of experimental
systems to try and answer the above question as to how steroid
hormones function to induce gene transcription.

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Various approaches to the study of hormone action

Receptor-DNA binding studies

In vitro studies of the interaction of the receptor to specific DNA fragments offer a way of determining whether, in vivo, unique DNA sequences exist around inducible genes that bind the receptor/hormone complex after its translocation into the nucleus. This type of study has recently gained the attention of many researchers following the report by Payvar et al. in 1981 (17) that the receptors for the glucocorticoid hormone recognize and bind to at least four widely separated regions of the mouse-mammary-tumour-virus (MMTV) proviral DNA. One of these regions was located 110-449 nucleotides upstream of the promotior for the MMTV transcription. The MMTV genome when integrated into host cells is transcribed by cellular RNA polymerase II (18) and the production of viral RNA is stimulated by glucocorticoid hormones such as dexamethasone (19). MMTV-infected cells therefore serve as models for the study of regulation of gene expression by steroid hormones (20). In another, unrelated receptor/DNA binding study involving cloned DNAs derived from in vitro deletions of the long terminal repeat (LTR) region of the MMTV genome known to have varying inducibility upon transfection of mouse cells, it has been shown that the glucocorticoid receptors bind preferentially to a restriction fragment covering 400-500 nucleotides of 3'-sequences of the LTR region (21). Similar reports have been made in the literature on the same system by Pfahl (22) and by Govindan and his colleagues but using electron-microscopic techniques (23).

Currently there are two main techniques for the study of the receptor/DNA interaction. The first method is a modification of the DNA competition assay of Kallos and Hollander (24). The receptor preparation is tagged with radioactive ligands such as the synthetic glucocorticoid [3H]triamcinolone acetonide or [3H]progesterone and incubated with calf thymus DNA linked to cellulose. The binding of the receptor to this DNA is competed out with specified cloned DNA fragments. The amount of radioactive receptor complex bound in the absence of any soluble competitor DNA is represented as 100%. Preference of the receptor for any specific cloned DNA is measured by that fragment's ability to serve as a better competitor for the receptor than free calf thymus DNA, which is characterized as a non-specific competitor. The second method is a nitrocellulose-filter binding assay where the DNA fragments are end-labelled with 32P04 and incubated with the receptor and thereafter filtered through nitrocellulose discs. DNA fragments bound to the receptor are retained on the filter whilst free labelled DNA can be filtered through. Again calf thymus DNA is used here to reduce non-specific DNA binding of the receptor. A number of discrepancies exist in these techniques. Some of the controversial points are whether the receptor binds to DNA ends or not and whether the receptor has a preference for single-stranded DNA as opposed to double-stranded DNA (25,26). Another source of conflicting results seems to be whether a purified receptor or crude cytosol is used for the experiment, as extensive purification of the receptor may remove components essential for the recognition of specific sequences on the DNA.