

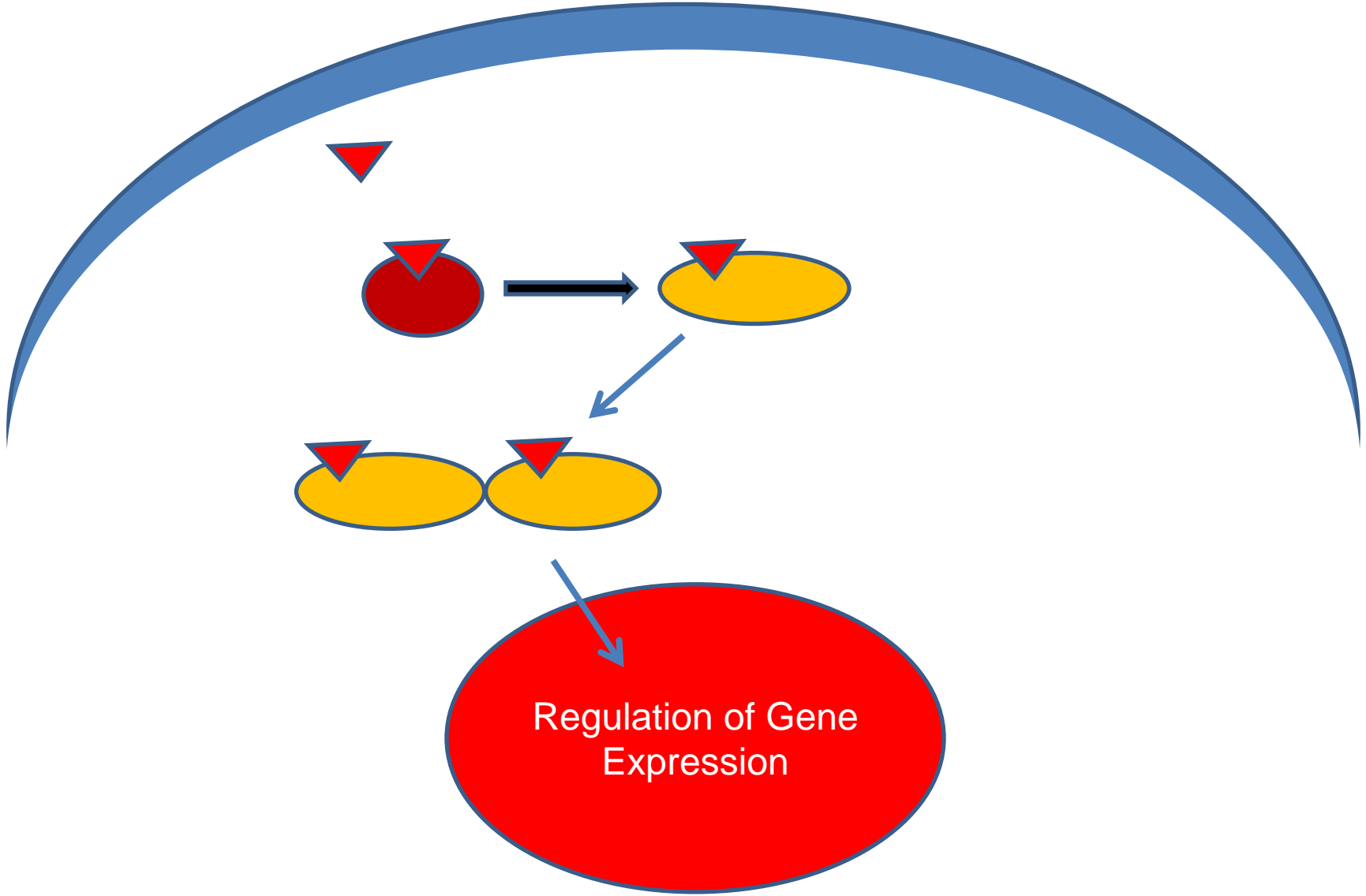
Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 21

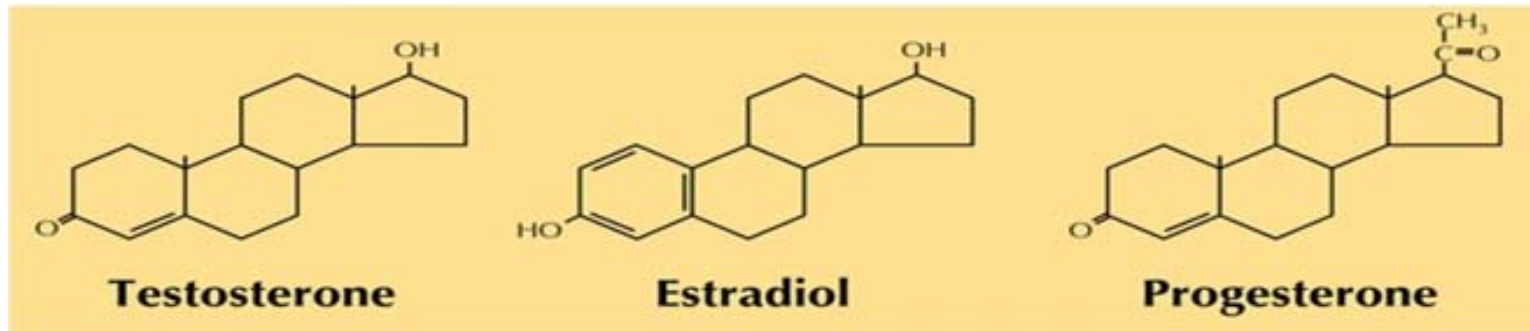
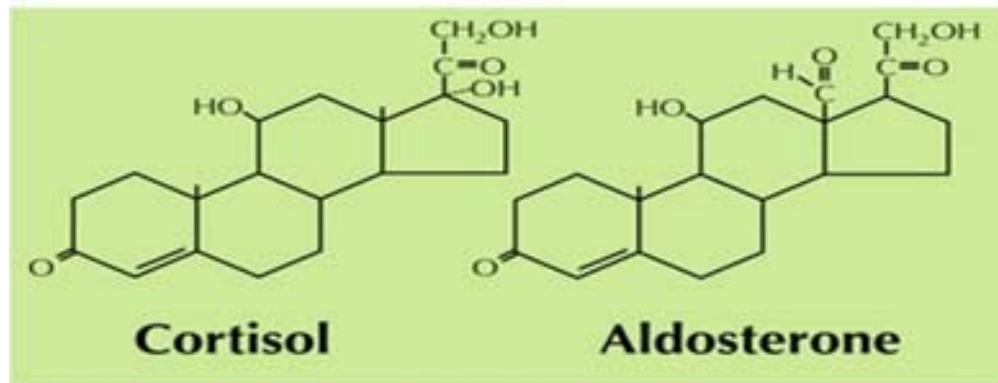
Regulation of gene expression by steroid hormones

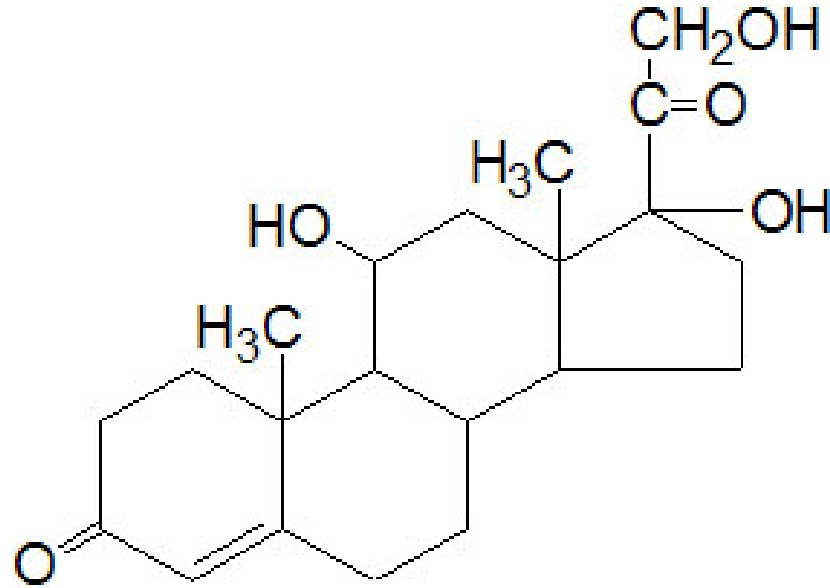
Mechanism of signal transduction by lipophilic hormones through intracellular receptors



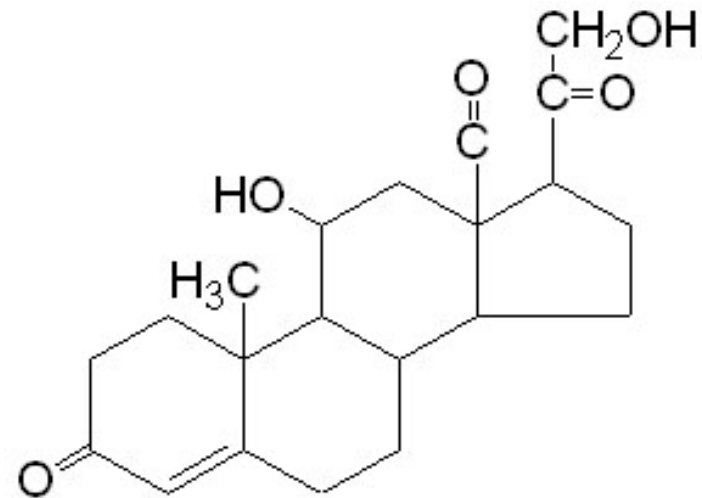
Steroid hormones are steroids that act as hormones.

Steroid hormones can be grouped into five groups by the receptors to which they bind: glucocorticoids, mineralocorticoids, androgens, estrogens, and progestagens.

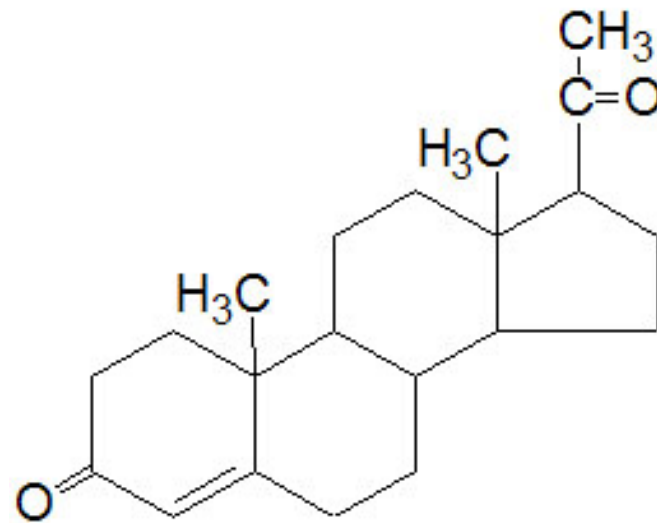




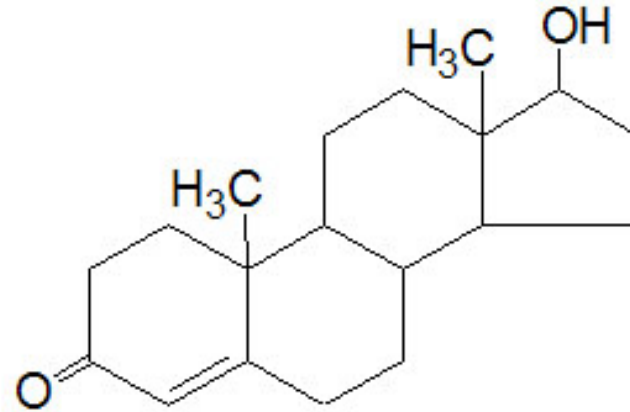
Cortisol: This glucocorticoid is synthesized from progesterone in the *zona fasciculata* of the adrenal cortex. It is involved in stress adaptation, elevation of blood pressure and Na⁺ uptake, numerous effects on the immune system



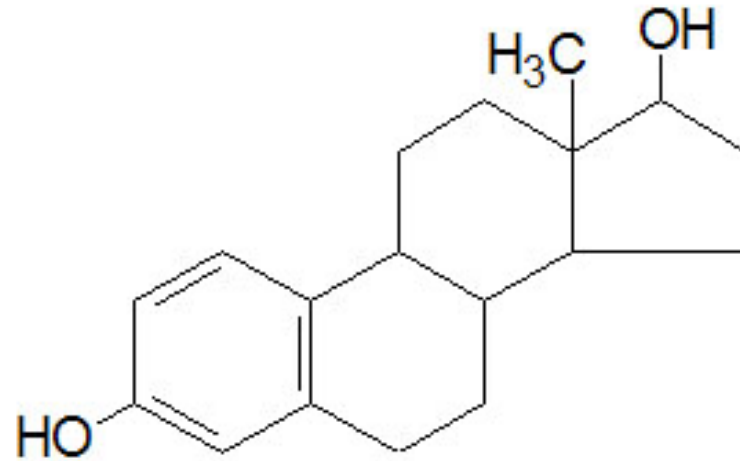
Aldosterone: the principal mineralocorticoid, produced from progesterone in the *zona glomerulosa* of adrenal cortex, raises blood pressure and fluid volume, increases Na⁺ uptake.



Progesterone: It is produced directly from pregnenolone and secreted from the *corpus luteum*. It is responsible for changes associated with luteal phase of the menstrual cycle. It is also involved in the differentiation of mammary glands



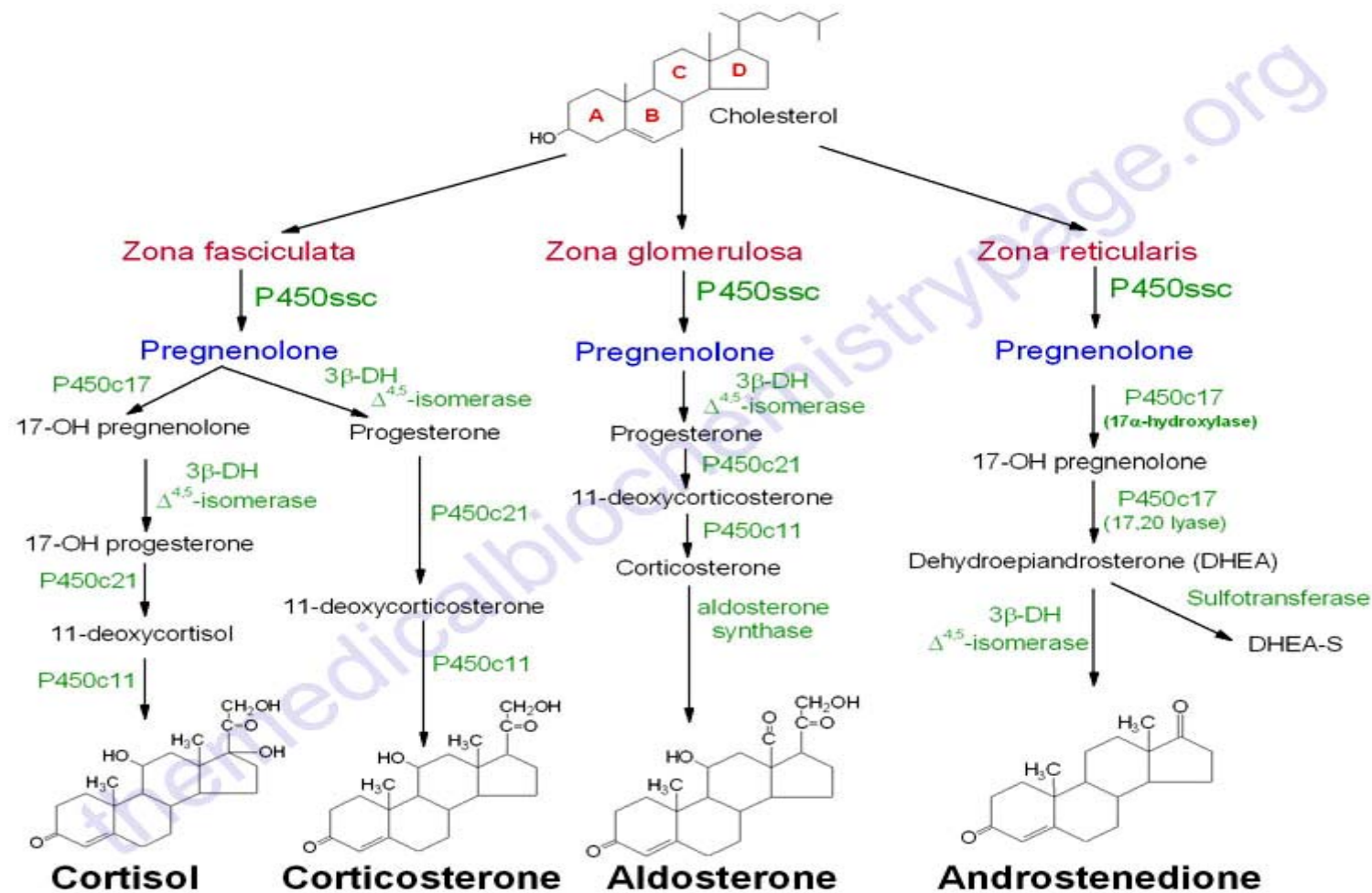
Testosterone: This is an androgen, male sex hormone synthesized in the testes. It is responsible for secondary male sex characteristics.



Estradiol: an estrogen, principal female sex hormone, produced in the ovary, responsible for secondary female sex characteristics

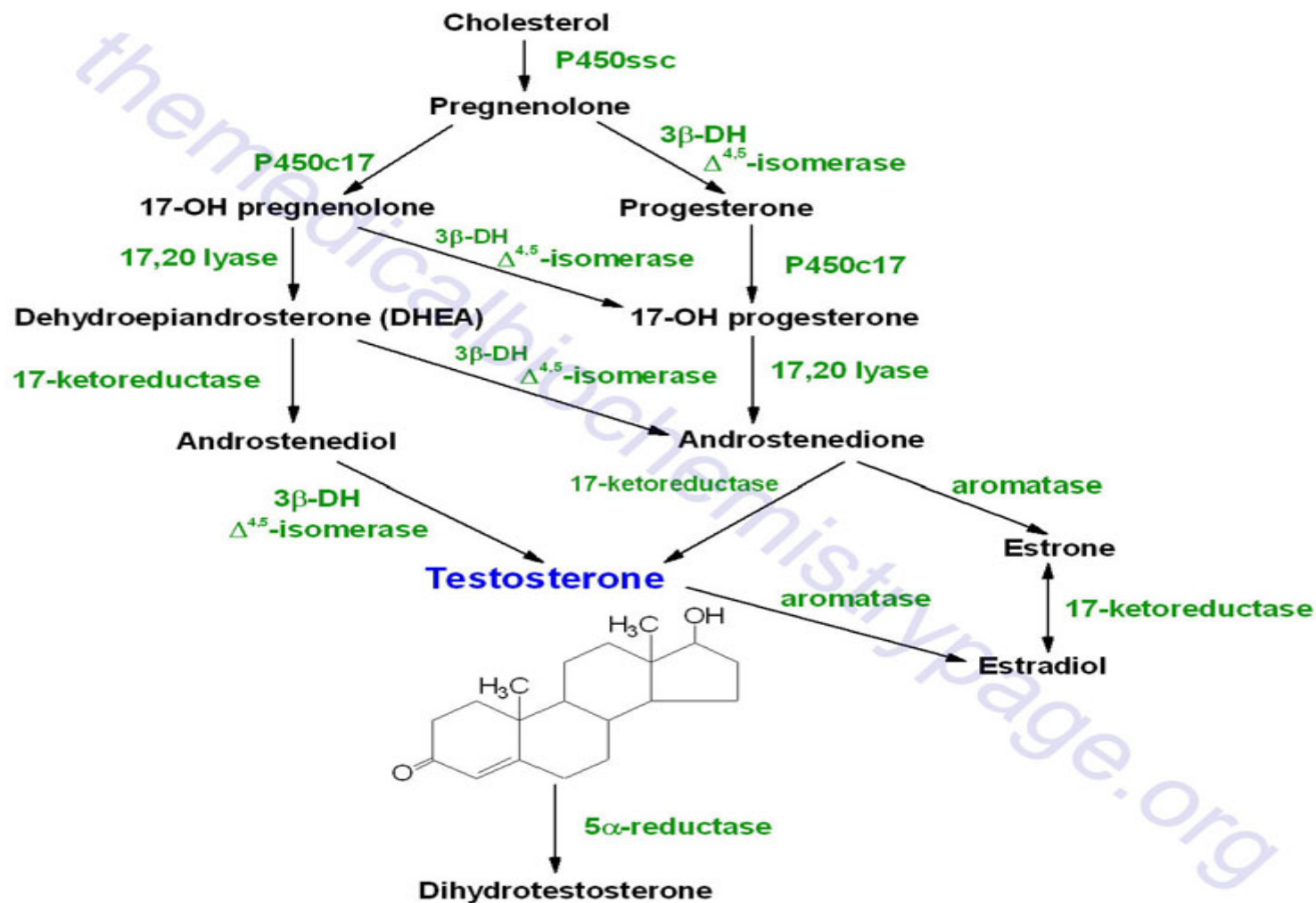
The natural steroid hormones are lipids and they are synthesized from cholesterol in the gonads and adrenal glands.

GLUCOCORTICOIDS AND MINERALOCORTICOIDS



The natural steroid hormones are lipids and they are synthesized from cholesterol in the gonads and adrenal glands.

GONADAL HORMONES



Synthetic steroid hormones

Glucocorticoids: prednisone, dexamethasone, triamcinolone

Mineralocorticoid: fludrocortisone

Androgens: oxandrolone, nandrolone (also known as **anabolic steroids**)

Estrogens: diethylstilbestrol (DES)

Progestins: norethindrone, medroxyprogesterone acetate

Steroid hormones had been known to exist since the early 20th century.

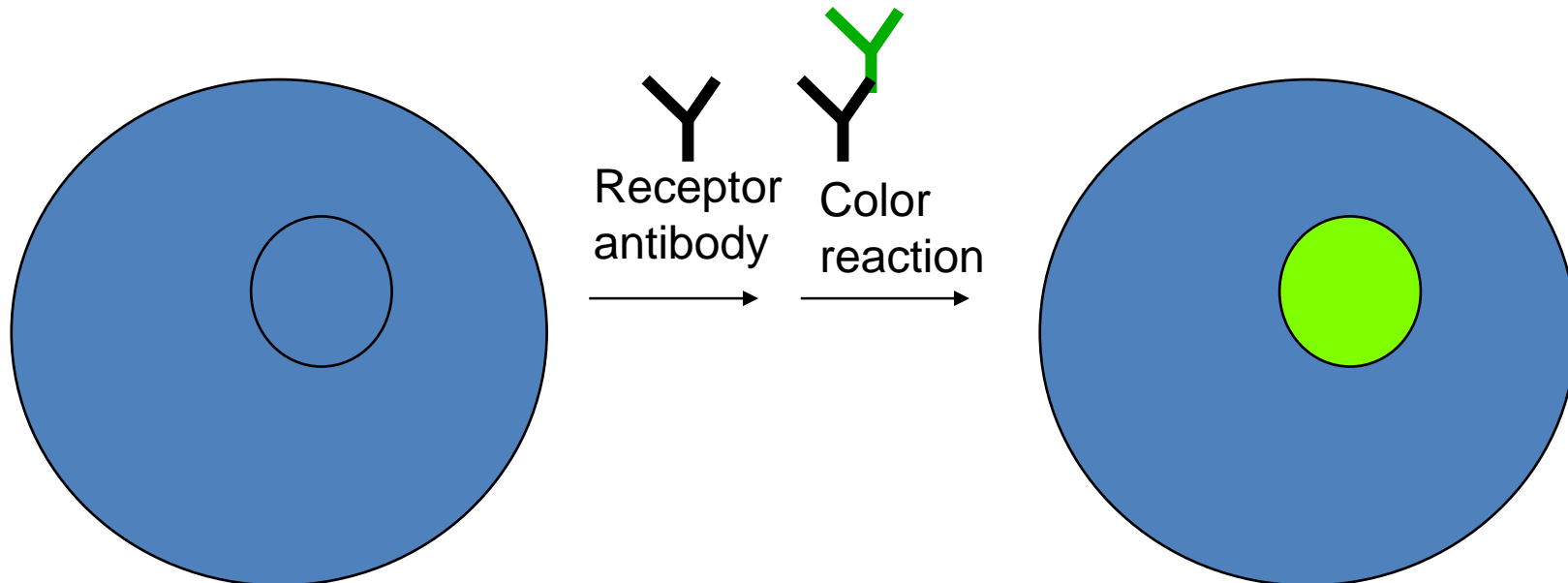
However, it was not until the early 1960s that the idea of specific hormone-binding molecules in the target tissues of these hormones began to emerge.

Analysis of the steroid hormone receptors had relied largely on biochemical techniques.

It is only after the genes encoding these receptors were cloned, it became possible to carry out detailed studies on the various functional domains of receptors.

Cellular Localization of Steroid Receptors

- Immunocytochemical studies indicated that all steroid receptors are nuclear.



Cellular Localization of Steroid Receptors

- It is generally thought that unoccupied steroid receptors can exist in the cytoplasm, while occupied receptors act in the nucleus on target DNA.
- When bound to hormone, cytoplasmic receptors move into the nucleus.

Purified steroid hormone receptors
bind to specific DNA sequences

Payvar, F., DeFranco, D., Firestone, G. L., Edgar, B., Wrange, O., Okret, S., Gustafsson, J. A., and Yamamoto, K. R. (1983).

Sequence-specific binding of glucocorticoid receptor to MTV DNA at sites within and upstream of the transcribed region.

Cell 35, 381-392.

Glucocorticoid receptor protein stimulates transcription initiation within murine mammary tumor virus (MTV) DNA sequences in vivo, and interacts selectively with MTV DNA in vitro.

We mapped and compared five regions of MTV DNA that are bound specifically by purified receptor; one resides upstream of the transcription start site, and the others are distributed within transcribed sequences between 4 and 8 kb from the initiation site.

Each region contains at least two strong binding sites for receptor, which itself appears to be a tetramer of 94,000 dalton hormone-binding subunits.

Such studies with purified steroid hormone receptors demonstrated that they are likely to be transcription factors

Cloning of steroid hormone receptors

Studies from a number of laboratories such as those headed by Elwood Jensen, Bert O'Malley, Keith Yamamoto and others led to the hypothesis that activated steroid hormone receptors were capable of binding to specific DNA sequences in the nuclei of their target cells and inducing the production of certain proteins in these cells.

By the end of the 1980s, when techniques for cDNA library screening and cloning and sequencing DNAs became routine, the first receptor cDNA, encoding the glucocorticoid receptor, was cloned in **Ron Evans** lab in the Salk Institute in La Jolla, California.

The cloning of cDNAs encoding receptors for estrogen, progesterone were also cloned in subsequent years.

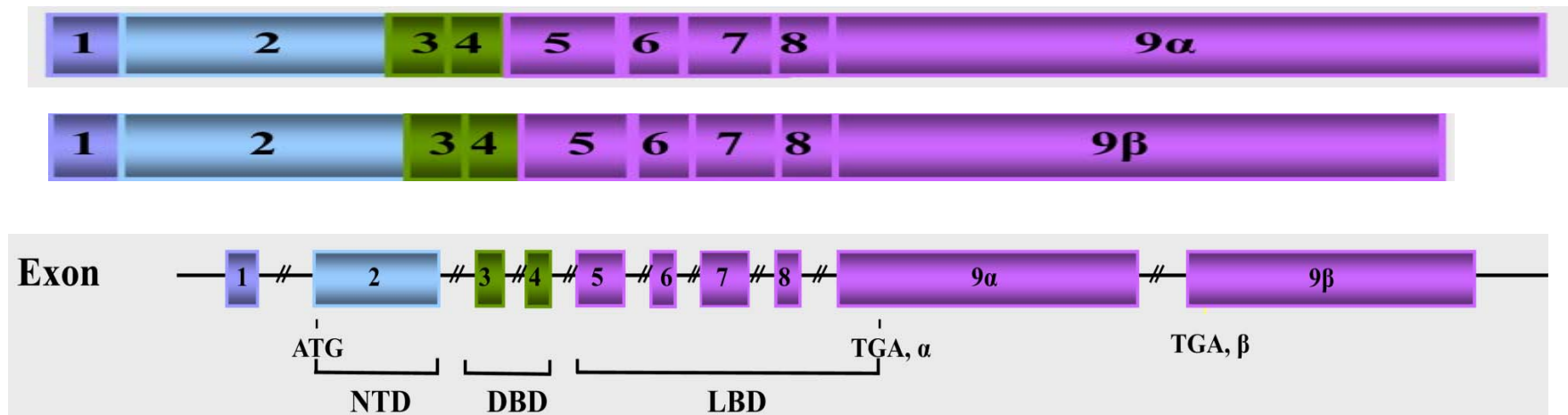
Nature. 1985 Dec 19-1986 Jan 1;318(6047):635-41.

Primary structure and expression of a functional human glucocorticoid receptor cDNA.

[Hollenberg SM](#), [Weinberger C](#), [Ong ES](#), [Cerelli G](#), [Oro A](#), [Lebo R](#), [Thompson EB](#), [Rosenfeld MG](#), [Evans RM](#).

Identification of complementary DNAs encoding the human glucocorticoid receptor predicted two protein forms, of 777 (alpha) and 742 (beta) amino acids, which differ at their carboxy termini.

The proteins contain a cysteine/lysine/arginine-rich region which may define the DNA-binding domain.



Nature. 1985 Dec 19-1986 Jan 1;318(6047):670-2.

Domain structure of human glucocorticoid receptor and its relationship to the v-erb-A oncogene product.

Weinberger C, Hollenberg SM, Rosenfeld MG, Evans RM.

We report here that both forms of the glucocorticoid receptor are related, with respect to their domain structure, to the v-erb-A oncogene product of avian erythroblastosis virus (AEV), which suggests that steroid receptor genes and the c-erb-A proto-oncogene are derived from a common primordial regulatory gene.

Therefore, oncogenicity by AEV may result, in part, from the inappropriate activity of a truncated steroid receptor or a related regulatory molecule encoded by v-erb-A.

This suggests a mechanism by which transacting factors may facilitate transformation.

We also identify a short region of hGR that is homologous with the Drosophila homoeotic proteins encoded by Antennapedia and fushi tarazu.

Nature. 1986 Dec 18-31;324(6098):641-6.

The c-erb-A gene encodes a thyroid hormone receptor.

[Weinberger C](#), [Thompson CC](#), [Ong ES](#), [Lebo R](#), [Gruol DJ](#), [Evans RM](#).

The cDNA sequence of human c-erb-A, the cellular counterpart of the viral oncogene v-erb-A, indicates that the protein encoded by the gene is related to the steroid hormone receptors.

Binding studies with the protein show it to be a receptor for thyroid hormones.

Nature. 1986 Dec 18-31;324(6098):635-40.

The c-erb-A protein is a high-affinity receptor for thyroid hormone.

[Sap J](#), [Muñoz A](#), [Damm K](#), [Goldberg Y](#), [Ghysdael J](#), [Leutz A](#), [Beug H](#),
[Vennström B](#).

Hormone binding and localization of the c-erb-A protein suggest that it is a receptor for thyroid hormone, a nuclear protein that binds to DNA and activates transcription.

In contrast, the product of the viral oncogene v-erb-A is defective in binding the hormone but is still located in the nucleus.

Nature. 1986 Mar 13-19;320(6058):134-9.

Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A.

[Green S](#), [Walter P](#), [Kumar V](#), [Krust A](#), [Bornert JM](#), [Argos P](#), [Chambon P](#).

We have cloned and sequenced the complete complementary DNA of the oestrogen receptor (ER) present in the breast cancer cell line MCF-7.

The expression of the ER cDNA in HeLa cells produces a protein that has the same relative molecular mass and binds oestradiol with the same affinity as the MCF-7 ER.

There is extensive homology between the ER and the erb-A protein of the oncogenic avian erythroblastosis virus.

Nature. 1986 Dec 18-31;324(6098):615-7.

A superfamily of potentially oncogenic hormone receptors.

[Green S](#), [Chambon P](#).

Clin Physiol Biochem. 1987;5(3-4):179-89.

Human steroid receptors and erb-A gene products form a superfamily of enhancer-binding proteins.

[Weinberger C](#), [Giguère V](#), [Hollenberg SM](#), [Thompson C](#), [Arriza J](#), [Evans RM](#).

Science. 1986 Aug 15;233(4765):767-70.

Molecular cloning of the chicken progesterone receptor.

[Conneely OM](#), [Sullivan WP](#), [Toft DO](#), [Birnbaumer M](#), [Cook RG](#),
[Maxwell BL](#), [Zarucki-Schulz T](#), [Greene GL](#), [Schrader WT](#), [O'Malley
BW](#).

Science. 1988 Apr 15;240(4850):327-30.

**Cloning of human androgen receptor complementary DNA and
localization to the X chromosome.**

[Lubahn DB](#), [Joseph DR](#), [Sullivan PM](#), [Willard HF](#), [French FS](#), [Wilson EM](#).

What was the strategy employed for cloning the genes encoding steroid hormone receptors

Screening cDNA libraries with

Receptor-specific antibodies

or

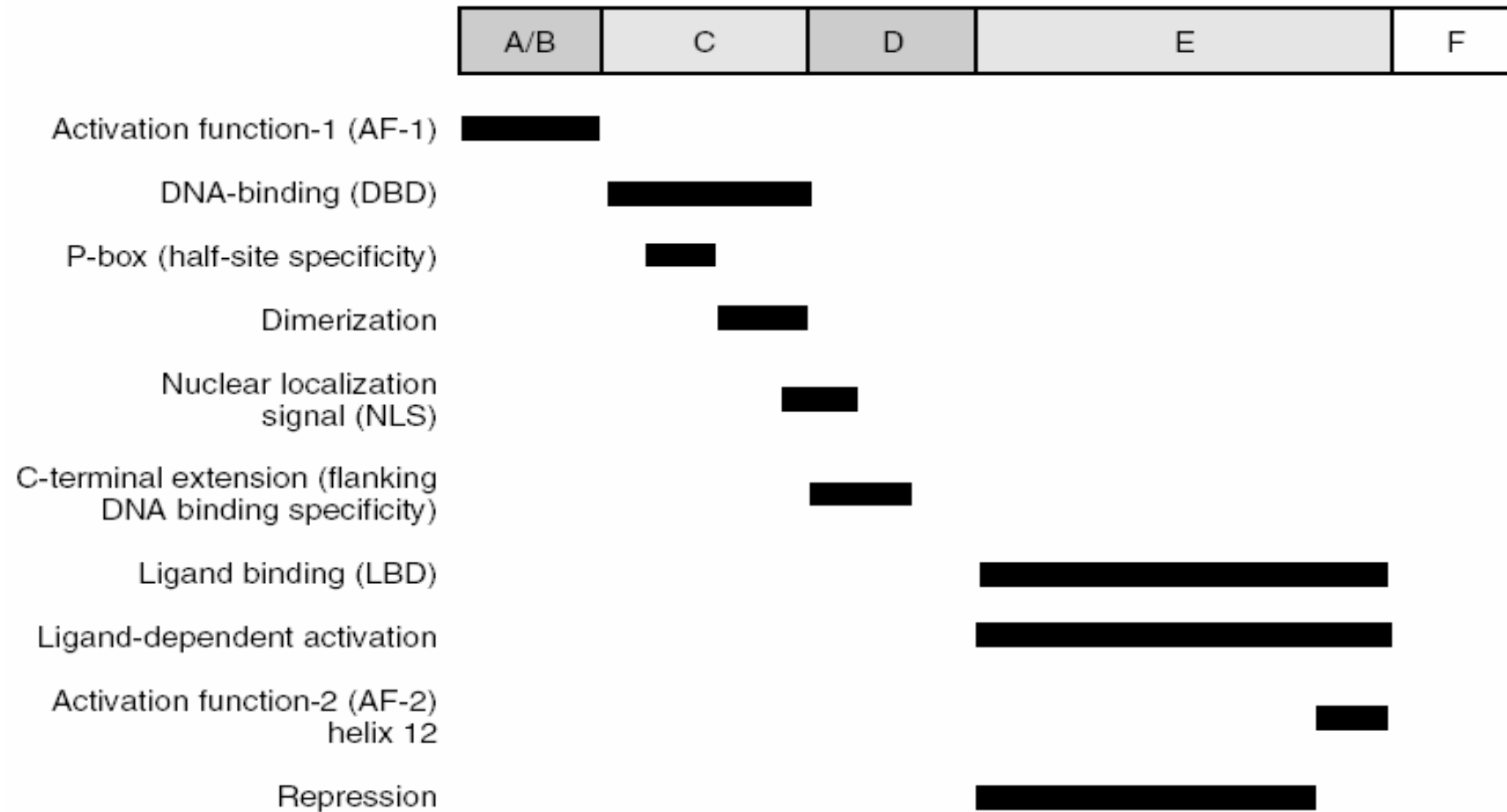
Oligonucleotides derived from amino acid sequence of the purified receptor

Three well conserved regions:

- Hormone binding domains (HBD / LBD) in carboxyl terminus
- DNA-binding domain (DBD) 5' to ligand binding domain
- A nonconserved hypervariable region, which may contribute to transcriptional activity of receptor



Domain structure of a steroid hormone receptor



Comparison of the deduced amino acid sequences encoded by many of the receptor cDNAs showed that they were members of a ligand-activated superfamily of transcription factors

The nuclear receptor superfamily

Type I receptors

They undergo nuclear translocation upon ligand activation and bind as homodimers to inverted repeat DNA half sites, called hormone response elements, or HREs.

Ex. Receptors activated by steroid ligands, such as glucocorticoid receptor, Mineralocorticoid receptor, estrogen receptor, progesterone receptor and androgen receptor.

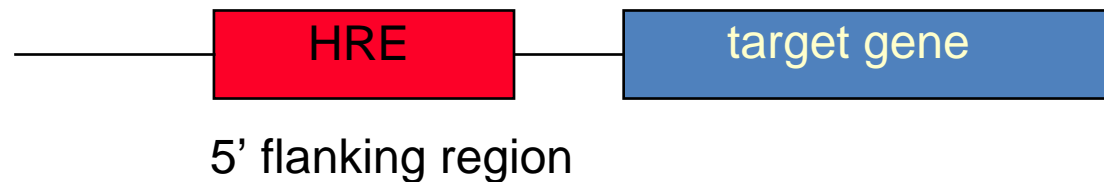
Type II receptors

These are often retained in the target cell nucleus regardless of the presence of ligand, and usually bind as heterodimers with RXR to direct repeats.

Ex. Receptors for thyroid hormone, all trans retinoic acid and vitamin D

Steroid Receptors bind to Hormone Response Elements (HREs) on DNA

- Following hormone binding, intracellular receptors act as transcription factors, binding to hormone response elements (HREs) on the 5' flanking region of target genes.



Identification of Functional Domains of Nuclear Receptors

The cis-trans cotransfection assay

The transient transfection-based HRE-reporter assay, developed by Ron Evans, became the tool of choice for investigating the mechanism by which nuclear receptors regulate their target genes.

In this assay, a cDNA encoding the receptor, or part of a receptor is transfected, into a suitable cell line along with a reporter gene, usually the luciferase gene, linked to a promoter controlled by 1 or more HREs specific for the receptor being studied.

The amount of luciferase enzyme assayed is proportional to the activity of the receptor.

Addition of a specific ligand activates the receptor and touches off a sequence of events, culminating in recruitment of general transcription factors and an increase in the rate of transcription of the reporter gene.

Steroid Receptors bind to Hormone Response Elements (HREs) on DNA

Hormone

Progesterone,
Androgen,
Glucocorticoid,
Mineralocorticoid

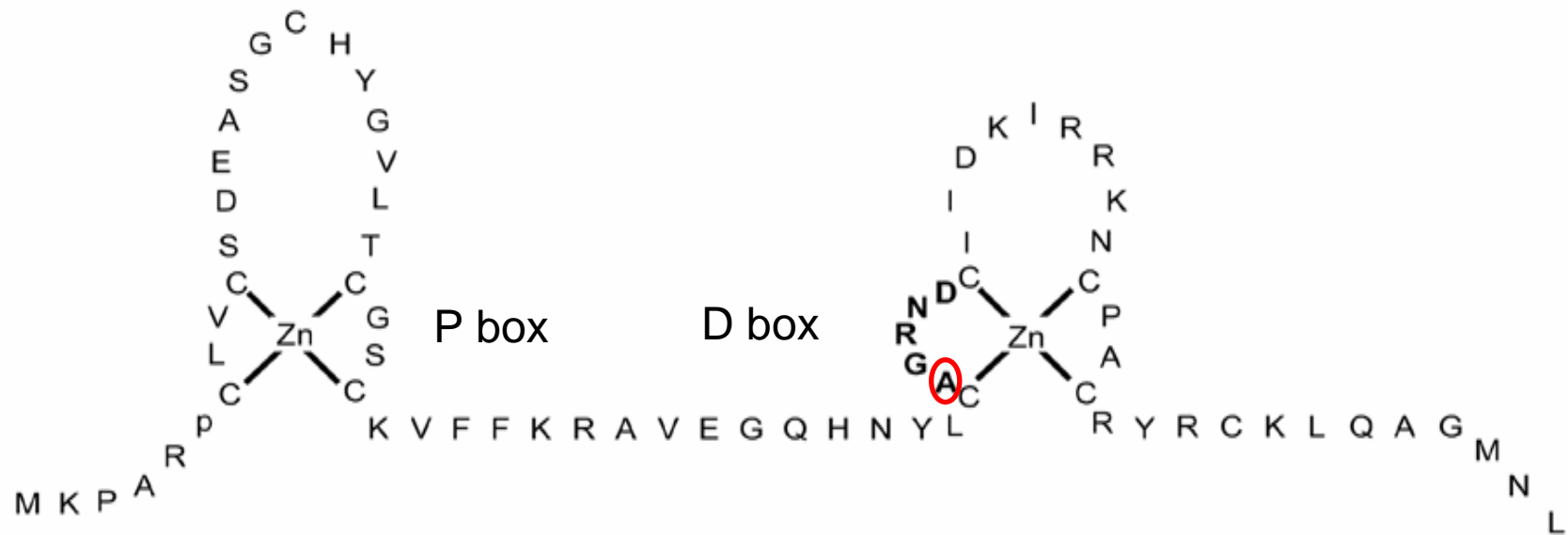
Consensus HREs

AGAACAAnnnTGTTCT

Estrogen

AGGTCAnnnTGACCT

GR DBD



Mutation of **A** at position **458** to T results in a receptor that is defective in dimerization.

Cell. 1989 Jun 30;57(7):1131-8.

Two amino acids within the knuckle of the first zinc finger specify DNA response element activation by the glucocorticoid receptor.

[Danielsen M](#), [Hinck L](#), [Ringold GM](#).

The specificity of target gene activation by steroid receptors is encoded within a small, cysteine-rich domain that is believed to form two zinc-coordinated fingers.

Here we show that the ability of glucocorticoid and estrogen receptors to discriminate between their closely related response elements resides in the two amino acids located between the two cysteines in the C-terminal half of the first finger.

Unexpectedly, chimeric glucocorticoid receptors harboring portions of the interfinger and/or second finger of the estrogen receptor have the ability to activate transcription from either a GRE- or ERE-containing promoter.

We surmise that whereas the "knuckle" region of the first finger may be the primary determinant of sequence recognition, the remainder of the DNA binding domain normally confers structural information required for preventing promiscuous HRE recognition.

Cell. 1989 Jun 30;57(7):1139-46.

Determinants of target gene specificity for steroid/thyroid hormone receptors.

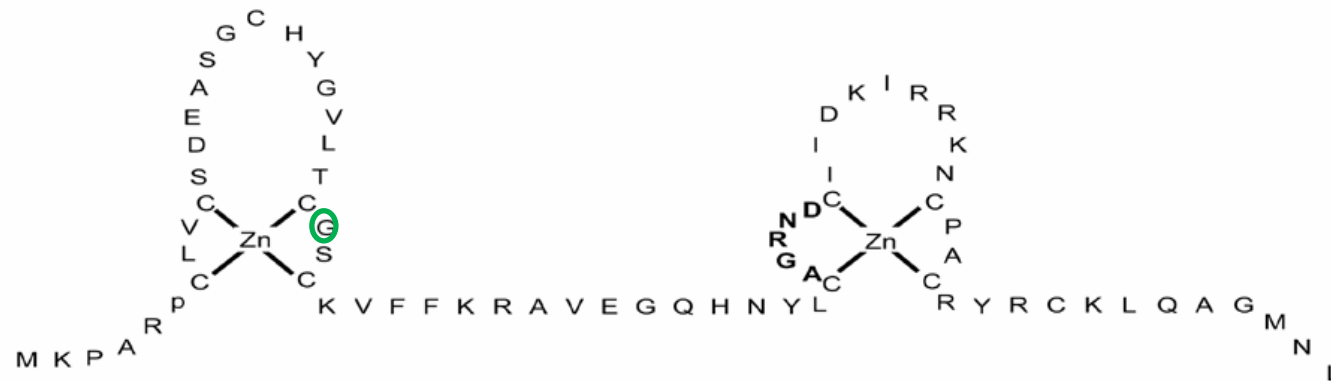
[Umesono K](#), [Evans RM](#).

The target gene specificity of the glucocorticoid receptor can be converted to that of the estrogen receptor by changing three amino acids clustered in the first zinc finger.

Remarkably, a single Gly to Glu change in this region produces a receptor that recognizes both glucocorticoid and estrogen response elements.

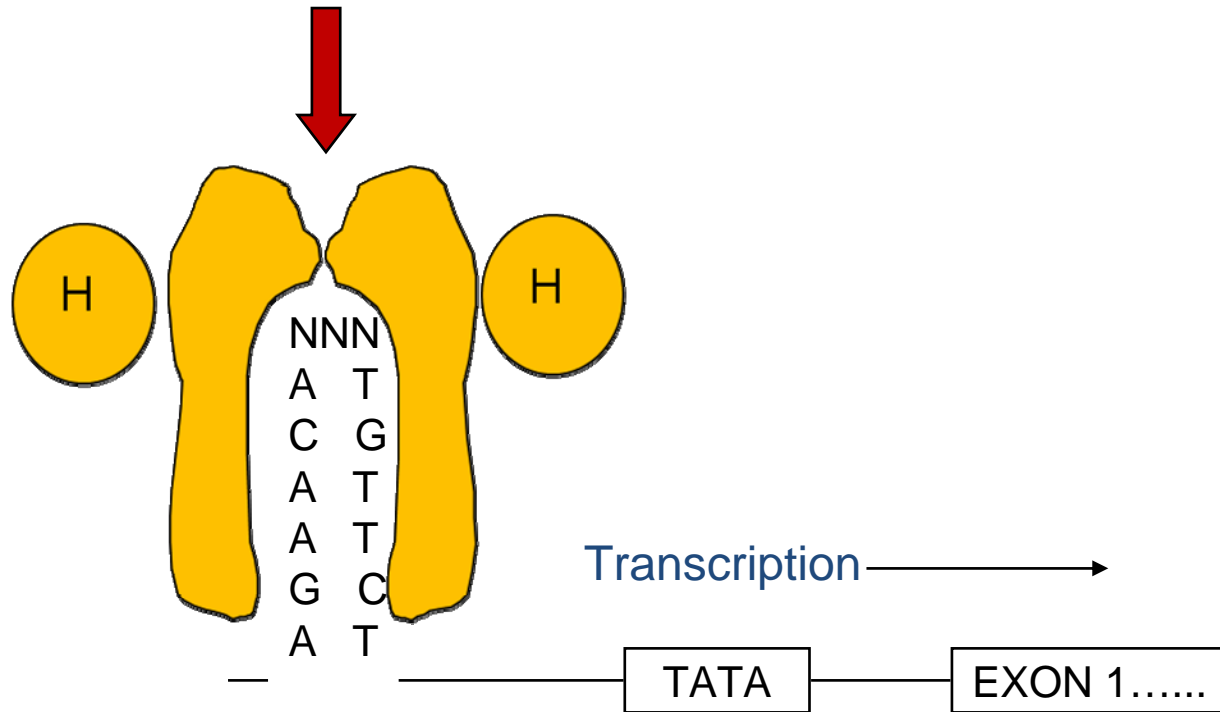
Further replacement of five amino acids in the stem of the second zinc finger transforms the specificity to that of the thyroid hormone receptor.

These findings localize structural determinants required for discrimination of HRE sequence and half-site spacing, respectively, and suggest a simple pathway for the coevolution of receptor DNA binding domains and hormone-responsive gene networks.



Palindromic Sequences Allow Binding of Receptors as Dimers

5' -AGAACA_nnTGTTCT- 3'



AGACA-N₃-TGTTCT GRE/PRE
AGGTCA-N₃-TGACCT ERE

Sharing of HREs by Different Steroid Receptors

There are many classes of steroid receptors, but only 3 consensus HRE's.

Many receptors recognize the same HRE!

How is specificity achieved

How does a cell know its being stimulated by PR and not GR)?

- Cell specific expression of receptors (don't express both PR and GR in same cell. But sometimes they are in the same cell!)
- Other transcriptional regulation elements (cofactors)

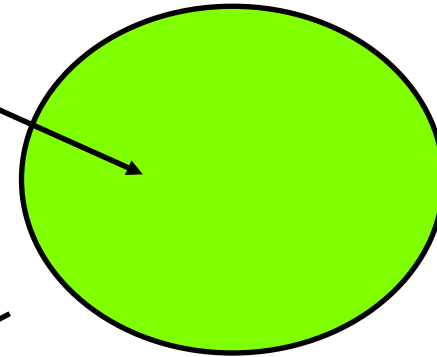
Evidence for HRE Specificity

Mader et al., 1989

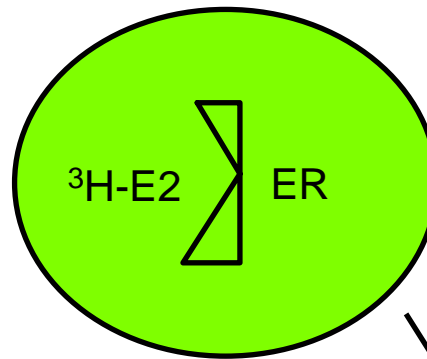
Estrogen Receptor cDNA



Transfect Cells



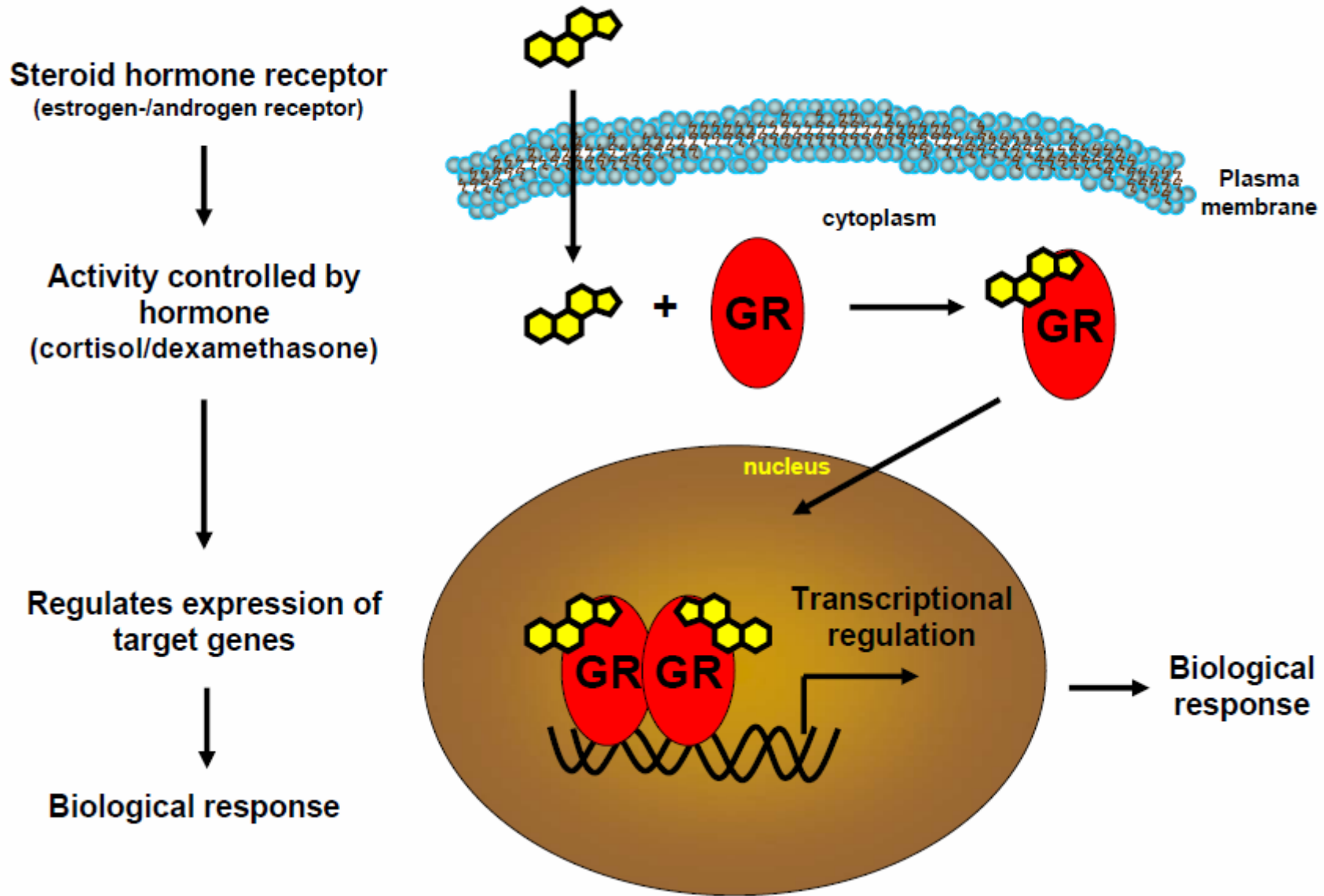
Expression



Genes with ERE= transcription

Genes with GRE= no transcription

Glucocorticoid Receptor Signaling : Transcriptional regulation



Nuclear Receptor Superfamily

Subfamily 1

NR1A1 (TR α) ←
NR1A2 (TR β) ←
NR1B1 (RAR α) ←
NR1B2 (RAR β) ←
NR1B3 (RAR γ) ←
NR1C1 (PPAR α) ←
NR1C2 (PPAR δ) ←
NR1C3 (PPAR γ) ←
NR1D1 (REV-ERB α) ←
NR1D2 (REV-ERB β) ←
NR1F1 (ROR α) ←
NR1F2 (ROR β) ←
NR1F3 (ROR γ) ←
NR1H2 (LXRb) ←
NR1H3 (LXRa) ←
NR1H4 (FXR) ←
NR1I1 (VDR) ←
NR1I2 (PXR) ←
NR1I3 (CAR) ←

Subfamily 2

NR2A1 (HNF4 α) ←
NR2A2 (HNF4 γ) ←
NR2A3 (HNF4 β) ←
NR2B1 (RXR α) ←
NR2B2 (RXR β) ←
NR2B3 (RXR γ) ←
NR2C1 (TR2) ←
NR2C2 (TR4) ←
NR2E1 (TLX) ←
NR2E3 (PNR) ←
NR2F1 (COUP-TF I) ←
NR2F2 (COUP-TF-II) ←
NR2F6 (EAR2) ←

Subfamily 3

NR3A1 (ER α) ←
NR3A2 (ER β) ←
NR3B1 (ERR α) ←
NR3B2 (ERR β) ←
NR3B3 (ERR γ) ←
NR3C1 (GR) ←
NR3C2 (MR) ←
NR3C3 (PR) ←
NR3C4 (AR) ←

Subfamily 4

NR4A1 (NGF-IB)
NR4A2 (Nurr1)
NR4A3 (Nor-1)

Subfamily 5

NR5A1 (SF-1)
NR5A2 (LRH-1)

Subfamily 6

NR6A1 (GCNF)

Subfamily 0

NR0B1 (DAX-1)
NR0B2 (SHP)

- Targets of marketed drugs - 35%
- Orphan NRs - 50%

← Marketed NR Drug

Nuclear Receptors

Steroid Receptors

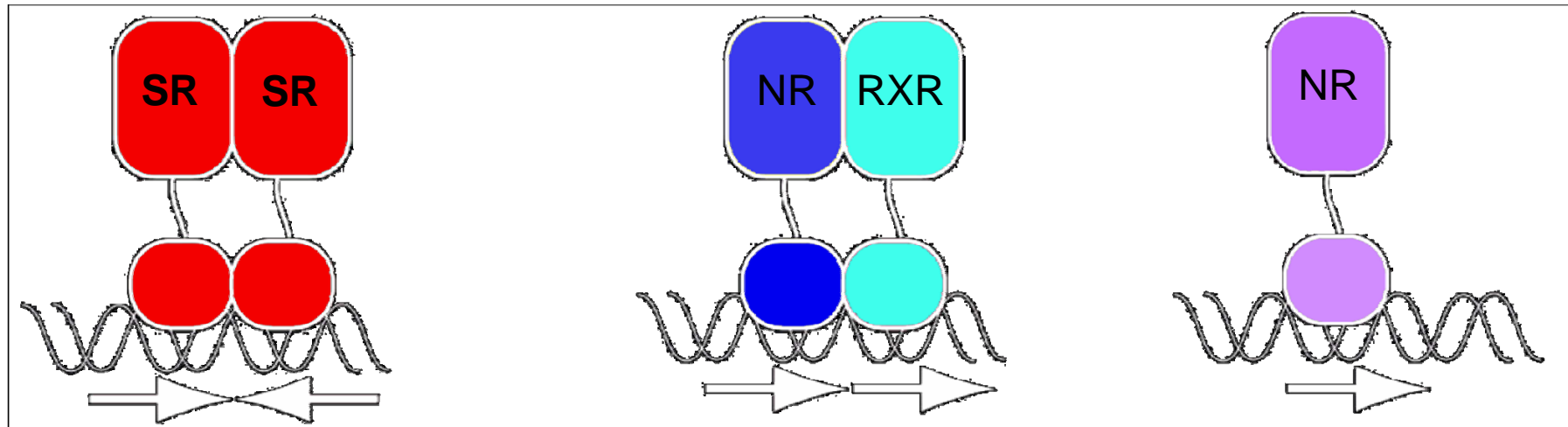
GR Glucocorticoid
PR Progesterone
AR Androgen
ER Estrogen

Class II Receptors

VDR, PPAR
 TR, FXR
 RXR, LXR
 RAR, PXR

Orphan Receptors

NGFI-B
 SF-1
 ERR
 ReVERB



AGAACA-N3-TGTTCT **GRE/PRE**
 AGGTCA-N3-TGACCT **ERE**

ACGGTCA-N1-5-AGGTCA

AAA-ACGGTCA NBRE
 TCA-AGGTCA SFRE

Albert Lasker Basic Medical Research Award

Pierre Chambon, Ronald Evans and Elwood Jensen



Pierre Chambon

Institute of Genetics and
Molecular Cellular Biology
Strasbourg, France



Ronald Evans

The Salk Institute for Biological
Studies, La Jolla, California



Elwood Jensen

University of Chicago
University of Cincinnati
College of Medicine

For the discovery of the superfamily of nuclear hormone receptors and elucidation of a unifying mechanism that regulates embryonic development and diverse metabolic pathways

<http://www.laskerfoundation.org/awards/2004basic.htm>

References

[Payvar, F., DeFranco, D., Firestone, G. L., Edgar, B., Wrange, O., Okret, S., Gustafsson, J. A., and Yamamoto, K. R. \(1983\). Sequence-specific binding of glucocorticoid receptor to MTV DNA at sites within and upstream of the transcribed region. Cell 35, 381-392.](#)

[Yamamoto, K. R. \(1985\). Steroid receptor regulated transcription of specific genes and gene networks. Annu Rev Genet 19, 209-252.](#)

Functional Domains of Nuclear Receptors

[Evans, R. M. \(1988\). The steroid and thyroid hormone receptor superfamily. Science 240, 889-895.](#)
[Green, S., and Chambon, P. \(1987\). Oestradiol induction of a glucocorticoid-responsive gene by a chimaeric receptor. Nature 325, 75-78.](#)

The Nuclear Receptor Superfamily

[Payvar, F., DeFranco, D., Firestone, G. L., Edgar, B., Wrange, O., Okret, S., Gustafsson, J. A., and Yamamoto, K. R. \(1983\). Sequence-specific binding of glucocorticoid receptor to MTV DNA at sites within and upstream of the transcribed region. Cell 35, 381-392.](#)

[Yamamoto, K. R. \(1985\). Steroid receptor regulated transcription of specific genes and gene networks. Annu Rev Genet 19, 209-252.](#)