Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 22

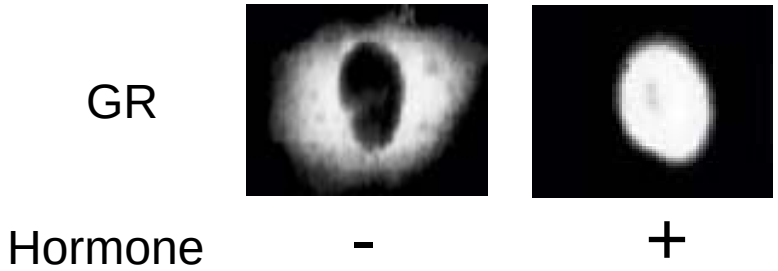
Regulation of gene expression by type II nuclear receptors

The nuclear receptor superfamily

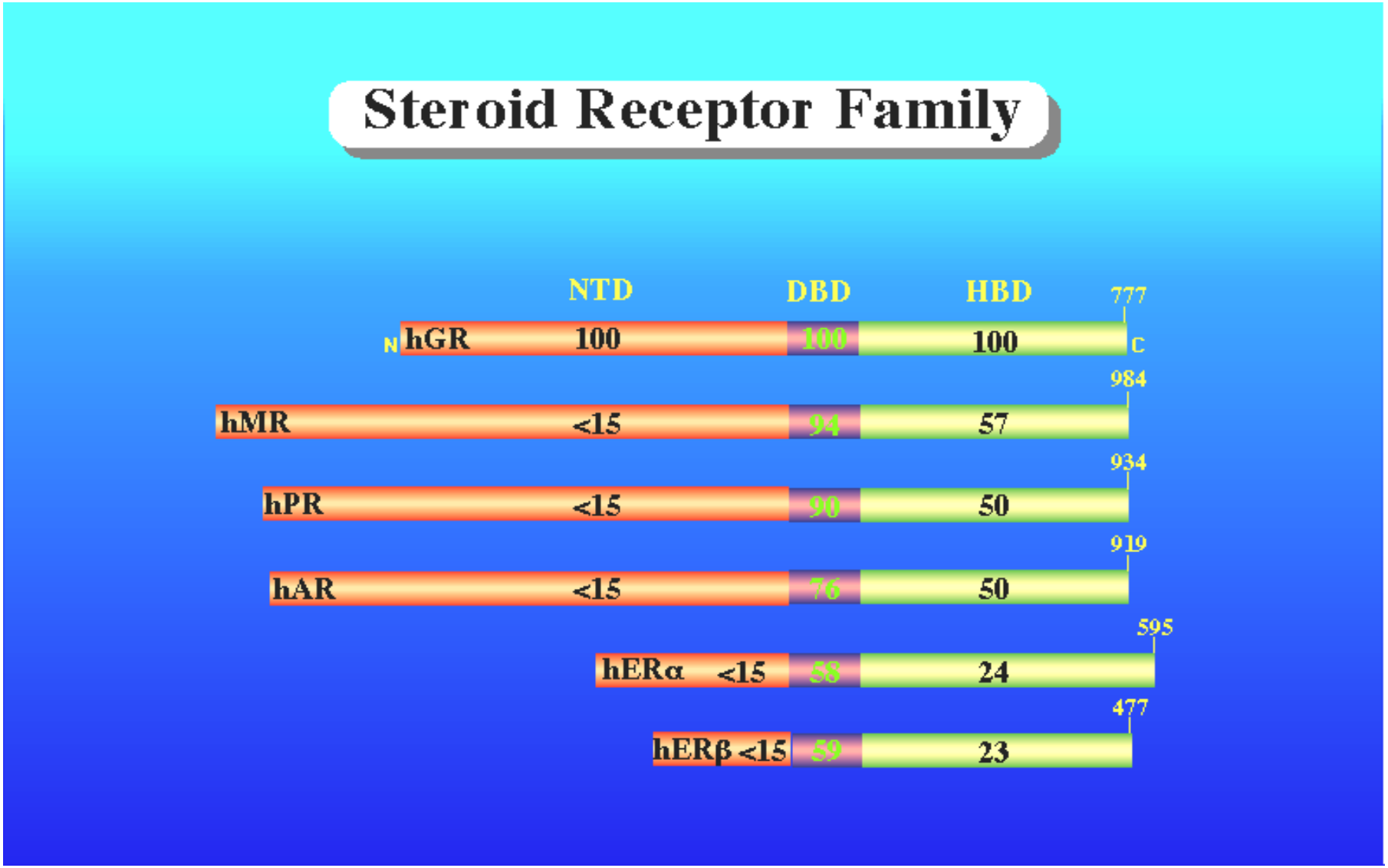
Type I receptors

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.



Steroid Receptor Family



AGAACA-N3-TGTTCT

AGGTCA-N3-TGACCT

The nuclear receptor superfamily

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, retinoic acid and vitamin D

THYROID HORMONE AND ITS RECEPTOR

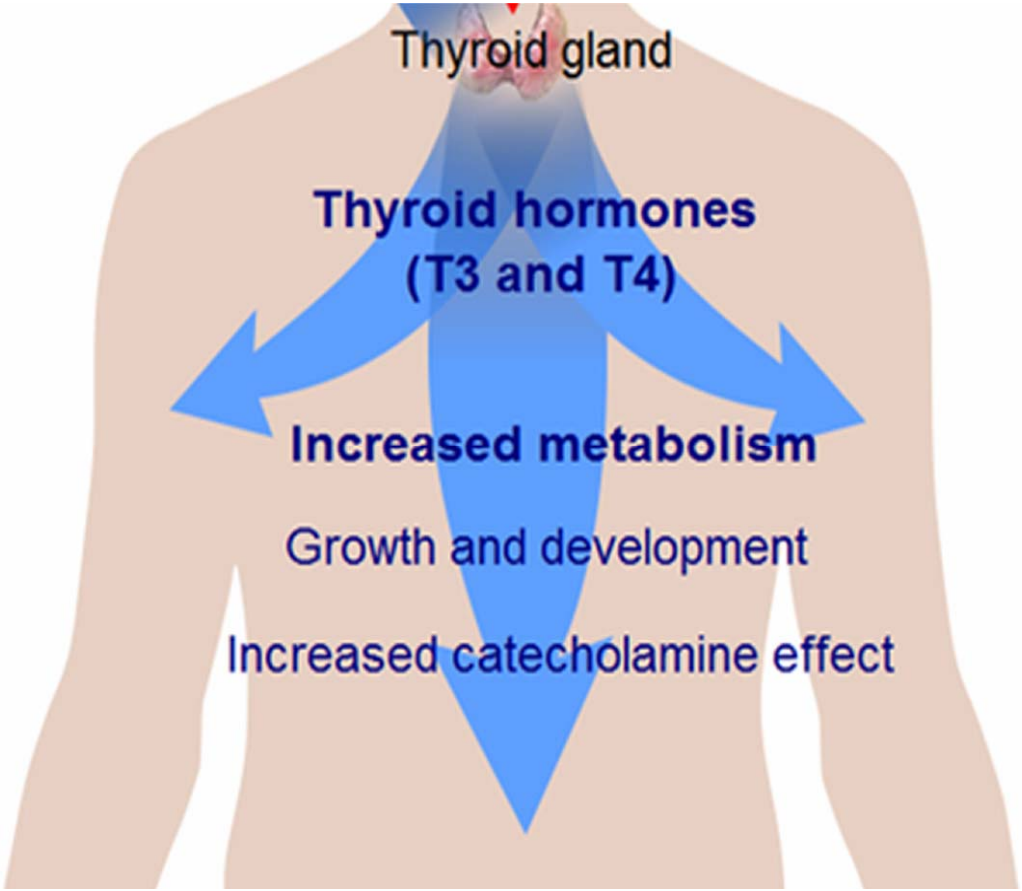
Ligand for thyroid hormone receptor

THYROXINE (T4)

↓ DEIODINASE

TRIIODOTHYRONINE (T3)

[Thyroxine](#) (T4) is produced in 20:1 ratio to [triiodothyronine](#) (T3)



[Adapted from](#) http://en.wikipedia.org/wiki/File:Thyroid_system.png

[Author: Mikael Häggström](#)

Historical links between the steroid and thyroid hormone receptor signaling systems

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

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

Nature. 1985 Dec 19-1986 Jan 1;318:635-641.

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

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

Nature. 1985 Dec 19-1986 Jan 1; 318:670-672.

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.

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.

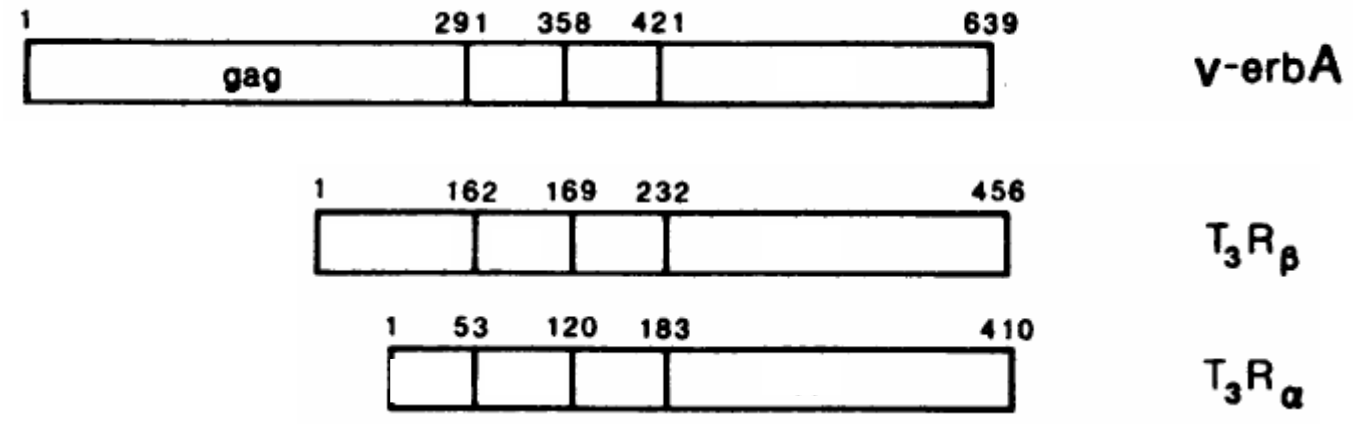
V-erbA is an oncogene expressed by the Avian Erythroblastosis Virus (AEV) which induces erythroleukemia and sarcomas in chickens .

V-erbA binds to the DNA but does not bind the hormone T3.

The finding that V-erbA is homologous to thyroid hormone receptor suggested that deregulation of thyroid hormone signalling can lead to cancer.

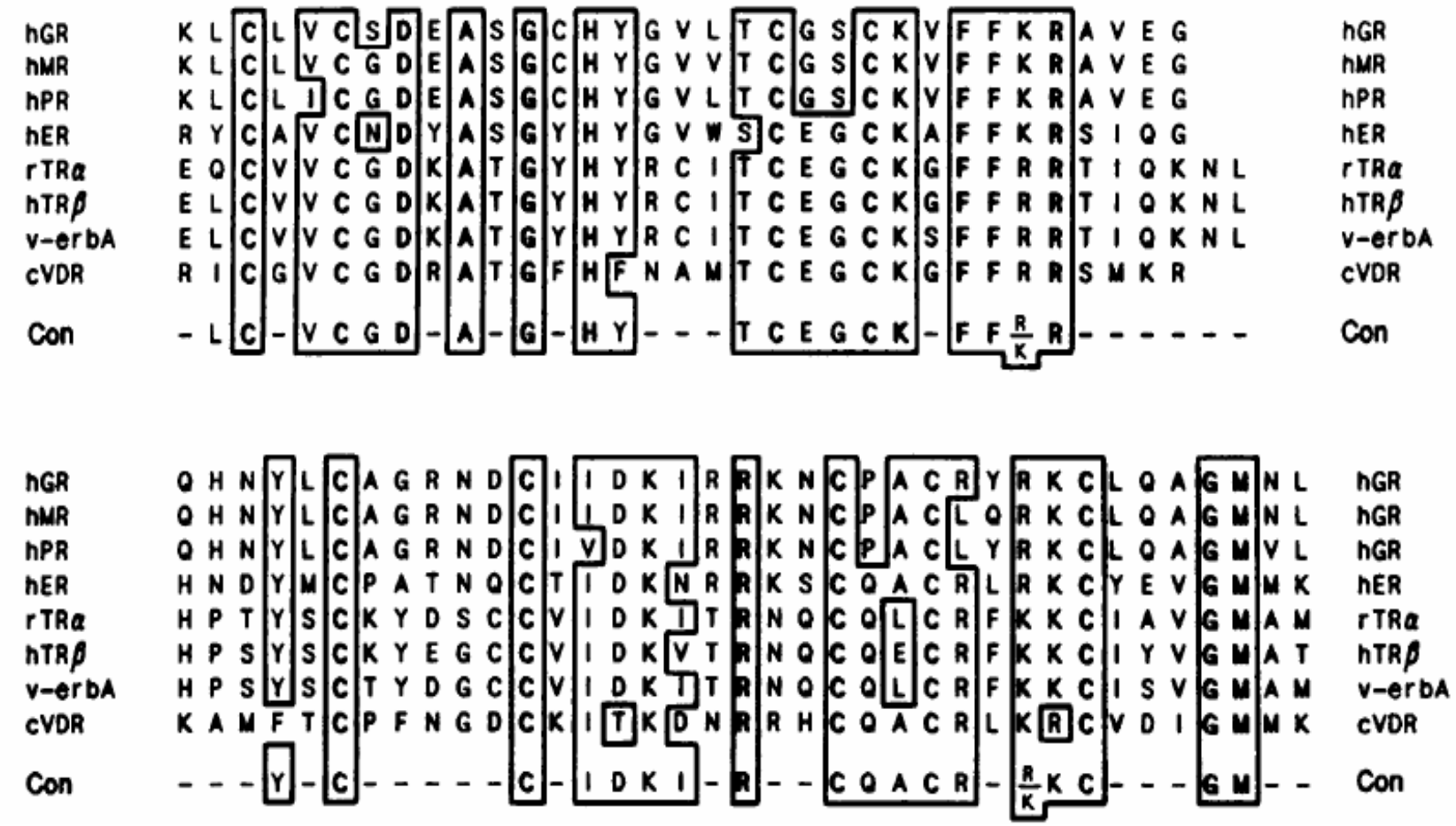
Hypervariable	DBD	LBD

Steroid hormone receptors



Although steroid and thyroid hormones are neither structurally nor biosynthetically related, the fact that their receptors shared structural homology suggested that there is a large superfamily of genes whose products are ligand-responsive transcription factors.

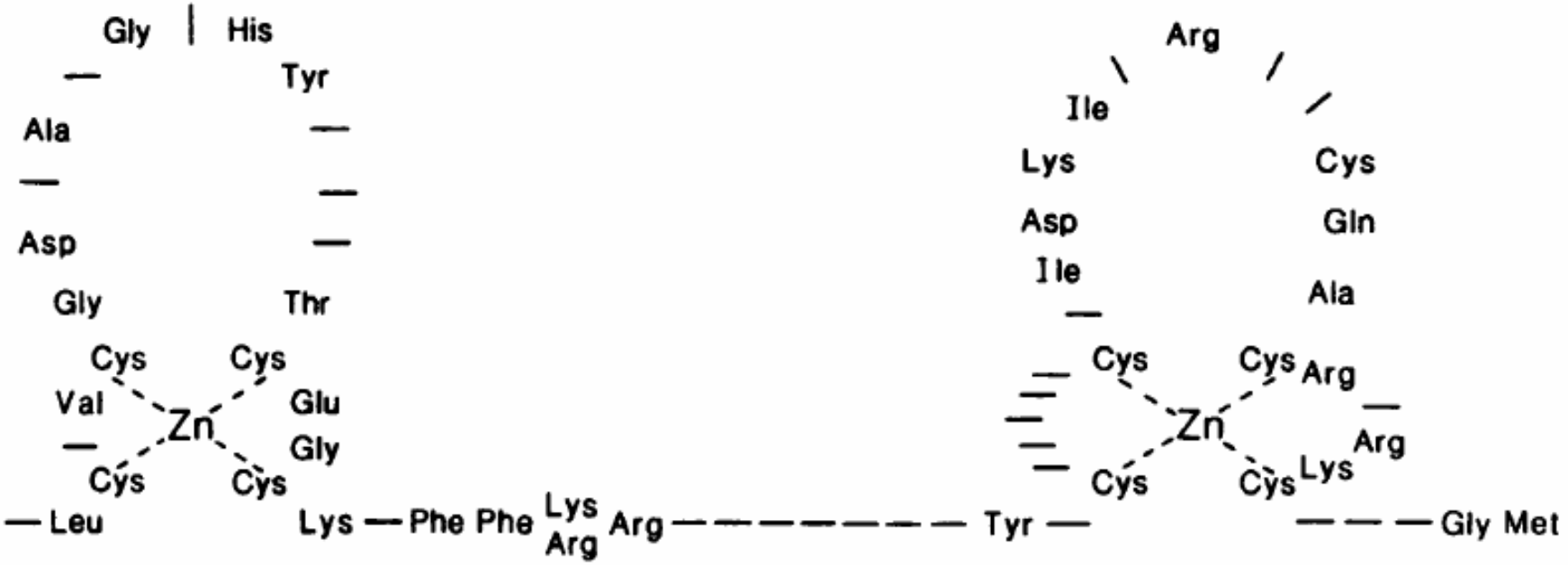
Thus the existence of a steroid/thyroid receptor superfamily was proposed



The Steroid and Thyroid Hormone Receptor Superfamily

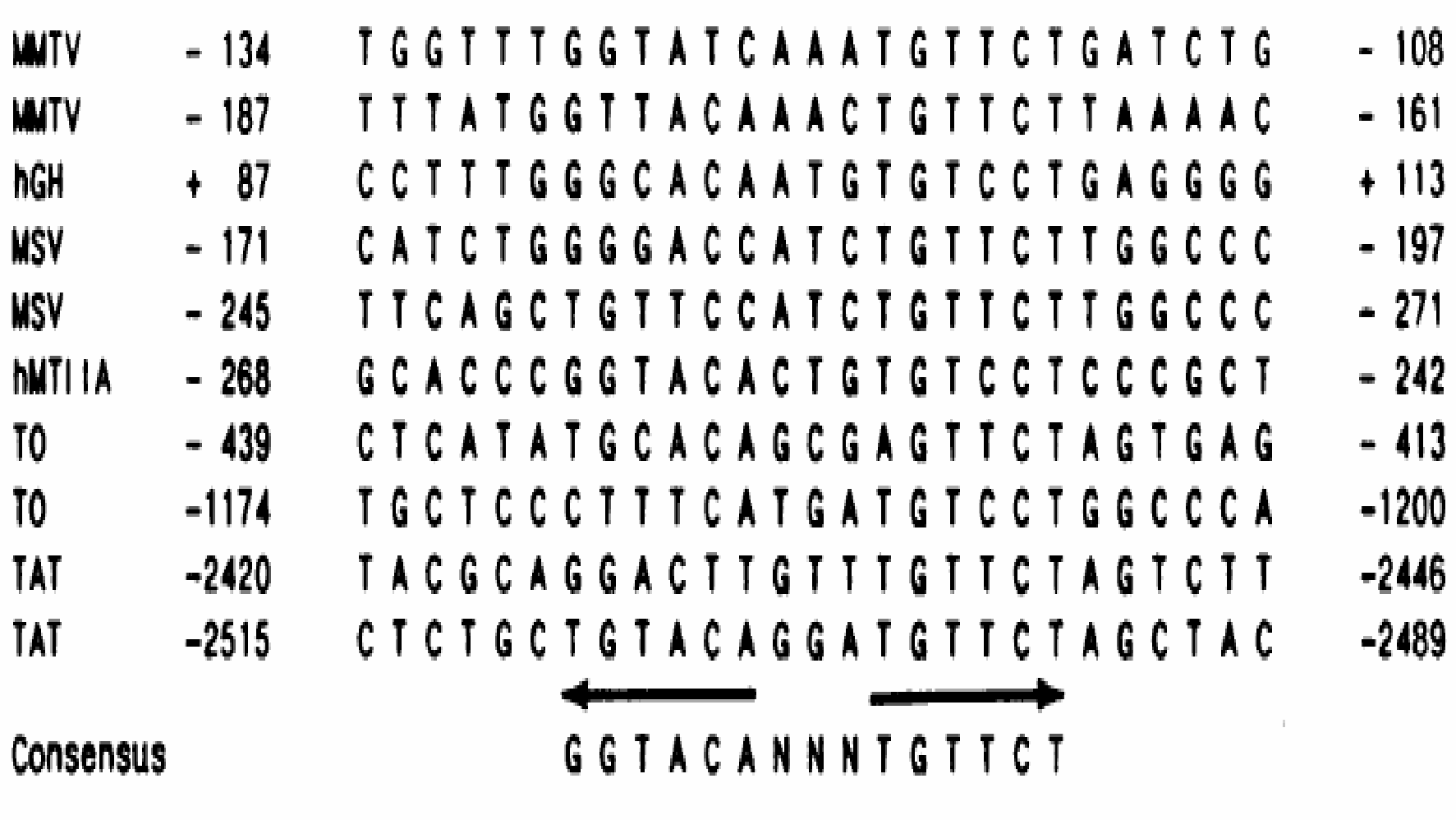
RONALD M. EVANS

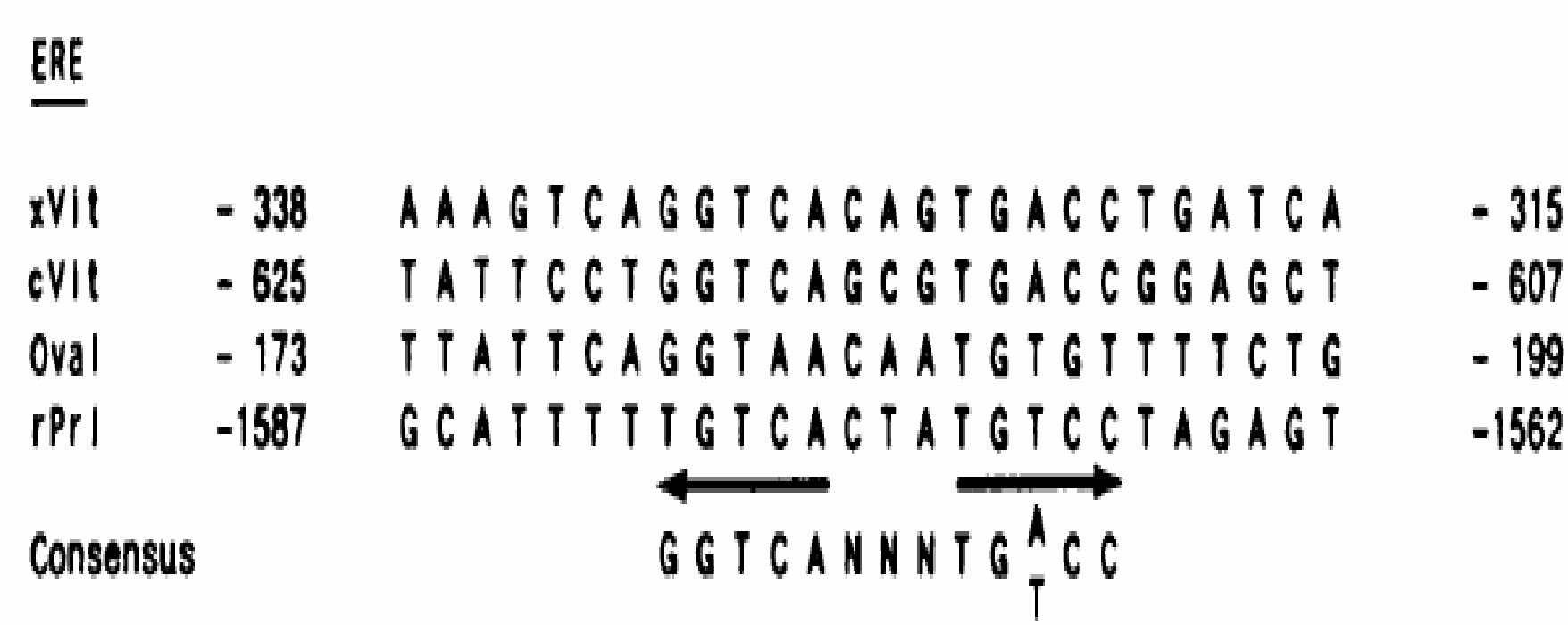
Science (1988) *240, 889–895.*

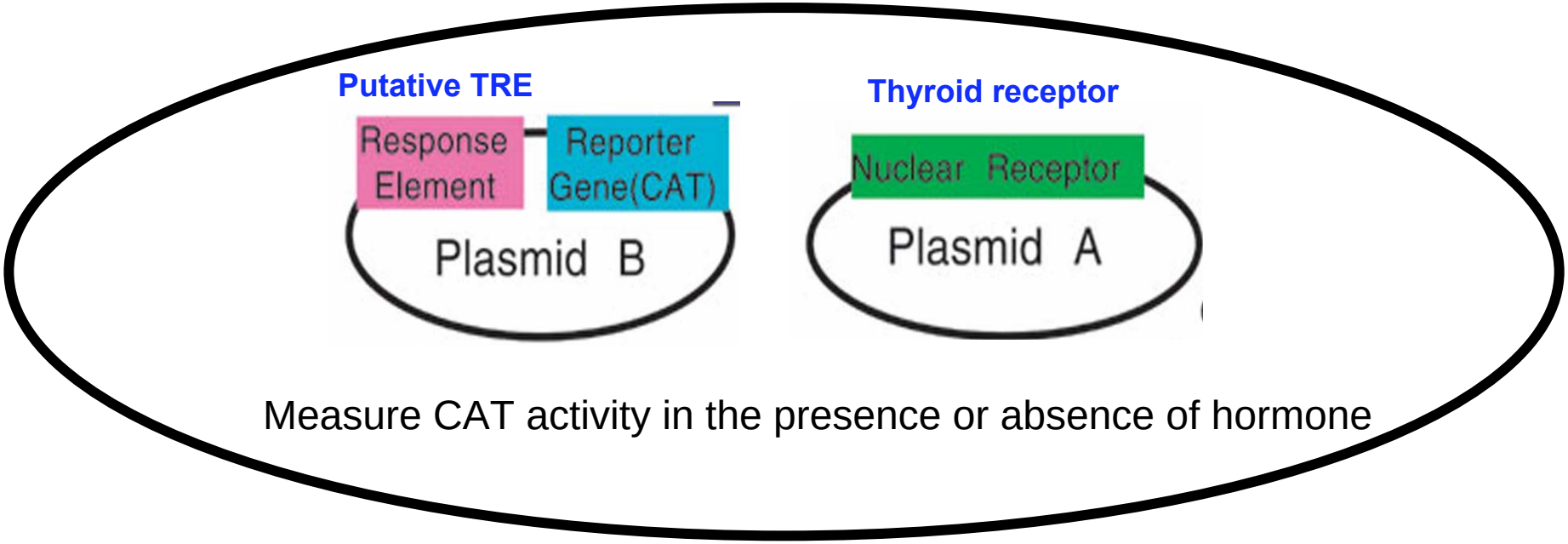


Science (1988) *240, 889–895.*

GRE


MMTV - 134 TGGTTTGGTATCAAATGTTCTGATCTG - 108
MMTV - 187 TTTATGGTTACAAACTGTTCTTAAAAC - 161
hGH + 87 CCTTTGGGCACAATGTGTCCTGAGGGG + 113
MSV - 171 CATCTGGGGACCATCTGTTCTTGGCCC - 197
MSV - 245 TTCAGCTGTTCCATCTGTTCTTGGCCC - 271
hMTIIA - 268 GCACCCGGTACACTGTGTCCTCCCGCT - 242
TO - 439 CTCATATGCACAGCGAGTTCTAGTGAG - 413
TO -1174 TGCTCCCTTTCATGATGTCCTGGCCCA -1200
TAT -2420 TACGCAGGACTTGTTTGTTCTAGTCTT -2446
TAT -2515 CTCTGCTGTACAGGATGTTCTAGCTAC -2489
Consensus GGTACANNNTGTTCT

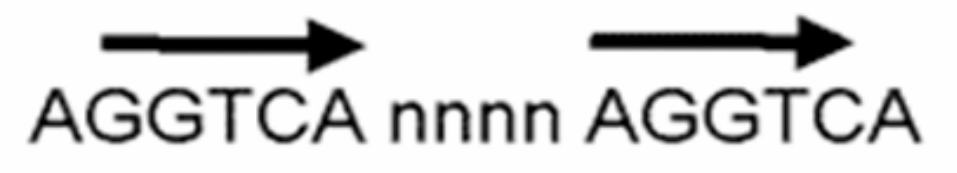

ERE
xVit - 338 A A A G T C A G G T C A C A G T G A C C T G A T C A - 315
cVit - 625 T A T T C C T G G T C A G C G T G A C C G G A G C T - 607
Oval - 173 T T A T T C A G G T A A C A A T G T G T T T T C T G - 199
rPrl -1587 G C A T T T T T G T C A C T A T G T C C T A G A G T -1562
Consensus G G T C A N N N T G A/T C C



CIS-TRANS CO-TRANSFECTION ASSAY

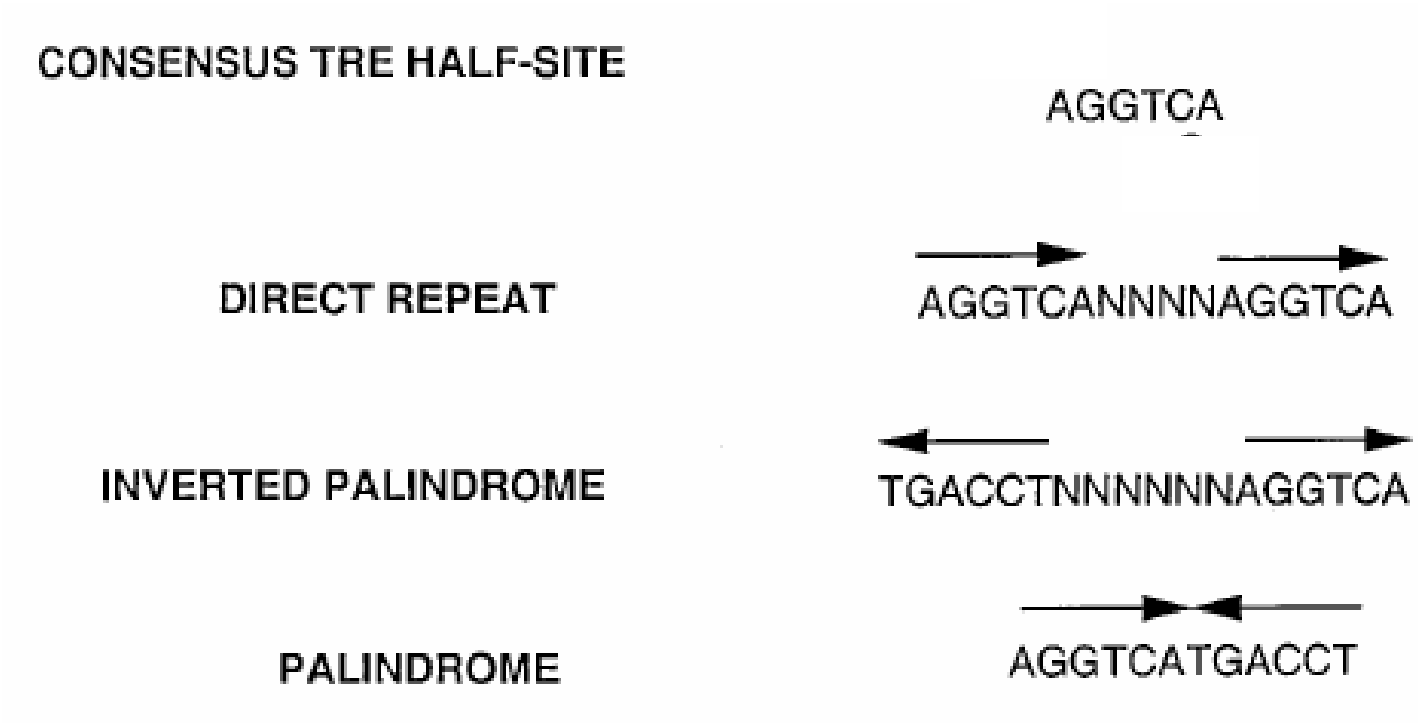
T3 - +

Thyroid hormone receptors bind to short, repeated sequences of DNA called thyroid or T3 response elements (TREs), composed of two AGGTCA "half sites" separated by four nucleotides.



DR4

Analysis of other thyroid hormone responsive promoters indicated that the half sites of a TRE can be arranged as direct repeats, palindromes or inverted repeats.



Cell. 1991 Jun 28;65(7):1255-66.

Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors.

Umesono K, Murakami KK, Thompson CC, **Evans RM**.

1958–1999

Thyroid hormone response elements (TREs) consist of direct repeat half sites seperated by a 4 bp spacer (AGGTCAnnnnAGGTCA).

The TRE can be converted into a retinoic acid response element (RARE) by increasing the spacing between the half-sites by 1 nucleotide (AGGTCAnnnnnAGGTCA), and the resulting retinoic acid response element is no longer a TRE.

Decreasing the half-site spacing by 1 nucleotide converts the TRE to a vitamin D3 response element (AGGTCAnnnAGGTCA), while eliminating response to T3.

This study suggests that a simple physiologic code exists in which half-site spacing plays a critical role in achieving selective hormonal response.

“3–4–5” rule

VDR: DR3

TR: DR4

RAR: DR5

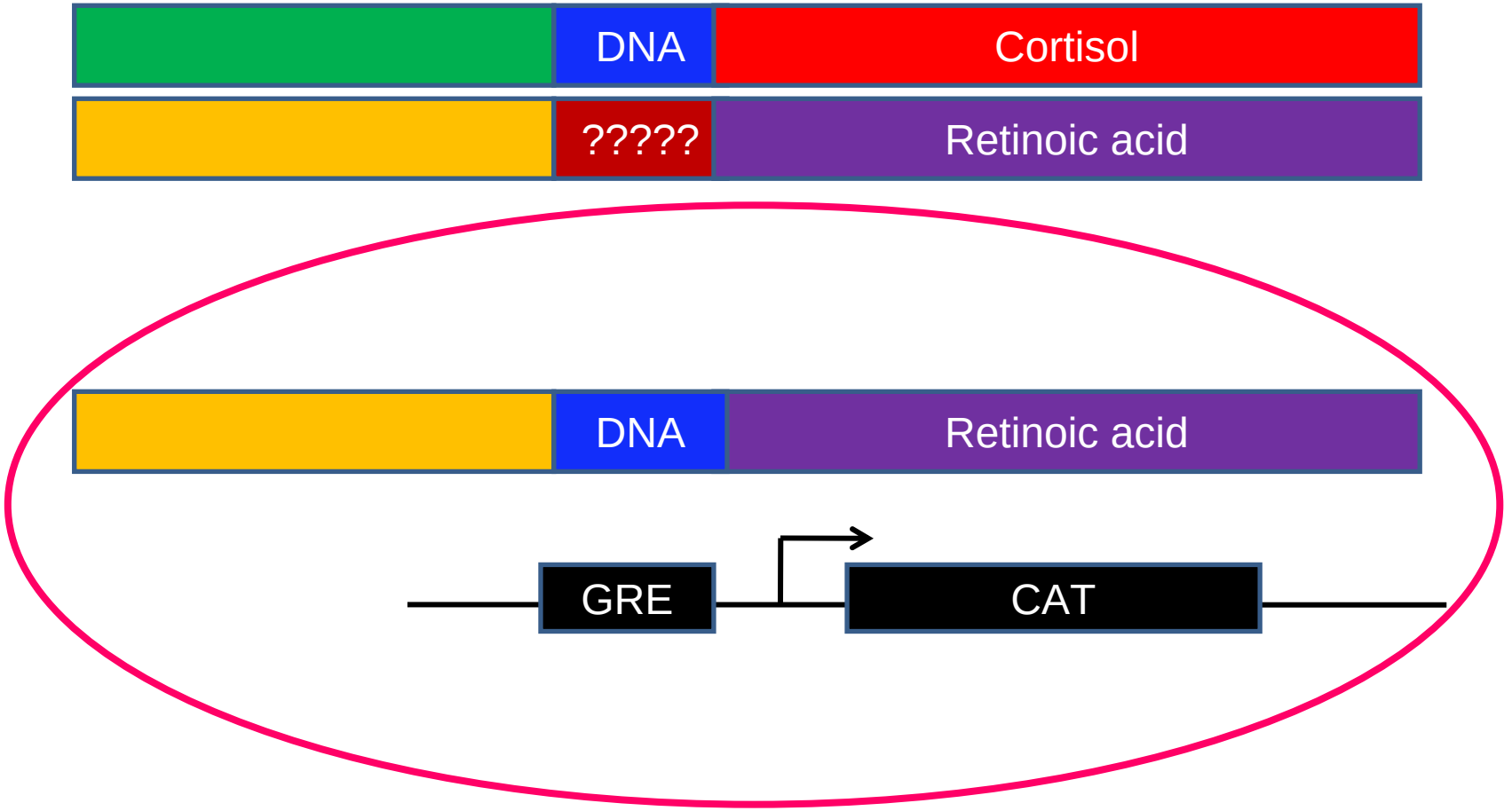
Endocrine Reviews (1994)15:391

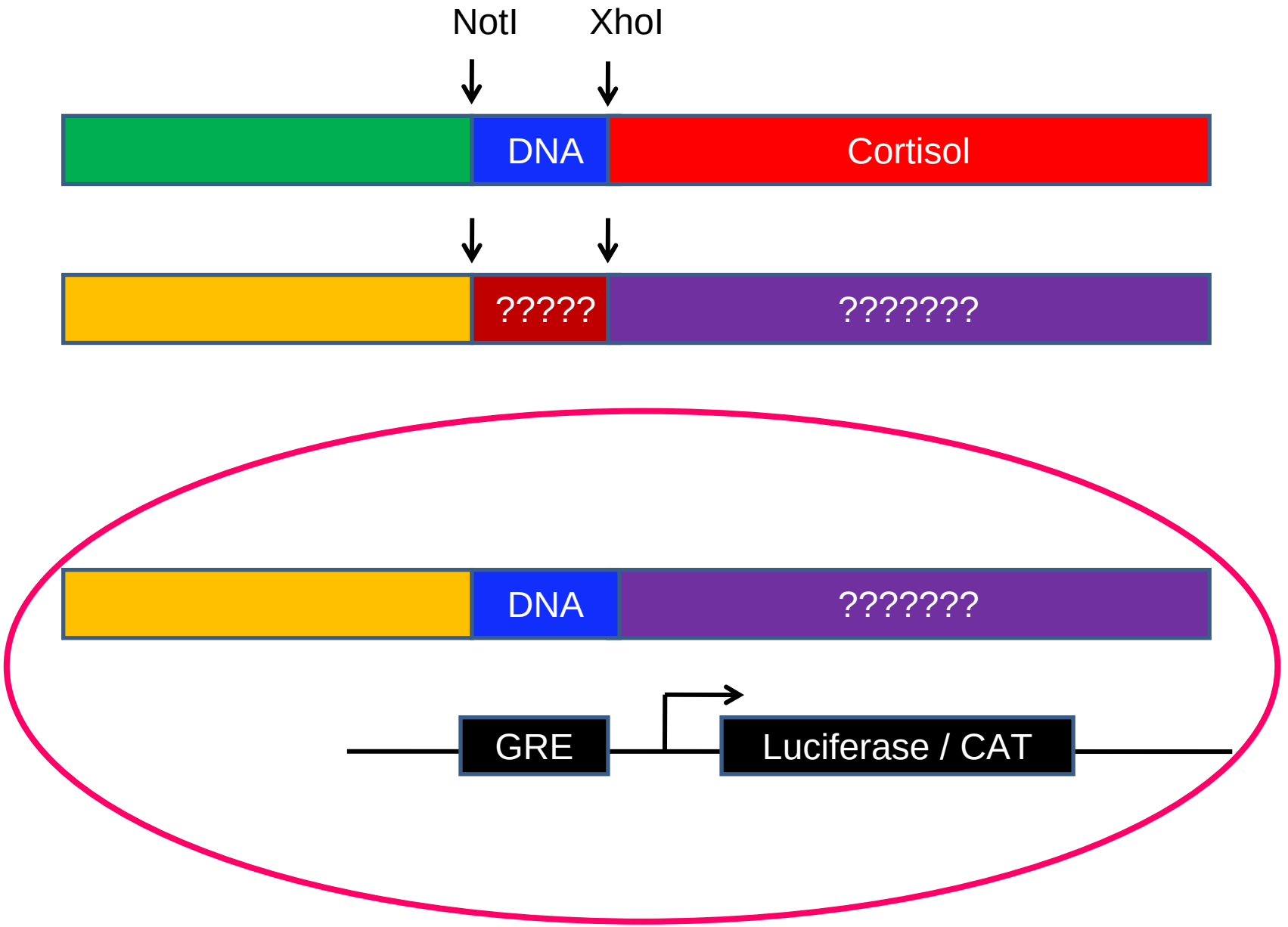
While the DNA binding specificities of a number of NRs whose ligands are known such as GR, ER, TR, VDR was being deciphered, efforts were also being made to clone and characterize other nuclear receptors such as the retinoic acid receptor (RAR)

Identification of a receptor for the morphogen retinoic acid

Vincent Giguere, Estelita S. Ong, Prudimar Segui & Ronald M. Evans

Nature (London) 330, 624 (1987).





Strategy for the identification of ligands for orphan receptors

While the DNA binding specificities of a number of NRs whose ligands are known such as TR, VDR was being deciphered, efforts were also being made to identify the ligands for orphan receptors. One such orphan receptor is RXR or retinoid X receptor.

RXR specifically responded to retinoids because high concentrations of all-*trans*-retinoic acid (ATRA) could activate RXRα,

Later, an isomer of ATRA, 9-*cis*-retinoic acid (9CRA), was found to be the ligand for RXRα, as well as for the two additional related subtypes, RXRβ (NR2B2) and RXRγ (NR2B3).

RAR: Receptor for all trans retinoic acid

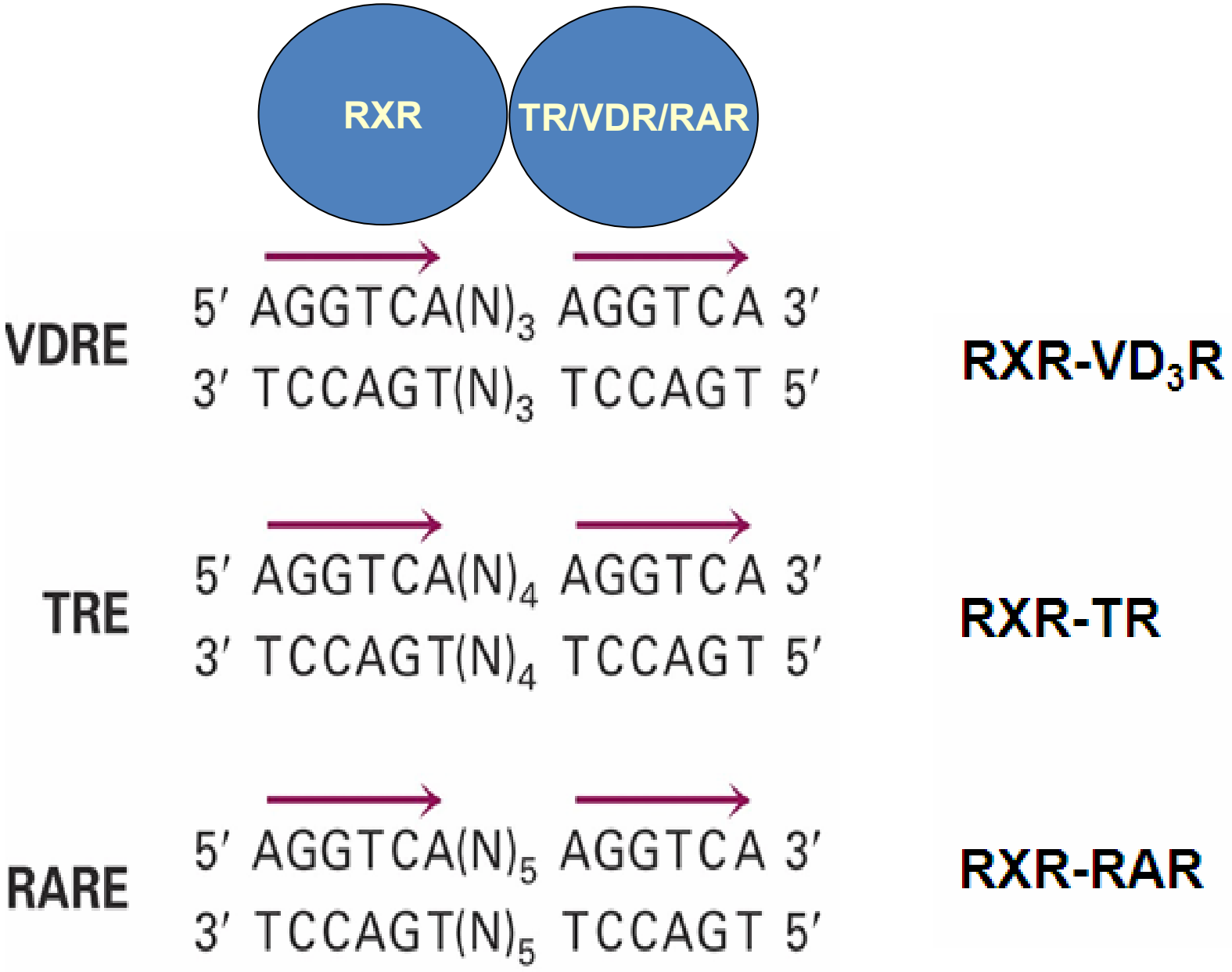
Nature. 1987 Dec 3-9;330(6147):444-50.
A human retinoic acid receptor which belongs to the family of nuclear receptors.
Petkovich M, Brand NJ, Krust A, Chambon P.

Nature. 1987 Dec 17-23;330(6149):624-9
Identification of a receptor for the morphogen retinoic acid.
Giguere V, Ong ES, Segui P, Evans RM.

RXR: Receptor for 9-cis retinoic acid

Nature. 1990 May 17;345(6272):224-9.
Nuclear receptor that identifies a novel retinoic acid response pathway.
Mangelsdorf DJ, Ong ES, Dyck JA, Evans RM.

RXRs were also independently identified as factors necessary for efficient binding to DNA of several members of the nuclear receptor superfamily and were shown to form heterodimers with these other nuclear receptors such as TR, VDR and RAR.


RXR
TR/VDR/RAR
VDRE
5′ AGGTCA(N)3 AGGTCA 3′
3′ TCCAGT(N)3 TCCAGT 5′
RXR-VD3R
TRE
5′ AGGTCA(N)4 AGGTCA 3′
3′ TCCAGT(N)4 TCCAGT 5′
RXR-TR
RARE
5′ AGGTCA(N)5 AGGTCA 3′
3′ TCCAGT(N)5 TCCAGT 5′
RXR-RAR


ER α,β
PR
AR
GR
MR
RAR α,β,γ
TR α,β
VDR
EcR
SF-1
LRH-1
DAX-1
SHP
TLX
PNR
NGFI-B α,β,γ
ROR α,β,γ
ERR α,β,γ
RVRα,β,γ
GCNF
TR 2,4
HNF-4
COUP-TFα,β,γ
RXR α,β,γ
PPAR α,β,γ
LXR α,β
FXR
PXR/SXR
CAR

“3– 4–5” rule:

VDR: DR3

TR: DR4

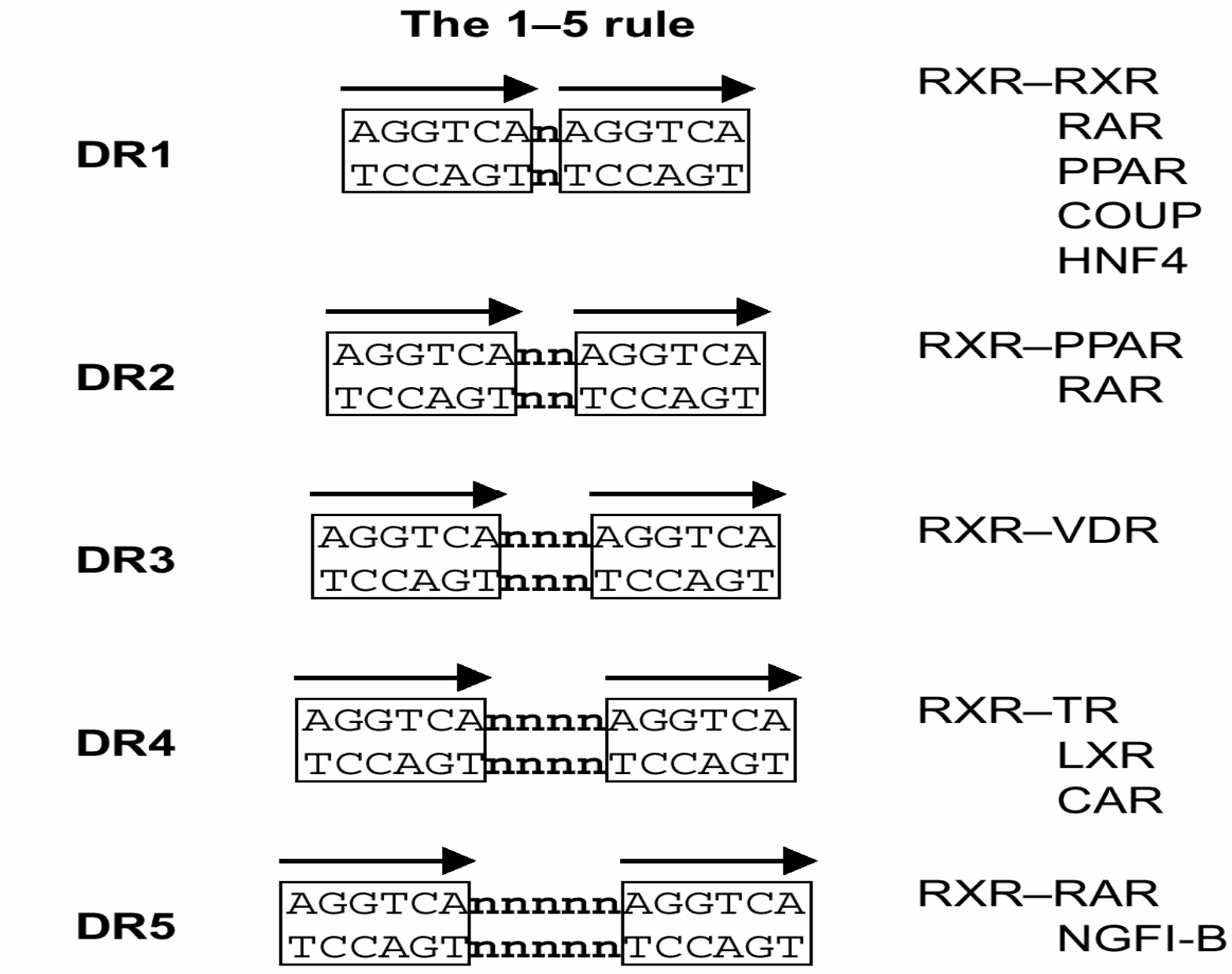
RAR: DR5

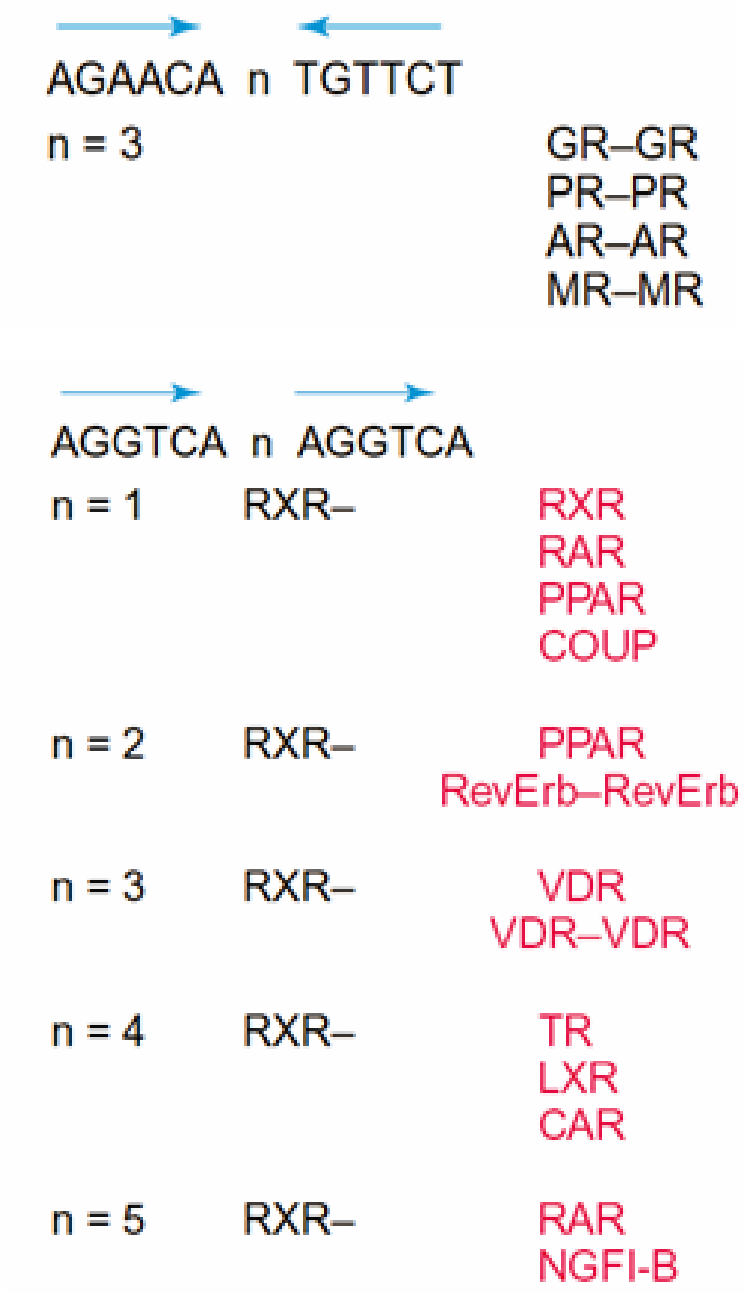
“1-2-3-4-5” rule

The 1-5 rule

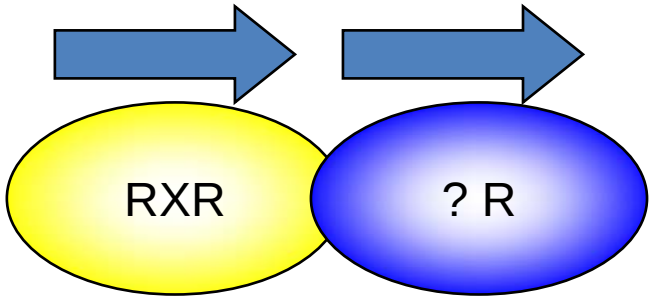
- direct repeats of AGGTCA with variable spacing (n)
  serve as binding sites for different NRs which have RXR as the common heterodimeric partner

- DRn where n determines partner
  - DR1 - RXR-RXR
  - DR2 - RXR-RAR
  - DR3 - RXR-VDR
  - DR4 - RXR-TR
  - DR5 - RXR-RAR
- RXR binds 1st half site, partner binds 2nd half site

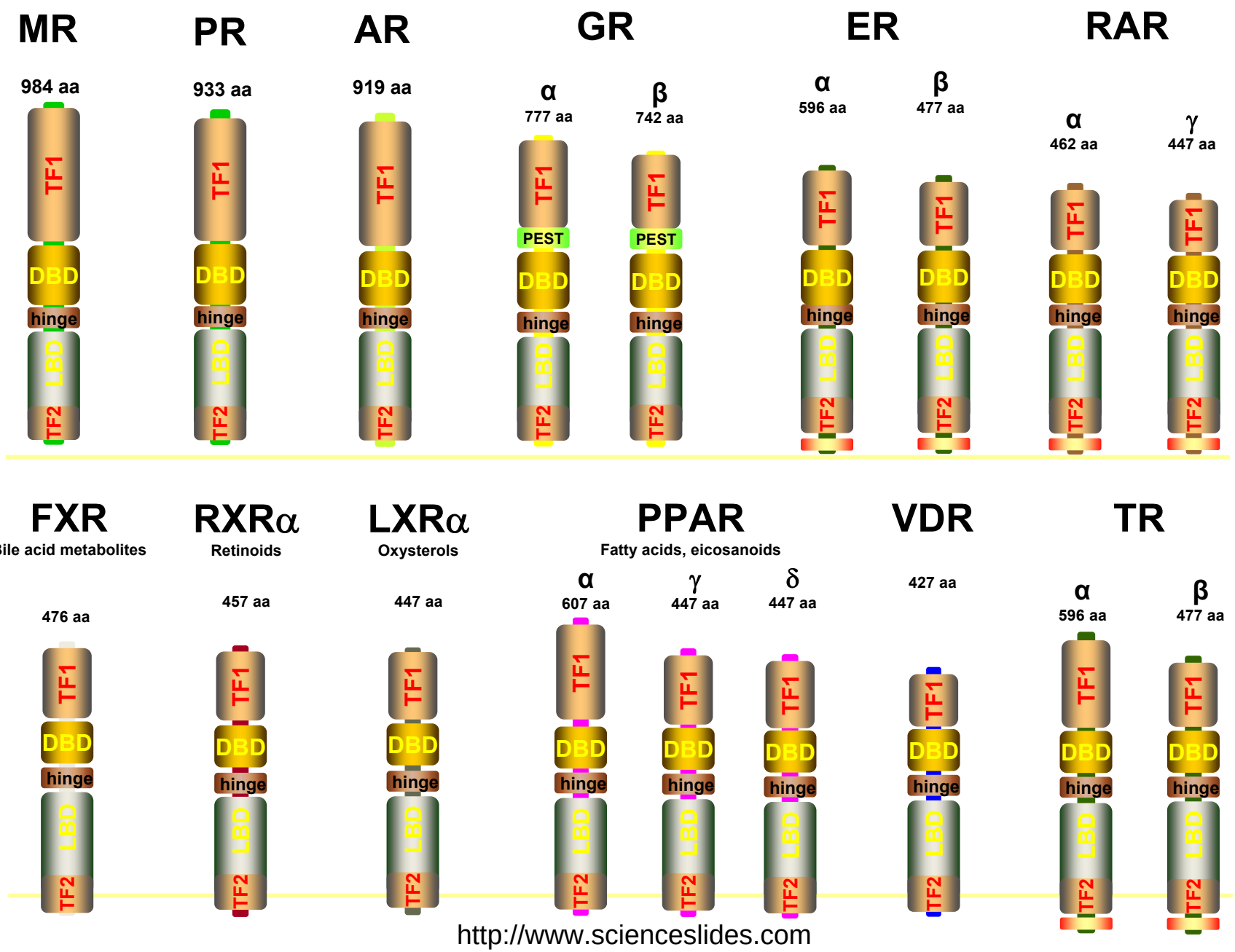

The 1–5 rule
DR1
AGGTCAnAGGTCA
TCCAGTnTCCAGT
RXR–RXR
RAR
PPAR
COUP
HNF4
DR2
AGGTCAnnAGGTCA
TCCAGTnnTCCAGT
RXR–PPAR
RAR
DR3
AGGTCAnnnAGGTCA
TCCAGTnnnTCCAGT
RXR–VDR
DR4
AGGTCAnnnnAGGTCA
TCCAGTnnnnTCCAGT
RXR–TR
LXR
CAR
DR5
AGGTCAnnnnnAGGTCA
TCCAGTnnnnnTCCAGT
RXR–RAR
NGFI-B


AGAACA n TGTTCT
n = 3
GR–GR
PR–PR
AR–AR
MR–MR
AGGTCA n AGGTCA
n = 1
RXR–
RXR
RAR
PPAR
COUP
n = 2
RXR–
PPAR
RevErb–RevErb
n = 3
RXR–
VDR
VDR–VDR
n = 4
RXR–
TR
LXR
CAR
n = 5
RXR–
RAR
NGFI-B

THUS RXR, IN ADDITION TO BINDING 9-CIS RA AND REGULATING THE EXPRESSION OF GENES THROUGH THE DR-1ELEMENT, IS A KEY HETERODIMERIC PARTNER FOR A NUMBER OF OTHER NUCLEAR RECEPTORS



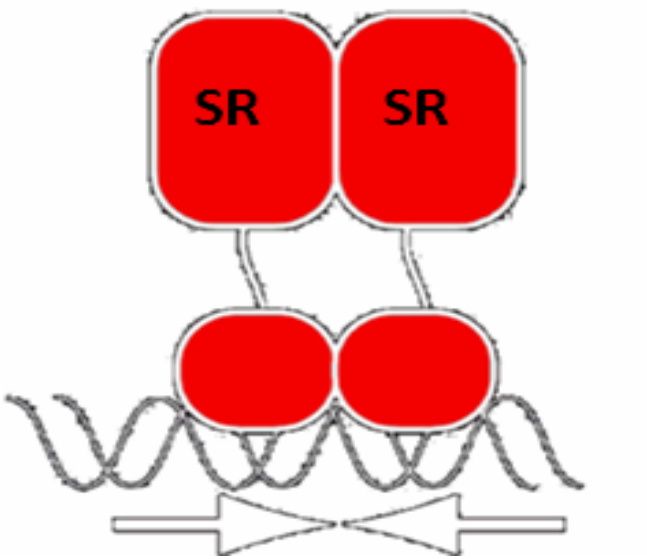
Nuclear receptor family



http://www.scienceslides.com

Type I
Steroid Receptors

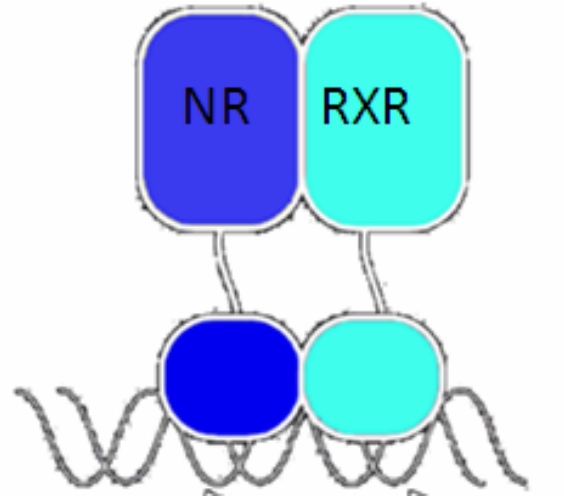
GR Glucocorticoid
PR Progesterone
AR Androgen
ER Estrogen



AGAACA-N3-TGTTCT **GRE/PRE**

AGGTCA-N3-TGACCT **ERE**

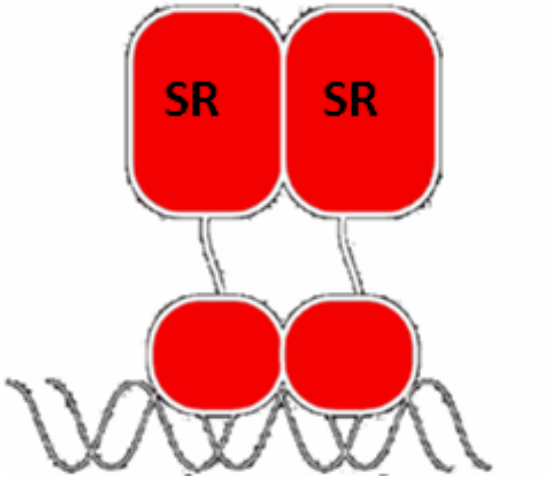
Type II *Receptors*



NR: RXR, PPAR,
VDR, TR, RAR

AGGTCA N(1-5)AGGTCA

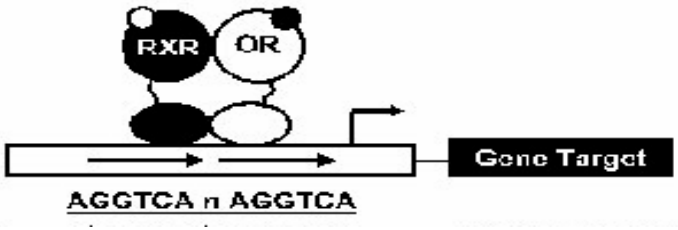
Type I Steroid Receptors



SR: GR, MR, AR, ER, PR

AGAACA(N3)TGTTCT

Steroid receptors	Nuclear receptors
Cytoplasmic or nuclear	Nuclear
Associated with HSPs in absence of ligands	Never associated with HSPs
DNA binding is ligand dependent	Can bind to DNA in both liganded and unliganded states
Homodimer	Heterodimer
Inverted repeats (DNA half site)	Directed repeats (DNA half site)



VDR	DR-3	LXRα,β	DR-4
TR	DR-4		
RARα,β,γ	DR-2 DR-5	FXR	IR-1
PPARα	DR-1		
PPARγ	DR-1	PXR	DR-3
PPARδ	DR-1	CAR	DR-5
RXRα,β,γ	DR-1		

Nuclear Receptor Superfamily

Subfamily 1

- NR1A1 (TRα) ←
- NR1A2 (TRβ) ←
- NR1B1 (RARα) ←
- NR1B2 (RARβ) ←
- NR1B3 (RARγ) ←
- NR1C1 (PPARα) ←
- NR1C2 (PPARδ)
- NR1C3 (PPARγ) ←
- NR1D1 (REV-ERBα)
- NR1D2 (REV-ERBβ)
- NR1F1 (RORα)
- NR1F2 (RORβ)
- NR1F3 (RORγ)
- NR1H2 (LXRb)
- NR1H3 (LXRa)
- NR1H4 (FXR)
- NR1I1 (VDR)
- NR1I2 (PXR)
- NR1I3 (CAR) ←

Subfamily 2

- NR2A1 (HNF4α)
- NR2A2 (HNF4γ)
- NR2A3 (HNF4β)
- NR2B1 (RXRα)
- NR2B2 (RXRβ)
- NR2B3 (RXRγ)
- NR2C1 (TR2) ←
- NR2C2 (TR4) ←
- NR2E1 (TLX) ←
- NR2E3 (PNR)
- NR2F1 (COUP-TF I)
- NR2F2 (COUP-TF-II)
- NR2F6 (EAR2)

Subfamily 3

- NR3A1 (ERα) ←
- NR3A2 (ERβ) ←
- NR3B1 (ERRα)
- NR3B2 (ERRβ)
- NR3B3 (ERRγ)
- 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%**

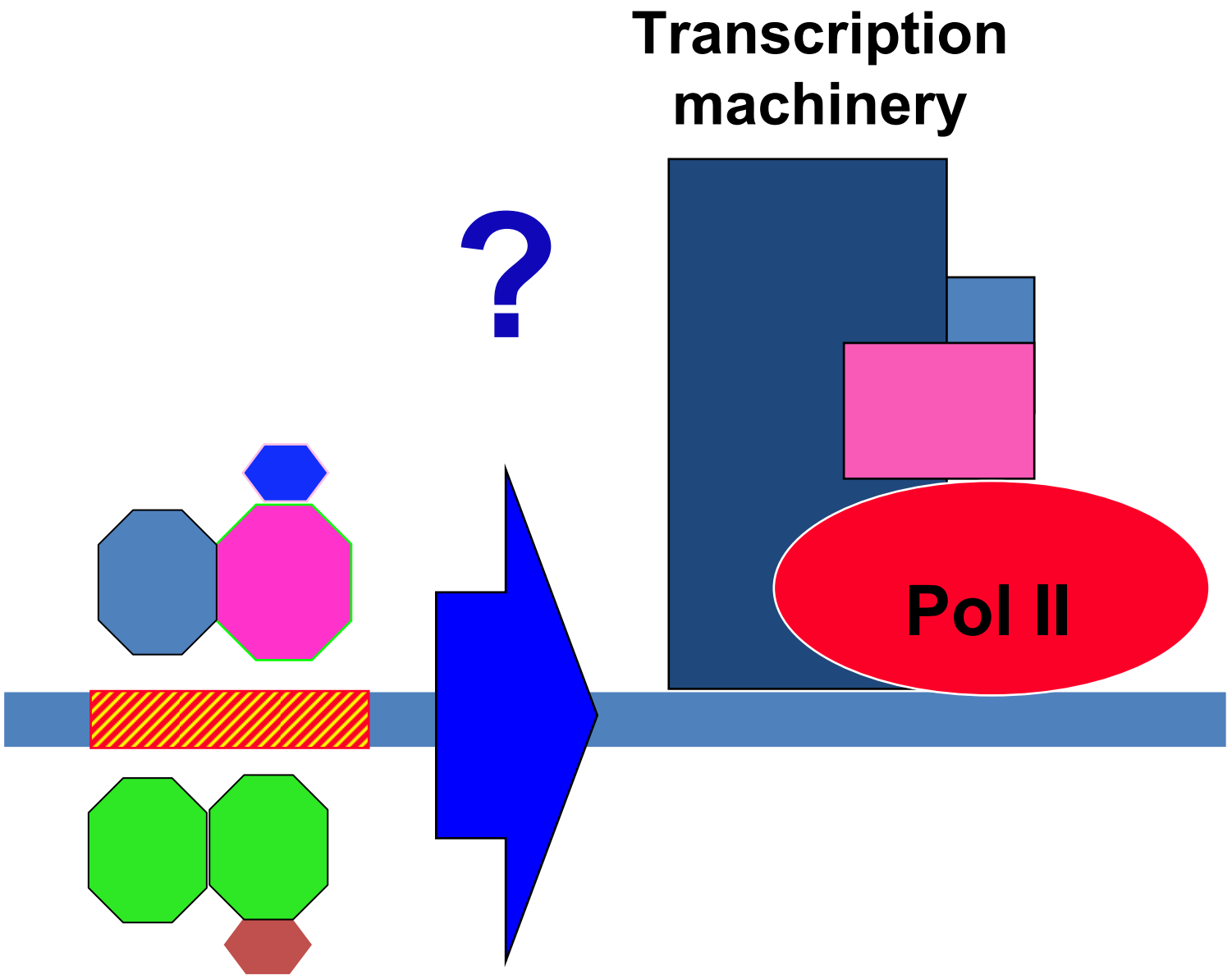
← **Marketed NR Drug**

In 1914, Kendall isolated thyroid hormone, the first lipophilic hormone, from more than 3 tons of porcine thyroid glands.

In 1915, Osborne and Mendel, and McCollum and Davis, purified the first dietary 'vital factor' (vitamin A) from cod liver oil, butter and egg yolk.

A few years later, Butenandt and Doisy discovered the female sex hormone estrogen, and Kendall and Reichstein isolated cortisol as the first adrenal steroid.

Following the cloning of genes encoding nuclear receptors and analysis of their domain structure, it is now clear that all these powerful molecules act by similar mechanisms involving their cognate receptors, which in turn became the founding members of the nuclear receptor superfamily.


Transcription machinery
?
Pol II

The Steroid and Thyroid Hormone Receptor Superfamily

RONALD M. EVANS

Science (1988) *240, 889–895.*

Mol Endocrinol. 2009 Jun;23(6):740-6. Epub 2009 May 7.
Minireview: Evolution of NURSA, the Nuclear Receptor Signaling Atlas.

McKenna NJ, Cooney AJ, DeMayo FJ, Downes M, Glass CK, Lanz RB, Lazar MA, Mangelsdorf DJ, Moore DD, Qin J, Steffen DL, Tsai MJ, Tsai SY, Yu R, Margolis RN, Evans RM, O'Malley BW.

Mol Cell Endocrinol. 2010 Jul 6. [Epub ahead of print]
What are nuclear receptor ligands?
Sladek FM.

Cell Mol Life Sci. 2000 May;57(5):809-27.
Origins and evolutionary diversification of the nuclear receptor superfamily.
Owen GI, Zelent A.

http://www.nursa.org/flash/gene/nuclearreceptor/start.html

NURSA

Nuclear Receptor Signalling Atlas