Eukaryotic Gene Expression: Basics & Benefits

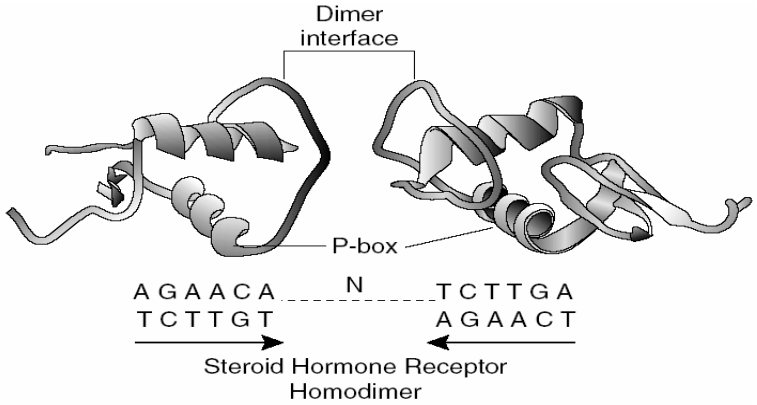
P N RANGARAJAN

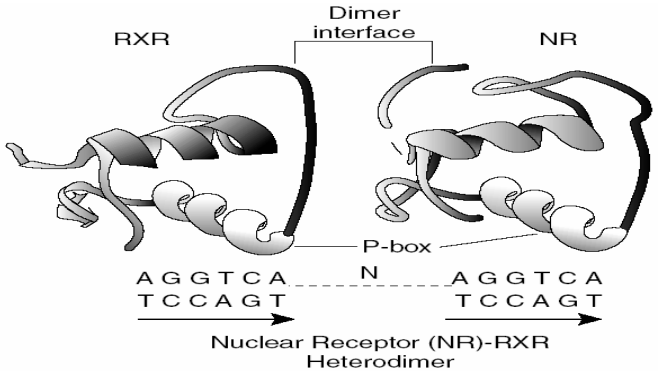
Lecture 23

Mechanism of transcriptional activation by nuclear receptors

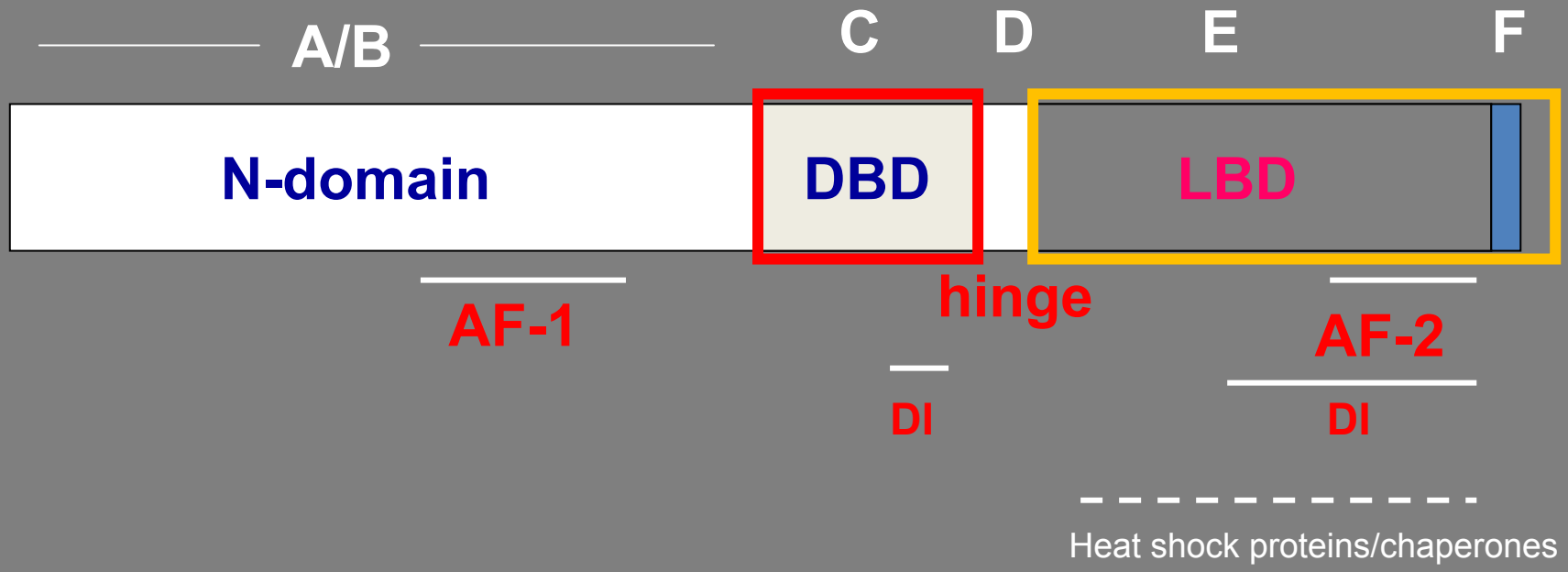
Nuclear Receptor Family

- Steroids
  - Estrogen (ER *α,β)*
  - Progesterone (PR)
  - Androgen (AR)
  - Glucocorticoids (GR)
- Non-steroidal lipophilic hormones
  - 1, 25-(OH)2-vitamin D3 (VDR)
  - All- *trans*-retinoic acid (RAR *α,β, γ)*
  - *9-cis*-retinoic acid (RXR *α,β, γ*)
  - Fatty acids (PPAR *α,β, γ)*
  - Thyroid hormone (TR *α,β)*
- Orphans (No ligand or ligand unknown)





Humans: 48 receptors; 23-ligands; 25-orphans


A/B
C
D
E
F
N-domain
DBD
LBD
AF-1
hinge
AF-2
DI
DI
Heat shock proteins/chaperones

TRANSCRIPTIONAL ACTIVATION BY LIGAND-BOUND NRs IS MEDIATED BY INTERACTIONS WITH NUCLEAR RECEPTOR CO-ACTIVATORS

The ability of nuclear receptors to alternate between activation and repression in response to specific molecular cues, is now known to be attributable to a diverse group of cellular factors known as the nuclear receptor coregulators.

-co-activators

-co-repressors

CO-ACTIVATORS

- Acetyltransferases, such as members of the SRC/p160 family
- Ubiquitin ligases, such as E6-AP
- ATP-coupled chromatin remodeling complexes, such as the SWI/SNF/BRG-1 complex
- Protein methylases, such as CARM-1 and PRMT-1
- RNA transcripts, such as SRA
- Cell cycle regulators such as cdc 25B
- RNA helicases such as p72
- And members of the TRAP/DRIP complex, which foster direct contact with components of the basal transcription machinery

Evidence for the existence of co-activators came from “squelching” experiments or competition for a common limiting factor.

Using the yeast two hybrid system, a number of proteins interacting with the ligand binding domain of nuclear receptors were identified.

The first authentic, common transcriptional coactivator was steroid receptor coactivator 1, or SRC-1

Other members of SRC/p160 family of coactivators include GRIP-1and p/CIP

The SRC/p160 family is defined by the presence in the N-terminus of tandem PAS and beta-H-LH motifs;

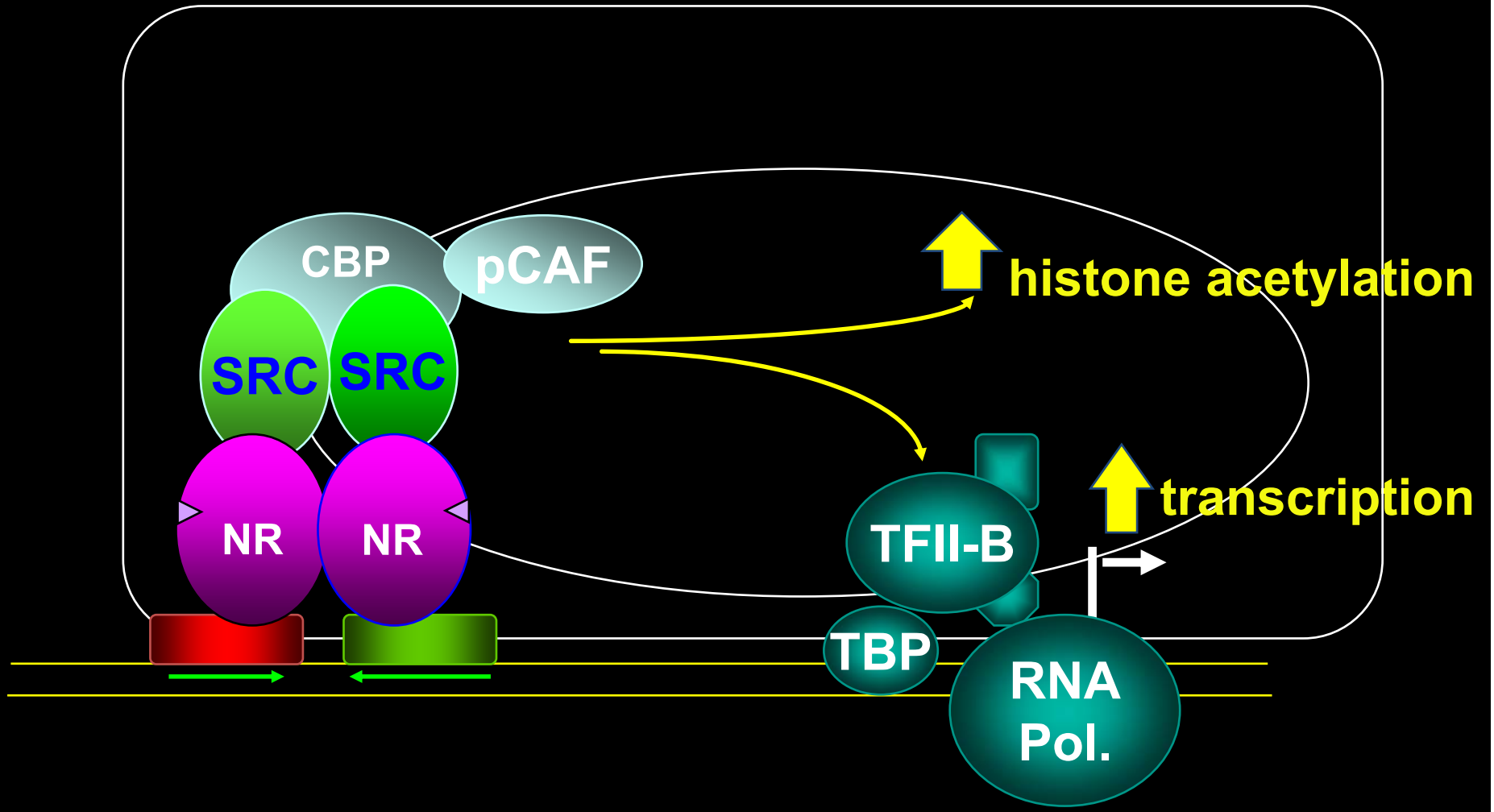
A centrally-located domain which binds the coactivators CBP and p300;

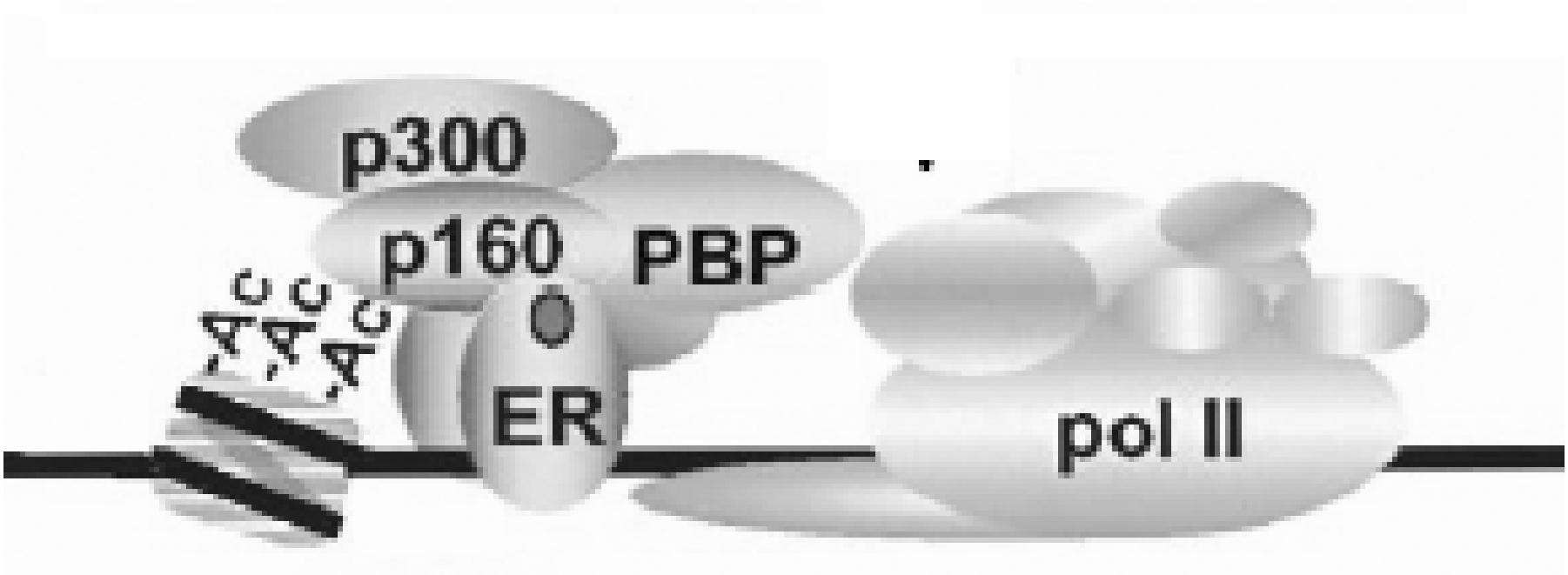
And a C-terminal region which mediates interaction with the CARM -1 coactivator.

Nuclear receptor co-activators

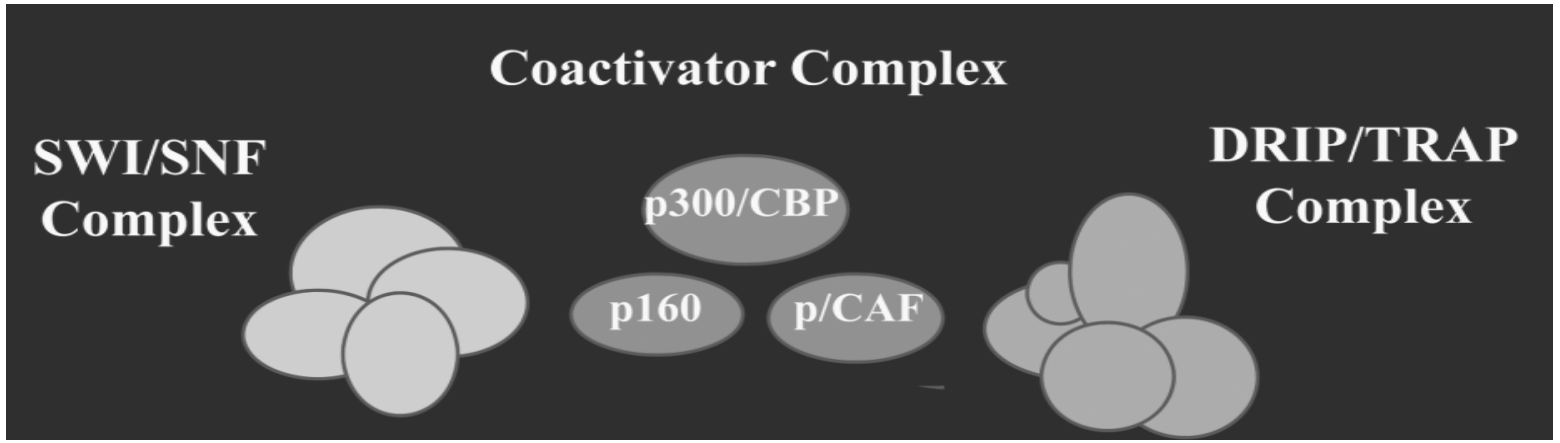
- Chromatin remodeling factor
  - Swi/Snf complex
- Histone acetyl transferase (HAT)
  - p160 family: SRC-1, GRIP-1, pCIP
  - p300/CBP
  - pCAF (p300/CBP-associated factor)
- Activation protein
  - TRAP/DRIP

In the presence of ligand, interactions between the AF-2 domain of the receptor and coactivators result in transcriptional activation




p300
p160
PBP
ER
pol II

Co-activators of glucocorticoid receptor



The human Glucocortiocid receptor α interacts with several other distinct chromatin modulators through its transactivation domains. These include:

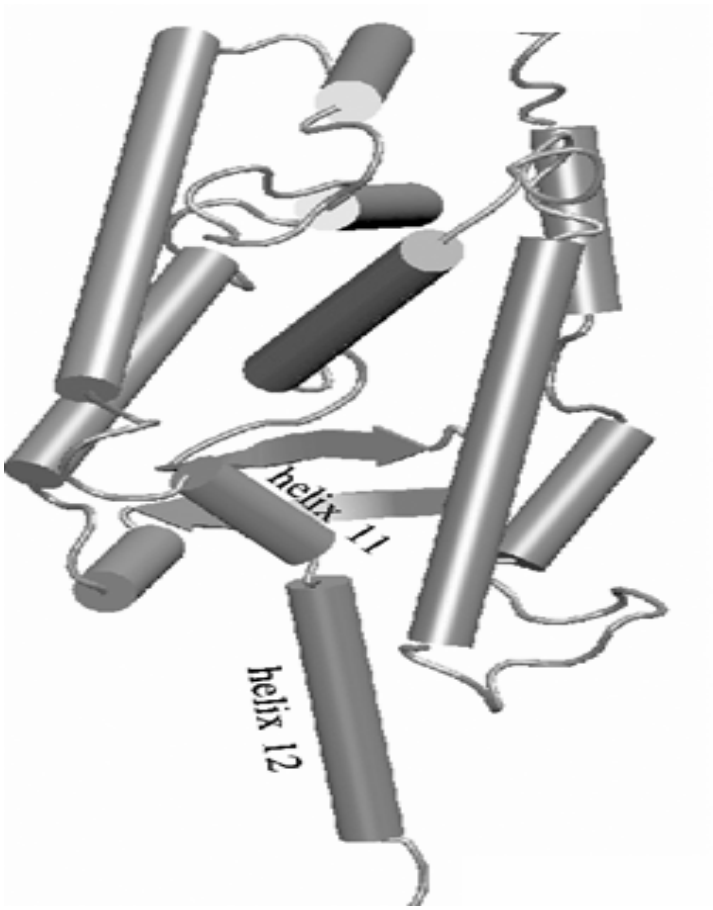
The mating-type switching/sucrose non-fermenting (SWI/SNF) complex

The p300/CBP (CREB-binding protein) serves as macromolecular docking “platform” for transcription factors from several signal transduction cascades, including nuclear receptors, CREB, AP-1, NF-κB, p53, Ras-dependent growth factor, and STATs.

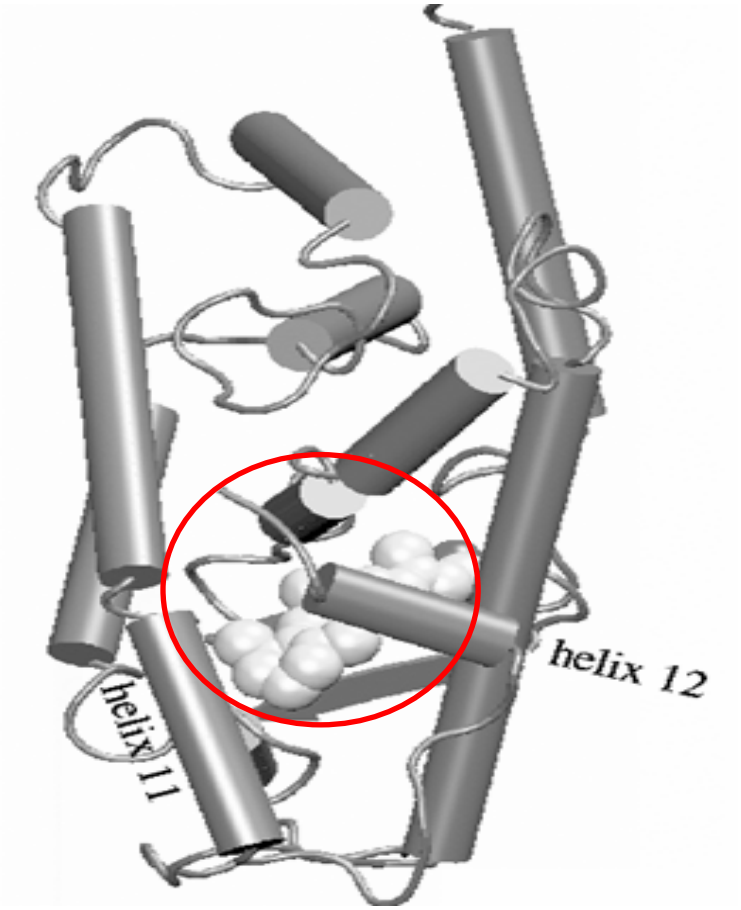
Vitamin D receptor-interacting protein (DRIP) / thyroid hormone receptor-associated protein (TRAP) complex.

HOW DOES THE AF-2 DOMAINS OF NRs INTERACT WITH COACTIVATORS?

Ligand binding induces a conformational change in the ligand binding domain of NRs thereby facilitating interaction with specific coactivators.



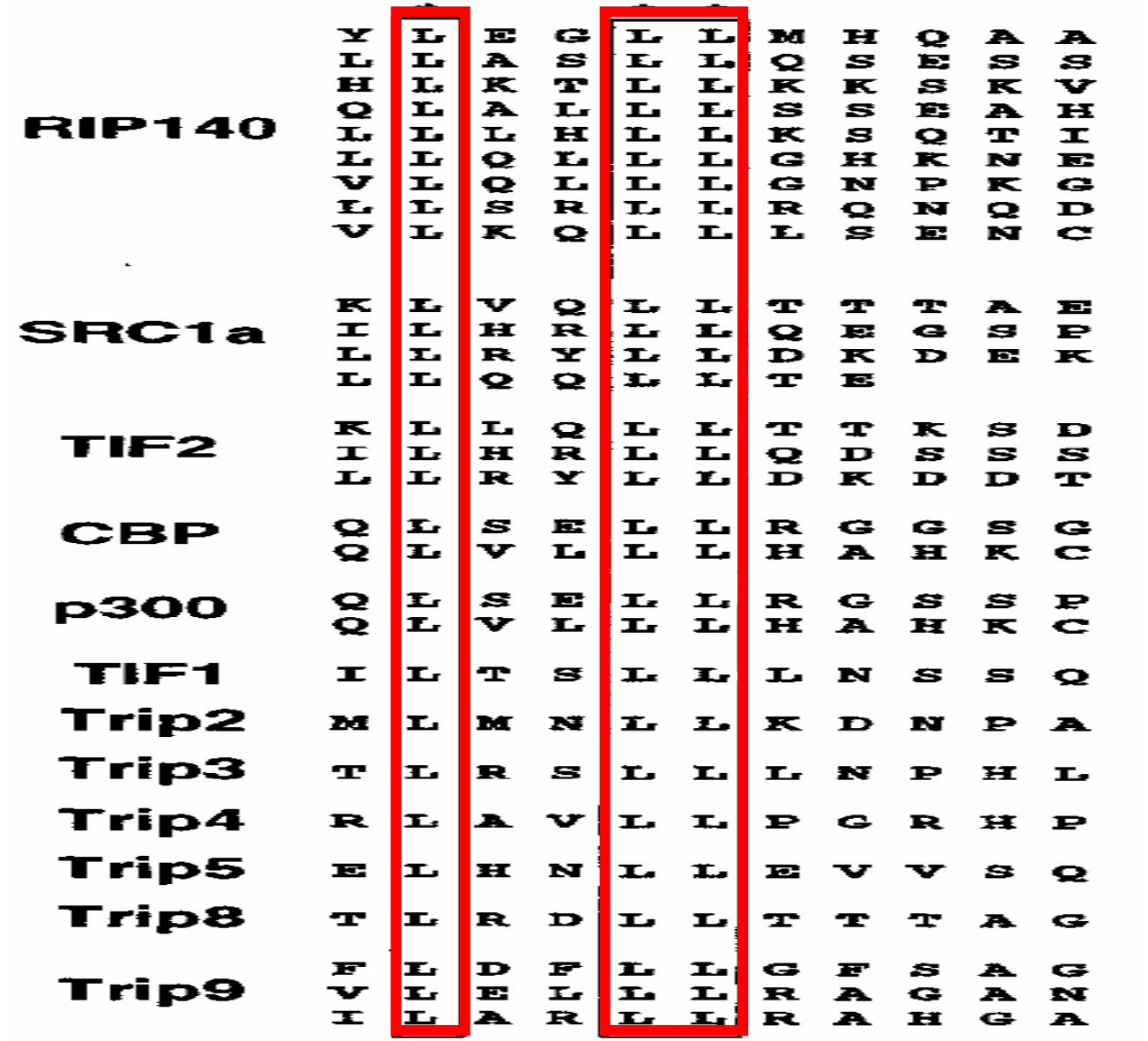
RAR (-RA)



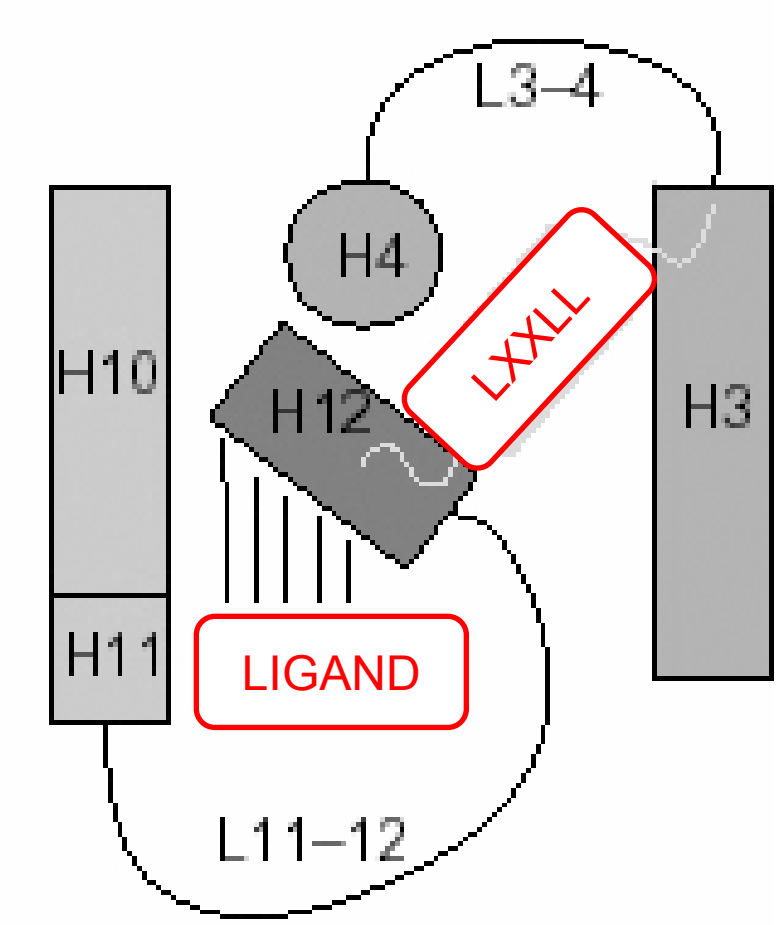
RAR(+RA)

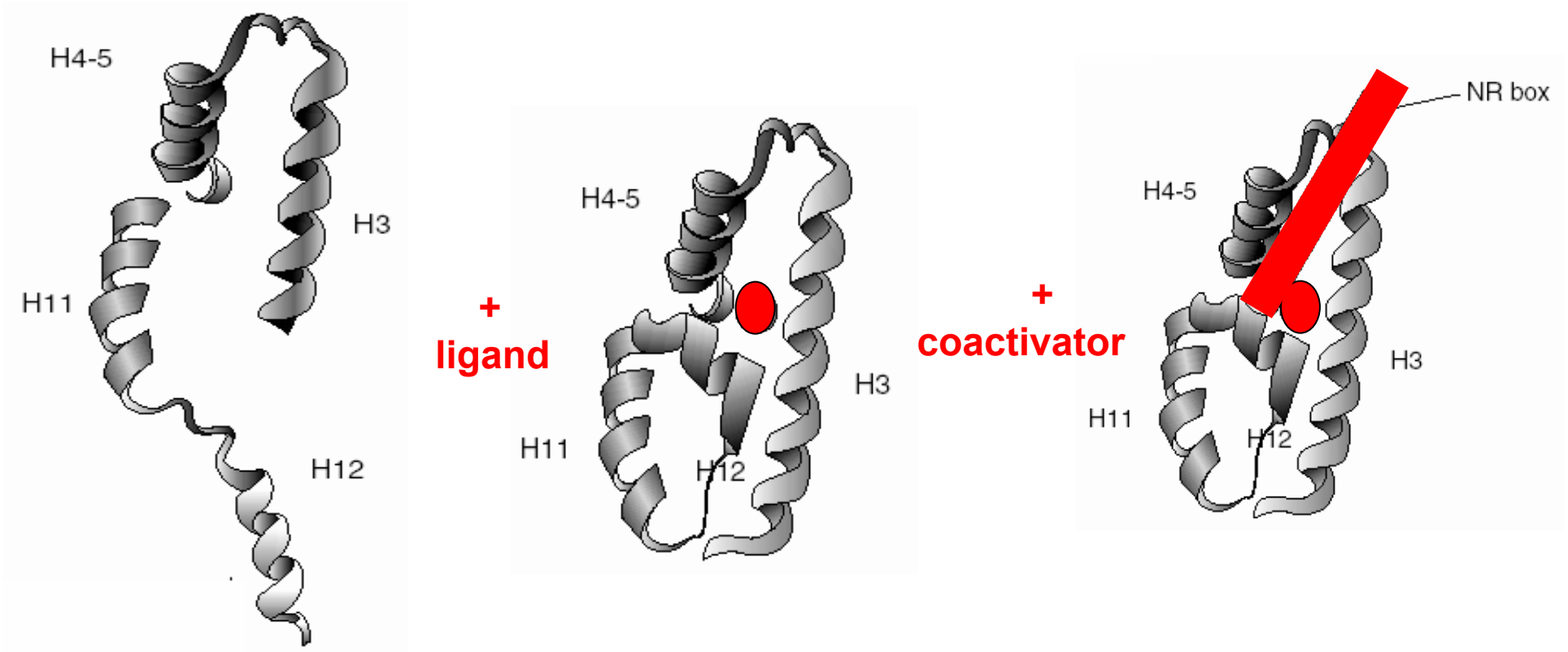
Nuclear receptor coactivators contain an alpha-helical motif known as the LXXLL motif, or nuclear receptor box, which interacts with AF-2 domain of ligand-bound nuclear receptors.

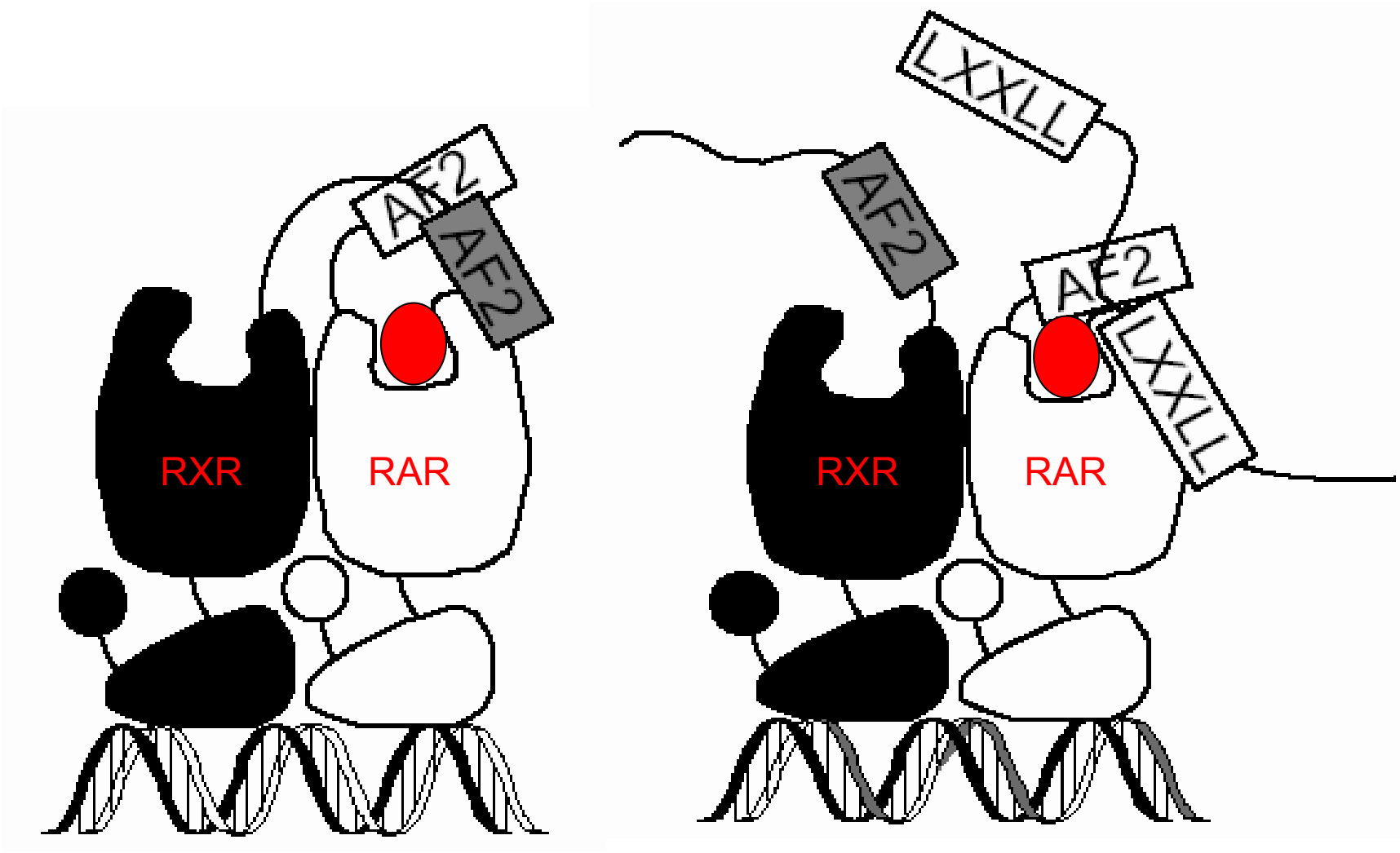
A number of nuclear receptor coactivators contain LXXLL motif



3 LXXLL BOXES


L3–4
H4
LXXLL
H10
H12
H3
H11
LIGAND
L11–12

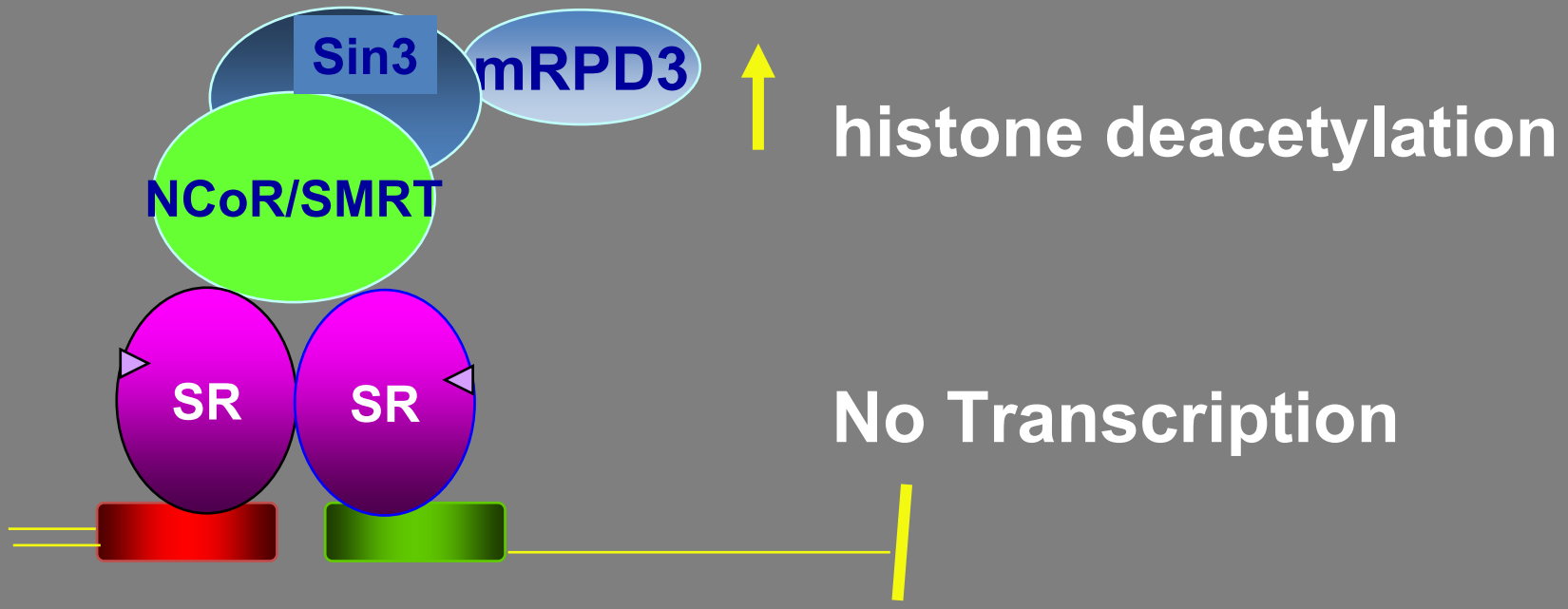

H4-5
H3
H11
H12
+
ligand
H4-5
H3
H11
H12
+
coactivator
NR box
H4-5
H3
H11
H12

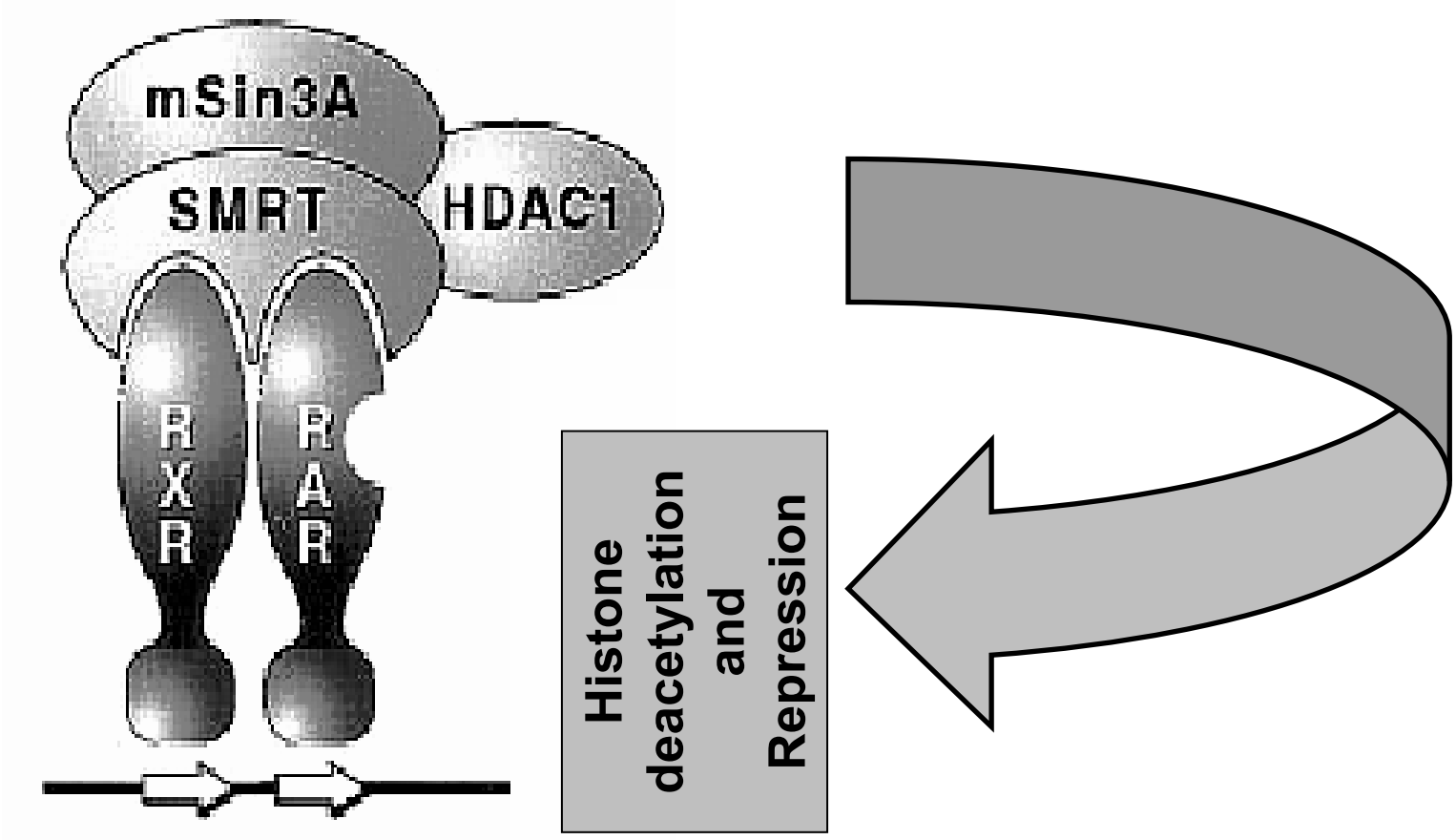

AF2
AF2
RXR
RAR
LXXLL
AF2
AF2
LXXLL
RXR
RAR

MECHANIMS OF TRANSCRIPTIONAL REPRESSION BY NUCLEAR RECEPTORS

- In the absence of ligand, nuclear receptors recruit co-repressors
  - N-CoR (nuclear receptor corepressor)
  - SMRT (silencing mediator for retinoid and thyroid hormone receptor)
- Co-repressors act as adaptors for histone deacetylation factors
  - Sin3
  - HDAC (histone deacetylase)

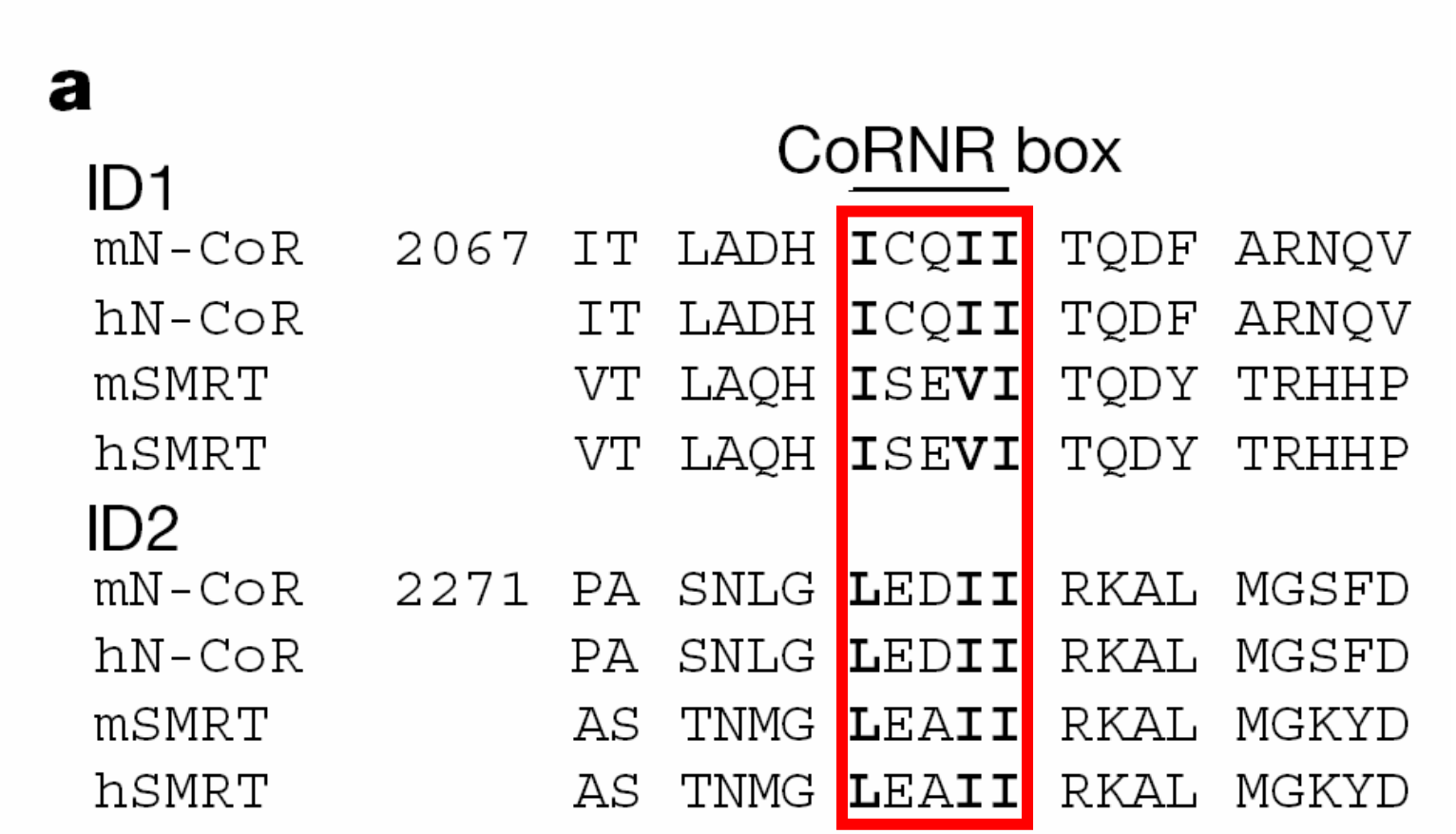
In the absence of ligand, the NRs interact with corepressors resulting in Inhibition of Transcription

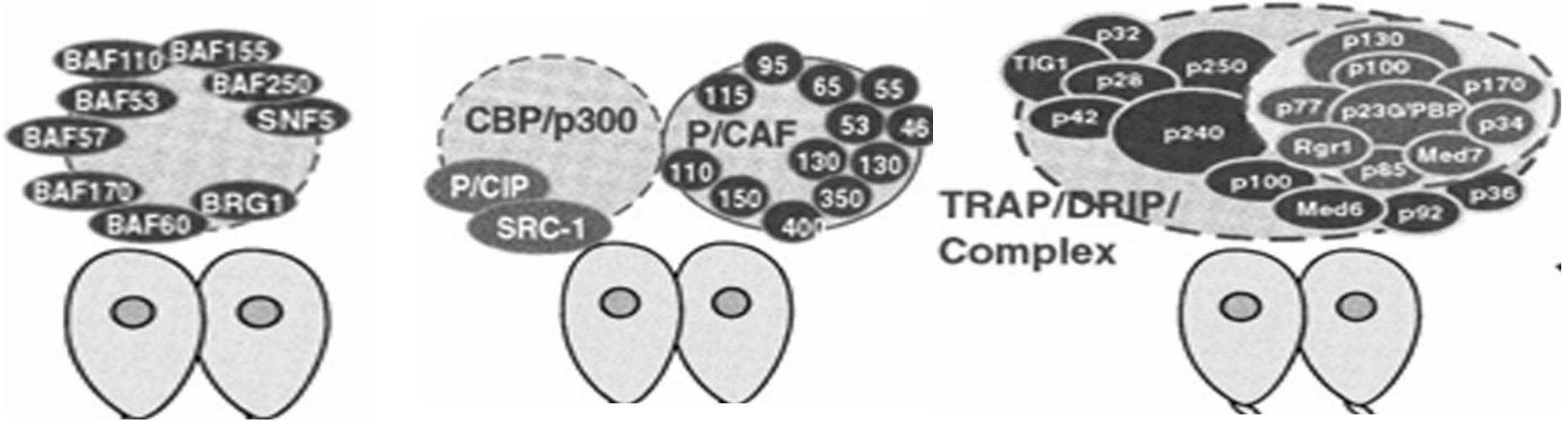


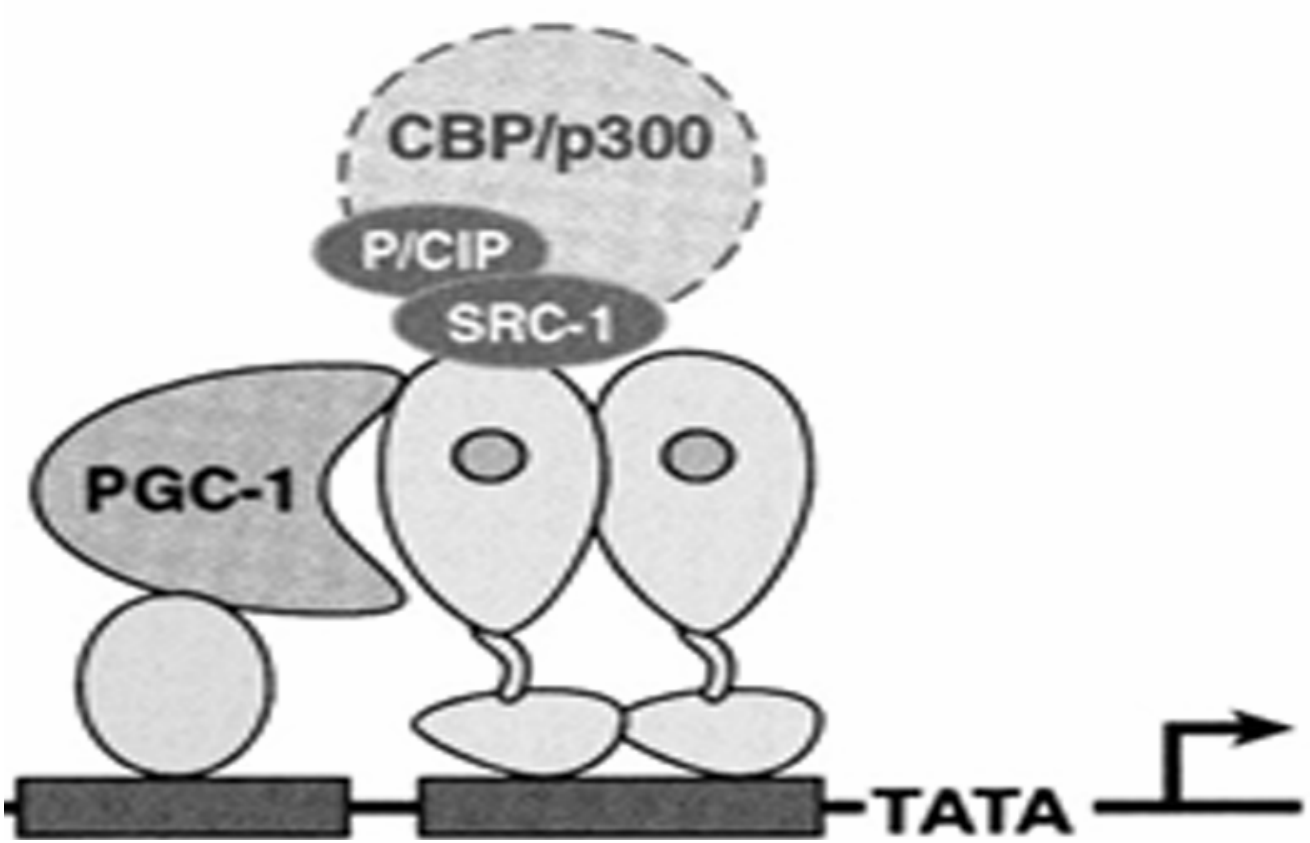

mSin3A
SMRT
HDAC1
RXR
RAR
Histone deacetylation and Repression

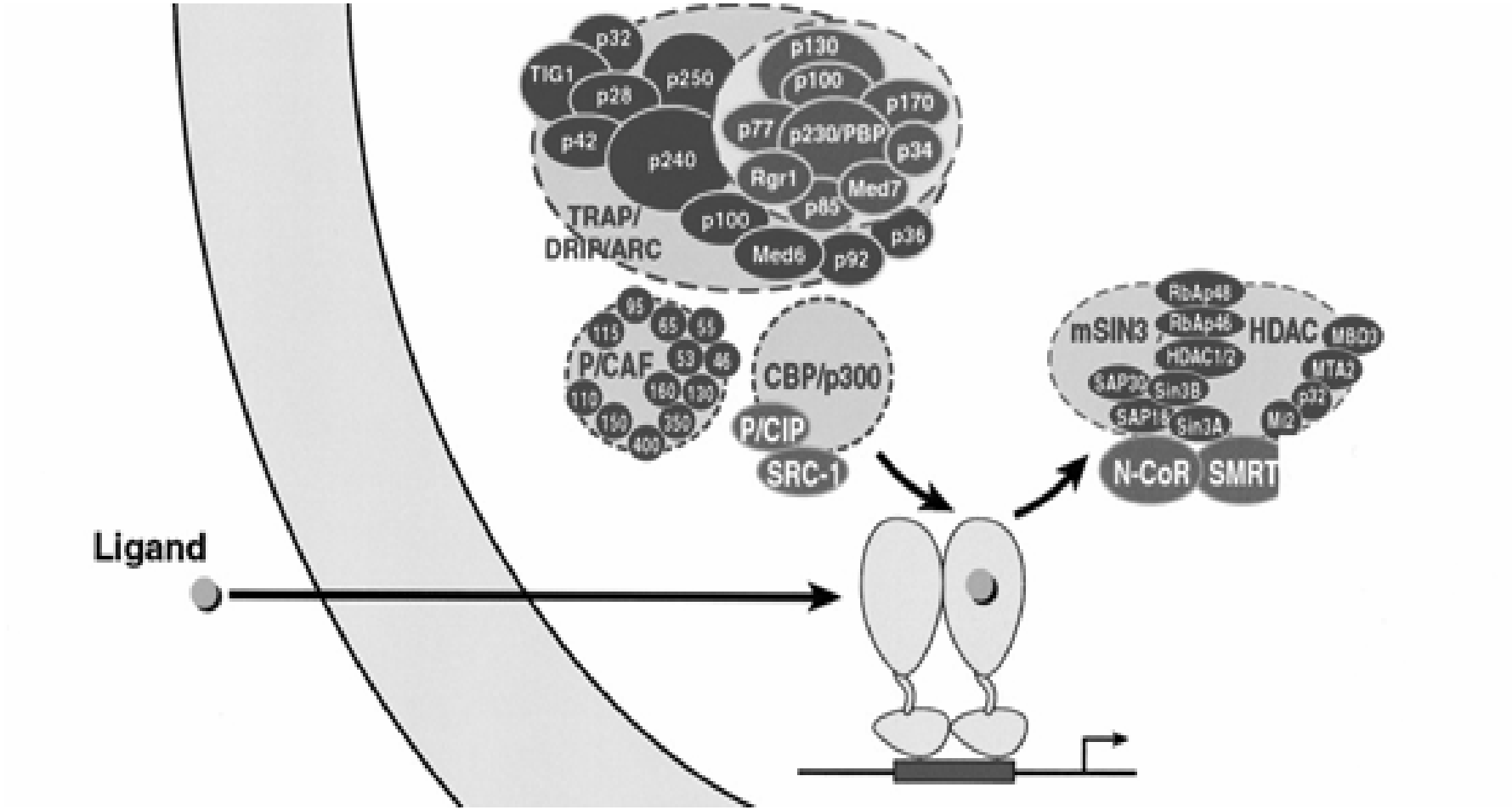
Corepressor binding to NRs is mediated by extended
LXXLL-like motifs (L-X-X-I/H-I-X-X-X-L/I),
termed
corepressor nuclear receptor boxes (CoRNR boxes),
which are located in the
COOH-terminal half of NCoR

Co-repressors interact with NRs through I/LxxI/VI motifs or CoRNR boxes



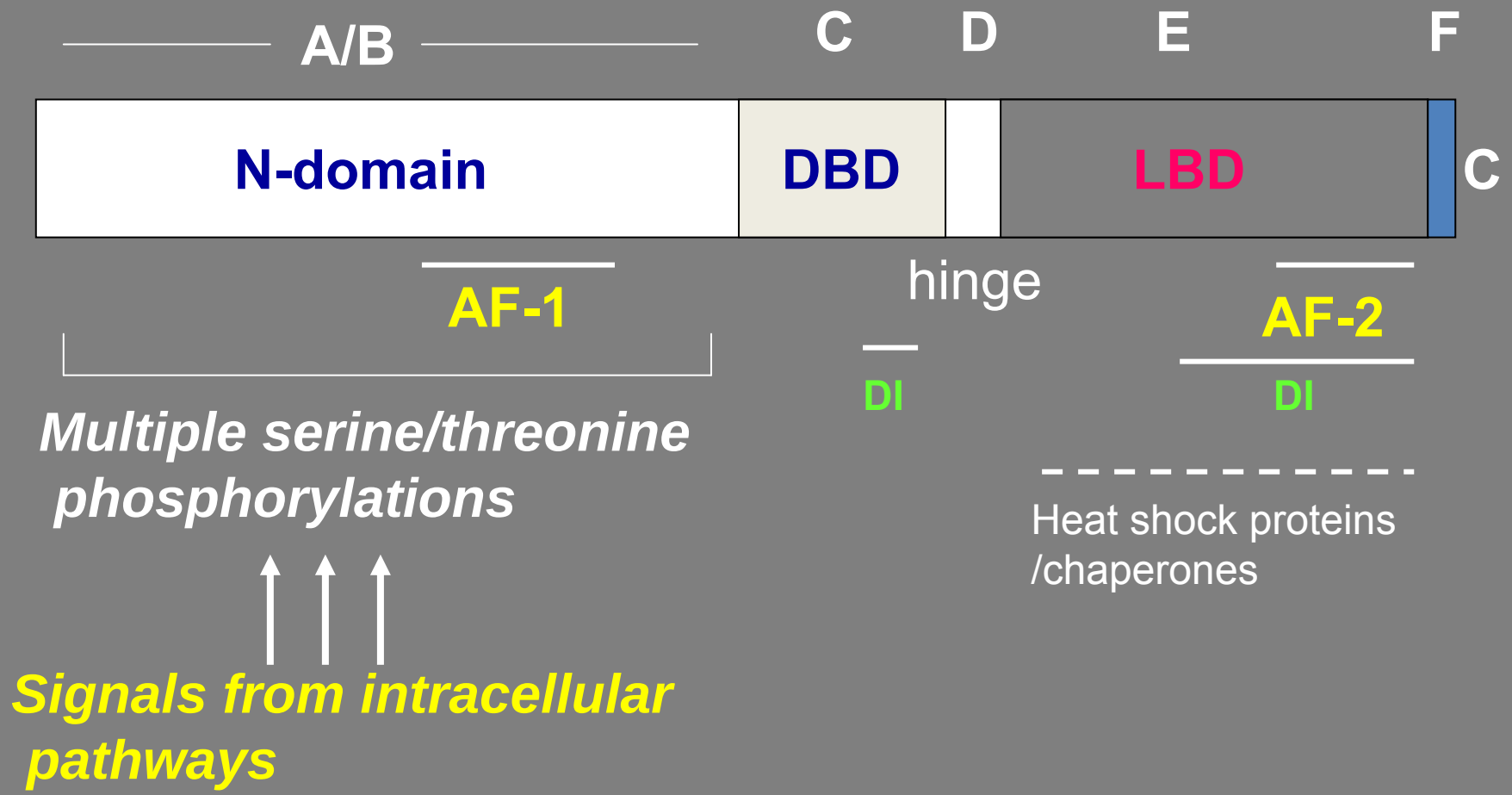

BAF110
BAF155
BAF250
BAF53
SNF5
BAF57
BAF170
BRG1
BAF60
CBP/p300
P/CIP
SRC-1
P/CAF
95
115
65
55
53
46
110
130
130
150
350
400
TRAP/DRIP/
Complex
p32
TIG1
p250
p130
p100
p28
p170
p77
p230/PBP
p34
p42
p240
Rgr1
Med7
p85
p100
Med6
p92
p36


CBP/p300
P/CIP
SRC-1
PGC-1
TATA


p32
TIG1
p28
p250
p42
p240
p130
p100
p170
p77
p230/PBP
p34
Rgr1
Med7
p85
p100
p36
Med6
p92
TRAP/
DRIP/ARC
95
115
85
55
P/CAF
53
46
110
180
120
150
250
400
CBP/p300
P/CIP
SRC-1
RbAp48
mSIN3
RbAp46
HDAC
MBD3
HDAC1/2
MTA3
SAP30
Sin3B
p32
SAP18
Sin3A
Mi2
N-CoR
SMRT
Ligand

REGULATION OF NUCLEAR RECPETOR FUNCITON BY POST-TRANSLATIONAL MODIFICATIONS

Role of Phosphorylation of Steroid Receptors



REGULATION OF GLUCOCORTICOID RECEPTOR FUNCTION BY PHOSPHORYLATION

The human Glucocortiocid receptorα has several phosphorylation sites, including S113, S141, S203, S211, S226 and S404, most of which are all located in the AF-1 domain of its N-terminal domain.

Phosphorylation of hGRα typically occurs after binding to the ligand, and may determine turnover, subcelluLar trafficking, target promoter specificity, cofactor interaction, strength and duration of receptor signaling, and receptor stability.

In addition, it modifies protein-protein interactions, which can stabilize the hypophosphorylated form of the receptor in the absence of ligand, as well as facilitate transcriptional activation by the hyperphosphorylation of GR via cofactor recruitment upon ligand binding. F

Therefore, phosphorylation of the GR is a versatile mechanism for modulating and integrating multiple receptor functions

There are several kinases that phosphorylate the hGRα *in vitro and in vivo.*

These include:

i)the yeast cyclin-dependent kinase P34cdc28

ii), the p38 mitogen-activated protein kinase (MAPK)

iii) the CNS-specific cyclin-dependent kinase 5 (CDK5)

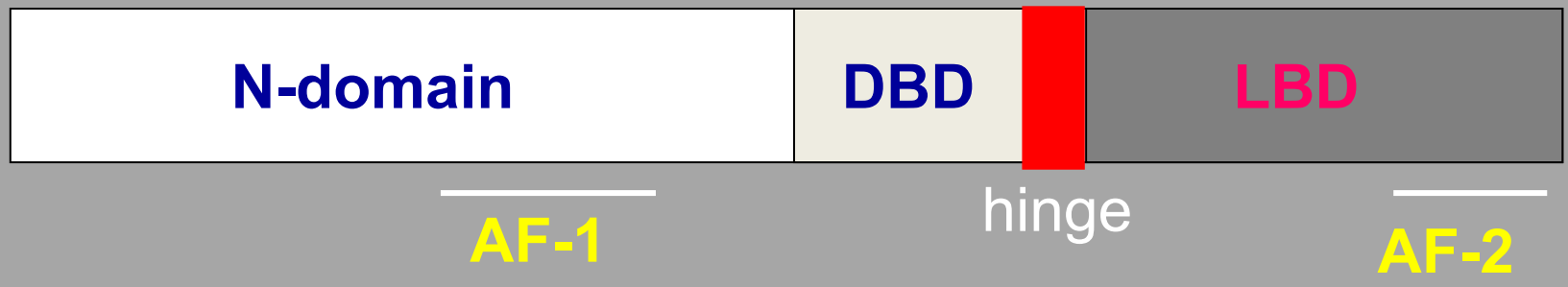
iv) the glycogen synthase kinase 3β (GSK-3β)

v) the c-Jun N-terminal kinase (JNK) (52,53), and either increase or decrease the transcriptional activity of hGRα.

REGULATION OF GLUCOCORTICOID RECEPTOR FUNCTION BY ACETYLATION

Acetylation of the GR occurs after ligand-binding and prior to nuclear translocation.

The acetylated GR is deacetylated by histone deacetylase 2 (HDAC2) and this deacetylation is necessary for the GR to be able to inhibit NF-κB activation of inflammatory genes.

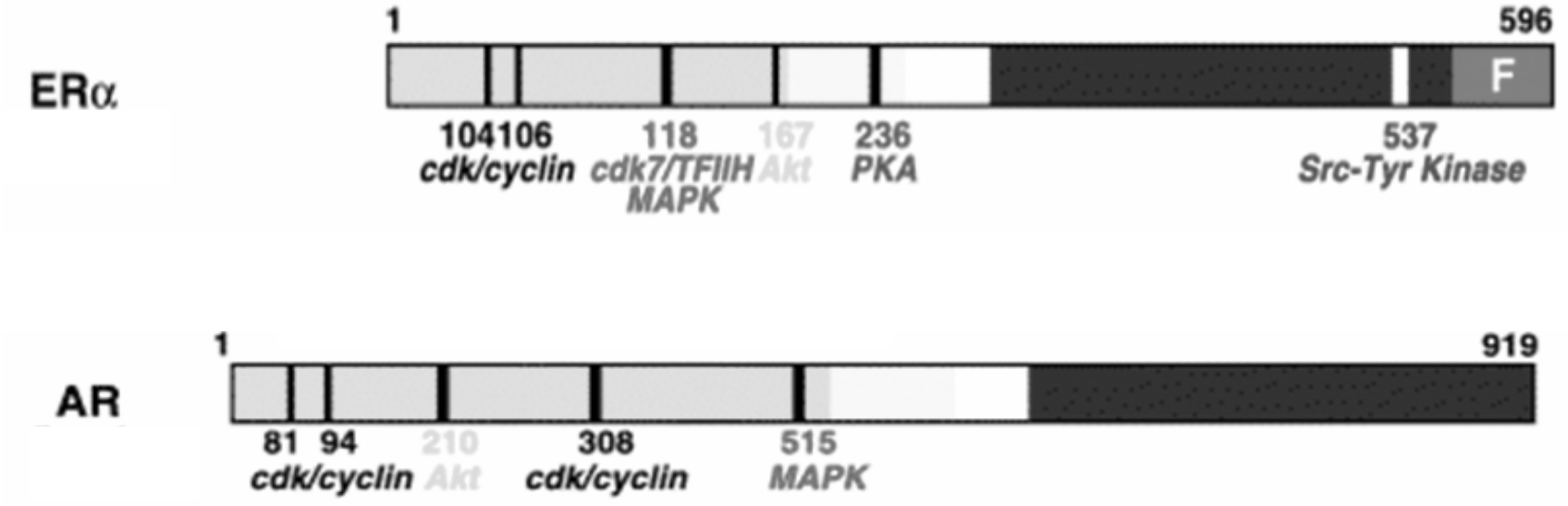


The site of acetylation (KKTK) of the GR is the lysine rich 'hinge' region 492–495.

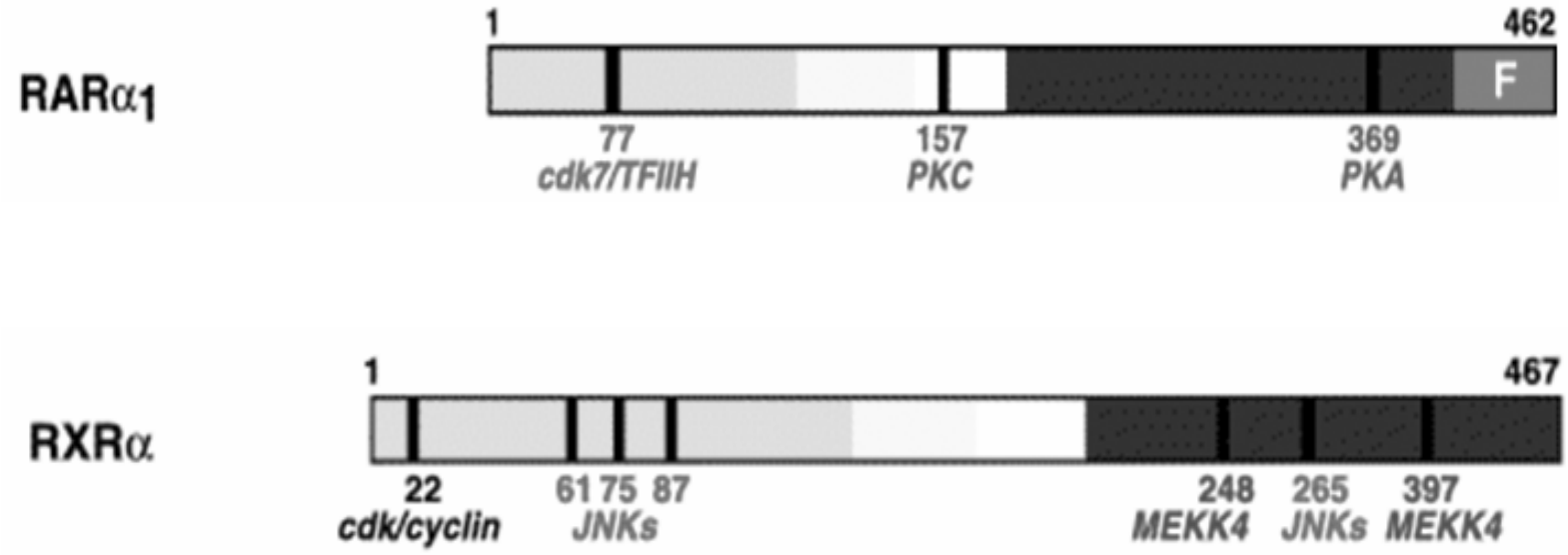
The transcriptional activity of Estrogen receptor (ER) can be induced by growth factors (EGF, IGF) through the Ras-Raf-MAPK pathway.

hER is phosphorylated by mitogen-activated protein kinase (MAPK) at Serine 118 located in AF-1.

Phosphorylation of Ser118 results in stimulation of AF-1 of ER resulting in ligand-independent activation of ER.



Phosphorylation of non-steroid nuclear receptors



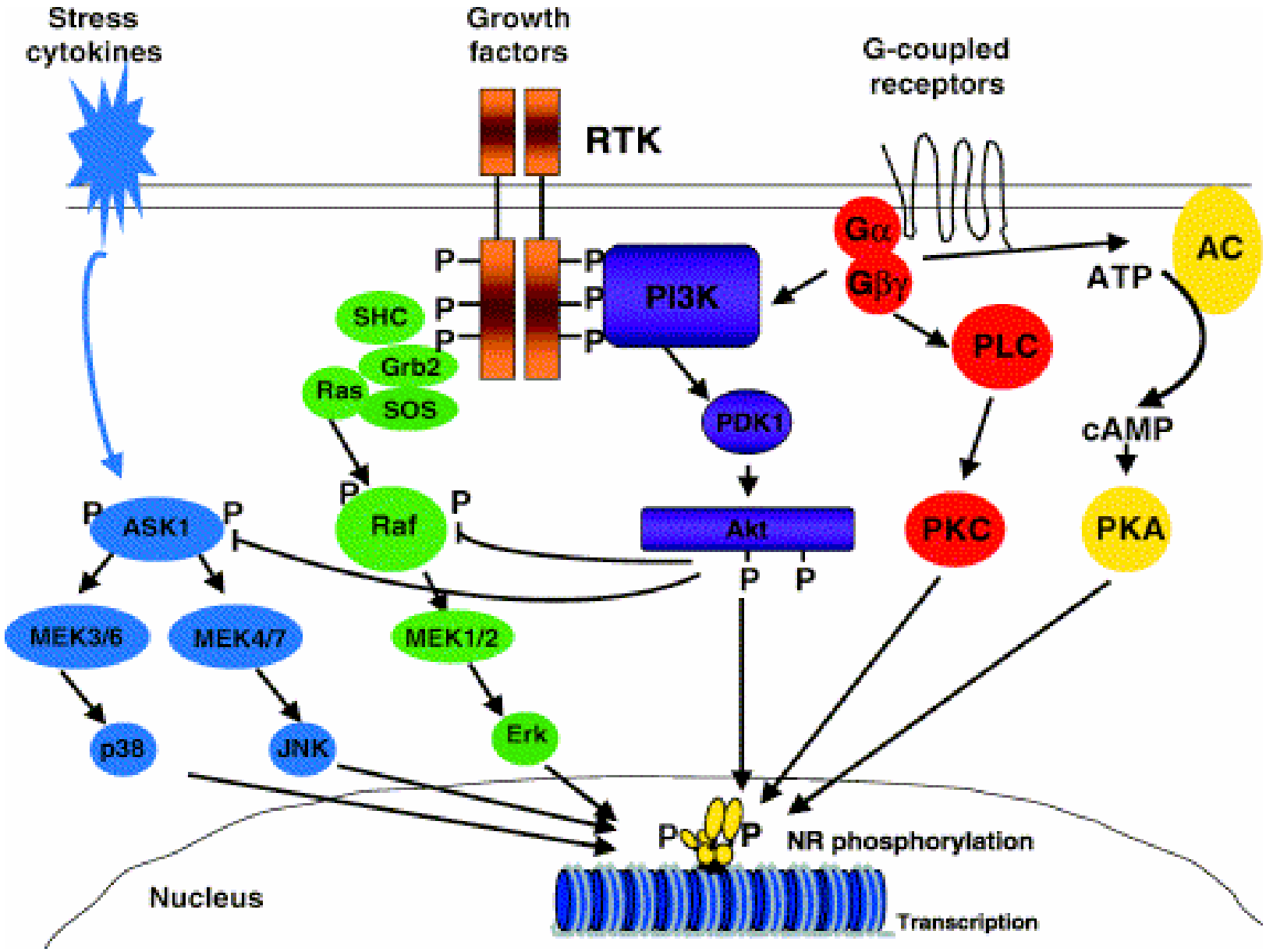
AF-1 of RARα1 and RARγ2 are phosphorylated by proline directed kinases

Phosphorylation of AF-1 in RARγ2 is required for RA-induced differentiation into primitive endoderm, whereas phosphorylation of AF-1 in RARα1 is required for differentiation into parietal endoderm.

AF-2 of RARα1 and RARγ2 are phosphorylated by PKA

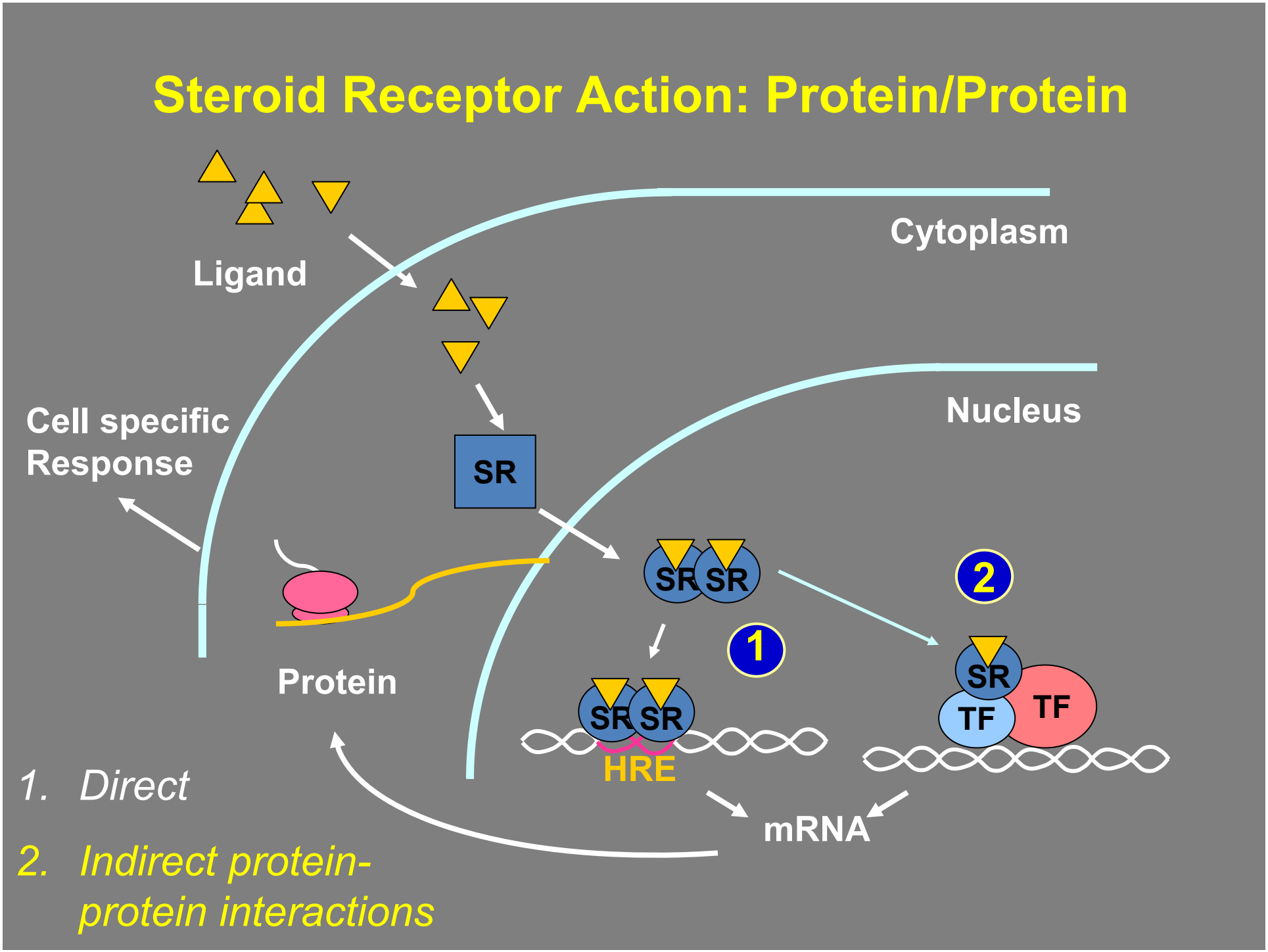
Phosphorylation of AF-2 in RARα1, but not in RARγ2, is required for differentiation into parietal endodermal cells.

Regulation of nuclear receptor activity by phosphorylation



Bastein and Rochette-Egly (2004) Gene 328, 1-16

Steroid Receptor Action: Protein/Protein



1. *Direct*
2. *Indirect protein-protein interactions*

NUCLEAR RECEPTORS: BENEFITS

The wealth of molecular data accumulated to date on functional interactions between nuclear receptors and coregulators has exciting implications for the development of novel pharmaceutical therapies for a wide range of diseases INCLUDING A VARIETY OF CANCERS.

Steroid hormones have been implicated in a variety of neoplastic diseases such as breast cancer, ovarian cancer and prostate cancer.

The interface between receptor AF-2 elements and the nuclear receptor box of co-regulators has been the subject of intense study for developing peptide-based agonists/antqgonists.

Role of Coactivators and Corepressors in Disease

- Tamoxifen is an estrogen antagonist in the presence of corepressors in reconstitution systems
- Decreased levels of N-CoR are detected in Tamoxifen-resistant MCF-7 breast cancer cells
- Suggests the levels of the coactivators or corepressors may modulate phenotype

The clinical use of retinoids in cancer therapies and chemoprevention

Trade name	Retinoid	Activity	Some Therapeutic applications
Tretinoin	ATRA	Pan-RAR	Promyelocytic leukemia, Leukoplakia (prevention), Actinic keratosis (prevention)
Alitretinoin,	*9-cis* retinoic acid	Pan-RAR	Kaposi's sarcoma
Panretin		Pan-RXR	Breast cancer
Isotretinoin	*13-cis* retinoic acid	Pan-RAR	Oral leukoplakia, Skin cancer, Head neck cancer (in combination with IFN), Neuroblastoma
Bexarotene	LDG1069	RXR	Cutaneous T-cell lymphoma (stage IA-IB, IIA), NSCLC
Fenretidine	4- HPR	RAR	Breast cancer
Acyclic retinoid	4-hydroxy -phenylretinamide polyprenoic acid	RAR, RXR, PPAR activities	Leukoplakia Ovarian cancer Hepatocellular carcinoma (prevention)

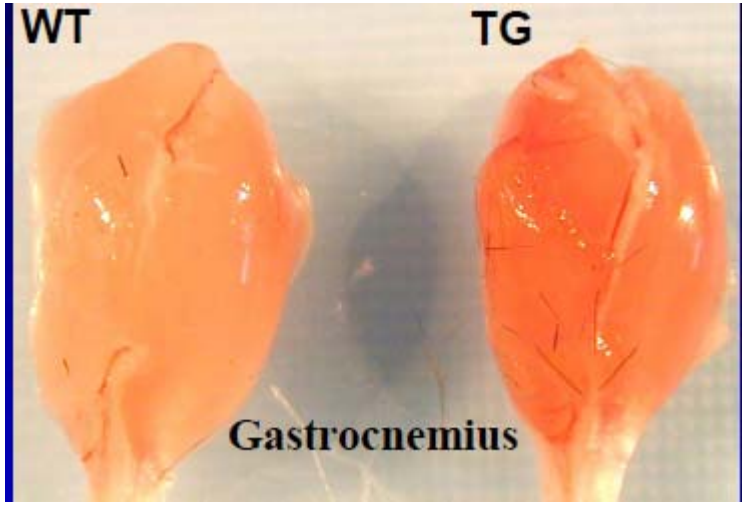
Abbreviations: ATRA, all trans retinoic acid4-HRP, 4-hydroxy-phenylretinamide; APL, promyelocytic leukemia; IFN, interferon; PPAR, peroxisome proliferator activated receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor.

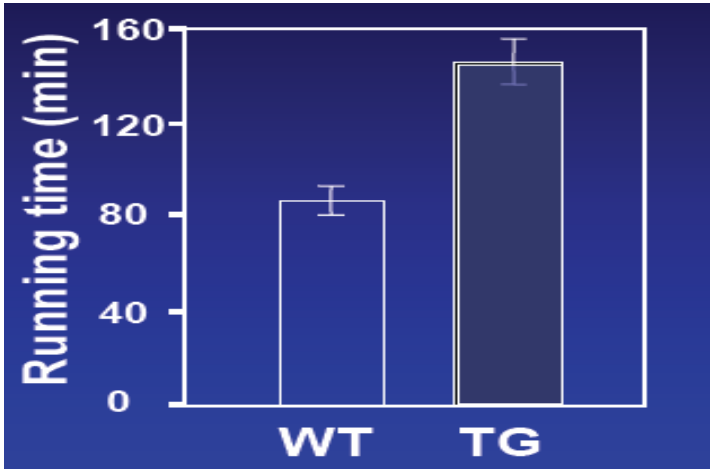
(Adapted from:Altucci and Gronemeyer, Nat. Rev Cancer, 2001 1:181)

Nuclear receptor		Ligand
Peroxisome proliferator-activated receptors	PPARα	Fatty acids Fibrates
	PPARδ	Fatty acids Carboprostacyclin
	PPARγ	Fatty acids Eicosanoids Thiazolidinediones
Liver X receptors	LXRα,β	Oxysterols
Farnesoid X receptor	FXR	Bile acids
Xenobiotic receptors	SXR/PXR	Xenobiotics Steroids
	CAR	Xenobiotics Phenobarbital

PPARδ and the Creation of the Marathon Mouse

A strain of mice was engineered to express an activated PPARδ transgene(termed VP-PPARδ)





Red Muscle Increased in Transgenic Mice

http://news.bbc.co.uk/2/hi/science/nature/3592976.stm

Researchers Identify Drugs that Enhance Exercise Endurance

http://www.youtube.com/watch?v=3ATMgC9pEw0

Nuclear Receptors and Lipid Physiology: Opening the X-Files

Ajay Chawla,[1]* Joyce J. Repa,[2]* Ronald M. Evans,[1]† David J. Mangelsdorf[2]†

SCIENCE (2001) 294: 1866

Orphan nuclear receptors--new ligands and new possibilities

Bruce Blumberg and Ronald M. Evans
Genes Dev. 1998 12: 3149-3155

www.NURSA.org