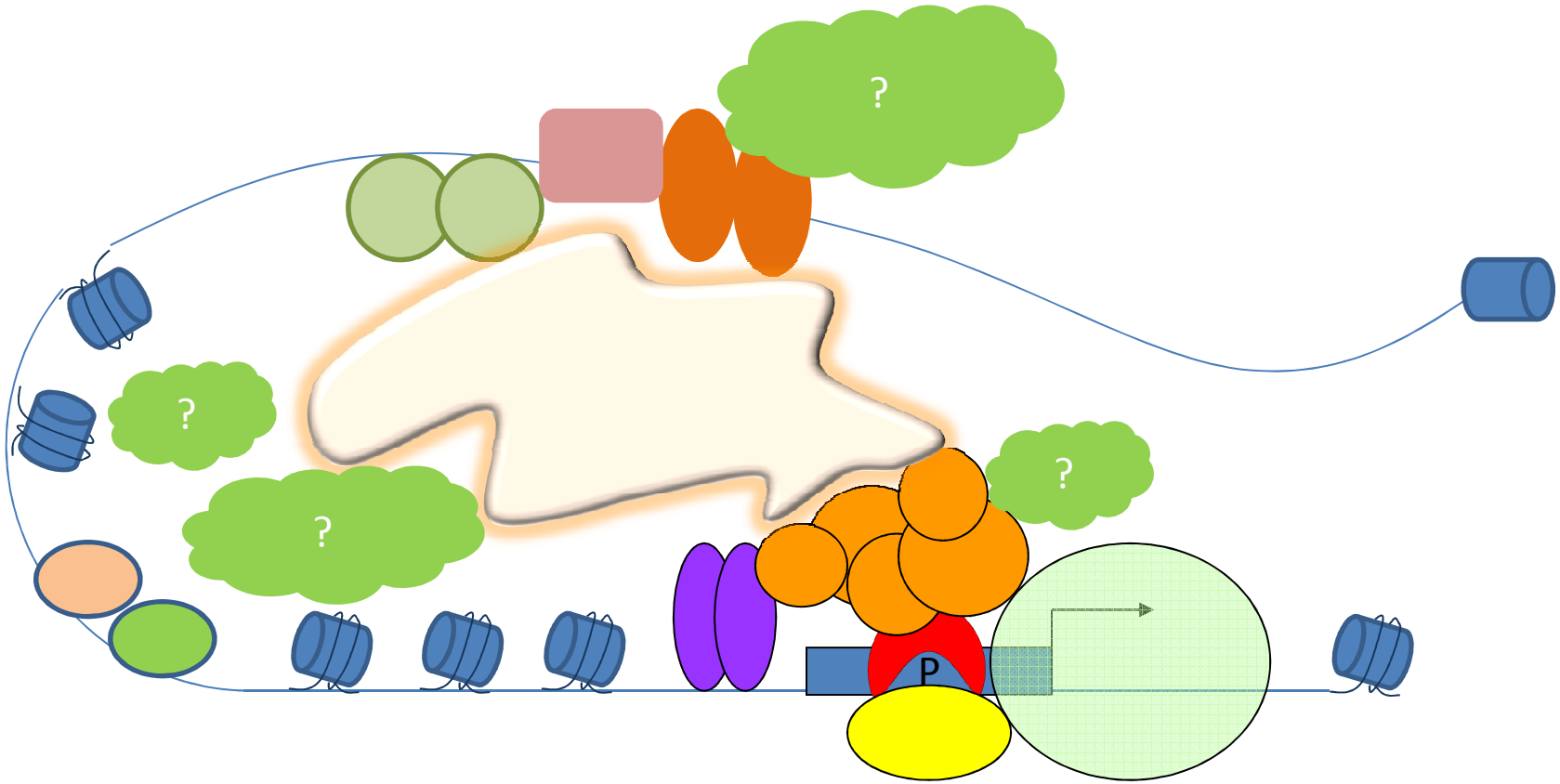
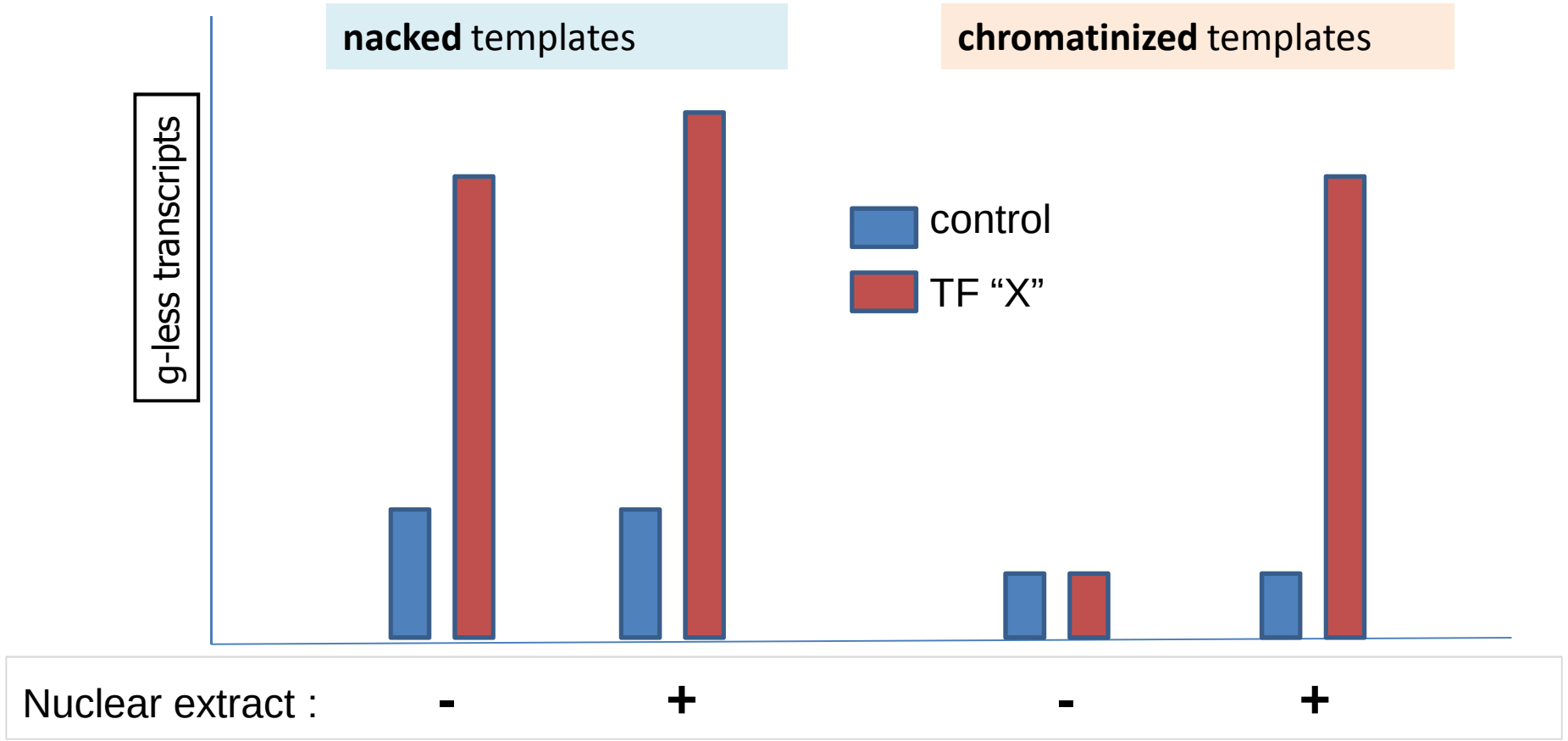
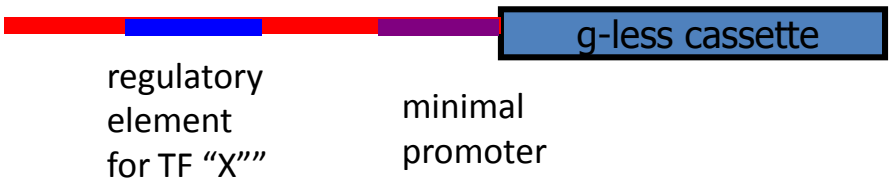
Our transcriptional tale still needs a little point (not so little indeed!) to be focused on ...



We still a lot of enzymatic activities to bring about transcription



"In vitro" transcription with RNA Pol II Holoenzyme



TRANSCRIPTIONAL REGULATION BY THE PHOSPHORYLATION-DEPENDENT FACTOR CREB

Bernhard Mayr and Marc Montminy

The transcription factor CREB — for 'cyclic AMP response element-binding protein' — functions in glucose homeostasis, growth-factor-dependent cell survival, and has been implicated in learning and memory. CREB is phosphorylated in response to various signals, but how is specificity achieved in these signalling pathways?

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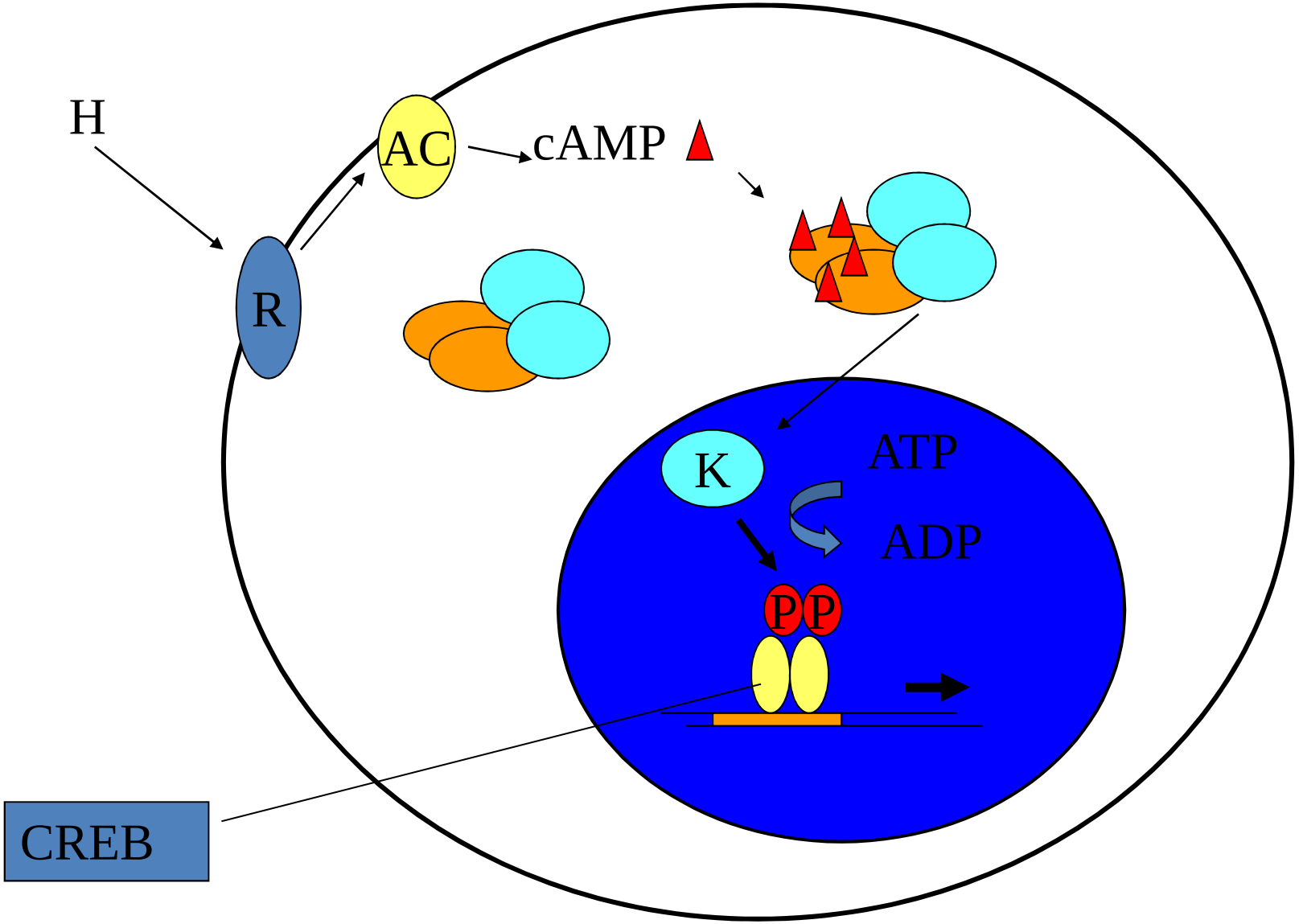
CREB – Cyclic AMP Response Element Binding protein



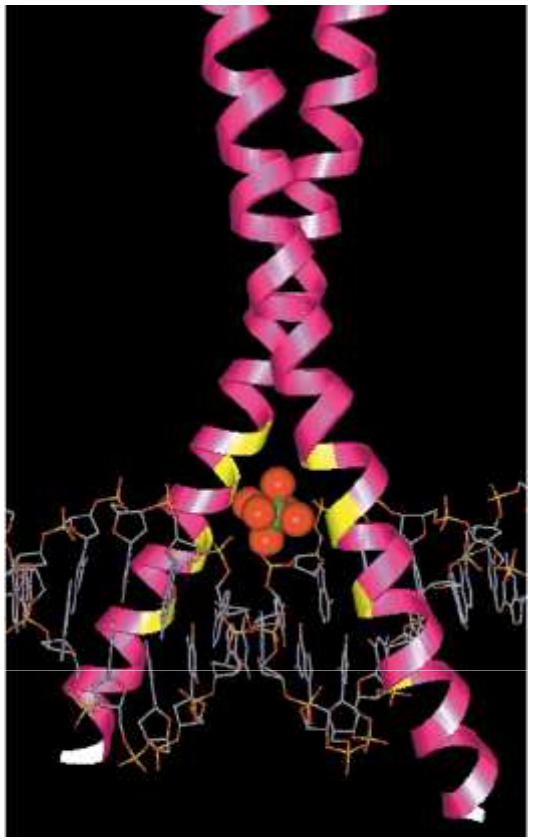


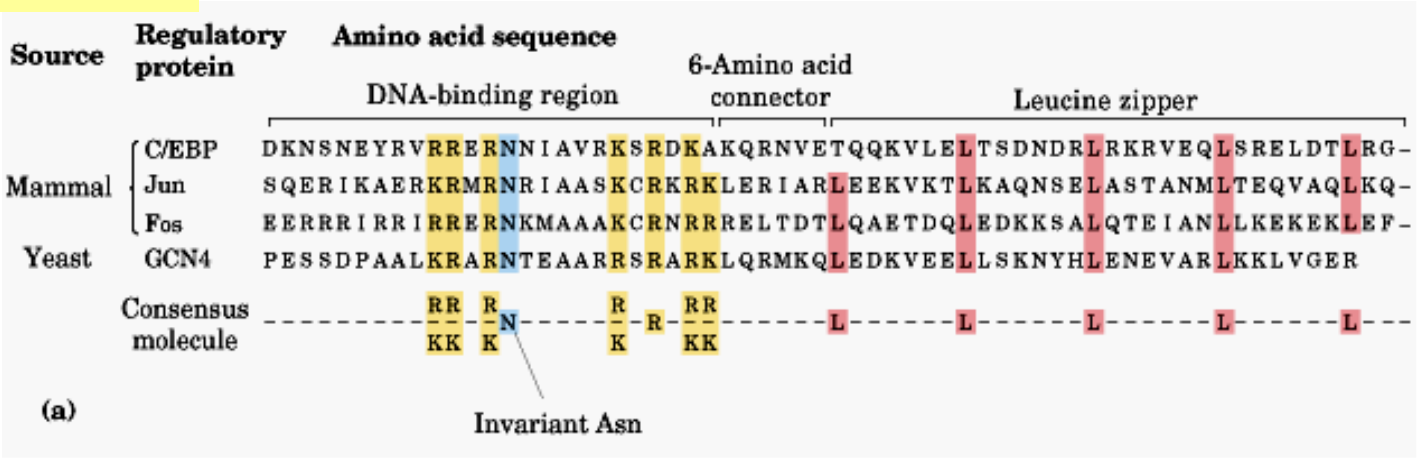
Figure 3

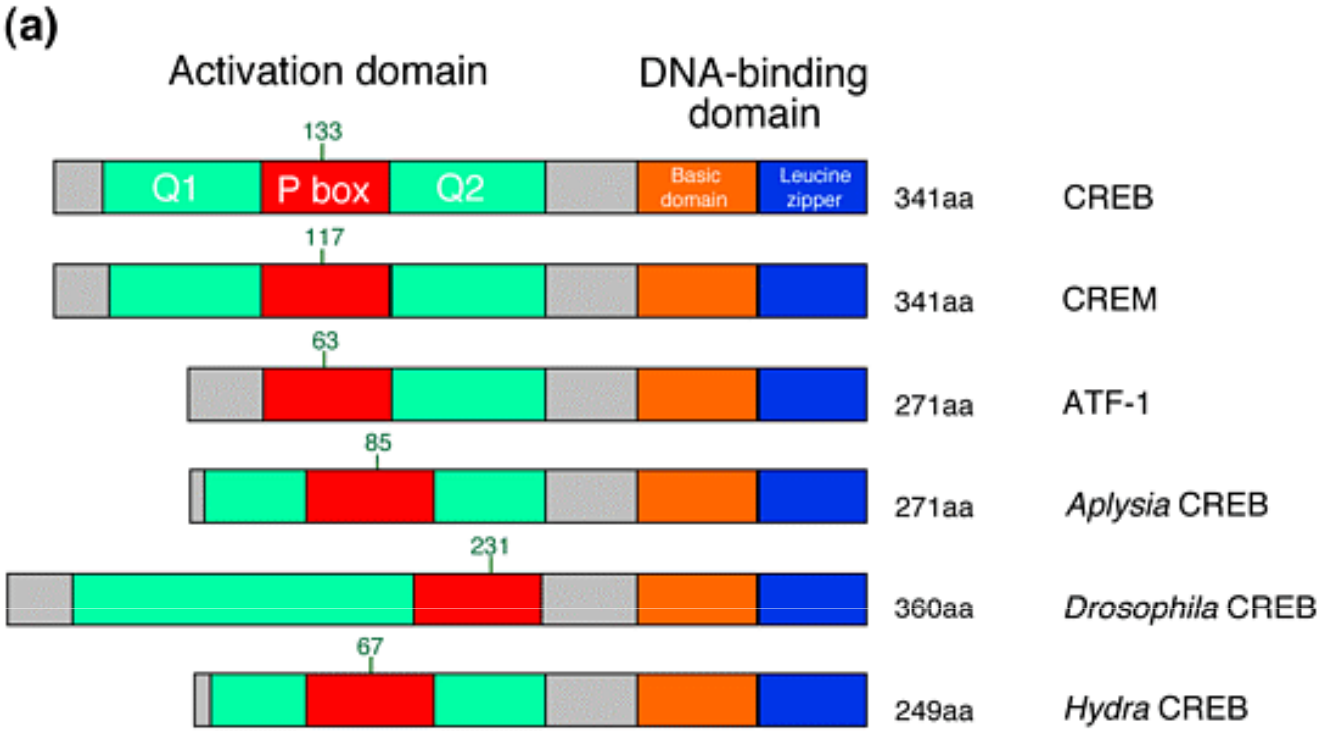
Structure of the CREB basic region/leucine zipper domain (amino acids 285–339) bound to the somatostatin CRE.

The cyclic AMP response element (CRE)-binding protein (CREB) bZIP domain is shown, with residues that function in DNA recognition highlighted in yellow. A magnesium ion (green) with surrounding water molecules (red) is located in the cavity between DNA and the CREB basic region.

from: Mayr & Montminy (2001), Nature Rev Mol Cell Biol 2:599.

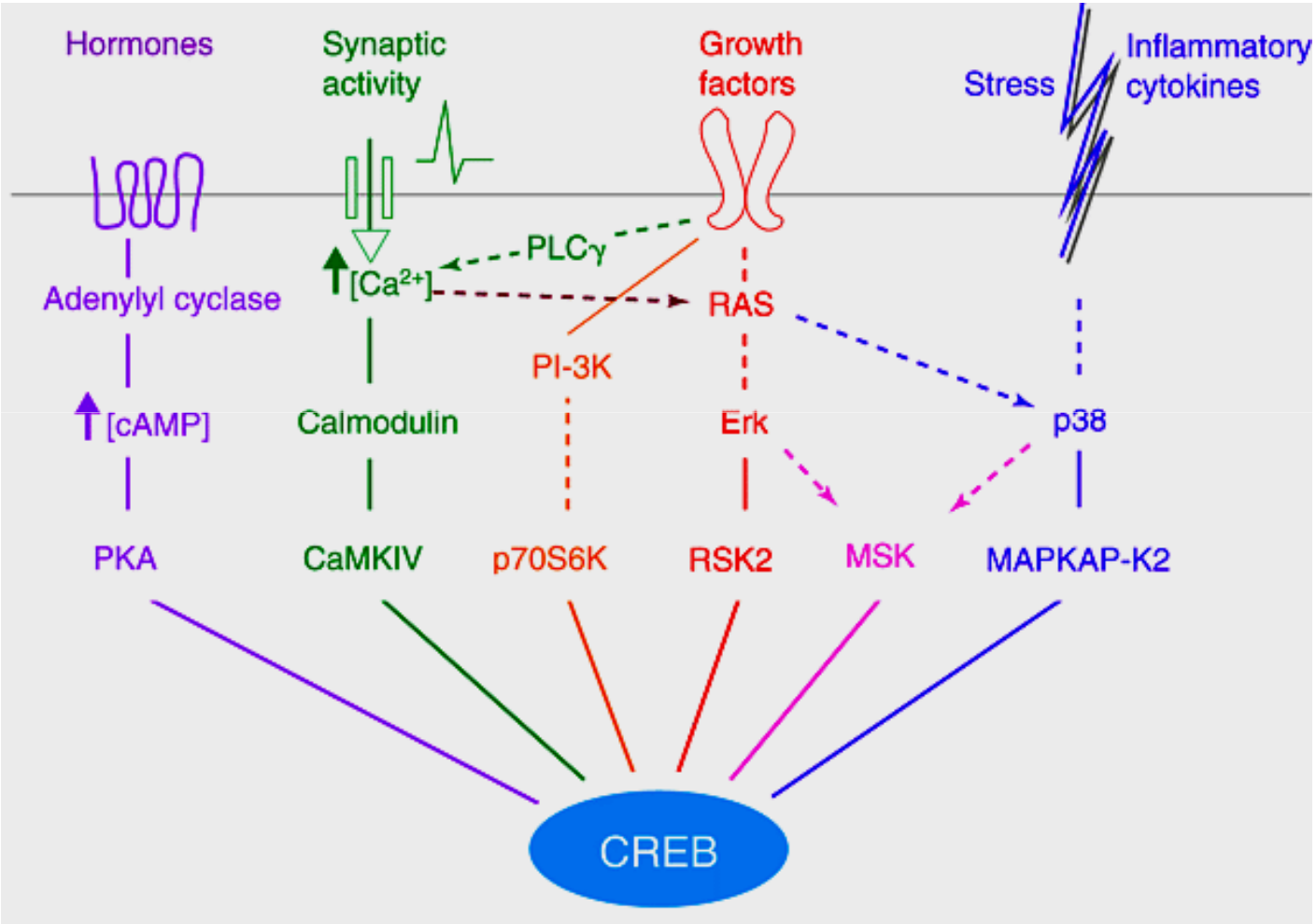
similar to:





KID=kinase interaction domain

A number of mechanisms are in place in order to control CRE activation, especially since it is traget of several pathways:



Activation by Protein Kinase A (and other kinases)

by phosphorylation on Ser 133

Ser 133 phosphorylation:
does not affect CREB localisation (almost all nuclear)
does not affect dimerization

Ser 133 phosphorylation led to the discovery of a protein interacting with the phospho- but not with the dephospho-CREB

This protein was called CBP for CREB binding protein and is the prototype **coactivator**

A homolog was cloned, p300, corresponding to a protein necessary for transactivation of the adenoviral E1A factor

Often they are indicated together “CBP/p300” or p300/CBP but they are distinct proteins encoded by distinct genes in Vertebrates.

These proteins are strictly necessary for CREB and other factors to activate transcription and represent prototypic “coactivators”

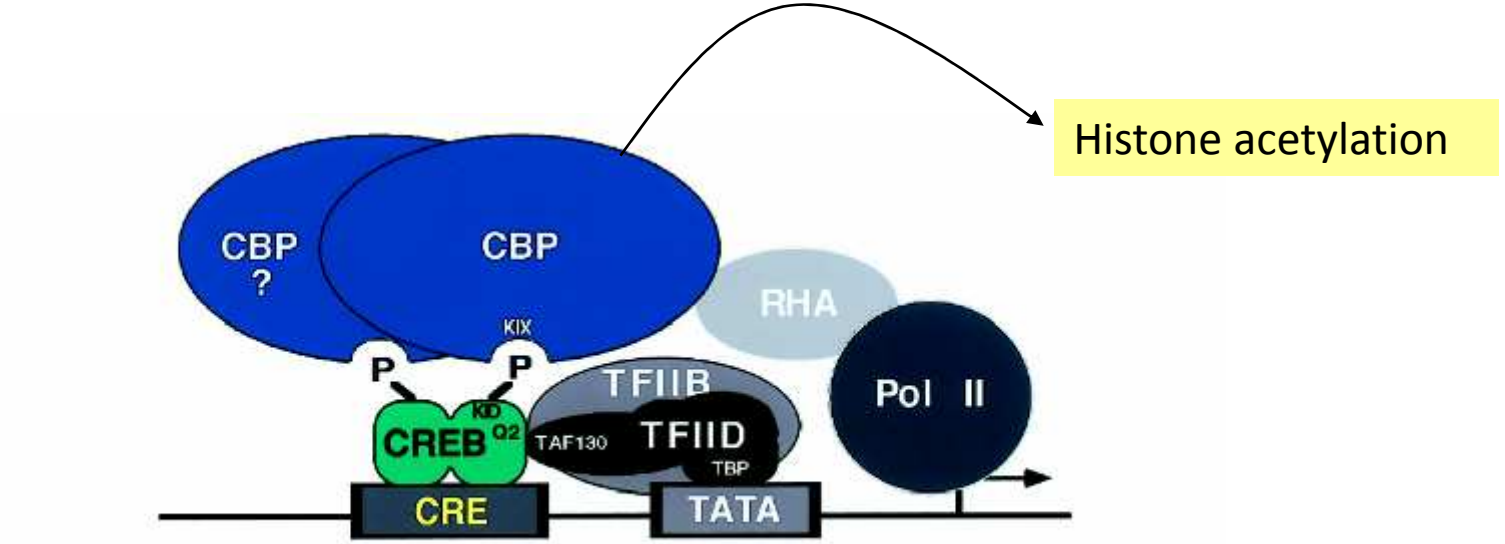
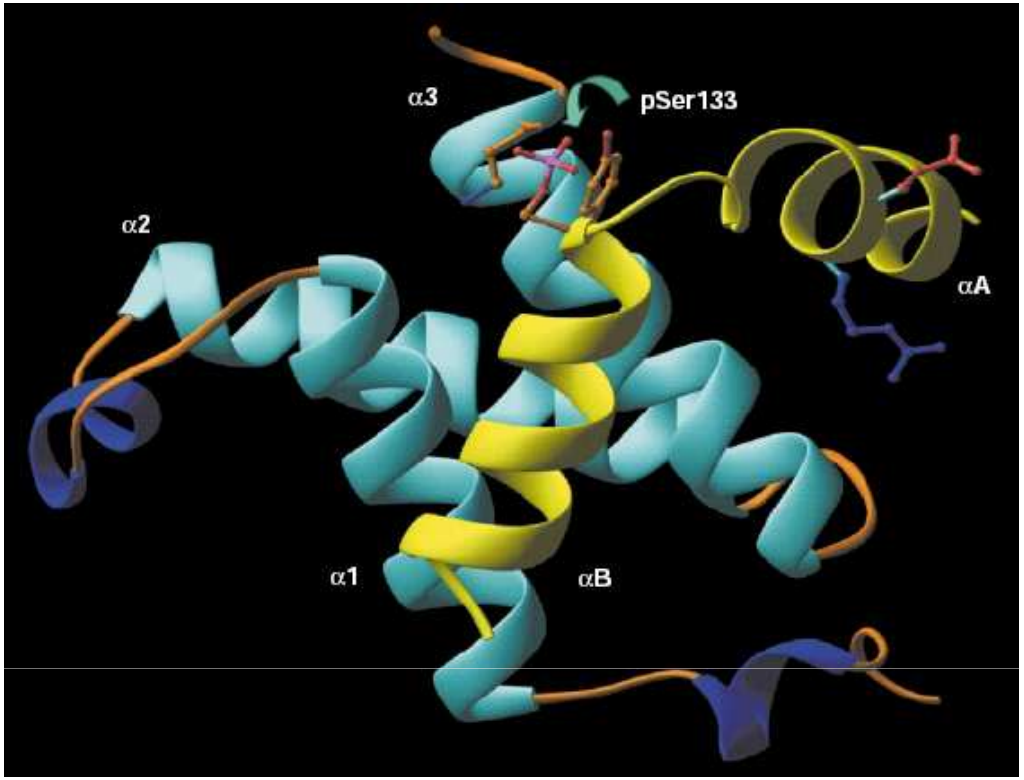


Figure 4 Multiple domains of CREB contribute to transcriptional activation. Different domains of CREB bind distinct coactivators and basal transcription factors to activate transcription. Shown is a CREB dimer bound to its cognate CaRE/CRE element on the promoter of a CREB target gene. Downstream of the CaRE/CRE is the TATA box, which binds the multiprotein TFIID basal transcription factor (via the TBP protein). Another factor within TFIID, TAF130, binds to the Q2 domain of CREB. The Q2 domain of CREB has also been shown to interact with TFIIB, which is a part of the basal transcription machinery as well. A distinct domain of CREB, the KID, contributes to signal-induced transcriptional activation. When phosphorylated at Ser133, the KID of CREB can bind to the KIX domain of the CBP. It is presently unclear whether CBP associates with Ser133–phosphorylated CREB as a dimer. CBP associates indirectly with Pol II via the RNA helicase A (RHA) protein. Therefore, recruitment of CBP to Ser133–phosphorylated CREB results in recruitment and stabilization of Pol II on the promoter of CREB target genes, whereas the Q2 domain interacts with other elements of the basal transcription machinery that are required for transcription, such as TFIID and TFIIB.

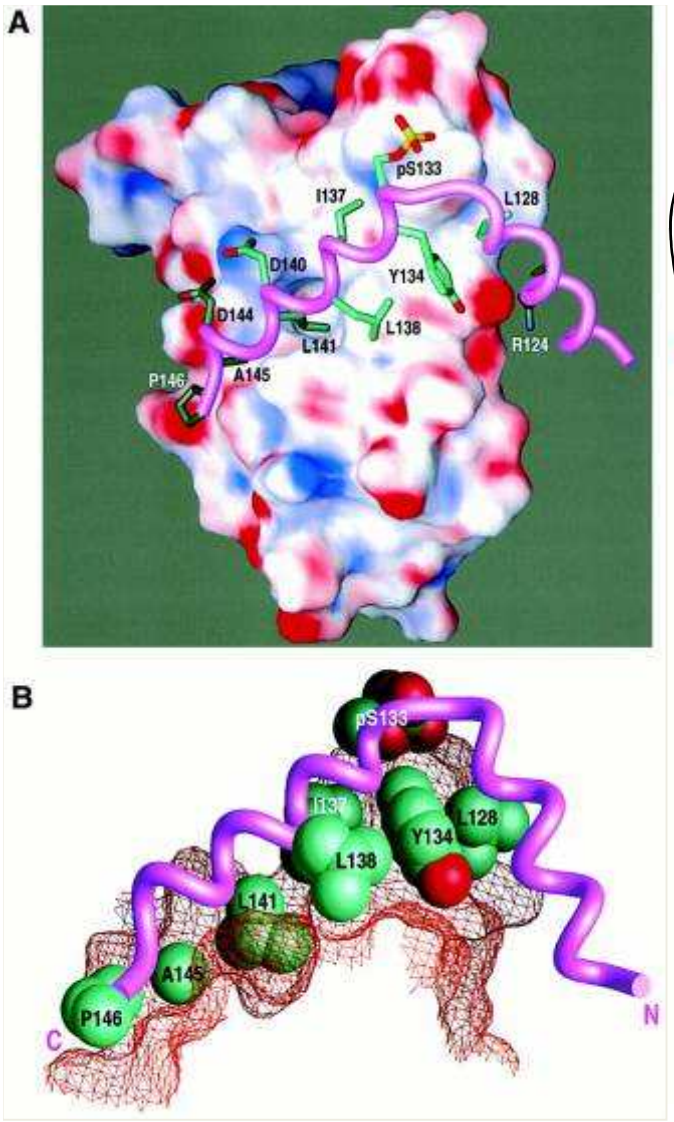


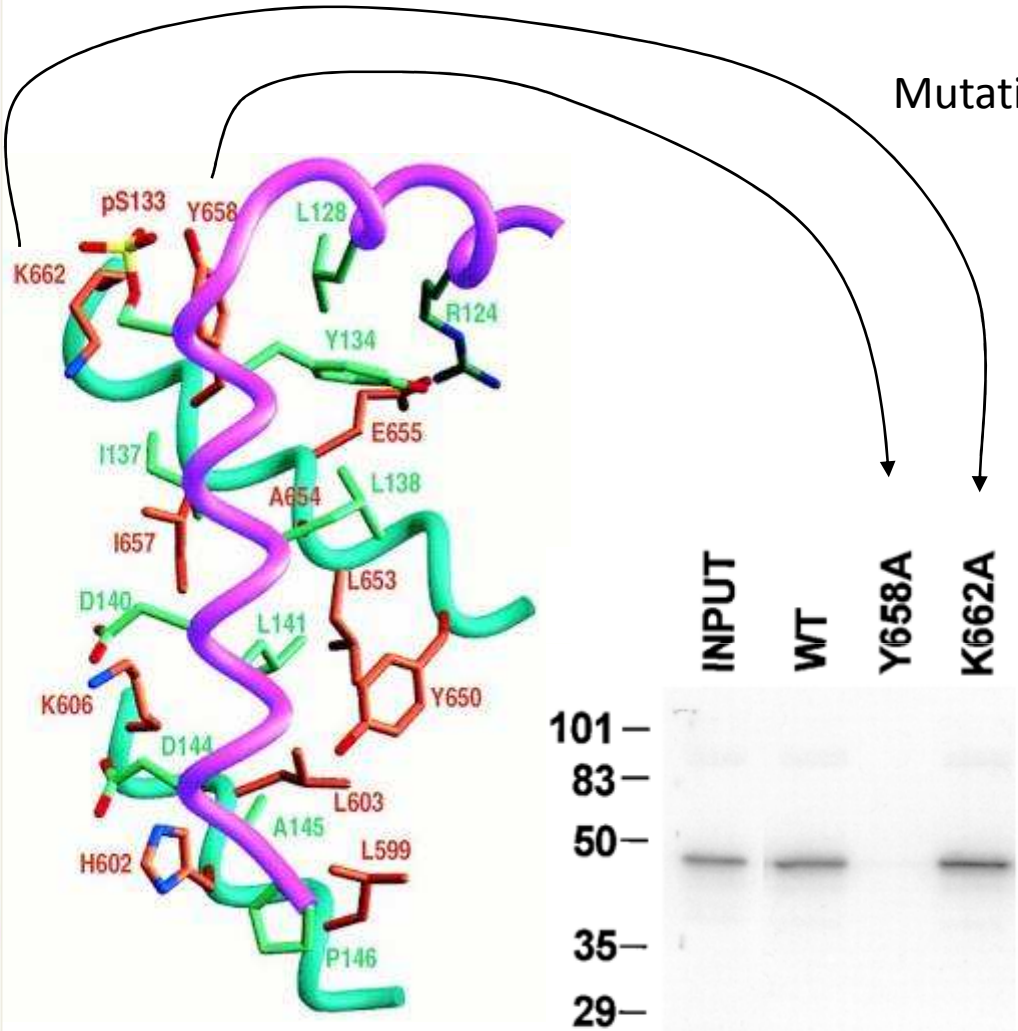
CREB KID=yellow

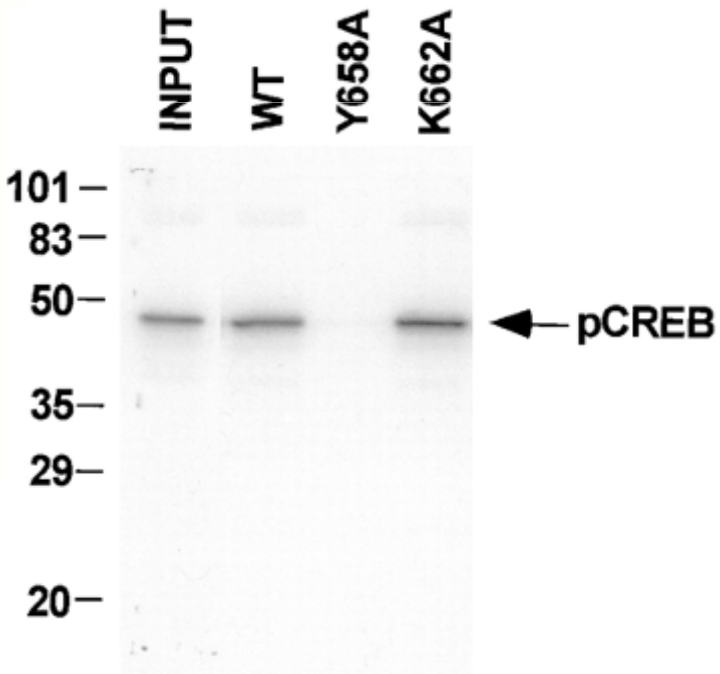
CBP KIX = cyano

Figure 4 | **Structure of the CREB–CBP complex using relevant interaction domains (KID and KIX, respectively).** The KIX domain of CBP (cyan) is a three-helix domain: helices α1 and α3 are nearly parallel and form a shallow hydrophobic groove and make all surface contacts with residues in the kinase-inducible domain (KID). Phosphorylated-Ser133 KID (yellow) forms a two-helix structure with αA and αB located perpendicular to one another.
Helix αB of the KID is amphipathic and forms most of the contacts with residues in KIX. Ser133 in the KID is located at the amino terminus of helix αB and makes two direct contacts: an ion pair with Lys 662 and a hydrogen bond with Tyr 658 in KIX. These contacts account for nearly half the free energy of complex formation.

from: Mayr & Montminy (2001), Nature Rev Mol Cell Biol 2:599.





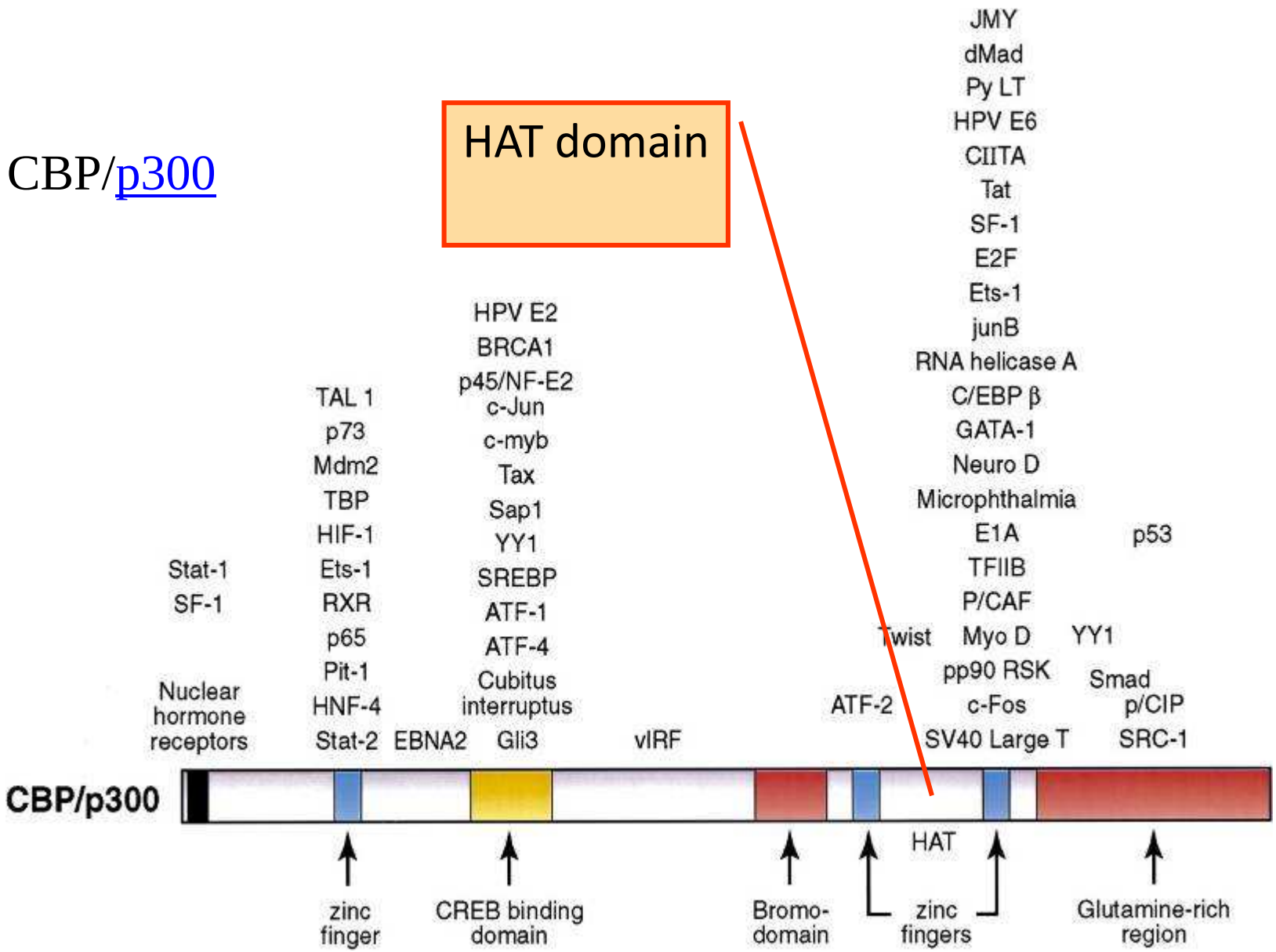


KIX Y658, but not K662, are essential for P-box interaction

GST-pull down experiment

CBP = CREB binding protein (265KDa) and p300 are in fact **general coactivators**

CBP/p300



Nuclear Receptors (NRs)

regulatory proteins that mediate cellular responses to:

Hormones

Vitamins

Retinoids

intracellular lipophilic molecules

xenobiotics

NRs ligands

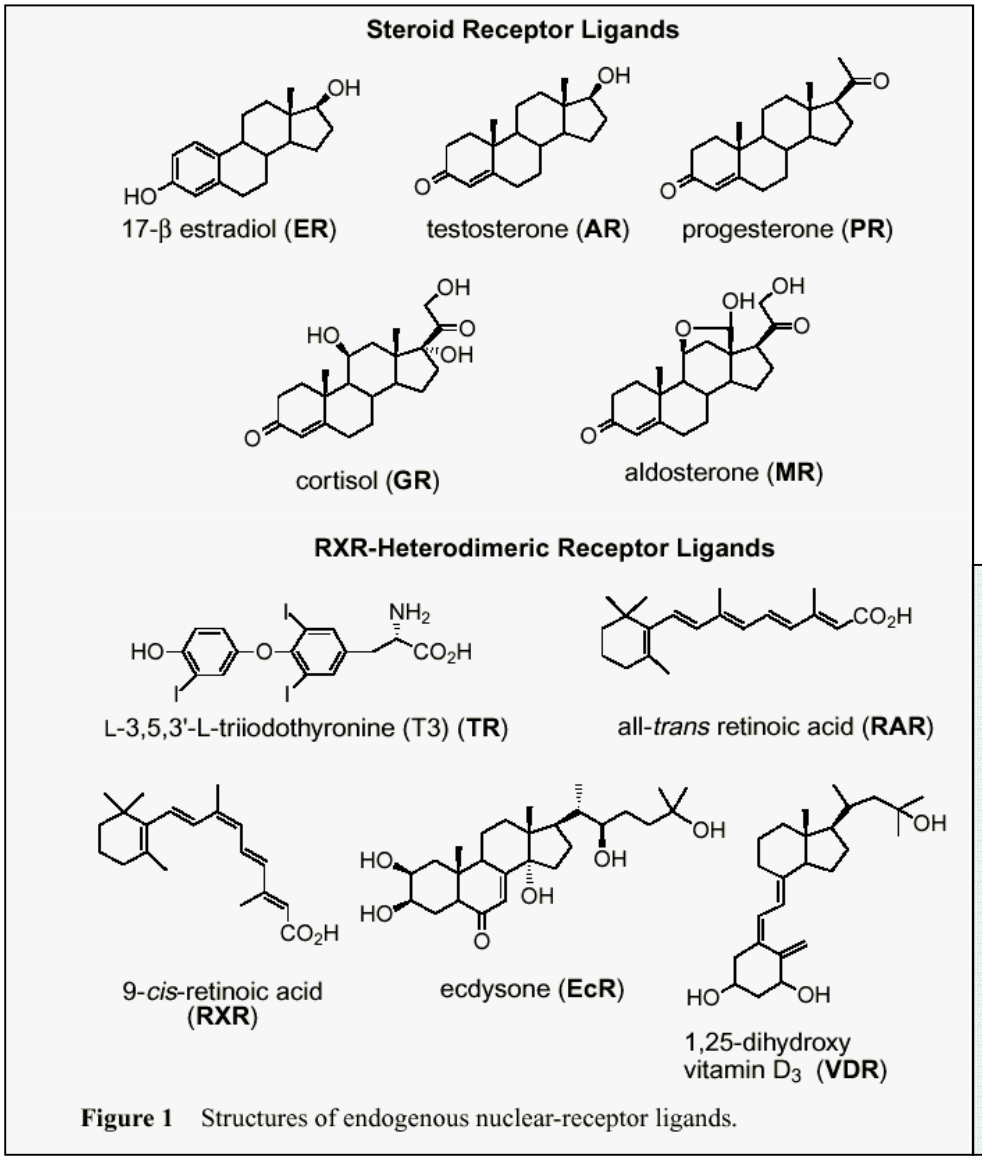


Figure 1 Structures of endogenous nuclear-receptor ligands.

1st subfamily:
"Steroid receptors

lypophilic endogenous molecules
(PPAR)

lypophilic exogneous compounds
(CAR, PXR)

Name	Abbreviation	Nomenclature	Ligand
Thyroid hormone receptor	TRa	NR1A1	Thyroid hormone
	TRb	NR1A2	Thyroid hormone
Retinoic acid receptor	RARα	NR1B1	Retinoic acid
	RARβ	NR1B2	Retinoic acid
	RARγ	NR1B3	Retinoic acid
Peroxisome proliferator-activated receptor			
	PPARα	NR1C1	Fatty acids, leukotriene B4, fibrates
	PPARβ	NR1C2	Fatty acids
	PPARγ	NR1C3	Fatty acids, prostaglandin J2,
Reverse erbA	Rev-erba	NR1D1	Orphan
	Rev-erbb	NR1D1	Orphan
RAR-related orphan receptor	RORα	NR1F1	Cholesterol, cholesteryl sulphate
	RORβ	NR1F2	Retinoic acid
	RORγ	NR1F3	Retinoic acid
Liver X receptor	LXRα	NR1H3	Oxysterols, T0901317, GW3965
	LXRβ	NR1H2	Oxysterols, T0901317, GW3965
Farnesoid X receptor	FXRα	NR1H4	Bile acids, Fexaramine
	FXRβ*	NR1H5	Lanosterol
Vitamin D receptor	VDR	NR1I1	1,25-dihydroxy vitamin D3,litocholic acid
Pregnane X receptor	PXR	NR1I2	Xenobiotics, PCN
Constitutive androstane rec	CAR	NR1I3	Xenobiotics, phenobarbital
Human nuclear factor 4	HNF4a	NR2A1	Orphan
	HNF4g	NR2A2	Orphan
Retinoid X receptor	RXRα	NR2B1	Retinoic acid
	RXRβ	NR2B2	Retinoic acid
	RXRγ	NR2B3	Retinoic acid
Testis receptor	TR2	NR2C1	Orphan
	TR4	NR2C2	Orphan
Tailless	TLL	NR2E2	Orphan
Photoreceptor-specific nuclear receptor			
	PNR	NR2E3	Orphan

(continued)

Name	Abbreviation	Nomenclature	Ligand
Chicken ovalbumin upstream promoter-transcription factor	COUP-TFI	NR2F1	Orphan
	COUP-TFII	NR2F2	Orphan
ErbA2-related gene-2	EAR2	NR2F6	Orphan
Oestrogen receptor	ERα	NR3A1	Oestradiol-17b, tamoxifen, raloxifene
	ERβ	NR3A2	Oestradiol-17b, various synthetic compounds
Oestrogen receptor-related rec.	ERRα	NR3B1	Orphan
	ERRβ	NR3B2	DES, 4-OH tamoxifen
	ERRγ	NR3B3	DES, 4-OH tamoxifen
Glucocorticoid receptor	GR	NR3C1	Cortisol, dexamethasone, RU486
Mineralocorticoid receptor	MR	NR3C2	Aldosterone, spirolactone
Progesterone receptor	PR	NR3C3	Progesterone, medroxyprogesterone acetate RU486
Androgen receptor	AR	NR3C4	Testosterone, flutamide bicalutamide
NGF-induced factor B	NGFIB	NR4A1	Orphan
Nur related factor 1	NURR1	NR4A2	Orphan
Neuron-derived orphan receptor 1	NOR1	NR4A3	Orphan
Steroidogenic factor 1	SF1	NR5A1	Orphan
Liver receptor homolog protein 1	LRH1	NR5A2	Orphan
Germ cell nuclear factor	GCNF	NR6A1	Orphan
DSS-AHC critical region on the chromosome, gene 1	DAX1	NR0B1	Orphan
Short heterodimeric partner	SHP	NR0B2	Orphan

Dysfunction of nuclear receptor signalling leads to proliferative, reproductive and metabolic diseases such as cancer, infertility, obesity and diabetes. Therefore:

Nuclear receptors are very important as **drug targets**

Pharmaceutical nuclear receptor **agonists** or **antagonists** are used in human therapy. Most known examples:

◊ tamoxifen for oestrogen receptors (targeted in breast cancer),

◊ flutamide-bicalutamide for androgen receptor (prostate cancer)

◊ thiazolidinediones for peroxisome proliferator-activated receptor-γ (PPARγ) (targeted in type II diabetes)

◊ dexamethasone for the glucocorticoid receptor (targeted in inflammatory diseases)

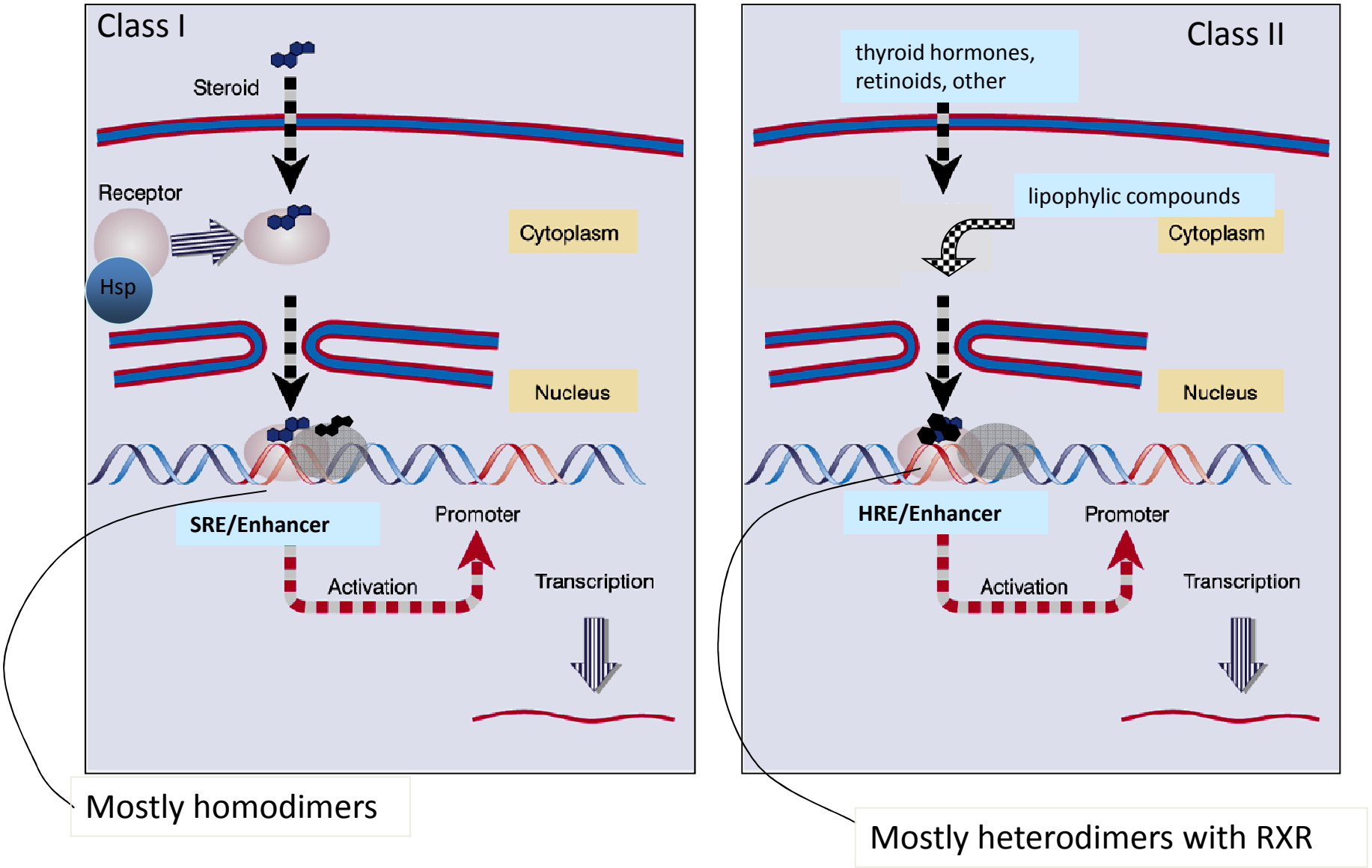
NRs classification

NUCLEAR RECEPTOR TYPE	NUCLEAR RECEPTOR MEMBERS
I (classical or steroid receptors)	Progestins receptor (PR) Estrogens receptor (ERα, ERβ) Androgens receptor (AR) Glucocorticoids receptor (GR) Mineralcorticoids receptor (MR)
II (RXR-heterodimeric receptors)	Thyroid hormone receptor (TRα, TRβ) All-*trans* retinoic acid receptor (RAR) 9-*cis* retinoic acid receptor (RXR) Vitamin D_3 receptor (VDR) Peroxisome proliferator receptor-γ (PPAR-γ)
III (Orphan nuclear receptors)	COUP-TFs X-linked orphan receptor (DAX-1) Rev-Erb

In H. sapiens there are 48 known nuclear receptor genes.

24 have known ligands 24 are orphan receptors

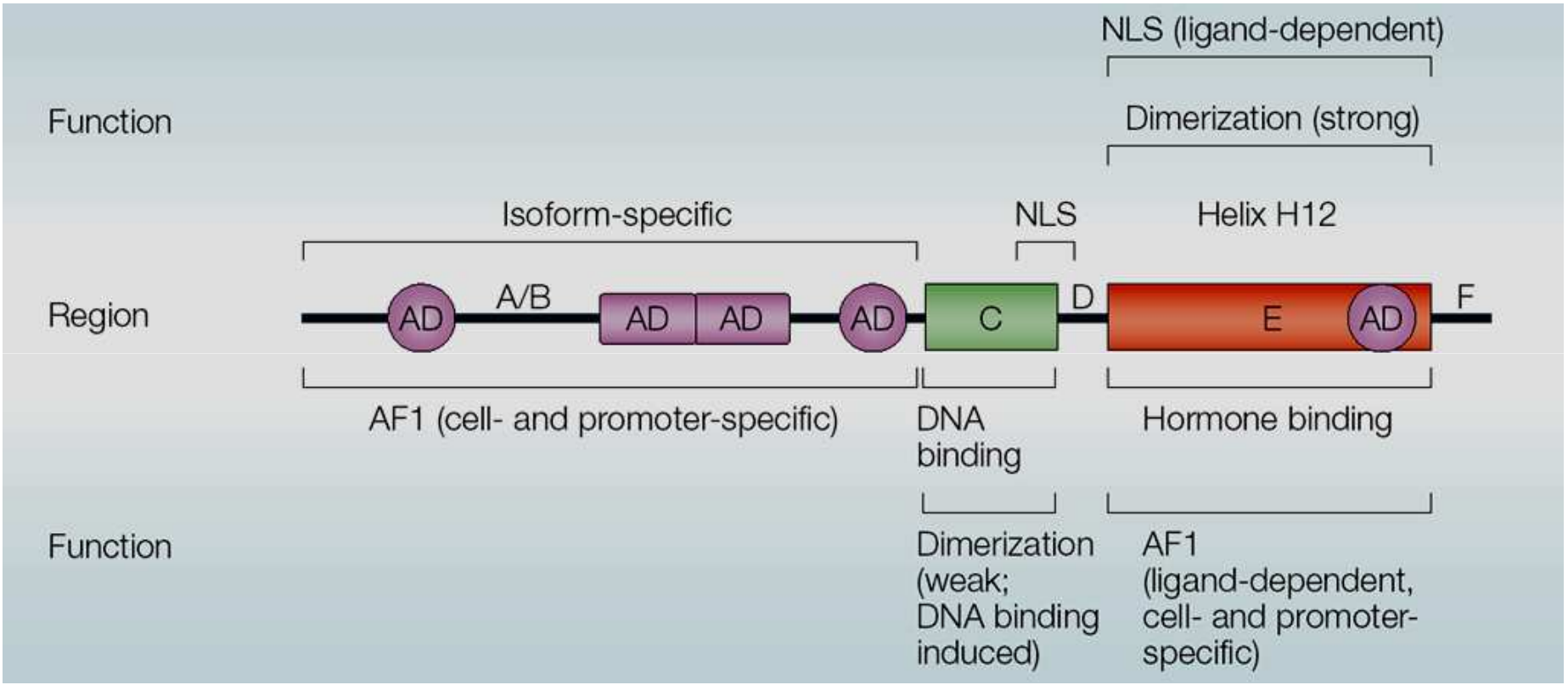
The simplest scheme of NRs mechanism of action



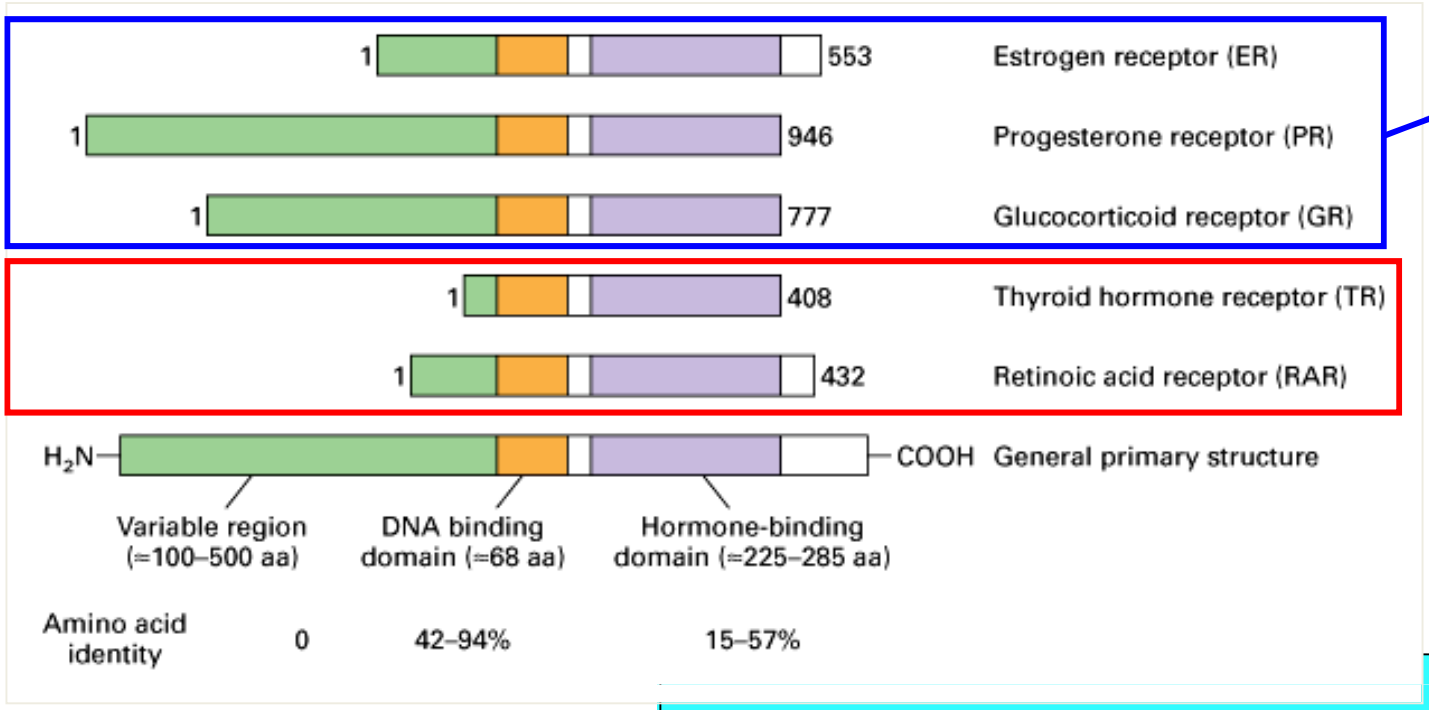
Mostly homodimers

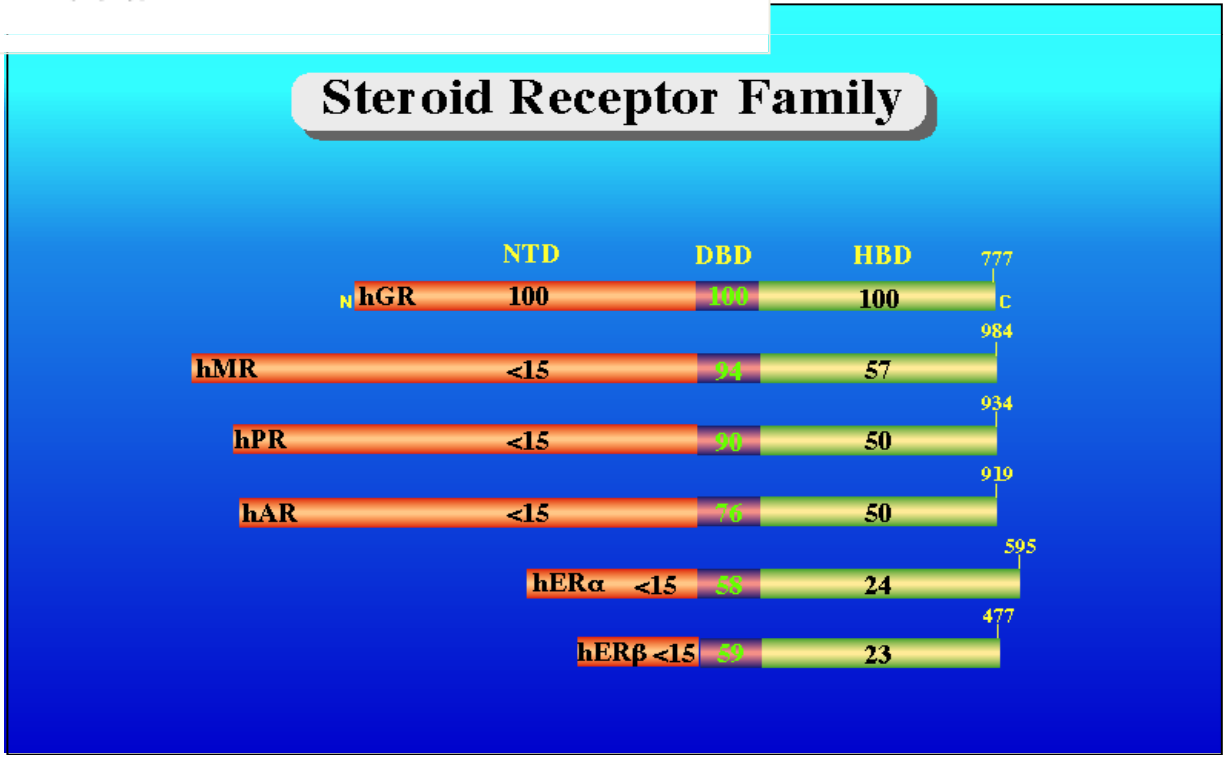
Mostly heterodimers with RXR

1 - Structure



Variable length in different NRs. In Class II this domain usually has little or no transactivating function





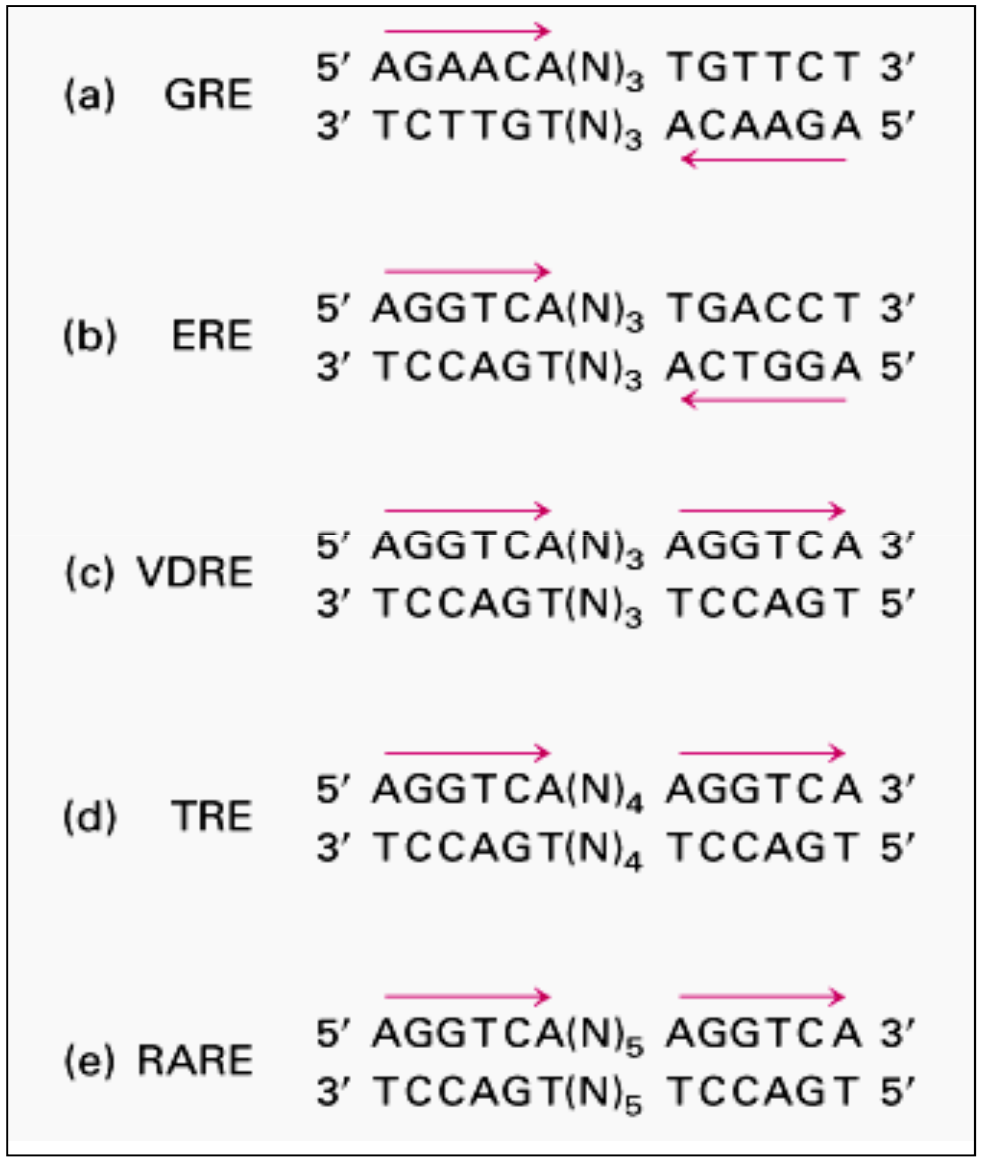
1st important difference:

Class 1 has longer N-term
Class 2 reduced

Class 1 A/B domain has transactivation activity

Class 2 A/B does not

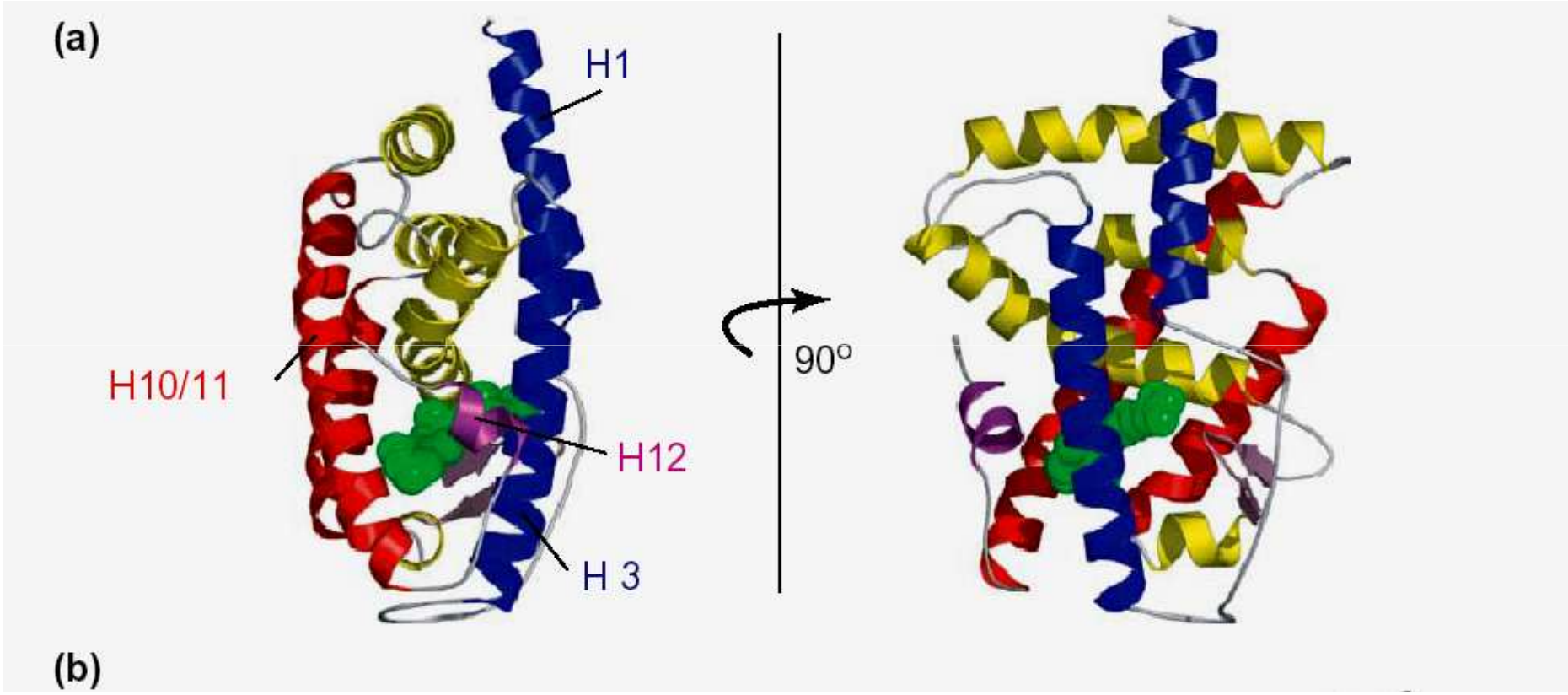
Hormone Response Elements: palindromic (Class1) or direct repeats (Class2)



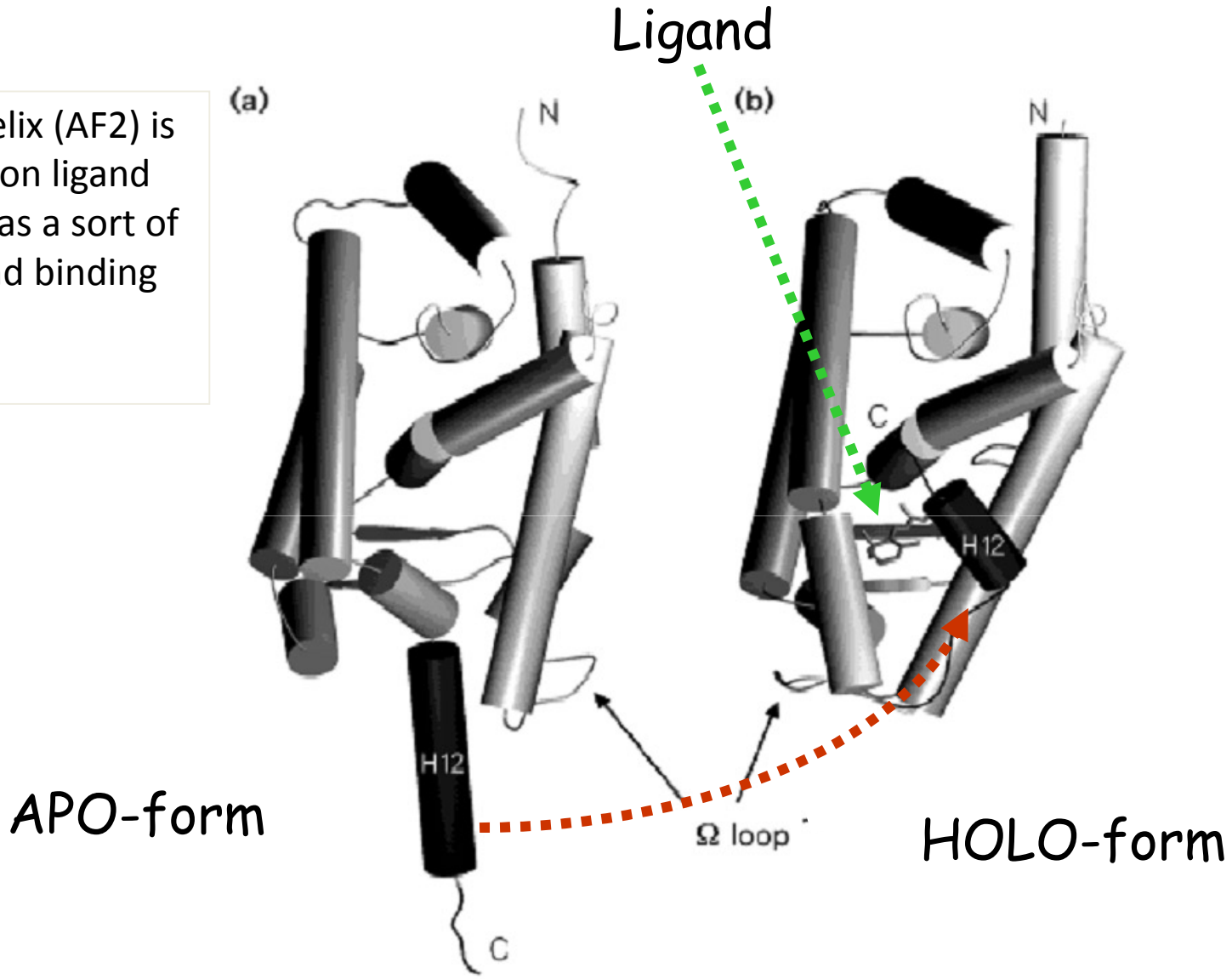
Class I

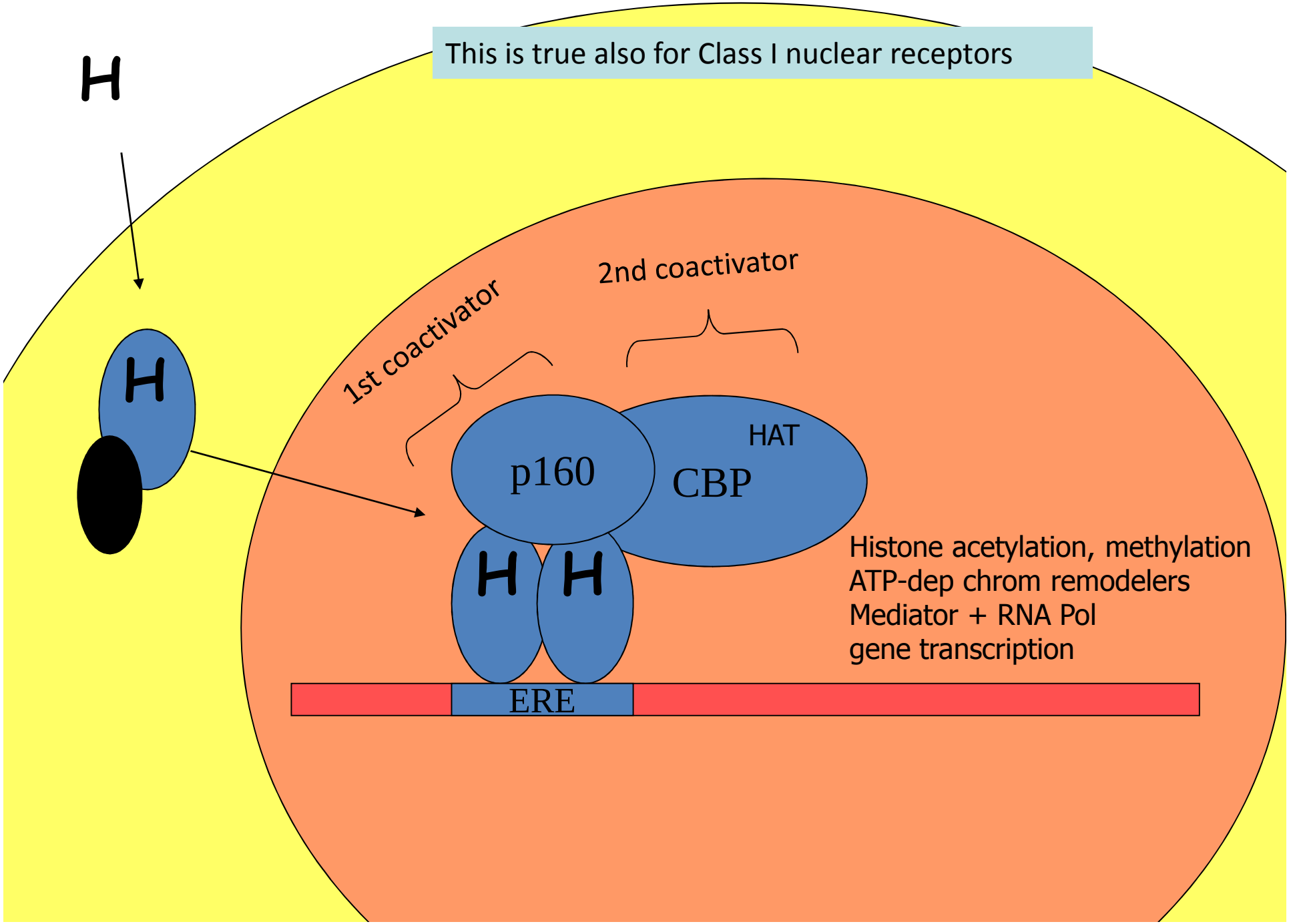
Class II

The Ligand-binding domain (LBD)



NR C-terminal helix (AF2) is re-positioned upon ligand binding, serving as a sort of "lid" on the ligand binding pocket




H
This is true also for Class I nuclear receptors
H
1st coactivator
2nd coactivator
p160
HAT
CBP
H
H
Histone acetylation, methylation
ATP-dep chrom remodelers
Mediator + RNA Pol
gene transcription
ERE

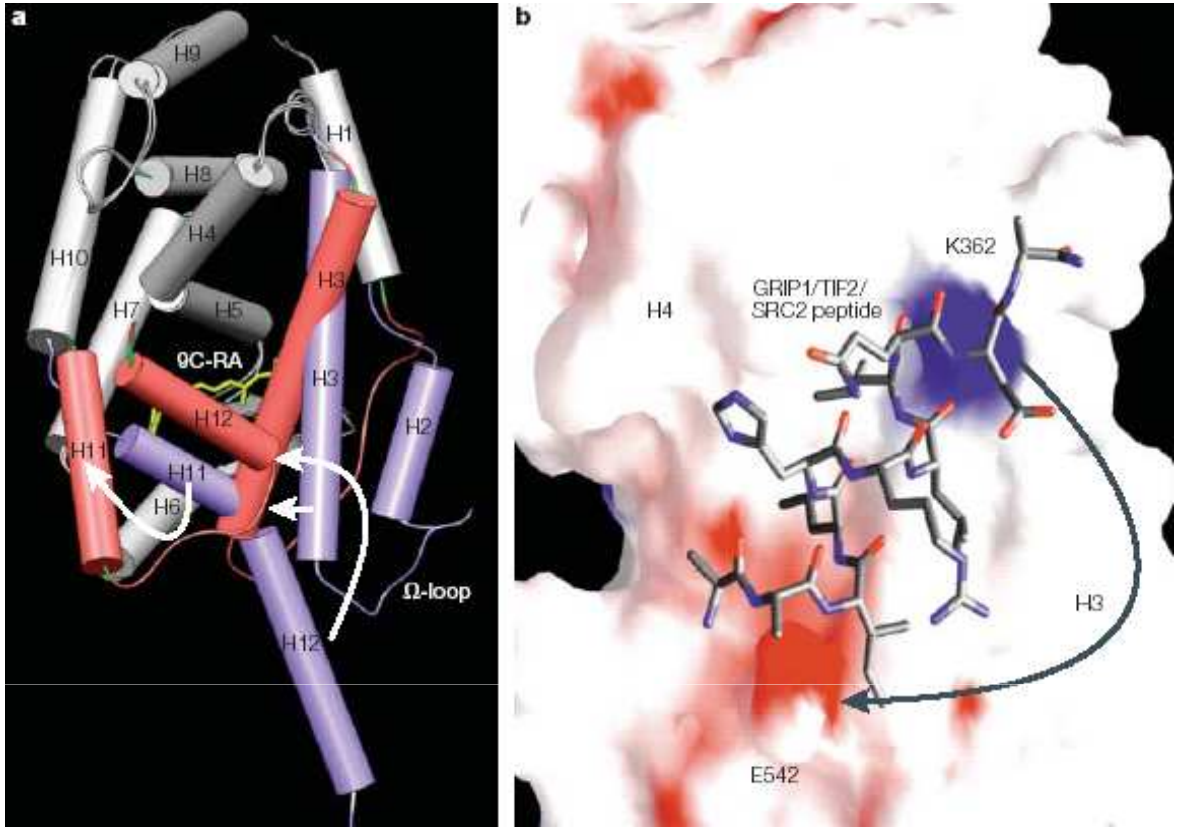
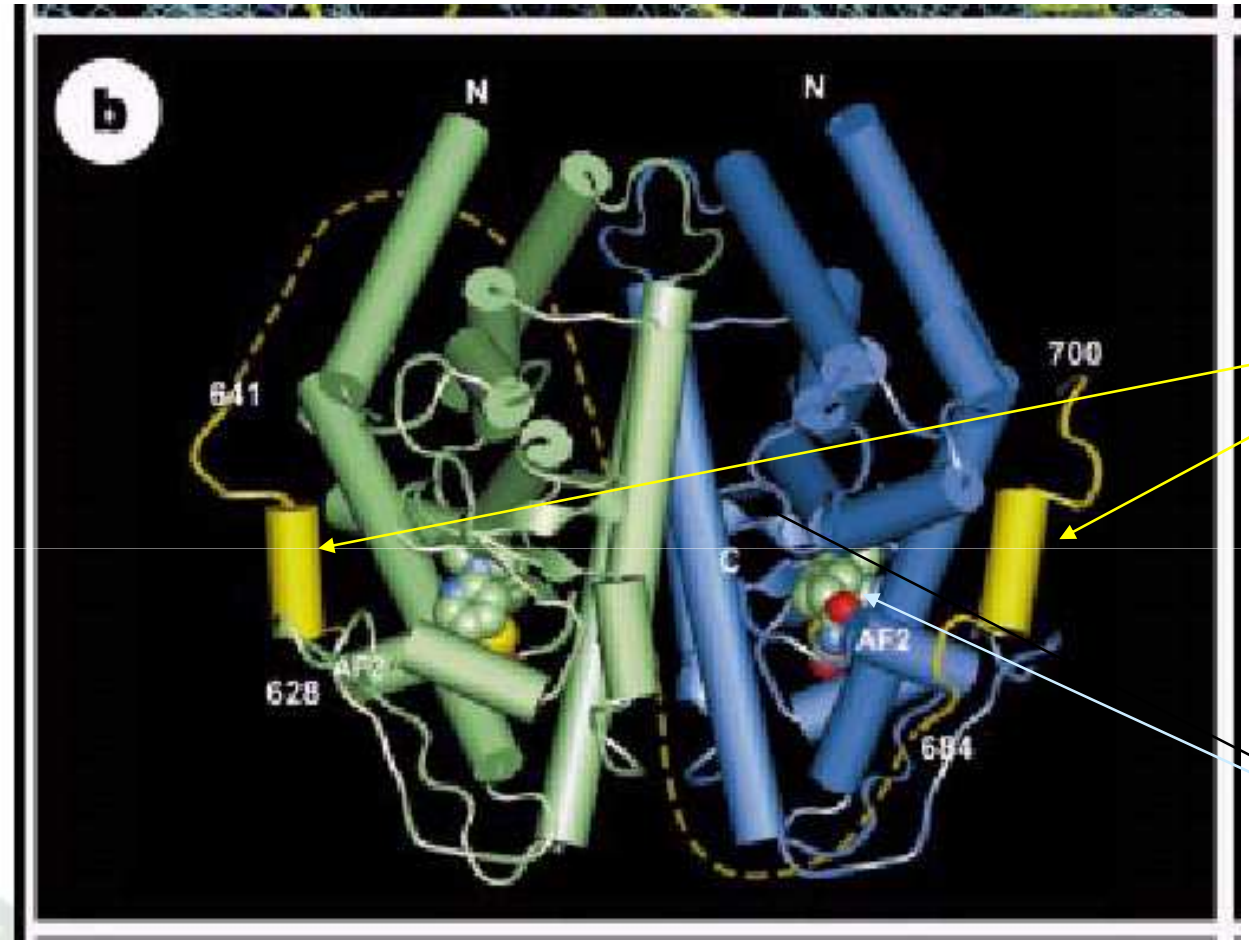


Figure 2 | Ligand binding induces a conformational change of the ligand-binding domain structure of nuclear receptors.

a | A comparison of the crystal structures of the apo-retinoid X receptor-α (RXRα) ligand-binding domain (LBD) with the holo-RXRα LBD complexed with 9-cis retinoic acid. The figure reveals the ligand-induced trans-conformation that generates the transcriptionally active form of the receptor. The coloured helices H2, H3, H11 and H12 (purple in the apo-form; red in the holo form) are relocalized during the conformational change. In this model, ligand binding induces a structural transition that triggers a mousetrap-like mechanism: pushed by the ligand, H11 is repositioned in alignment with H10 and the concomitant swinging of H12 unleashes the omega-loop, which flips underneath H6, carrying along the amino-terminal part of H3. In its final position, H12 seals the ligand-binding cavity as a lid and further stabilizes ligand binding by contributing to the hydrophobic pocket.

b | The co-activator nuclear-receptor box LxxLL peptide-binding surface on the ERα–diethylstilbestrol (DES) complex is shown in white. A charge clamp (that is, charged amino acids that interact with both ends of the peptide and increase the strength of the interaction; the two ends of the clamp are indicated by the arrow) controls the binding of the LxxLL-containing nuclear-receptor-box peptide of co-activator proteins. The two residues constituting the charge clamp are indicated by blue and red surfaces. Oestrogen receptor (ER) residues E542 (red) and K362 (blue) stabilize co-activator binding in addition to the hydrophobic interactions established by the leucines. The regions of the surface that correspond to helices H3 and H4 of the receptor LBD are indicated.

The PPARγ LBD complexed with SRC1 helix and rosiglitazone



LXXLL helix from the SRC1 coactivator

rosiglitazone

from: Nolte et al., 1998, Nature 395:137.

To activate transcription of target genes, liganded NRs interact with proteins called “co-activators”.

Interaction of co-activators with the LBD of NRs is mediated by a common motif “**LXXLL**”

Parker pdf

which is flanked by charged residues interacting with opposite charges in the nuclear receptor LBD, making a sort of **“charged clamp”**

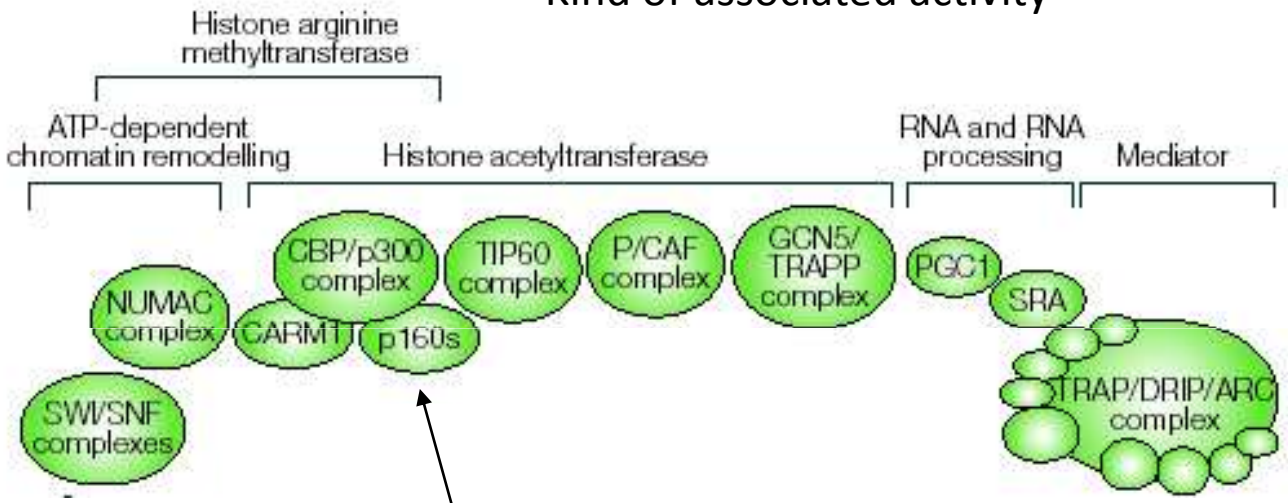
Table II. Nuclear receptor co-activators

CO-ACTIVATOR		INTERACTING NUCLEAR RECEPTORS
SRC-1/NcoA-1	Steroid receptor co-activator-1/Nuclear receptor co-activator-1	PR, GR, ER, TR, RXR, HNF-4, PPARγ, RAR
TIF2/GRIP1/NcoA-2	Transcriptional intermediary factor-2/ Glucocorticoid receptor-interacting protein-1/ Nuclear receptor coactivator-2	PR, ER, RAR, RXR
p/CIP/AIB-1/ ACTR/ TRAM-1/ RAC3/SRC-3	p300/CBP co-integrator associated protein/ Amplified in breast cancer-1/ Activator of the TR and RAR/ TR activator molecule/ Receptor associated co-activator-3/Steroid receptor co-activator-3	ER, TR, RAR
CBP/p300	CREB Binding Protein	RAR, RXR, ER, TR
p/CAF/hGCN5	p300/CBP-associated factor	PR, TR, ACTR, RAR
DRIP/TRAP/ARC	Vitamin D_3 receptor interacting proteins/ Thyroid hormone receptor associated proteins/ Activator-recruited complex	TR, VDR
PC2	Positive cofactor 2	TR
PC4	Positive cofactor 4	TR
BRG-1	Brahma-related gene 1	ER, GR
SRA	Steroid Receptor RNA activator	PR
ARA-70		AR
TIF-1	Transcriptional intermediary factor-1	ER, RAR, RXR

P160 family

NR coactivators have been purified as complexes that bind to activated receptors:

Kind of associated activity

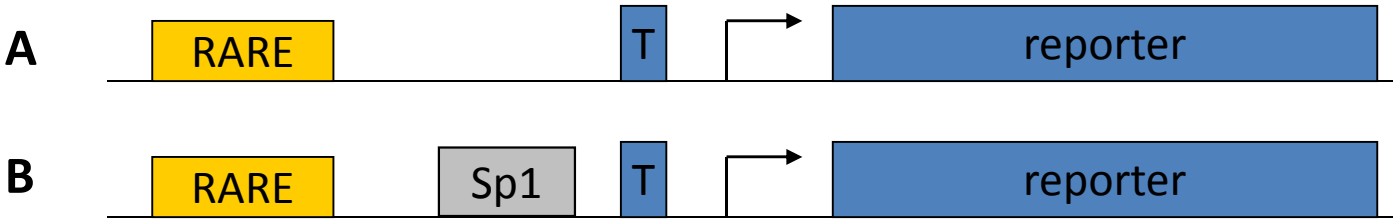


P160 coactivators: several homologous proteins (see Table)

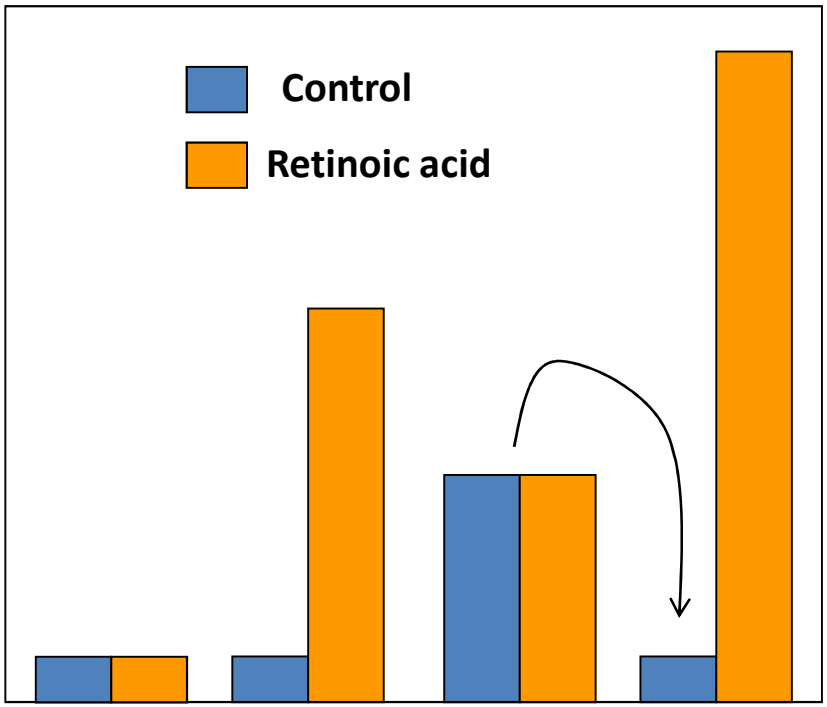
Class 2 are **nuclear** in apo-form as well

Often found to be bound to their cognate elements in the absence of ligands.

So… what does the ligand do to switch the receptor from an inactive (will see……) to **active** status ?



Transfection in cells that do not possess RAR



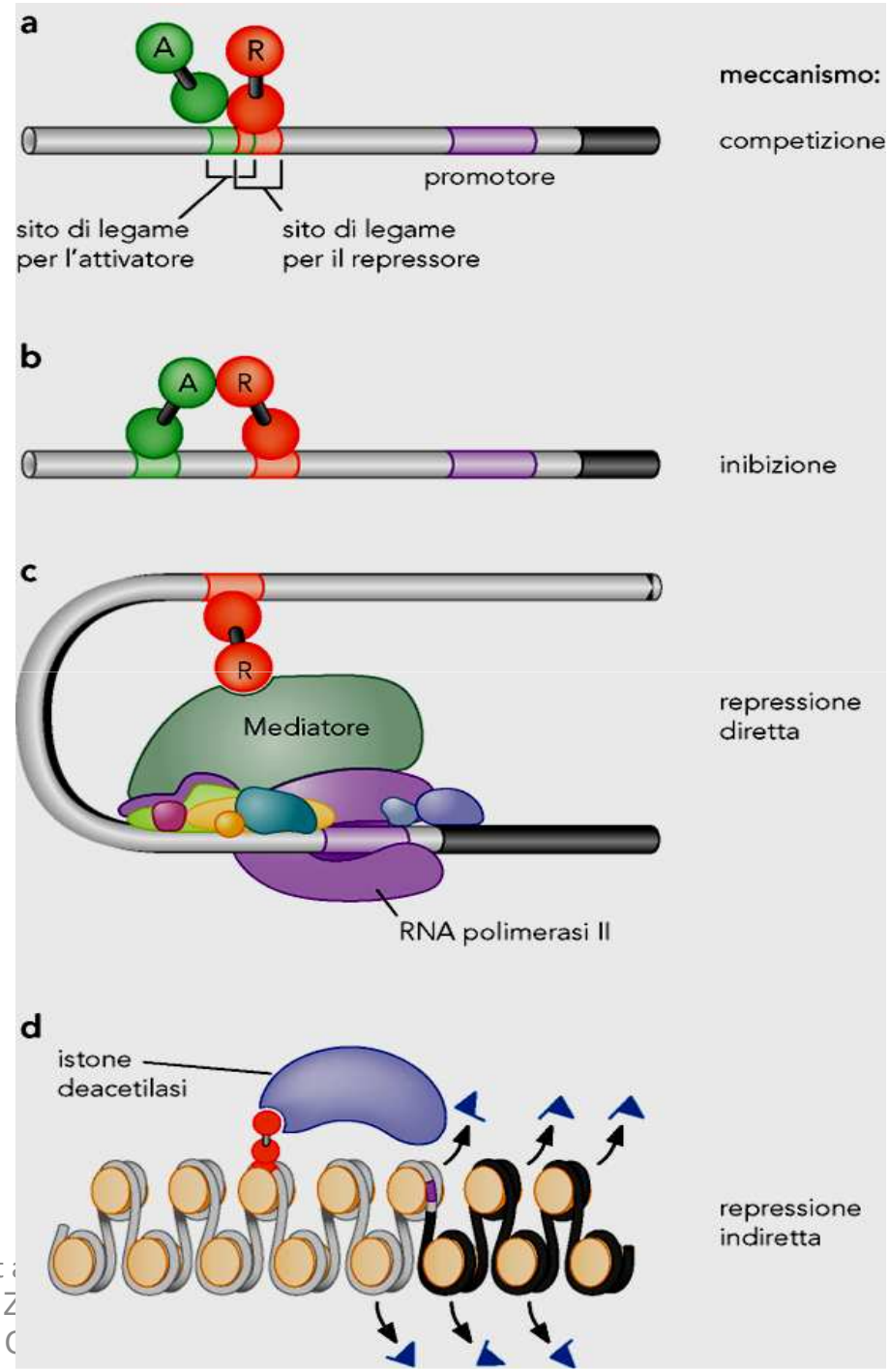
Vector expressing RAR:

In the absence of retinoic acid, the RAR is a **repressor**

Trying to find the reasons for this, a novel class of coregulators was identified: the “corepressor” complexes.

These proteins are found in general in complexes with Histone deacetylases (HDACs) and other enzymes that modify chromatin in the sense of heterochromatin.

Different ways a factros can repress transcription form a target gene. Model d) is the one that explains results of nuclear receptors.



Watson et a
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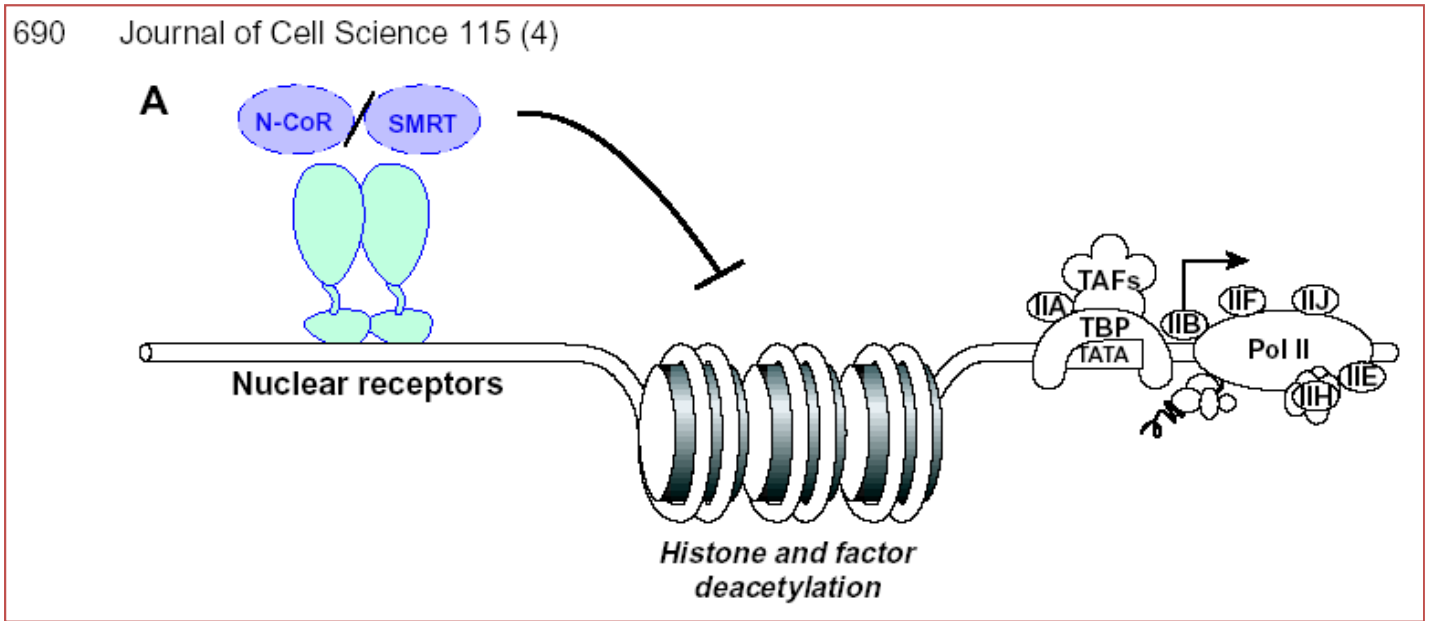
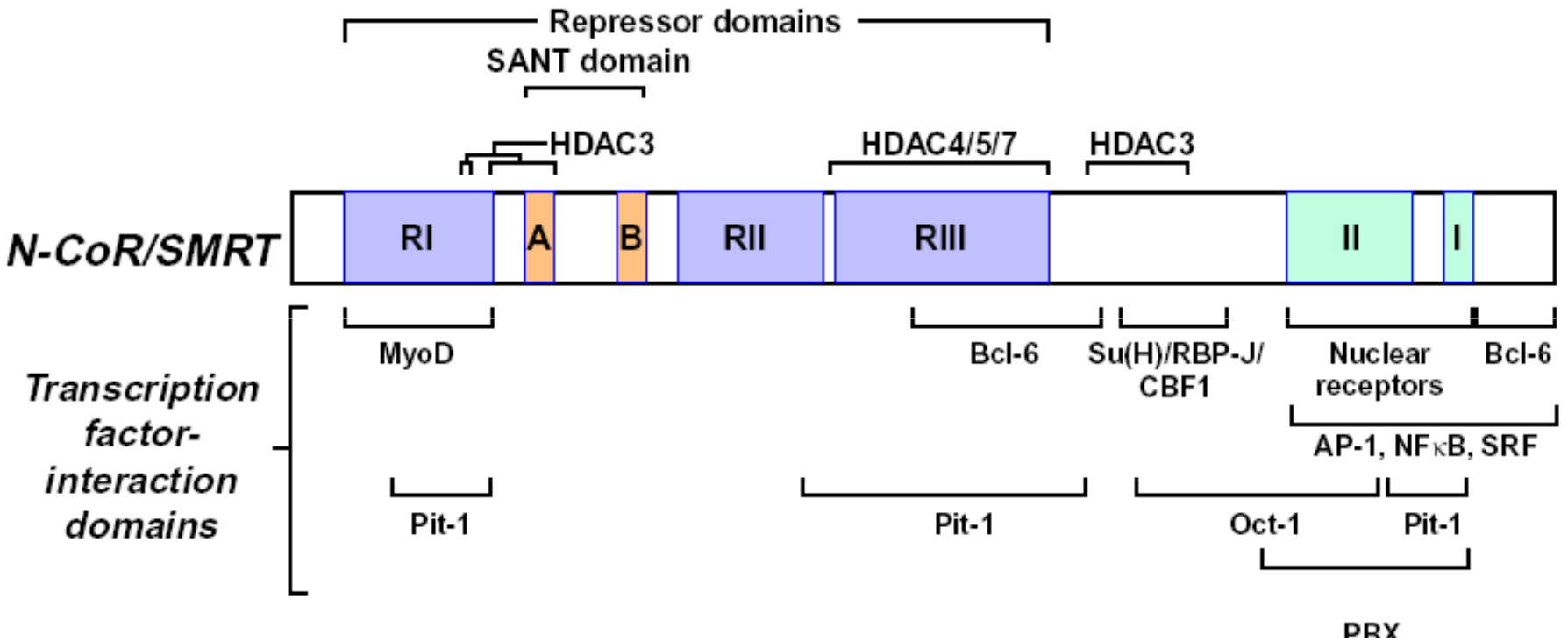
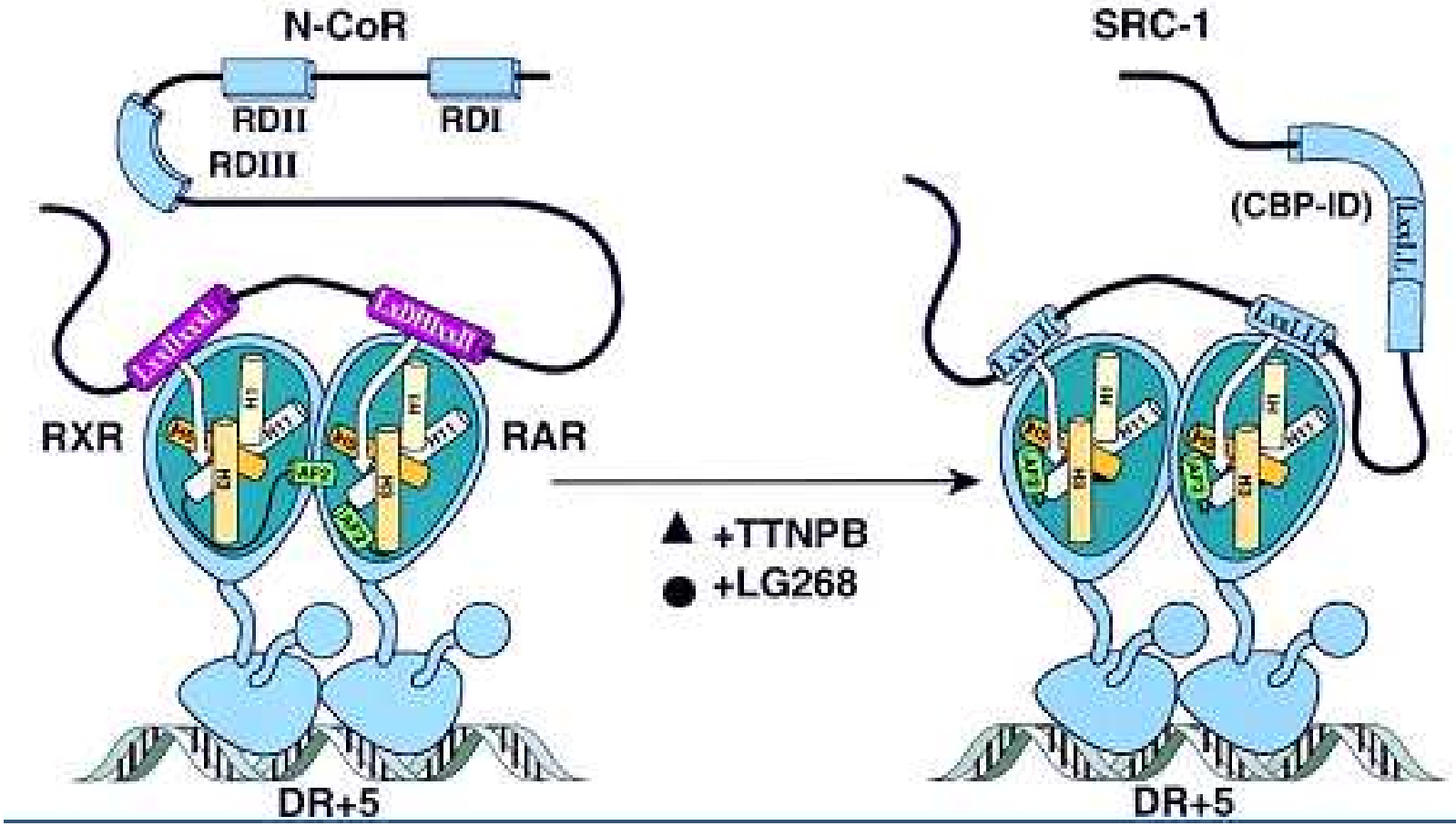
Table III. Nuclear receptor co-repressors

CO-REPRESSOR		INTERACTING NUCLEAR RECEPTORS
NCoR/RIP-13	Nuclear Receptor co-repressor/ Retinoid X receptor interacting protein-13	TRα, RARα, RAR γ, Rev-Erb, COUP-TFs, DAX-1
SMRT/TRAC2	Silencing mediator for retinoic acid and thyroid hormone receptors/ T_3 receptor-associated cofactor 2	RARα, RARγ, TR:RXR, PPARγ, ER
TRUP/SURF-3/PLA-X/L7a	Thyroid receptor uncoupling protein	RAR, TR
SUN-CoR	Small ubiquitous nuclear co-repressor	TR, RevErb
Alien		TR





Very large proteins (250-300 kDa)



Model of the ligand-dependent **exchange** of corepressor for coactivator. The two related N-CoR interaction helices are suggested to cooperatively be recruited into the helix 3, 5, 6 binding pocket of RXR/ /RAR or RXR/T3R heterodimers on DNA, with no requirement for the conserved glutamic acid residues of the AF2 helix. Ligand binding induces exchange for coactivators, which contain the short LXXLL helical motifs, requiring the conserved glutamic acid residue of the AF-2 helix for effective orientation and positioning into the receptor binding pocket.

modified from: Perissi et al., 1999, Genes Dev. 13:3198.

A number of corepressor complexes exist

most of them are multisubunit, multi-functional

many enzymatic activities are associated with corepressor complexes

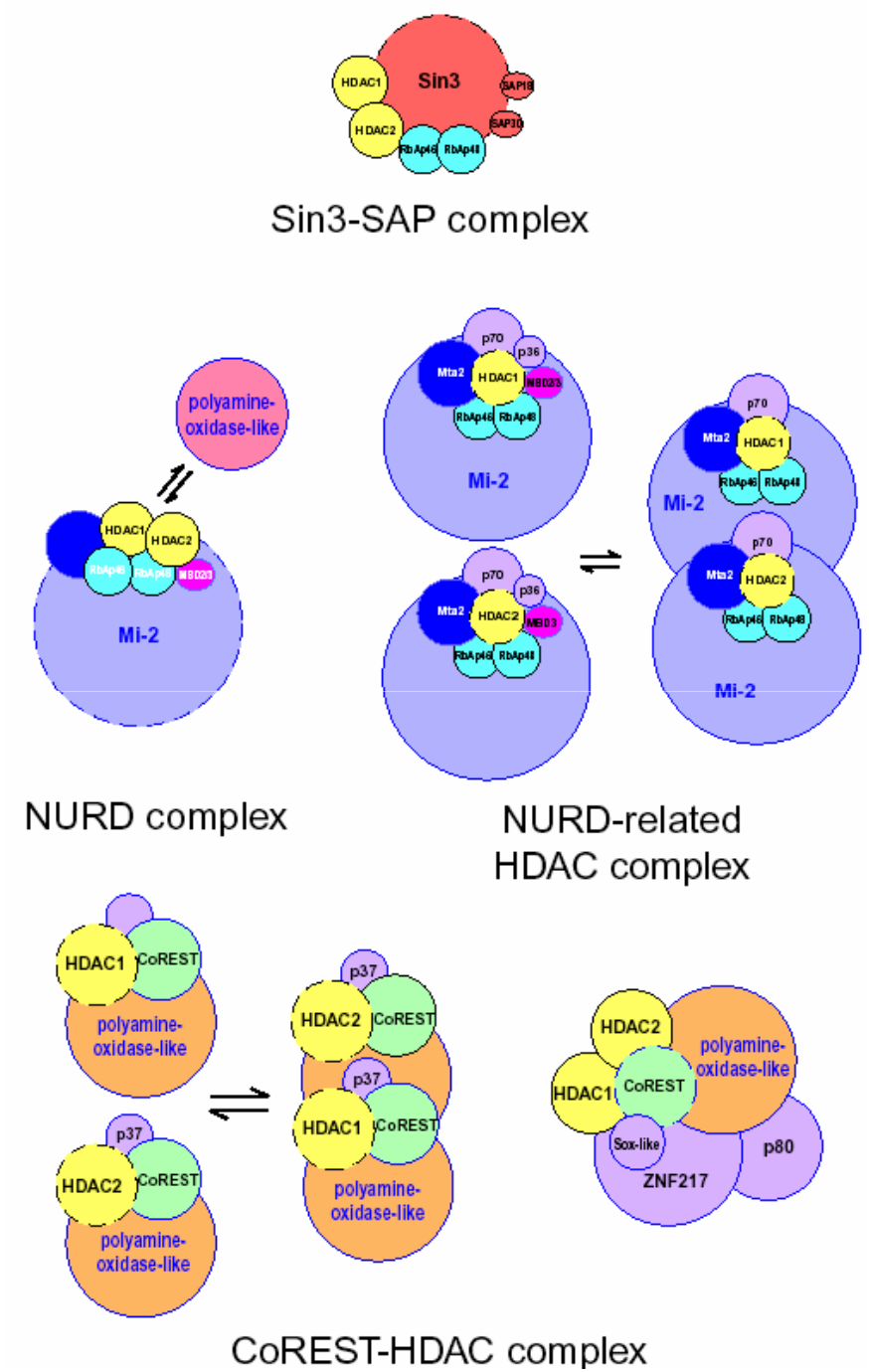


Fig. 2. Other HDAC-containing co-repressor complexes identified by biochemical purification, including the Sin3-SAP (Sin-associated proteins) complex (Zhang et al., 1997; Zhang et al., 1998c), the NURD (nucleosome remodeling and histone deacetylation) complex (Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998b; Zhang et al., 1999), a NURD-related HDAC-complex (Humphrey et al., 2001) and a CoREST-HDAC complex (Humphrey et al., 2001; You et al., 2001). Dashed outlines indicate the molecule that was used to purify each complex.

Review

Deconstructing repression: evolving models of co-repressor action

Valentina Perissi, Kristen Jepsen*, Christopher K. Glass‡ and Michael G. Rosenfeld**

Abstract | A crucial aspect of development, homeostasis and prevention of disease is the strict maintenance of patterns of gene repression. Gene repression is largely achieved by the combinatorial action of various enzymatic complexes — known as co-repressor complexes — that are recruited to DNA by transcription factors and often act through enzymatic modification of histone protein tails. Our understanding of how co-repressors act has begun to change over recent years owing to the increased availability of genome-scale data. Here, we consider specific strategies that underlie repression events — for example, those mediated by the nuclear receptor co-repressor (NCoR, also known as NCOR1) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT, also known as NCOR2) co-repressor complexes — and discuss emerging themes in gene repression.

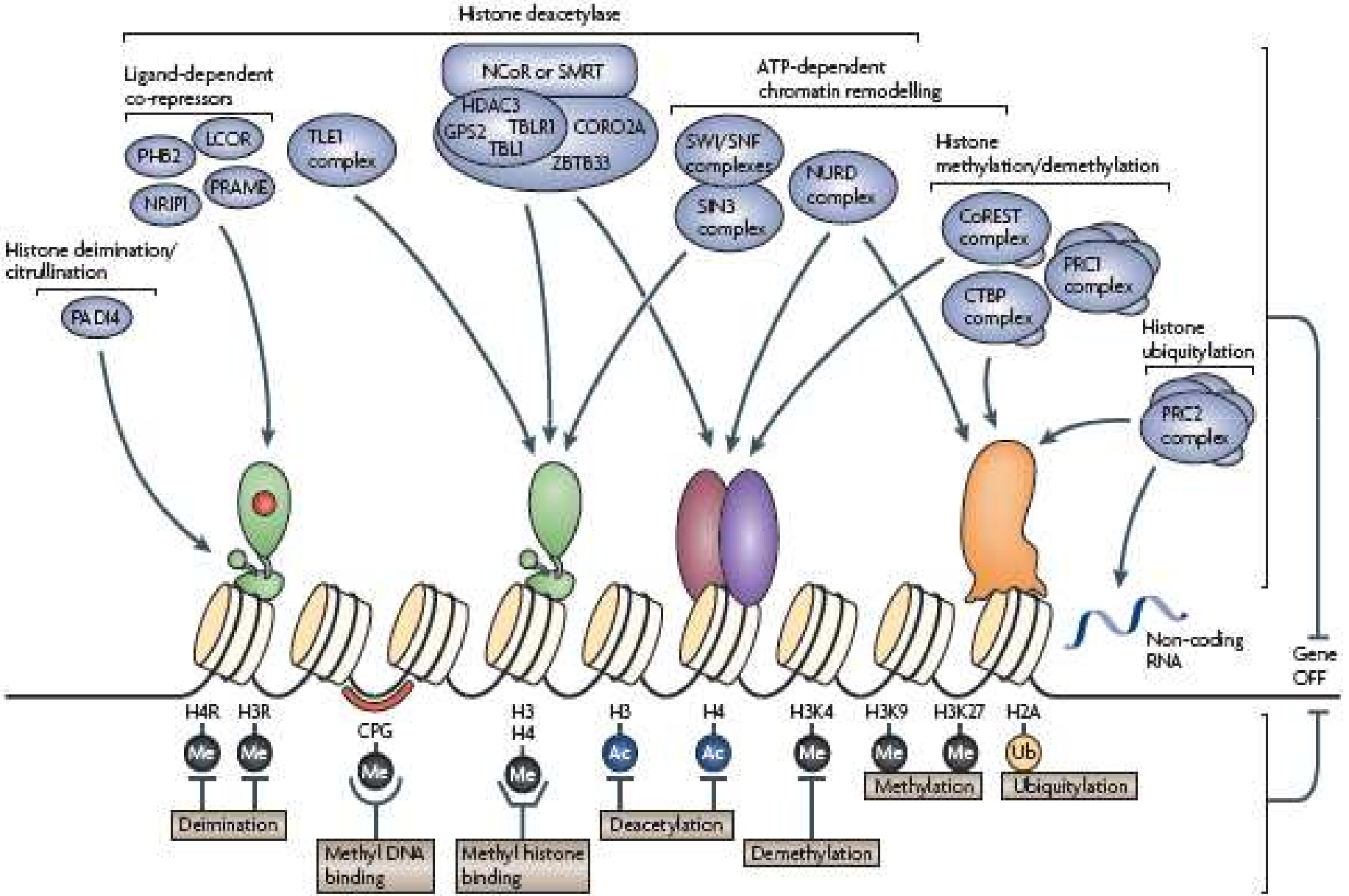

Histone deacetylase
Ligand-dependent co-repressors
PHB2
LCOR
NRIP1
PRAME
TLE1 complex
NCoR or SMRT
HDAC3
GPS2
TBLR1
TBL1
CORO2A
ZBTB33
ATP-dependent chromatin remodelling
SWI/SNF complexes
SIN3 complex
NURD complex
Histone methylation/demethylation
CoREST complex
CTBP complex
PRC1 complex
Histone deimination/ citrullination
PADI4
Histone ubiquitylation
PRC2 complex
Non-coding RNA
Gene OFF
H4R
H3R
CPG
H3
H4
H3
H4
H3K4
H3K9
H3K27
H2A
Me
Ac
Ub
Deimination
Methyl DNA binding
Methyl histone binding
Deacetylation
Demethylation
Methylation
Ubiquitylation

Figure 1 | **Co-repressor complexes and associated enzymatic activities.** A putative repressed transcription unit is represented, with various co-repressor complexes (grey). The co-repressors can be recruited by different transcription factors (green shapes with or without a red circle, which represents a ligand), including dimeric transcription factors (brown and purple ovals) and monomeric transcription factors (orange shape). The bars at the top of the figure group together complexes that carry similar enzymatic functions. At the bottom, below the nucleosomal DNA, are the histone tail modifications that are mediated by the above complexes. Also shown at the bottom of the figure is the recruitment of these complexes by specific modifications, such as DNA methylation (for example, at a methylated CpG island (red curve)) or histone tail methylation, or by non-coding RNA. CoREST, co-repressor for RE1 silencing transcription factor (also known as neural-restrictive silencing factor and RCOR1); CORO2A, coronin 2A (also known as IR10); CTBP, carboxy-terminal-binding protein; GPS2, G-protein-pathway suppressor 2; HDAC3, histone deacetylase 3; LCOR, ligand-dependent co-repressor; NCoR, nuclear receptor co-repressor (also known as NCOR1); NRIP1, nuclear receptor-interacting protein 1 (also known as RIP140); NURD, nucleosome remodelling and histone deacetylation; PADI4, peptidyl arginine deiminase type 4; PHB2, prohibitin 2; PRAME, preferentially expressed antigen in melanoma; PRC, polycomb repressive complex; SMRT, silencing mediator of retinoic acid and thyroid hormone receptor (also known as NCOR2); SWI/SNF, switch/sucrose non-fermentable; TBL1, transducin β-like 1 (also known as TBL1X); TBLR1, transducin β-like-related 1 (also known as TBL1XR1); TLE1, transducin-like enhancer of split 1; ZBTB33, zinc finger and BTB domain-containing 33 (also known as Kaiso).

Table 1 | **The components of co-repressor complexes and their associated enzymatic activities**

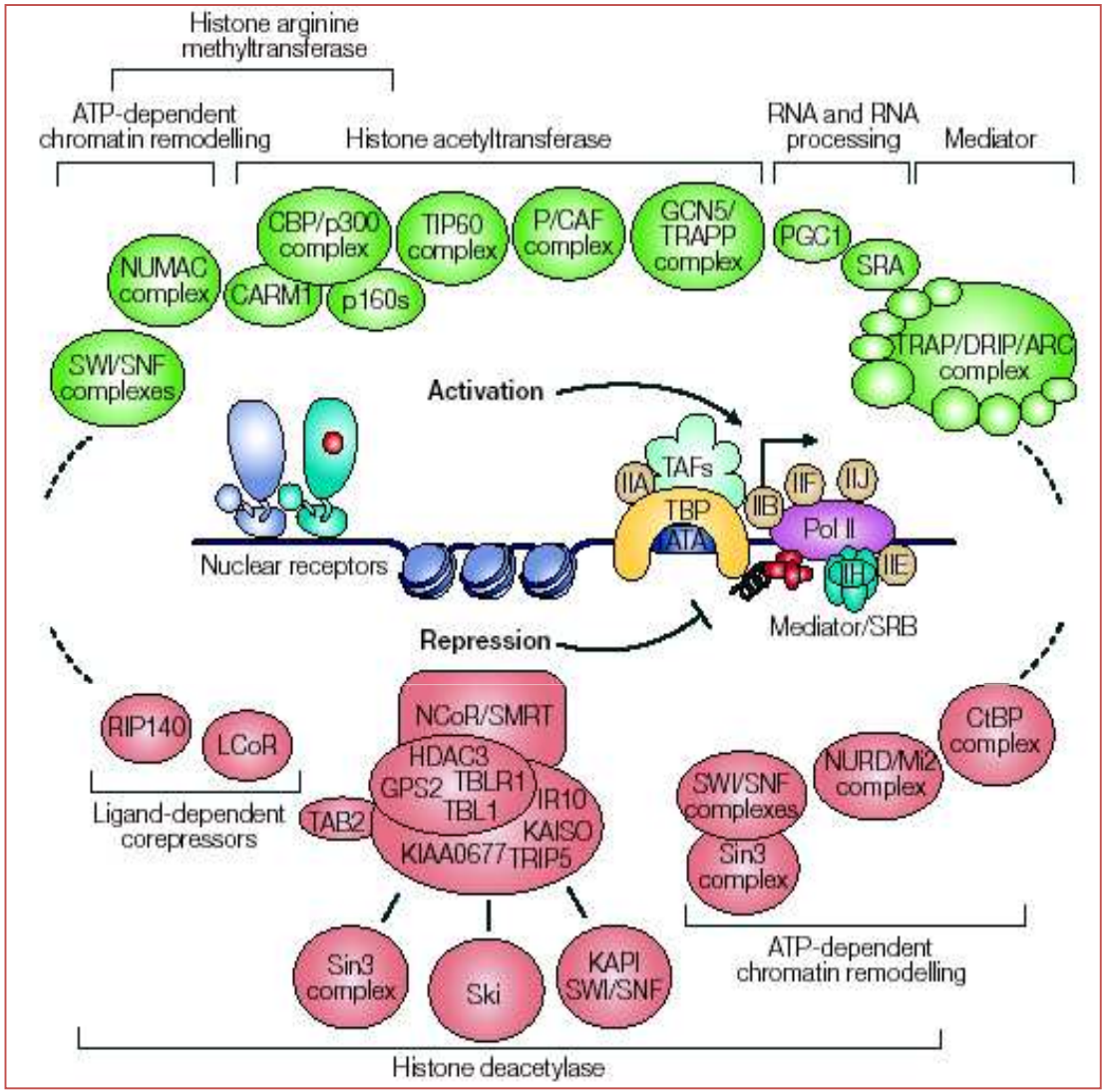
Co-repressor	Complex	Enzymatic activity/function	Refs
BAF proteins	SWI/SNF	DNA and histone binding	129,130
BMI1	PRC1	H2A ubiquitylation cofactor	131
BRG1	SWI/SNF	ATPase	129,130
CBX4	PRC1	H3K27 trimethylation	131,132
CHD4	NURD	ATPase	133
CoREST	CoREST	*	134
CORO2A	NCoR, SMRT	Actin binding	‡
CTBP1, CTBP2	CoREST	NAD-dependent dehydrogenase	134,135
EED, EZH2	PRC2	H3K27 methylation	131,132,136
GPS2	NCoR, SMRT	Kinase inhibition	‡
HDAC1, HDAC2	SIN3, NURD, CoREST	Deacetylation	24,133,137
HDAC3	NCoR, SMRT	Deacetylation	24
HDAC4–HDAC11	§	Deacetylation	22,24
KAP1	NCoR, SMRT	Histone binding/scaffold	138
LSD1	CoREST, NURD	H3K4 demethylation	134,139
MBD3	NURD	Methyl DNA binding	133,140
MTA1–MTA3	NURD	*	133
NCoR, SMRT	NCoR, SMRT	*	‡
PARP1	TLE1, CTCF	Poly-ADP ribosylation	141

continued...

...continued from previous slide.

Table 1 | **The components of co-repressor complexes and their associated enzymatic activities**

Co-repressor	Complex	Enzymatic activity/function	Refs
NCoR, SMRT	NCoR, SMRT	*	‡
PARP1	TLE1, CTCF	Poly-ADP ribosylation	141
PHC1, PHC2	PRC1	*	131,132
RBAP46, RBAP48	SIN3	H4 binding	142
RING1	PRC1	H2AK119 ubiquitylation	131,132
SAP18	SIN3	*	142
SAP30	SIN3	DNA binding/bending	142
SIN3	SIN3, CoREST	*	137,142
SIRT1–SIRT5	§	NAD-dependent deacetylation	21,143
SUZ12	PRC2	H3K27 methylation stimulation	131,132,136
TAB2	NCoR, SMRT	Ubiquitin binding/shuttling	‡
TBL1, TBLR1	NCoR, SMRT	Ubiquitylation	‡
TLE1–TLE5	TLE	Histone binding/oligomerization	12
ZBTB33	NCoR	Methyl DNA binding	140



Coactivator and corepressor complexes are required for nuclear receptor-mediated transcriptional regulation. The regulation of a general transcription unit by nuclear receptors requires a vast number of co-regulatory complexes that have various functions and enzymatic activities. Coactivator complexes (green) include factors that contain ATP-dependent chromatin remodelling activity, histone arginine methyltransferases, histone acetyl-transferases9, as well as factors that are involved in RNA processing and components of the so-called Mediator complex that mediate the interaction with the RNA polymerase II (pol II) machinery. Conversely, corepressors (red) include ATP-dependent chromatin remodelling complexes, basal corepressors, such as NCoR and SMRT, which function as platforms for the recruitment of several subcomplexes that often contain histone deacetylase activity and specific corepressors, such as LCoR and RIP140, which are surprisingly able to recruit general corepressors on ligand induction. This schematic representation is useful to underline the numerous regulatory complexes that are involved in nuclear-receptor mediated regulation; however, it is important to keep in mind that it does not illustrate the dynamics of their recruitment to the regulated transcription unit (see FIG. 3). IIA, IIB, IIE, IIF, IIH, IIJ, general transcription factors A, B, E, F, H, J; HDAC, histone deacetylase; LCoR, ligand-dependent nuclear-receptor corepressor; NCoR, nuclear-receptor corepressor; RIP140, receptor-interacting protein-140; SMRT, silencing mediator of retinoic acid and thyroid hormone receptors; TAF, TBP-associated factor; TBP, TATA-binding protein.

(From: Perissi & Rosenfeld, Nat. Rev. Mol. Cell Biol., 2005, 6:543-554.

This model was finely worked out since it was discovered that a class of pharmacological **antagonists** of steroid hormones, called SRMs, make receptors simulating the empty status of strict NRs such as RAR or TR.

In fact, when one of such molecules occupies the ligand binding site in place of hormone, then the receptor interact preferably with corepressors instead than with coactivators

This was lead discovered working with Tamoxifen and Estrogen Receptor....

Binding of antagonists induce a specific conformational change

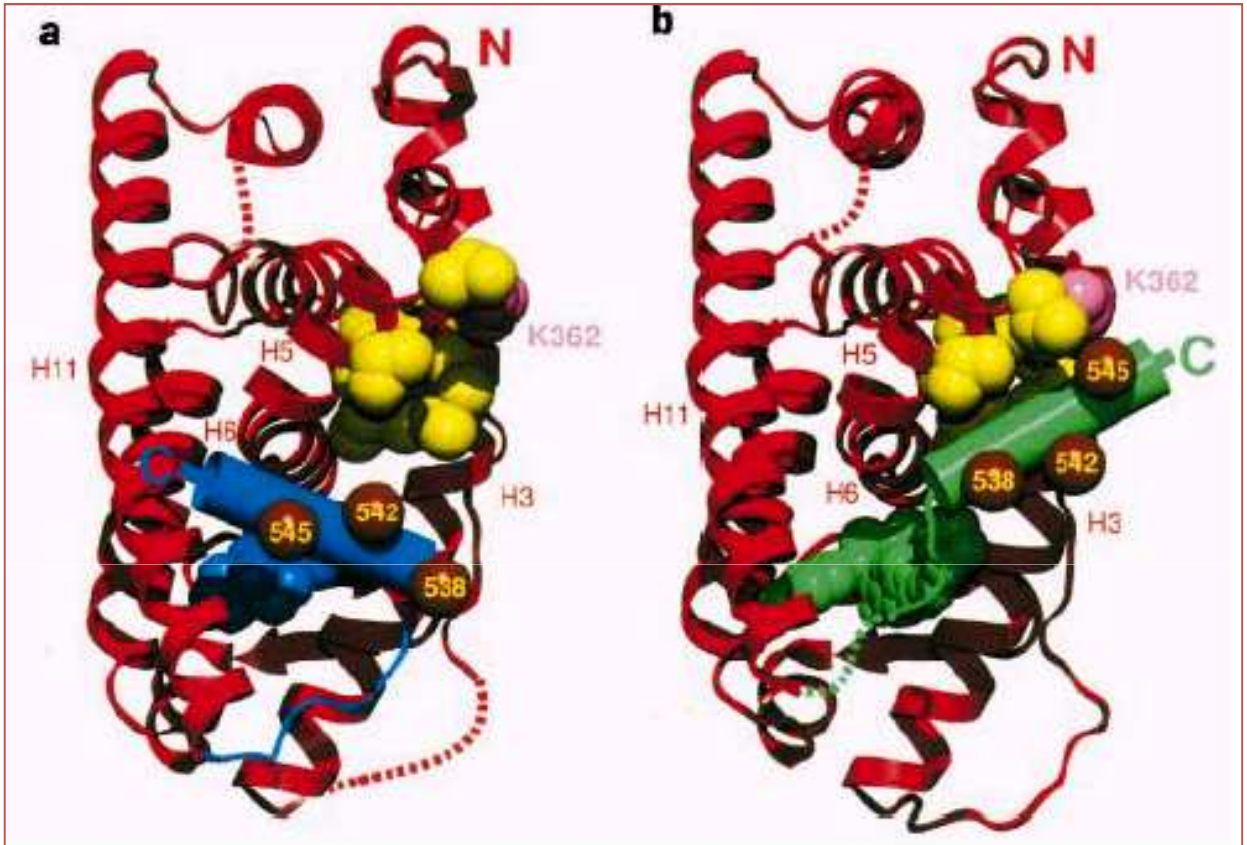
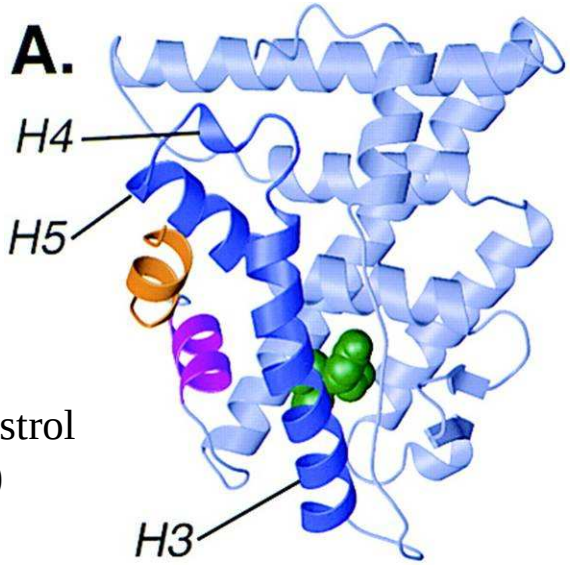


Figure 3 Positioning of helix H12. Position is shown in **a**, the ER LBD-E_2 complex; and **b**, the ER LBD-RAL complex. H12 is drawn as a cylinder and coloured blue (E_2 complex) or green (RAL complex). The remainder of the ER LBD is shown in red. Dotted lines indicate unmodelled regions of the structures. Hydrophobic residues located in the groove between H3 and H5 (yellow) and Lys 362 (K362, pink) are depicted in space-filling form. The locations of Asp 538, Glu 542 and Asp 545 are highlighted (brown spheres) along with the helices that interact with H12 in the two complexes.

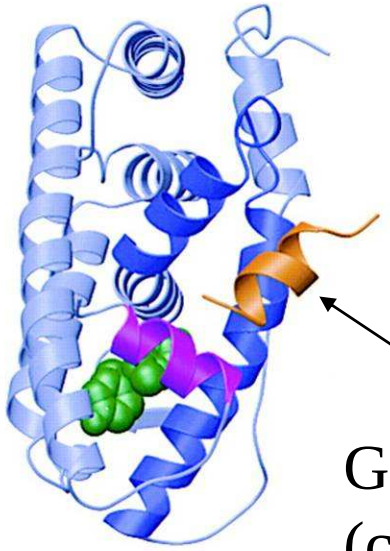
Cn3D RAL

Cn3D E2

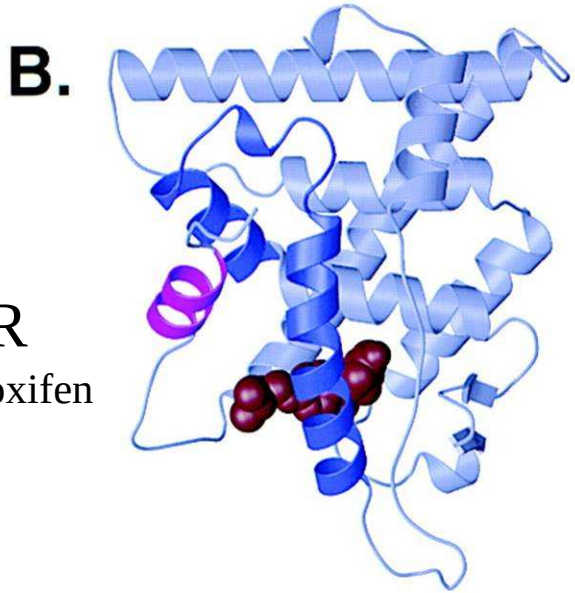
Brzozowski et al., 1997, Nature 389: 753.



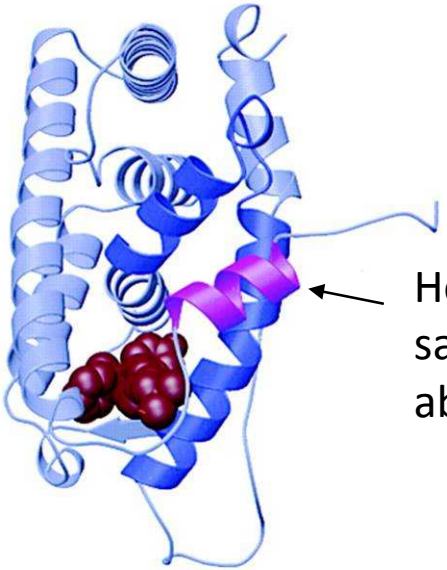
DES-ER
(diethylstilbestrol
is estrogenic)



GRIP NR box II
(coactivator LxxLL)



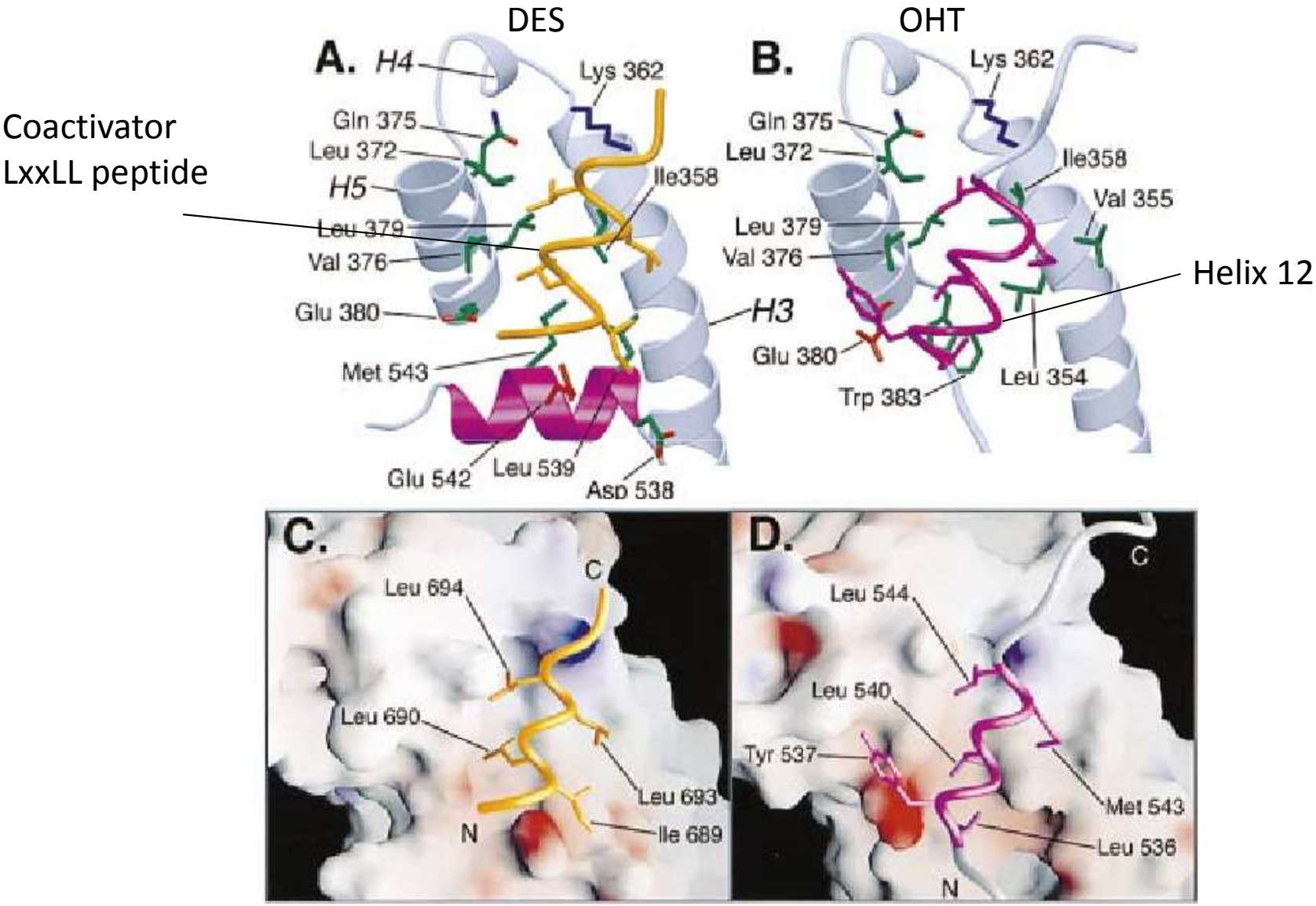
OHT-ER
4-OH-Tamoxifen



Helix 12 occupies the same position as LxxLL above

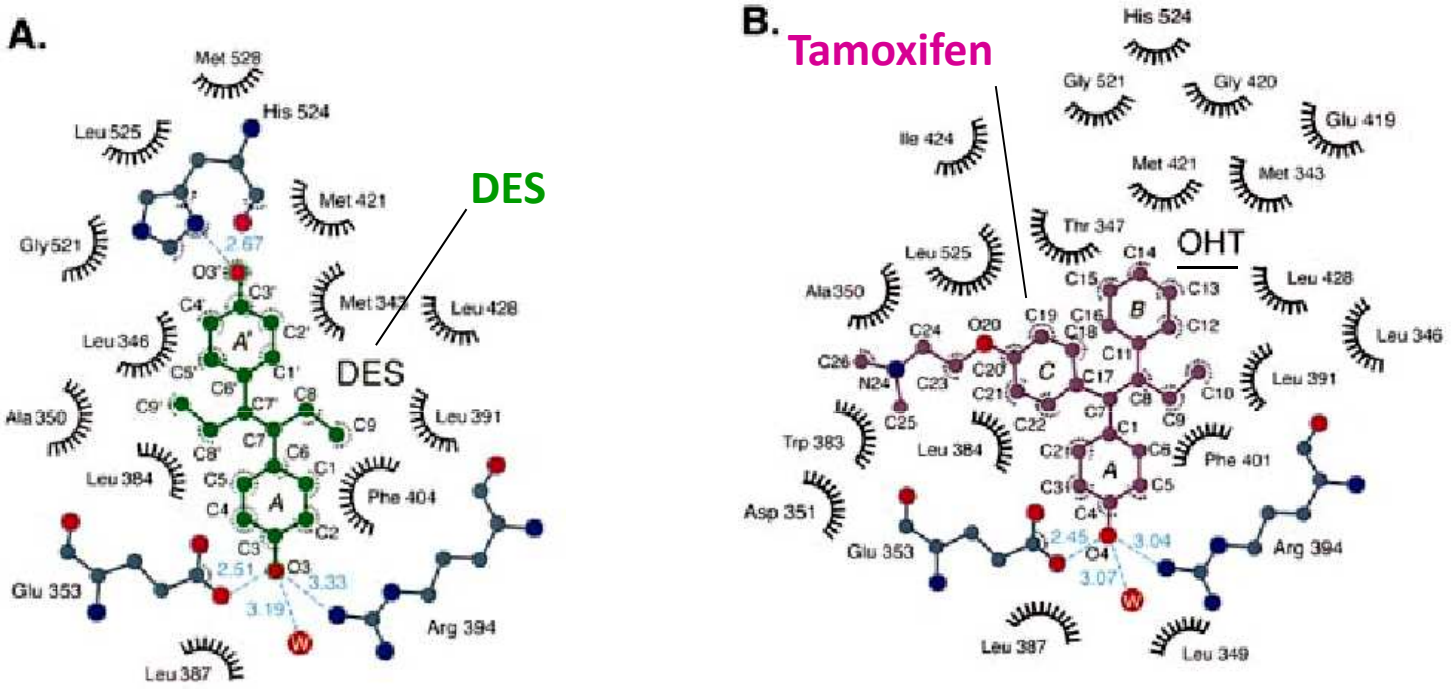
from Shiau et al., 1998, Cell 95: 927.

In the antagonist-bound structure, helix 12 occupies the position that is normally taken by the coactivator LxxLL motif



Shiau et al., 1998, Cell 95: 927.

The hydrophobic ligand binidng pocket of ER



Tamoxifen (right) has a hydrophobic chain that pushes away aa.aa. Ala350 and others, repositioning Helix 12.

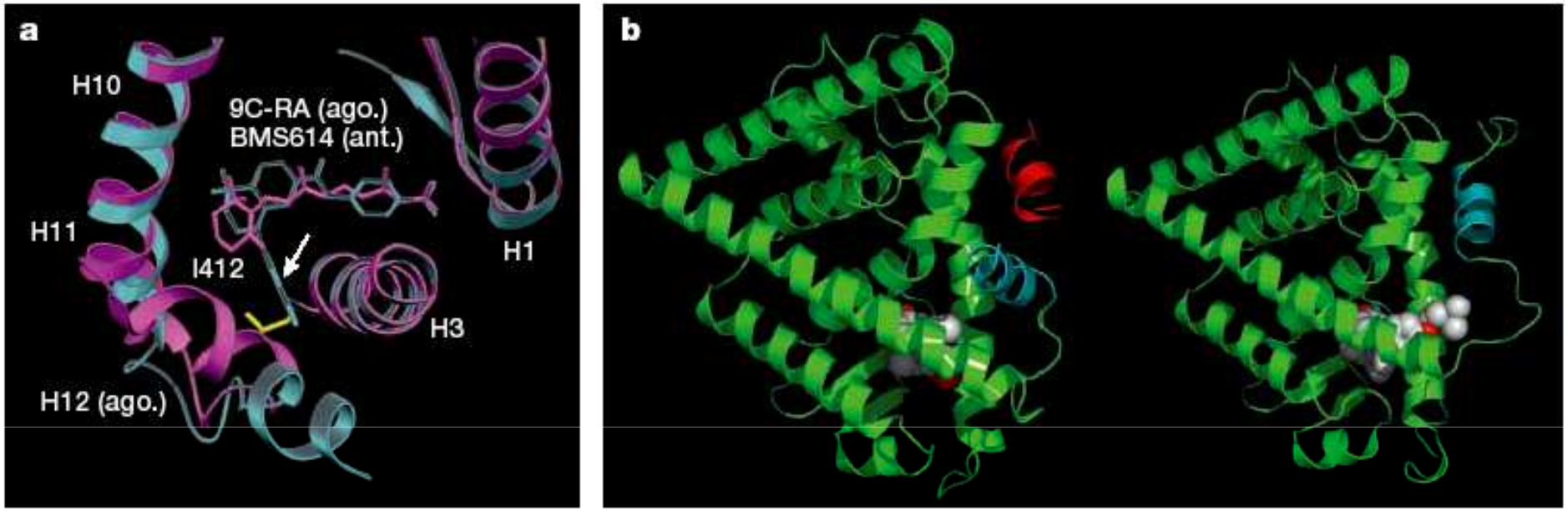


Figure 4 | **Structural basis of antagonist action. a** | Superposition of the ligand-binding sites in the complex between retinoic acid receptor-α (RARα) and the RARα-selective antagonist BMS649 (grey; PDB: 1dkf) and the RARγ-retinoic acid complex (pink; PDB: 2lbd). The superposition illustrates the steric clash between the antagonist BMS614 (white arrow) and Ile412 (yellow) of the receptor. **b** | Depiction of the human ERα ligand-binding domain complexed with the agonist diethyl stilbesterol (left; PDB: 3erd) and 4-hydroxy-tamoxifen (right; PDB: 3ert). The protein is depicted in green, except for helix 12, which is coloured cyan and the GRIP co-activator peptide, which is coloured red. Ligand atoms are coloured white (carbon), red (oxygen) and blue (nitrogen). This figure was created with PyMol molecular graphics system.

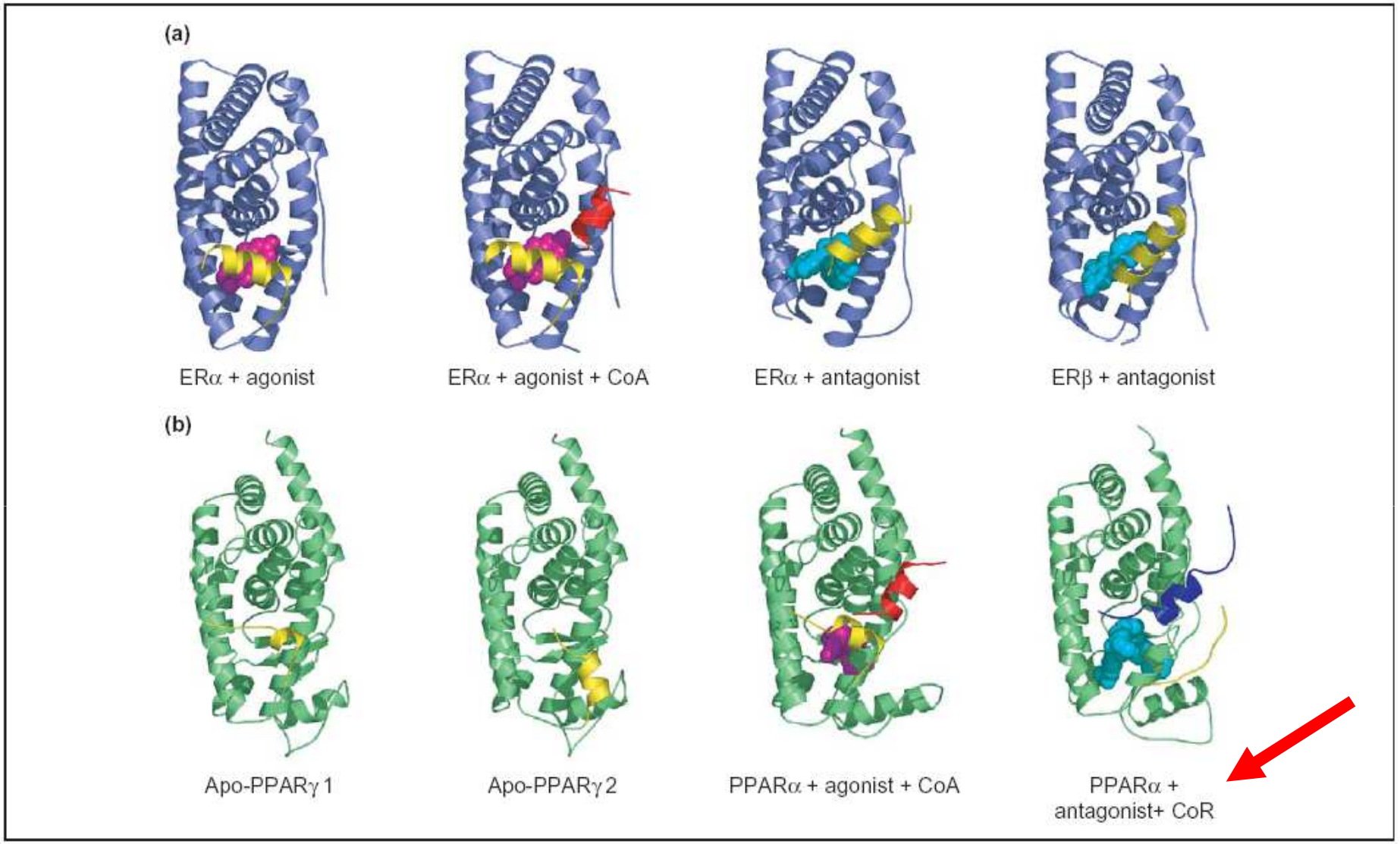
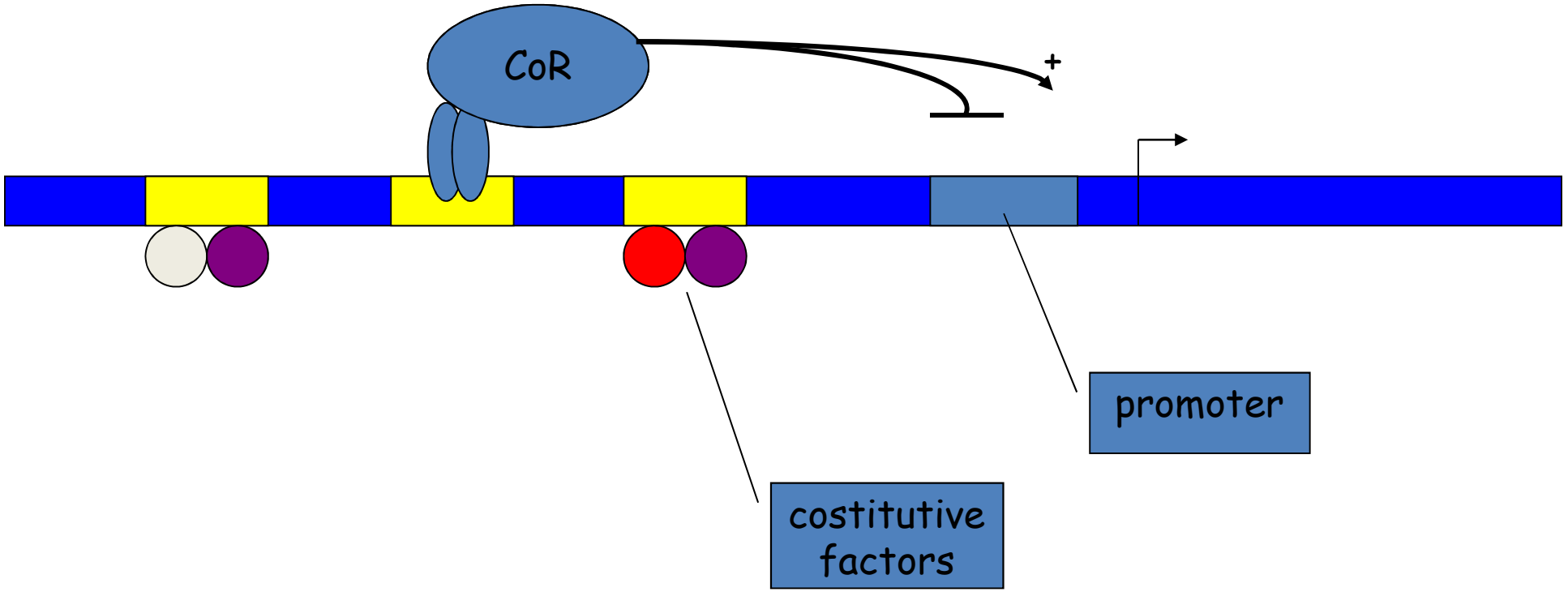


Figure 2. Effect of ligands and co-regulators on the position of helix 12. The top row of structures shows the oestrogen receptor (ER) bound to agonists and antagonists (PDB codes 1ERE, 1ERR, 1GWR and 1QKM) [19,63]. Note that in the antagonist-bound structures helix 12 adopts a position very similar to that of the co-activator in the agonist-bound structure. The bottom row of structures shows peroxisome proliferator-activated receptors (PPARs) in the absence of ligand (left two structures; PDB code 1PRG), bound to agonist and co-activator (PDB code 2PRG) and to antagonist and co-repressor (PDB code 1KKQ) [20,49]. Note that in the absence of ligand, helix 12 can adopt both the active position and an alternative position. In the presence of antagonist and co-repressor, helix 12 unravels. ERs and PPARs are shown in light blue and green, respectively; helix 12 in yellow; agonists and antagonists in magenta and cyan, respectively; and co-activator and co-repressor peptides in red and dark blue, respectively.

On-off genes:

A corepressor-coactivator exchange takes place under activation



First question: in view of the number of proteins found in all corepressor/coactivator complexes,

are all these proteins contemporaneously present at the same promoters?

Cell, Vol. 115, 751–763, December 12, 2003, Copyright ©2003 by Cell Press

Estrogen Receptor-α Directs Ordered, Cyclical, and Combinatorial Recruitment of Cofactors on a Natural Target Promoter

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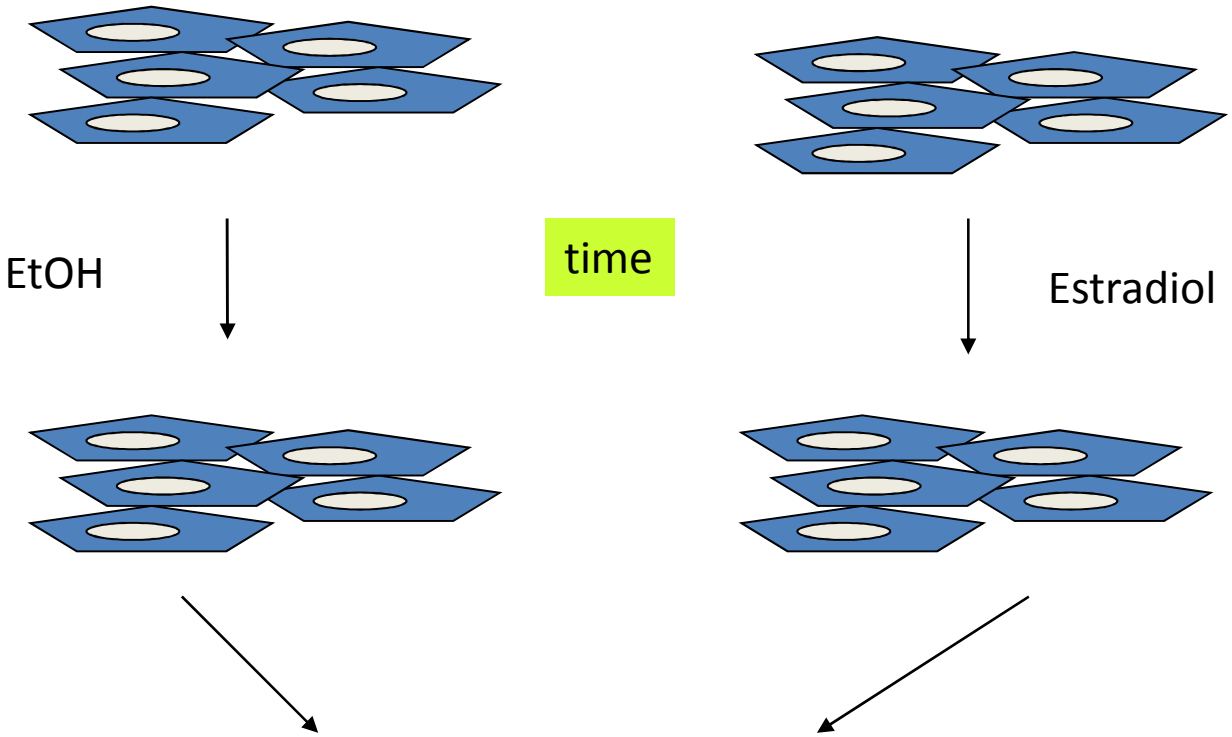
D-69117 Heidelberg
Germany

Summary

Transcriptional activation of a gene involves an orchestrated recruitment of components of the basal transcription machinery and intermediate factors, concomitant with an alteration in local chromatin structure generated by posttranslational modifications of histone tails and nucleosome remodeling. We provide here a comprehensive picture of events resulting in transcriptional activation of a gene, through evaluating the estrogen receptor-α (NR3A1) target pS2 gene promoter in MCF-7 cells. This description integrates chromatin remodeling with a kinetic evaluation of cyclical networks of association of 46 transcription factors with the promoter, as determined by chromatin immunoprecipitation assays. We define the concept of a "transcriptional clock" that directs and achieves the sequential and combinatorial assembly of a transcriptionally productive complex on a promoter. Furthermore, the unanticipated findings of key roles for histone deacetylases and nucleosome-remodeling complexes in limiting transcription implies that transcriptional activation is a cyclical process that requires both activating and repressive epigenetic processes.



Formaldehyde fix., chromatin extr., Immunoprecipitation with several antibodies against coregulator proteins, PCR one target gene (pS2)

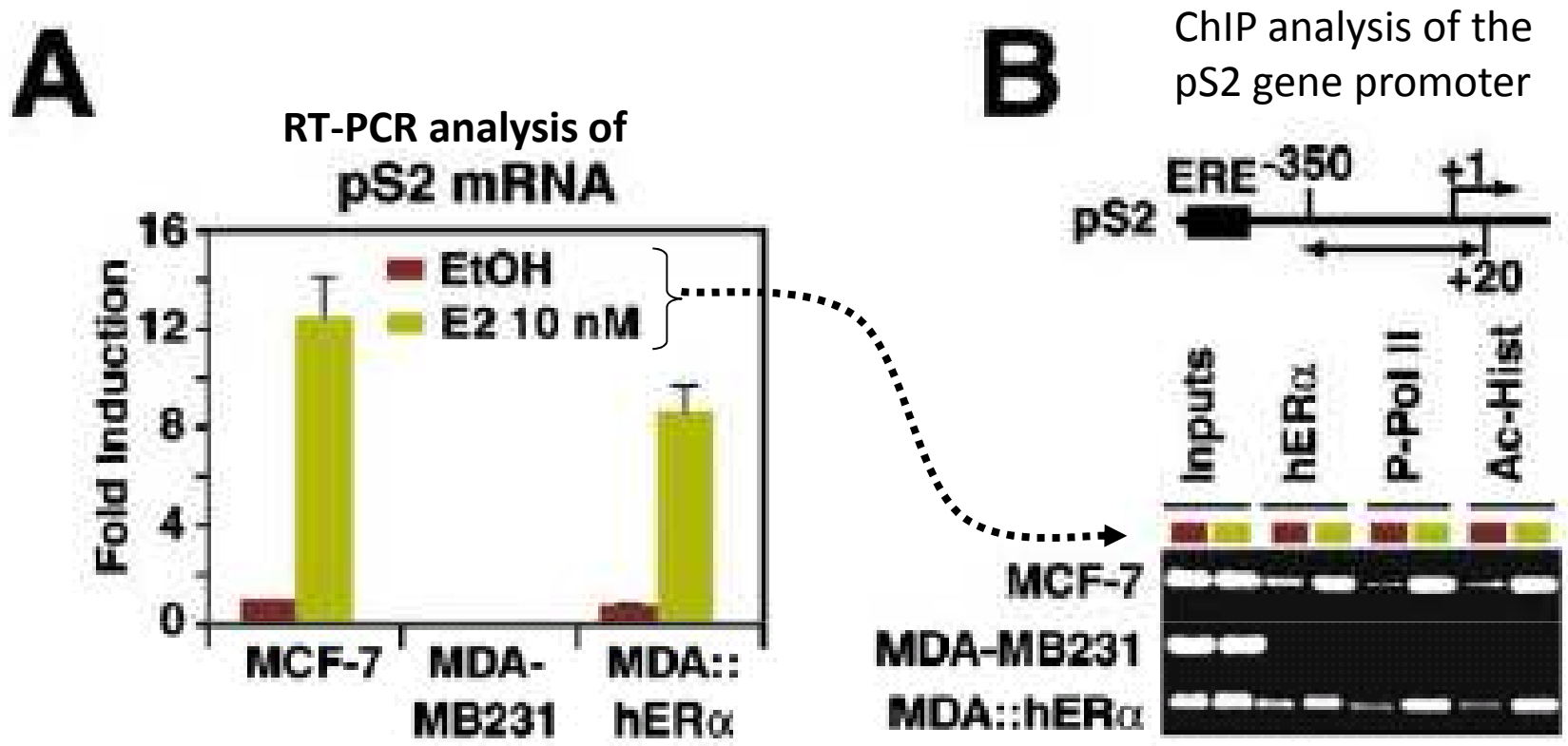


Figure 1. In Vivo Identification of the Transcription Factors Involved in pS2 Gene Activity

(A) The expression of the pS2 gene was monitored by real-time PCR on reverse-transcribed mRNA from hER positive (MCF-7) or negative (MDA-MB231) cells or MDA-MB231 cells stably expressing hER. After 52 hours of culture in stripped-media, cells were treated for **3 hr** with 10 nM Estradiol (E2) or ethanol (EtOH) as vehicle control. The pS2 mRNA levels were normalized against invariant GAPDH mRNA, as measured by real-time PCRs.

(B) (B) Chromatin immunoprecipitations (ChIP) determining the recruitment of hER, Phosphorylated Pol II (P-Pol II) and acetylated histones (Ac-Hist) to the pS2 promoter after 3 hr treatment with 10 nM E2 or EtOH.

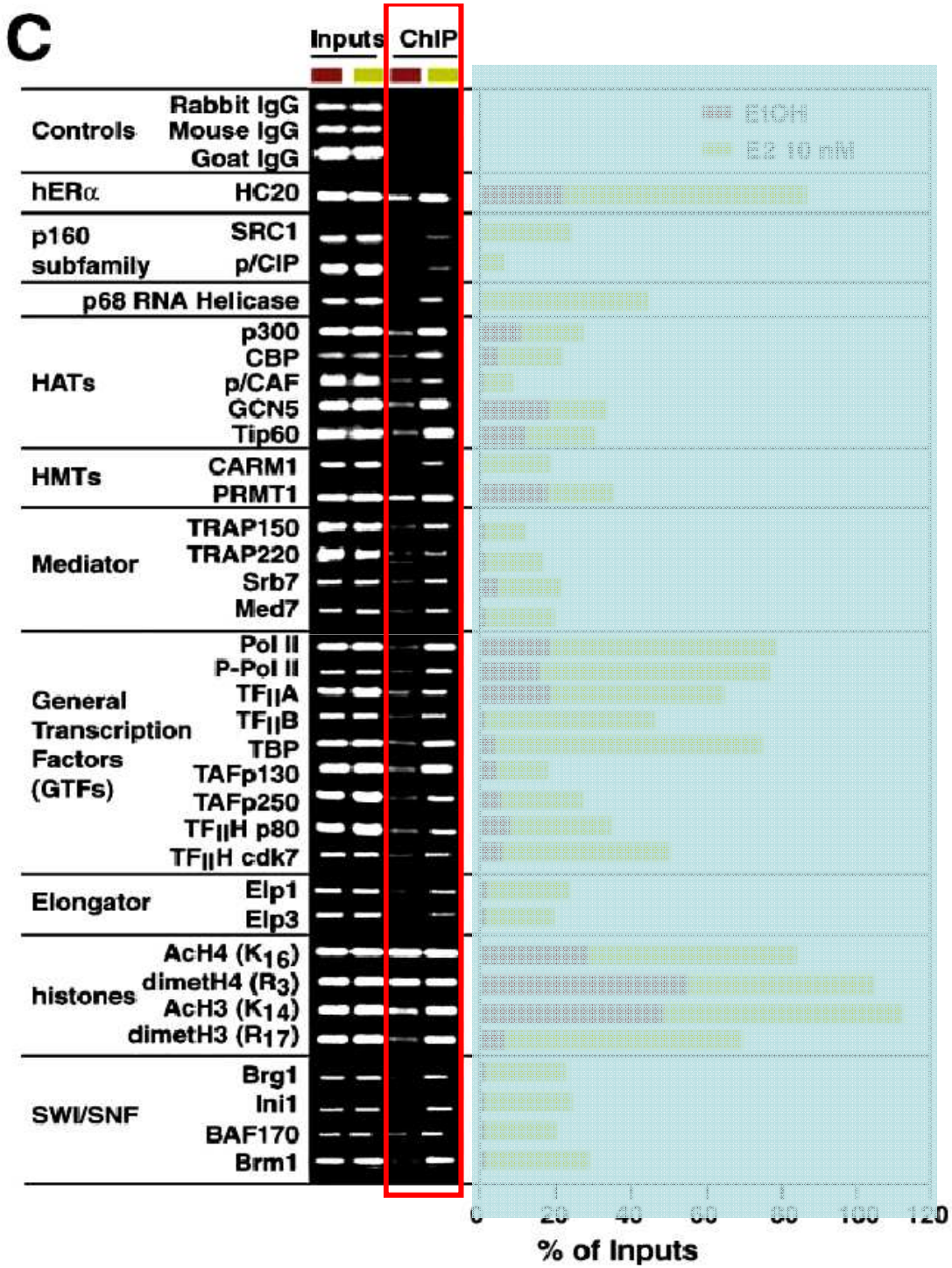


Figure 1. In Vivo Identification of the Transcription Factors Involved in pS2 Gene Activity

(C) ChIP experiments performed using chromatin prepared from untreated or **2 hr** 10 nME2-treated cells, quantified by real-time PCR (values in the right hand image).

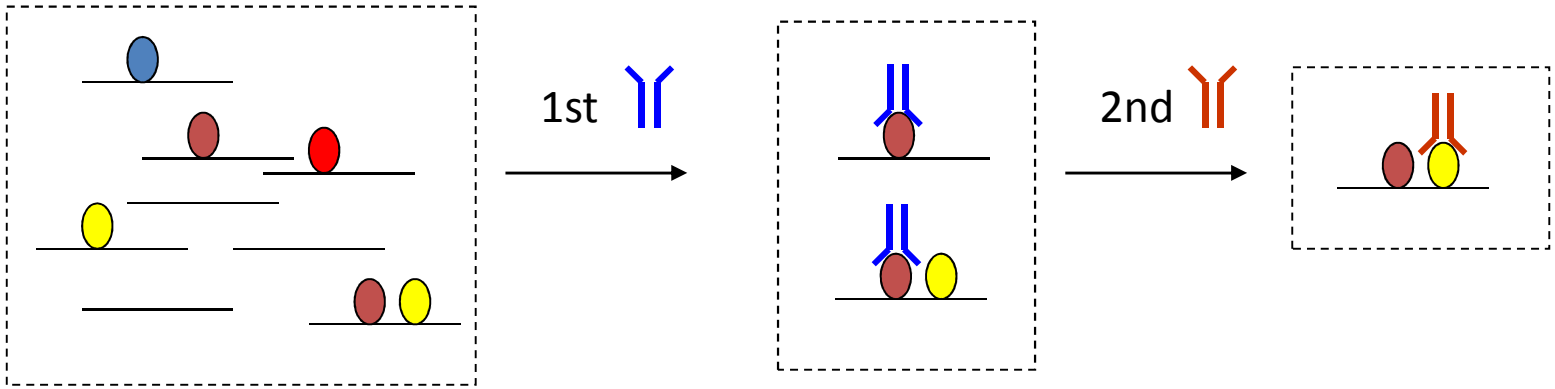
Are **all** these proteins present at the **same** gene promoter at the **same** time in the **same** cell ?

Are they present in different cells in the same population ?

Are they present at different times, i.e. is there a dynamics of these factors ?

To answer this question: the **Re-ChIP assay.**

The chromatin is ChIPped, then it is re-precipitated using a second antibody



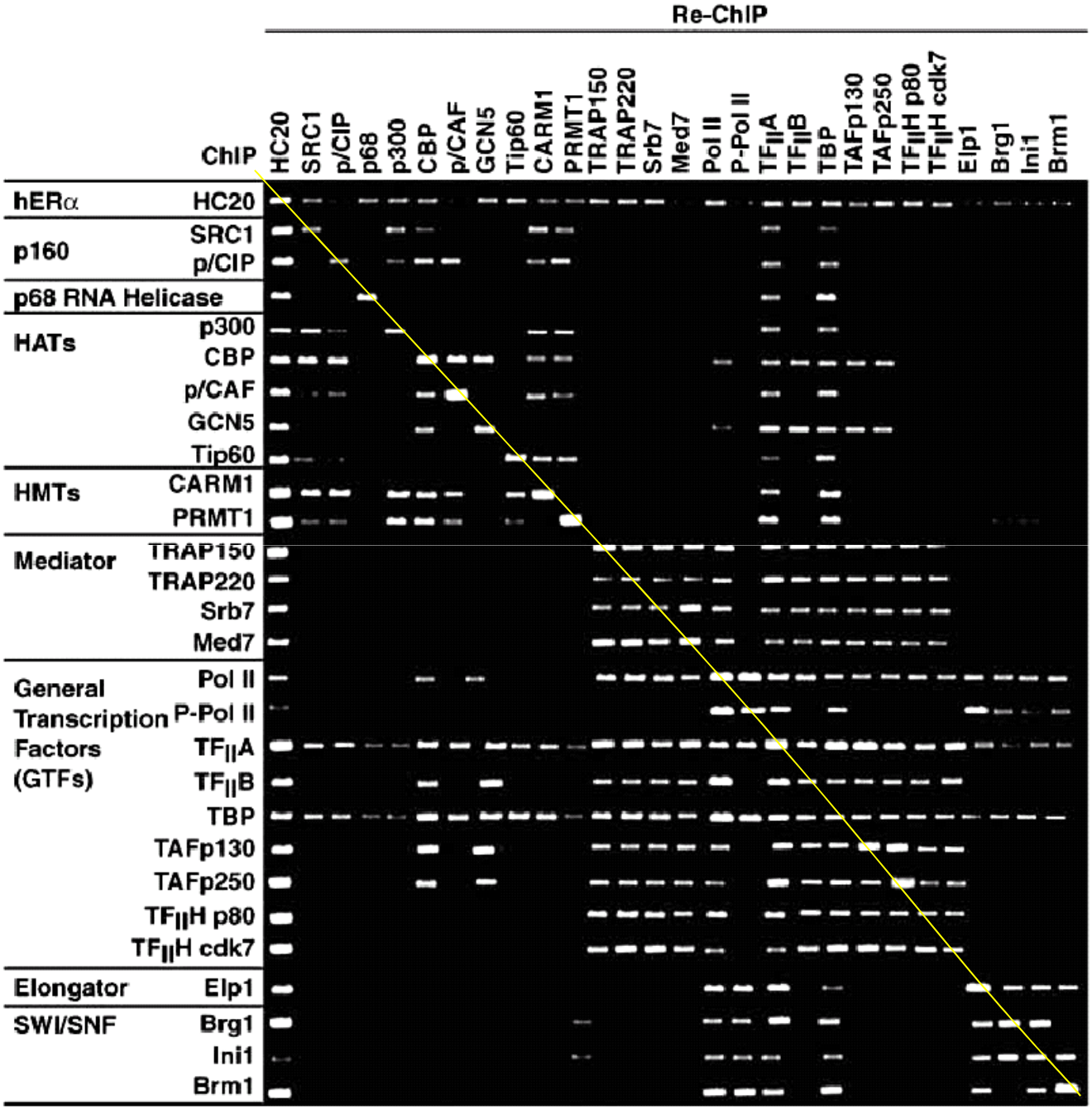
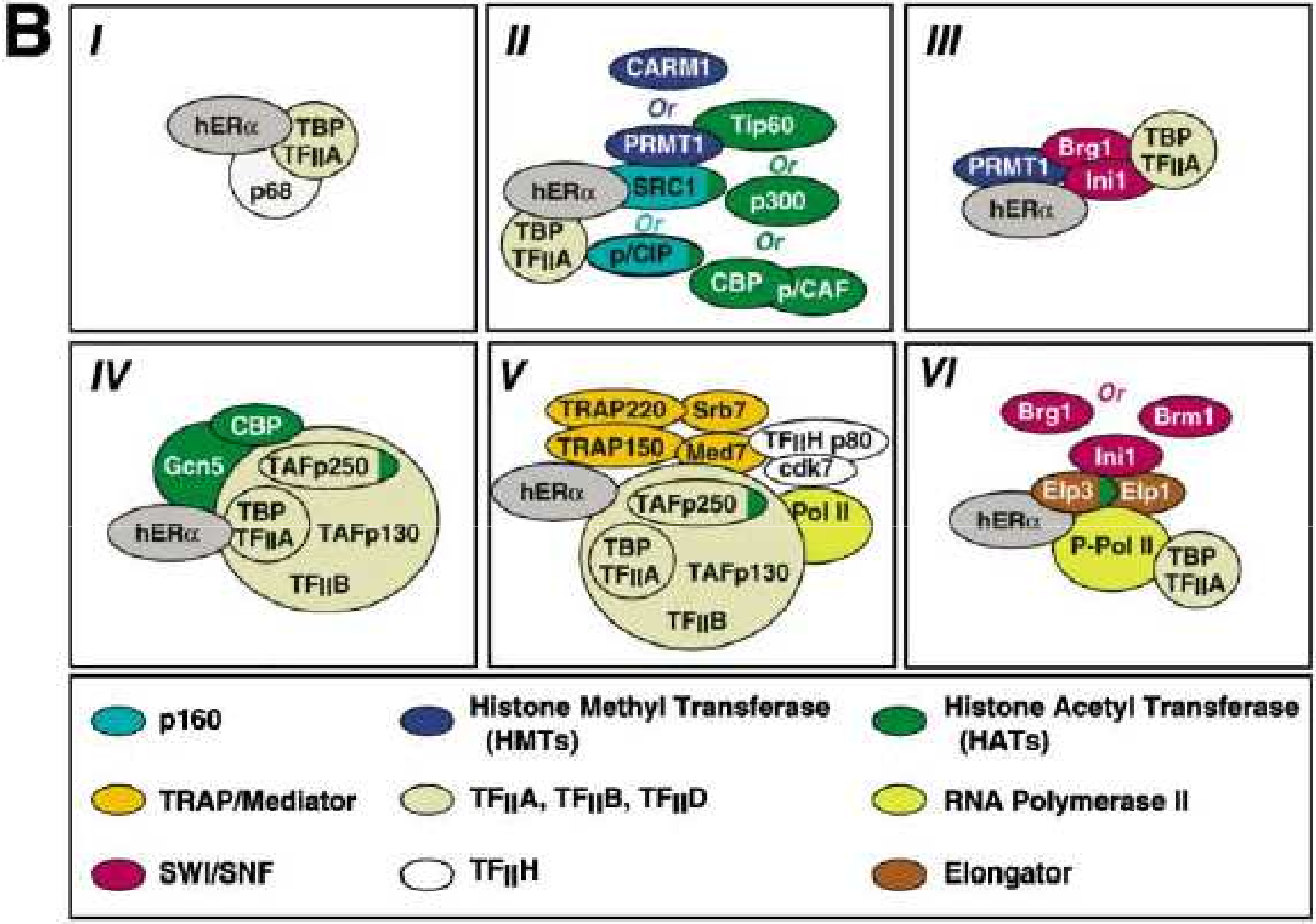


Figure 2. Re-ChIP Screening of the Factors Recruited to the pS2 Promoter

(A) Chromatin prepared from cells treated for 3 hr with 10 nM Estradiol (E2) was subjected to the ChIP procedure with the antibodies shown at the left side, and again immunoprecipitated using antibodies shown at the top of the image.



(B) Scheme illustrating the complexes indicated through the comprehensive Re-ChIP analysis of transcription factors on the pS2 promoter.

Conclusions:

Discrete complexes are present at a given gene promoter, in a mutually exclusive way.

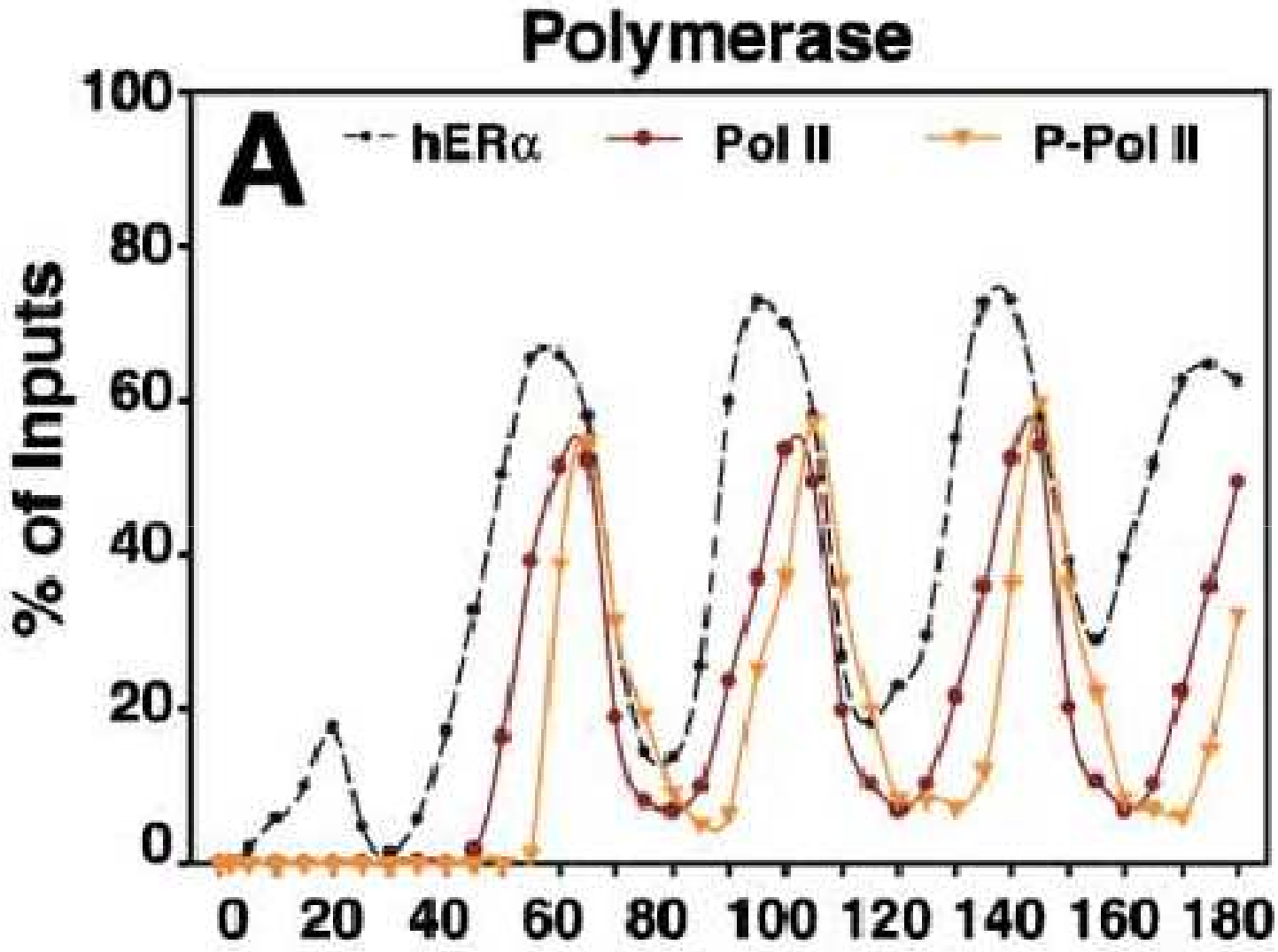
…still

-Are they in different cells of the same population ?

Or

Are they present at different times? i.e. are they dynamically cycling in a timely orderer fashion ?

To measure this, we need **synchronized** transcription in response to the stimulus,



Minuti dopo estradiolo

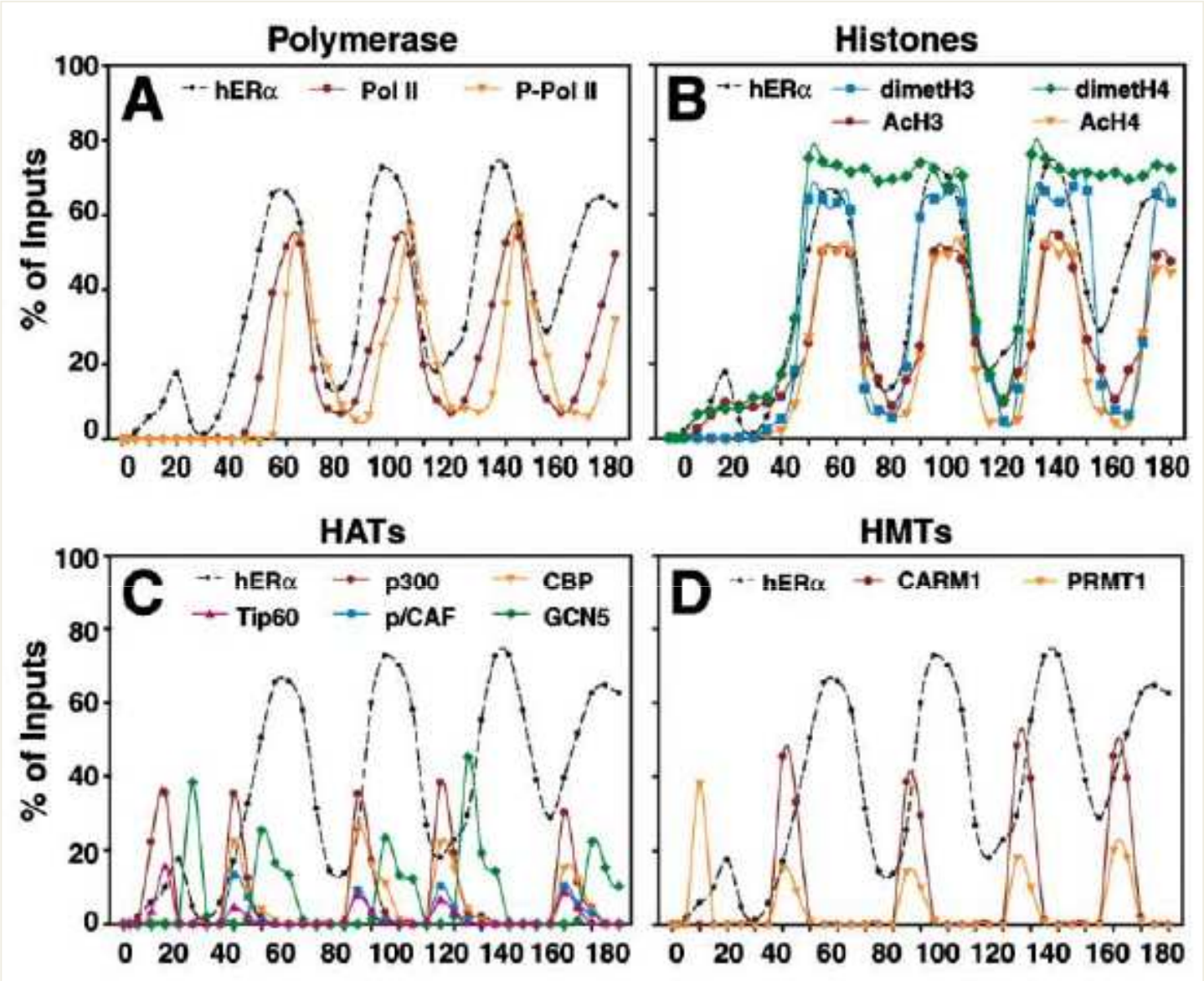
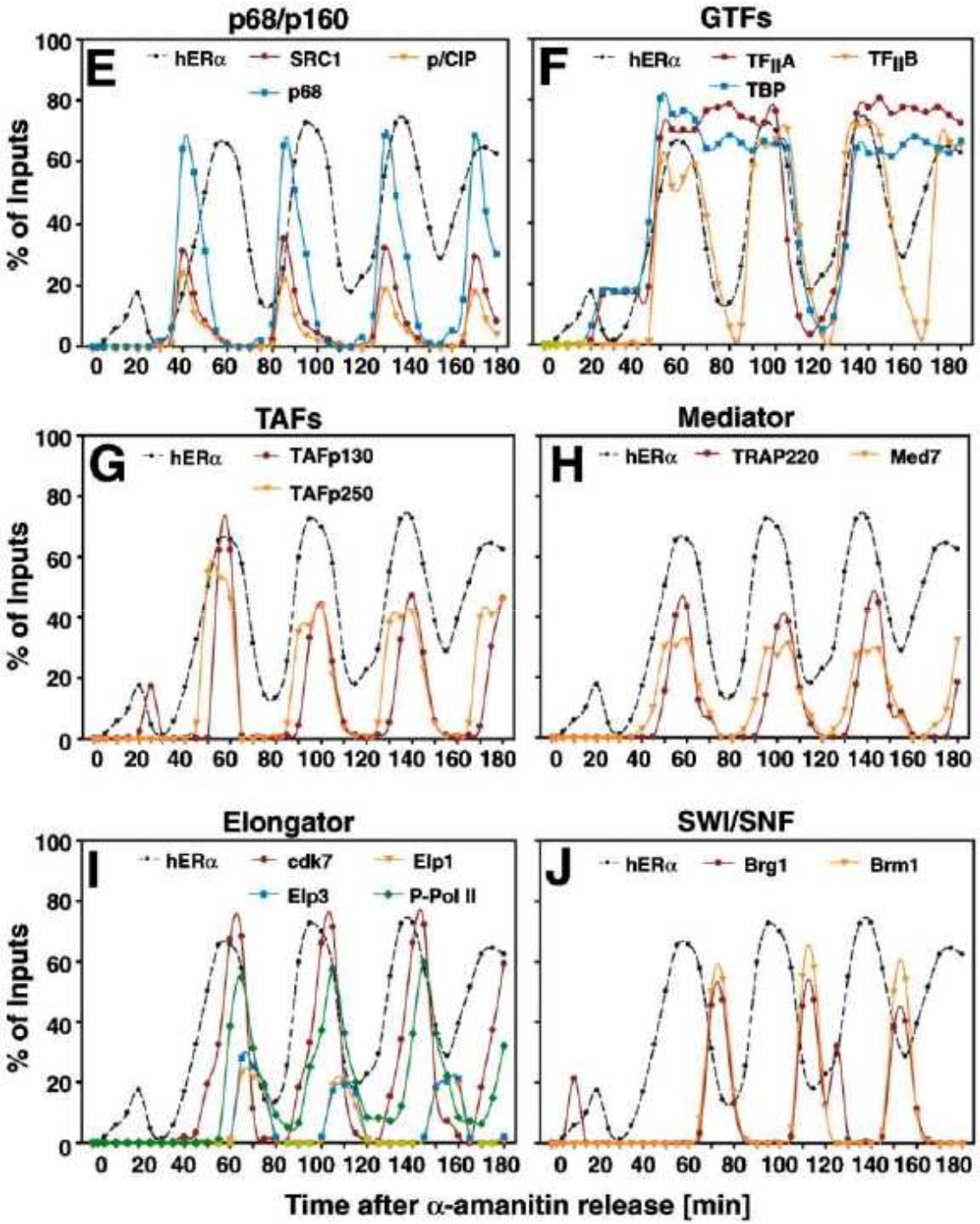


Figure 3. Dynamics of Cofactor Recruitment Directed by E2-Liganded hER on the pS2 Promoter. Kinetic ChIP experiments were performed using specified antibodies as shown within the images. Chromatin was prepared on sampled cells at 5 minutes intervals. The amount of immunoprecipitated pS2 promoter was quantified by real-time PCR. Values, expressed as % of the inputs, are the mean of three separate experiments, and have a SD 2%. All ChIP were performed from a single chromatin preparation for each time point.


p68/p160
GTFs
E
hERα
SRC1
p/CIP
p68
F
hERα
TFIIA
TFIIB
TBP
TAFs
Mediator
G
hERα
TAFp130
TAFp250
H
hERα
TRAP220
Med7
Elongator
SWI/SNF
I
hERα
cdk7
Elp1
Elp3
P-Pol II
J
hERα
Brg1
Brm1
% of Inputs
0 20 40 60 80 100
0 20 40 60 80 100 120 140 160 180
Time after α-amanitin release [min]

No corepressor

Proteasome 20S component

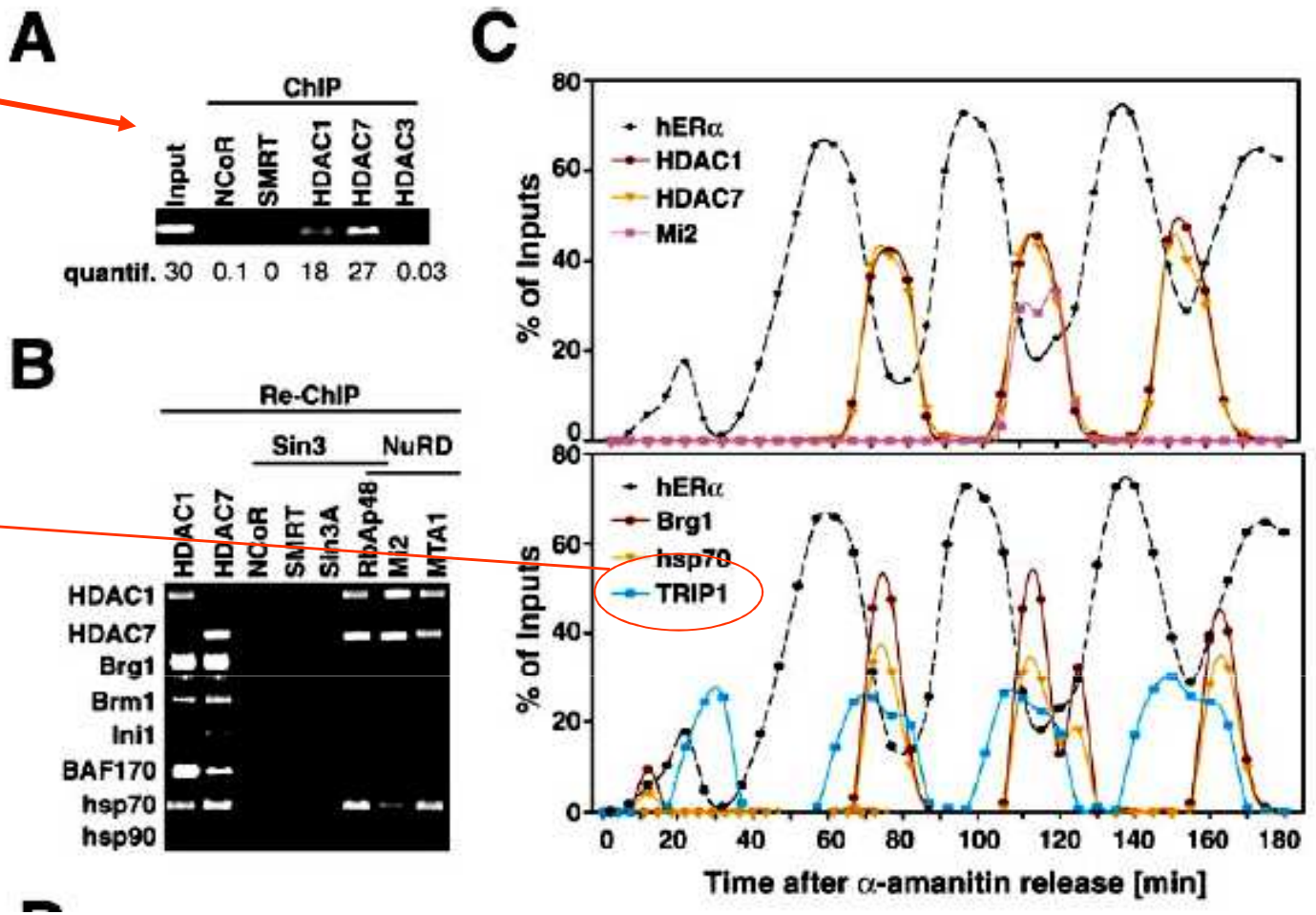


Figure 5. Recruitment of "Repressive Complexes," hsp and **Proteasome Subunits** on Active pS2 Promoter

(A) ChIP assays performed on chromatin prepared from cells treated with 10 nM E2 for 3 hr.

(B) Re-ChIP analysis: Chromatin prepared from cells treated for 3 hr with 10 nM E2 was subject to ChIP with antibodies shown at the left side, and then purified complexes were again subject to ChIP using antibodies shown at the top of the image.

(C) Kinetic ChIP experiments on chromatin prepared from cells after a-amanitin treatment and following 10 nM E2 treatment. Results are expressed as in Figure 2.

Ubiquitinazione e proteasoma sono analogamente coinvolti anche nello scambio corepressore – coattivatore durante l'attivazione in situazioni "on-off".

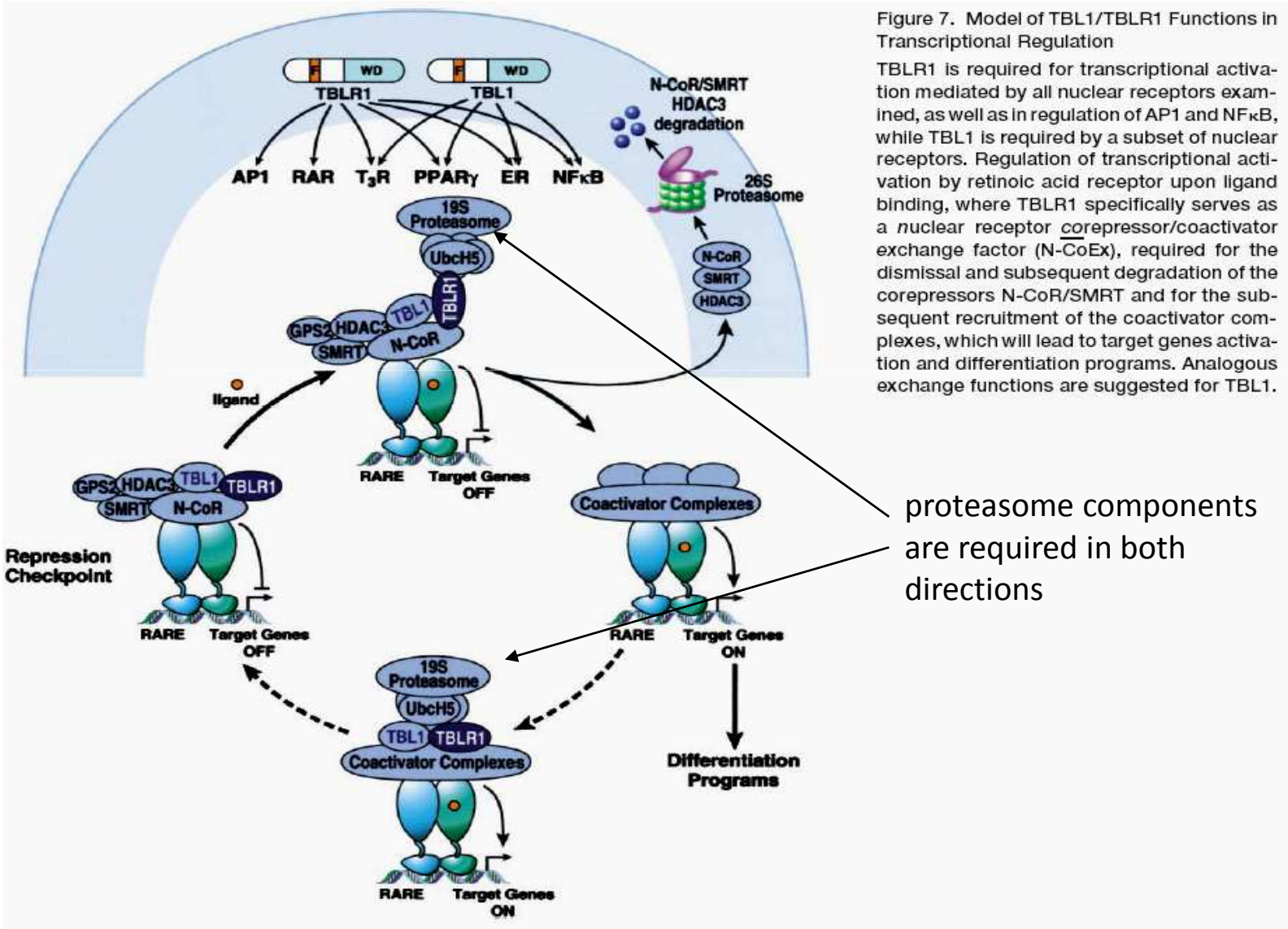


Figure 7. Model of TBL1/TBLR1 Functions in Transcriptional Regulation

TBLR1 is required for transcriptional activation mediated by all nuclear receptors examined, as well as in regulation of AP1 and NFκB, while TBL1 is required by a subset of nuclear receptors. Regulation of transcriptional activation by retinoic acid receptor upon ligand binding, where TBLR1 specifically serves as a *n*uclear receptor *co*repressor/coactivator exchange factor (N-CoEx), required for the dismissal and subsequent degradation of the corepressors N-CoR/SMRT and for the subsequent recruitment of the coactivator complexes, which will lead to target genes activation and differentiation programs. Analogous exchange functions are suggested for TBL1.

proteasome components are required in both directions

Second question: in view of the number of proteins found in all corepressor/coactivator complexes,

why are all these proteins needed to regulate transcription ?

Many components of coactivator and corepressor complexes mediate responses to other signaling pathways

CONTROLLING NUCLEAR RECEPTORS: THE CIRCULAR LOGIC OF COFACTOR CYCLES

Valentina Perissi and Michael G. Rosenfeld

Abstract | Nuclear receptors regulate many biologically important processes in development and homeostasis by their bimodal function as repressors and activators of gene transcription. A finely tuned modulation of the transcriptional activities of nuclear receptors is crucial for determining highly specific and diversified programmes of gene expression. Recent studies have provided insights into the molecular mechanisms that are required to switch between repression and activation functions, the combinatorial roles of the multiple cofactor complexes that are required for mediating transcriptional regulation, and the central question of how several different signalling pathways can be integrated at the nuclear level to achieve specific profiles of gene expression.

Review

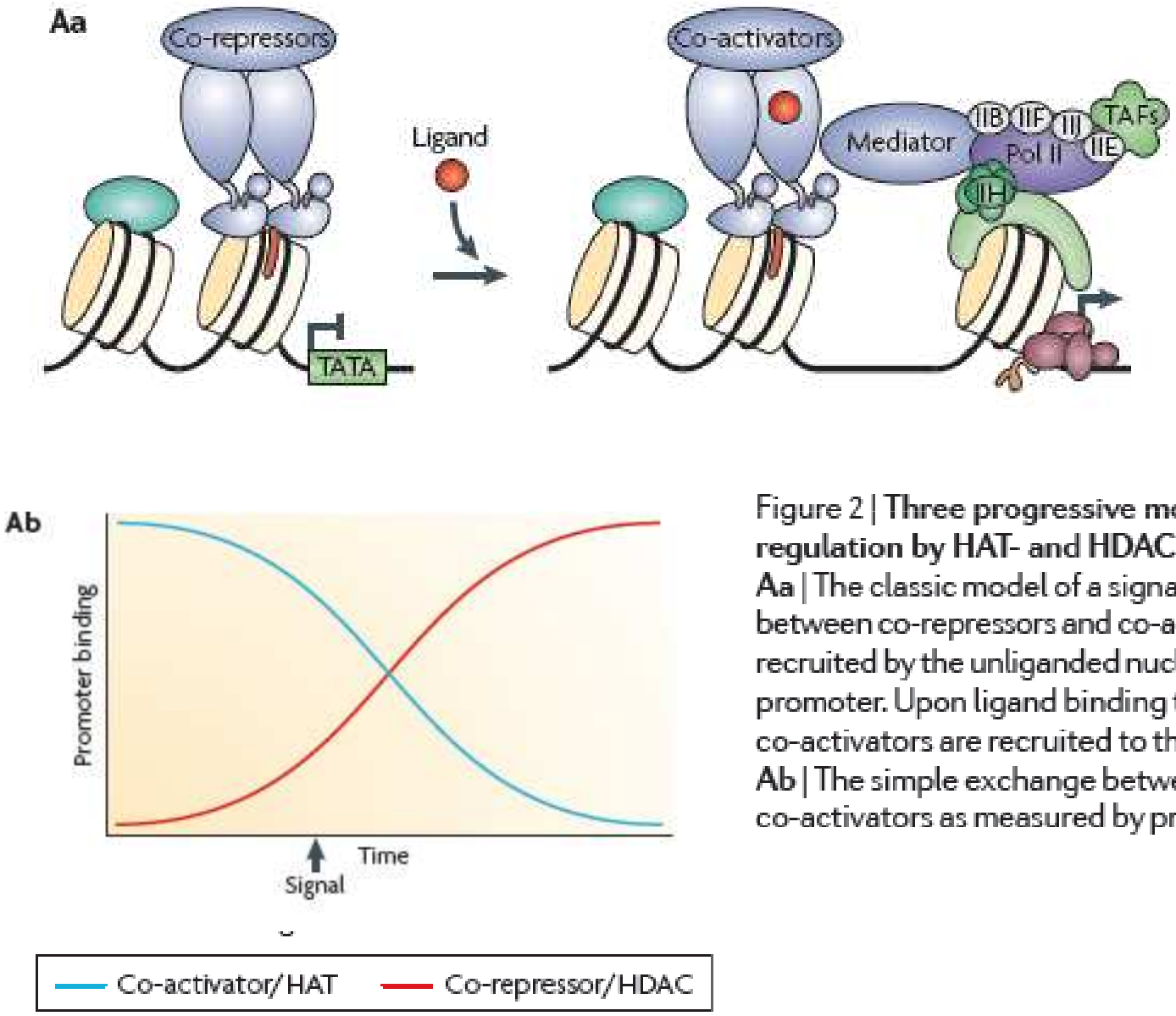
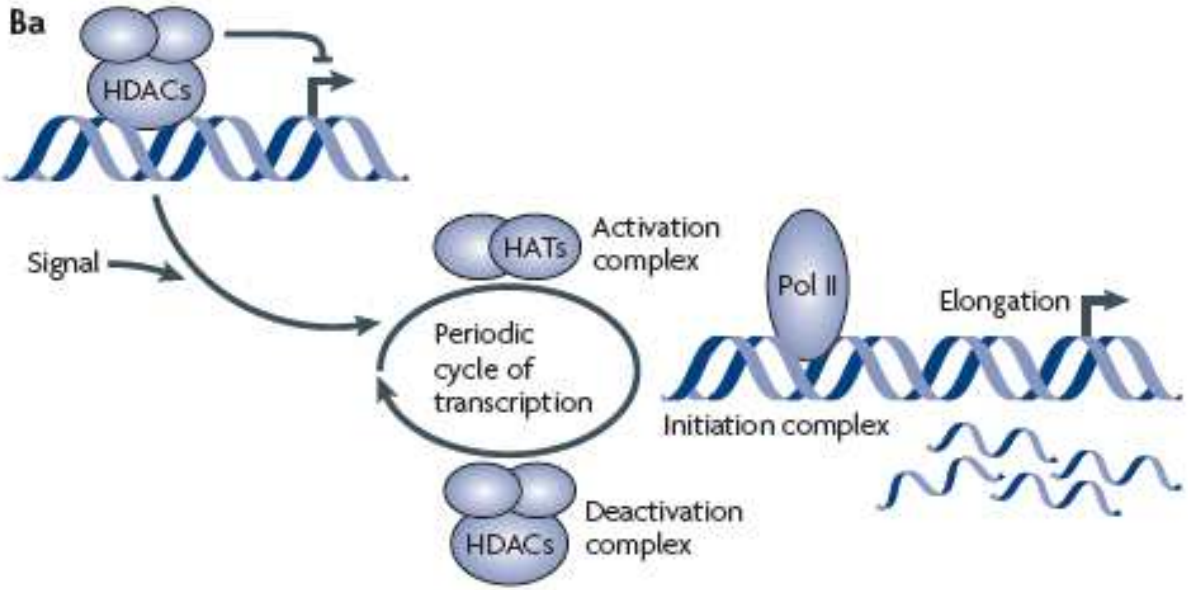
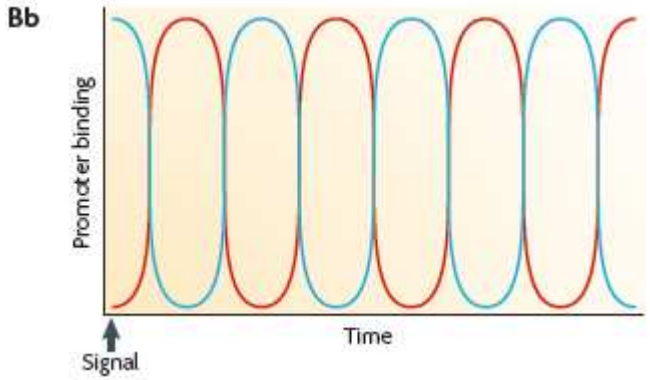


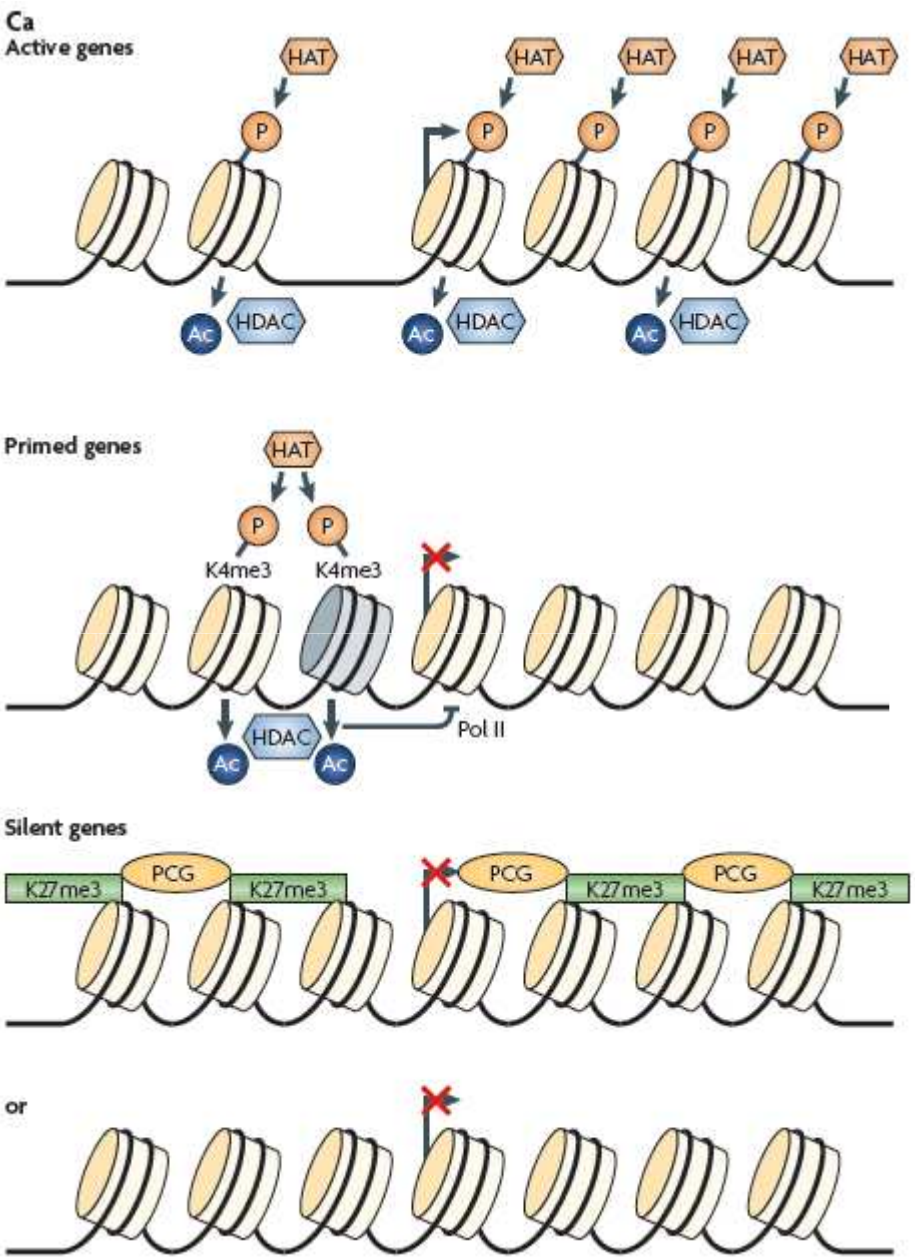
Figure 2 | **Three progressive models of transcriptional regulation by HAT- and HDAC-containing complexes.** **Aa** | The classic model of a signal-dependent switch between co-repressors and co-activators. Co-repressors are recruited by the unliganded nuclear receptor to a repressed promoter. Upon ligand binding they are dismissed and co-activators are recruited to the active transcription unit. **Ab** | The simple exchange between co-repressor and co-activators as measured by promoter occupancy.

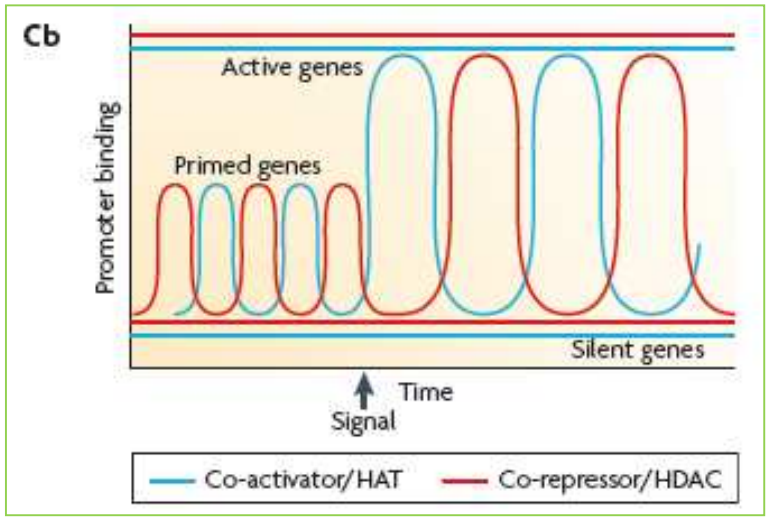




Ba | Ligand-induced regulation of hormone-dependent genes with cyclic recruitment and dismissal of co-repressor and co-activator complexes. **Bb** | In this case, co-repressors and co-activators are continuously exchanged on the promoter. **Ca** | Summary of a recent

Recent genome-wide mapping of HATs and HDACs using ChIP-Seq allowed modelling of three types of genes:





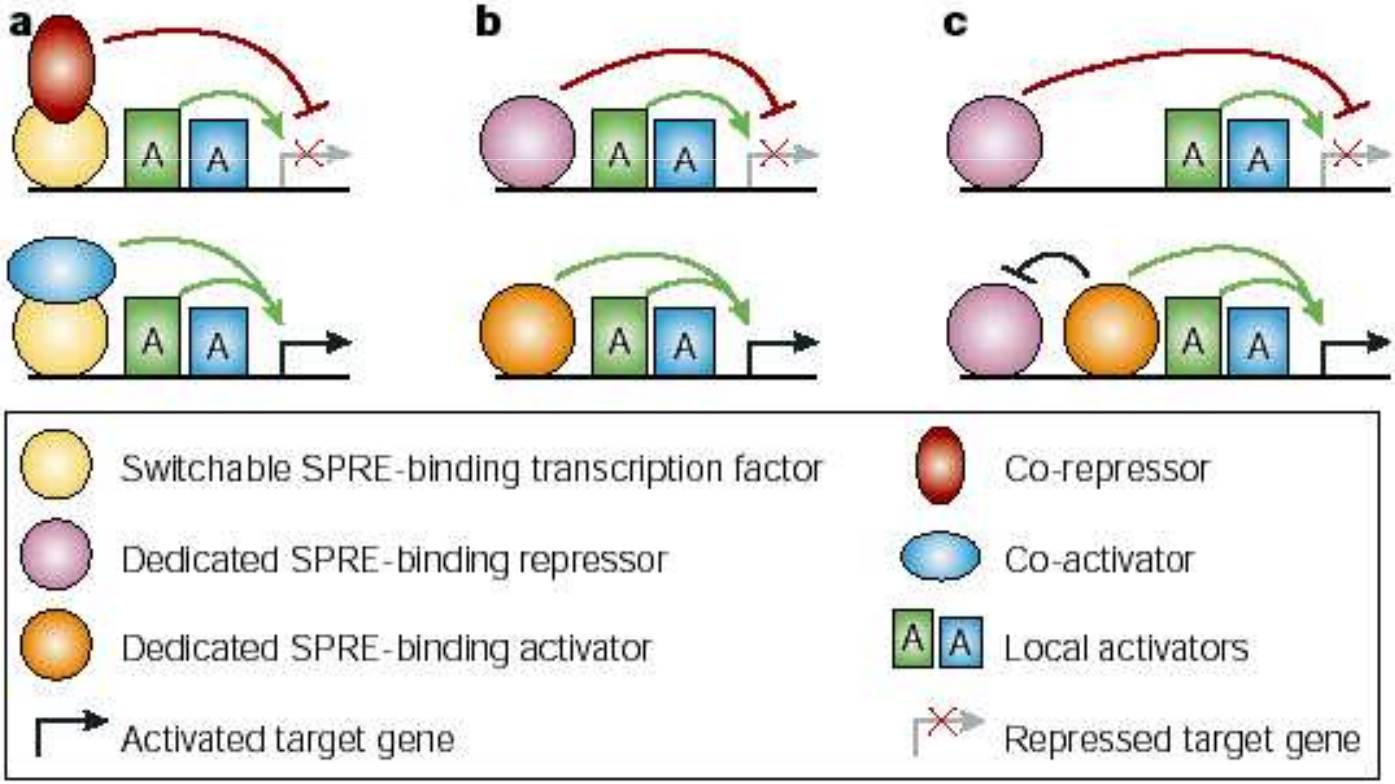
: **Ca** | Summary of a recent genome-wide location analysis for histone deacetylases (HDACs) and histone acetyltransferases (HATs). Three classes of regulated genes have been identified: silent, primed and active genes[59]. **Cb** | Co-activators and co-repressors cycle on and off both primed and active genes. Ac, acetylation; K4me3, trimethylation at lysine 4; K27me3, trimethylation at lysine 27; IIB/IIE/IIF/IIH/IIJ, transcription factor IIB/IIE/IIF/IIH/IIJ; P, phosphorylation; PCG, polycomb group complex; Pol II, RNA polymerase II; TAF, TATA box-binding protein (TBP)-associated factor. Part c is modified, with permission, from REF. 59 © (2009) Elsevier.

How frequent are these models?

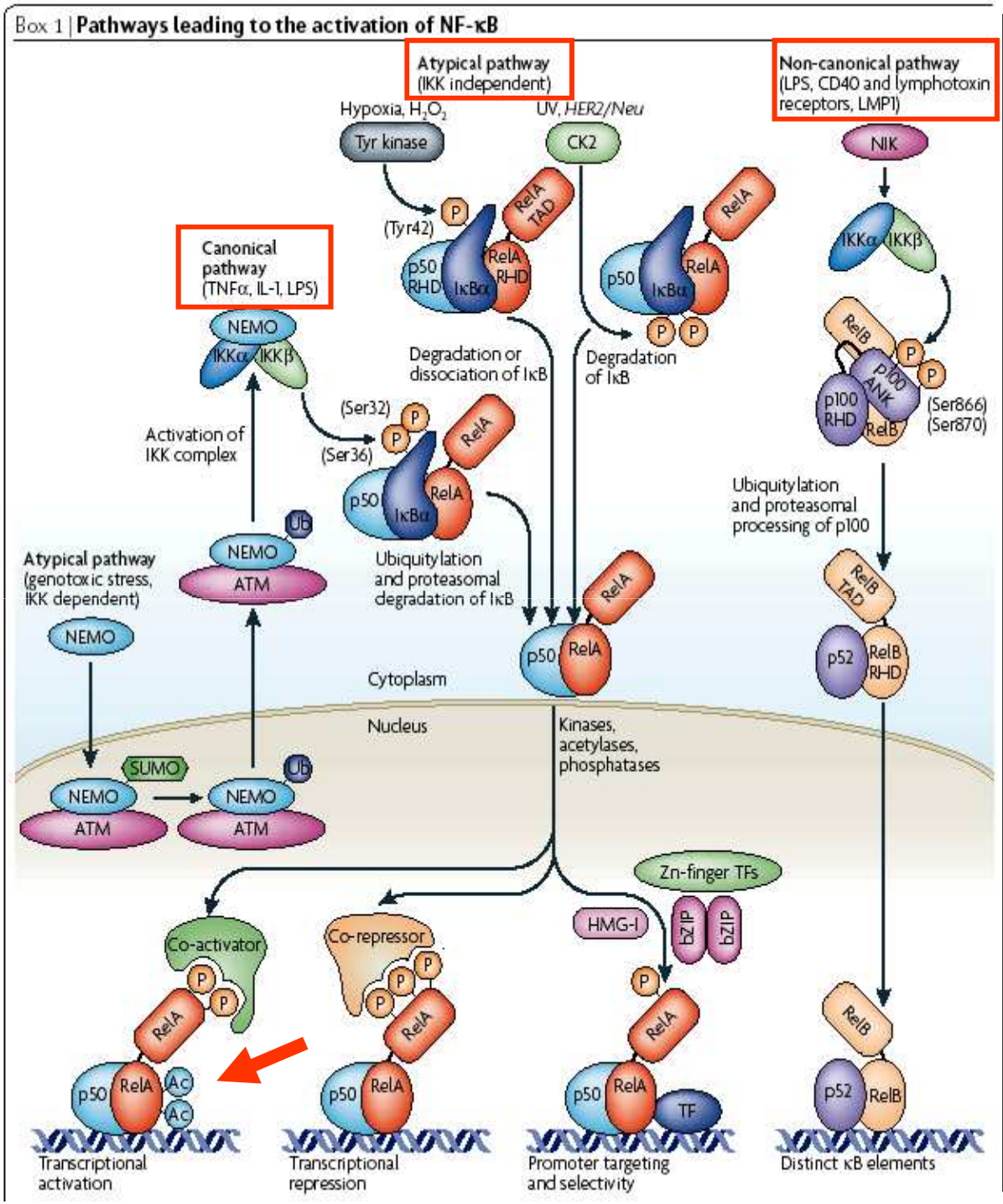
Seven major types of pathways in mammals:

- Wingless-related (Wnt)
- Transforming growth factor β (TGF β - Smad)
- Hedgehog (Hh)
- Receptor Tyrosine Kinase (RTK)
- Janus kinases (JAK) – STAT
- Notch
- Nuclear receptors

4/5 of these pathway types involve transition from a REPRESSED to an ACTIVATED status.



from: Pires-daSilva, 2003, Nature Rev Genet. 4: 39



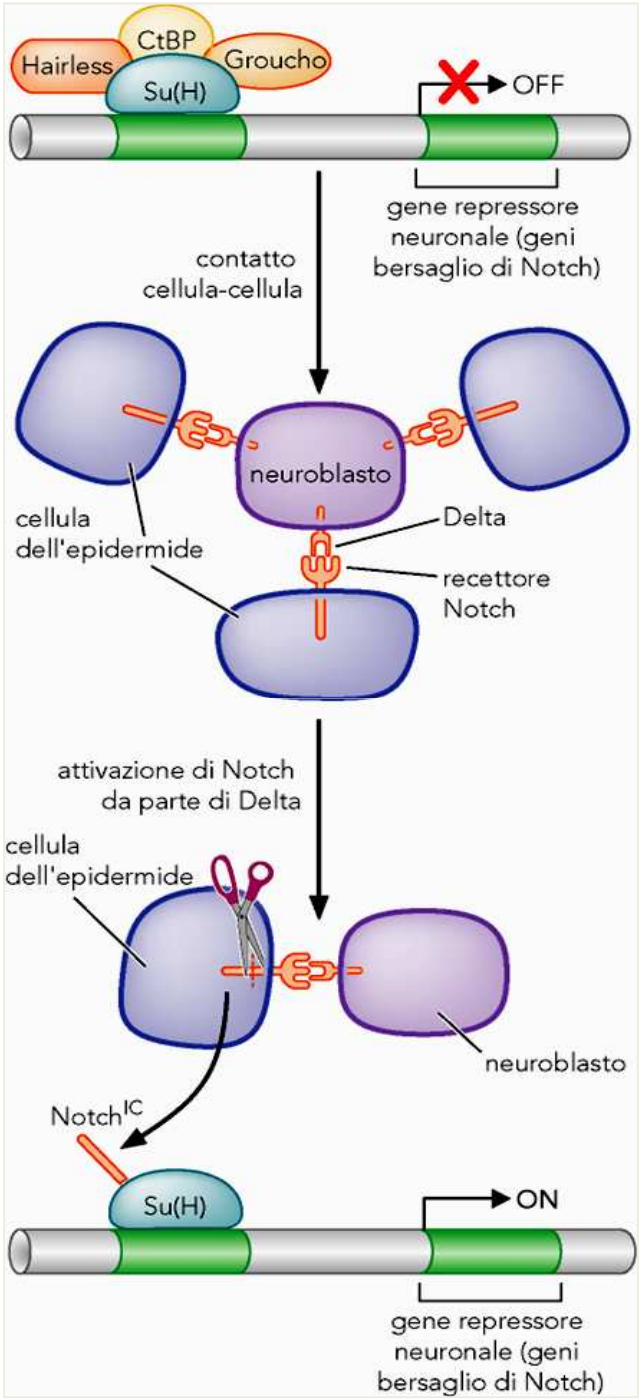
The canonical pathway is induced by tumour necrosis factor-α (TNFα), interleukin-1 (IL-1) and many other stimuli, and is dependent on activation of IKKβ. This activation results in the phosphorylation (P) of IκBα at Ser32 and Ser36, leading to its ubiquitylation (Ub) and subsequent degradation by the 26S proteasome. Release of the NF-κB complex allows it to relocate to the nucleus. Under some circumstances, the NF-κB–IκBα complex shuttles between the cytoplasm and the nucleus (not shown). IKK-dependent activation of NF-κB can occur following genotoxic stress. Here, NF-κB essential modifier (NEMO) localizes to the nucleus, where it is sumoylated and then ubiquitylated, in a process that is dependent on the ataxia telangiectasia mutated (ATM) checkpoint kinase. NEMO relocates back to the cytoplasm together with ATM, where activation of IKKβ occurs. IKK-independent atypical pathways of NF-κB activation have also been described, which include casein kinase-II (CK2) and tyrosine-kinase-dependent pathways. The non-canonical pathway results in the activation of IKKα by the NF-κB-inducing kinase (NIK), followed by phosphorylation of the p100 NF-κB subunit by IKKα. This results in proteasome dependent processing of p100 to p52, which can lead to the activation of p52–RelB heterodimers that target distinct κB elements. Phosphorylation of NF-κB subunits by nuclear kinases, and modification of these subunits by acetylases and phosphatases, can result in transcriptional activation and repression as well as promoter-specific effects.

Notch signalling: a simple pathway becomes complex

Sarah J. Bray

Abstract | A small number of signalling pathways are used iteratively to regulate cell fates, cell proliferation and cell death in development. Notch is the receptor in one such pathway, and is unusual in that most of its ligands are also transmembrane proteins; therefore signalling is restricted to neighbouring cells. Although the intracellular transduction of the Notch signal is remarkably simple, with no secondary messengers, this pathway functions in an enormous diversity of developmental processes and its dysfunction is implicated in many cancers.

Nat. Rev. Mol. Cell. Biol. (2006), 7: 678-689.



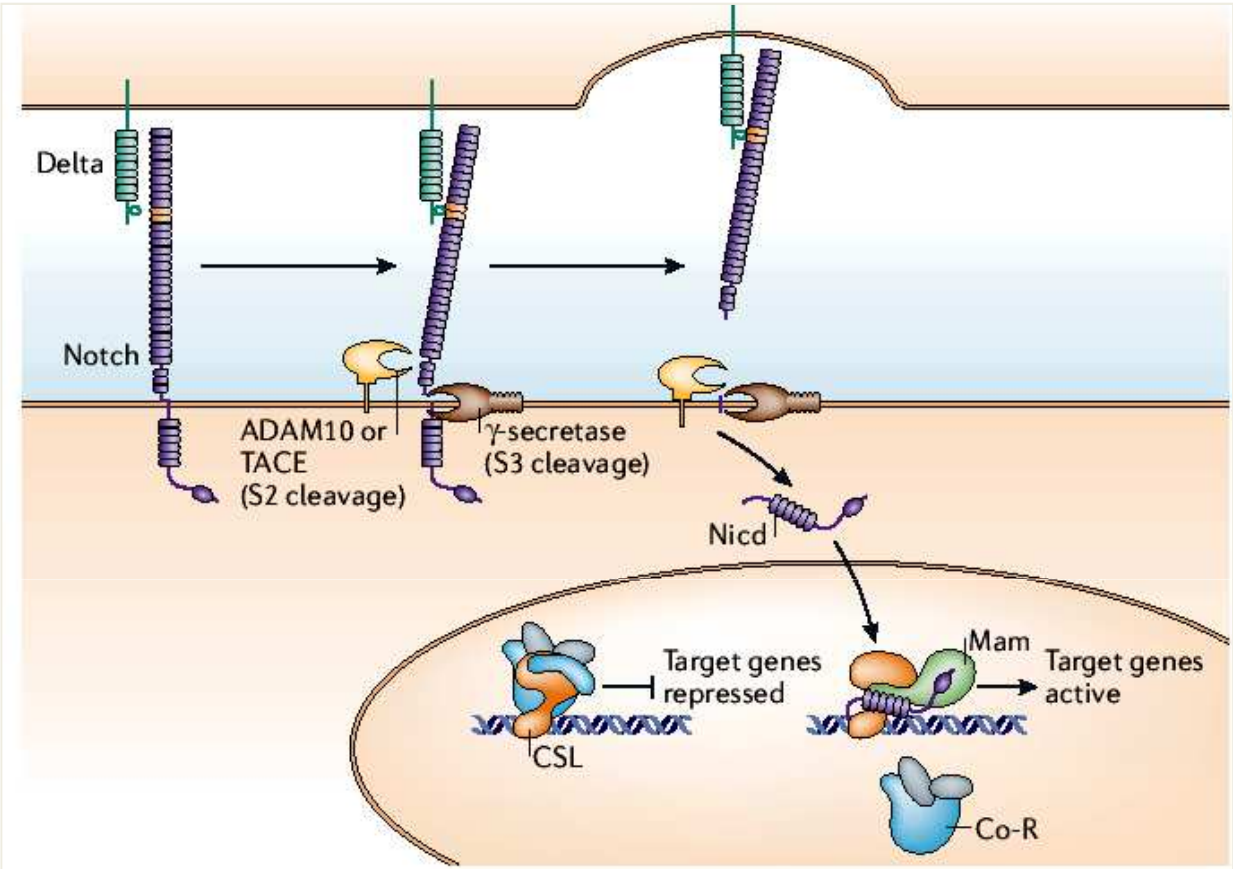


Figure 1 | **The core Notch pathway.** Binding of the Delta ligand (green) on one cell to the Notch receptor (purple) on another cell results in two proteolytic cleavages of the receptor. The ADAM10 or TACE (TNF-α-converting enzyme; also known as ADAM17) metalloprotease (yellow) catalyses the S2 cleavage, generating a substrate for S3 cleavage by the γ-secretase complex (brown). This proteolytic processing mediates release of the Notch intracellular domain (Nicd), which enters the nucleus and interacts with the DNA-binding CSL (CBF1, Su(H) and LAG-1) protein (orange). The co-activator Mastermind (Mam; green) and other transcription factors (see also FIG. 4) are recruited to the CSL complex, whereas co-repressors (Co-R; blue and grey) are released.

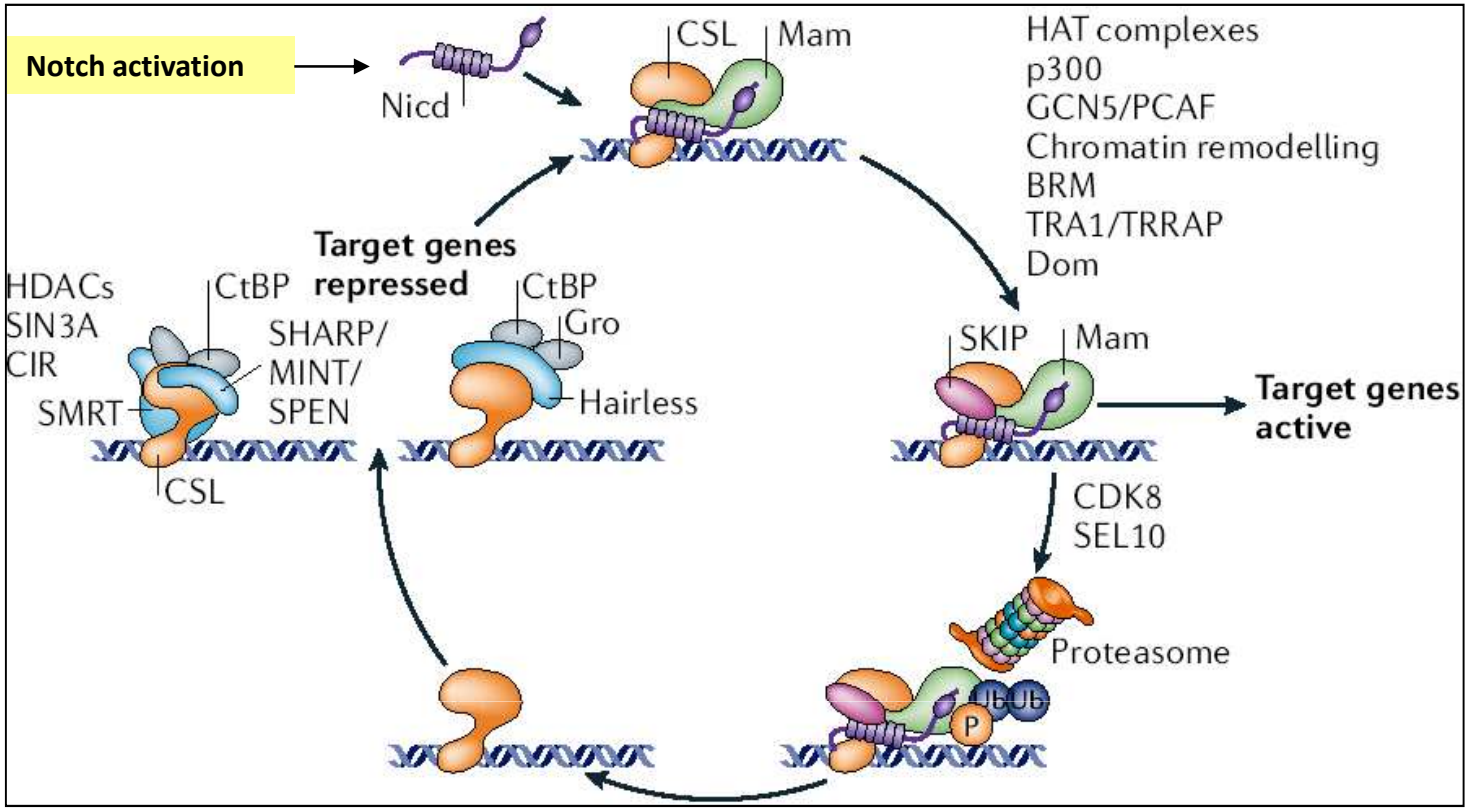
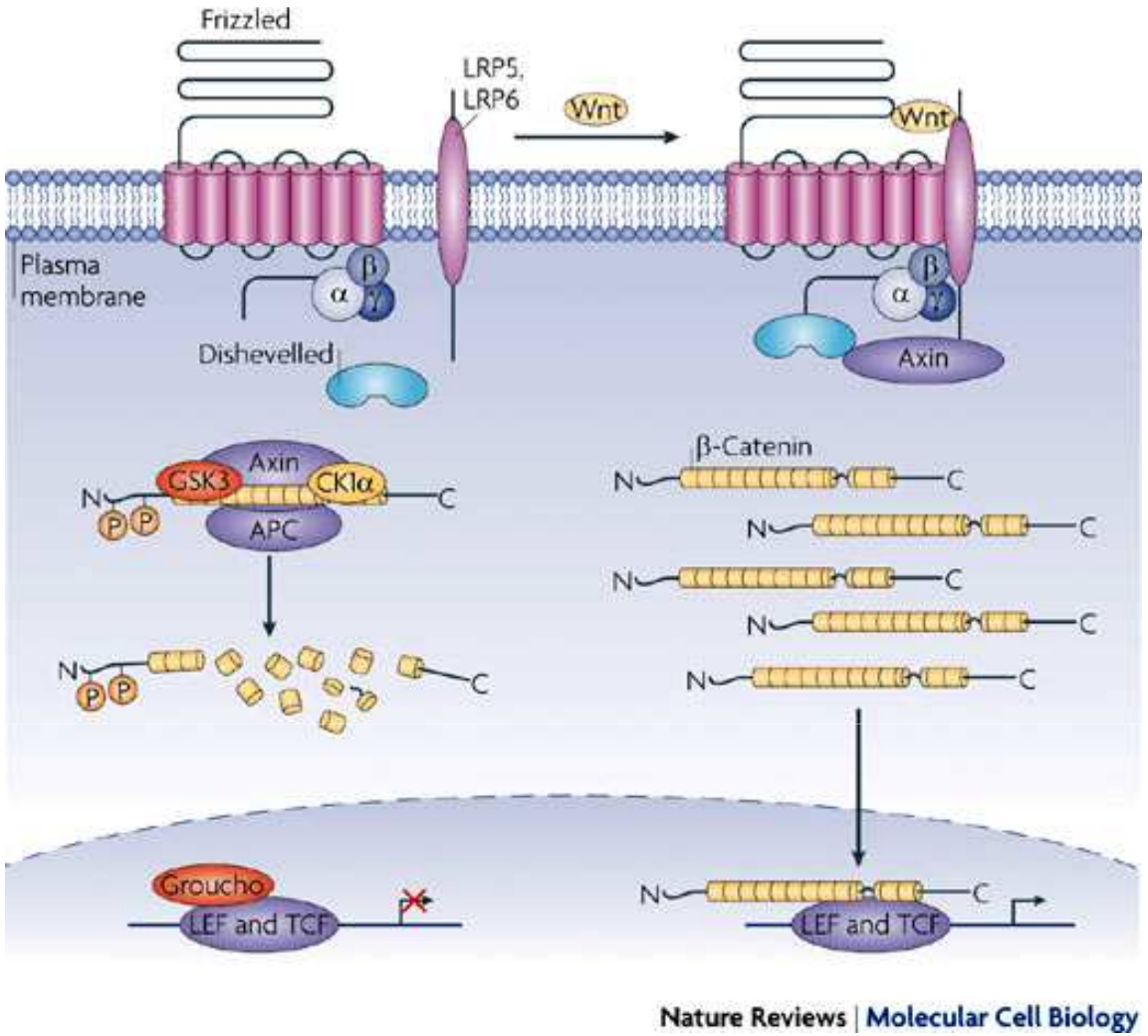


Figure 4 | **Nuclear cycle of CSL.** The Notch intracellular domain (Nicd, purple) forms a trimeric complex with the DNA-binding protein CSL (CBF1, Su(H) and LAG-1; orange) and the co-activator Mastermind (Mam, green). SKIP (Ski-interacting protein), a protein that interacts with the ankyrin repeat domain of Nicd and with CSL is also present. Histone acetyl transferases (HATs; p300 and/or PCAF/GCN5) and chromatin remodelling complexes (BRM, TRA1/TRRAP and Dom) are recruited and contribute to activation and elongation of transcription at target genes. Kinases such as cyclin dependent kinase-8 (CDK8) and the SEL10 E3 ligase modify Nicd, making it a substrate for proteosomal degradation. In the absence of Nicd, CSL is associated with co-repressors, the precise composition of which might vary according to species and cell type. Two putative co-repressor complexes are illustrated: a mammalian complex containing SMRT, SHARP (also known as MINT and SPEN) and CtBP, and a Drosophila melanogaster complex containing Hairless, CtBP and Groucho (Gro). Although not depicted, SKIP might also be part of the repression complex. The co-repressors recruit histone deacetylases (HDACs; such as HDAC1, HDAC3, HDAC4 and RPD3) and other cofactors (SIN3A and CIR). Target genes are repressed until more Nicd is produced to re-initiate the cycle. P, phosphate; Ub, ubiquitin.



The post-transcriptional control of β-catenin protein stability is central to the Wnt–β-catenin pathway. Adenomatous polyposis coli (APC) and Axin are scaffold proteins that form the -catenin degradation complex. Phosphorylation of β-catenin, by the degradation complex kinases glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CK1), forms the signal for recognition of β -catenin by SKP1–cullin 1–F-box (SCF^{-TrCP}) E3 ligase (not shown) and consequent degradation by the ubiquitin–proteasome pathway. Therefore, under resting conditions, a low level of β-catenin protein exists and Wnt target genes are repressed by the Groucho family of transcriptional repressors, which bind to lymphoid enhancer factor (LEF) and T cell factor (TCF) proteins. Upon Wnt activation of the Frizzled–low density lipoprotein receptor-related protein 5 (LRP5) or Frizzled–LRP6 receptor complexes, heterotrimeric G proteins and Dishevelled proteins are activated and lead to the recruitment of Axin to the LRP5 or LRP6 co-receptor. This inhibits the degradation complex, promotes the accumulation of β-catenin and its translocation to the nucleus where it displaces Groucho from LEF and TCF and acts as a transcriptional co-activator to modulate context-dependent transcription.