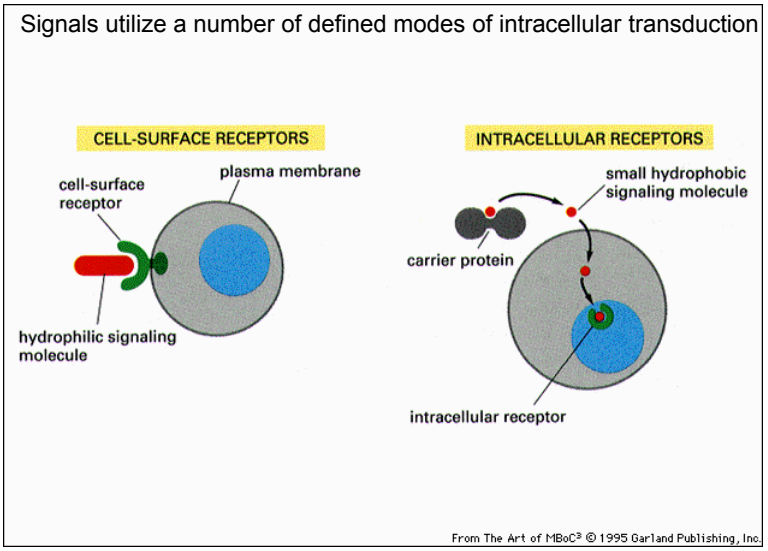
Many perceptive and intraceptive signals **converge** to gene regulation

Response requires **integration** of different signals

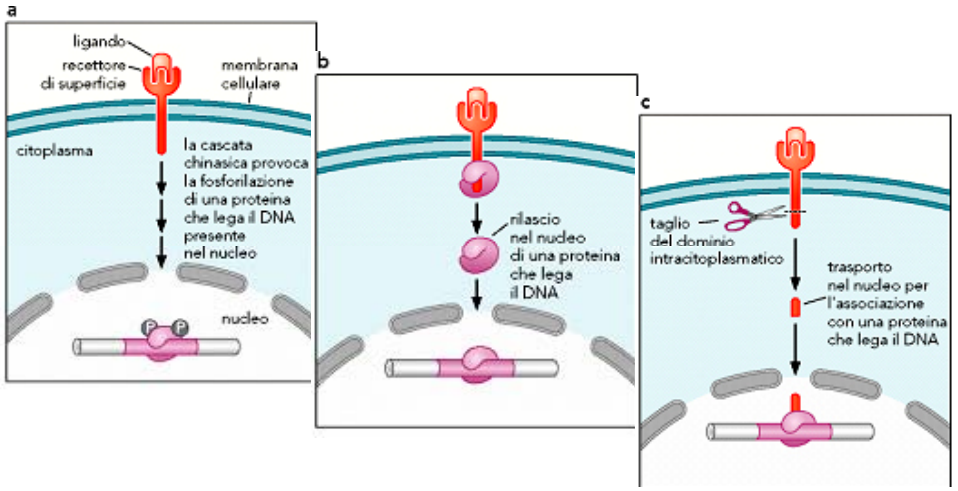
Signals utilize a number of defined modes of intracellular transduction



The activity of many transcription factor is controlled by signal transduction pathways

“Sensor” signals → quantitative modulation of genes

Receptors of differentiation/growth signals → “on-off”



Integration at several levels

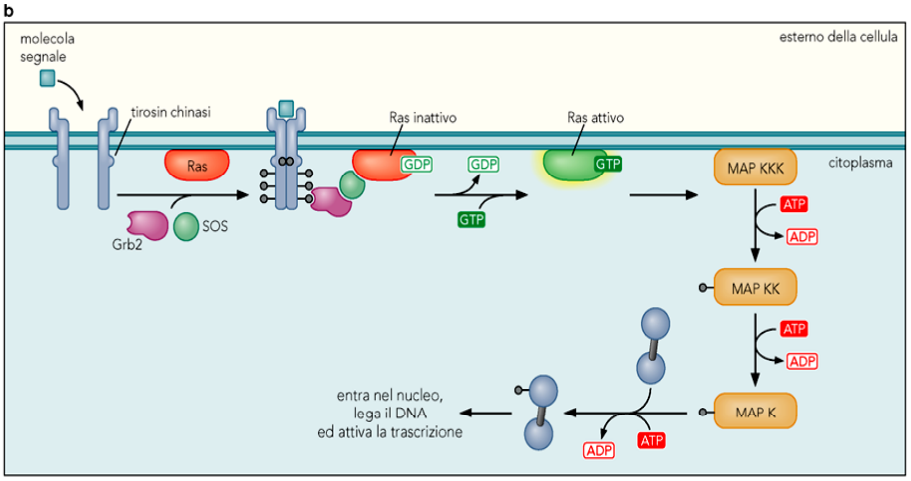
1st level – cross-talk between signaling pathways

2nd level – transcription factor interaction at enhancers and promoters

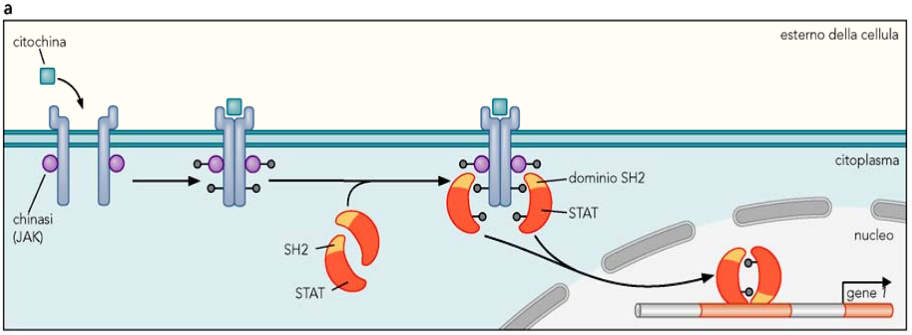
3rd level – regulation of coactivators and corepressors

Several pathways are revised in the following slides:
MAP Kinases
STAT
SMAD
NF-kB
Notch
Wnt / β-catenin
cAMP-CREB

b


molecola
segnale
esterno della cellula
tirosin chinasi
Ras inattivo
Ras attivo
Ras
GDP
GDP
GTP
GTP
MAP KKK
citoplasma
ATP
ADP
Grb2
SOS
MAP KK
ATP
ADP
entra nel nucleo,
lega il DNA
ed attiva la trascrizione
MAP K
ADP
ATP

a


citochina
esterno della cellula
citoplasma
dominio SH2
STAT
chinasi
(JAK)
SH2
STAT
nucleo
gene

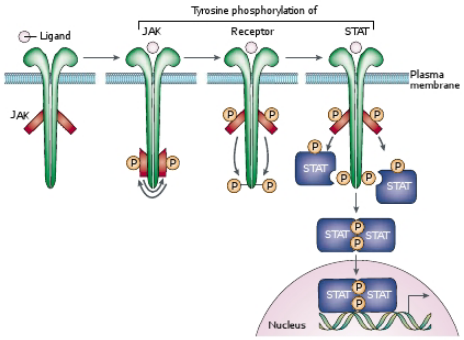
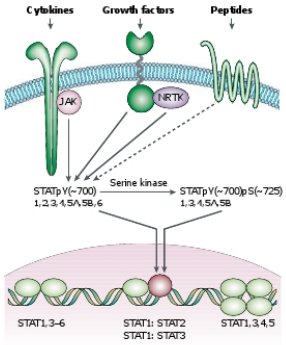


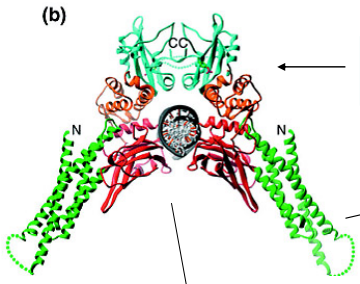
Figure 1 | **Canonical JAK–STAT pathway.** Sequential tyrosine phosphorylations triggered by cytokine–receptor interaction. Receptor dimerization allow s transphosphorylation and activation of Janus kinases (JAKs). This is follow ed by phosphorylation of receptor tails and the recruitment of the signal transducers and activators of transcription (STAT) proteins through their Src-homology-2 domains. STAT tyrosine phosphorylation then occurs. Dimerization of activated (tyrosine phosphorylated) STAT is follow ed by nuclear entry.

Box 1 | Variations in mechanisms of STAT activation



Tyrosine phosphorylation of signal transducers and activators of transcription (STAT) proteins at or around residue 700 occurs in response to cytokine receptors through Janus kinases (JAKs). However, at least several dozen receptors with intrinsic tyrosine kinase activity (RTKs), such as those for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), seem to be able to mediate the activation of STAT proteins[149–151]. Apparently, this activation can be direct (as in the case of STAT1 activation by PDGF receptor) or indirect. The latter case involves the recruitment of complexes of proteins to the phosphorylated RTK. Non-receptor tyrosine kinases (NRTKs), such as Src — the first tyrosine kinase to be discovered — are among the recruited proteins. STAT3 and Src can interact independently and STAT3 probably becomes phosphorylated by Src on the EGF and PDGF receptors. Furthermore, it is clear that seven-transmembrane (7TM) receptors can, after binding their peptide or short polypeptide ligands, also activate STAT proteins[152–154]. It has been proposed again that the tyrosine kinase involved is Src — or perhaps the JAKs become activated by associating with 7TM receptors[155–157]. STAT1, STAT3, STAT4, STAT5 and STAT6 homodimerize. STAT1 and STAT2, and STAT1 and STAT3 can form heterodimers, and several STAT proteins can form tetramers (or potentially higher order complexes).

One final comment on STAT activation is needed. Direct recruitment of latent STAT proteins to activated cytokine receptors might be the most common and is certainly conceptually the simplest mechanism of cytokine activation, but pre-association of STAT1 and STAT2 with the interferon-α (IFN-α) receptor before ligand stimulation has also been described. Furthermore, it is known that, after IFN-α treatment, STAT2 must be phosphorylated before STAT1, at least in some cell types[6,121]. So, in this case, the general model for phosphotyrosine-dependent recruitment might not operate. However, the exact role of pre-associated STAT2 at the IFN-α receptor and that of inducible receptor phosphorylation in the IFN-α pathway remains uncertain[158]. STATpY, tyrosine-phosphorylated STAT; pS, serine phosphorylated.

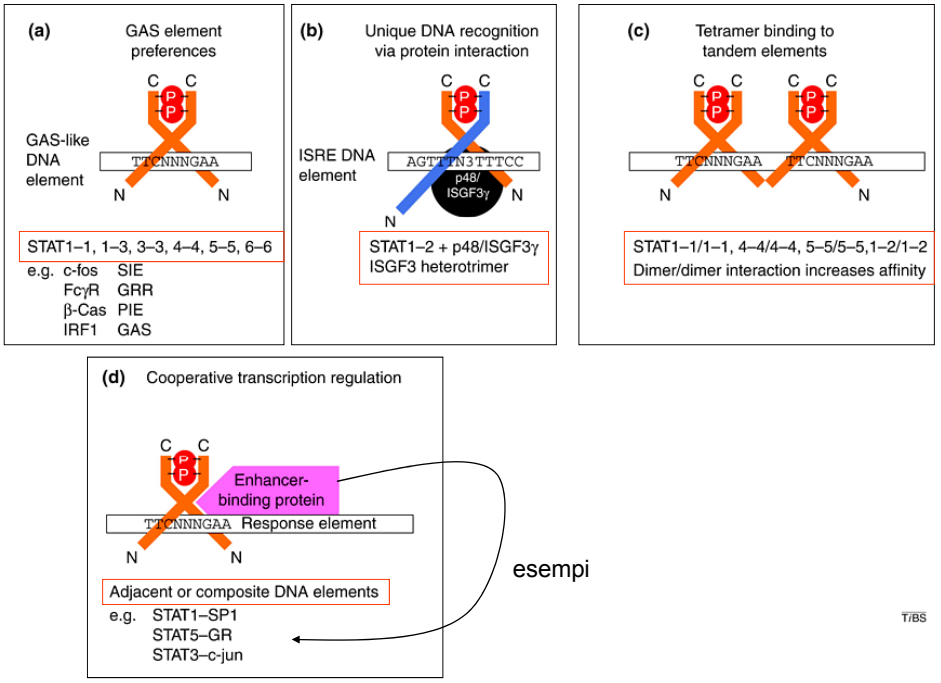


STAT proteins dimerize using reciprocal interaction P-Tyr / SH2 domains

Tetramerization domain

The DNA binding domain is an Ig-like fold similar to that of p53 and NF-κB

STAT proteins recognize very different DNA elements, depending on protein-protein contacts and arrangments.



TGFβ–SMAD signal transduction: molecular specificity and functional flexibility

Bernhard Schmierer and Caroline S. Hill

Abstract | Ligands of the transforming growth factor-β (TGFβ) superfamily of growth factors initiate signal transduction through a bewildering complexity of ligand–receptor interactions. Signalling then converges to nuclear accumulation of transcriptionally active SMAD complexes and gives rise to a plethora of specific functional responses in both embryos and adult organisms. Current research is focused on the mechanisms that regulate SMAD activity to evoke cell-type-specific and context-dependent transcriptional programmes. An equally important challenge is understanding the functional role of signal strength and duration. How are these quantitative aspects of the extracellular signal regulated? How are they then sensed and interpreted, and how do they affect responses?

Nat. Rev. Mol. Cell Biol. (2007) **8**:970-982.

The **Smad** proteins are a family of regulated transcription factors found in Vertebrates, insects and nematodes

Smads are the only substrates of TGF-β receptors (and related)

In this class of receptors, 2 types of receptors are brought together by the ligand, indicated as Type I and Type II receptors

A third type of receptors, called Type III, showing low affinity and constituted by "betaglycans", cooperate in ligand binding

Type I and Type II receptors are **Serine/Threonine kinases**

Inactive Type I receptors have inactive kinase. After ligand binding and dimerization, Type II phosphorylates Type I activation loop

Activated Type I receptors phosphorylate C-terminal Serines in **Smads**

Smads directly assemble complexes that translocate to nucleus and activate transcription of target genes and/or repress other genes.

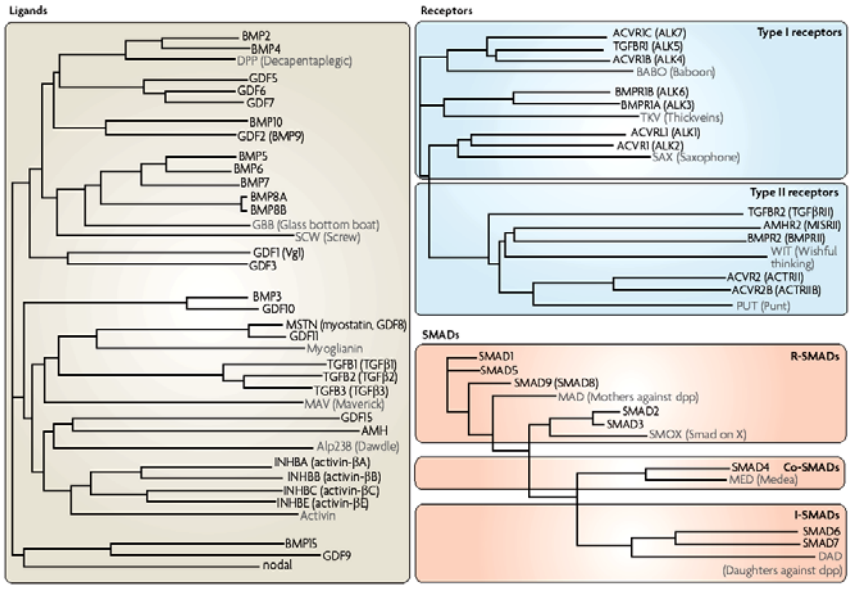


Figure 1 | **Ligands, receptors and SMADs.** Phylogenetic trees[141] derived from protein alignments of the core components of TGFβ signalling pathways in humans and *Drosophila melanogaster*. Human proteins are shown in black and *D. melanogaster* proteins are in grey. Protein symbols are given with alternative names in parentheses. For the ligands, the putative, mature, fully processed forms were used to construct the tree. ACVR, activin receptor; ALK, activin receptor-like kinase; AMH, anti-Muellerian hormone; AMHR2, AMH receptor-2; BMP, bone morphogenetic protein; BMPR, BMP receptor; GDF, growth and differentiation factor; I-SMAD, inhibitory SMAD; R-SMAD, receptor-regulated SMAD; TGF, transforming growth factor; TGFBR, TGFβ receptor.

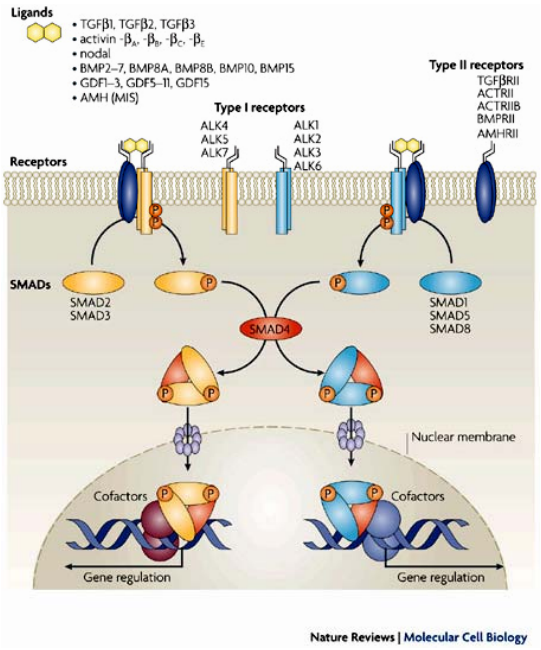
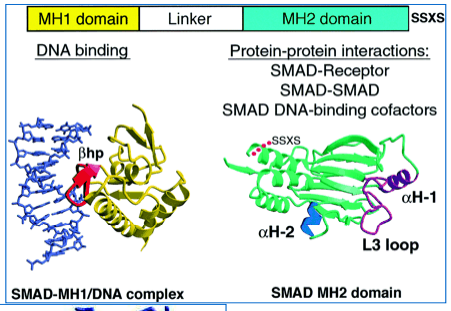


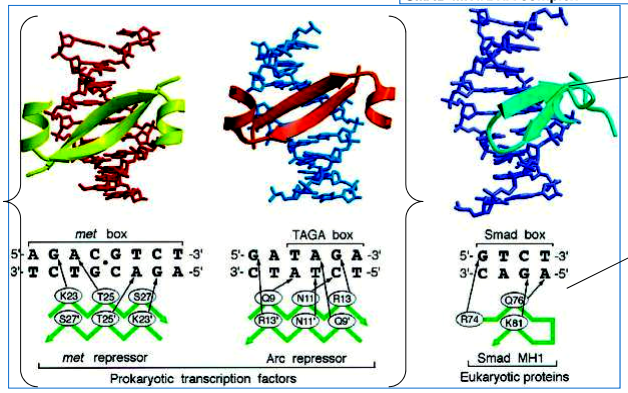
Fig 2 Core signalling in the mammalian TGF –SMAD pathways.

Binding of ligands to type II receptors and recruitment of type I receptors involves high combinatorial complexity. Traditionally, ligands have been split into two groups: SMAD2-and SMAD3-activating TGF s, activins and nodal on the one hand, and SMAD1, SMAD5 and SMAD8-activating BMPs, GDFs and AMH on the other hand. Note that this concept does not accurately reflect reality. The pathway only splits into two distinct branches downstream of type I receptors: ALK4, ALK5 and ALK7 specifically phosphorylate SMAD2 and SMAD3, whereas ALK1, ALK2, ALK3 and ALK6 specifically phosphorylate SMAD1, SMAD5 and SMAD8. Complex formation of the phosphorylated receptor regulated SMADs with SMAD4 causes nuclear accumulation of active SMAD complexes,which directly regulate gene transcription in conjunction with transcription factors,chromatin-remodelling complexes and histone-modifying enzymes.

ACTR, activin receptors; ALK, activin receptor-like kinase; AMH, anti-Muellerian hormone; BMPs, bone morphogenetic proteins; GDFs, growth and differentiation factors; TGF s, transforming growth factor-ligands.

Smad protein structure





DBD is a 2-strand β-sheet

Very few specific protein-DNA contacts

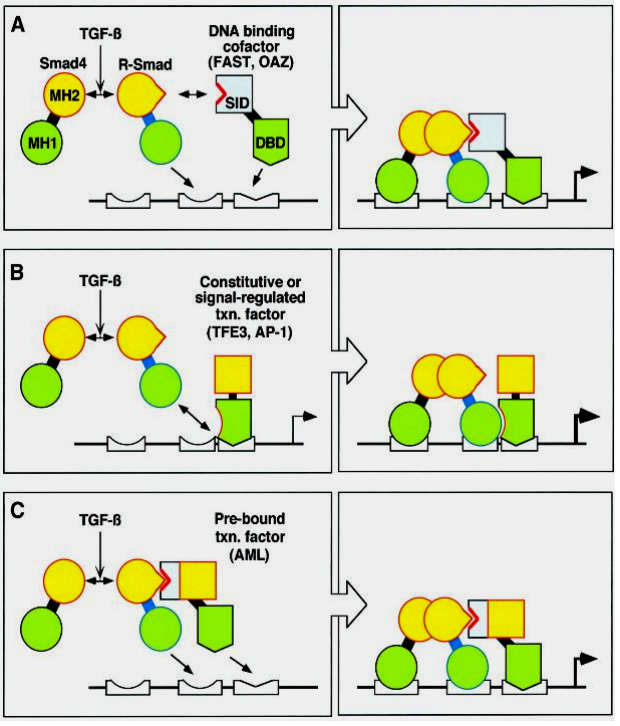
The short SBEs only allow low-affinity binding, and interaction with other DNA-binding factors is frequently required for the specific recruitment of SMADs to response elements. Numerous transcription factors have been shown to interact with SMADs.

The first SMAD-interacting transcription factor to be identified was FOXH1 (forkhead box H1), a winged-helix transcription factor that specifically recruits a complex of phosphorylated SMAD2 and SMAD4 to an ARE, such as that found in the X. laevis Mix2 promoter.

A distinct group of SMAD2-interacting transcription factors is the Mix family of homeodomain proteins. The Mix family members Mixer, Milk and Bix3 share the same SMAD2-interaction motif with FOXH1, but contain a distinct DNA-binding domain and exhibit a different expression pattern. Therefore, they induce a different set of nodal-responsive genes to that regulated by FOXH1.

Smads acquire specificity for DNA elements by heterodimerization and specific interactions with other transcription factors.

R-Smad = receptor regulated Smad



Integrating cell-signalling pathways with NF-κB and IKK function

Neil D. Perkins

Abstract | Nuclear factor (NF)-κB and inhibitor of NF-κB kinase (IKK) proteins regulate many physiological processes, including the innate- and adaptive-immune responses, cell death and inflammation. Disruption of NF-κB or IKK function contributes to many human diseases, including cancer. However, the NF-κB and IKK pathways do not exist in isolation and there are many mechanisms that integrate their activity with other cell-signalling networks. This crosstalk constitutes a decision-making process that determines the consequences of NF-κB and IKK activation and, ultimately, cell fate.

Nat. Rev. Mol. Cell. Biol. (2007) 8: 49-62.

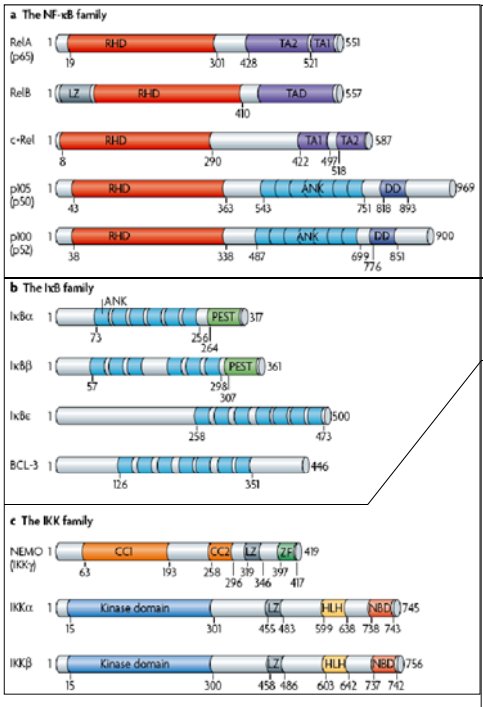


Figure 1 | The mammalian members of the NF-κB, IκB and IKK families.

a | In mammalian cells, there are five nuclear factor (NF)-κB family members, RelA (p65), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2). p50 and p52 (not shown) are derived from the longer precursor proteins p105 and p100, respectively. All NF-κB family members contain an N-terminal Rel-homology domain (RHD) that mediates DNA binding and dimerization and contains the nuclear-localization domain. The Rel subfamily, RelA, RelB and c-Rel, contain unrelated C-terminal transcriptional activation domains (TADs). TA1 and TA2 are subdomains of the RelA transactivation domain.

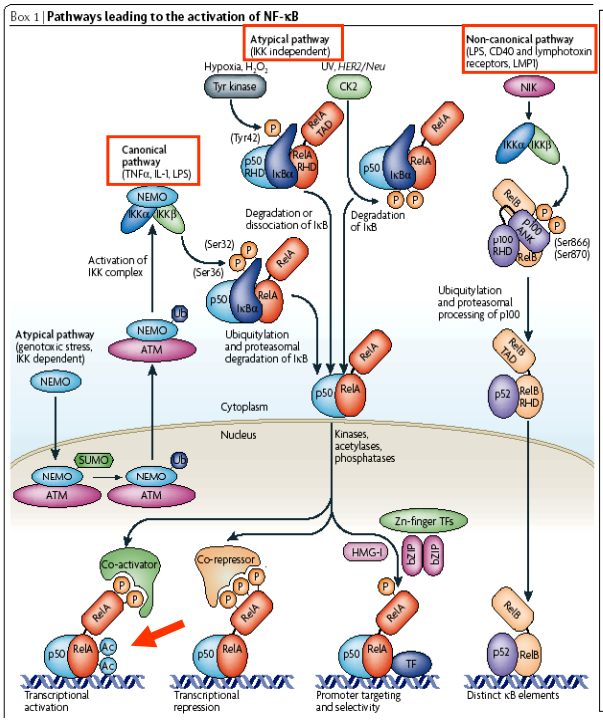
b | The inhibitor of NF-κB (IκB) family consists of IκBα, IκBβ, IκBε and BCL-3. Like p105 and p100, the IκB proteins contain ankyrin-repeat motifs (ANK) in their C termini.

c | The three core subunits of the IκB kinase (IKK) complex are shown: the catalytic subunits IKKα and IKKβ and the regulatory subunit called the NF-κB essential modifier (NEMO, also known as IKKγ). The principal structural motifs of each protein are shown, together with amino-acid numbers corresponding to the human proteins, although some definitions of where a domain begins and ends might differ between publications.

CC, coiled-coil; DD, region with homology to a death domain; HLH, helix–loop–helix;

LZ, RelB-transactivation-domain containing a putative leucine-zipper-like motif;

NBD, NEMO-binding domain; PEST, domain rich in proline (P), glutamate (E), serine (S) and threonine (T); ZF, zinc-finger domain.



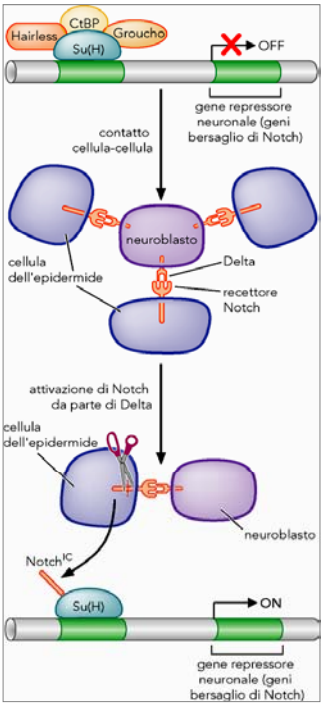
The canonical pathway is induced by tumour necrosis factor-α (TNFα), interleukin-1 (IL-1) and many other stimuli, and is dependent on activation of IKKβ. This activation results in the phosphorylation (P) of IκBα at Ser32 and Ser36, leading to its ubiquitylation (Ub) and subsequent degradation by the 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.



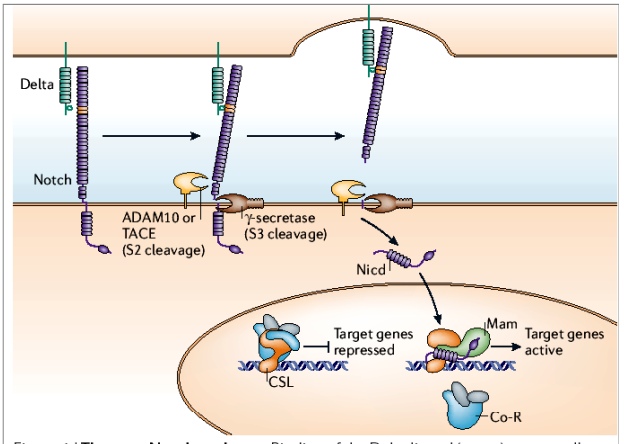
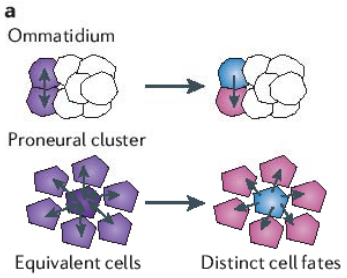
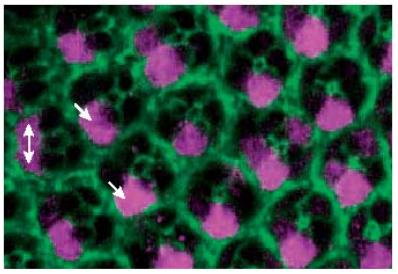


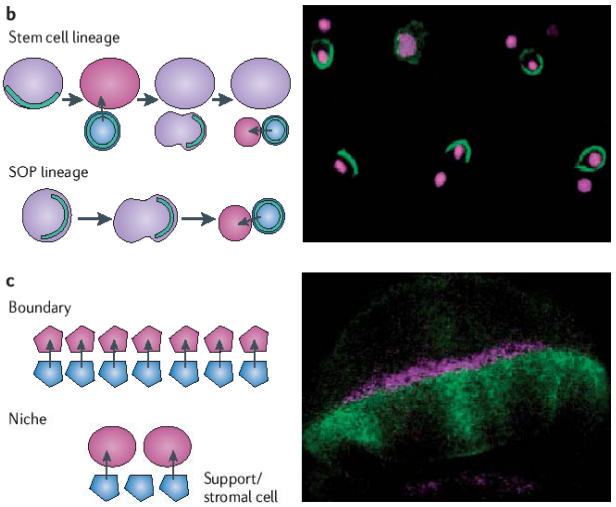
Figure 1 | **The core Notch pathway.** Binding of the Delta ligand (green) on one cell to the Notch receptor (purple) on another cell results in two proteolytic cleavages of the receptor. The ADAM10 or TACE (TNF-α-converting enzyme; also known as ADAM17) metalloprotease (yellow) catalyses the S2 cleavage, 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.





Lateral inhibition

Notch signalling amplifies small or weak differences within roughly equivalent populations of cells. The diagrams (**a**) represent Notch signalling (black arrows) in ommatidia (upper) and neural preclusters (lower) that resolves equivalent (purple) cells into distinct fates (blue and pink; cells with the highest Notch activity are coloured pink). A confocal image shows Notch activity (E(spl) mδ0.5 expression; pink) in developing ommatidia of a fly eye (the green staining marks cell membranes and the thin peripheral cells demarcate each ommatidium). At early stages, mδ0.5 expression is sometimes detected in both posterior photoreceptors (see double arrow) before signalling is refined, and then it is only detected in one (R4) cell (see single arrows).



b. Lineage decisions
Notch signalling between two daughter cells is dependent on **asymmetrical inheritance of Notch regulators** (for example, Numb). Diagrams (**b**) illustrate segregation of regulators (green) in progeny from a hypothetical stem cell lineage (upper) and the *Drosophila melanogaster* sensory organ precursors (SOP) lineage (lower). Thin black arrows indicate the direction of Notch signalling, pink cells acquire highest Notch activation. The confocal image shows Numb distribution (green, by Partner of Numb (PON)-GFP) in SOP lineages (nuclei are pink). Confocal bottom row left; A Numb crescent is evident prior to division. Confocal bottom row right; Numb is present in one of two daughter cells.

c. Boundaries/inductive
Notch signalling occurs between two populations of cells and can establish an organizer and/or segregate the two groups. Diagrams (**c**) of signalling at a boundary (upper) or between stromal and progenitor cells (lower). Black arrows indicate the direction of Notch signalling, pink cells have Notch activation. The confocal image is of the fly wing primordium, in which Notch activity, as measured by Wg expression (pink), is detected at the boundary of Serrate-expressing cells (green).

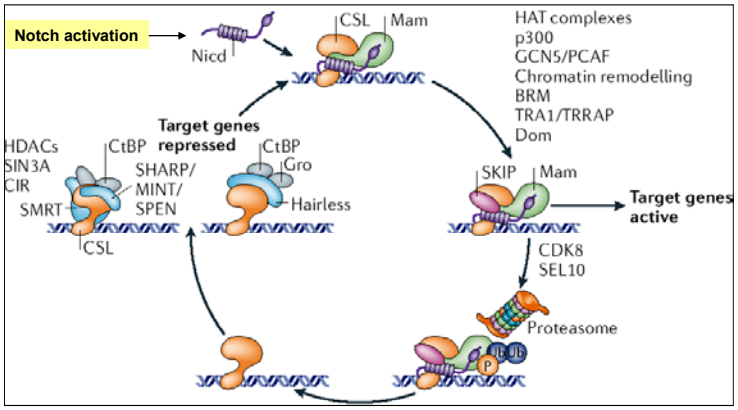
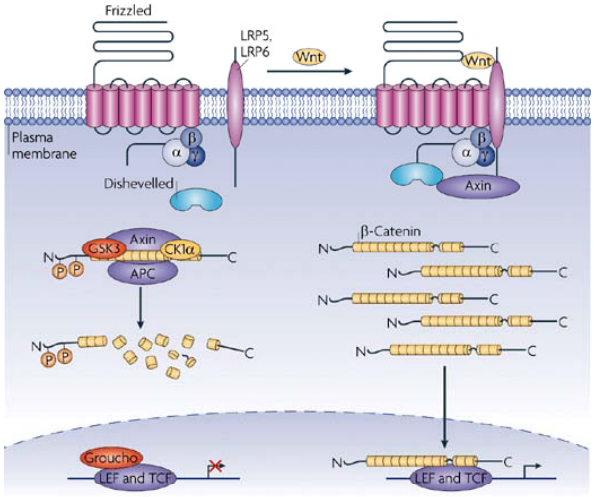


Figure 4 | **Nuclear cycle of CSL.** The Notch intracellular domain (Nicd, purple) forms a trimeric complex with the DNA-binding protein CSL (CBF1, Su(H) and LAG-1; orange) and the co-activator Mastermind (Mam, green). SKIP (Ski-interacting protein), a protein that interacts with the ankyrin repeat domain 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.

Wnt / β-catenin



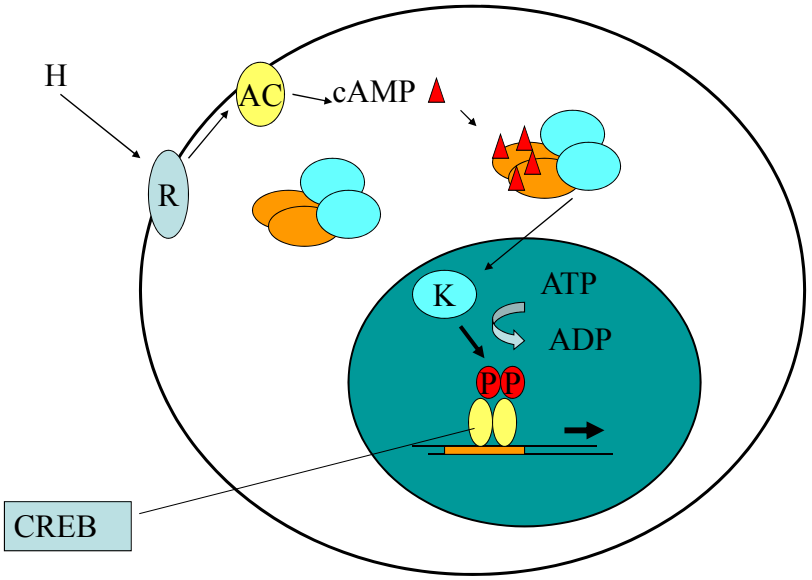
Nature Reviews | Molecular Cell Biology

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.

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?



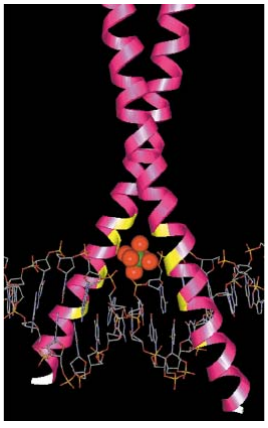


Figure 3
Structure of the CREB basic region/leucine zipper domain (amino acids 285–339) bound to the somatostatin CRE.
The cyclic AMP response element (CRE)-binding protein (CREB) bZIP domain is shown, with residues that function in DNA recognition highlighted in yellow. A magnesium ion (green) with 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.

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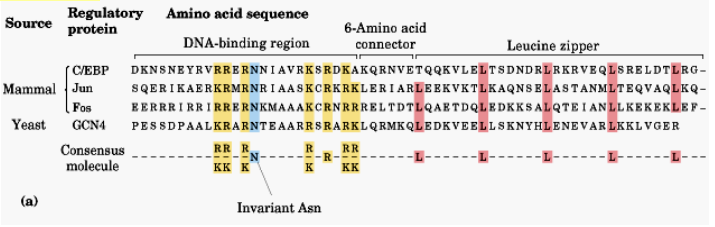
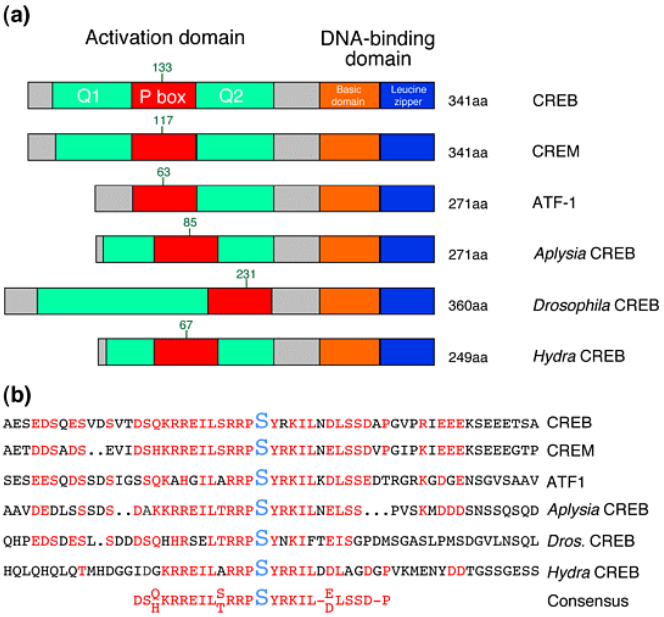




KID=kinase interaction domain

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”

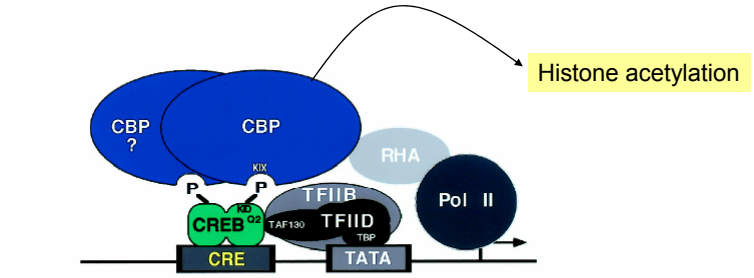
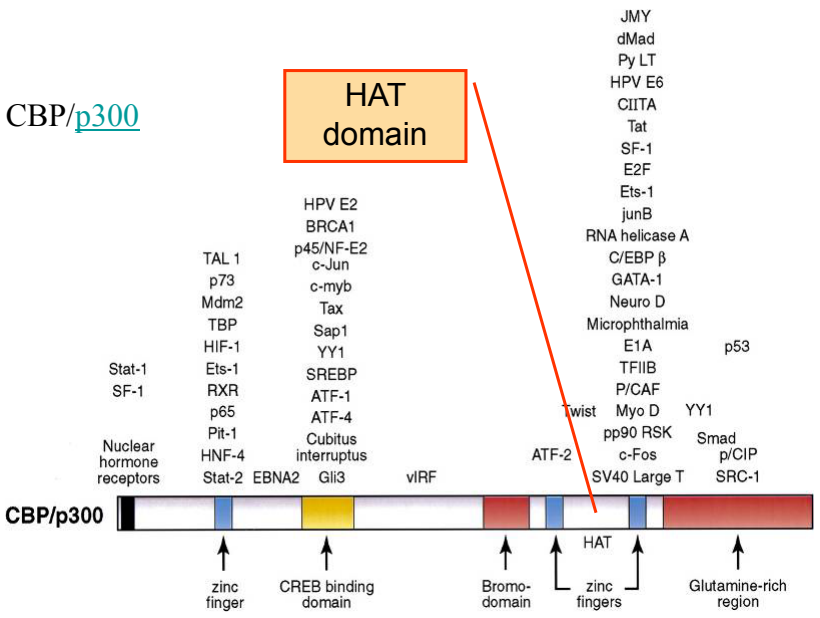
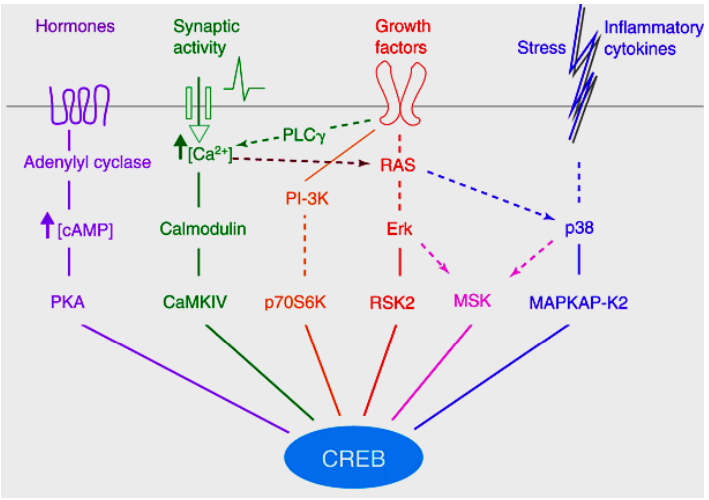


Figure 4 Multiple domains of CREB contribute to transcriptional activation. Different domains of CREB bind distinct coactivators and basal transcription factors to activate transcription. Shown is a CREB dimer bound to its cognate CaRE/CRE element on the promoter of a CREB target gene. Downstream of 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.

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



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

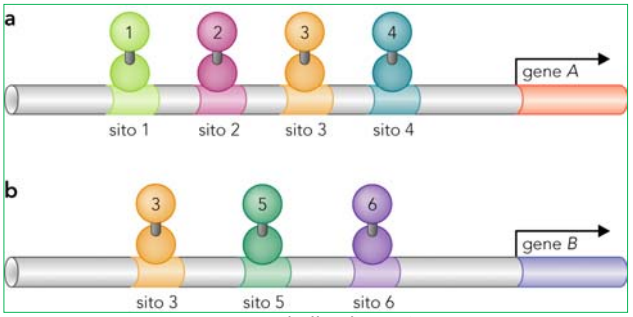


Integration by combinatorial binding to enhancers and promoters

Il controllo trascrizionale viene realizzato con la combinazione di un numero limitato (ancorchè assai grande) di fattori trascrizionali leganti il DNA.

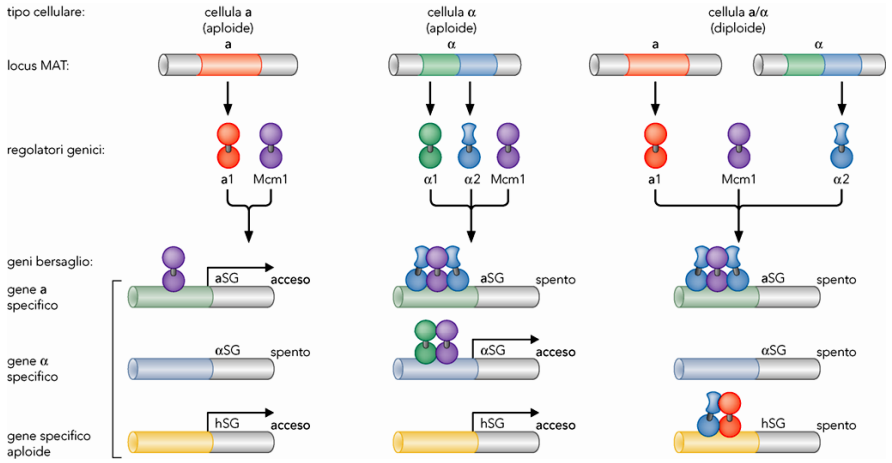
Ogni regione di controllo è formata dalla giustapposizione di diversi elementi in un ordine spaziale specifico.

I fattori trascrizionali interagiscono fisicamente o / e funzionalmente tra di loro



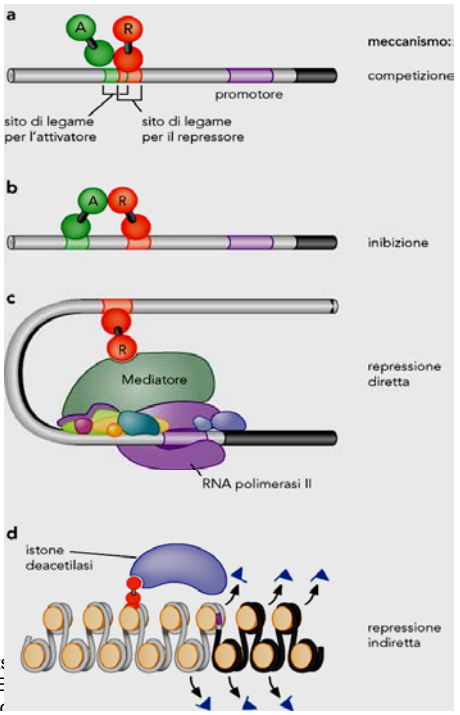
Zanichelli editore S.p.A.

Proteins encoded by the MAT locus in S. cerevisiae



Watson et al., BIOLOGIA MOLECOLARE DEL GENE, Zanichelli editore S.p.A.

Diverse modalità di repressione della trascrizione osservate



Il risultato trascrizionale (attivazione o repressione) non è intrinseco al fattore trascrizionale stesso, ma è frutto del contesto genico nel quale esso agisce, come risultato delle interazioni con altri fattori.

Wats
MOLE
Zapi

Combinatorial control of gene expression

Attila Reményi[1,2,4], Hans R Schöler[1,3] & Matthias Wilmanns[2]

Revealing the molecular principles of eukaryotic transcription factor assembly on specific DNA sites is pivotal to understanding how genes are differentially expressed. By analyzing structures of transcription factor complexes bound to specific DNA elements we demonstrate how protein and DNA regulators manage gene expression in a combinatorial fashion.

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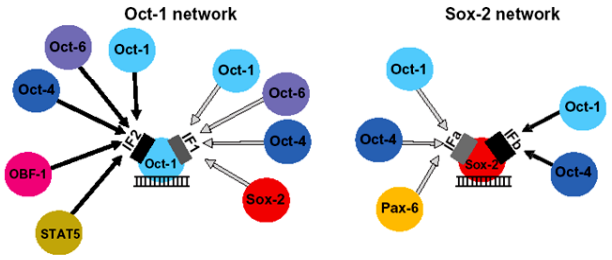
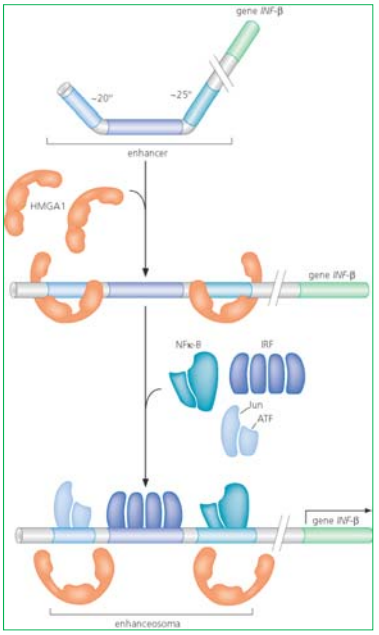
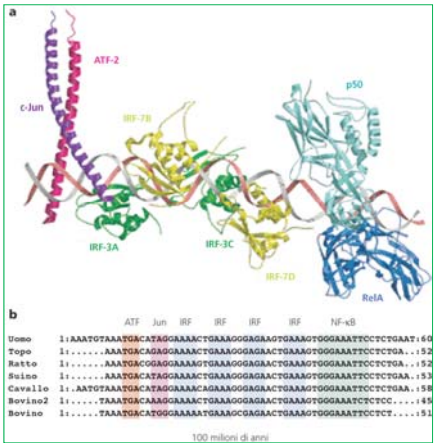


Figure 3 Interaction diagram of Oct-1 and Sox-2. Transcription factors are depicted as protein molecules with surface patches that can interact with a whole array of different partners provided that the protein is bound to a specific DNA element. DNA-bound Oct-1 and Sox-2 are depicted schematically with protein-protein interaction surface patches that are instrumental in binding to other partners. IF1 and IF2 on the Oct-1–DNA complex denote two interfaces of Oct-1 that are accessible and used for interaction on various DNA. Similarly, IFa and IFb designate interfaces of Sox-2 that are used for interaction on different DNA sites.

Transcription factor interaction is specific and assisted by architectural proteins..

This example refers to the enhanceosome of the IFN-β gene. HMGA-1 proteins bind to specific sequences and bent DNA in order to allow proper interaction between different transcription factors bound to DNA

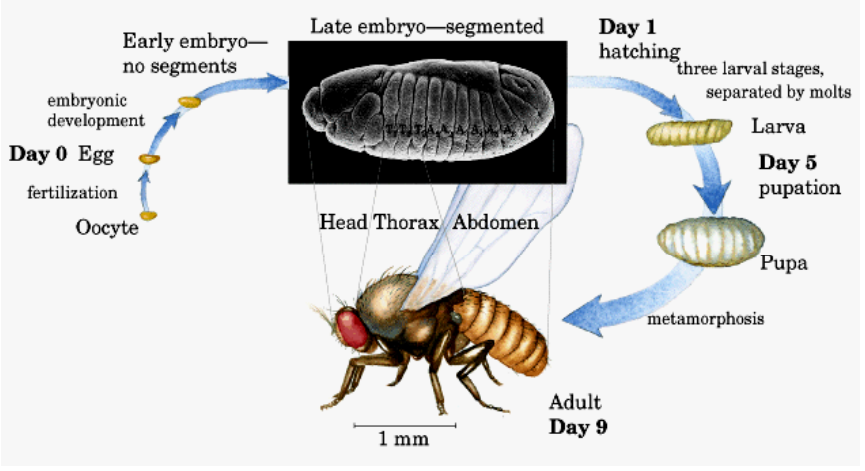


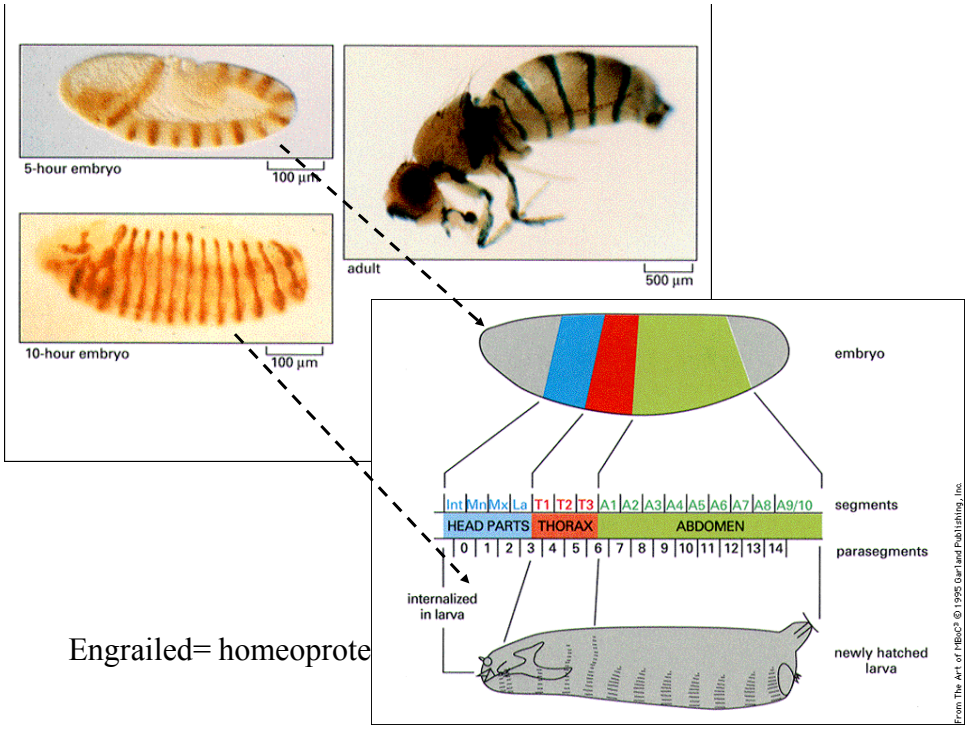


Molti dei concetti di “composizionalità” e “combinatorialità” degli elementi di regolazione trascrizionale deriva da

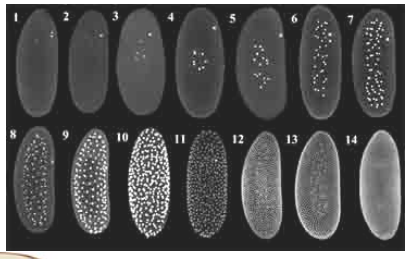
biologia molecolare dello sviluppo

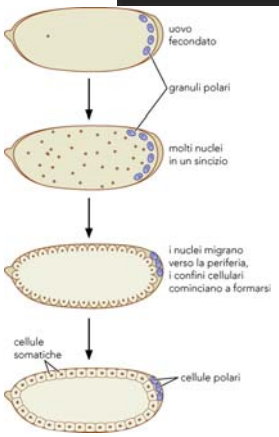
in particolare dal modello di *Drosophila melanogaster*

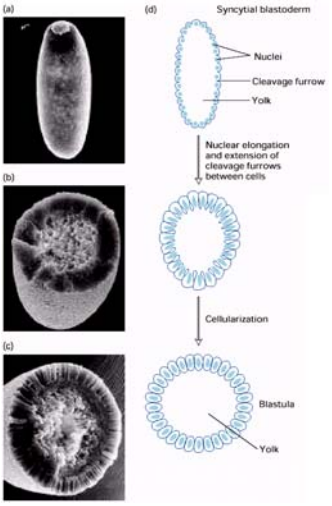




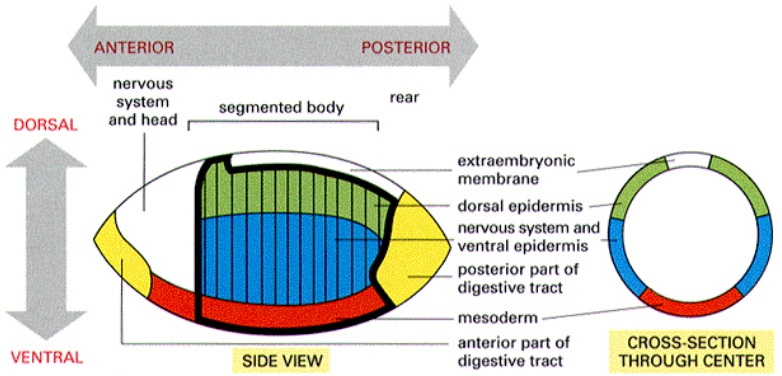
Engrailed= homeoprote

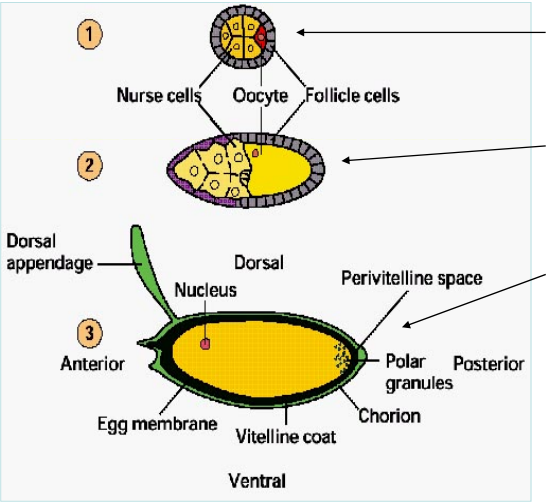






Very early, the developmental axes are defined, as well as territories.





Each developing unit, or follicle, consists of a developing oocyte, nurse cells and a layer of somatic cells called follicle cells.

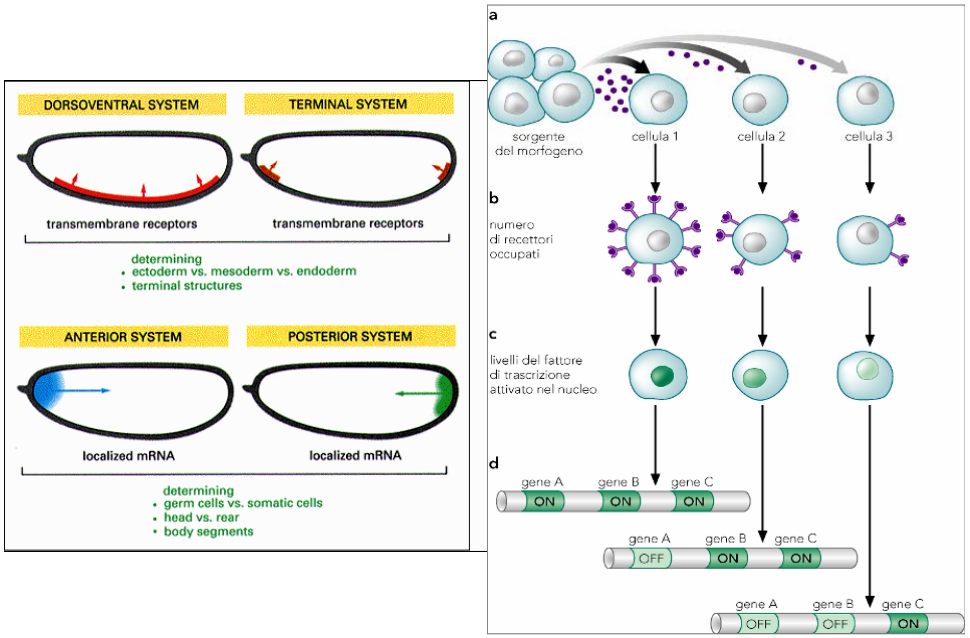
Stage 1: Early in oogenesis, the oocyte is about the same size as the neighboring nurse cells.

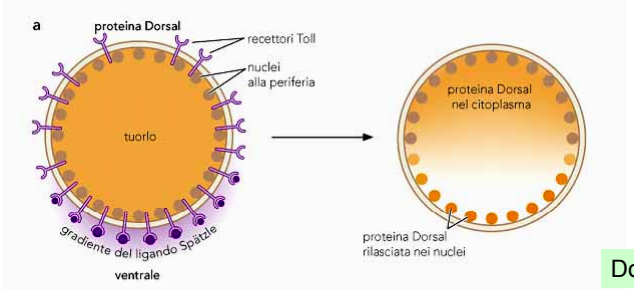
Stage 2: The nurse cells begin to synthesize mRNAs and proteins necessary for oocyte maturation, and the follicle cells begin to form the egg shell.

Stage 3: The mature egg is surrounded by the vitelline coat and chorion, which compose the egg shell. The nurse cells and follicle cells have been discarded, but some of the mRNAs synthesized by nurse cells, which become localized in discrete spatial domains of the oocyte, function in early patterning of the embryo.

Polar granules are disinct cytoplasmic structures located in the posterior region of the egg. This is the region in which germ cells arise.

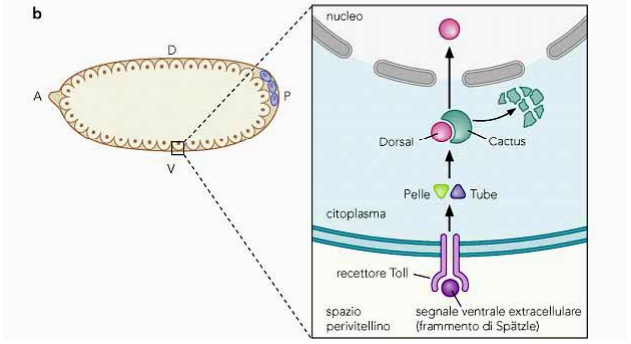
Several **morphogenetic gradients** are present



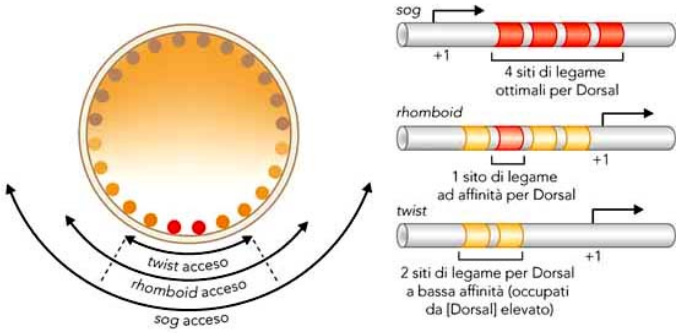


Dorso-ventral axis

Dorsal is NFkB homologue

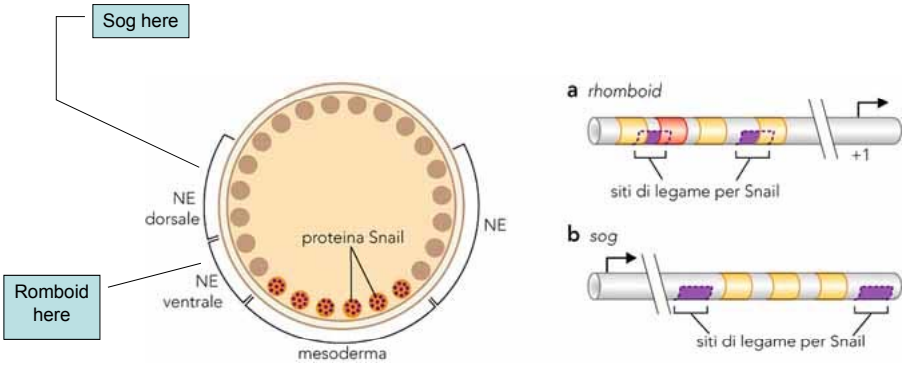


geni regolati da dorsal



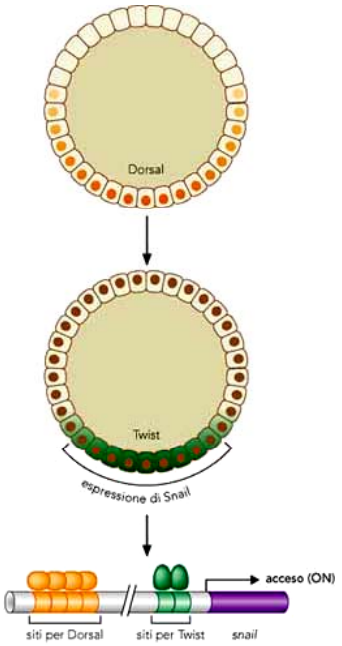
Twist 5' contains 2 low affinity sites for Dorsal (bound only were Dorsal is higher)
Rhomboid 5' enhancer cotains several sites: only one is high-affinity: it is on at high or intermediate levels of Dorsal.
Soh intronic enhancer contains 4 high-affinity dorsal sites: on in all cells where dorsal is present

In ventral cells, twist also stimulate expression of the Snail repressor:

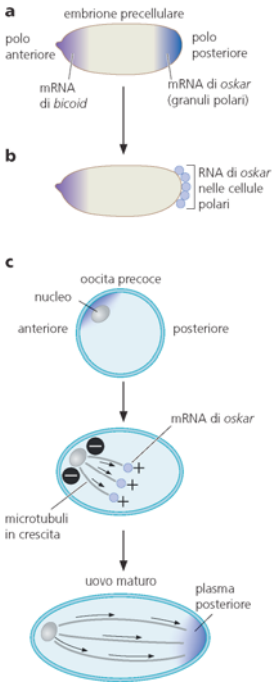


Snail è il repressore primario dei geni epiteliali (es. E-cadherin)

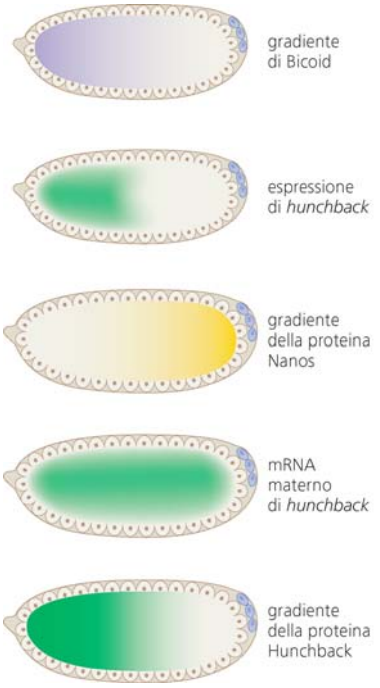
Snail is activated by **synergy** between Twist and dorsal

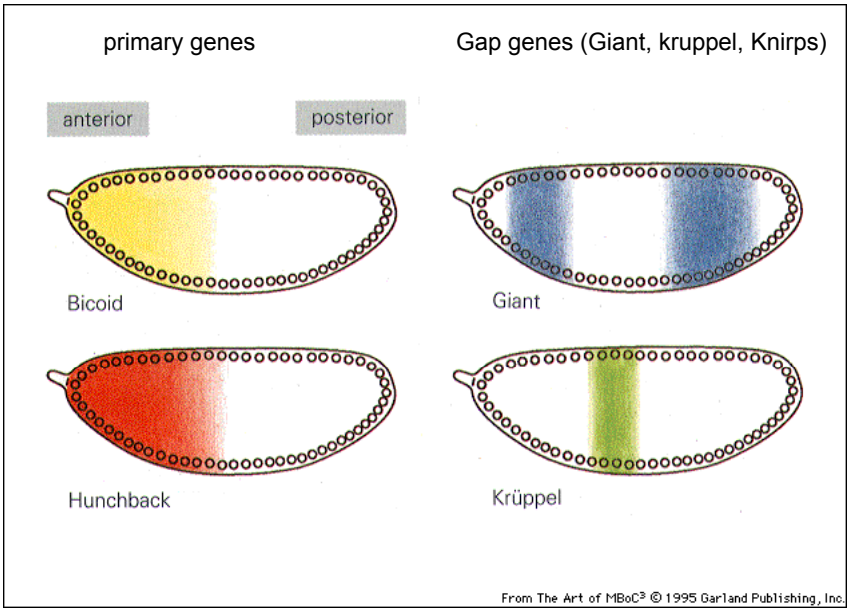


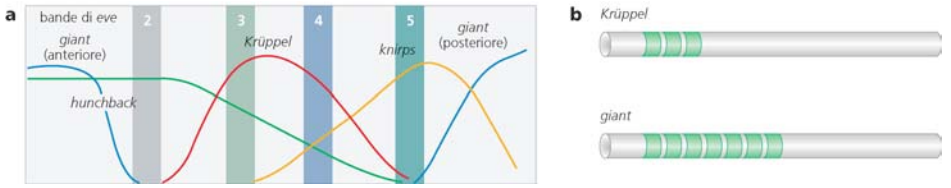
Antero-posterior axis



nanos è un repressore traduzionale di hunchback

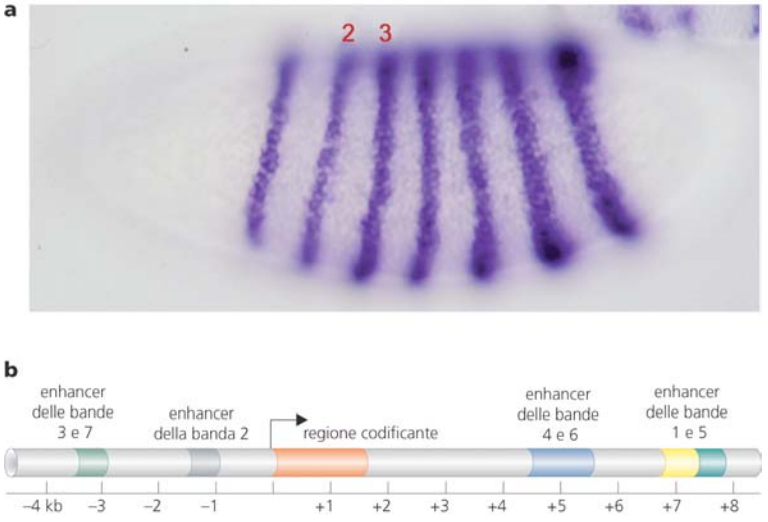


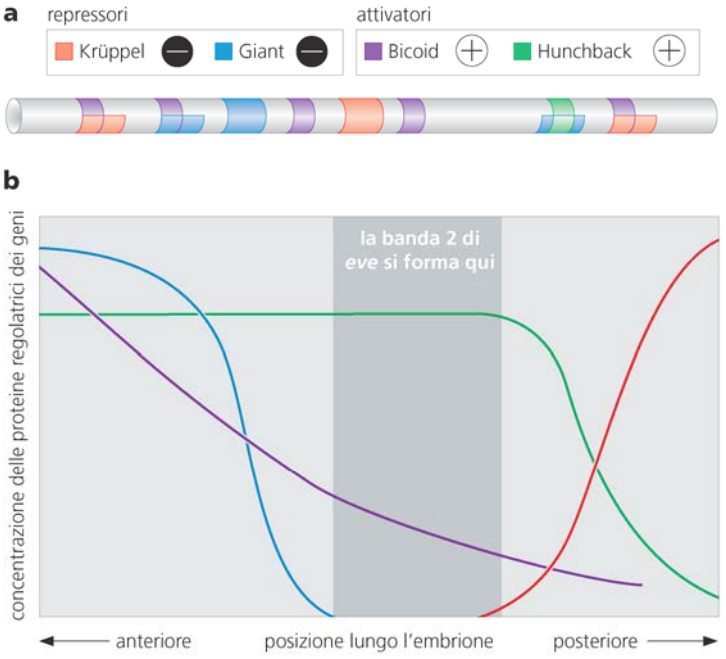


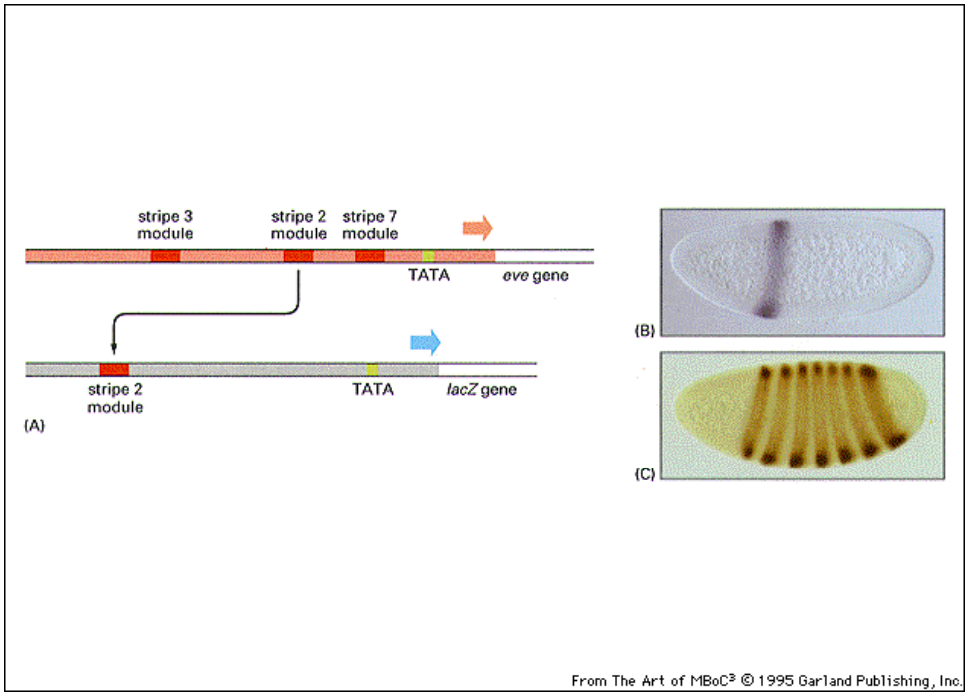


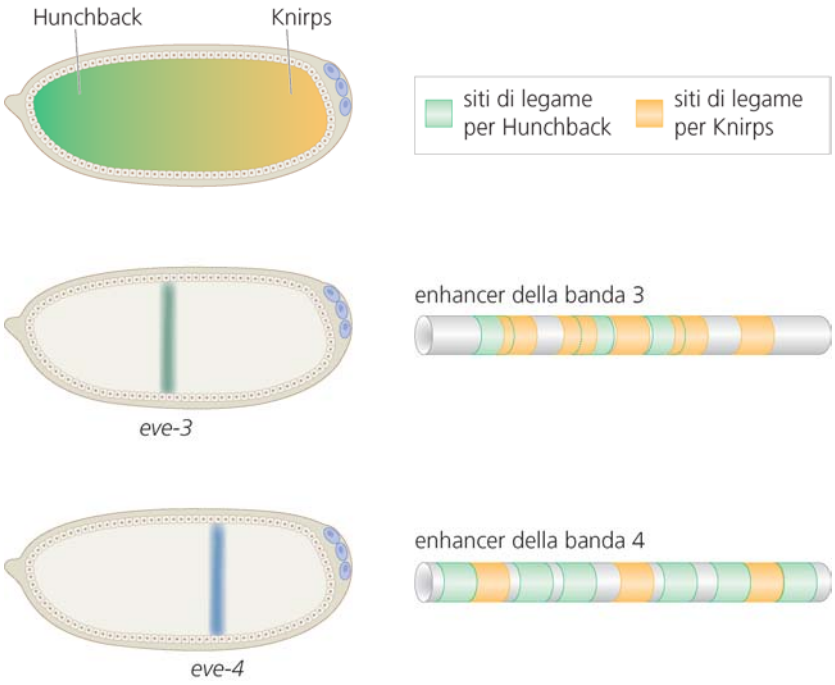
Hunchback is a repressor:
Kruppel has few sites, requires higher levels
Giant has more sites: lower levels are enough

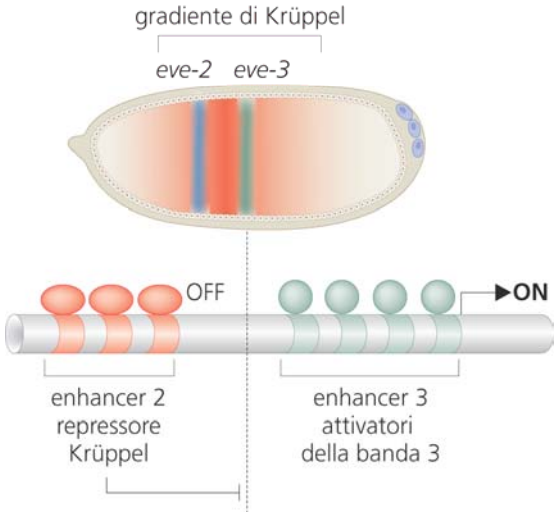
Eve (even-skipped) is the first “pair-rule” segmental gene: it has more than 12Kb essential regulatory sequences
Each enhancer is regualted by the exact combination of factors present in stripes

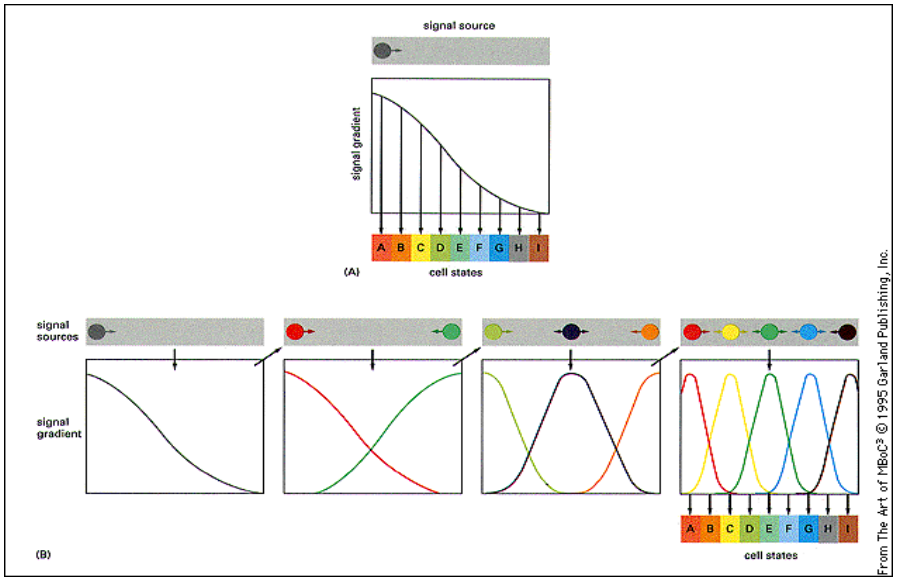



a
repressori
Krüppel
Giant
attivatori
Bicoid
Hunchback
b
concentrazione delle proteine regolatrici dei geni
la banda 2 di
eve si forma qui
anteriore
posizione lungo l'embrione
posteriore


stripe 3
module
stripe 2
module
stripe 7
module
TATA
eve gene
stripe 2
module
TATA
lacZ gene
(A)
(B)
(C)
From The Art of MBoC³ © 1995 Garland Publishing, Inc.


Hunchback
Knirps
siti di legame per Hunchback
siti di legame per Knirps
enhancer della banda 3
eve-3
enhancer della banda 4
eve-4


gradiente di Krüppel
eve-2
eve-3
OFF
ON
enhancer 2
repressore
Krüppel
enhancer 3
attivatori
della banda 3

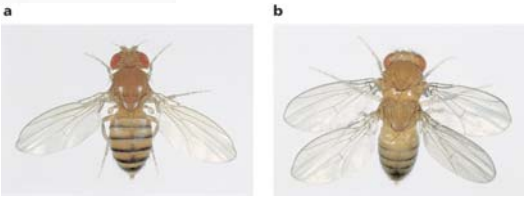


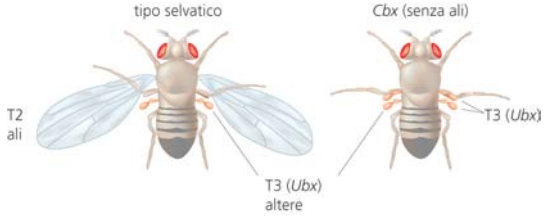
Risposte secondarie a gradienti primari possono generare segmentazione

Homeotic gene mutations

Antennapedia (Antp)

Ultrabitorax (Ubx)





Contrabitorax (Cbx)

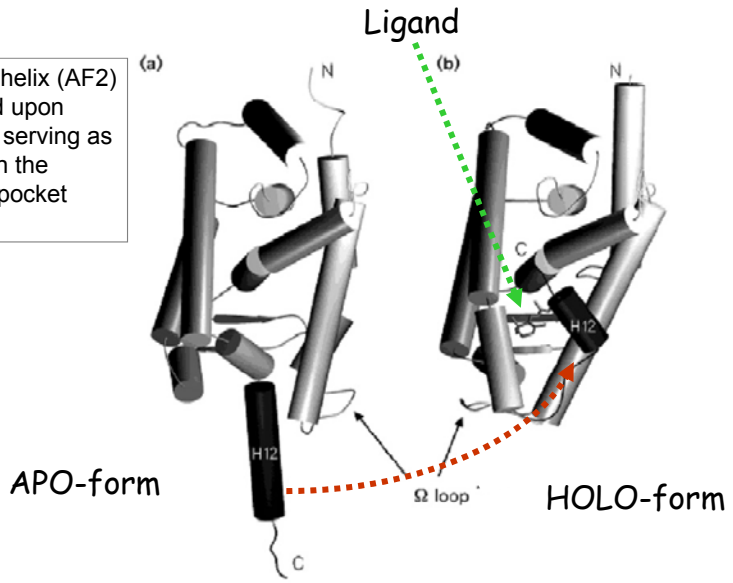
The activity of a given transcription factor can be modulated (up to inverted) by action of cross-talkin signals that affect co-activator or co-repressor activity, by different mechanisms (degradation, phosphorylation, nucleo-cytoplasmatic distribution, intranuclear localization, and other).

One example is given by the inversion of steroid antagonistic drug by activation of different signal transduction pathway.

The case of anti-androgen and anti-estrogen reversion due to activation of the inflammatory.

All starts from the conformational change that is induced by ligand binding to the C-terminal domain of nuclear receptors.....

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



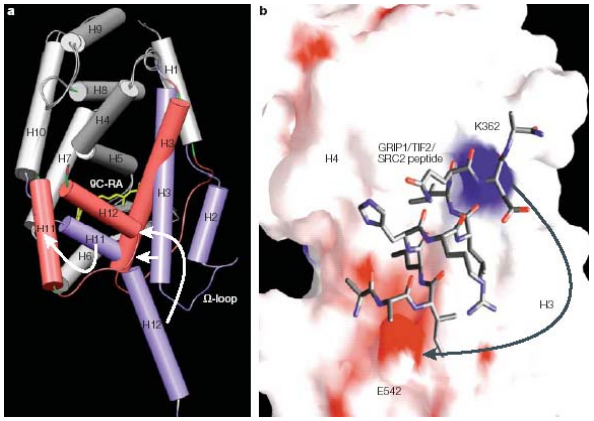


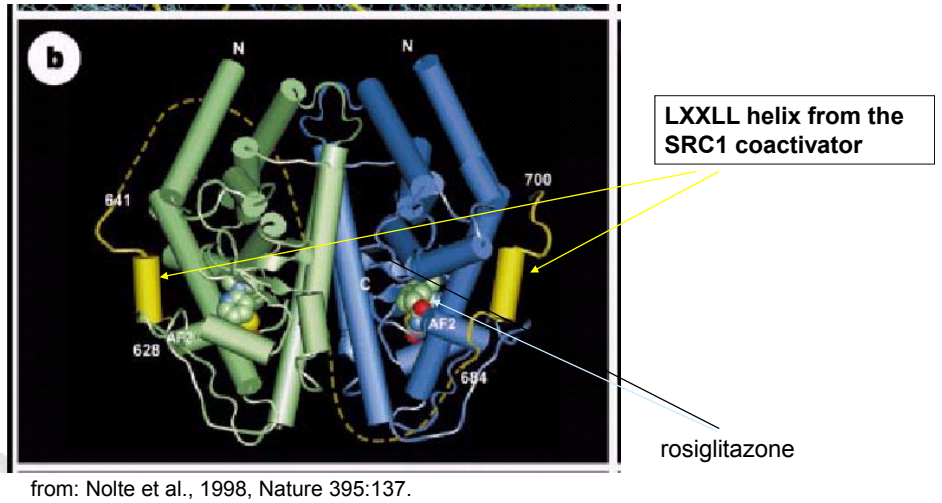
Figure 2 | Ligand binding induces a conformational change of the ligand-binding domain structure of nuclear receptors.

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

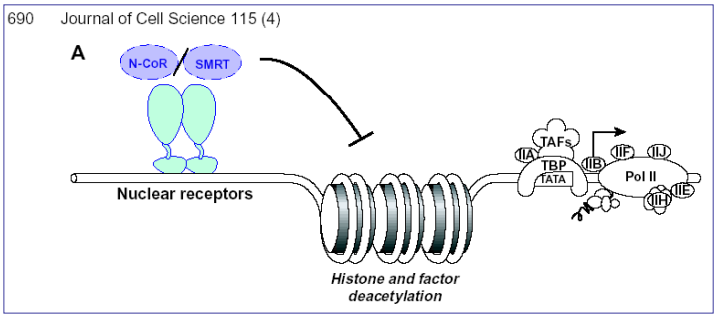


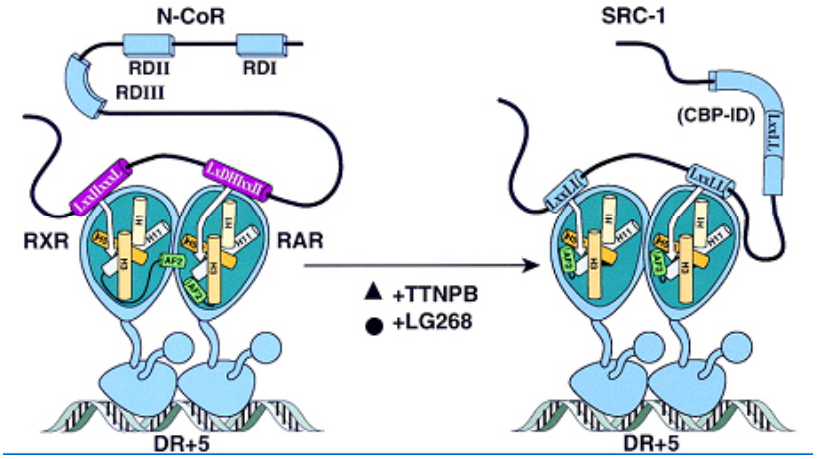
from: Nolte et al., 1998, Nature 395:137.

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, RARγ, 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

The apo-form of certain NR binds to co-repressors, instead than co-activators





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.

Binding of antagonists induce a specific conformational change

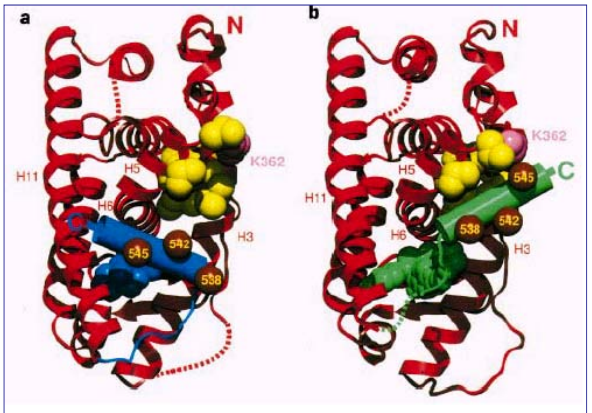
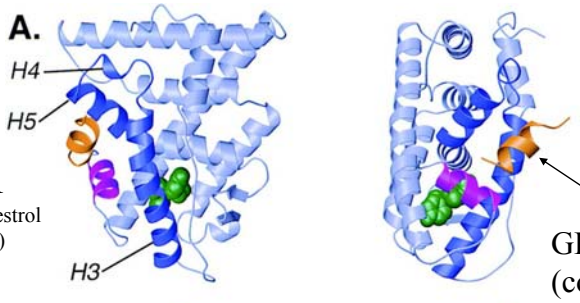


Figure 3 Positioning of helix H12. Position is shown in **a**, the ER LBD–E_2 complex; and **b**, the ER LBD–RAL complex. H12 is drawn as a cylinder and coloured blue (E_2 complex) or green (RAL complex). The remainder of the ER LBD is shown in red. Dotted lines indicate unmodelled regions of the structures. 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

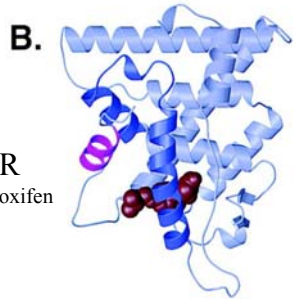
Cn3D E2

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

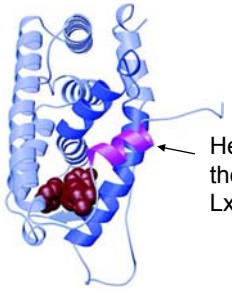


DES-ER
(diethylstilbestrol is estrogenic)

GRIP NR box II
(coactivator LxxLL)



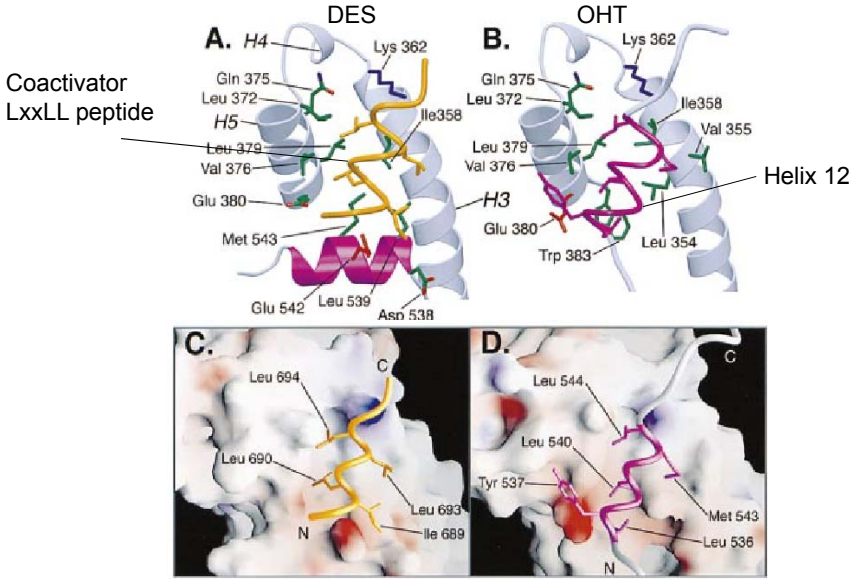
OHT-ER
4-OH-Tamoxifen



Helix 12 occupies the same position as LxxLL above

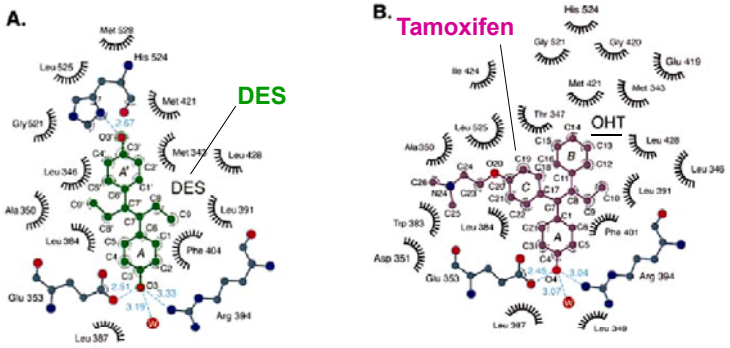
from Shiau et al., 1998, Cell 95: 927.

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.

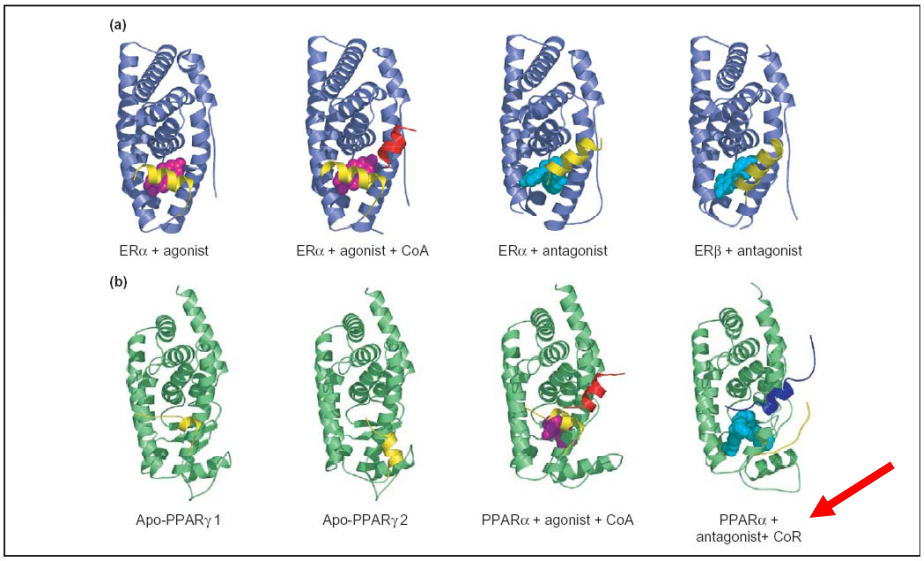


Figure 2. Effect of ligands and co-regulators on the position of helix 12. The top row of structures shows the oestrogen receptor (ER) bound to agonists and antagonists (PDB codes 1ERE, 1ERR, 1GWR and 1QKM) [19,63]. Note that in the antagonist-bound structures helix 12 adopts a position very similar 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.

ChIP studies have demonstrated that Tamoxifen- or Raloxifen-bound ER, as well as flutamide- or bicalutamide-bound AR, **interact with NCoR** instead of co-activators.

For this reason, genes that are normally activated by estrogen or androgen are repressed by anti-estrogen or anti-androgen

However, this action may critically depend upon other signal pathways....

Macrophage/Cancer Cell Interactions Mediate Hormone Resistance by a Nuclear Receptor Derepression Pathway

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A short résumé of the paper is included (Rev Zhu 2006)

SUMMARY

Defining the precise molecular strategies that coordinate patterns of transcriptional responses to specific signals is central for understanding normal development and homeostasis as well as the pathogenesis of hormone-dependent cancers. Here we report specific prostate cancer cell/macrophage interactions that mediate a switch in function of selective androgen receptor antagonists/modulators (SARMs) from repression to activation in vivo. This is based on an evolutionarily conserved receptor N-terminal L/HX$_7$LL motif, selectively present in sex steroid receptors, that causes recruitment of TAB2 as a component of an N-CoR corepressor complex. TAB2 acts as a sensor for inflammatory signals by serving as a molecular beacon for recruitment of MEKK1, which in turn mediates dismissal of the N-CoR/HDAC complex and permits derepression of androgen and estrogen receptor target genes. Surprisingly, this conserved sensor strategy may have arisen to mediate reversal of sex steroid-dependent repression of a limited cohort of target genes in response to inflammatory signals, linking inflammatory and nuclear receptor ligand responses to essential reproductive functions.