Nuclear Receptors

Genome-wide localization of Estrogn Receptor-binding sites: mostly far away from promoters, ERE in a fraction of these loci, accompanied by other TF binding sequences, depending on the regulatory class of genes.

Recent mapping of Retinoic Acid Receptor showed very similar patterns

Ligand-regulated TFs (Class I: steroid hormone receptors; Class II: T3, VitD, RA+Rds, lipophylic, xenobiotics; Class III:orphan)

Ligand binding to LBD modulates the LBD surface, changing the relative affinity of receptors for coactivators and corepressors

Nuclear Receptors (NRs)

regulatory proteins that mediate cellular responses to:

Hormones

Vitamins

Retinoids

intracellular lipophilic molecules

xenobiotics

NRs: nuclear receptors are a large family if regulatory proteins that mediate cellular responses to: Hormones, Vitamins, Retinoids, intracellular lipophilic molecules, xenobiotics

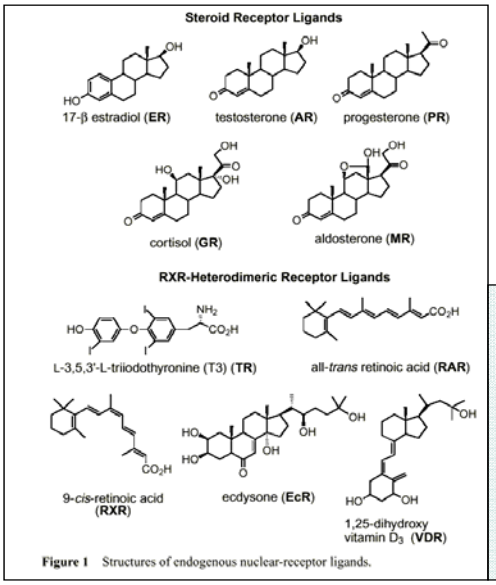


Figure 1 Structures of endogenous nuclear-receptor ligands.

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
Retnoid 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)

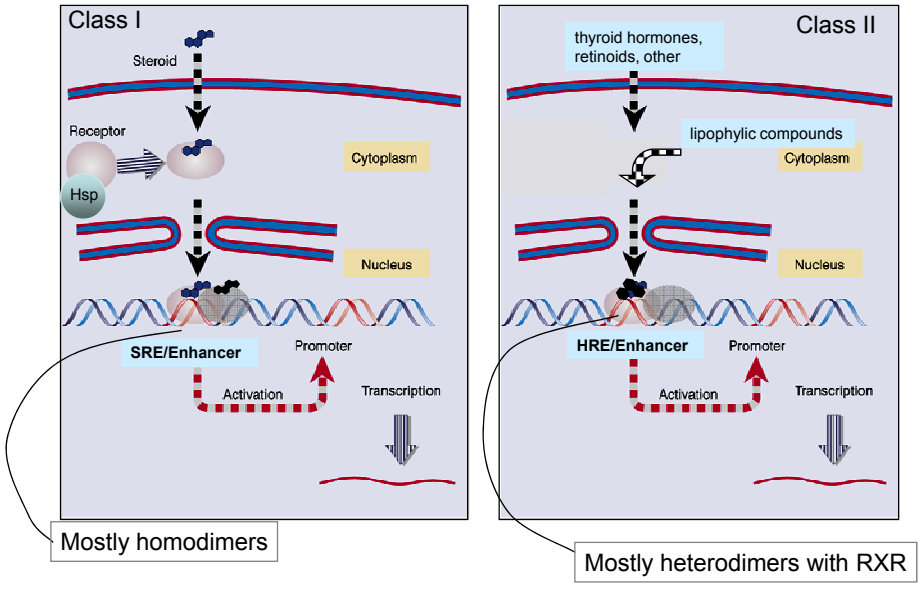
NRs classification

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

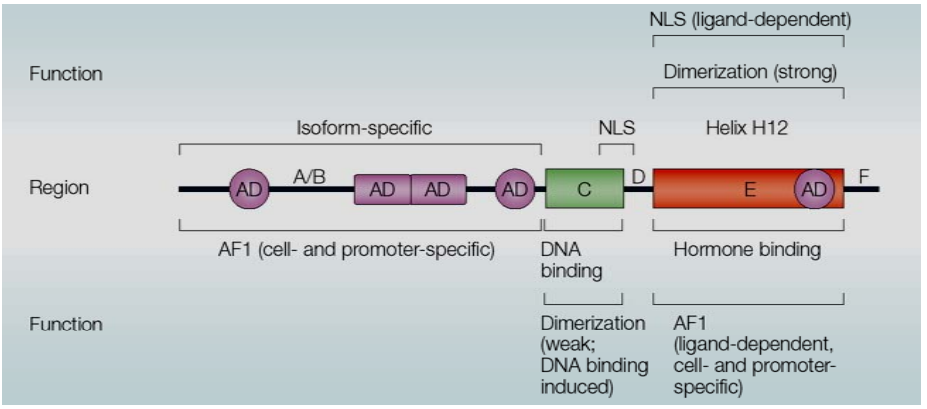
In H. sapiens there are 48 known nuclear receptor genes.

24 have known ligands 24 are orphan receptors

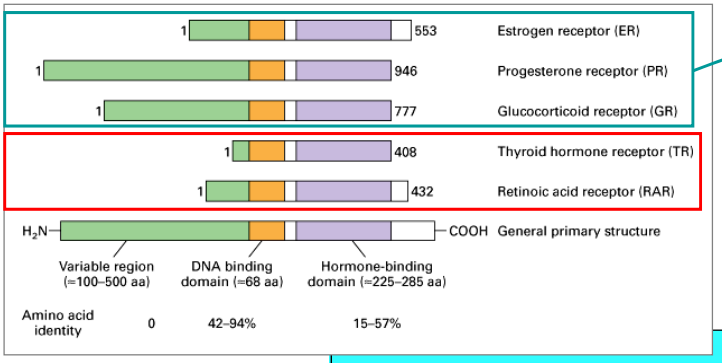
The simplest scheme of NRs mechanism of action

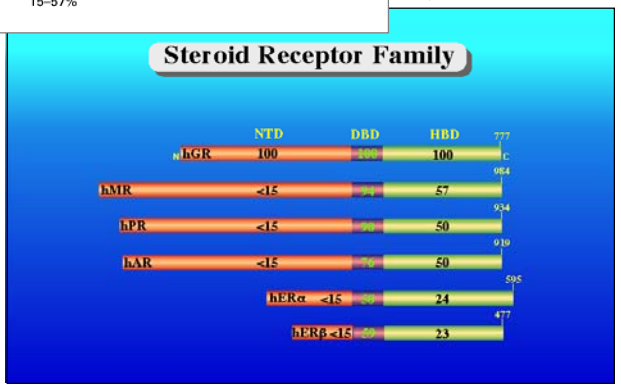


1 - Structure



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





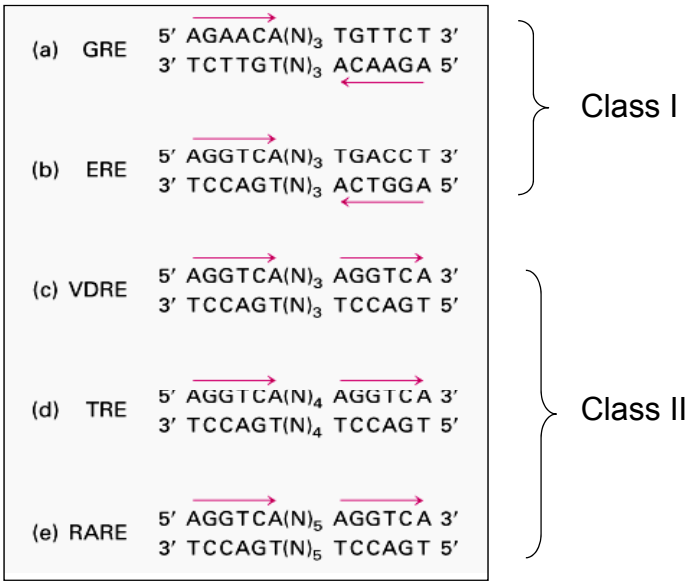
1st important difference:

Class 1 has longer N-term
Class 2 reduced

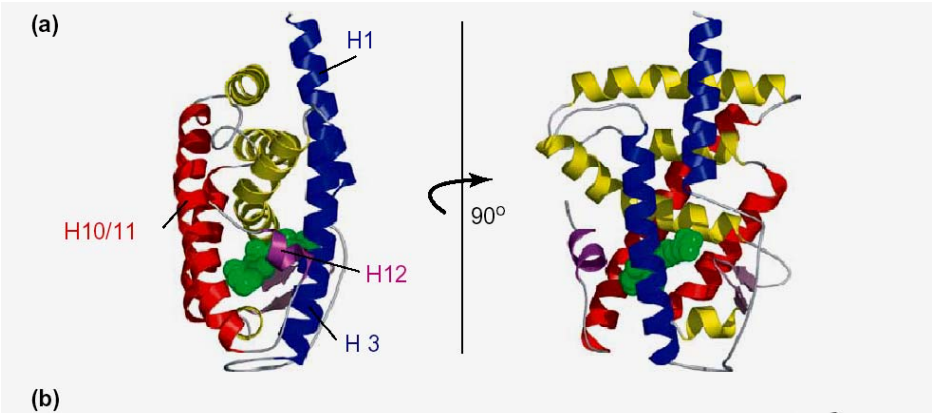
Class 1 A/B domain has transactivation activity

Class 2 A/B does not

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

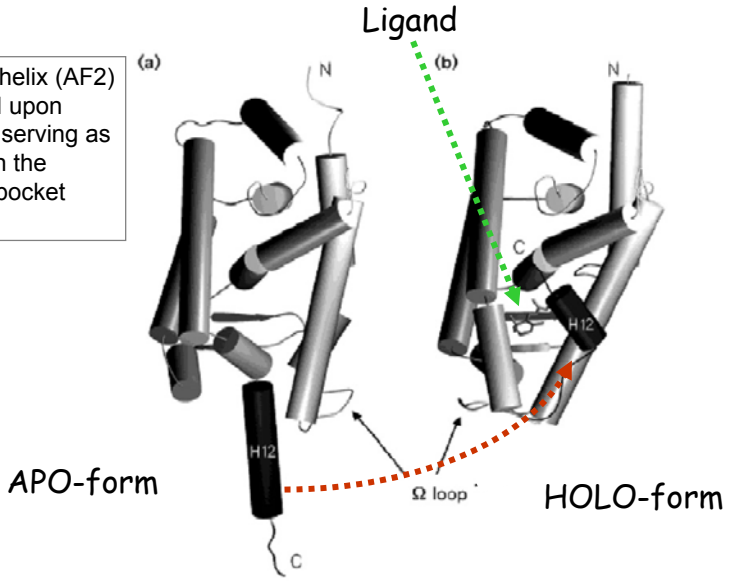


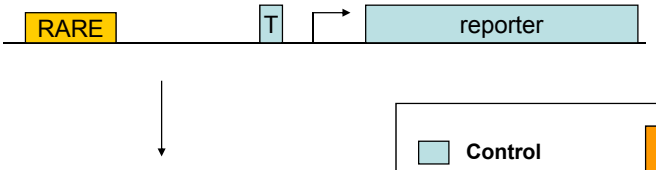
The Ligand-binding domain (LBD)



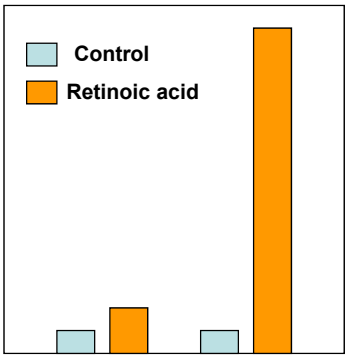
Cn3D model

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



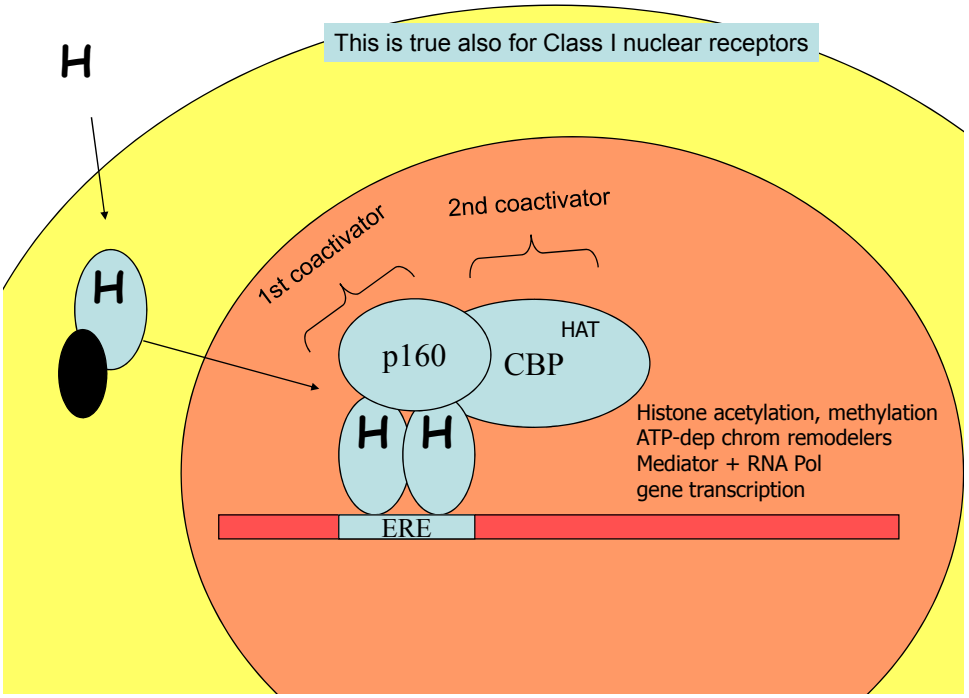


In vitro transcription with
RNAPol, TFIID, A, B, F, H, RAR, RXR



Nuclear extract - - + +

The function of nuclear receptor needs cofactors (we call these cofactors in general “coregulators” (either “Coactivators” or “corepressors”).



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**”

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

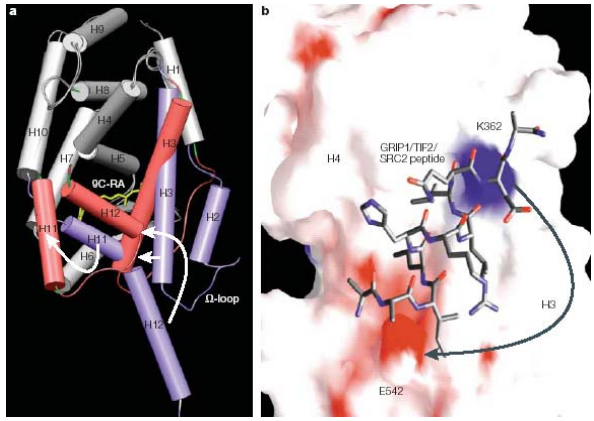


Figure 2 | Ligand binding induces a conformational change of the ligand-binding domain structure of nuclear receptors.

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.

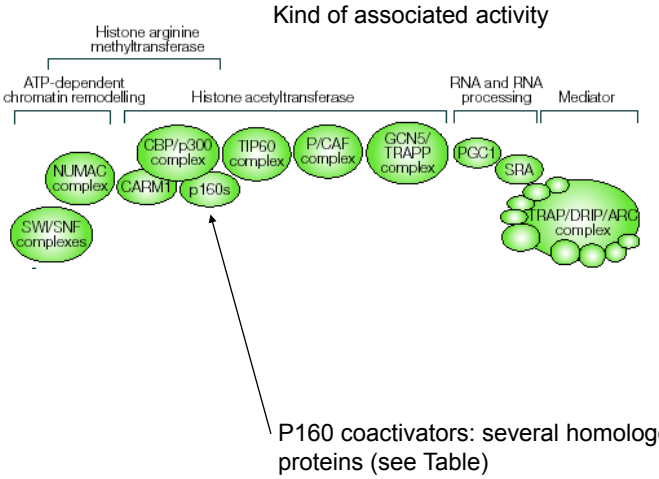
The figure reveals the ligand-induced trans-conformation that generates the transcriptionally active form of the receptor. The coloured helices H2, H3, H11 and H12 (purple in the apo-form; red in the holo form) are relocalized during the conformational change. In this model, ligand binding induces a 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.

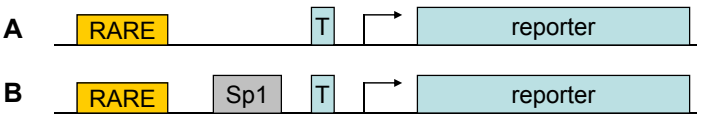
b | The co-activator nuclear-receptor box LxxLL peptide-binding surface on the ERα–diethylstilbestrol (DES) complex is shown in white. A charge clamp (that is, charged amino acids that interact with both ends of the peptide and increase the strength of the interaction; the two ends of the clamp are indicated 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.

Table II. Nuclear receptor co-activators

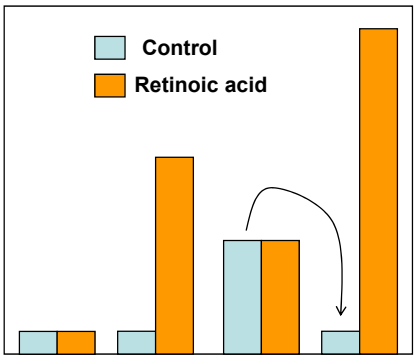
CO-ACTIVATOR		INTERACTING NUCLEAR RECEPTORS	
SRC-1/NcoA-1	Steroid receptor co-activator-1/Nuclear receptor co-activator-1	PR, GR, ER, TR, RXR, HNF-4, PPARγ, RAR	P160 family
TIF2/GRIP1/NcoA-2	Transcriptional intermediary factor-2/ Glucocorticoid receptor-interacting protein-1/ Nuclear receptor coactivator-2	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	

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





Transfection in cells that do not possess RAR

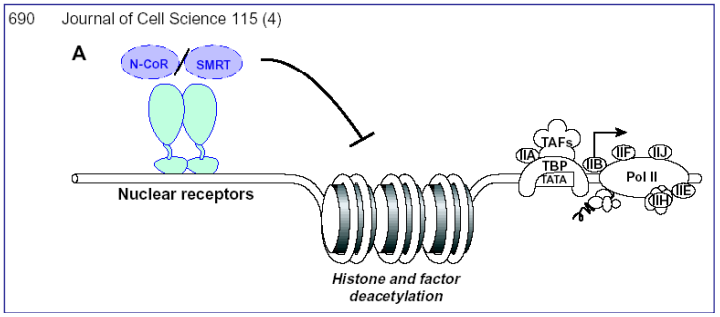


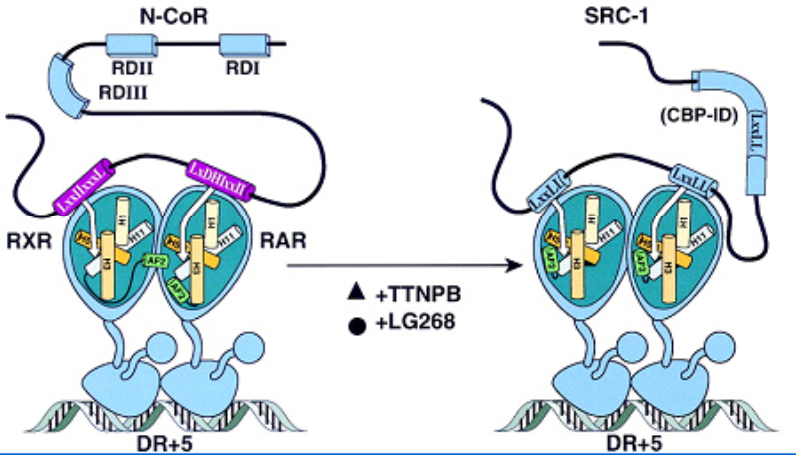
Vector expressing RAR: - + - +

Reporter A Reporter B

Table III. Nuclear receptor co-repressors

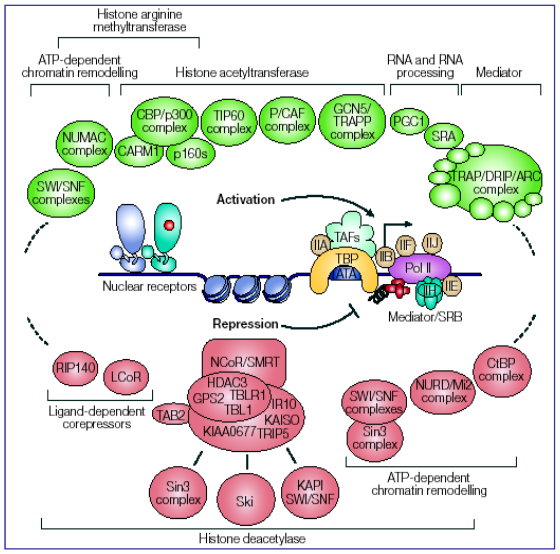
CO-REPRESSOR		INTERACTING NUCLEAR RECEPTORS
NCoR/RIP-13	Nuclear Receptor co-repressor/ Retinoid X receptor interacting protein-13	TRα, RARα, RARγ, Rev-Erb, COUP-TFs, DAX-1
SMRT/TRAC2	Silencing mediator for retinoic acid and thyroid hormone receptors/ T_3 receptor-associated cofactor 2	RARα, RARγ, TR:RXR, 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





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.

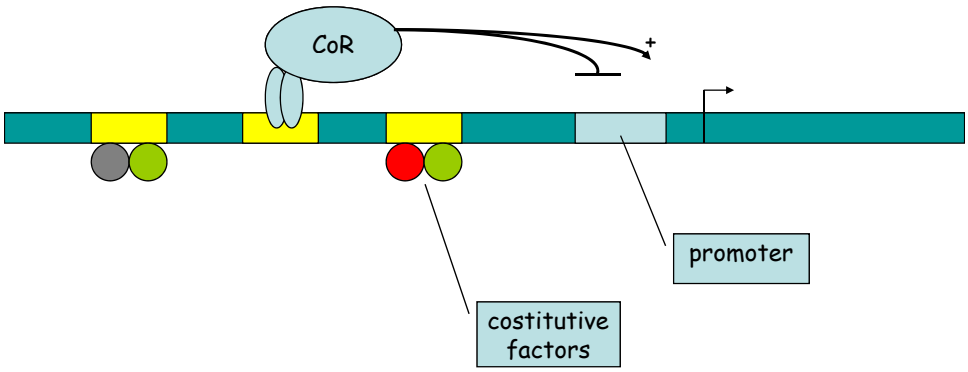


Coactivator and corepressor complexes are required for nuclear receptor-mediated transcriptional regulation. The regulation of a general transcription unit by nuclear receptors requires a vast number of co-regulatory complexes that have various functions and enzymatic activities. Coactivator complexes (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.

On-off genes:

A corepressor-coactivator exchange takes place under activation



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

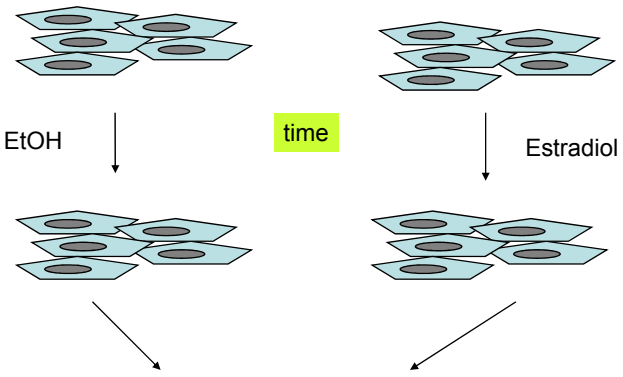
Estrogen Receptor-α Directs Ordered, Cyclical, and Combinatorial Recruitment of Cofactors on a Natural Target Promoter

Raphaël Métivier,[1,2,4,*] Graziella Penot,[1,2] Michael R. Hübner,[1] George Reid,[1] Heike Brand,[1] Martin Kos,[1,3] and Frank Gannon[1,*]
[1]European Molecular Biology Laboratory (EMBL)
Meyerhofstrasse 1
D-69117 Heidelberg
Germany

ARTICOLO

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.



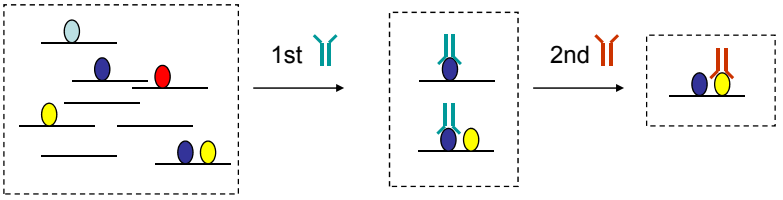
Are **all** these proteins present at the **same** gene promoter at the **same** time in the **same** cell ?

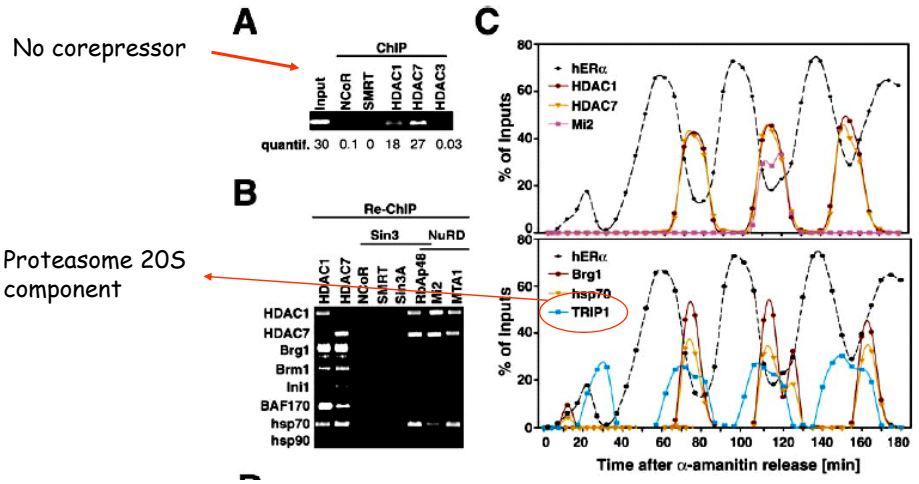
Are they present in different cells in the same population ?

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





Molecular Cell, Vol. 11, 695–707, March, 2003, Copyright ©2003 by Cell Press

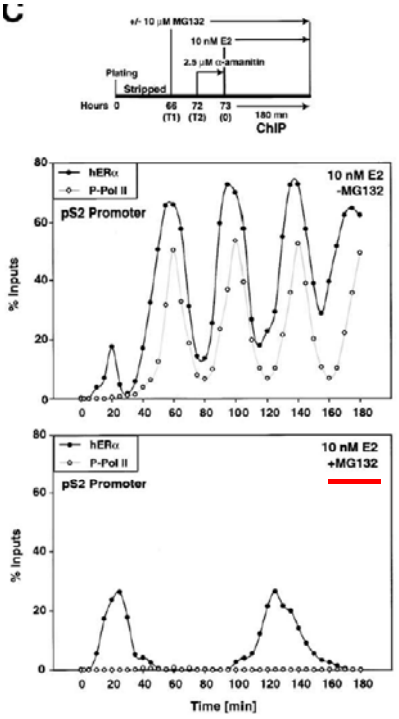
Cyclic, Proteasome-Mediated Turnover of Unliganded and Liganded ERα on Responsive Promoters Is an Integral Feature of Estrogen Signaling

George Reid,[1] Michael R. Hübner,[1] Raphaël Métivier,[1] Heike Brand, Stefanie Denger, Dominique Manu, Joël Beaudouin, Jan Ellenberg, and Frank Gannon*
European Molecular Biology Laboratory
Meyerhofstrasse 1
D-69117 Heidelberg
Germany

Summary

We present an integrated model of hERα-mediated transcription where both unliganded and liganded receptors cycle on estrogen-responsive promoters. Using ChIP, FRAP, and biochemical analysis we evaluate hERα at several points in these cycles, establishing the ubiquitination status and subnuclear distribution of hERα, its mobility, the kinetics of transcriptional activation, and the cyclic recruitment of E3 ligases and the 19S regulatory component of the proteasome. These experiments, together with an evaluation of the inhibition of transcription and proteasome action, demonstrate that proteasome-mediated degradation and hERα-mediated transactivation are inherently linked and act to continuously turn over hERα on responsive promoters. Cyclic turnover of hERα permits continuous responses to changes in the concentration of estradiol.

from: Reid et al. (2003) Mol Cell 11: 695-707.



Liganded ER cycling depend on **proteasome** activity. Proteasome inhibitors also inhibit transcription

Figure 5. Inhibition of Proteasome Activity Also Prevents the Cycling of Transcriptionally Active hER on Responsive Promoters

(C) Transcription from the pS2 promoter was synchronized by treatment for 1 hr with -amanitin following 6 hr pretreatment with MG132 or vehicle control. Quantitative ChIP assays were then performed following the addition of E2 and the removal of -amanitin. Values from three independent experiments, performed in duplicate (SEM 2%) are shown. Following an initial unproductive cycle, liganded hER cycles on the pS2 promoter and recruits phosphorylated polymerase II. Inhibition of proteasome activity both reduces and delays association of liganded hER with the pS2 promoter.

from: Reid et al. (2003) Mol Cell 11: 695-707.

Unliganded ER cycles on the pS2 promoter in a proteasome-dependent fashion

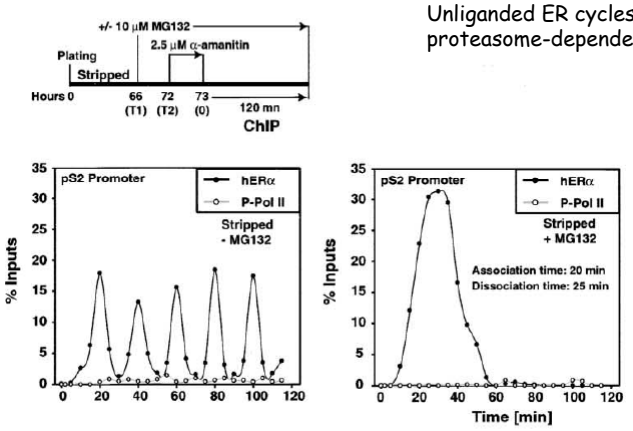


Figure 4. Inhibition of Proteasome Activity Prevents Cycling of Transcriptionally Inactive hERα on Responsive Promoters

(A) Transcription from the pS2 promoter was synchronized by treatment for 1 hr with α-amanitin following 6 hr pretreatment with MG132 or vehicle control. Quantitative ChIP assays were then performed following removal of α-amanitin. Values from three independent experiments, performed in duplicate (SEM <2%), are shown. Inhibition of proteasome activity precludes cycling of unliganded hERα on the pS2 promoter.

from: Reid et al. (2003) Mol Cell 11: 695-707.

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

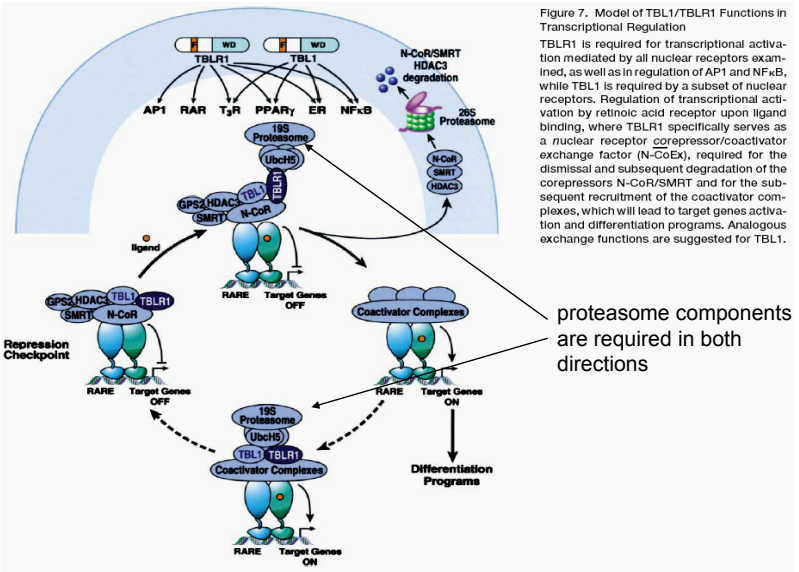


Figure 7. Model of TBL1/TBLR1 Functions in Transcriptional Regulation

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 nuclear receptor corepressor/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

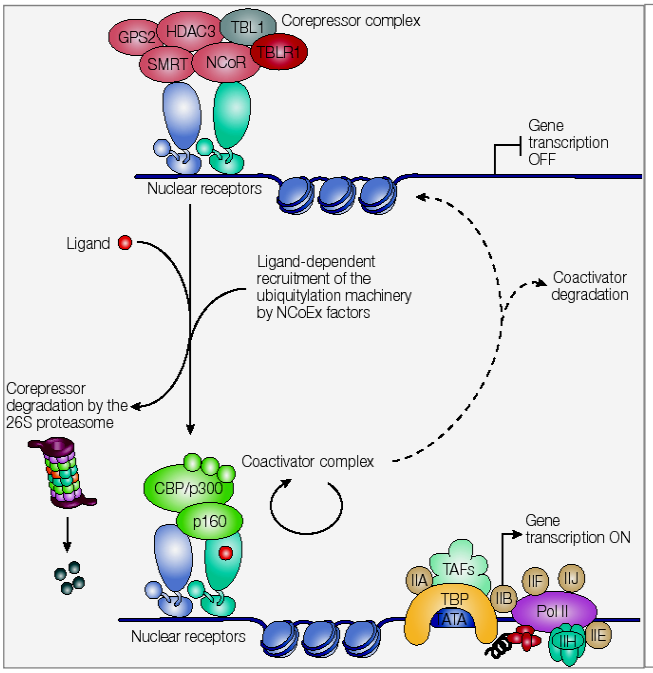


Figure 4 | Ubiquitin-dependent exchange of corepressors for coactivators. During the transition from gene repression to gene activation on ligand stimulation, there is a required exchange among cofactor complexes. This molecular switch is regulated by conformational changes in the nuclear receptor, which results in a different affinity for cofactor complexes, and by the recruitment of the ubiquitylation machinery, which has a fundamental role in the dismissal of the corepressor machinery. The diagram shows a generic, unliganded nuclear receptor heterodimer that recruits the NCoR and SMRT corepressor complexes to repress transcription. The nuclear corepressor exchange (NCoEx) factors TBL1 and TBLR1 are required to recruit the ubiquitylation machinery following ligand stimulation, thereby allowing the dismissal and degradation of the corepressor complex and the recruitment of coactivator complexes. Ubiquitin-dependent protein degradation events have also been associated with cycling of the receptor itself on the promoter and with coactivator turnover. IIA, IIB, IIE, IIF, IIH, IIJ, general transcription factors A, B, E, F, H, J; CBP/p300; the co-activators CREB-binding protein and p300; GPS2, G-protein pathway suppressor-2; HDAC, histone deacetylase; NCoR, nuclear-receptor corepressor; p160, a co-activator of the NCoA family; pol II, RNA polymerase II; SMRT, silencing mediator of retinoic acid and thyroid hormone receptors; TAF, TBP-associated factor; TBP, TATA-binding protein.

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 1

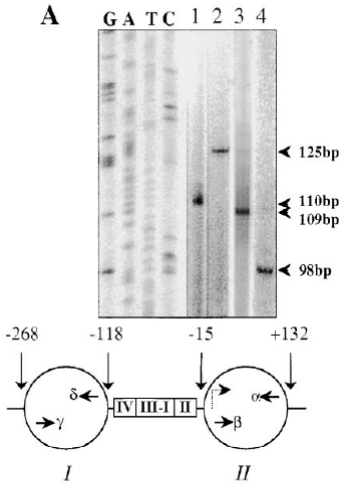
Cell, Vol. 103, 667–678, November 10, 2000, Copyright ©2000 by Cell Press

Ordered Recruitment of Chromatin Modifying and General Transcription Factors to the IFN-β Promoter

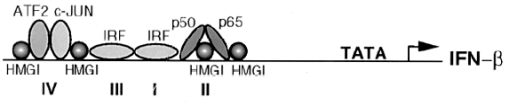
Theodora Agalioti,*§ Stavros Lomvardas,*§ Bhavin Parekh,† Junming Yie,* Tom Maniatis,† and Dimitris Thanos*‡
*Department of Biochemistry and Molecular Biophysics
Columbia University
630 West 168th Street
New York, New York 10032
†Department of Molecular and Cell Biology
Harvard University
7 Divinity Avenue
Cambridge, Massachusetts 02138

Summary

Here, we show that the IFN-β enhanceosome activates transcription by directing the ordered recruitment of chromatin modifying and general transcription factors to the IFN-β promoter. The enhanceosome is assembled in the nucleosome-free enhancer region of the IFN-β gene, leading to the modification and remodeling of a strategically positioned nucleosome that masks the TATA box and the start site of transcription. Initially, the GCN5 complex is recruited, which acetylates the nucleosome, and this is followed by recruitment of the CBP-PolII holoenzyme complex. Nucleosome acetylation in turn facilitates SWI/SNF recruitment by CBP, resulting in chromatin remodeling. This program of recruitment culminates in the binding of TFIID to the promoter and the activation of transcription.



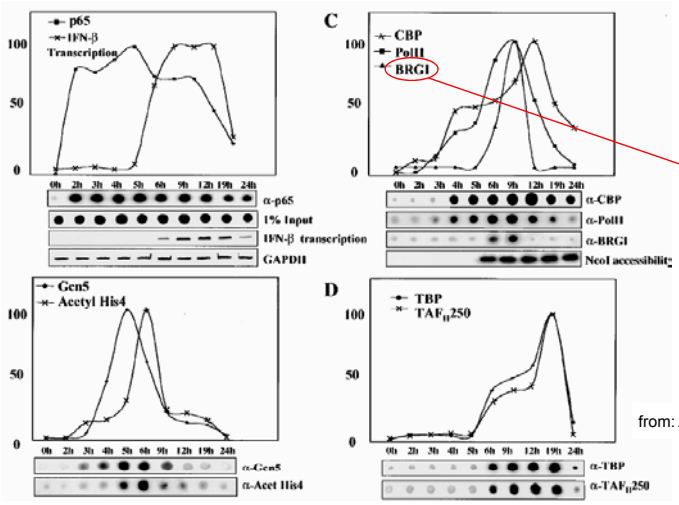
Chromatin digestion with MNase, followed by linker-mediated PCR demonstrates that 2 positioned nucleosomes flank the IFN-β enhancer "in vivo"



from: Agalioti et al. (2000) Cell 103: 667-678.

Figure 1. The IFN-β Enhancer Is Nucleosome-Free In Vivo and In Vitro

(A) The positions of the nucleosomes at the IFN-β promoter were mapped by ligation-mediated PCR using the indicated IFN-β primers: Lane 1 corresponds to the boundary of nucleosome II at −15 using primer α; lane 2 corresponds to the boundary of nucleosome II at +132 using primer β; lane 3 corresponds to the boundary of nucleosome I at −118 using primer γ; lane 4 corresponds to the boundary of nucleosome I at −268 using primer δ. The bottom part of the Figure shows a diagrammatic illustration of nucleosome organization at the IFN-β promoter region and the relative location of the primers.



ChIP analysis shows ordered recruitment of cofactors “in vivo”.

SWI-SNF-like ATP-dependent chromatin remodelling

from: Agalioti et al. (2000) Cell 103: 667-678.

Figure 5. The IFN-b Enhanceosome Directs Ordered Recruitment of Histone acetyltransferases, SWI/SNF, and general transcription factors to the IFN-b promoter in vivo. The immunoprecipitations were repeated two times for p65, three times for hGCN5, three times for acetyl-Histone 4, three times for CBP, two times for PolII. two times for hBRG1, three times for TBP and two times for TAFII250. Shown are the results of one immunoprecipitation using all antibodies. The variability from experiment to experiment was small with respect to the kinetics of recruitment of individual factors. (A) HeLa cells were either mock- or virus-infected with Sendai virus for the indicated amounts of time. Cross-linked chromatin was immunoprecipitated with a p65 antibody and the IFN-b promoter was detected by dot blot hybridization of partially amplified PCR products. The bottom part of the Figure shows the abundance of the IFN-b mRNA as detected by RT-PCR, along with the abundance of GAPDH mRNA as a control. The radioactive bands were quantitated with a phosphorimager and, after subtracting the background the net values, were plotted as percentage of factor recruitment or transcription and correspond to the highest amount of recruitment or transcription obtained at one of the time points. For example, in the case of p65, 100% recruitment is the amount of IFN-b promoter recovered at the 6 hr time point. (B) Same as in (A), but the antibodies used were against hGCN5 or acetyl-Histone 4. (C) Same as in (A), but the antibodies were against the large subunit of PolII, CBP or hBRG1. The bottom part of the Figure depicts nucleosome II remodeling as judged by NcoI accessibility (see Figure 2A). (D) Same as in (A) but the antibodies used were against TBP or TAFII250.

from: Agalioti et al. (2000) Cell 103: 667-678.

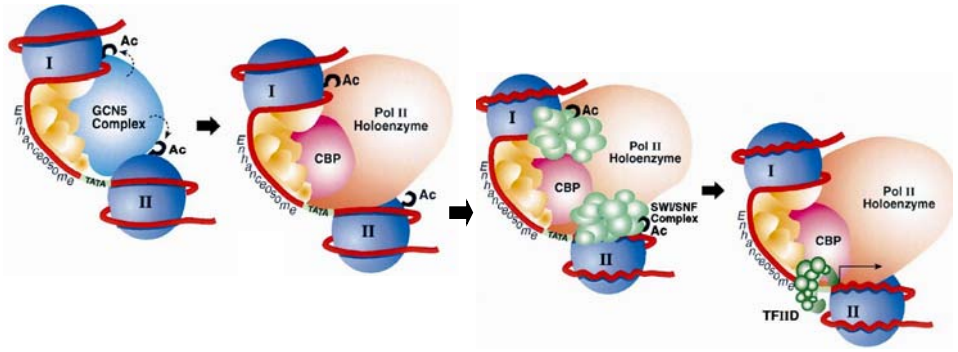


Figure 6. Model Depicting the Ordered Recruitment of Chromatin-Modifying and Basal Factors to the IFN-beta Promoter.
Shortly after virus infection, the enhanceosome assembles on the nucleosome-free IFN-b enhancer and recruits the GCN5 complex, which acetylates nucleosomes I and II (acetylated histone N-termini are shown as hooks). GCN5 departs from the promoter and the CBP-PolII holoenzyme complex is recruited by the enhanceosome. Next, SWI/SNF associates with the promoter via its interactions with CBP. This recruitment is stabilized by the acetylated histone N-termini, which presumably interact with the bromodomain of BRG1/BRM proteins present in the SWI/SNF complex. SWI/SNF remodels the nucleosomes (DNA shown as ruffled lines), thus allowing recruitment of TFIID, completion of preinitiation complex assembly at the core promoter and initiation of transcription.

Table 1 | **Numbers of signalling molecules in selected pathways**

Signalling molecules	Species			
	Human	Fly	Worm	Yeast
Ligand				
RTK	48	3	4	0
TGF-β	29	6	4	0
Wnt	18	7	5	0
Notch	3	2	2	0
STAT	7	1	1	0
Receptor				
RTK	25	6	1	0
Wnt	12	6	5	0
NHR	59	25	270	1

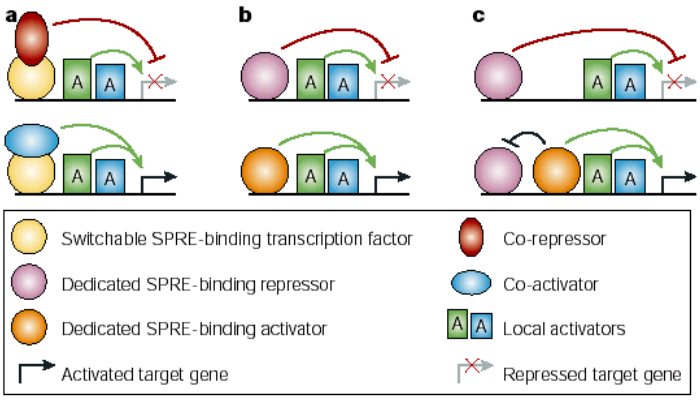
NHR, nuclear hormone receptor; RTK, receptor tyrosine kinase; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor-β; Wnt, wingless related. The table

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

Seven major types of pathways:

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

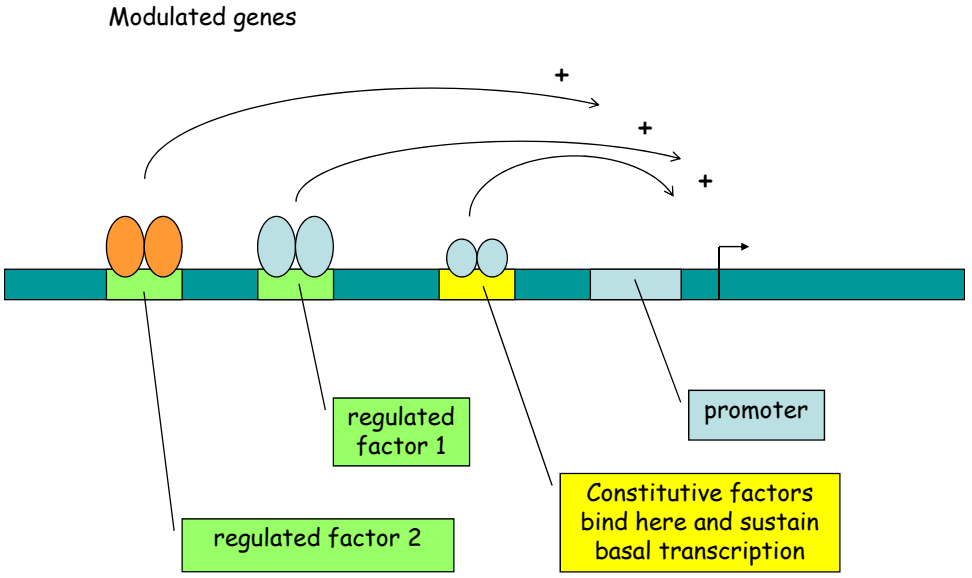
4/5 of these pathway types involve transition from a REPRESSED to an ACTIVATED status.



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

The mechanism requiring proteasome-mediated corepressor-coactivator exchange may be a general mechanism for **on-off** genes

What about **basally active**, modulated genes?



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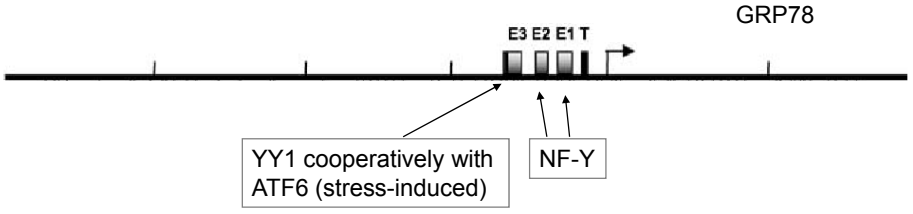
Constitutive Nucleosome Depletion and Ordered Factor Assembly at the *GRP78* Promoter Revealed by Single Molecule Footprinting

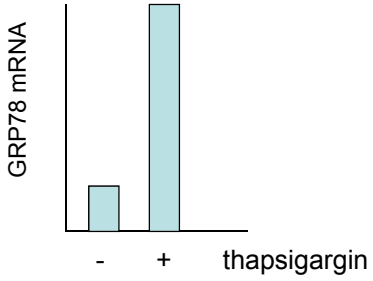
Einav Nili Gal-Yam[1,2], Shinwu Jeong[1,2], Amos Tanay[3], Gerda Egger[1,2], Amy S. Lee[2], Peter A. Jones[1,2*]

1 Department of Urology, USC/Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California, United States of America, 2 Department of Biochemistry and Molecular Biology, USC/Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California, United States of America, 3 Center for Studies in Physics and Biology, Rockefeller University, New York, New York, United States of America

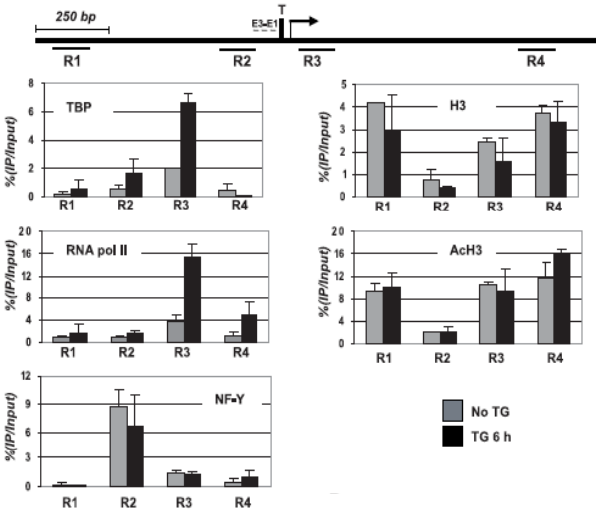
Chromatin organization and transcriptional regulation are interrelated processes. A shortcoming of current experimental approaches to these complex events is the lack of methods that can capture the activation process on single promoters. We have recently described a method that combines methyltransferase M.SssI treatment of intact nuclei and bisulfite sequencing allowing the representation of replicas of single promoters in terms of protected and unprotected footprint modules. Here we combine this method with computational analysis to study single molecule dynamics of transcriptional activation in the stress inducible *GRP78* promoter. We show that a 350–base pair region upstream of the transcription initiation site is constitutively depleted of nucleosomes, regardless of the induction state of the promoter, providing one of the first examples for such a promoter in mammals. The 350–base pair nucleosome-free region can be dissected into modules, identifying transcription factor binding sites and their combinatorial organization during endoplasmic reticulum stress. The interaction of the transcriptional machinery with the *GRP78* core promoter is highly organized, represented by six major combinatorial states. We show that the TATA box is frequently occupied in the noninduced state, that stress induction results in sequential loading of the endoplasmic reticulum stress response elements, and that a substantial portion of these elements is no longer occupied following recruitment of factors to the transcription initiation site. Studying the positioning of nucleosomes and transcription factors at the single promoter level provides a powerful tool to gain novel insights into the transcriptional process in eukaryotes.

E = ERSE = Endoplasmic Reticulum Stress response Element

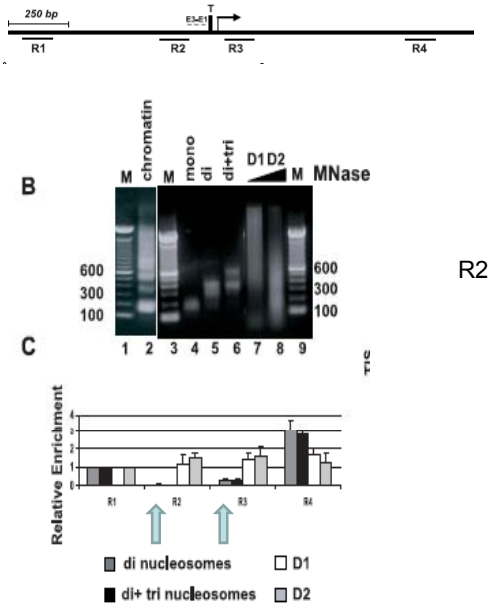




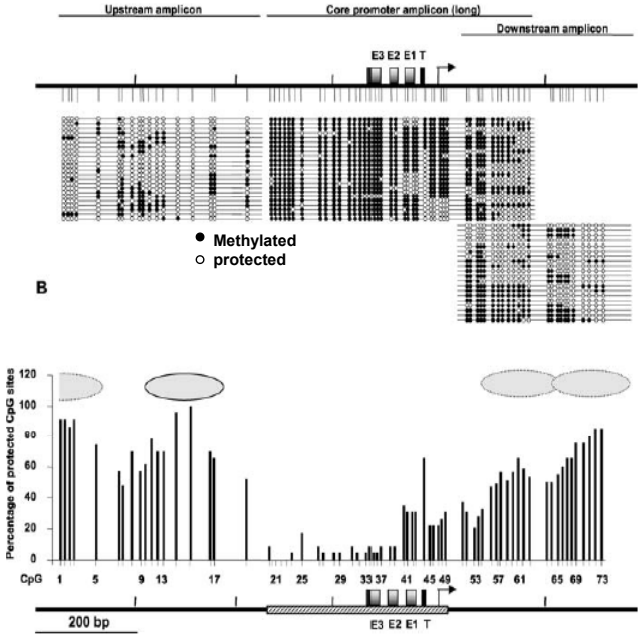
GRP78=BiP a chaperon of the E.R. important in dealing with protein unfolding



(A) ChIP analysis of the *GRP78* promoter was performed on noninduced or TG-induced LD419 cells using antibodies against TBP, RNA polII, NF-Y, total H3, and acetylated H3-K9/14. Precipitated DNA was quantified by real-time PCR using primers specific for the indicated four regions of the promoter. The enrichment at each region is plotted as percentage of input. The data are representatives of experiments performed from two or three independent chromatin preparations.



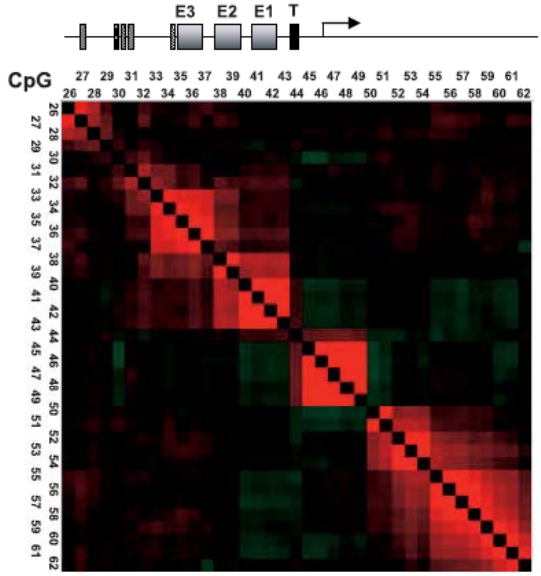
R2 and R3 are free of nucleosomes



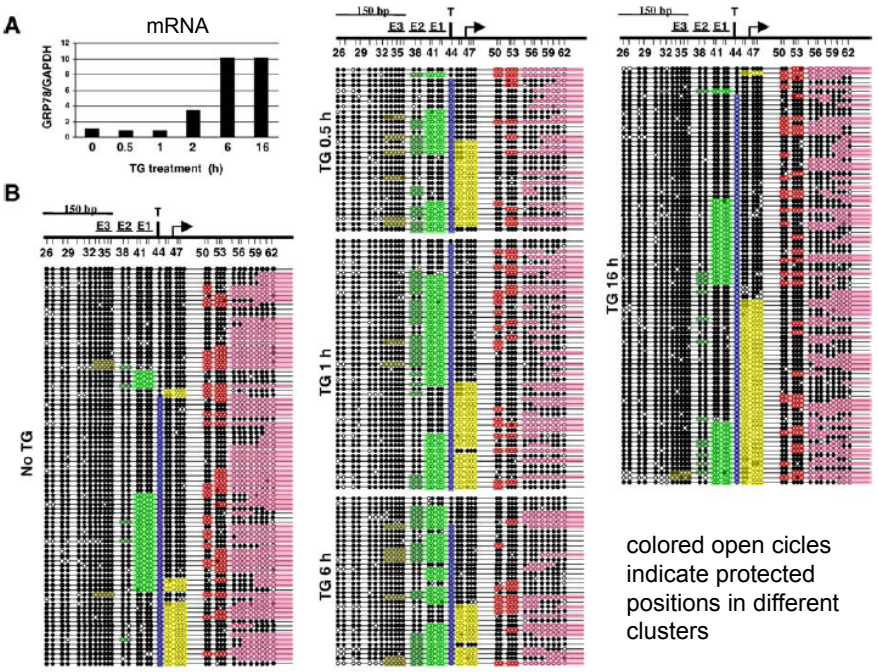
Ex-vivo methylation protection assay

Intact nuclei are treated with M.SssI followed by DNA extraction, bisulfite conversion of the DNA, and PCR amplification of the studied region. The PCR products are cloned and single clones are sequenced, providing **protection** patterns for single promoter molecules.

Figure 2. The Nucleosome-Free Region on the *GRP78* Core Promoter Is Minimally 350 bp Long

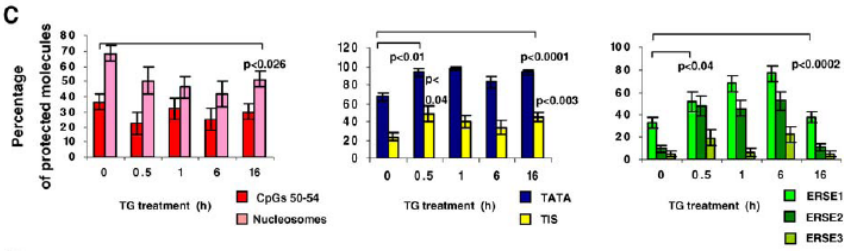


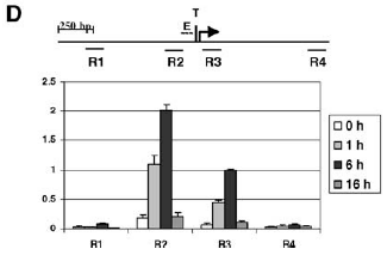
Several homogeneous CpG clusters identified (red boxes indicate more frequent coexistance of protection in the 294 alleles sequenced)



colored open cicles indicate protected positions in different clusters

(C) Protection levels of nucleosomal modules (left), TATA and TIS (middle), and ERSEs (right) were calculated (see Materials and Methods) and are shown at the different time points.





ATF6 enrichment at the ERSE region shown by ChIP analyses on LD419 cells harvested at 0, 1, 4, and 16 h after TG stress induction. DNA was quantified by real-time PCR using primers specific for the indicated four regions of the promoter (as in Figure 1A).

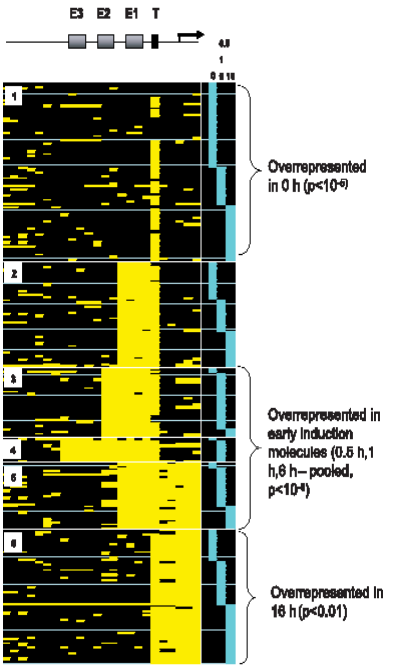


Figure 5. Few Combinatorial Modes of GRP78 Promoter Organization

Shown are clustered protection patterns for the 294 sampled promoters (rows, see Materials and Methods). Only few modes of promoter organization are observed, including clusters representing high levels of TATA binding (cluster 1), cassette like loading of the ESREs (clusters 2–4), recruitment of factors to the TIS (cluster 5), and release of the ESRE modules (cluster 6). Statistical enrichment analysis (Materials and Methods) confirms that specific modes of activity (clusters) are overrepresented in specific phases of the ER-stress activation process, enabling us to arrange the clusters in a chronological order. The designation of each row (¼ protection pattern of one promoter molecule) to the time point from which it originated is marked by the blue boxes on the right. The early induction time points (1, 0.5, and 6 h) are pooled.

Conclusions. The GRP78 promoter is always nucleosome-free. Short-time after induction (stimulation by E.R. stress) there is occupancy of the three E1 E2 & E3 elements, while at late time points only the region where transcription initiates is occupied.

This is the paradigm of a always transcribed, modulated promoter.