Ch 3 - L 5.3

Promoter activation modes

Activation modes

Different categories of Promoters are regulated following different modalities. Aside from those that are mainlyregulated by Enhancer contacts, we observe two general modes of activation:

TATA+, LCG are usually covered by nucleosomes when silent. When an inductive stimulus arrives, nucleosomes are remodeled and basal apparatus can bind. We will refer to these as «on-off» genes.

HCG on the contrary are basally transcribed, show a permanent NFR, but their rate of transcription can be «modulated».

HCG promoters

- Many HCG promoter undermethylated even if not transcribed («poised»)
- High density of CG-containing TFBS (transcription factor binding sites) for constitutive TFs (Sp1, Nrf-1, E2F, ETS and other)
- «intrinsic» promoter activity due to reduced nucleosome occupancy
- Do **not** require ATP-dependent remodellers for activity

Nonetheless, the level of transcription can be «modulated».

Let’s see an example of this....

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

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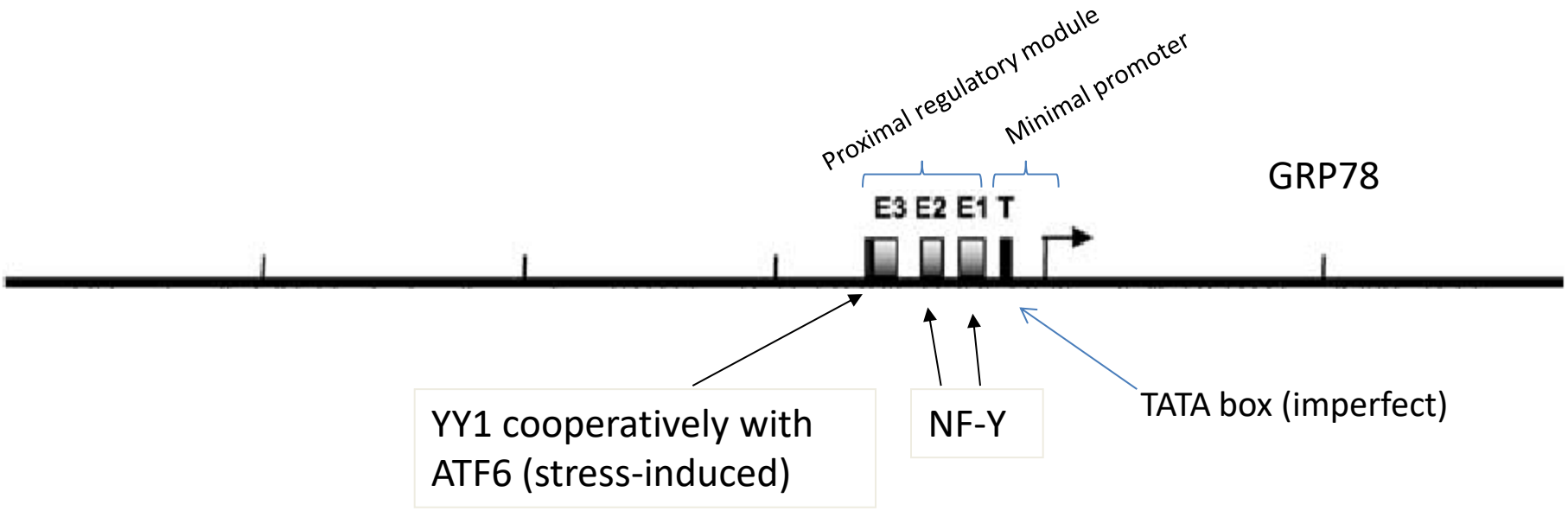
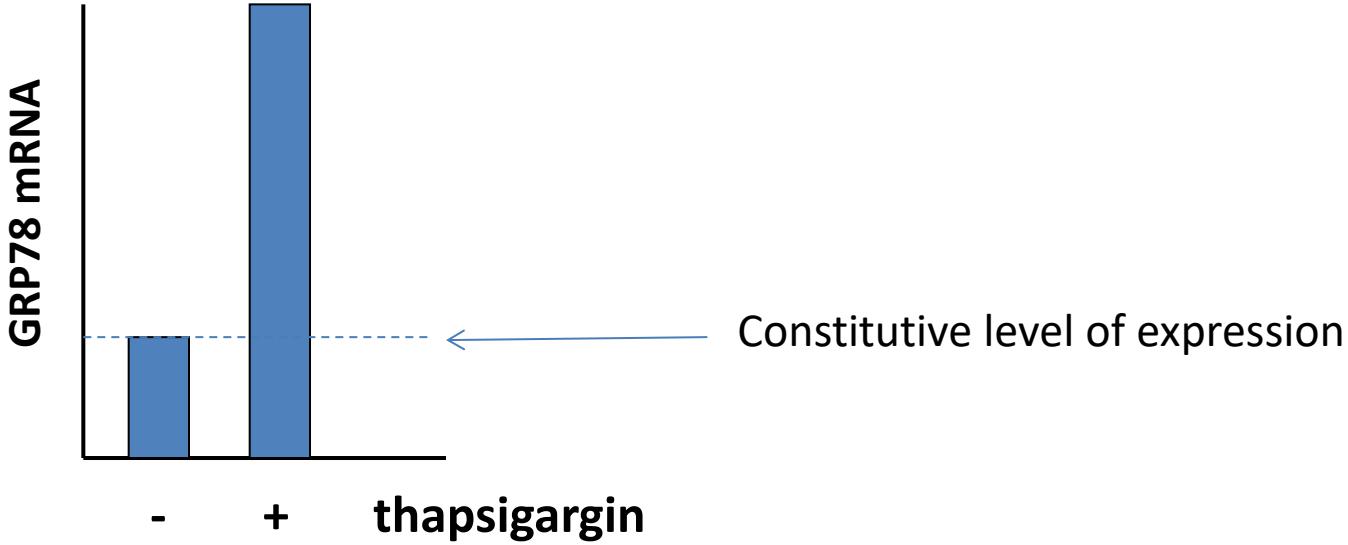
Chromatin organization and transcriptional regulation are interrelated processes. A shortcoming of current experimental approaches to these complex events is the lack of methods that can capture the activation process on single promoters. We have recently described a method that combines methyltransferase 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





Authors studied the position of nucleosomes, PIC and Transcription Factors
at basal status
after stress induction
in the promoter + proximal regulatory module of this gene

Method: methylation protection

Ex-vivo methylation protection assay

1) Intact nuclei are treated with **M.SssI**
2) DNA extraction, bisulfite conversion of the DNA (converts C→U)
3) PCR amplification of the studied regions
4) PCR products are cloned and single clones are sequenced (ante-NGS)
5) C→T mutations provide protection patterns for single promoter molecules

Description

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The CpG Methyltransferase, M.SssI, methylates all cytosine residues (C^5) within the double-stranded dinucleotide recognition sequence 5'...CG...3' (1).

Product Source

The CpG Methyltransferase(M.SssI) is isolated from a strain of *E. coli.* which contains the Methyltransferase gene from *Spiroplasma* sp. strain MQ1 (2,3).

This region studied in induction

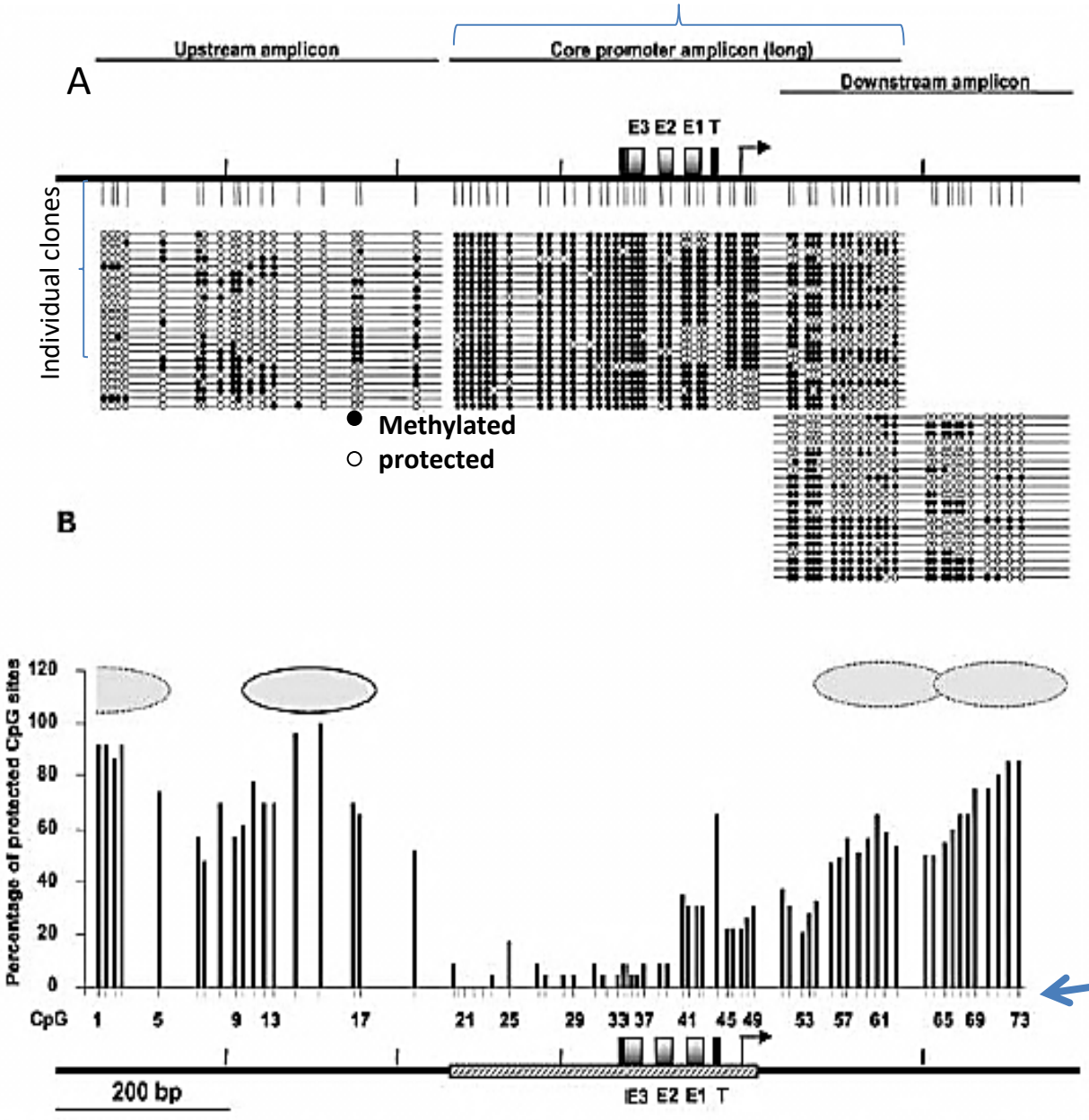


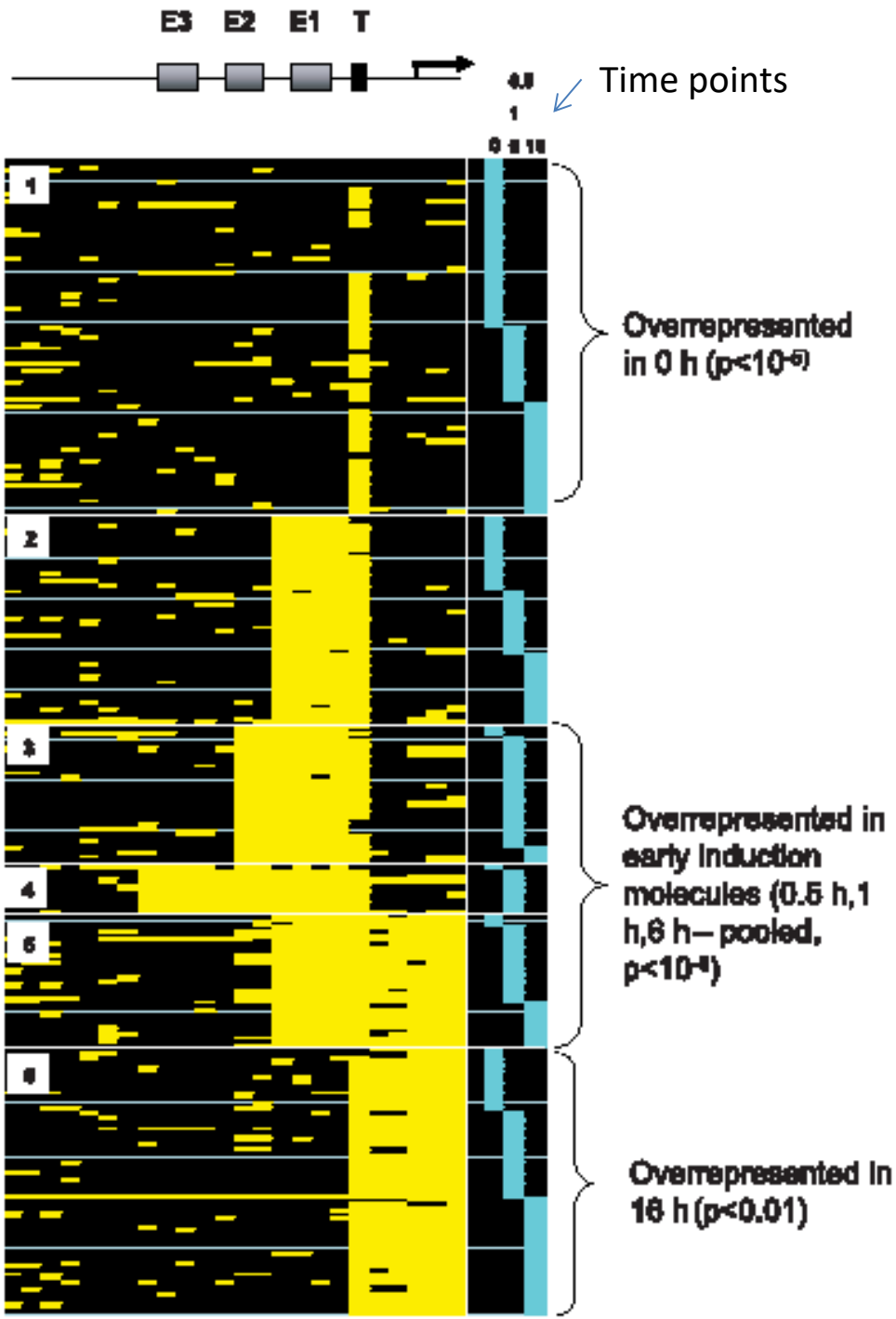
Figure 2. The Nucleosome-Free Region on the *GRP78* Core Promoter Is Minimally 350 bp Long

basal status analysis

A-Map of the promoter; E1, E2, E3 regulatory elements. T=TATA

Each bar is a single allele, showing accessible (methylated) and covered (unmethylated) CpGs.

Each small bar (I) ia a CpG position. CpG are numbered 1 to 73

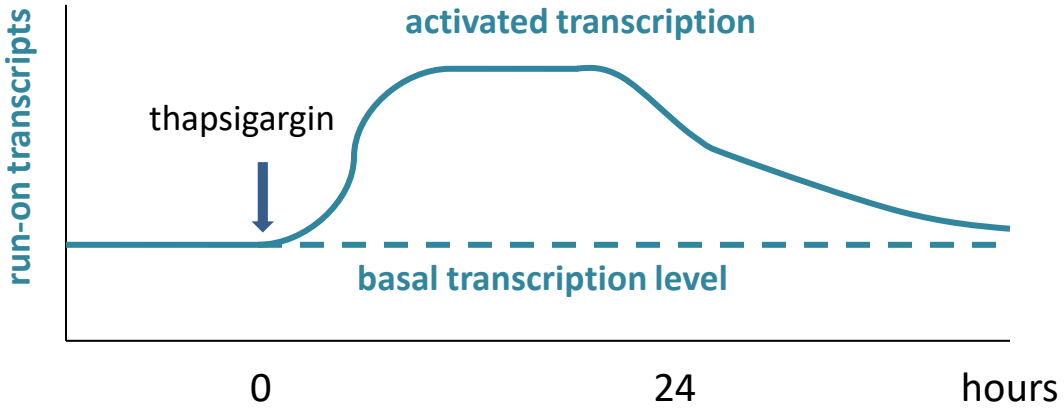


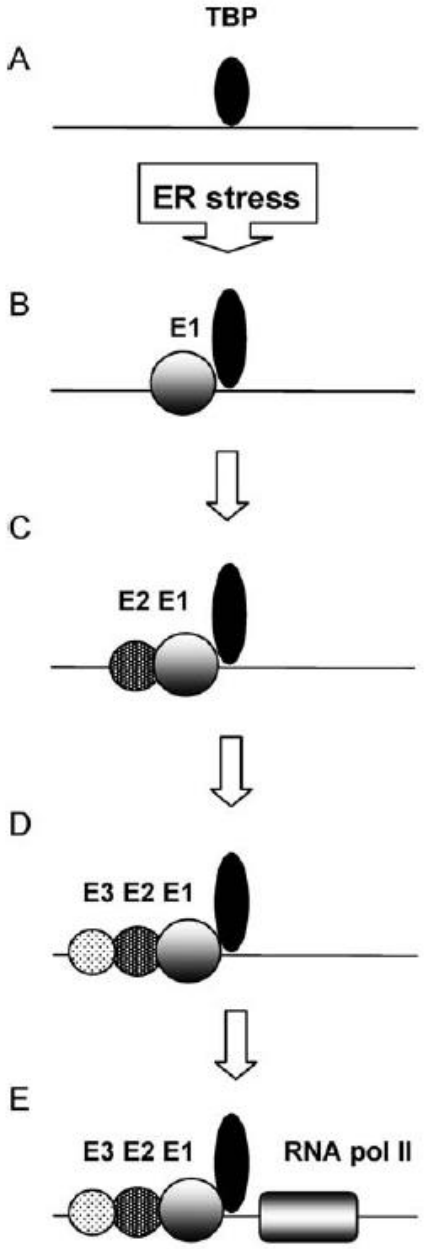
Map of protected region at different time points after Thapsigargin (Yellow dots). Each line is one allele studied.

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», but modulated promoter.

Concluding…
GRP78 is an Housekeeping gene that is always basally activated (A in figure) and transcribed at low level. TBP and basal factors are always bound to this promoter, and rare alleles show the presence of RNA Pol II molecules traveling on the gene. The promoter is always nucleosome-free.
After induction by thapsigargin, a progressively more extended region gets protected from methylation in vitro. These are Transcription Factors binding to regulatory elements (B, C, D). After a while, a consistent RNA Pol II footprint is seen in the transcribed region, testifying frequent initiation and increased transcription (E). This "activated status" is quite stable even when factors are no longer present and is self-sustaining.

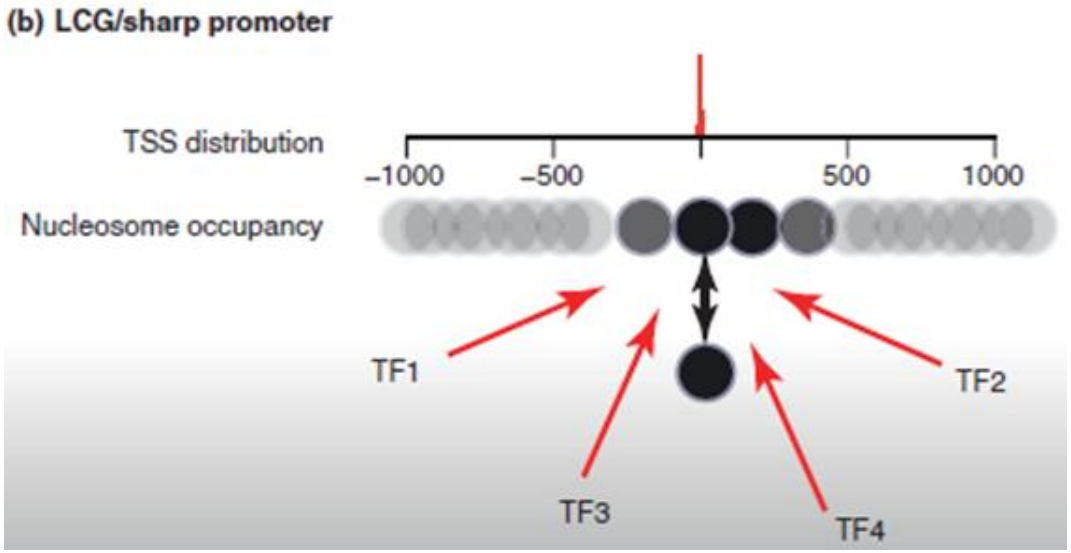




On-off genes

Usually LCG, TATA-plus

They possess intrinsic tendency to be «covered» i.e. repressed by nucleosome positioning

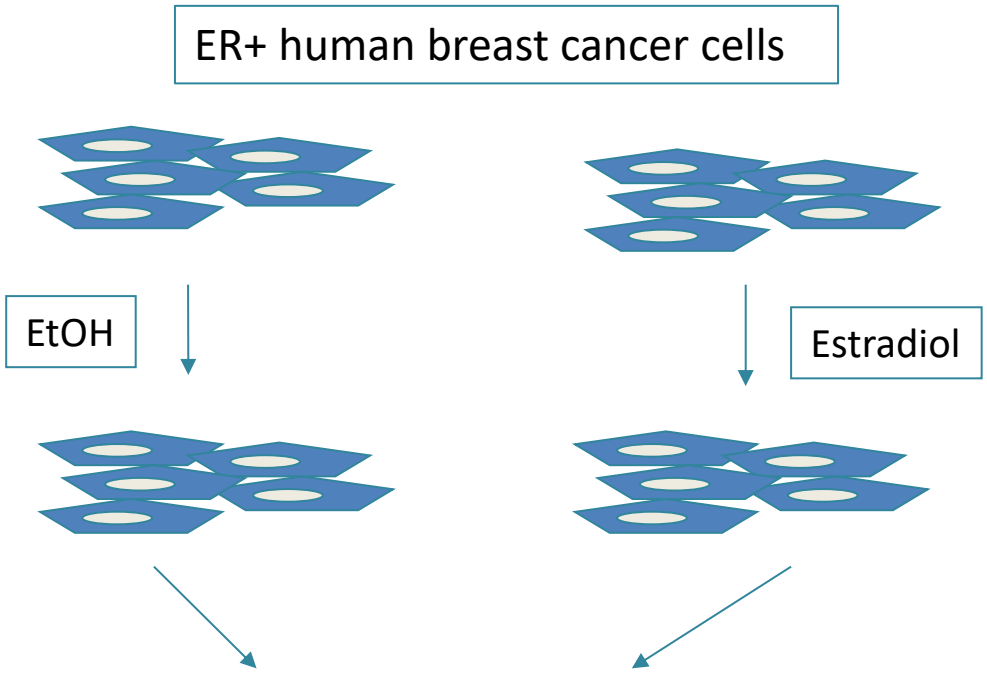


This system **requires ATP**, since activation requires ATP-dependent chromatin remodelers.

Many ATP-dependent chromatin remodelling enzyme families in Mammals:

SWI/SNF – ISWI – CHD - INO80 - SWR1

How is an «on-off» gene activated ?



Expression analysis (microarrays, RNA-Seq)
ER binding analysis (ChIP-chip, ChIP-Seq)

The pS2/TTF1 gene is estrogen-responsive and is one of the few genes that contains an ER-binding site close to the promoter. (From Métivier et al., 2003)

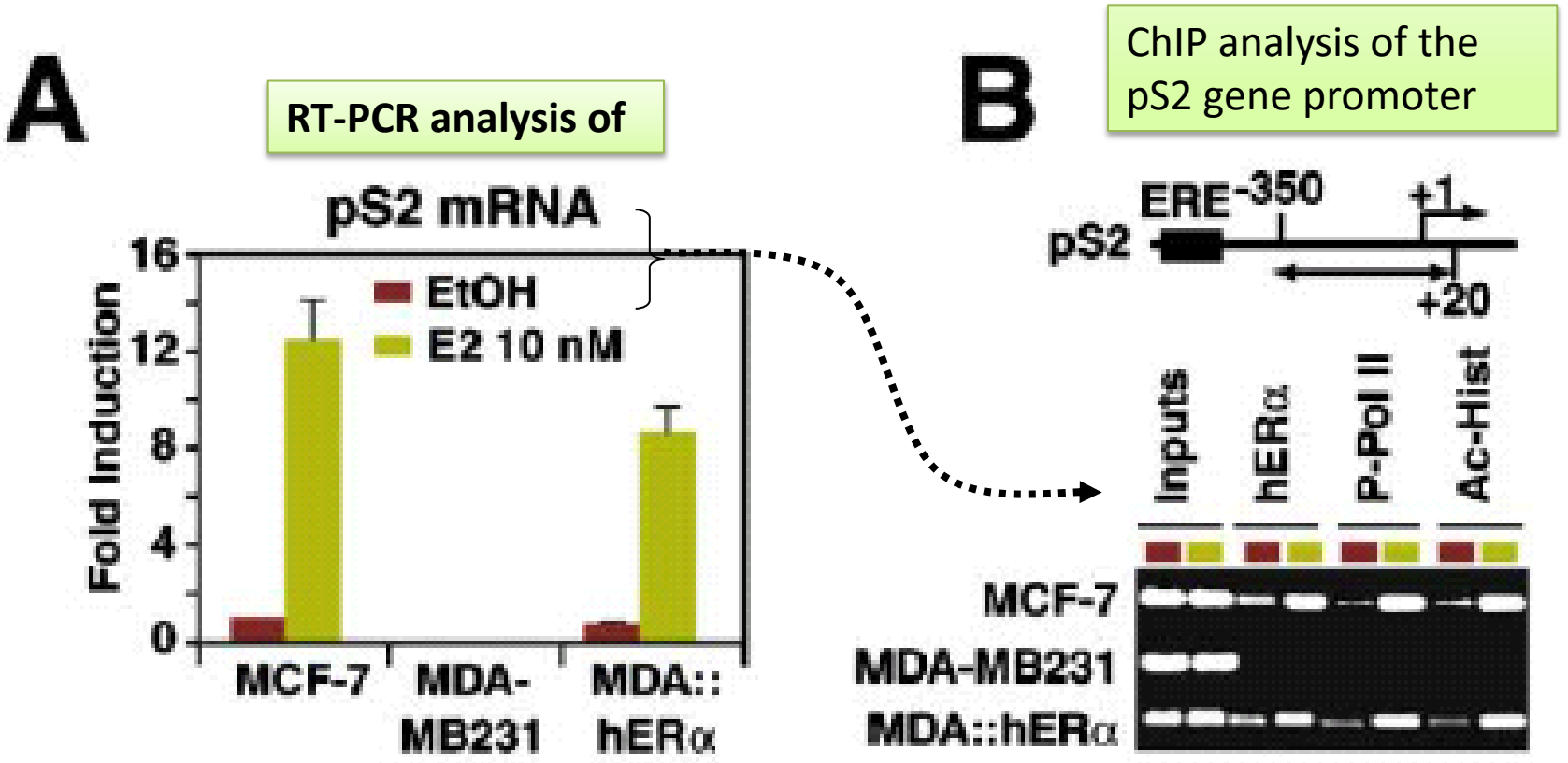


Figure 1. In Vivo Identification of the Transcription Factors Involved in pS2 Gene Activity

(A) Cells were treated for **3 hr** with 10 nM Estradiol (E2) or ethanol (EtOH) as vehicle control. The pS2 mRNA levels were normalized against invariant GAPDH mRNA, as measured by real-time PCRs.

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

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

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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.

- MCF-7 cells are starved of estrogen for several days
- 2 hours before treatment, they are added of **α-amanitin** (blocks transcription)
- Cells are then washed and treated with estradiol
- ChIP analysis for several factors is run at **5 minute intervals** on the pS2/TFF1 gene promoter

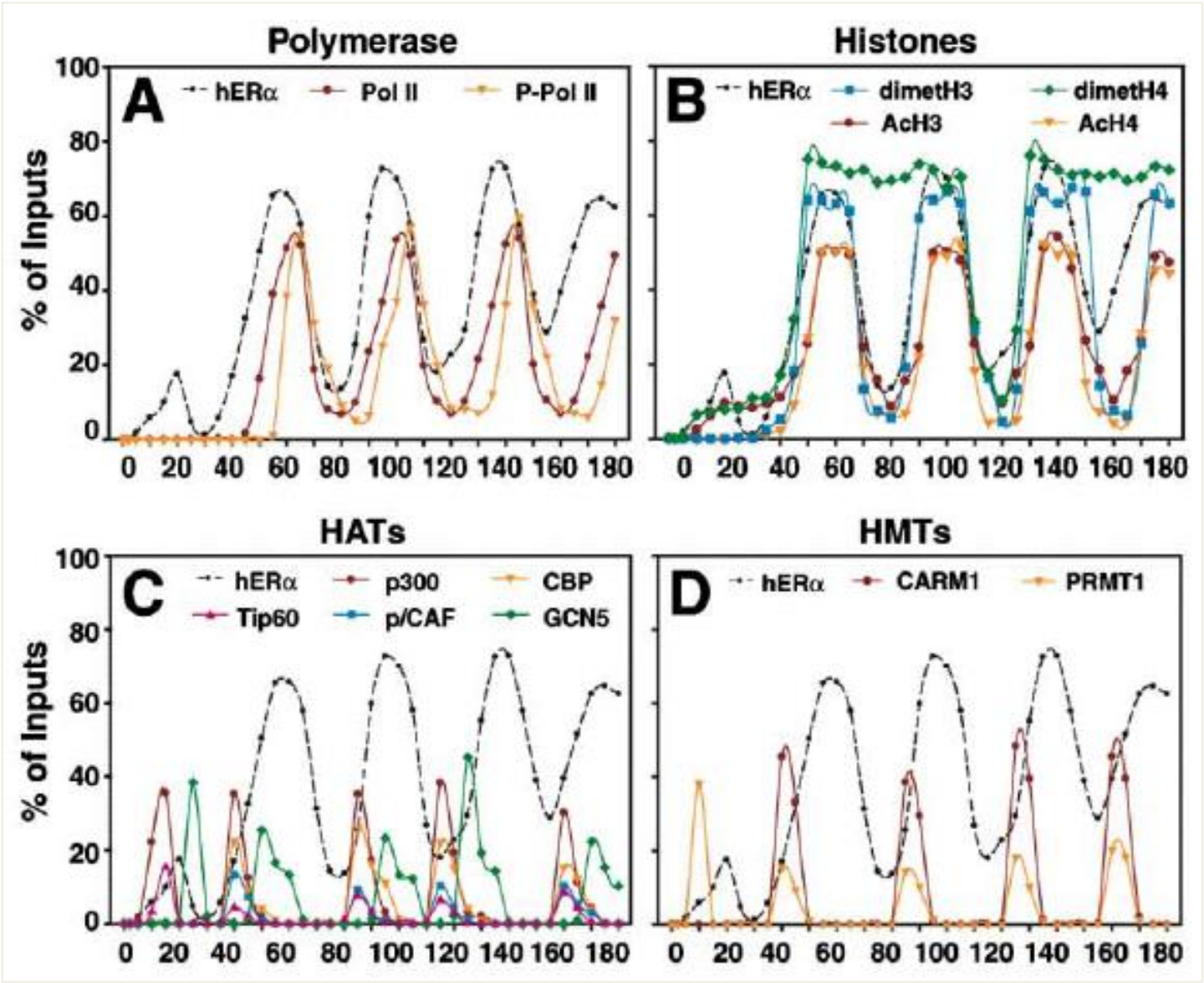
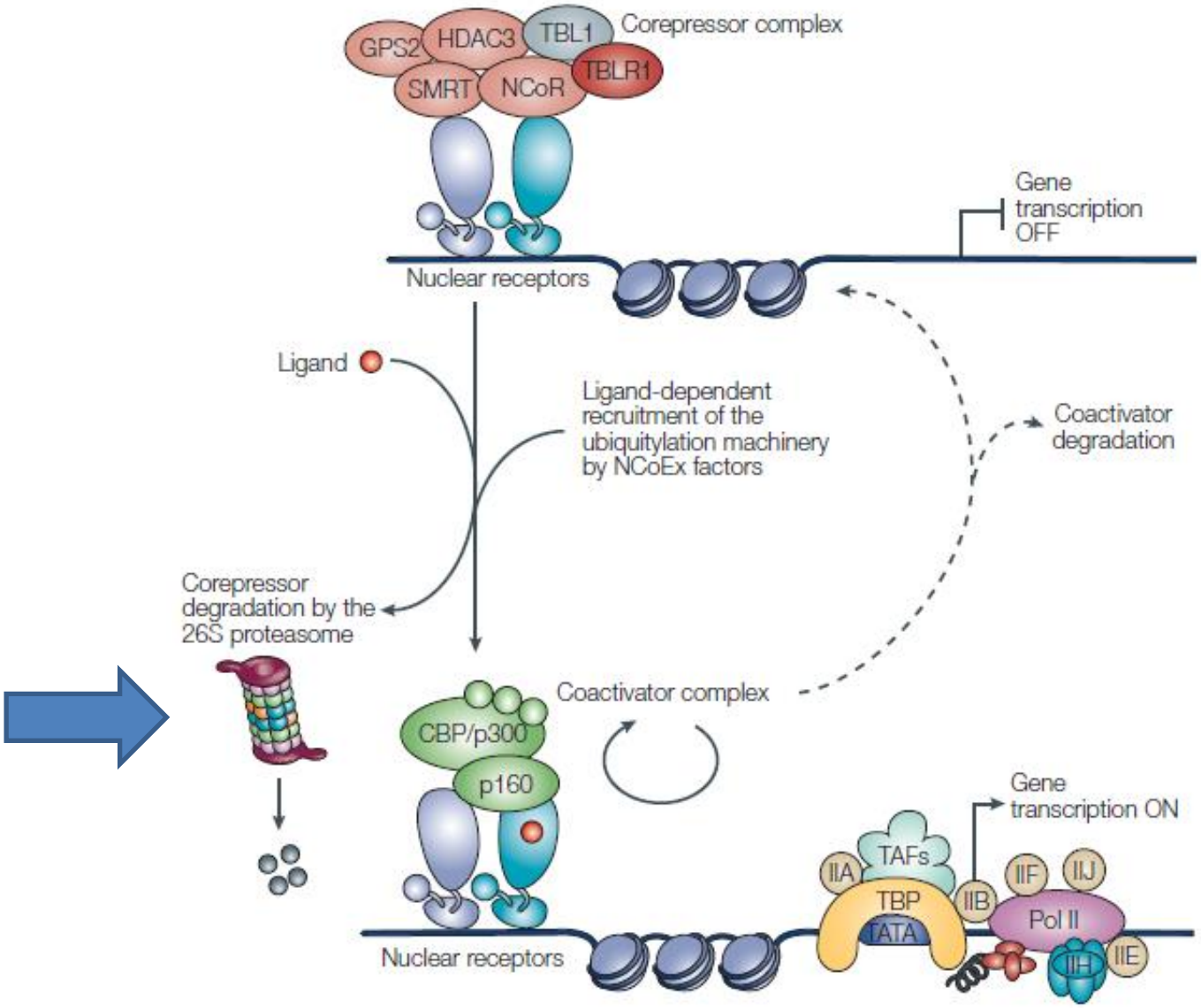


Figure 3. Dynamics of Cofactor Recruitment Directed by E2-Liganded hER on the pS2 Promoter. Kinetic ChIP experiments were performed using specified antibodies as shown within the images. Chromatin was prepared on sampled cells at 5 minutes intervals. The amount of immunoprecipitated pS2 promoter was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

Important to note that this dynamic is dramatically ***inhibited by proteasome inhibitors***.

The role of Ubiquitin has been demonstrated in this dynamics.

In other words, the «on-off» kinetics seen in these experiments does not mean simply association-dissociation, but active displacement of components from the complexes, mediated by the proteasome.



Perissi & Rosenfeld 2005

Conclusions

Micro-chromatin environments are extremely dynamic, contrary to constitutive HC or constitutive “on” loci

These promoters tend to return quickly to «repressed» status.

This model is more or less demonstrated for a number of genes and regulators showing «transient» immediate transcriptional response.