In previous lectures we have defined the heterochromatic (HC) status (silenced) as opposed to the euchromatic (EC) status (active)

We try now to answer two questions:

1) is there physical separation between adjacent HC and EC regions (as in the case of the MAT locus) ?

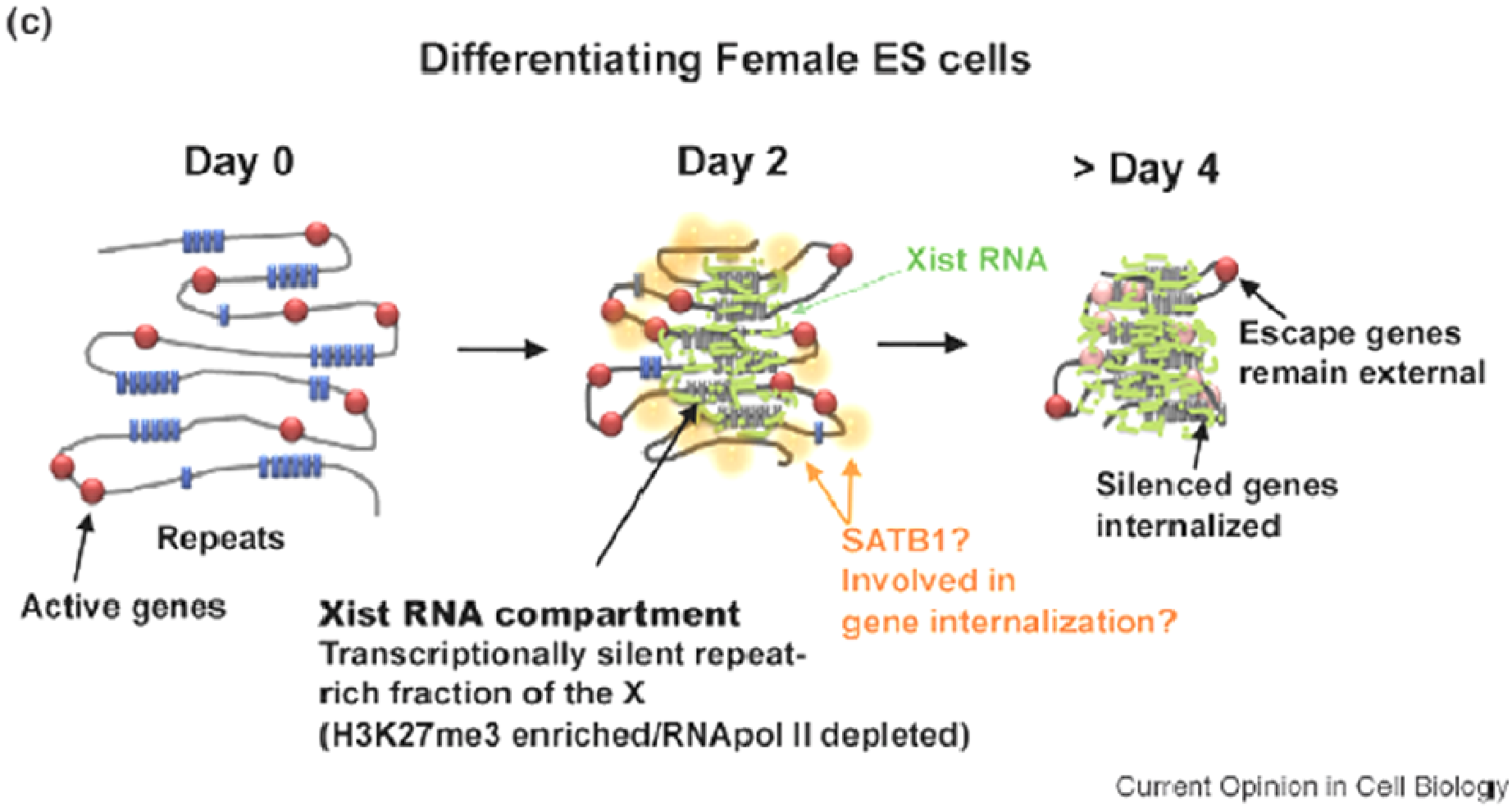
2) what happens when a gene transits from HC to EC, i.e. when it gets activated (and vice-versa) ?

(even though note that constitutive HC differs from the repressed status of a gene that can be activated, as well as a constitutively active gene (constitutively EC) differs from a gene that is transiently activated)

The study of chromosome X inactivation has given an important suggestion.

In fact, we know that several genes in the Xi escape inactivation (pseudo-autosomal regions) and are indeed active

Localization by DNA/RNA FISH has showed that they stay “outside” the inactivation core (the part coated with Xist RNA, dense of heterochromatic marks).



from Chow, Current Opinion in Cell Biology 2009, 21:359–366

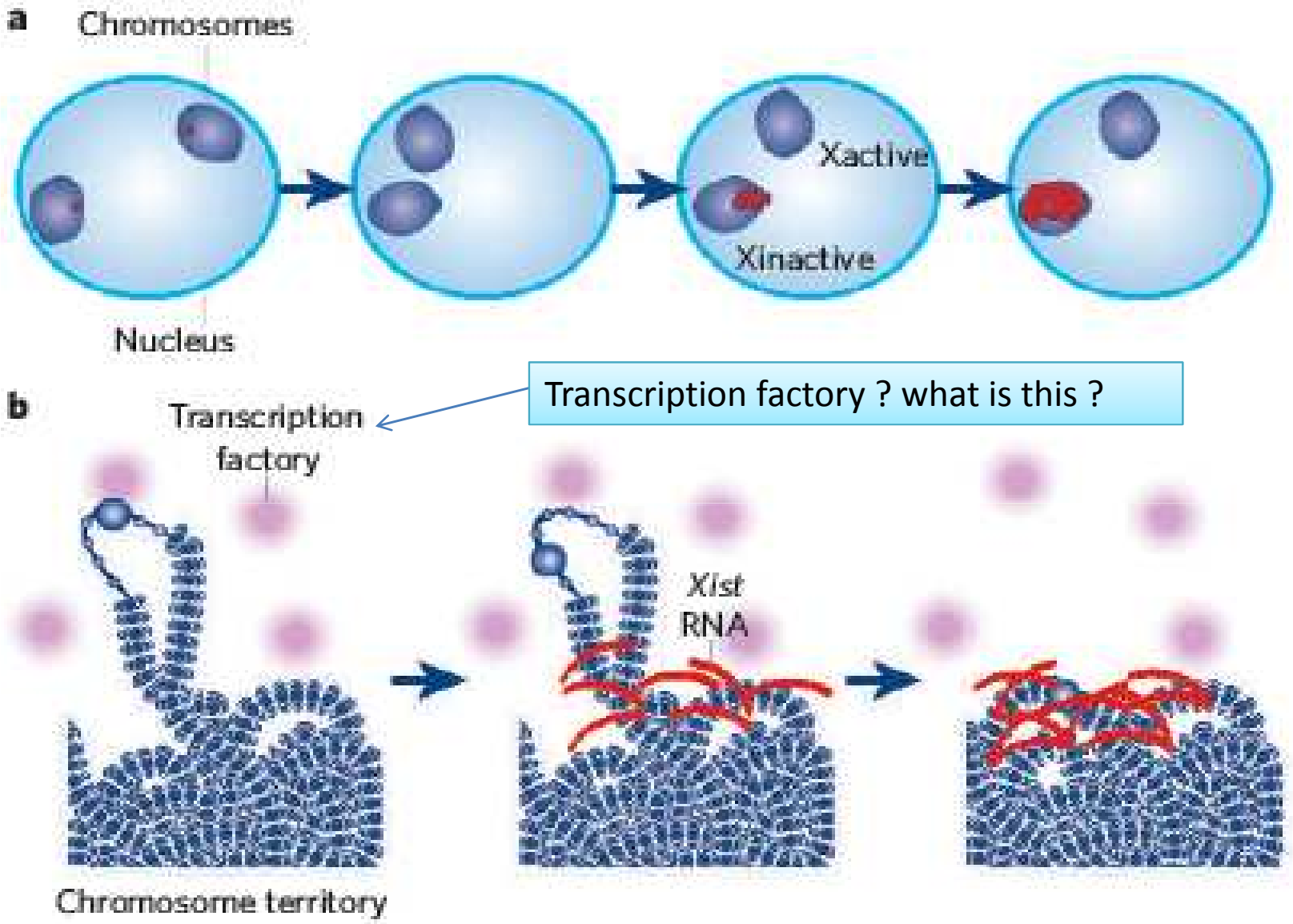


Figure 1 | Events of nuclear reorganization during X-chromosome inactivation. **a**, Soon after female embryonic stem cells start to differentiate,

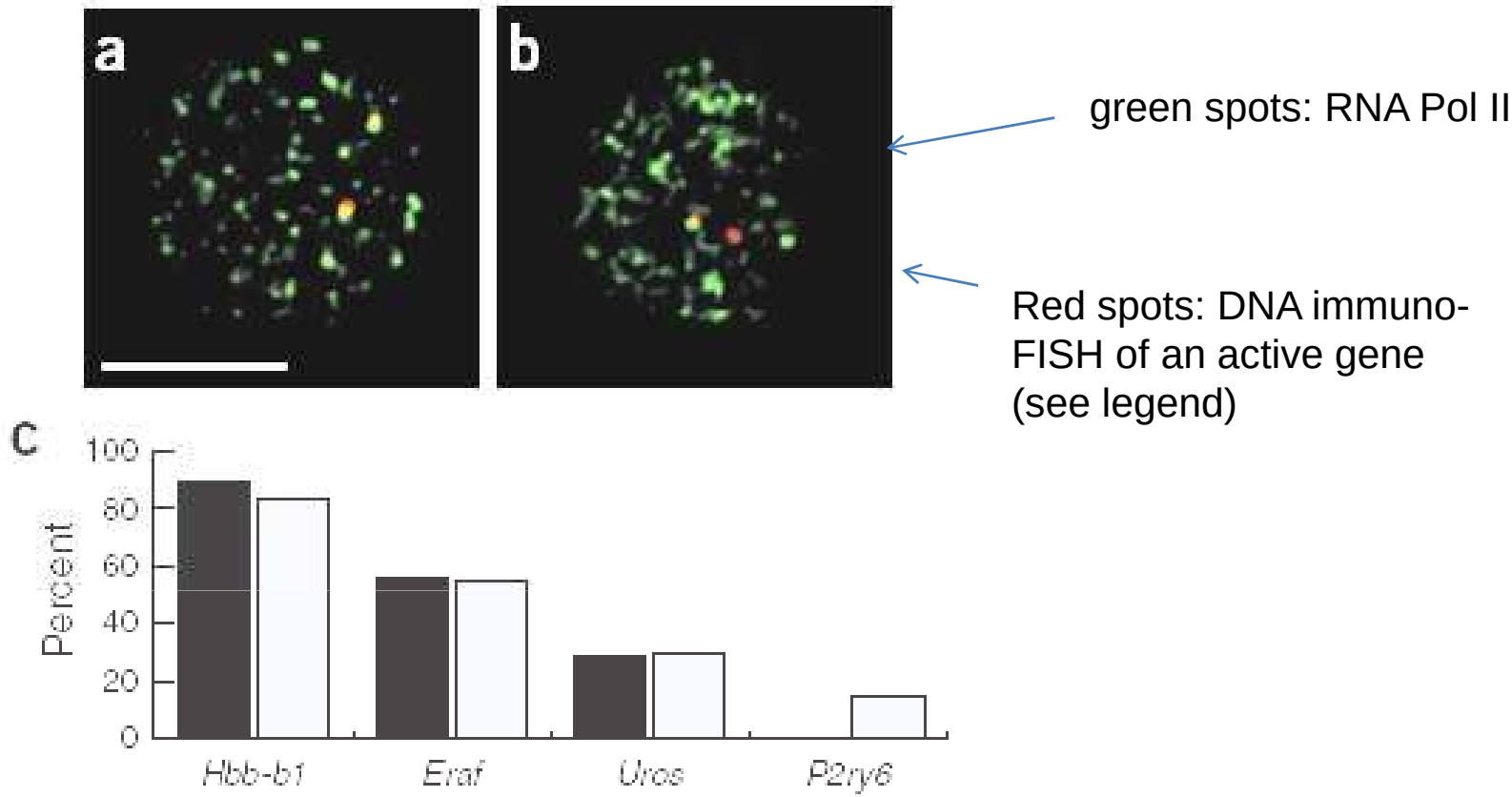


Figure 4 Actively transcribed genes associate with RNAP II foci. (**a**) RNA immuno-FISH of *Hbb-b1* transcription (red) with RNAP II staining (green) in anemic spleen erythroid cells. Scale bar, 5 μm. (**b**) DNA immuno-FISH of *Eraf* (red) with RNAP II staining (green). (**c**) Comparison of the percentage of alleles exhibiting a gene transcription signal by RNA FISH (black), with the percentage of loci that overlap with an RNAP II focus by DNA FISH (gray) for *Hbb-b1* ($n = 83$), *Eraf* ($n = 59$), *Uros* ($n = 47$) and *P2ry6* ($n = 79$).

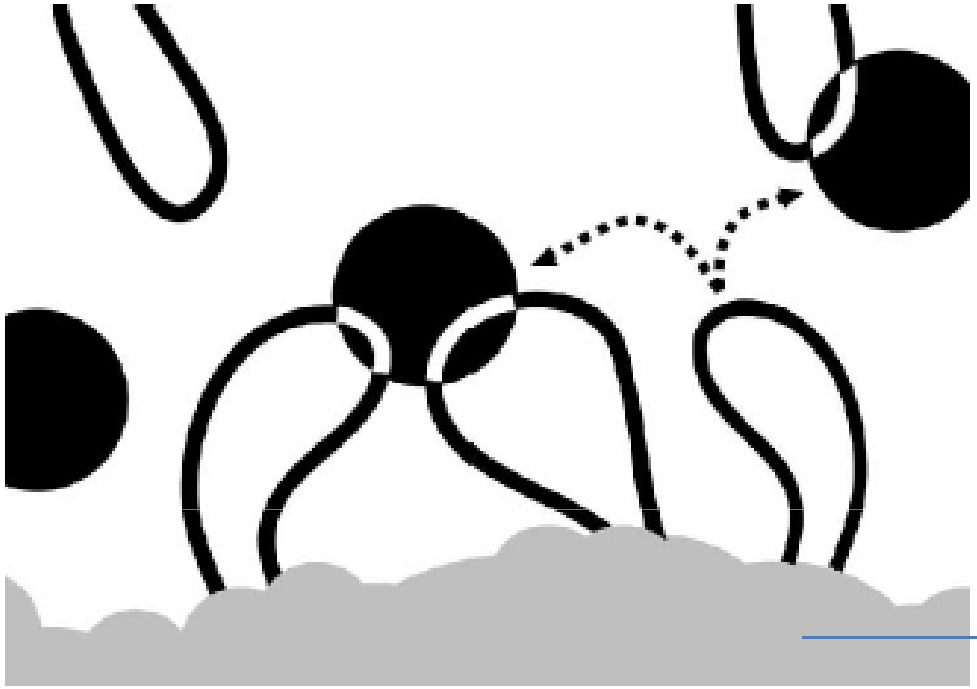
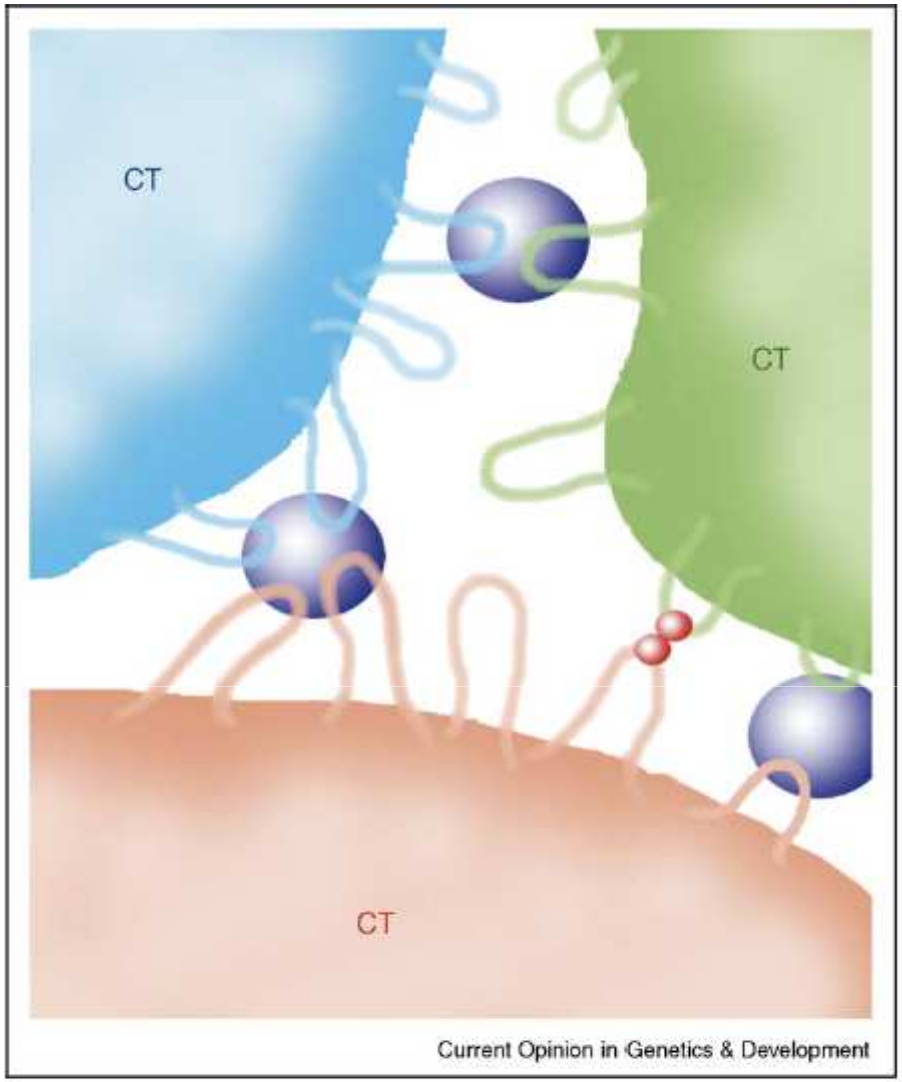


Figure 8 Model of dynamic associations of genes with transcription factories. Schematic representation of chromatin loops (black) extruding from a chromosome territory (gray). Transcribed genes (white) in RNAP II factories (black circles). Potentiated genes (free loops) that are not associated with RNAP II factories are temporarily not transcribed. Potentiated genes can migrate to a limited number of preassembled RNAP II factories to be transcribed (dotted arrows). We propose that both *cis* and *trans* associations are possible.



Nuclear organization. Three different chromosome territories (CTs) are shown. Potentially active genes in *cis* and *trans* dynamically engage transcription factories (blue spheres). Most 'active' genes spend the majority of their time outside transcription factories and are transcriptionally inactive. Other types of regulatory interactions (red spheres) might possibly sequester genes or inhibit mobility and factory contact.

Is there a biochemical assay for this phenomenon ?

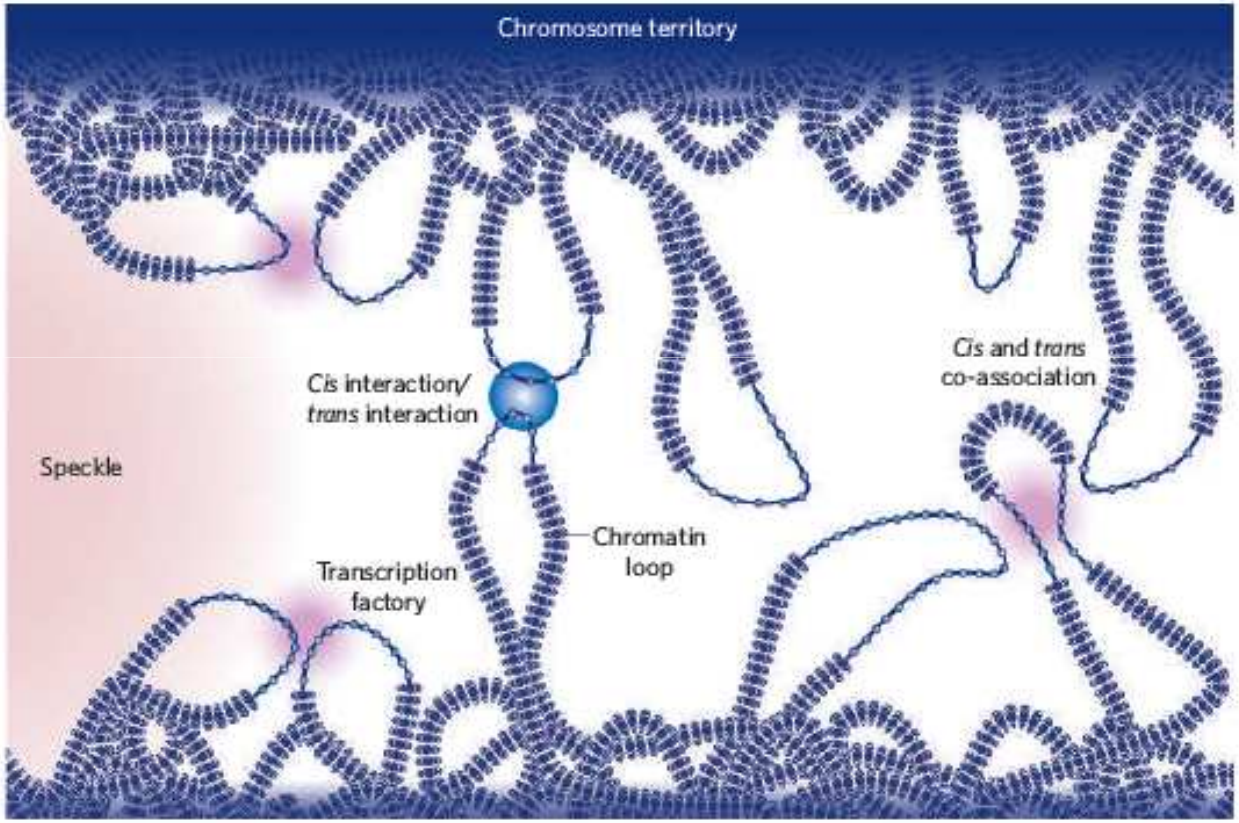


Figure 2 | Colocalization of genes in the nucleus for expression or coregulation. Active genes on decondensed chromatin loops that extend outside chromosome territories can colocalize both *in cis* and *in trans* at sites in the nucleus with local concentrations of Pol II (namely transcription factories; dark pink) and adjacent to splicing-factor-enriched speckles (pale pink). Interactions can also occur between regulatory elements and/or gene loci and lead to coregulation in *trans* (blue circle).

review

Nuclear organization of the genome and the potential for gene regulation

Peter Fraser[1] & Wendy Bickmore[2]

Much work has been published on the *cis*-regulatory elements that affect gene function locally, as well as on the biochemistry of the transcription factors and chromatin- and histone-modifying complexes that influence gene expression. However, surprisingly little information is available about how these components are organized within the three-dimensional space of the nucleus. Technological advances are now helping to identify the spatial relationships and interactions of genes and regulatory elements in the nucleus and are revealing an unexpectedly extensive network of communication within and between chromosomes. A crucial unresolved issue is the extent to which this organization affects gene function, rather than just reflecting it.

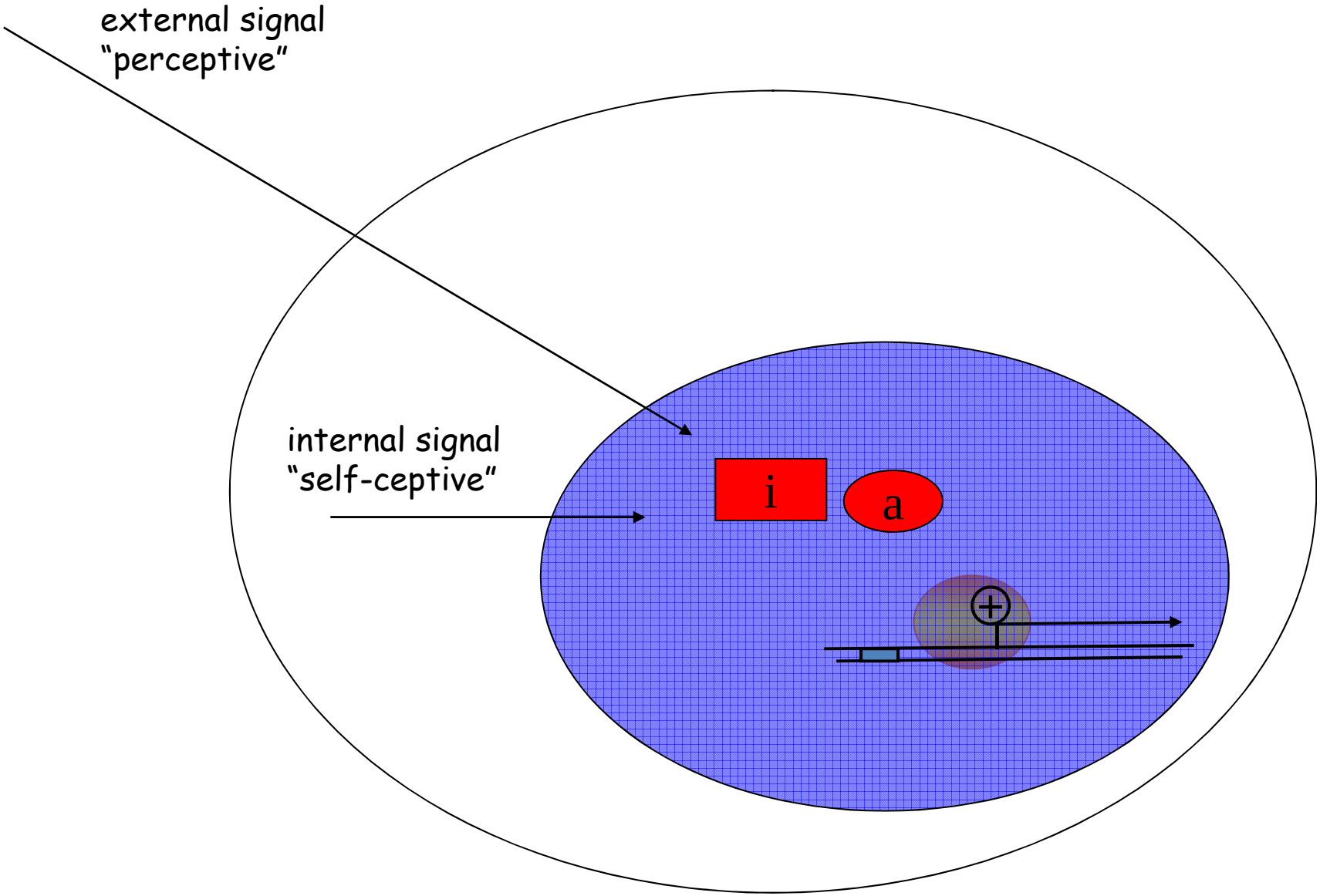
NATURE|Vol 447|24 May 2007|

pagg. 413-417

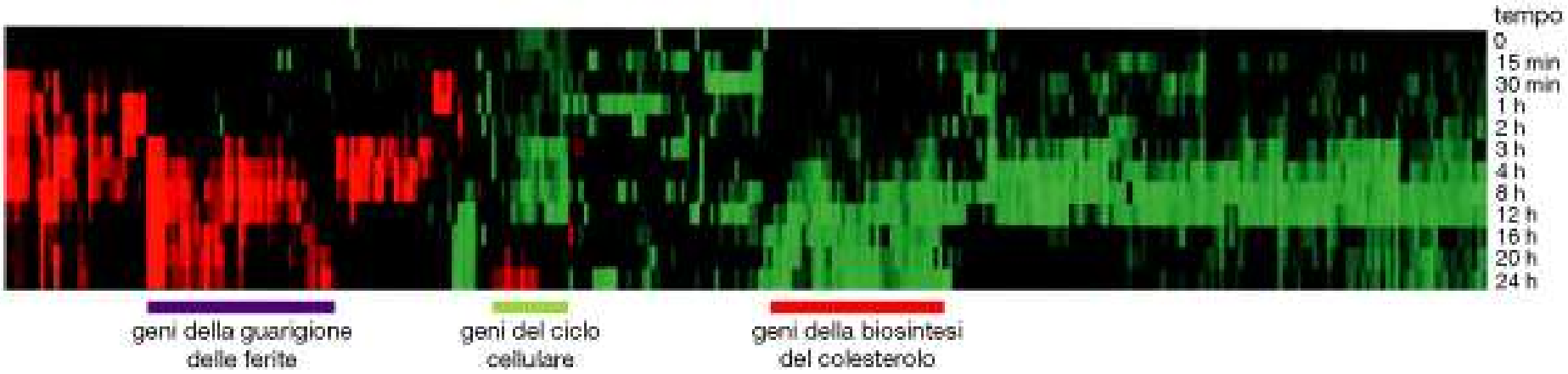
Conclusion 1

Transcribed regions (EC) are physically separated from adjacent HC by “boundaries” (or insulators) and from the bulk of HC by “looping” out towards transcription factories.

- Constitutively expressed genes ("housekeeping")
- Regulated genes (positive and negative feedbacks)
- **Inducible genes ("on-off")**
  repressible genes ("off-on" ?)
- Silenced genes (heterochromatic, CpG-methylated)


external signal
"perceptive"
internal signal
"self-ceptive"
i
a

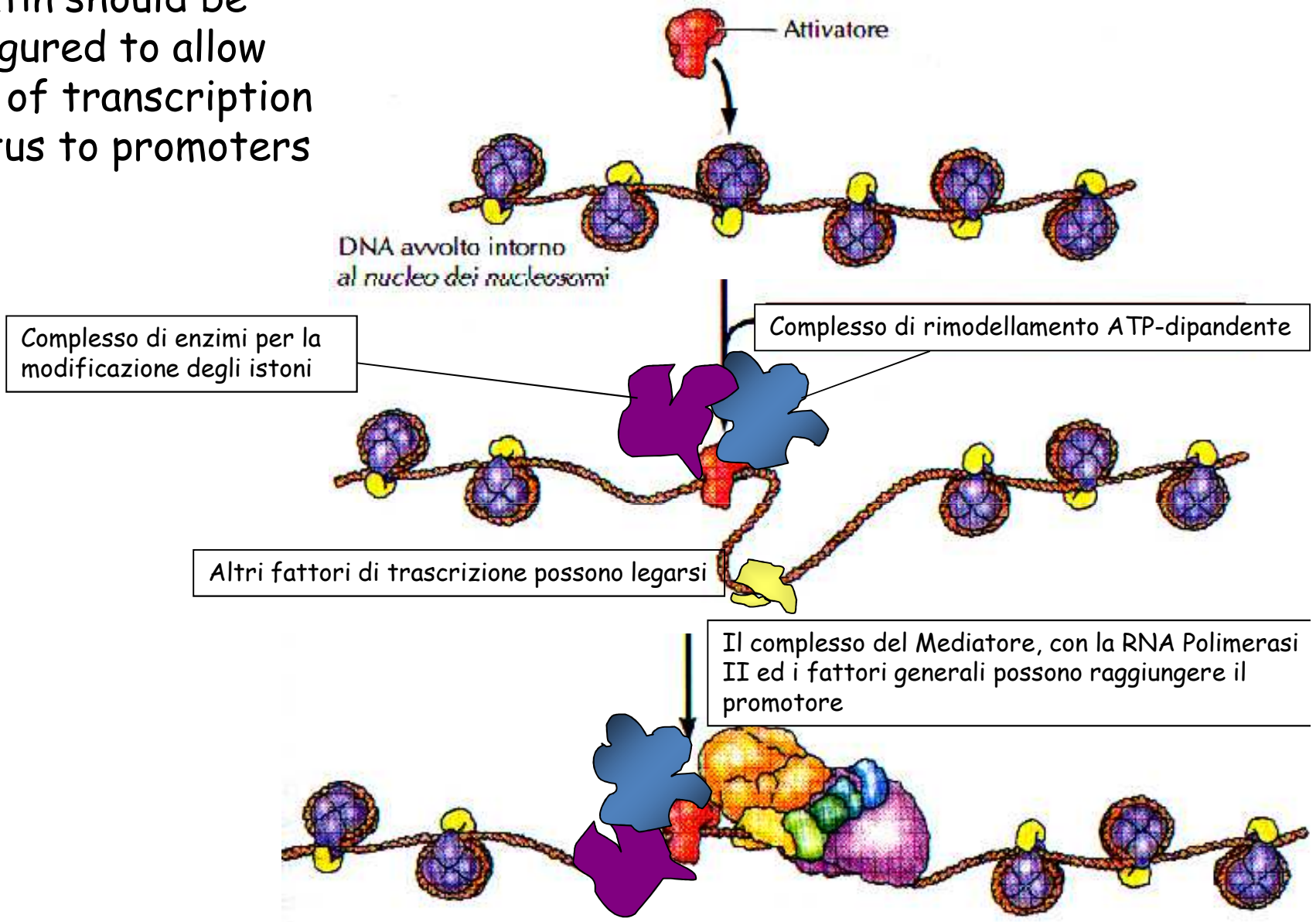
1 example:



human fibroblasts: 48 hrs serum starvation, then serum back for the indicated times
RNA extracted at time points labelled with red dye
RNA extracted at time = 0 labelled with green dye

early response

Chromatin should be reconfigured to allow binding of transcription apparatus to promoters



modified from Cooper "The Cell", Sinauer Ed. 1997

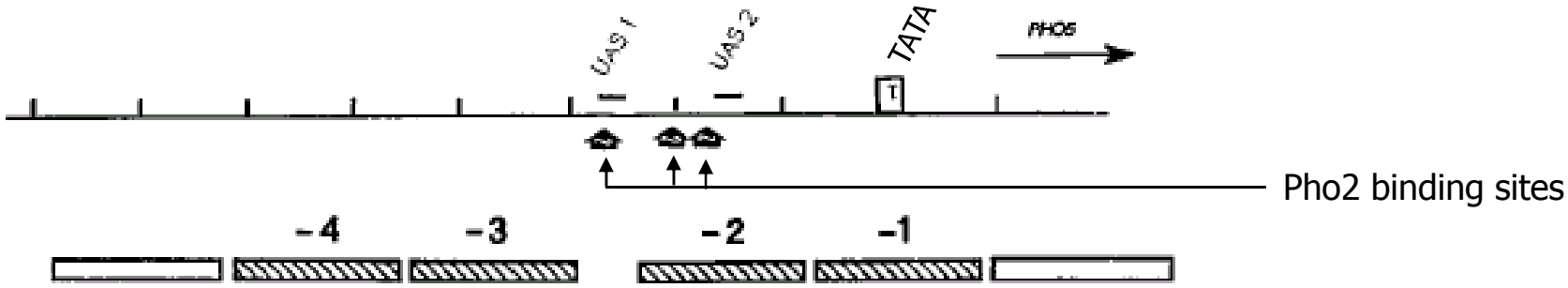
One extremely well studied model system for gene activation (switch from repressed to activated status) is represented by the PHO5 gene in S. cerevisiae.

PHO5 encodes for a protein phosphatase playing a role in phosphate homeostasis

When the ortophosphate level in the cell falls below a treshold value, the PHO5 promoter:

- changes its chromatic status – nucleosomes are **mobilized**
- becomes DNaseI hypersensitive
- transcription starts

mRNA and protein is produced → phosphates are removed from storage proteins and the intracellular level is restored



Pho4 is the P-sensitive inducer, whereas Pho2 is constitutive

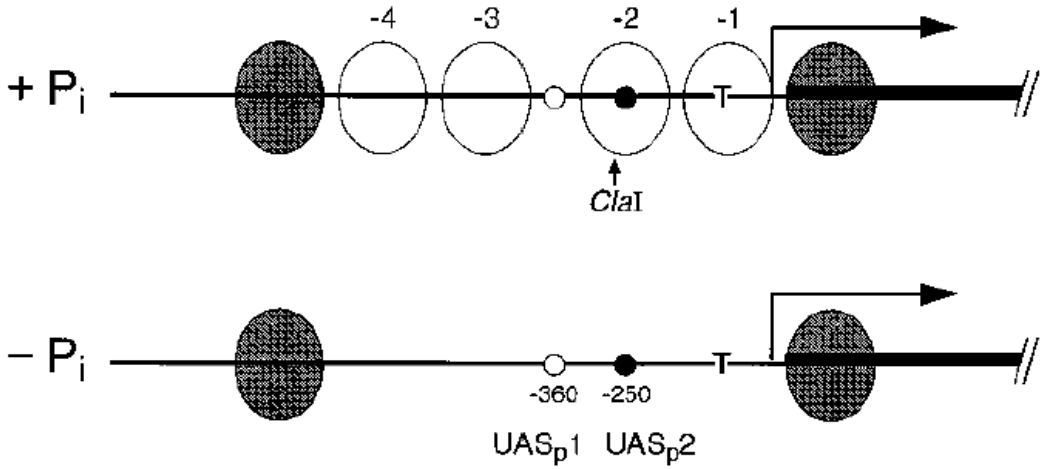


Figure 1. Chromatin Structure at the *PHO5* Promoter

Nucleosomes −1, −2, −3, and −4 are remodeled upon activation of the promoter by phosphate starvation conditions (Almer et al., 1986). The small circles mark UASp1 (open) and UASp2 (solid), which are Pho4-binding sites found by in vitro (Vogel et al., 1989) and in vivo (Venter et al., 1994) footprinting experiments. The positions are listed relative to the coding sequence (solid bar). T denotes the TATA box (Rudolph and Hinnen, 1987). The location of a ClaI site at −275 relative to the coding region is shown.

Molecular Cell, Vol. 11, 1599–1607, June, 2003, Copyright ©2003 by Cell Press

Histones Are First Hyperacetylated and Then Lose Contact with the Activated *PHO5* Promoter

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D-80336 München
Germany

Summary

We have analyzed the histone modification status of the *PHO5* promoter from yeast by the ChIP technology and have focused on changes occurring upon activation. Using various acetylation-specific antibodies, we found a dramatic loss of the acetylation signal upon induction of the promoter. This turned out to be due, however, to the progressive loss of histones altogether. The fully remodeled promoter appears to be devoid of histones as judged by ChIP analyses. Local histone hyperacetylation does indeed occur, however, prior to remodeling. This can explain the delay in chromatin remodeling in the absence of histone acetyltransferase activity of the SAGA complex that was previously documented for the *PHO5* promoter. Our findings shed new light on the nucleosomal structure of fully remodeled chromatin. At the same time, they point out the need for novel controls when the ChIP technique is used to study histone modifications in the context of chromatin remodeling in vivo.

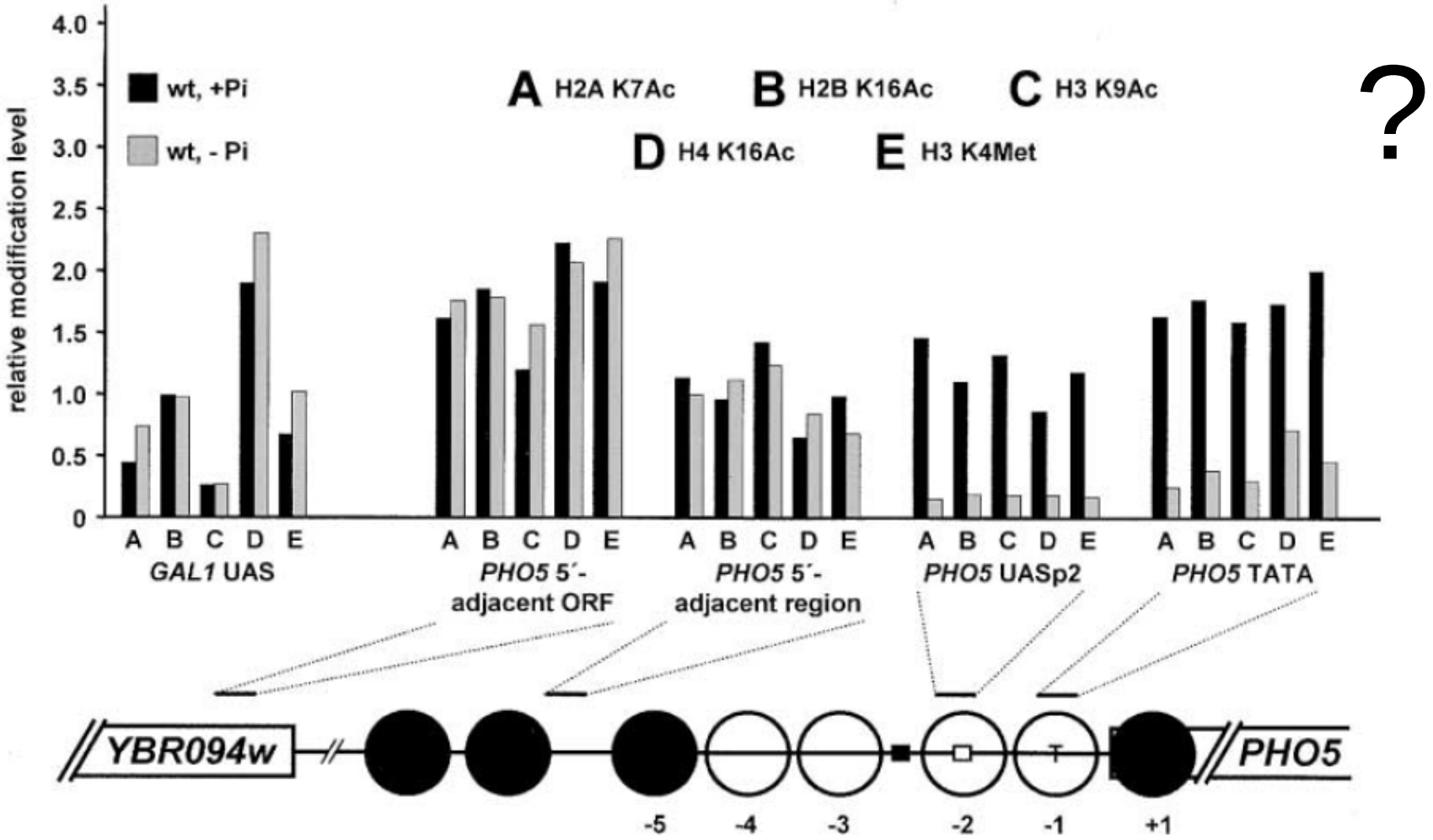


Figure 1. ChIP Analysis of Histone Acetylation at the *PHO5 Promoter upon Chromatin* Remodeling and Transcriptional Activation Positions of the PCR fragments used for ChIP analysis of the *PHO5 promoter are shown* with respect to the nucleosomal organization of the repressed promoter. Nucleosomes, which are remodeled at the active promoter, are indicated by open circles (Almer et al., 1986). UASp1 (black box), UASp2 (gray box), and the TATA box (T) are also indicated. Strain 8136 (wt) was phosphate starved for 4 hr, and the chromatin was fixed by formaldehyde treatment. Chromatin fragments were precipitated with antibodies specific for acetylated histones as indicated on top (labeled A–E). The amounts of coimmunoprecipitated DNA determined by quantitative PCR were normalized to the respective input DNA and are shown in arbitrary units. Black bars show ChIP signals of the repressed promoter, and gray bars show ChIP signals after 4 hr induction. Standard deviations were less than 15%.

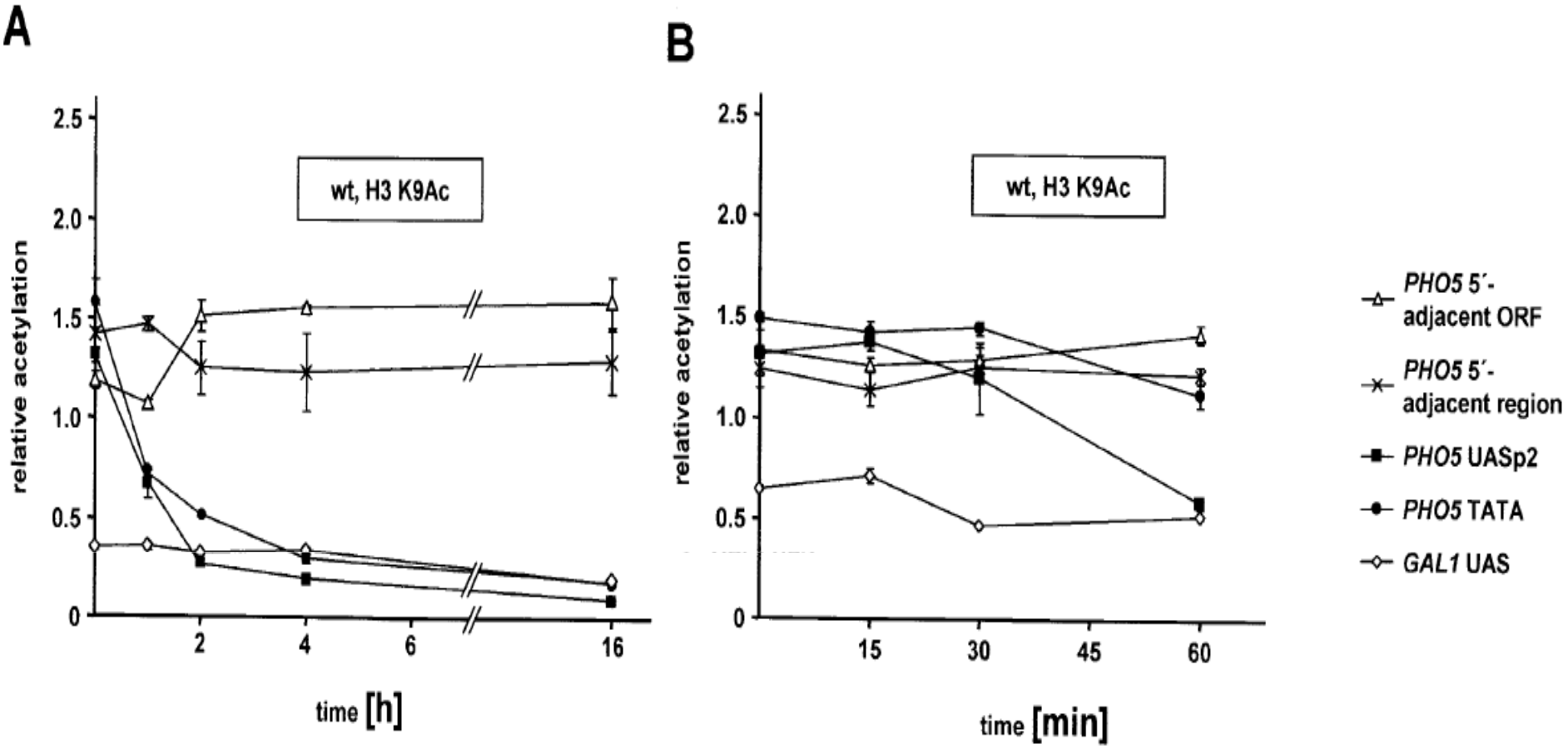
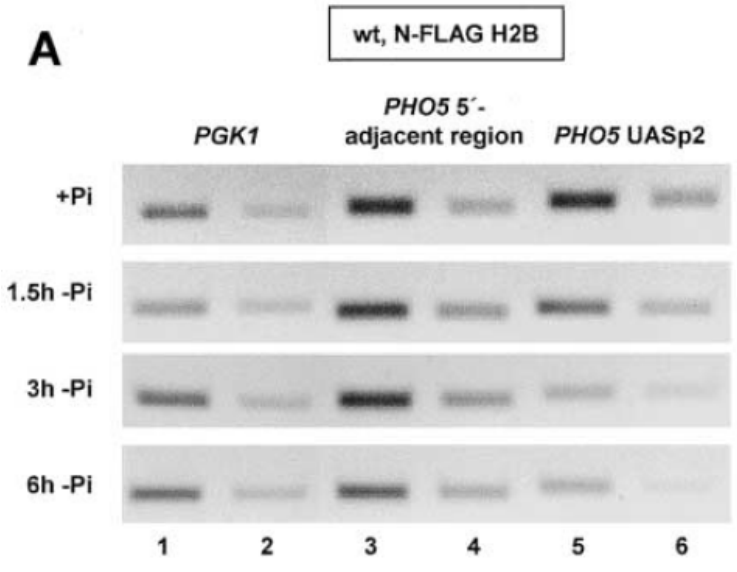


Figure 2. Time Course of H3 K9 Acetylation in a Wild-Type Strain during *PHO5* Induction. Acetylation of H3 K9 was determined by ChIP analysis. Relative acetylation levels at *PHO5* and the control regions are plotted against the induction time.
In (A) the time course of acetylation was determined over a 16 hr period and in (B) at early times of induction.

May be nucleosomes are removed from this region....?



H2B is lost from the promoter

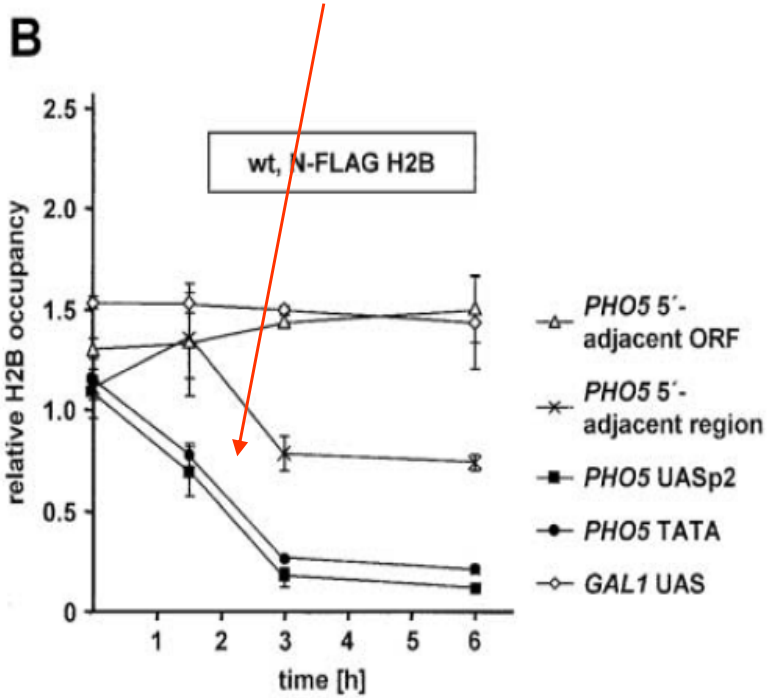
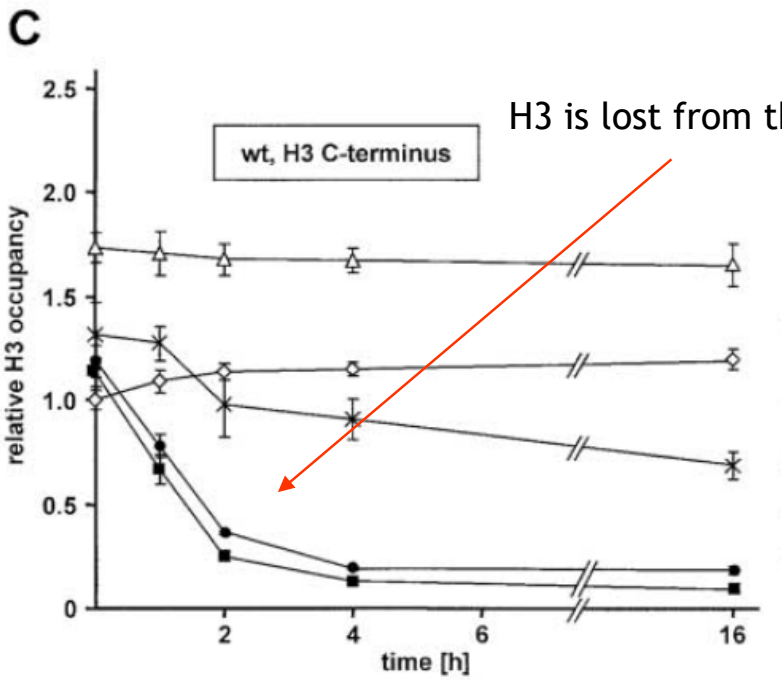


Figure 3. Histones Are Lost from the Remodeled *PHO5* Promoter
(A) Strain YZS276 (Flag-H2B) was subjected to ChIP analysis at various time points after induction by phosphate starvation. Histone H2B occupancy at *PHO5* and the *PGK1* promoter was determined by immunoprecipitation with an anti-FLAG antibody. IP samples were analyzed using two different concentrations of the respective template DNA with each primer pair (2.5-fold dilution of template DNA in each even numbered lane) to confirm that the PCR reaction was in the linear range.
(B) Histone H2B occupancy was determined by ChIP employing real-time PCR for the quantification of DNA in the precipitates.
(C) Strain 8136 (wt) was induced by phosphate starvation, and H3 occupancy was measured at various time-points after induction using ChIP with an antibody against the C terminus of histone H3.



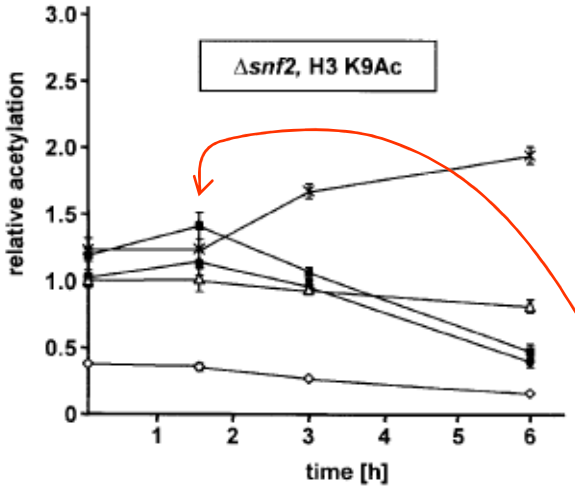
H3 is lost from the promoter

ATP-dependent chromatin remodelers.
Enzymes that induce a topological change into nucleosomal DNA, altering DNA/histone interaction and/or the positioning of nucleosomes.

Chromatin remodeling activities rely on quite large multiprotein **complexes** that are recruited to gene promoters by interaction with transcription factors

Using of a **snf2** defective strain: Snf2 is one of the major ATP-dependent chromatin remodelling enzymes in yeast.

B

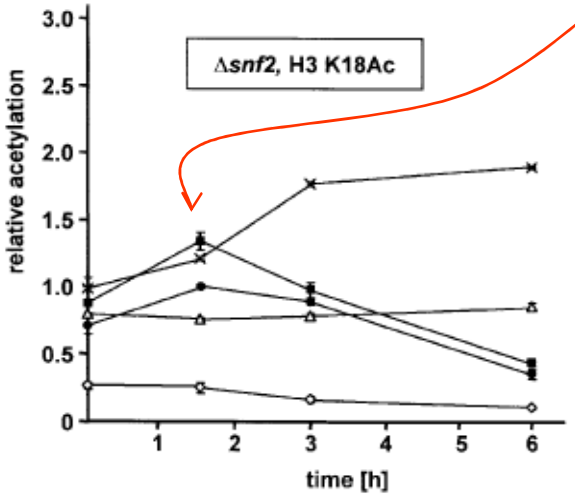


H3K9 acetylation / H3 occupancy

time [h]	0	1.5	3
PHO5 5´-adjacent ORF	1.0	1.0	0.9
PHO5 UASp2	1.0	1.3	1.9
PHO5 TATA	1.0	1.1	1.7

Transient increase in H3K9 and H3K18 acetylation is now appreciated

C



H3K18 acetylation / H3 occupancy

time [h]	0	1.5	3
PHO5 5´-adjacent ORF	1.0	0.9	1.0
PHO5 UASp2	1.0	1.7	2.4
PHO5 TATA	1.0	1.4	2.4

Figure 4. Histone H3 hyper-acetylation at the Induced *PHO5* Promoter in a *snf2* Strain. Cells from strain 8141 (*snf2*) were induced by phosphate starvation, and the levels of histone H3 (A), H3 acetylated at lysine 9 (B), and at lysine 18 (C) were followed over time by ChIP. Acetylation levels normalized with respect to histone occupancy are listed in the tables. They were calculated by dividing the values for acetylated H3 by the H3 occupancy values. 0 time values were set to be 1.0.

Other ATP-dep. chromatin remodeling enzymes exist in yeast: removal of nucleosome is therefore delayed, but not abrogated.

This demonstrated that there are both specifi action at the promoter to activate:
ATP-dependent remodeling and histone modification (acetyation)

ATP-dependent chromatin remodelling enzyme families (Mammals))

SWI/SNF

ISWI

CHD

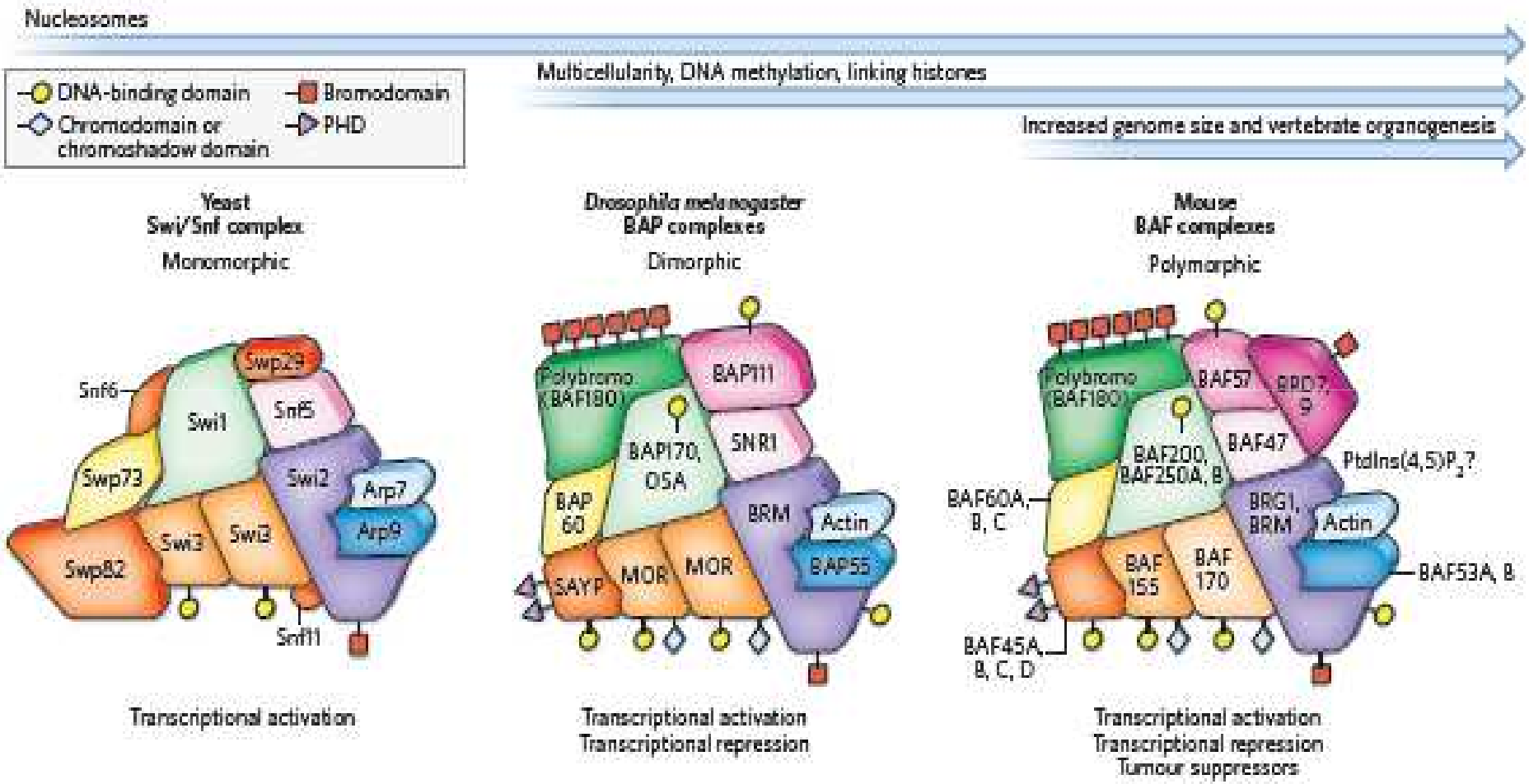
INO80

NATURE|Vol 463|28 January 2010|doi10.1038/nature08911

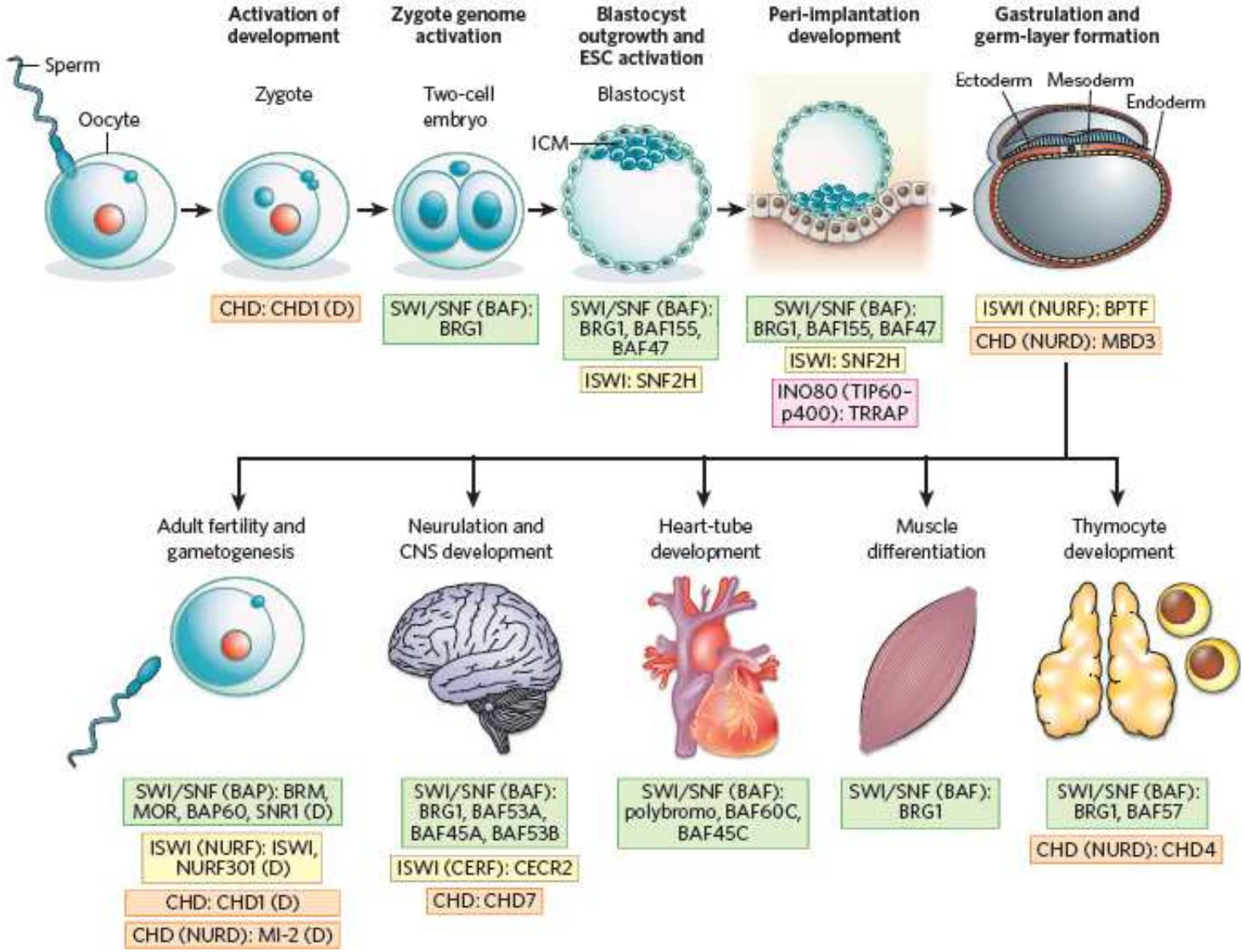
Chromatin remodelling during development

Lena Ho[1] & Gerald R. Crabtree[1]

New methods for the genome-wide analysis of chromatin are providing insight into its roles in development and their underlying mechanisms. Current studies indicate that chromatin is dynamic, with its structure and its histone modifications undergoing global changes during transitions in development and in response to extracellular cues. In addition to DNA methylation and histone modification, ATP-dependent enzymes that remodel chromatin are important controllers of chromatin structure and assembly, and are major contributors to the dynamic nature of chromatin. Evidence is emerging that these chromatin-remodelling enzymes have instructive and programmatic roles during development. Particularly intriguing are the findings that specialized assemblies of ATP-dependent remodellers are essential for establishing and maintaining pluripotent and multipotent states in cells.


Nucleosomes
Multicellularity, DNA methylation, linking histones
Increased genome size and vertebrate organogenesis
DNA-binding domain
Bromodomain
Chromodomain or chromoshadow domain
PHD
Yeast
Swi/Snf complex
Monomorphic
Swp29
Snf6
Swi1
Snf5
Swp73
Swi2
Arp7
Arp9
Swi3
Swi3
Swp82
Snf11
Transcriptional activation
Drosophila melanogaster
BAP complexes
Dimorphic
Polybromo (BAF180)
BAP111
BAP170, OSA
SNR1
BAP 60
BRM
Actin
BAP55
SAYP
MOR
MOR
Transcriptional activation
Transcriptional repression
Mouse
BAF complexes
Polymorphic
Polybromo (BAF180)
BAF57
BRD7, 9
BAF200, BAF250A, B
BAF47
PtdIns(4,5)P2?
BAF60A, B, C
BRG1, BRM
Actin
BAF53A, B
BAF 155
BAF 170
BAF45A, B, C, D
Transcriptional activation
Transcriptional repression
Tumour suppressors

ATP-dependent chromain remodelers play a very important role, as demonstrated by the fact that they are individually essential for development



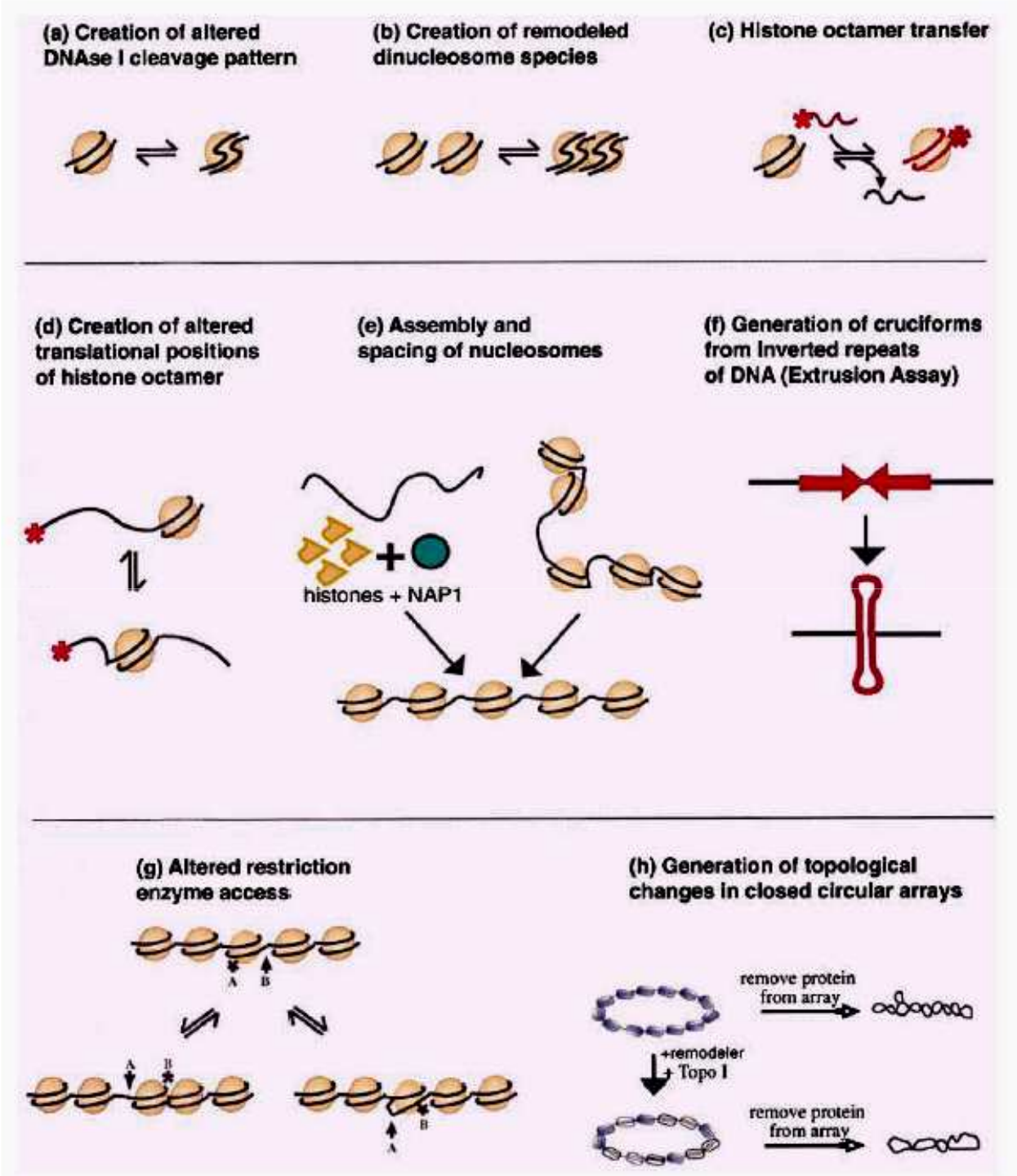


Figure 2. Biochimical Activities of ATPDependent Remodeling Complexes
Each panel depicts a known activity of at least one remodeling complex (see text).
(A) The 10 bp pattern generated by DNaseI on a positioned nuclesome is disrupted. Some DNA sites become hypersensitive, and some become less accessible to DnaseI.
(B) A nucleosomal species is generated that has the size of a dinucleosome and has a disrupted DNaseI cleavage pattern.
(H) Treatment of a closed circular nucleosomal array with TopoI followed by deproteinization gives one negative supercoil per nucleosome. A remodeler can reduce this number of supercoils without loss of the histone octamers.

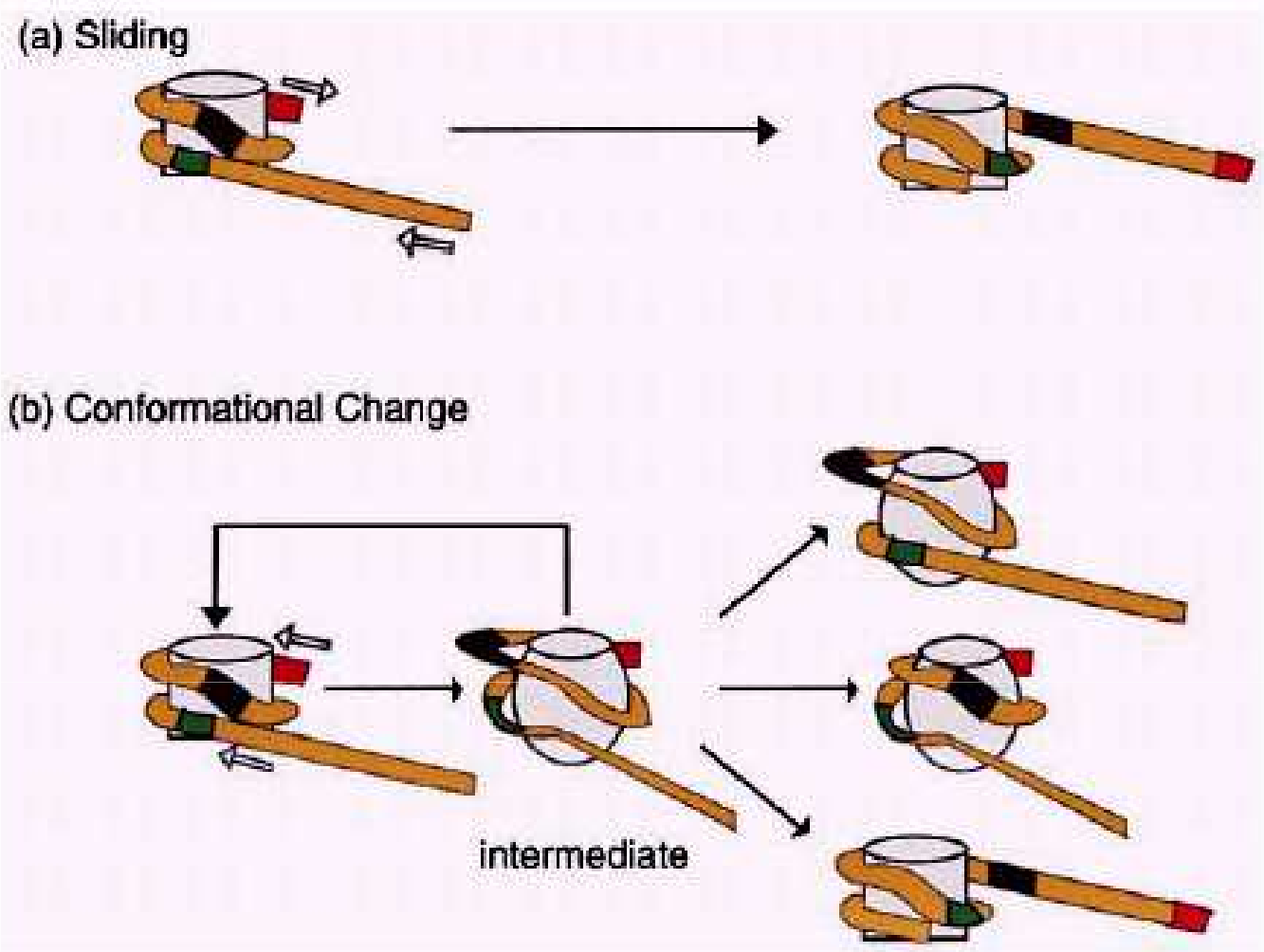
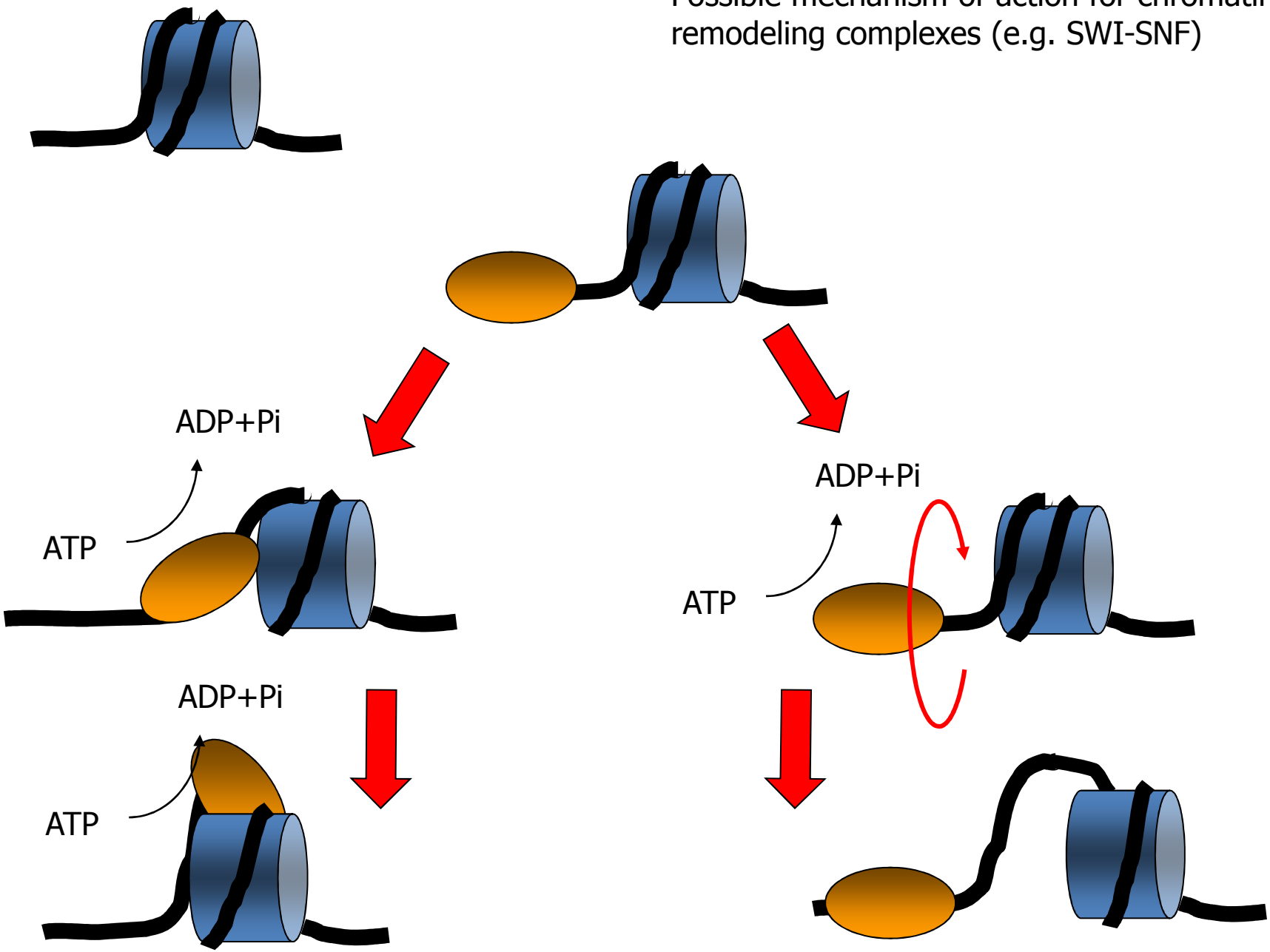


Figure 3. Two Models for the Mechanism of ATP-Dependent Nucleosome Remodeling

The structures are depicted for the intermediate, and products in (B) are hypothetical and could involve changes in the conformation of DNA, histones, or both.

Possible mechanism of action for chromatin remodeling complexes (e.g. SWI-SNF)



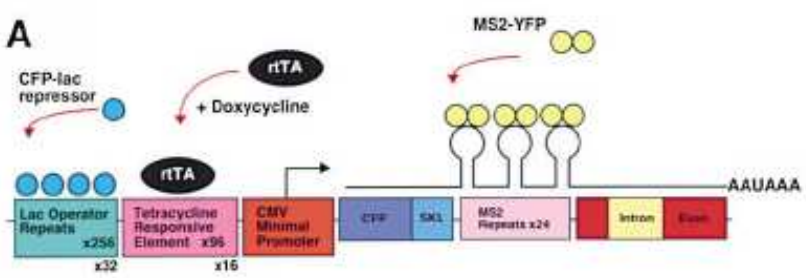
This, as usual, is the friendly yeast system, where apparently everything is so easy....

What about mammalian cells?

Cell, Vol. 116, 683–698, March 5, 2004, Copyright ©2004 by Cell Press

From Silencing to Gene Expression: Real-Time Analysis in Single Cells

Susan M. Janicki,[1] Toshiro Tsukamoto,[2] Simone E. Salghetti,[1] William P. Tansey,[1] Ravi Sachidanandam,[1] Kannanganattu V. Prasanth,[1] Thomas Ried,[3] Yaron Shav-Tal,[4] Edouard Bertrand,[5] Robert H. Singer,[4] and David L. Spector[1,*]



Summary

We have developed an inducible system to visualize gene expression at the levels of DNA, RNA and protein in living cells. The system is composed of a 200 copy transgene array integrated into a euchromatic region of chromosome 1 in human U2OS cells. The condensed array is heterochromatic as it is associated with HP1, histone H3 methylated at lysine 9, and several histone methyltransferases. Upon transcriptional induction, HP1α is depleted from the locus and the histone variant H3.3 is deposited suggesting that histone exchange is a mechanism through which heterochromatin is transformed into a transcriptionally active state. RNA levels at the transcription site increase immediately after the induction of transcription and the rate of synthesis slows over time. Using this system, we are able to correlate changes in chromatin structure with the progression of transcriptional activation allowing us to obtain a real-time integrative view of gene expression.

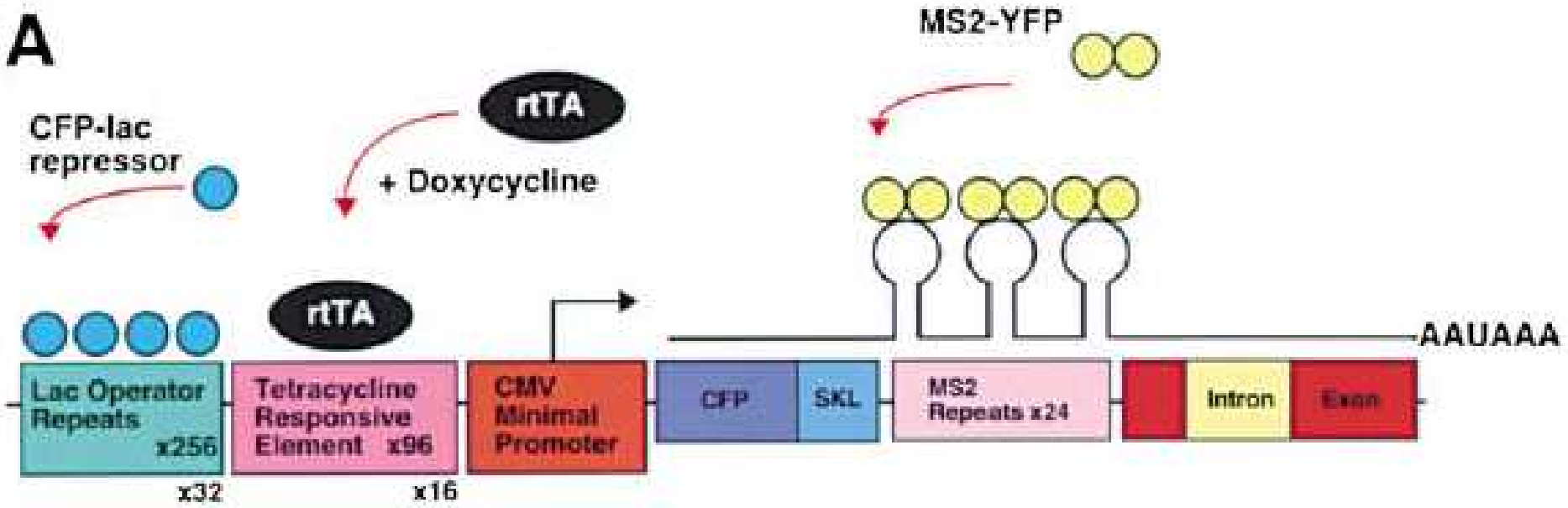
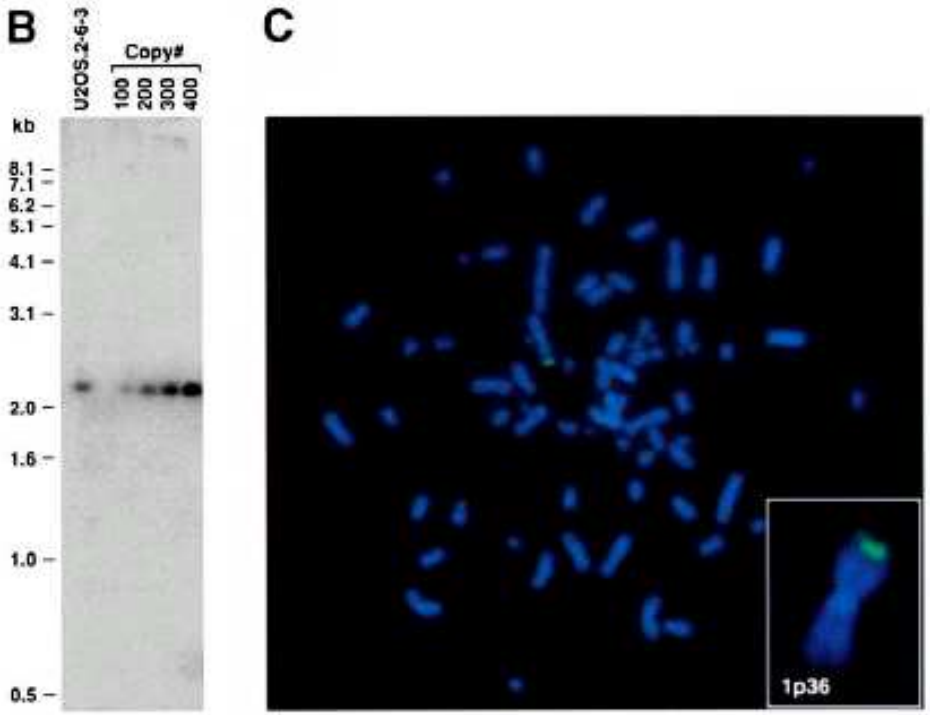
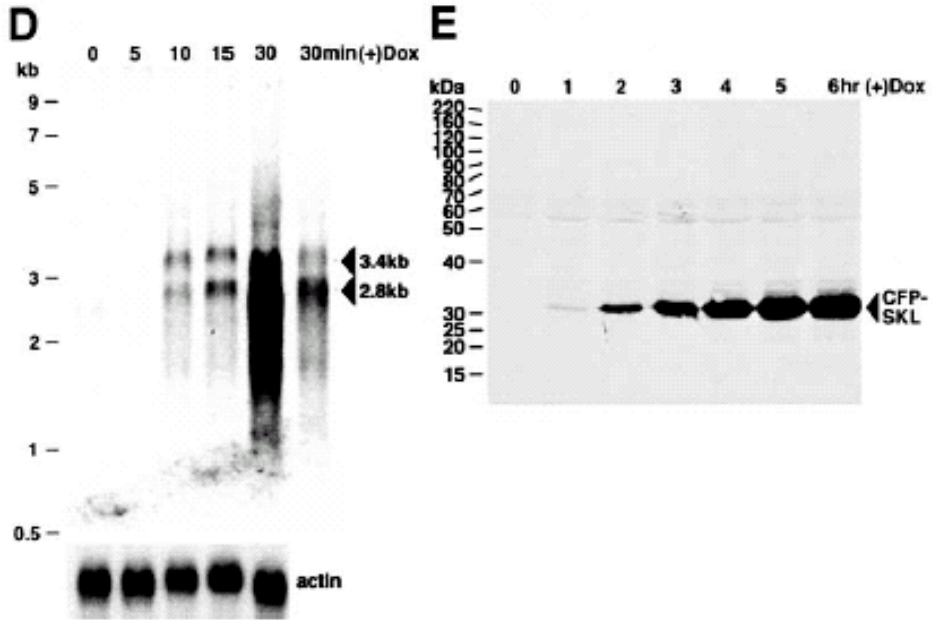


Figure 1. Characterization of Human U2OS 2-6-3 Cells

(A) Schematic representation of the gene expression plasmid, p3216PECMS2. The plasmid is composed of 256 copies of the *lac* operator, 96 tetracycline response elements, a minimal CMV promoter, CFP fused to the peroxisomal targeting signal SKL, 24 MS2 translational operators (MS2 repeats), a rabbit -globin intron/exon module, and a cleavage/polyadenylation signal. Expression of CFP-lac repressor allows the DNA to be visualized and expression of pTet-On (rtTA) in the presence of doxycycline (dox) drives expression from the CMV minimal promoter. When MS2-YFP (YFP fused to the MS2 coat protein) dimerizes and interacts with the stem loop structure of the translational operator, it allows the transcribed RNA to be visualized.





(B) Quantitative Southern blot of clone 2-6-3 genomic DNA. A 2.4 kb fragment is produced when clone 2-6-3 genomic DNA and p3216PECMS2β are digested with NcoI which cuts at the 5′ end of CFP and within the β-globin intron. Comparison of known quantities of plasmid DNA equal to 100, 200, 300, and 400 copies per cell showed that 2-6-3 cells contain ~200 stably integrated copies of p3216PECMS2β.

(C) DNA fluorescence in situ hybridization (DNA FISH) of 2-6-3 cells shows that there is a single integration site in the euchromatic region of chromosome 1p36.

(D) Northern blot time course analysis of RNA isolated 0, 5, 10, 15, and 30 min after the induction of transcription. The last lane shows a lighter exposure of the 30 min time point. Pre-mRNA transcripts run at 3.4 kb and spliced mRNA at 2.8 kb. The probe recognizes the MS2 repeats. Actin was probed as a loading control.

(E) Immunoblot time course analysis of CFP-SKL expression 0, 1, 2, 3, 4, 5, and 6 hr after the addition of doxycycline.

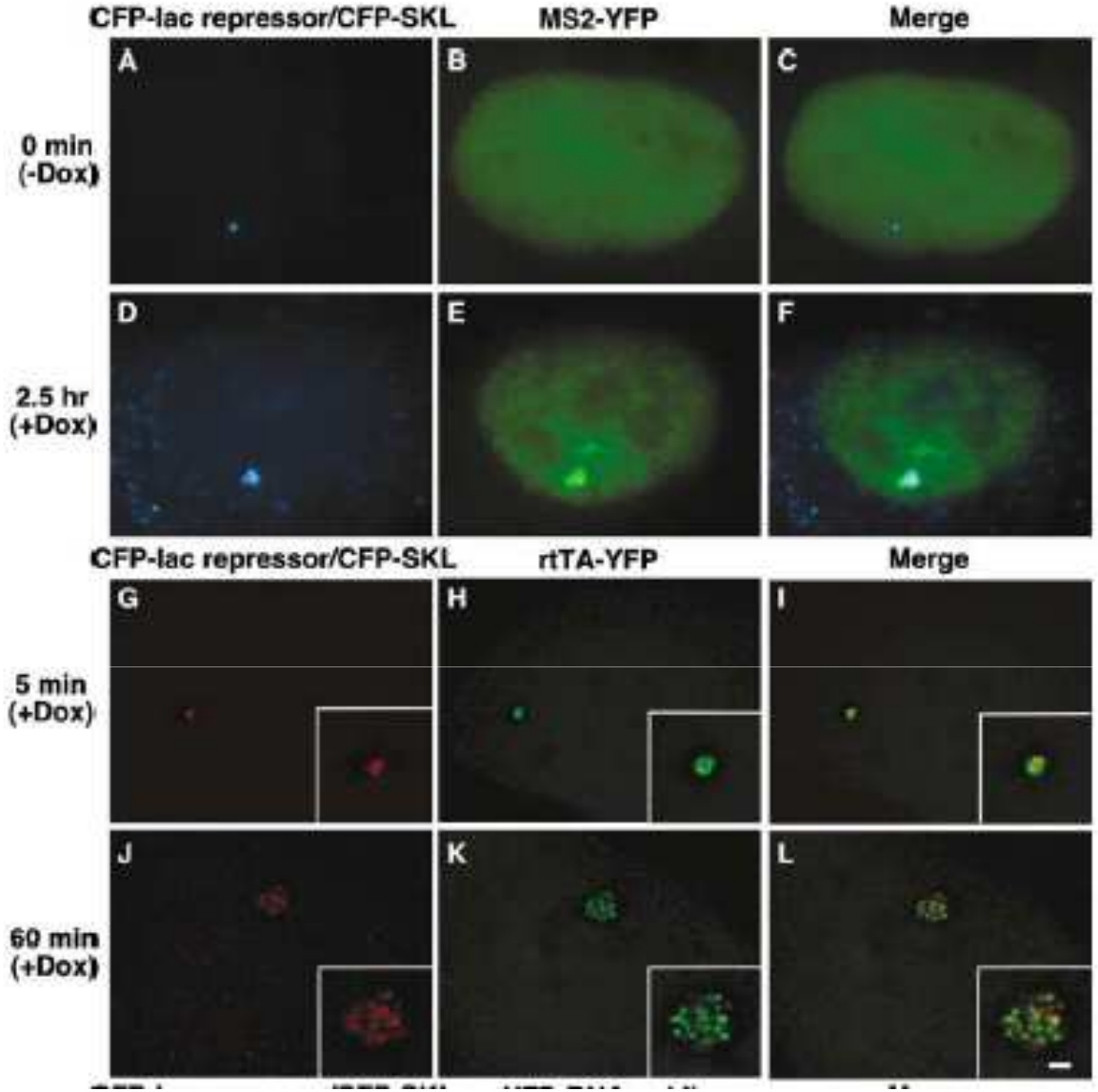


Figure 2. Visualization of DNA, RNA, and Protein in Living Cells

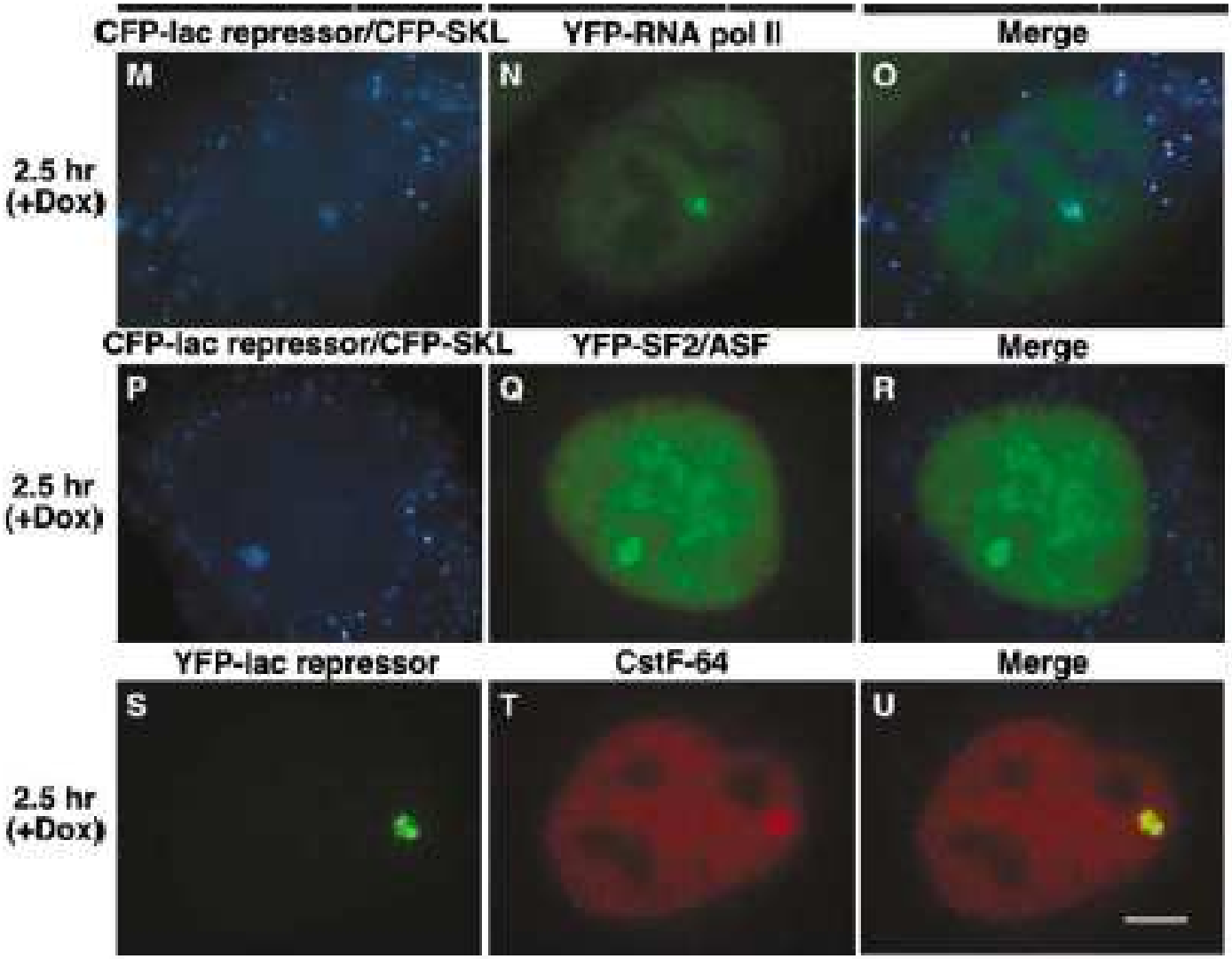
(A–F) U2OS 2-6-3 cells were transiently transfected with pSV2-CFP-lac repressor, pTet-ON (rtTA) and MS2-YFP, and imaging was begun 2.5 hr posttransfection.

(A–C) At 0 min () dox, CFP-lac repressor marks the locus (A) and MS2-YFP is diffusely distributed throughout the nucleus (B).

(D–F) 2.5 hr after the addition of Dox, the locus is highly decondensed and CFP-SKL is seen in the cytoplasmic peroxisomes (D). MS2-YFP accumulates at the site of the decondensed locus and is present in a particulate pattern throughout the nucleoplasm (E).

(G–L) Image stacks of cells expressing pSV2-CFP-lac repressor (pseudocolored red) and EYFP-rtTA-N1 (pseudocolored green) were collected and deconvolved in cells fixed 5 min (G–I) and 60 min (J–L) after the induction of transcription. Single sections from deconvolved stacks are shown.

Transient co-transfection of CFP-lac repressor, rtTA and MS2-YFP



(M–U) Factors involved in gene expression colocalize with the decondensed locus. YFP-RNA polymerase II (M–O), YFP-SF2/ASF (P–R), and Cstf64 (S–U) are present at the active locus. Scale bar is equal to 5 m. Scale bar in enlarged insets is equal to 1 m.

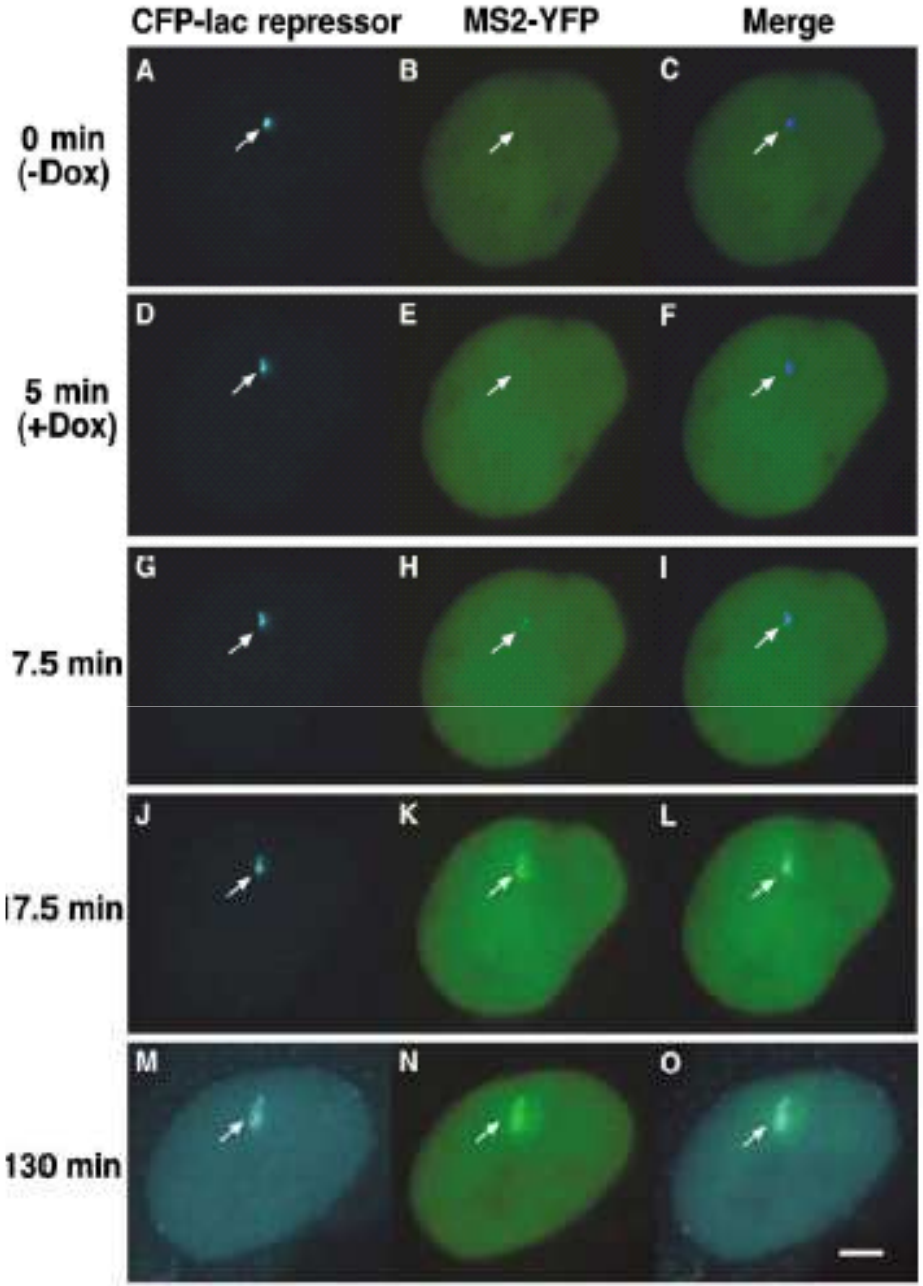
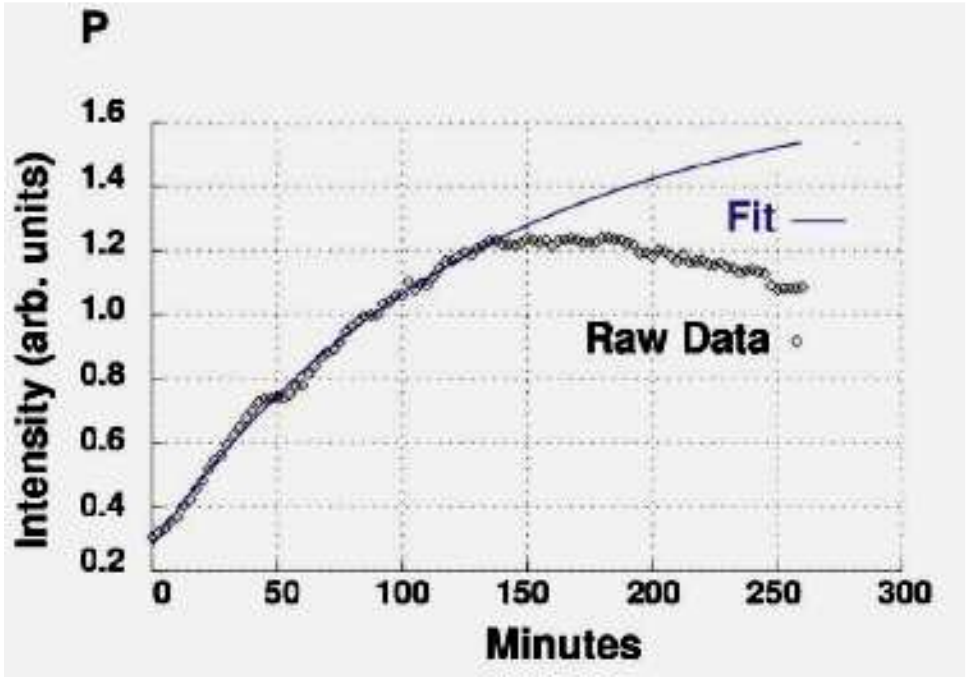


Figure 3. Kinetics of RNA Synthesis

(A–O) Still images from a time series of U2OS 2-6-3 cells during transcriptional activation showing the relationship between the chromatin of the locus, marked by CFP-lac repressor, and the RNA, marked by MS2-YFP. Also see Supplemental Data and Supplemental Movie S1 available on *Cell* website. Scale bar is equal to 5 μm.

(P) Quantitative analysis and modeling of RNA levels at the locus. The intensity of the MS2-YFP signal at the locus as it associates with the synthesized RNA was measured every 2.5 min. There is an initial linear increase after the induction of transcription which slows over time (following the form A*(1-exp(-B*t))). The fit shows a deviation from this increase at 140 min after which a decrease predominates. There is a delay in the increase at 50 min.



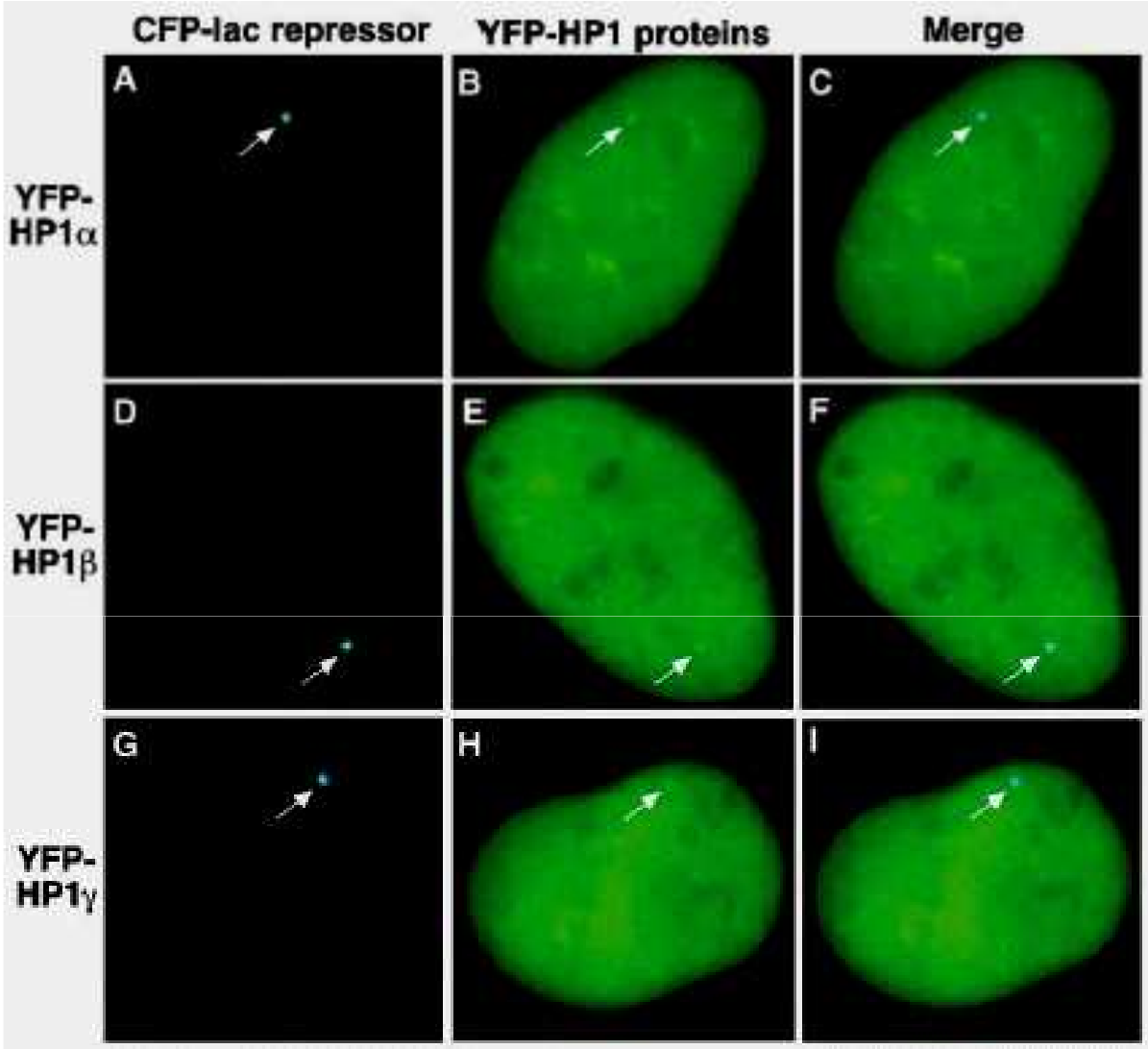
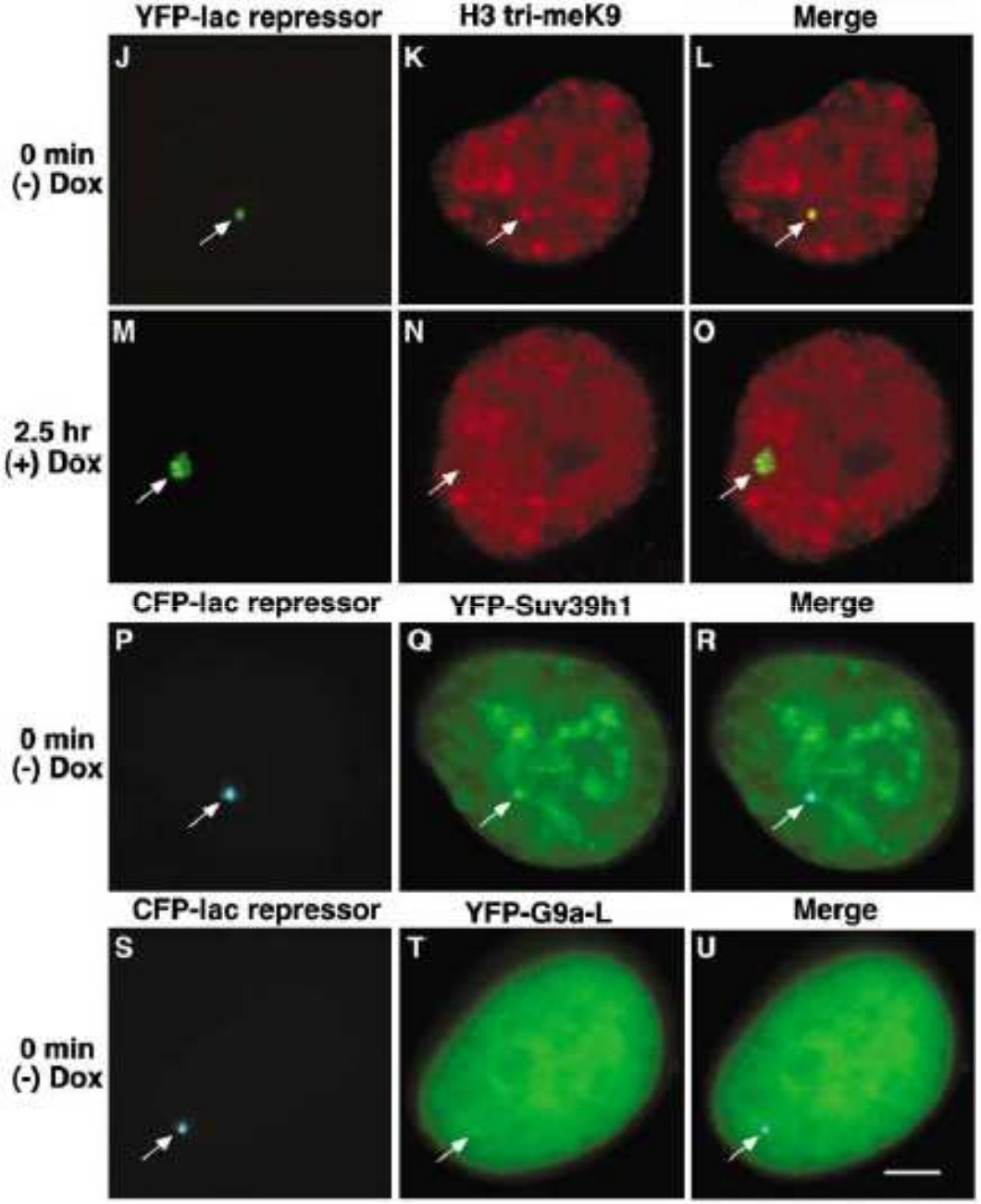


Figure 4. Characterization of the Condensed Heterochromatic Locus

YFP-HP1α (A-C), YFP-HP1β (D-F), and YFP-HP1γ (G–I) colocalize with the condensed locus, marked by CFP-lac repressor and the histone H3 is trimethylated on lysine 9 (H3 trimeK9) (J-L). The H3 lysine 9 modification is not detected after the induction of transcription (M–O; 2.5 hr postdox). The histone H3 K9 methyltransferases YFP-Suv39h1 (P–R) and YFP-G9a-L (S–U) are present at the condensed locus. Scale bar is equal to 5 μm.


YFP-lac repressor
H3 tri-meK9
Merge
J
K
L
0 min
(-) Dox
M
N
O
2.5 hr
(+) Dox
CFP-lac repressor
YFP-Suv39h1
Merge
P
Q
R
0 min
(-) Dox
CFP-lac repressor
YFP-G9a-L
Merge
S
T
U
0 min
(-) Dox

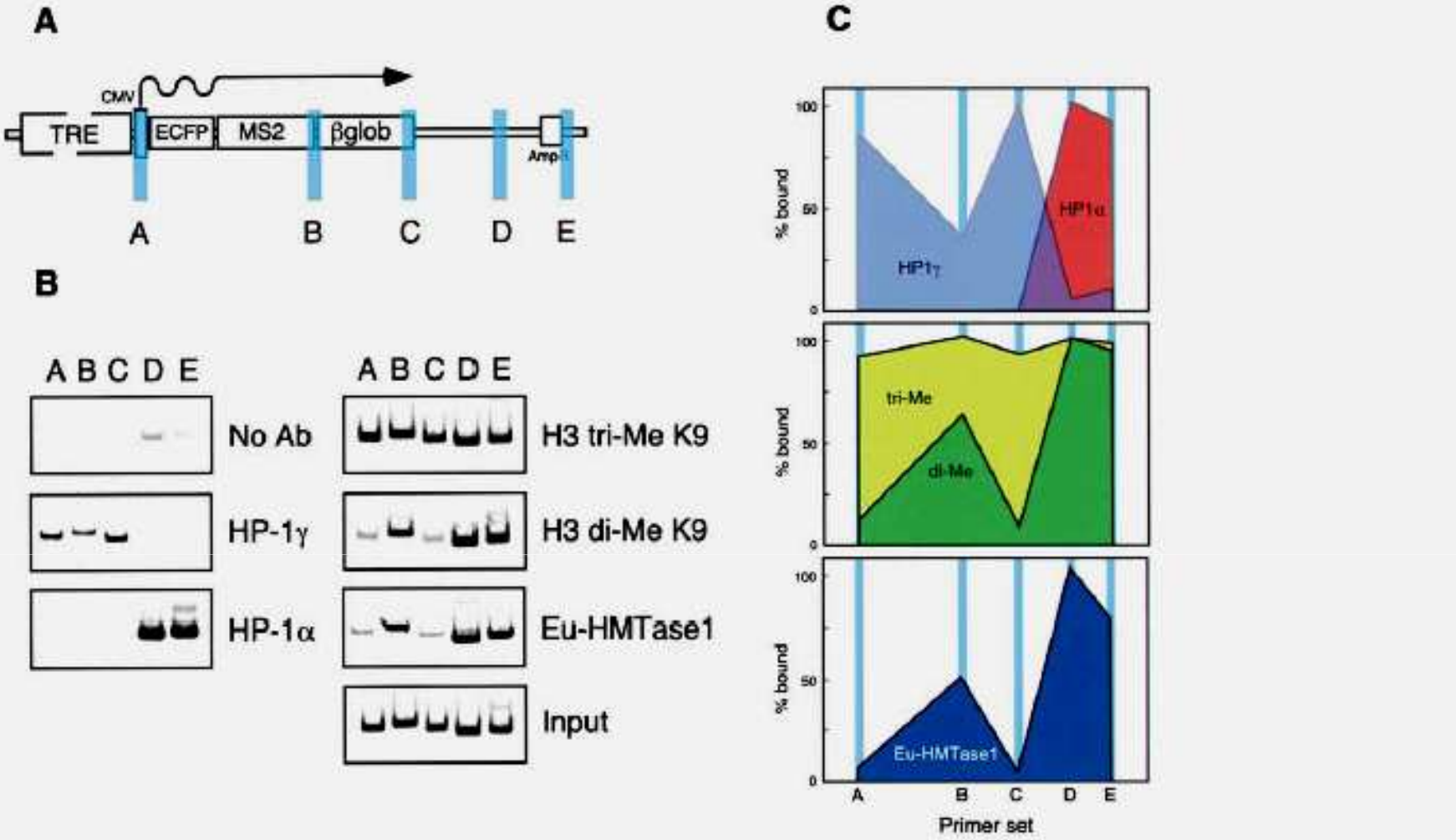


Figure 5. Chromatin Immunoprecipitation (ChIP) Analysis of Heterochromatin Proteins and Modifications on the Inactive Gene Expression Plasmid

Diagram (A) depicts the gene expression plasmid and the location of the primer pairs used: (a) promoter, (b) beginning of β-globin intron/exon module, (c) end of β-globin intron/exon module, (d) and (e) bacterial plasmid sequence.

(B) Results of the ChIP analysis showing the localization of HP1α, HP1γ, histone H3 di- and tri-MeK9, and Eu-HMTase1 on the gene expression plasmid.

(C) The levels of the associated factors and modifications are plotted.

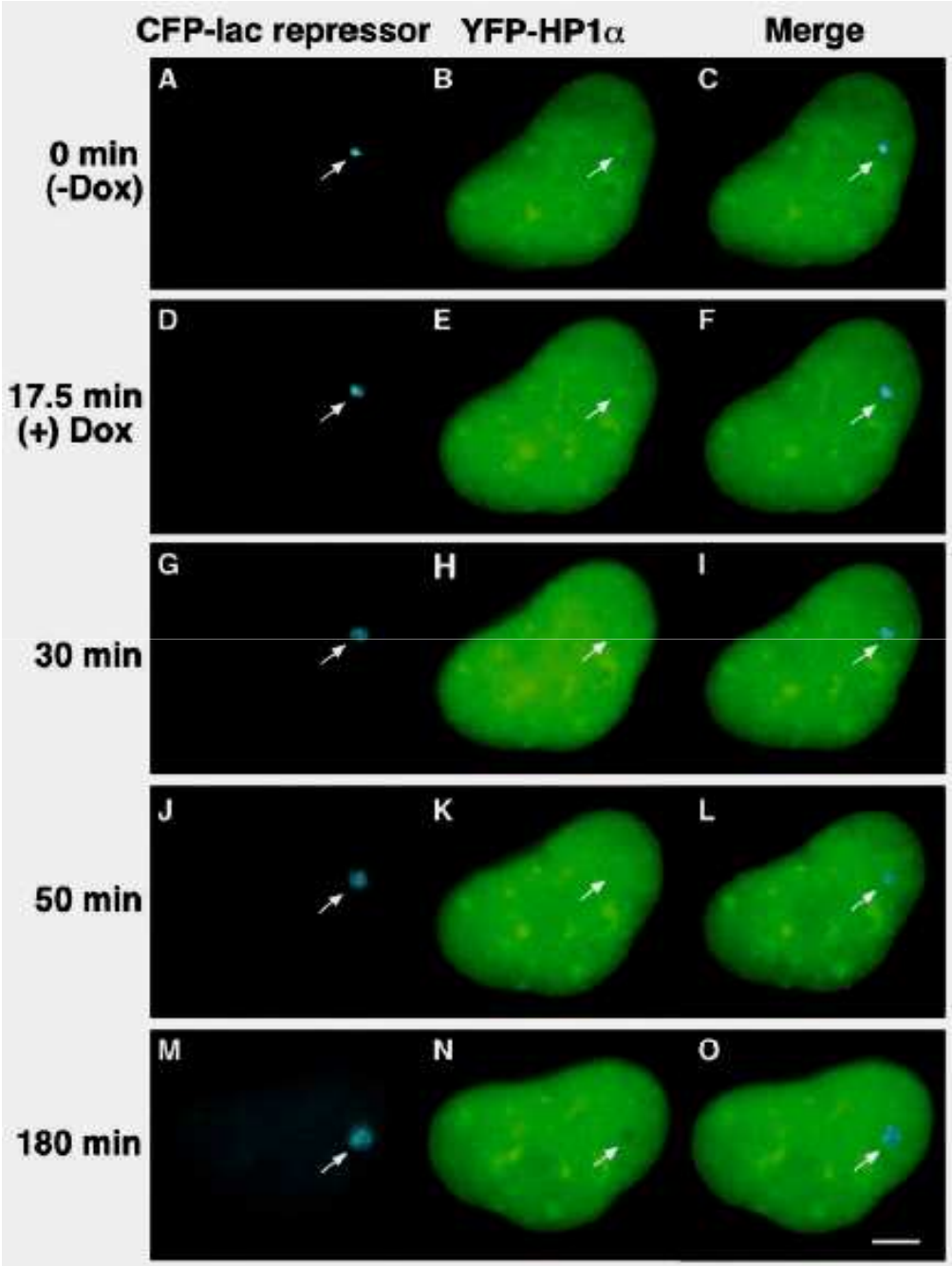


Figure 6. Dynamics of YFP-HP1α Depletion from the Locus during Transcriptional Activation

YFP-HP1α colocalizes with the condensed locus (0 min, A–C) and the condensed regions during the early time points of transcriptional activation (D–F, 17.5 min). It is seen in punctate structures at the 30 min time point (G–I) and appears smooth and diffuse by 50 min (J–L). 180 min postinduction, a dark region (HP1α depleted) that colocalizes with the decondensed locus, is seen in the YFP-HP1α image. Also see Supplemental Data and Supplemental Movie S2 available on *Cell* website. Scale bar is equal to 5 μm.

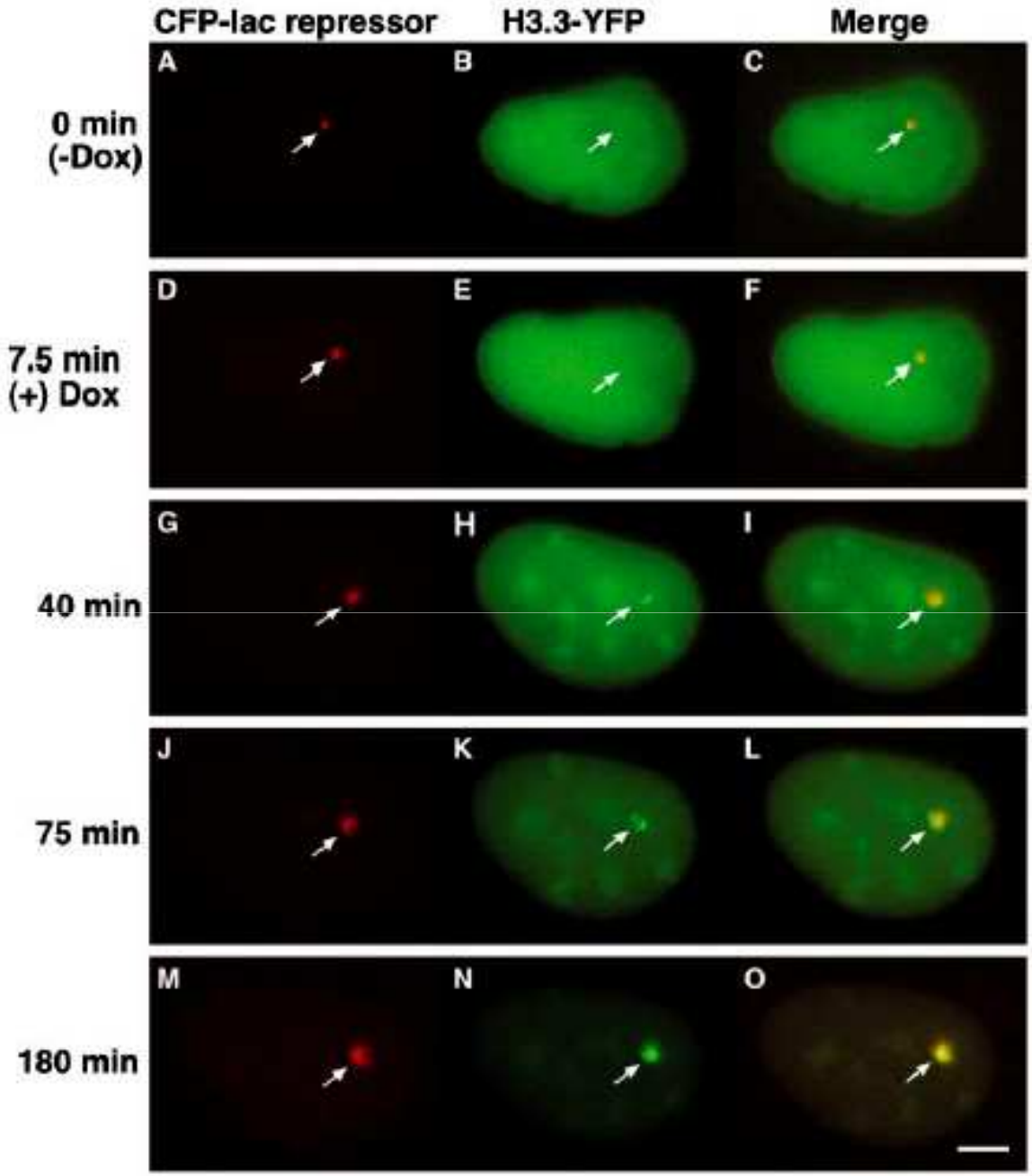


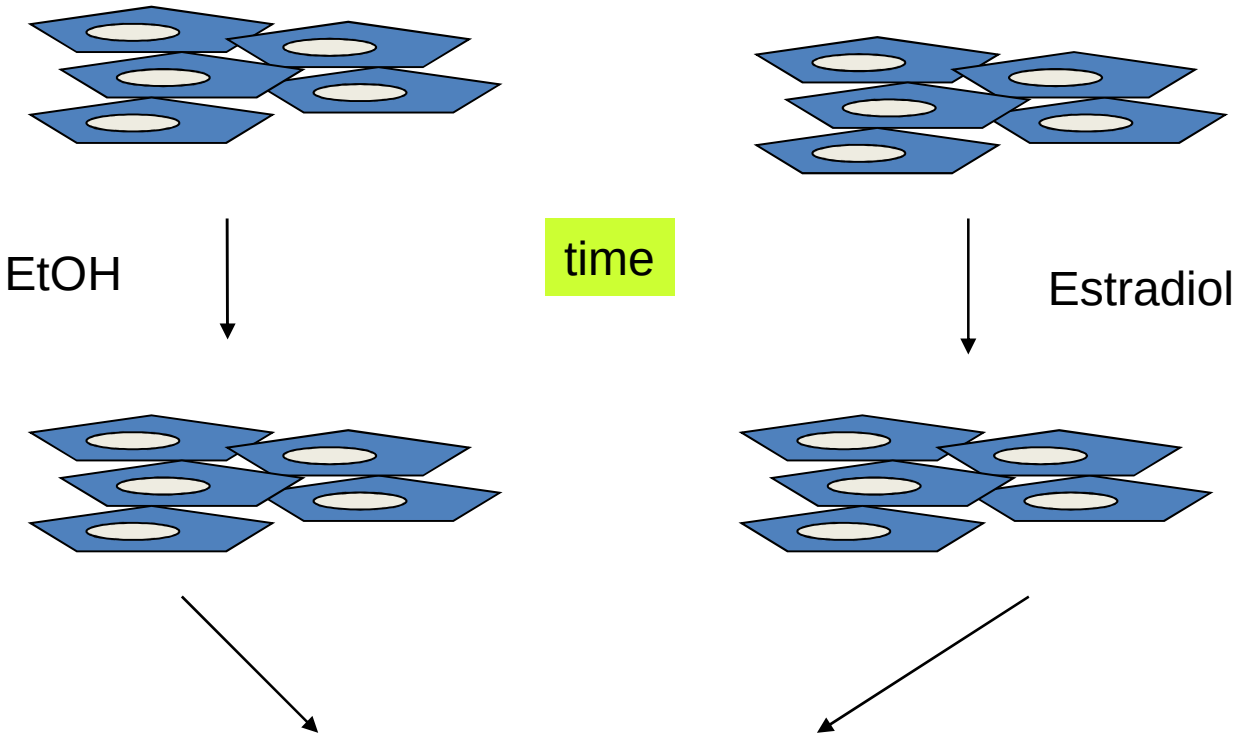
Figure 7. Analysis of the Deposition of the Histone Variant, H3.3, at the Locus during Transcriptional Activation

H3.3-YFP is not enriched at the condensed locus (A–C). A small spot adjacent to the locus is seen immediately after induction (D–F; 7.5 min). Significant deposition begins around the periphery of the decondensing locus (G–I, 40 min; J–L, 75 min) and eventually H3.3-YFP appears in a concentrated region that does not completely colocalize with CFP-lac repressor (pseudocolored red) (M–O; 180 min). Also see Supplemental Data and Supplemental Movie S3 available on *Cell* website. Scale bar is equal to 5 μm.

This study demonstrates “in vivo” that a regulatable transgene kept in the “off” status is heterochromatic.

Upon induction, the heterochromatic state is loosened, HP1 is lost and also the methyl-K9H3 is lost, most likely by **histone exchange.**

This is artificial.... what about a real-life system ?



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

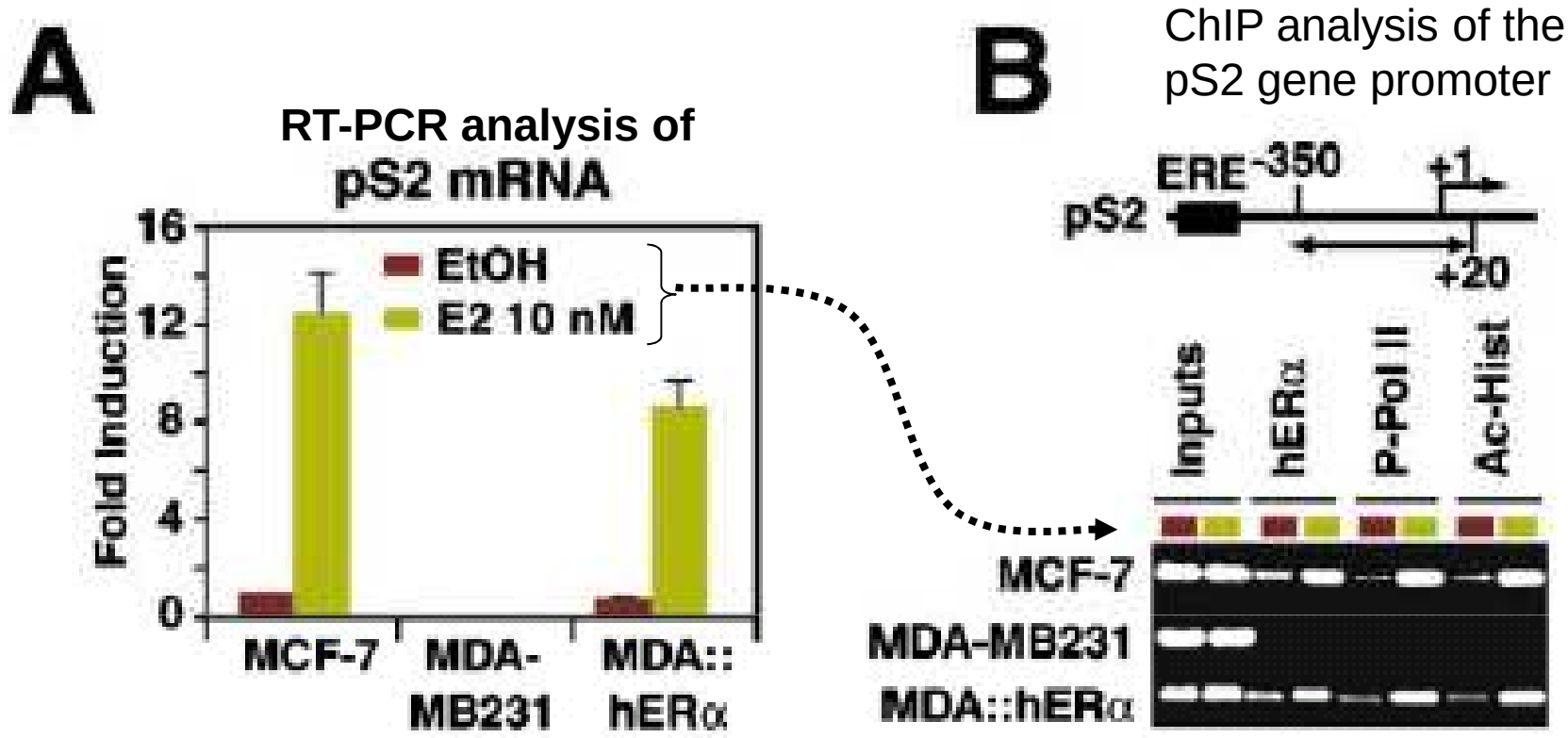


Figure 1. In Vivo Identification of the Transcription Factors Involved in pS2 Gene Activity

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

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

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

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

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Germany

Summary

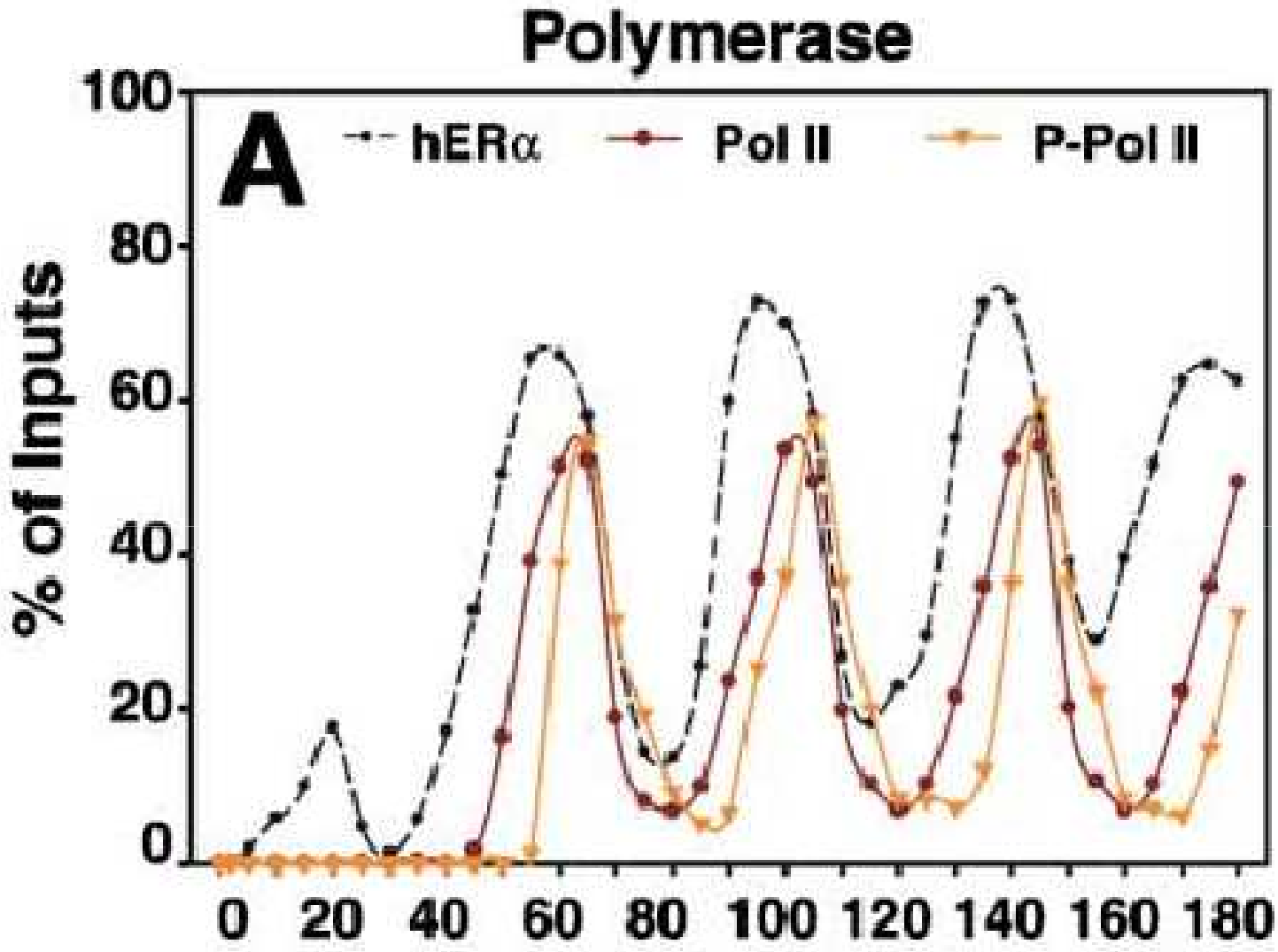
Transcriptional activation of a gene involves an orchestrated recruitment of components of the basal transcription machinery and intermediate factors, concomitant with an alteration in local chromatin structure generated by posttranslational modifications of histone tails and nucleosome remodeling. We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

MCF-7 cells are starved of estrogen for several days

2 hours before treatment, they are added of a-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 gene promoter



Minuti dopo estradiolo

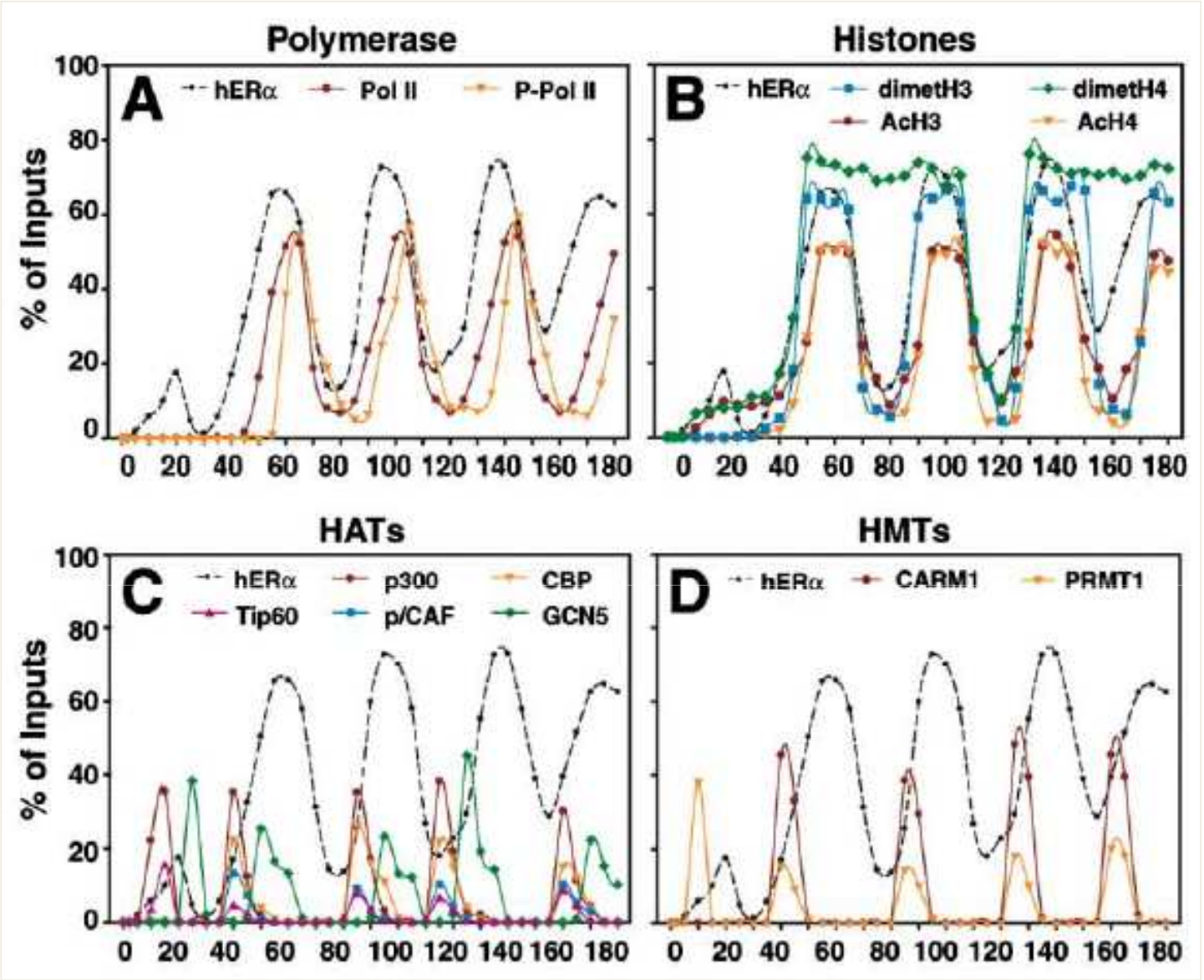
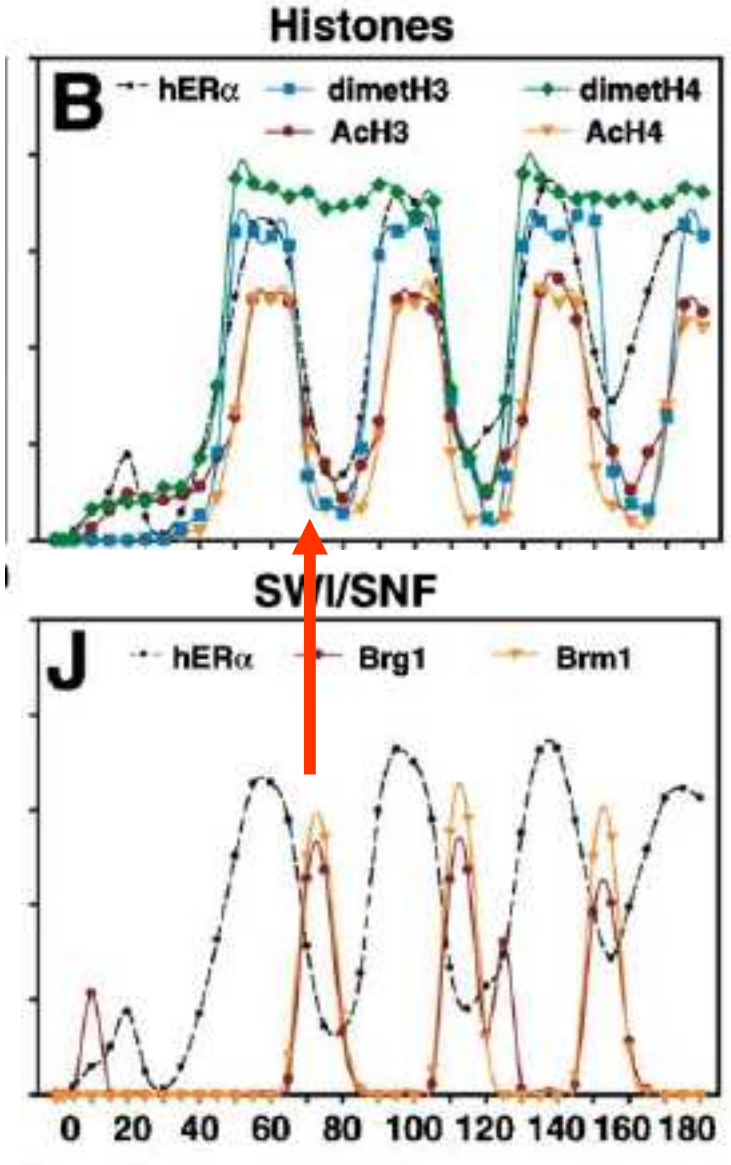


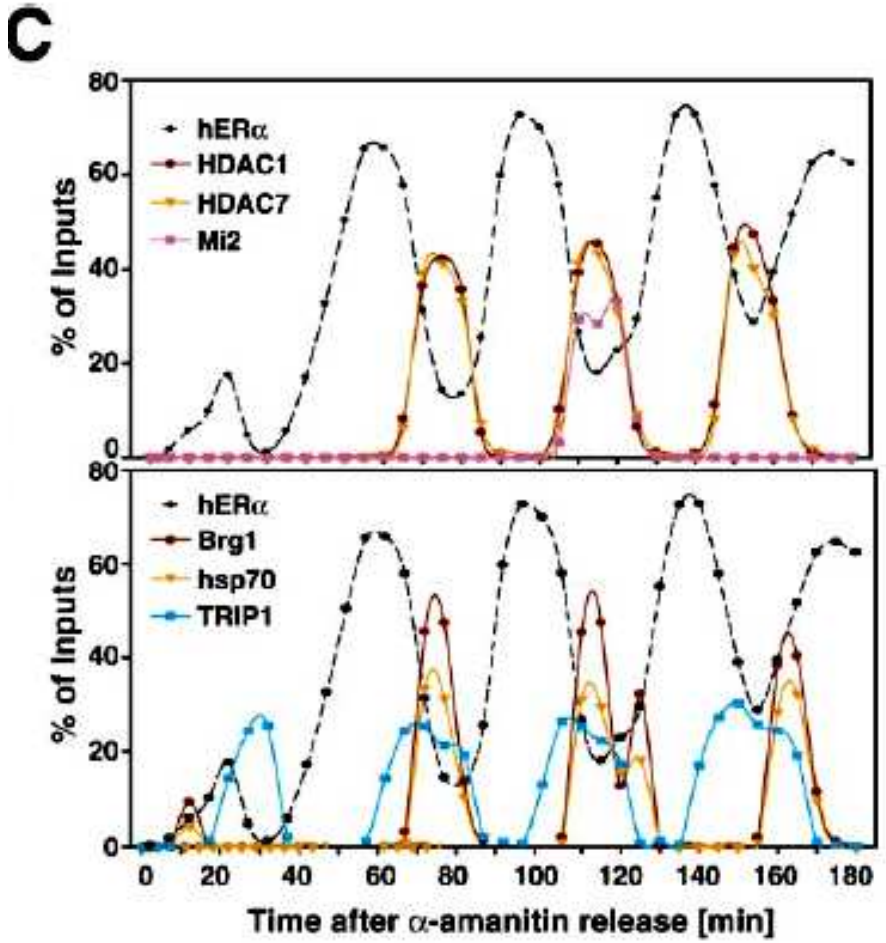
Figure 3. Dynamics of histone modification directed by E2-Liganded hER on the pS2 Promoter. Kinetic ChIP experiments were performed using specified antibodies as shown within the images. Chromatin was prepared on sampled cells at 5 minutes intervals. The amount of immunoprecipitated pS2 promoter was quantified by real-time PCR.



From Figure 3

Deacetlation of histones occurs at the end of each cycle and is accompanied by the recruitment of SWI-SNF ATPases.

Are HDACs also transiently associated?


C
hERα
HDAC1
HDAC7
Mi2
% of Inputs
hERα
Brg1
hsp70
TRIP1
% of Inputs
0
20
40
60
80
100
120
140
160
180
Time after α-amanitin release [min]

YES

Conclusion 3

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

What about house-keeping genes ?

OPEN ACCESS Freely available online PLoS GENETICS

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

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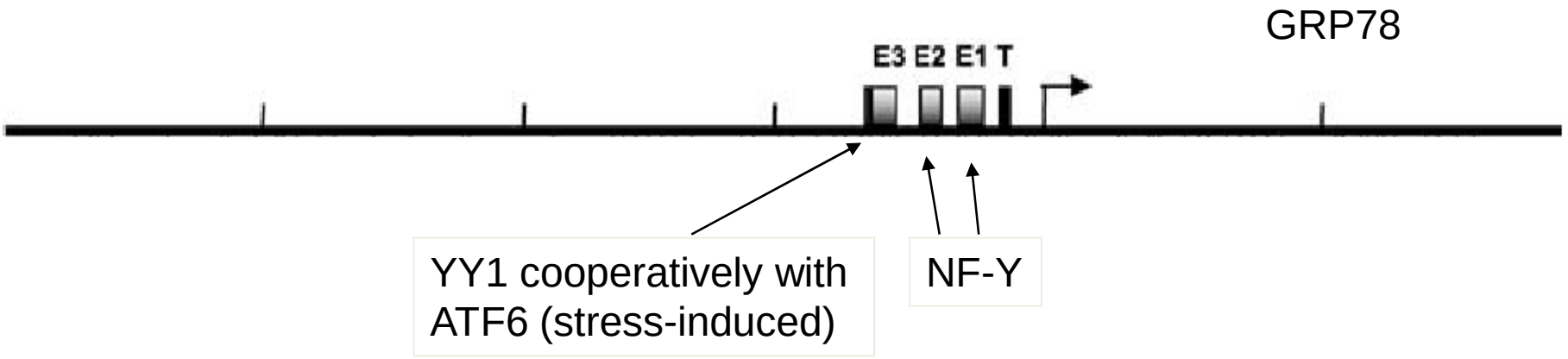
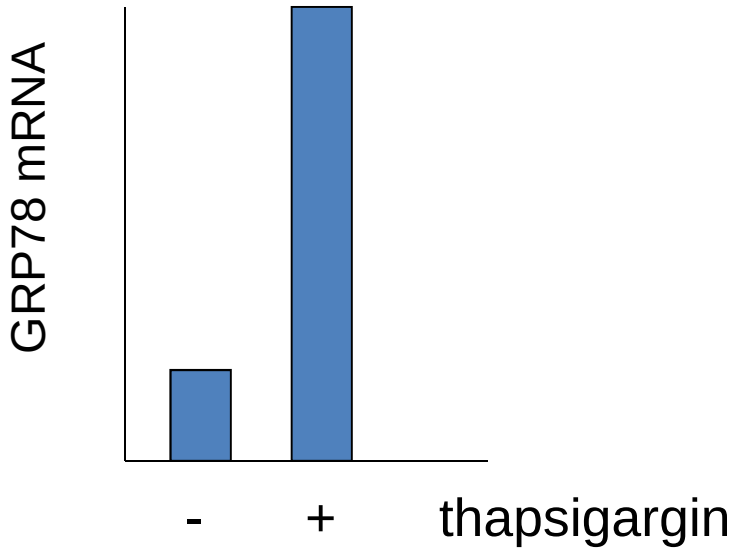
1 Department of Urology, USC/Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California, United States of America, **2** Department of Biochemistry and Molecular Biology, USC/Norris Comprehensive Cancer Center, Keck School of Medicine, University 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





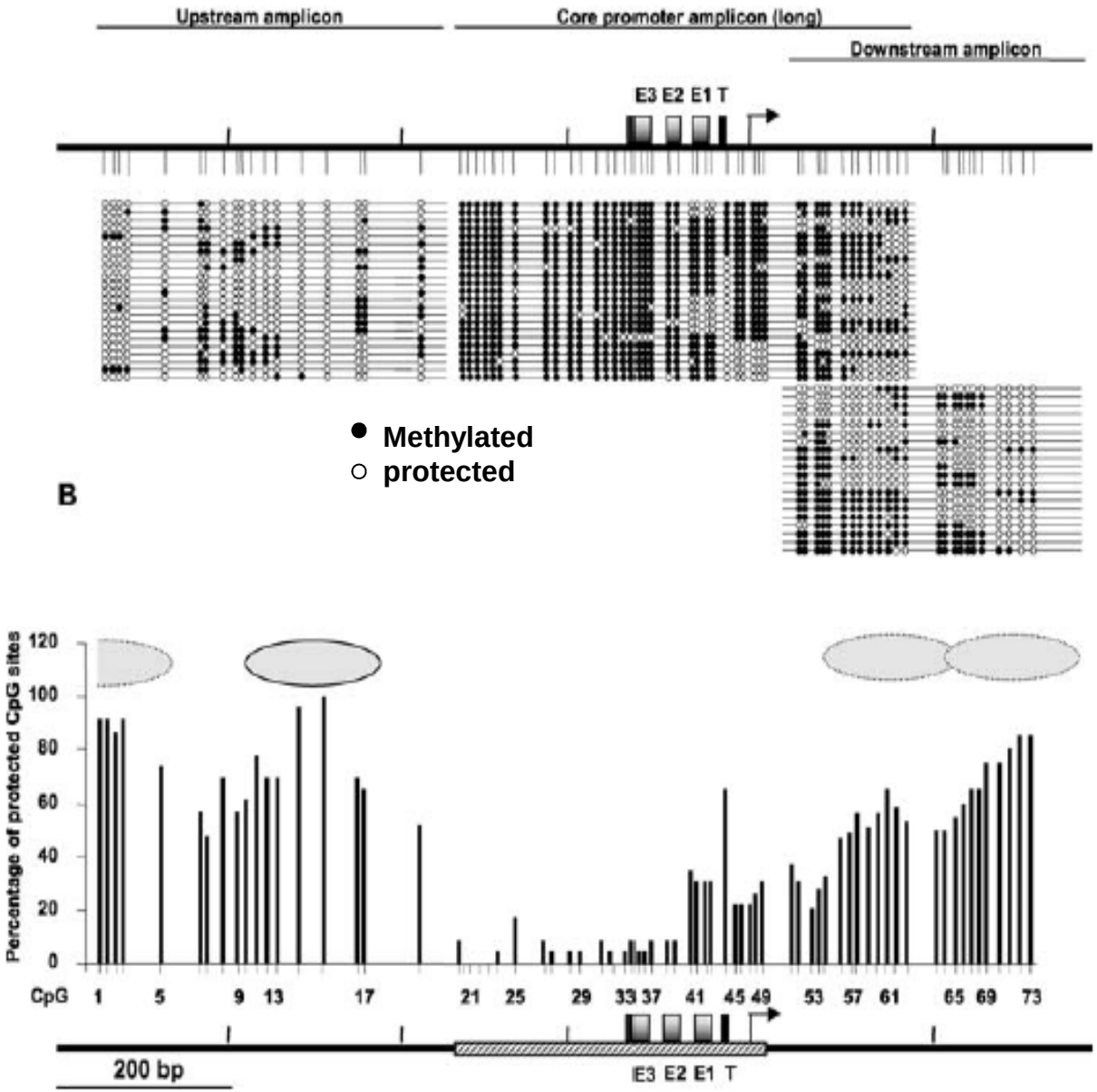


Figure 2. The Nucleosome-Free Region on the *GRP78* Core Promoter Is Minimally 350 bp Long

Ex-vivo methylation protection assay

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

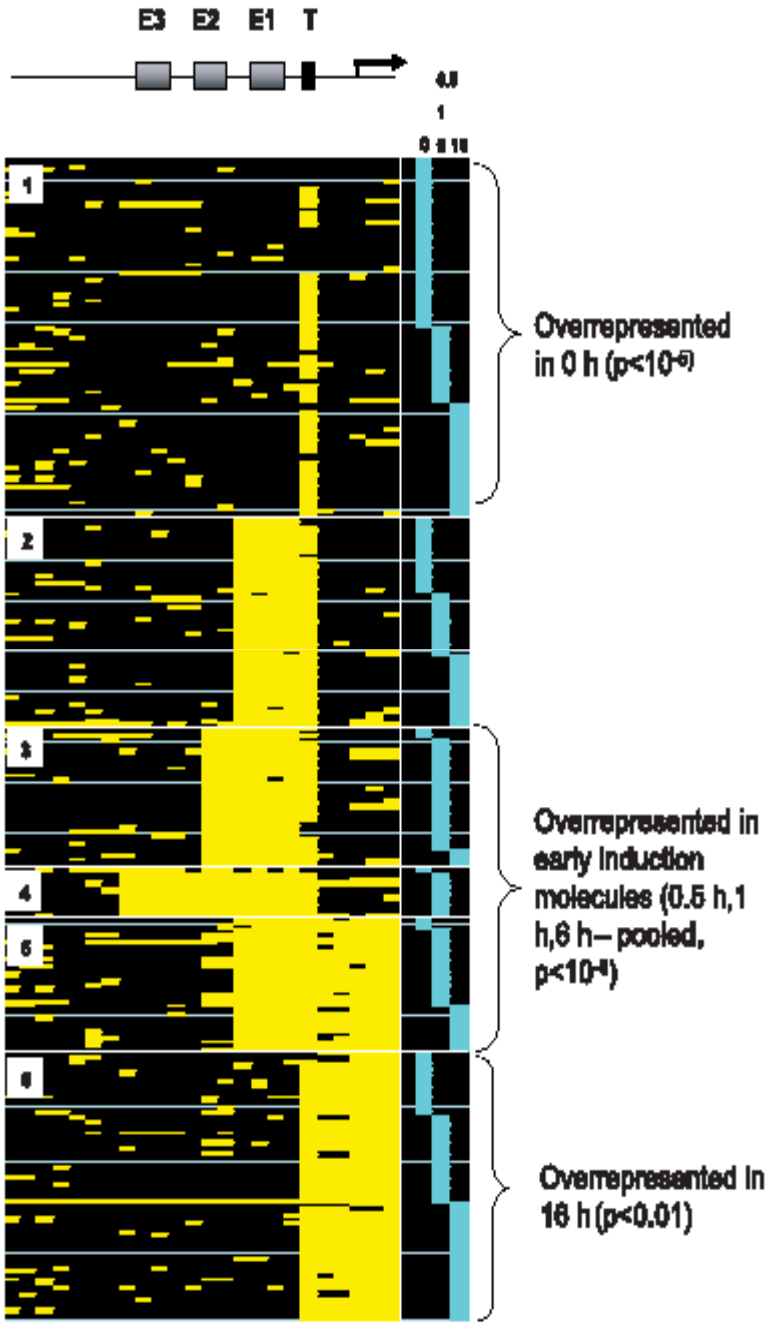


Figure 5. Few Combinatorial Modes of GRP78 Promoter Organization

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.