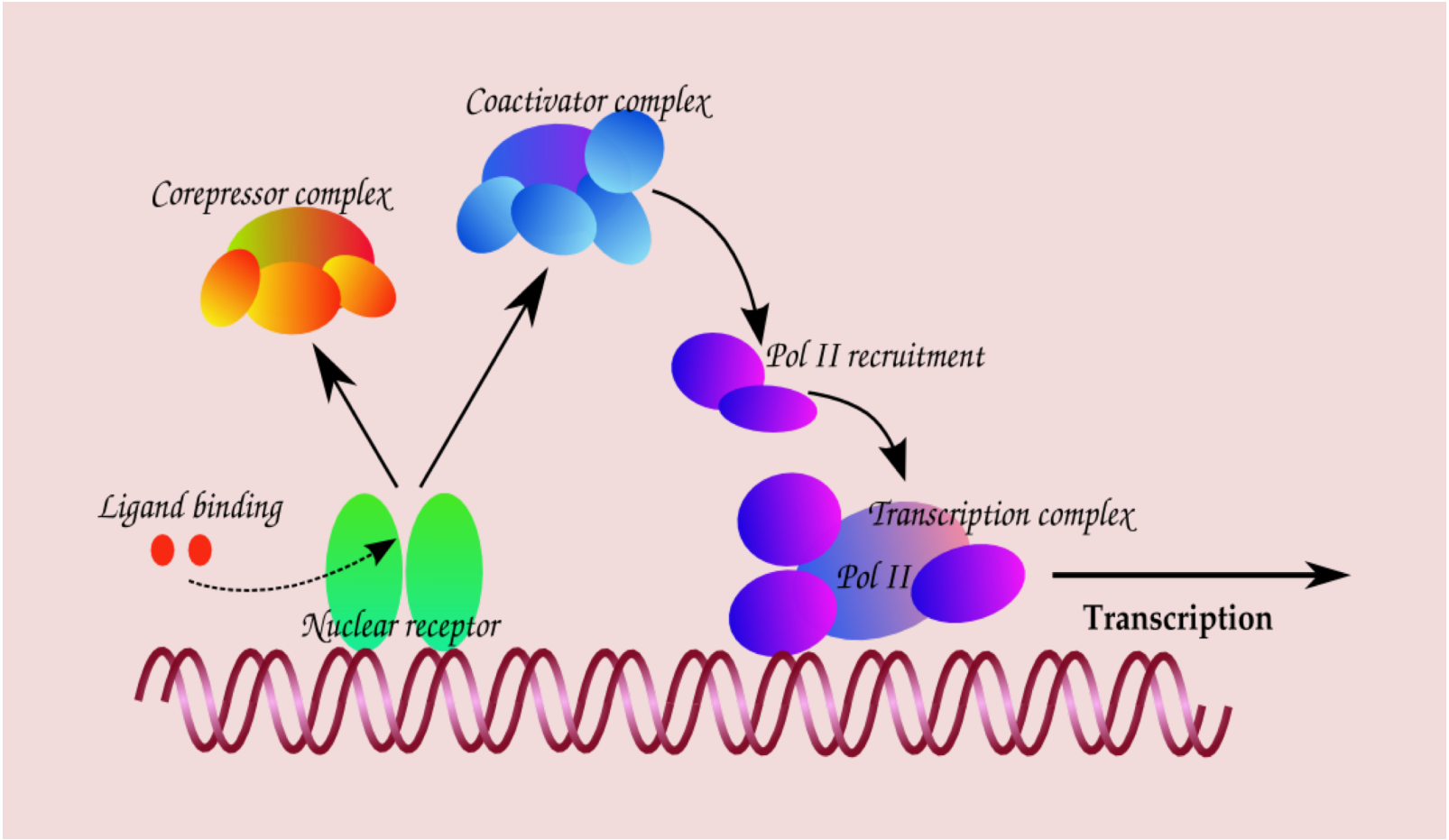
L4.4 – Transcriptional regulation
(Distal sites and combinatorial regulation)

AGENDA

1. Dynamics of TF/cofactors recruitment
2. Enhancer definition & characteristics
3. Enhancer selection

Corepressor/Coactivator Exchange Model



Molecular Cell
Review

Transcription Dynamics

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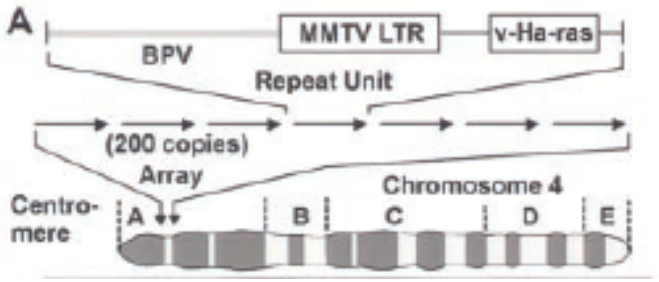
All aspects of transcription and its regulation involve dynamic events. The basal transcription machinery and regulatory components are dynamically recruited to their target genes, and dynamic interactions of transcription factors with chromatin—and with each other—play a key role in RNA polymerase assembly, initiation, and elongation. These short-term binding dynamics of transcription factors are superimposed by long-term cyclical behavior of chromatin opening and transcription factor-binding events. Its dynamic nature is not only a fundamental property of the transcription machinery, but it is emerging as an important modulator of physiological processes, particularly in differentiation and development.

The Glucocorticoid Receptor: Rapid Exchange with Regulatory Sites in Living Cells

James G. McNally,* Waltraud G. Müller,* Dawn Walker, Ronald Wolford, Gordon L. Hager†

Steroid receptors bind to site-specific response elements in chromatin and modulate gene expression in a hormone-dependent fashion. With the use of a tandem array of mouse mammary tumor virus reporter elements and a form of glucocorticoid receptor labeled with green fluorescent protein, targeting of the receptor to response elements in live mouse cells was observed. Photobleaching experiments provide direct evidence that the hormone-occupied receptor undergoes rapid exchange between chromatin and the nucleoplasmic compartment. Thus, the interaction of regulatory proteins with target sites in chromatin is a more dynamic process than previously believed.



The cell line 3134 contains a large tandem array of a mouse mammary tumor virus/Harvey viral ras (MMTV/v-Ha-ras) reporter (*8*). The

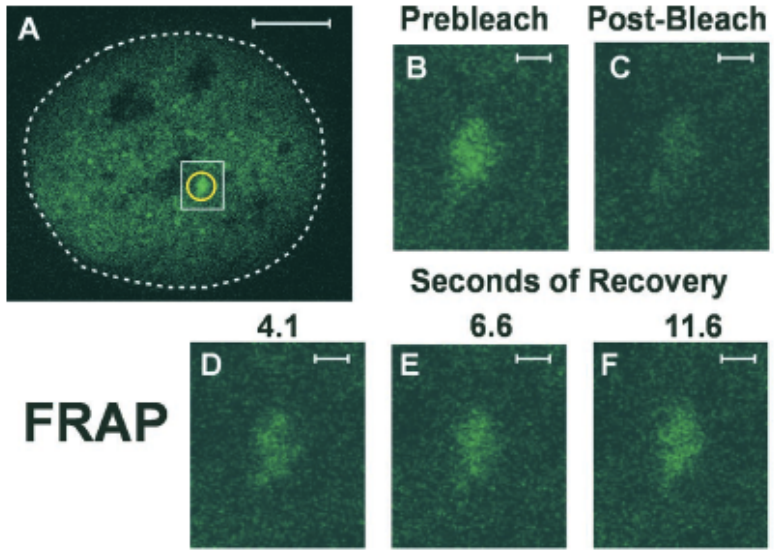
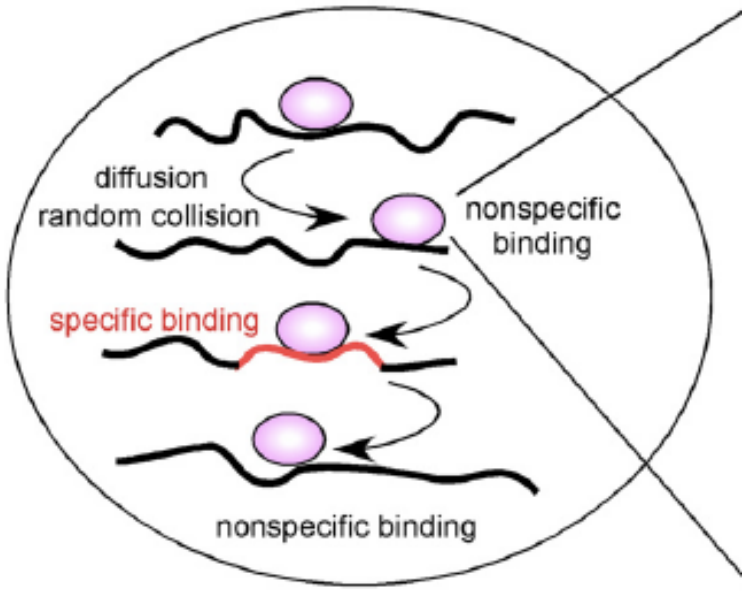


Fig. 5. GFP-GR undergoes rapid exchange with the LTR in the continuous presence of ligand. (**A** to **F**) GFP-GR bound to the LTR array was subjected to FRAP (*12*). Cells were grown and imaged as described in Figs. 3 and 4. The time-lapse/bleach mode was used on the Leica TCS NT SP confocal microscope. Settings were configured to produce a prebleach image (A) first and then focus a 1.5-μm diameter beam (indicated by yellow circle) on the ar



How Transcription Factors Find Their Targets: 3D Genome Scanning

The basis of all transcriptional activity and regulation is the recruitment of transcription complexes to target genes. The basal transcription machinery associates with well-defined binding sites in promoter regions, and regulatory factors bind to specific sites in control elements in the vicinity and, at times, at long distances away, from target genes. Specific binding sites for both the basal machinery, as well as gene-specific regulators, are exceedingly sparse in the genome compared to the number of nonspecific binding sites with which a given transcription factor (TF) may interact. Conservatively, assuming an average mammalian core promoter size of ~150 nt, promoter regions make up less than 0.1% of the human genome, and many TFs have only a few specific binding sites in the genome. How then do TFs find, often rapidly and in response to tightly controlled physiological signaling cascades, their few specific binding sites in the vast sea of nontarget sites in the genome? The key to efficient recruitment of the transcription machinery to its target site are two fundamental dynamic properties of TFs: their ability to rapidly diffuse through the nucleus and their propensity to very transiently bind to chromatin.

Figure 1. TFs Find Their Specific Binding Sites by Random Scanning of the Genome in 3D

A TF (purple) diffuses through the nuclear space and by random collision associates with chromatin. Most encounters are at nontarget sites resulting in highly transient interactions. Occasionally, a specific binding site (orange) is encountered, and prolonged binding occurs. At each encounter a TF might undergo local motion on the chromatin fiber by either sliding along the DNA, hopping locally or by directed, motor driven motion.

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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

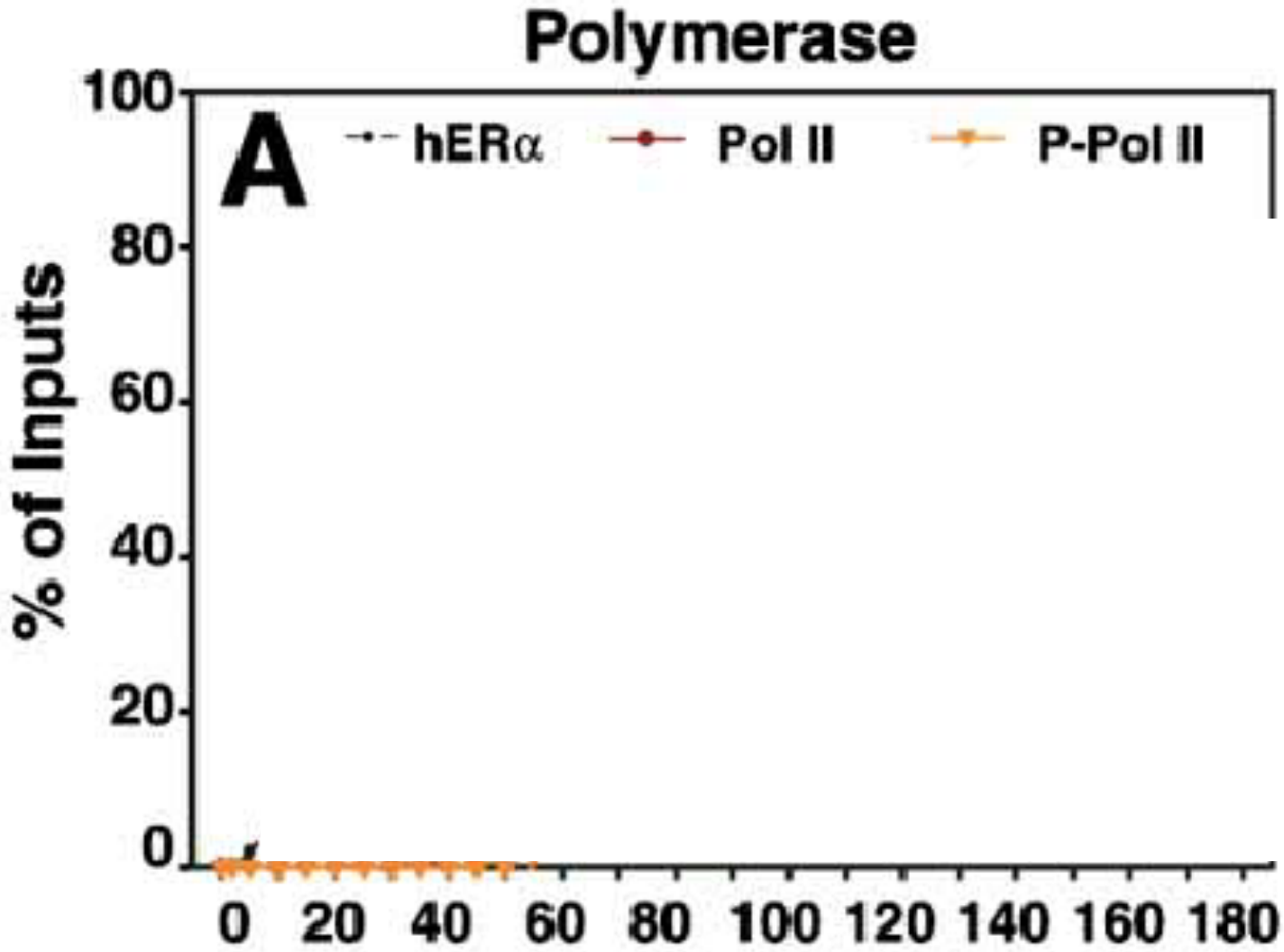
Transcriptional activation of a gene involves an orchestrated recruitment of components of the basal transcription machinery and intermediate factors, concomitant with an alteration in local chromatin structure generated by posttranslational modifications of histone tails and nucleosome remodeling. We 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


Polymerase
A
hERα
Pol II
P-Pol II
% of Inputs
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
Minuti dopo estradiolo

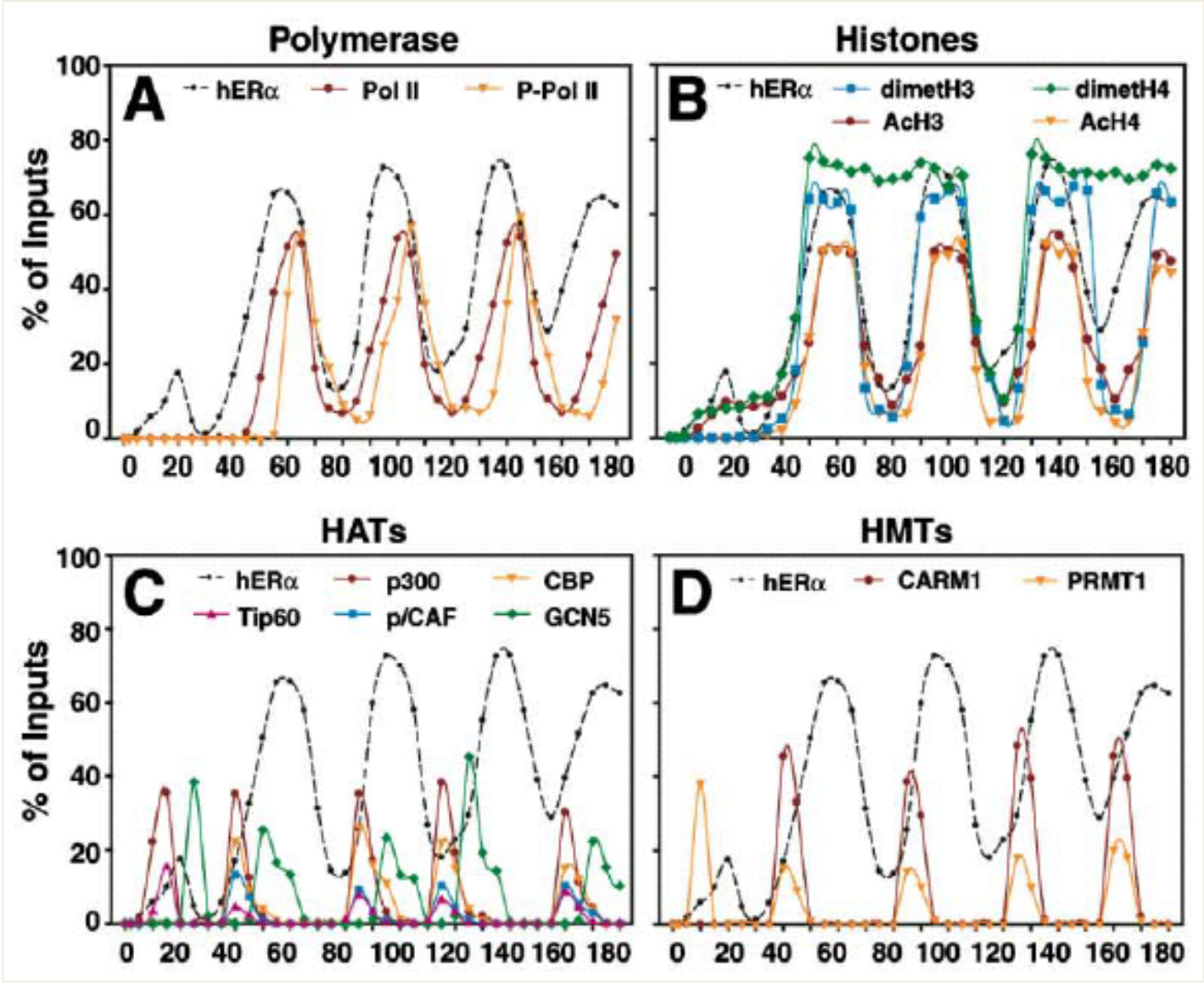
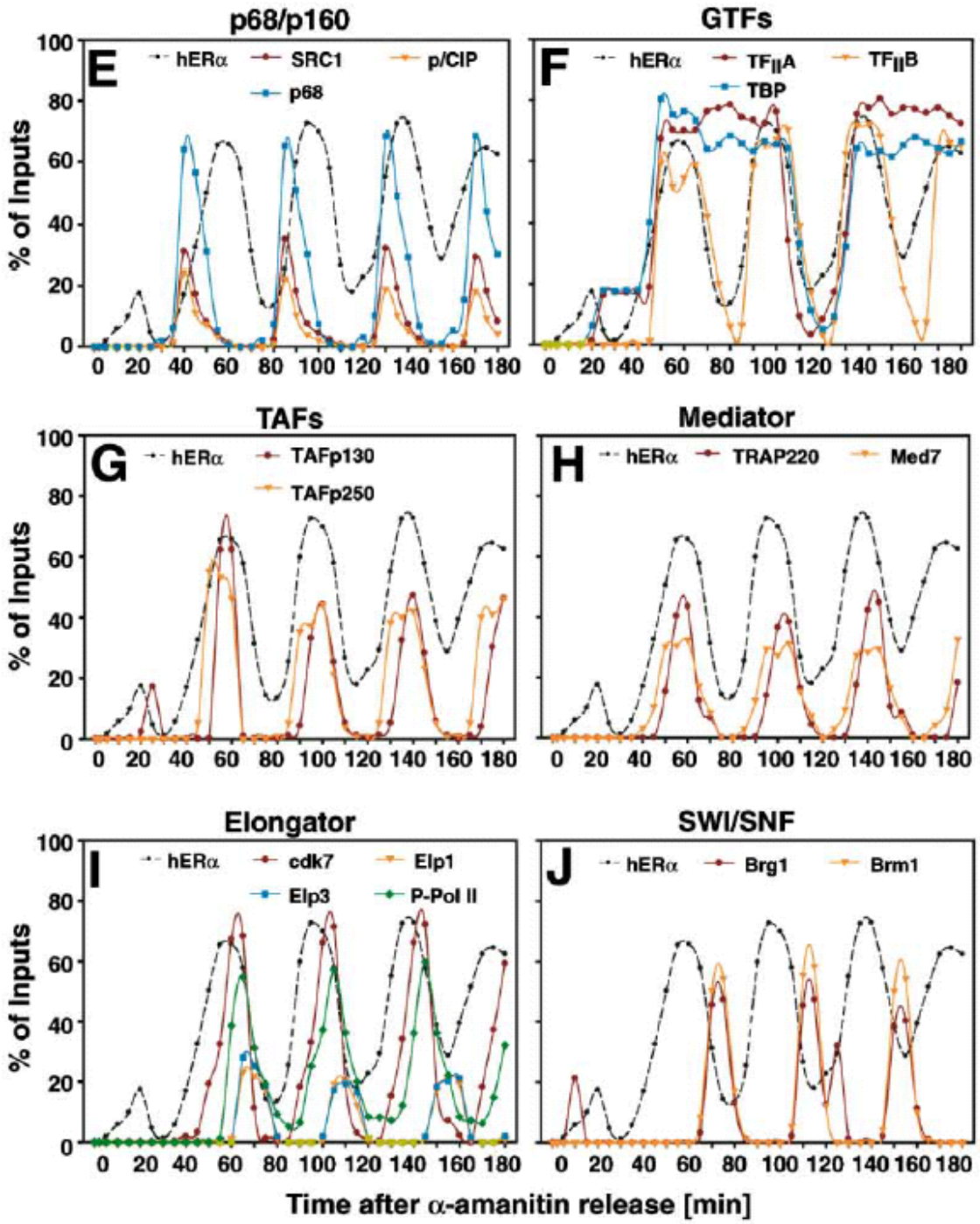


Figure 3. Dynamics of Cofactor Recruitment Directed by E2-Liganded hER on the pS2 Promoter. Kinetic ChIP experiments were performed using specified antibodies as shown within the images. Chromatin was prepared on sampled cells at 5 minutes intervals. The amount of immunoprecipitated pS2 promoter was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


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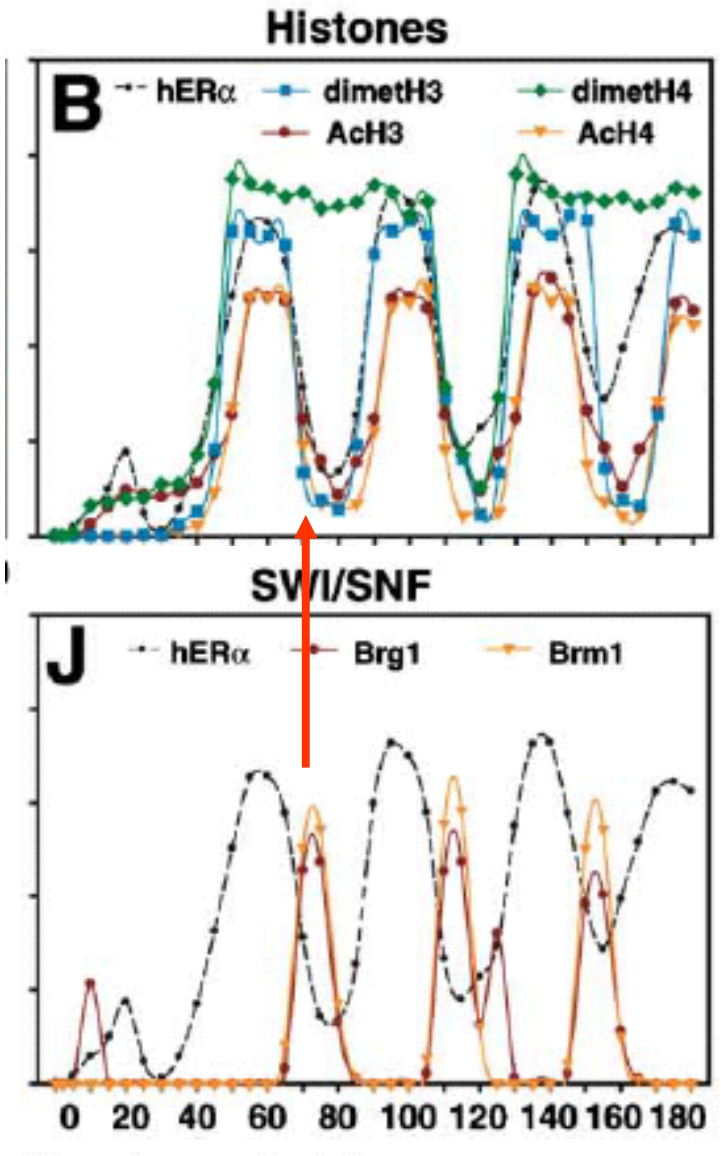
% of Inputs
0 20 40 60 80 100 120 140 160 180
0 20 40 60 80 100
Time after α-amanitin release [min]



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

Later on, recruitment of the corepressor NCoR was demonstrated

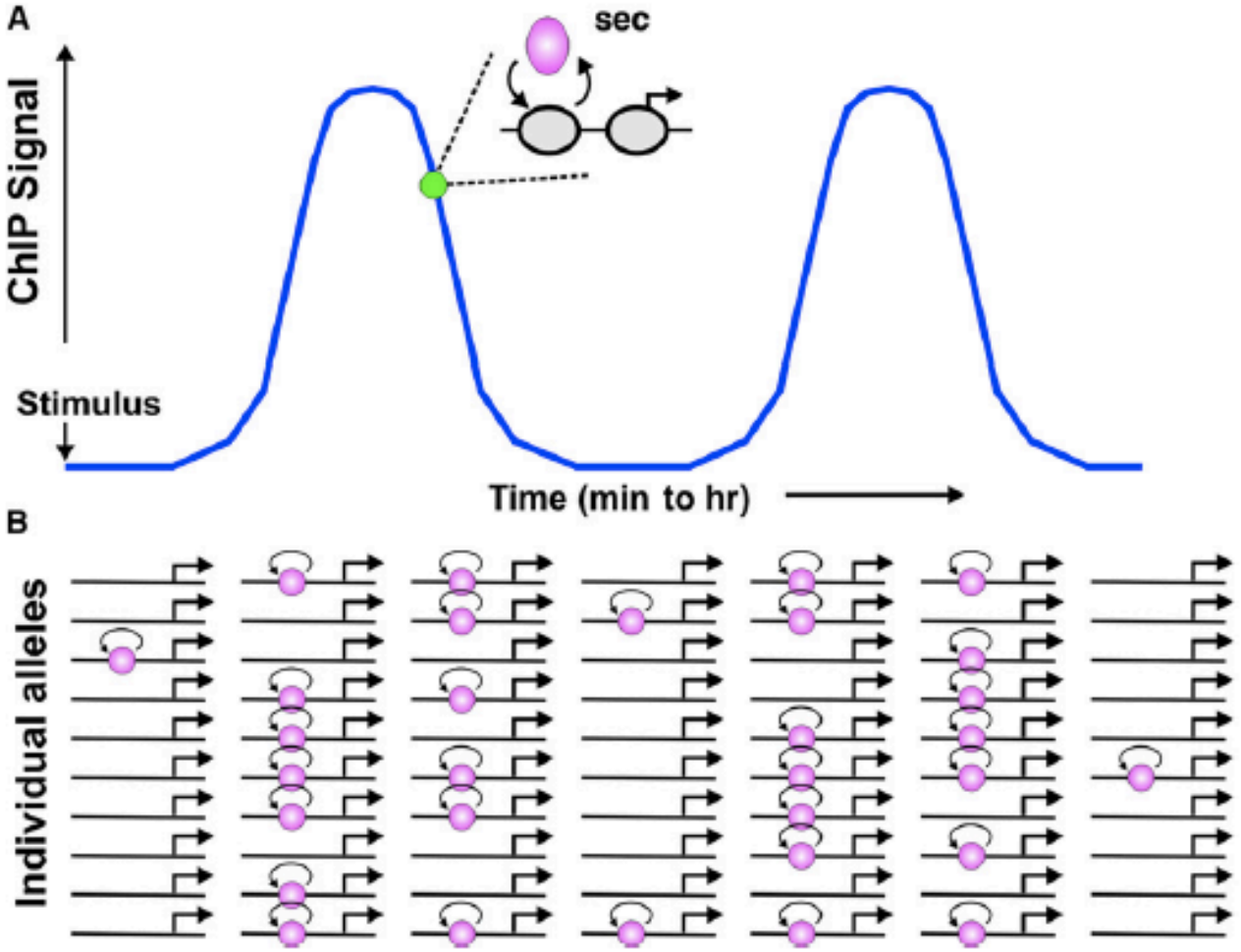


Figure 4. Integrated View of Transcription Factor Dynamics

(A) Most factors that have been studied in living cells exchange rapidly, on a time scale of seconds, with their recognition elements in chromatin ("hit-and-run"). The frequency and transient duration of these binding events can also fluctuate on a longer time scale, by a variety of mechanisms.

(B) Depicted here are a set of ten abstracted alleles with one binding element. After a transcriptional stimulus, the number of interaction events increases. If this element is sampled across the population by a methodology such as ChIP, more of the events will be captured in a given time. If secondary mechanisms are triggered that decrease the interaction frequency, the ChIP signal will decrease, and an oscillatory process may ensue. However, if real-time residence times could be examined at a specific allele (green circle), one would observe rapid exchange.

Hager, McNally and Misteli, Mol Cell Review

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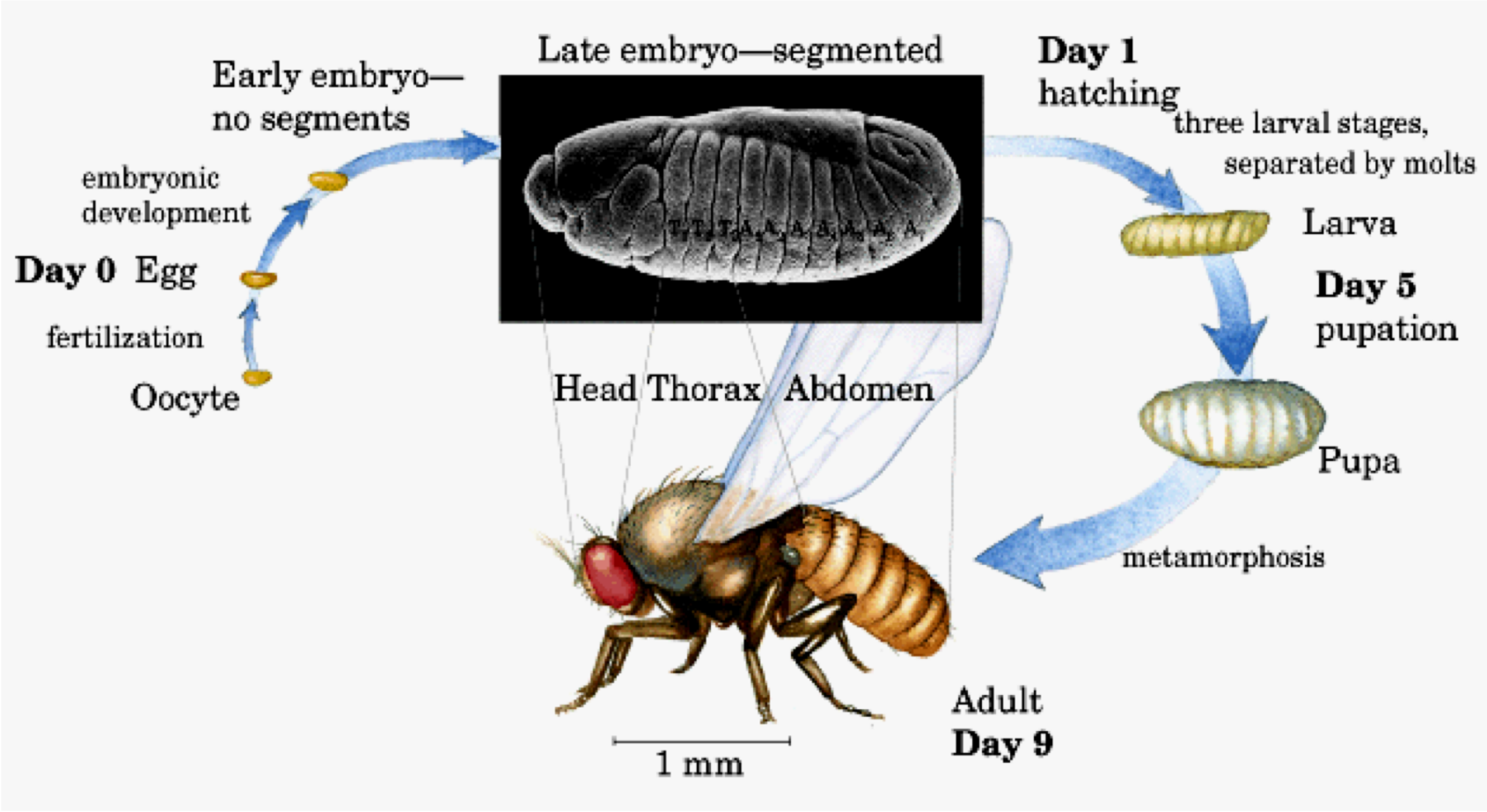
2. Enhancer definition & characteristics
3. Enhancer selection

The primary knowledge about enhancers came from studies on patterning during early *Drosophila* development.



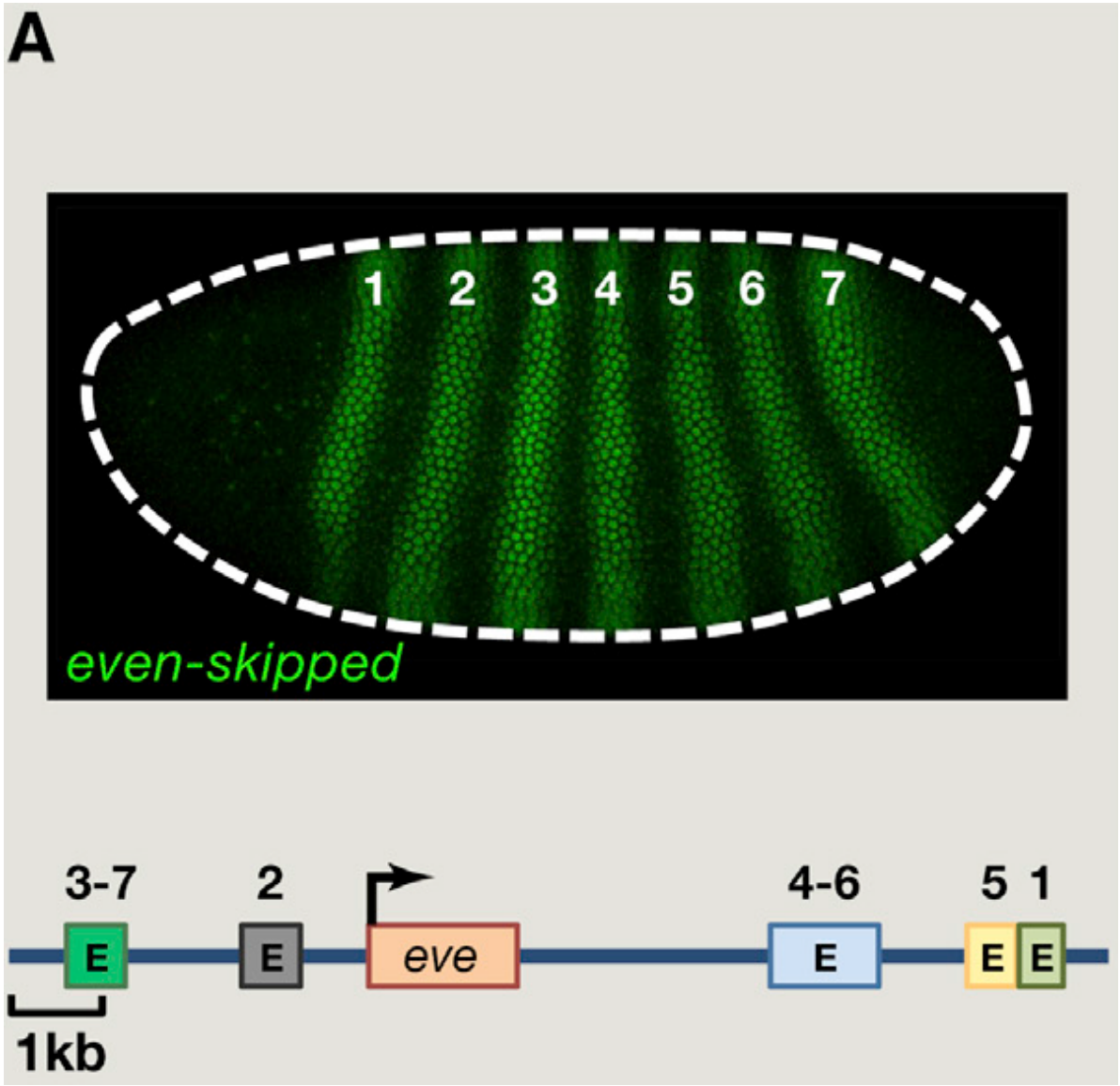
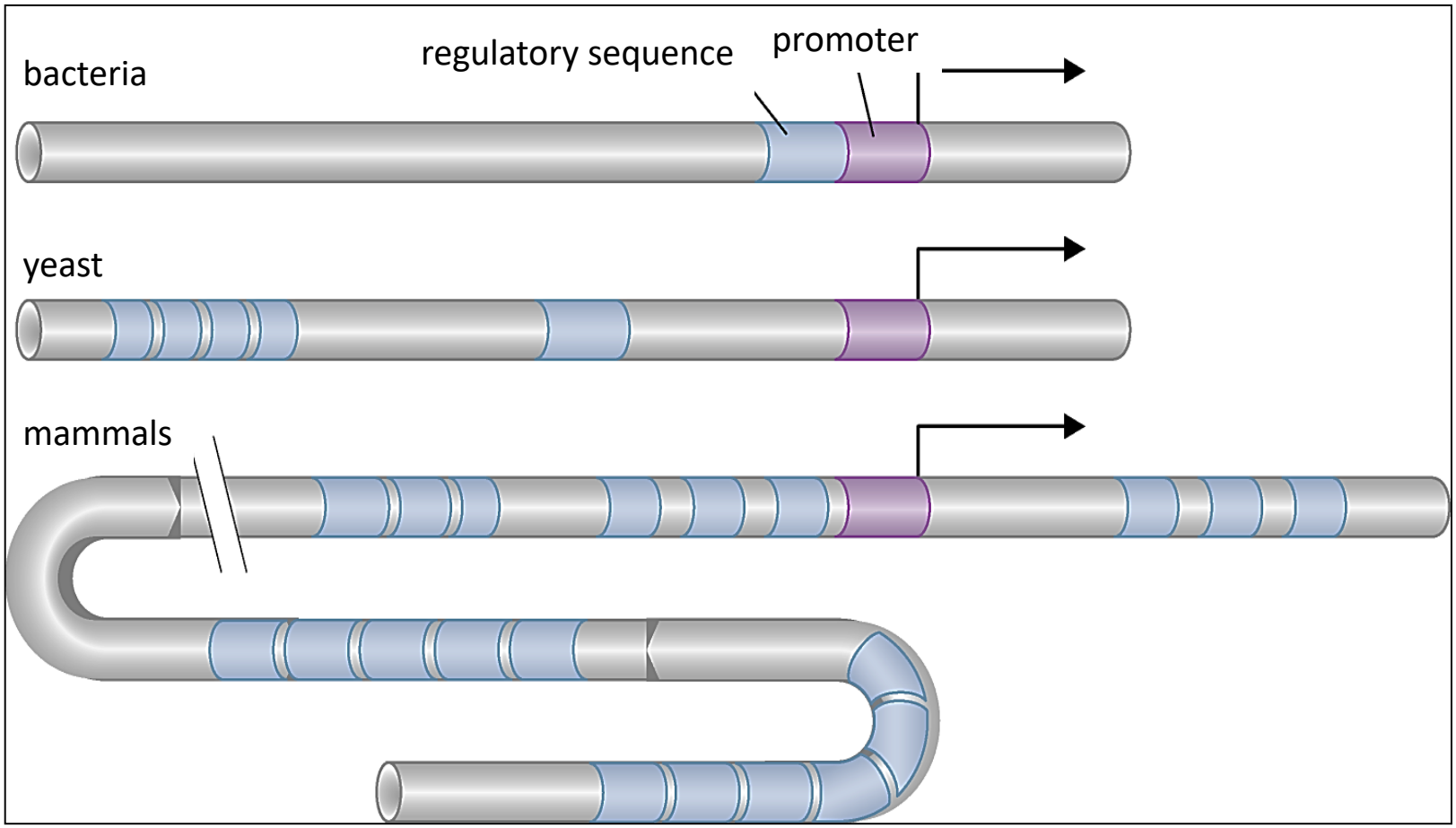


Figure 1. Organization of cis-Regulatory DNAs in Metazoan Genomes

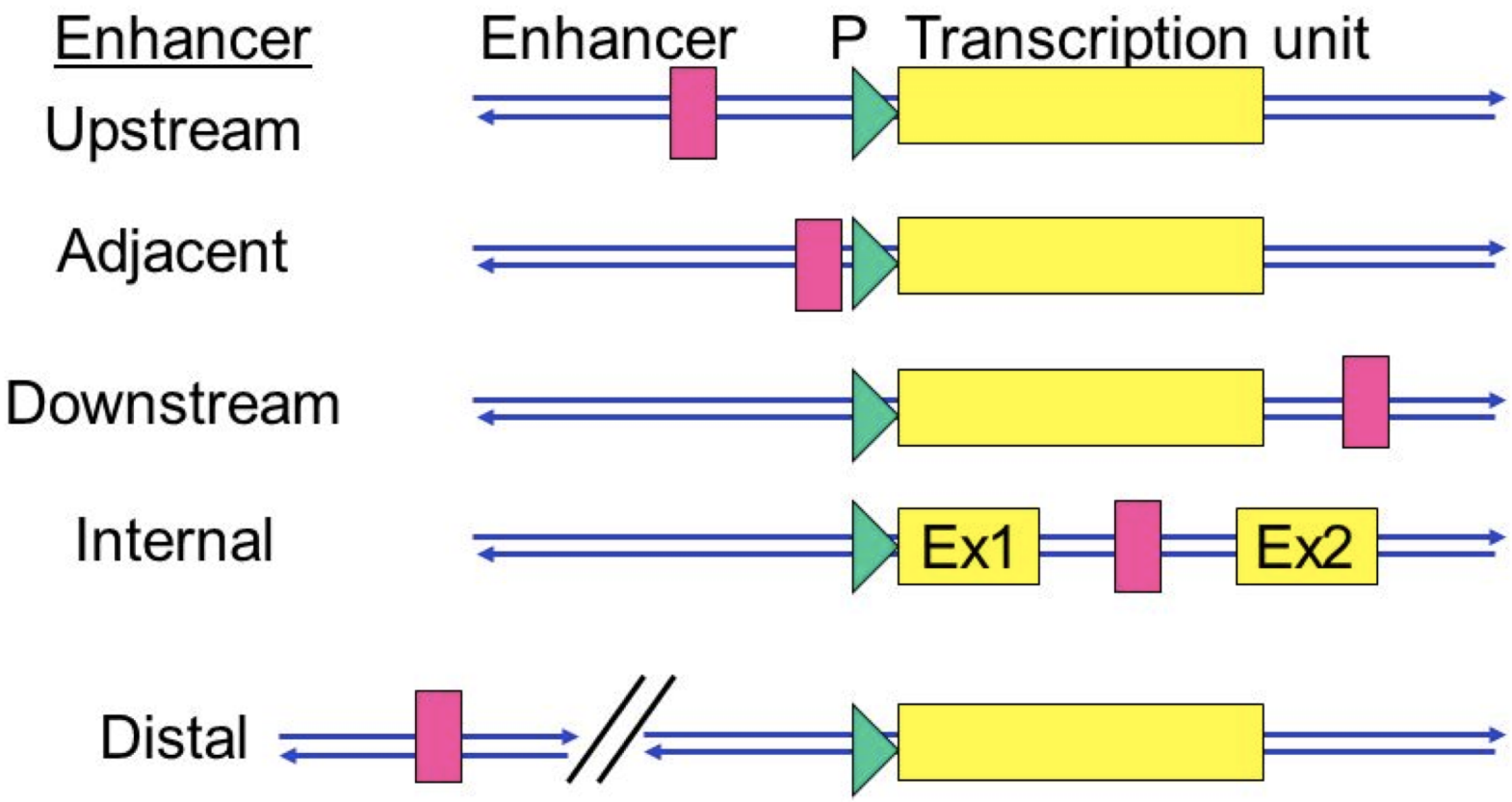
Metazoan genes are regulated by multiple enhancers.
(A) Organization of the even-skipped (eve) locus in the Drosophila genome. The eve gene is just 3 kb in length but is regulated by individual stripe enhancers (E) located in both 50 and 30 flanking regions. The eve stripe enhancers function in an additive fashion to produce seven stripes of gene expression in the early Drosophila embryo

Levine et al., 2014

While in Yeasts the majority of regulatory sequences (UAS) are preset very proximal to gene promoter, as far we move towards higher organisms we see and increase in the number of regulatory sequences (or «modules»), as well as the increase of their distance and positional randomness in respect to promoters.

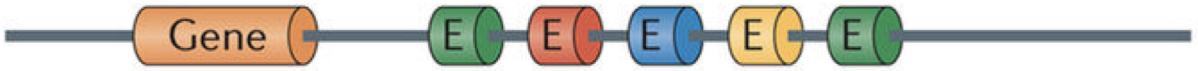


Enhancers can occur in a variety of positions with respect to genes

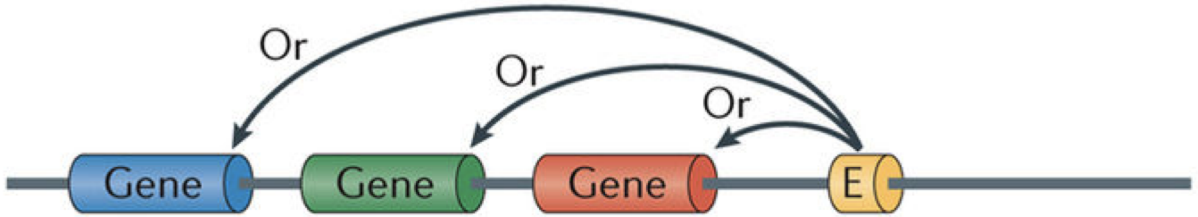


a One gene, multiple enhancers, one tissue

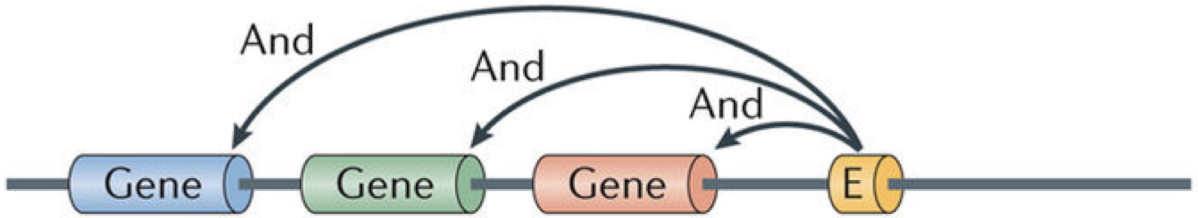
b One gene, multiple enhancers, more tissues



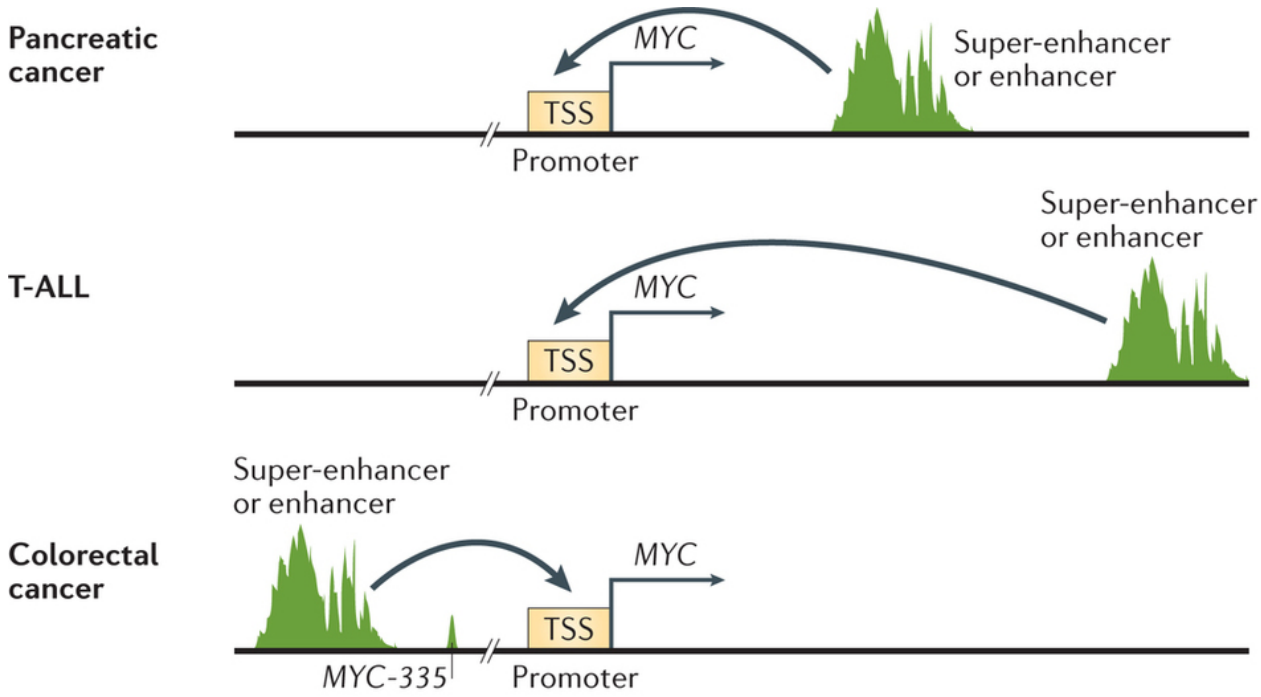
c Gene competition for a shared enhancer: winner takes all



d Gene competition for a shared enhancer: we are all winners



Different enhancers can control the same gene in specific cell types or disease states



Nature Reviews | Cancer

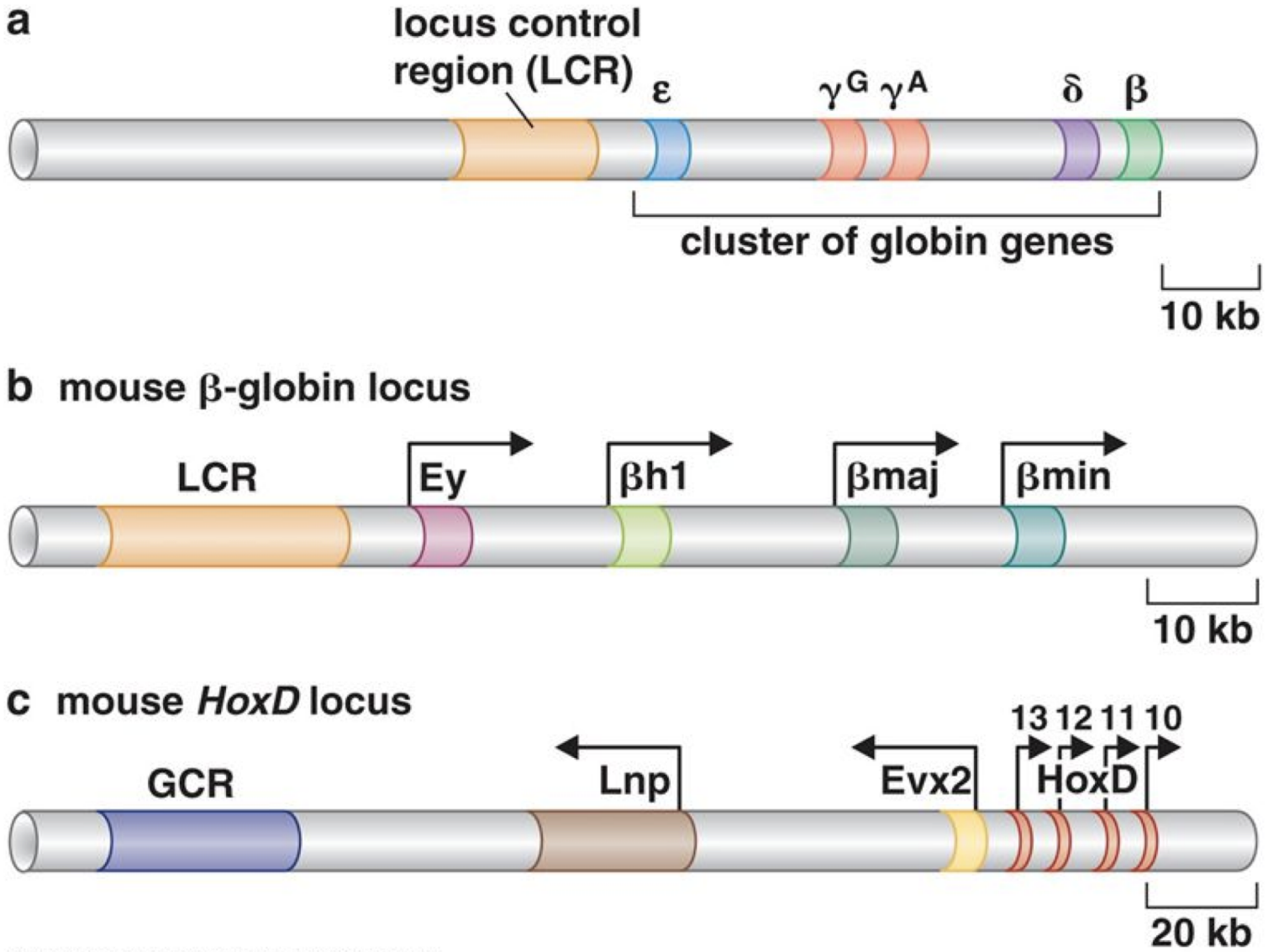
Definitions of Enhancers:

- **Functional**: DNA elements that regulates the transcription of a gene from a distance and independently of their orientation

- **Technical:** DNA elements that are characterized by a chromatin/cofactor profile corresponding to that of functional enhancers (i.e. p300 binding, H3K4me1, H3K27ac)

Super-enhancers: large clusters of enhancers (up to 50kb)
TFBSs hotspots
strong enrichment for Med1
they overlap with other large scale regulatory domains (LCRs)

Appropriate regulation of some groups of genes requires locus control regions



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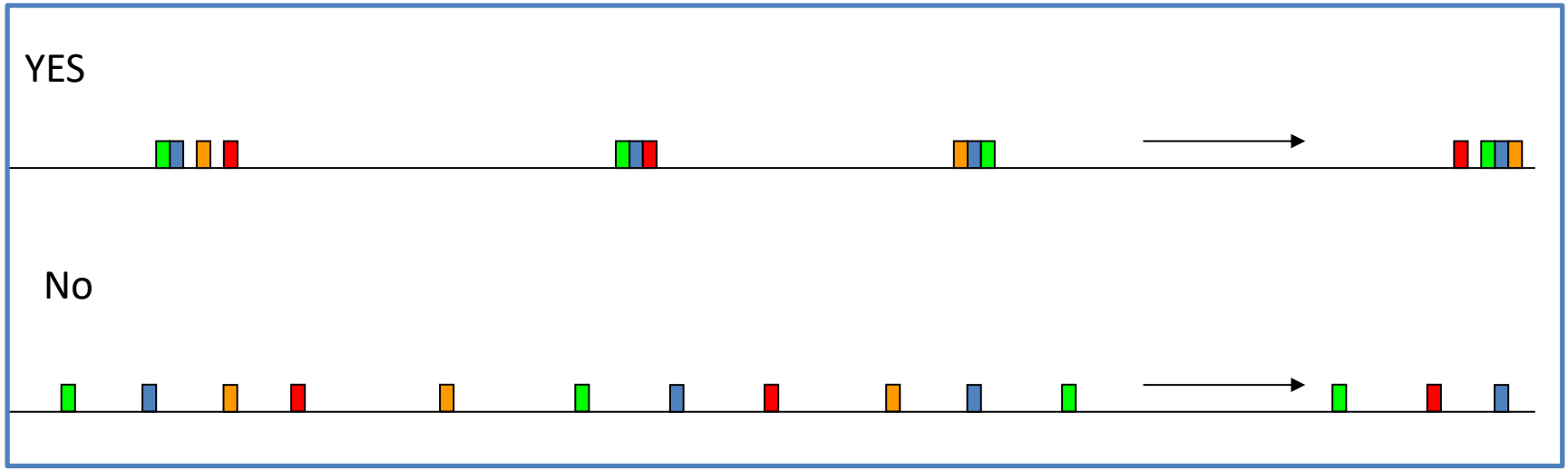
BREAK

Genomic regions that functions as transcriptional enhancers are enriched in closely spaced REs for sequence-specific transcription factors



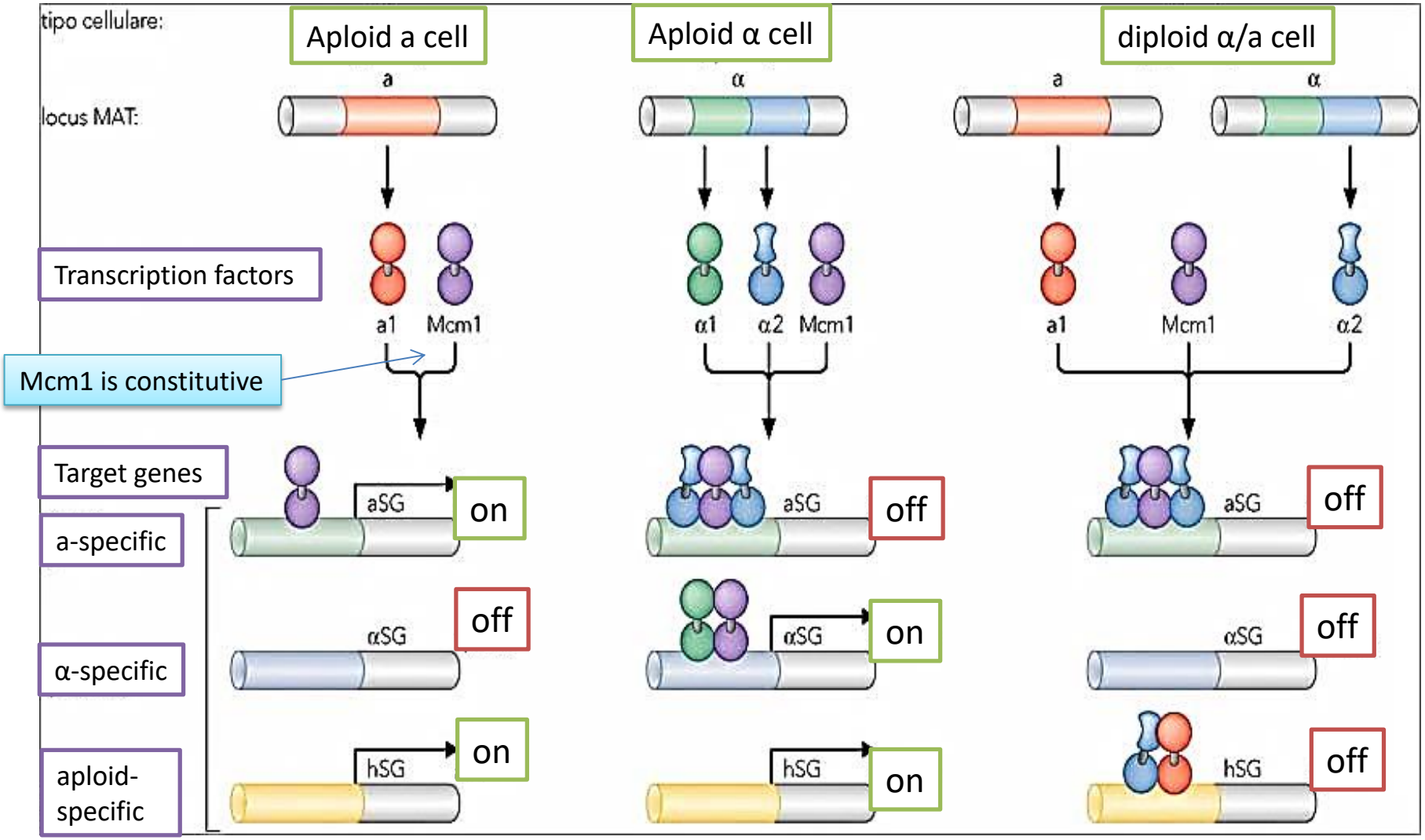
- Combinatorial binding integrates multiple regulation
- Compositional binding increases fine-tuning
- Cooperativity will determine transcriptional outcome

cooperativity

The binding of one Transcription Factor increases the probability of binding for a second TF and so forth

Factor added	Transcription
None	1
TF-A	2
TF-A + TF-B	3
TF-C	2
TF-A + TF-B + TF-C	25

The yeast **MAT locus** encodes TFs a1, α1, α2. Activation or repression comes from the **combination** and **composition** of th different factors, on differently composite DNA elements.



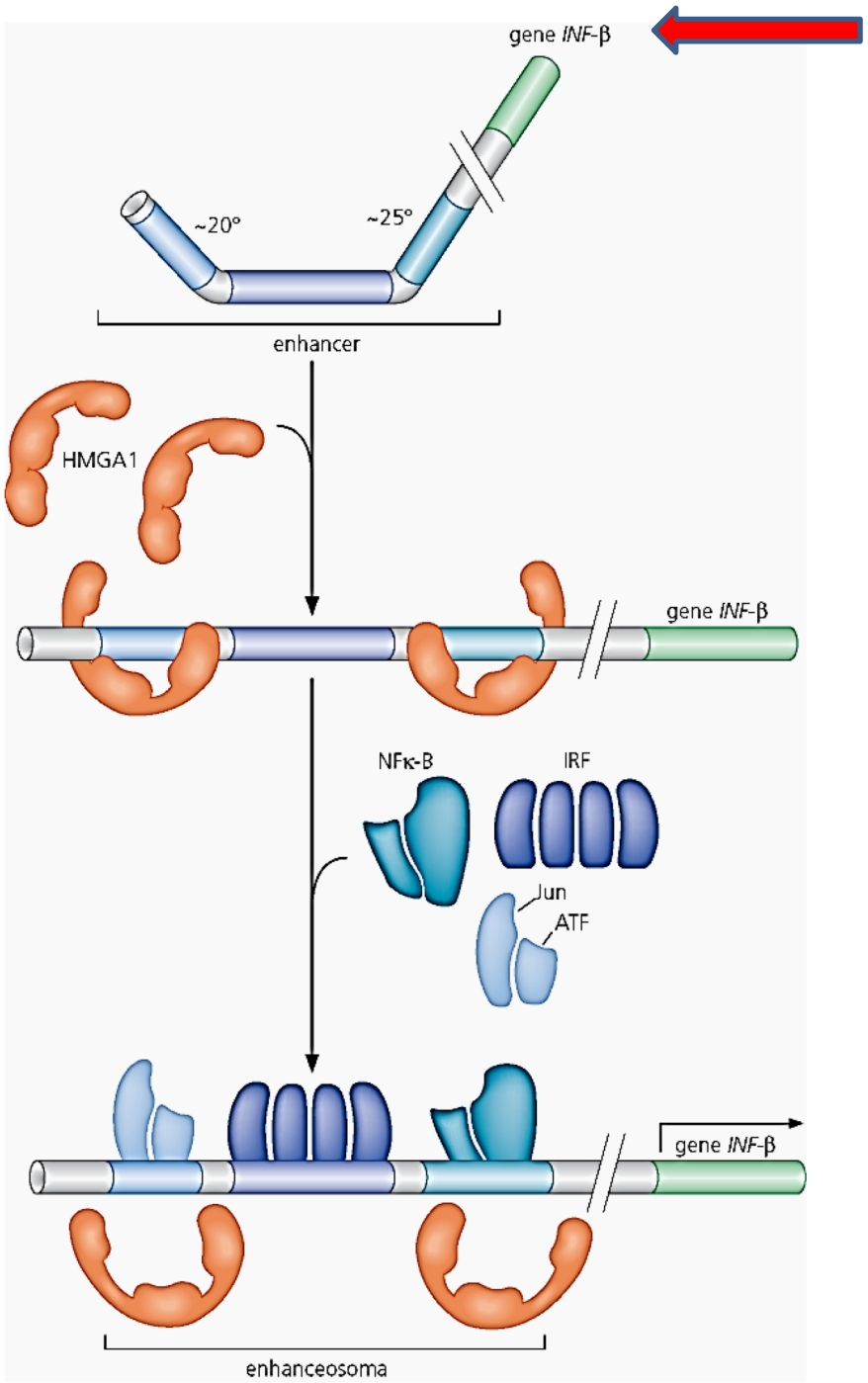
This old example illustrates «**combinatorial**» effect

Compositionality

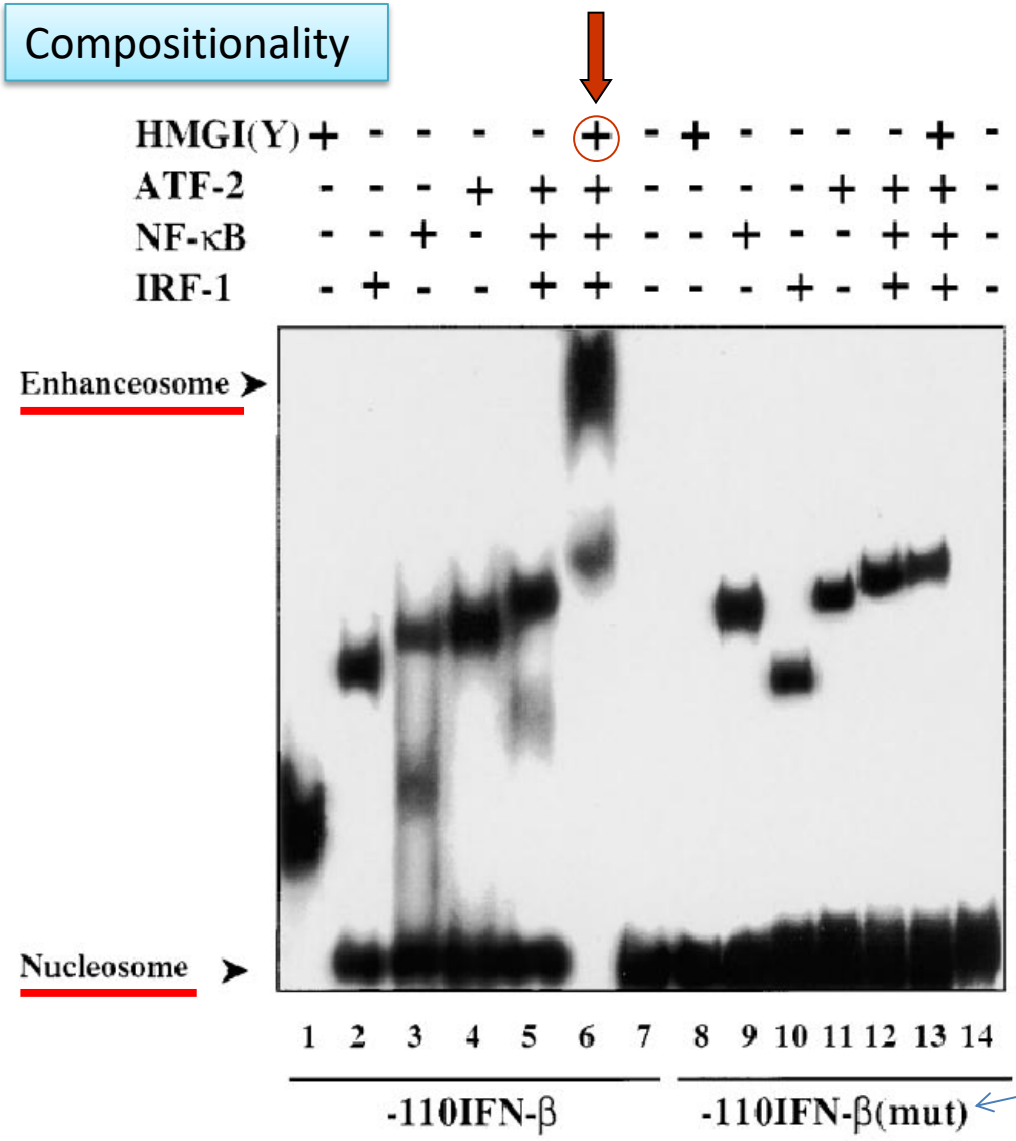
TFs binding may be favoured by the local 3D conformation

Old example

the INF-β enhancer:



Compositionality



How it is done:
Enhancer DNA with nucleosome is added of recombinant proteins.

Band-shift (**EMSA**) analysis shows binding of ATF-2, NF-kB and IRF-1 to the enhancer.

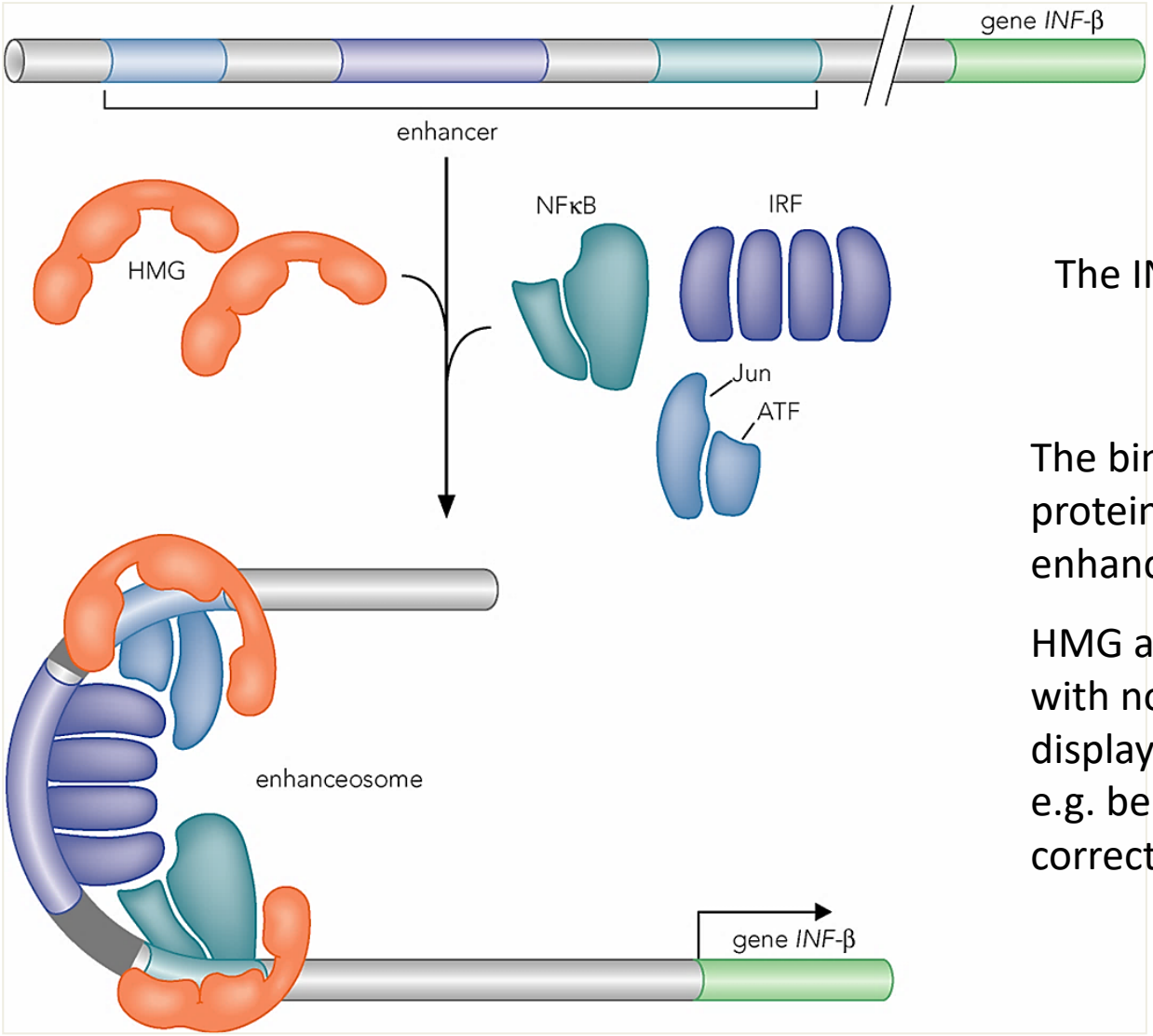
The enhanceosome is formed only in the presence of the architectural protein HMGI(Y) to 4 specific sites.

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

Mutated in HMGI(Y) site

(D) Assembly of the IFN-β enhanceosome on nucleosomal IFN-β promoter fragments. An IFN-β promoter fragment (−143 to +183) (lanes 1–7) or an identical-sized fragment bearing mutations in all HMG I(Y) binding sites (lanes 8–14) were reconstituted into a nucleosome, gel purified, and used in EMSA experiments along with recombinant IFN-β activators in the presence or in the absence of HMG I(Y). The following amounts of recombinant proteins were used: HMG I(Y) 10 ng, IRF-1 30 ng, NF-κB 20 ng, ATF-2/c-Jun 50 ng. The bottom part of the Figure depicts a diagrammatic illustration of the enhanceosome bound to the IFN-β nucleosomal promoter fragment.

Compositionality



The INF-β "enhanceosome"

The binding of multiple different proteins to adjacent sites in enhancers is required.

HMG are DNA-binding proteins with no transactivating domain, but displaying "architectural" functions, e.g. bending the DNA and allowing correct interaction among TFs.

Is any kind of TF cooperation is allowed or there are specific rules?

Do we find any kind of combination of TFBS ?

ARTICLE

doi:10.1038/nature11245

Architecture of the human regulatory network derived from ENCODE data

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Transcription factors bind in a combinatorial fashion to specify the on-and-off states of genes; the ensemble of these binding events forms a regulatory network, constituting the wiring diagram for a cell. To examine the principles of the human transcriptional regulatory network, we determined the genomic binding information of 119 transcription-related factors in over 450 distinct experiments. We found the combinatorial, co-association of transcription factors to be highly context specific: distinct combinations of factors bind at specific genomic locations. In particular, there are significant differences in the binding proximal and distal to genes. We organized all the transcription factor binding into a hierarchy and integrated it with other genomic information (for example, microRNA regulation), forming a dense meta-network. Factors at different levels have different properties; for instance, top-level transcription factors more strongly influence expression and middle-level ones co-regulate targets to mitigate information-flow bottlenecks. Moreover, these co-regulations give rise to many enriched network motifs (for example, noise-buffering feed-forward loops). Finally, more connected network components are under stronger selection and exhibit a greater degree of allele-specific activity (that is, differential binding to the two parental alleles). The regulatory information obtained in this study will be crucial for interpreting personal genome sequences and understanding basic principles of human biology and disease.

Transcription Factors + transcription-related factors by ChIP-Seq.

The sum of chromatin sites bound by a given TF in a given cell type under a specific experimental condition is called «**cistrome**»

Using data from 5 cell lines, the ENCODE project has identified:

- Total 7.5 million «peaks»
  (40% of these within 2.5Kbp from TSS -> 60% are far from TSSes).

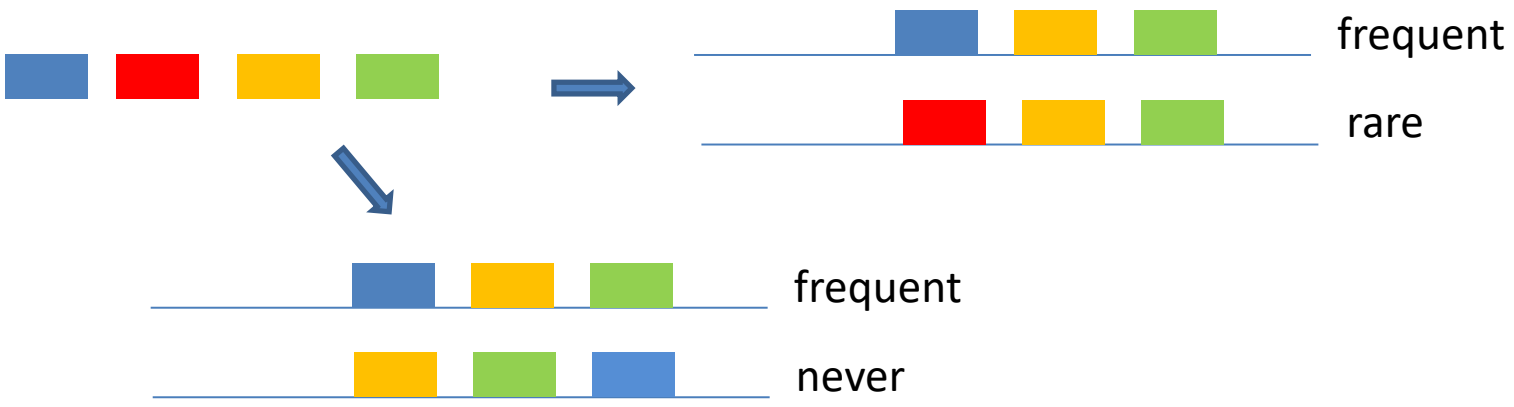
This allowed to estimate around 400,000 putative enhancers in the human genome (Gerstein et al., 2012). (some recent estimates reach up to one million).

Therefore, **enhancers largely outnumber promoters**.

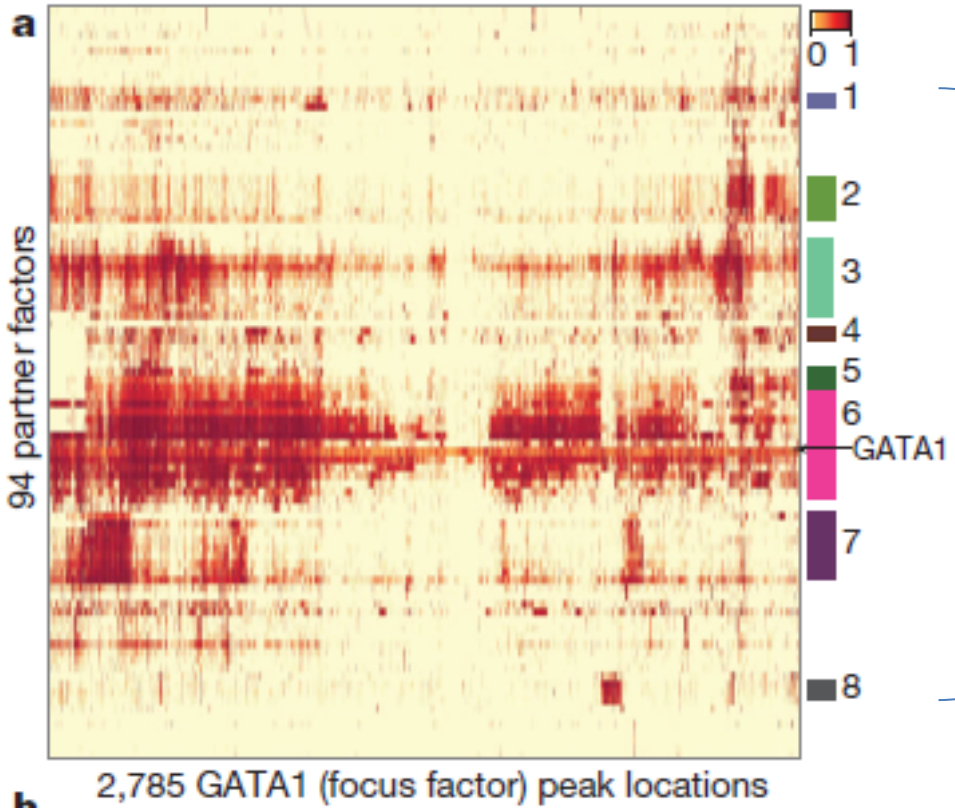
The combinatorial rule of Transcription Factor Binding Sites at enhancers

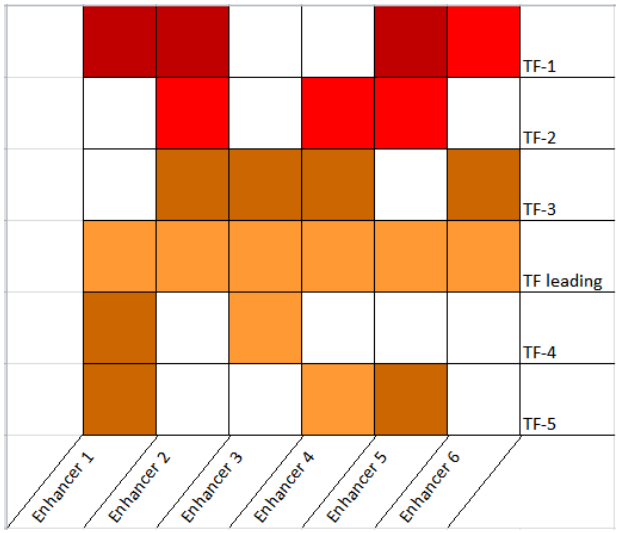
Gerstein paper clearly indicates that different «combinatorial» groups exist or, in other words, not all the possible combinations are seen.

This implies co-evolution of regulatory modules. This conclusion is further emphasized by the obervation that in a given conserved module, different TFBS are often arranged (ordered) in the same way.



One example from Gernstein paper: GATA1





The «focus» factor here is GATA1. Merging all the experiments, 94 partner factors were found.
Taking into account co-presence, several clusters are defined.

AGENDA

1. Cyclic cofactor recruitment
2. Enhancer definition & characteristics
3. Enhancer selection

Rapid and Pervasive Changes in Genome-wide Enhancer Usage during Mammalian Development

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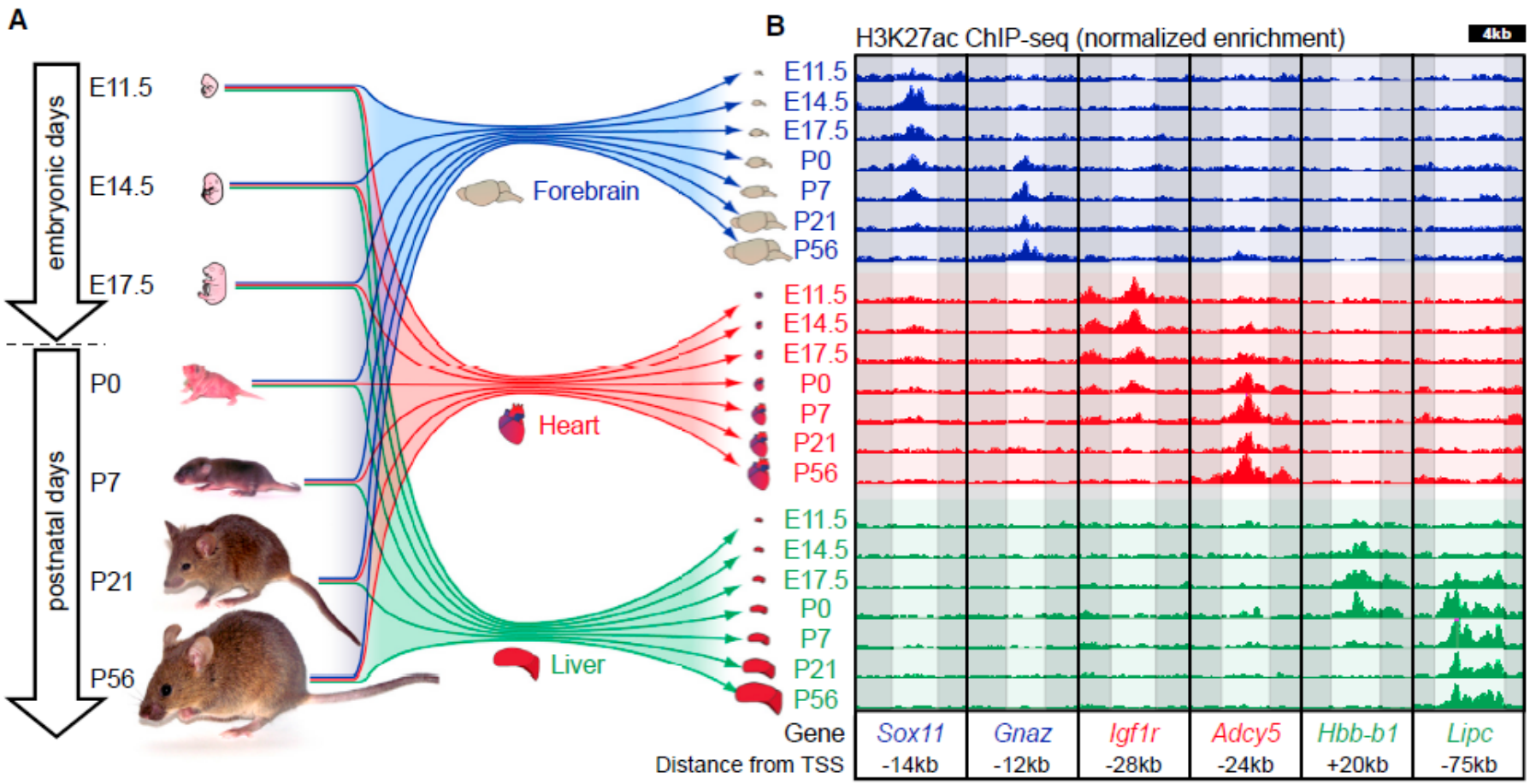
Enhancers are distal regulatory elements that can activate tissue-specific gene expression and are abundant throughout mammalian genomes. Although substantial progress has been made toward genome-wide annotation of mammalian enhancers, their temporal activity patterns and global contributions in the context of developmental in vivo processes remain poorly explored. Here we used epigenomic profiling for H3K27ac, a mark of active enhancers, coupled to transgenic mouse assays to examine the genome-wide utilization of enhancers in three different mouse tissues across seven developmental stages. The majority of the ~90,000 enhancers identified exhibited tightly temporally restricted predicted activity windows and were associated with stage-specific biological functions and regulatory pathways in individual tissues. Comparative genomic analysis revealed that evolutionary conservation of enhancers decreases following midgestation across all tissues examined. The dynamic enhancer activities uncovered in this study illuminate rapid and pervasive temporal in vivo changes in enhancer usage that underlie processes central to development and disease.



(B) Representative examples of putative enhancers exhibiting dynamic **H3K27ac** signal across tissues and time points.

This study demonstrates that enhancer show temporally restricted and tissue-specific patterns of activity and are associated with stage-specific biological functions.

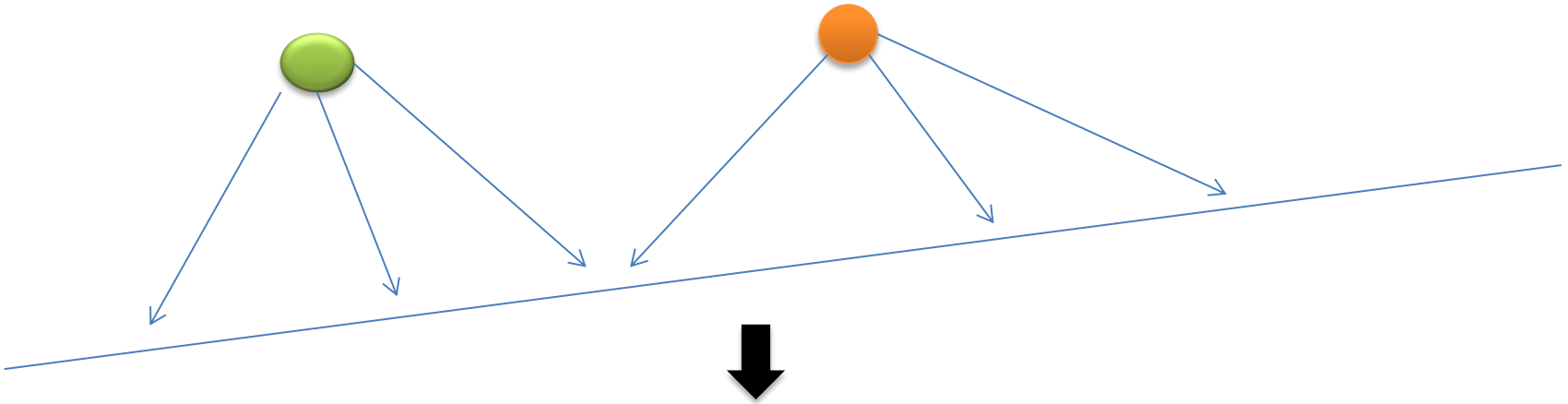
Textbook

The selection and function of cell type-specific enhancers

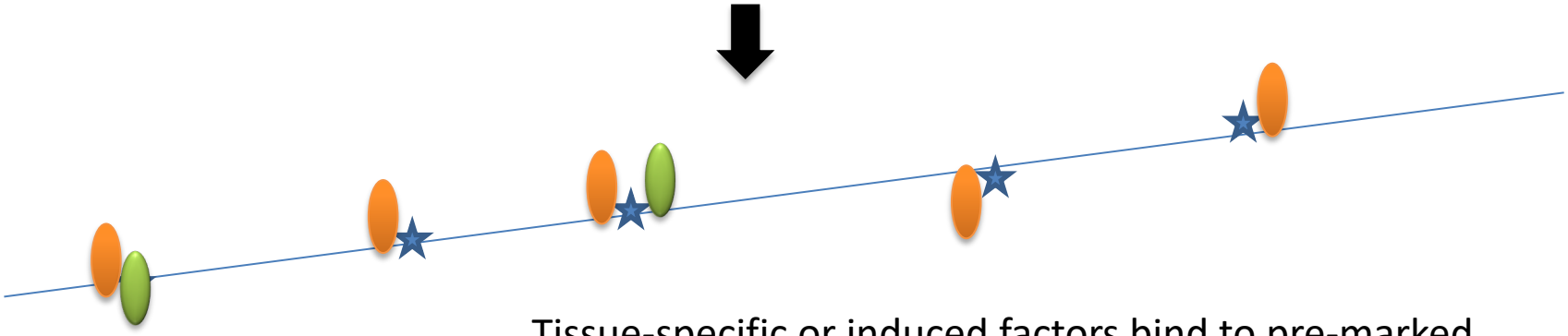
Sven Heinz[1], Casey E. Romanoski[2], Christopher Benner[1] and Christopher K. Glass[2,3]

Abstract | The human body contains several hundred cell types, all of which share the same genome. In metazoans, much of the regulatory code that drives cell type-specific gene expression is located in distal elements called enhancers. Although mammalian genomes contain millions of potential enhancers, only a small subset of them is active in a given cell type. Cell type-specific enhancer selection involves the binding of lineage-determining transcription factors that prime enhancers. Signal-dependent transcription factors bind to primed enhancers, which enables these broadly expressed factors to regulate gene expression in a cell type-specific manner. The expression of genes that specify cell type identity and function is associated with densely spaced clusters of active enhancers known as super-enhancers. The functions of enhancers and super-enhancers are influenced by, and affect, higher-order genomic organization.

Primary factors «prime» enhancer sequences in differentiating cells



Marks remain at enhancer maintaining a «poised» status (H3.3, H2A.z, H3K4me2 ?)



Tissue-specific or induced factors bind to pre-marked enhancers and activate transcription from neighboring genes

In a given cell type and at a specific developmental/functional time, potential enhancers can be:

- **Inactive**
- **Primed**
- **Poised**
- **Active**

Their status is defined by:

- Accessibility (DNaseI, FAIRE)
- Histone PTMs
- Presence of «mobile» histone isoforms H3.3/H2A.Z
- Presence of the acetyltransferase p300/CBP
- Transcription of eRNA

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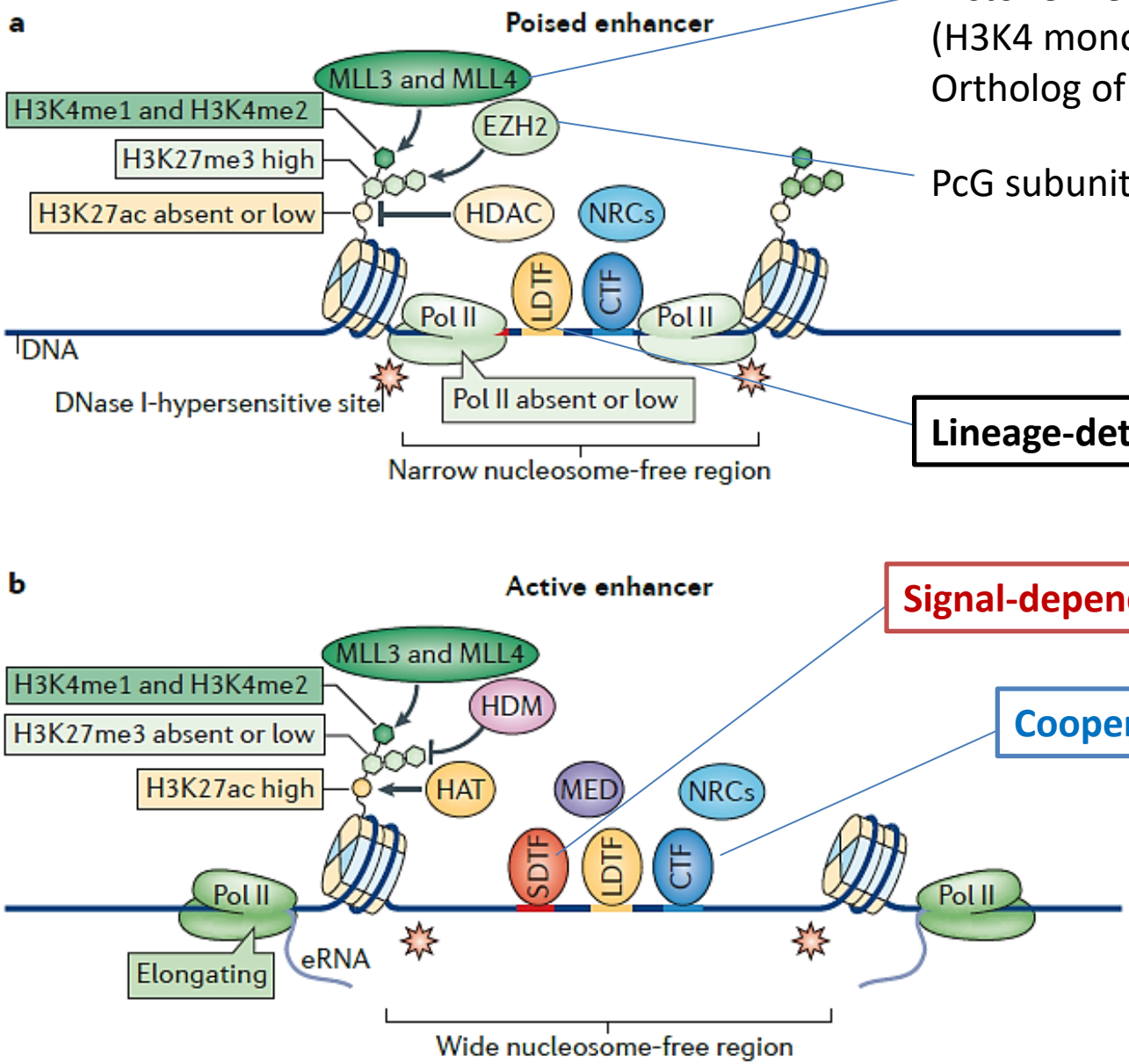


Histone metyl transferase
(H3K4 mono- di-)
Ortholog of Tritorax

PcG subunit

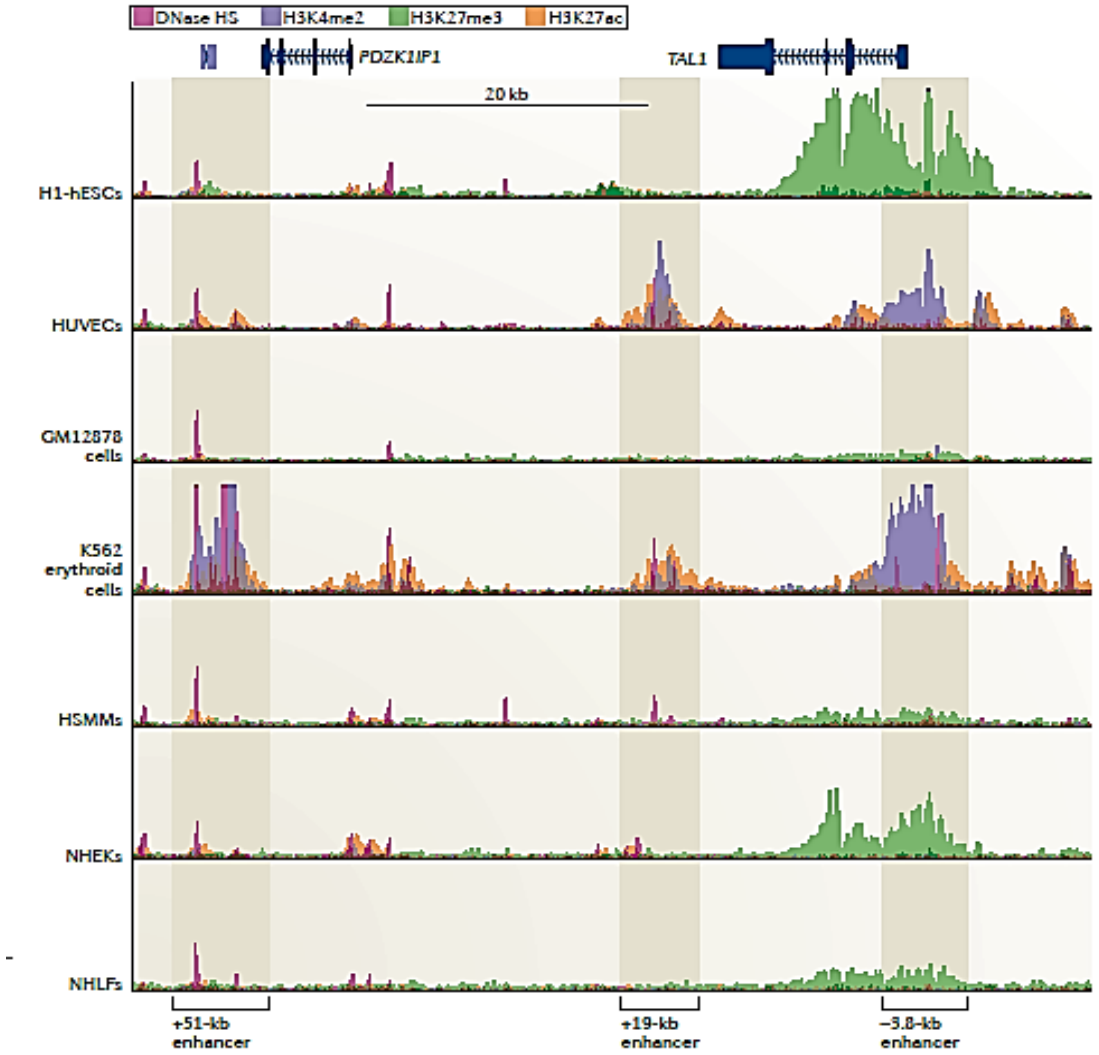
Lineage-determining TF

Signal-dependent TF

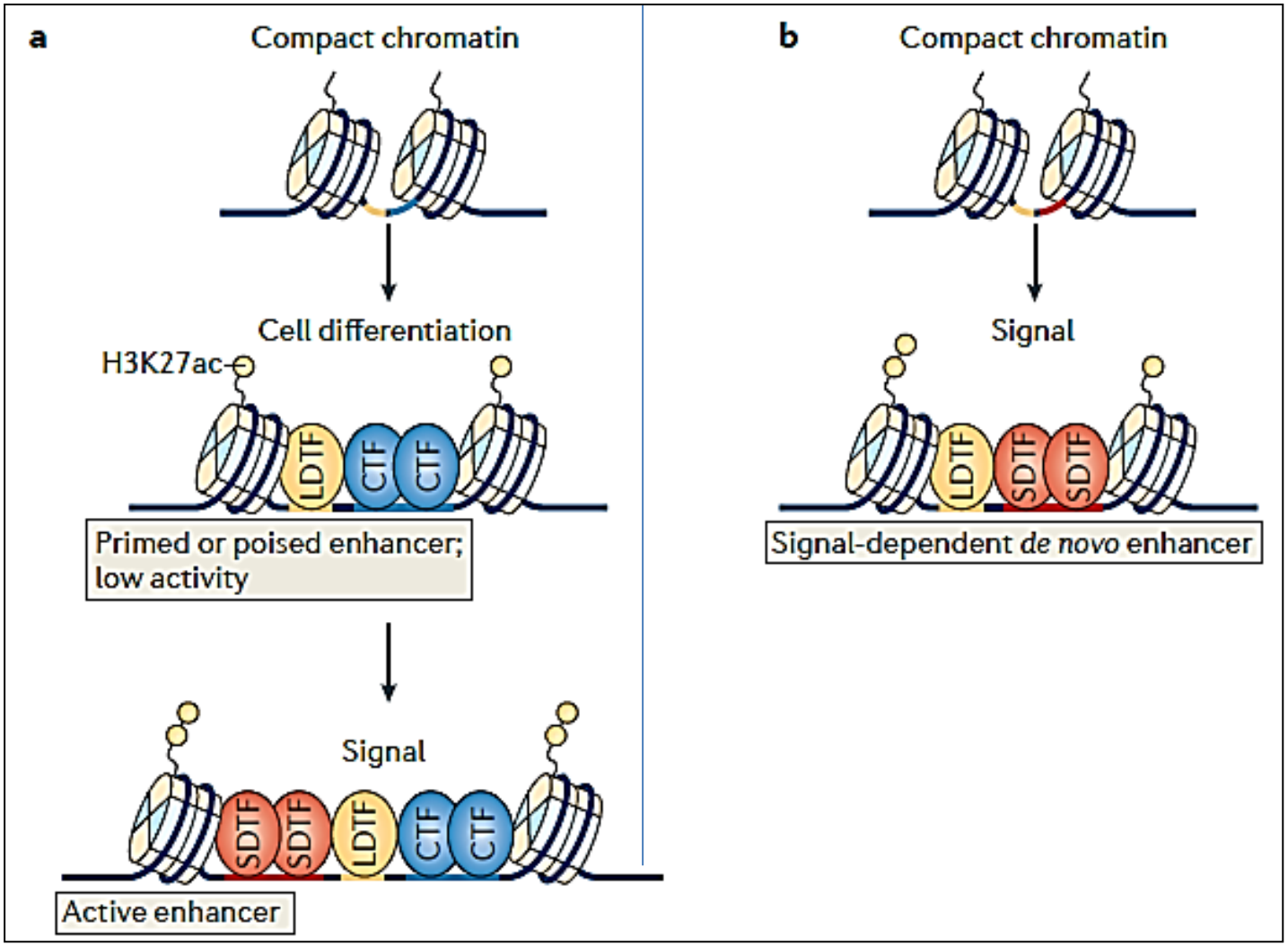
Cooperating TF

Heinz et al., 2015

Textbook



Cell type-specific enhancers are marked by specific epigenomic features and chromatin accessibility. Genomic features of a ~60 kb region of human chromosome 1 centered around the *TAL1* gene in 7 cell lines. Enhancers known to be responsible for *TAL1* transcription in endothelial cells (the −3.8 kb and +19 kb enhancers in HUVEC cells) and erythroid cells (the +51 kb enhancer in K562 cells) exhibit cell type-specific DNase HS, H3K4me2 and H3K27ac signals. In cell types where TAL1 is not expressed, the promoter and gene body are devoid of DNase HS and histone modifications indicative of enhancer activation (H3K4me2, H3K27ac



Enhancer activatin can follow a progressive way, stepping all intermediate states, or in some cases can be activated «ex-novo» following certain stimulations.

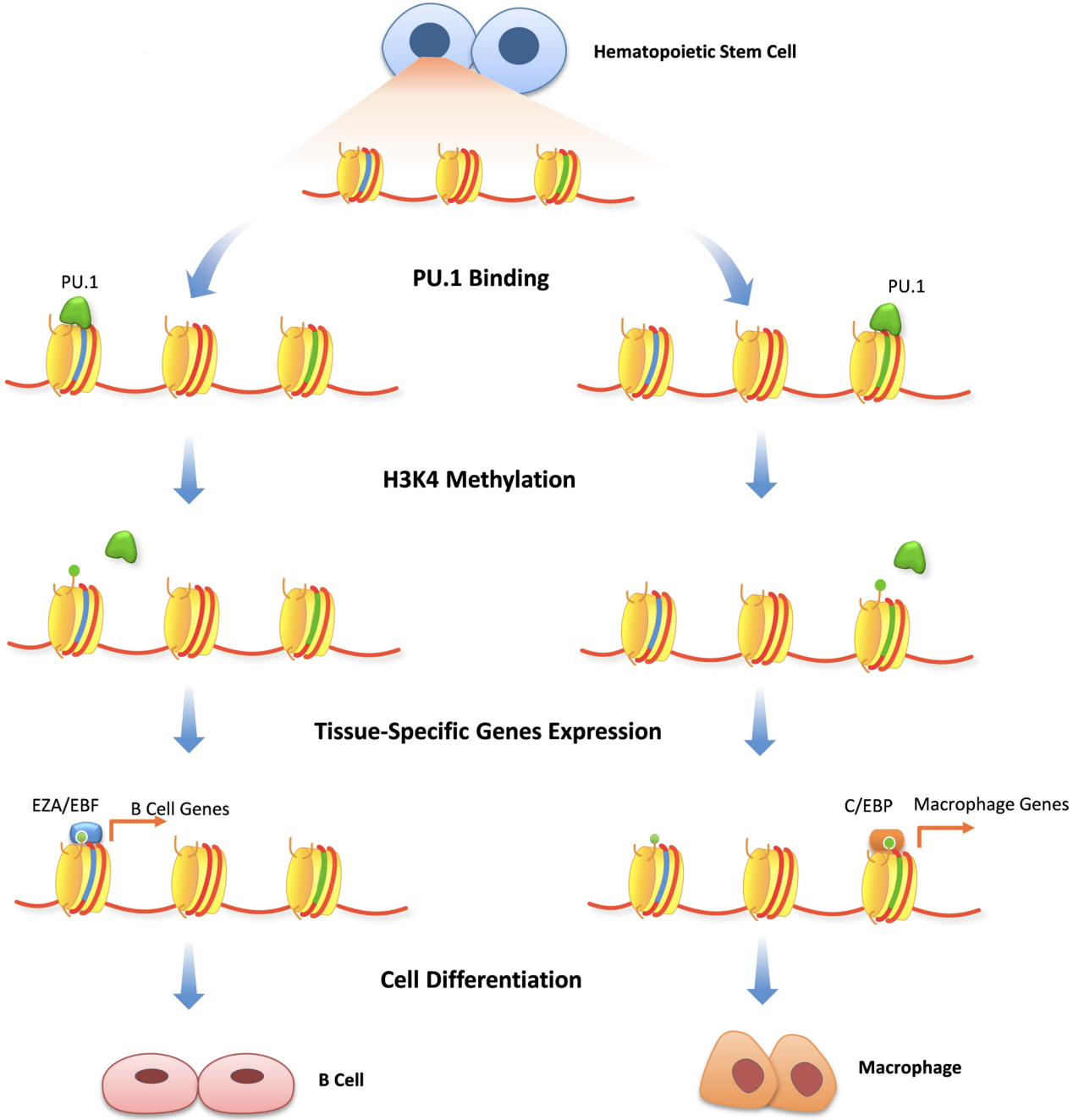
A certain number of Transcription Factors have the special property of being capable of interacting on DNA even when it is thightly bound to heterochromatic nucleosomes.

These TFs are defined «**Pioneer Factors**».

The family called Fox(nm) has been particularly studied, e.g. FoxA1.

The binding of these factors to their sites in chromatin is believed to be one of the first events to bring repressed enhancers to the primed or poised status. For example, this has been proposed for enhancers responding to AR and ER in prostate and breast cancer, respectively.

However, one potential caveat comes from the fact that the recognition sequence is very short and degenerated, posing the problem of how these factors recognize the correct sequences among thousands.


Hematopoietic Stem Cell
PU.1 Binding
PU.1
PU.1
H3K4 Methylation
Tissue-Specific Genes Expression
EZA/EBF
B Cell Genes
C/EBP
Macrophage Genes
Cell Differentiation
B Cell
Macrophage

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Chromosome-Wide Mapping of Estrogen Receptor Binding Reveals Long-Range Regulation Requiring the Forkhead Protein FoxA1

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Summary

Estrogen plays an essential physiologic role in reproduction and a pathologic one in breast cancer. The completion of the human genome has allowed the identification of the expressed regions of protein-coding genes; however, little is known concerning the organization of their *cis*-regulatory elements. We have mapped the association of the estrogen receptor (ER) with the complete nonrepetitive sequence of human chromosomes 21 and 22 by combining chromatin immunoprecipitation (ChIP) with tiled microarrays. ER binds selectively to a limited number of sites, the majority of which are distant from the transcription start sites of regulated genes. The unbiased sequence interrogation of the genuine chromatin binding sites suggests that direct ER binding requires the presence of Forkhead factor binding in close proximity. Furthermore, knockdown of *FoxA1* expression blocks the association of ER with chromatin and estrogen-induced gene expression demonstrating the necessity of FoxA1 in mediating an estrogen response in [illegible]s.

FOXA1 is a key determinant of estrogen receptor function and endocrine response

Antoni Hurtado[1,2], Kelly A Holmes[1,2], Caryn S Ross-Innes[1], Dominic Schmidt[1] & Jason S Carroll[1]

Estrogen receptor-α (ER) is the key feature of most breast cancers and binding of ER to the genome correlates with expression of the Forkhead protein FOXA1 (also called HNF3α). Here we show that FOXA1 is a key determinant that can influence differential interactions between ER and chromatin. Almost all ER-chromatin interactions and gene expression changes depended on the presence of FOXA1 and FOXA1 influenced genome-wide chromatin accessibility. Furthermore, we found that CTCF was an upstream negative regulator of FOXA1-chromatin interactions. In estrogen-responsive breast cancer cells, the dependency on FOXA1 for tamoxifen-ER activity was absolute; in tamoxifen-resistant cells, ER binding was independent of ligand but depended on FOXA1. Expression of FOXA1 in non-breast cancer cells can alter ER binding and function. As such, FOXA1 is a major determinant of estrogen-ER activity and endocrine response in breast cancer cells.

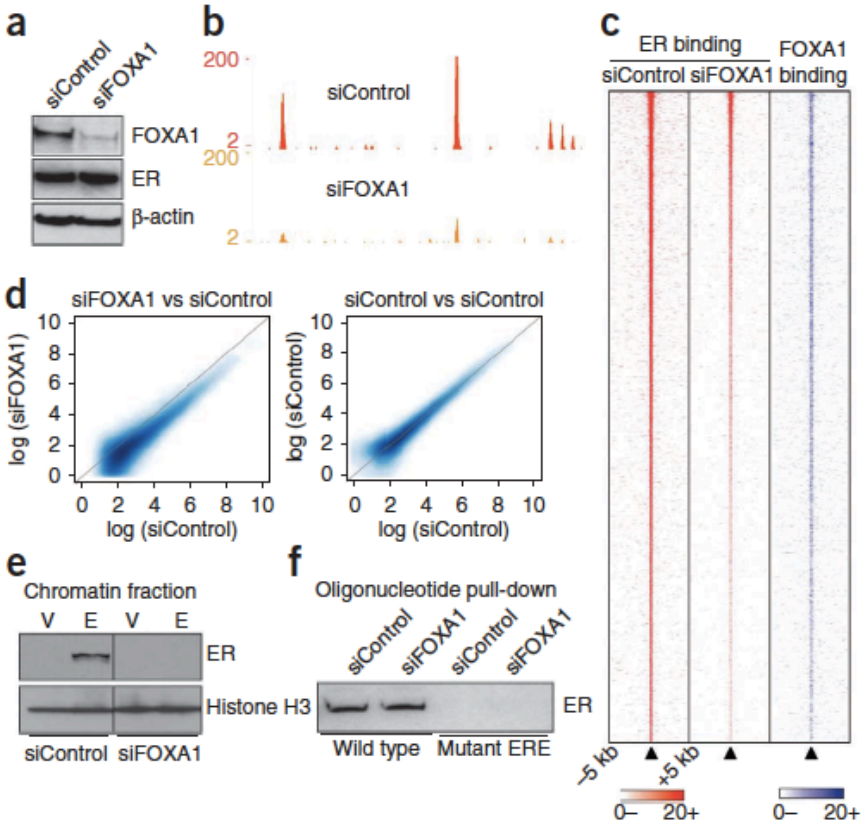

Figure 2 Binding of ER to chromatin and transcriptional activity requires FOXA1. (**a**) Protein blot of cells transfected with siControl or siFOXA1. (**b**) An example of ER binding in cells transfected with siControl or siFOXA1. (**c**) Heatmap showing the signal intensity of ER binding in cells transfected with siControl or siFOXA1 in a window of ±5 kb. Signal intensity for FOXA1 at the equivalent genomic region is also shown. The heatmap represents binding events ranked from the strongest to weakest ER binding (in the siControl condition), and the adjacent columns represent the signal from the corresponding genomic region but under the different experimental conditions. (**d**) Smoothened scatterplot comparing ER binding intensity in cells transfected with siControl and those transfected with siFOXA1. As a control, a scatterplot representing two different siControl experiments is shown. (**e**) Cells were transfected with siControl or siFOXA1, treated with vehicle (V) or estrogen (E) and were fractionated to enrich for the chromatin fraction, which was protein blotted. Histone H3 was used as a loading control. The uncropped protein blot is in **Supplementary Figure 2**. (**f**) Oligonucleotide pull-down using total protein from cells transfected with siControl or siFOXA1. A double-stranded, biotin-labeled oligonucleotide containing a perfect ERE sequence or a mutant sequence was used and protein enriched by the oligonucleotide was protein blotted. (**g**) Gene expression microarray analysis following transfection of siControl or siFOXA1 and treatment with vehicle or estrogen for 6 h.

Hurtado et al., Nat Genetics 2011

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Article

Cell

Steroid Receptors Reprogram FoxA1 Occupancy through Dynamic Chromatin Transitions

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The estrogen receptor (ER), glucocorticoid receptor (GR), and forkhead box protein 1 (FoxA1) are significant factors in breast cancer progression. FoxA1 has been implicated in establishing ER-binding patterns though its unique ability to serve as a pioneer factor. However, the molecular interplay between ER, GR, and FoxA1 requires further investigation. Here we show that ER and GR both have the ability to alter the genomic distribution of the FoxA1 pioneer factor. Single-molecule tracking experiments in live cells reveal a highly dynamic interaction of FoxA1 with chromatin in vivo. Furthermore, the FoxA1 factor is not associated with detectable footprints at its binding sites throughout the genome. These findings support a model wherein interactions between transcription factors and pioneer factors are highly dynamic. Moreover, at a subset of genomic sites, the role of pioneer can be reversed, with the steroid receptors serving to enhance binding of FoxA1.

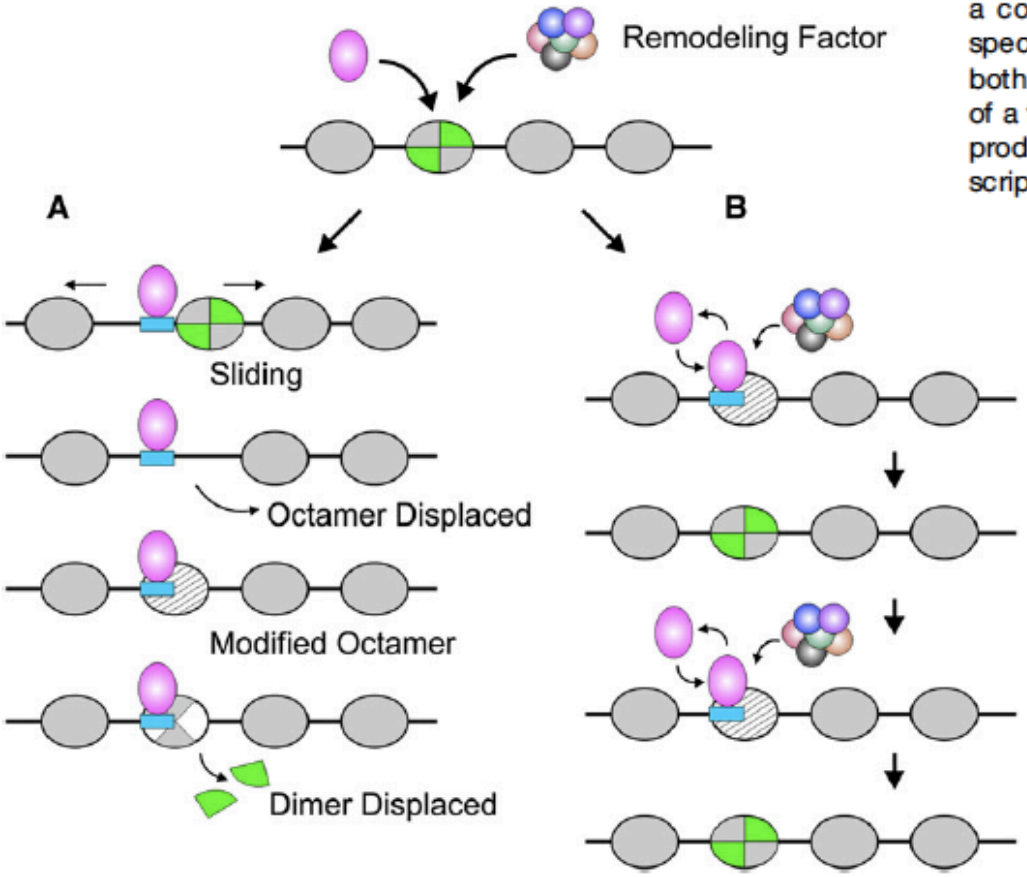

Figure 2. Transcription Factor/Template Interactions during Chromatin Remodeling

(A) Local nucleosome reorganization, giving rise to enhanced transcription factor access, is commonly discussed in terms of altered static states. Evidence has been advanced to support several specific mechanisms, including sliding to a new position, octamer displacement, modified octamer structure, and partial octamer dissociation. These modified nucleosome states would in turn accommodate factor binding events not compatible with the unaltered state.

(B) A dynamic view of local transitions suggests that remodeling is a continuous process. Remodeling complexes are targeted to specific nucleosomes by a given transcription factor. However, both the remodeling process itself and commensurate binding of a factor are transient events. Constant repetition of this cycle produces a shift in the equilibrium distribution of both the transcription factor and nucleosome components.