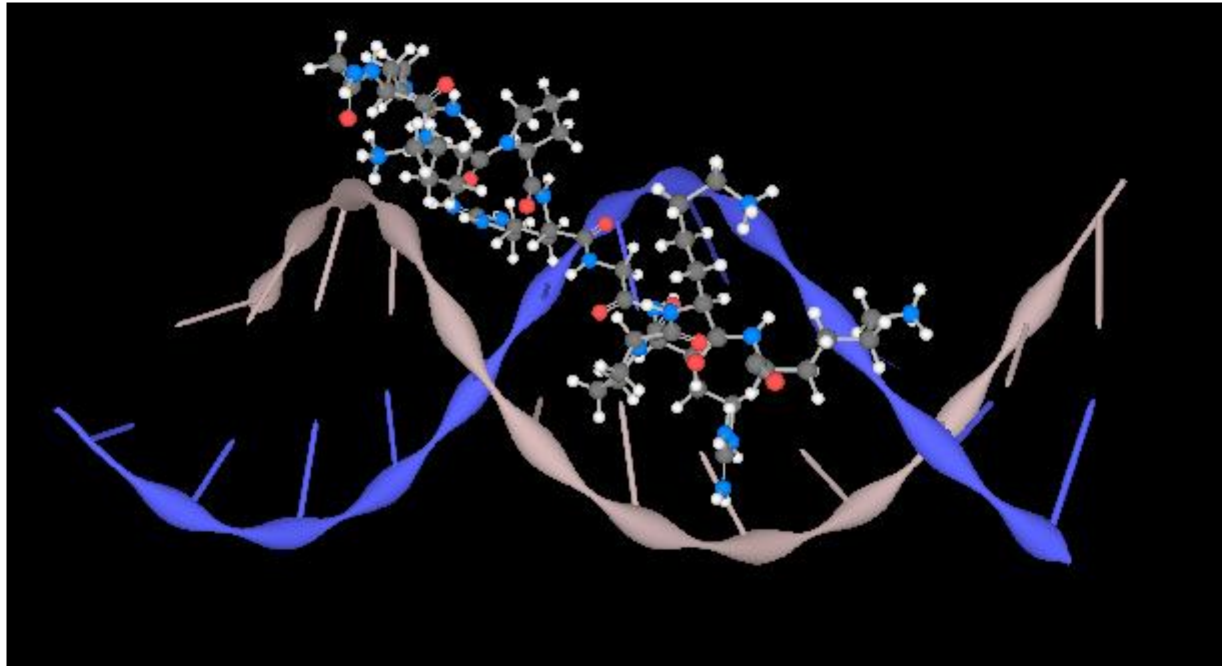
L3.3 - Enhancer activity

From previous lessons and from Lambert's Review (Textbook)

- The Human TF repertoire: TFs in families, classification on type of DBD, most represented C2H2-ZF
- Motifs hunting algorithms
- Cell- and tissue-specificity of TFs
- Human TFBSs in Genetics and Diseases: 80% of GWAS maps in noncoding regions

Figures?



AT-hook doman - PDBe › 2ezg

Agenda:

- Different and cell type-specific enhancer activation
- How are enhancers selected and activated during development
- Effects of enhancer activation

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.

First issue is that, when scientists explored data from ENCODE, FANTOM and other big studies conducted in a wide range of different cell types and tissues, by integrating TFBS with histone modifications and RNA-Seq data, they could conclude that about half a million Enhancers exist (perhaps up to one million, some Authors say) in the Human genome

This greatly outnumbers the regions labelled as «Promoters», by a factor close to ten.

ENCODE

- 450,000 elements in the Human Genome display enhancer features, in different cell types.
- Estimated up to one million.
- Accentuated **cell-type specificity**.

Only a small fraction of these Enhancers are either active, or marked for activation in each cell type.

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

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

some Author make one class of these (Primed, Poised)

Their status is defined by:

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

Different functional states of Enhancers

a

Poised enhancer

MLL3 and MLL4

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

H3K4me1 and H3K4me2

EZH2

PcG subunit

H3K27me3 high

H3K27ac absent or low

HDAC

NRCs

LDTF

CTF

Pol II

Pol II

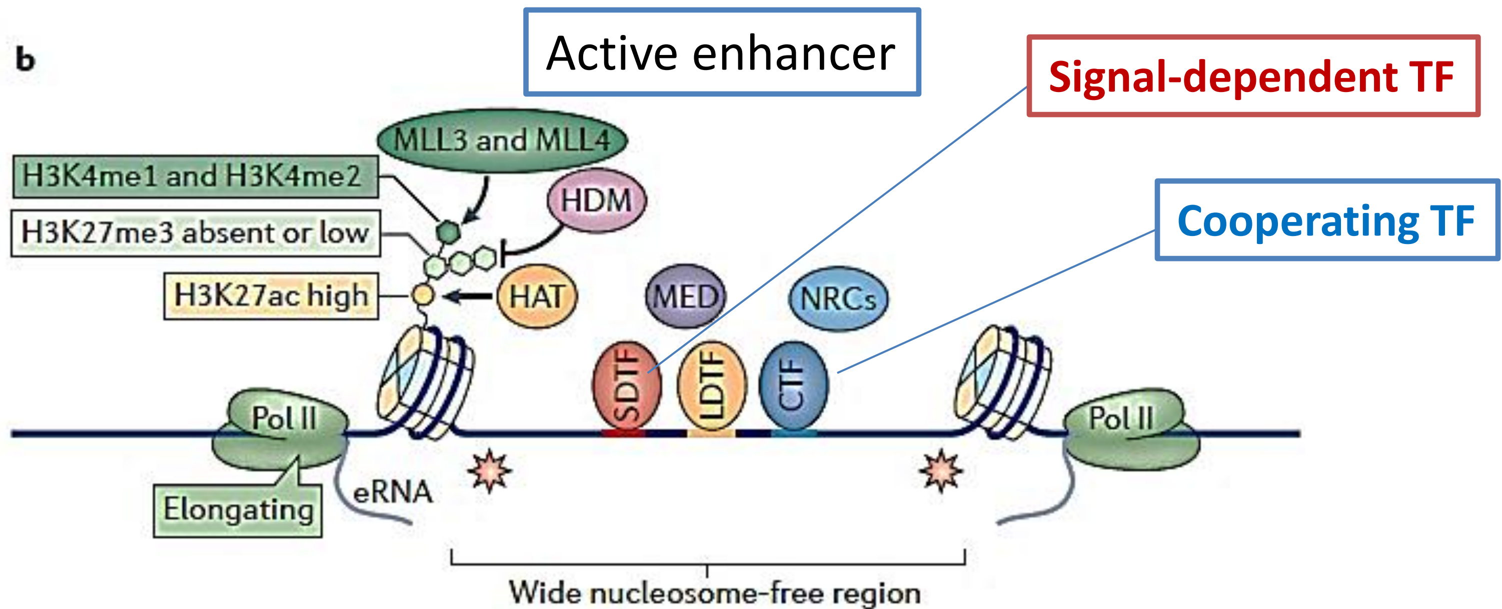
DNA

DNase I-hypersensitive site

Pol II absent or low

Lineage-determining TF

Narrow nucleosome-free region



Heinz et al., 2015

a

Poised enhancer

MLL3 and MLL4

H3K4me1 and H3K4me2

EZH2

H3K27me3 high

H3K27ac absent or low

HDAC

NRCs

LDTF

CTF

Pol II

Pol II

DNA

DNase I-hypersensitive site

Pol II absent or low

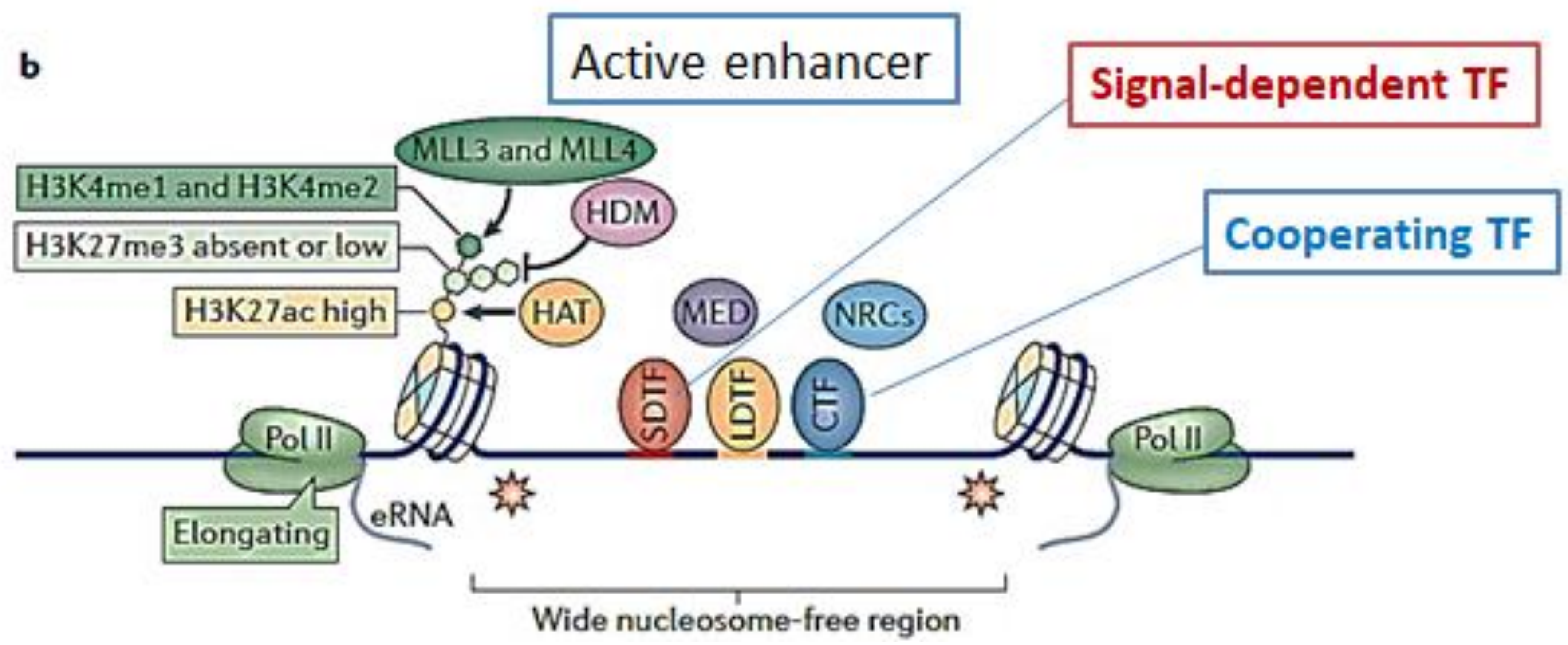
Narrow nucleosome-free region

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

PcG subunit

Lineage-determining TF

Nucleosome Remodelling Complex


b
Active enhancer
Signal-dependent TF
Cooperating TF
MLL3 and MLL4
H3K4me1 and H3K4me2
HDM
H3K27me3 absent or low
H3K27ac high
HAT
MED
NRCs
SDTF
LDTF
CTF
Pol II
Pol II
Elongating
eRNA
Wide nucleosome-free region

In the Lambert's review, they divide TFs conceptually:

LDTFs – Lineage Determining Transcription Factors

There are examples where the same LDTF binds to different sets of Enhancers in different cell types, since it is driven by other cell-specific factors (PU.1 in macrophages vs B-cells) (combinatorial rule).

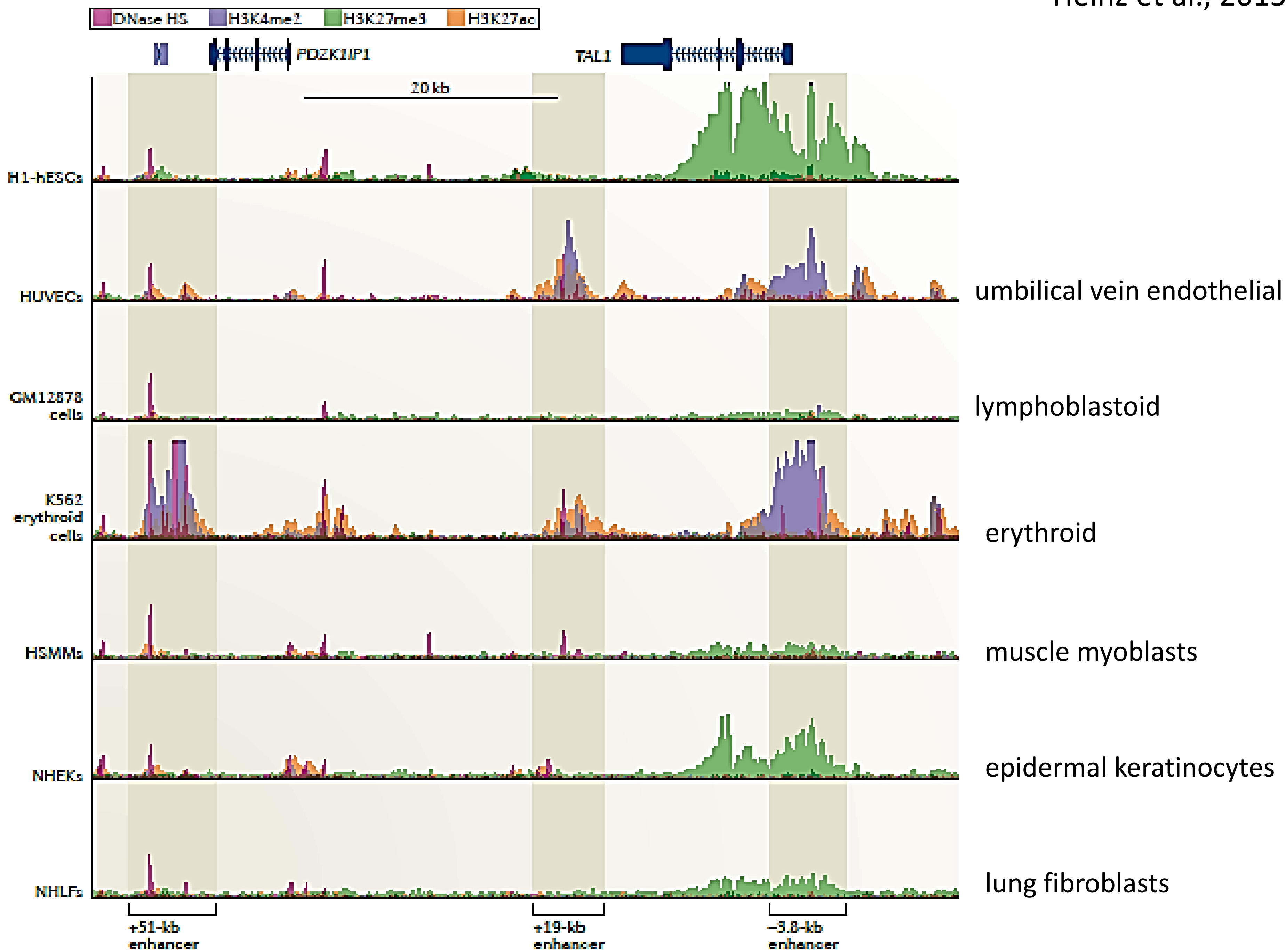
SDTFs – Signal-Dependent TFs

examples are NF-kB and Nuclear Receptors

CFTs – Collaborating TFs

any other TF, also constitutive / ubiquitous TFs, collaborating with LDTFs and SDTFs to achieve maximum response at Enhancers

Heinz et al., 2015



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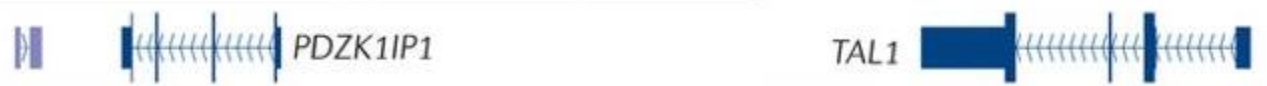
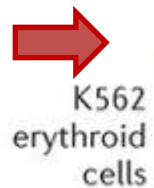


20 kb

H1-hESCs

HUVECs

GM12878 cells

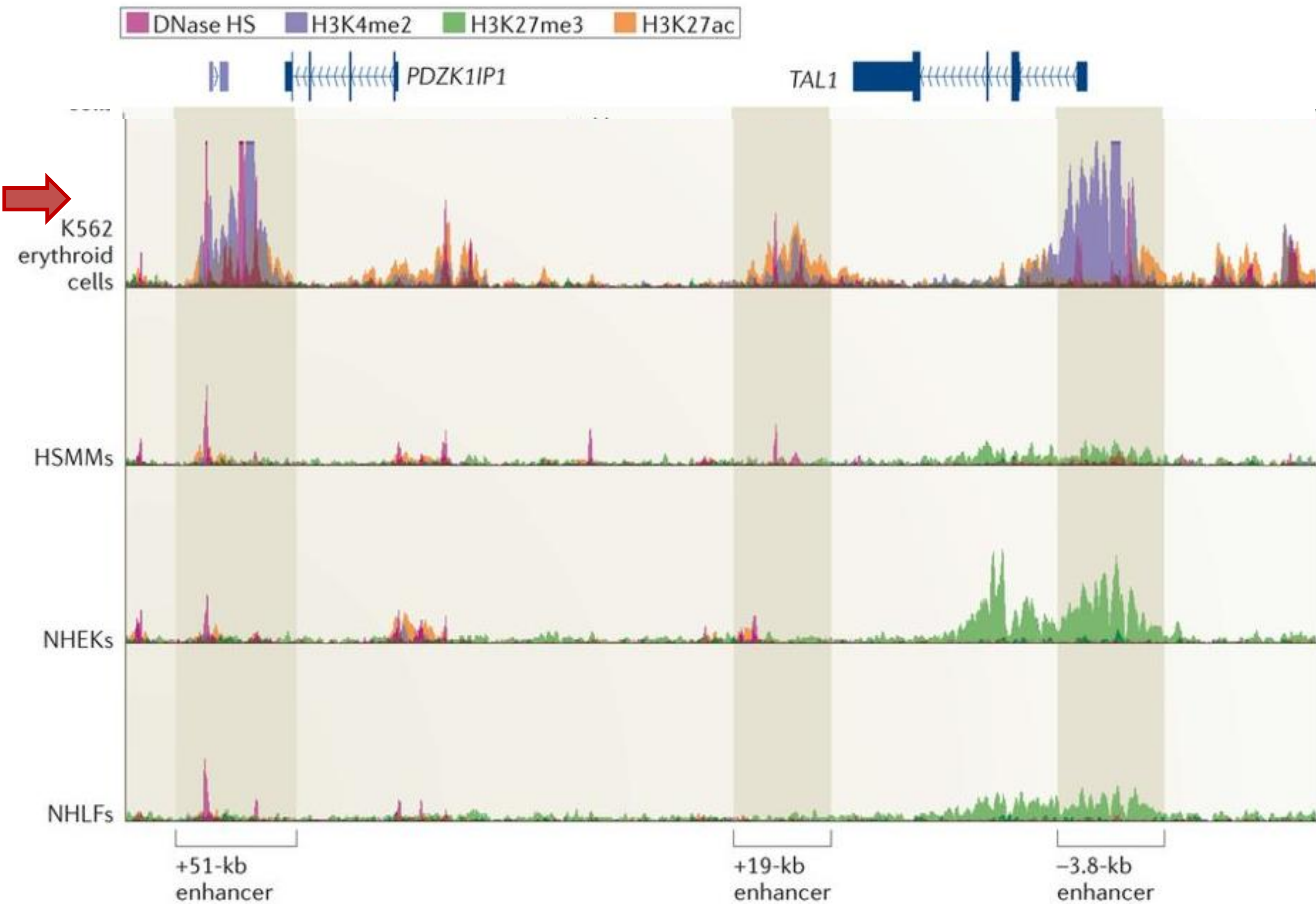


K562 erythroid cells

+51-kb enhancer

+19-kb enhancer

−3.8-kb enhancer


DNase HS
H3K4me2
H3K27me3
H3K27ac
PDZK1IP1
TAL1
K562
erythroid
cells
HSMMs
NHEKs
NHLFs
+51-kb
enhancer
+19-kb
enhancer
−3.8-kb
enhancer

How is Enhancer state developmentally determined ?

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

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Sengthavy Phouanenavong,[1] Ingrid Plajzer-Frick,[1] Malak Shoukry,[1] Veena Afzal,[1] John L.R. Rubenstein,
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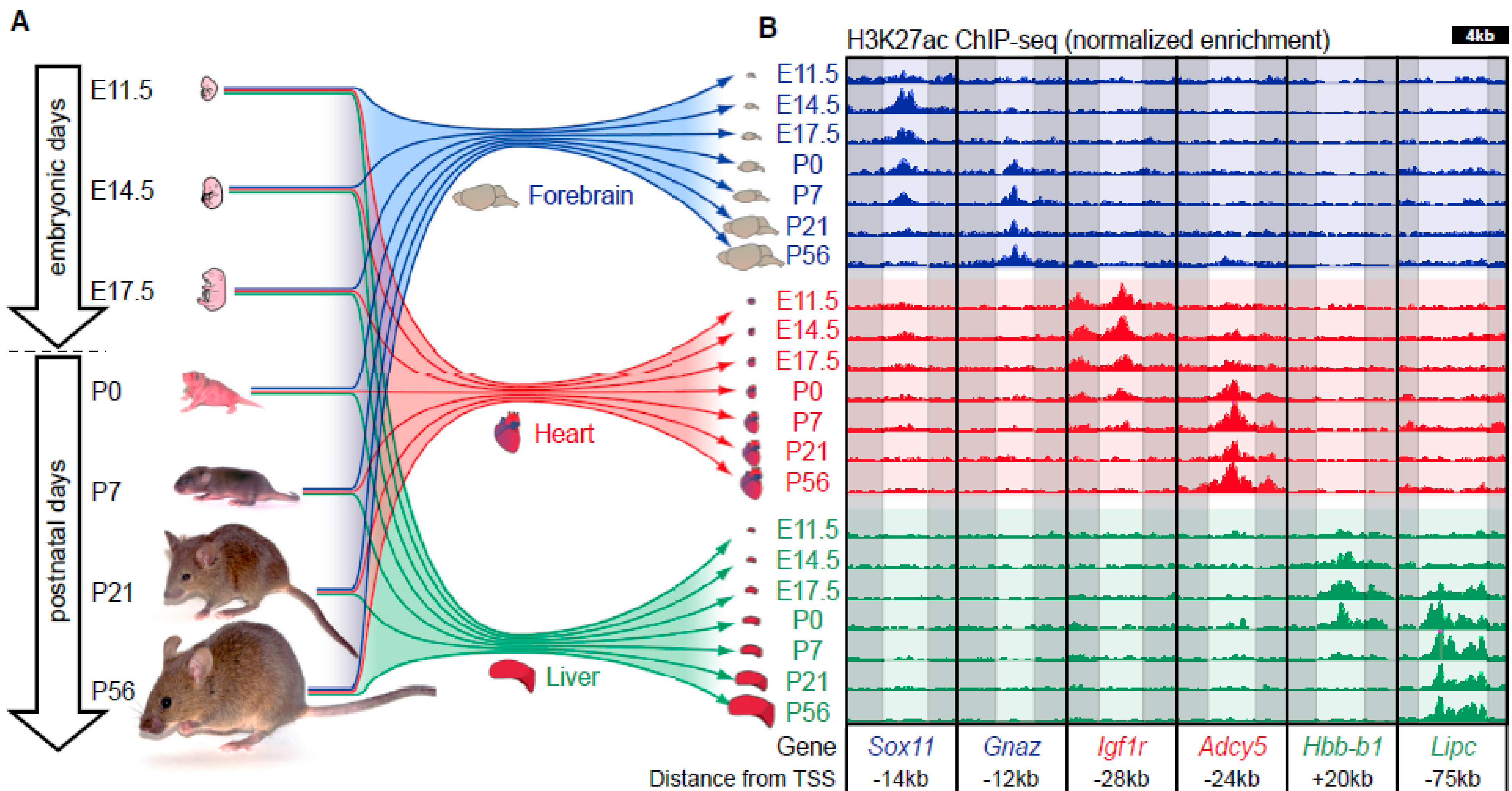
Enhancers are distal regulatory elements that can activate tissue-specific gene expression and are abundant throughout mammalian genomes. Although substantial progress has been made toward genome-wide annotation of mammalian enhancers, their temporal activity patterns and global contributions in the 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.

Another way is to examine enhancer dynamics through development

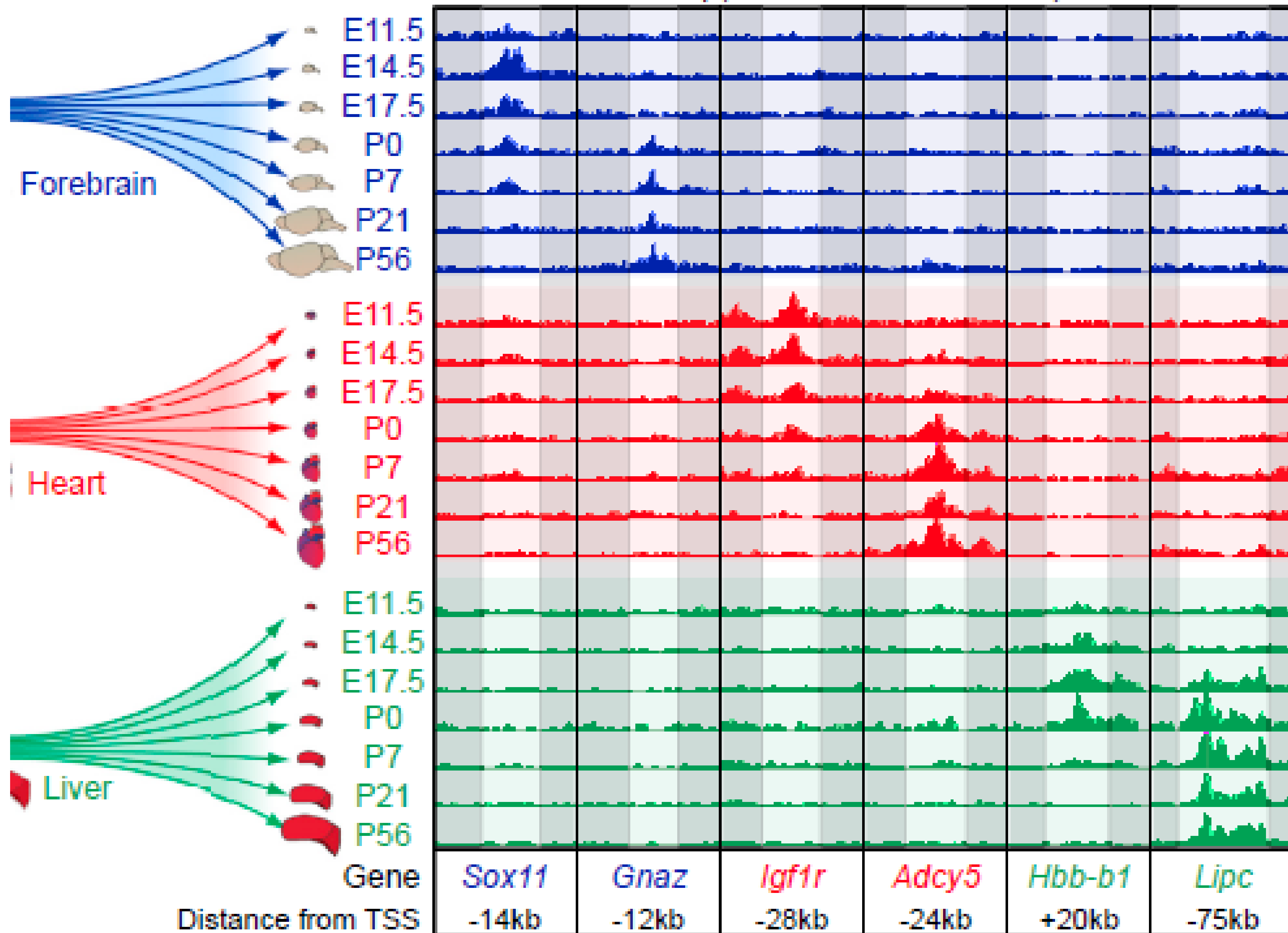
Cell *155*, 1521–1531, December 19, 2013 ©2013 Elsevier Inc.

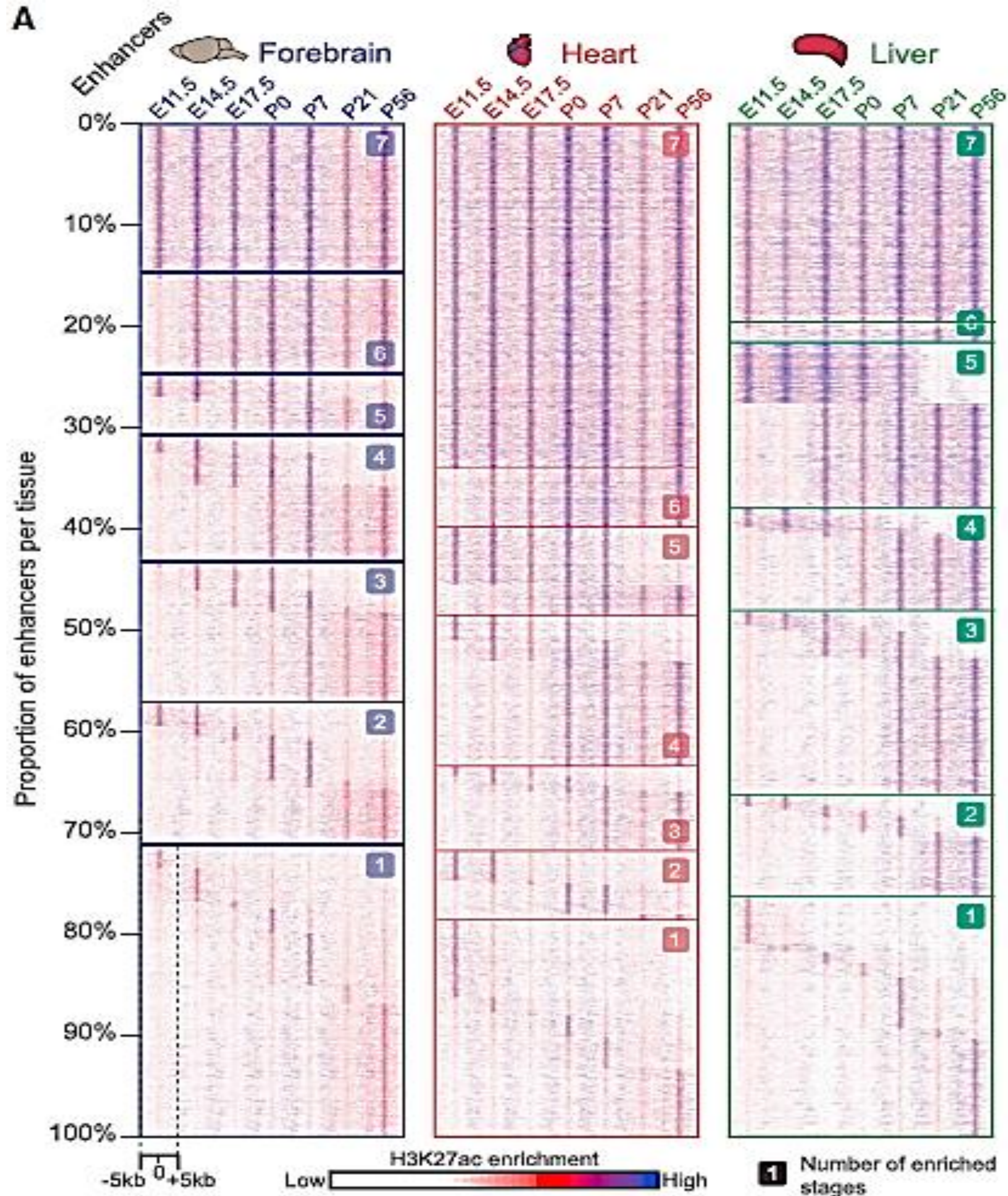
Cell



(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.


B
H3K27ac ChIP-seq (normalized enrichment)
4kb
Forebrain
Heart
Liver
E11.5
E14.5
E17.5
P0
P7
P21
P56
Gene
Distance from TSS
Sox11
-14kb
Gnaz
-12kb
Igf1r
-28kb
Adcy5
-24kb
Hbb-b1
+20kb
Lipc
-75kb

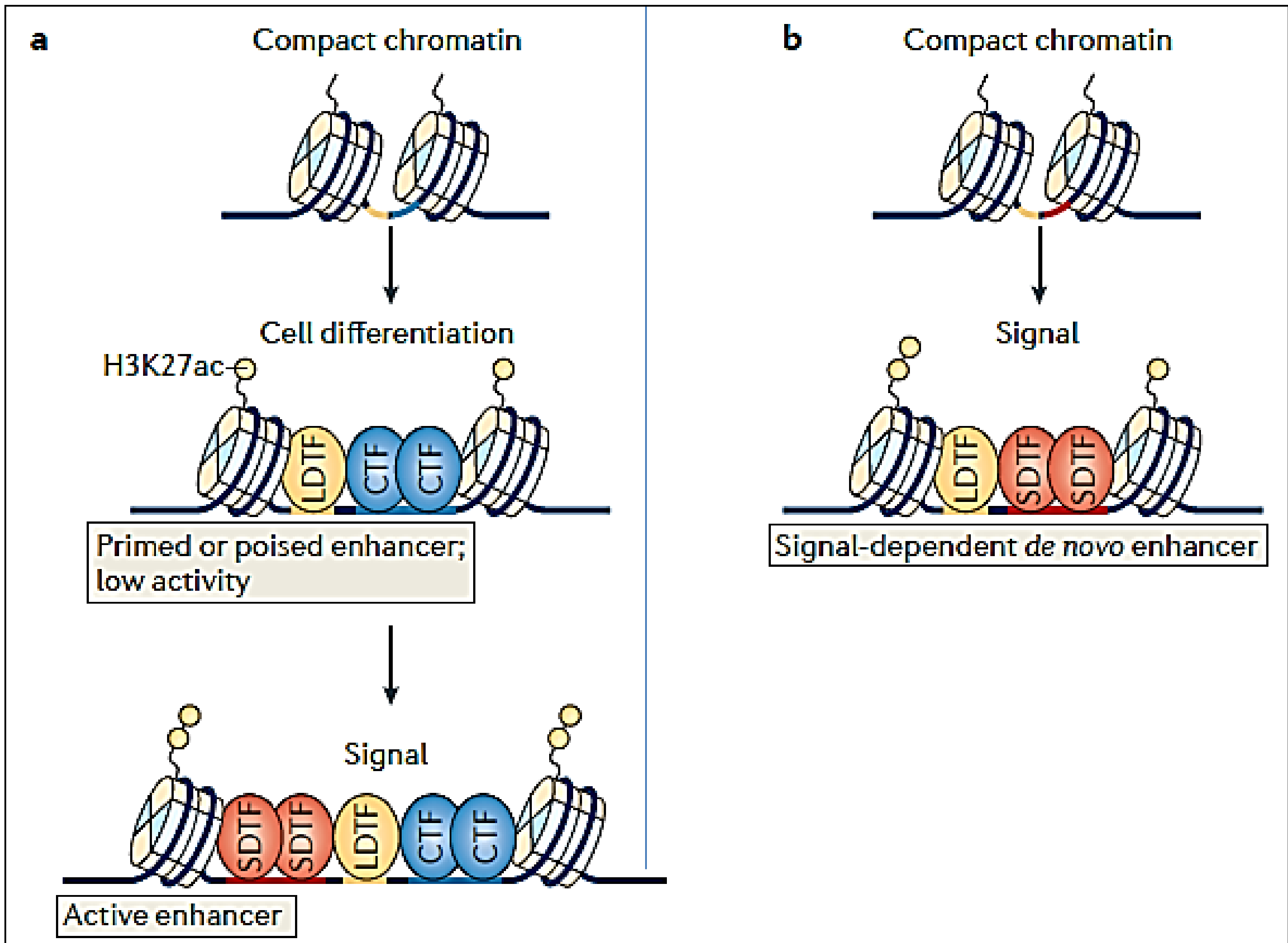

A
Enhancers
Forebrain
Heart
Liver
E11.5
E14.5
E17.5
P0
P7
P21
P56
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Proportion of enhancers per tissue
7
6
5
4
3
2
1
-5kb
0
+5kb
H3K27ac enrichment
Low
High
Number of enriched stages

How are cell-specific enhancers selected and established ?

In ESC many enhancers are in «bivalent state»: nucleosomes display both «activating» marks (H3K4me1/2) and «repressive» marks (H3K27me3). This situation represents a unstable permissive condition.

When ESC are induced to differentiate, establishment of repressive marks will predominate at enhacers controlling master pluripotency genes (among which Sox2, Oct4, Nanog) and repressing of these TF will also repress all pluripotency genes progressively.

Other enhancers controlling lineage-specific genes, on the contrary, lose repressive marks and become overt active enhancers.



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.

Cooperative action with other factors ?

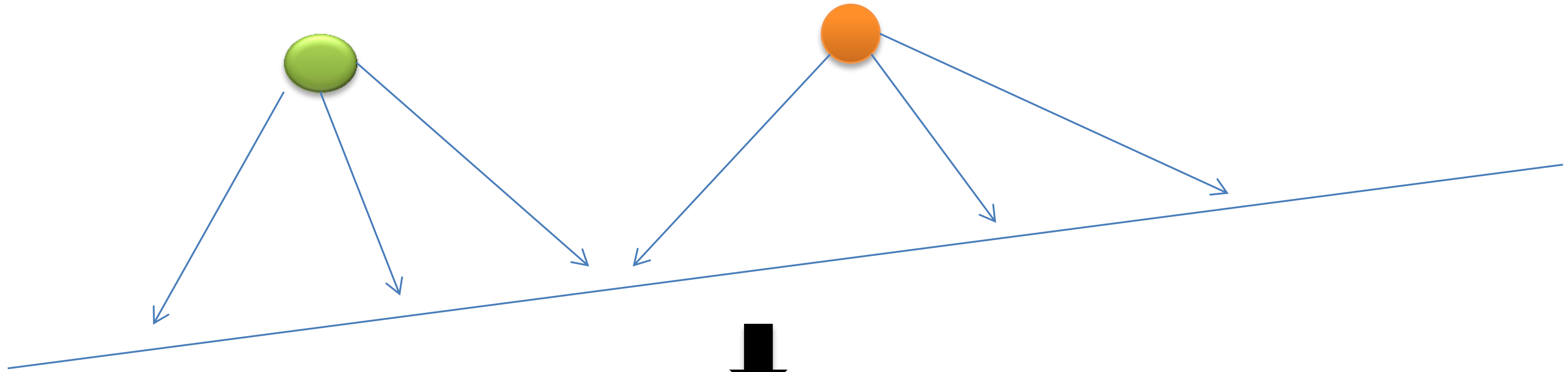
There a a number of not completely understood questions.

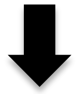
First: does future activation of enhancer require the primary factor (e.g. pioneer factor) to be expressed at all stages ?

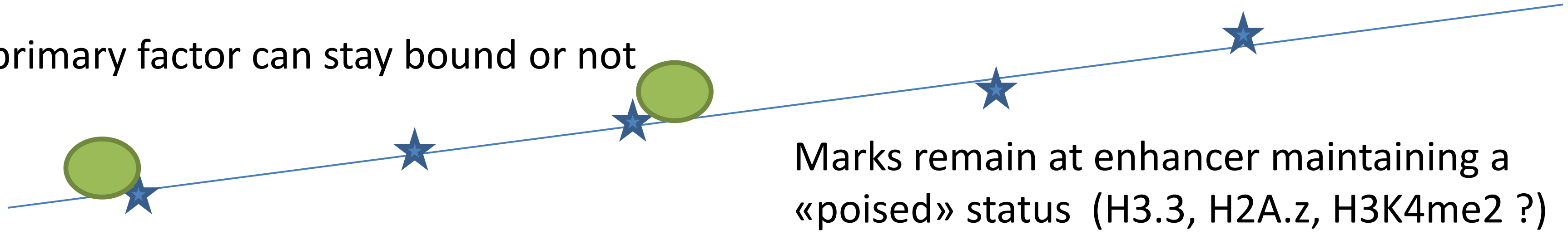
Certain «pluripotent factors» such as Nanog, Sox2 etc. are actually transiently expressed and it may well be that some pioneer factors, once marked the Enhancers by histone PTMs and H isoforms, can be replaced by other tissue-specific factors.

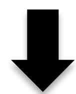
More detailed analysis, which should include all developmental steps of a cell lineage, are be very difficult to perform also since 1) difficult to synchronize; 2) stochasticity of factors in different cells.

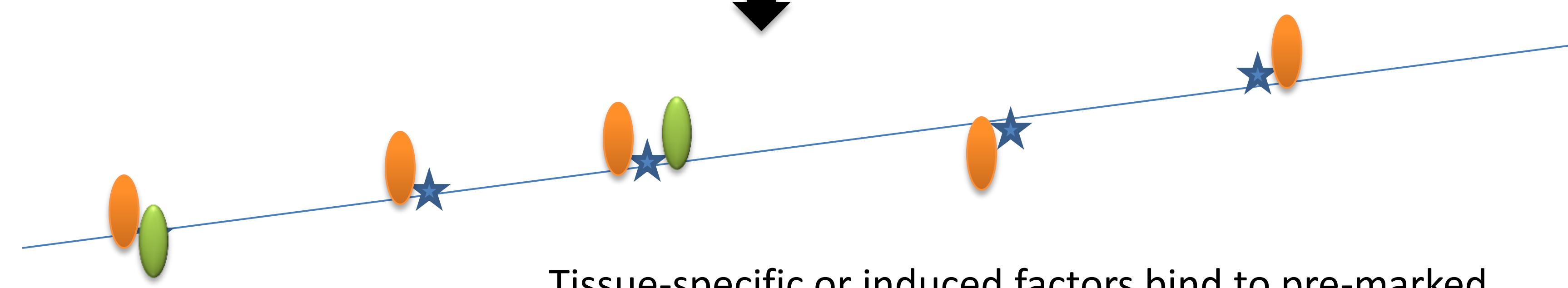
Primary factors «prime» enhancer sequences in differentiating cells







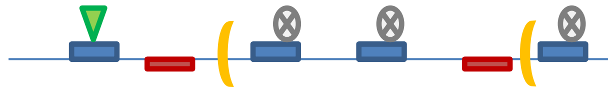




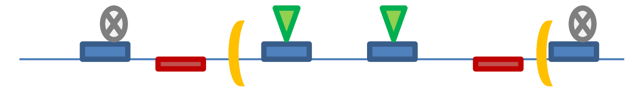
Enhancer
Gene
Histone PTM act.
Histone PTM repr.
insulator

Pioneer or primary factor
Tissue-specific TF
Gene transcript

Cell fate switch

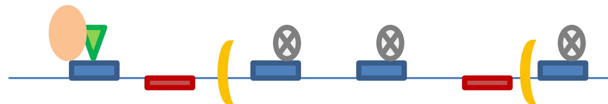


Cell type A

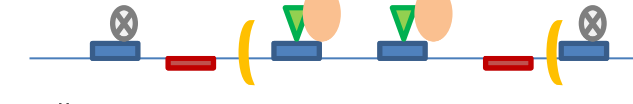


Cell type B

Pioneer or primary TFs bind

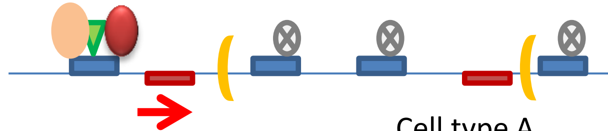


Cell type A

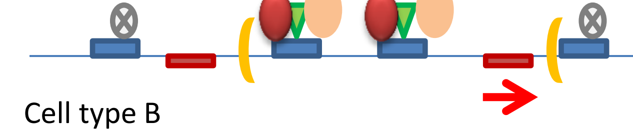


Cell type B

Tissue-specific TFs bind and...



Cell type A



Cell type B

activate transcription from promoters within chromatin domain

In other reviews you may find this classification:

Pioneer Factors:
transcription factors able to recognize their cognate DNA sequence even in compacted chromatin. Their binding is followed by histone modification at the nuclosomes flanking the enhancer

Tissue-specific Factors:
TFs that are expressed in precursors or in differetiated cells. Also called lineage-specific factors. They will bind pre-marked enhancers and activate them.

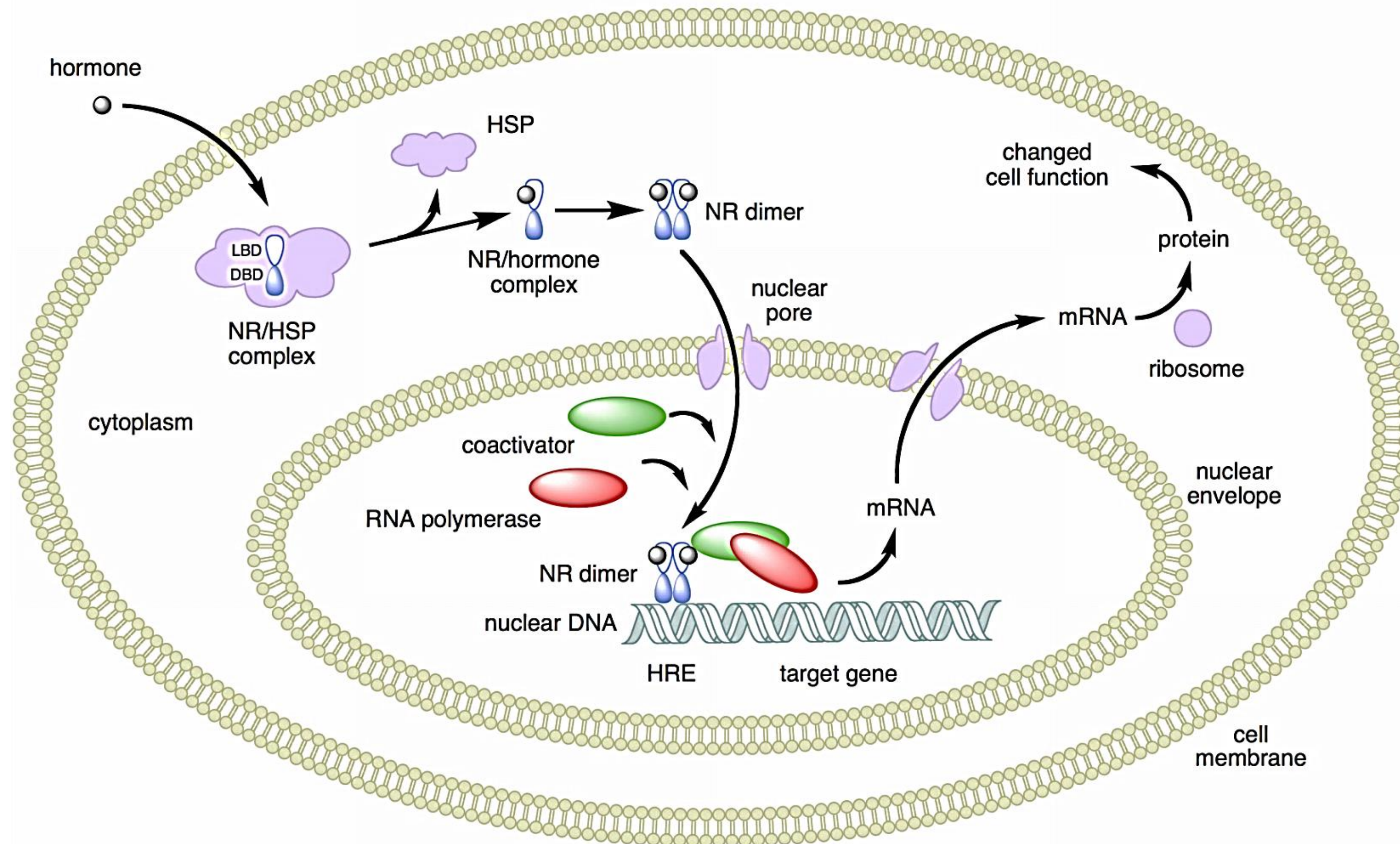
Signal-dependent factors:
TFs that are expressed or activated following a specific endogenous or exogenous stimulus. They will bind to and activate pre-marked enhancers (sometimes also to novel enhancers).

Example from steroid hormone receptors

For Steroid Sex Hormones such as estrogens and androgens, a model has been proposed where Pioneer Factor FoxA1, in collaboration with Tissue-specific TFs, marks the enhancers that will be activated by Nuclear Receptors (ERα and AR).

ChIP studies on both ERα (in breast cancer cells) and AR (in prostate cancer cells) clearly showed that the majority of the several thousand TFBS were in distal position respect the regulated genes, i.e. at Enhancers.

In **Human most of the** the genes responding primarily (protein synthesis- -independent) to steroid hormones are of this kind.
Let’s consider the Nuclear Receptor Superfamily of Transcription Factors



Nuclear Receptor activity, Type I and Type II, orphan receptors

Nuclear Receptor are a class of TFs that have been intesively studied

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)

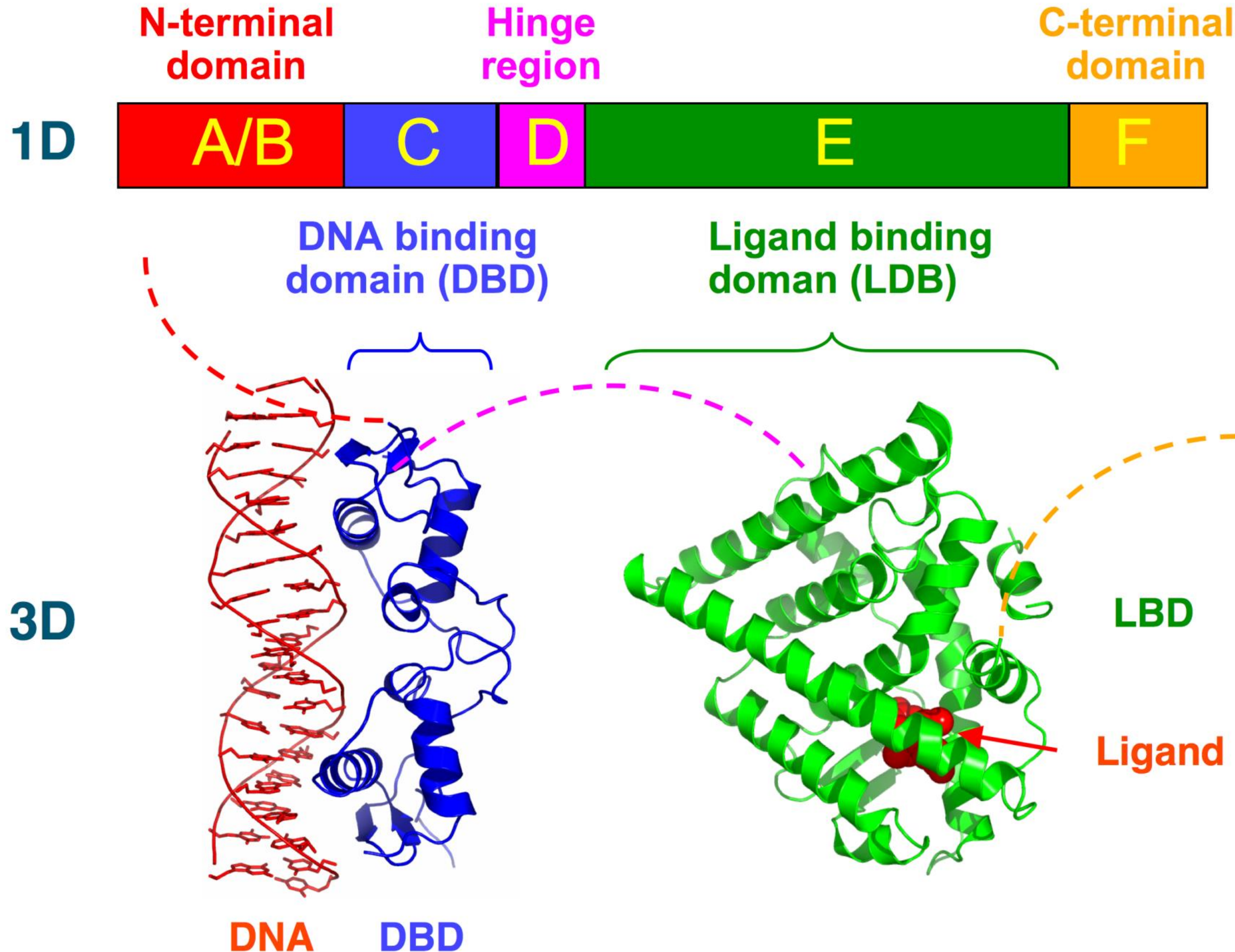
NRs classification

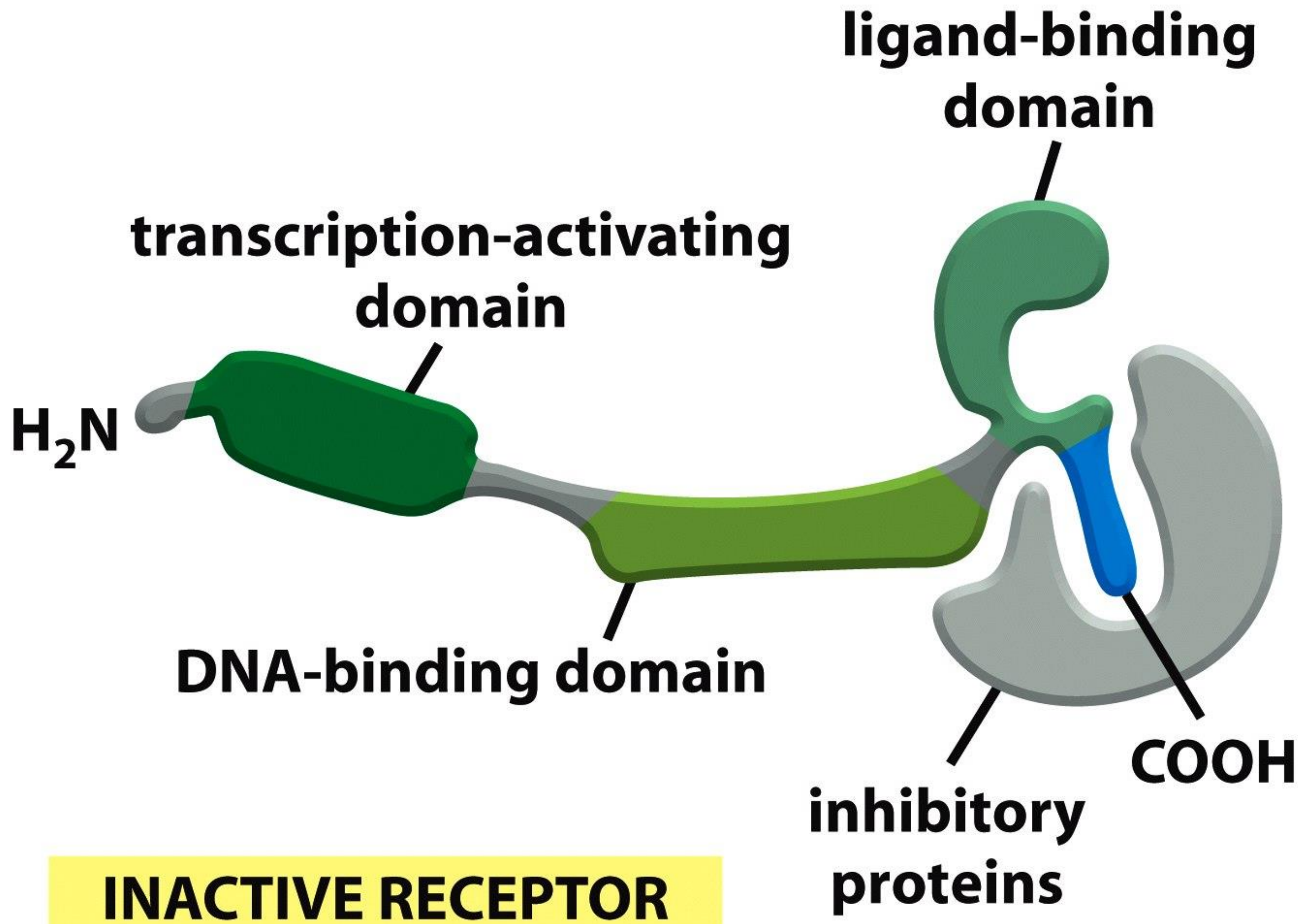
NUCLEAR RECEPTOR TYPE	NUCLEAR RECEPTOR MEMBERS
I (classical or steroid receptors)	Progestins receptor (PR) Estrogens receptor (ERα, ERβ) Androgens receptor (AR) Glucocorticoids receptor (GR) Mineralcorticoids receptor (MR)
II (RXR-heterodimeric receptors)	Thyroid hormone receptor (TRα, TRβ) All-*trans* 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

Structural Organization of Nuclear Receptors





hsp=heat shock proteins
(chaperons)

Figure 15-14b *Molecular Biology of the Cell* (© Garland Science 2008)

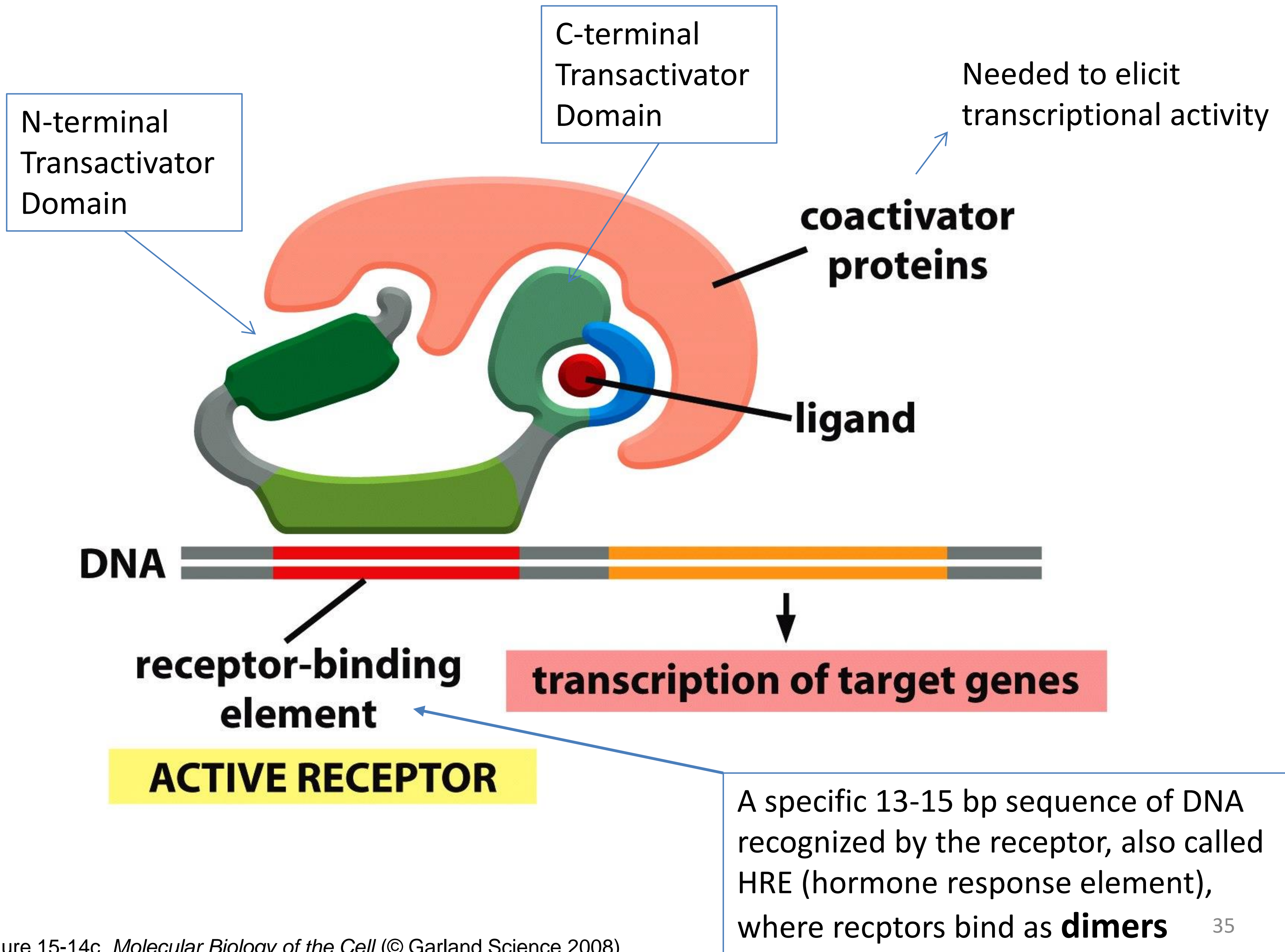
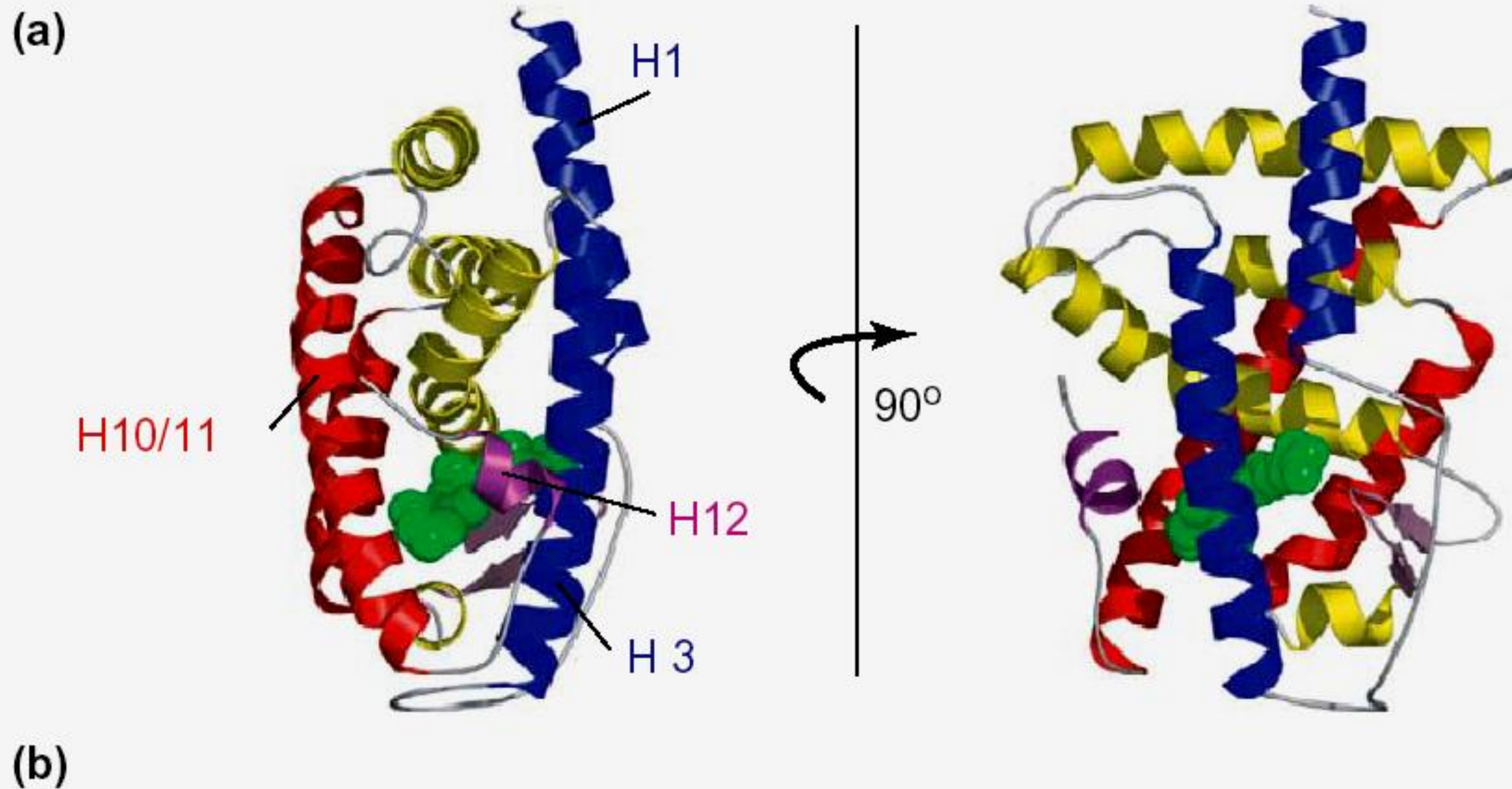


Figure 15-14c *Molecular Biology of the Cell* (© Garland Science 2008)

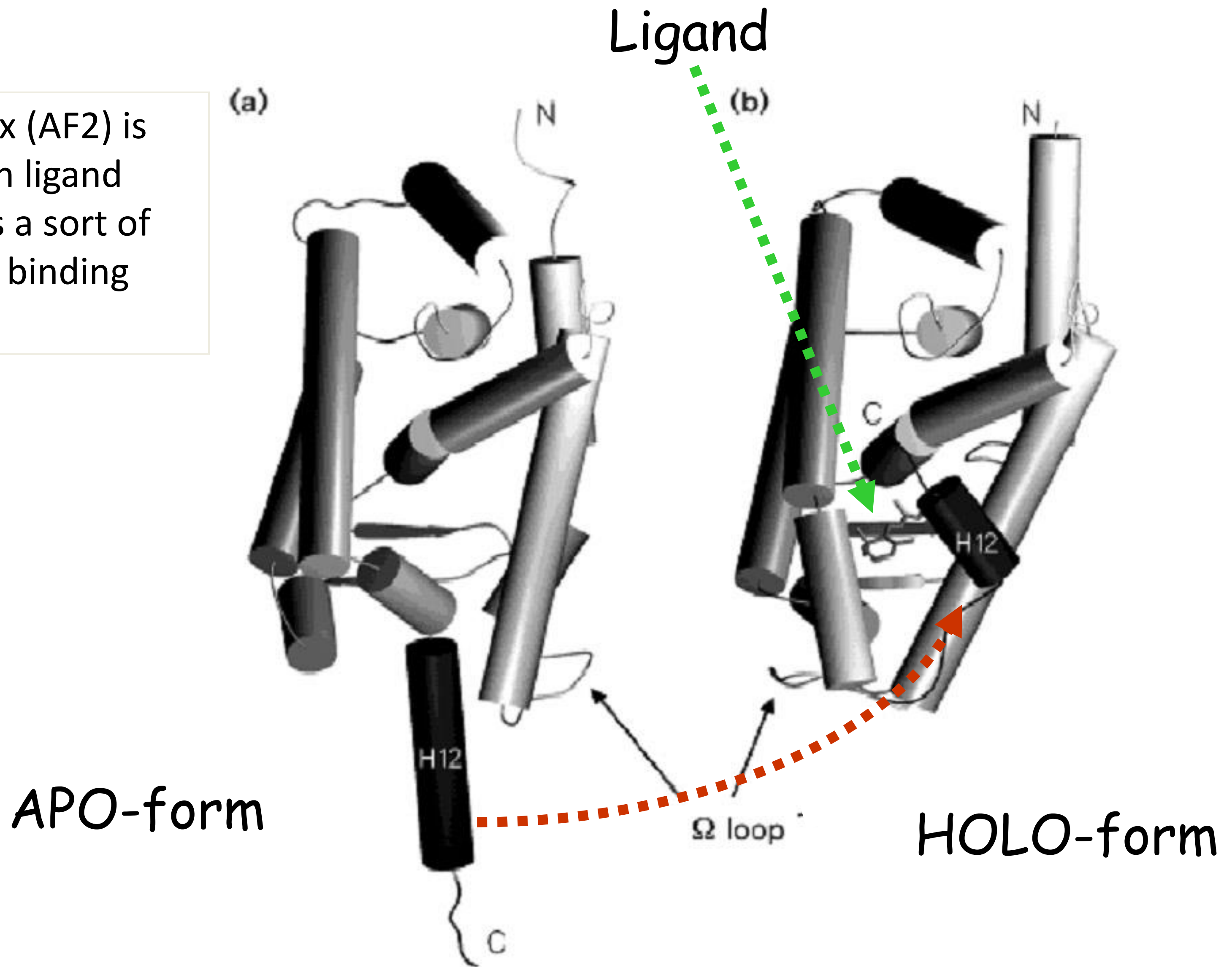
The most interaction of the Steroid Receptors with Co-Activators is mapped to the ligand-binding domain (LBD)



The Ligand-binding domain (LBD)

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

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

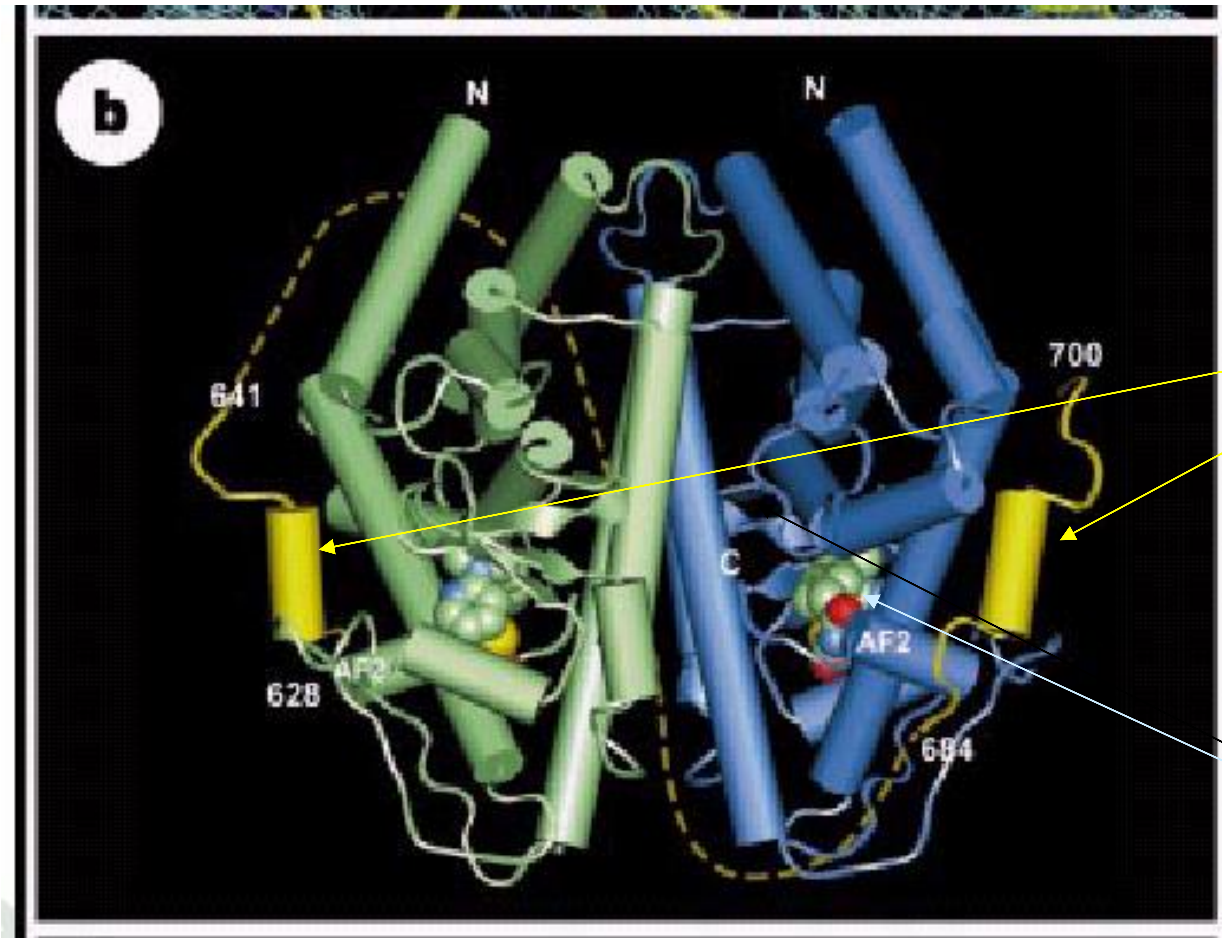


Interaction of co-activators with the LBD of NRs is mediated by a common motif “**LXXLL**”

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

-LXXLL-

The PPARγ LBD complexed with SRC1 helix and rosiglitazone



LXXLL helix from the SRC1 coactivator

rosiglitazone

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

Residence time

The pictures emerging from these schemes are quite static, since developmental times are long
(same for reprogrammed cells in culture).

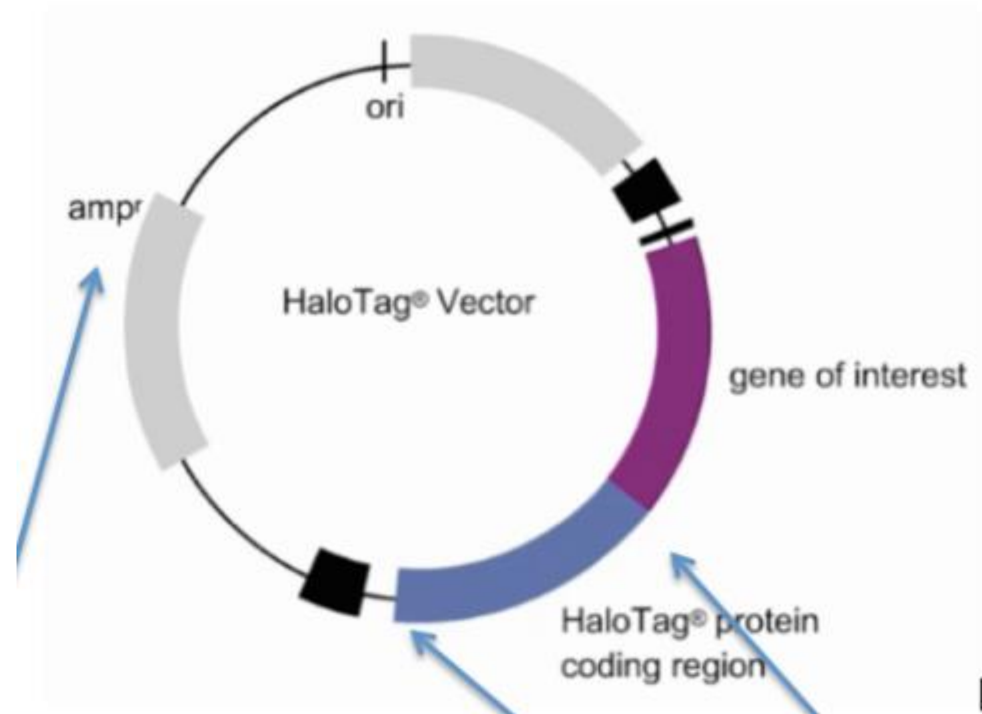
Do Transcription Factors reside at enhancers for such long times ?

Halo-TAG-realtime movies of single nuclei of ER, GR, p53 and other SDTFs demonstrated very short residence times

Is residence time of Pioneer Factors really longer than Signal-dependent TFs ?

Since LDTFs or Pioneer Factors are supposed to «keep alive» the enhancer programmes, the hypothesis is that these factors could be relatively stable on chromatin.
However …….

In vivo studies on single molecule can be performed using the Halo-Tag method

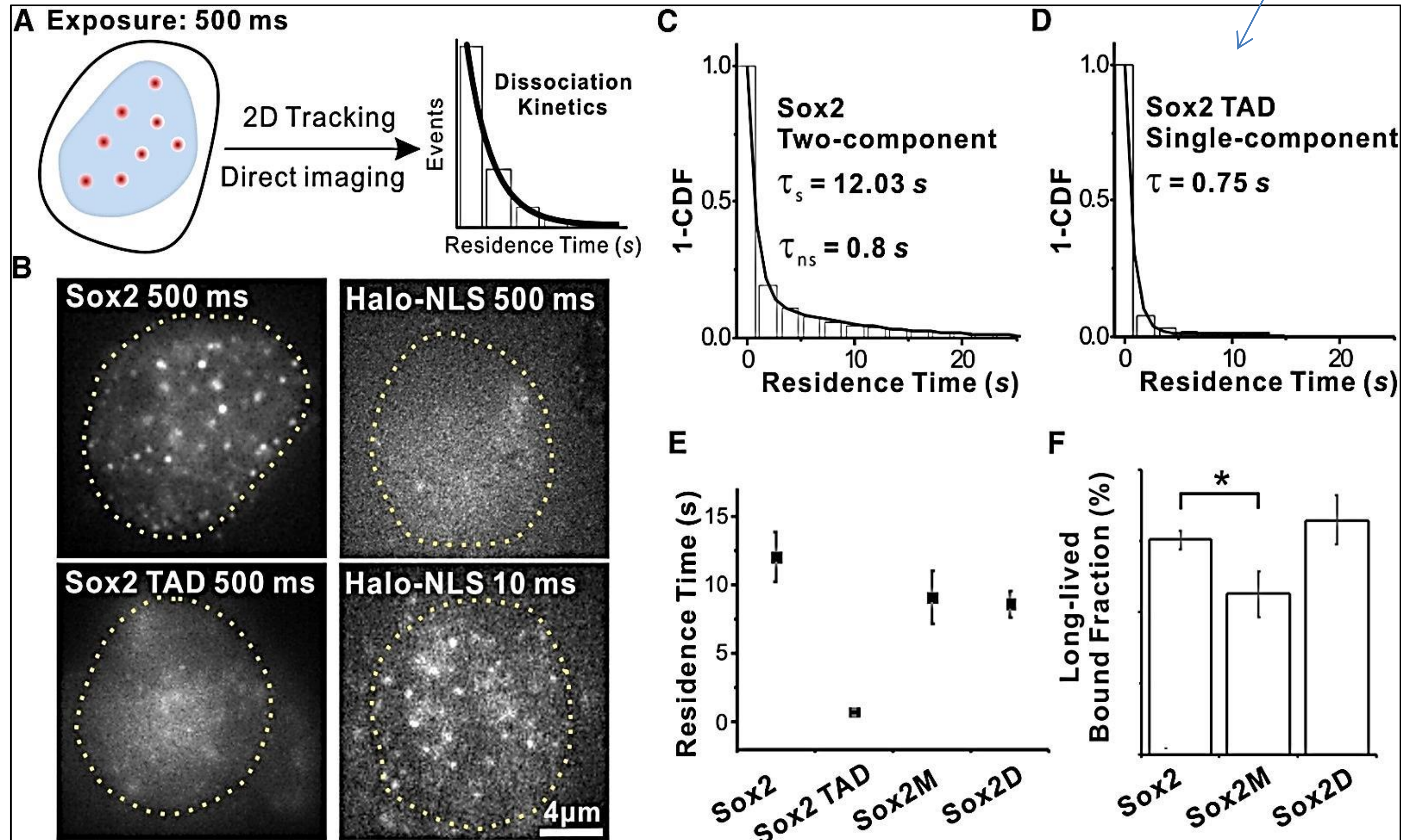


Halo-tag is a 297 residue peptide (33 kDa) derived from a bacterial enzyme. It binds to, and covalently attached to, a chloroalkane linker. Chloroalkane-fluorescent molecules are available. In this way, once the recombinant portein is expressed in cells, addition of the chloroalkane-fluorescent probe will result in covalently labelled Halo Tag.
Halo-Tag is fused to any protein of interest using a plasmid like this one.

Halo-Tag-Sox2

Dynamic single-molecule imaging

Transcriptional Activating Domain only



Chen et al., Cell (2014), 156:1254

Please cite this article in press as: Swinstead et al., Steroid Receptors Reprogram FoxA1 Occupancy through Dynamic Chromatin Transitions, Cell (2016), http://dx.doi.org/10.1016/j.cell.2016.02.067

Article

Cell

Steroid Receptors Reprogram FoxA1 Occupancy through Dynamic Chromatin Transitions

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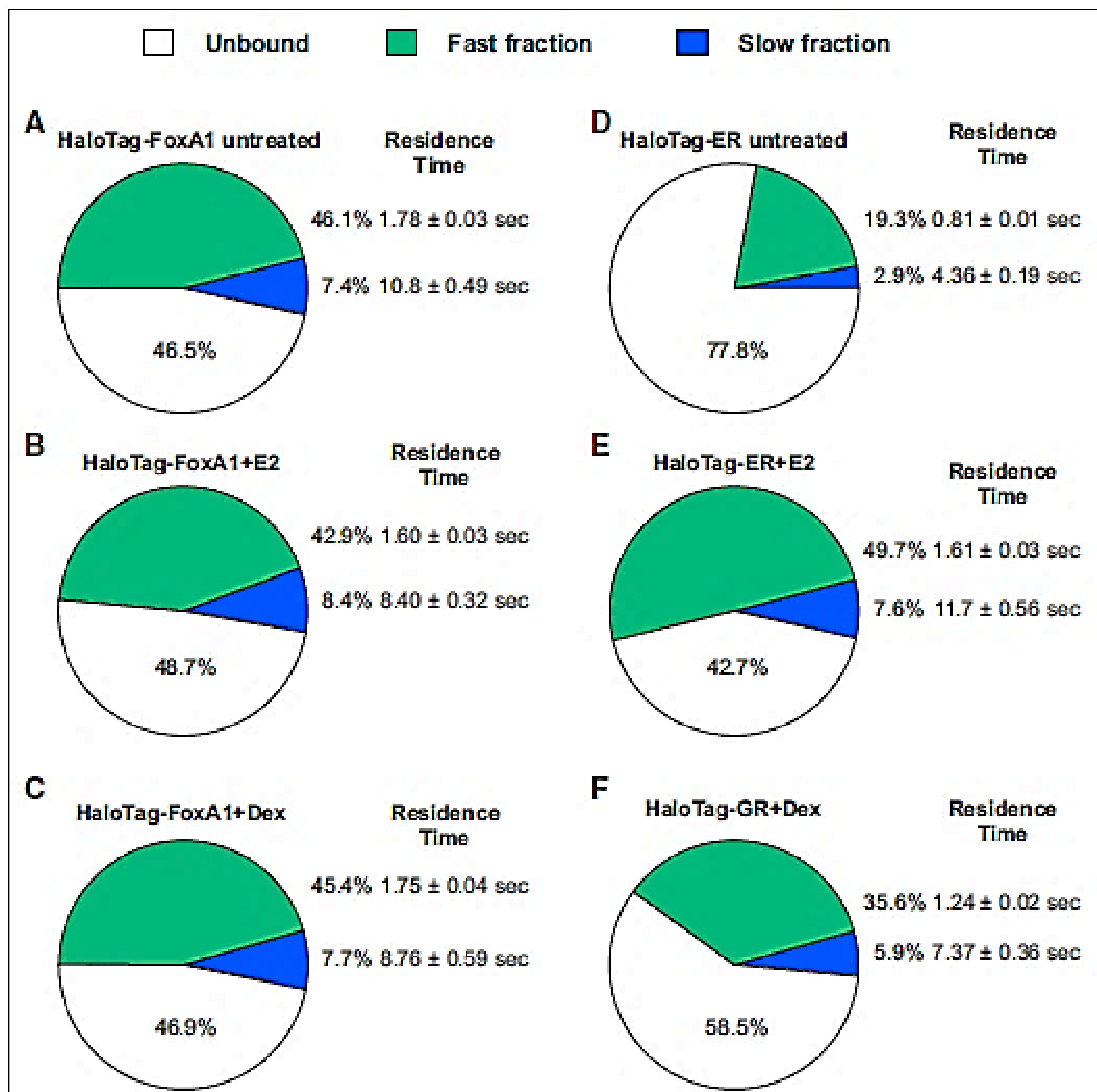
The estrogen receptor (ER), glucocorticoid receptor (GR), and forkhead box protein 1 (FoxA1) are significant factors in breast cancer progression. FoxA1 has been implicated in establishing ER-binding patterns though its unique ability to serve as a pioneer factor. However, the molecular interplay between 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.

Times of residence of the Pioneer Factor FoxA1 are also very short.

Movie 2



Conclusion of these studies is that the residence time of TFs, either pioneer factor or Signal-dependent TFs, are very short.
In addition, whenever two (or more) components are tested, this results in increased number of foci and slightly increased residence times.

However, since residence times observed are always in the order of seconds, the model devises a **highly dynamic** interplay between TFs, cofactors, coregulators and chromatin, in order to keep enhancers in the active status.

Enhancer activation.

TF binding and H3K4me1/me2 are required but not sufficient

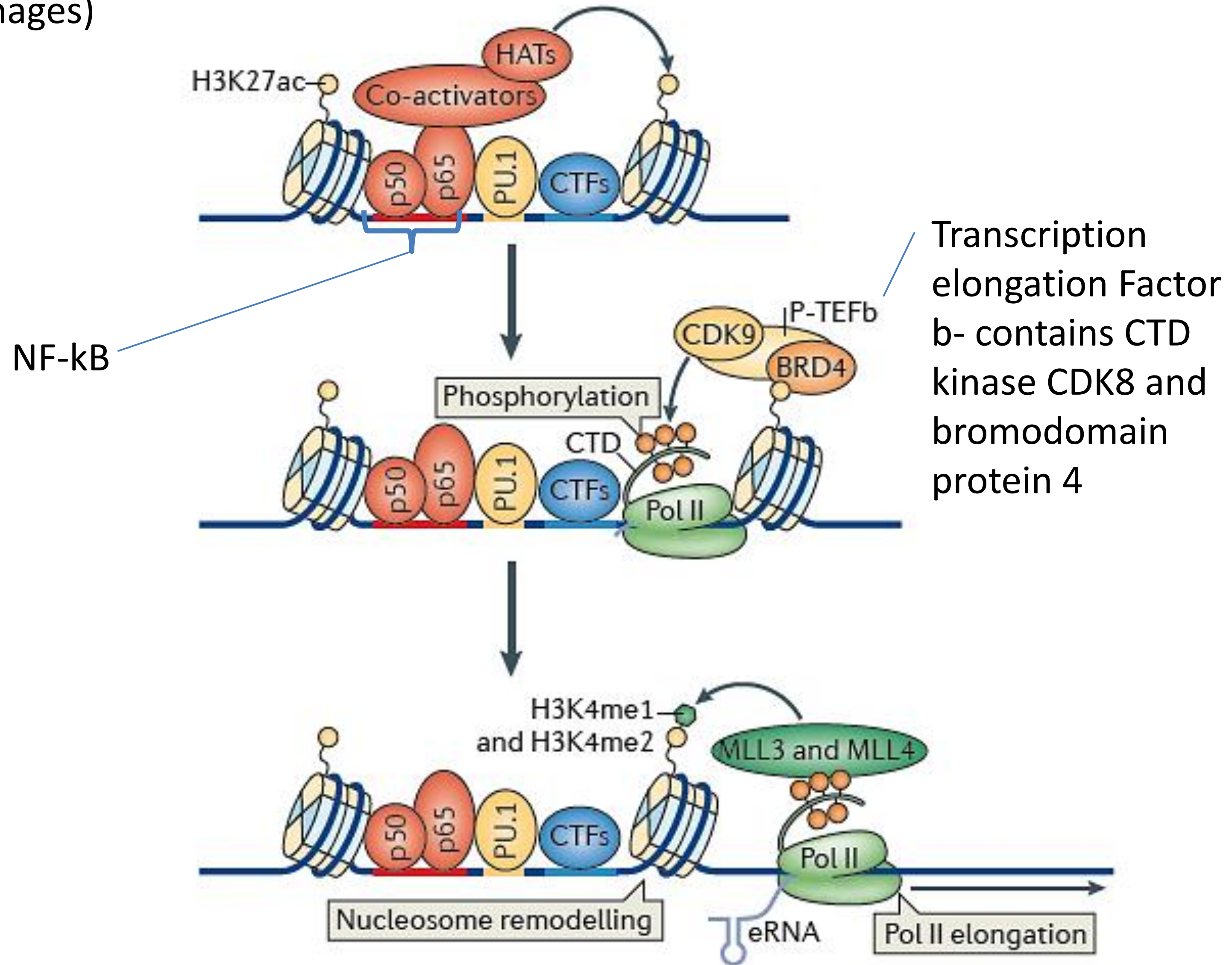
No activity if they do not show e.g. H3K27ac.

There are several components, defined **co-regulators** (co-activator and co-repressor), that are recruited by TFs, and whose activity is necessary to make an active Enhancer.

Among the others, the HAT enzyme p300/CBP, that binds to several TFs including both LDTFs, SDTFs and CTFs, is considered an essential mark for enhancer activity.

Among others, the Mediator, BRG1, MLL, BRD4 are also found at active enhancers

Activation of eRNA transcription
(example in macrophages)



Heinz et al., 2015

Enhancer transcription

Production of unstable short trancripts in both directions from the Enhancer (called eRNA) is considered today as an essential mark of enhancer activity.

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ARTICLES

Widespread transcription at neuronal activity-regulated enhancers

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We used genome-wide sequencing methods to study stimulus-dependent enhancer function in mouse cortical neurons. We identified ~12,000 neuronal activity-regulated enhancers that are bound by the general transcriptional co-activator CBP in an activity-dependent manner. A function of CBP at enhancers may be to recruit RNA polymerase II (RNAPII), as we also observed activity-regulated RNAPII binding to thousands of enhancers. Notably, RNAPII at enhancers transcribes bi-directionally a novel class of enhancer RNAs (eRNAs) within enhancer domains defined by the presence of histone H3 monomethylated at lysine 4. The level of eRNA expression at neuronal enhancers positively correlates with the level of messenger RNA synthesis at nearby genes, suggesting that eRNA synthesis occurs specifically at enhancers that are actively engaged in promoting mRNA synthesis. These findings reveal that a widespread mechanism of enhancer activation involves RNAPII binding and eRNA synthesis.

In the nervous system, hundreds of genes are induced in response to sensory experience-dependent neuronal activation.

Exposure of **primary neuronal cultures** to an elevated level of potassium chloride (KCl) leads to membrane depolarization and an influx of calcium through L-type voltage-sensitive calcium channels.

The resulting increase in intracellular calcium level then triggers several calcium dependent signalling pathways that ultimately lead to changes in gene expression.

We used this in vitro neuronal culture system to characterize neuronal activity-regulated enhancers

- **Enhancers identified using ChIP-Seq with CBP antibodies**
- **RNA Pol II colocalizes with several enhancers (ChIP-Seq)**

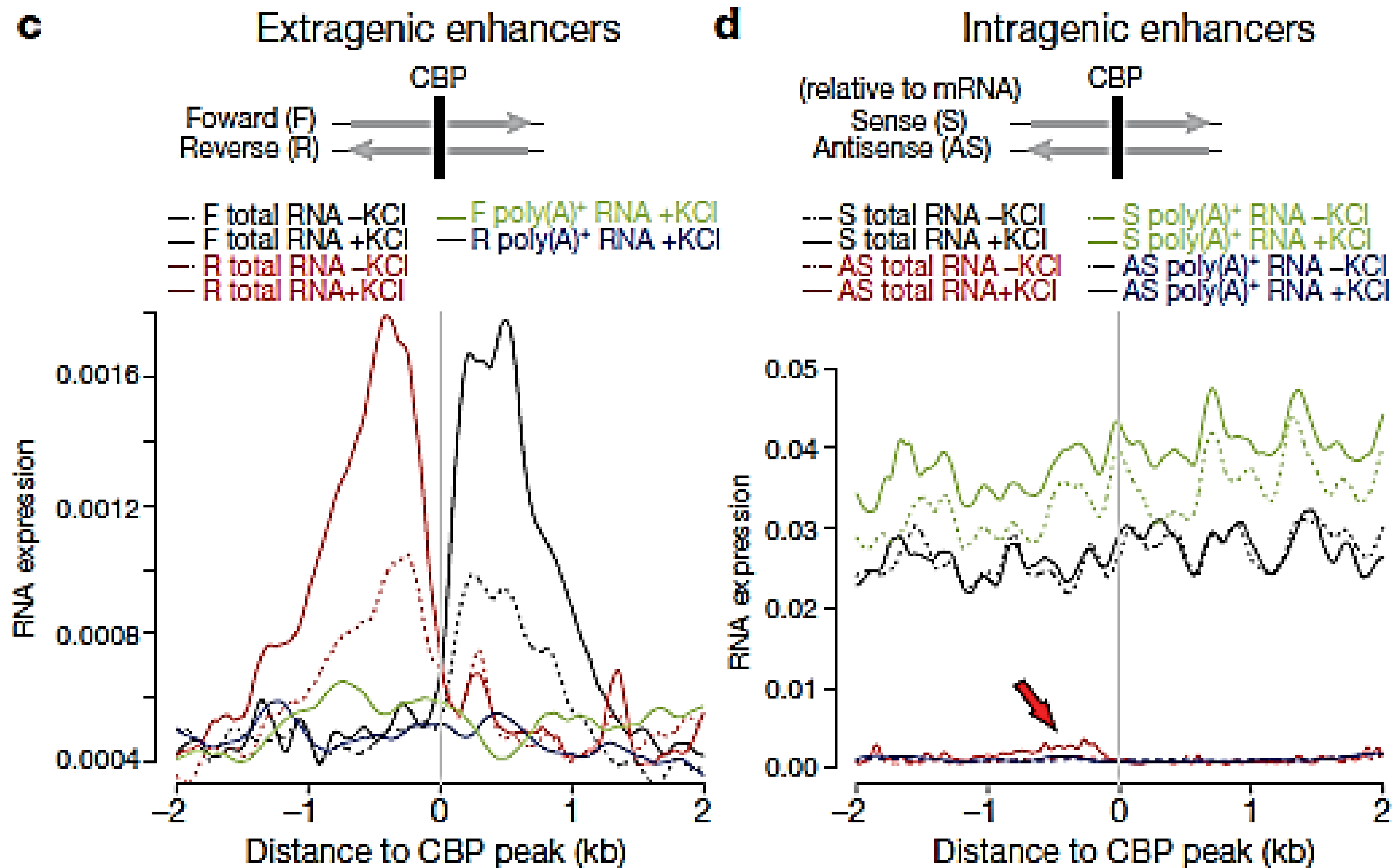

Figure 4 | Enhancers bind RNA polymerase II (RNAPII) and produce eRNAs.
In c, F and R denote forward (1) and reverse (2) genomic strands.
In d, enhancers are aligned oriented relative to the gene in which they reside to allow for sense and antisense RNA-Seq reads to be shown separately. Although sense eRNAs cannot be detected due to overlapping mRNA transcription, the red arrow indicates a local increase in antisense RNA expression attributable to eRNAs. Note different scales on the y axis in c and d.

eRNA are induced after stimulation

eRNA are **quantitatively correlated** with enhancer-regulated mRNA

eRNA transcription confirmed by subsequent studies including ENCODE

(described in one of the 2012 ENCODE articles by Djebali et al., Nature 2012)

eRNA unstable

Difficult to map using common RNA-Seq technologies

«Nascent» RNA methods required (such as GRO-seq).

eRNA are unstable, long noncoding transcripts in both directions around enhancers

eRNA are mainly poly(A-)

eRNA transcription is increased in active enhancers, i.e. when the enhancer contacts a promoter and activates transcription

There is indication that knockdown of eRNA can influence enhancer function ?

LETTER

doi:10.1038/nature12210

Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation

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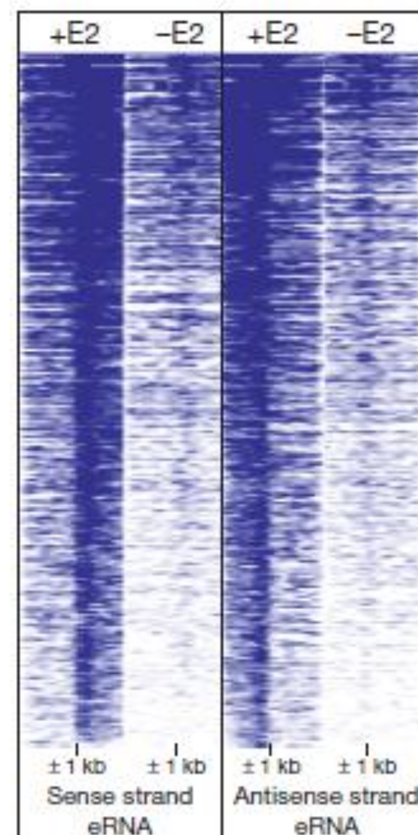
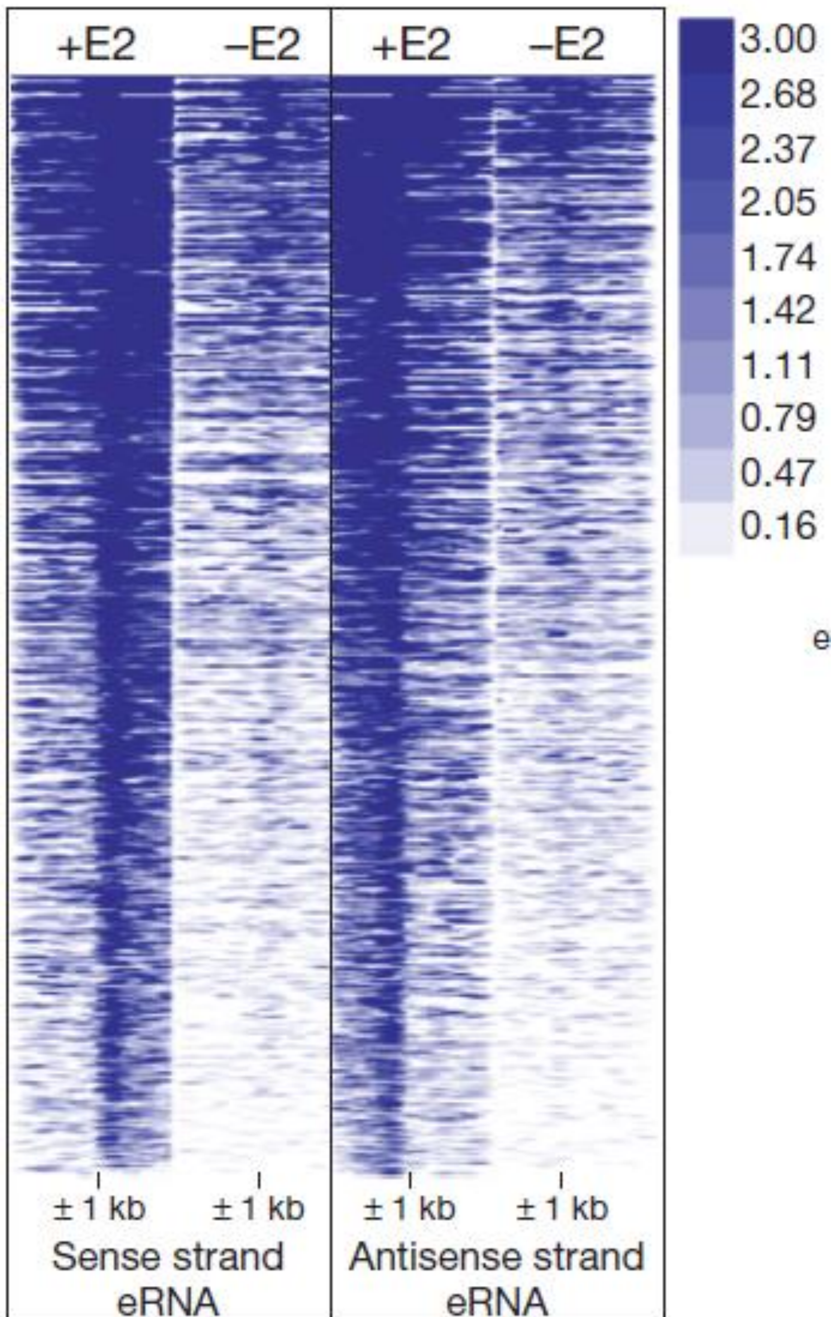
The functional importance of gene enhancers in regulated gene expression is well established[1–3]. In addition to widespread transcription of long non-coding RNAs (lncRNAs) in mammalian cells[4–6], bidirectional ncRNAs are transcribed on enhancers, and are thus referred to as enhancer RNAs (eRNAs)[7–9]. However, it has remained unclear whether these eRNAs are functional or merely a reflection of enhancer activation. Here we report that in human breast cancer cells 17β-oestradiol (E2)-bound oestrogen receptor α (ER-α) causes a global increase in eRNA transcription on enhancers adjacent to E2-upregulated coding genes. These induced eRNAs, as functional transcripts, seem to exert important roles for the observed ligand-dependent induction of target coding genes, increasing the strength of specific enhancer–promoter looping initiated by ER-α binding. Cohesin, present on many ER-α-regulated enhancers even before ligand treatment, apparently contributes to E2-dependent gene activation, at least in part by stabilizing E2/ER-α/eRNA-induced enhancer–promoter looping. Our data indicate that eRNAs are likely to have important functions in many regulated programs of gene transcription.





Strand-specific RNA-Seq

Variation of common RNA-Seq, called **GRO-Seq**

It pictures only RNAs that are being transcribed

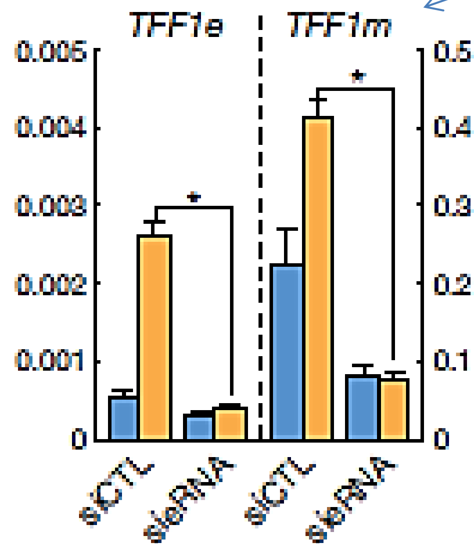
In this representation, each line is an enhancer, and the density of reads is depicted in the -1Kb to +1Kb interval, using a scale of blue color.
The lines are then ordered from the most transcribed to the less transcribed

Do eRNAs have any functional role ?

One approach is to knock them down and see if transcription of the connected genes is affected

eRNA at enhancer

mRNA



eRNA knock-out using siRNA/LNA abrogates the enhancer effect on transcription

LNA= Locked Nucleic Acid

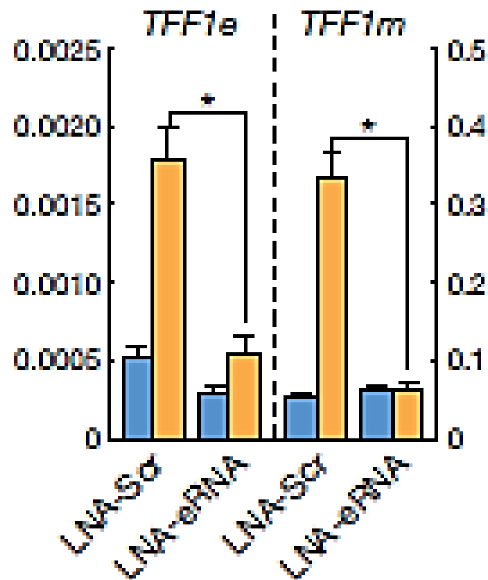


Figure 2 | Importance of eRNA for target gene activation. a, b, siRNA/LNA knockdown of eRNAs. Efficacy and effects on coding gene transcription were assessed by qPCR for the TFF1, FOXC1 and CA12 eRNAs and corresponding coding transcription units. Lower case 'e' and 'm' after gene names denote eRNA and gene mRNA, respectively. CTL, control; Scr, scramble. (Li et al., 2013)