Ch 3 – L 4.1

Developmental and signal-dependent specification of Enhancers

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.

ENCODE conference talk (2015): video in Moodle Ch3 book, L4 (*https://cmb.i-learn.unito.it/mod/book/view.php?id=12747&chapterid=625*)

Agenda:

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

NUMBERS

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

Making calculations on sequencing depth, number of cell types/tissues and developmental stages examined, some Authors estimate this number increase up to **one million** estimated.

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

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

Accentuated **cell-type specificity**. This goes in close parallel with gene expression, which we know to be not only cell/tissue specific, but also to be widely regulated in different contexts, including pathological states, and by many signalling pathways.

Different functional states of Enhancers

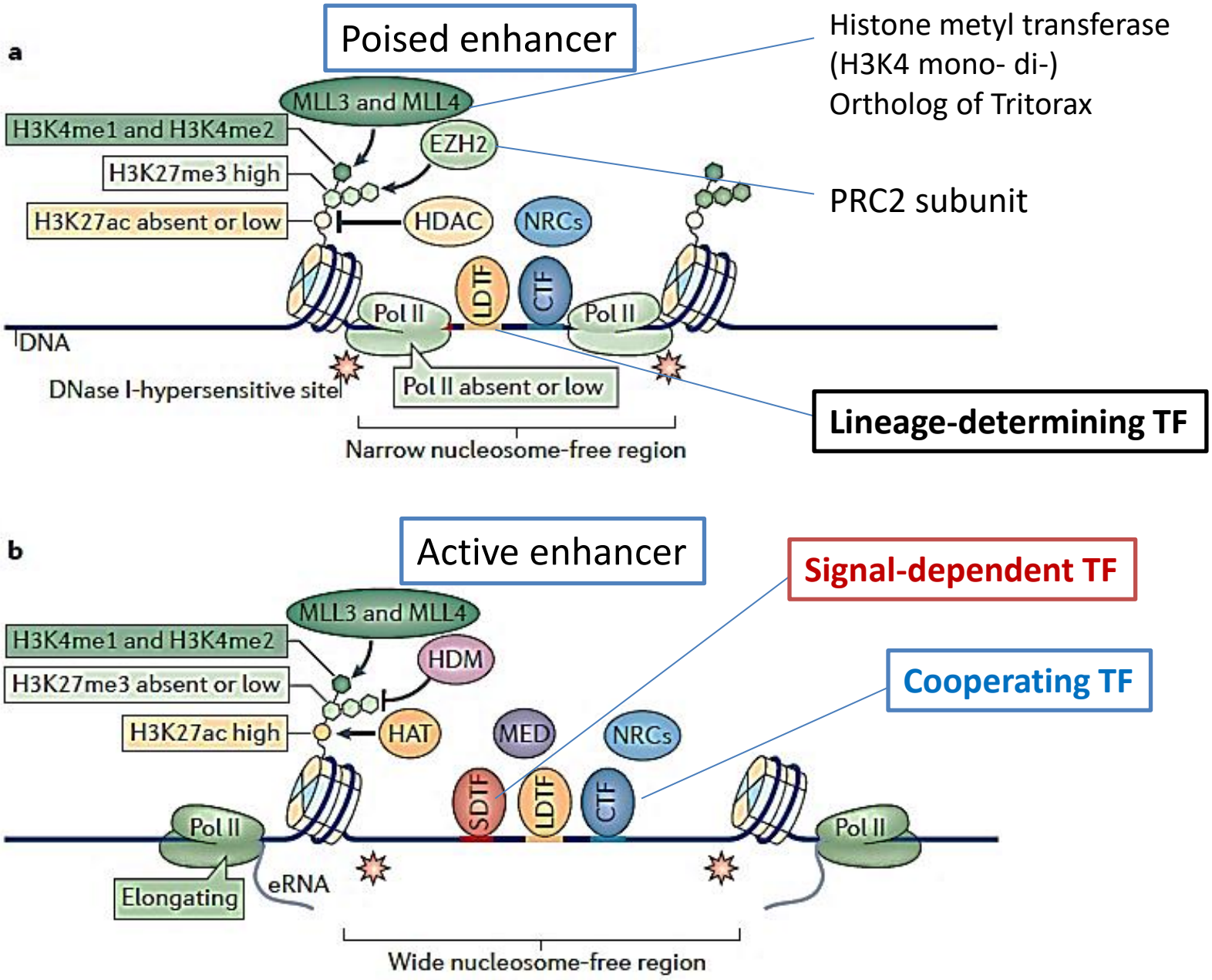
In a given cell type and at a specific developmental/functional time, potential enhancers can be:

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

some Author make one class of these

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



NRC Nucleosome Remodelling Complex

Heinz et al., 2015

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

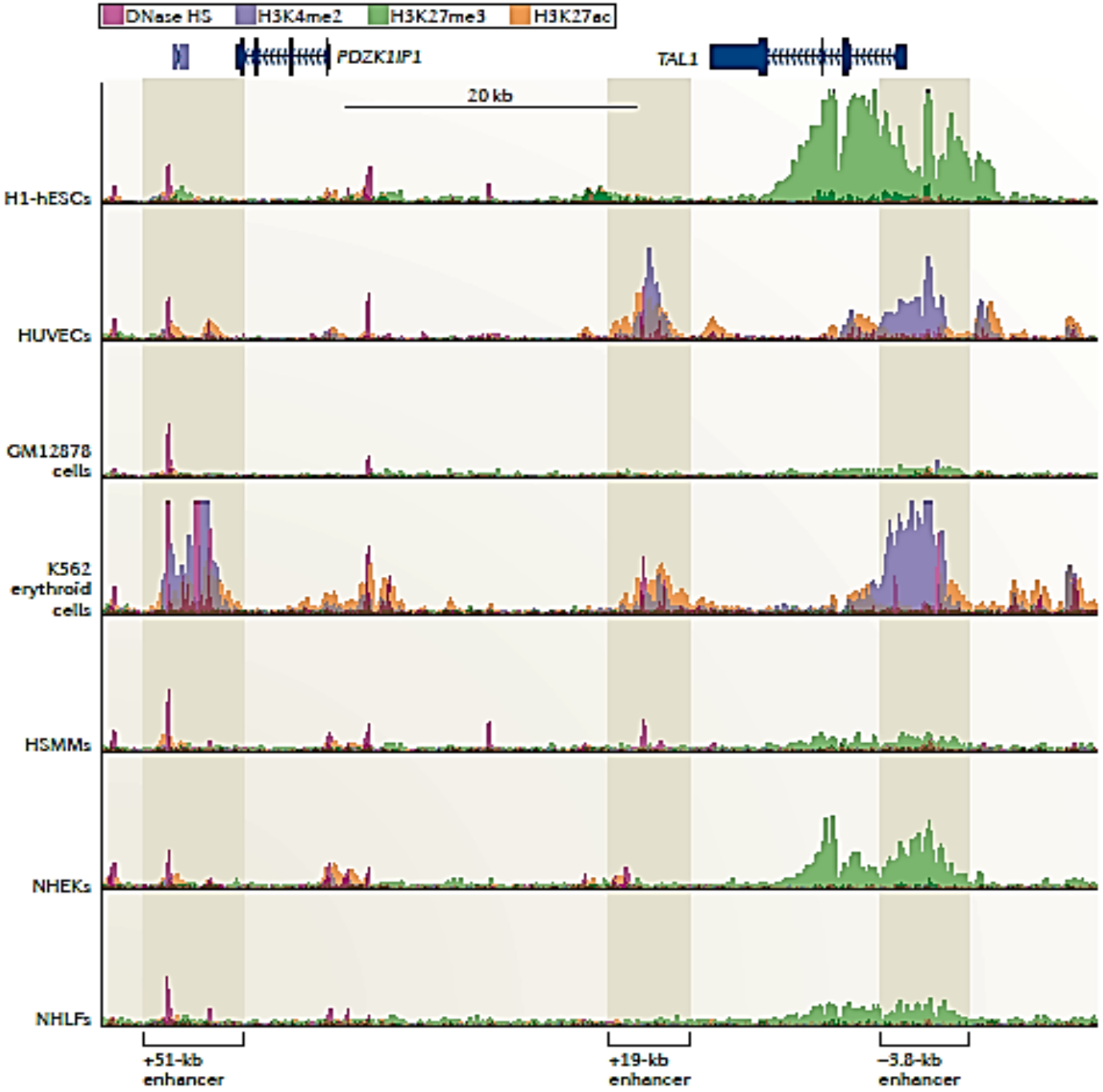
In other reviews you may find this classification:

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).

Heinz et al., 2015



umbilical vein endothelial

lymphoblastoid

erythroid

muscle myoblasts

epidermal keratinocytes

lung fibroblasts

How is Enhancer state developmentally determined ?

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

Alex S. Nord,[1] Matthew J. Blow,[1,2] Catia Attanasio,[1] Jennifer A. Akiyama,[1] Amy Holt,[1] Roya Hosseini,[1] Sengthavy Phouanenavong,[1] Ingrid Plajzer-Frick,[1] Malak Shoukry,[1] Veena Afzal,[1] John L.R. Rubenstein,[1] Edward M. Rubin,[1,2] Len A. Pennacchio,[1,2,*] and Axel Visel[1,2,4,*]

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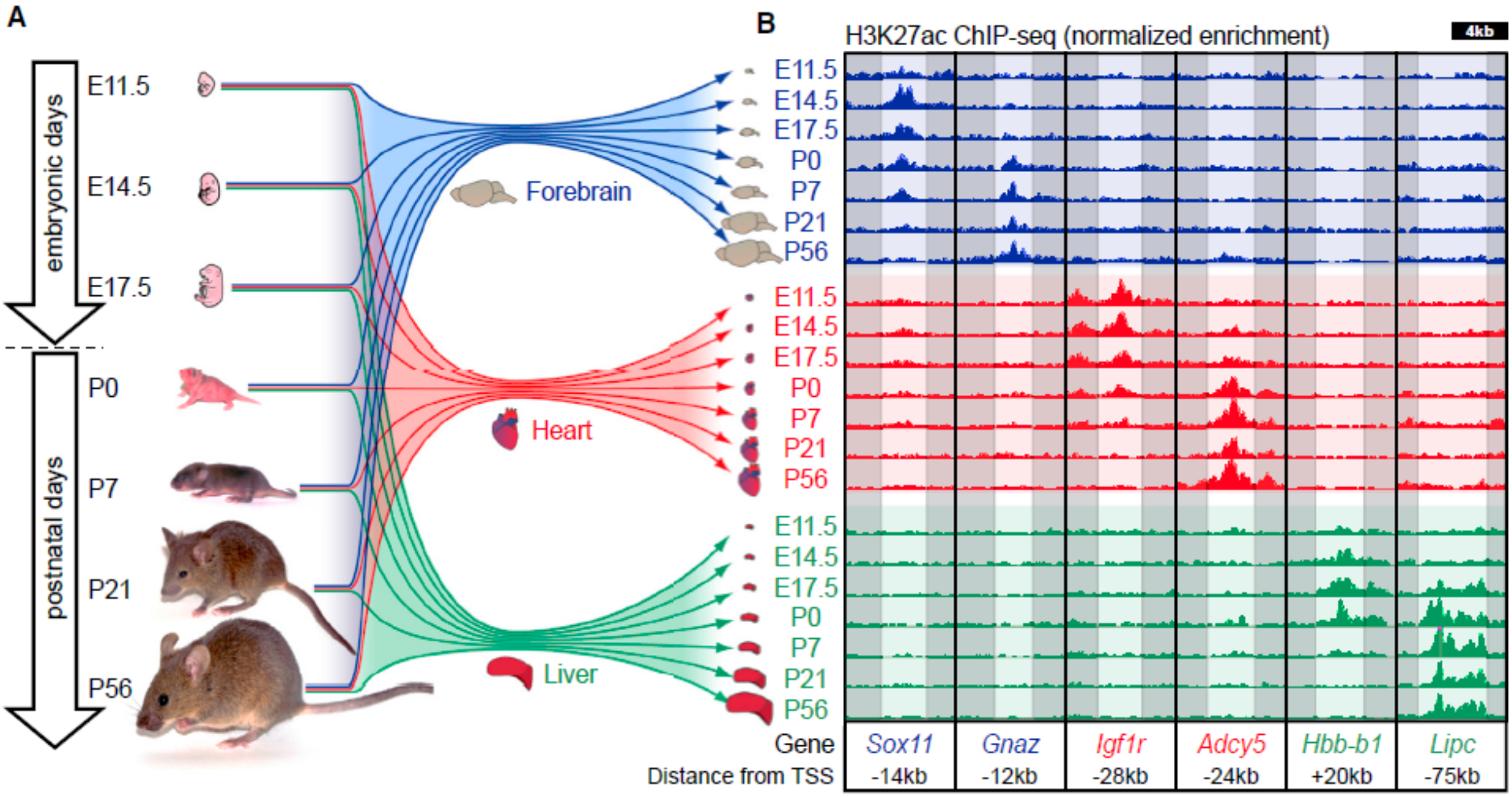
Enhancers are distal regulatory elements that can activate tissue-specific gene expression and are abundant throughout mammalian genomes. Although substantial progress has been made toward genome-wide annotation of mammalian enhancers, their temporal activity patterns and global contributions in the 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.

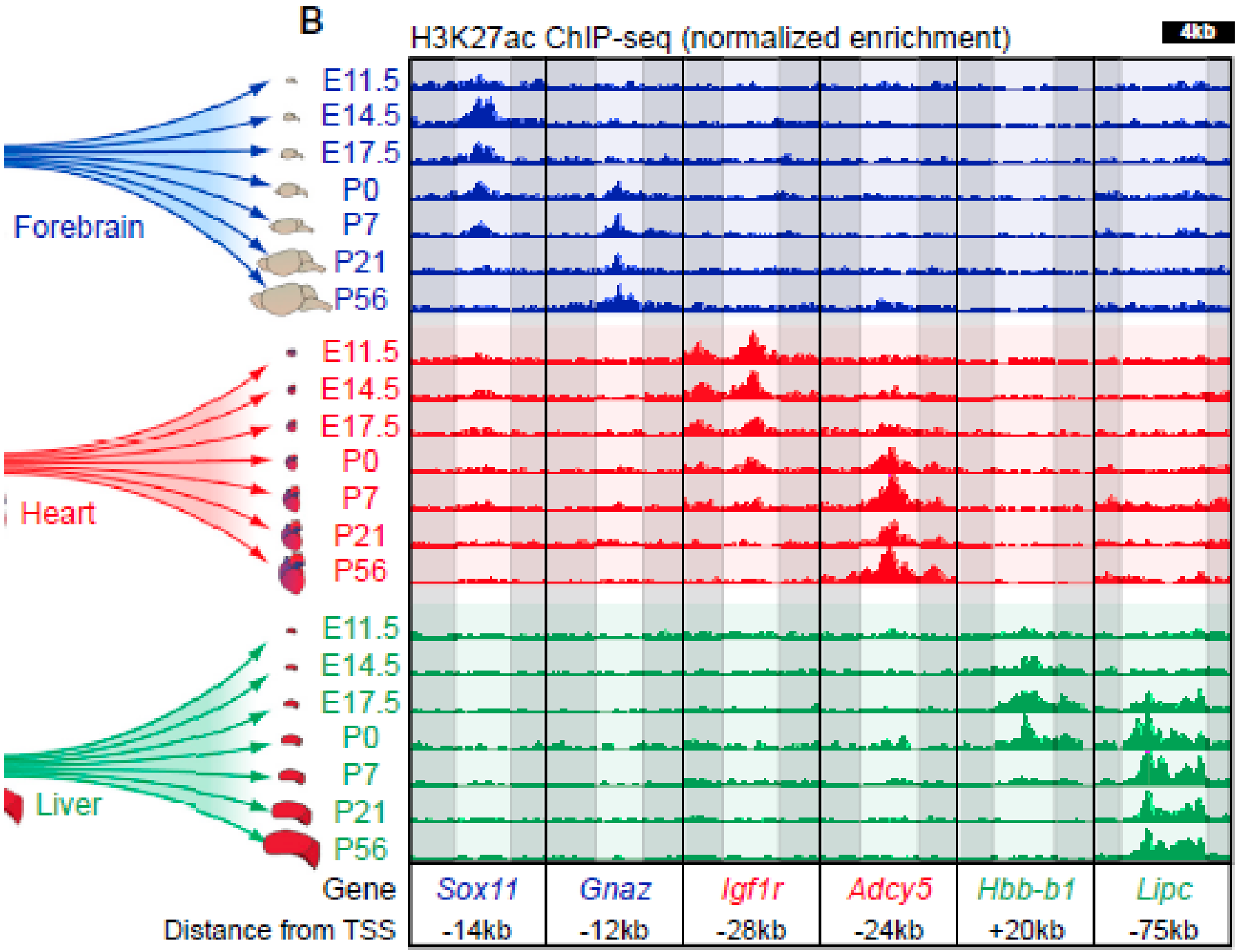
Another way is to examine enhancer dynamics through development

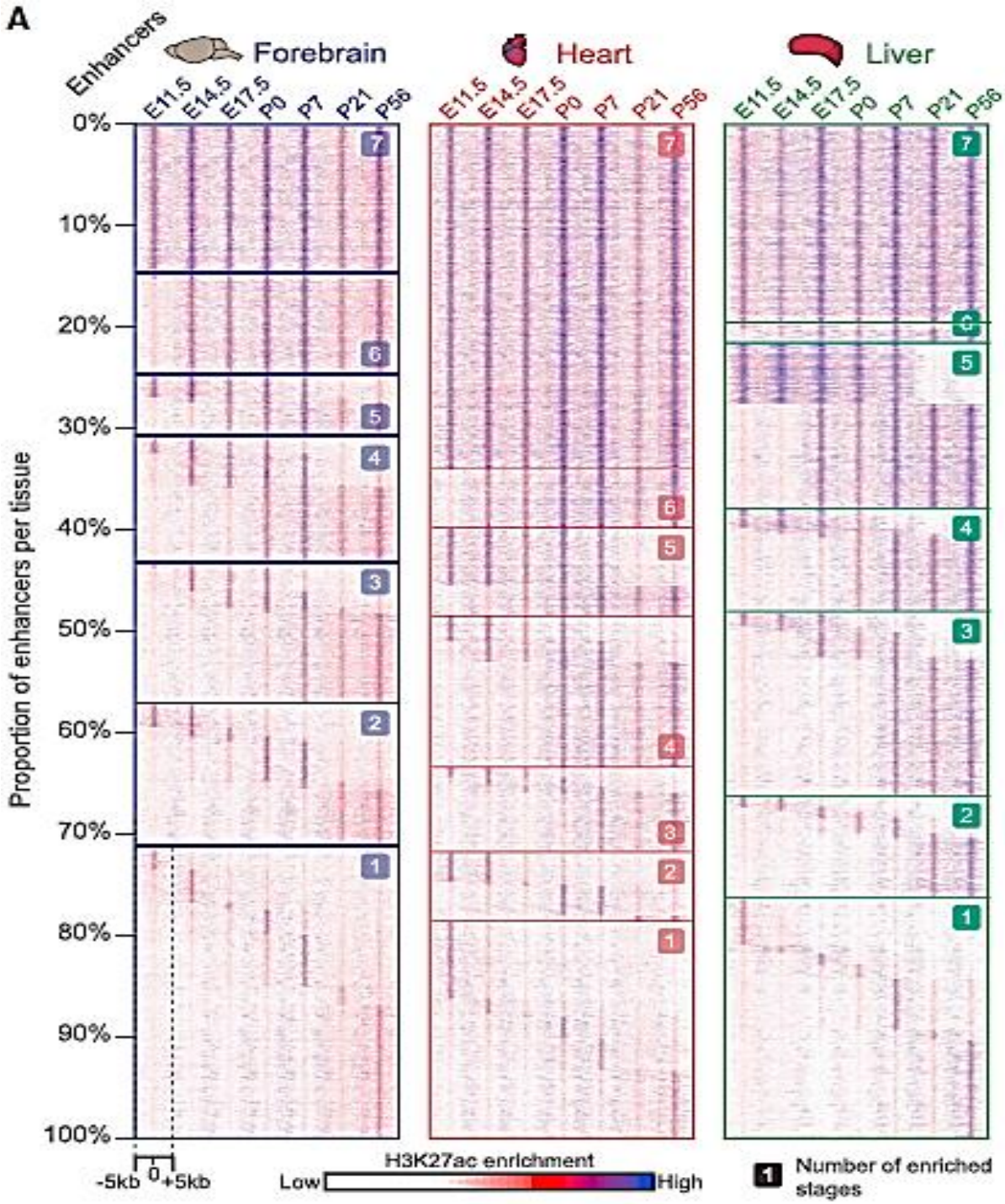
Cell *155*, 1521–1531, December 19, 2013 ©2013 Elsevier Inc. 1521



(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
E11.5
E14.5
E17.5
P0
P7
P21
P56
Heart
E11.5
E14.5
E17.5
P0
P7
P21
P56
Liver
E11.5
E14.5
E17.5
P0
P7
P21
P56
Gene
Sox11
Gnaz
Igf1r
Adcy5
Hbb-b1
Lipc
Distance from TSS
-14kb
-12kb
-28kb
-24kb
+20kb
-75kb

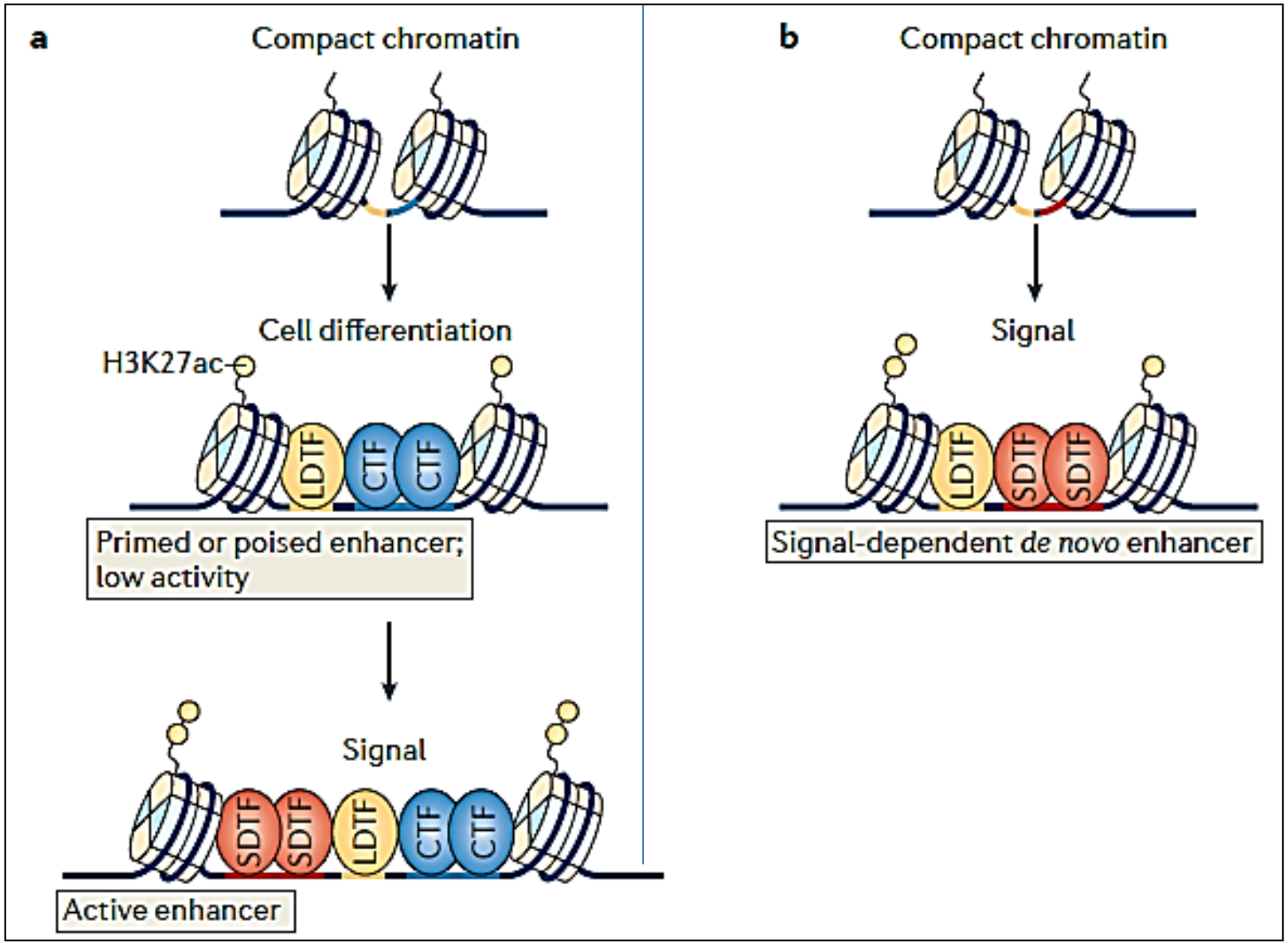

A
Enhancers
Forebrain
Heart
Liver
E11.5
E14.5
E17.5
P0
P7
P21
P56
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Proportion of enhancers per tissue
7
6
5
4
3
2
1
-5kb
0
+5kb
H3K27ac enrichment
Low
High
Number of enriched stages

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.

«**Pioneer Factors**»
Transcription Factors capable of interacting on DNA even when it is in heterochromatic or «bivalent» regions

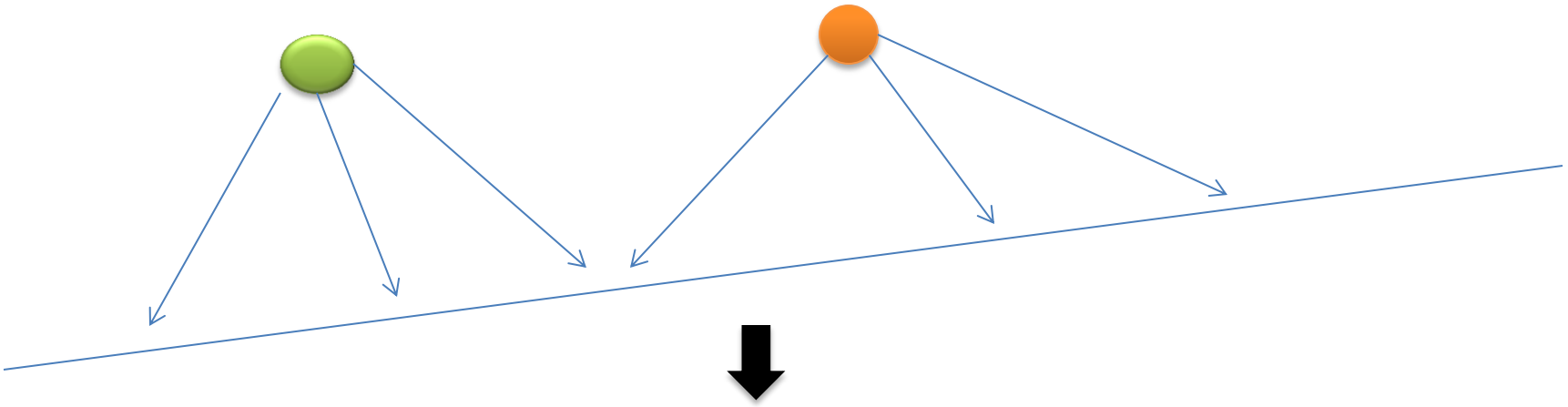
Pioneer Factor binding may be one of the first events to bring undetermined enhancers to the primed or poised status

Example: FoxA1 has been proposed to prime Enhancers responding to AR and ER in prostate and breast cancer, respectively.

Caveat: the recognition sequence is very short and degenerated: how do these factors recognize the correct sequences among thousands.

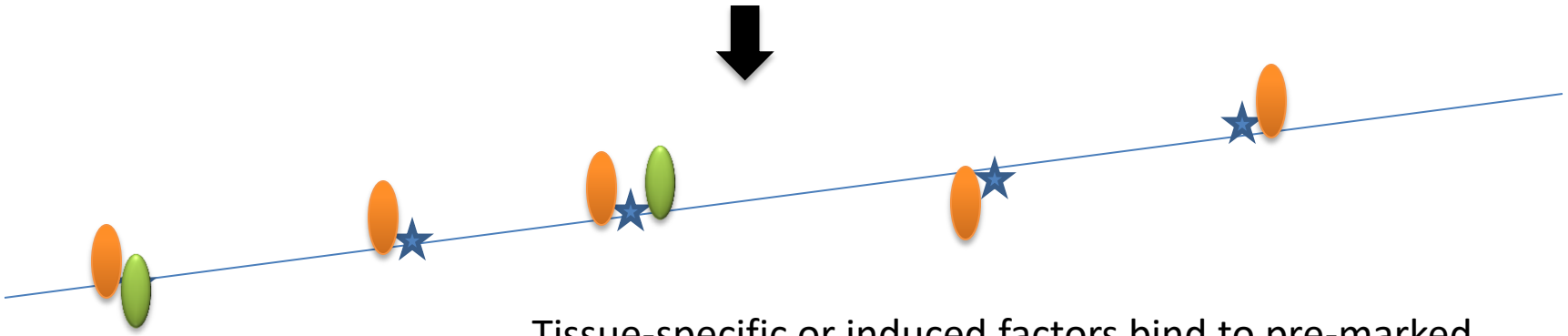
Dr Chris Glass says: Cooperative action with other factors

Primary factors «prime» enhancer sequences in differentiating cells

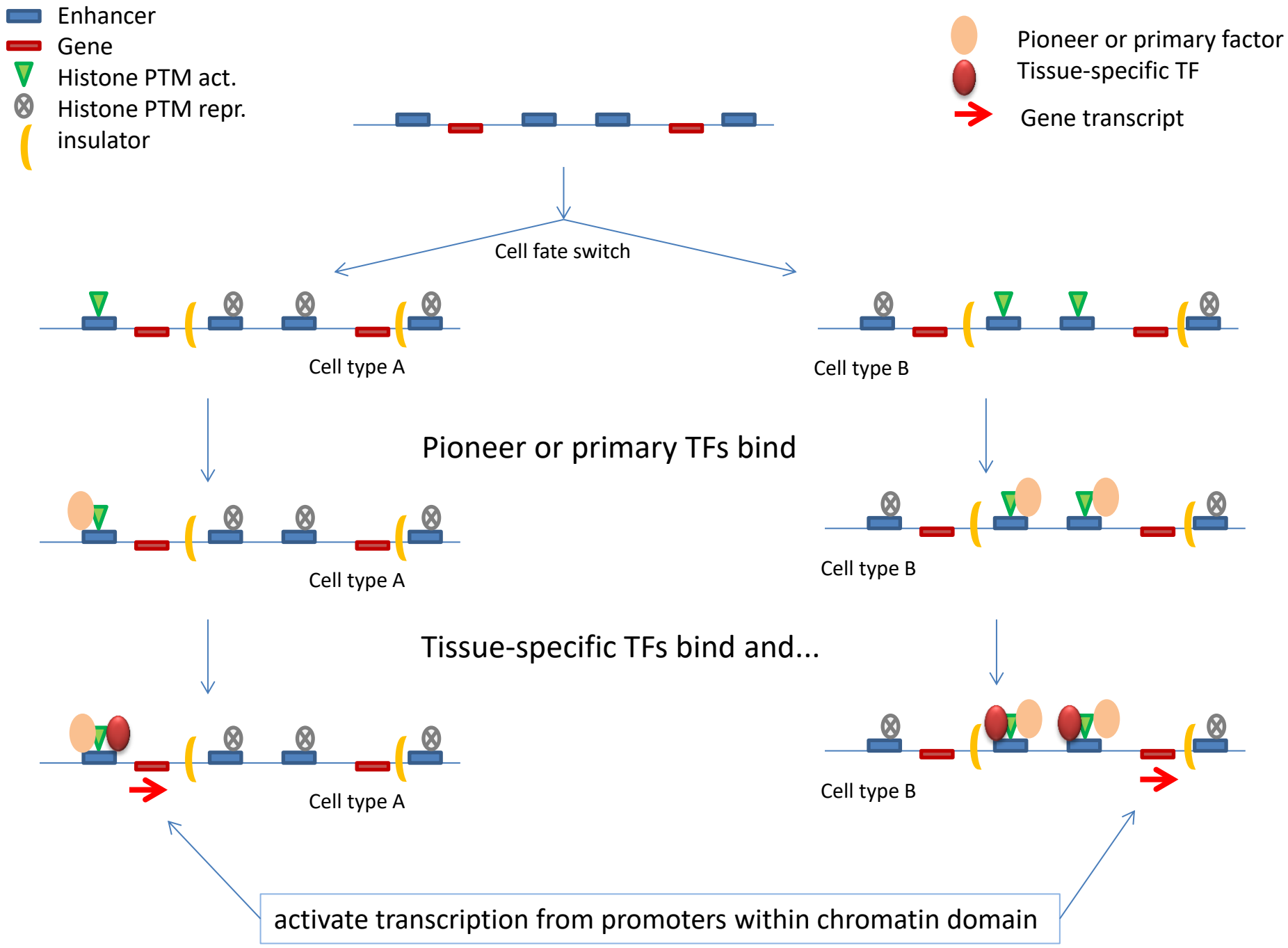


primary factor can stay bound or not

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


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 addition to the PU-1 example described by Dr. C Glass in macrophages and B-cells, other contexts have been well-described.

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.

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.