Ch 3 – L 4.2

Long-distance interaction and TADs

Does an Enhancer interact with any promoter at which distance ?

- interaction seems quite specific
- interaction is much more frequent within defined chromatin domains

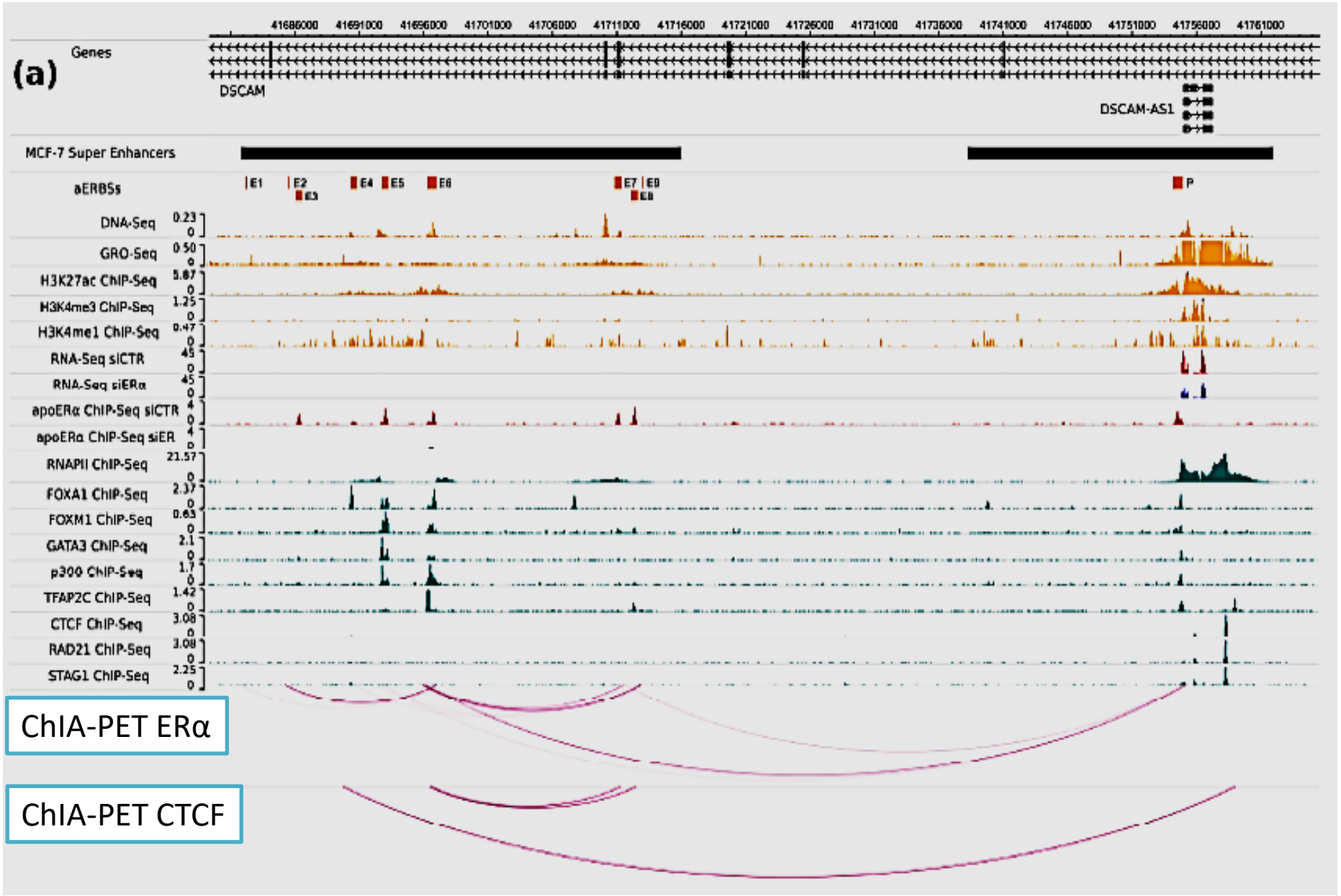
Exceptions are known, where E can even interact with a P placed on a different chromosome (e.g. the Olfactory receptor genes E and Ps)

- The fact that there is a decreasing frequency with the distance testifies that limitations exist for this kind of interactions

- Mapping of a number of insulator binding proteins, the most known of them being CTCF (CCCTC-binding Factor), has shown that te chromosomes are organized in domains.

- Within the domains, the frequency of long-range interaction is higher, and studies of PTMS have shown that often these domains are coherent as far as the status of chromatin is considered.

- Using the HiC methods, several laboratories have traced maps of domains in several cell types.



Miano V, Int J Mol Sci. 2018 Feb 16;19(2). pii: E593. doi: 10.3390/ijms19020593

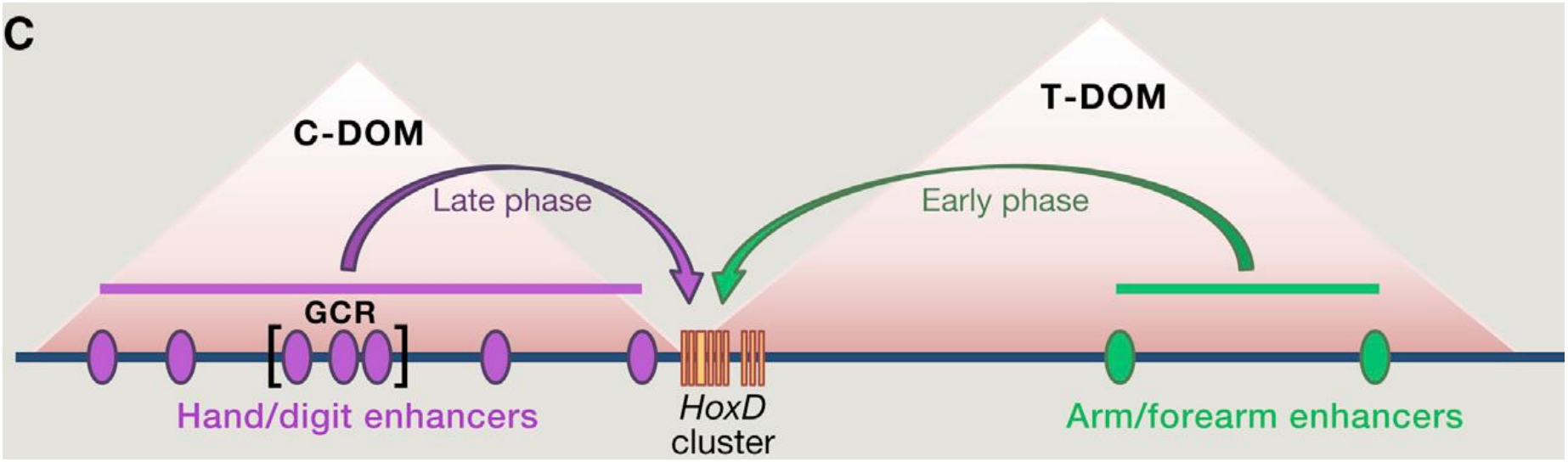
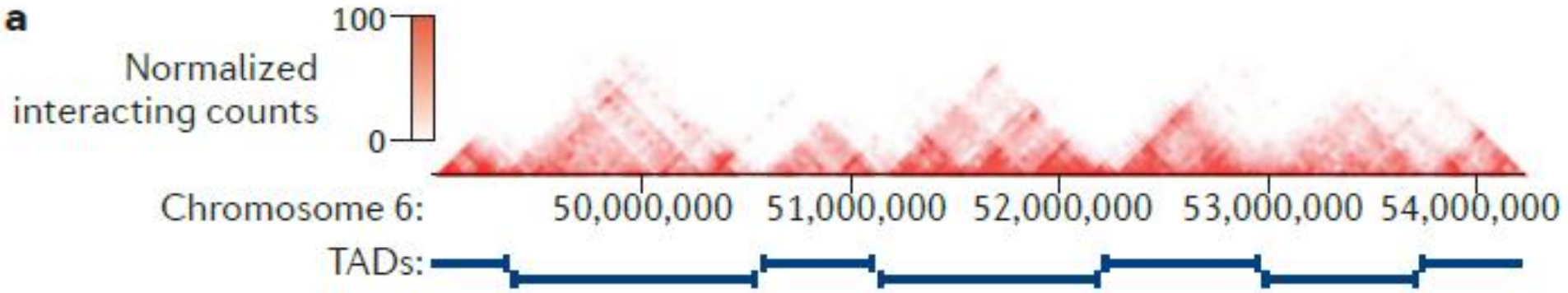


Figure 1. Organization of cis-Regulatory DNAs in Metazoan Genomes

(C) Organization of the Hoxd complex in mice.
The complex is regulated by a series of flanking enhancers (purple and green ovals) located in two neighboring TADs. The telomeric TAD (T-DOM) regulates linked Hoxd genes in the developing arm and forearm, whereas the centromeric TAD (C-DOM) regulates expression in the hand and the digits.

Levine et al., 2014

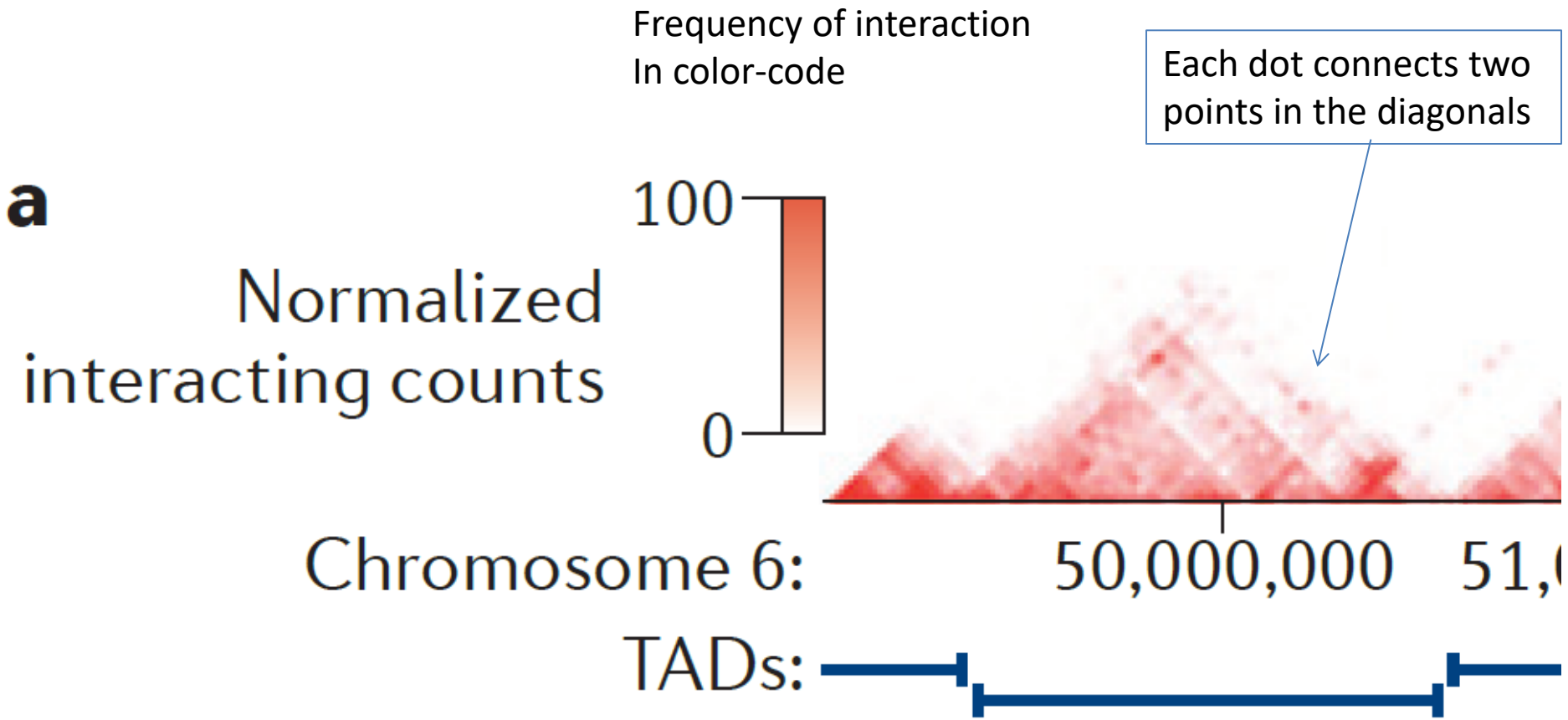
TADs are studied mainly using Hi-C



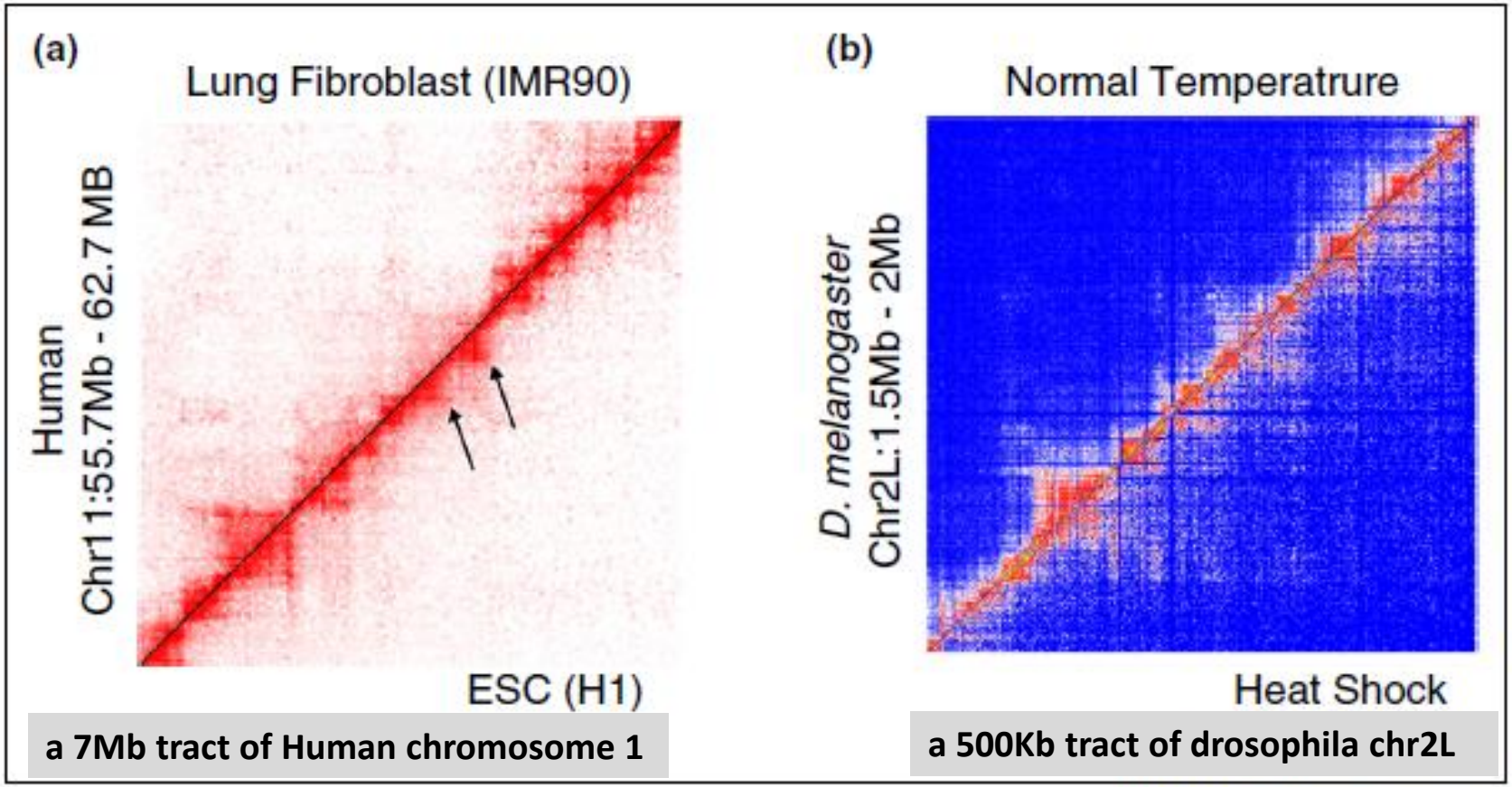
a | Hi-C profiles reveal that the mammalian genome is organized into **topologically associating domains (TADs)**: regions that show high levels of interaction within the region and little or no interaction with neighbouring regions. The heat map represents normalized Hi-C interaction frequencies.

Pombo & Dillon, 2015

Hi-C results are expressed as **interacting frequency**



Developmental changes in TADs organization



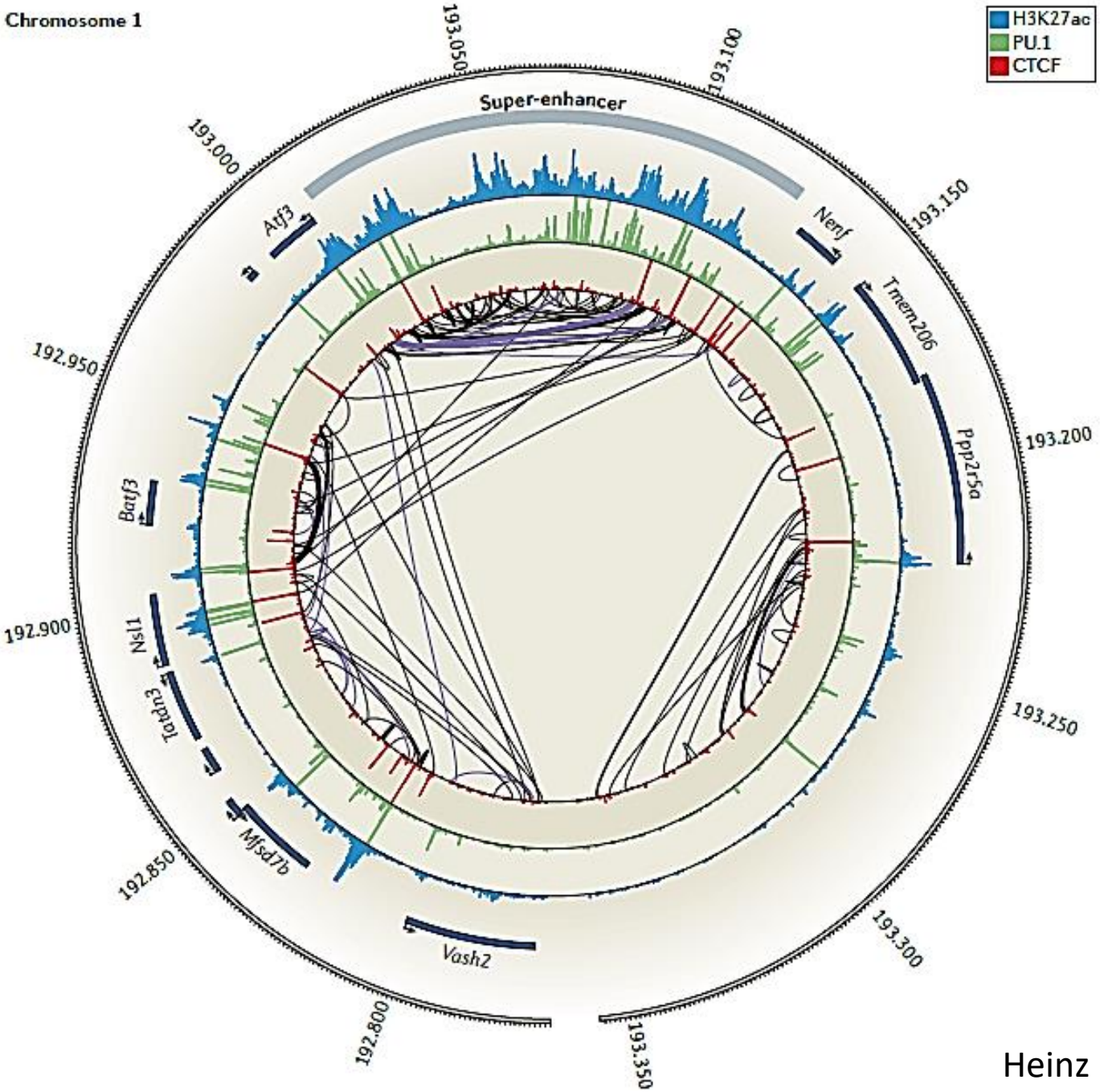
a 7Mb tract of Human chromosome 1

a 500Kb tract of drosophila chr2L

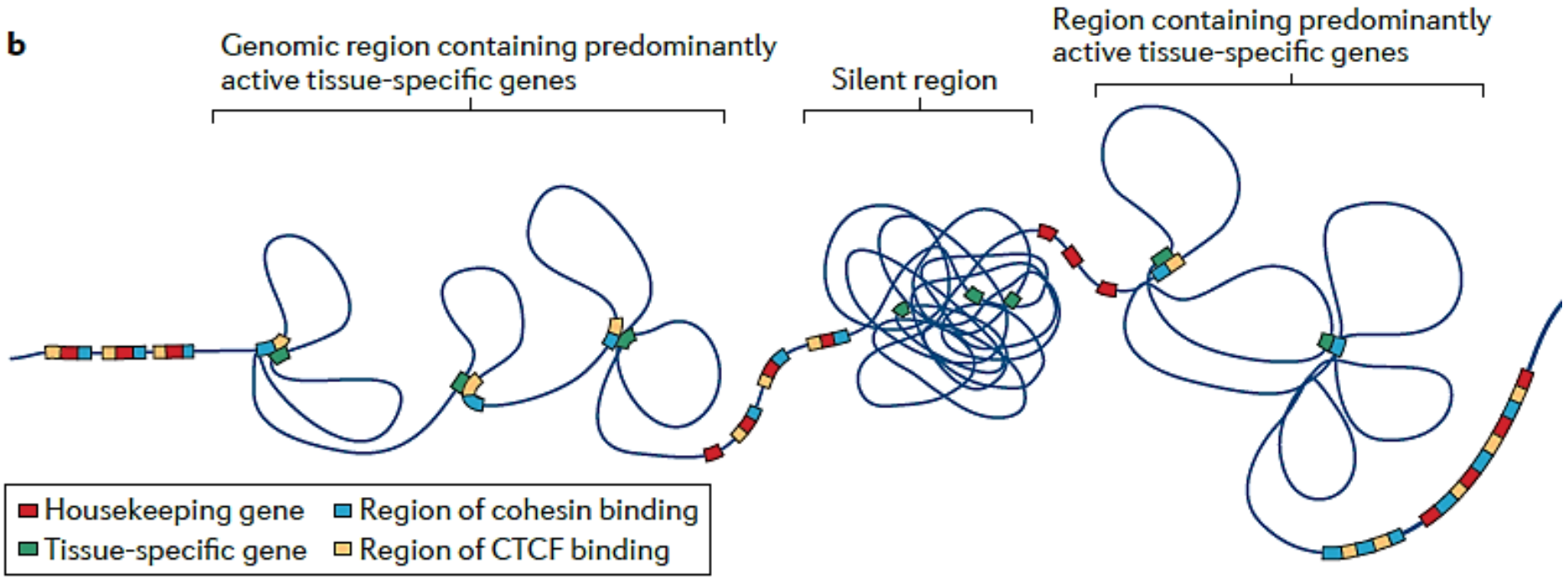
Changes to domain organization. (a) **Hi–C** of human embryonic stem cells (H1 ESC — bottom right) compared to lung fibroblast cells (IMR90 — top left) [47]. Arrows indicate TAD structure changes. (b) **Hi–C** of D. melanogaster under heat shock (bottom right) compared to normal temperature (top left)

from Rowley & Corces, 2016

TAD



Heinz et al. 2015



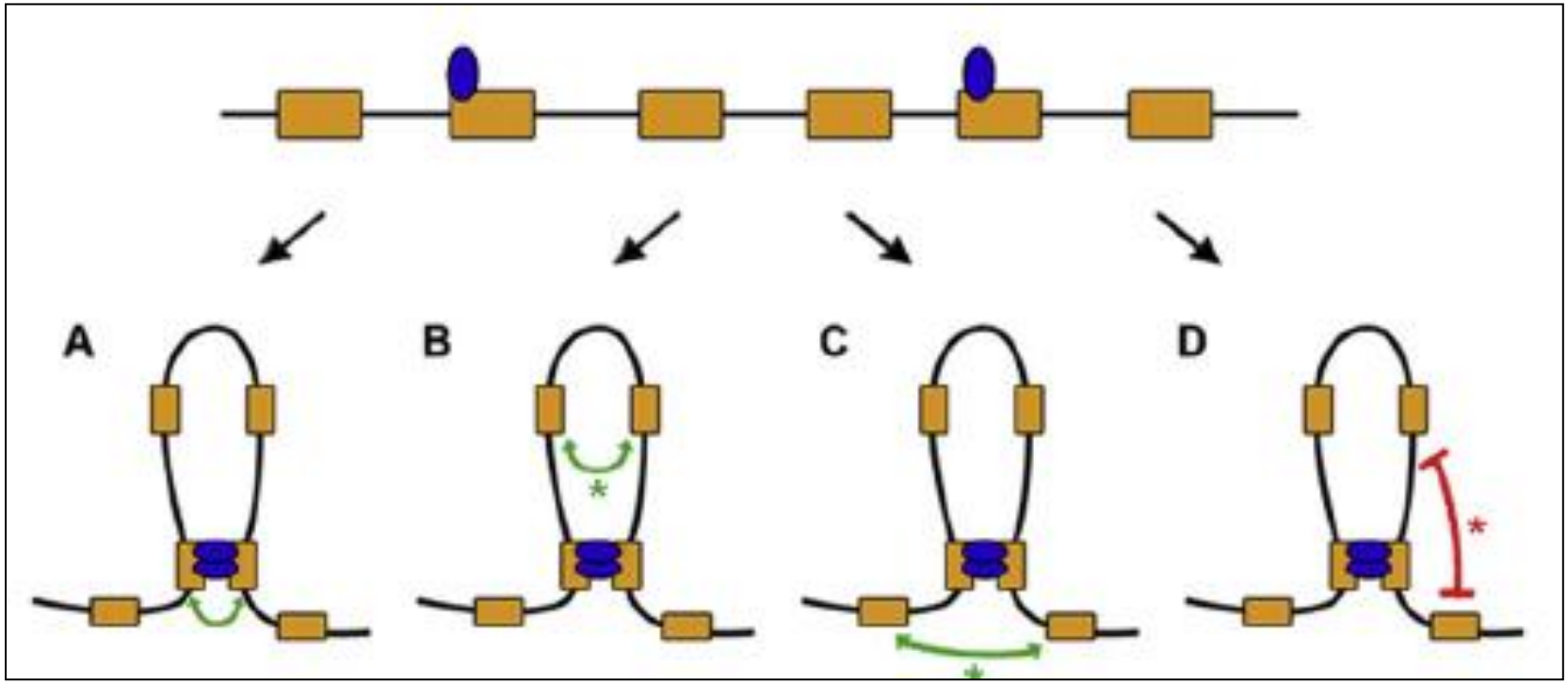
Schematic of putative TAD structures. The central regions of TADs show high levels of chromatin interaction and coincide with the presence of tissue-specific genes and their associated enhancers, the interactions of which with their cognate promoters are facilitated by the presence of **cohesin** and CCCTC-binding factor (**CTCF**). The border regions between TADs are enriched for housekeeping genes, which are often clustered together and generally lack the widely dispersed distal enhancers that are found around tissue-specific genes. The border regions show high levels of CTCF and cohesin binding, although only CTCF seems to prevent interactions between TADs.

Pombo & Dillon, 2015

TADs are **bordered by highly transcribed regions** (housekeepers).

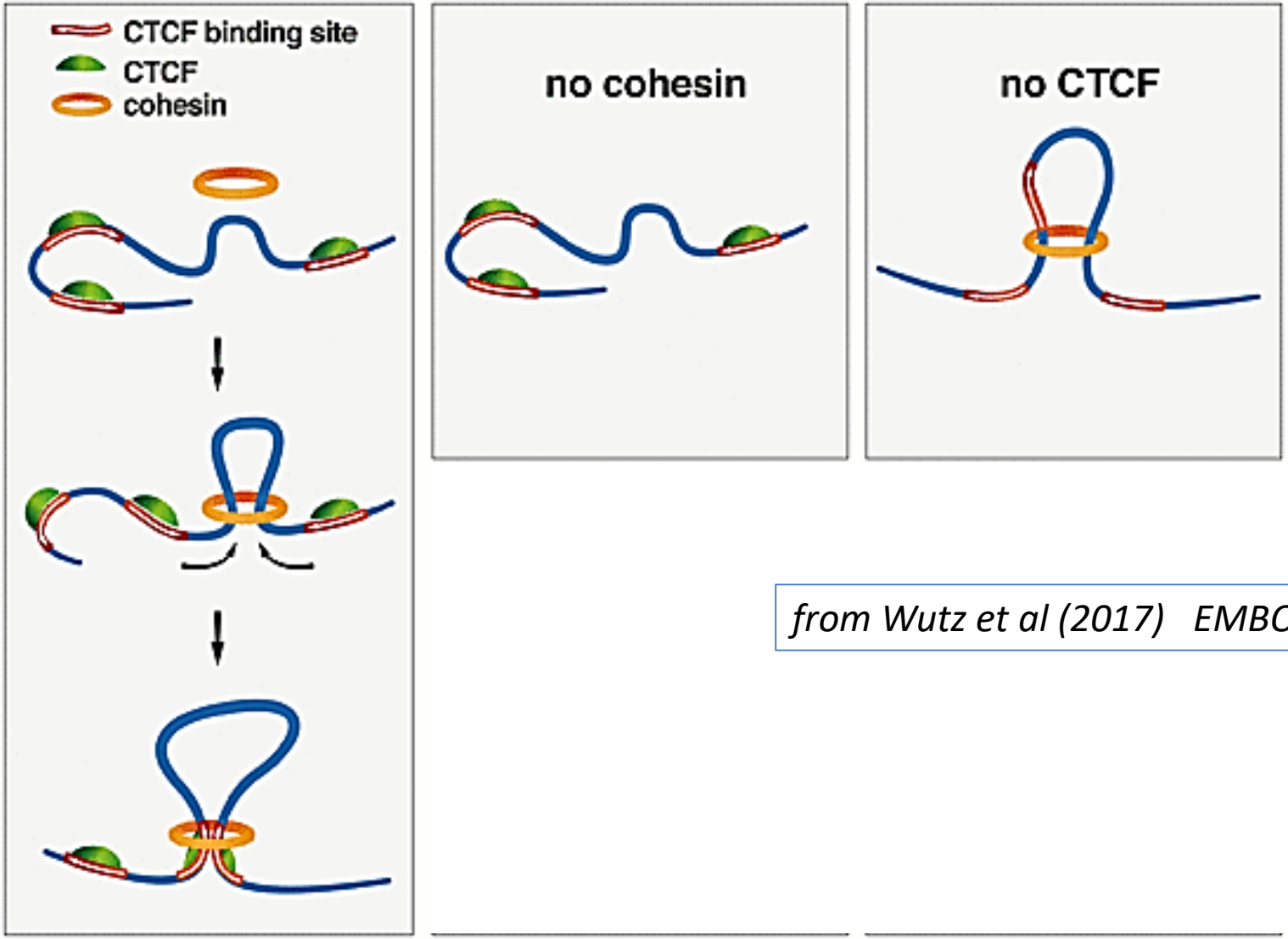
Large loops of chromain are due to **specific proteins** that bind DNA at specific sequences and interact reciprocally, called «**Architectural Proteins**»

The most studied AP in Mammals is **CTCF**, a methylation-sensitive factor, that interacts with **cohesins**



?

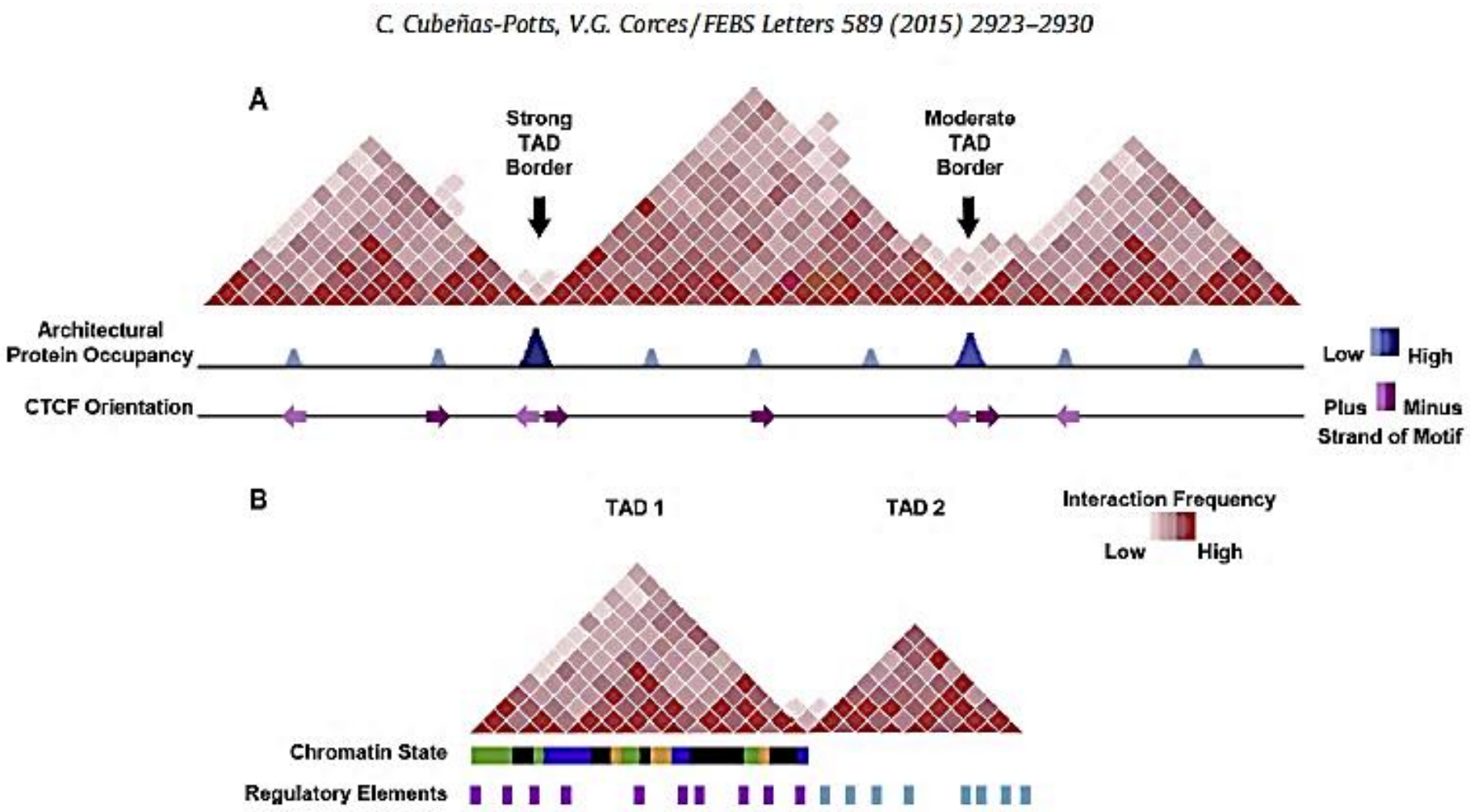
From Cabenas-Potts & Corces 2015



from Wutz et al (2017) EMBO J, 36:3573.

A model has been proposed where unidentified forces drive DNA loops into the cohesin ring, then slide more DNA into it until a CTCF binding site is reached.
Cohesin loading inhibitors WAPL/PDS5A/B Knock-Down increases the extension of the loop, possibly by inhibiting CTCF-cohesin interaction.

Borders can be stronger or weaker, i.e. extra-TAD interactions are sometimes permitted



TADs

- The importance of «domains» is also evident when studying the effect of integration position on the activity of a reporter transgene

- analysis of transgenic mice carrying a transposable reporter gene cassette (*sleeping beauty*). This transposon moves using a cut-and-paste strategy. Transposase expressed only in haploid spermatids. https://www.nature.com/articles/ng.790

- As a result, spermatocyte carry the reporter at different locations and transmit this to the progeny.

- The reporter transgene is expressed with very variable patterns in the embryo.

One way to study this is to make transgenic animals carrying reporter genes

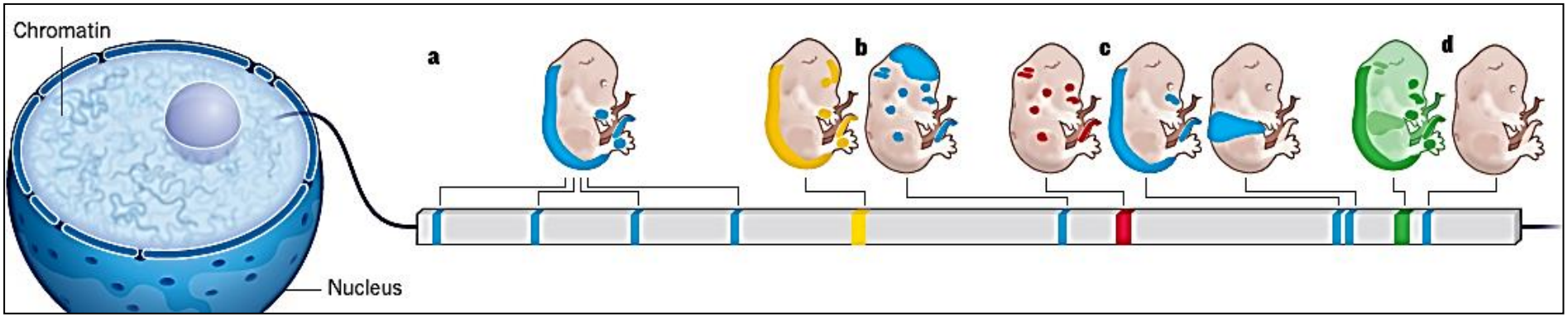


Figure 2 | The mammalian regulatory jungle. A model of three hypothetical genes (yellow, red and green) and their hypothetical expression pattern at a given stage of embryonic development are shown. Embryos coloured blue show the activity of a given reporter gene integrated at different chromosomal locations (adapted from ref. 16). They illustrate that genomic context critically determines expression patterns. Thus, **a**, various insertion sites may display comparable expression patterns despite being spread over a large chromosomal interval. Note that these reporter genes incorporate most of the regulatory activities acting on the downstream gene shown in yellow. **b**, Often, the reporter gene incorporates the enhancer activities that control the expression of one the nearest genes (red gene). **c**, Tissue-specific reporter gene expression can sometimes be seen at sites close to housekeeping genes (green gene). In addition, two closely linked integration sites may show very distinct expression patterns that reveal highly localized regulatory circuits. **d**, At some chromosomal sites, the reporter gene is inactive and apparently not capable of capturing enhancer activity.

De Laat & Duboule, 2013

LCR and Super-Enhancers

Development or cell-fate programming enhancers are grouped together to define large regulatory regions that are called **Super-Enhancers** (SE).

Active Ses: very high H3K27ac signal over several thousand bp + several centers of eRNA transcription (*see Henriques RP3*).

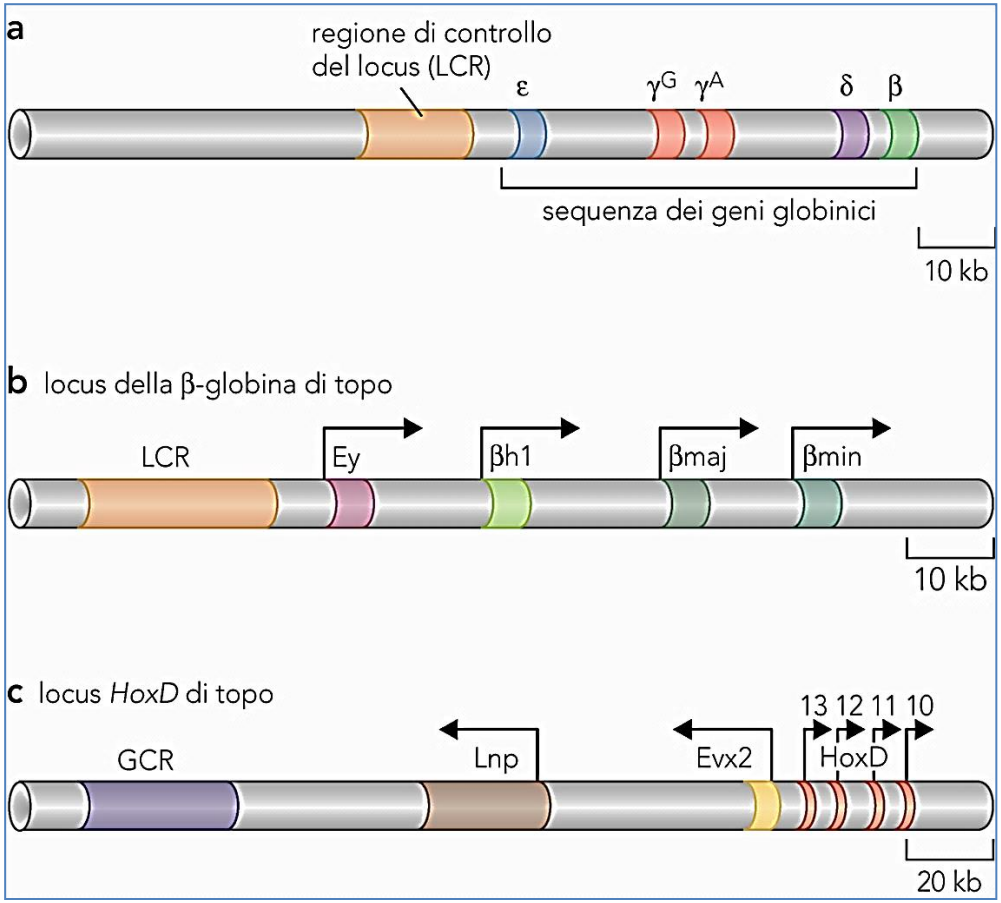
SE are essential regulators of cell-specific genes.

Some typical SEs were known before genome-wide technologies in particular loci, as for example the globin locus, were they were dubbed LCR for Locus Control Region.

We know examples in which a single enhancer (composite, called LCR- modern name «super-enhancer») controls groups of genes, often in an «exclusive» fashion

Gene clusters that derive from gene duplication events are often controlled by one or more common enhancer module, which interacts in a exclusive fashion with one or the other promoter. These common control regions are called LCR (*locus control region*).

LCRs control the sequential and exclusive use of one promoter at the time. An example is given by the globin gene clusters, containing embryonic, phoetal and adult versions of the globin proteins. Another example is given by the gene clusters encoding homeoproteins, that are expressed following a precise spatial order in the body.



Molecular Cell, Vol. 10, 1453–1465, December, 2002, Copyright ©2002 by Cell Press

Looping and Interaction between Hypersensitive Sites in the Active β-globin Locus

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The Netherlands

Summary

Eukaryotic transcription can be regulated over tens or even hundreds of kilobases. We show that such long-range gene regulation in vivo involves spatial interactions between transcriptional elements, with intervening chromatin looping out. The spatial organization of a 200 kb region spanning the murine β-globin locus was analyzed in expressing erythroid and nonexpressing brain tissue. In brain, the globin cluster adopts a seemingly linear conformation. In erythroid cells the hypersensitive sites of the locus control region (LCR), located 40–60 kb away from the active genes, come in close spatial proximity with these genes. The intervening chromatin with inactive globin genes loops out. Moreover, two distant hypersensitive regions participate in these interactions. We propose that clustering of regulatory elements is key to creating and maintaining active chromatin domains and regulating transcription.

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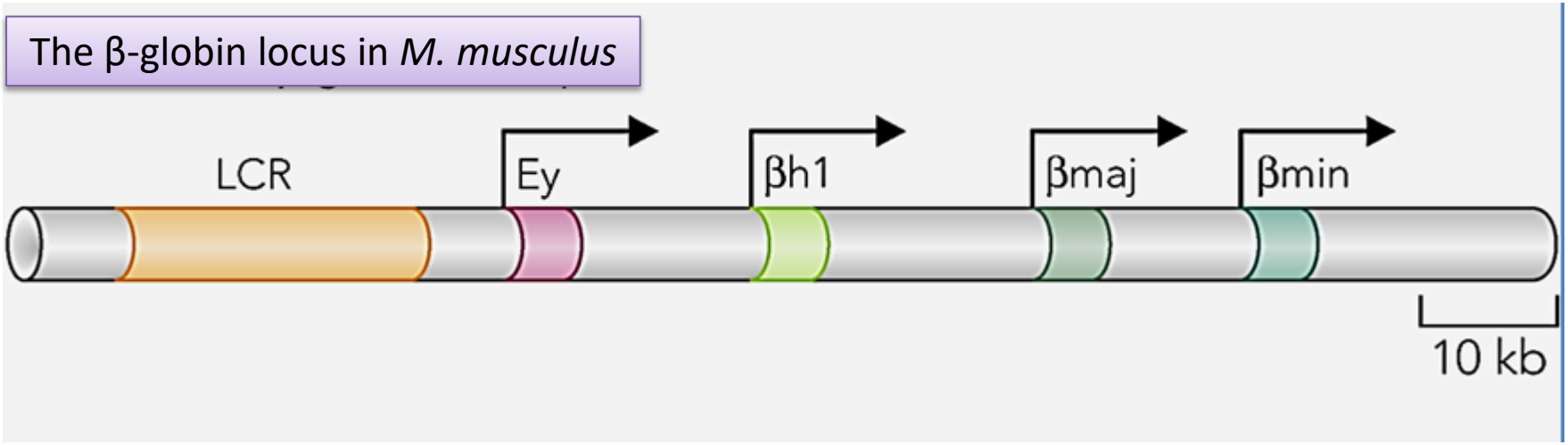

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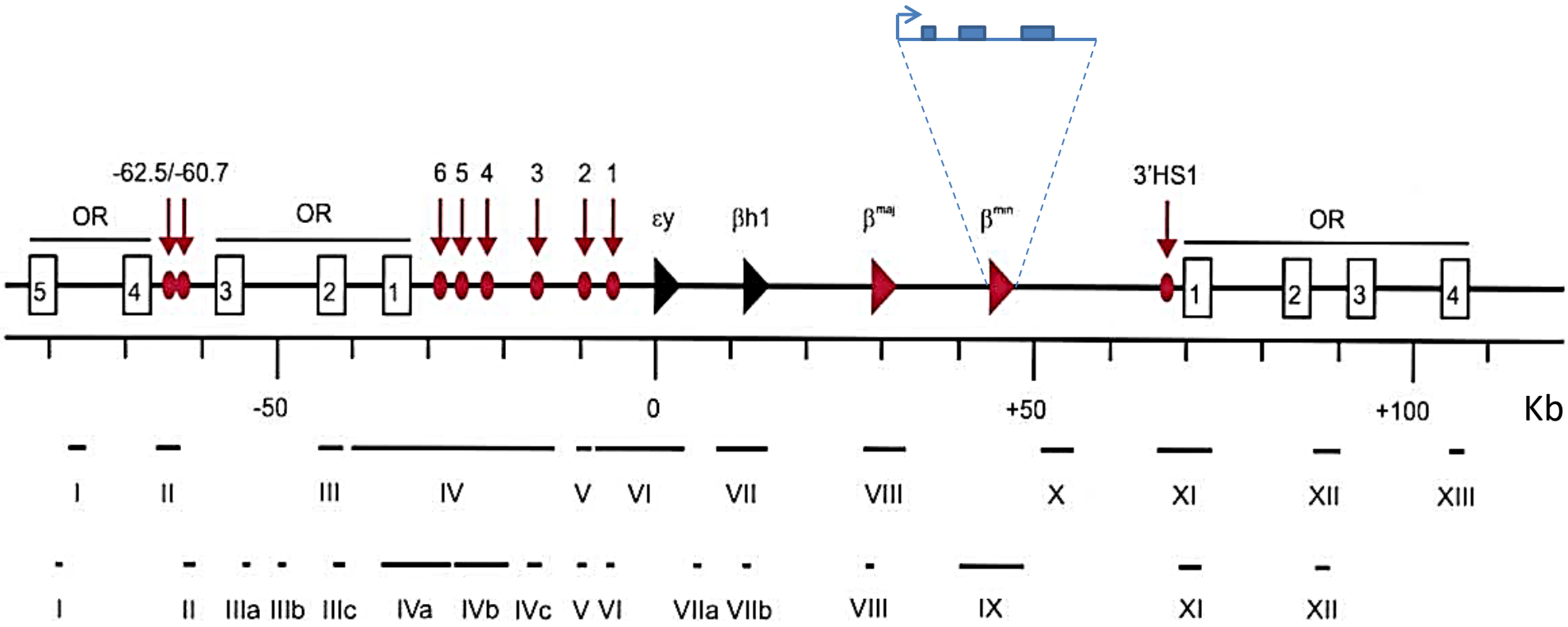


Figure 1. 3C Technology in the Murine beta-globin Locus

(A) Schematic presentation of the murine **beta-globin** locus. Red arrows and ellipses depict the individual HS. The globin genes are indicated by triangles, with **active genes (maj and min) in red** and **inactive genes (y and h1) in black**. The white boxes indicate the olfactory receptor (OR) genes (5OR1-5 and 3OR1-4). The two sets of restriction fragments (BglII and HindIII) that were used for 3C analysis are shown below the locus. The individual fragments are indicated by Roman numerals. Identical numbering between BglII and HindIII indicates that two fragments colocalize. Distances are in kb counting from the site of initiation of the y gene.

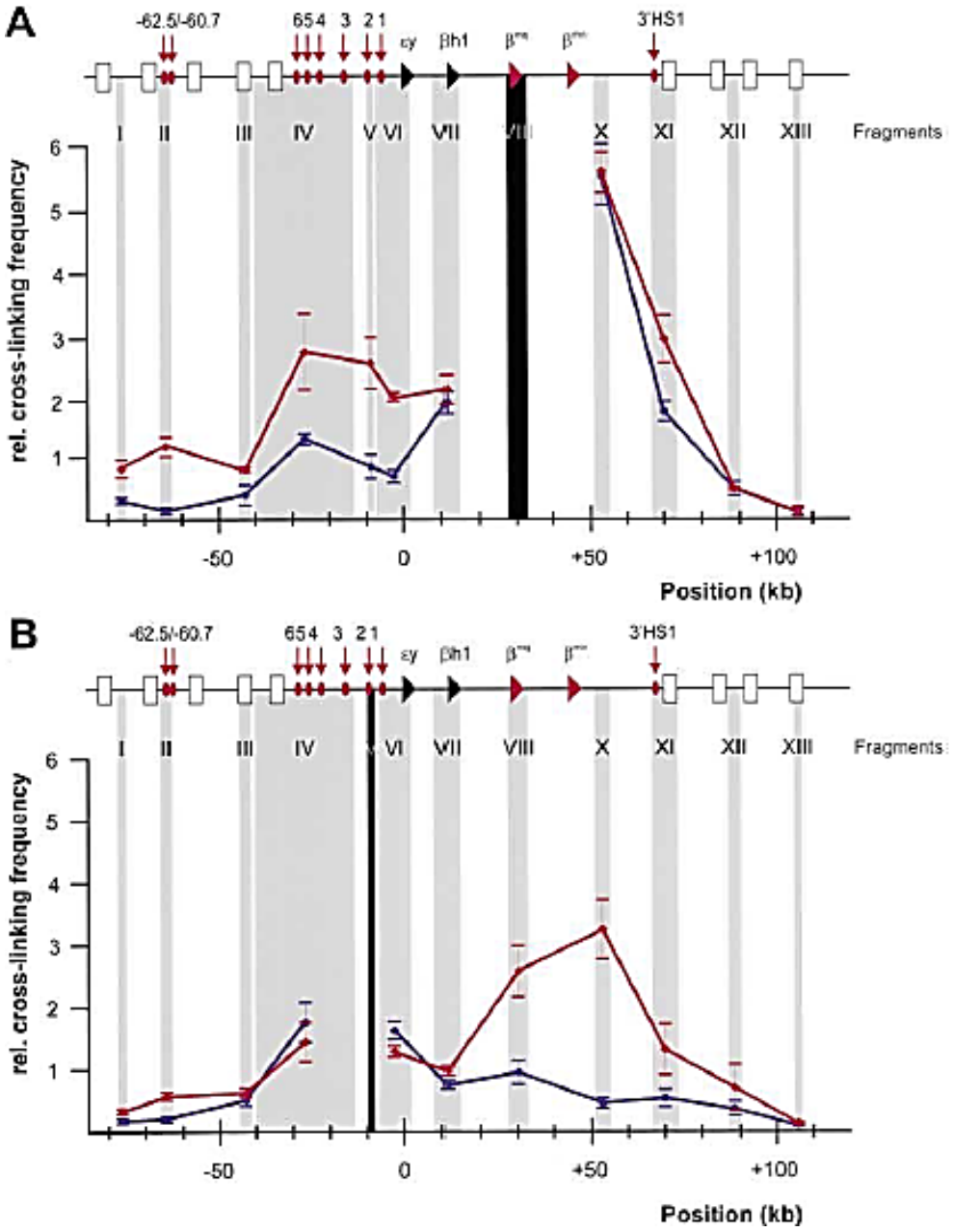
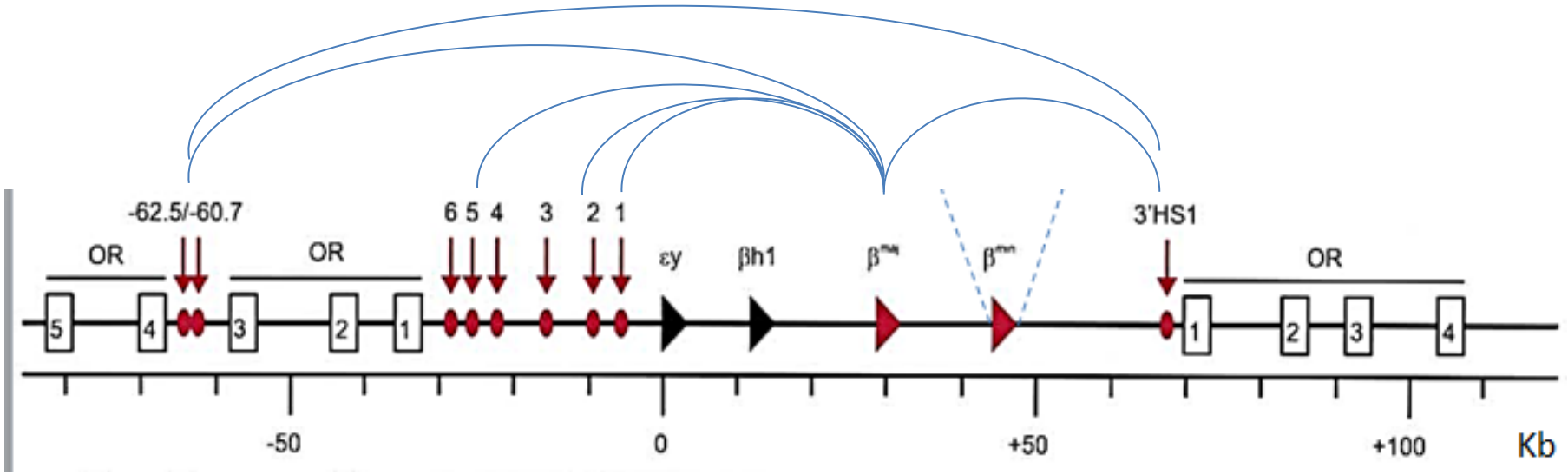
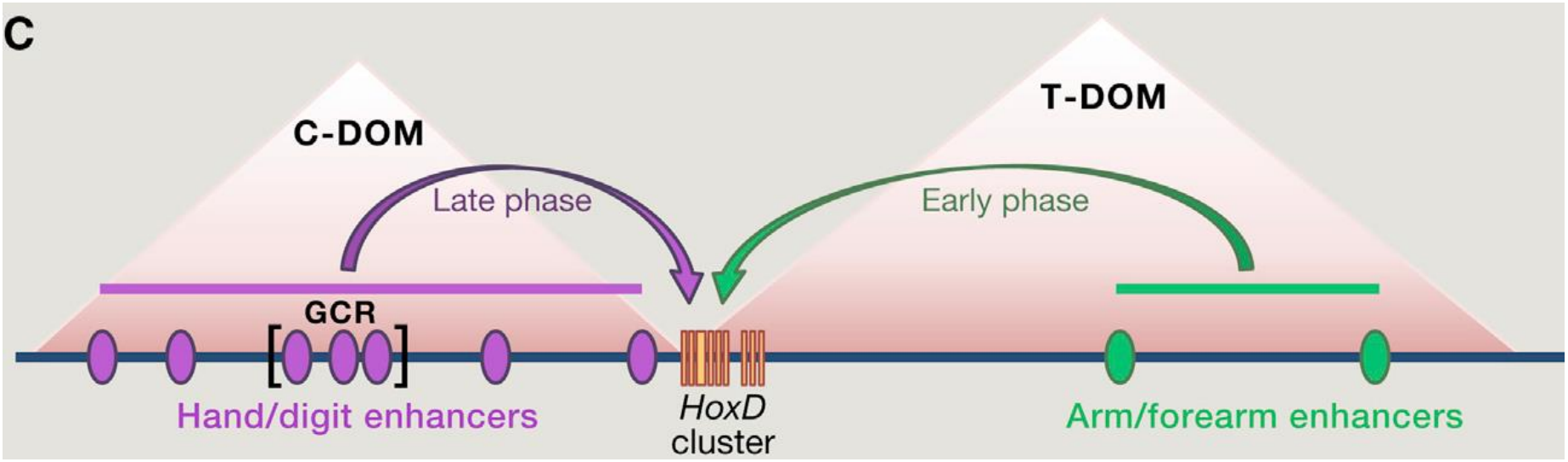


Figure 3. **Erythroid-Specific** Interaction and Looping between the LCR and an Active beta--globin Gene. Relative crosslinking frequencies observed in fetal liver are shown in red. For comparison, data obtained in brain are depicted in blue. Standard error of the mean is indicated. Crosslinking frequency with a value of 1 arbitrarily corresponds to the crosslinking frequency between two neighboring CalR control fragments (with restriction sites analyzed being 1.5 kb apart). Scaling on the *y* axis (from 0 to 6) allows direct comparison with Figures 2 and 4–6.
(**A**) Fixed BglII fragment VIII (maj) versus the rest of the locus. (**B**) Fixed BglII fragment V (5HS2) versus the rest of the locus. (**C**) Fixed BglII fragment VII (h1) versus the rest of the locus.

This data demonstrated multiple «looping» involving components of the super-enhancer LCR, other enhancers and promoters of the active B genes
(the example below shows only some of the contacts verified)





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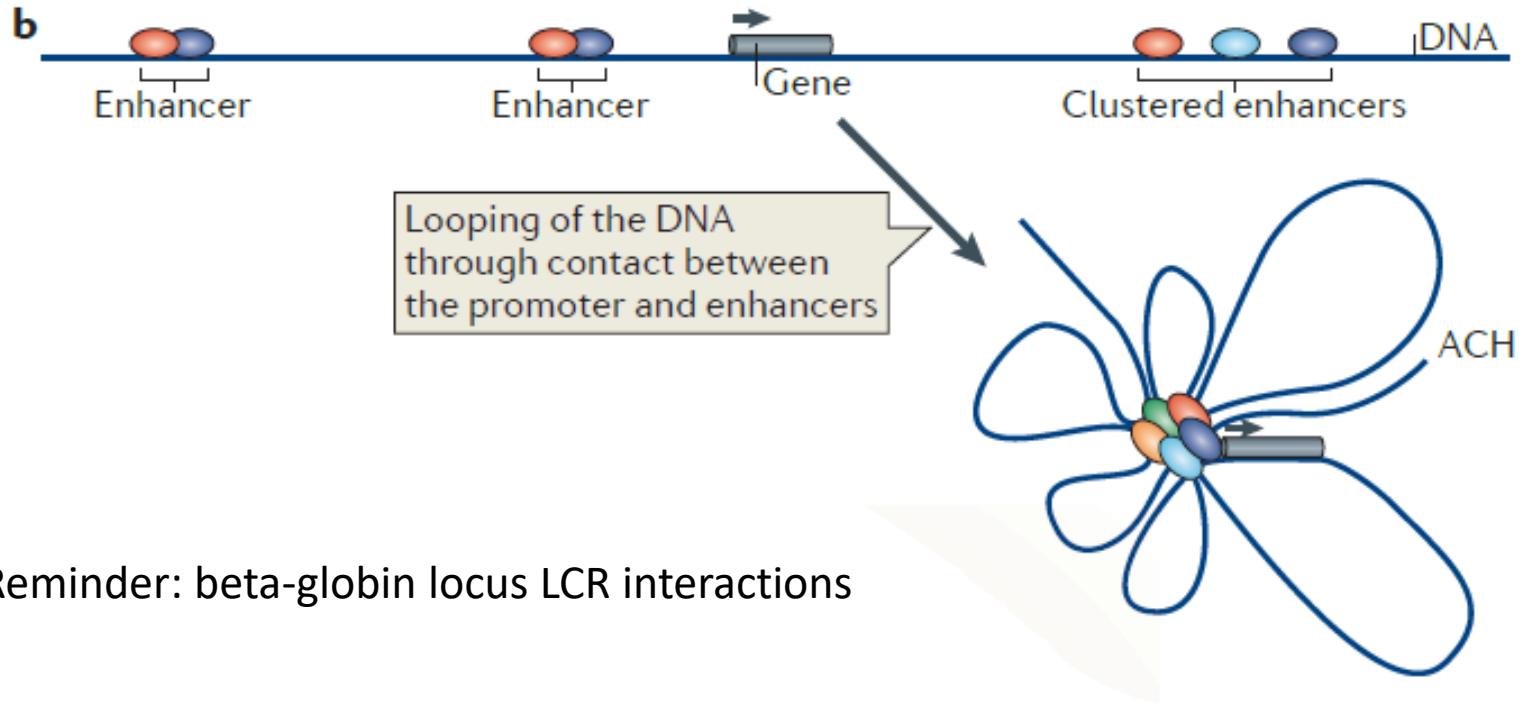
Levine et al., 2014

The concept of transcriptional «hub»



Reminder: beta-globin locus LCR interactions

This model has been verified at LCR (super-enhancers)

Pombo & Dillon, 2015