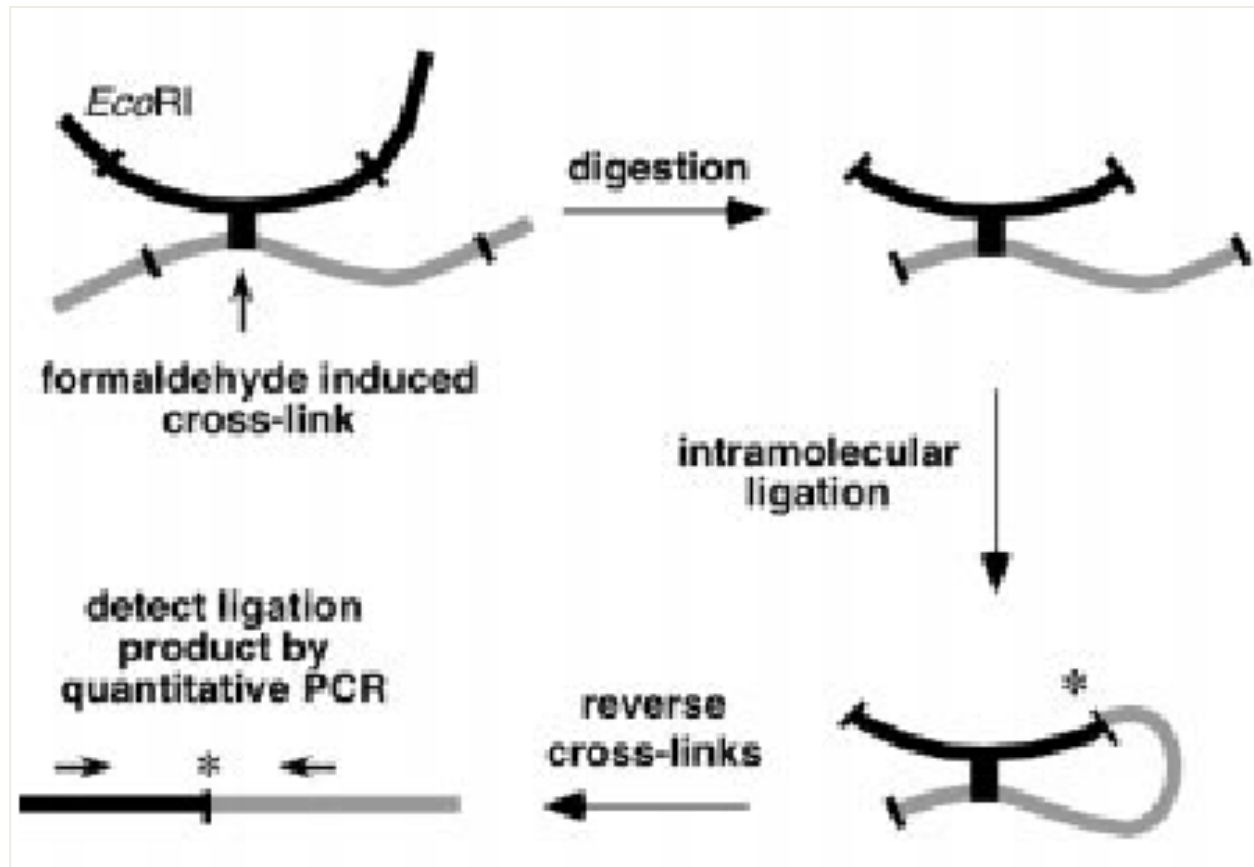# L3.4

Enhancer functioning

The next levels of complexity:

- Long-range interaction with promoters

- Chromatin domains, insulators

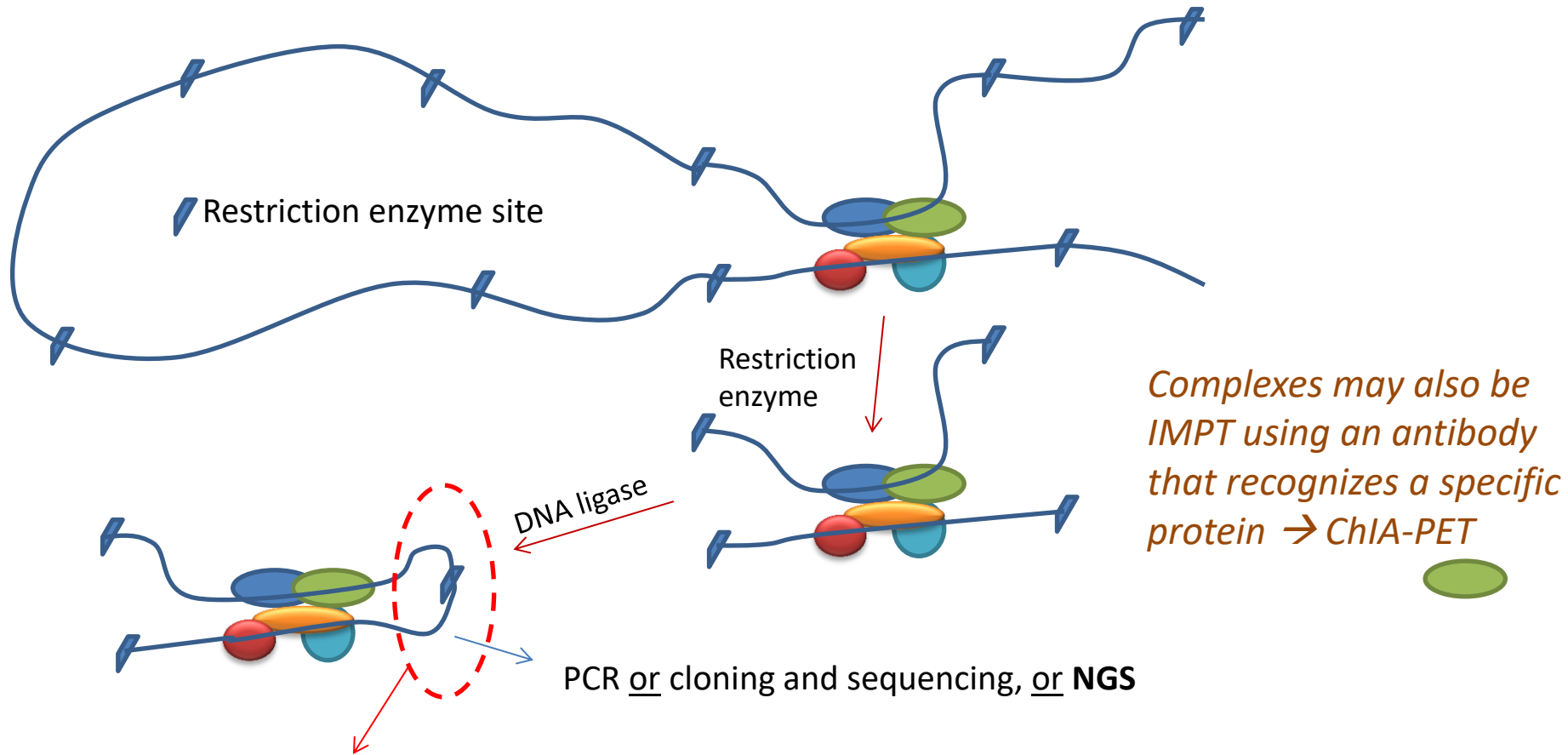- Mediators of interaction, co-regulators

Key methods to allow appreciating the functioning of Enhancers were Long-Range interaction methods

The first historically and technically is 3C

A number of different methods have been developed to scale up this approach genome-wide
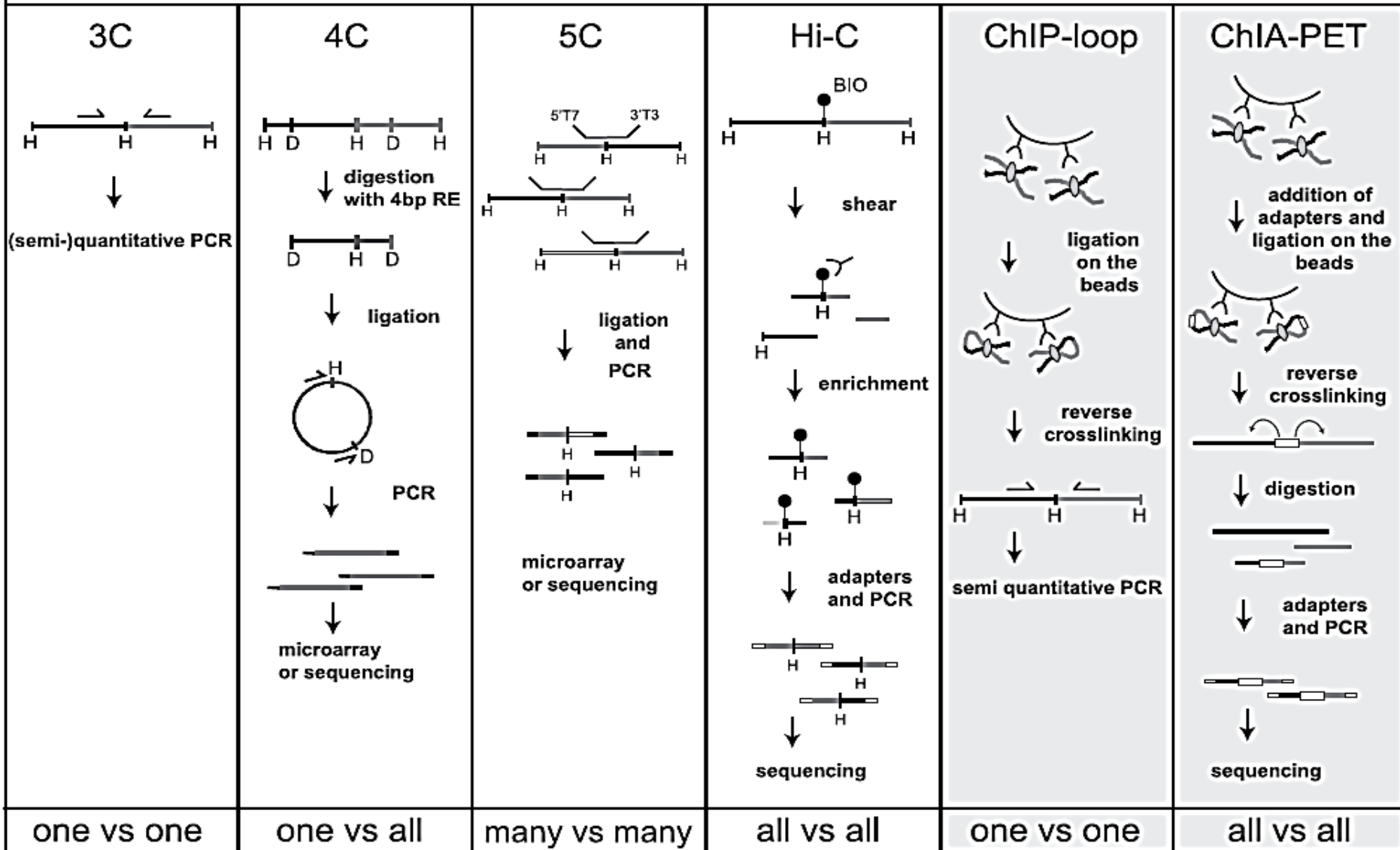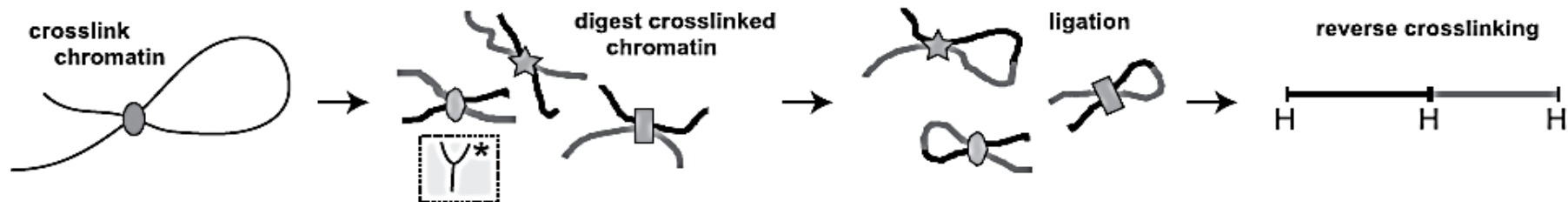
Long-range interactions are studied with 3C (Chromatin Conformation Capture) or different genome-wide scale variants (4C, 5C, Hi-C, ChIA-PET).

Restriction enzyme site

Restriction enzyme

DNA ligase

Complexes may also be IMPT using an antibody that recognizes a specific protein → ChIA-PET

PCR or cloning and sequencing, or **NGS**
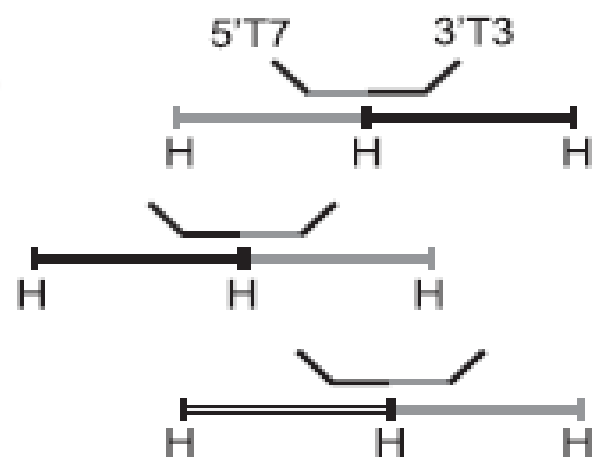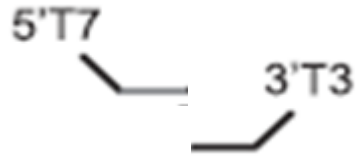
PCR for single interaction.
Generate libraries to NGS for genome-wide studies

Note: from this scheme nucleosomes are omitted

Top workflow

crosslink chromatin → digest crosslinked chromatin → ligation → reverse crosslinking

Y*

H   H   H

3C

H   H   H

↓

(semi-)quantitative PCR

one vs one

4C

H D   H D   H

↓ **digestion with 4bp RE**

D   H   D

↓ **ligation**

H (circle) D

↓ **PCR**

↓

microarray or sequencing

one vs all

5C

5'T7   3'T3

H   H   H

H   H   H

H   H   H

↓ **ligation and PCR**

H   H

H   H

microarray or sequencing

many vs many

Hi-C

BIO

H   H   H

↓ **shear**

H

H

↓ **enrichment**

H

H   H

H

↓ **adapters and PCR**

H

H

H

↓

sequencing

all vs all

ChIP-loop

↓ **ligation on the beads**

↓ **reverse crosslinking**

H   H   H

↓

semi quantitative PCR

one vs one

ChIA-PET

↓ **addition of adapters and ligation on the beads**

↓ **reverse crosslinking**

↓ **digestion**

↓ **adapters and PCR**
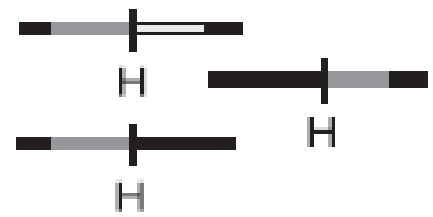
↓

sequencing

all vs all

Chromosome conformation capture carbon copy (5C)

Oligonucleotides partially overlapping all restriction sites (H) in the genome are made, with 5'- and 3' common extensions.
When they hybridize to a junction, can be ligated together, so that they «carbon copy» the junction.

ligation and PCR

microarray or sequencing

HiC

This is an «all versus all» method.

After digestion and before ligation, sticky ends are filled using biotinylated nucleotides, so that ligation junctions remain marked with Biotin and can be enriched using streptavidin beads.
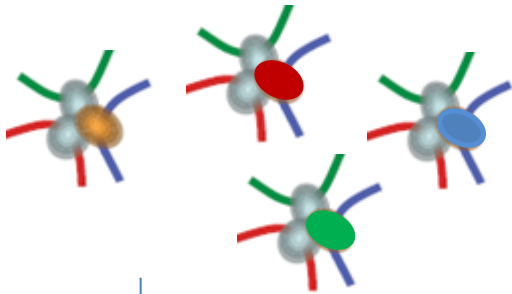
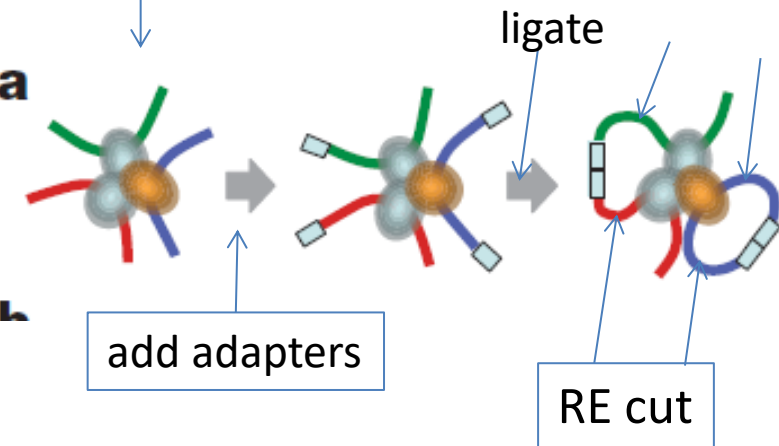After this step, fragments are processed, amplified and NGSequenced as in other methods.

BIO

H   H   H

shear

H

H

enrichment

H

H

H

adapters and PCR

H   H

H

sequencing

ChIA-PET
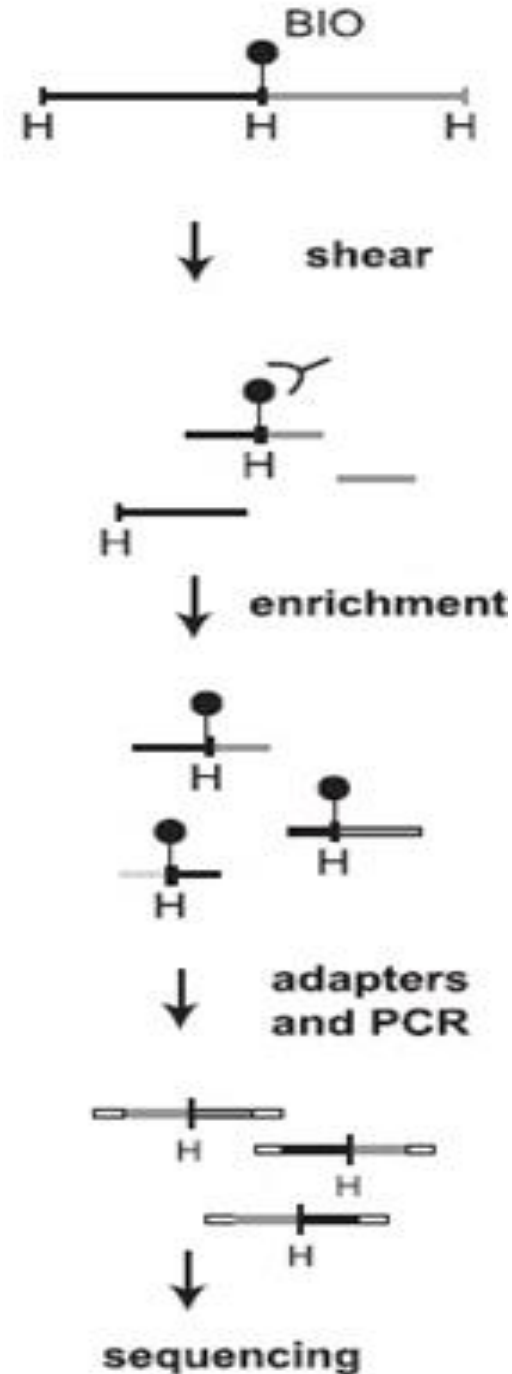
Formaldehyde
Sonication

Uses only complexes containing a specific protein, i.e. ChIPped complexes

mapping

ChIP with anti-●

ligate

add adapters

a

add adapters

RE cut
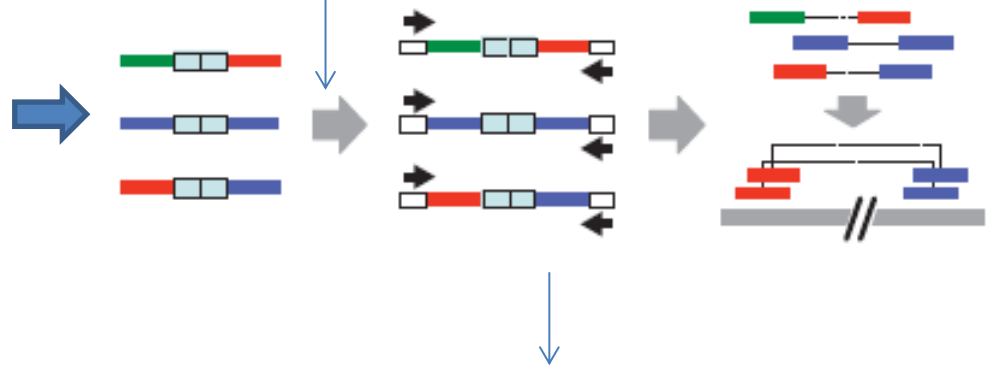
PCR amplify and mass-sequencing

LETTER

The long–range interaction landscape of gene promoters

Amartya Sanyal[1]*, Bryan R. Lajoie[1]*, Gaurav Jain[1] & Job Dekker[1]

The vast non-coding portion of the human genome is full of functional elements and disease-causing regulatory variants. The principles defining the relationships between these elements and distal target genes remain unknown. Promoters and distal elements can engage in looping interactions that have been implicated in gene regulation1. Here we have applied chromosome conformation capture carbon copy (5C) to interrogate comprehensively interactions between transcription start sites (TSSs) and distal elements in 1% of the human genome representing the ENCODE pilot project regions. 5C maps were generated for GM12878, K562 and HeLa-S3 cells and results were integrated with data from the ENCODE consortium. In each cell line we discovered >1,000 long-range interactions between promoters and distal sites that include elements resembling enhancers, promoters and CTCF-bound sites. We observed significant correlations between gene expression, promoter–enhancer interactions and the presence of enhancer RNAs.
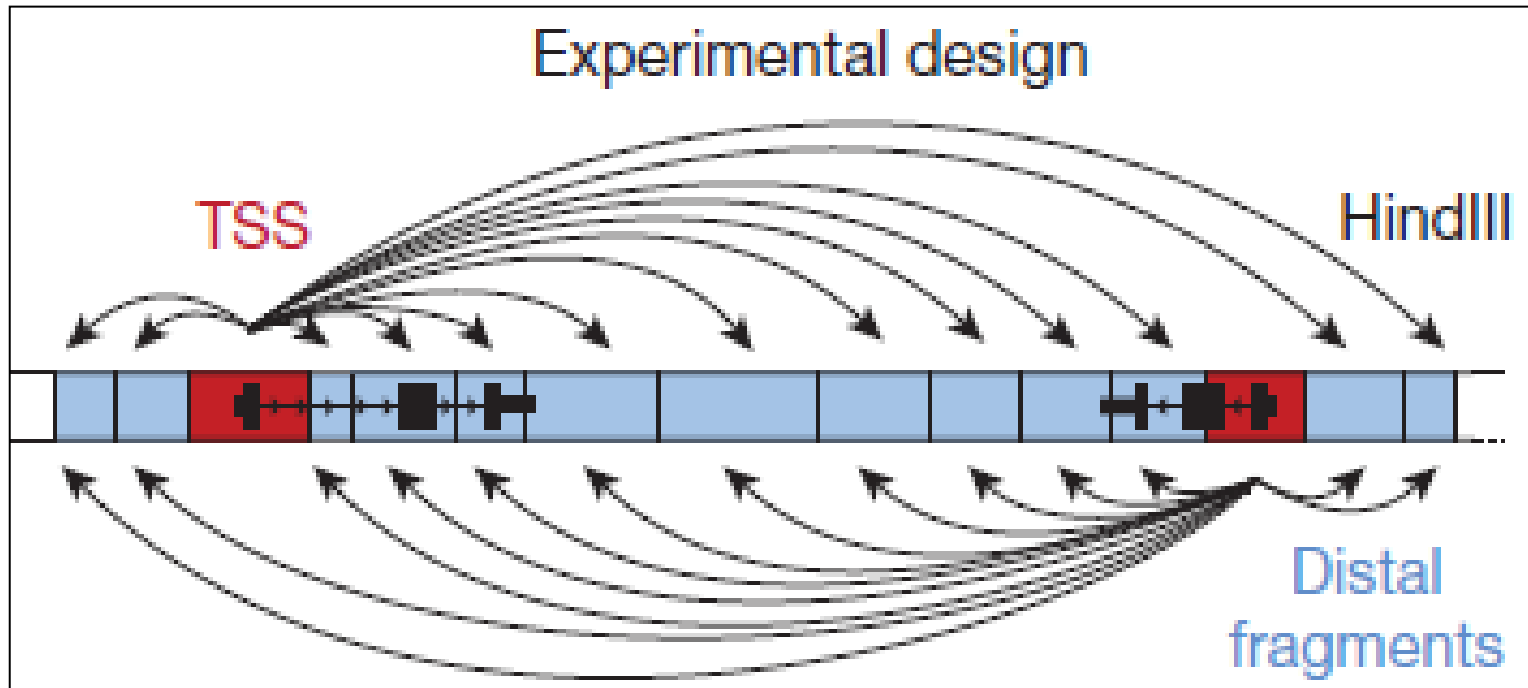
Reverse 5C primers were designed for HindIII fragments that contain a TSS (red; according to the GENCODE v720) and forward 5C primers for all other 'distal' HindIII fragments (blue)
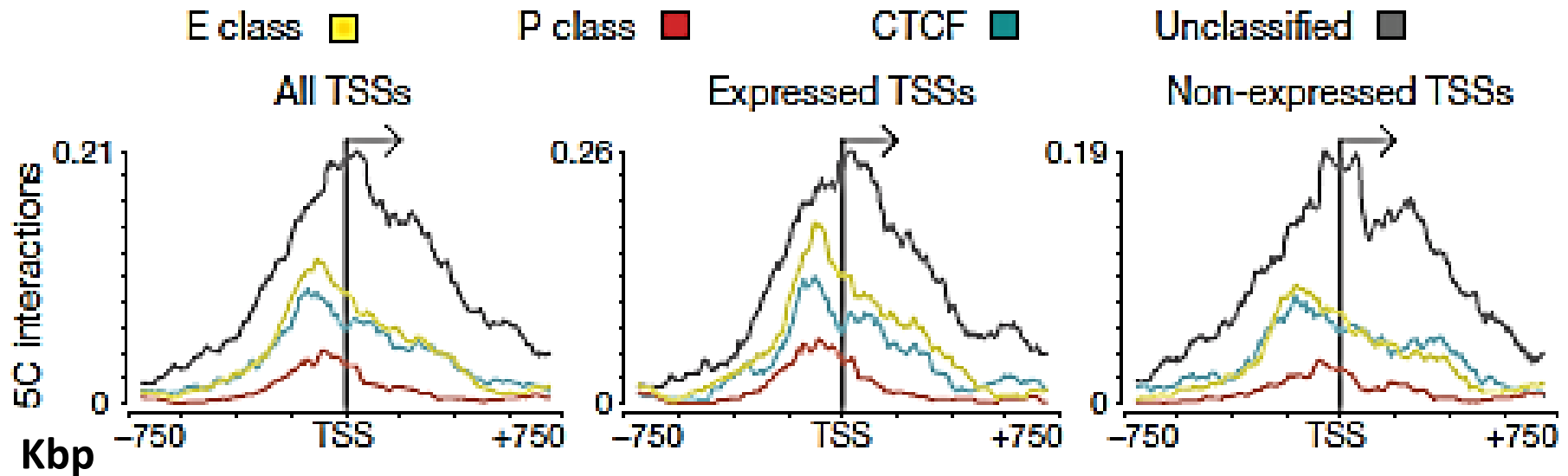
results

Long-range interactions show <u>marked asymmetry</u> with a bias at 120 kilobases upstream of TSS

Long-range interactions often not blocked by sites bound by CTCF and cohesin

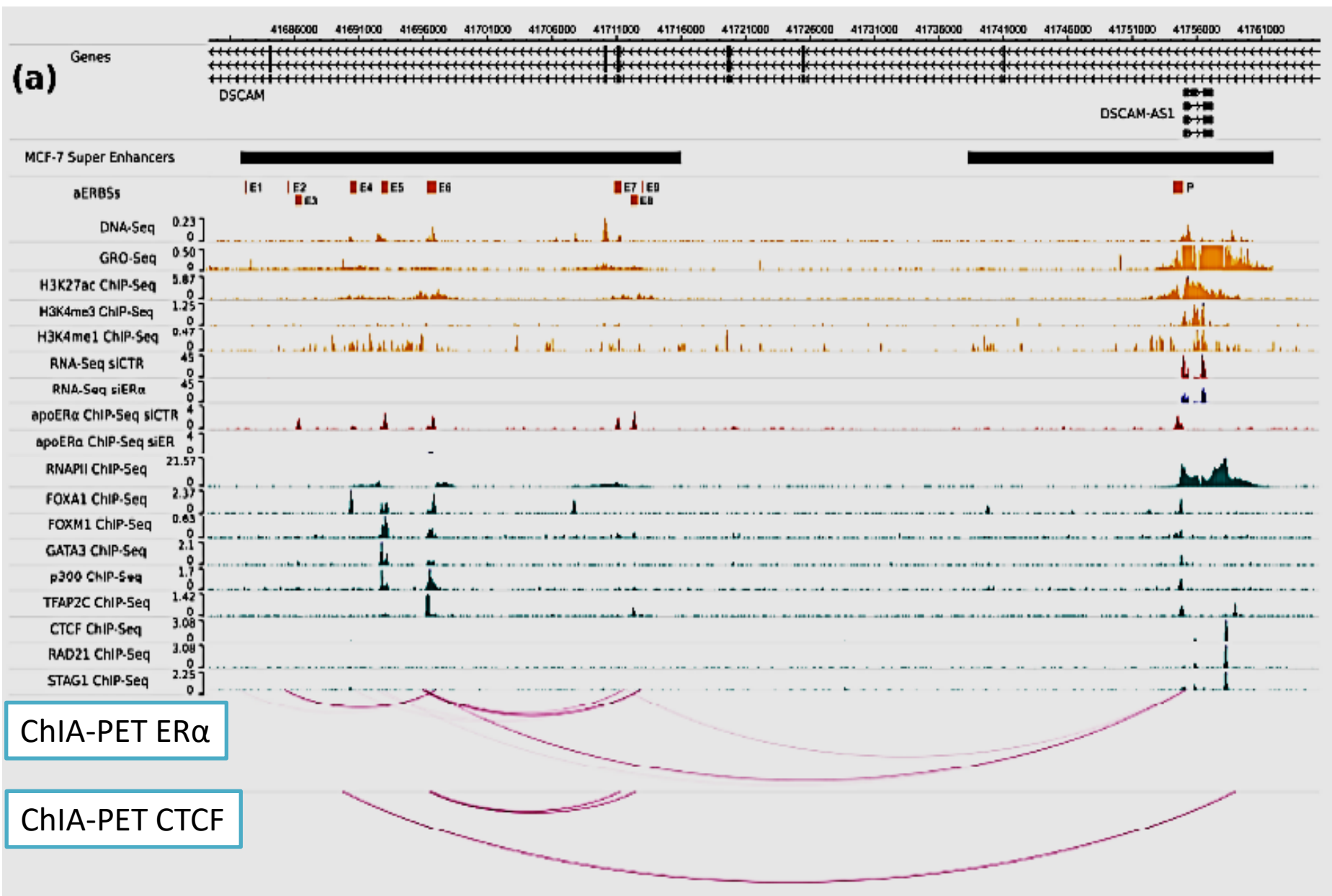Only 7% of looping interactions are with the nearest gene

Promoters and distal elements are engaged in multiple long-range interactions to form complex networks.

Enhancers are asymmetric in respect to TSS

E class ☐   P class ■   CTCF ■   Unclassified ■

All TSSs | Expressed TSSs | Non-expressed TSSs

5C interactions

0.21 ... 0.26 ... 0.19

−750   TSS   +750   −750   TSS   +750   −750   TSS   +750

Kbp

the long-range interaction landscape is asymmetric, with interactions of E, P and CTCF classes peaking around 120 kb upstream of the TSS. This asymmetry of interactions reveals an <u>unanticipated directionality</u> in long-range interactions with TSSs.
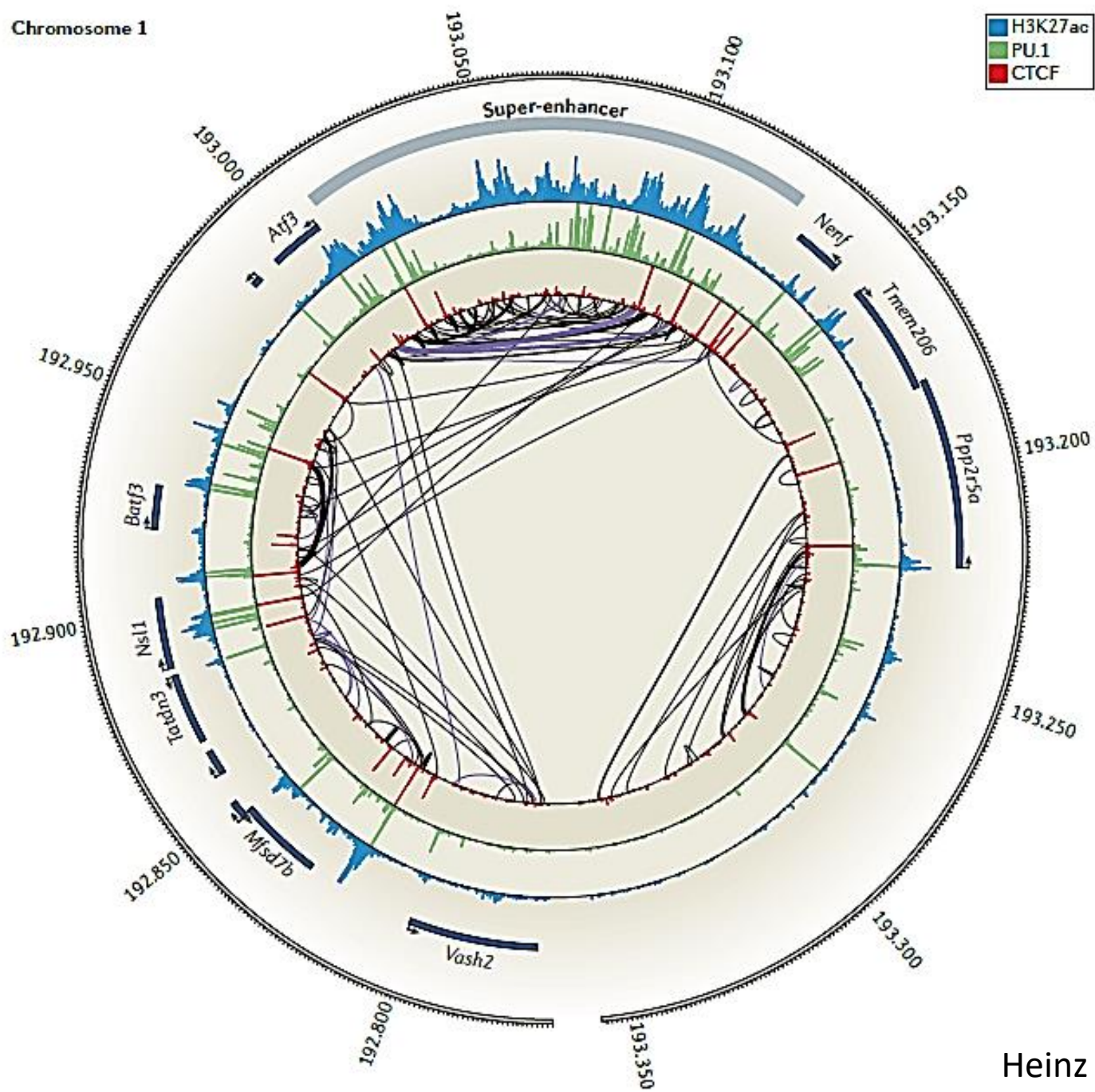
- The fact that there is a decreasing frequency with the distance testifies that limitations exist for this kind of interactions

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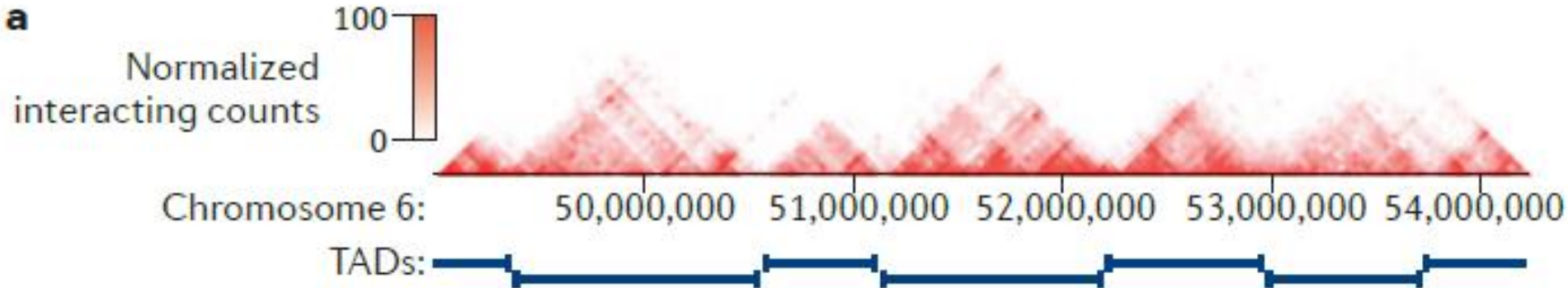
Miano V, Int J Mol Sci. 2018 Feb 16;19(2). pii: E593. doi: 10.3390/ijms19020593

TAD

Chromosome 1

H3K27ac
PU.1
CTCF

Super-enhancer

Atf3 *Ncrf* *Tmem206* *Ppp2r5a* *Batf3* *Nsl1* *Tatdn3* *Mfsd7b* *Vash2*
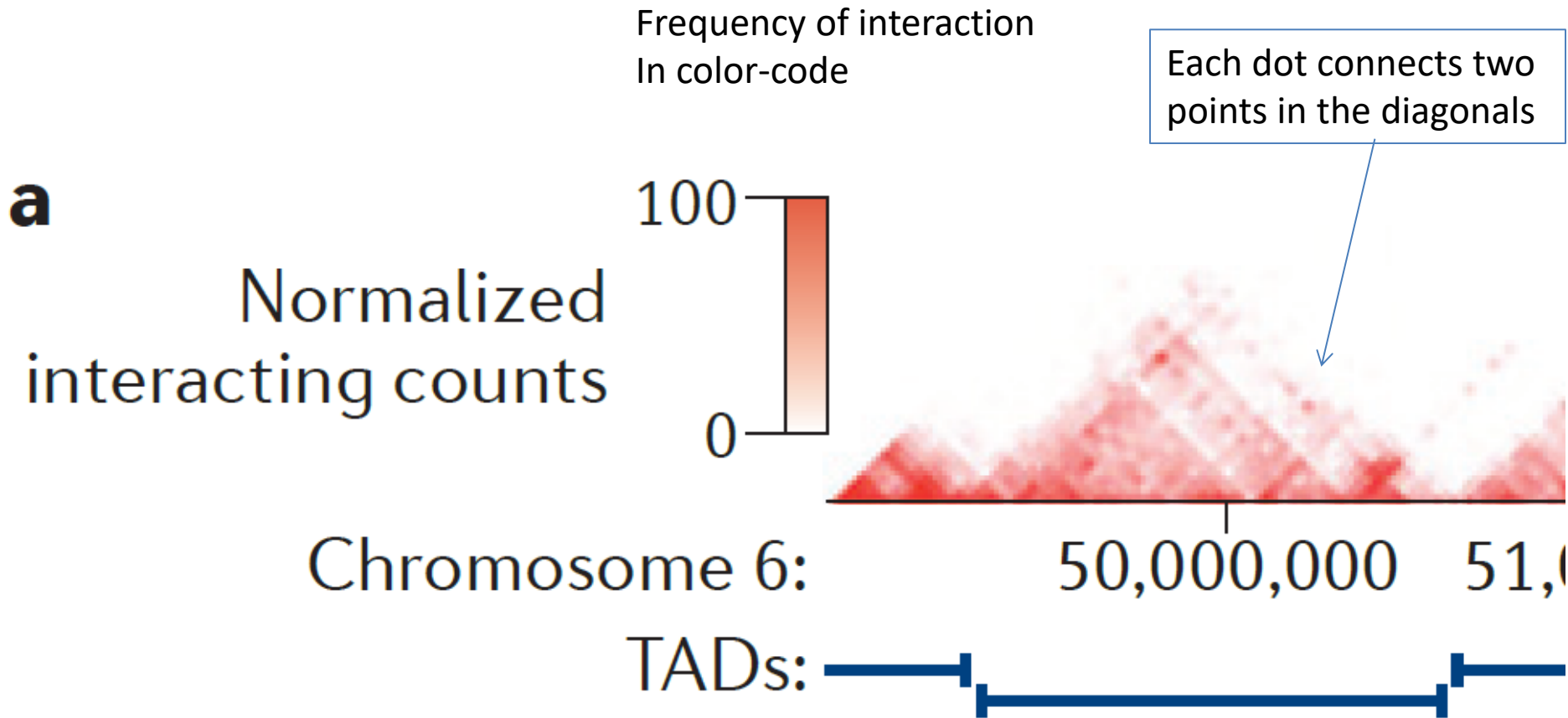
Heinz et al. 2015

TAD

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

Frequency of interaction
In color-code

Each dot connects two
points in the diagonals

a

Normalized
interacting counts
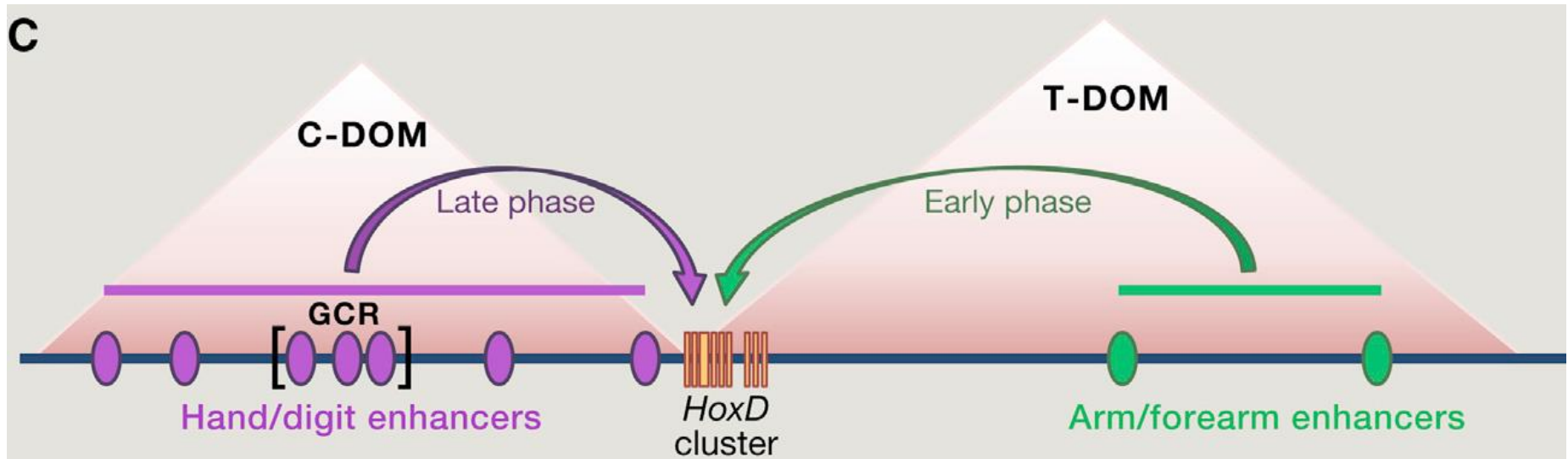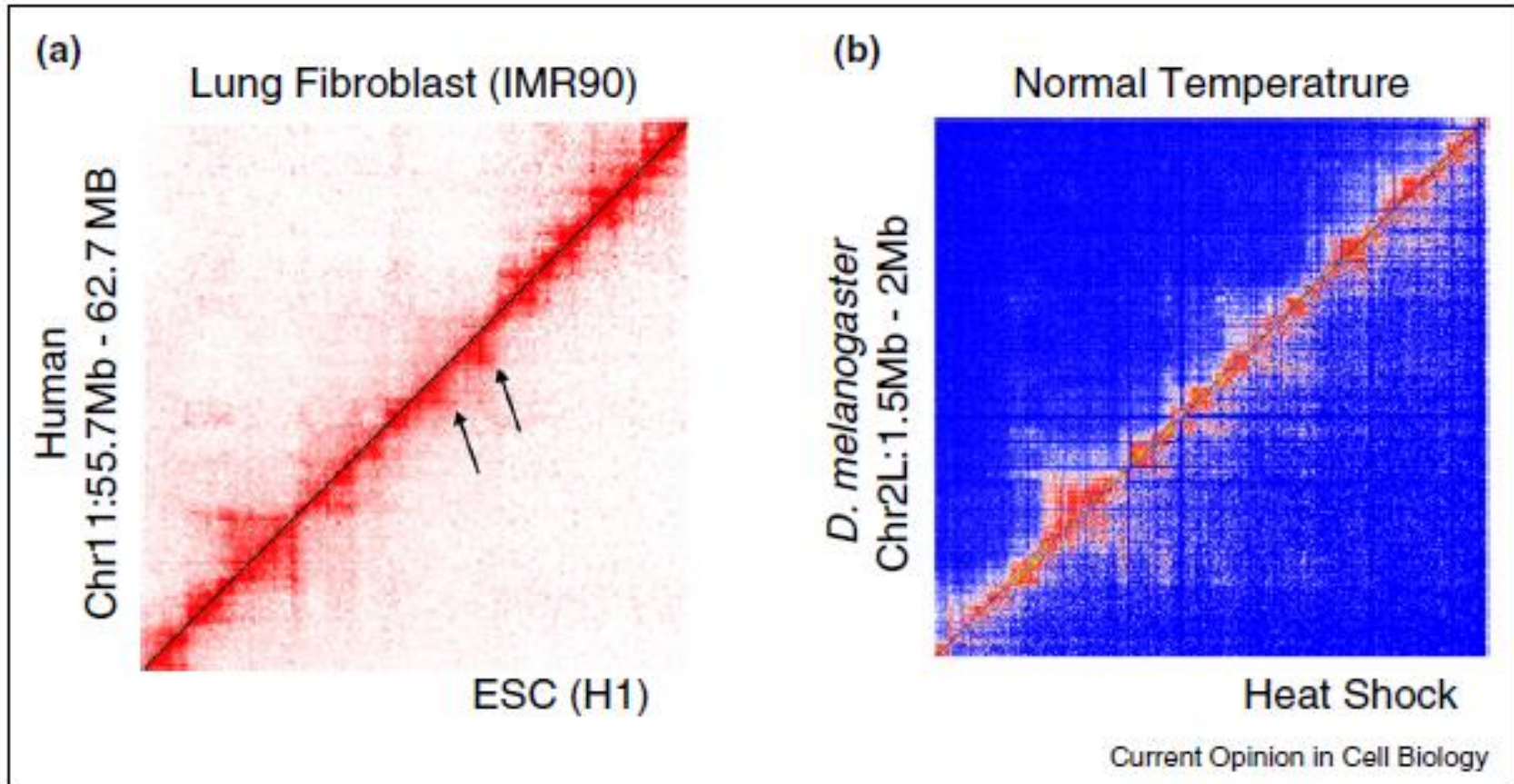
100

0

Chromosome 6:

50,000,000   51,0

TADs:

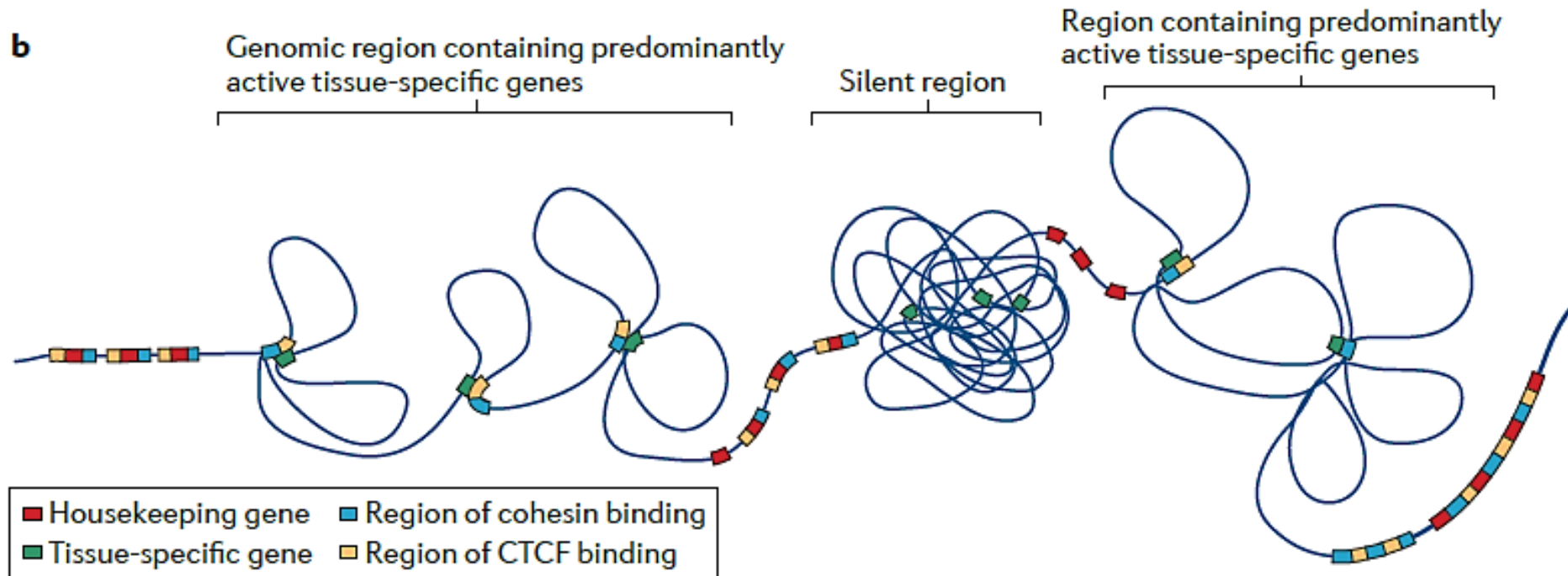Figure 1. Organization of cis-Regulatory DNAs in Metazoan Genomes

(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

Developmental changes in TADs organization

(a) Lung Fibroblast (IMR90)

Human Chr1 1:55.7Mb - 62.7 MB

ESC (H1)

(b) Normal Temperatrure

D. melanogaster Chr2L:1.5Mb - 2Mb

Heat Shock

Current Opinion in Cell Biology

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)

Rowley & Corces, 2016

b

Genomic region containing predominantly active tissue-specific genes

Silent region

Region containing predominantly active tissue-specific genes

■ Housekeeping gene   ■ Region of cohesin binding
■ Tissue-specific gene □ Region of CTCF binding

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 defined by interactions and are bordered by highly transcribed regions (housekeepers).

The large loops of chromain that define TADs are due to specific proteins that bind DNA at specific sequences and interact reciprocally.

They are defined «**Architectural Proteins**» and the sequences of DNA that are recognized by APS are called APBS.

By far, the most studied AP in Mammals is CTCF, but others exist. CTCF also interacts with **cohesins**, which are supposed to stabilize loops and that some studies have shown to be essential for enhancer activity.

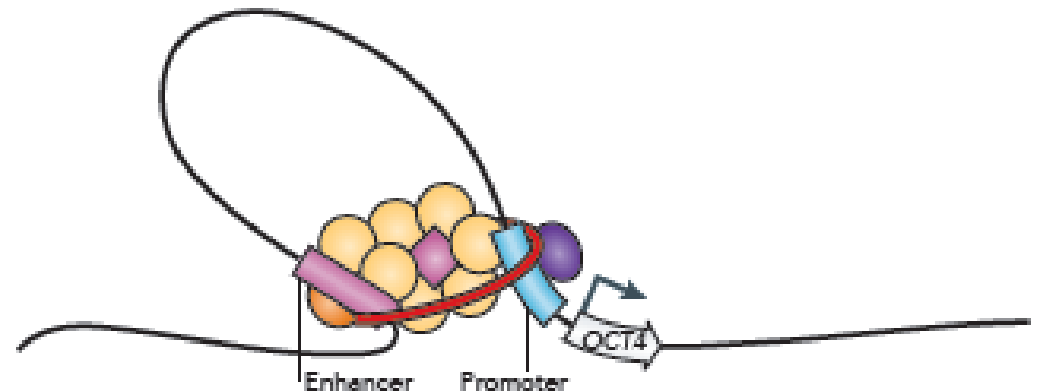From Cabenas-Potts & Corces 2015
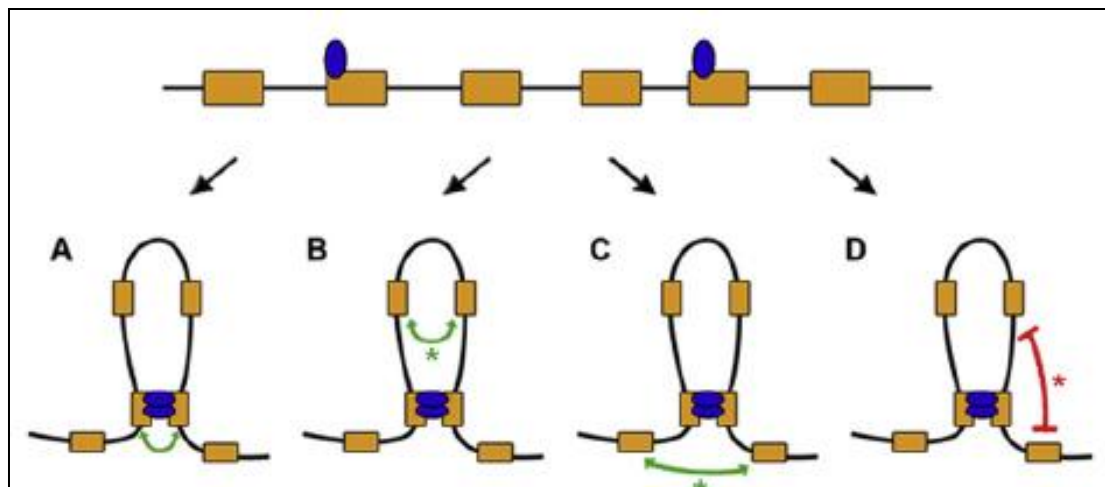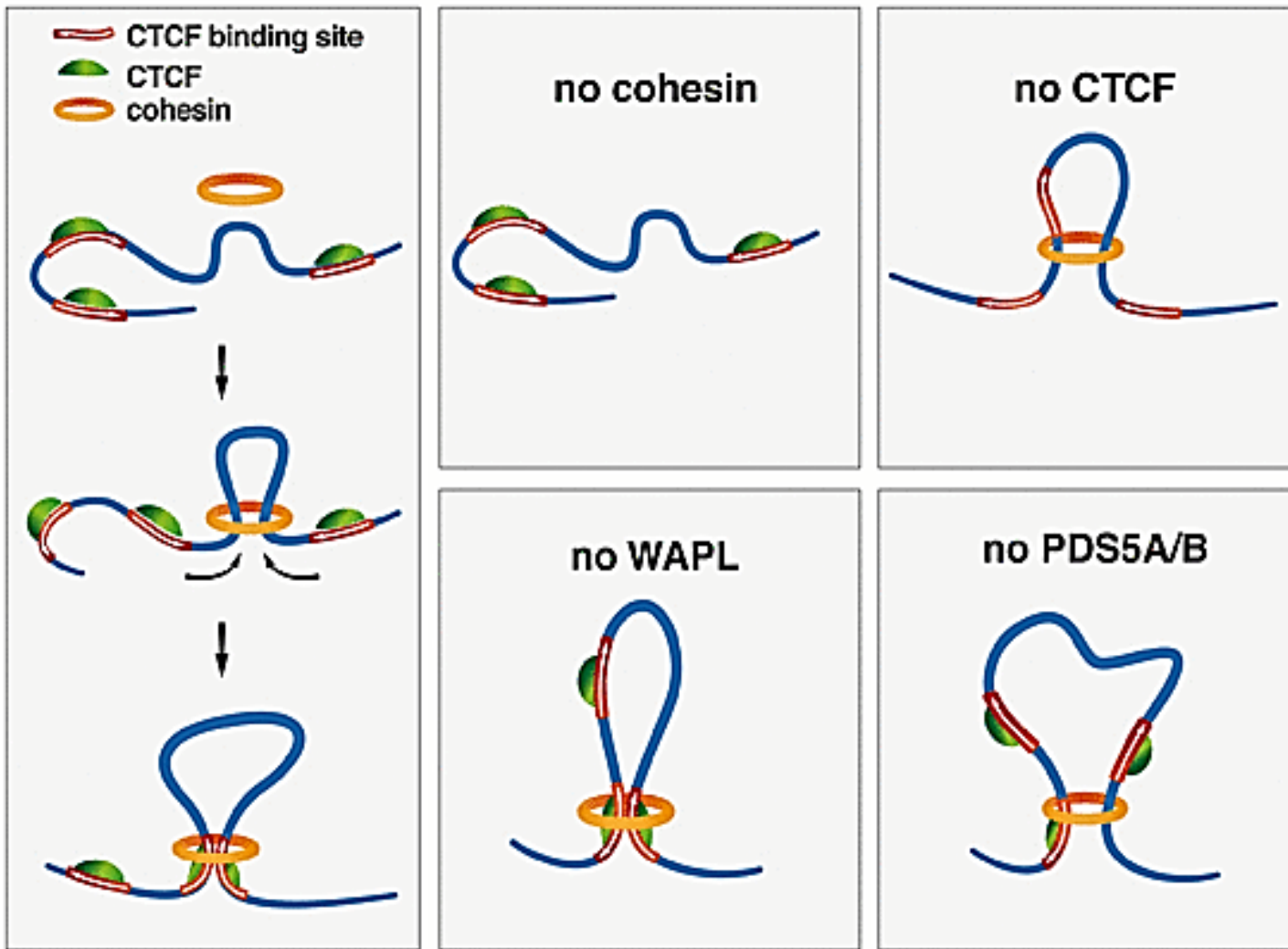
from Wutz et al (2017)
EMBO J, 36:3573.

A model has been proposed where unidentified forces drive DNA loops into the cohesin ring, then side 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, pssobly by inhibiting CTCF-cohesin interaction.

Cohesin stabilizes long-range interactions.

Cohesins mediate looping also in a CTCF-independent fashion, in ESC. In this case, the cohesin loading factor NIPBL and Mediators are found in complex with cohesins at enhancers.

Question: does DNA methylation at insulators regulate CTCF binding and, as a consequence, TAD organization?


c Embryonic stem cells

Enhancer   Promoter   OCT4

RNA polymerase II

Mediator

H3.3/H2A.Z

Cohesin

DNase I hypersensitive sites
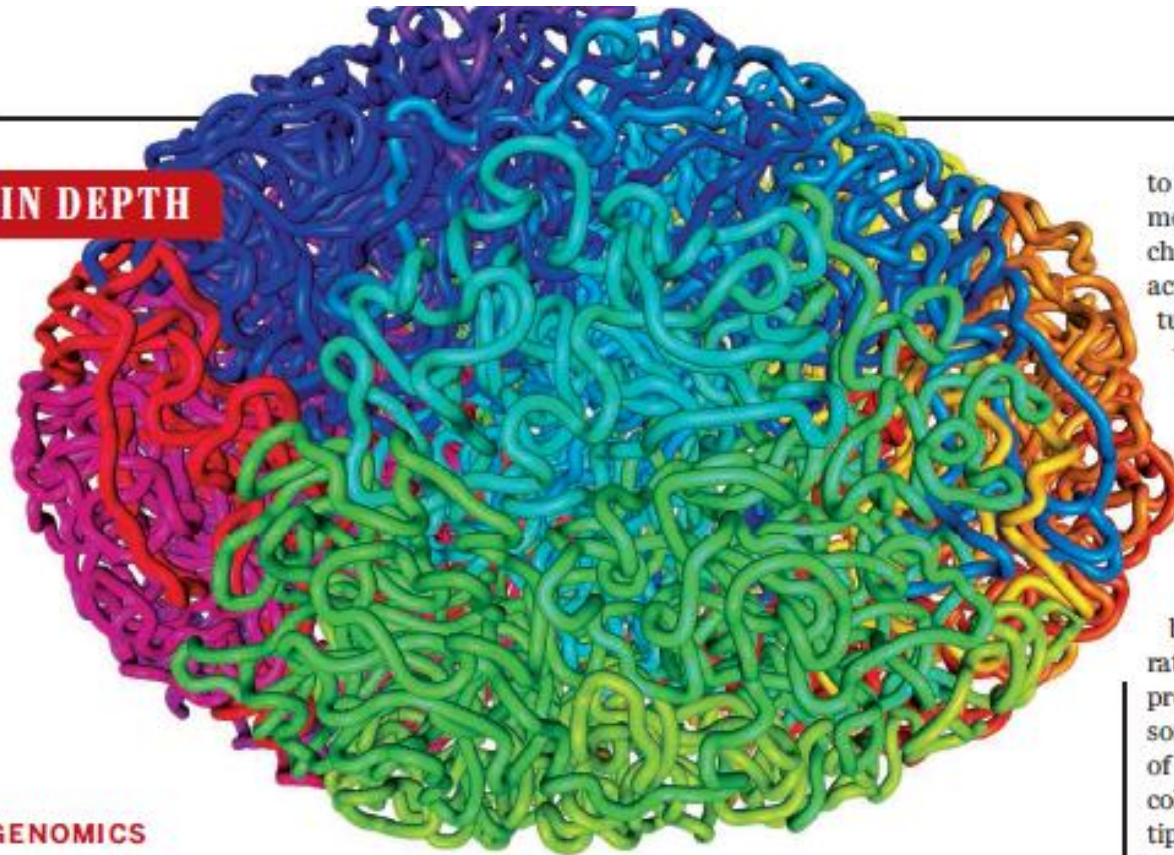
Co-activator or transcription factor

CTCF

Question: does DNA methylation at insulators regulate CTCF binding and, as a consequence, TAD organization?

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

C. Cubeñas-Potts, V.G. Corces/FEBS Letters 589 (2015) 2923–2930
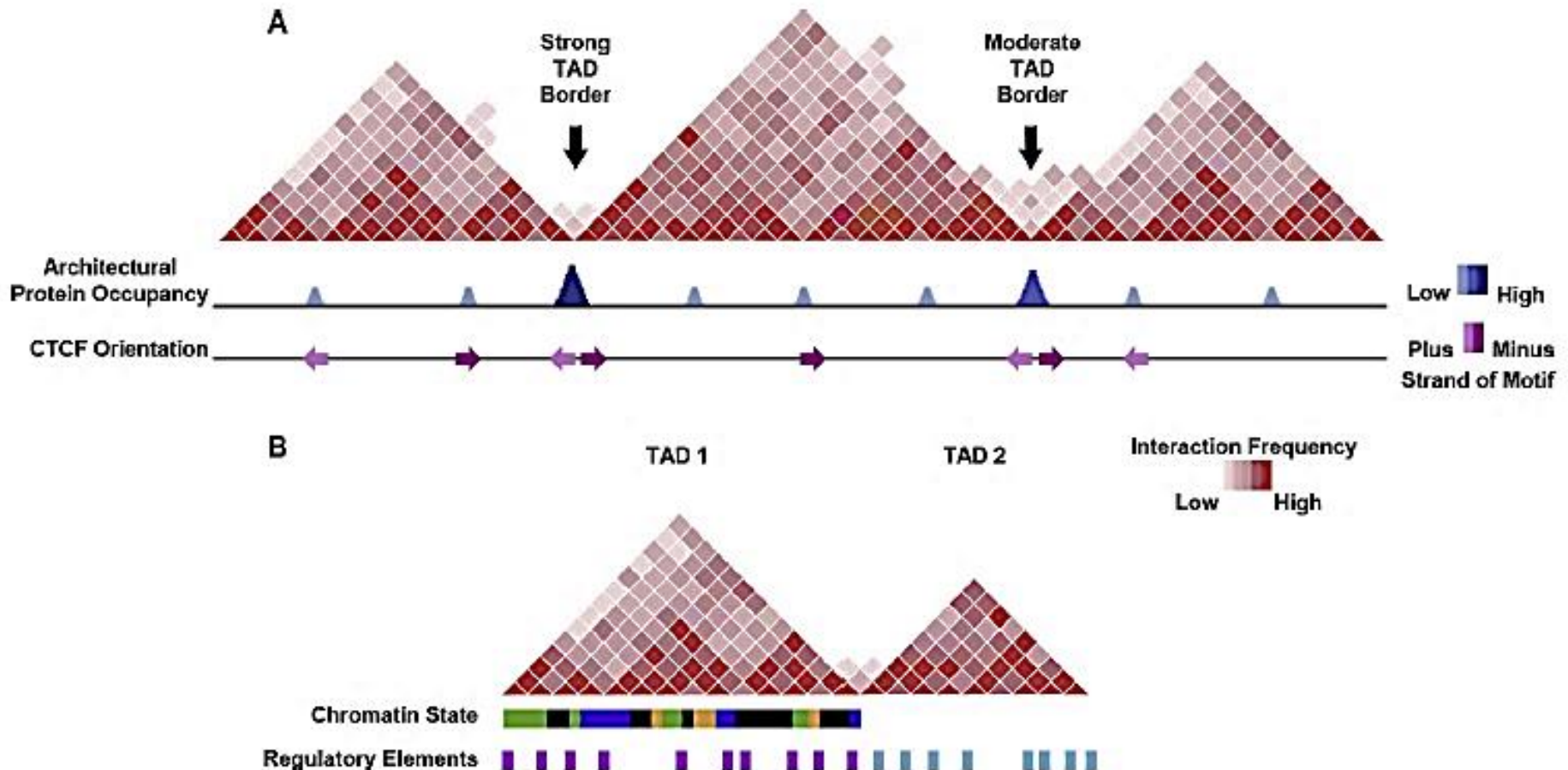
GENOMICS

Inching toward the 3D genome

Maps of DNA's loops and folds advance—but may disagree

By **Elizabeth Pennisi**

different and better ideas," says Job Dekker, a biologist at the University of Massachu-

to millions of bases long, and six compartments, regions where DNA with similar chemical modifications and levels of gene activity come together. Some of the structures are common to all the tested cell types, whereas others are unique to each kind of cell. "It opens up a new way of looking at biology," says Vishy Iyer, a molecular biologist at the University of Texas, Austin.

There's one wrinkle, as the *Genes & Development* paper showed: A different nucleome mapping technique based on direct observation of the DNA rather than on computational models can produce conflicting results. Iain Williamson and Wendy Bickmore of the University of Edinburgh in the United Kingdom and colleagues applied fluorescent labels to multiple pieces of DNA, using a different fluorescent probe for each one so that they could easily identify pieces that were close to each other. The researchers looked at a 1-million-base-long region of mouse chromosome 2, which contains a cluster of *Hox* genes that are key in development. For comparison, they analyzed the same DNA region using a computational technique similar to Hi-C

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.

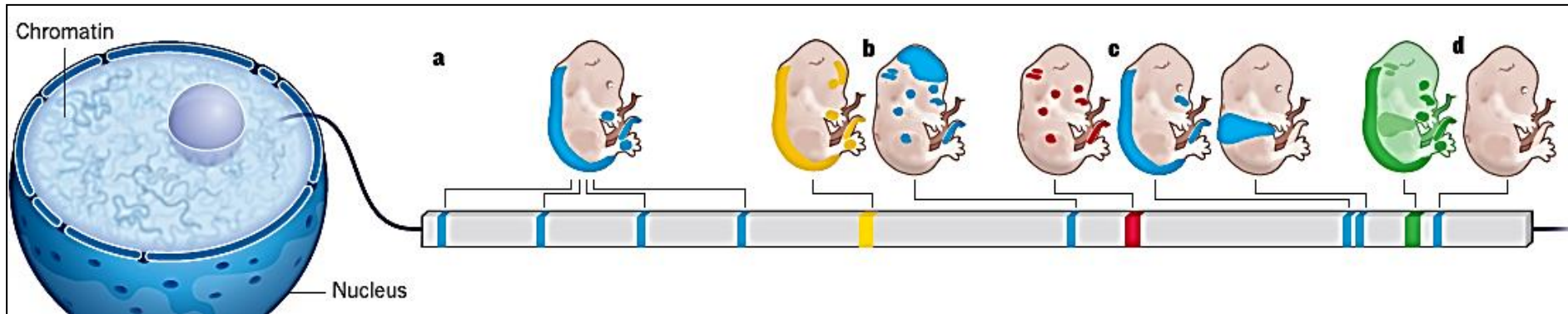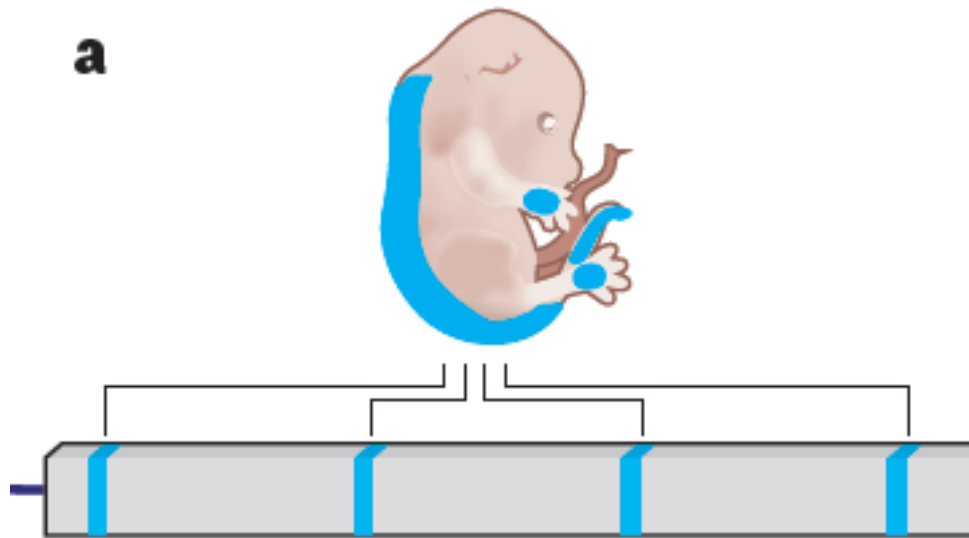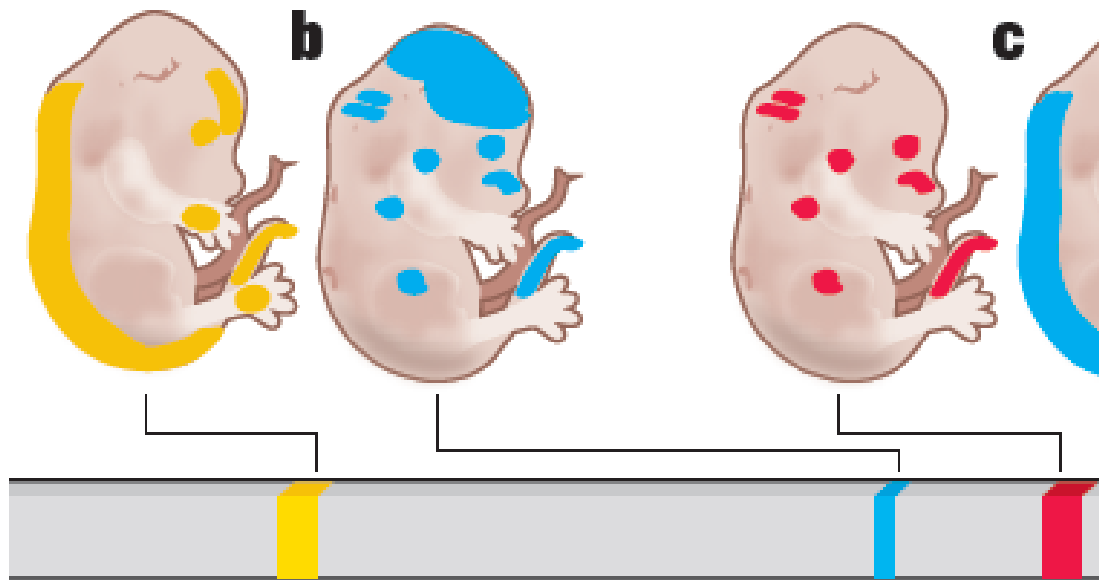One way to study this is to make transgenic animals carrying reporter genes

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.
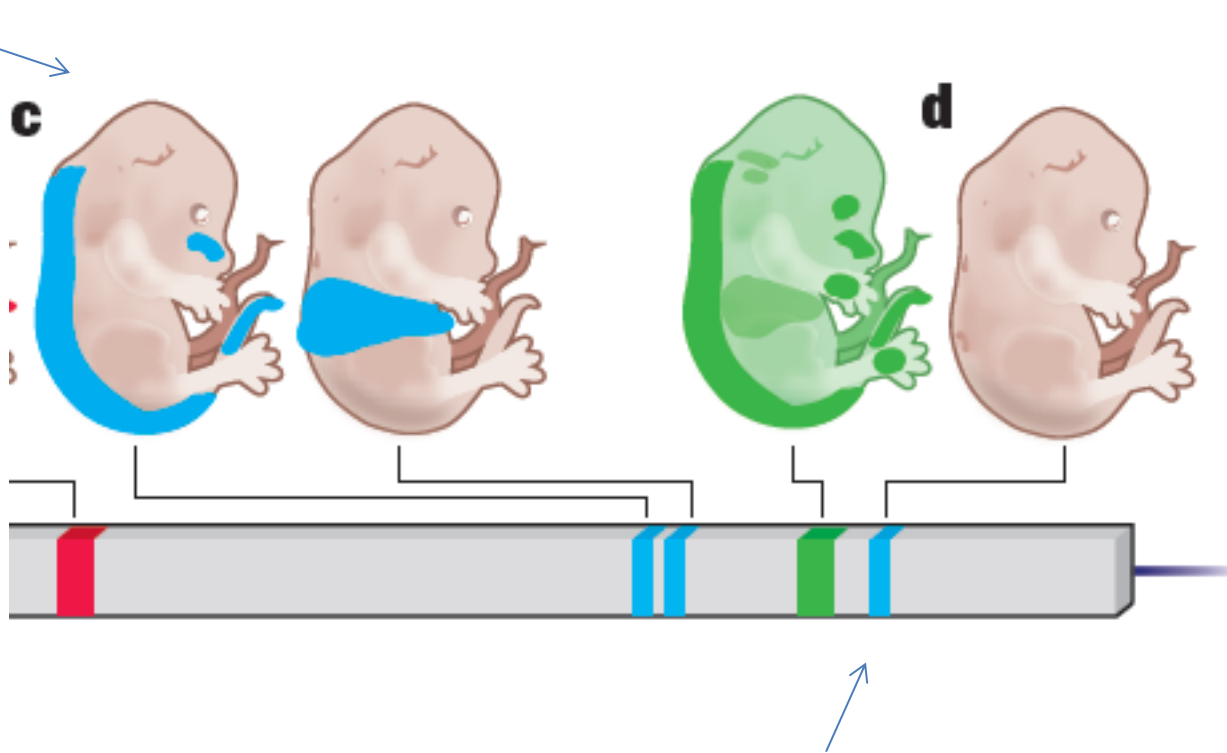
De Laat & Duboule, 2013

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.

LCR and Super-Enhancers

Apparently, development or cell-fate programming enhancers are grouped togeter to define Large regulatory regions that are called **Super-Enhancers** (SE).

In active SEs, a very high signal of H3K27ac is seen spanning several thousand bp; several centers of eRNA transcription may be present.
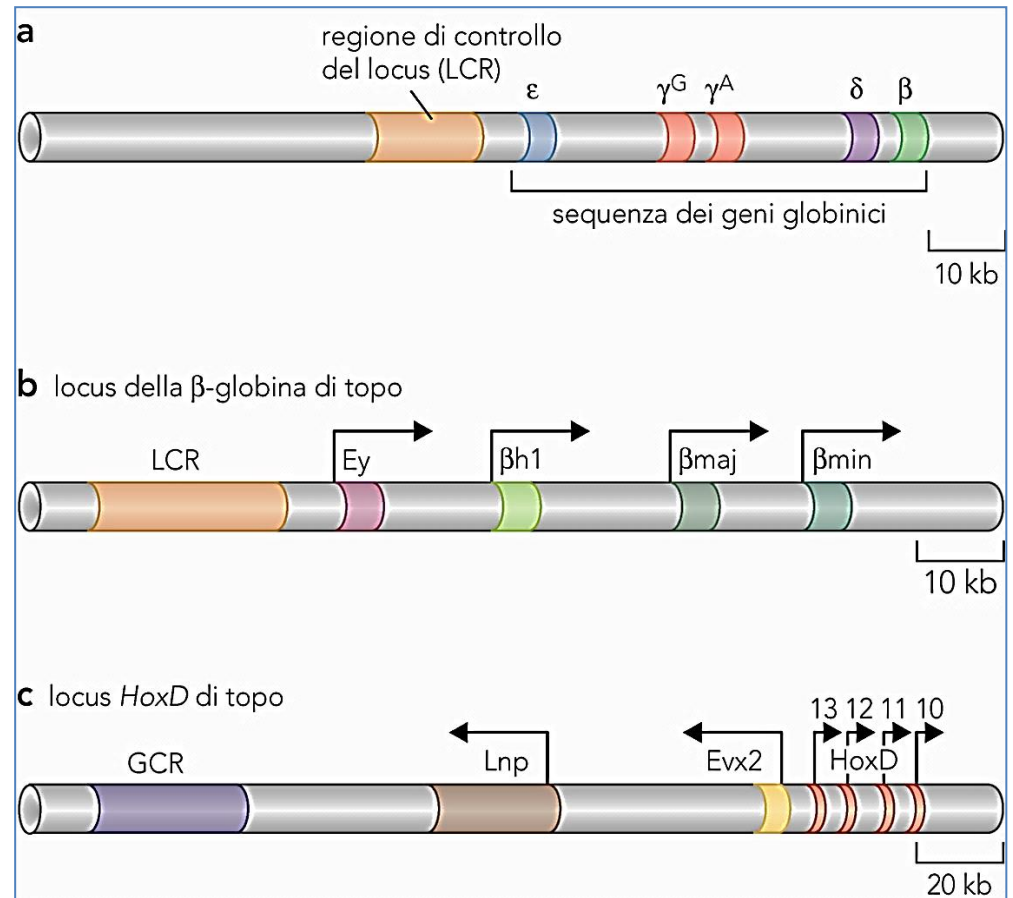
It is thought that these SE are essential regulators of cell-specific genes.

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.

a — regione di controllo del locus (LCR) — ε — γ^G γ^A — δ β — sequenza dei geni globinici — 10 kb

b locus della β-globina di topo — LCR — Ey — βh1 — βmaj — βmin — 10 kb

c locus *HoxD* di topo — GCR — Lnp — Evx2 — HoxD — 13 12 11 10 — 20 kb

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

Bas Tolhuis,[2] Robert-Jan Palstra,[2] Erik Splinter,
Frank Grosveld, and Wouter de Laat[1]
Department of Cell Biology and Genetics
Faculty of Medicine
Erasmus University, Rotterdam
P.O. Box 1738
3000DR Rotterdam
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.

Gene clusters that derive from gene duplication events are often controlled by one or more common enhancer module. These common control regions are called LCR (*locus control region*).

Some 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.

The β-globin locus in *M. musculus*

LCR   Ey   βh1   βmaj   βmin

10 kb
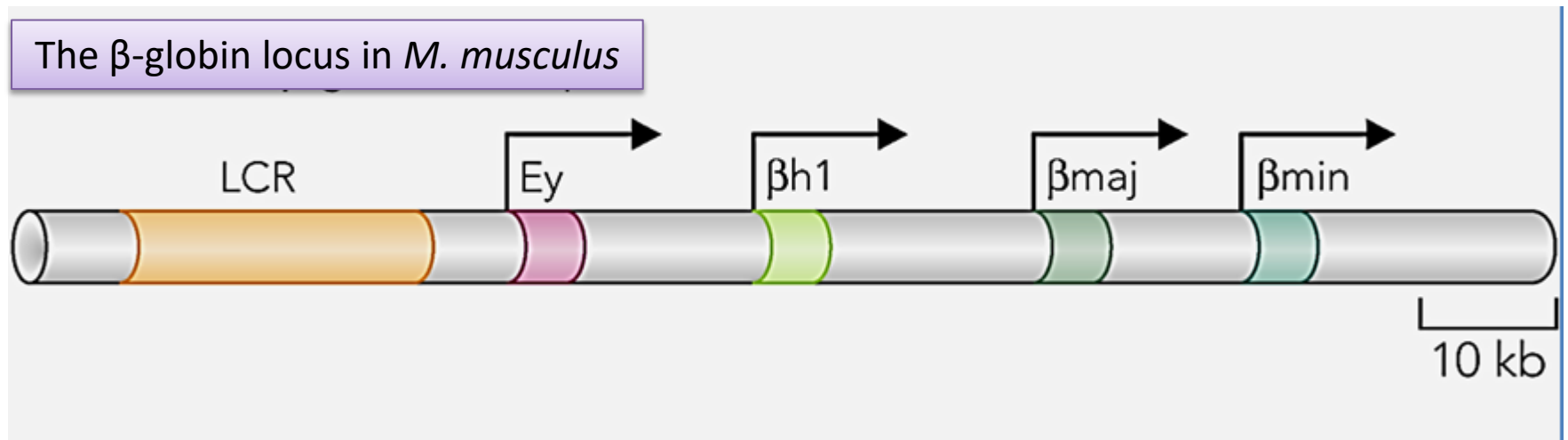
Another example is given by the gene clusters encoding homeoproteins, which are expressed following a precise spatial order in the body.
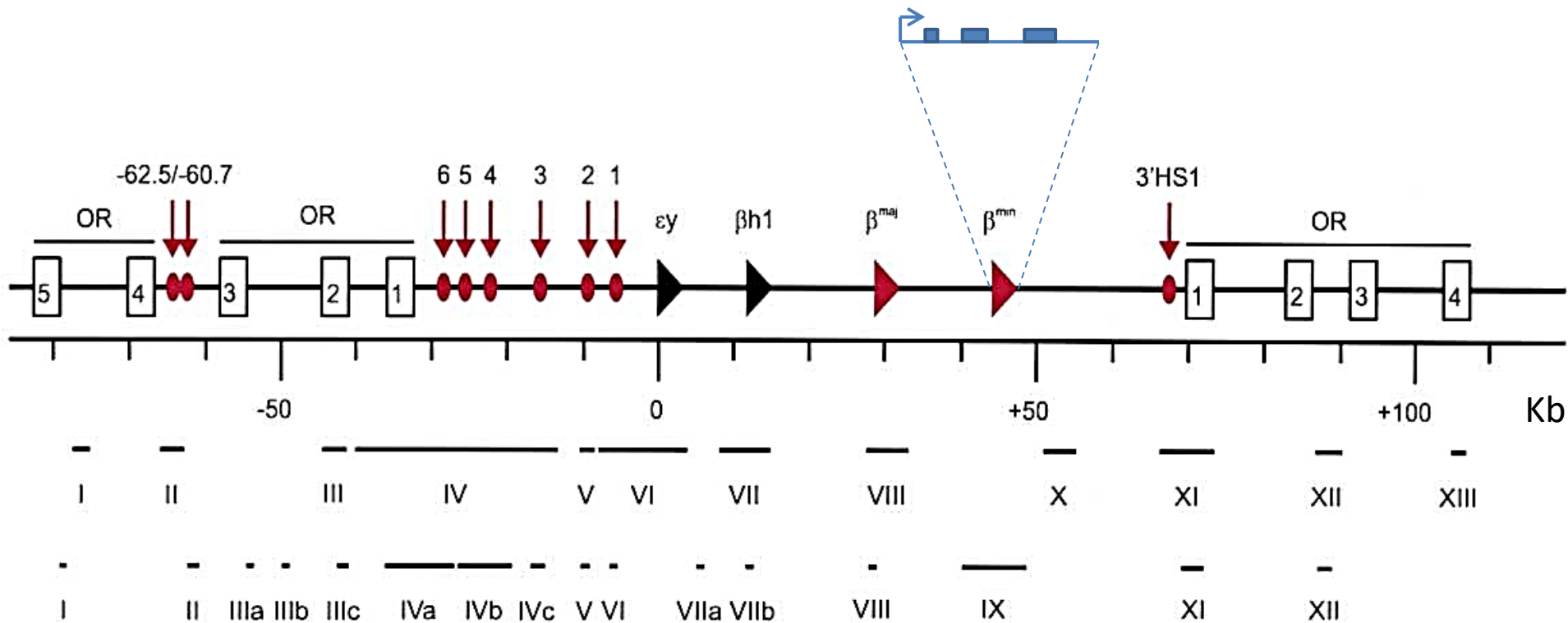
Figure 1. 3C Technology in the Murine beta-globin Locus
(A) Schematic presentation of the murine **beta-globin** locus. Red arrows and ellipses depict the individual HS. The globin genes are indicated by triangles, with **active genes (maj and min) in red** and **inactive genes (y and h1) in black**. 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.

Embryo 14.5dpc

Liver (expressing) Brain (non-expressing)

cross-linking/digestion

intramolecular ligation

reverse cross-links/quantitative PCR

DNA fragments

Control (random)

mix equimolar/digestion
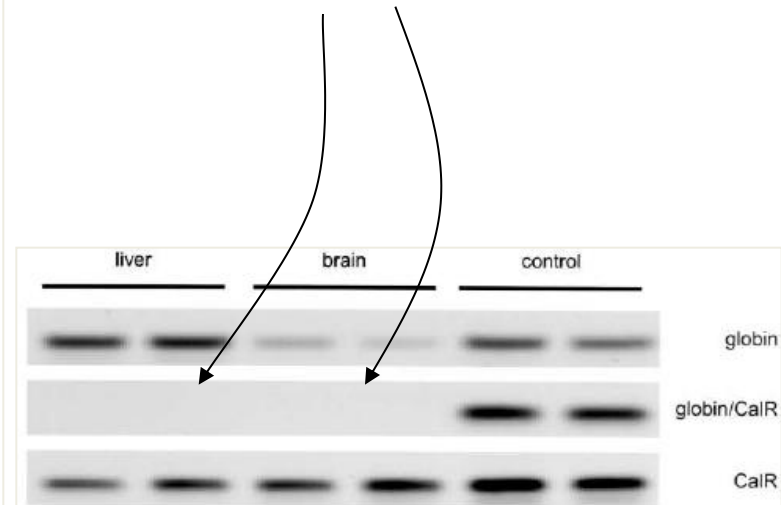
intermolecular ligation

add to gDNA/quantitative PCR

globin

globin/CalR

CalR

In 3C analysis, high-dilution ligase step guarantees intramolecular ligation and virtually no intermolecular ligation

liver   brain   control

globin

globin/CalR

CalR

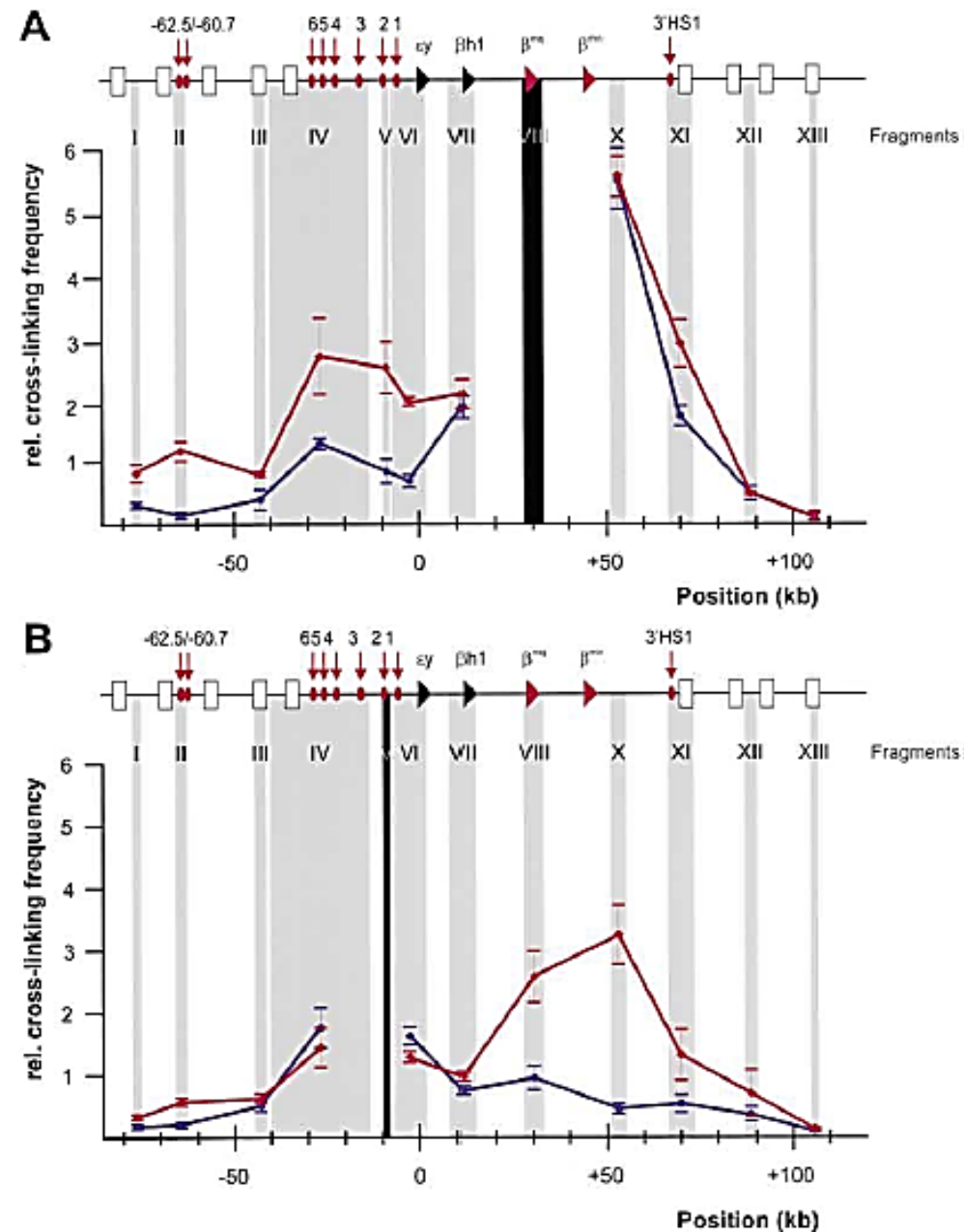$$X(gl) = \frac{[A(gl) / A(CalR)]_{tissue}}{[A(gl) / A(CalR)]_{control}}$$

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.
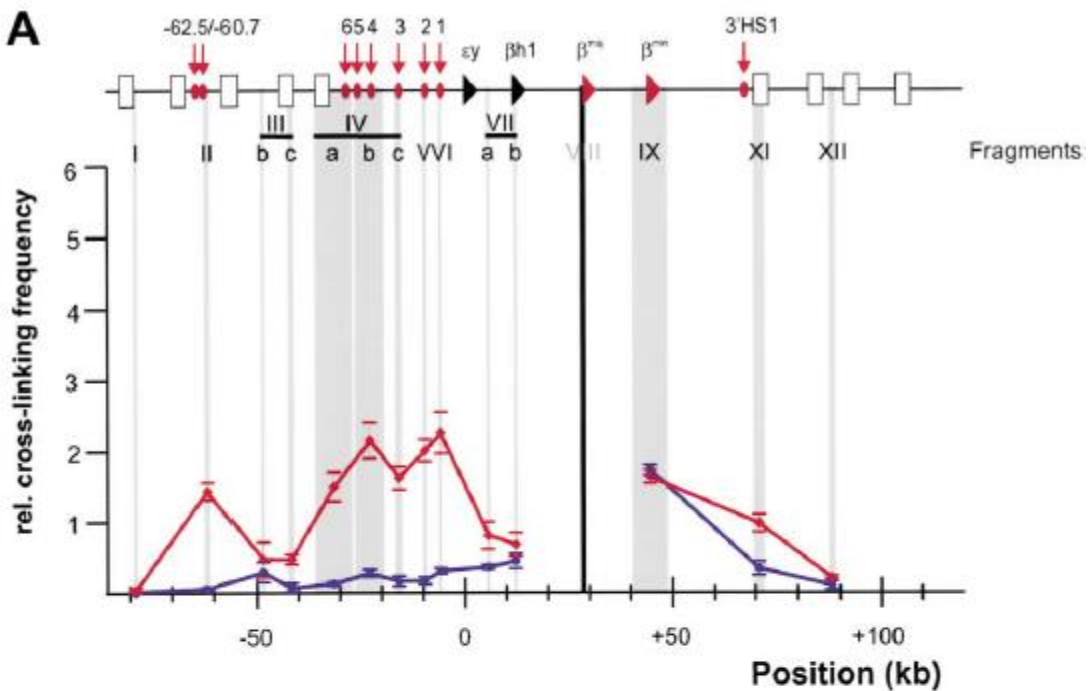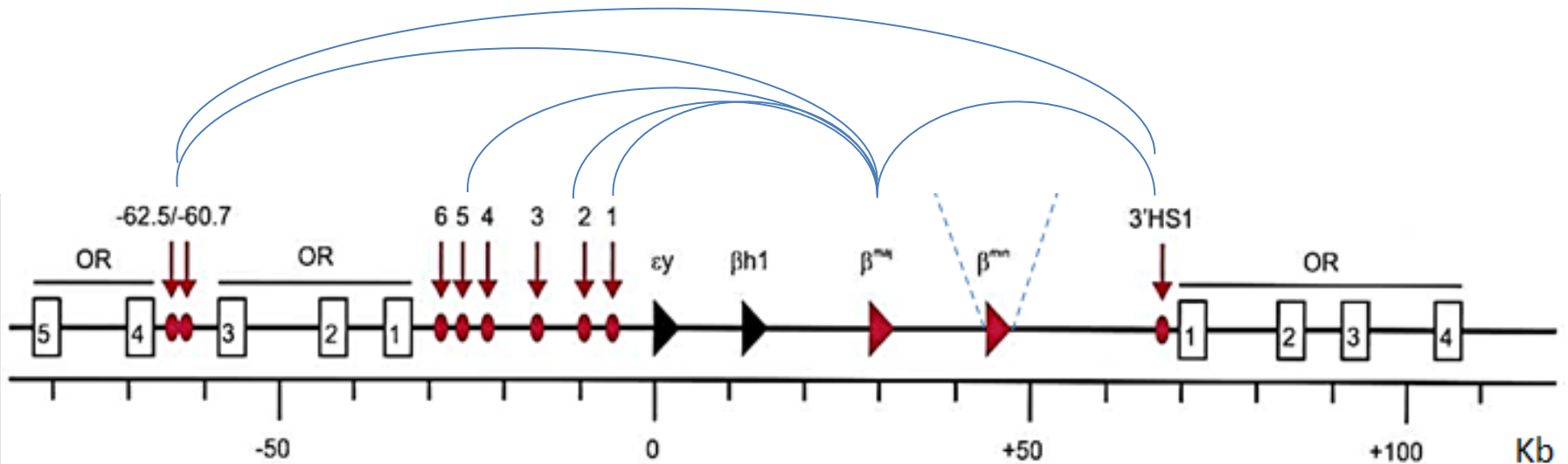
Figure 4. **Erythroid**-Specific Interactions between the Active beta-globin Genes and Individual Hypersensitive Sites in the LCR. Relative crosslinking frequencies observed in fetal liver (red) and brain (blue) are shown. Standard error of the mean is indicated. Crosslinking frequency with a value of 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 other figures.

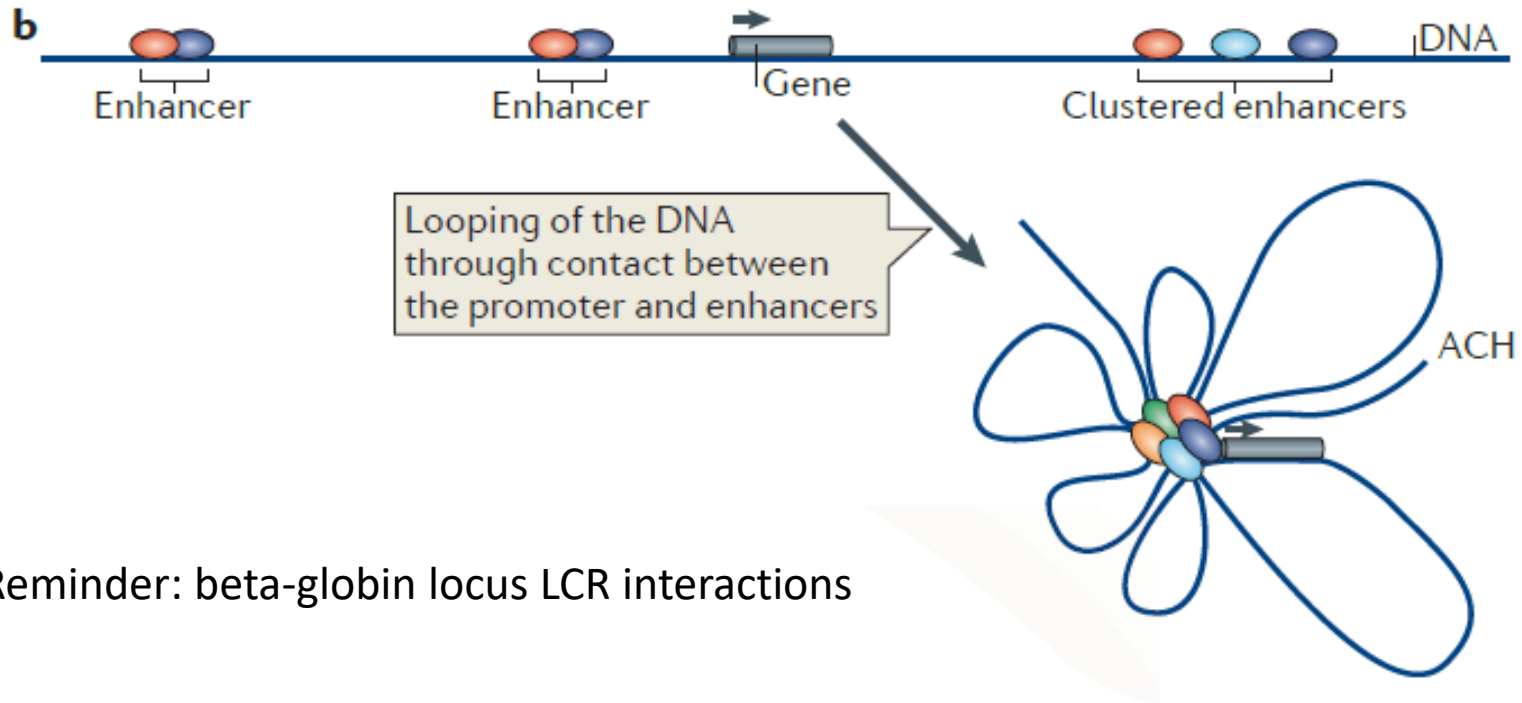(A) Fixed HindIII fragment VIII Bmaj versus the rest of the locus.

(B) Fixed HindIII fragment IX (Bmin) versus the rest of the locus.
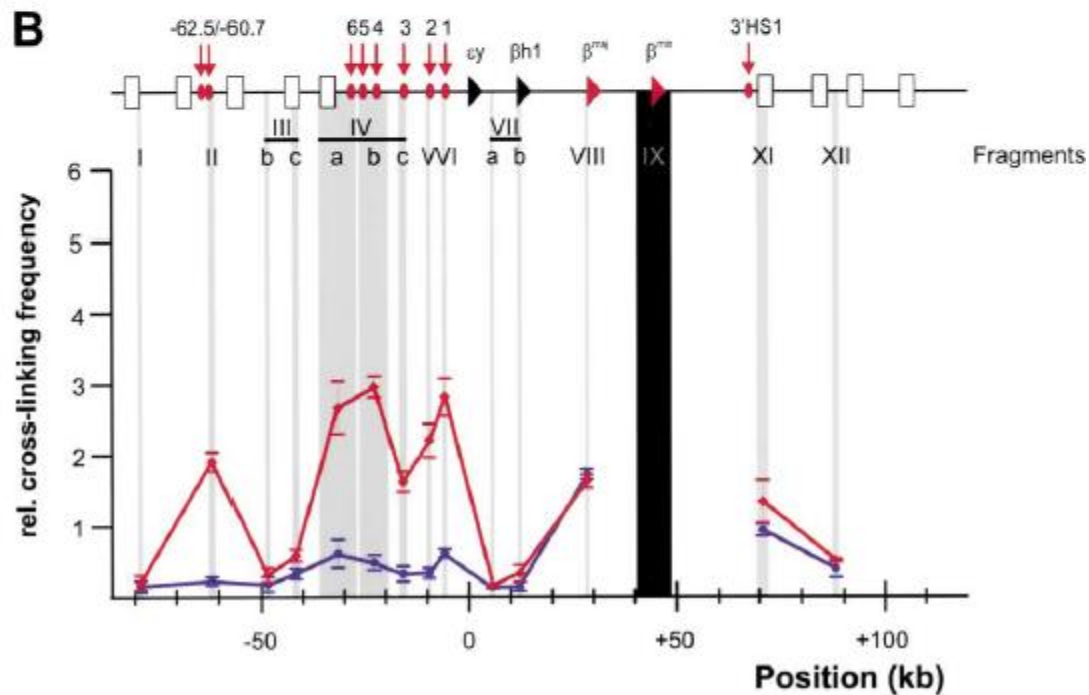
This data demonstrated multiple «looping» involving components of the super-enhancer LCR, other enhancers and promoters of the active B genes
(the example below shows only some of the contacts verified)

The concept of transcriptional «hub»

b

Enhancer   Enhancer   Gene   Clustered enhancers   DNA

Looping of the DNA
through contact between
the promoter and enhancers

ACH

Reminder: beta-globin locus LCR interactions

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

Pombo & Dillon, 2015

Looping mechanisms

Pombo & Dillon, 2015

a

H3K27ac
H3K4me1 and H3K4me2
SDTF SDTF LDTF CTFs
Enhancer
Pol II
eRNA

Looping-promoting factors:
- Mediator complex
- Non-specific RNA-binding proteins?
- Cohesin

Co-activators
CTFs
Promoter
Pol II
mRNA
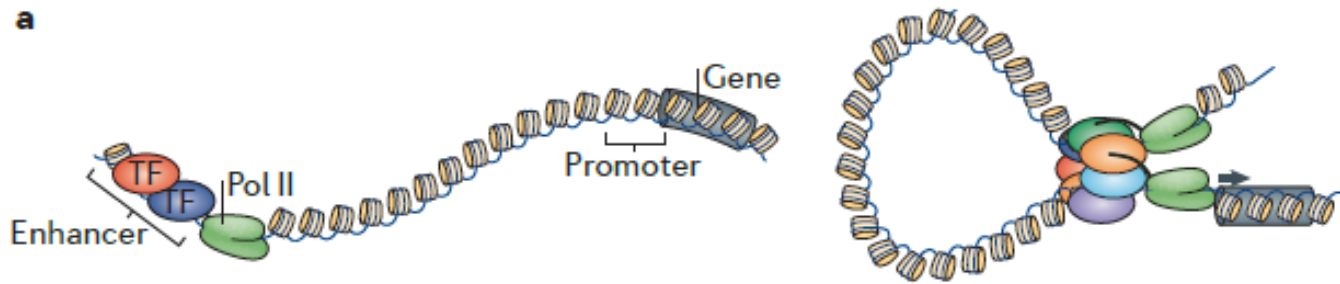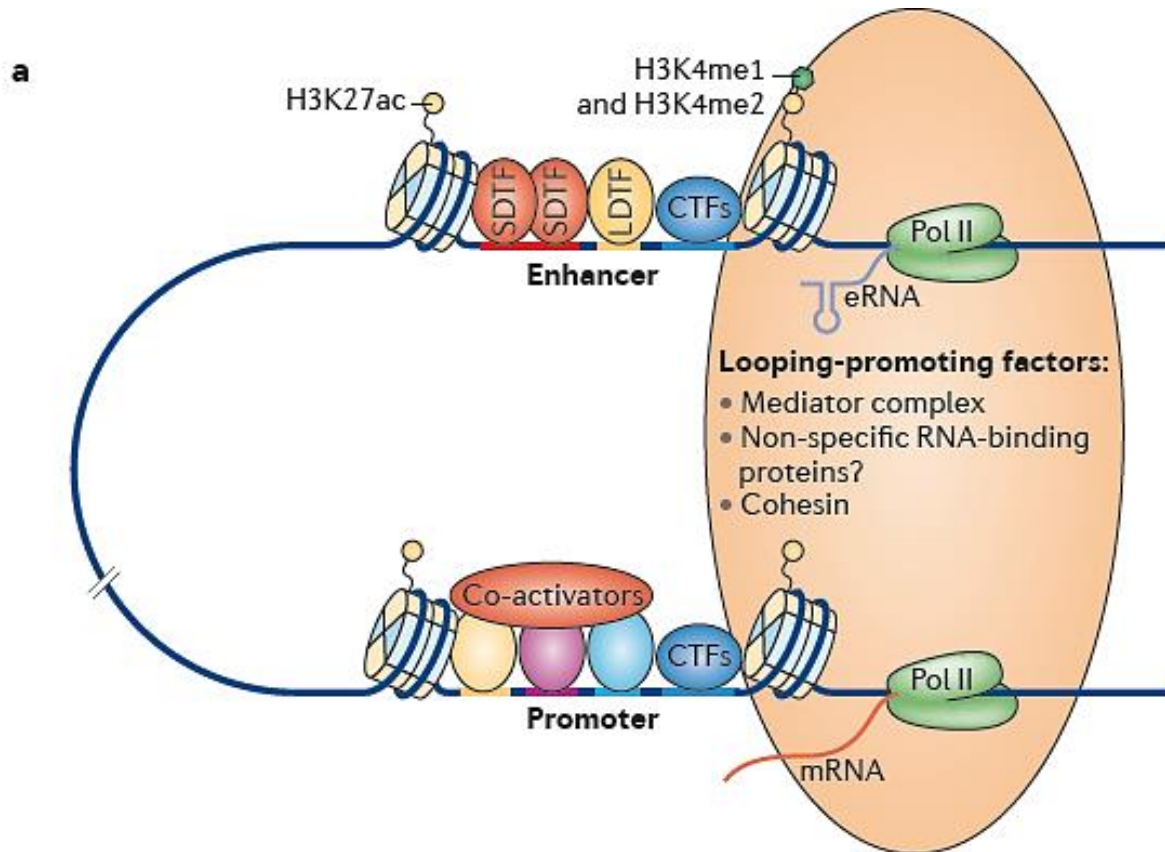
What is the molecular mechanism of looping ?

Heinz et al., 2015

Mediator, as well as a number of coactivator complexes, are multi-subunit proteins.

Interaction of individual subunits with a series of Transcription Factors was demonstrated (CoIP+ reconstruction of transcription in vitro)

Even though interaction of Mediator subunits were observed with basal PIC components, there is a need of proximal TFBS for a promoter to interact with an enhancer.

Sequence element

Transcription factor

Enhancer

Mediator

CoActivators

Pol II+Basal complex

P

Transcribed sequence

Proximal regulatory elements
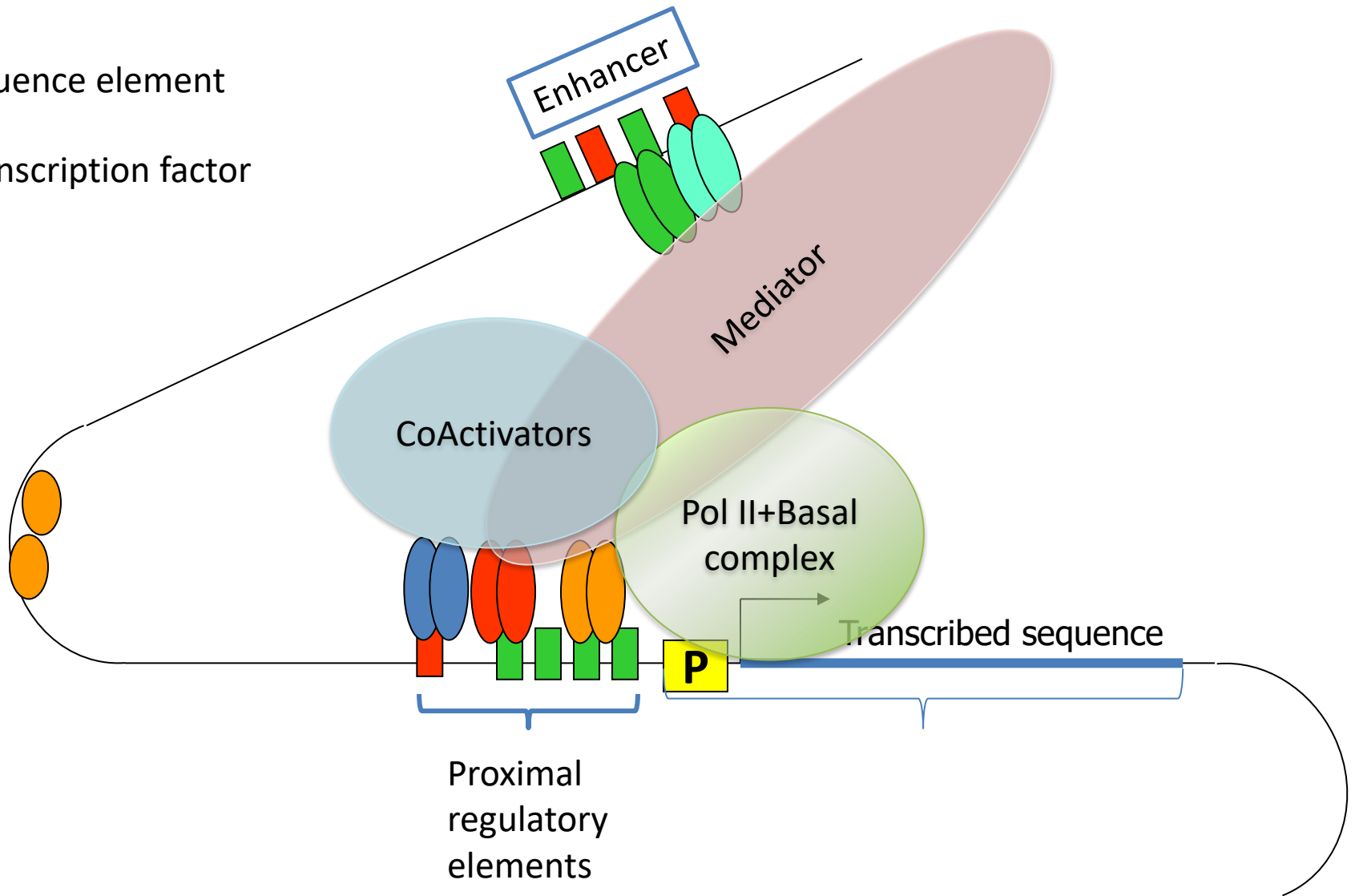
Coactivators and Corepressors:

Large protein complexes exhibiting several functions:

- Interaction with Transcription Factors (sequence-specific TF)

- Histone PTM writers/erasers (in particular, HAT histone acetyl transferase)

- Chromatin remodeling factors

  - (ATP-dependent chromatin remodelers are a class of proteins that «remodel» nucleosomes over DNA, usually in ATP-dependent fashion)

CoA and **CoR** may be recruited:

a) directly by Transcription Factors

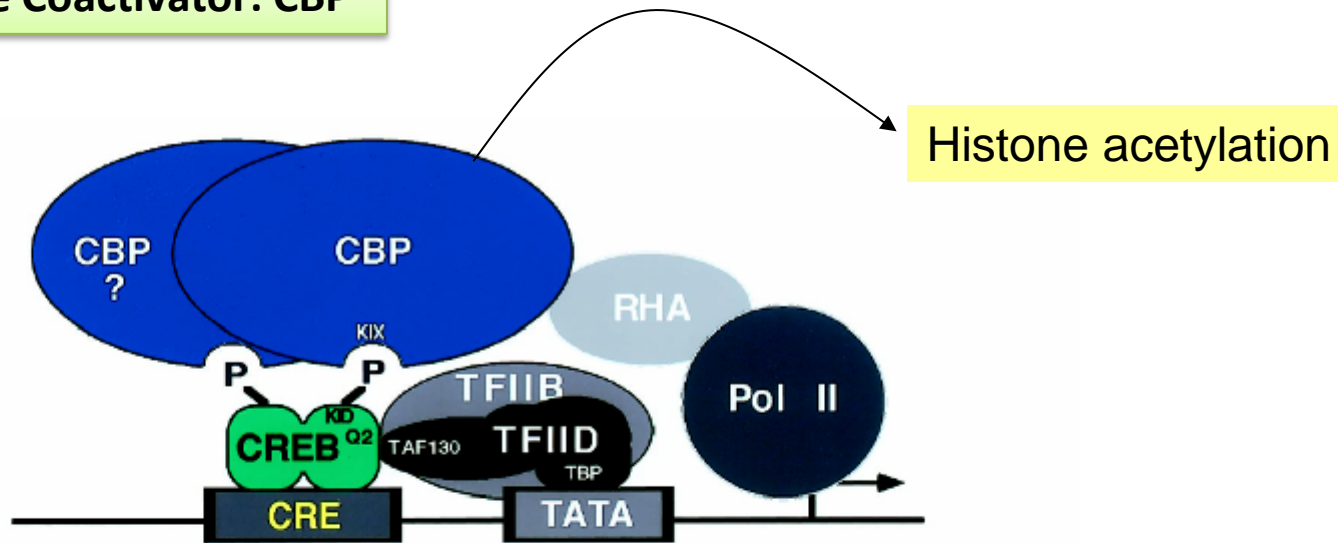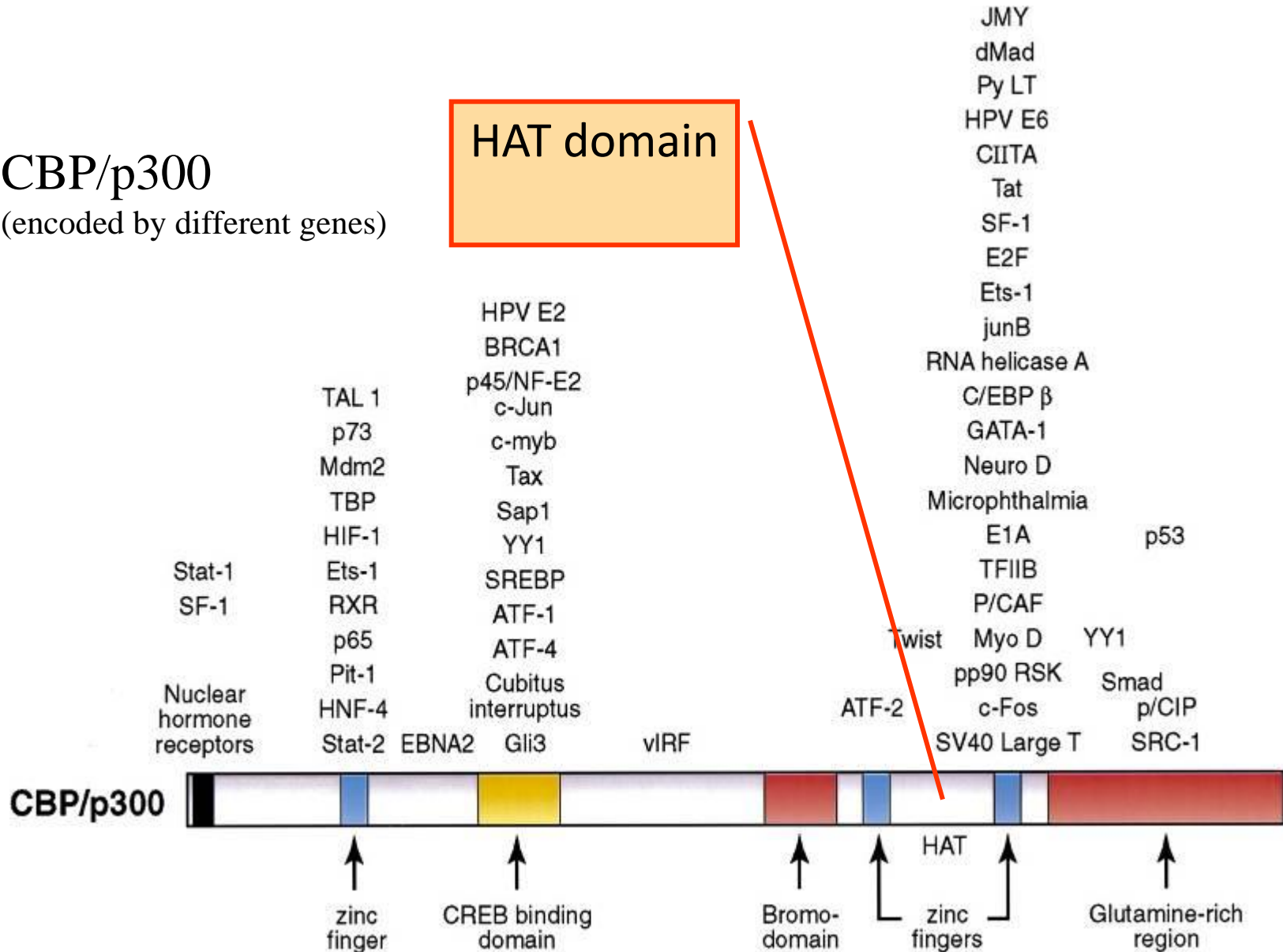b) through interaction with PIC or Mediator subunits

Figure 4 Multiple domains of CREB contribute to transcriptional activation. Different domains of CREB bind distinct coactivators and basal transcription factors to activate transcription. Shown is a CREB dimer bound to its cognate CaRE/CRE element on the promoter of a CREB target gene. Downstream of the CaRE/CRE is the TATA box, which binds the multiprotein TFIID basal transcription factor (via the TBP protein). Another factor within TFIID, TAF130, binds to the Q2 domain of CREB. The Q2 domain of CREB has also been shown to interact with TFIIB, which is a part of the basal transcription machinery as well. A distinct domain of CREB, the KID, contributes to signal-induced transcriptional activation. When phosphorylated at Ser133, the KID of CREB can bind to the KIX domain of the CBP. It is presently unclear whether CBP associates with Ser133–phosphorylated CREB as a dimer. CBP associates indirectly with Pol II via the RNA helicase A (RHA) protein. Therefore, recruitment of CBP to Ser133–phosphorylated CREB results in recruitment and stabilization of Pol II on the promoter of CREB target genes, whereas the Q2 domain interacts with other elements of the basal transcription machinery that are required for transcription, such as TFIID and TFIIB.

CBP/p300
(encoded by different genes)

HAT domain

JMY
dMad
Py LT
HPV E6
CIITA
Tat
SF-1
E2F
Ets-1
junB
RNA helicase A
C/EBP β
GATA-1
Neuro D
Microphthalmia

HPV E2
BRCA1
p45/NF-E2
c-Jun
c-myb
Tax
Sap1
YY1
SREBP
ATF-1
ATF-4
Cubitus
interruptus

TAL 1
p73
Mdm2
TBP
HIF-1
Ets-1
RXR
p65
Pit-1
HNF-4

Stat-1
SF-1

Nuclear
hormone
receptors

Stat-2   EBNA2   Gli3

vIRF

E1A   p53
TFIIB
P/CAF
Twist   Myo D   YY1
pp90 RSK   Smad
ATF-2   c-Fos   p/CIP
SV40 Large T   SRC-1

CBP/p300

zinc
finger

CREB binding
domain

Bromo-
domain

HAT

zinc
fingers

Glutamine-rich
region

Co-activators participating in transcriptional activation by Nuclear Receptors

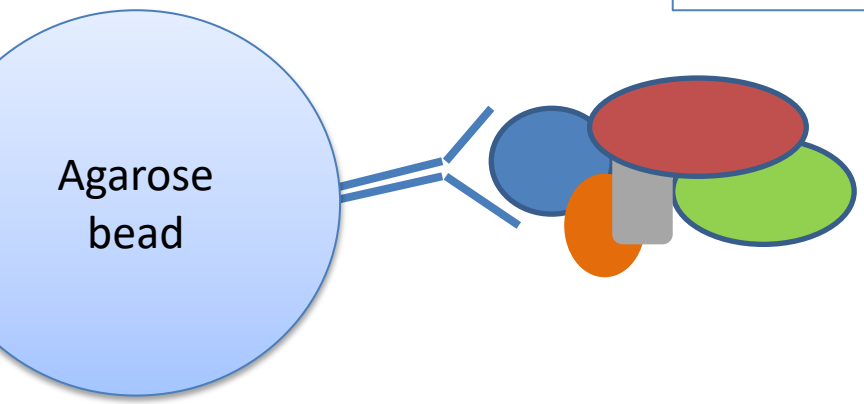From: Perissi & Rosenfed, 2005, Nature Rev Mol Cell Biol, 6: 542-554
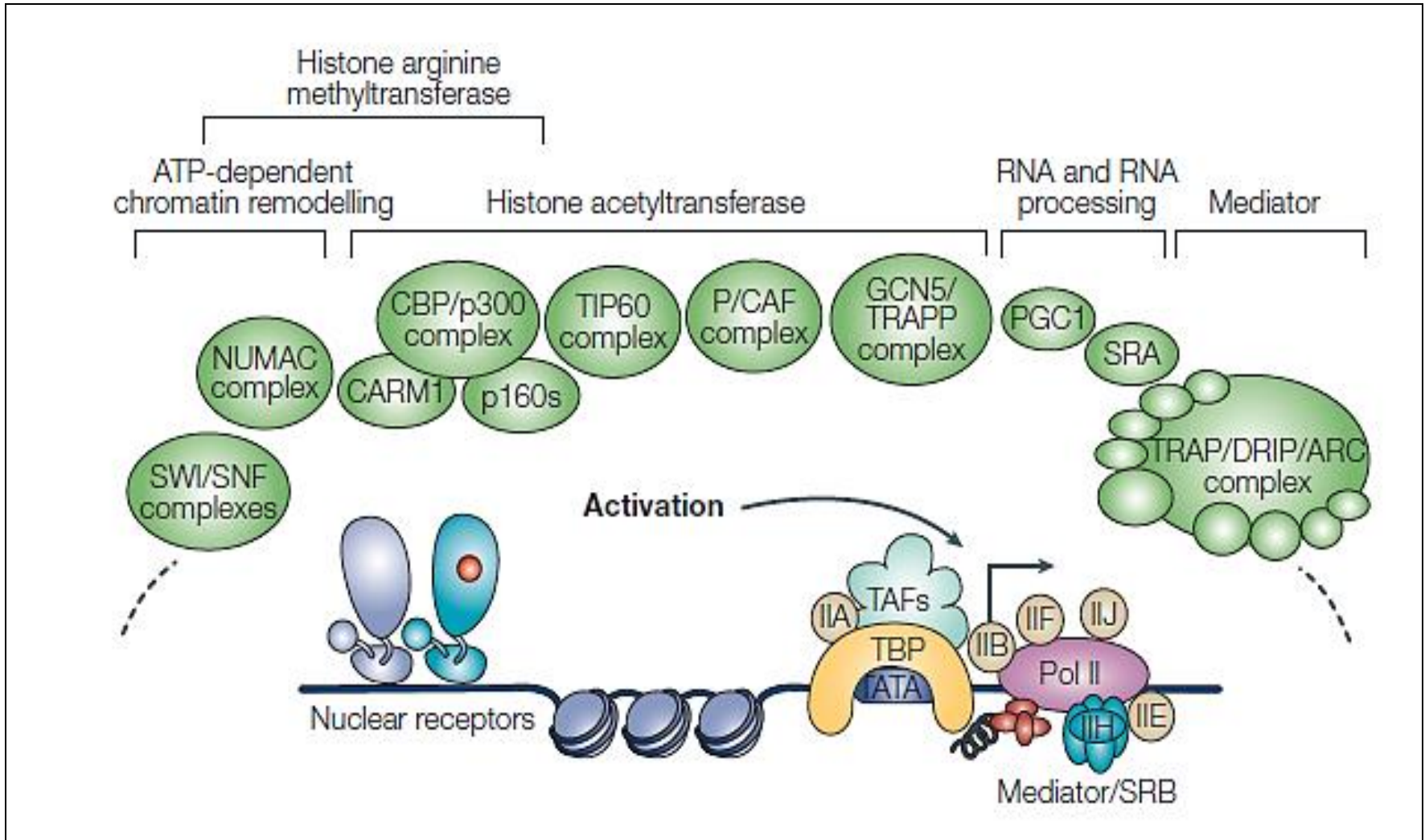
Mediator

Cells or tissue

Nuclei

2000-2005

Nuclear extract (high salt)

Immunoprecipitation using anti-TF Ab
(or other component)
or
using Tagged proteins and Ab against
Tag (e.g. FLAG or HA epitope)

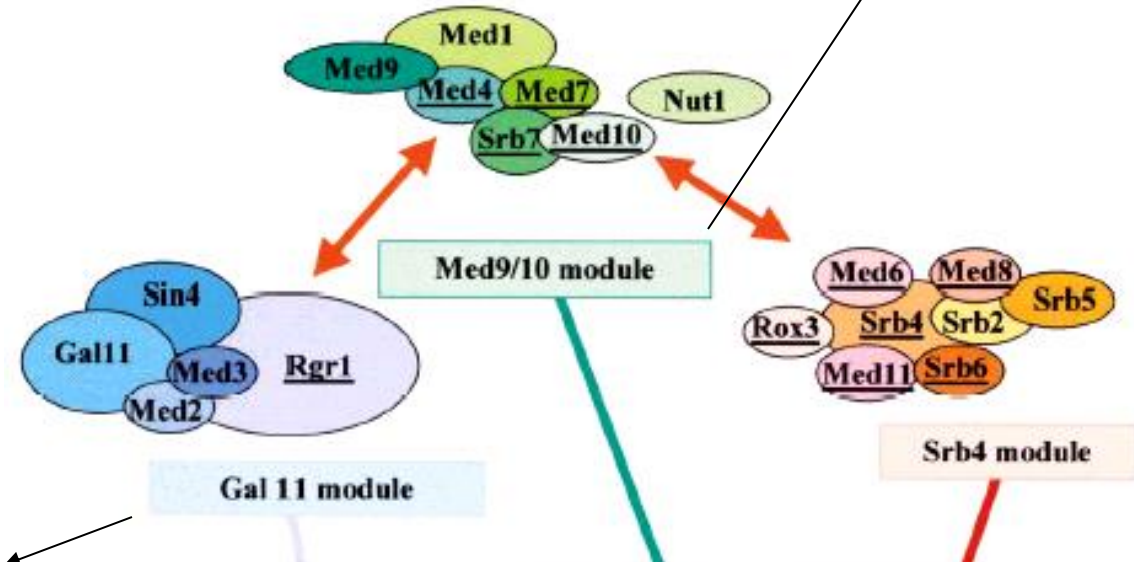Agarose
bead

Mass spectrometry (identify)
Immunoblotting (validate)

Subunit compositions of mediator complexes.

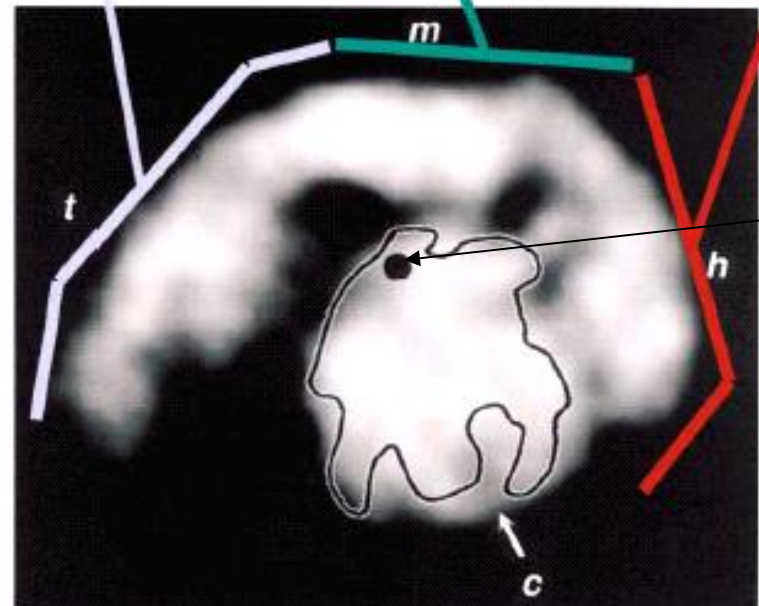| Unified subunit designation	DRIP Mediator-D	ARC Mediator-A	TRAP/SMCC Mediator-T/S	PC2 Mediator-P	CRSP Mediator-C	NAT Mediator-N	hMediator Mediator-S	Murine Mediator Mediator-M	S. cerevisiae	C. elegans	Drosophila
		CBP/p300									
Med240	DRIP250	ARC250	TRAP240				ND				dTRAP240
Med230	DRIP240	ARC240	TRAP230			p230	ND	p160a	Nut1	Sop-1	dTRAP230
Med220	DRIP205	ARC205	TRAP220	(TRAP220)	CRSP200		ND	p160b	Gal11		dTRAP220
Med150	DRIP150	ARC150	TRAP170	TRAP170	CRSP150	p150	ND	Rgr1/p110	Rgr1		dTRAP170
Med130	DRIP130	ARC130	TRAP150	TRAP150b	CRSP130	p140/hSur2	hSur2			Sur-2	CG3695
Med105		ARC105/TIG-1									
Med100	DRIP100	ARC100	TRAP100	TRAP100			ND		Sin4		dTRAP100
Med97	DRIP97		TRAP97			p95		Ring3/p96a	Srb4		
Med95	DRIP92	ARC92	TRAP95	TRAP95		p90	ND	p96b	Med1		dTRAP95
			TRAP93								
Med78	DRIP77	ARC77	TRAP80	TRAP80	CRSP77		ND	p78			dTRAP80
Med70	DRIP70-2	ARC70			CRSP70	p70	ND		Med2		
		ARC42		p37				p55	Pgd1/Hrs1		
Cdk8	(Cdk8)	(Cdk8)	hSrb10			p56/Cdk8	Cdk8		Srb10		dCdk8
Med36	DRIP36	ARC36		p36	CRSP34	p45	ND	p34	Med4		CG8609
Med34	DRIP34	ARC34	hMed7	hMed7	CRSP33	p37	Med7	Med7/p36	Med7	ceMed7	dMed7
						p36			Srb5		
Med33	DRIP33	ARC33	hMed6	(hMed6)		p33	ND	Med6/p32	Med6	ceMed6	CG9473
		ARC32	hTRF	hTRF			ND	TRF/p28a	Med8		
Cyclin C			hSrb11			p31/Cyclin C	Cyclin C		Srb11		
						p30		p28b	Rox3		
						p23			Srb2		
			hSoh1	hSoh1		p22					
						p21			Med9/Cse2		
Med17	hSrb7		hSrb7	hSrb7		p17	ND	Srb7/p21	Srb7	ceSrb7	CG17397
Med10	hMed10		hNut2	hNut2		p14	ND		Med10/Nut2	ceMed10	dNut2
									Med11		
									Srb6		

Mediator

Med1
Med9
Med4
Med7
Nut1
Srb7
Med10

Interactcs with CTD
Both activator and repressor signal
Transmitter to Pol

Med9/10 module

Sin4
Gal11
Med3
Rgr1
Med2

Gal 11 module

Med6
Med8
Srb5
Rox3
Srb4
Srb2
Med11
Srb6

Srb4 module
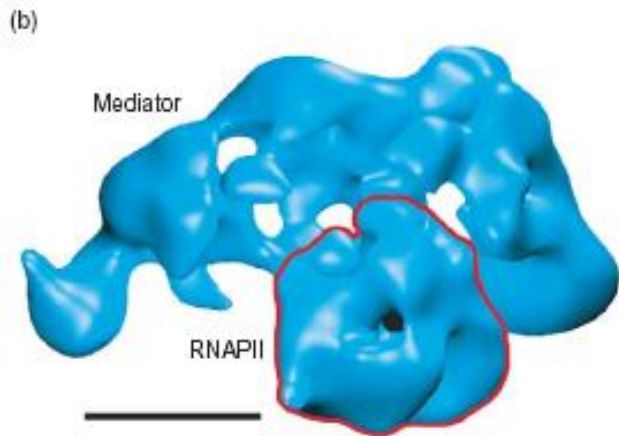
Role in contacting
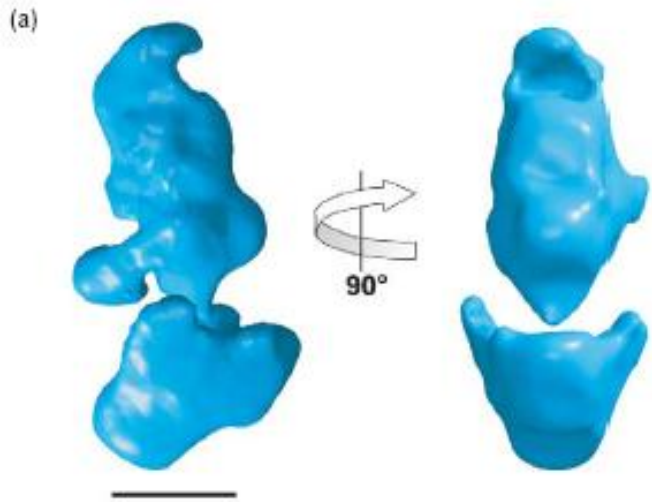trans-activators

m

t

h

CTD

c

Figure 1. Structure of the yeast Mediator and holoenzyme complexes.

(a) A 3D reconstruction of the yeast Mediator structure was calculated from images of individual particles imaged in an electron microscope after preservation in stain. Mediator has a compact, roughly triangular shape. A large domain at the bottom is linked by a thin connection to the top portion of the structure. The resolution of the reconstruction is 35- A° , and the scale bar represents 100 A° .

(b) (b) Structure of the Mediator–RNA polymerase II holoenzyme complex calculated from electron microscope images of individual particles preserved in stain. Previous characterization of the polymerase and Mediator structures led to identification of the Mediator and RNA polymerase II (red outline) portions of the holoenzyme structure. In the holoenzyme, Mediator adopts an extended conformation, embracing the central polymerase density. The resolution of the reconstruction is w35 A° , and the scale bar represents 100 A° .
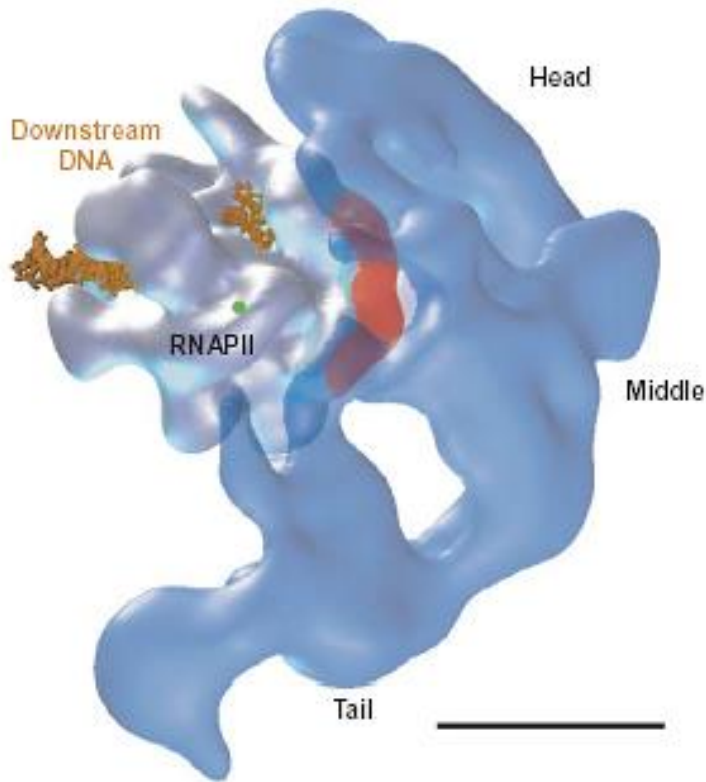
Figure 3. Interaction of Mediator and RNA polymerase II (RNAPII) in the holoenzyme complex. The precise orientation of RNAPII in the holoenzyme complex was established by 2D cross-correlation analysis between holoenzyme and RNAPII projections. The figure shows a cryoelectron microscopy reconstruction of polymerase fitted into the extended Mediator structure in the orientation determined by cross-correlation analysis. Multiple contacts between Mediator and RNAPII are established in the holoenzyme complex, involving mostly the head and middle domains, and distributed around the Rpb3–Rpb11 polymerase subunits (highlighted in red). The small green circle indicates the point where the carboxyterminal domain of Rpb1 (the largest polymerase subunit), crucial for Mediator polymerase interaction, emanates from the surface of the enzyme. The bacterial homolog of the Rpb3–Rpb11 complex, the a2 homodimer, is involved in transcription regulation in bacteria, suggesting a conservation between prokaryotes and eukaryotes of the RNA polymerase surface involved in regulation. The scale bar represents 100 A° .

Different forms of Mediator exist in different cell types/developmental stage and possibly gene context, depending on the kind of TFs bound.
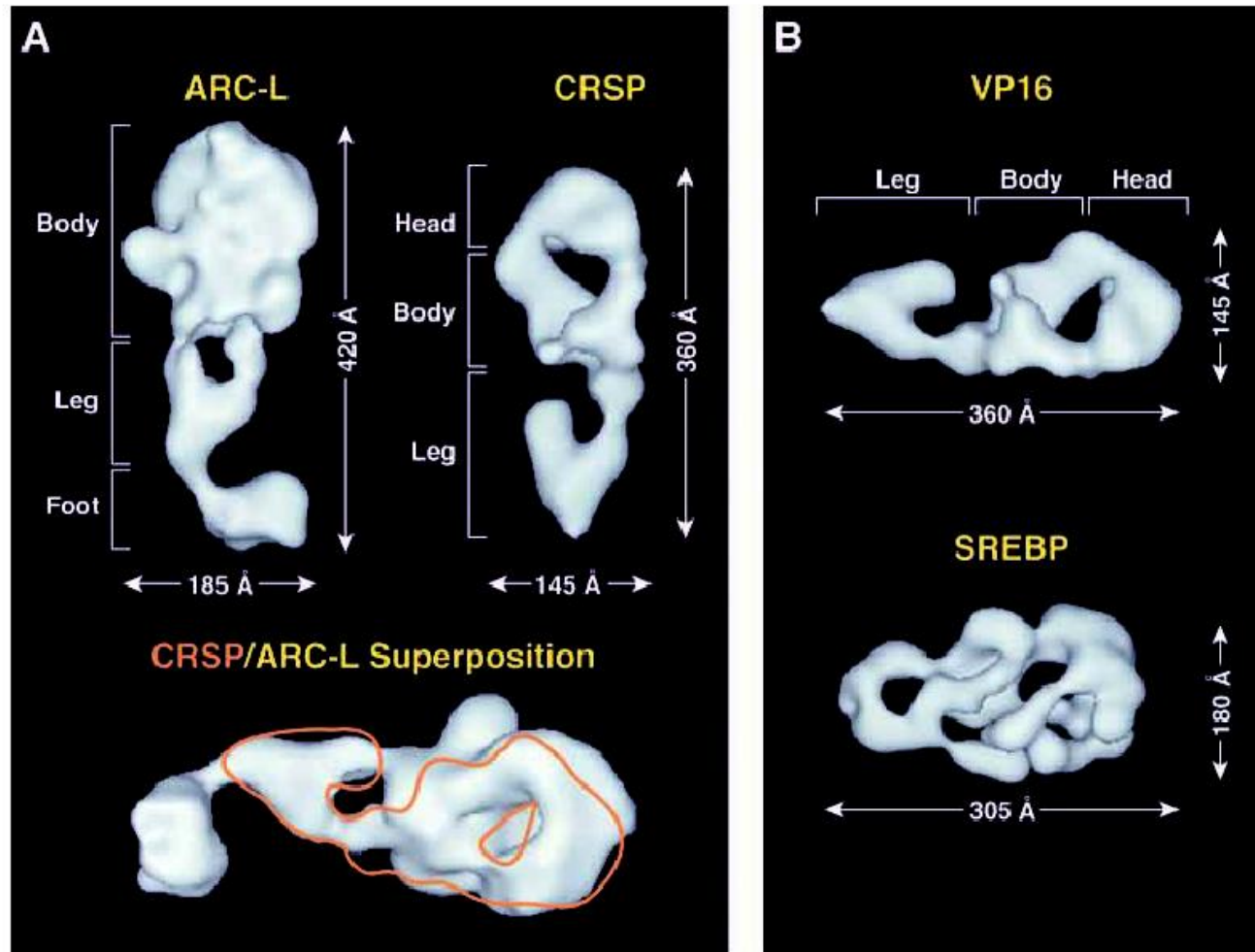

Fig. 2. Conformations of the mammalian mediator complexes. (A) EM composites of the ARC-L and CRSP complexes, which illustrate the size and structural differences between the two. (B) EM composites showing the distinctly different structural conformations adopted by CRSP when isolated via affinity interactions with either the VP16 or SREBP activator. EM composites were generously provided by Dylan Taatjes and Bob Tjian (Naar et al., 2002; Taatjes et al., 2002).

Take-home message:

Mediator is a large, multi-subunit protein complex. showing both common and facutative subunits.

The composition, shape and MW is dictated by the context where Mediator operates, i.e. TFs bound to Enhancers and Promoters involved in any specific interaction.

RNA in the loop ?

Model of ncRNA-a function as described by Lai et al. (2013).
An ncRNA-a interacts with the multisubunit Mediator complex to facilitate the formation of a long-range DNA loop, bringing the enhancer-like ncRNAa locus into physical proximity with its target locus. This then leads to robust expression of the target gene.
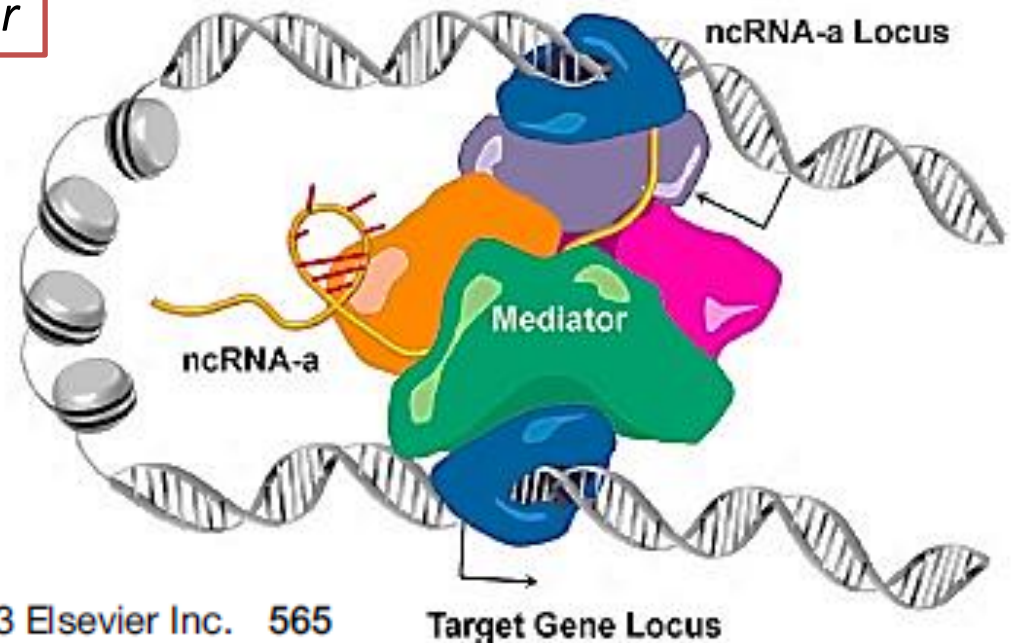
ncRNA-a = noncoding RNA activator

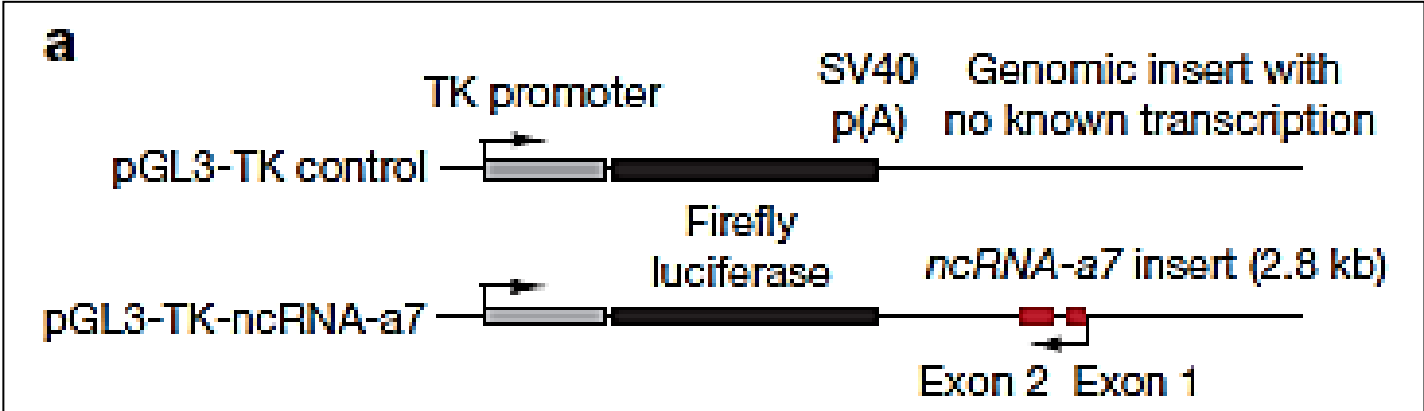Figure 1. Schematics of lncRNA-Directed cis-Regulatory Mechanisms

LETTER

Activating RNAs associate with Mediator to enhance chromatin architecture and transcription

Fan Lai[1], Ulf A. Orom[2], Matteo Cesaroni[1], Malte Beringer[3], Dylan J. Taatjes[4], Gerd A. Blobel[5] & Ramin Shiekhattar[1]
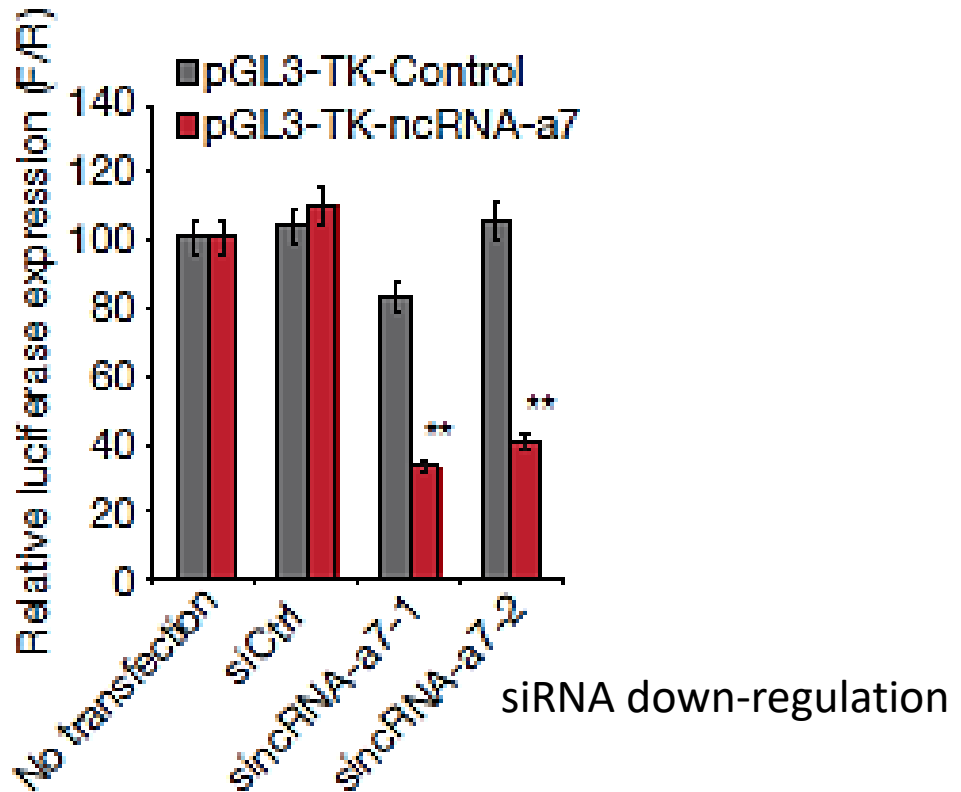
In previous work they found lncRNAs with enhancer-like properties:
A class of lncRNAs, termed **ncRNA-activating** (ncRNA-a), that function to activate their neighbouring genes using a cis-mediated mechanism.

They systhematically siRNA noncoding RNAs and identified neighbouring down-regulated genes

1st question: is activation ncRNA dependent ?

a

TK promoter — SV40 p(A) — Genomic insert with no known transcription

pGL3-TK control

Firefly luciferase

pGL3-TK-ncRNA-a7 — ncRNA-a7 insert (2.8 kb)

Exon 2   Exon 1

HEK293 cells
(Human embryonal kidney)

■ pGL3-TK-Control
■ pGL3-TK-ncRNA-a7

Relative luciferase expression (F/R)

No transfection, siCtrl, sincRNA-a7-1, sincRNA-a7-2
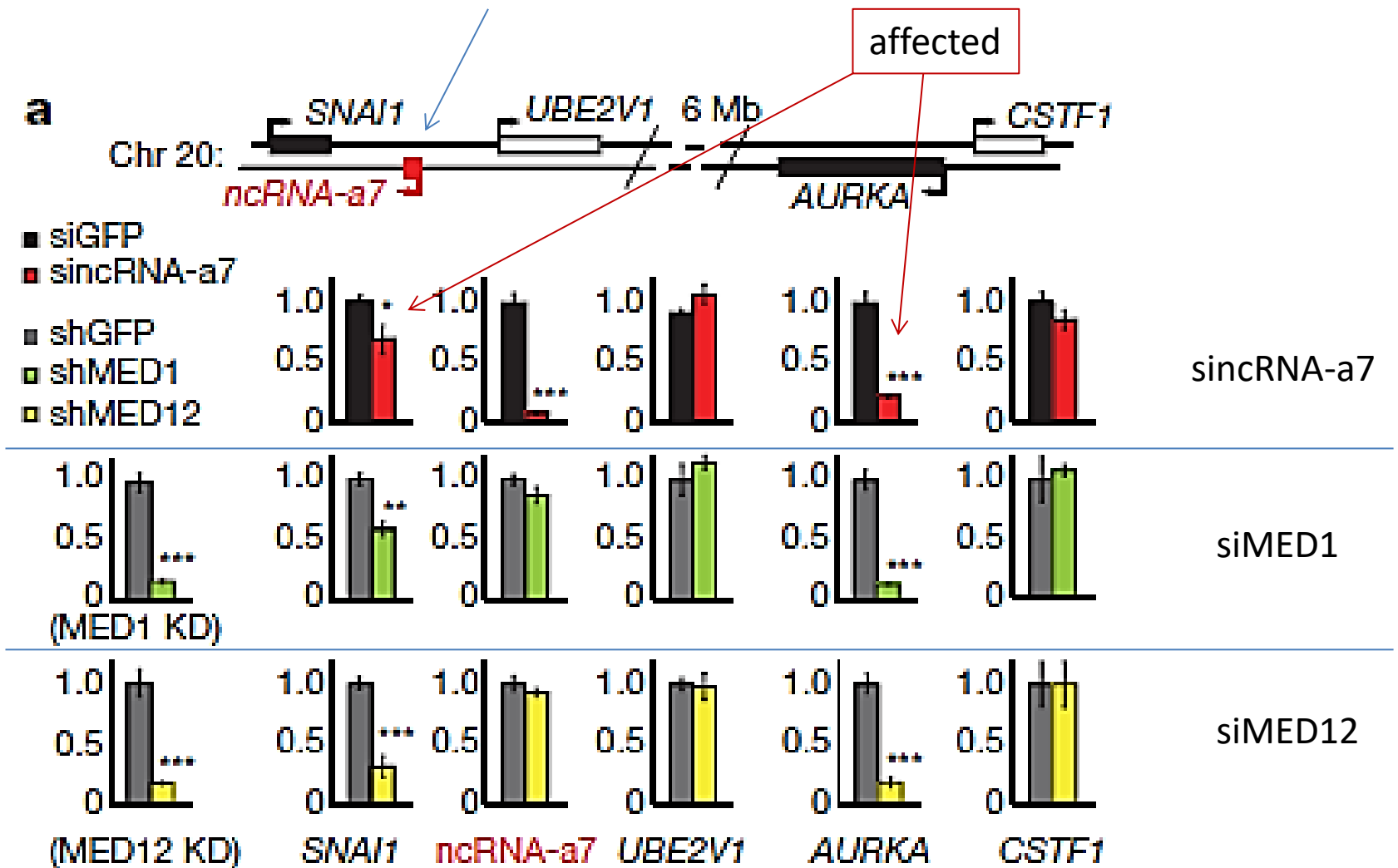
** **

siRNA down-regulation

2nd question: which component of the transcriptional machinery is involved ?

Screening protein components for function in gene activity
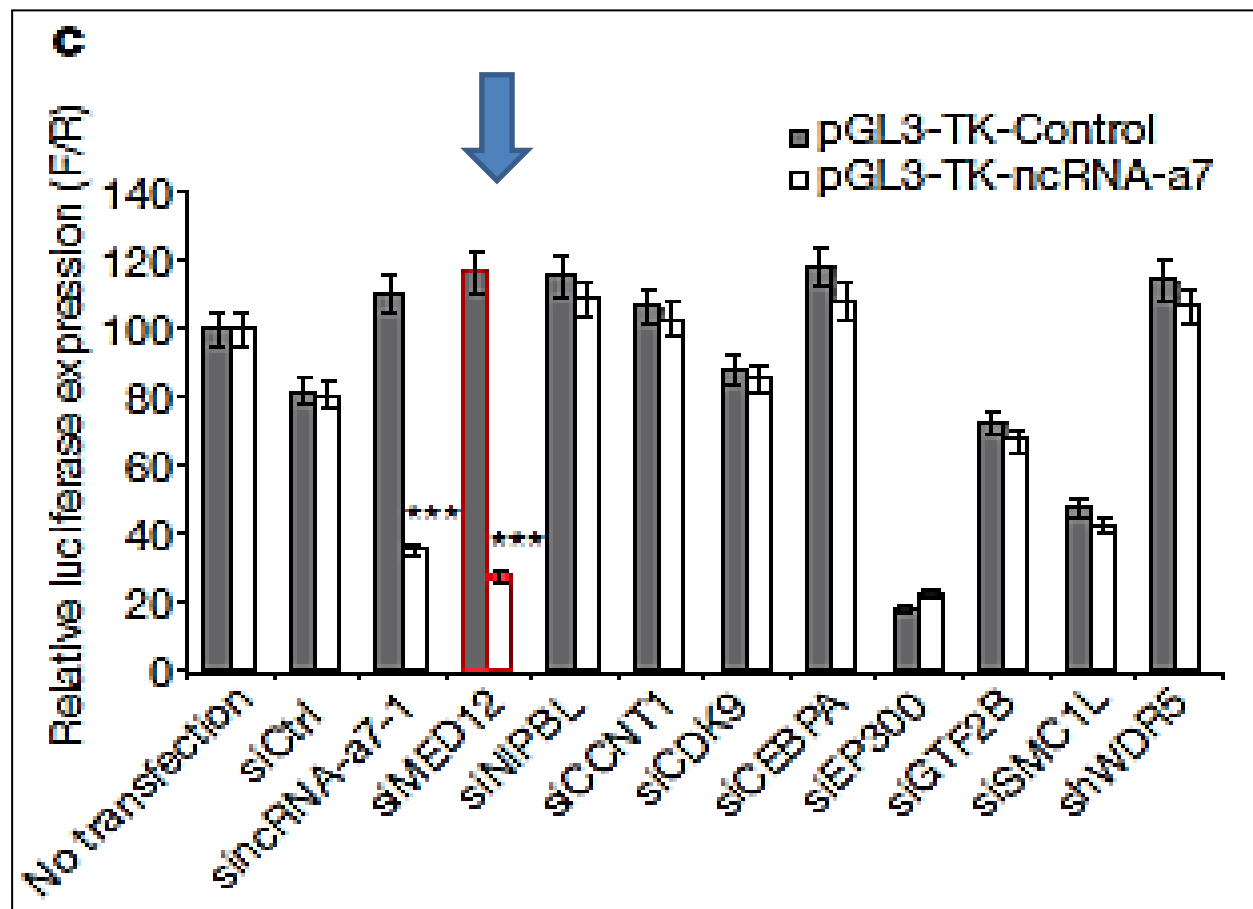
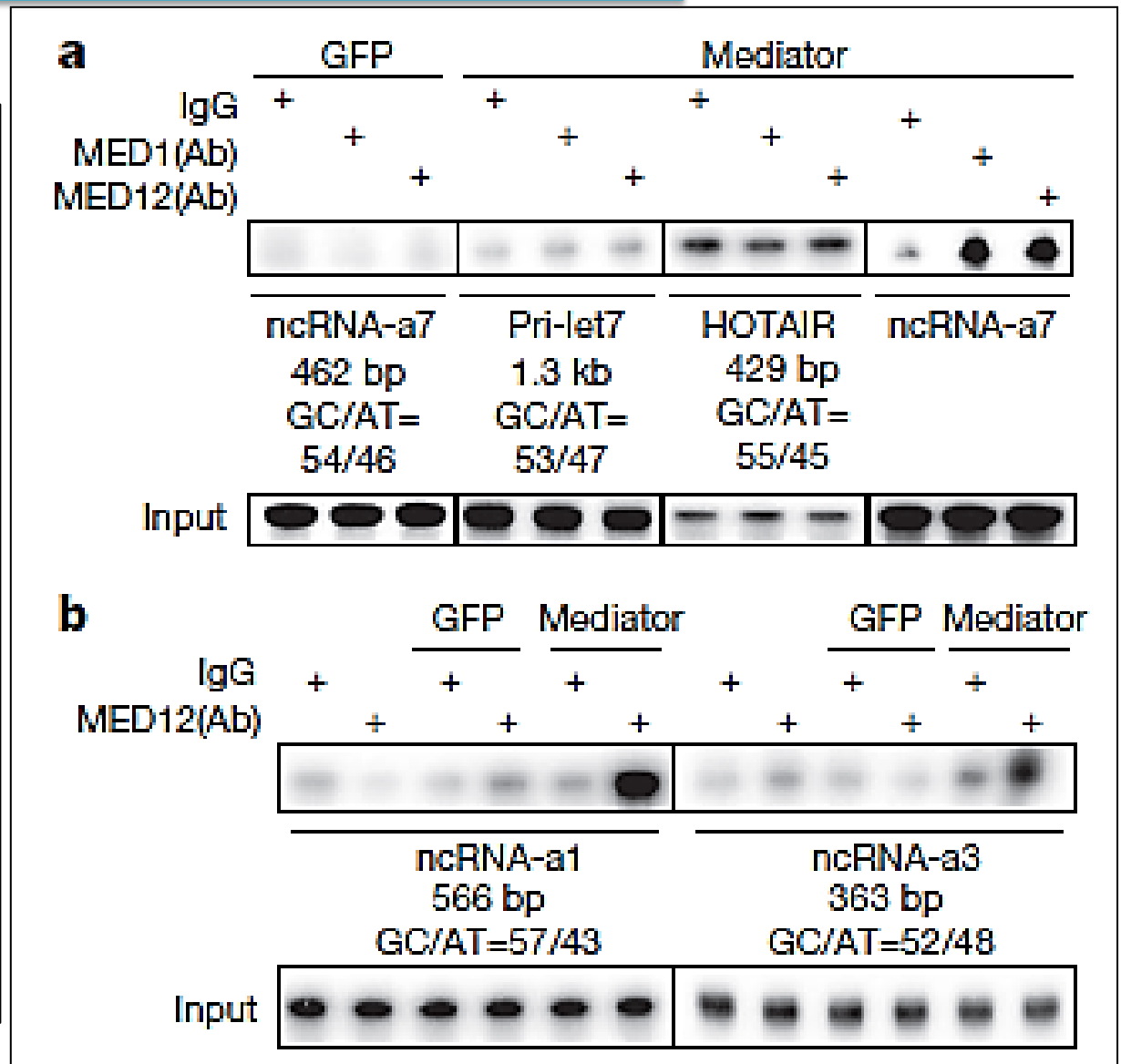MED12 is the only protein, among those tested, that affects RNA-a function
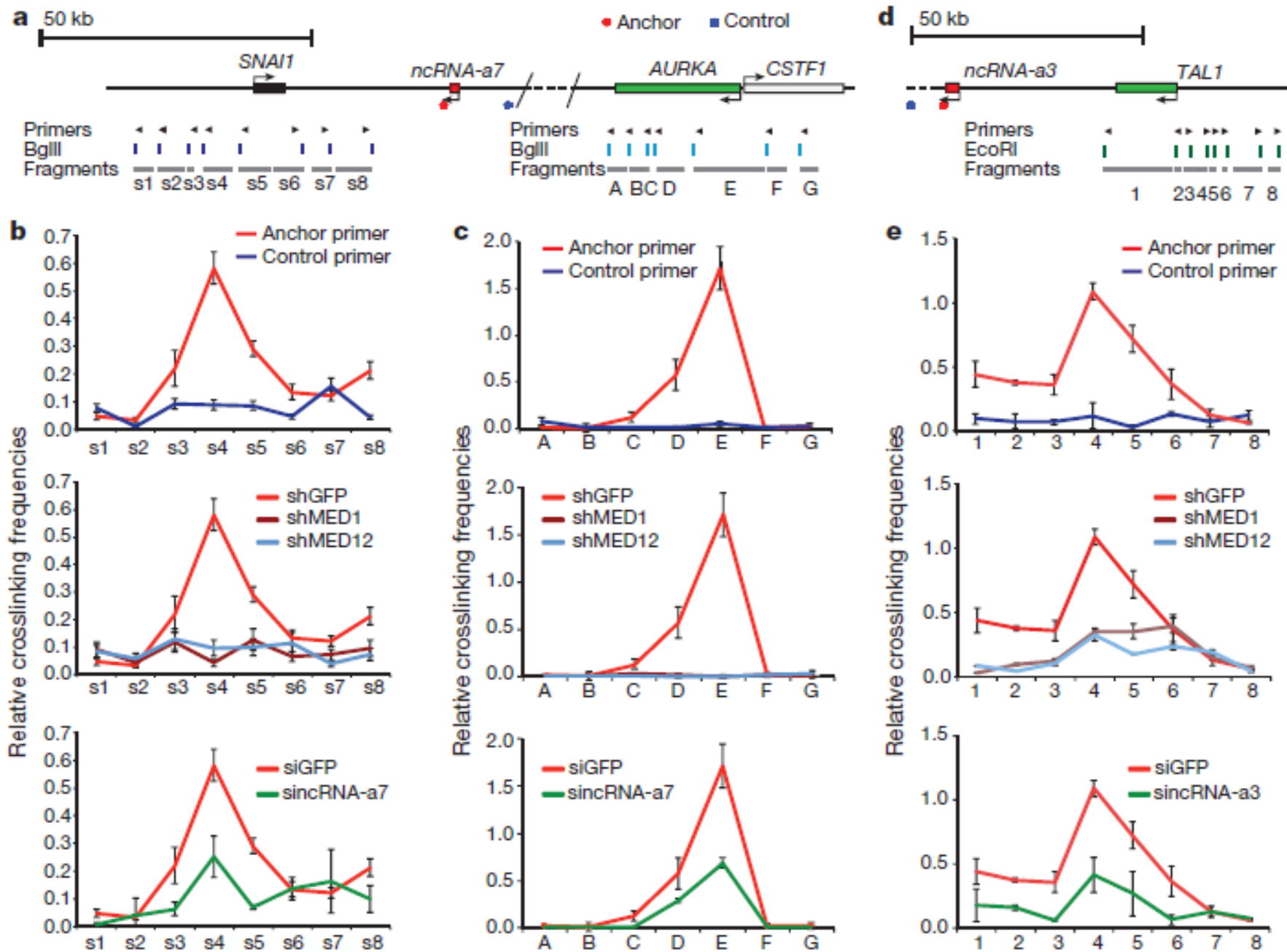
Authors demonstrated ncRNA-a/MED binding

RIP (RNA immunoprecipitation) performed using IgG or MED1-Ab or MED12-Ab, using in vitro transcribed ncRNA-a7 and controls.

Mediator purified using FLAG-tagged Med12

Controllo: FLAG-GFP

a 50 kb

SNAI1   • Anchor   ■ Control   ncRNA-a7   AURKA   CSTF1

Primers
BglII
Fragments   s1 s2s3s4 s5 s6 s7 s8

Primers
BglII
Fragments   A BC D   E   F   G

d 50 kb

ncRNA-a3   TAL1

Primers
EcoRI
Fragments   1   23456 7 8

b
Relative crosslinking frequencies
— Anchor primer
— Control primer

— shGFP
— shMED1
— shMED12

— siGFP
— sincRNA-a7

c
— Anchor primer
— Control primer

— shGFP
— shMED1
— shMED12

— siGFP
— sincRNA-a7

e
— Anchor primer
— Control primer

— shGFP
— shMED1
— shMED12

— siGFP
— sincRNA-a3

Conclusions

A new action of ncRNA was discovered

ncRNA-a binds to Mediator

 These ncRNAs are involved in looping interactions