Ch 3 – L3.1

how TF/enhancers work

How do TFs work at enhancers ?
How do they regulate the activity of promoters/RNA Pol ?

There are several questions we may examine:

- how does a TF find the right places to bind in chromatin ?
- how a TF interacts with an enhancer and for how long ?
- how do enhancers interact with promoters?
- are there proteins cooperating with TF ?
- do TFs induce any change in chromatin ?
- ...

how does a TF find the right places to bind in chromatin ?

from the point of view of cell biology, this is still open question

we know from molecular biology that:

- a TF never binds to all its possible binding sites in the genome
- TF binding sites are cell-type specific

Second, most of a TF binding sites are **pre-marked in chromatin**
(*see the video form Christofer Glass and your Textbook (Heinz 2016*)

One class of TF is defined as Lineage-determining TFs (LDTF) or Pioneer Factors -- able to bind to undetermined chromatin

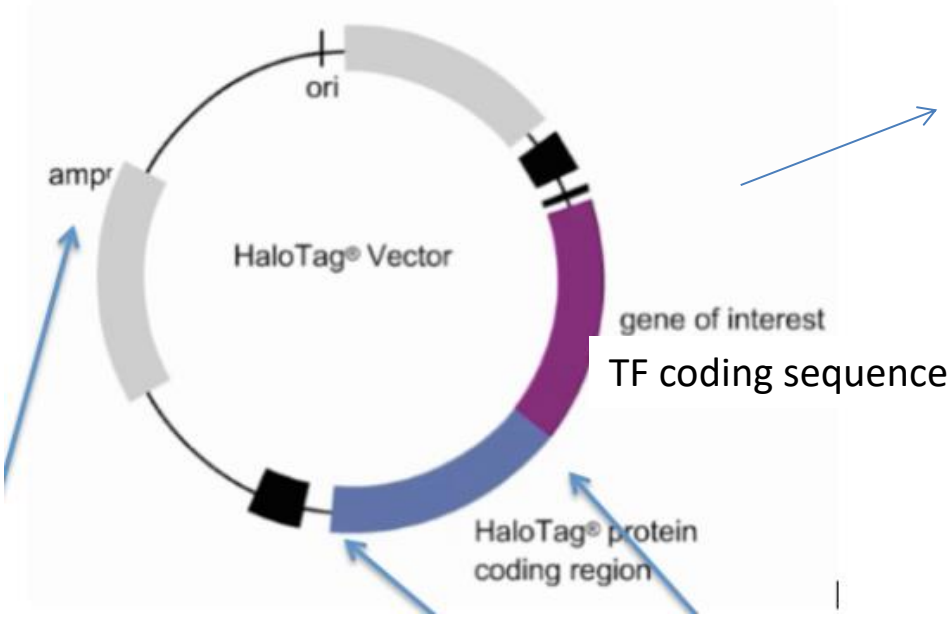
- **how do TF interact with an enhancer and for how long ?**

ChIP-seq studies depict a quite static situation, since developmental times are long (same for reprogrammed cells in culture), but also after a stimulus.

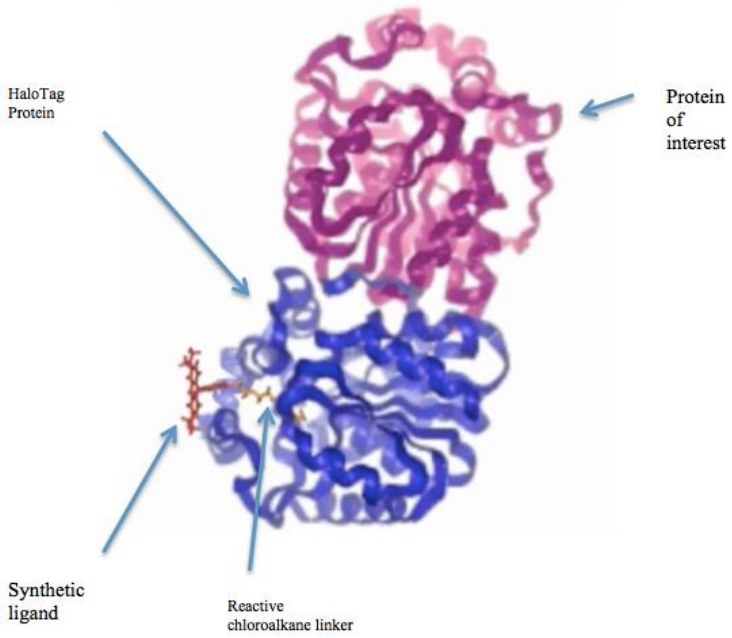
Do Transcription Factors reside at enhancers for such long times ?

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

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



- transfect in your favourite cells
- cell express TF-halotag
- add chloroalkane-linker-fluor
- TF-Halo binds fluor covalently

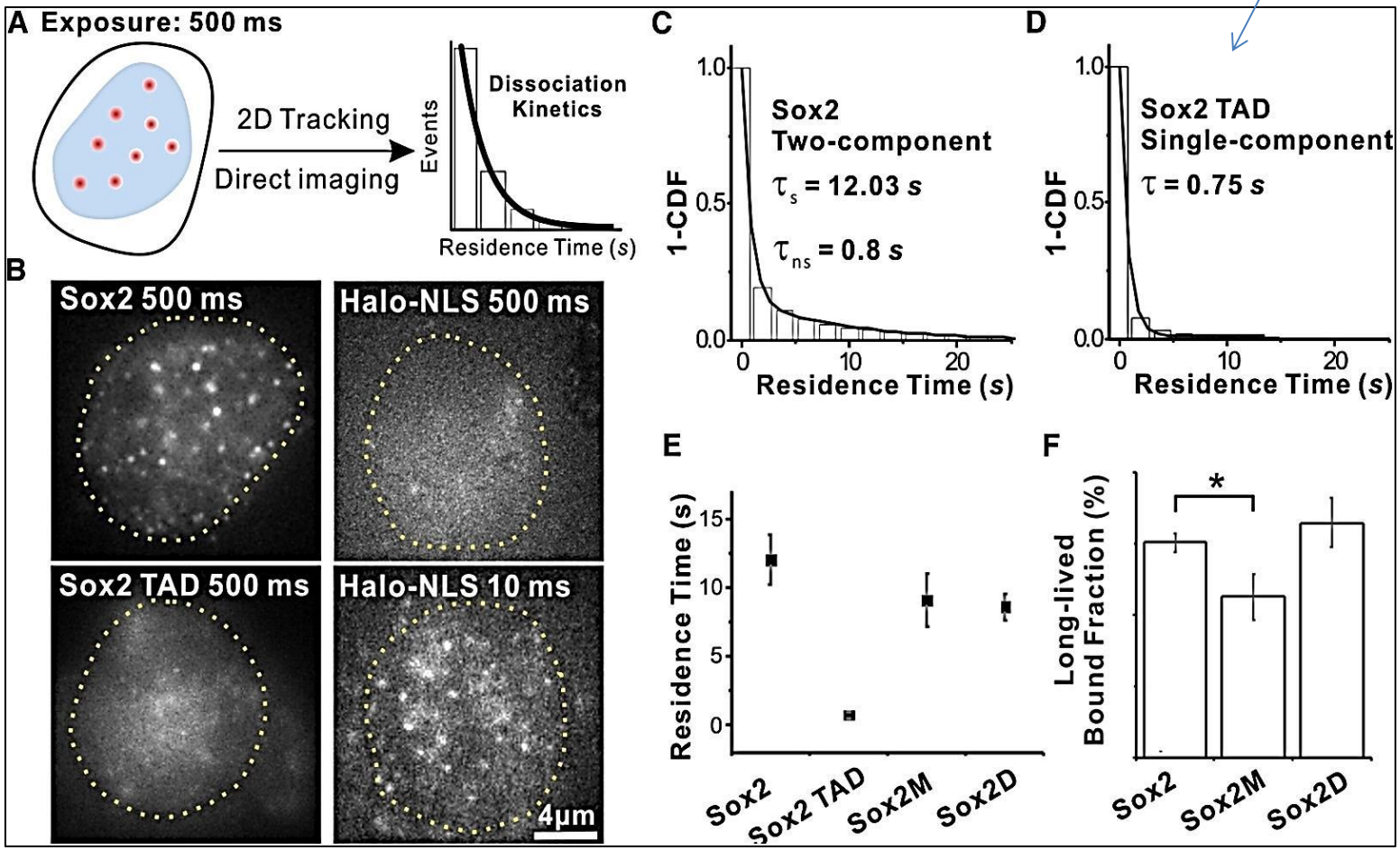


HaloTag is a self-labeling protein tag of 297 aa (33 kDa) derived from a bacterial enzyme. It binds to, and covalently attaches to, a chloroalkane linker. Chloroalkane-fluorescent molecules are available. In this way, once the recombinant protein is expressed in cells, addition of the chloroalkane-fluorescent probe will result in covalently labelled Halo Tag.

Halo-Tag-Sox2

Dynamic single-molecule imaging

Transcriptional Activating Domain only



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

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Article

Cell

Steroid Receptors Reprogram FoxA1 Occupancy through Dynamic Chromatin Transitions

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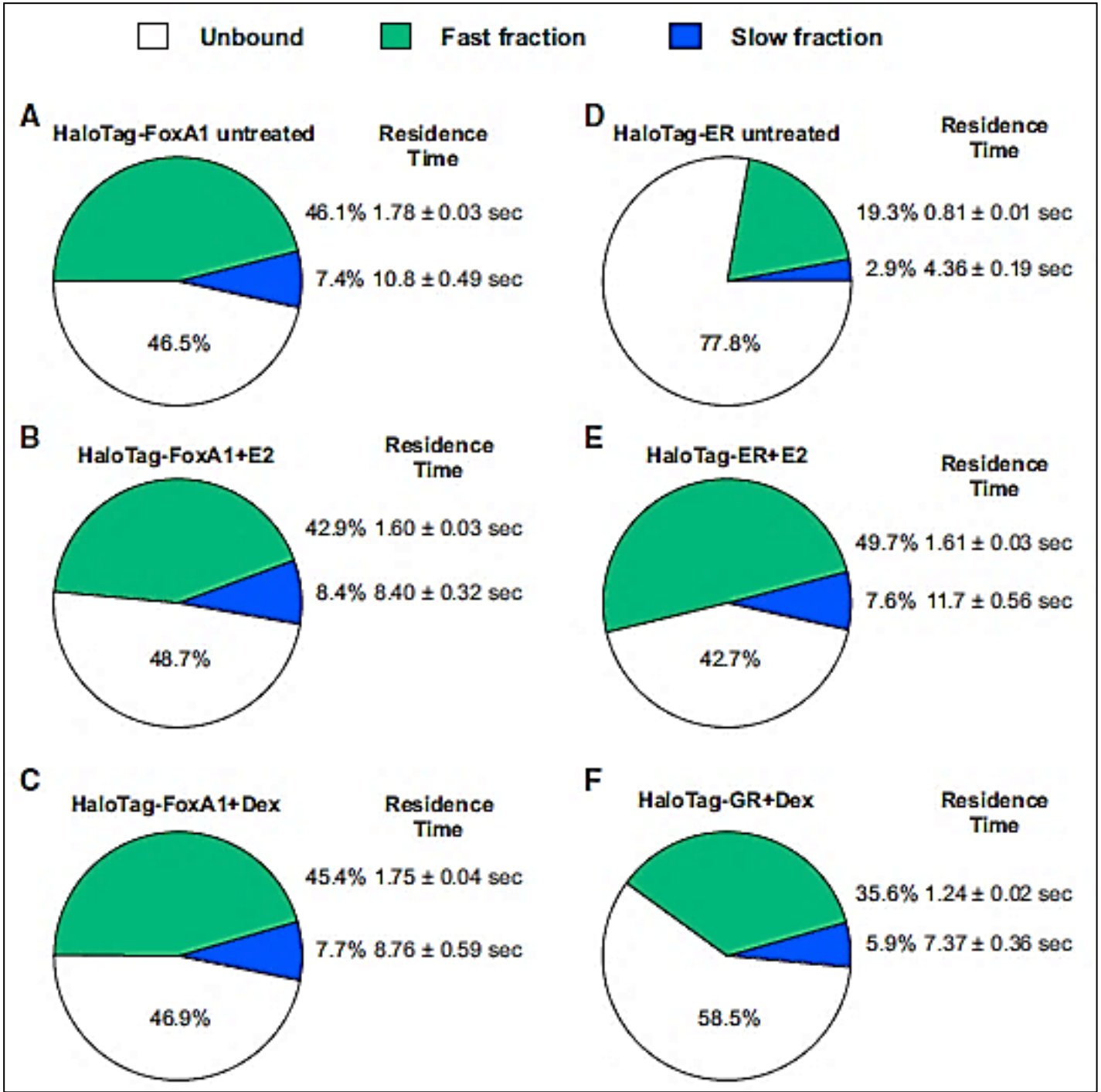
The estrogen receptor (ER), glucocorticoid receptor (GR), and forkhead box protein 1 (FoxA1) are significant factors in breast cancer progression. FoxA1 has been implicated in establishing ER-binding patterns though its unique ability to serve as a pioneer factor. However, the molecular interplay between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

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

[Movie 2]

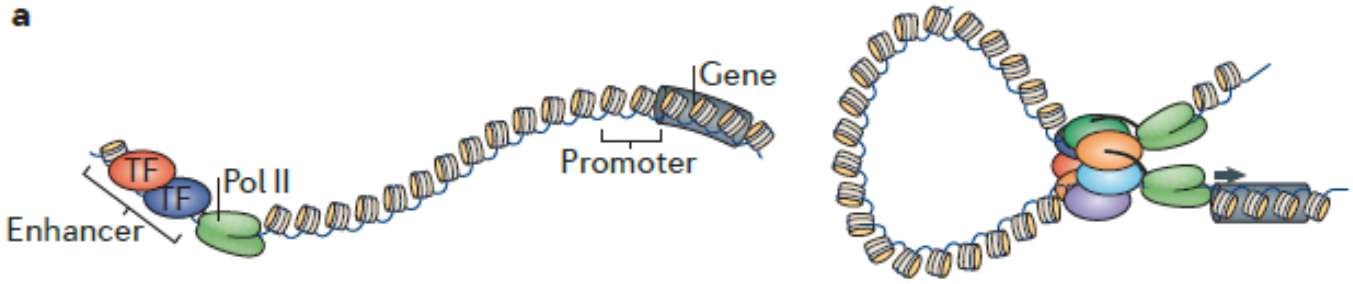


Conclusion of these studies is that the residence time of TFs, either pioneer factor or Signal-dependent TFs, are very short.
In addition, whenever two (or more) components are tested, this results in increased number of foci and slightly increased residence times.

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.

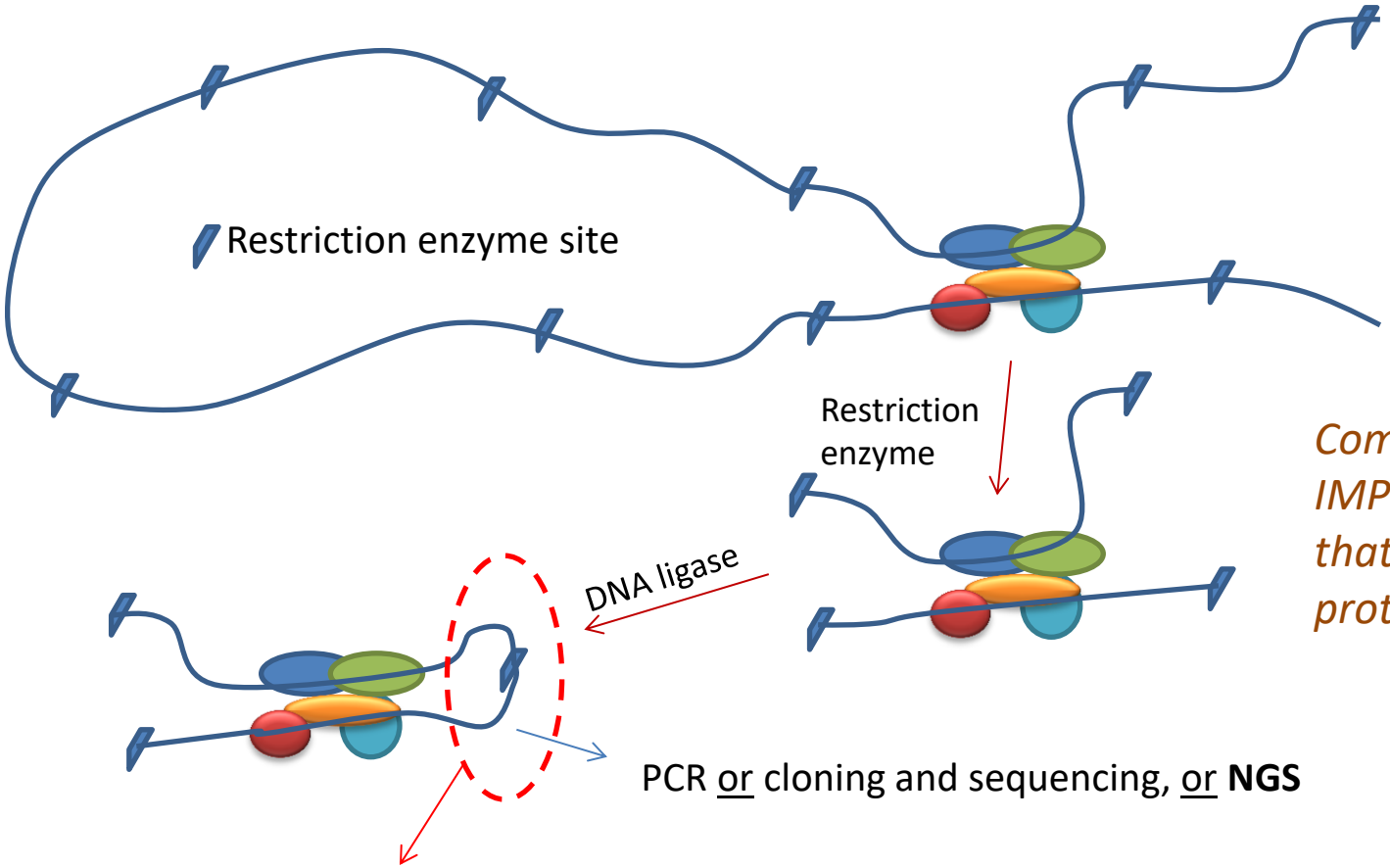
- **how do enhancers interact with promoters?**

Chromatin looping mechanisms



Pombo & Dillon, 2015

Long-range interactions are studied with 3C (Chromatin Conformation Capture) or different genome-wide scale variants (4C, 5C, Hi-C, ChIA-PET).

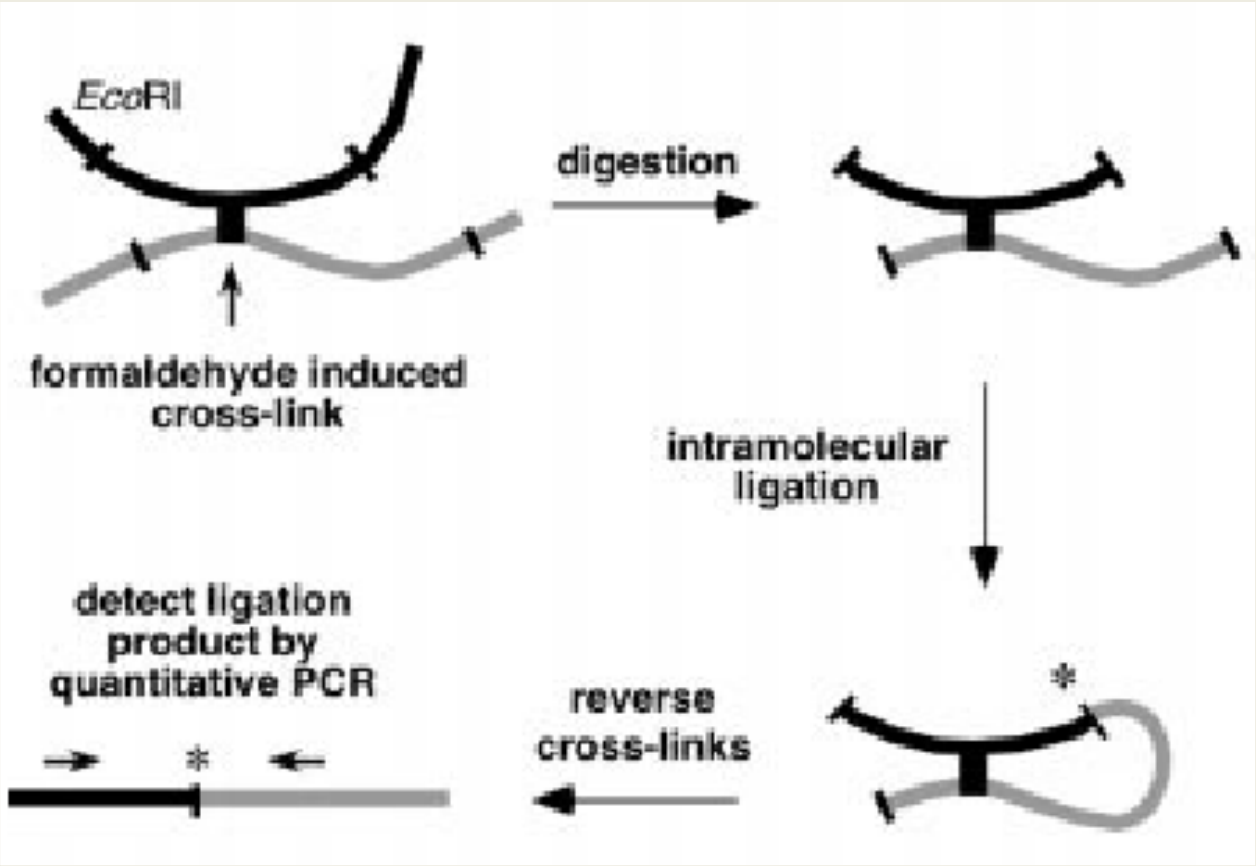


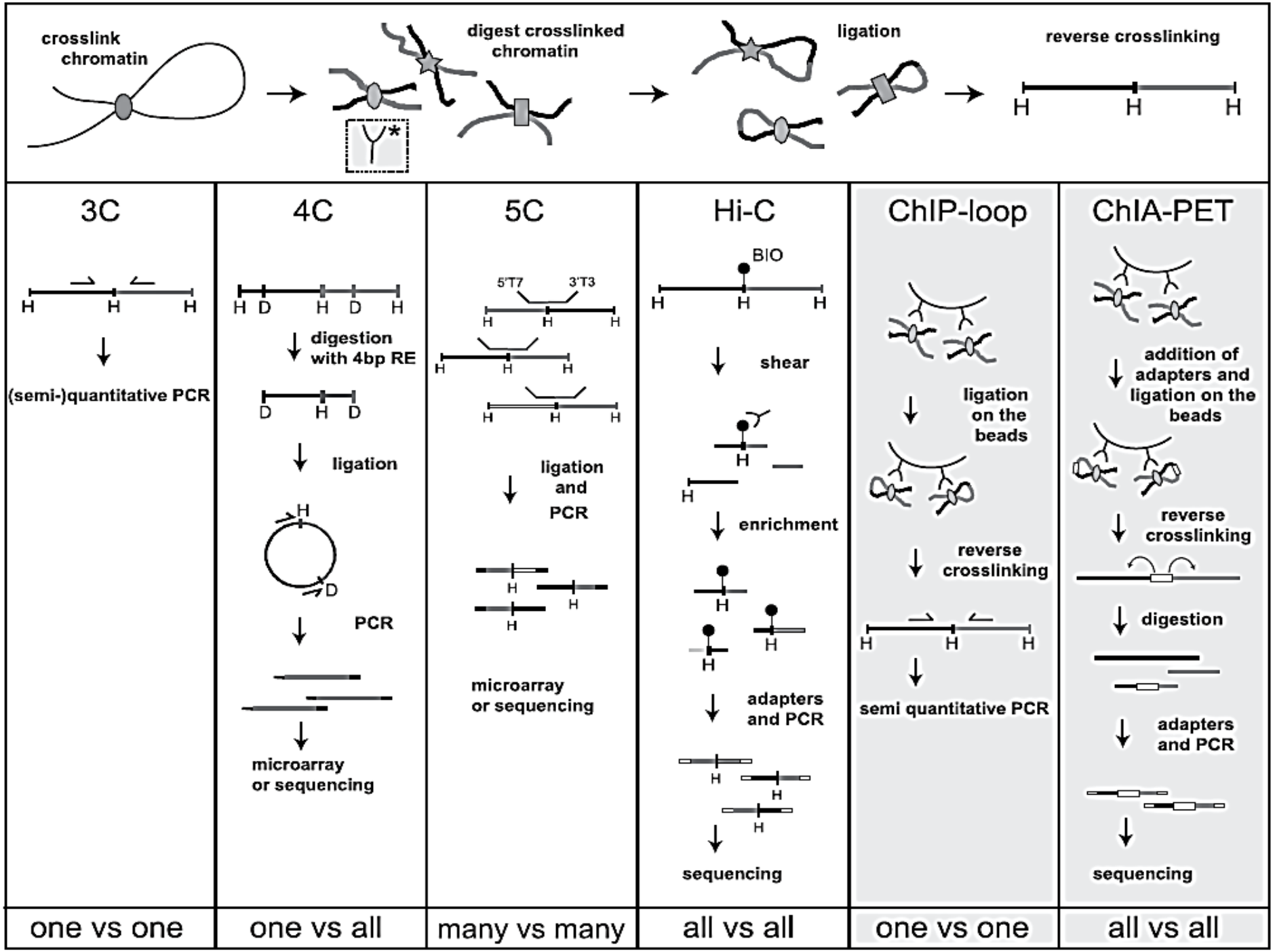
Complexes may also be IMPT using an antibody that recognizes a specific protein → ChIA-PET

PCR for single interaction.
Generate libraries to NGS for genome-wide studies

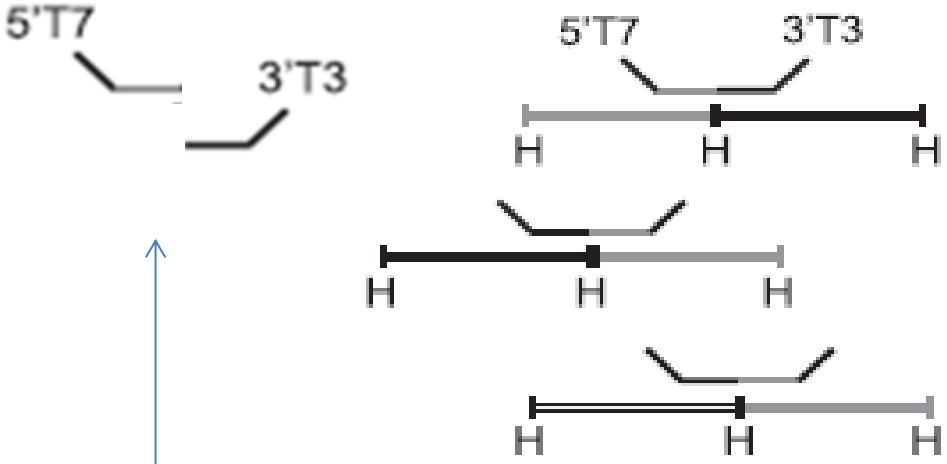
Note: from this scheme nucleosomes are omitted

The first historically and technically is 3C

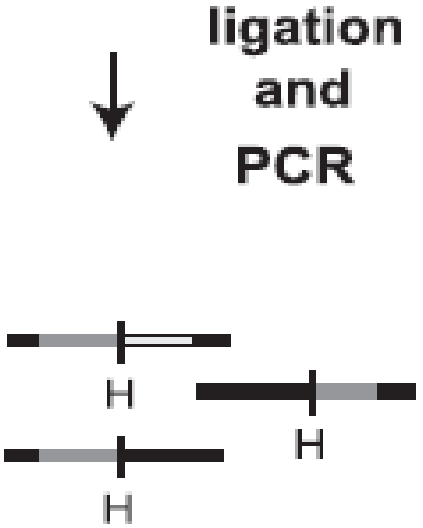



crosslink chromatin
digest crosslinked chromatin
ligation
reverse crosslinking
H H H
3C
(semi-)quantitative PCR
4C
H D H D H
digestion with 4bp RE
D H D
ligation
H
D
PCR
microarray or sequencing
5C
5'T7
3'T3
ligation and PCR
microarray or sequencing
Hi-C
BIO
shear
enrichment
adapters and PCR
sequencing
ChIP-loop
ligation on the beads
reverse crosslinking
semi quantitative PCR
ChIA-PET
addition of adapters and ligation on the beads
reverse crosslinking
digestion
adapters and PCR
sequencing
one vs one
one vs all
many vs many
all vs all
one vs one
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.



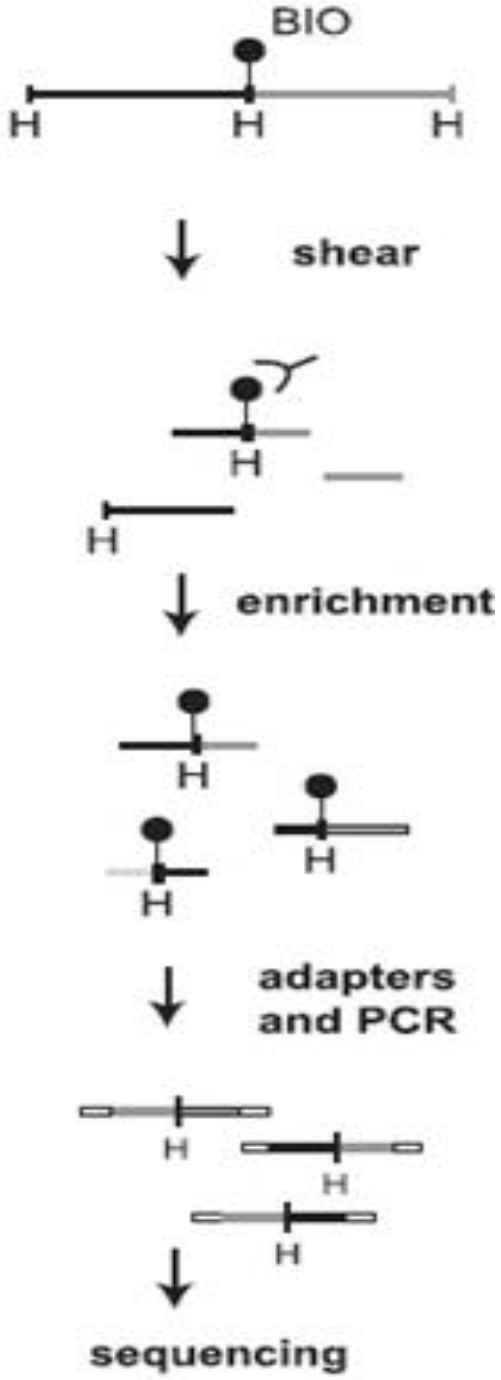
microarray or sequencing

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.

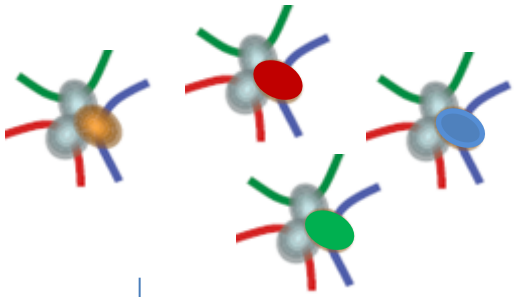
After this step, fragments are processed, amplified and NGSequenced as in other methods.



ChIA-PET

Formaldehyde
Sonication

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



ChIP with anti-

a

b

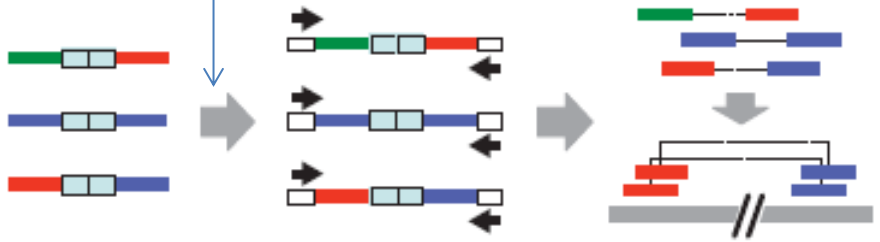
add adapters

ligate

RE cut

add adapters

mapping



PCR amplify and mass-sequencing

LETTER

ENCODE paper

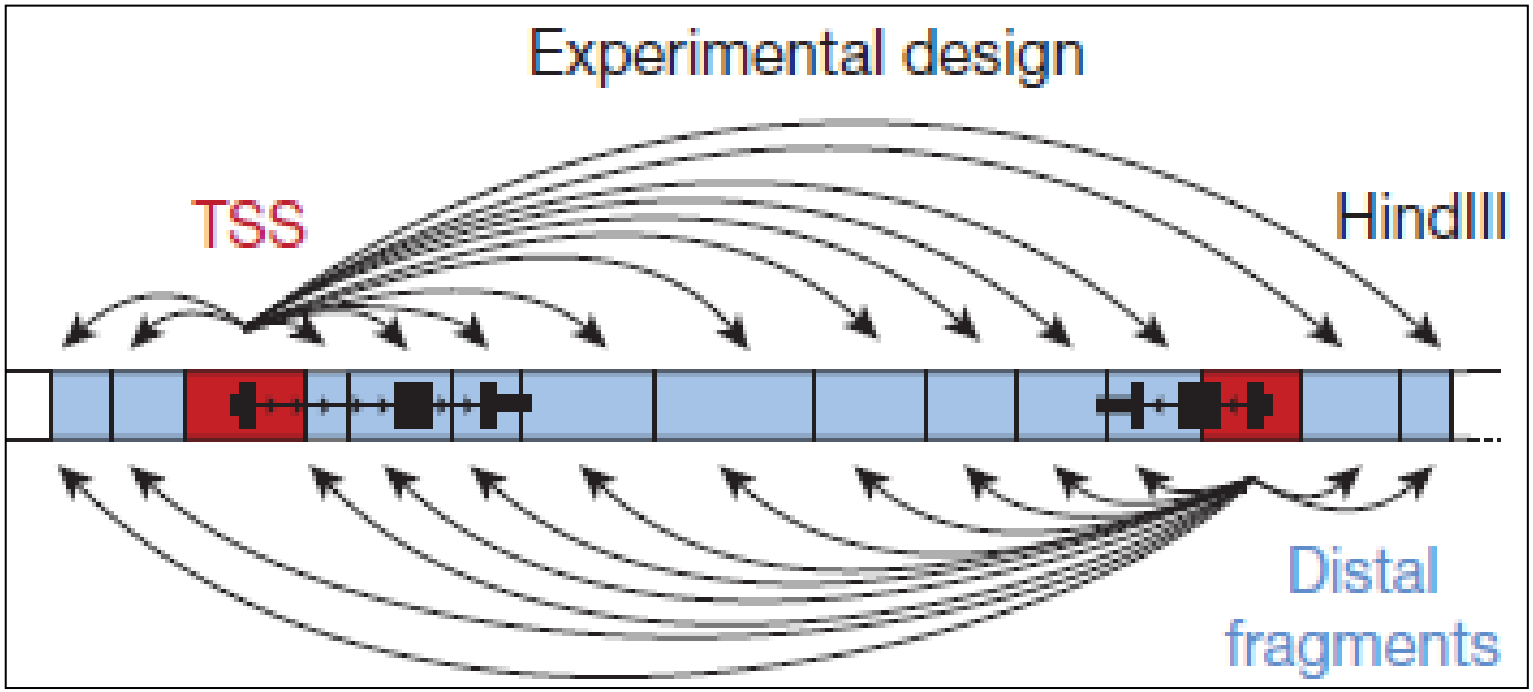
doi:10.1038/nature11279

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.

Nature. 2012 Sep 6; 489(7414): 109–113.



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

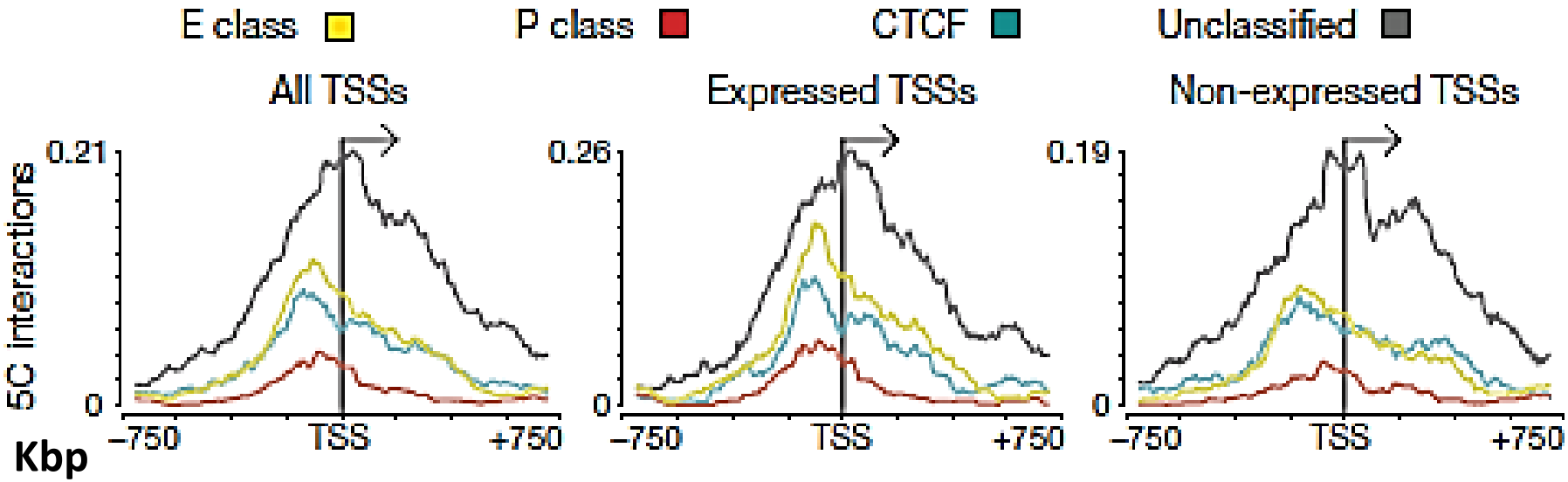
Long-range interactions show marked asymmetry with a bias at 120 kilobases upstream of TSS

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

Only 7% of looping interactions are with the nearest gene

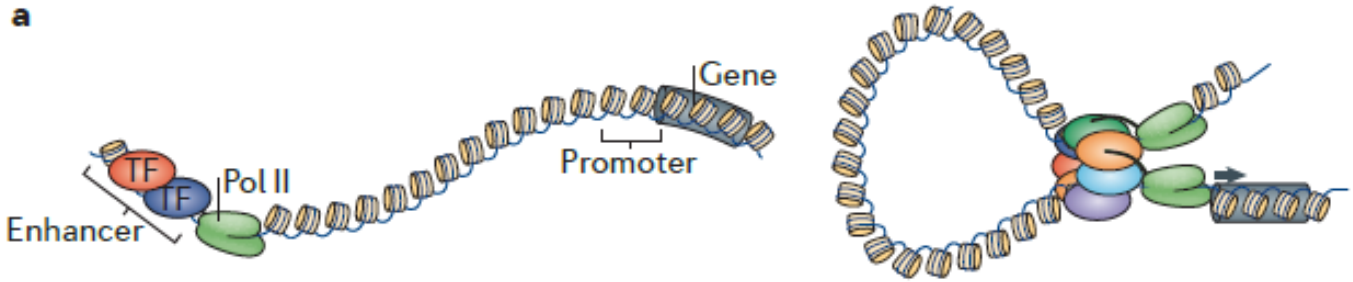
Promoters and distal elements are engaged in multiple long-range interactions to form complex networks.

Enhancers are asymmetric in respect to TSS

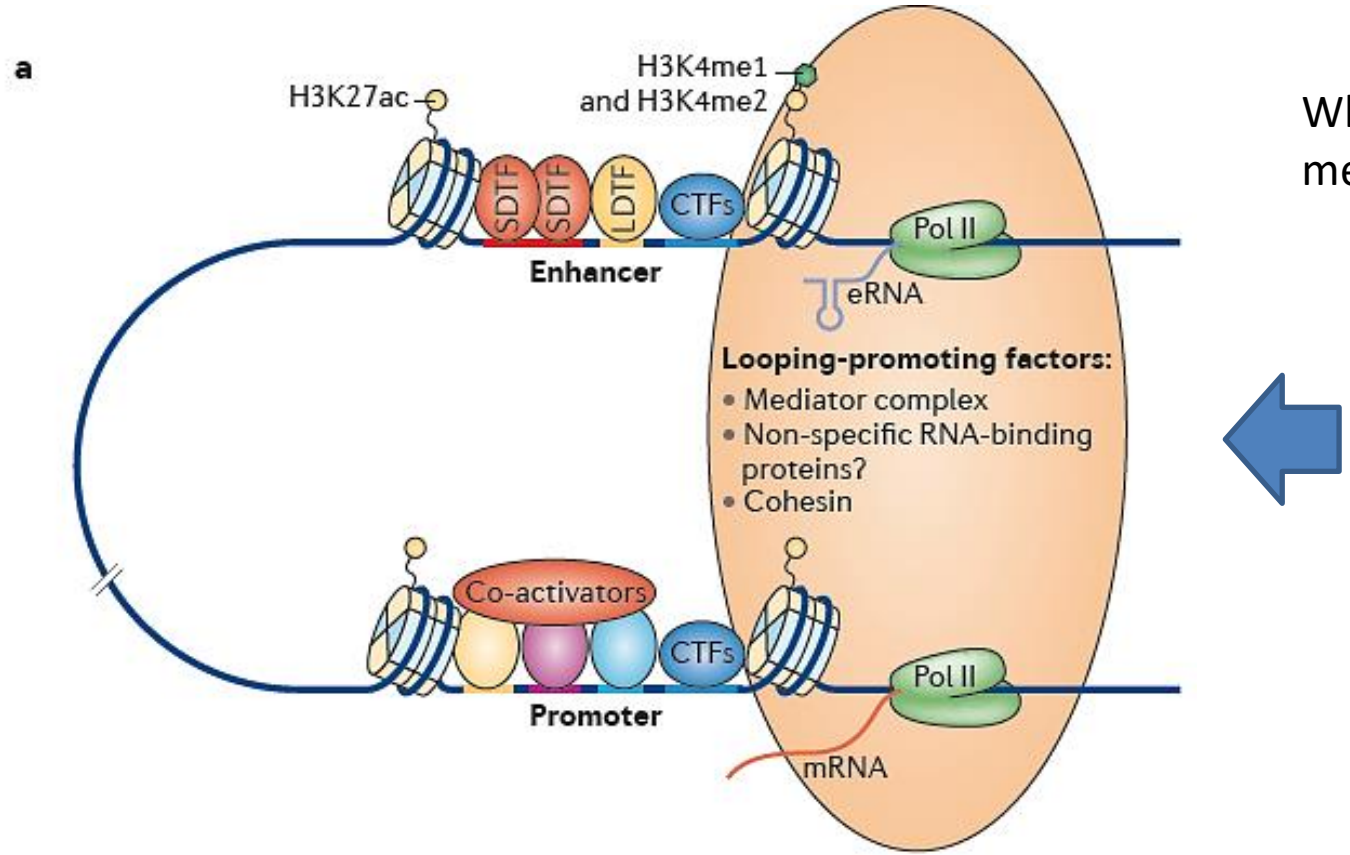


the long-range interaction landscape is asymmetric, with interactions of E, P and CTCF classes peaking around 120 kb upstream of the TSS. This asymmetry of interactions reveals an unanticipated directionality in long-range interactions with TSSs.

How do Enhancers interact with their target promoters ?



Pombo & Dillon, 2015



What is the molecular mechanism of looping ?

Heinz et al., 2015

Cohesin stabilizes long-range interactions.