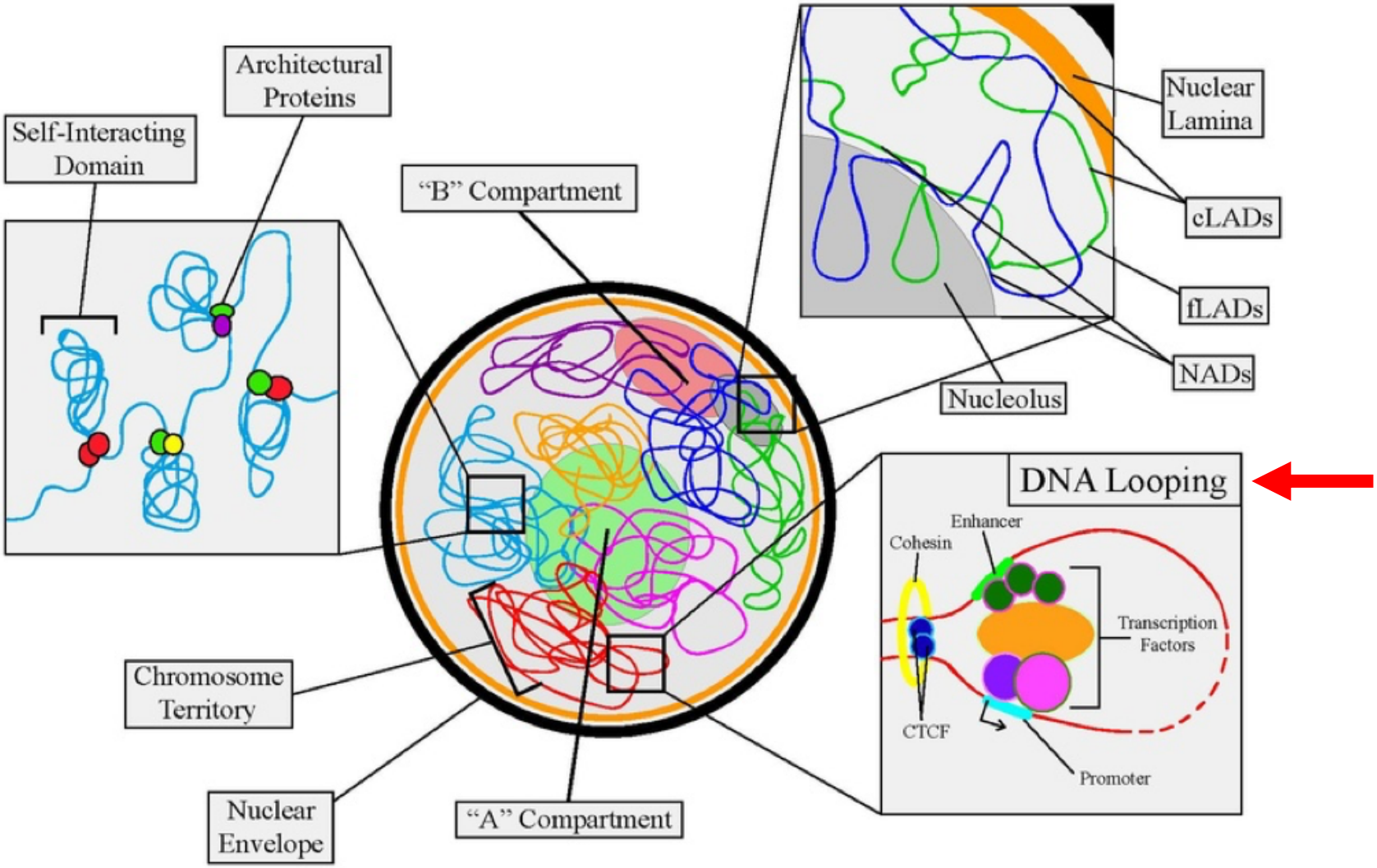
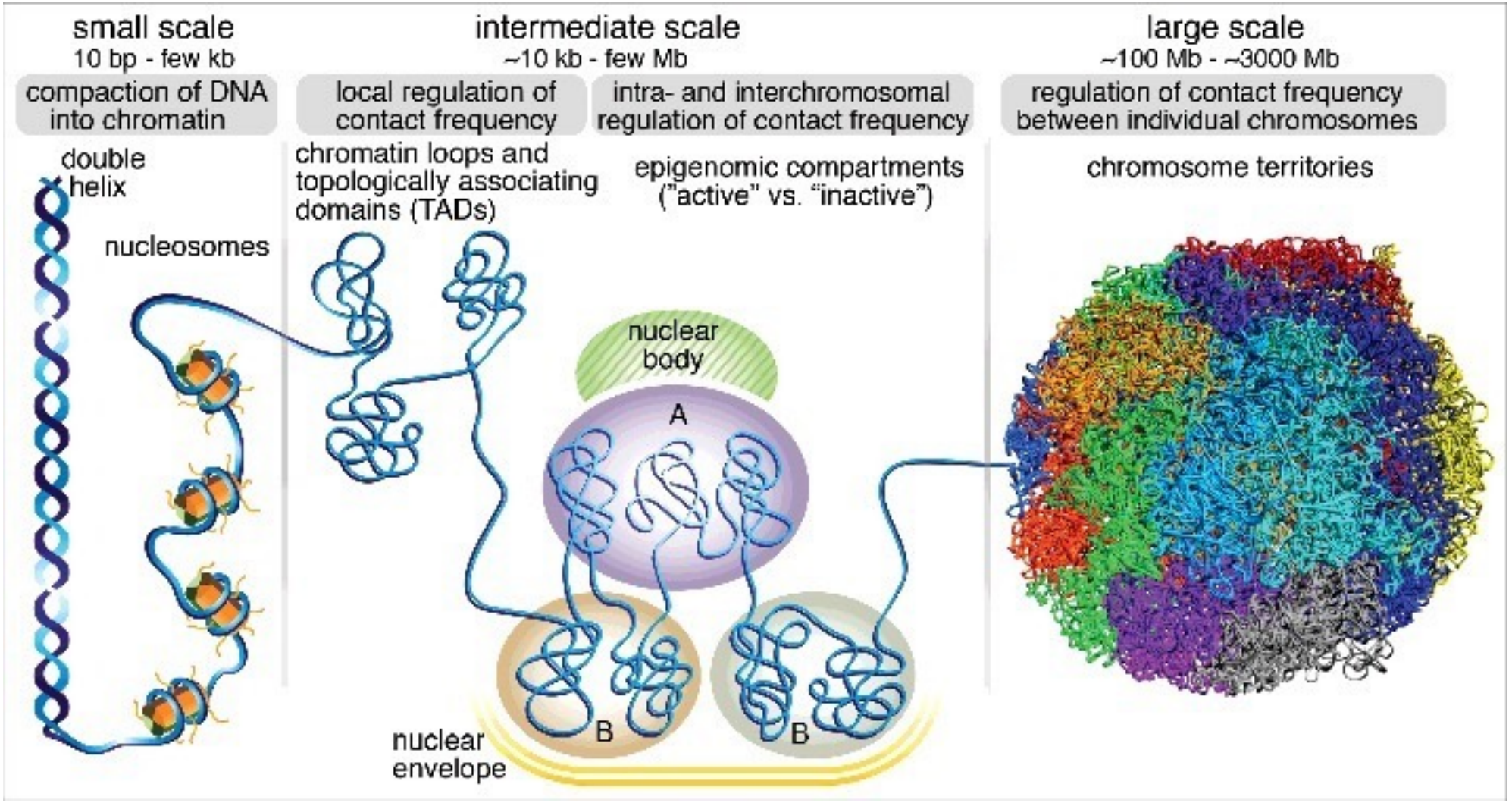
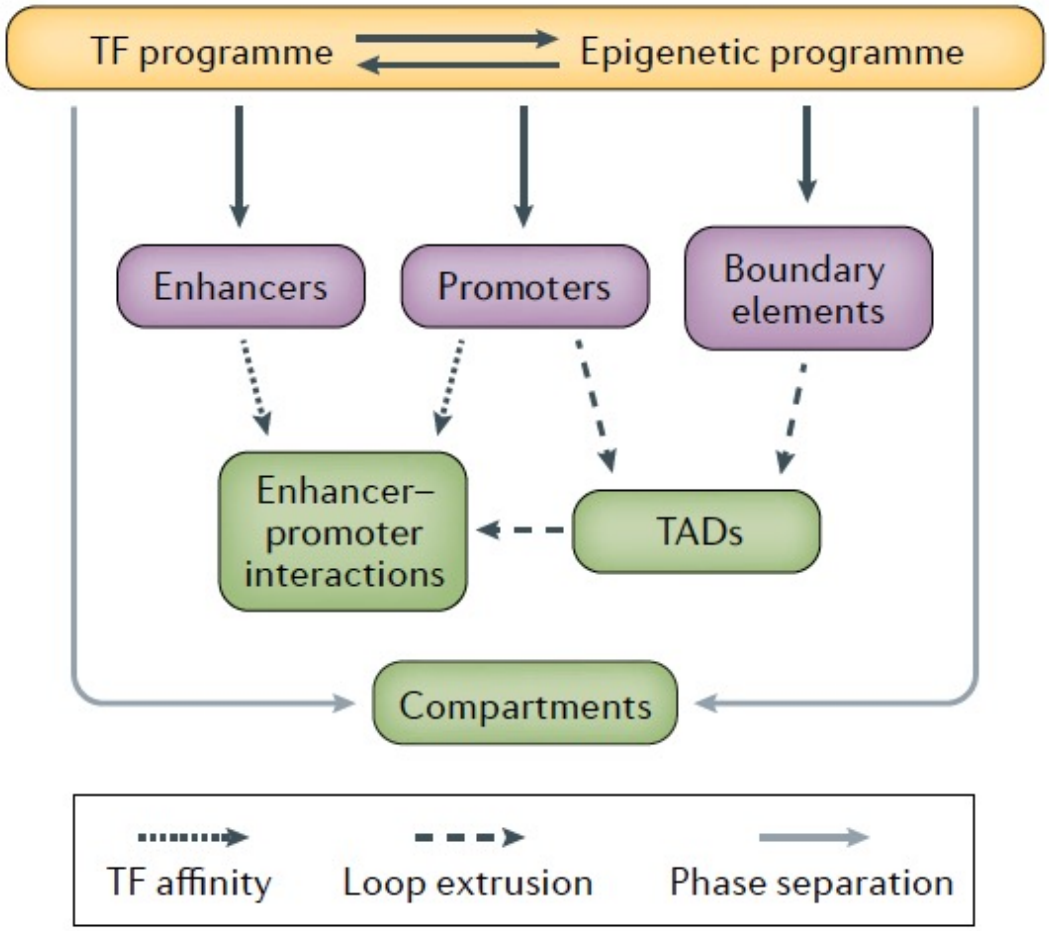
Transcriptional regulation II

Functional elements are physically connected to each other



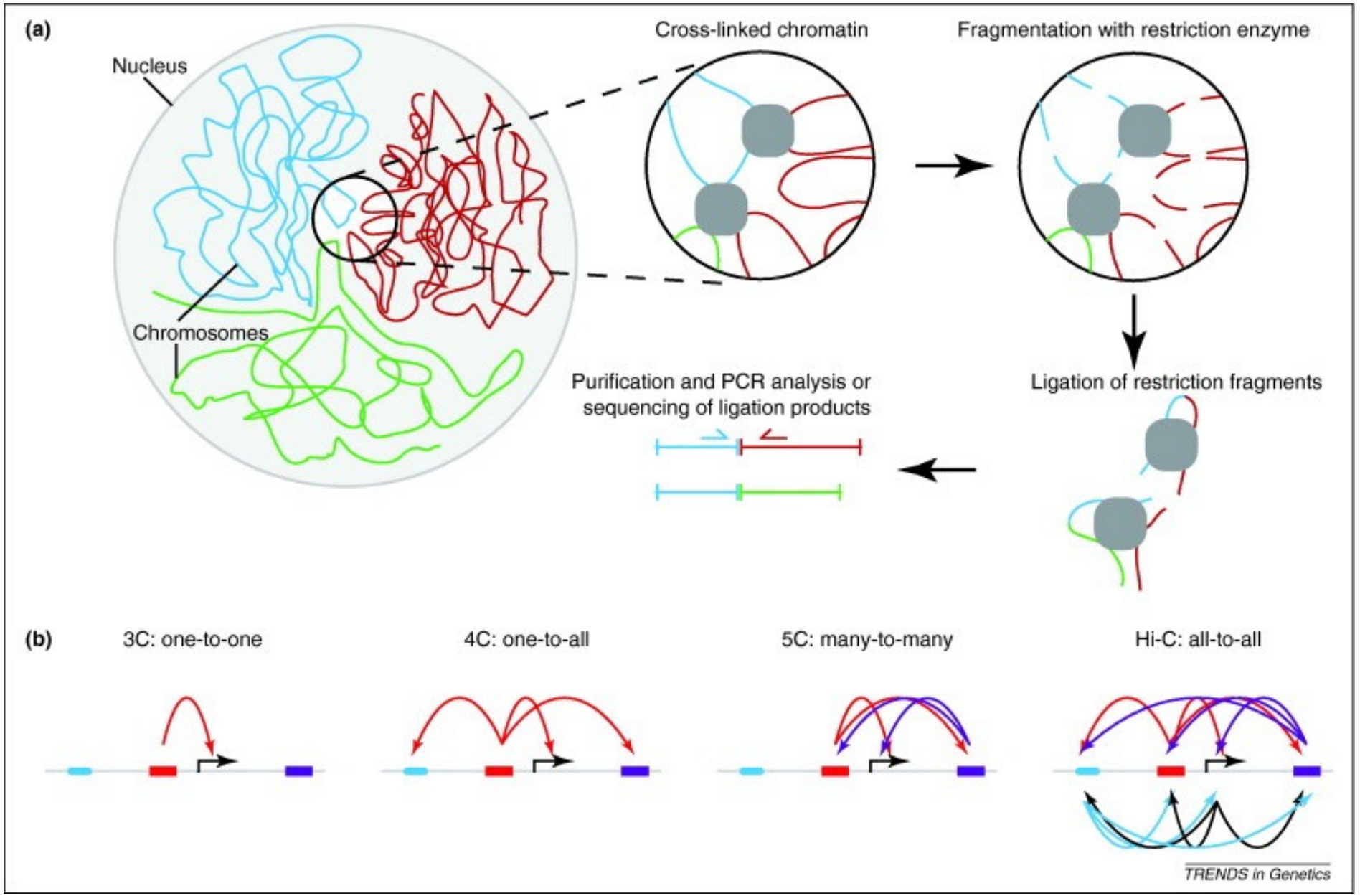

small scale
10 bp - few kb
compaction of DNA into chromatin
double helix
nucleosomes
intermediate scale
~10 kb - few Mb
local regulation of contact frequency
chromatin loops and topologically associating domains (TADs)
intra- and interchromosomal regulation of contact frequency
epigenomic compartments ("active" vs. "inactive")
nuclear body
A
B
B
nuclear envelope
large scale
~100 Mb - ~3000 Mb
regulation of contact frequency between individual chromosomes
chromosome territories

The relationship between genome structure and function

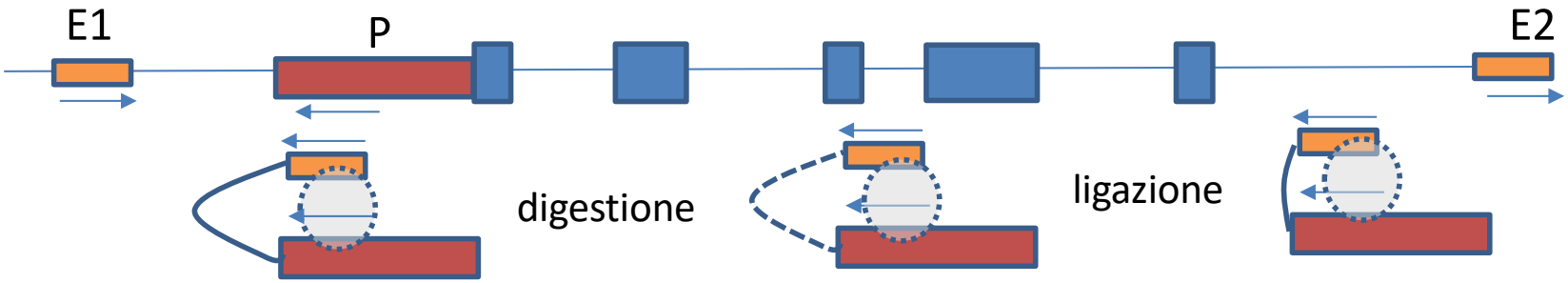


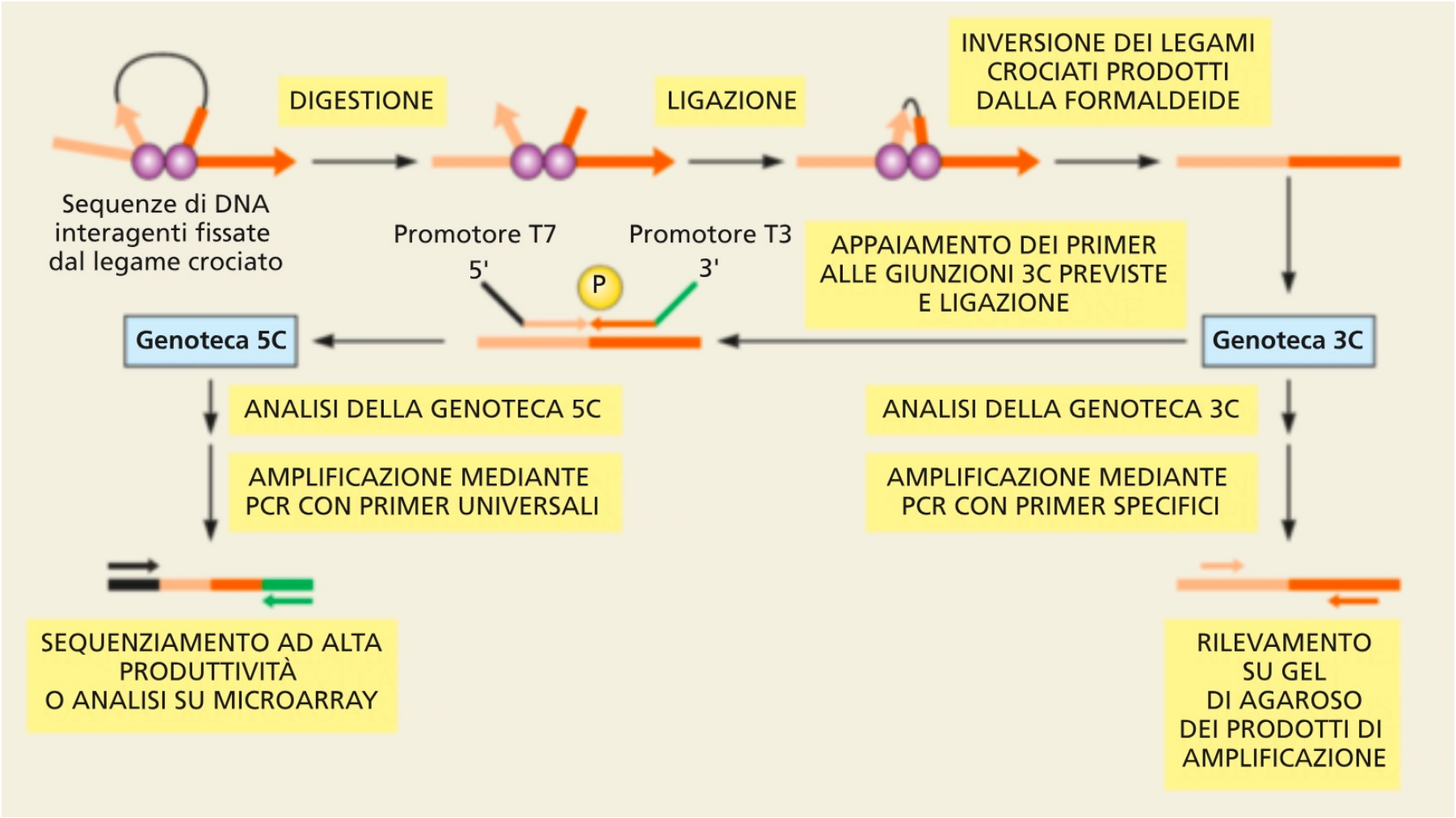
The activity of the regulatory elements of the genome is related to the TF programme and the epigenetic programme in the cell nucleus. Together, the sequence, order and activity of these regulatory elements instruct the processes underlying higher- order chromatin structures. CTCF- binding boundary elements demarcate the boundaries of cohesin mediated loop extrusion and thereby control the positions of TAD borders; active gene promoters might act in a similar way. Within TADs, the process of loop extrusion brings enhancers and promoters into close physical proximity; this may facilitate specific enhancer–promoter interactions, which are stabilized by affinity between their bound TFs and cofactors. Dependent on their chromatin state, regions of euchromatin and heterochromatin form spatially separated compartments via a process of phase separation.

Chromosome conformation capture

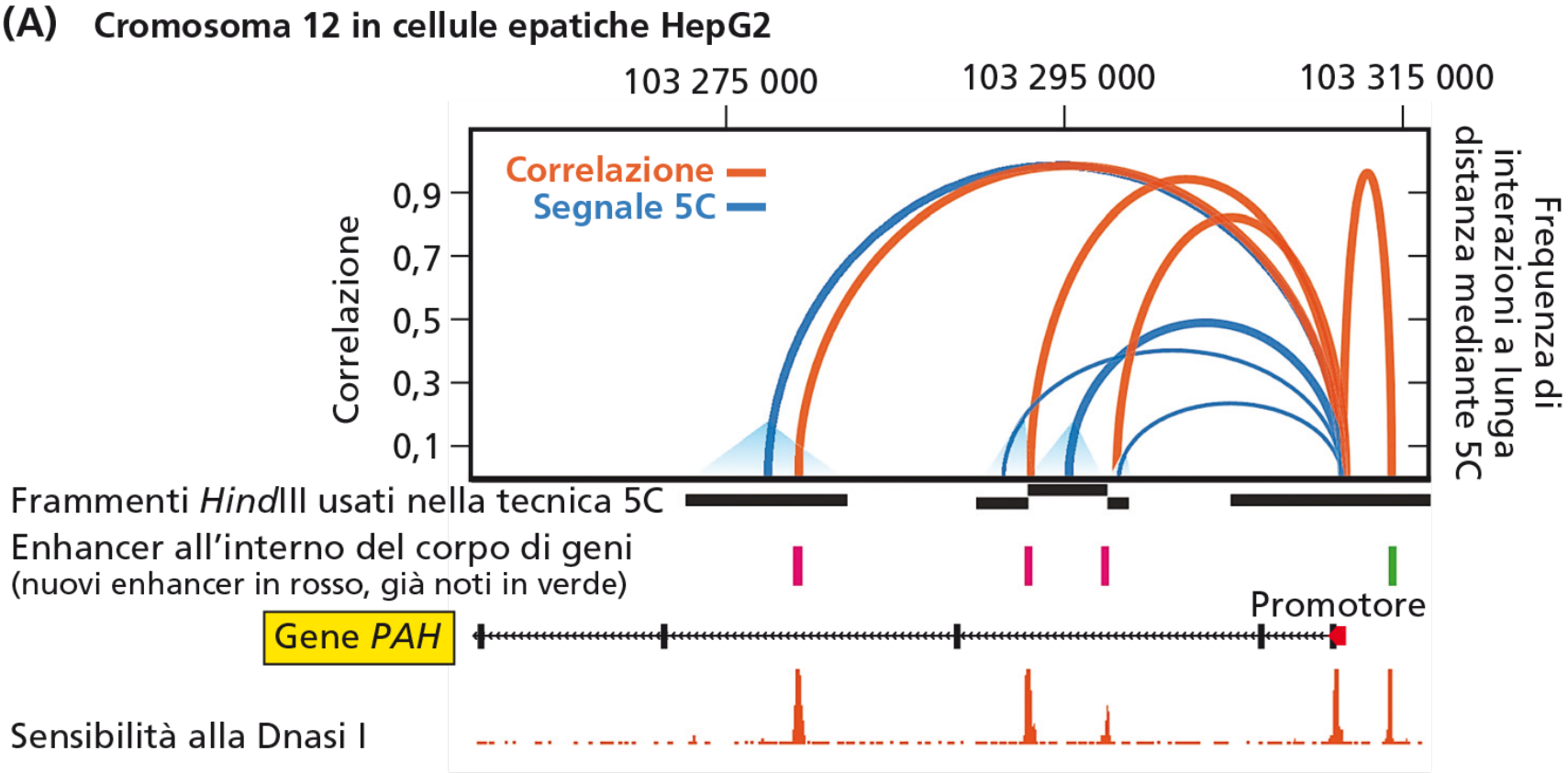


Chromosome Conformation Capture

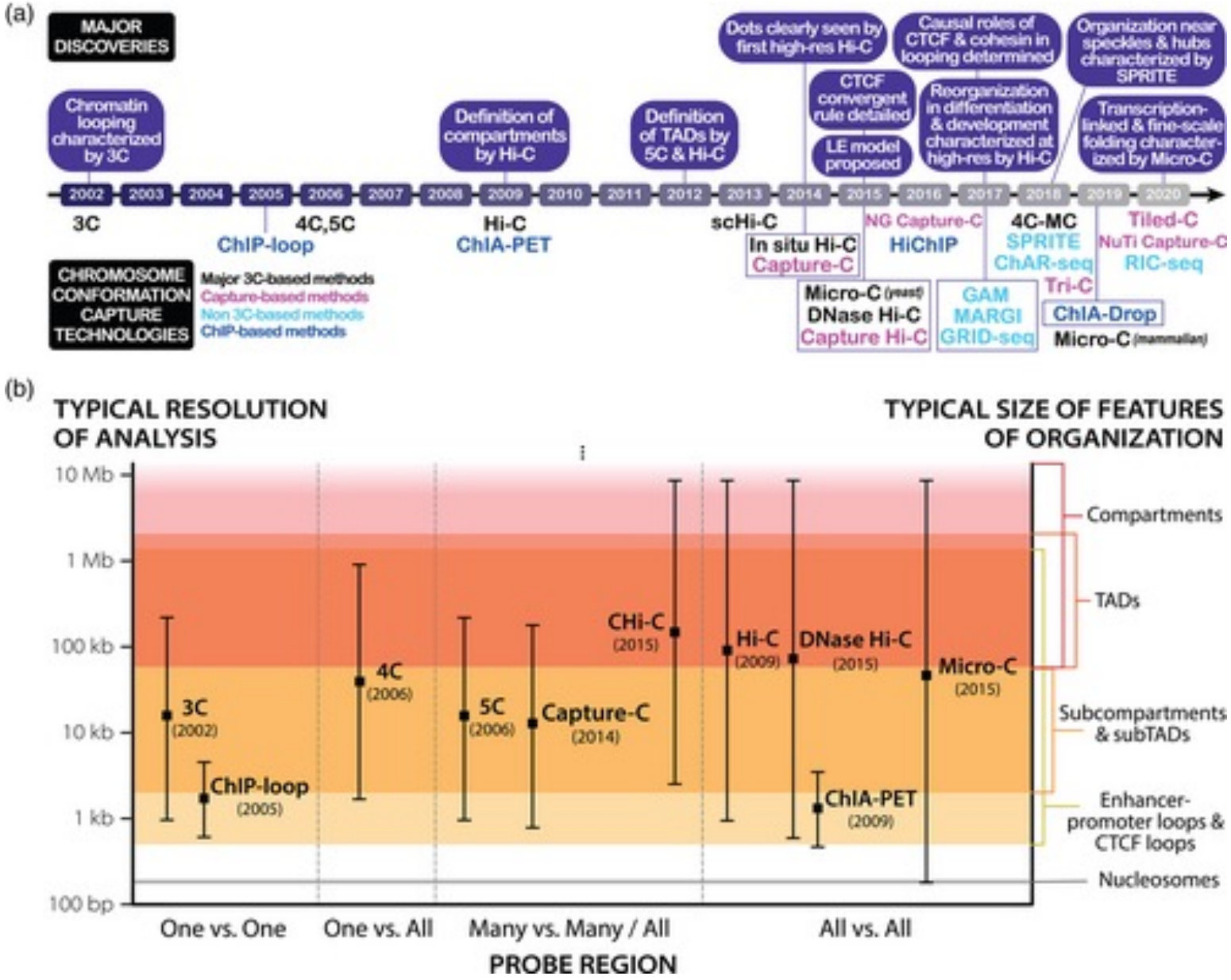


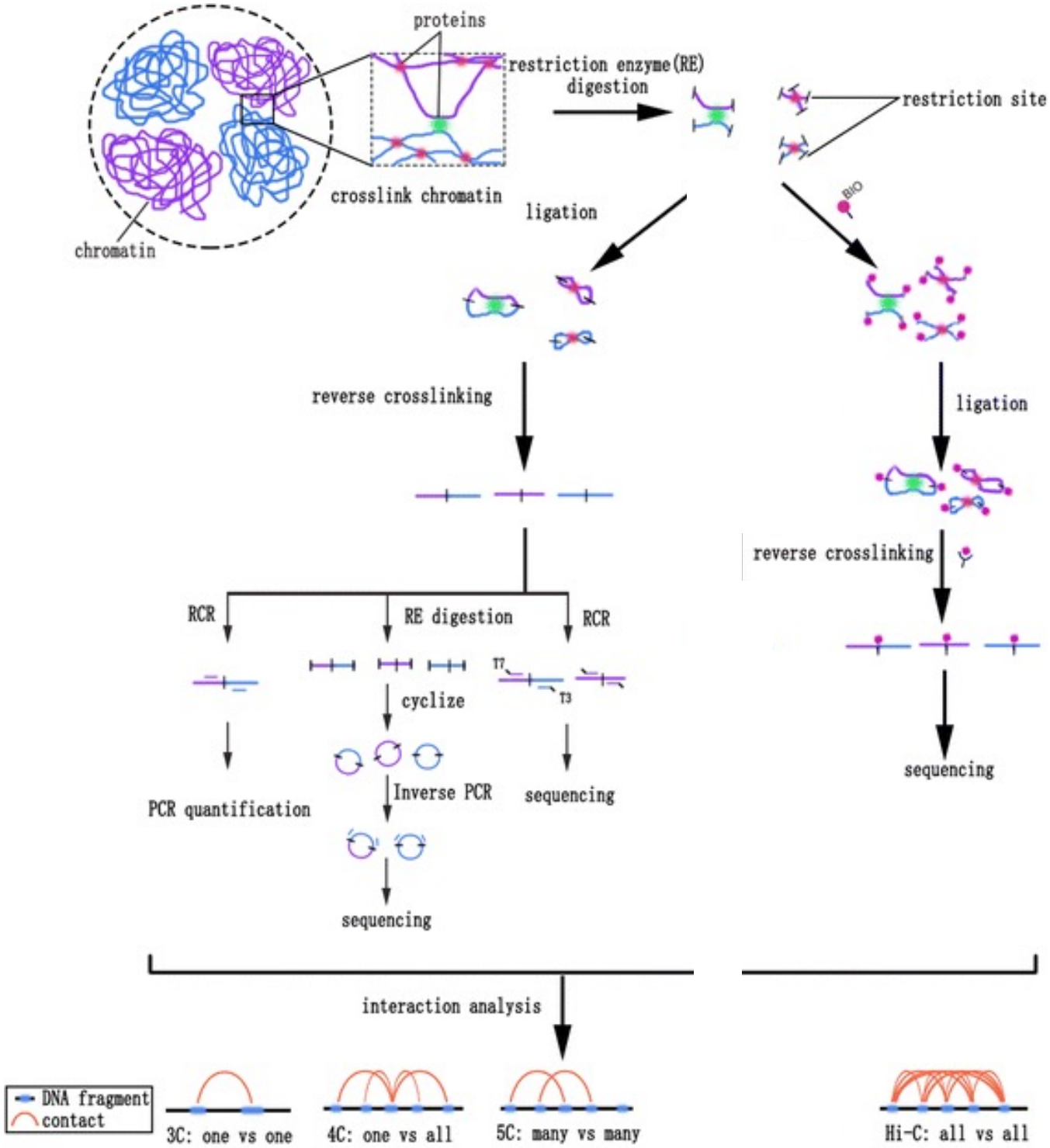


Connessioni tra i DHS e il promotore del gene PAH

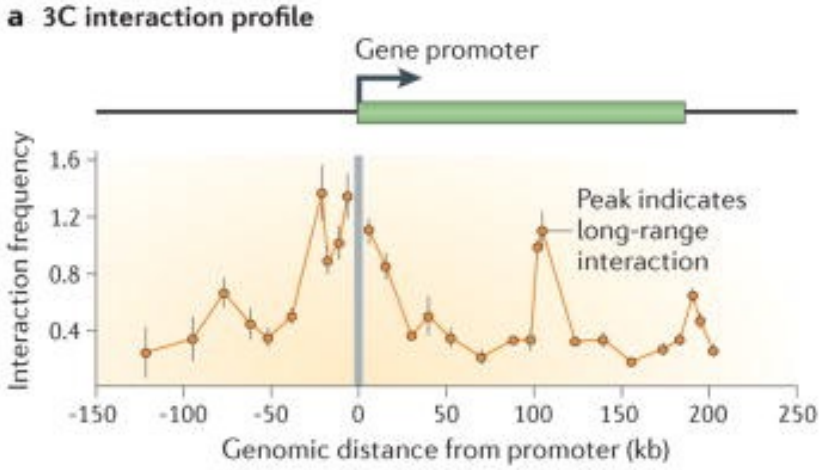


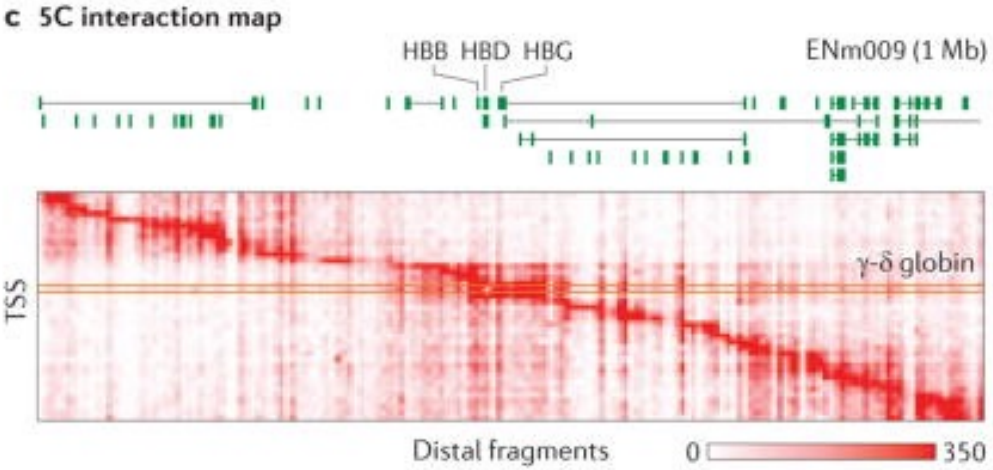
Timeline and comparison of major chromosome conformation capture techniques.

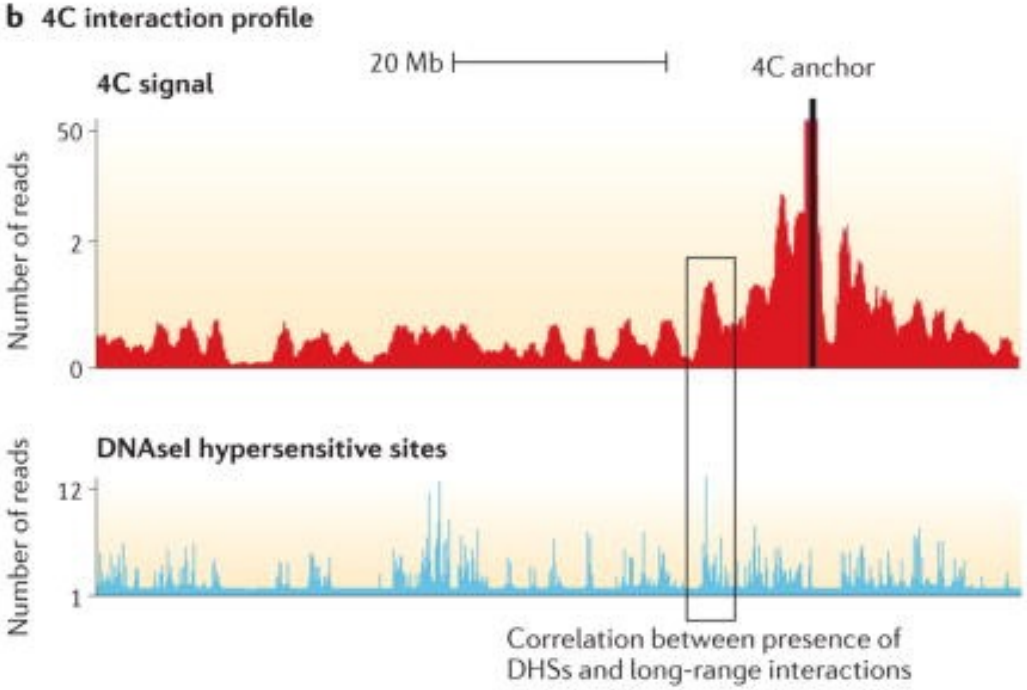


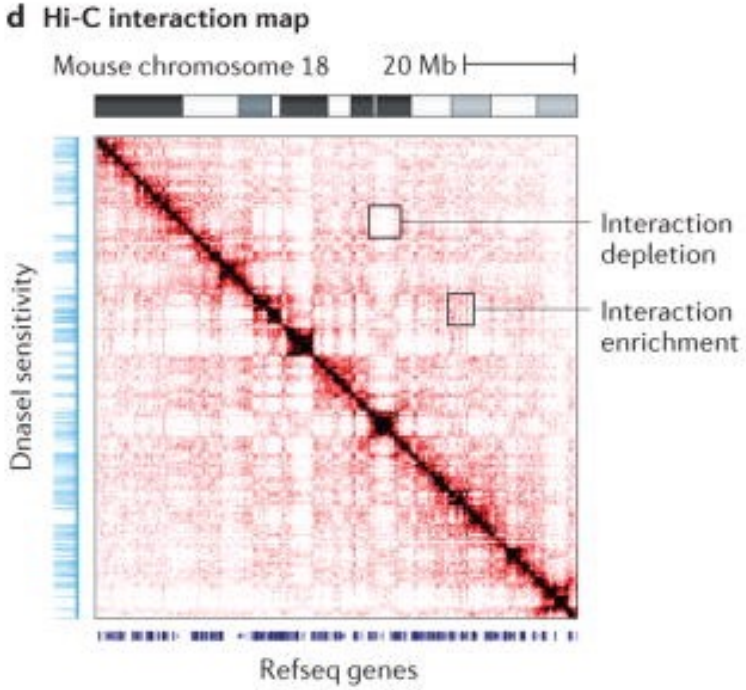

proteins
restriction enzyme(RE) digestion
restriction site
crosslink chromatin
chromatin
ligation
BIO
reverse crosslinking
ligation
reverse crosslinking
RCR
RE digestion
RCR
T7
T3
cyclize
PCR quantification
Inverse PCR
sequencing
sequencing
sequencing
interaction analysis
DNA fragment
contact
3C: one vs one
4C: one vs all
5C: many vs many
Hi-C: all vs all

3C, 4C, 5C and Hi-C datasets

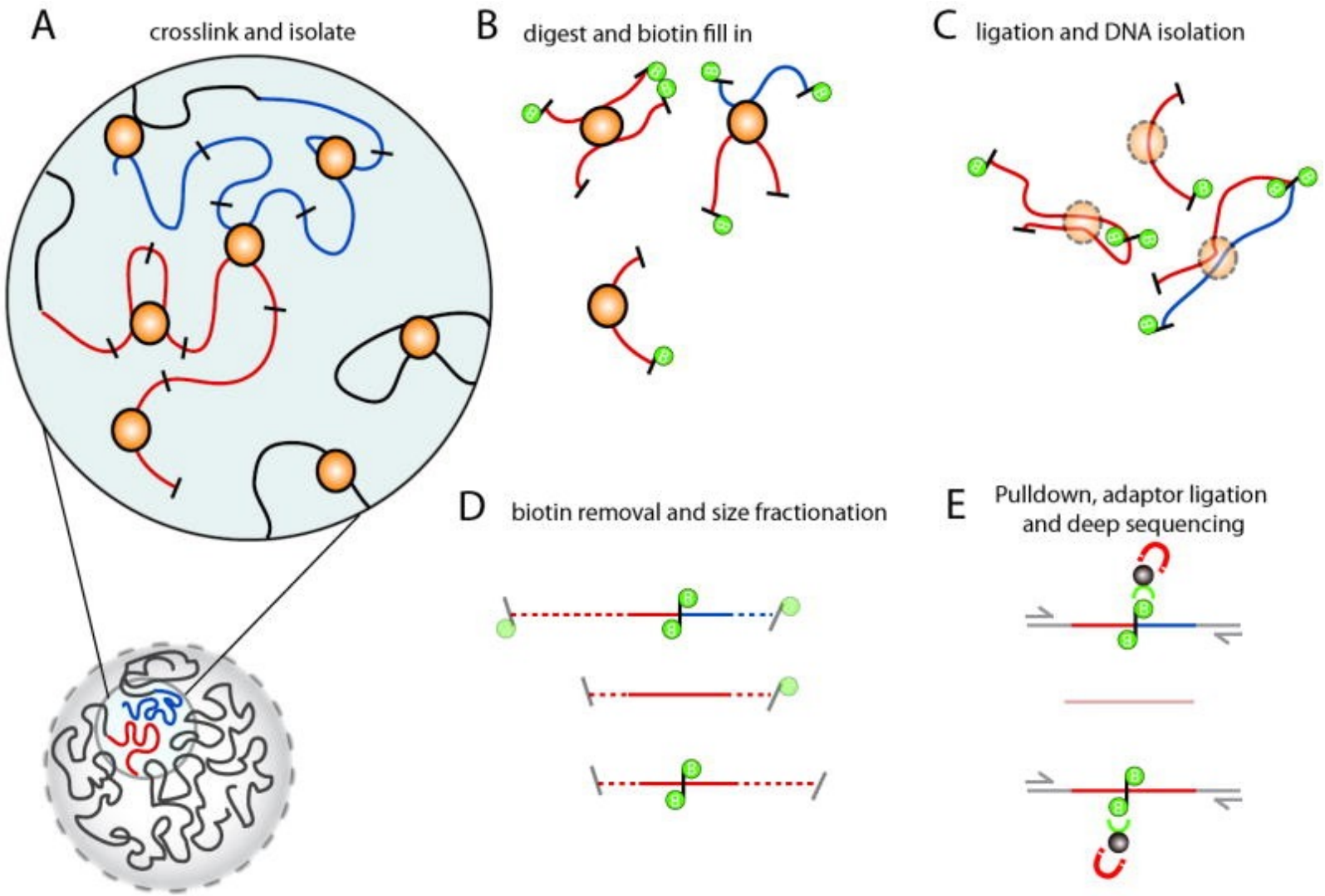




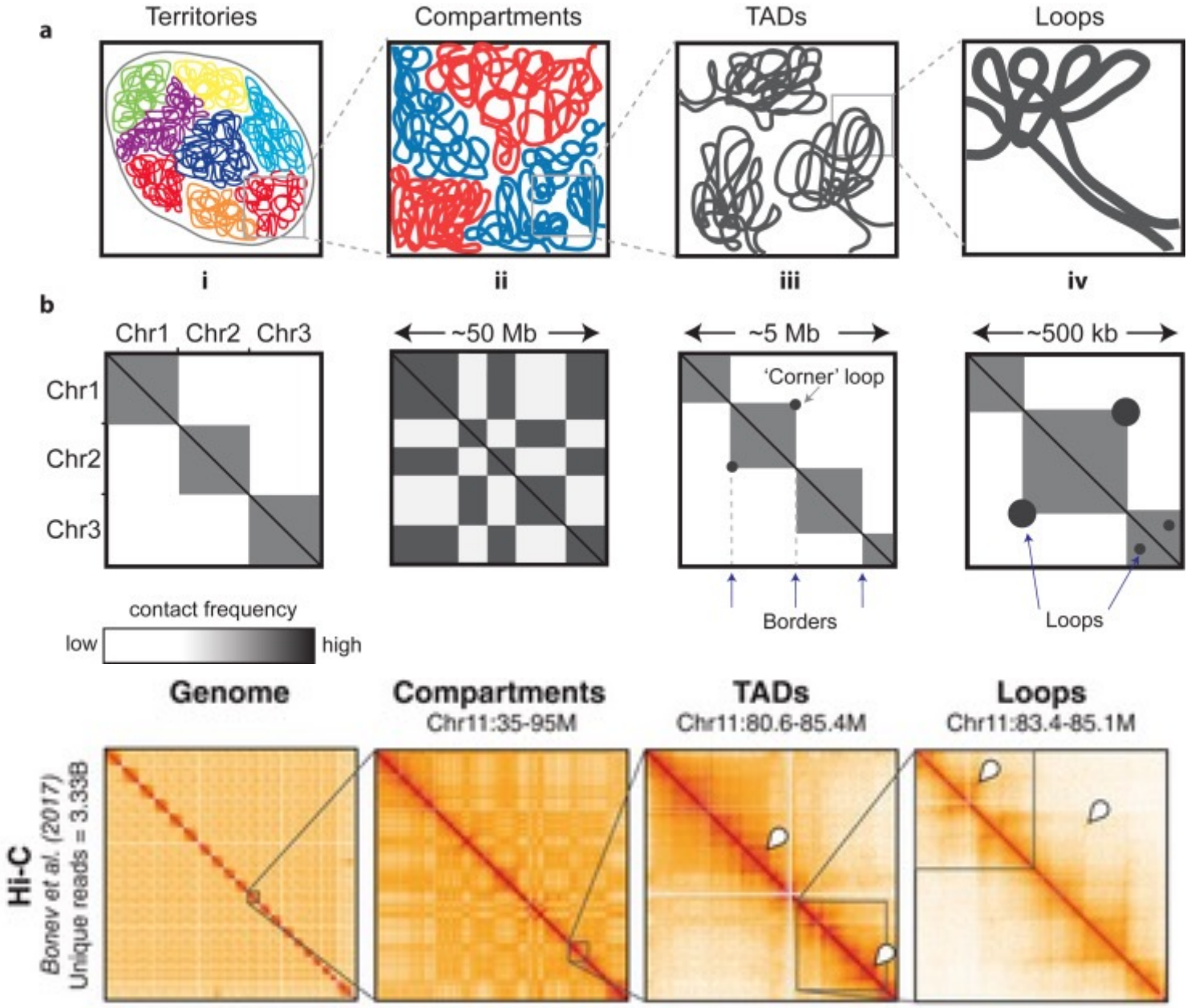


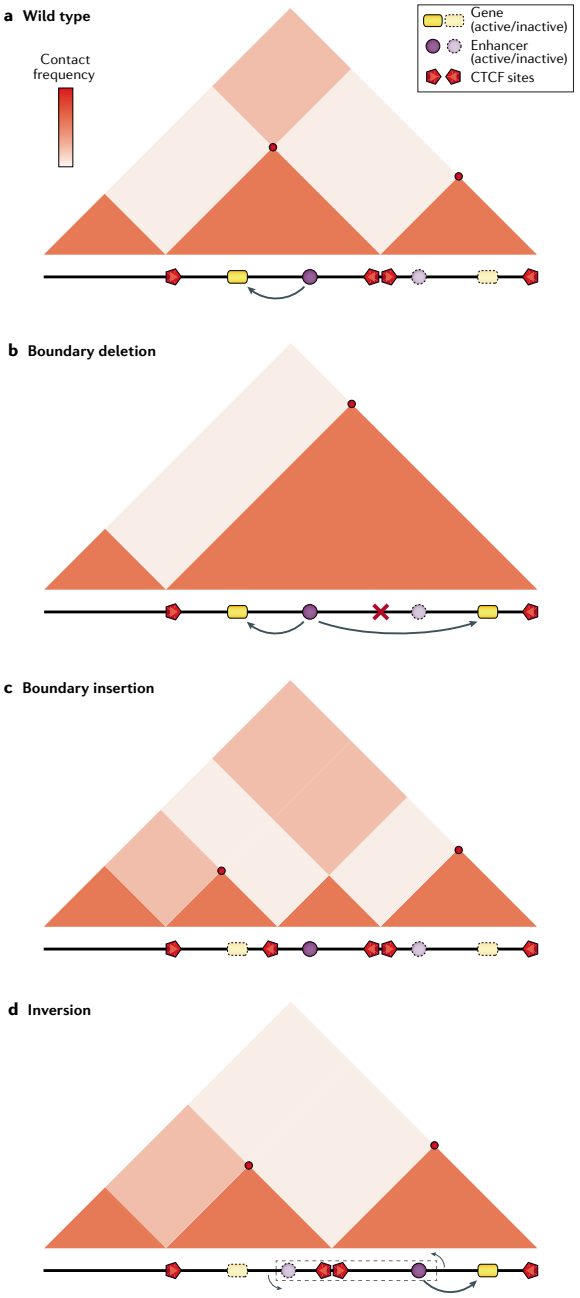


Hi-C: A comprehensive technique to capture the conformation of genomes



Hi-C: A comprehensive technique to capture the conformation of genomes





NATURE REVIEWS | GENETICS

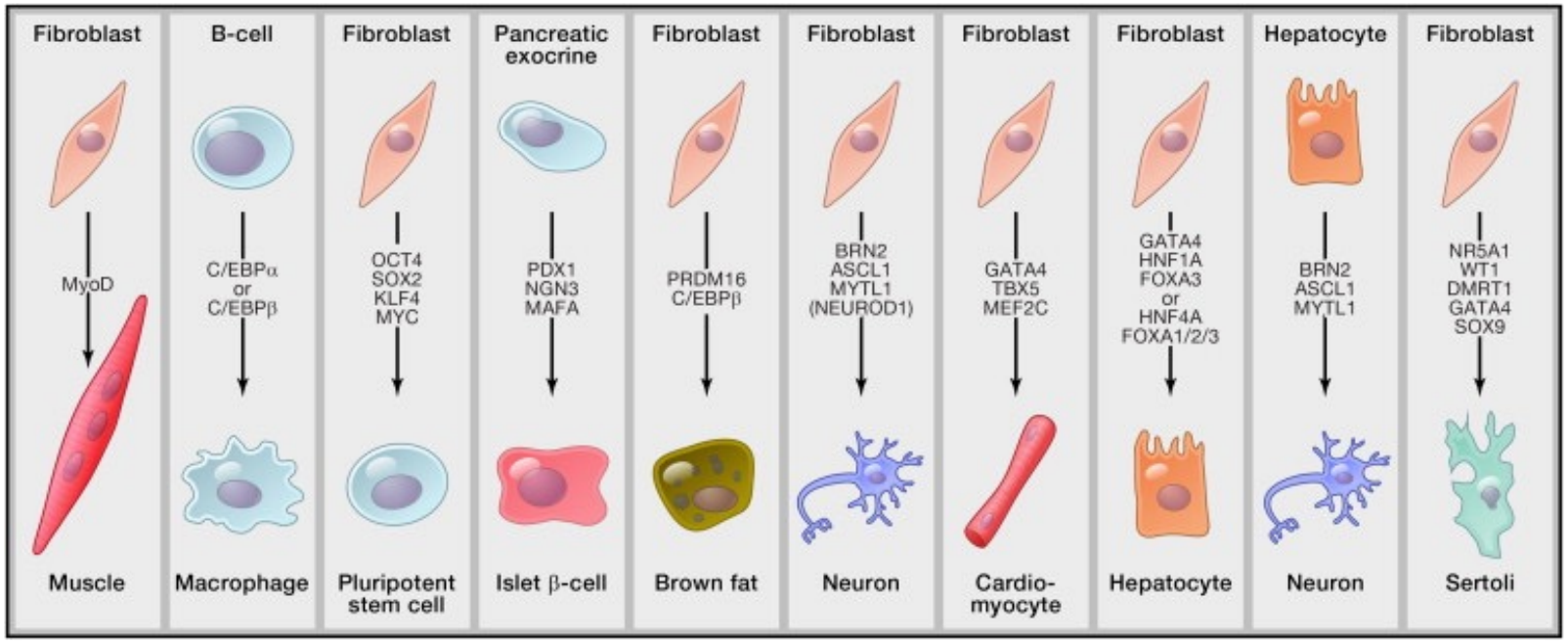
The relationship between the linear order of the regulatory elements and the organization of the genome.

In a hypothetical region of the genome, an active enhancer activates an upstream gene in the middle topologically associating domain (TAD). **b** | Deletion of the downstream boundary of the middle TAD (red cross) causes two TADs to merge; the active enhancer now also activates the downstream gene. **c** | Insertion of a new boundary element within the middle TAD causes the formation of an extra TAD, with the active enhancer and the upstream gene now located in two separate TADs. The enhancer can no longer interact with and activate the upstream gene promoter. **d** | Inversion of the region highlighted by the dashed box repositions the active enhancer in a new TAD. It can no longer interact with the upstream gene promoter, but instead activates the downstream promoter. Note that in each scenario the changes in activity within the domains alter their long-range compartmentalization. CTCF, CCCTC-binding factor.

Master Transcriptional Regulators

The set of genes that are transcribed largely defines the cell. The gene expression program of a specific cell type includes RNA species from genes that are active in most cells (**housekeeping genes**) and genes that are active predominantly in one or a limited number of cell types (**cell-type-specific genes**).

Transcription factors that have dominant roles in the control of specific cell states are capable of reprogramming cell states when ectopically expressed in various cell types.



Transcription factors

- Transcription factors can be separated into two classes based on their regulatory responsibilities: **control of initiation** versus **control of elongation**. This distinction is not absolute, as some transcription factors may contribute to control of both initiation and elongation.

- Transcription factors typically bind cofactors, which are protein complexes that contribute to activation (**coactivators**) and repression (**corepressors**).

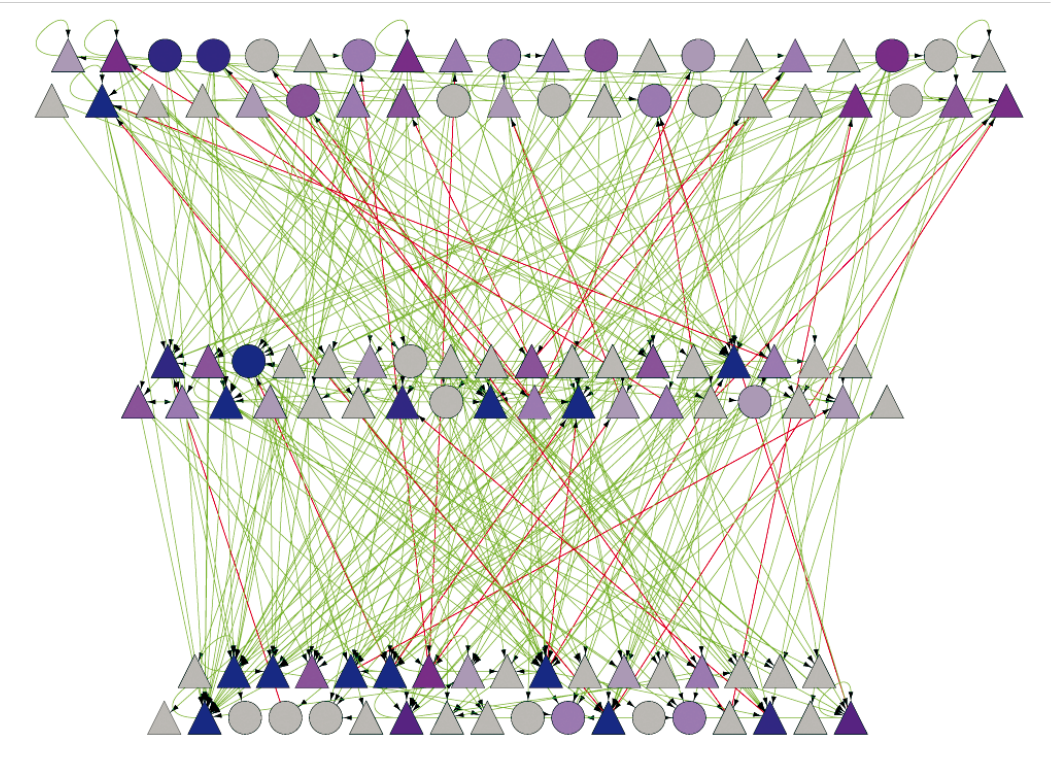
- These coactivators include **histone modifiers** (e.g. HAT) and the **Mediator complex**, among others.

Transcription factors form a vast network

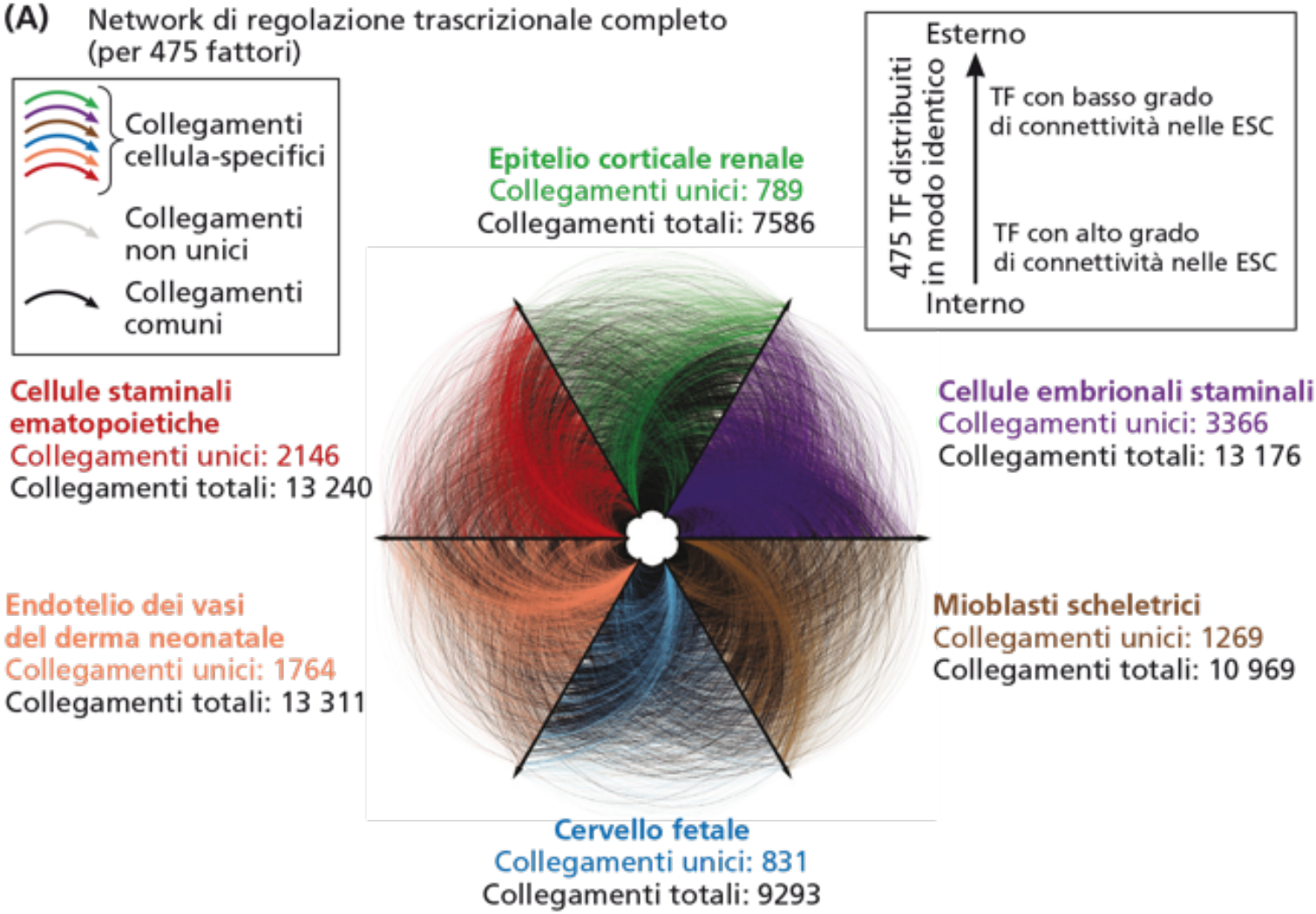
In addition to contributing to the annotation of functional DNA elements, the ENCODE project has enabled analysis of the connections between these linear elements, such as the interaction network among transcription factors and long-range chromatin interactions.

The analysis of target sites for 119 transcription factors examined by the ENCODE project using ChIP-seq has revealed the presence of a hierarchical interaction network. At the top of the pyramid are the most powerful transcription factors, while further down are factors that are more regulated than regulatory.

www.regulatorynetworks.org: links between specific transcription factors in certain cell types

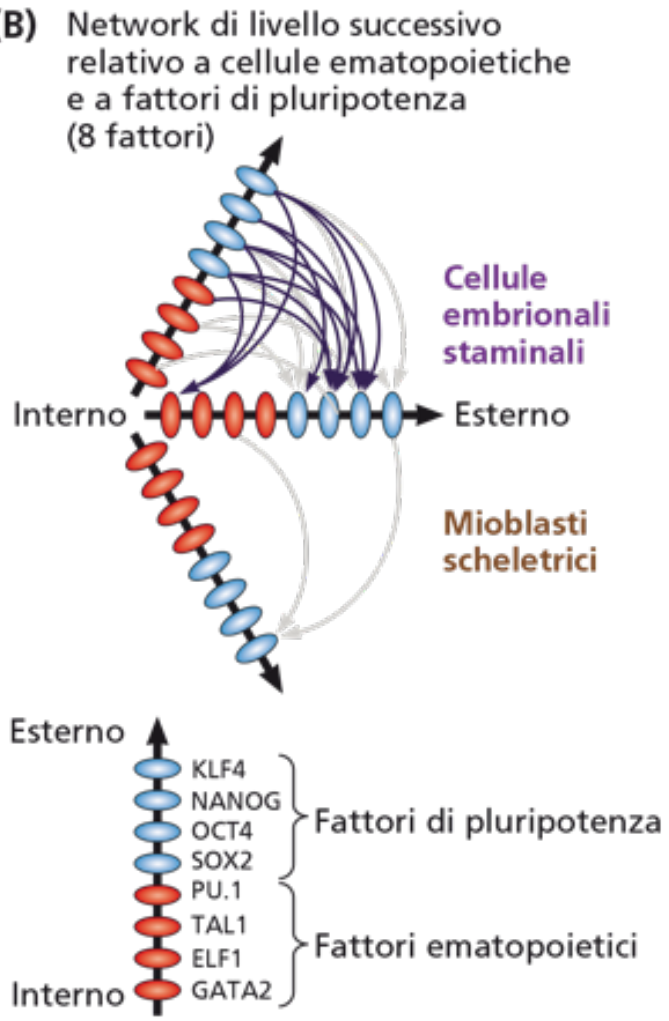


Connection networks among transcription factors show high cell specificity



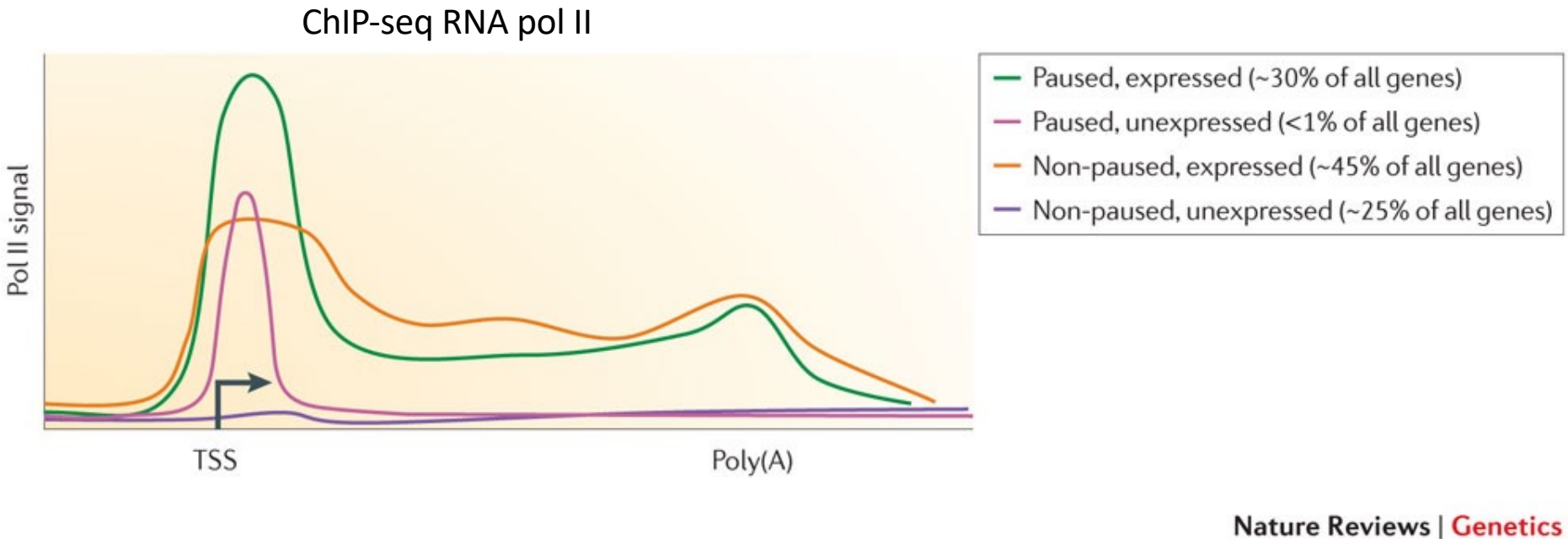
475 transcription factors in 6 connection networks of different cell types.

Connection networks among transcription factors show high cell specificity



Transcriptional control by RNA pol II pausing

Whereas traditional models focused solely on the events that brought RNA Pol II to a gene promoter to initiate RNA synthesis, emerging evidence points to the pausing of Pol II during early elongation as a widespread regulatory mechanism in higher eukaryotes.

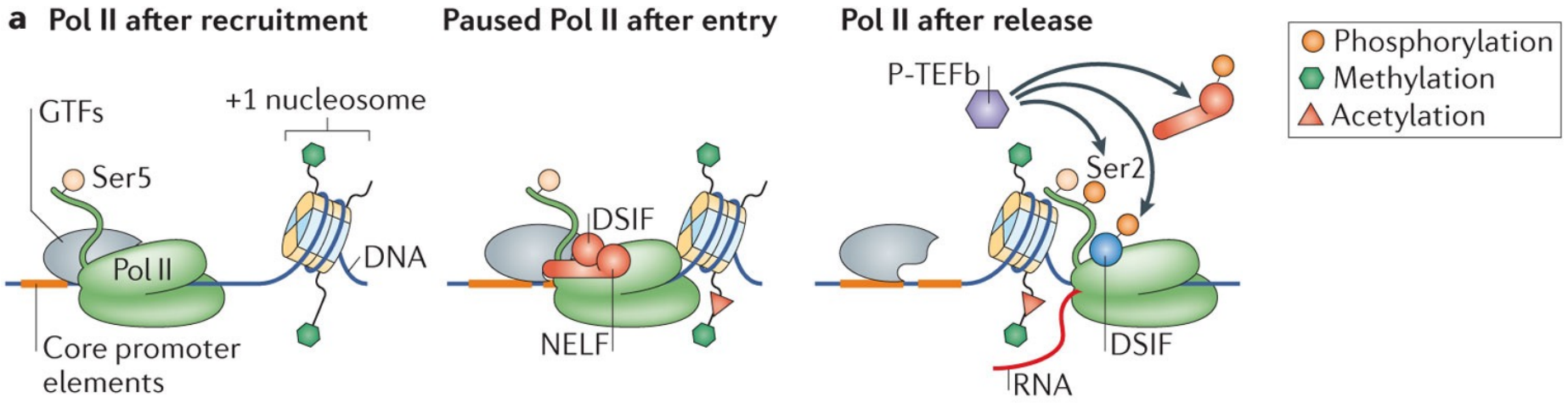


Transcriptional control by RNA pol II pausing

Once the recruited RNA Pol II molecules initiate transcription, they generally transcribe a short distance, typically 20–80 bp, and then pause. This process is controlled by the factors **DSIF** and **NELF**, which are physically associated with the paused RNA Pol II. The paused polymerases may transit to active elongation through pause release, or may ultimately terminate transcription with release of the small RNA species.

Pause release and subsequent elongation occur through recruitment and activation of **positive transcription elongation factor b (P-TEFb)**, which phosphorylates the Ser2 of paused polymerase and its associated pause control factors.

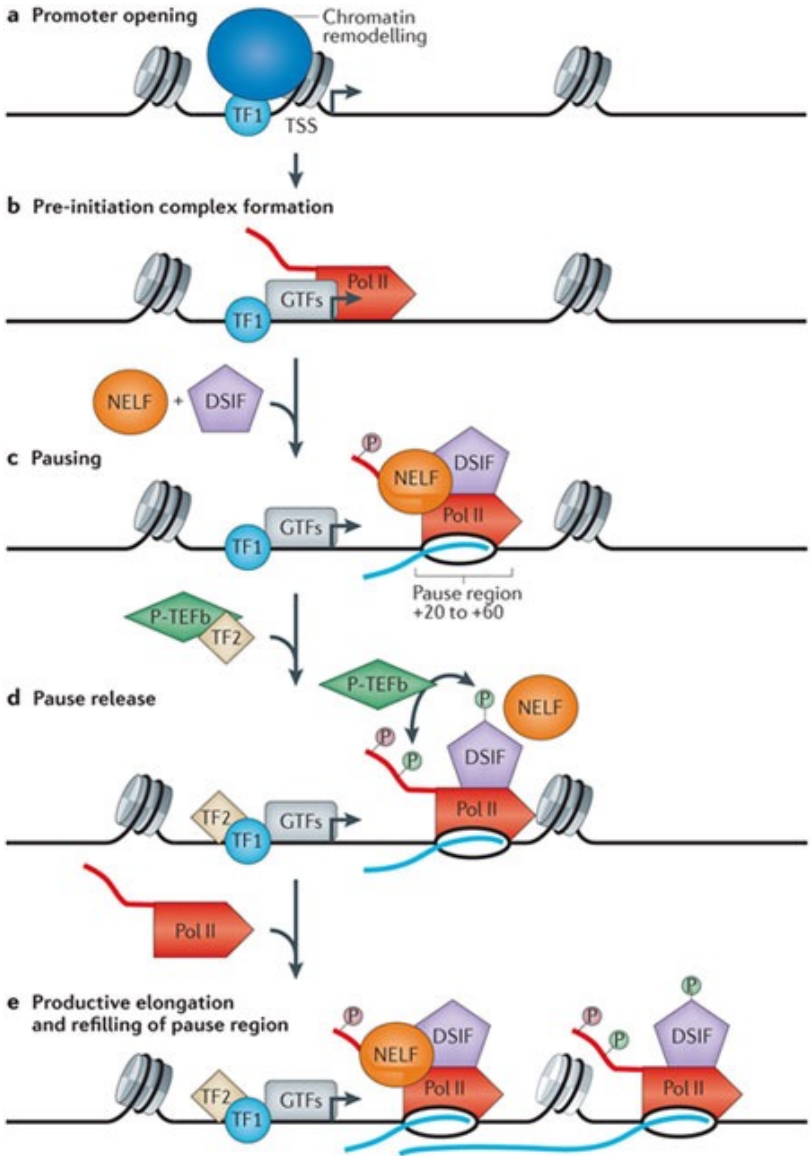
Transcription factors may stimulate P-TEFb-mediated release of RNA polymerase II from these pause sites and thus contribute to the control of transcription elongation



Establishment and release of paused Pol II

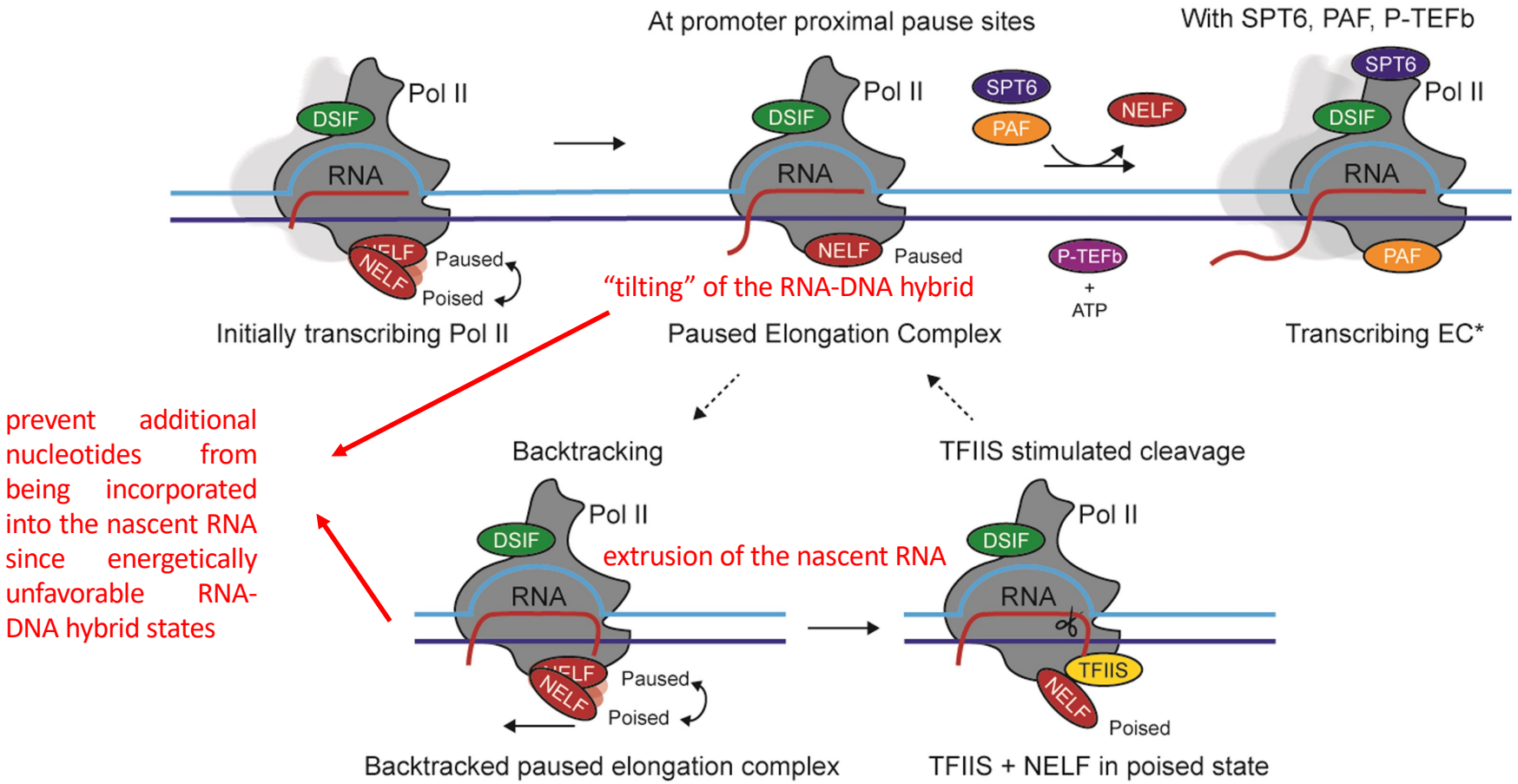
a. Promoter opening often involves binding of transcription factor (TF) that brings in chromatin remodellers to remove nucleosomes from around the TSS and to render the promoter accessible for recruitment of the transcription machinery.

b. Pre-initiation complex formation involves the recruitment of a set of general transcription factors and Pol II. This step precedes the initiation of RNA synthesis. **c. Pol II pausing** occurs shortly after transcription initiation and involves the association of pausing factors DSIF and NELF. The paused Pol II is phosphorylated on its CTD domain. **d. Pause release** is triggered by the recruitment of the P-TEFb kinase, either directly or indirectly by a transcription factor (TF2). P-TEFb kinase phosphorylates the DSIF–NELF complex to release paused Pol II. **e. Phosphorylation of DSIF–NELF** dissociates NELF from the elongation complex and transforms DSIF into a positive elongation factor that associates with Pol II throughout the gene.



Model for function of NELF poised and paused states during early transcription elongation

Promoter proximal pausing is a general feature of transcription elongation affecting virtually all genes and is important for retaining open chromatin at gene promoters and for regulating rapid gene expression in response to environmental and developmental cues. Pausing is the consequence of both the underlying DNA sequence and the function of DSIF and NELF. Additional factors, such as **cap-binding complex**, histone chaperone FACT, and BRD4, can play a role in pause regulation



Resolution of these non-productive states by Pol II is greatly stimulated by the association of transcription factor (TF) IIS with the transcription elongation complex. TFIIS binds near the Pol II active site and stabilizes a Mg2+ ion, which supports cleavage of extruded RNA by Pol II and restoration of the active site to a state compatible with elongation.

7SK RNA regulated p-TEFb activity

7SK RNA forms the core of a small nuclear ribonucleoprotein (snRNP) complex that minimally contains P-TEFb, and the proteins HEXIM1/2 and LARP7, which control the activity of P-TEFb and stabilize 7SK RNA. The P-TEFb kinase is inactive when present in the 7SK snRNP complex and becomes active when it dissociates from the complex.