# Transcriptional regulation I

# Eukaryotic Transcriptional Regulation

**1. Level of Chromatin (DNA accessibility)**

- Histone modifications
- Histone modifying enzymes & remodeling complexes
- Nucleosome composition
- DNA methylation

**2. Level of DNA (Interaction with basal transcription machinery)**

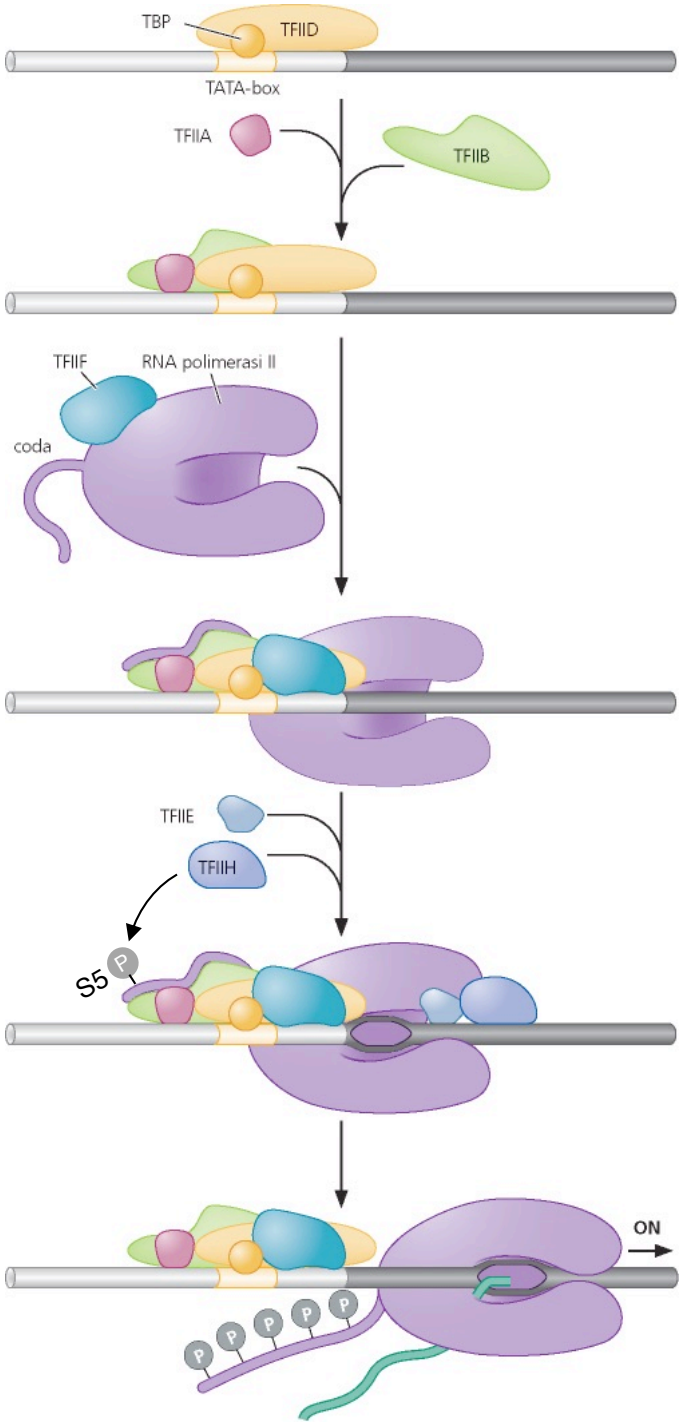
- Regulatory sequences (enhancers, silencers)
- Transcription factors (activators, repressors)

**3. Level of Regulatory RNA (Interaction with DNA, RNA or protein)**

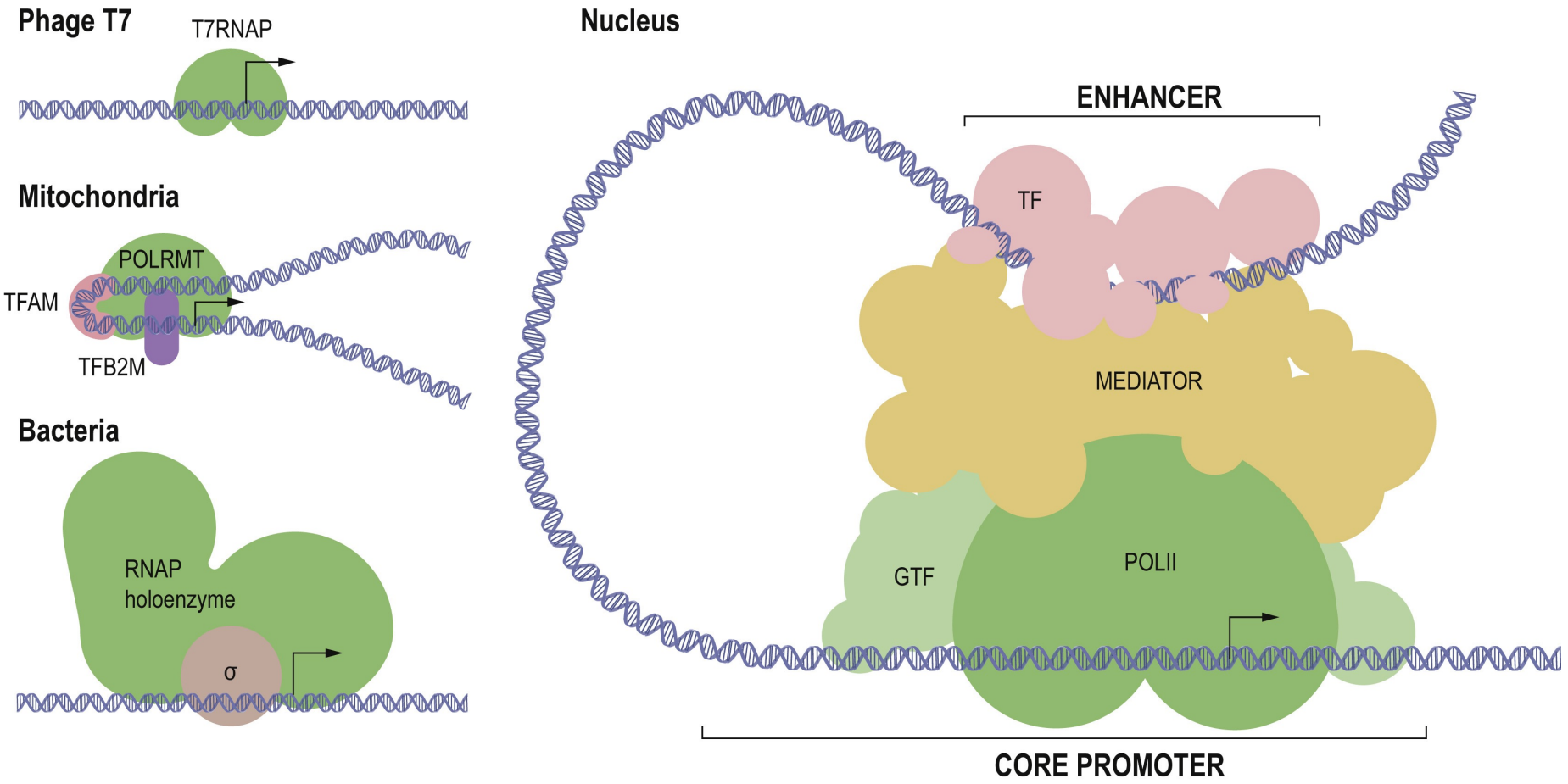
- Small and long non-coding RNAs

# Transcription initiation in eukaryotes

At the transcription start site, Pol II initiation is regulated by a protein assembly known as the **pre-initiation complex (PIC)** containing **TFIIA**, **TFIIB**, **TFIID**, **TFIIE**, **TFIIF**, **TFIIH**, **Pol II** and **Mediator**

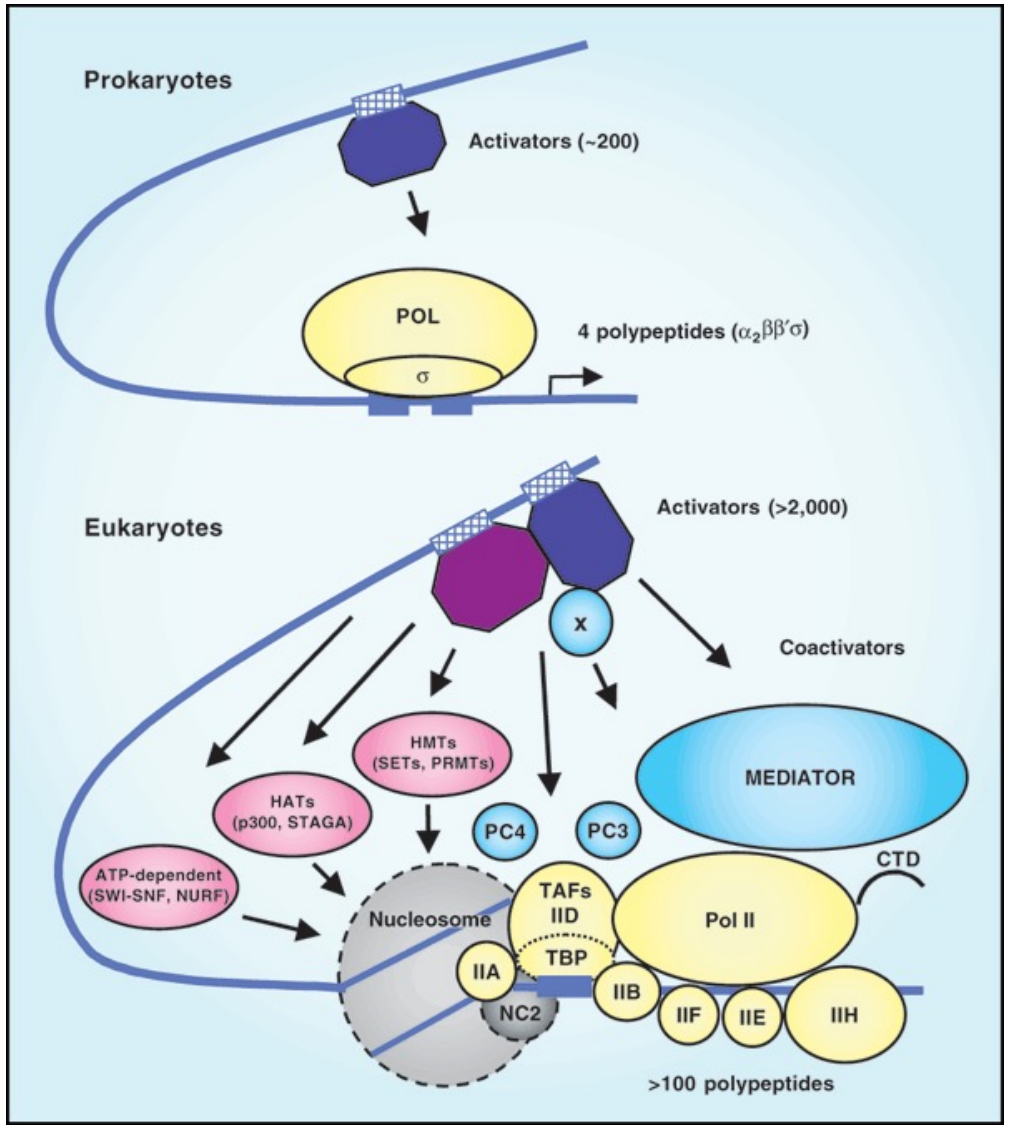


# Schematic comparison of transcription initiation systems

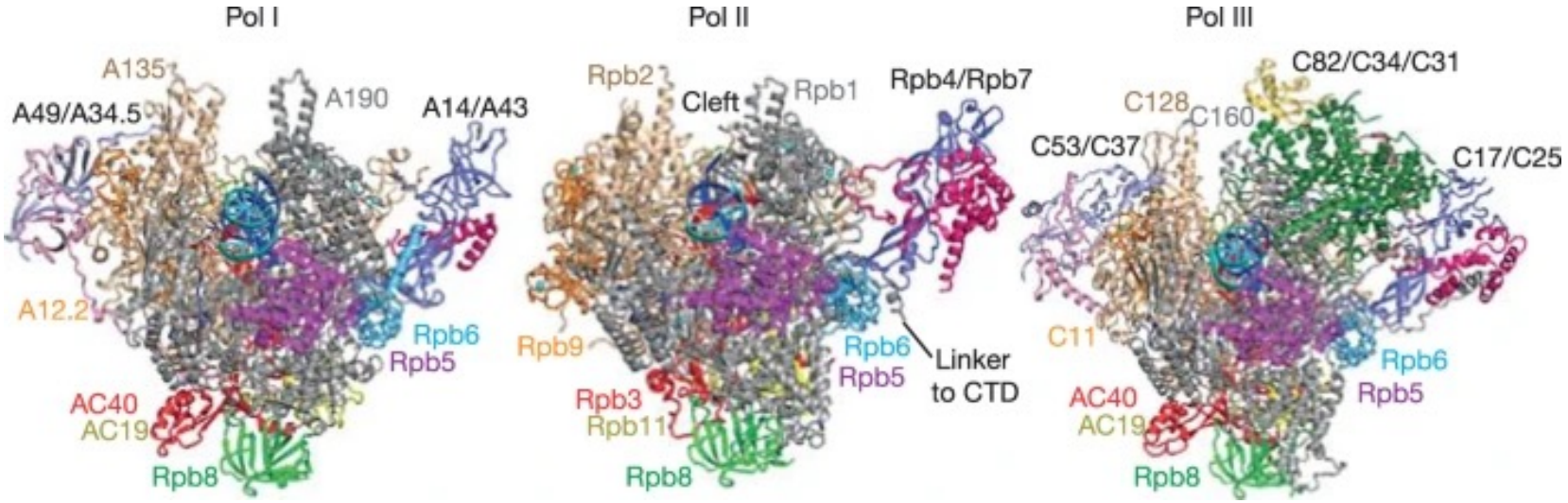


# Comparison of transcription activation mechanisms in prokaryotes and eukaryotes

The ultimate purpose of DNA-binding activators is to mediate RNA polymerase recruitment and function. But whereas activators interact directly with RNA polymerase subunits in prokaryotes, the effects of activators on RNA polymerase II in eukaryotes are mostly indirect and involve interactions with diverse cofactors that modify chromatin structure and facilitate recruitment of RNA polymerase II and general initiation factors



# The RNA-polimerases

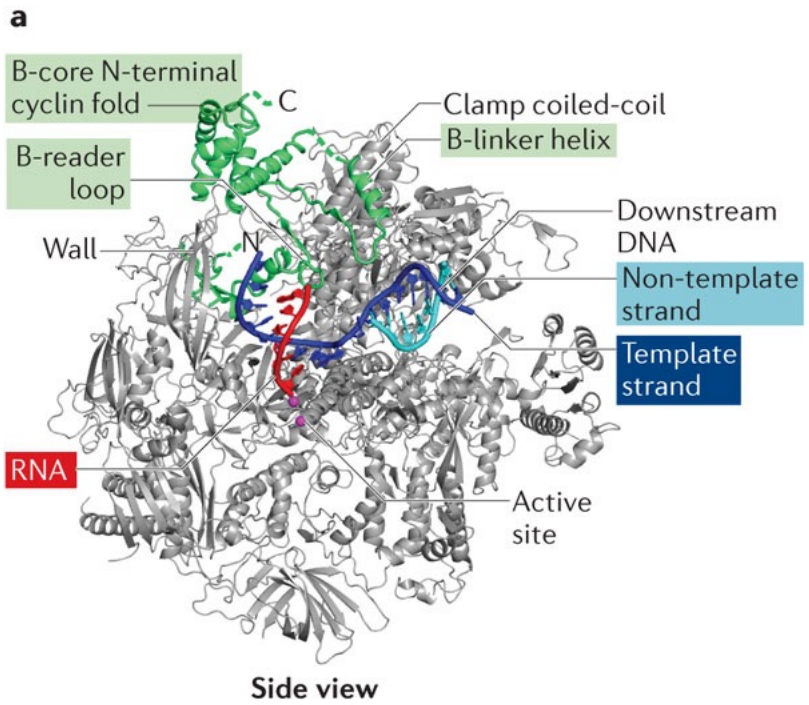



rRNA

mRNA
Variety of ncRNA

tRNA
Small ribosomal RNA

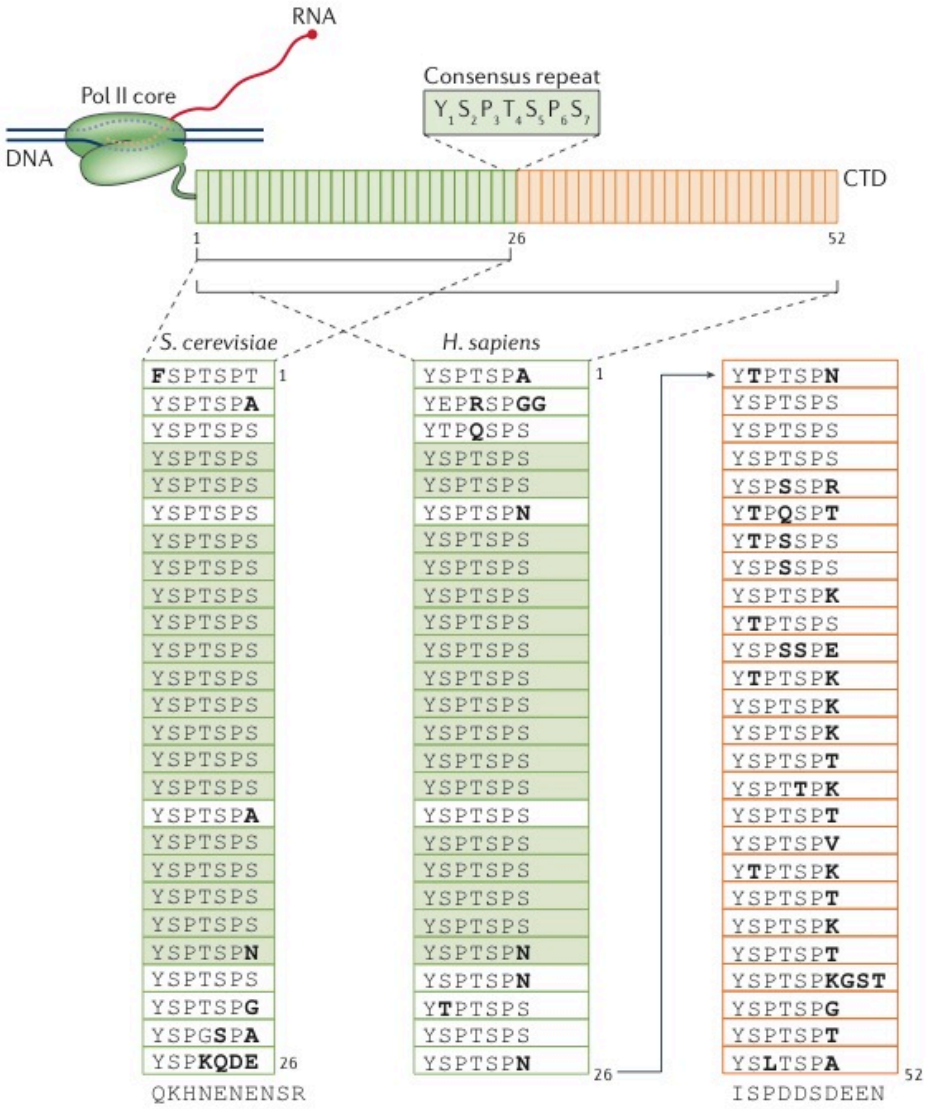
# RNA Pol II




| Factor | Gene name | | Mass (kDa) | | Uniprot accession number | |
|---|---|---|---|---|---|---|
| | Yeast | Human | Yeast | Human | Yeast | Human |
| *Pol II (RNAP*): transcribing enzyme* | | | | | | |
| RPB1 | *RPO21* | *POLR2A* | 191.6 | 217.2 | P04050 | P24928 |
| RPB2 | *RPB2* | *POLR2B* | 138.8 | 133.9 | P08518 | P30876 |
| RPB3 | *RPB3* | *POLR2C* | 35.3 | 31.4 | P16370 | P19387 |
| RPB4 | *RPB4* | *POLR2D* | 25.4 | 16.3 | P20433 | O15514 |
| RPB5‡ | *RPB5* | *POLR2E* | 25.1 | 24.6 | P20434 | P19388 |
| RPB6‡ | *RPO26* | *POLR2F* | 17.9 | 14.5 | P20435 | P61218 |
| RPB7 | *RPB7* | *POLR2G* | 19.1 | 19.3 | P34087 | P62487 |
| RPB8‡ | *RPB8* | *POLR2H* | 16.5 | 17.1 | P20436 | P52434 |
| RPB9 | *RPB9* | *POLR2I* | 14.3 | 14.5 | P27999 | P36954 |
| RPB10‡ | *RPB10* | *POLR2L* | 8.3 | 7.6 | P22139 | P62875 |
| RPB11 | *RPB11* | *POLR2J* | 13.6 | 13.3 | P38902 | P52435 |
| RPB12‡ | *RPB12* | *POLR2K* | 7.7 | 7.0 | P40422 | P53803 |
| Total (12 subunits) | | | 513.6 | 516.7 | | |

# The carboxy-terminal domain (CTD)

This domain consists of heptapeptide repeats with the consensus sequence YSPTSPS and forms the **carboxy-terminal domain (CTD)** of the largest subunit of Pol II RNA polymerase B1 (RPB1). The CTD is not required for the catalytic functions of Pol II, but it is crucial for the regulation of multiple steps in transcription, as well as for the coupling of transcription to a number of co- transcriptional processes.



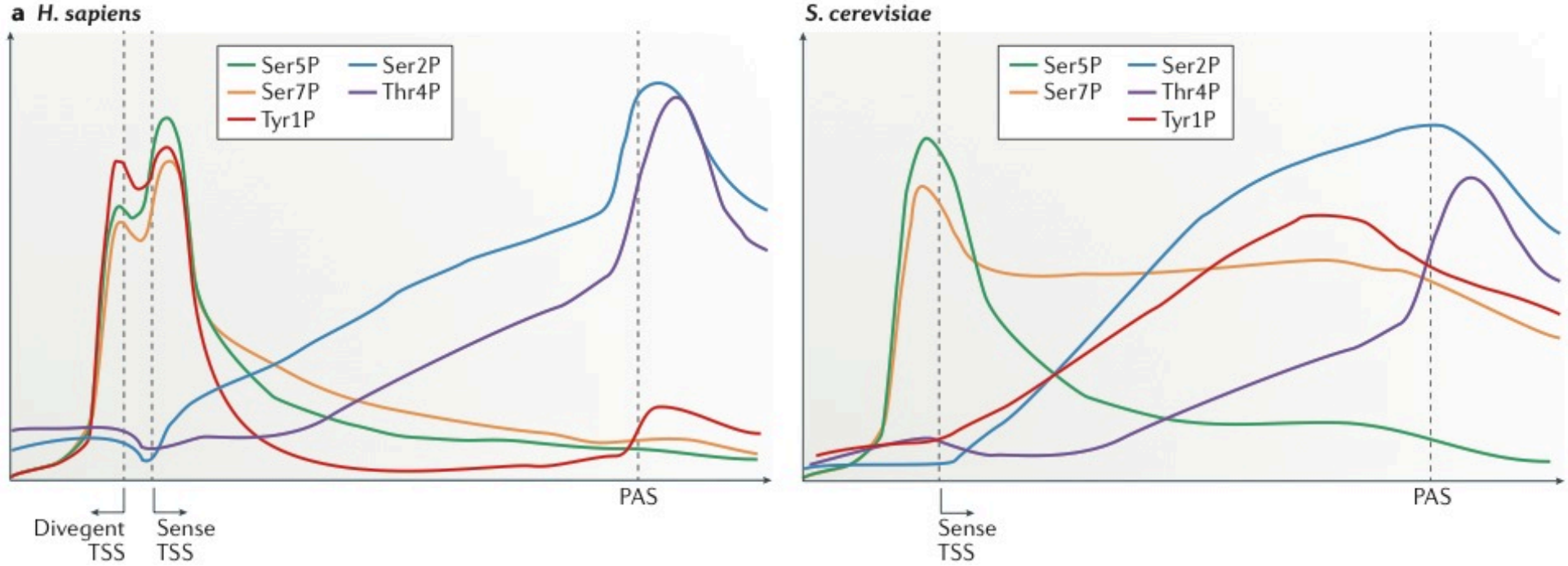
# Post-translational modifications of the CTD and their associated processes

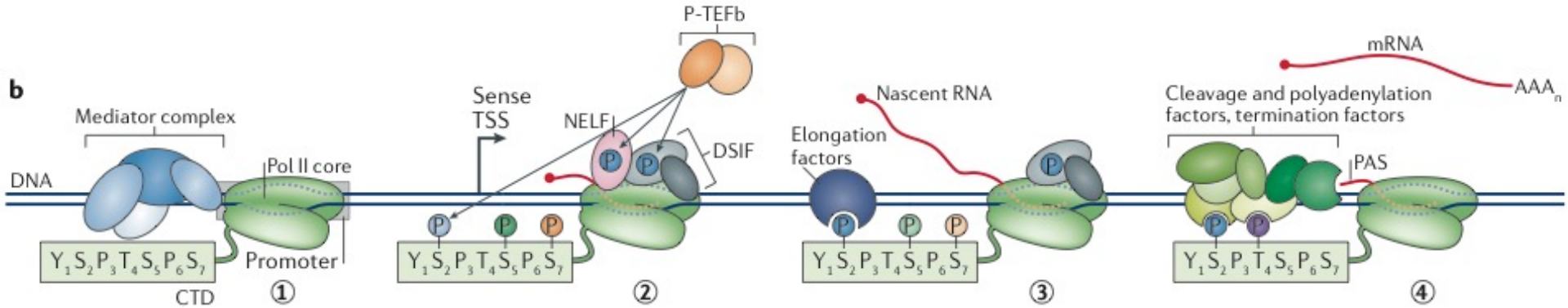
| Post-translational modification | Position in the CTD | Organisms | Associated process or processes |
|---|---|---|---|
| Ser5 phosphorylation | Multiple repeats | • *Saccharomyces cerevisiae*<br>• *Schizosaccharomyces pombe*<br>• *Homo sapiens* | Transcription initiation, mRNA capping and splicing, non-coding RNA transcription termination and chromatin modification |
| Ser2 phosphorylation | Multiple repeats | • *S. cerevisiae*<br>• *S. pombe*<br>• *H. sapiens* | Transcription elongation, promoter-proximal pause and release, splicing, transcription termination and DNA topology |
| Ser7 phosphorylation | Multiple repeats | • *S. cerevisiae*<br>• *S. pombe*<br>• *H. sapiens* | snRNA expression, interaction with the Integrator complex and P-TEFb recognition |
| Thr4 phosphorylation | Multiple repeats | • *S. cerevisiae*<br>• *S. pombe*<br>• *Gallus gallus*<br>• *H. sapiens* | Transcription elongation and termination, post-transcriptional splicing, processing of histone mRNA and chromatin remodelling |
| Tyr1 phosphorylation | Multiple repeats | • *S. cerevisiae*<br>• *S. pombe*<br>• *G. gallus*<br>• *H. sapiens* | Inhibition of recruitment of transcription termination factors, CTD stability, antisense and enhancer transcription |
| Arg methylation | Arg1,810 of human RPB1 | • *Mus musculus*<br>• *H. sapiens* | snRNA and snoRNA regulation, R-loop resolution and transcription termination |
| Lys methylation | Lys7 in the non-consensus region of human CTD | • *H. sapiens*<br>• *M. musculus*<br>• *Drosophila melanogaster*<br>• *Caenorhabditis elegans* | Supports nucleosome occupancy at promoters; negatively regulates gene expression |
| Lys acetylation | Lys7 in the non-consensus region of murine CTD; Lys7 in repeats 39, 42, 47 and 49 of human CTD | • *H. sapiens*<br>• *M. musculus* | Induction of growth-factor response genes, transcription elongation; maintains balance between Lys methylation and acetylation and affects mRNA expression levels |
| *O*-GlcNAcylation | Ser5 and/or Ser7 in multiple repeats | *H. sapiens* | Pre-initiation complex assembly |
| Ubiquitylation | RPB1 Lys residues 859, 1866, 1873, 1887, 1908, 1922 | *M. musculus* | RPB1 degradation |

CTD, carboxy-terminal domain of RNA polymerase II; P-TEFb, positive transcription elongation factor B; RPB1, RNA polymerase B1; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA.

# Transcription regulation by the CTD code

Average chromatin immunoprecipitation (ChIP) profiles of phosphorylated residues of the carboxy-terminal domain (CTD) of RNA polymerase II (Pol II) across protein-coding genes in humans (*Homo sapiens)* and in budding yeast (*Saccharomyces cerevisiae)* .






1. **Recruitment of the core Pol II enzyme** to the promoter with an unphosphorylated CTD that interacts with the Mediator complex. The Mediator complex has a high affinity for unphosphorylated CTD and upon phosphorylation of Ser5 of the CTD, this affinity is lost and Pol II escapes from the promoter.

2. **Pol II during promoter-proximal pausing**. At this step, Pol II is highly phosphorylated at Ser5 and Ser7, is paused downstream of the transcription start site (TSS) and is bound by **negative elongation factor (NELF)** and **DRB sensitivity-inducing factor (DSIF).** The arrival of **positive elongation factor B (P-TEFb)** leads to the phosphorylation of NELF, DSIF and Ser2. These phosphorylation events are followed by the release of NELF and the transition into productive transcription elongation.

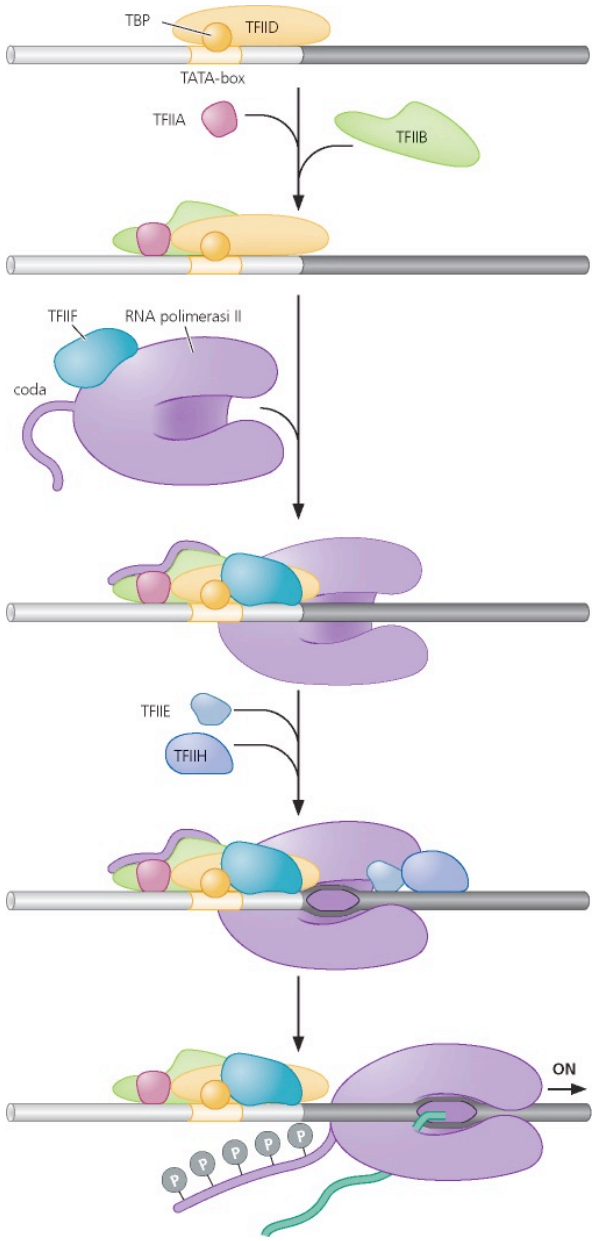
3. **During productive elongation**, the CTD contains lower levels of Ser5P and Ser7P and higher levels of Ser2P, which promotes the recruitment of many transcription elongation, chromatin-modifying and RNA-processing factors that regulate co-transcriptional processes.

4. **Pol II transitions from transcription elongation to termination**. Ser2P and Thr4P levels peak, thereby promoting the recruitment of cleavage and polyadenylation factors, as well as termination factors that release Pol II from the DNA.

# General Transcription Factors

| Factor | Gene name | | Mass (kDa) | | Uniprot accession number | | Copies |
|---|---|---|---|---|---|---|---|
| | Yeast | Human | Yeast | Human | Yeast | Human | |
| *TFIIA[§]: TBP stabilization and counteracts repressive effects of negative co-factors* | | | | | | | |
| Large subunit | *TOA1* | *GTF2A1* | 32.2 | 41.5 | P32773 | P52655 | 1 |
| Small subunit | *TOA2* | *GTF2A2* | 13.5 | 12.5 | P32774 | P52657 | 1 |
| Total (2 subunits) | | | 45.7 | 54.0 | | | |
| *TFIIB: Pol II recruitment, TBP binding and TSS selection* | | | | | | | |
| TFIIB (TFB*) | *SUA7* | *GTF2B* | 38.2 | 34.8 | P29055 | Q00403 | 1 |
| *TFIID: Pol II recruitment and promoter recognition* | | | | | | | |
| TBP (TBP*): recognition of the TATA box | *TBP* | *TBP* | 27.0 | 37.7 | P13393 | P20226 | 1 |
| TAF1 | *TAF1* | *TAF1* | 120.7 | 212.7 | P46677 | P21675 | 1 |
| TAF2 | *TAF2* | *TAF2* | 161.5 | 137.0 | P23255 | Q6P1X5 | 1 |
| TAF3 | *TAF3* | *TAF3* | 40.3 | 103.6 | Q12297 | Q5VWG9 | 1 |
| TAF4 | *TAF4* | *TAF4* | 42.3 | 110.1 | P50105 | O00268 | 2 |
| TAF5 | *TAF5* | *TAF5* | 89.0 | 86. | | | 2 |
| TAF6 | *TAF6* | *TAF6* | 57.9 | 72. | | | 2 |
| TAF7 | *TAF7* | *TAF7* | 67.6 | 40. | | | 1 |
| TAF8 | *TAF8* | *TAF8* | 58.0 | 34. | | | 1 |
| TAF9 | *TAF9* | *TAF9* | 17.3 | 29.0 | Q05027 | Q16594 | 2 |
| TAF10 | *TAF10* | *TAF10* | 23.0 | 21.7 | Q12030 | Q12962 | 2 |
| TAF11 | *TAF11* | *TAF11* | 40.6 | 23.3 | Q04226 | Q15544 | 1 |
| TAF12 | *TAF12* | *TAF12* | 61.1 | 17.9 | Q03761 | Q16514 | 2 |
| TAF13 | *TAF13* | *TAF13* | 19.1 | 14.3 | P11747 | Q15543 | 1 |
| TAF14[‖] | *TAF14* | NA | 27.4 | NA | P35189 | NA | 3 |
| Total (14–15 subunits) | | | 1,200[¶] | 1,300[¶] | | | |
| *TFIIE: recruitment of TFIIH and open DNA stabilization* | | | | | | | |
| TFIIEα (TFE*) | *TFA1* | *GTF2E1* | 54.7 | 49.5 | P36100 | P29083 | 1 |
| TFIIEβ | *TFA2* | *GTF2E2* | 37.0 | 33.0 | P36145 | P29084 | 1 |
| Total (2 subunits) | | | 91.7 | 82.5 | | | |

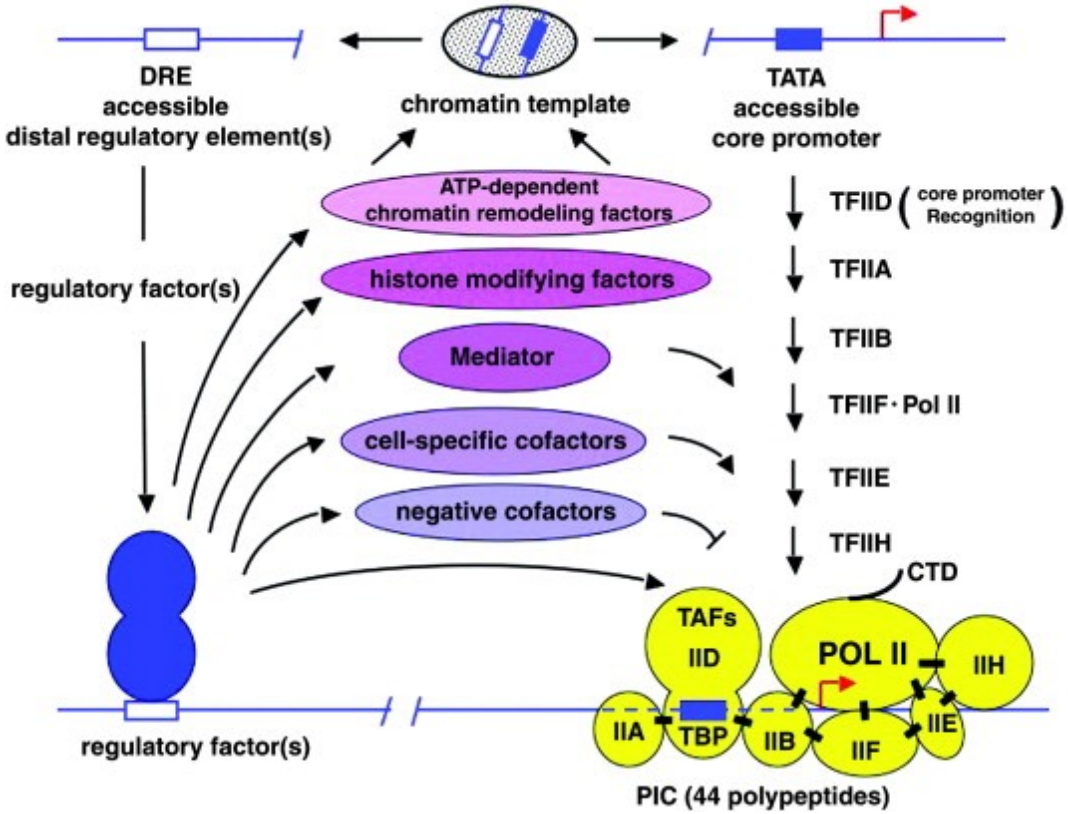
TAFs recognize DNA elements in the promoter region



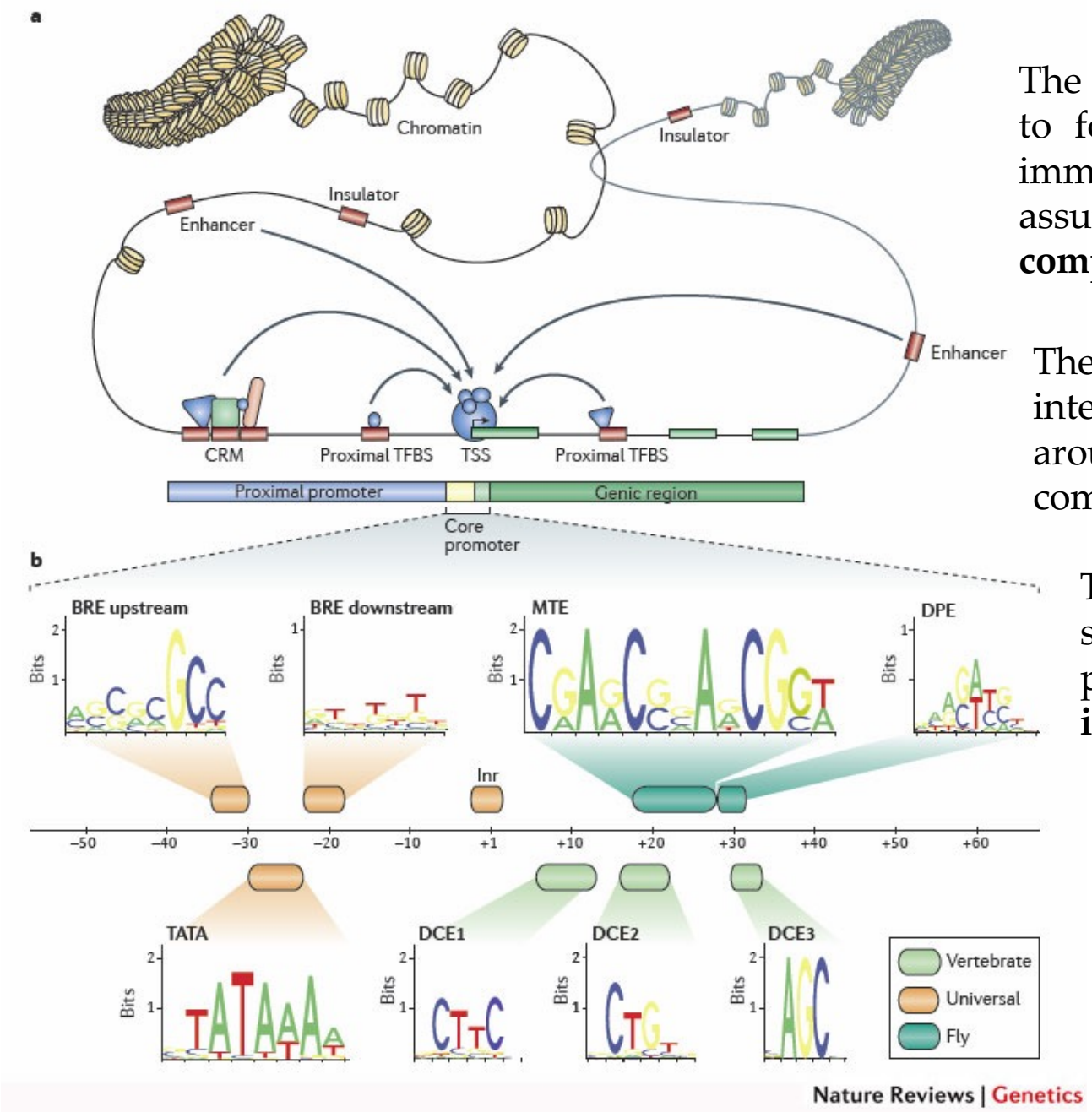
# PIC assembly pathway for genes with a TATA-containing core promoter

Assembly of a PIC containing RNA polymerase II and general initiation factors (yellow) is nucleated by binding of TFIID to the core promoter.

A model for the regulation of PIC assembly and function involves, sequentially: (i) **binding of regulatory factors to distal control elements**; (ii) **regulatory factor interactions with cofactors that modify chromatin structure** to facilitate additional factor interactions; and (iii) **regulatory factor interactions with cofactors that act after chromatin remodeling** to facilitate, through direct interactions, recruitment or function of the general transcription machinery.



# Promoter elements and regulatory signals in Metazoa




The term '**core promoter**' is often used to focus on the DNA region in the immediate vicinity of the TSS, which is assumed to dock the **pre-initiation complex (PIC)**

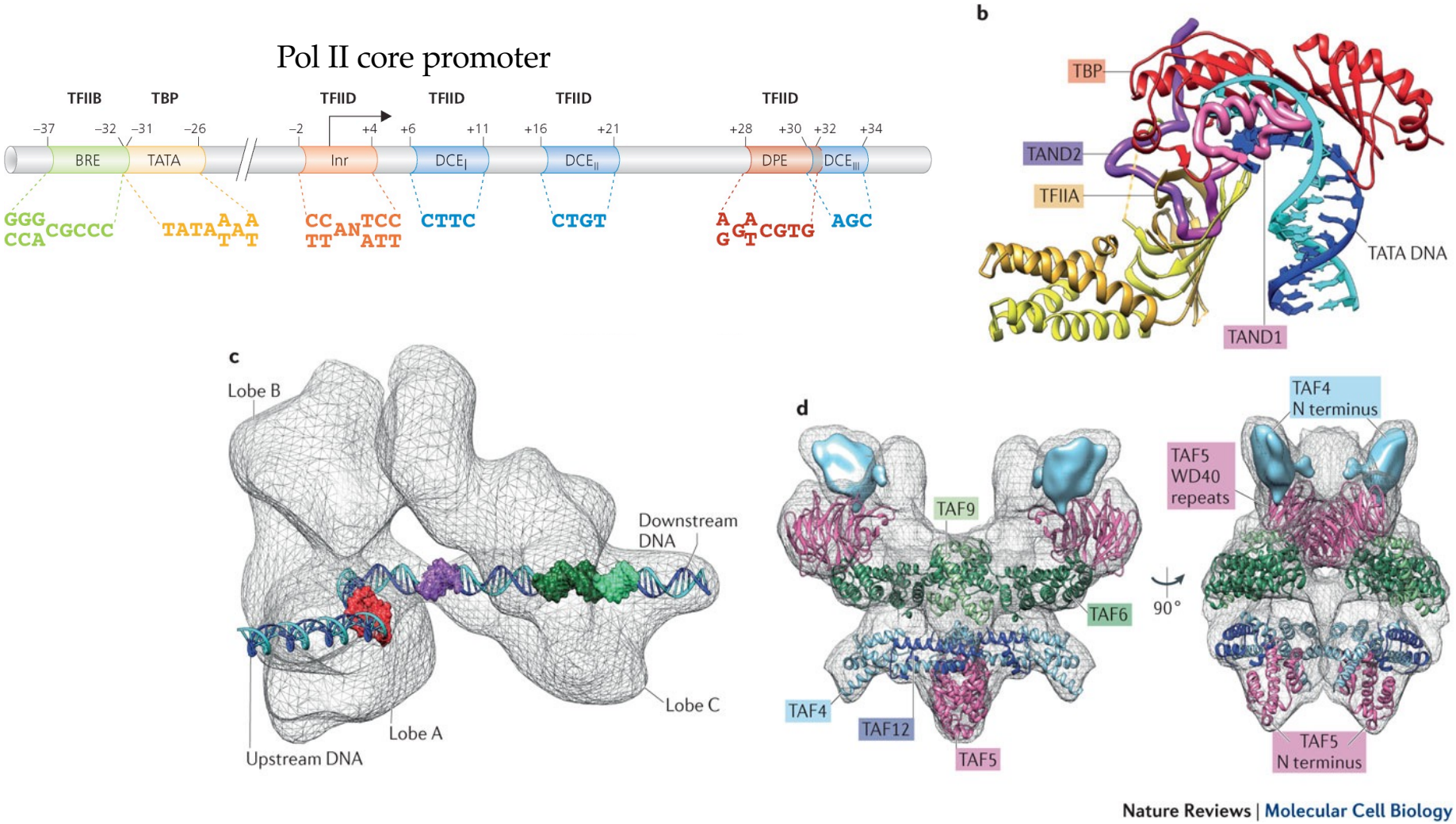
The core promoter consists of several interchangeable sequence elements around the TSS, which bind core components of the PIC.

The region around the TSS has several over-represented sequence patterns; the **TATA box** and **initiator** (**Inr**) are the most studied

**BRE**, B recognition elements
**DCE**, downstream core element;
**DRE**, DNA recognition element;
**MTE**, motif ten element.

# TFIID and promoter specificity

It was originally thought that TFIID was recruited to Pol II promoters through the TATA-binding activity of TBP. A conserved TATA box is, however, found only in 10%–20% of yeast and human promoters. Analysis of core promoter sequences led to the identification of several additional core promoter elements that are recognized by TAFs. TFIID also binds to transcription factors, which help to recruit TFIID to promoters and may facilitate enhancer–promoter interactions.

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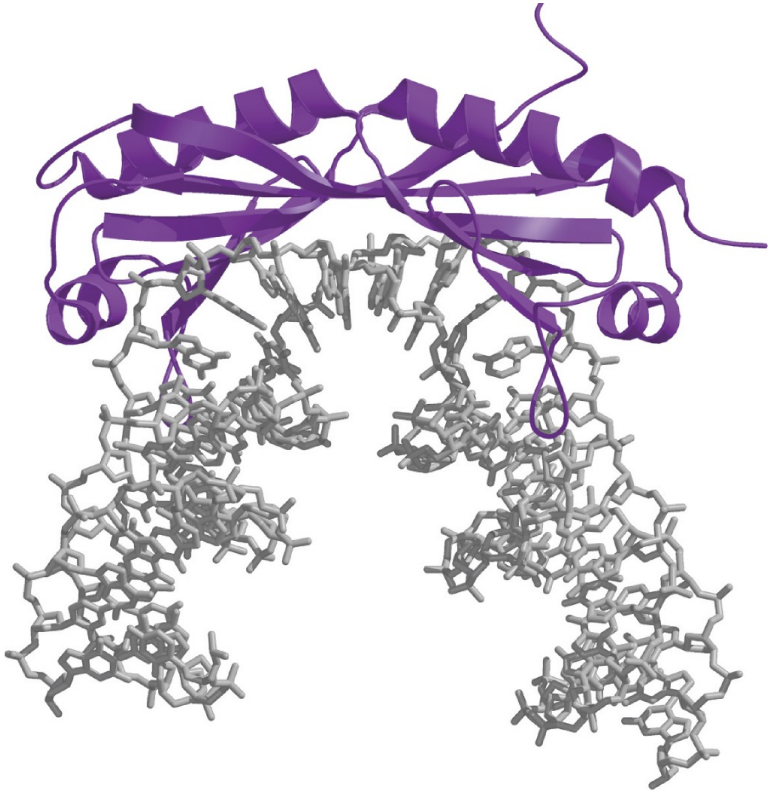
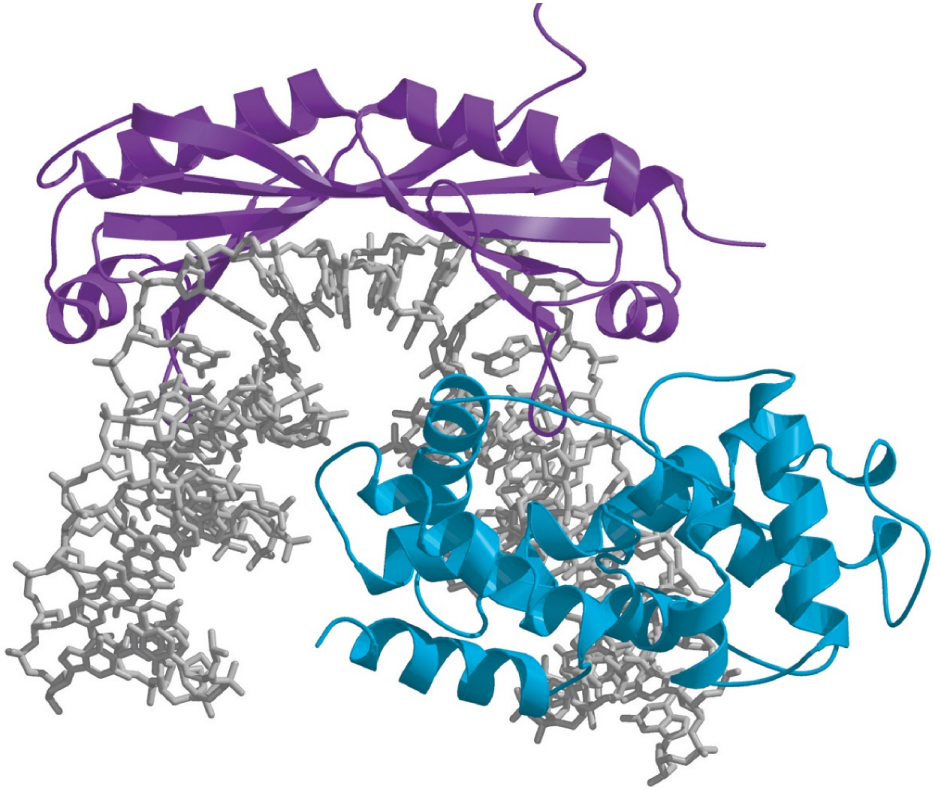
# The TBP

**TBP-DNA complex**



**TBP-DNA + TFIIB complex**



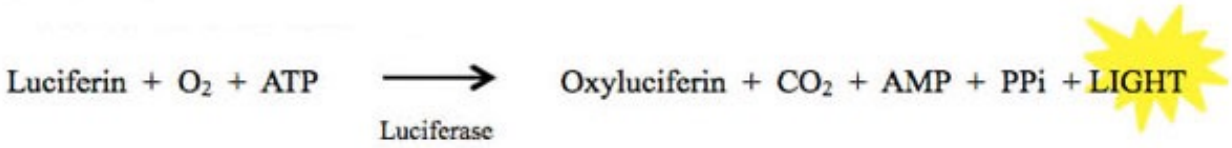
The TBP protein is a monomer with a symmetric axis of symmetry. It binds to the minor groove of DNA and has the ability to bend it, **creating an angle of approximately 80°.** This bending facilitates the binding of other factors to the promoter. For example, it allows TFIIB to interact with both DNA and TBP.

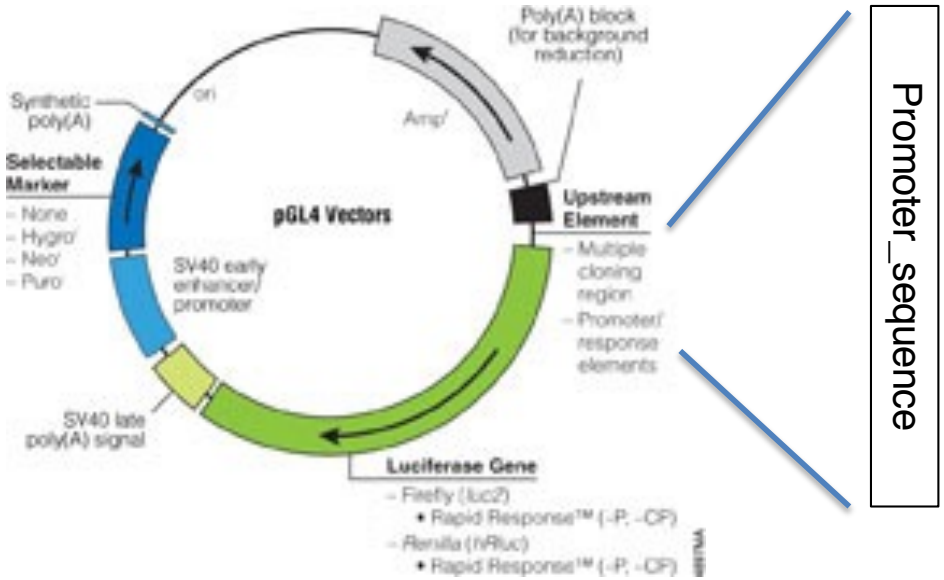
1. **How do we identify a promoter?**

2. **How do we study its activity?**

# The Luciferase reporter assay

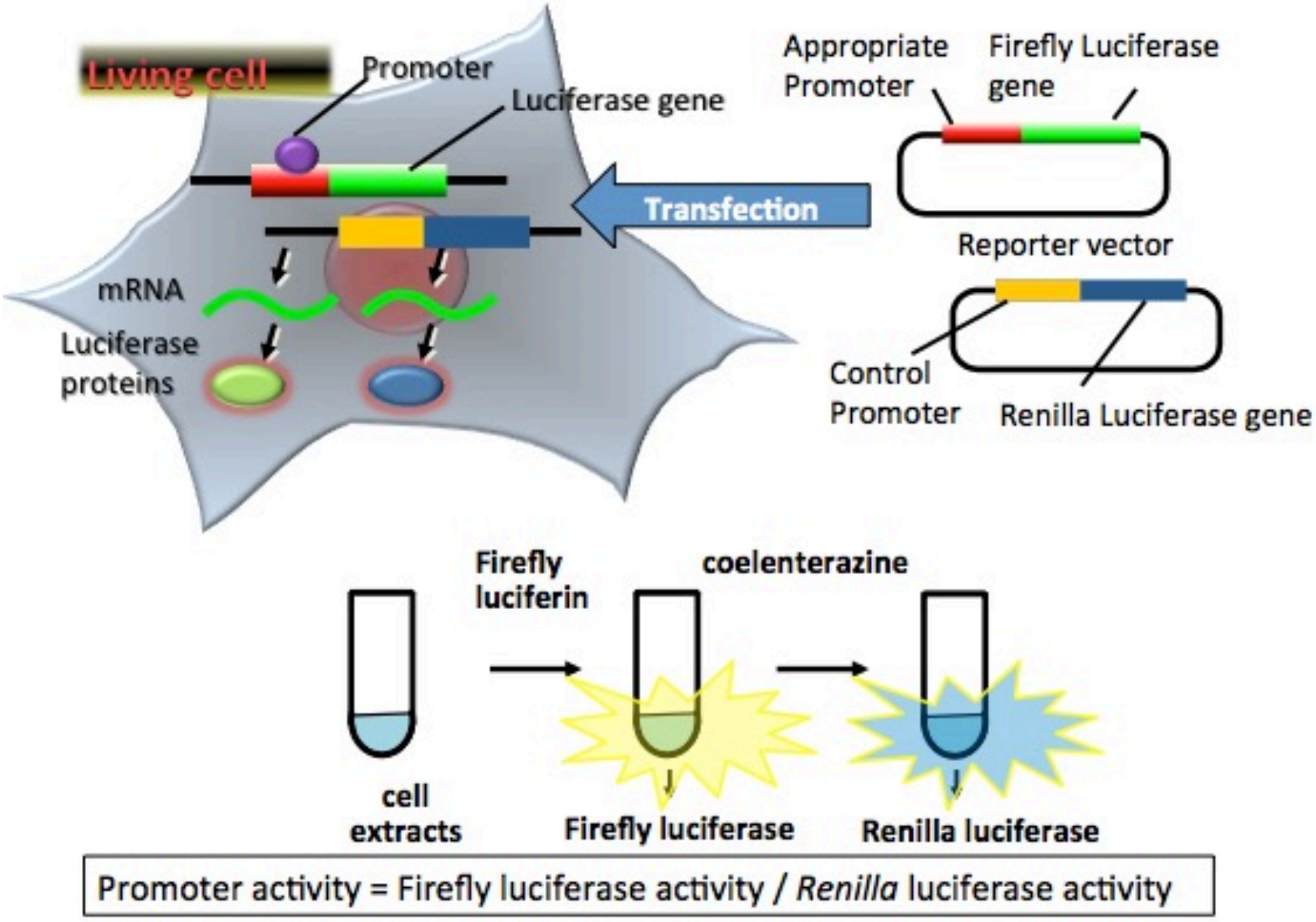
The luciferase reporter assay is commonly used as a tool to study gene expression at the transcriptional level. It is widely used because it is convenient, relatively inexpensive, and gives quantitative measurements instantaneously.



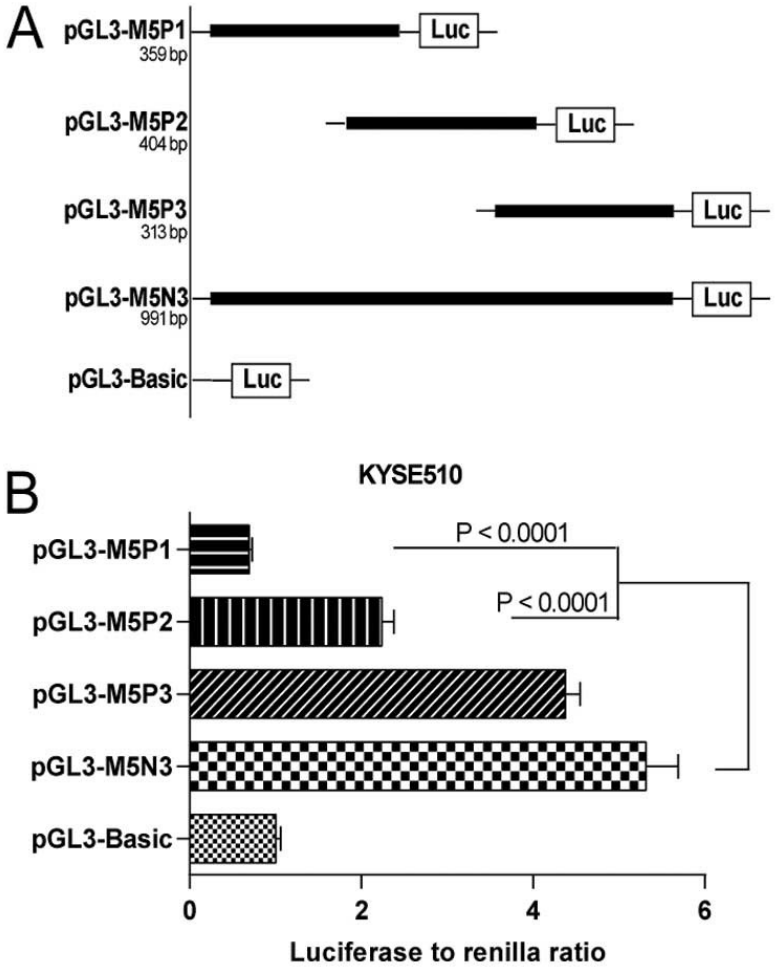




Promoter_sequence

# The Luciferase reporter assay



# The Luciferase reporter assay




C M5N3 - GenBank KF765385
chr2:202,122,236-202,123,227 (hg19)

GGGTCTAGGGCTCAGAGCTTTGGAGAACAGACCTCAGTAGCACCAACACTCCAGGAT
CAATGCTACAAAGACACGGGTTACAACTAAACTGGAGAACATGGCCAAGGATGGGAA
CTCAGCCTGAGCAGGGCTGAGCCGAGCAGGGCTAAGCCAAGTAGGGCTGAGCCAGAA
CACTTCCTCCTTTTTTCTGAACAATCTACCTACATTTCAGCTACAGGGCTGGCTTTAC
CCAGTCCGGCGGGAGGGAGGAGAGGGCTGGTCTGTGACTTCAGTGCTGAGGTTTGAT
CAAGGCAAAGGGAAACTTCCTATTCCCAGACCCTTTGCAAGAAAGAATGGCATATTA
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CTCTTCTGAATGGTTGGAAATTGGGCATCTGTTCCTTTAAACAGGAAACATTTCTTG
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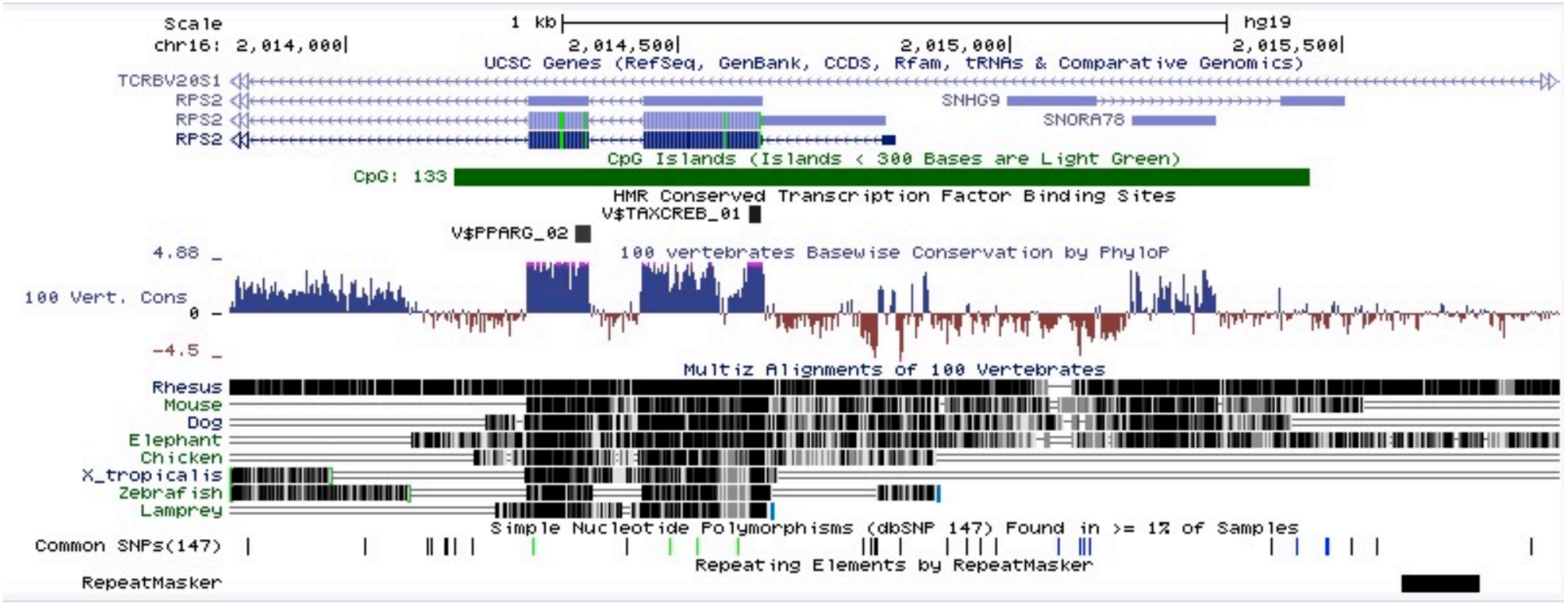
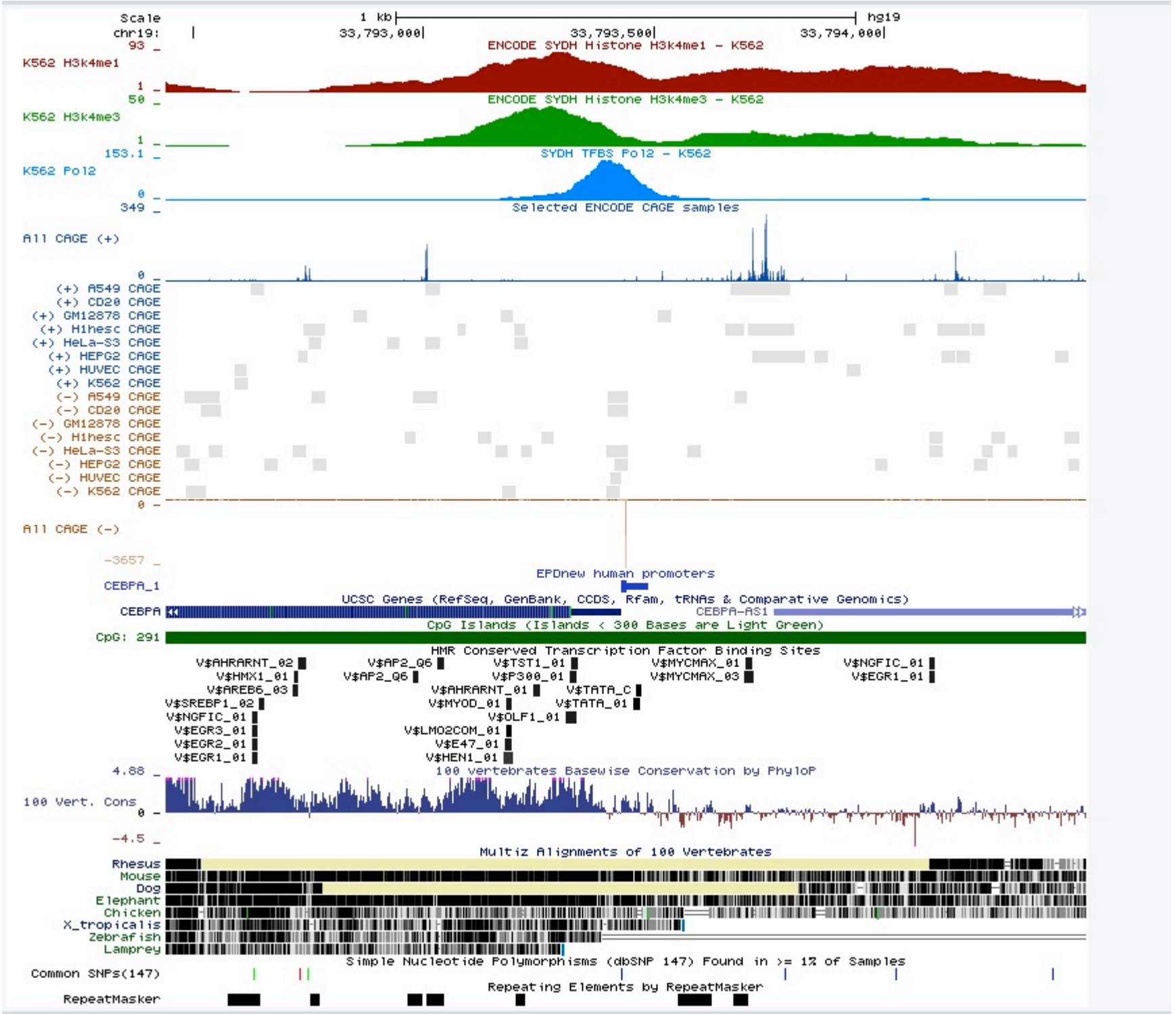
ATCTGCCCTTCTG

# Any promoter should be study in its genomic context!




Scale
chr19:
1 kb
33,793,000
33,793,500
33,794,000
hg19
ENCODE SYDH Histone H3k4me1 – K562
K562 H3k4me1
ENCODE SYDH Histone H3k4me3 – K562
K562 H3k4me3
SYDH TFBS Pol2 – K562
K562 Pol2
Selected ENCODE CAGE samples
All CAGE (+)
(+) A549 CAGE
(+) CD20 CAGE
(+) GM12878 CAGE
(+) H1hesc CAGE
(+) HeLa-S3 CAGE
(+) HEPG2 CAGE
(+) HUVEC CAGE
(+) K562 CAGE
(-) A549 CAGE
(-) CD20 CAGE
(-) GM12878 CAGE
(-) H1hesc CAGE
(-) HeLa-S3 CAGE
(-) HEPG2 CAGE
(-) HUVEC CAGE
(-) K562 CAGE
All CAGE (-)
EPDnew human promoters
CEBPA_1
UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)
CEBPA
CEBPA-AS1
CpG Islands (Islands < 300 Bases are Light Green)
CpG: 291
HMR Conserved Transcription Factor Binding Sites
V$AHRARNT_02
V$AP2_Q6
V$TST1_01
V$MYCMAX_01
V$NGFIC_01
V$HMX1_01
V$AP2_Q6
V$P300_01
V$MYCMAX_03
V$EGR1_01
V$AREB6_03
V$AHRARNT_01
V$TATA_C
V$SREBP1_02
V$MYOD_01
V$TATA_01
V$NGFIC_01
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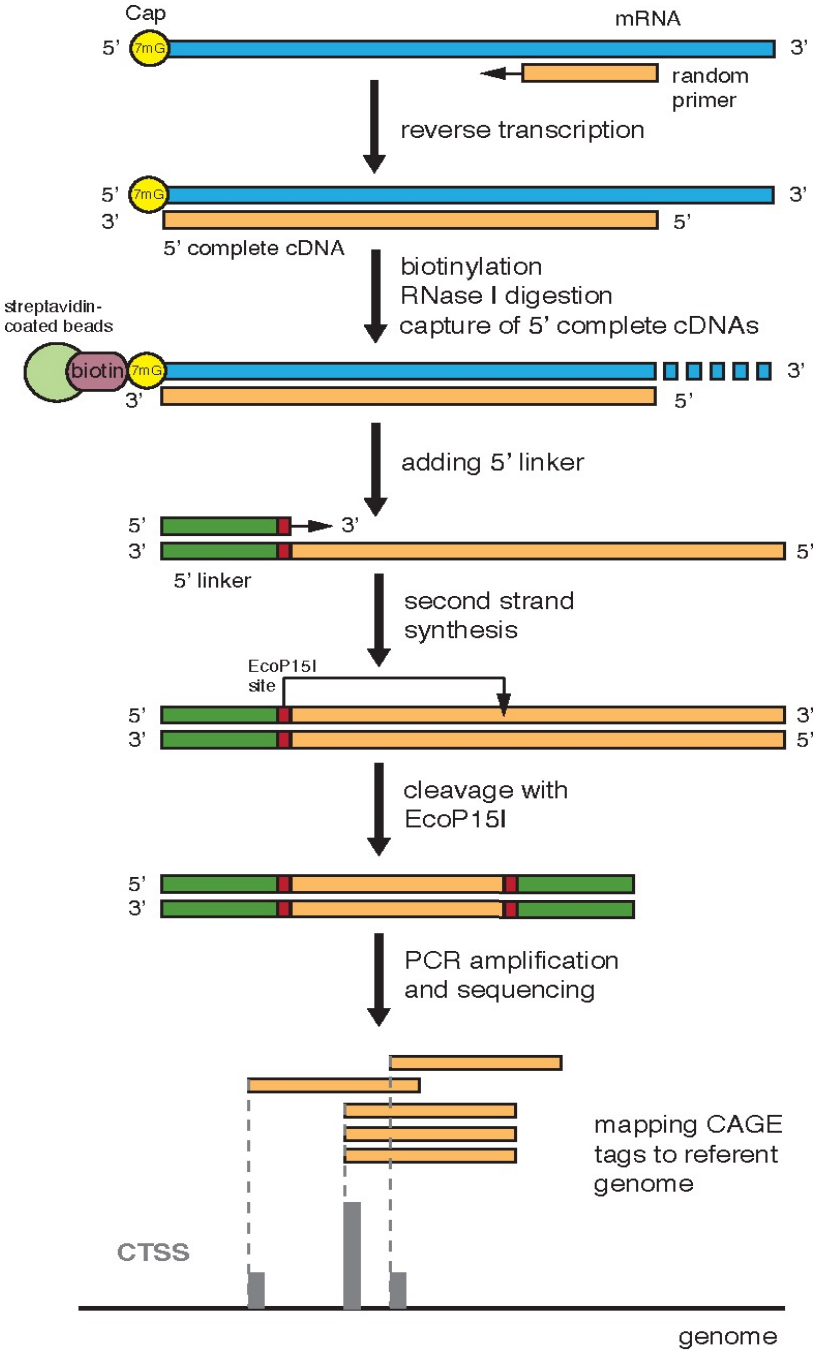
V$HEN1_01
100 vertebrates Basewise Conservation by PhyloP
100 Vert. Cons
Multiz Alignments of 100 Vertebrates
Rhesus
Mouse
Dog
Elephant
Chicken
X_tropicalis
Zebrafish
Lamprey
Simple Nucleotide Polymorphisms (dbSNP 147) Found in >= 1% of Samples
Common SNPs(147)
Repeating Elements by RepeatMasker
RepeatMasker

# CAGE-seq for TSS indentification

Cap Analysis of Gene Expression (CAGE) is a high-throughput method for transcriptome analysis that utilizes *cap trapping* , a technique based on the biotinylation of the 7-methylguanosine cap of Pol II transcripts, to pulldown the 5′-complete cDNAs reversely transcribed from the captured transcripts.

A linker sequence is ligated to the 3′ end of the cDNA and a specific restriction enzyme is used to cleave off a short fragment from the 5′ end of the double stranded cDNA. Resulting fragments are then amplified and sequenced using massive parallel high-throughput sequencing technology, which results in a large number of short sequenced tags that can be mapped back to the referent genome to infer the exact position of the transcription start sites (TSSs) used for transcription of captured RNAs

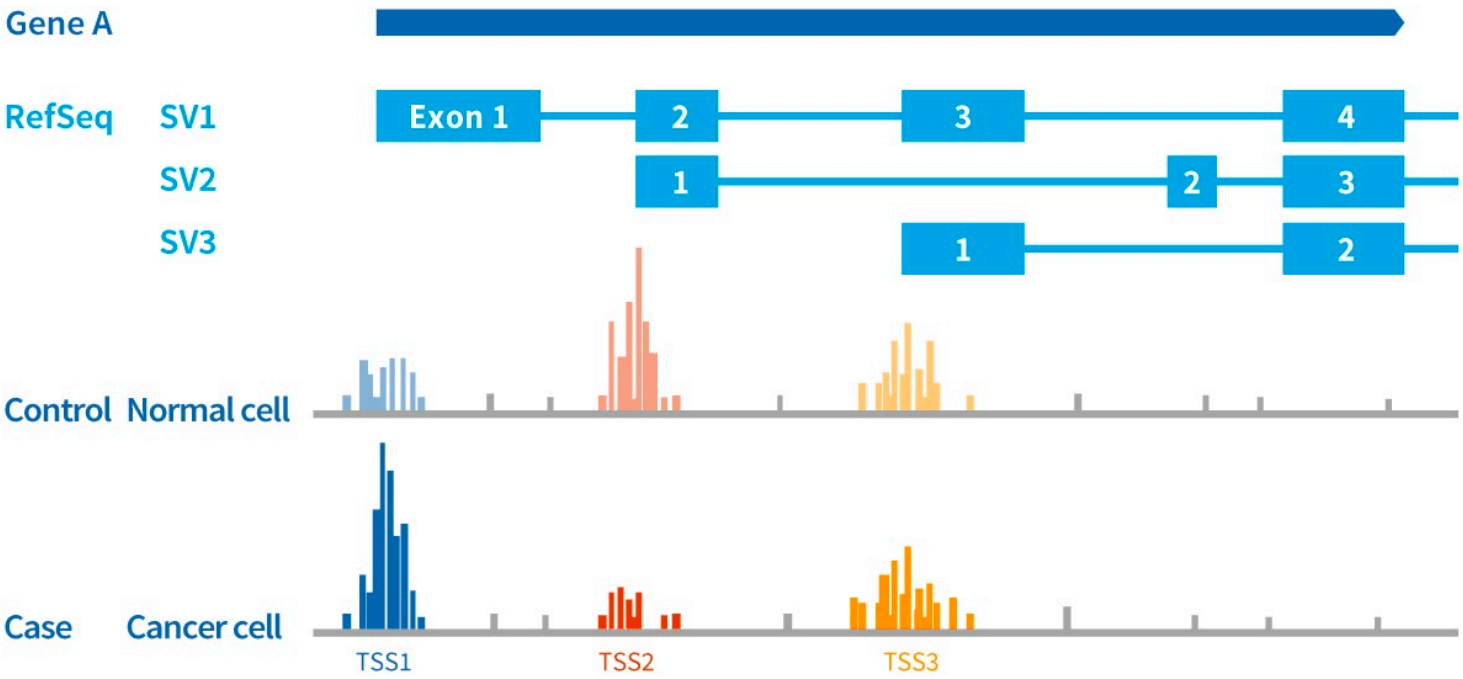
The number of CAGE tags supporting each TSS gives the information on the relative frequency of its usage and can be used as a measure of expression from that specific TSS.

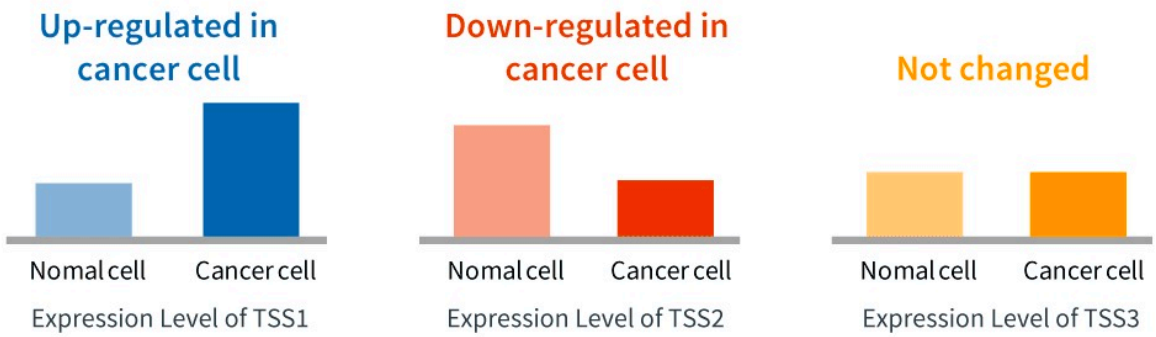


# Gene Expression Analysis - Alternative Transcription Start Sites -

e.g.) Detect the expression level of the whole genome of normal cells and cancer cells by CAGE

Comprehensively detect the differentially expressed transcript at each TSS



# Promoter types in Metazoa

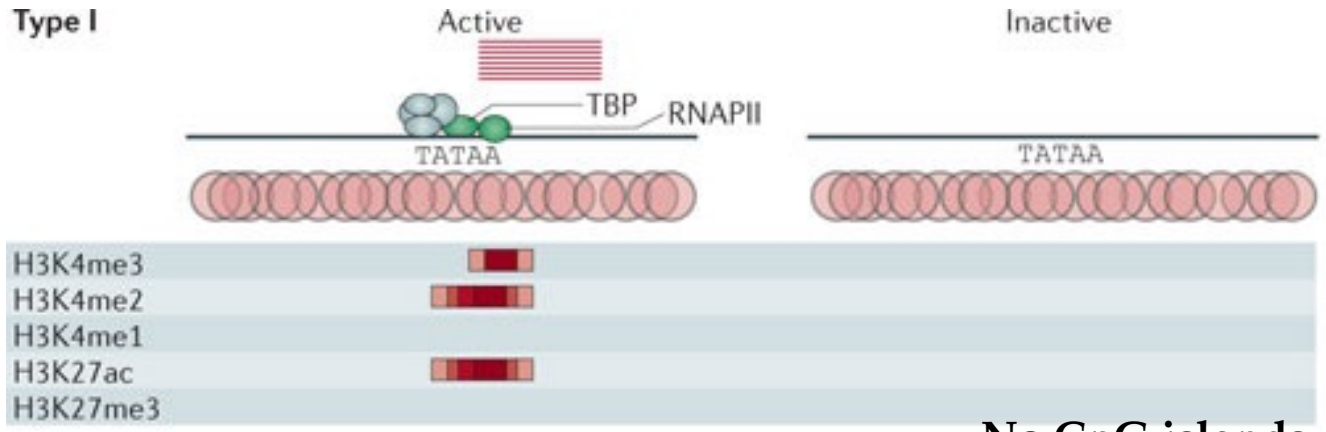
| Promoter type | Dominant gene function | Common properties | Vertebrate-specific |
|---|---|---|---|
| *Major promoters* | | | |
| Type I ('adult') | Tissue-specific expression in adult peripheral tissues | Sharp ('focused') TSS, TATA-box enrichment, disordered nucleosomes | Mostly no CpG islands |
| Type II ('ubiquitous') | Broad expression throughout organismal cycle | Broad ('dispersed') TSS, ordered nucleosome configuration | CpG islands, TATA-depleted |
| Type III ('developmentally regulated') | Differentially regulated genes, often regulators in multicellular development and differentiation | Polycomb repression-regulated genes, broad H3K27me3 marks | Large CpG islands extending into the body of gene |
| *Minor promoters* | | | |
| TCT promoter | Highly expressed genes of translational apparatus | Sharp, pyrimidine-stretch ('TCT') initiator sequence, often full TATA box, ubiquitous-promoter-like nucleosome configuration | CpG island overlapping |

DPE, downstream promoter element; DRE, DNA recognition element; H3K27me3, histone H3 lysine 27 trimethylation; TSS, transcription start site.

# Promoter types in Metazoa

| Promoter type | Dominant gene function | Common properties | Vertebrate-specific |
|---|---|---|---|
| *Major promoters* | | | |
| Type I ('adult') | Tissue-specific expression in adult peripheral tissues | Sharp ('focused') TSS, TATA-box enrichment, disordered nucleosomes | Mostly no CpG islands |

Genes that are specifically expressed in peripheral terminally differentiated tissues (e.g. liver or skeletal muscle) have **type I promoters** with a pattern of histone modification that is distinct from that of most other genes. H3K4me3 is generally only present downstream of the TSS, and there is no RNAPII binding at these promoters when the genes are not active.

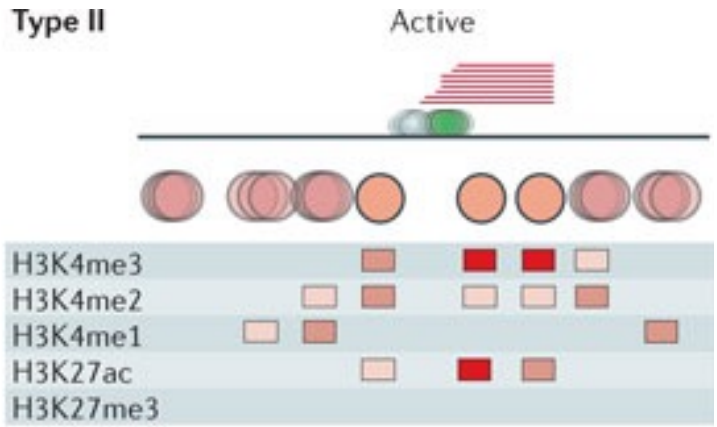



**No CpG islands**

# Promoter types in Metazoa

| Promoter type | Dominant gene function | Common properties | Vertebrate-specific |
|---|---|---|---|
| Type II ('ubiquitous') | Broad expression throughout organismal cycle | Broad ('dispersed') TSS, ordered nucleosome configuration | CpG islands, TATA-depleted |

Ubiquitously expressed genes have H3K4me3 throughout their **type II promoters** across all tissues. Across classes of genes in vertebrates, H3K4me3 distribution is almost identical with the span of CpG islands. Ubiquitously expressed genes generally have short CpG islands, and the H3K4me3 mark and CpG island typically only overlap the 5′ end of the gene.

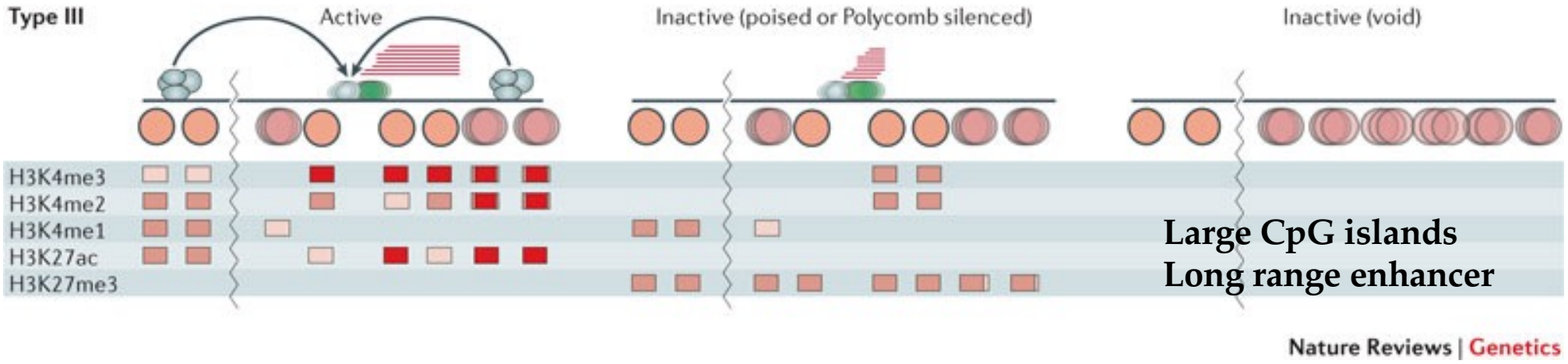



**Short CpG islands**

# Promoter types in Metazoa

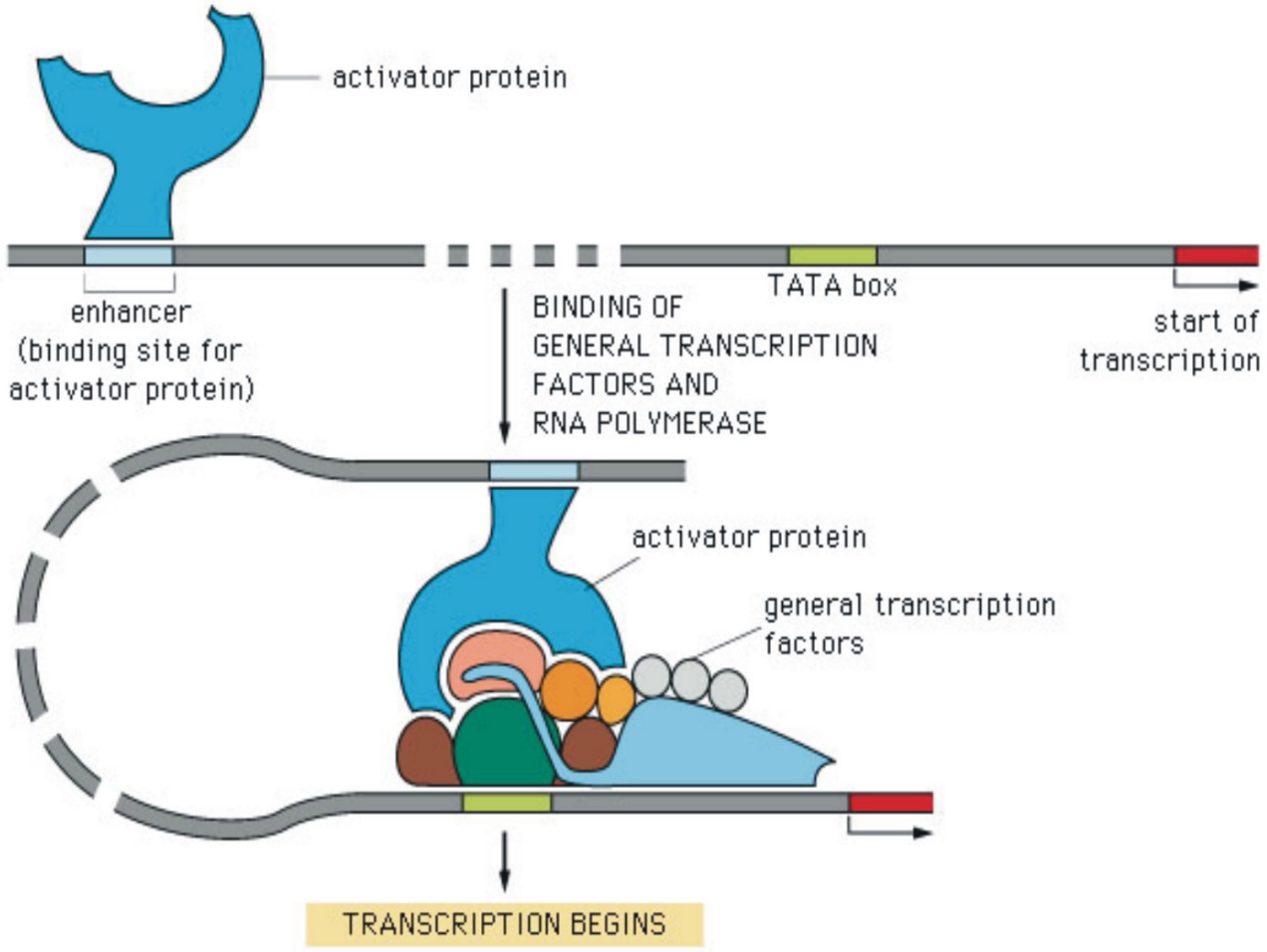
| Promoter type | Dominant gene function | Common properties | Vertebrate-specific |
|---|---|---|---|
| Type III ('developmentally regulated') | Differentially regulated genes, often regulators in multicellular development and differentiation | Polycomb repression-regulated genes, broad H3K27me3 marks | Large CpG islands extending into the body of gene |

Developmentally regulated genes (with **type III promoters**) have a number of features that are associated with repression by **polycomb group proteins (PcG)**. These features include multiple large CpG islands, wide distribution of bound PcG proteins and both H3K27me3 and H3K4me3. Because of the presence of both of these marks, which are associated with repression and activation, respectively, these are described as **bivalent promoters**. The large CpG islands often extend into the body of the genes and are closely tracked by H3K4me3, which is thus not restricted to promoter regions in developmental genes.



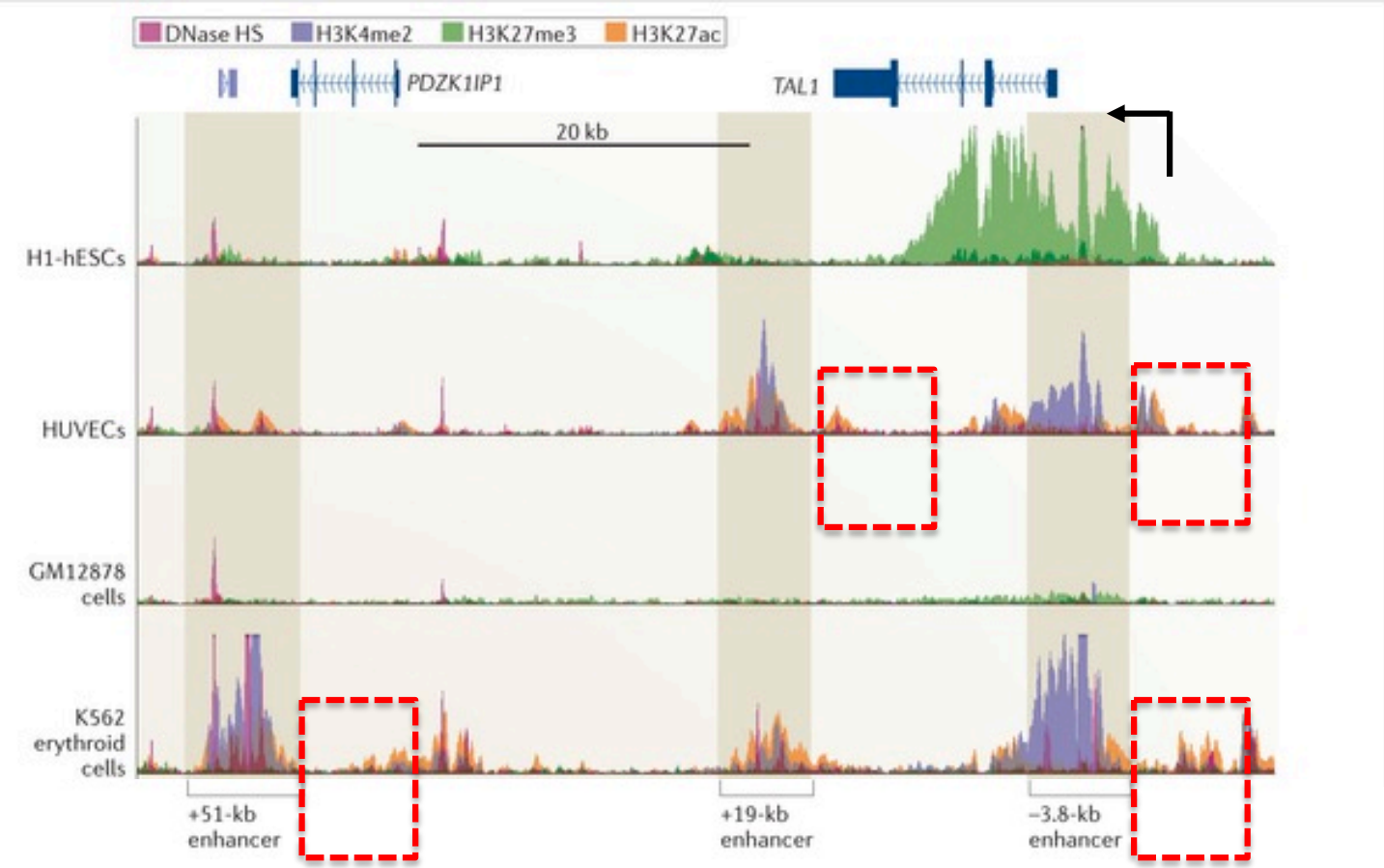
# Enhancers

**Enhancer** is a short (50-1500 bp) region of DNA that can be bound by proteins (transcriptional activators) to increase transcription of a particular gene.



# Enhancers

Genomic regions that function as transcriptional enhancers are enriched in closely spaced recognition motifs for sequence-specific transcription factors. Cell type-specific enhancers are marked by specific epigenomic features and chromatin accessibility. In particular, enhancers display enrichment of H3K4me1 or H3K4me2 and depletion of H3K4me3 compared with promoters




It is estimated that the human genome contains approximately 150,000 enhancers, but 'only' about 10,000–50,000 of them are active in any given cell type

Enhancer activation begins with the binding of transcription factors and local nucleosome remodelling. Enhancer states can broadly be classified as:

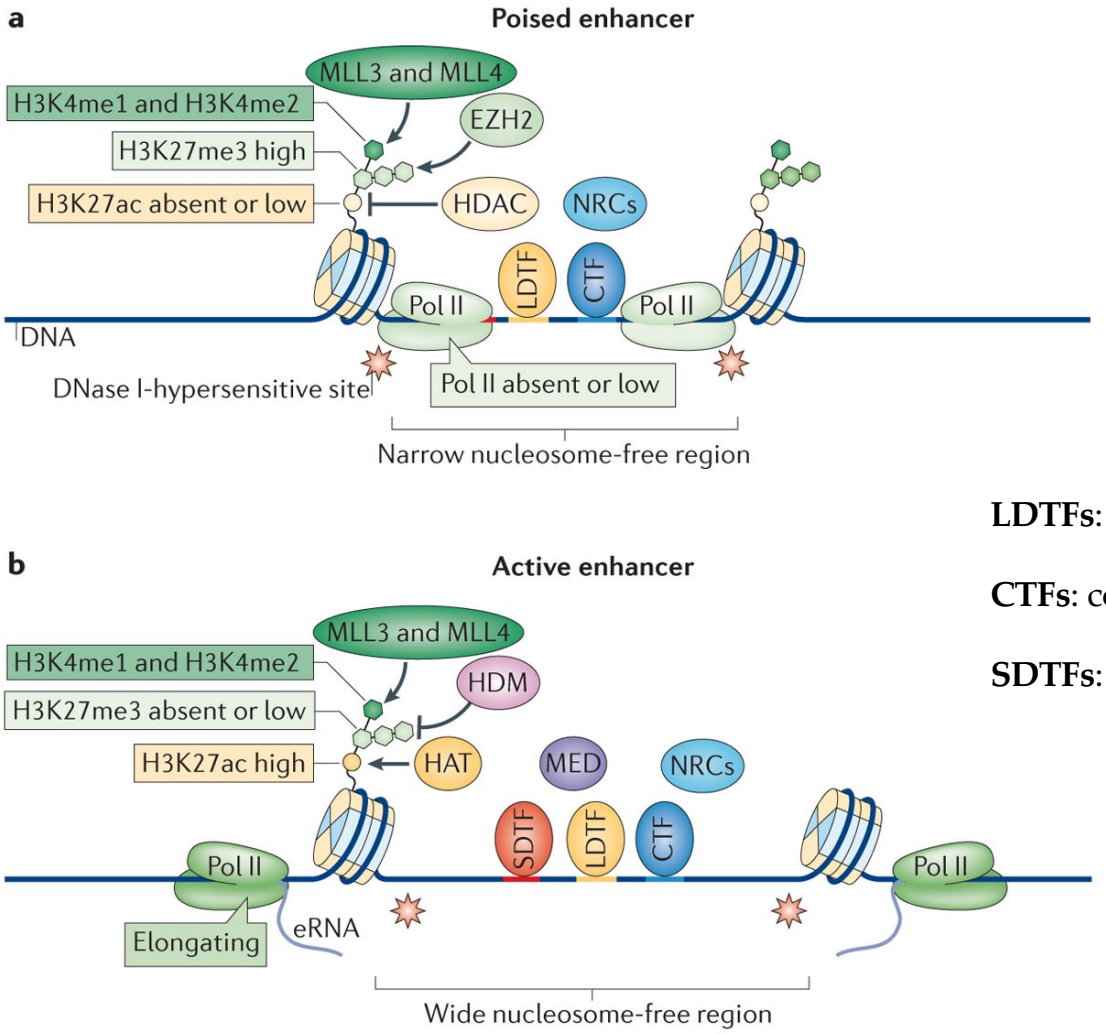
**Inactive enhancers:** are essentially buried in compact chromatin and are devoid of transcription factor binding and histone modifications.

**Primed/Poised enhancers: Primed enhancer** are characterized by closely bound sequence-specific transcription factors that establish a nucleosome-free region of open chromatin. However, they may require additional cues to accomplish their function, which may include signal-dependent activation, the recruitment of additional transcription factors and the eventual recruitment of co-activators that lead to enhancer activation. **Poised enhancers** can be defined as primed enhancers that also contain repressive epigenetic chromatin marks

**Active enhancers:** present co-activator complexes containing histone demethylase (HDM) complexes that remove H3K27me3 marks, histone acetyltransferases (HATs) that deposit H3K27 acetylation (H3K27ac) marks, and the **Mediator complex (MED)**. The transformation to elongating Pol II results in bidirectional transcription — a hallmark of active enhancers — and the generation of **enhancer RNAs (eRNAs)**, which is closely coupled to enhancer activity.

**Importantly, enhancers are mobile in 3D space, and therefore have the potential to interact with multiple promoters over time**

# Enhancers




**LDTFs**: lineage determining transcription factors

**CTFs**: collaborating transcription factors

**SDTFs**: signal-dependent transcription factors

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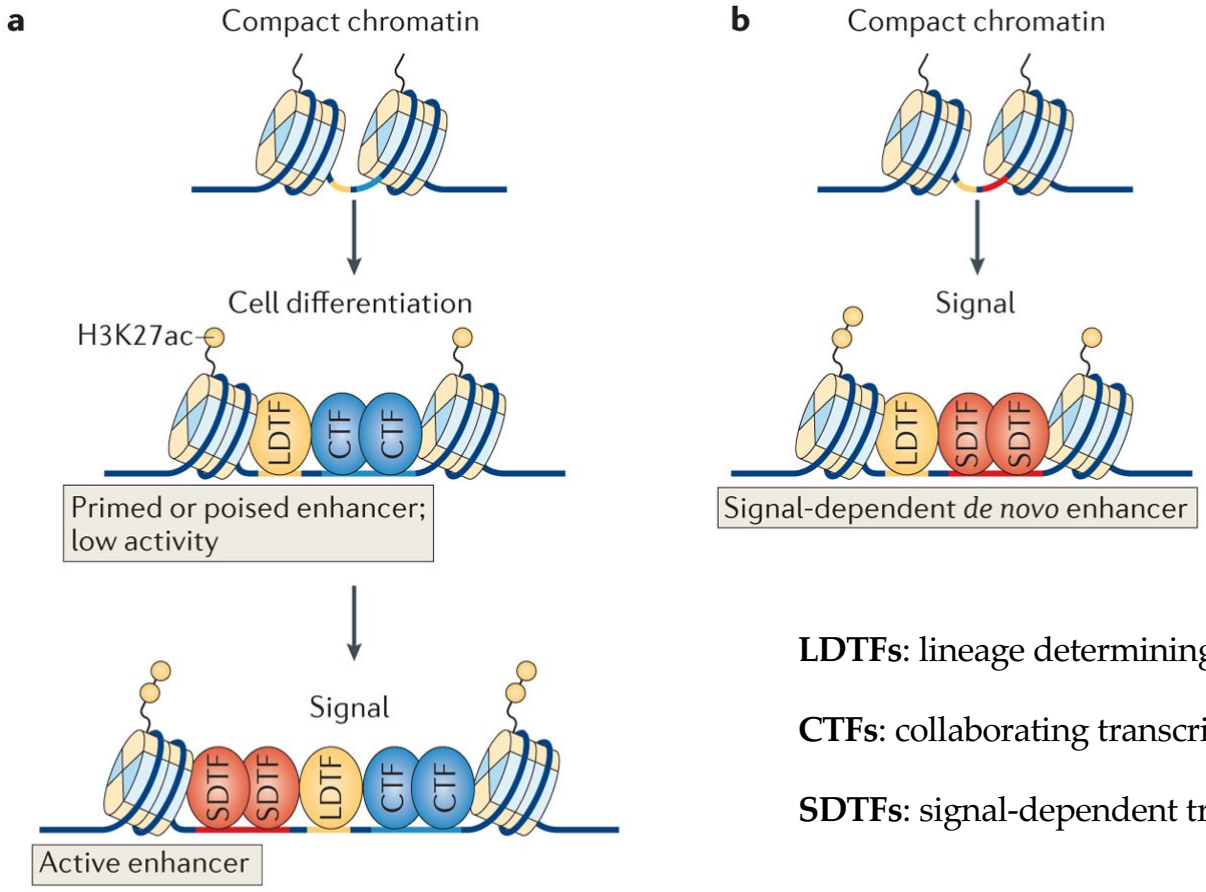
# Cell type-specific enhancer selection and activation

lineage-determining transcription factors (LDTFs)
collaborating transcription factors (CTFs)
signal-dependent transcription factors (SDTFs).




**LDTFs**: lineage determining transcription factors

**CTFs**: collaborating transcription factors

**SDTFs**: signal-dependent transcription factors

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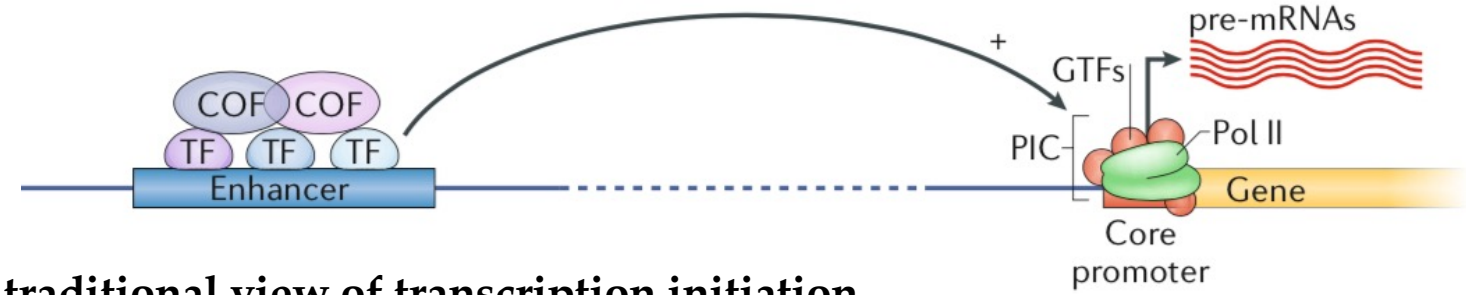
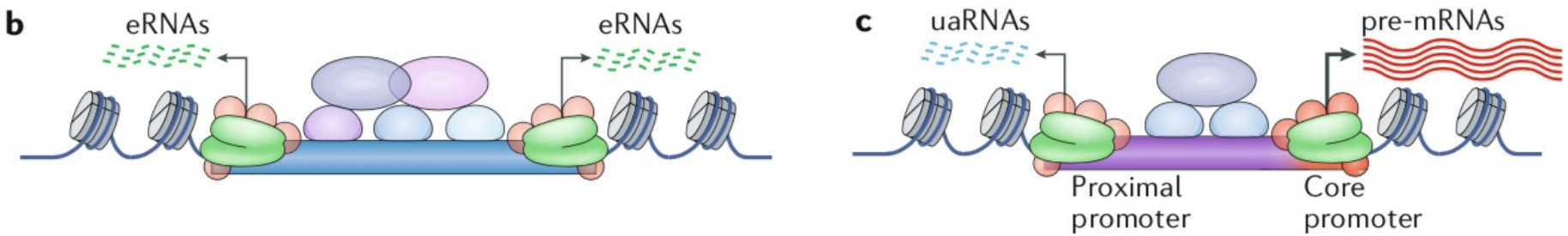
# Widespread transcription of mammalian genome




**The traditional view of transcription initiation**

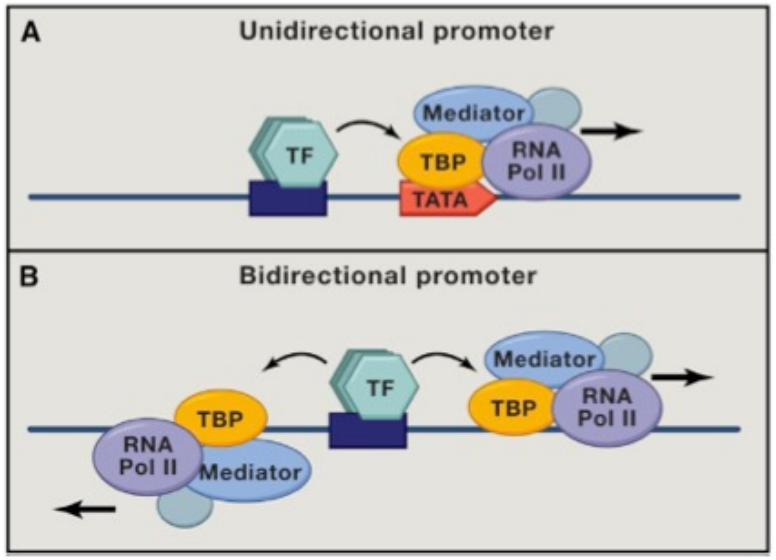
The traditional view of gene expression and the role of enhancers and core promoters has been challenged by the observation that many genomic positions outside annotated gene starts initiate transcription, including positions within enhancers.




**The modern view of transcription initiation**

# Divergent transcription from promoter and enhancer produces non coding RNAs

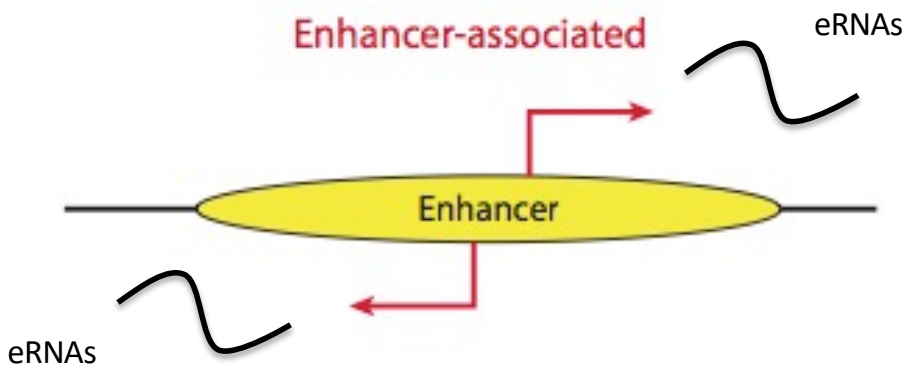
Most mammalian promoters lack a TATA element (TATA-less). For these promoters, TBP is recruited through sequence-specific transcription factors (such as Sp1) and components of the TFIID complex that have little sequence specificity. Thus, in the absence of strong TATA elements, TBP-complexes are recruited on both sides of the transcription factors to form preinitiation complexes in both orientations. Since transcription factors with transcription activation domains also bind at enhancer sites, these are also sites of divergent transcription.




Wu and Sharp, 2013 Cell

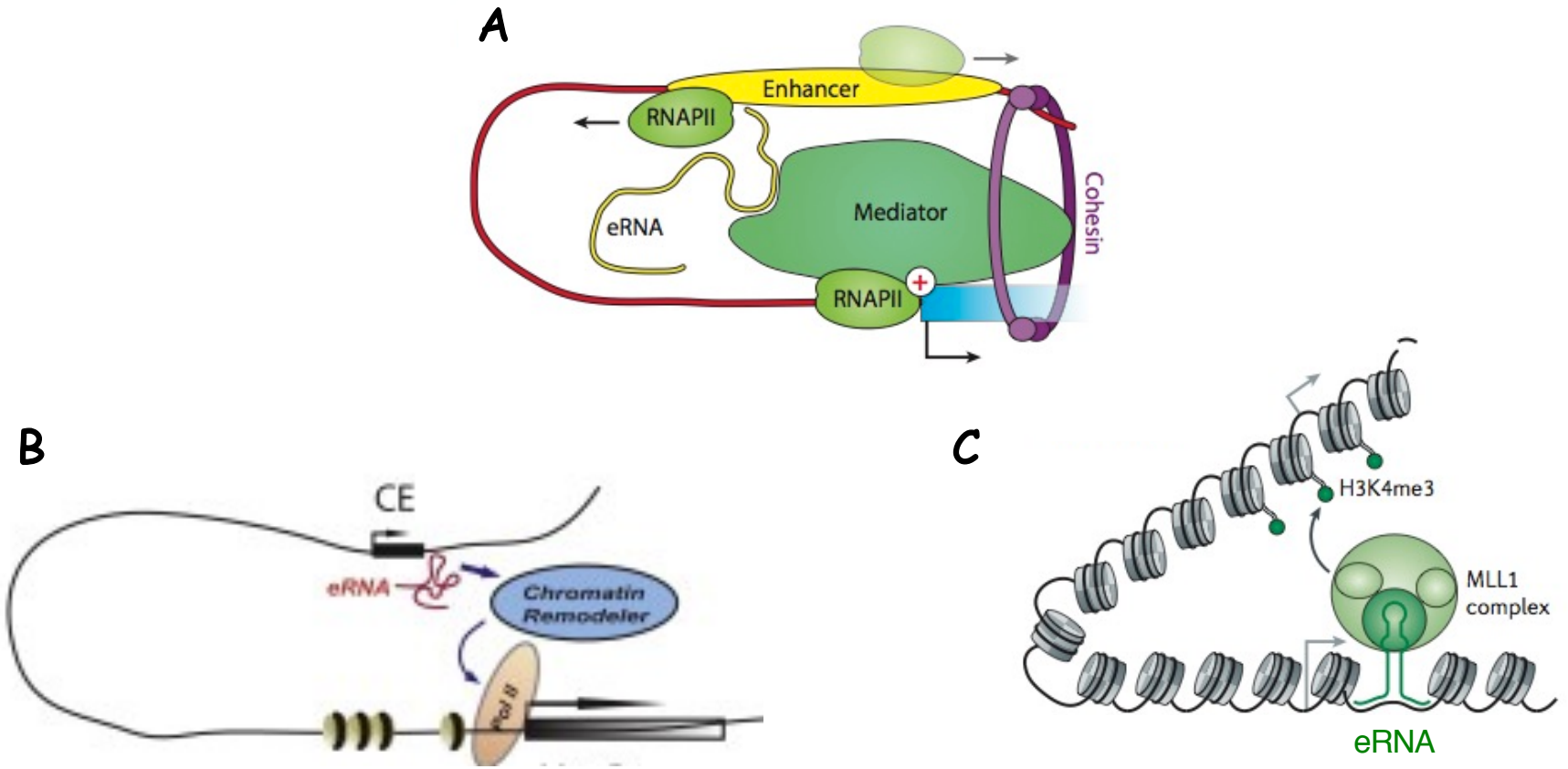
# Enhancer RNAs (eRNAs)

- eRNAs are produced from transcriptional enhancers
- eRNA transcription correlates with the activation of the neighboring protein-coding genes (*cis* –acting)
- The majority of eRNAs are reported to be monoexonic and not polyadenylated (2–5 kb in length), but few cases of spliced and polyadenylated eRNAs have been described.



# Enhancer RNAs

eRNAs **cooperate with the the Mediator and Cohesin complexes (A)** to promote enhancer–promoter interactions (chromatin looping) or they **recruit chromatin remodeling (B) or chromatin modifying complexes (C)** to the targeted promoters.



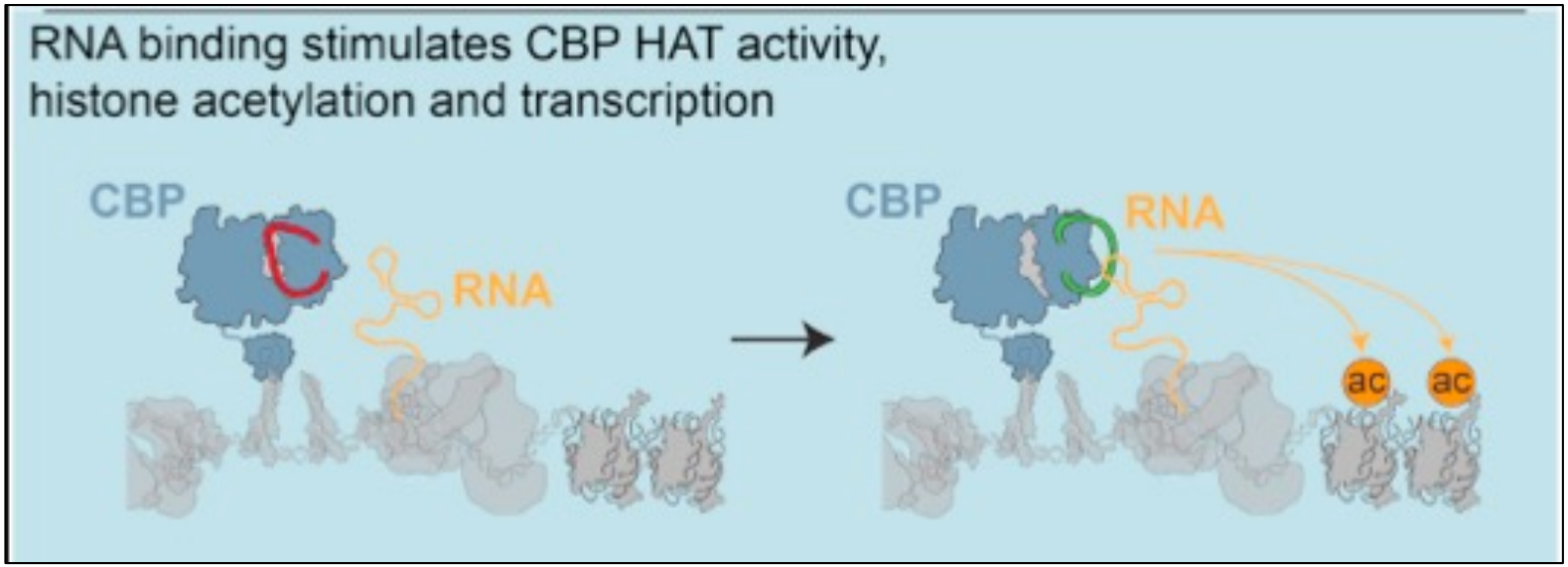
# eRNA Binding to CBP Stimulates Histone Acetylation and Transcription

CBP/p300 are histone acetyltransferase (HAT) whose binding is a signature of enhancers.

eRNA binding region in the HAT domain of CBP allows RNA to stimulate CBP's HAT activity.

At enhancers where CBP interacts with eRNAs, stimulation manifests in RNA-dependent changes in the histone acetylation mediated by CBP, such as H3K27ac, and by corresponding changes in gene expression.

**By interacting directly with CBP, eRNAs contribute to the unique chromatin structure at active enhancers, which, in turn, is required for regulation of target genes.**




Bose et al., 2017. Cell

# The mediator complex

**Mediator** is a multiprotein complex that is implicated in regulating many fundamental processes involved in transcription including:

- **transcription initiation**,
- **transcription elongation**,
- **chromatin architecture**,
- **enhancer-promoter gene looping**

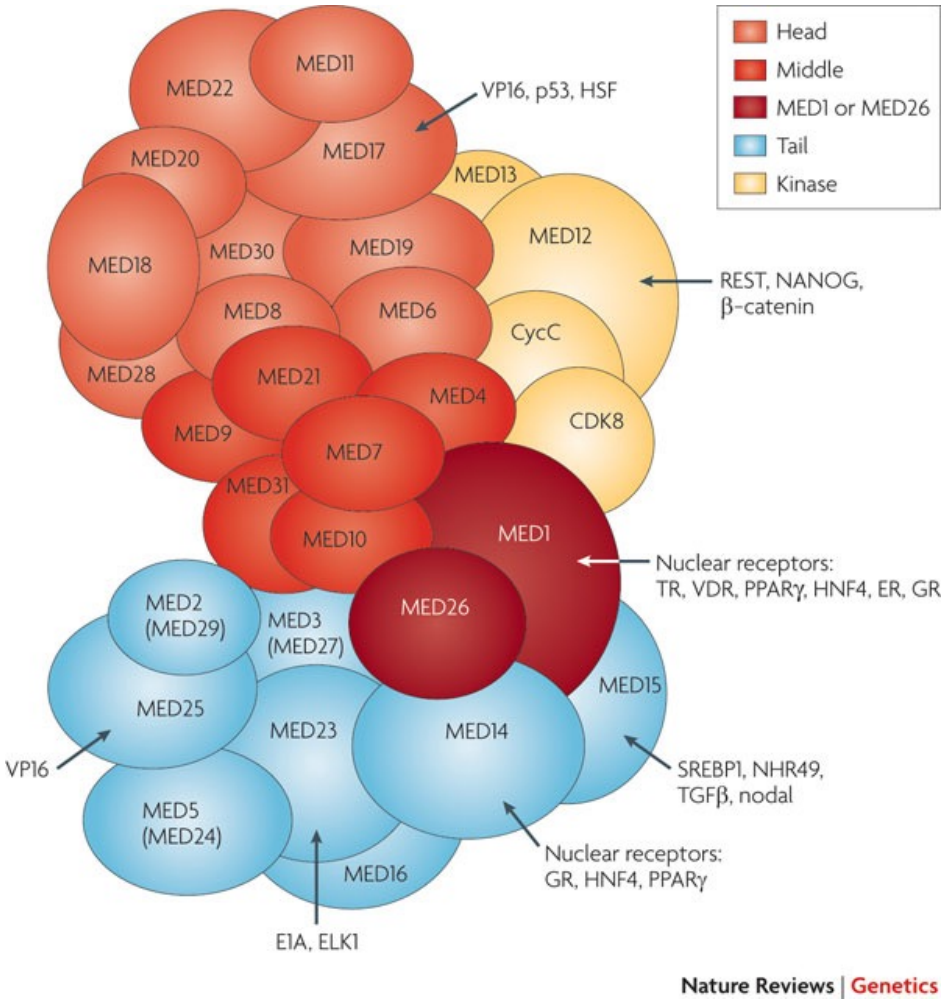
# The mediator complex

The human Mediator complex has 30 subunits and is 1.2 MDa in size.

Mediator is universally required for the expression of almost all genes

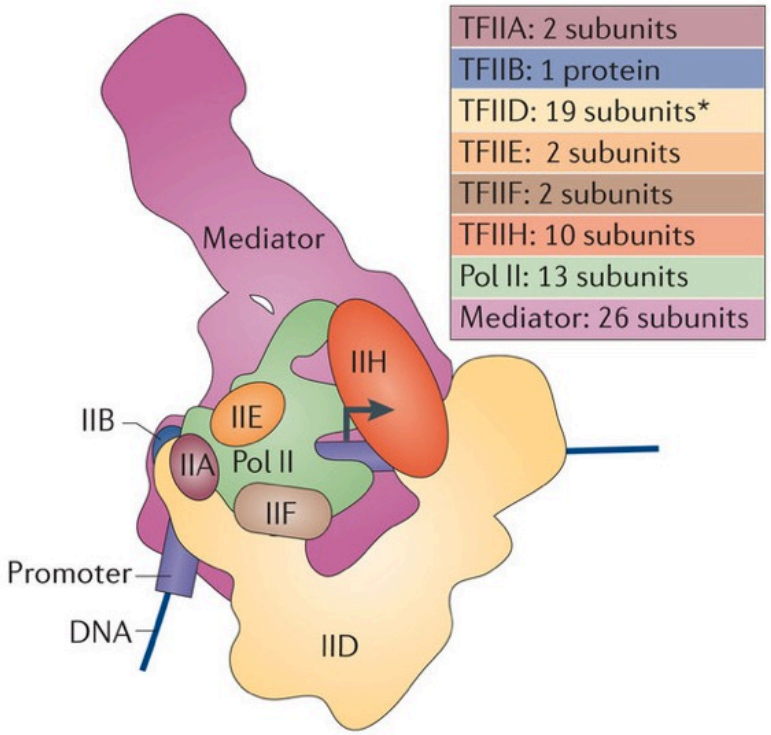
A fundamentally important characteristic of Mediator is that its subunit composition can change; subunits can be lost or added to affect its biological function. individual subunits reside in one of four distinct modules designated '**head**', '**middle**', '**tail** and '**kinase**'

Whereas the head, middle and tail modules form a relatively stable 'core' structure, the kinase module associates reversibly with the Mediator complex



# Mediator has key roles in the assembly of the PIC

For gene-specific transcription, Pol II must be recruited to specific sites on the genome. This is generally controlled by sequence-specific, DNA-binding TFs. Although TFs do not directly bind to Pol II, one mechanism by which they can promote Pol II recruitment is by binding to the Mediator complex. Mediator enables Pol II recruitment via interaction with the CTD of the Pol II. The large size of Mediator is likely to promote stable PIC formation by allowing the complex to directly interact with multiple PIC factors. Moreover, Mediator helps to regulate the recruitment and/or the activity of the PIC components.

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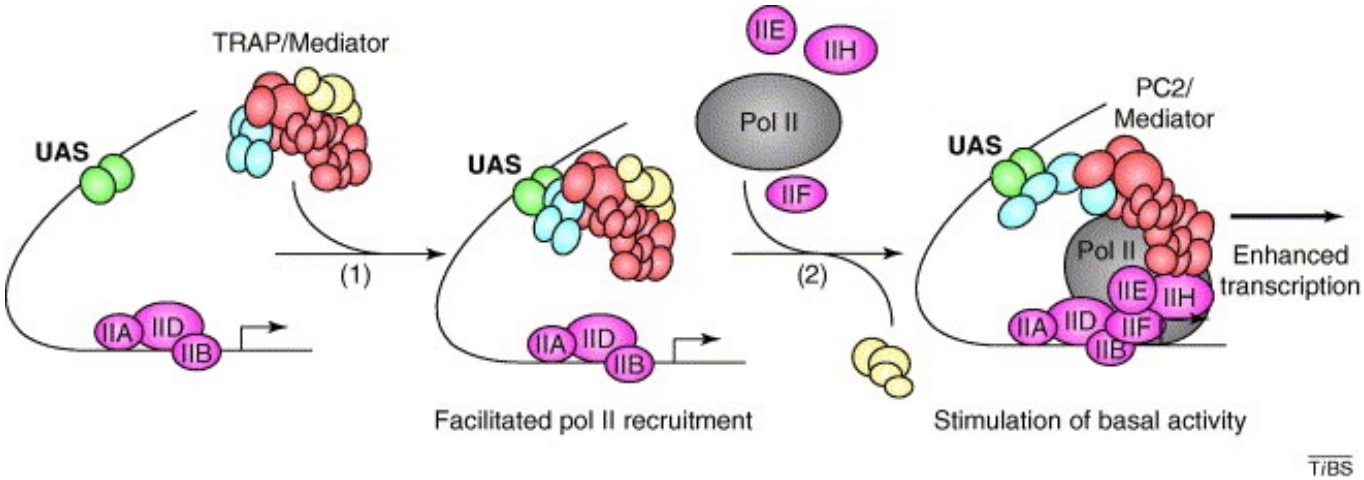
# Model for the Mediator-dependent transcriptional activation pathway

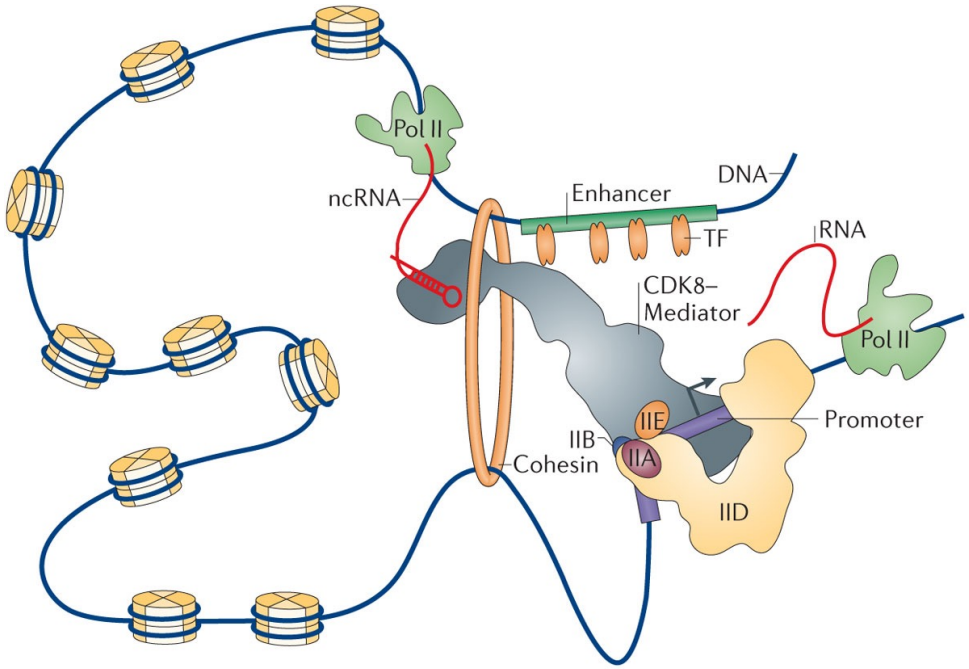
Mediator function is proposed to be manifested at two distinct levels. (1) First, in concert with the activators, **Mediator** might promote PIC assembly (**recruitment**). (2) Second, following structural or topological conversion, Mediator could modulate the (basal) function of pol II in the PIC (**post-recruitment**).




By inserting itself between the activators and the general transcription factors, the Mediator affords additional opportunities to fine-tune the diverse regulatory inputs received both from the DNA-binding factors and, most likely, from other signals and to present an <u>appropriately calibrated output to the pol II machinery</u>.

# Mediator and chromatin architecture

The 3D organization of the genome has basic roles in coordinating gene expression programmes. A common theme is the formation of **DNA loops** that enable interaction between linearly separated DNA sequences. Mediator has a central role in the formation and/or stabilization of looped DNA structures in eukaryotes. In mammalian cells, long-range looping interactions between enhancer and promoter sequences appear to be important for driving high-level and cell type-specific gene expression.

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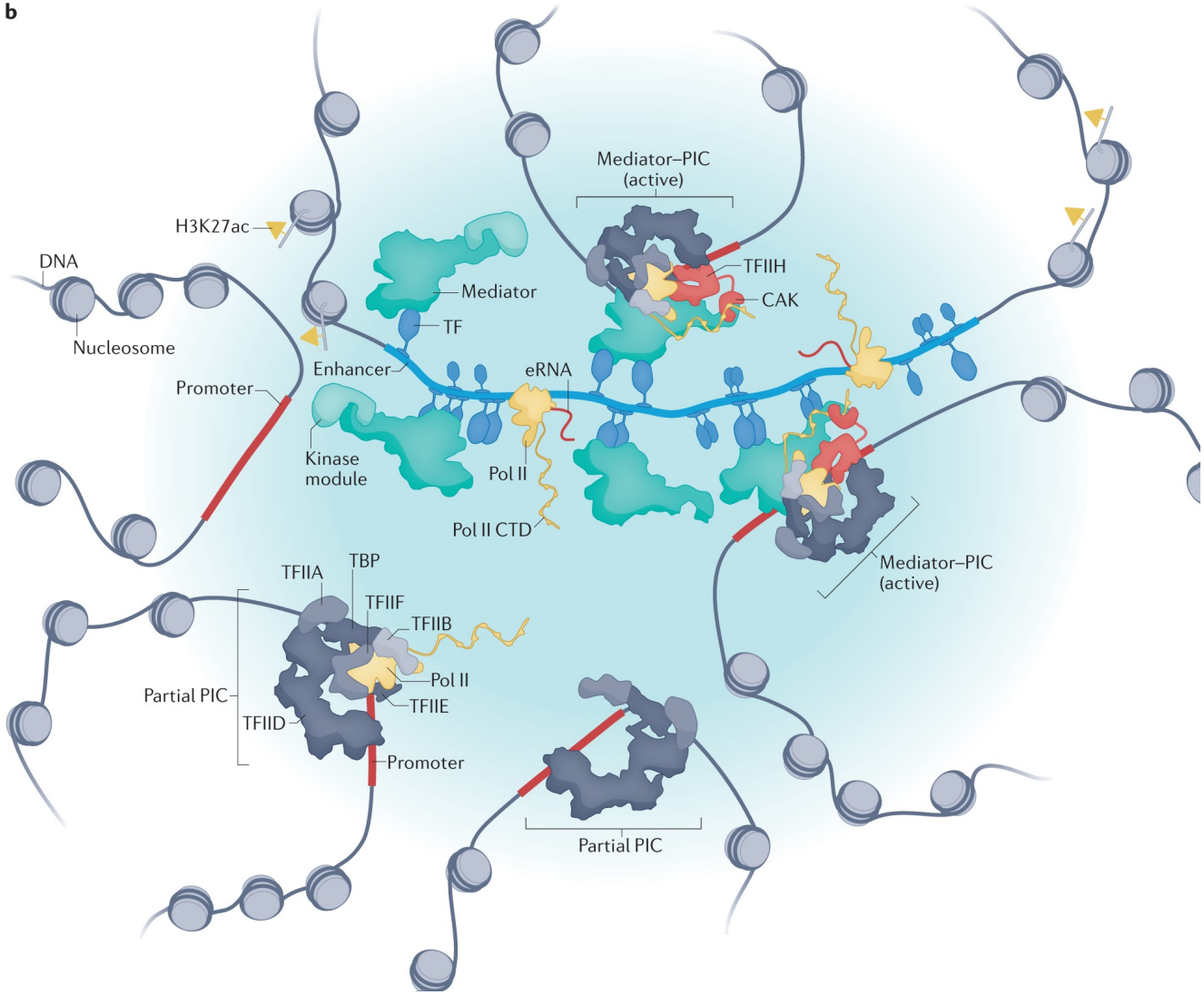
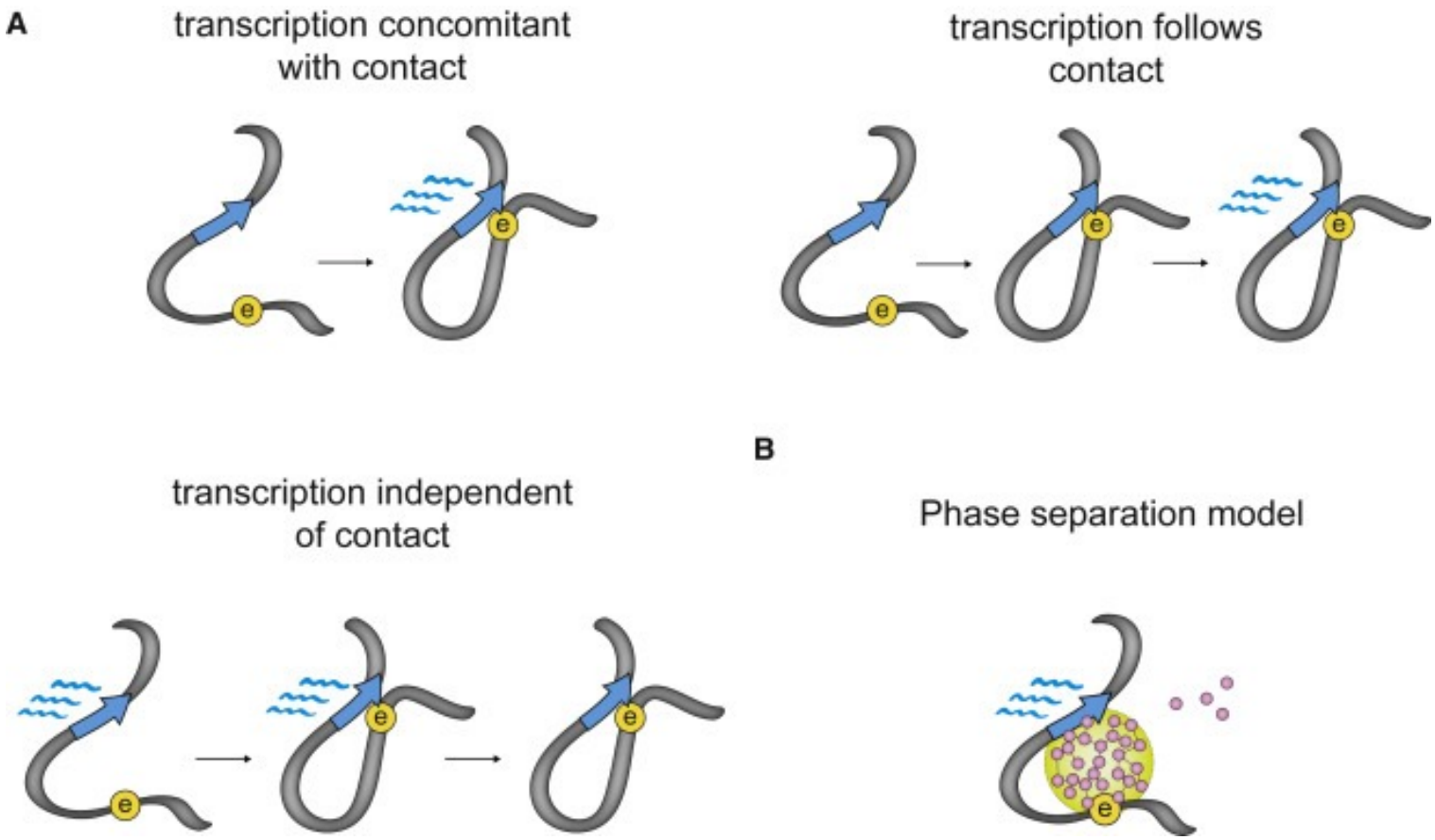
# Transcription hubs or condensates

Enhancer sequences are characterized by clusters of TF-binding sites, and, consequently, active enhancers will be bound by an array of TFs. Because typical mammalian enhancers encompass several hundred base pairs, and because TFs recruit Mediator to enhancers it is plausible that multiple Mediator complexes could simultaneously occupy an active enhancer




a model has emerged in which active enhancers and promoters are juxtaposed in cell nuclei, whereas transcriptionally silent regions are sequestered elsewhere.

Clustering of enhancers and promoters through spatial proximity will result in high local concentrations of bound factors such as chromatin remodellers, TFs, Mediator and Pol II



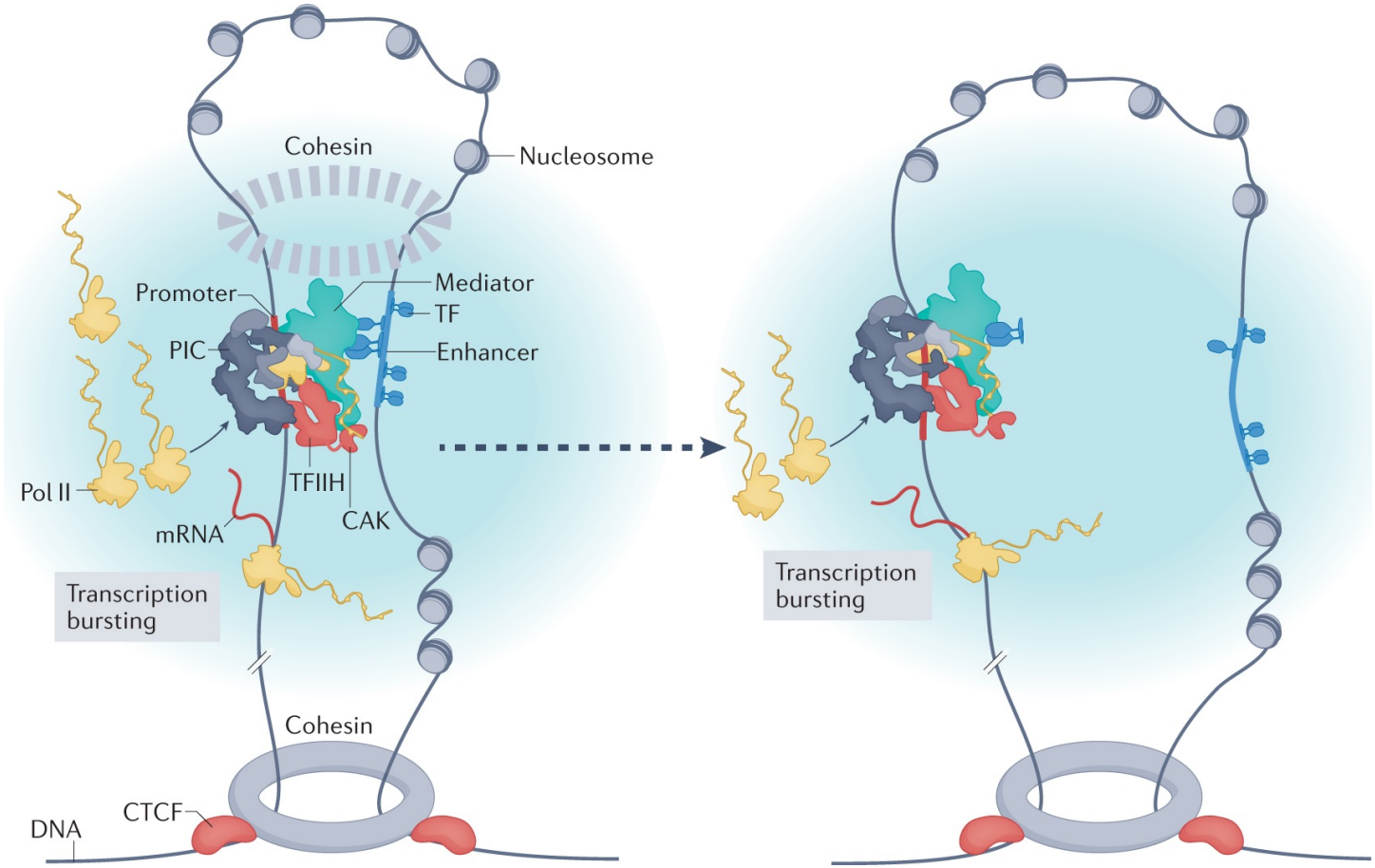

**Alternative Hypotheses on Promoter-Enhancer Communication**

(A) Transcription of RNA could either occur together with, follow, or be completely unrelated to enhancer-promoter interactions. This could possibly depend on the specific enhancer-promoter pair, or the stability of their interactions.

(B) Sub-micrometer-sized phase-separated compartments could alternatively lead to functional communication and RNA production without the need of actual physical interactions between an enhancer and its target promoter.

# Transcription bursting

A basic requirement for bursting is reinitiation, which may be facilitated by a PIC scaffold complex that remains at the promoter after the initial round of Pol II transcription.
The scaffold PIC retained TFIIA, TFIID, TFIIE, TFIIH and Mediator — that is, Pol II, TFIIF and TFIIB were missing. Furthermore, the PIC scaffold was stabilized by a TF, and TF binding resulted in a higher rate of transcription reinitiation

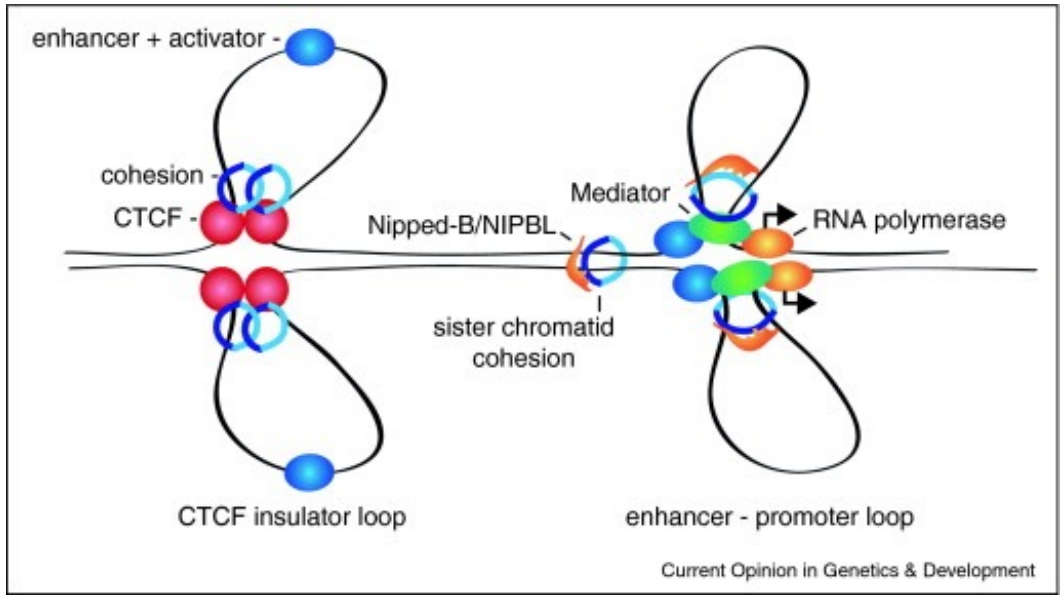


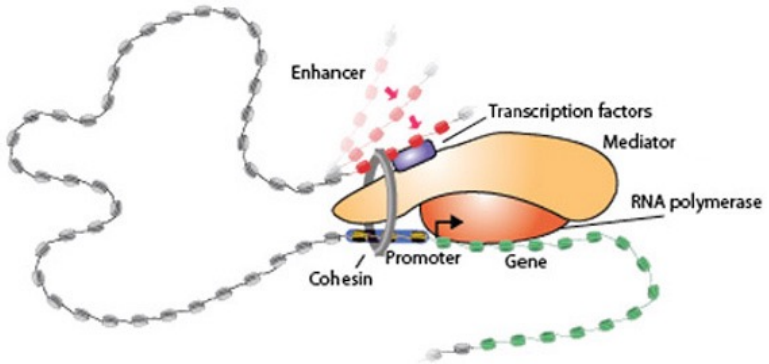

In mammalian cells, genomic regions of 1 Mb or more in size are organized into topologically associating domains (TADs), which are formed and maintained by CTCF and cohesin

# Cohesin connects gene expression and chromatin architecture

Recruitment of **Mediator** to the enhancer, or to other upstream elements, results in the formation of a chromatin loop that brings together the enhancer and the promoter of the gene to be transcribed. Subsequent recruitment of **cohesin** could potentially stabilize enhancer–promoter interactions by embracing the base of the chromatin loop.

Although **cohesin** is recruited to active promoters, it also becomes associated with the DNA-binding factor **CTCF**, which has been implicated in the formation of insulator elements. Thus, cohesin is thought to have roles in transcription activation at some genes and in silencing at others.



# CTCF links the architecture of the genome to its function

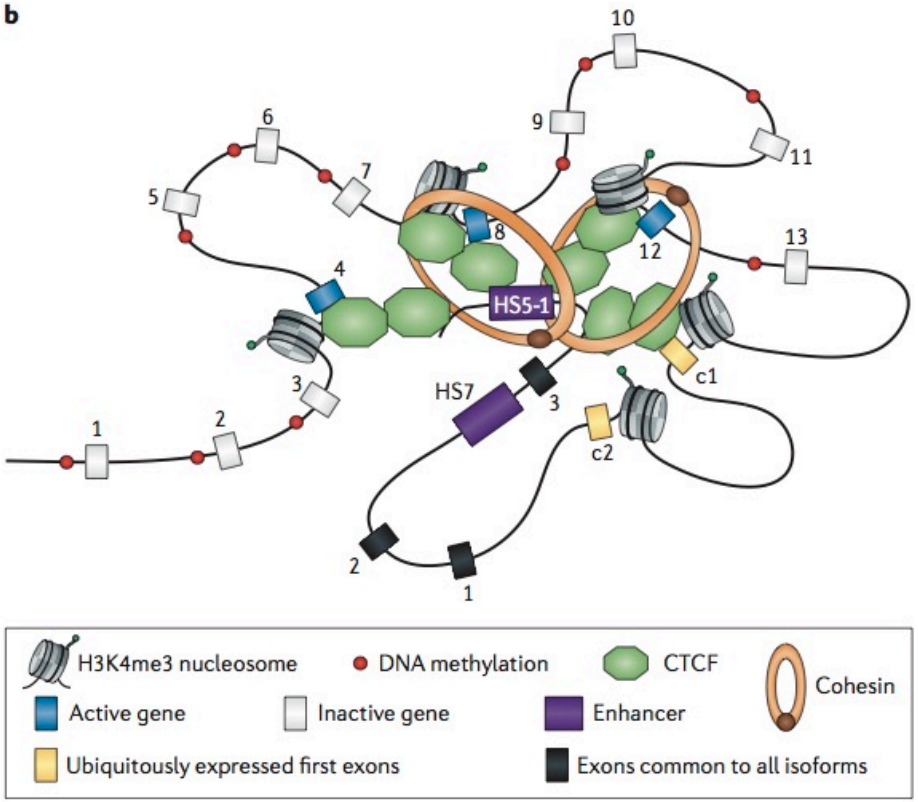
The eukaryotic genome is organized in the three-dimensional nuclear space in a specific manner that is both a cause and a consequence of its function. This organization is partly established by a special class of architectural proteins, of which **CCCTC-binding factor (CTCF)** is the best characterized.

CTCF creates boundaries between topologically associating domains in chromosomes and, within these domains, facilitates interactions between transcription regulatory sequences.

# CTCF links the architecture of the genome to its function

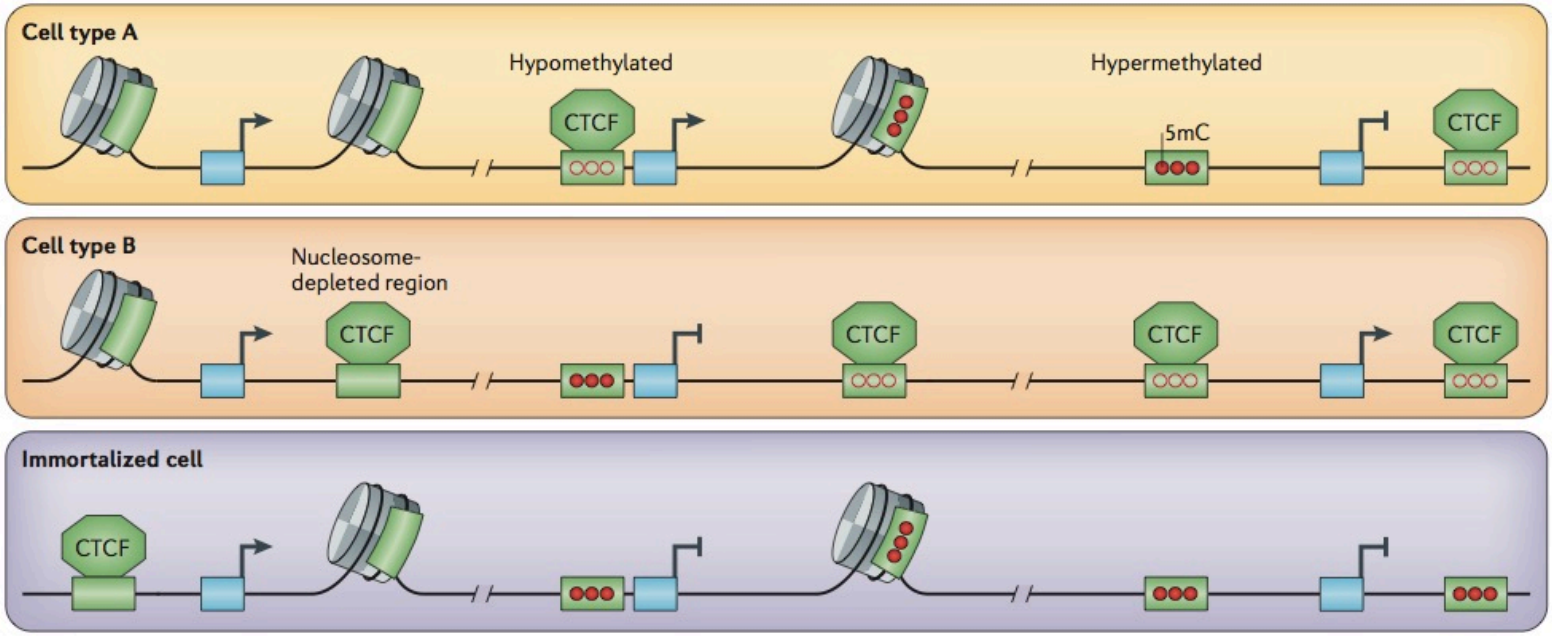
CTCF was initially classified as **insulators** -that is, a protein with the ability to interfere with enhancer–promoter communication.

CTCF mediates both inter- and intra-chromosomal interactions between distant sites in the genome. As a result of these interactions, CTCF elicits specific functional outcomes that are context dependent, determined by the nature of the two sequences brought together and by the proteins with which they interact (‘architectural’ protein)



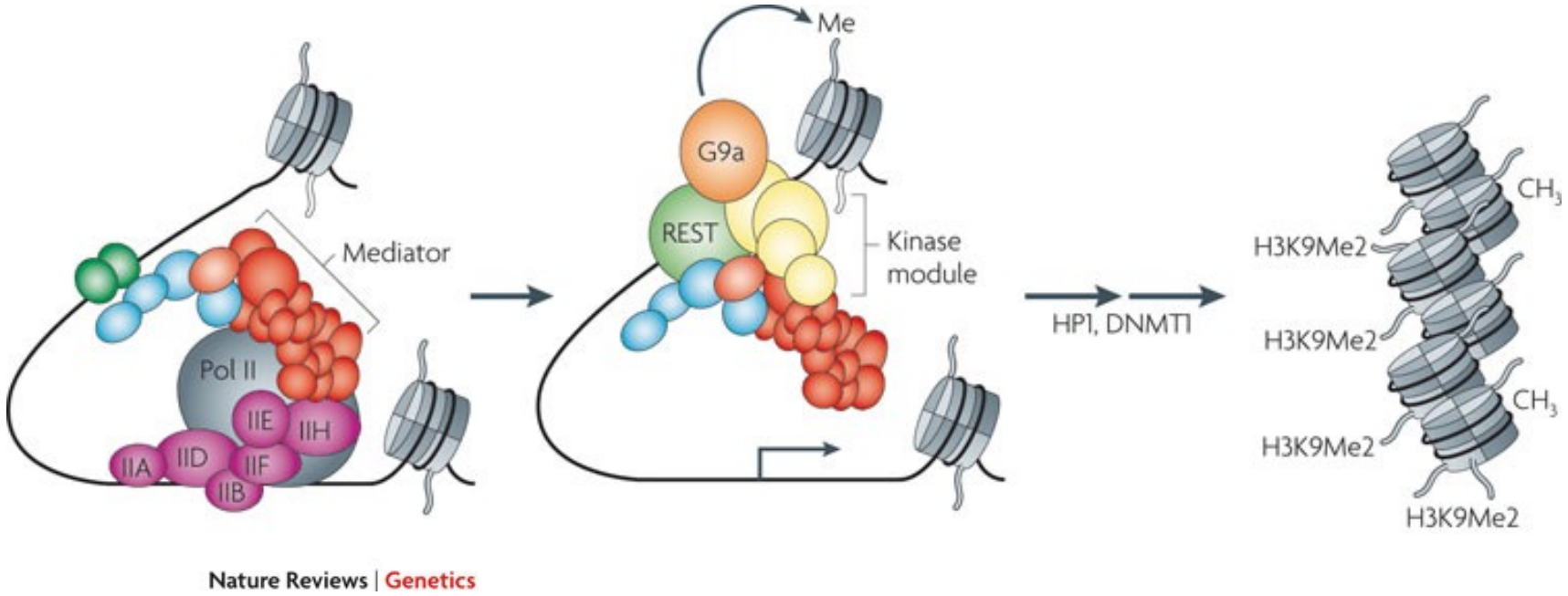
# Regulation of CTCF binding to DNA

Constitutive binding sites of CTCF are present in non-methylated and nucleosome-free regions. Cell-type-specific CTCF binding is partly regulated by differential DNA methylation and nucleosome occupancy across different cell types. This suggests that cells can use ATP-dependent chromatin remodelling complexes to regulate nucleosome occupancy at specific CTCF-binding sites and control the interaction of this protein with DNA. In addition, the methylation status of cell-type-specific CTCF-binding sites may be determined by a combination of activities of *de novo* methyltransferases and TET enzymes that regulate the presence and levels of 5mC at specific sites. Immortalized cancer cell lines contain high levels of 5mC at CTCF-binding sites, which correlates with the low CTCF occupancy in these cells.



# The mediator complex can also contribute to epigenetic silencing

Mediator can contribute to **long-term silencing** of neuronal genes in extraneuronal cells. Starting with an active **PIC**, **REST** binding to the cognate site commits the gene to a heterochromatin fate as development proceeds. A ternary complex containing REST, the histone methyltransferase G9a and the intact Mediator is first assembled. The multipartite interactions are anchored through **the MED12 subunit of the kinase module**. Following G9a action and dimethylation of histone H3 lysine 9 (H3K9) to H3K9me2, heterochromatin protein 1 (HP1) and DNA methyltransferase 1 (DNMT1) are recruited to the site. Ultimately, the gene is embedded in transcriptionally inert heterochromatin marked by H3K9me2 and methylated DNA (.

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# MED12 and G9a Interaction