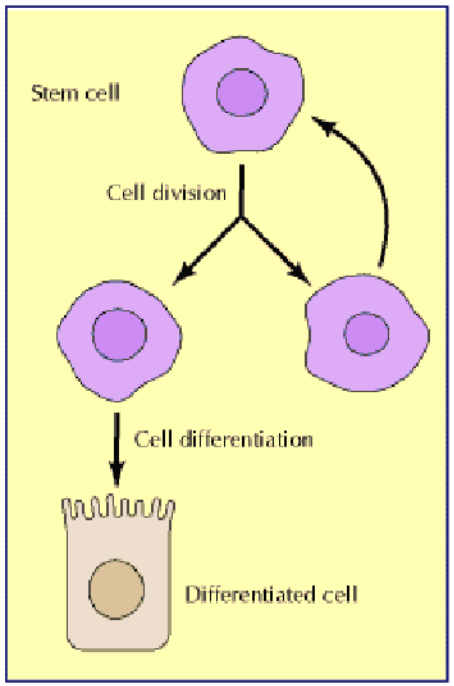
DIFERENCIACIÓN CELULAR

La diferenciación celular es el proceso por el que una célula cambia sus características de forma permanente. Implica la adquisición de las características propias, funcionales y morfológicas.



CAUSAS DE LA DIFERENCIACIÓN

Mantenimiento del genoma, pero distinta expresión génica.
Excepciones, ej. linfocitos.

¿Qué es la diferenciación celular?

Proceso por el que las células adquieren una forma y una función determinada durante el desarrollo embrionario o la vida de un organismo pluricelular, especializándose en un *tipo celular*.

La morfología de las células cambia notablemente durante la diferenciación, pero el material genético, o genoma permanece prácticamente inalterable.

Una célula capaz de diferenciarse en todos los tipos celulares de un organismo se llama **totipotente**.

En los mamíferos, solo el zigoto y las células embrionarias jóvenes son totipotentesUna célula capaz de diferenciarse en varios tipos celulares se llama **pluripotente**.

Estas células se llaman **células madre** en los animales.

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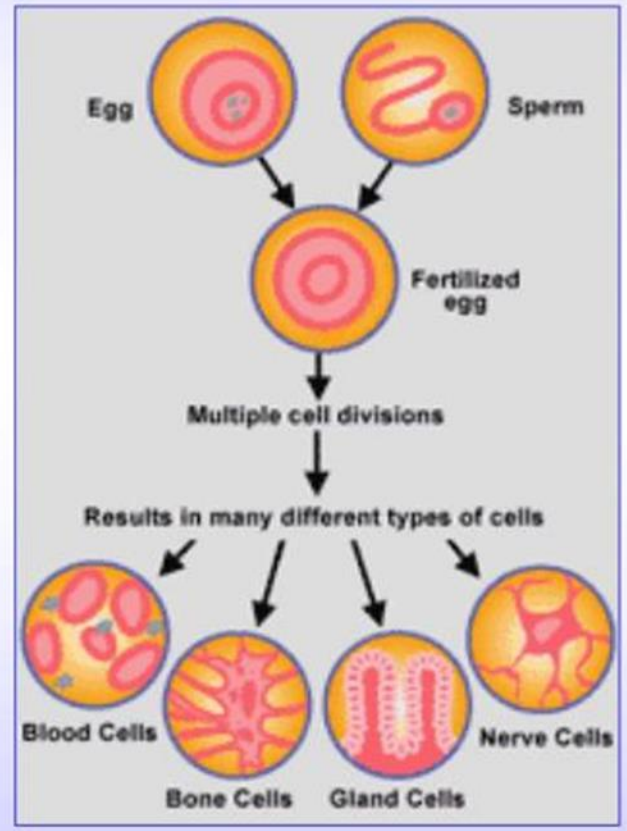


Diferenciación Vs. Proliferación

Diferenciación

Proceso que le sirve a la célula
A "definirse" morfológicamente,
Está relacionado con una serie de
Eventos moleculares que determinan
que tipo de célula será.

Proliferación

Proceso que le sirve a la célula
A "perpetuar su especie", ya que
La célula al dividirse duplica
Su material genético.
Para ello ocurre una serie de
Eventos moleculares que
Determinan en que momento
ocurrirá.
>>>>Ciclo celular<<<<

La diferenciación celular es regulada por la expresión de genes específicos.

Señal de transducción

Hormonas, factores de crecimiento

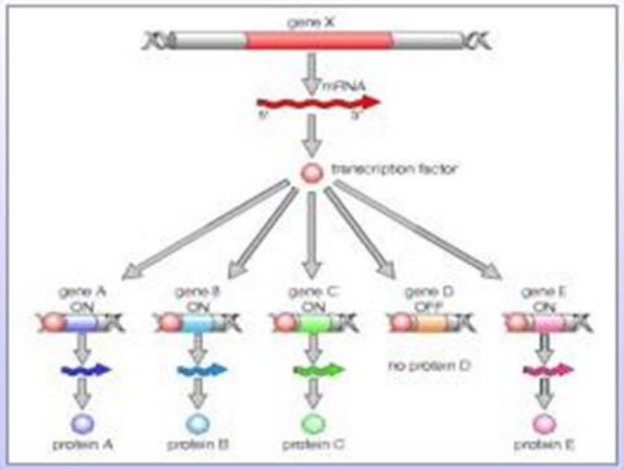
Producción de una combinación
De factores de transcripción (FT)

Activación de un receptor intracelular
Que actúa como FT

Factores de transcripción que
Se unen a regiones del ADN

Se encienden o apagan genes (Actividad génica ON-OFF)

DIFERENCIACION



Factor de transcripción: Es una proteína que hace que algunos genes Se activen ("ON") y Otros se apaguen ("OFF")

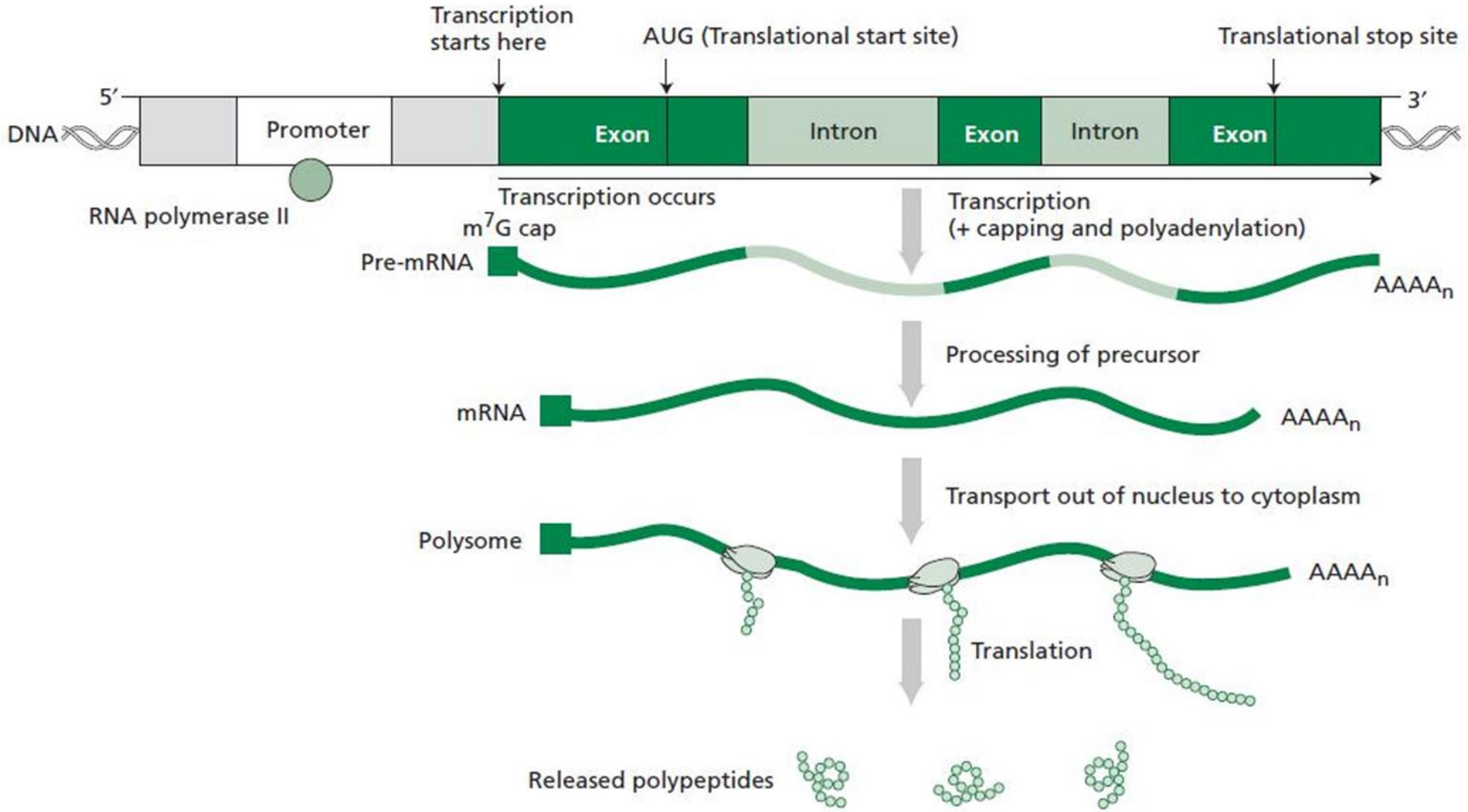
14

Si la célula va a proliferar siendo el "mismo tipo de célula", su ciclo proseguirá a un ritmo que le permita utilizar los nutrientes y factores del medio para asegurar su replicación.

En cambio, si las condiciones externas cambian, o si está "programada" para diferenciarse, deberá cambiar el patrón de genes que será transcrito (transcriptoma) y, en consecuencia, el conjunto de proteínas que utilizará para funcionar (proteoma).

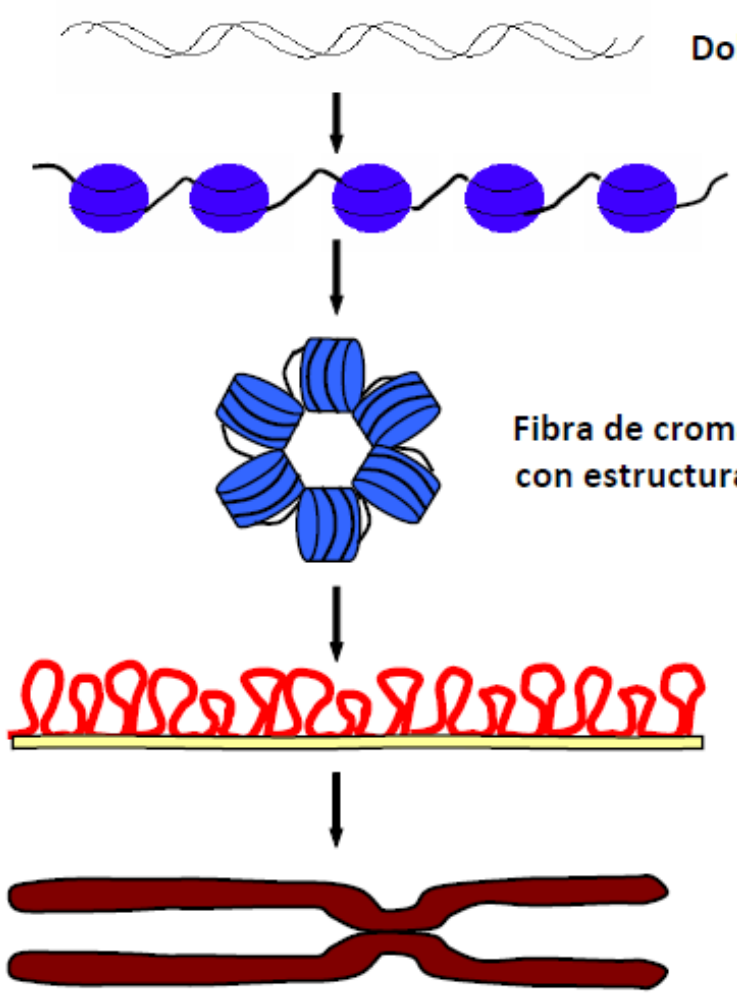
ACRONIMOS USADOS EN LA PRESENTACION

ACRONIMO	SIGNIFICADO	ACRONIMO	SIGNIFICADO
CBP	Proteína que se une a CREB (proteína que se une al elemento de respuesta a AMPc	PRE	Elemento de respuesta a Polycomb
CpG	Islas de dinucleótidos de citosina y guanina	RNA pol II	RNA polimerasa II
CTCF	Factor que se une a CCCTC	TAFs	Factores asociados a TBP
DNMT	DNA metil transferasa	TBP	Proteína que se une a TATA
HAT	Histona acetil transferasa	TFIIs	Factores de transcripción para la RNA pol II
HDAC	Histona desacetilasa		



Es importante recordar que para llegar a tener una proteína funcional, los genes deben ser transcritos (1), los mRNA editados (2) a su forma madura, transportados al citosol (3) y traducidos en los ribosomas (4), para que luego las proteínas, en su caso, sean madurados y dirigidas al compartimiento donde llevarán a cabo sus funciones.

INTRODUCCION. NIVELES DE ORGANIZACIÓN DEL DNA EUCARIOTICO



Doble hélice del DNA (2 nm)

Estructura de los nucleosomas (10 nm)

Fibra de cromatina de 30 nm con estructura de solenoide

Sección extendida de un cromosoma (300 nm)

Cromosoma metafásico (2 cromátidas de 700 nm cada una)

Empaquetamiento del DNA eucariótico

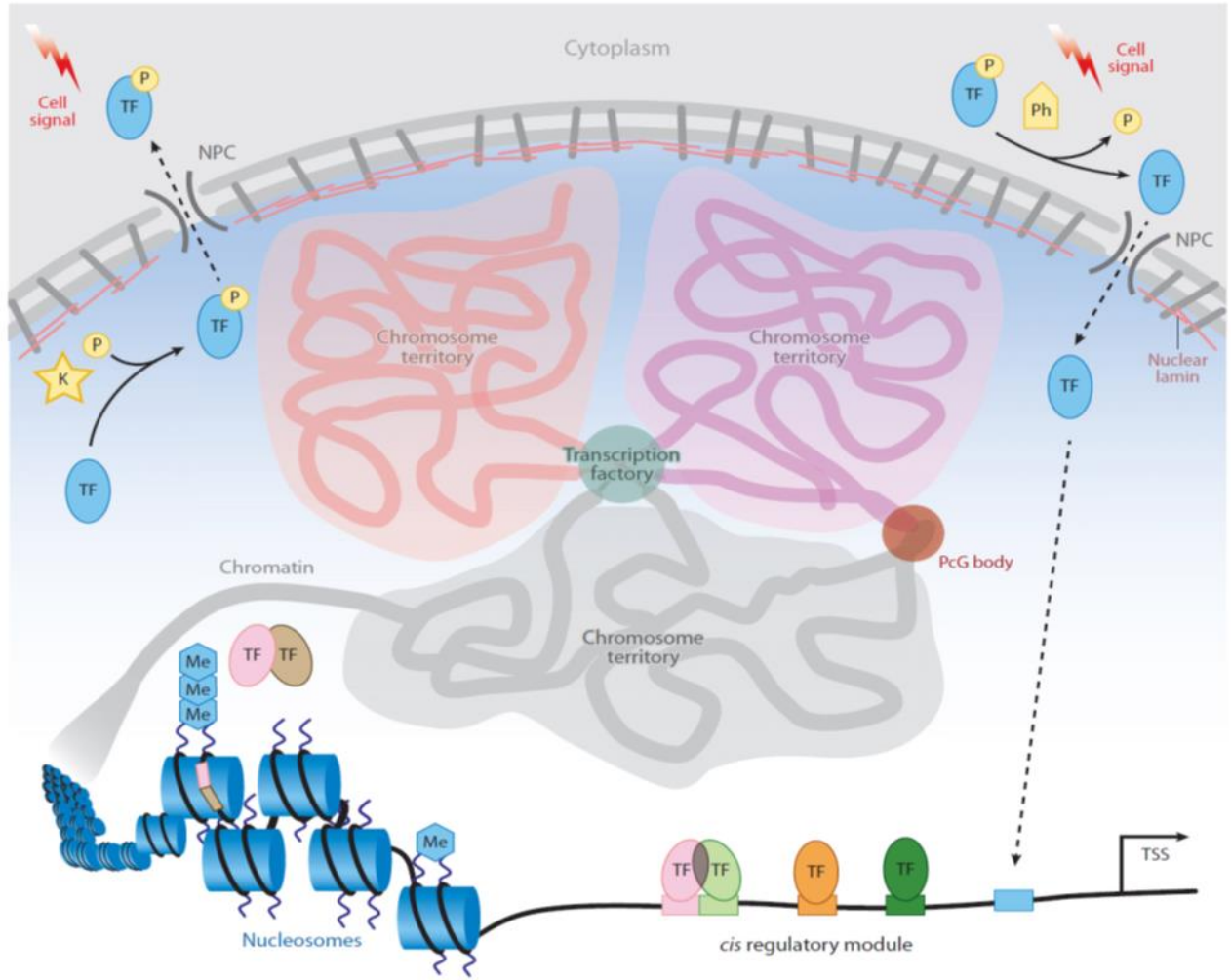


Figure 1

Overview of eukaryotic gene regulation. Within the nucleus of a cell, chromosomes occupy defined spatial regions called territories. Interactions between adjacent territories can correlate with transcriptionally active chromatin in transcription factories or silenced chromatin in Polycomb Group (PcG) bodies. Posttranslational modification of transcription factors (TFs), such as phosphorylation (P), can influence nuclear import (*dashed arrows*) through nuclear pore complexes (NPCs) in response to extracellular signals. Posttranslational modifications of histones, such as methylation (Me), can also correlate with the transcriptional state of associated genes. The position of nucleosomes can restrict access of TFs by occluding binding sites (*colored rectangles*). Lastly, TF recognition of specific binding sites, either as monomers or as a part of a complex with other proteins, also contributes to proper recruitment or release of RNA polymerase from the transcriptional start site (TSS). Abbreviations: K, kinase; Ph, phosphatase.

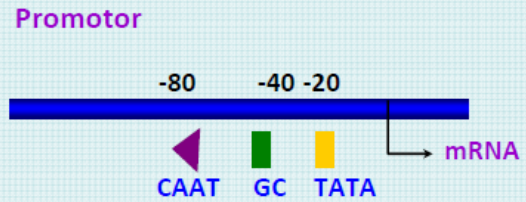
Organización de los elementos de control en genes eucarióticos

Organización en **módulos de secuencias cortas altamente conservadas**

→ un mismo gen está regulado por varios TFs; acción modular

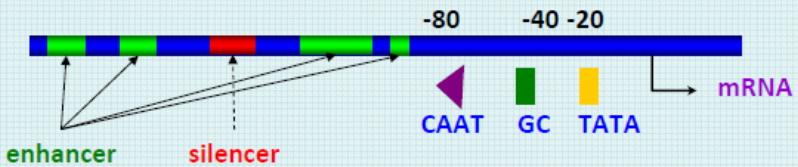
Promotor

- núcleo del promotor
- promotor regulador



Elementos upstream con diversos elementos de respuesta

- enhancers
- silencers



** Prediccion bioinformática de los promotores eucarióticos*

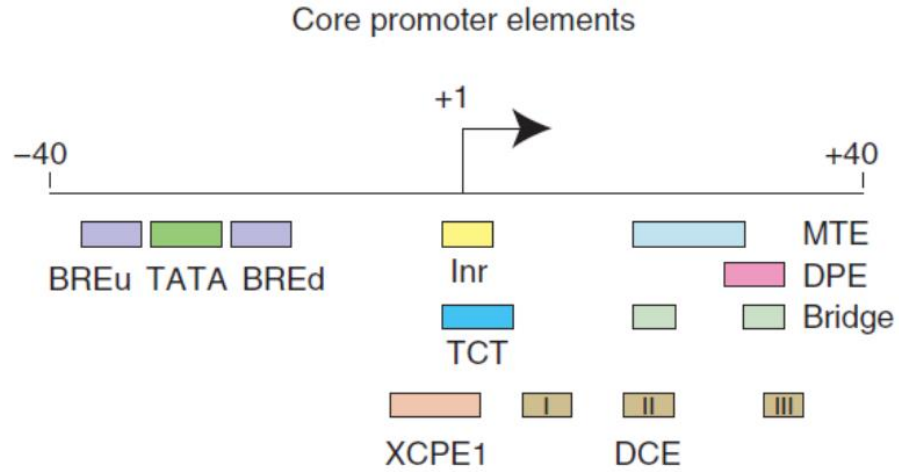
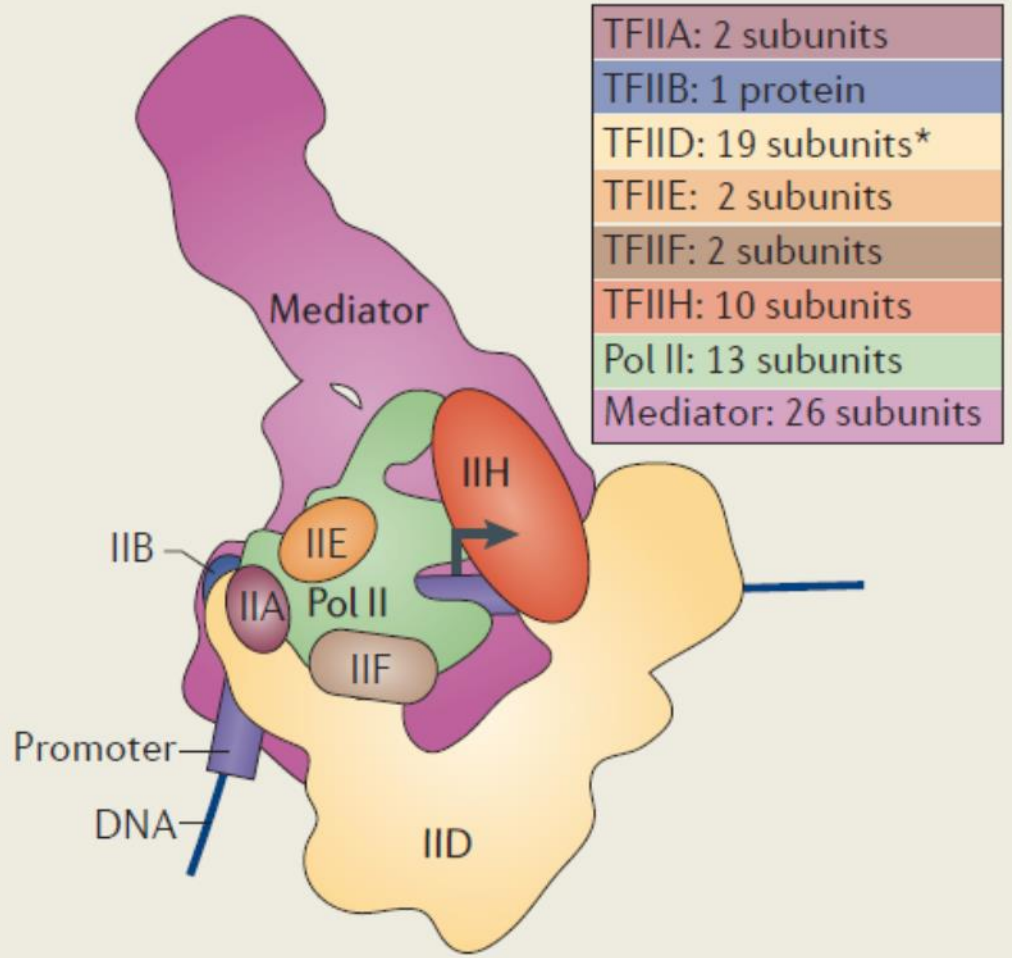


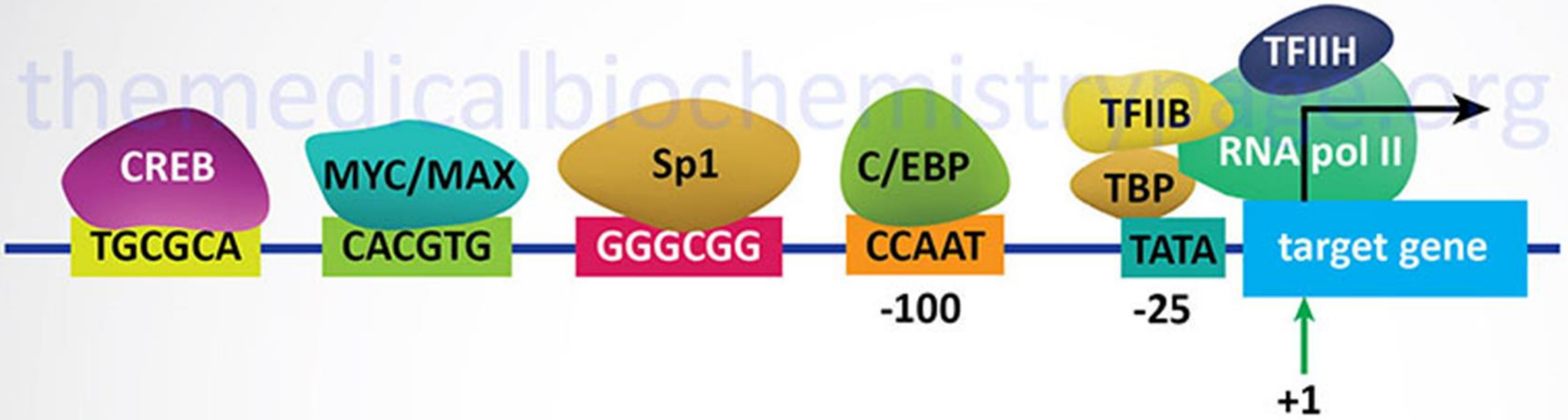
FIGURE 1 | Some core promoter elements for transcription by RNA polymerase II. These elements include the BREu and BREd (TFIIB recognition element, upstream and downstream), TATA box, Inr (Initiator), MTE (motif ten element), DPE (downstream core promoter element), Bridge, TCT (polypyrimidine initiator), XCPE1 (X core promoter element 1), and DCE (downstream core element). The locations of the motifs are drawn roughly to scale. The BREu, TATA, Inr, MTE, DPE, and TCT motifs have been found in both *Drosophila* and humans. These motifs are typically found in focused core promoters, although there are probably Inr-like elements in dispersed promoters. There are no universal core promoter elements that are found in all promoters. Moreover, it is likely that many other core promoter motifs remain to be discovered. The functional properties of a core promoter are determined by the presence or absence of specific core promoter motifs. For example, some enhancers will activate transcription from DPE-dependent core promoters but not from TATA-dependent core promoters.

Box 3 | **The pre-initiation complex**

The eukaryotic RNA polymerase II (Pol II) enzyme transcribes most non-coding RNA genes and all protein-coding genes. Unlike bacterial polymerases, however, Pol II relies on an array of auxiliary factors that ensure transcription initiation occurs at the correct site in the genome, and that ensure that RNA processing events result in the production of a mature transcript[190]. At the transcription start site, Pol II initiation is regulated by a protein assembly known as the pre-initiation complex (PIC)[191] (see the figure). Each of the factors that constitute the PIC (transcription factor IIA (TFIIA), TFIIB, TFIID, TFIIE, TFIIF, TFIIH, Pol II and Mediator) have varied functional and structural roles[192], but much remains unknown about how the PIC functions as a unit. Biophysical studies have begun to determine the overall structure and architecture of the PIC. Although current results suggest different architectures for human and yeast PICs[193,194], this remains an area of active investigation[195]. Finally, even though the PIC can function in various forms (for example, the PIC contains simpler TFIID assemblies in differentiated cells), it represents a fundamental intermediate in transcription initiation that is targeted by an array of general and gene-specific regulatory factors.



The asterisk (see the figure) indicates that TFIID contains a TATA-binding protein (TBP) and 13 different TBP-associated factors (TAFs), 5 of which dimerize (TAF4, TAF5, TAF6, TAF9 and TAF12)[196].

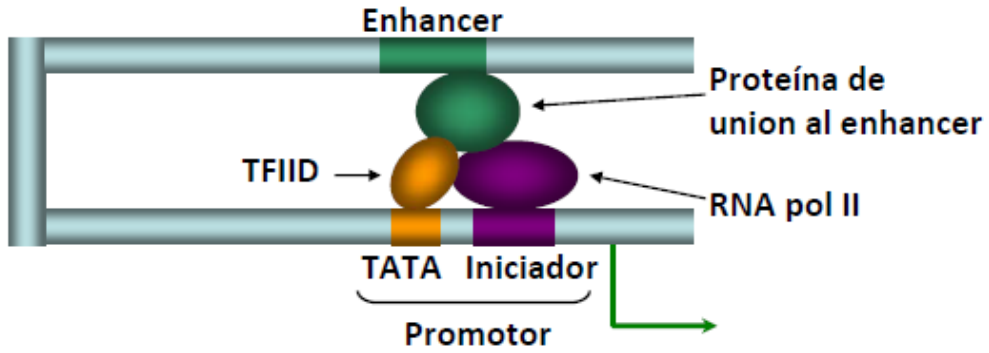


El control depende de secuencias de DNA llamadas promotores, que permiten la unión de factores de transcripción, para que la RNA polimerasa II (en el caso del mRNA) entre e inicie la transcripción (1), pero también de otros elementos que se unen en otras secuencias más o menos cercanas (2).

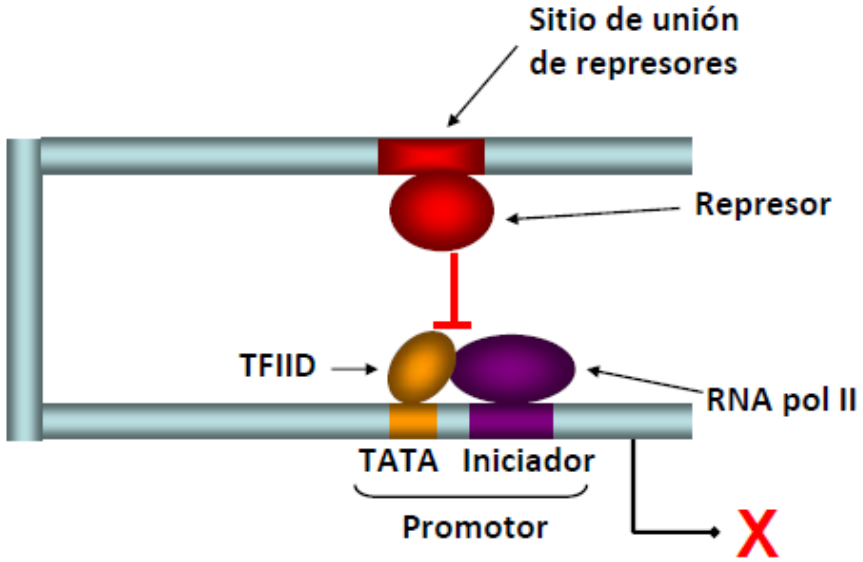
ENHANCERS

Se definen como elementos genómicos que activan la transcripción de un grupo de promotores, independientemente de su posición con respecto a éstos (corriente abajo o corriente arriba hasta 106 kpb de distancia) dentro del llamado dominio regulador, en el cual pueden incluso existir promotores que el enhancer no puede activar.

La activación específica de los promotores depende de las propiedades del complejo que éste puede formar con los factores de transcripción (enhanceosoma) y las características del promotor.



SILENCERS



Es un elemento cuya actividad es la opuesta al enhancer, y funciona a nivel de la reconfiguración de cromatina. Por ejemplo, los elementos de respuesta a Polycomb (PREs), que inician el ensamblaje de los complejos represores Polycomb poseen dos actividades:

Previenen la comunicación entre un enhancer y un promotor cuando se ubican entre ellos (actividad de bloqueo de enhancers), y el desenrrollamiento de la cromatina (actividad de barrera).

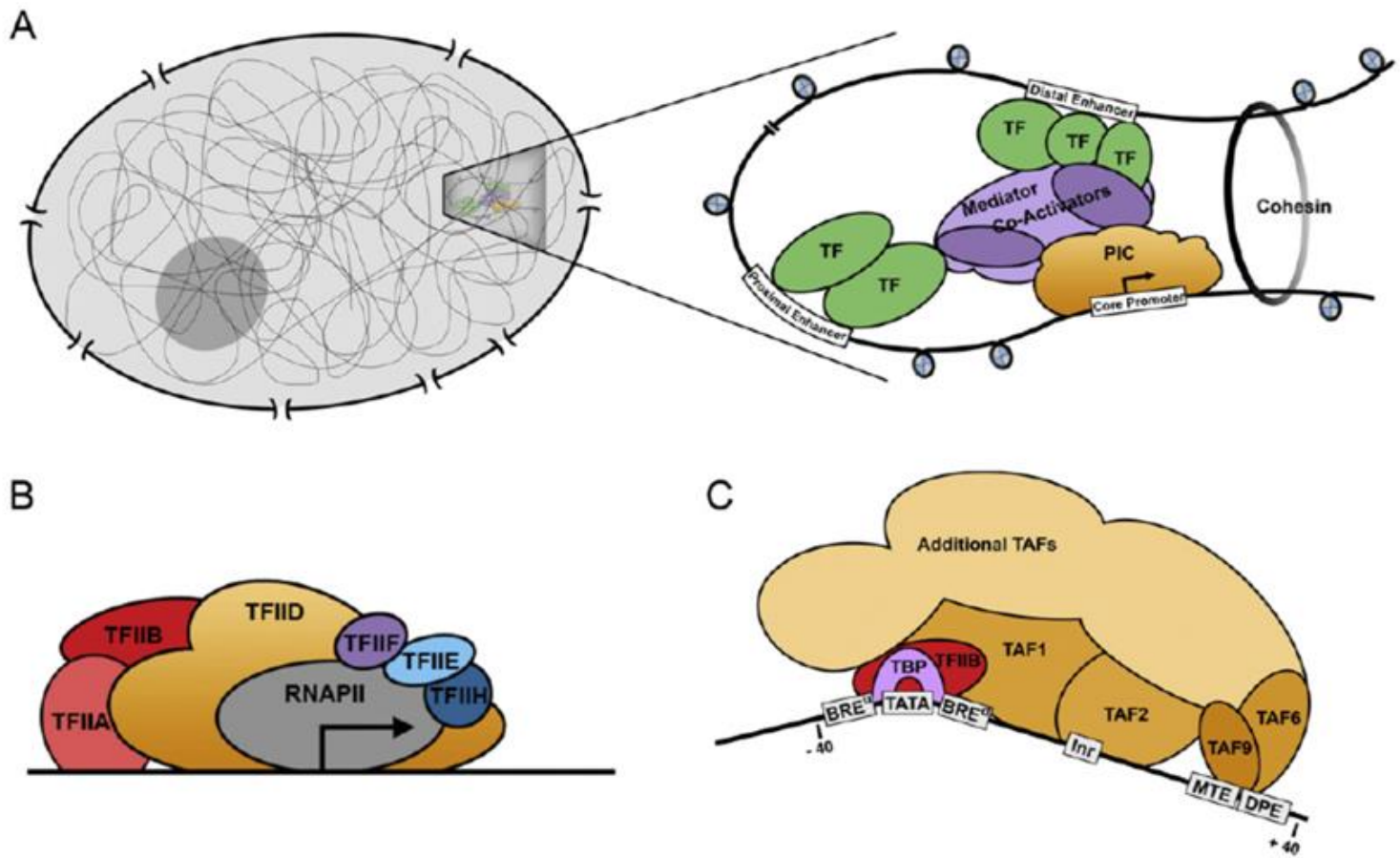
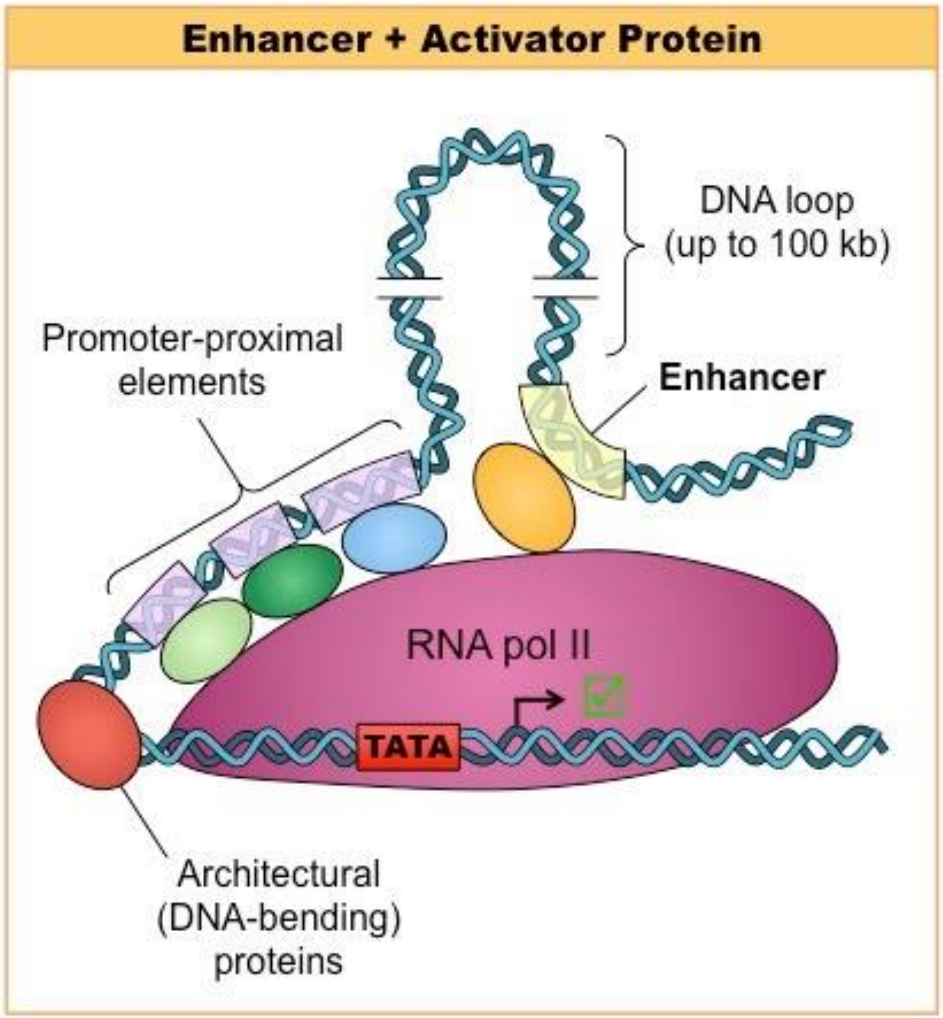
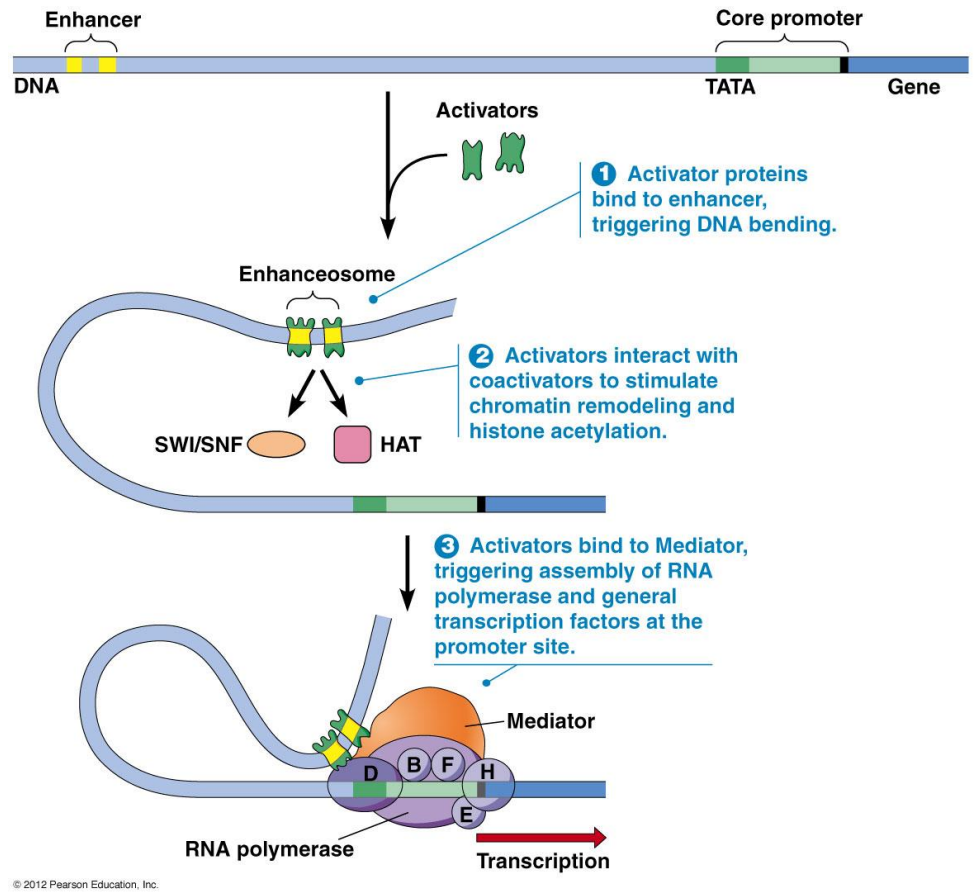
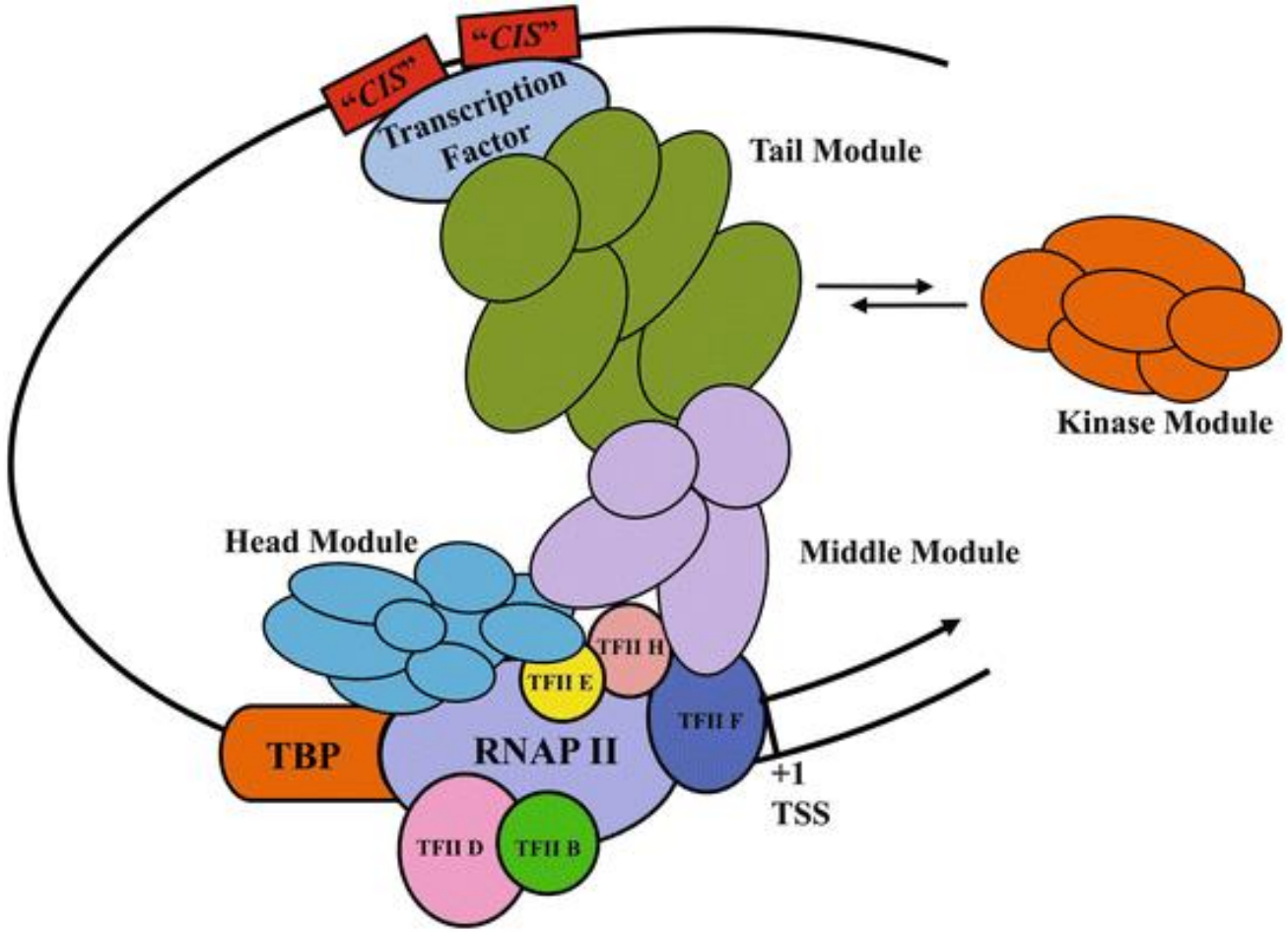


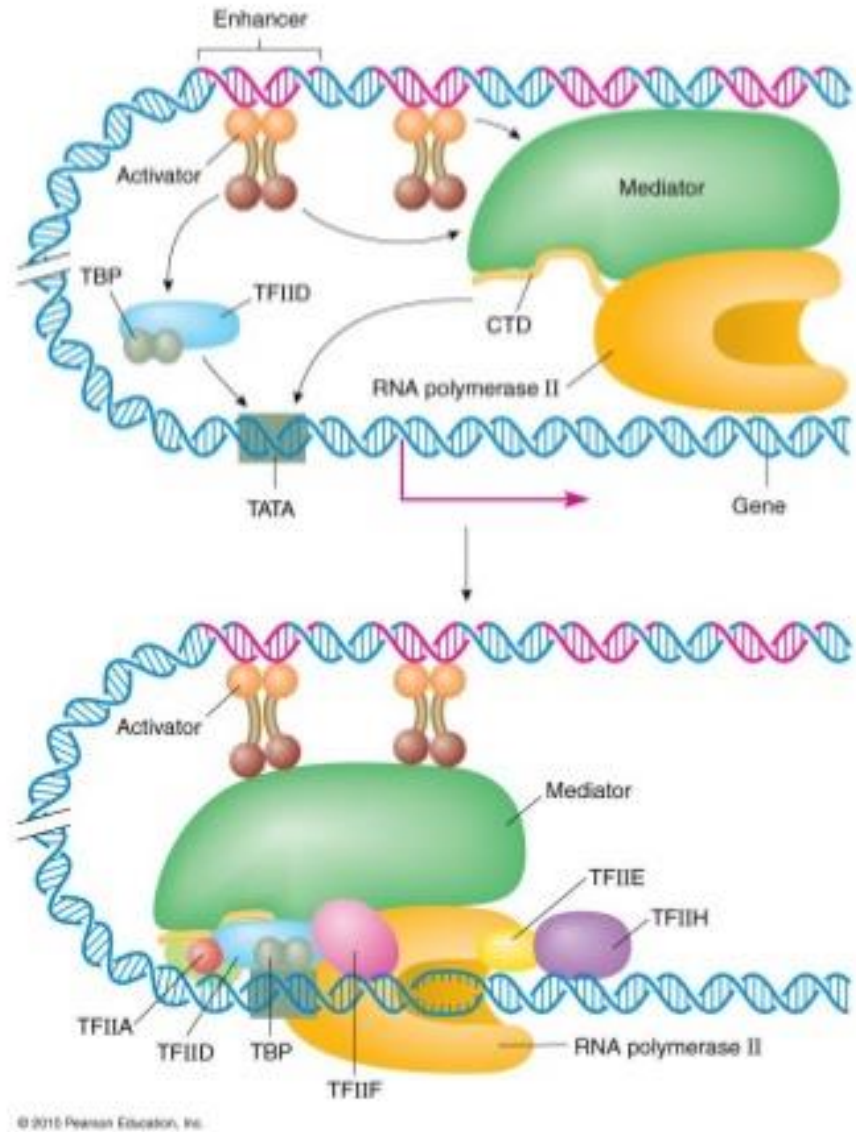
Fig. 2. The core promoter can be studied from different angles in multiple resolutions. A. Zooming in on global genomic interactions in the nucleus, one can study long-range interactions, such as those between enhancers and promoters, by analyzing chromatin looping, cohesion function, interactions of transcription factors (TFs) with co-activators and cis-regulatory modules and interactions of the preinitiation complex (PIC) components with their target promoters. B. Zooming in on the basal transcription machinery, one can study the assembly and composition of the PIC at different Pol II-promoters and the 3D structure of different PIC components. C. Zooming in on the DNA-binding PIC components (TFIIB and TFIID), one can focus on the alternative protein components at different Pol II-promoters, on the core promoter composition of specialized transcription programs, and on the interactions of different PIC components with specific core promoter elements.



Los complejos formados pueden incluir activadores que son los que se unen directamente al enhancer y, en su caso, coactivadores, que se unen al activador y al aparato transcripcional básico; este complejo puede recibir el nombre de enhanceosoma.




"CIS"
"CIS"
Transcription Factor
Tail Module
Kinase Module
Head Module
Middle Module
TFII H
TFII E
TFII F
TBP
RNAP II
TFII D
TFII B
+1
TSS


Enhancer
Activator
Mediator
TBP
TFIID
CTD
RNA polymerase II
TATA
Gene
Activator
Mediator
TFIIE
TFIIH
TFIIA
TFIID
TBP
TFIIF
RNA polymerase II
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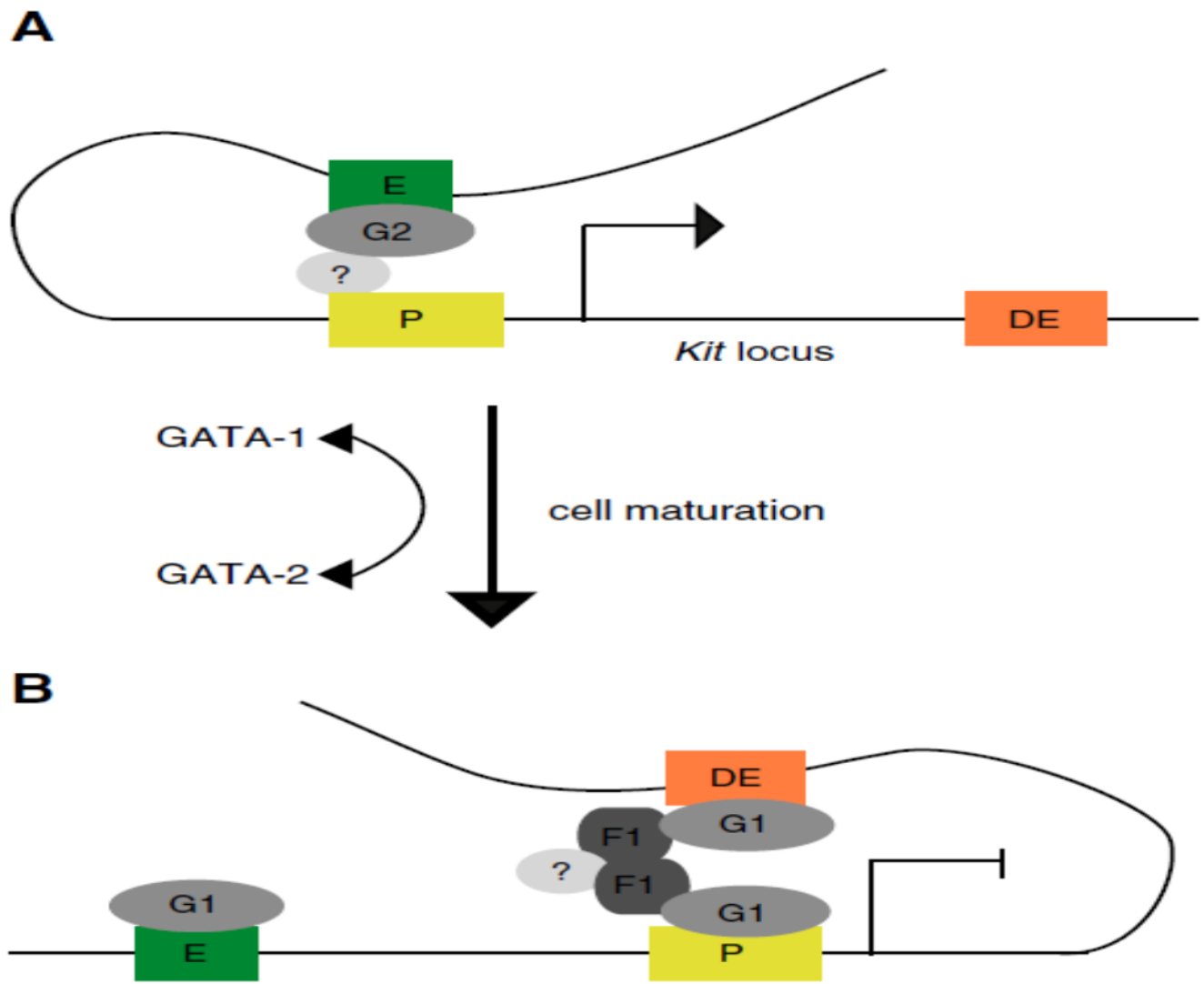
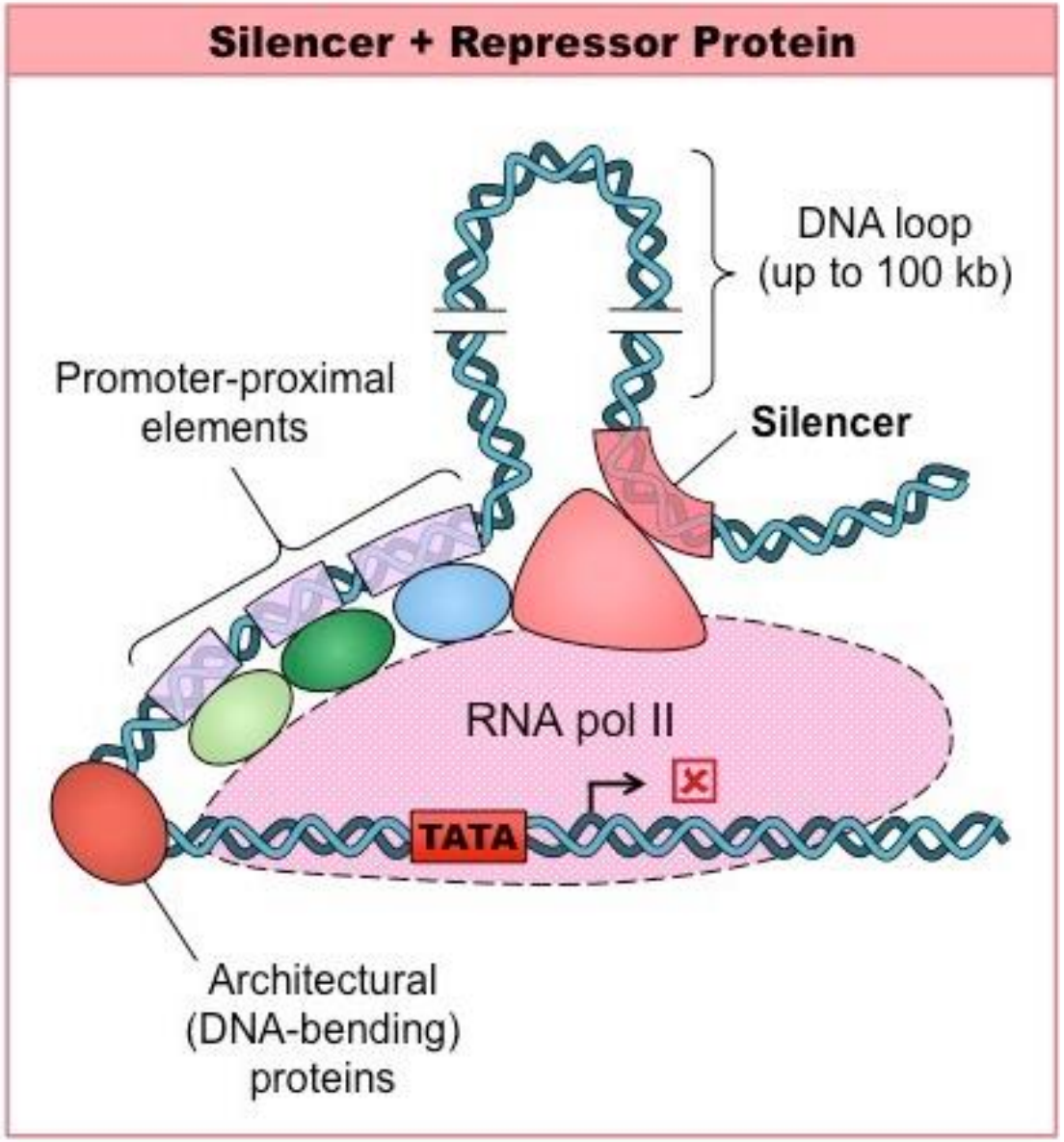
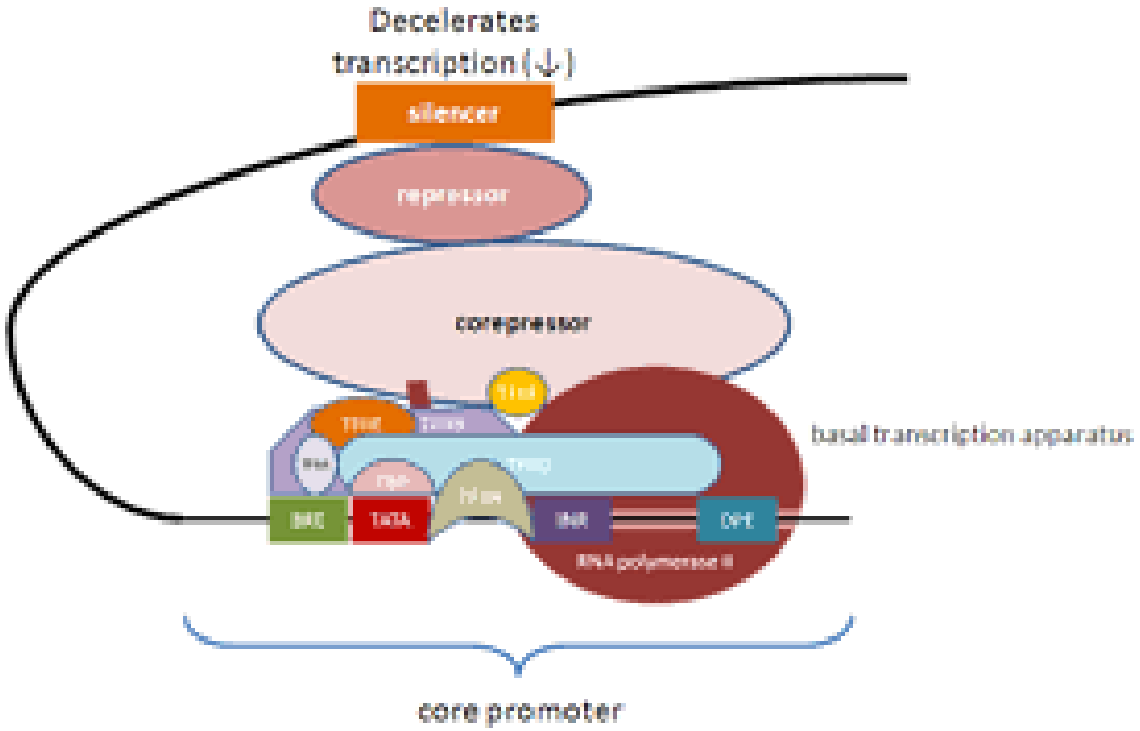


Fig. 3. GATA transcription factors regulate Kit expression by changing loop conformations. (A) In immature erythrocytes the Kit locus is expressed by recruitment of GATA-2 and other unknown factors (?), which mediate looping between an upstream enhancer (E) and the Kit promoter (P). (B) Upon cell maturation GATA-2 is replaced by GATA-1, which binds to a downstream element (DE). GATA-1 together with FOG-1 (F1) and other unknown factors mediate looping between the DE and the promoter, blocking enhancer–promoter looping and thereby down regulating Kit transcription.



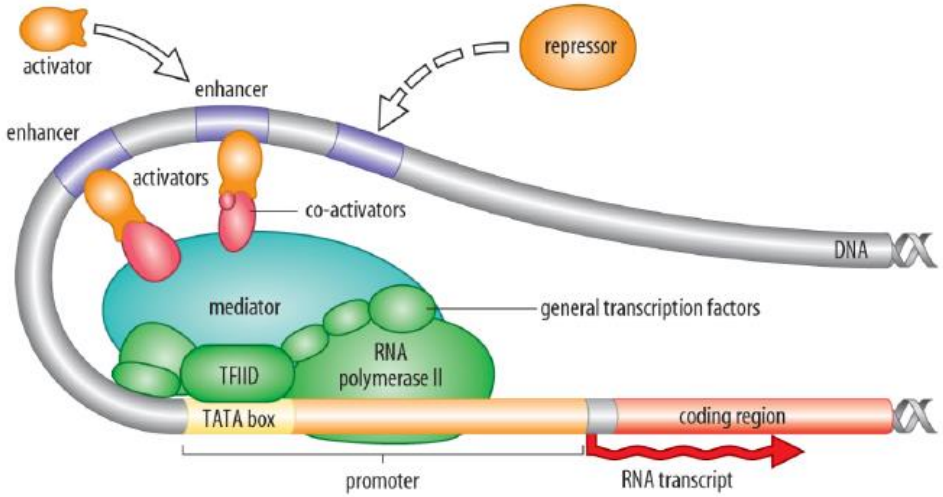
Los silencers (silenciadores) permiten la unión de represores (y en ocasiones correpresores) que interaccionan con el aparato transcripcional básico, disminuyendo la posibilidad de formar los complejos de preiniciación y abierto del promotor, lo que lleva a una disminución de la tasa transcripcional.



Regulation of transcription initiation

What happens during transcription initiation?

The assembly of the initiation complex at the promoter site of a gene.

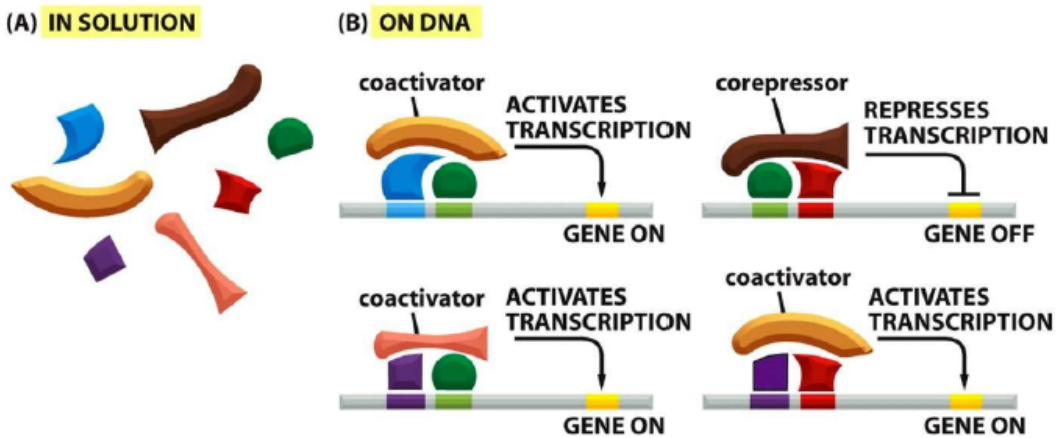


Let's complicate things 2

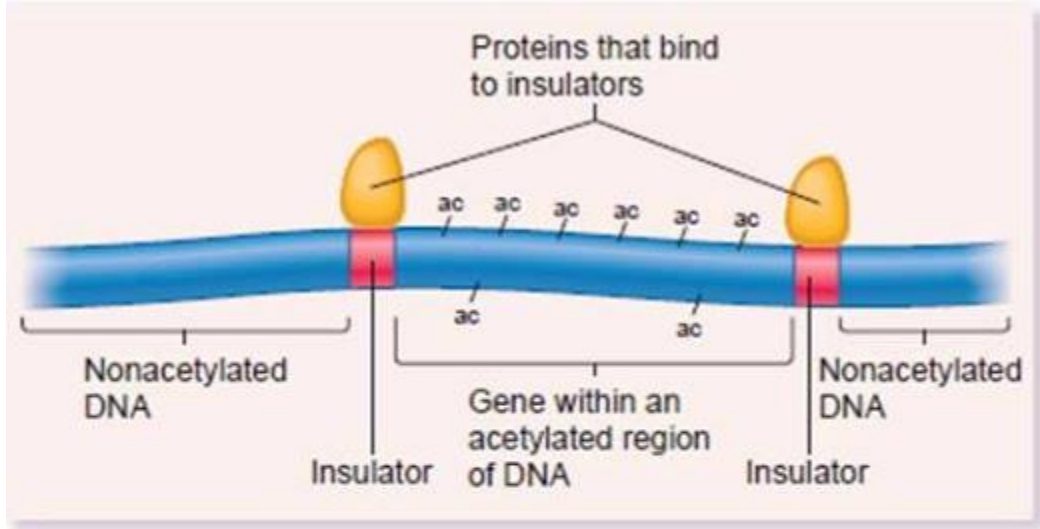
Co-activator / Co-repressor.

Co-activators and co-repressors provide an additional layer of regulation

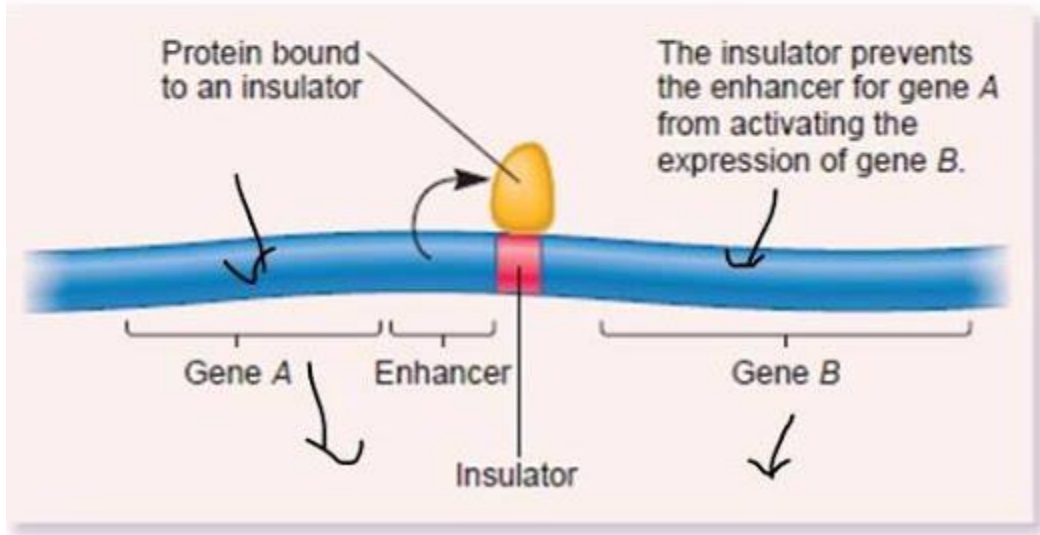
How? Why?



Gene insulators as gene silencers



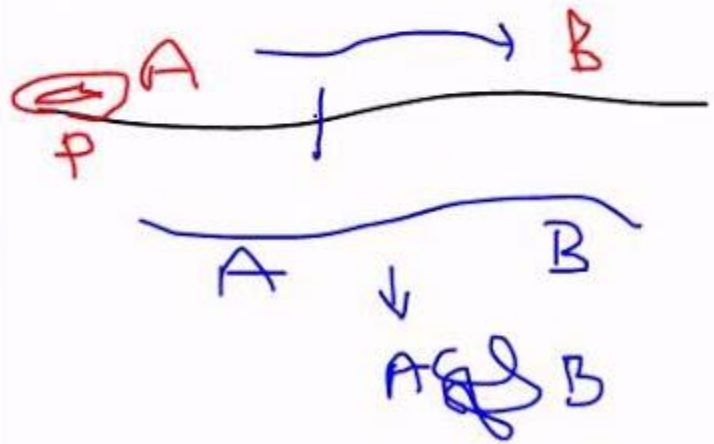
(a) Insulators as a barrier to changes in chromatin structure



(b) Insulator that blocks the effects of a neighboring enhancer

- An **insulator is a segment of DNA that functions as a** boundary between two genes.

- An insulator is so named because it protects, or "insulates," a gene from the regulatory effects of a neighboring gene.



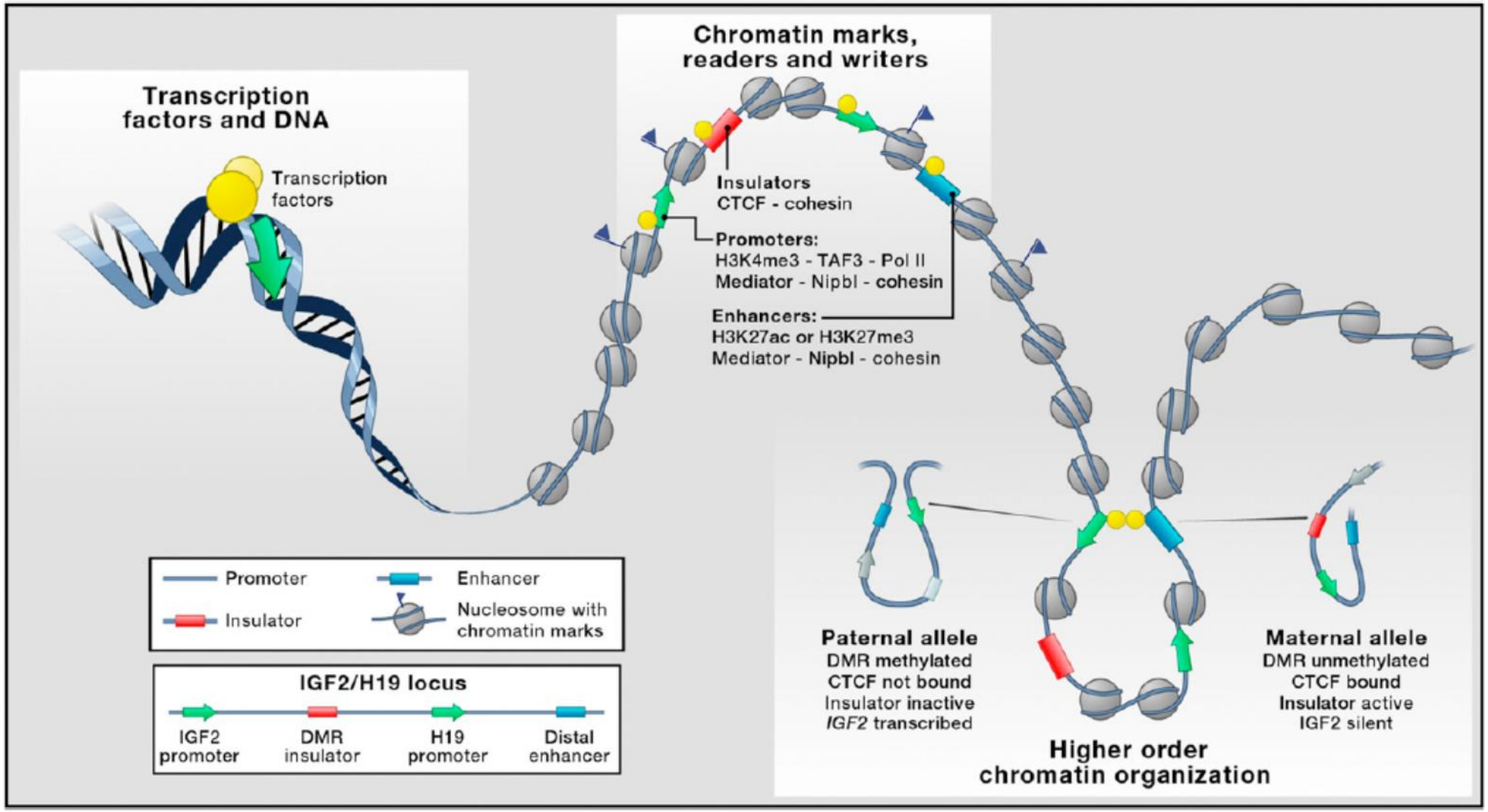
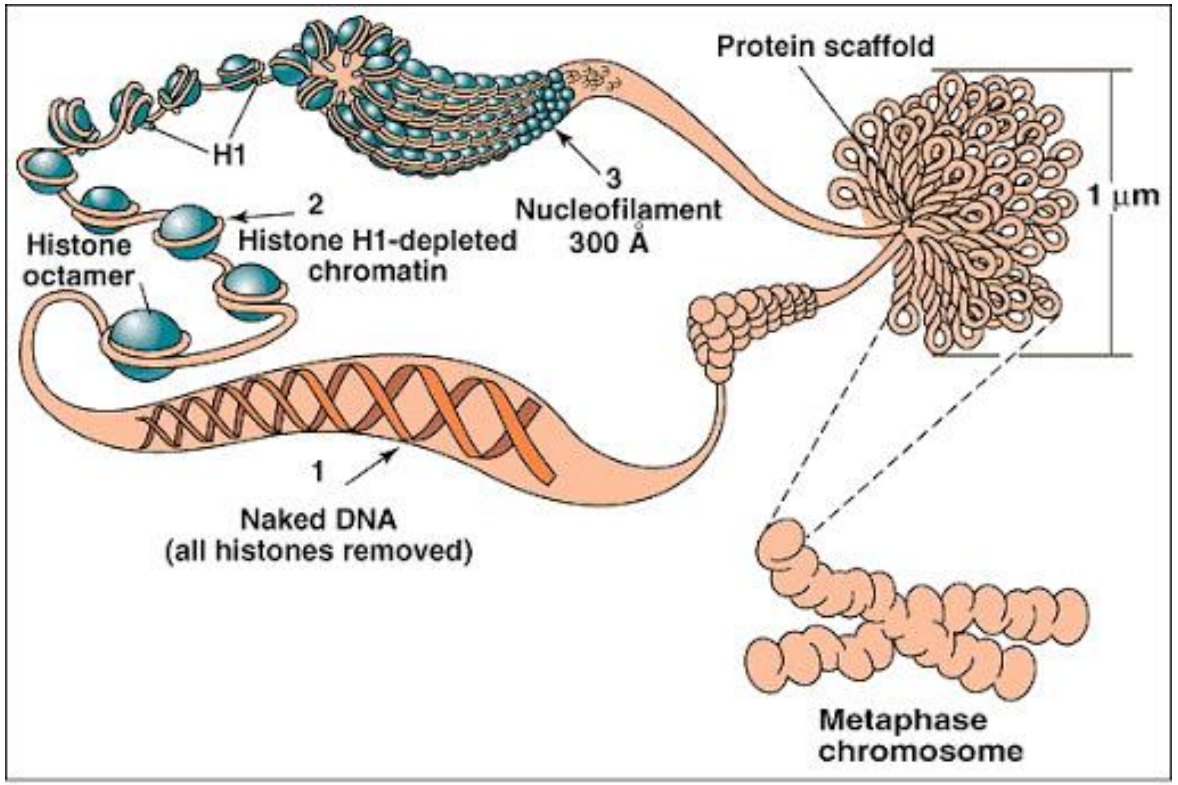
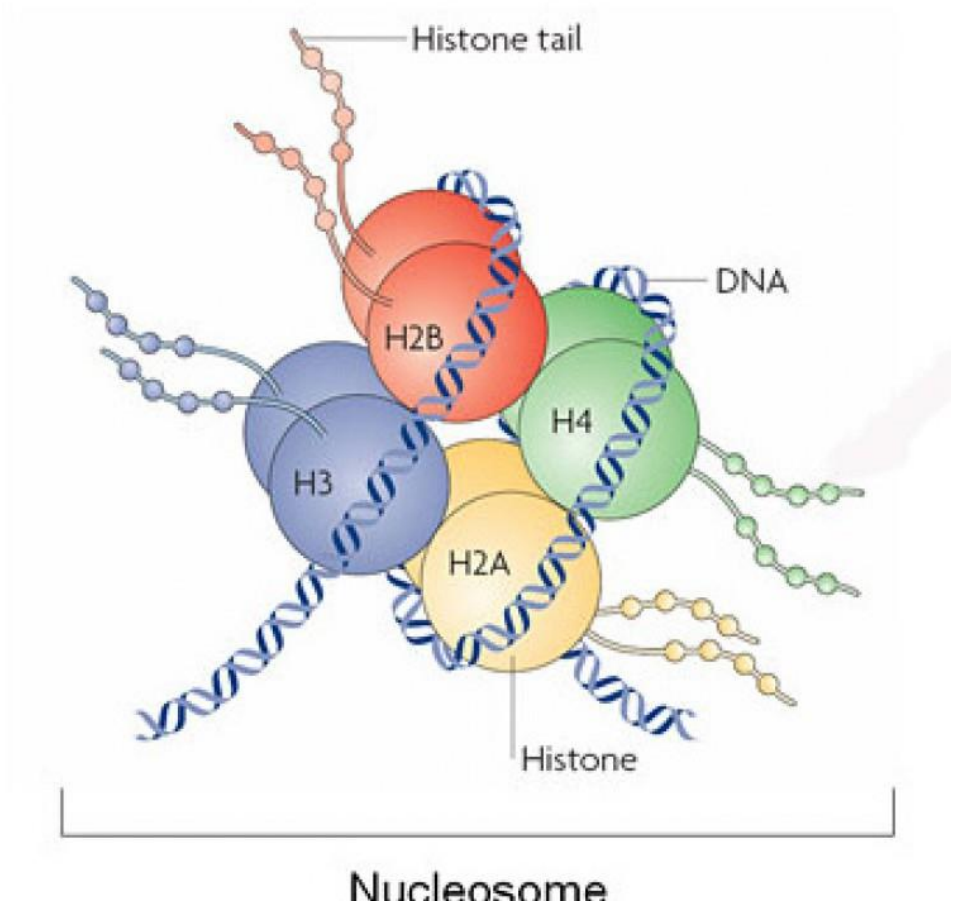


Figure 1. Chromatin Modifications at Regulatory Elements—from Marks to Function

Models for gene regulation have moved from an early focus on transcription factors and DNA to encompass the full context of chromatin (left). Regulatory elements are marked by patterns of DNA methylation, histone marks, and interacting proteins that link chromatin modifications to the regulation of transcription (center). Regulatory elements are often separated by considerable distances in the linear sequence of metazoan genomes. Transcriptional control is thought to involve interactions between regulatory elements in three-dimensional nuclear space (right). To illustrate this, the figure depicts regulatory elements of the imprinted *IGF2/H19* locus and their interactions as detailed in the section "CTCF and Cohesin Regulate Complex Loci."



El nivel estructural más fino de la cromatina interfásica es el nucleosoma, constituido en su región central, por un octámero de histonas $(H2A)_2(H2B)_2(H3)_2(H4)_2$, que sirve como soporte de alrededor de 140 pares de nucleótidos.



Los nucleosomas se separan entre sí por una región interconectora (linker) de 60 pares de nucleótidos, que se recubre por una molécula de histona H1.

La estabilidad de los nucleosomas depende de la conformación de las histonas, la cual puede ser modificada por la adición de varios grupos, particularmente acilos (metilos o acetilos).

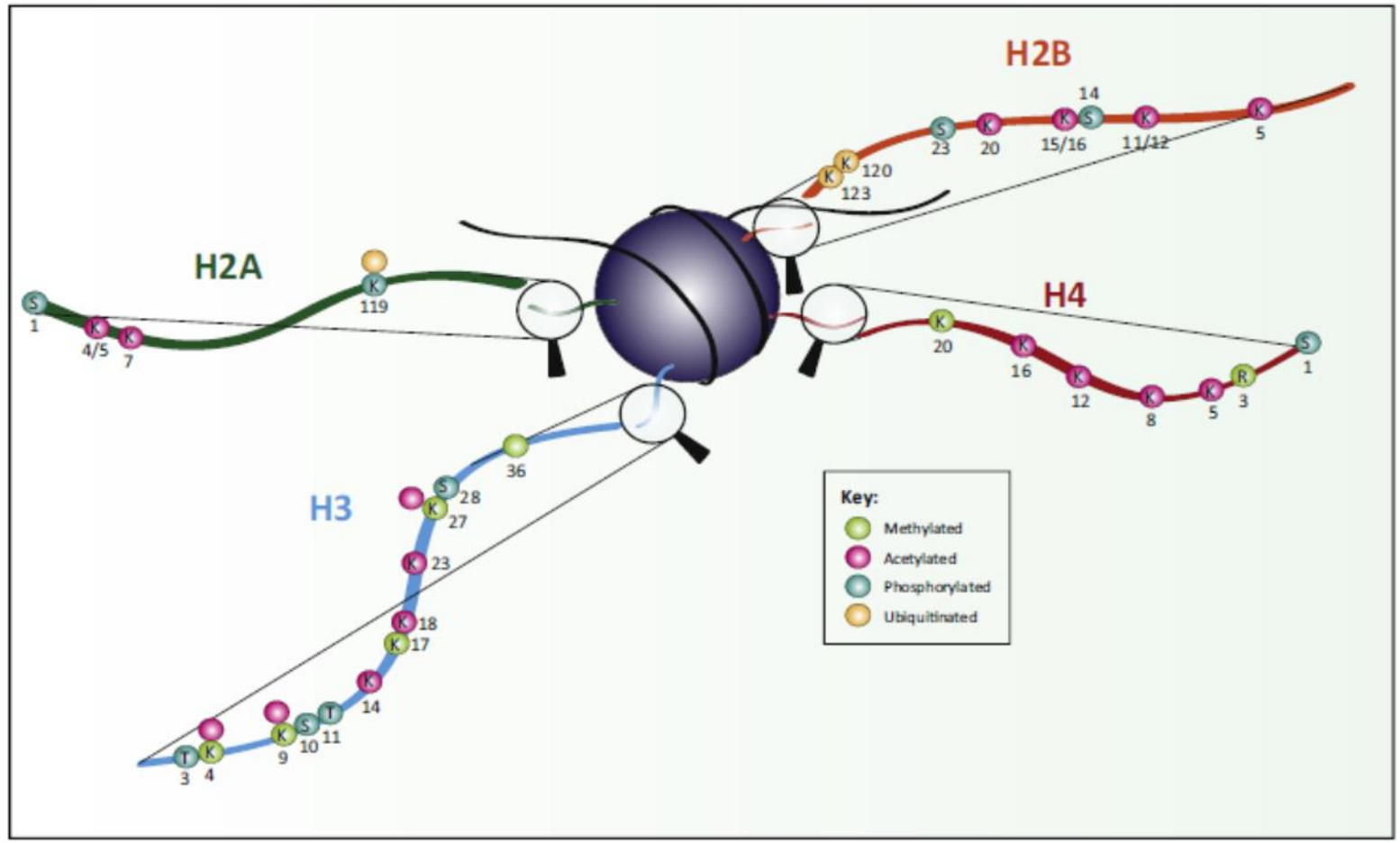
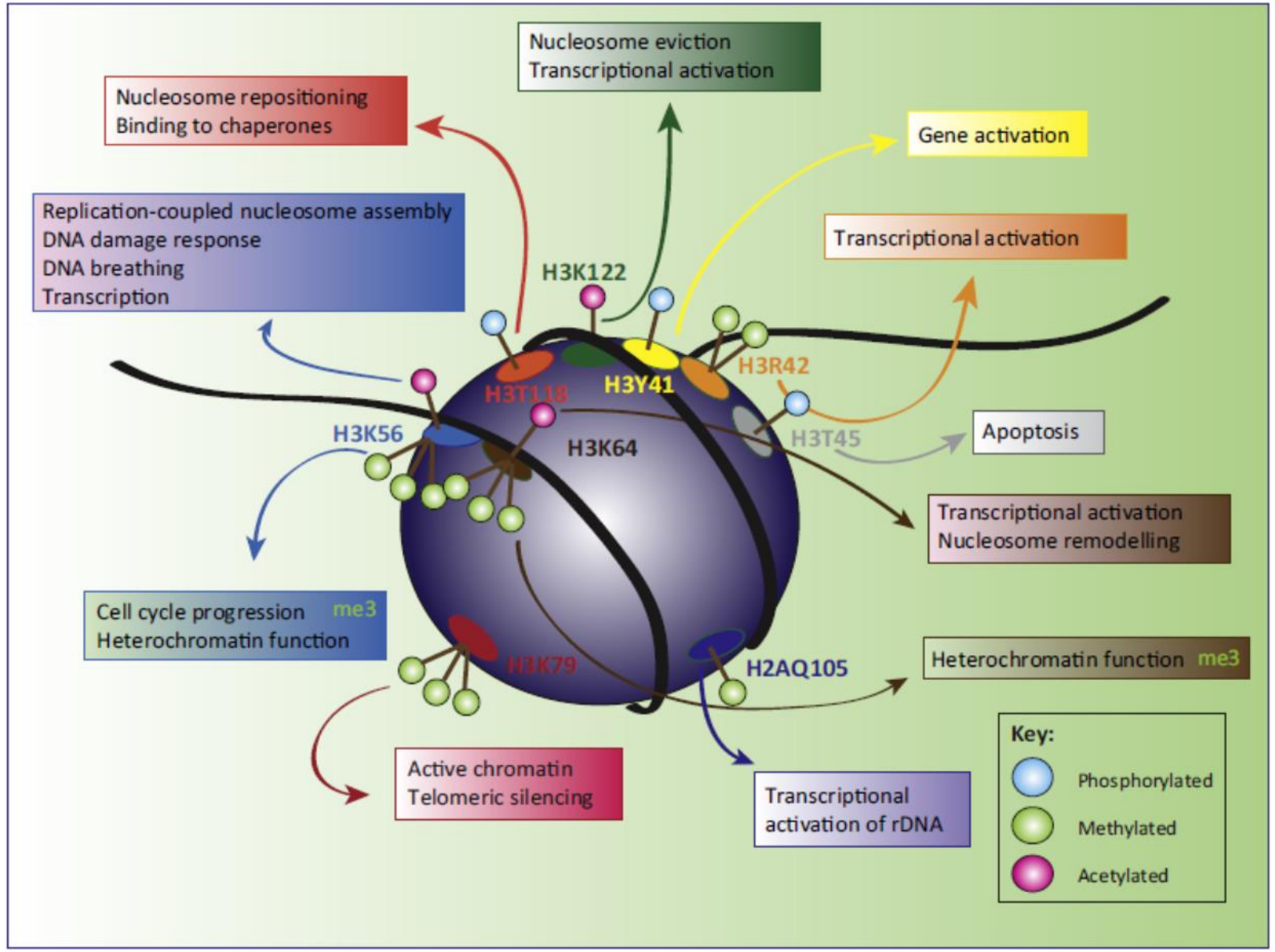


Figure 1. Schematic Showing Post-Translational Modifications of the Histone Tails. The location of each modification is shown in black and the amino acid modified at each position is also shown (K = lysine, R = arginine, S = serine, T = threonine). Colours depict how each residue is modified (green = methylated, pink = acetylated, turquoise = phosphorylated, beige = ubiquitinated).

Table 1. Histone-modifying enzymes.

Acetyltransferase	**Substrates**	**Deacetylases**	**Substrates**
HAT1	H4 (K5, K12)	SirT2–3	H4K16
GCN5, PCAF	H3 (K9, K14, K18)	**Lysine demethylases**	**Substrates**
CBP, P300	H3 (K14, K18), H4 (K5, K8), H2AK5, H2B (K12, K15)	LSD1/BHC110	H3 (K4, K9)
TIP60/PLIP	H4 (K5, K8, K12, K16), H3K14	JHDM1a-b	H3K36
HBO1	H4 (K5, K8, K12)	JHDM2a-b	H3K9
		JMJD2A/JHDM3A, JMJD2B-C	H3 (K9, K36)
Lysine methyltransferases	**Substrates**	JMJD2D	H3K9
SUV39H1–2	H3K9	JARID1A-D	H3K4
G9a	H3K9	UTX	H3K27
EuHMTase/GLP	H3K9	JMJD3	H3K27
ESET/SETDB1	H3K9	**Serine/threonine kinases**	**Substrates**
CLL8	H3K9	Haspin	H3T3
MLL1–5	H3K4	MSK1–2	H3S28
SET1A-B	H3K4	CKII	H4S1
ASH1	H3K4	Mst1	H2BS14
SET2	H3K36	Rsk2	H3S10
NSD1	H3K36	**Ubquitilases**	**Substrates**
SYMD2	H3K36	Bmi/Ring1a	H2AK119
DOT1	H3K79	RNF20/RNF40	H2BK120
Pr-SET7/8	H4K20	**Arginine methyltransferases**	**Substrates**
SUV4 20H1–2	H4K20	CARM1	H3 (R2, R17, R26)
EZH2	H3K27	PRMT4	H4R3
SET7/9	H3K4	PRMT5	H3R8, H4R3
RIZ1	H3K9		

* Only human enzymes are shown with their substrate histones and the sites of modification. A series of isoforms with the same substrate specificity are indicated by the name of the enzymes followed by hyphenated numbers or letters.



Trends in Genetics

Figure 3. Schematic of a Nucleosome with Its Coating DNA, Detailing the Locations and Functions of Key Modifications within the Globular Domains of the Histones. Methyl marks are shown in light green, acetyl marks in pink, and phosphorylated residues in light blue.

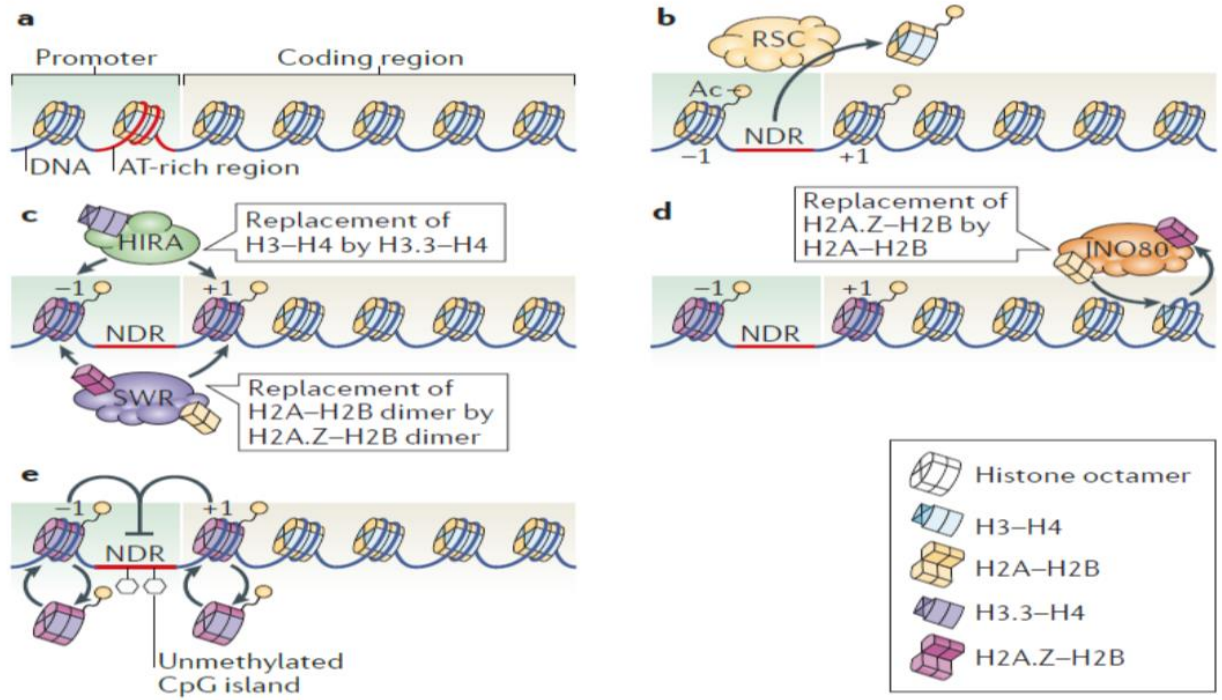
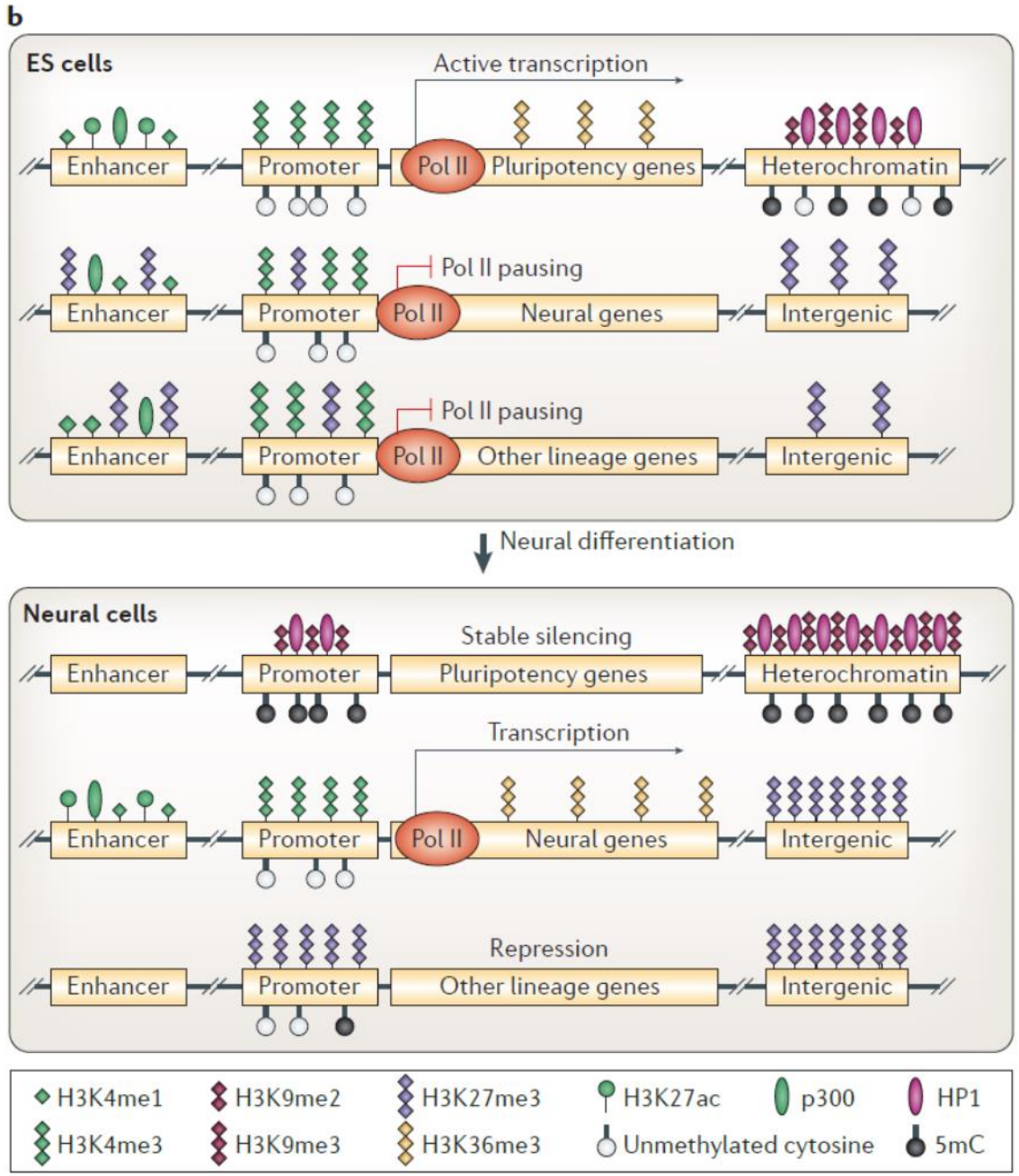
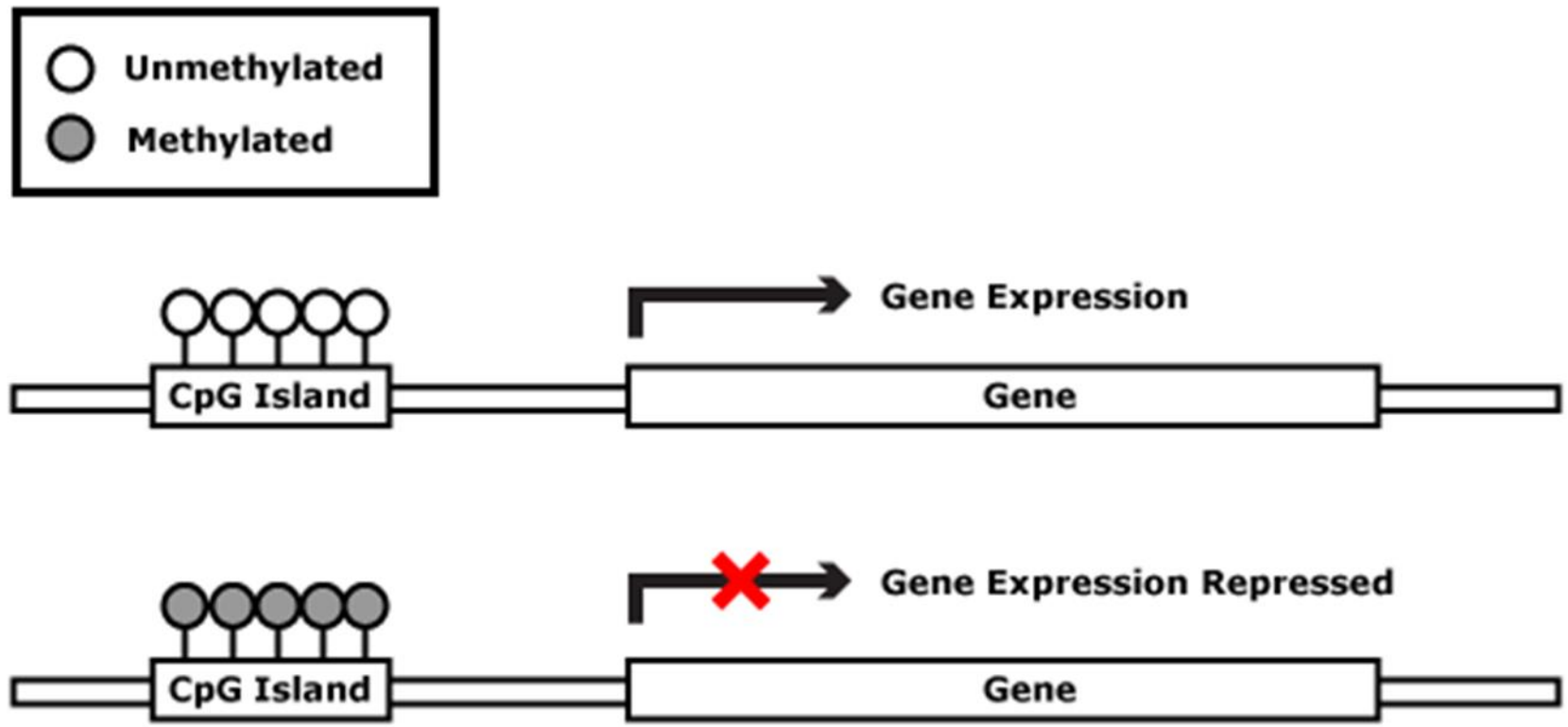


Figure 2 | **Role of histone exchange in transcription initiation.** **a** | Nucleosome distribution over a gene before transcription initiation is shown. Promoter DNA is usually AT-rich (red line), which does not favour the bending of DNA around the nucleosome. **b** | In addition, action of the chromatin remodeller RSC removes these nucleosomes and creates a nucleosome-depleted region (NDR) over the promoter. RSC also ensures the maintenance of the NDR in a histone acetylation (Ac)-dependent manner. **c** | The creation of the NDR aids SWR activity in replacing the canonical H2A–H2B dimer with the variant dimer H2A.Z–H2B over the positioned −1 and +1 nucleosomes. Acetylation of histones over the promoter ensures that SWR activity is targeted over this region. In addition, the replacement of H3–H4 by the H3.3–H4 variant, which is mediated by the histone chaperone HIRA, in these nucleosomes creates the unstable bivariant combination that leads to transcription elongation. **d** | The retention of H2A.Z-marked nucleosomes over the promoter is ensured by H2A.Z acetylation, which prevents its removal by the chromatin remodeller inositol-requiring protein 80 (INO80). Conversely, the misincorporation of the variant H2A.Z over the unacetylated nucleosomes in the coding regions is prevented by the action of INO80. **e** | The unstable bivariant nucleosomes lead to further histone exchange of the −1 and +1 nucleosomes, allowing for multiple rounds of transcription. Moreover, H2A.Z variant incorporation into nucleosomes prevents DNA methylation at CpG islands in plants, which ensures active transcription.


b
ES cells
Active transcription
Enhancer
Promoter
Pol II
Pluripotency genes
Heterochromatin
Pol II pausing
Enhancer
Promoter
Pol II
Neural genes
Intergenic
Pol II pausing
Enhancer
Promoter
Pol II
Other lineage genes
Intergenic
Neural differentiation
Neural cells
Stable silencing
Enhancer
Promoter
Pluripotency genes
Heterochromatin
Transcription
Enhancer
Promoter
Pol II
Neural genes
Intergenic
Repression
Enhancer
Promoter
Other lineage genes
Intergenic
H3K4me1
H3K9me2
H3K27me3
H3K27ac
p300
HP1
H3K4me3
H3K9me3
H3K36me3
Unmethylated cytosine
5mC


Unmethylated
Methylated
Gene Expression
CpG Island
Gene
Gene Expression Repressed
CpG Island
Gene

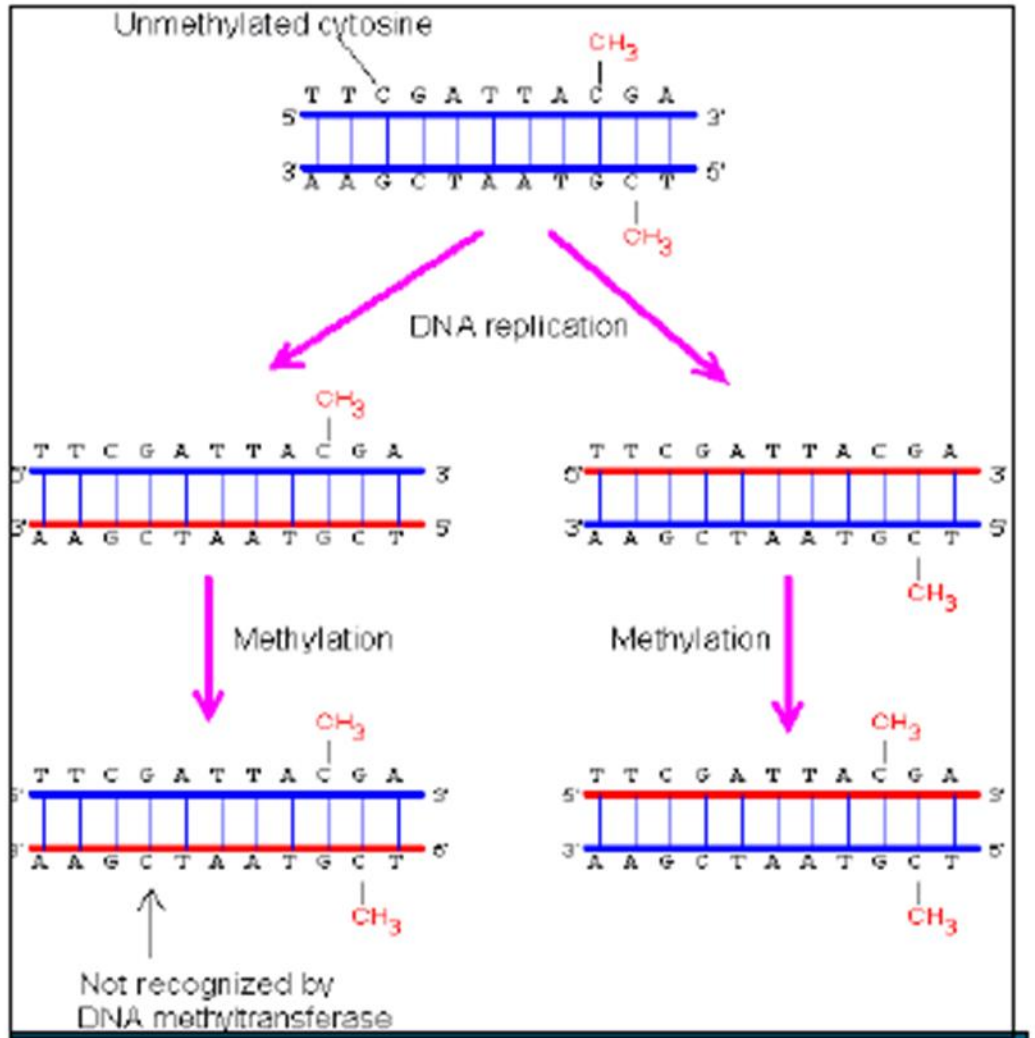
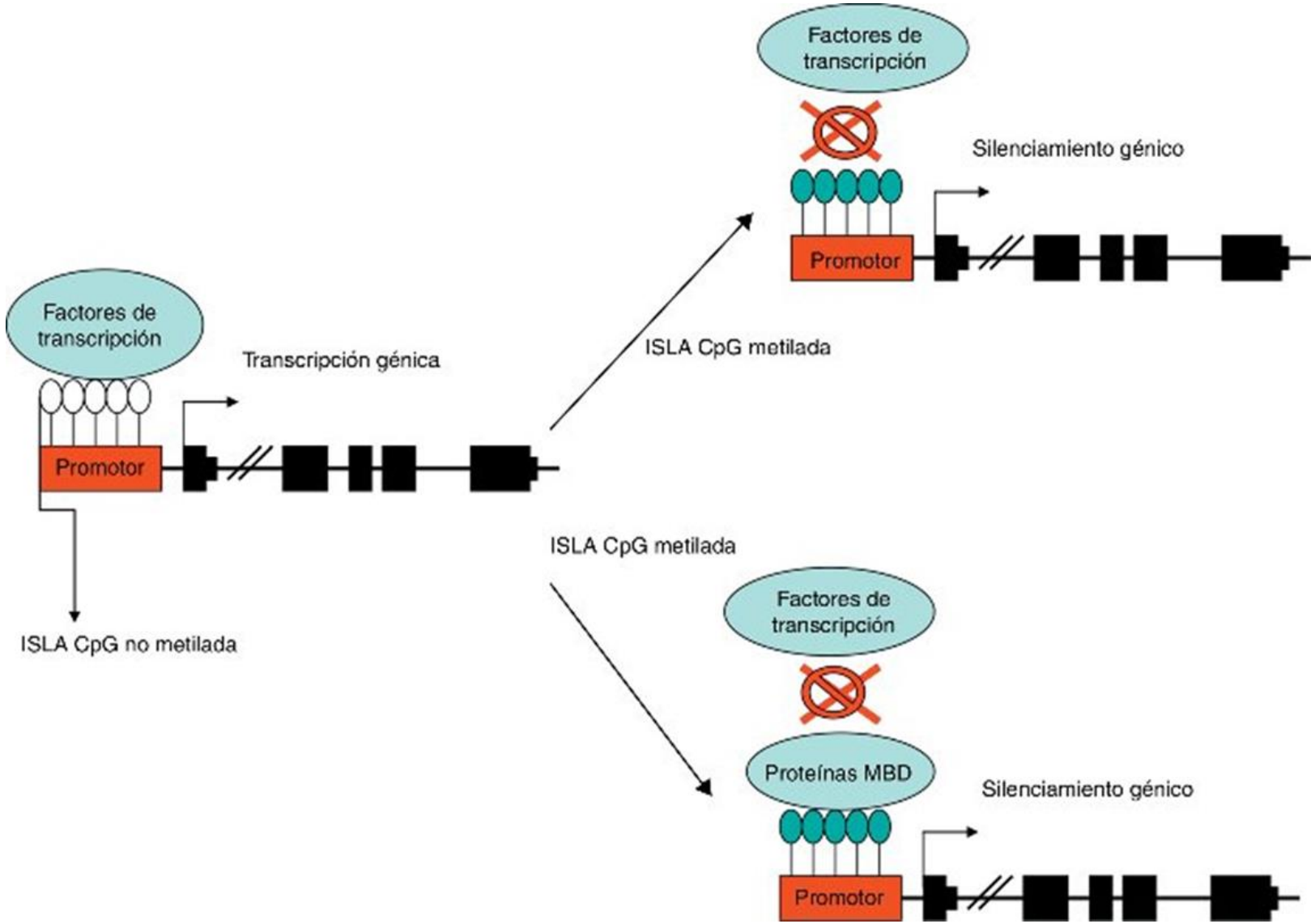


Figura 2. Metilación de la citosina en los segmentosCpG. Obsérvese que después de la replicación del DNA, una metilación de novo puede ser heredada. Extraído de http://www.web-books.com/MoBio/Free/Ch7F2.htm>>>>CpG

Tab. 1. Proteins participating in chromatin remodelling. MBD: m^5CpG-binding domains, MeCP1: m^5CpG-binding proteins, HDAC: histone deacetylase, HAT: histone acetyltransferase.

Proteins modifying chromatin structure		Function
DNA metyl-transferases	DNMT1	maintains DNA-methylation pattern during replication [10, 19, 35]
	DNMT2	has very weak methylating activity [19, 35]
	DNMT3A	involved in *de novo* acquisition of DNA-methylation pattern [10, 19, 35]
	DNMT3B	
	DNMT3L	involved in maternal genomic imprinting affects activity of DNMT3A and 3B [35]
m^5CpG-binding proteins	MBD1	m^5CpG-binding domains that act as transcription repressors [10, 19]
	MBD2	
	MBD3	component of the chromatin remodelling protein complex Mi-2/NuRD [10, 19]
	MBD4	DNA glycosylase involved in DNA mismatch repair [19]
	MeCP1	MBD2-NuRD complex binding to methylated promoters that represses transcription [10]
	MeCP2	forms a complex with HDAC, co-repressor protein (Sin3a) and functions as a transcription
	Kaiso complex	uncharacterized protein lacking MBD domain but that binds to methylated DNA [10, 19]
Histone-modification enzymes	HDAC1, HDAC2	deacetylate histones [19]
	H3K4 MTases	methylate H3 histone K4 [19]
	Suv39h1, Suv39h2	methylate H3 histone K9 and K27 [19]
	G9a, Eu-HMTase1, ESET/SETDB1	methylate H3 histone K9 [19, 63-65]
	P300/CBP, PCAF, AF250, Gcn5	acetylate histones [19]
	p160 family	nuclear receptor that has histone acetyltransferase activity [19]
ATP-dependent remodelling proteins	Mi-2/NuRD	protein complexes that have ATPase activity introducing conformational changes in nucleosomal DNA [19]
	SWI/SNF/Brm	
	ISWI	


Factores de transcripción
Silenciamiento génico
Promotor
ISLA CpG metilada
Factores de transcripción
Transcripción génica
Promotor
ISLA CpG no metilada
ISLA CpG metilada
Factores de transcripción
Proteínas MBD
Silenciamiento génico
Promotor

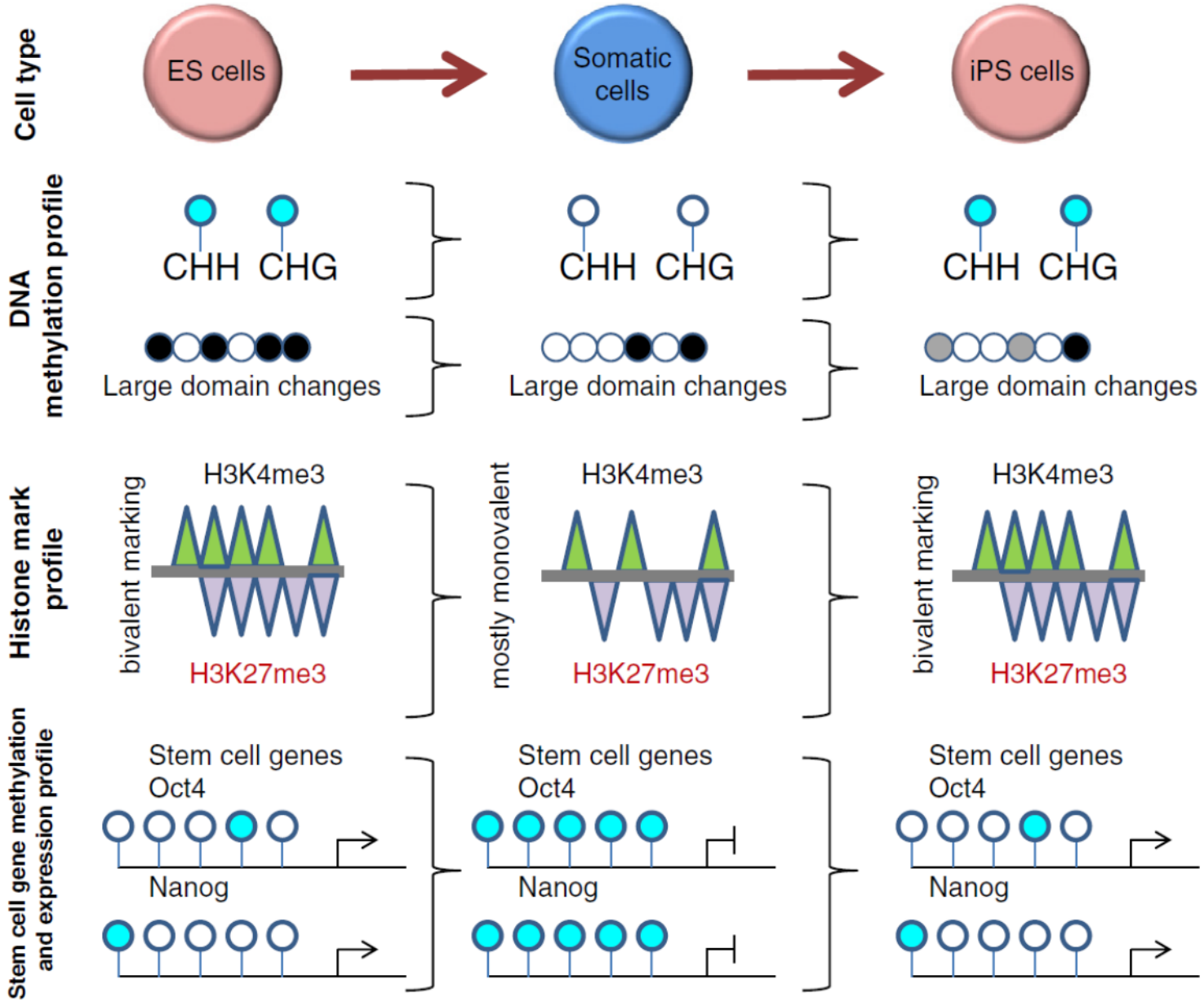


Fig. 1. Global cellular epigenetic profiles present during ES cells as it differentiates to somatic cells and then further to iPS cells. DNA methylation occurs in three different sequence context: CG, CHG or CHH methylation (H stands for C, A, or T) [3]. ES cells, and iPS cells show a higher frequency of non-CG methylation than somatic cells [8,9]. During transition from ES cell to somatic cells large regions alter DNA methylation, however, iPS cells often show an aberrant pattern [8]. Most bivalent marks (H3K4me3 and H3K27me3) in ES cells become monovalent in somatic cells and return to bivalent again in iPS cells [9,72,73]. Stem cell genes also show changes in the DNA methylation profile changing from significantly hypomethylated in ES and iPS cells to become hypermethylated in somatic cells. While upregulated in ES and iPS cells, stem cell genes remain silenced in somatic cells.

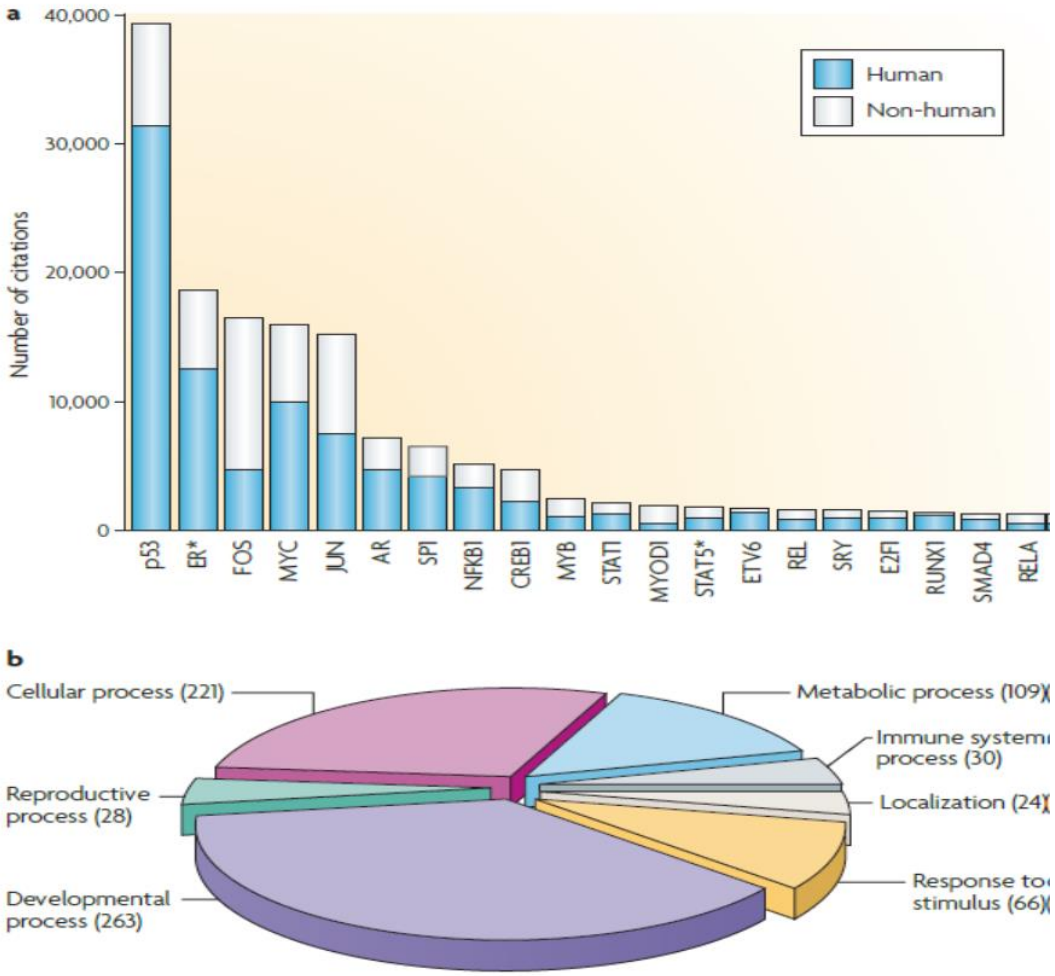


Figure 1 | **Current state of knowledge about transcription factors in the human genome. a** | For the top 20 most cited transcription factors (TFs) in PubMed the number of studies performed in humans (blue bars) and in all other organisms (grey bars) is shown. ER* combines the citations for ERS1 and ERS2, which were indistinguishable in the literature search; similarly, STAT5* includes citations for both STAT5A and STAT5B. **b** | Summary of biological processes regulated by TFs. Annotations were obtained from the Gene Ontology database, excluding those based only in electronic annotation. Numbers of annotated TFs are given in parentheses; each gene can be annotated with more than one function.

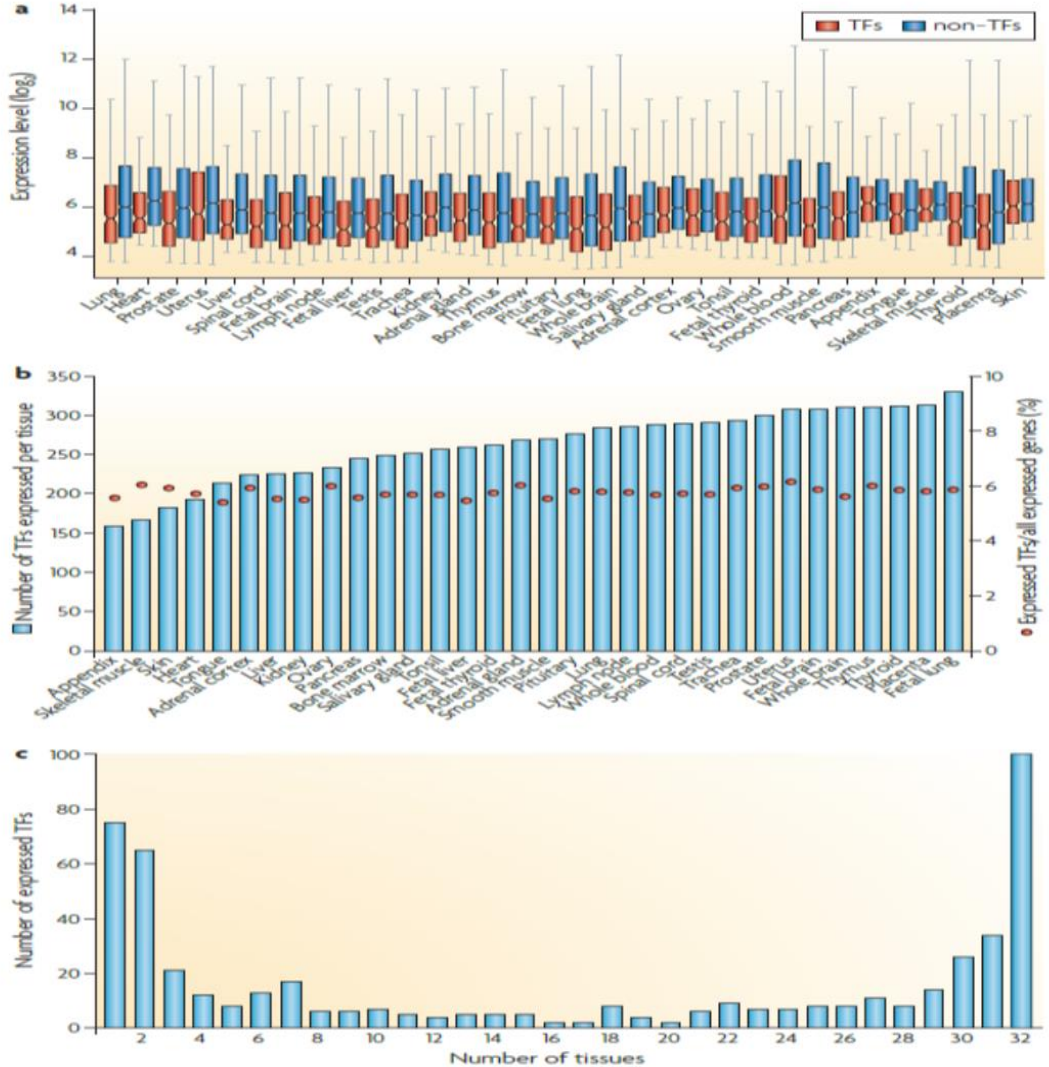


Figure 3 | **Expression level of transcription factors in 32 human organs and tissues. a** | Distribution of gene expression levels for transcription factors (TFs) (red) and non-TFs (blue) in different tissue types, shown as a box plot. In all samples, non-TF genes have higher average expression than TFs. **b** | Numbers of TFs expressed in each sample (blue bars) and the proportion of expressed TFs versus all expressed genes, given as a percentage (red points). The numbers of expressed regulators vary widely, ranging from about 150 in the appendix to over 300 in the fetal lung. However, in all tissues, TFs constitute ~6% of expressed genes. **c** | Number of tissues in which transcription factors are expressed (see also Supplementary information S1 (PDF)). Regulators are either expressed generally (30–32 samples) or specifically (1–3 samples). The U-shaped distribution in which factors are expressed in most tissues or in few tissues is robust against outliers (data not shown).