Alternative Splicing

Genomics & Gene Regulations

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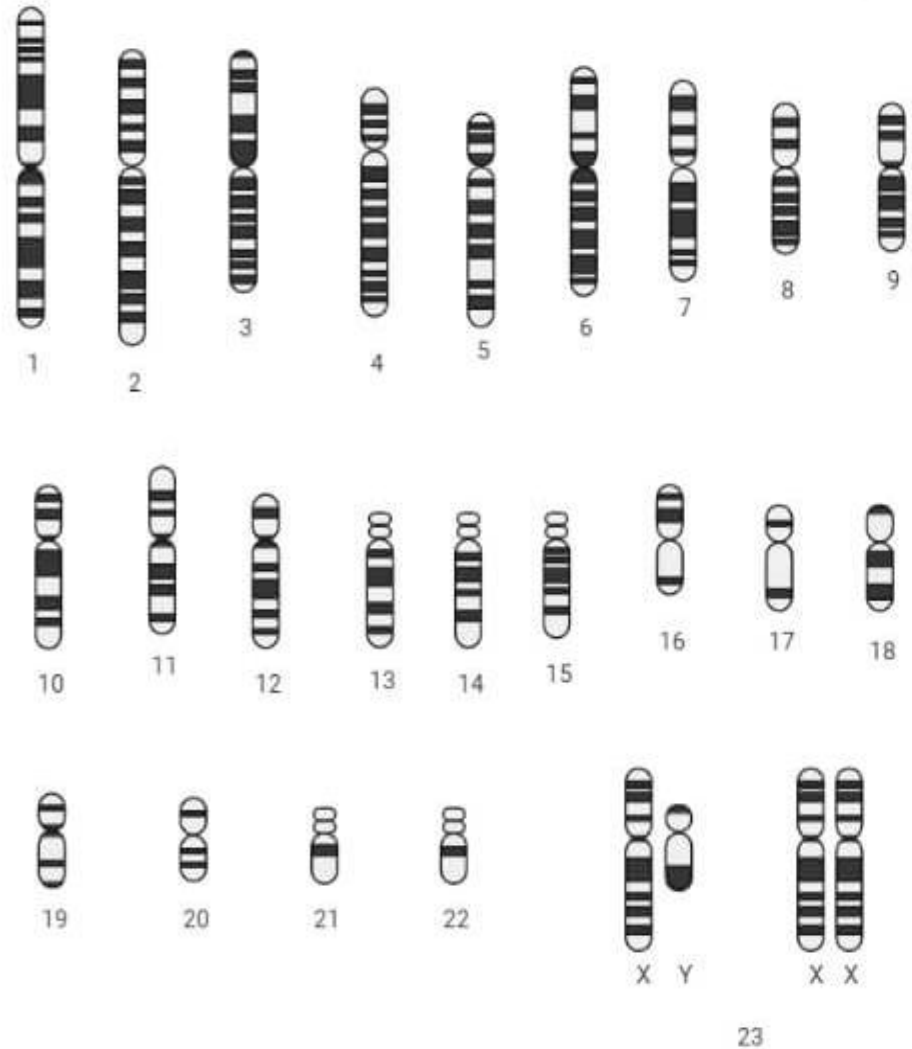
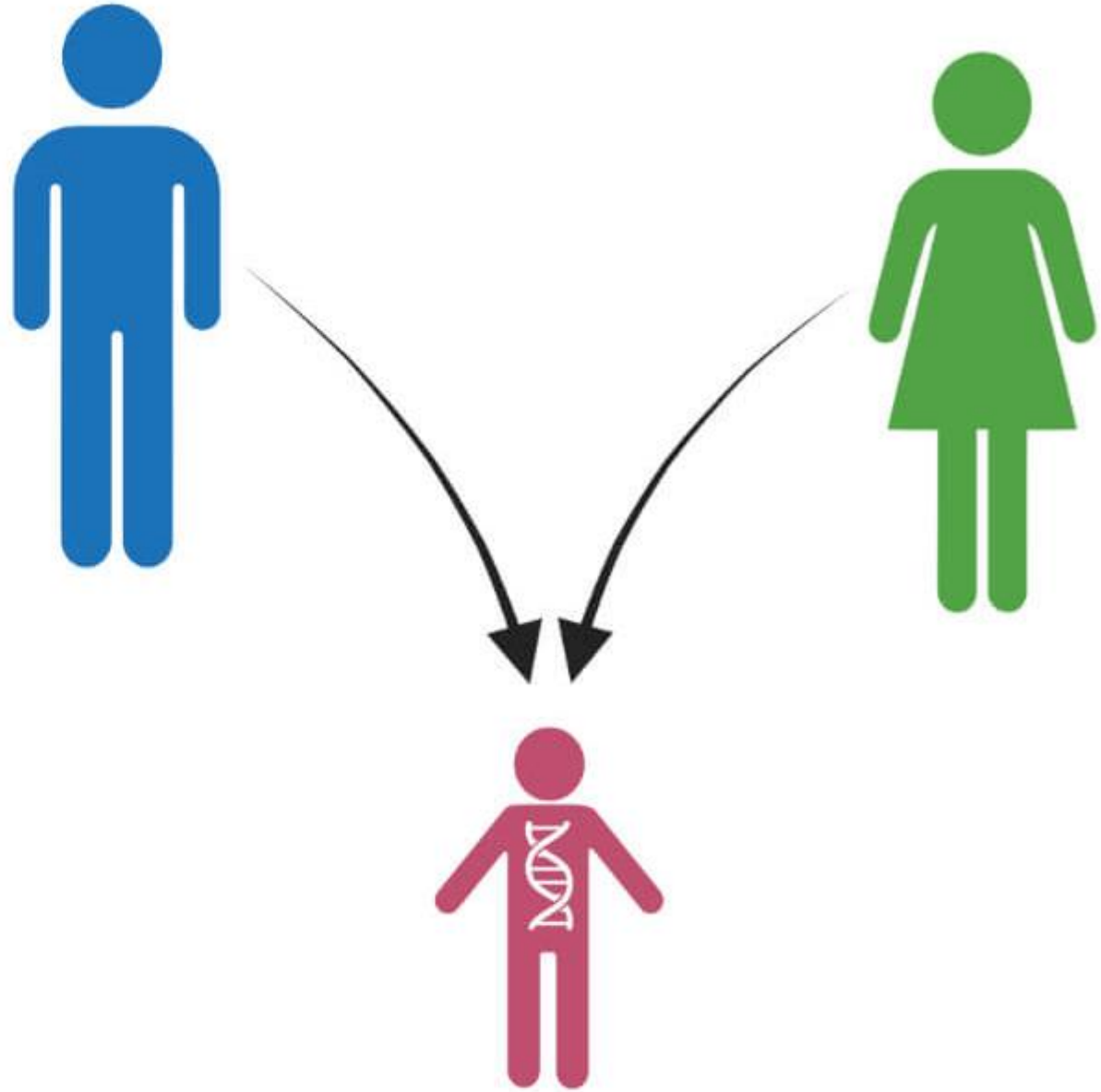
University of Tehran

Heredity

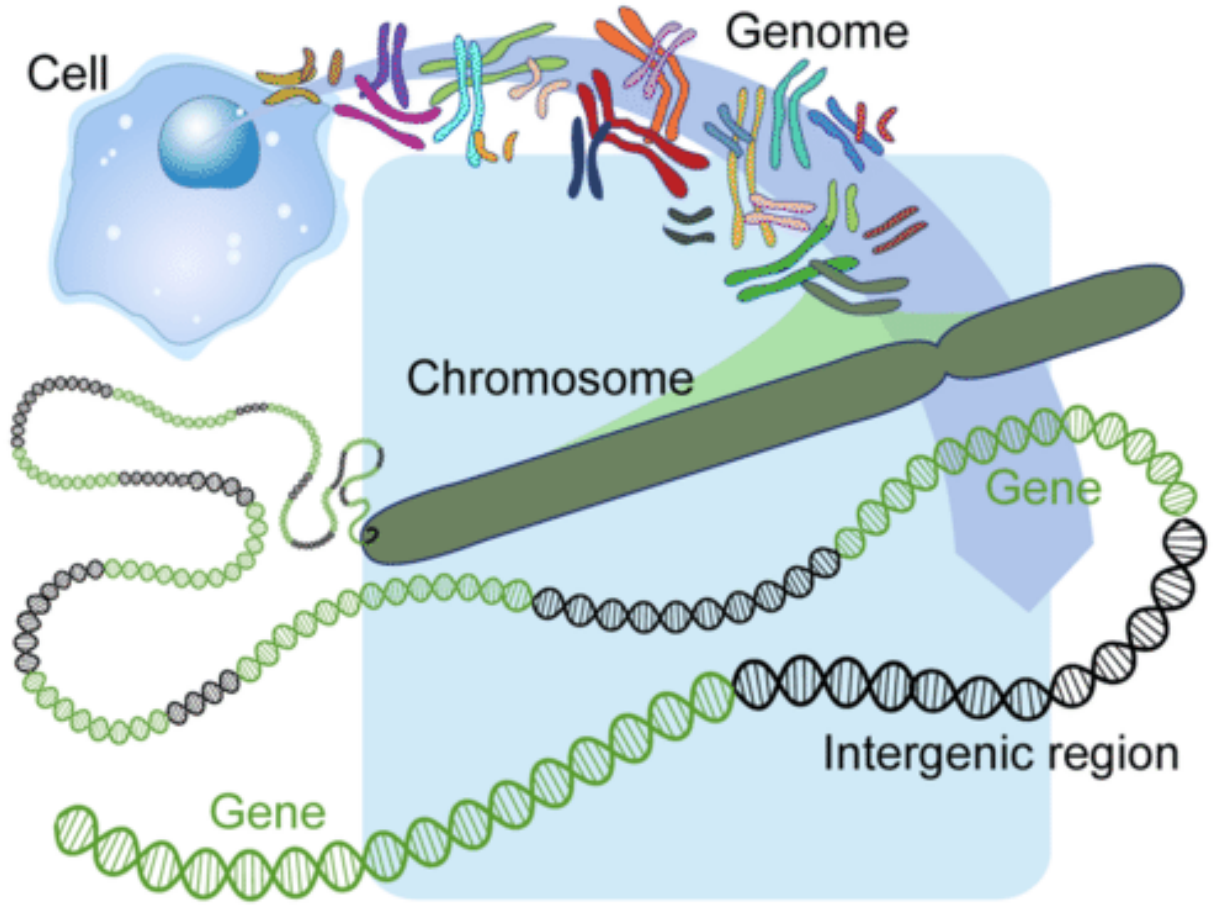




Genome

Genome is all the genetic information of an organism.

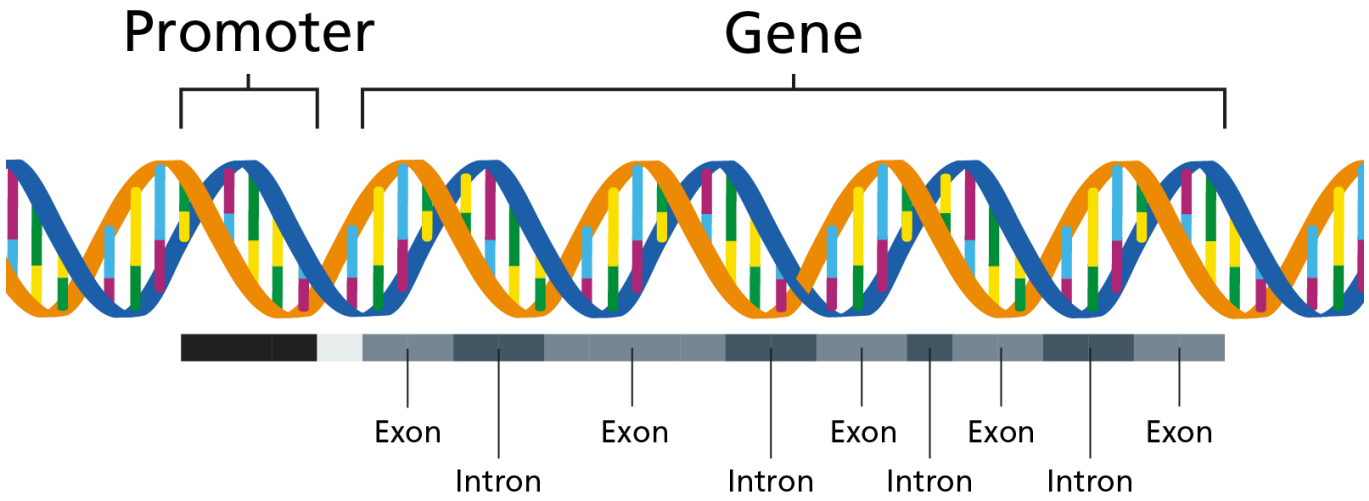
It consists of nucleotide sequences of DNA.



https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_Introductory_Biology_%28CK-12%29/03%3A_Genetics/3.08%3A_Human_Genome

Gene

The basic unit of heredity passed from parent to child.

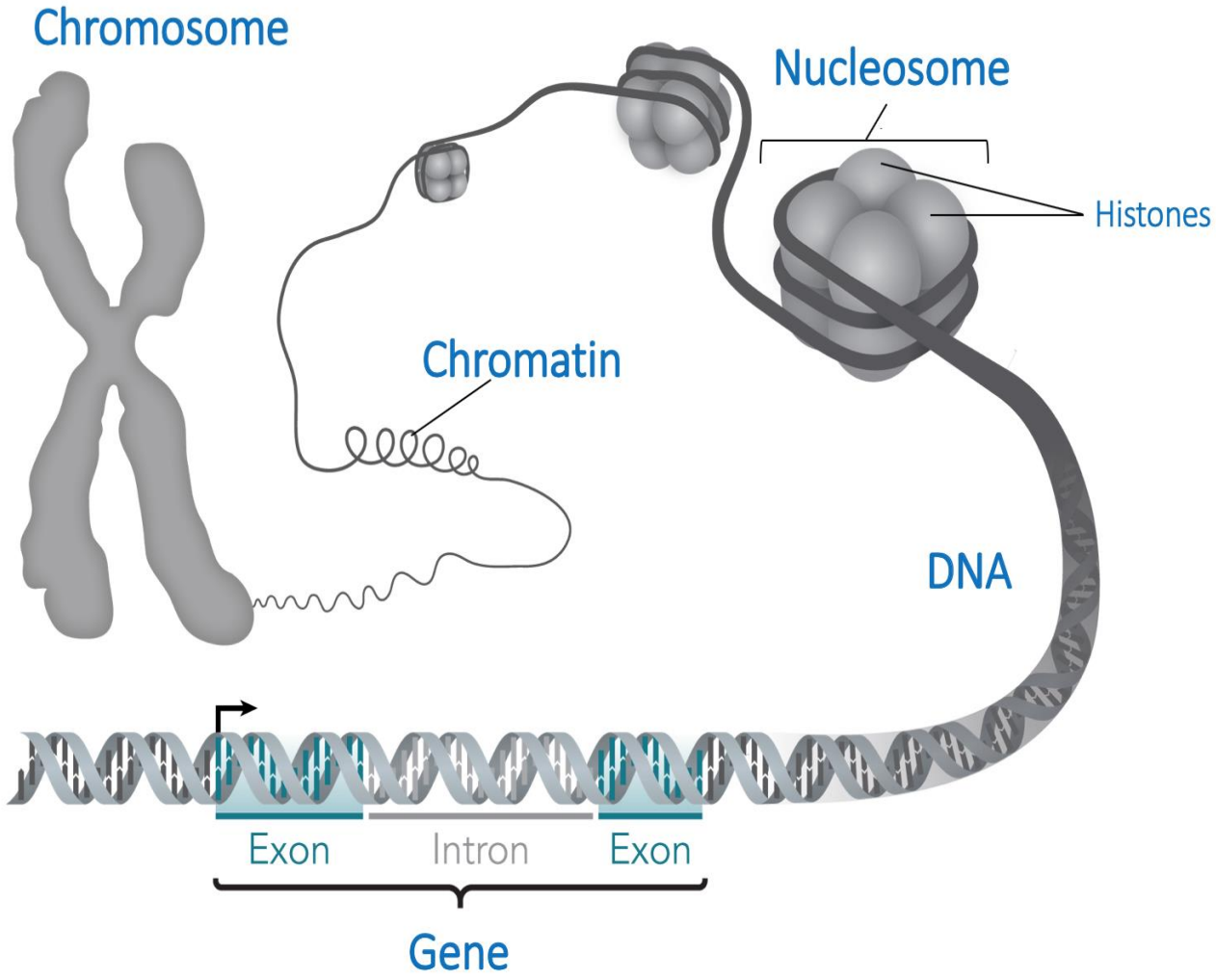


https://www.genomicseducation.hee.nhs.uk/genotes/knowledge-hub/gene/

Intron & Exon

An intron is any nucleotide sequence within a gene that is not expressed in the final RNA product but contains important regulatory information.

Exons are The non-intron sequences of the gene.



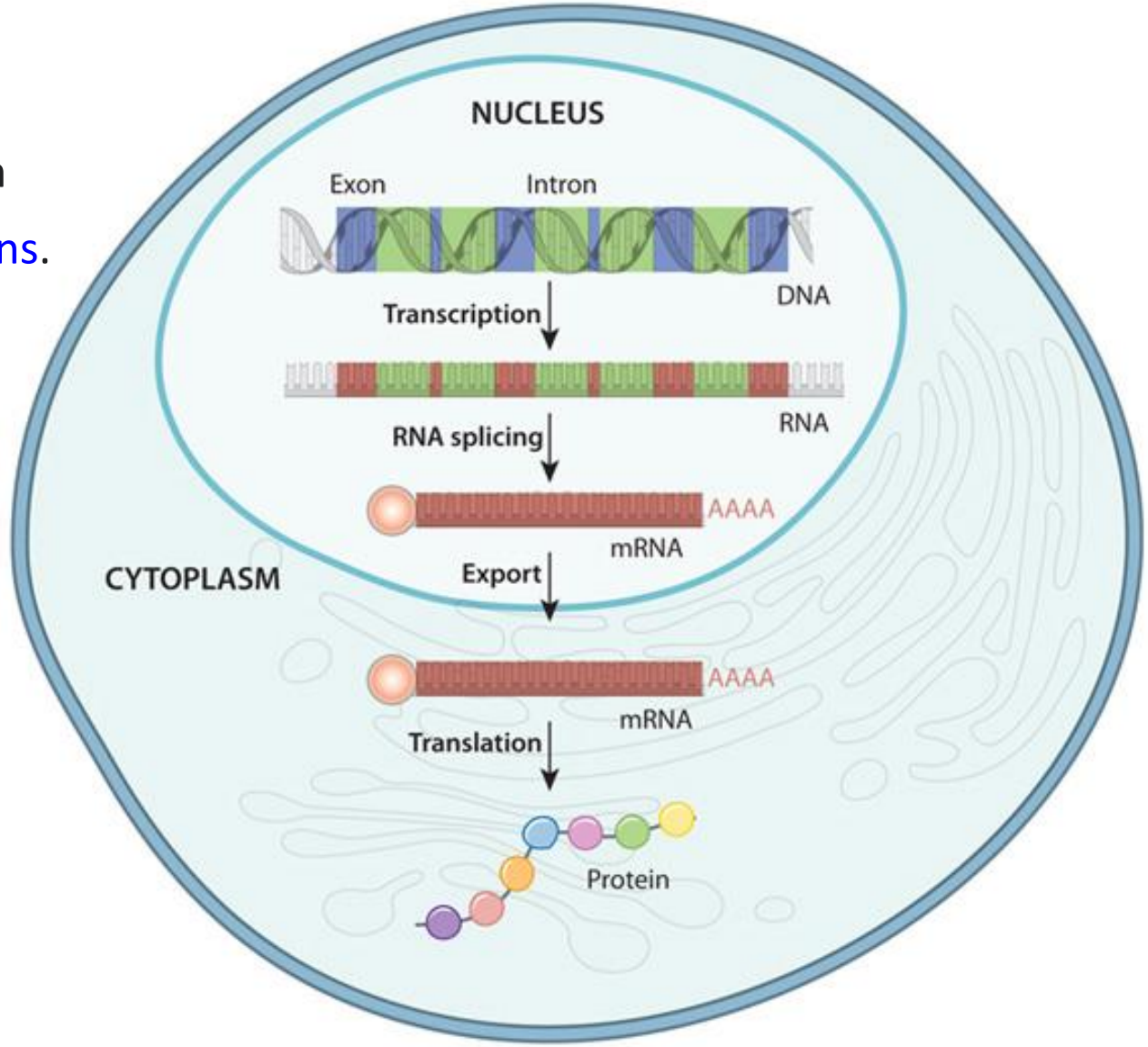
https://www.wizeprep.com/online-courses/19594/chapter/8/core/1/1

Central Dogma

Flow of genetic information within a biological system

Flow of information from DNA to RNA and into proteins.

The process of transcription, splicing,
and translation is called gene expression,
the central dogma of molecular biology

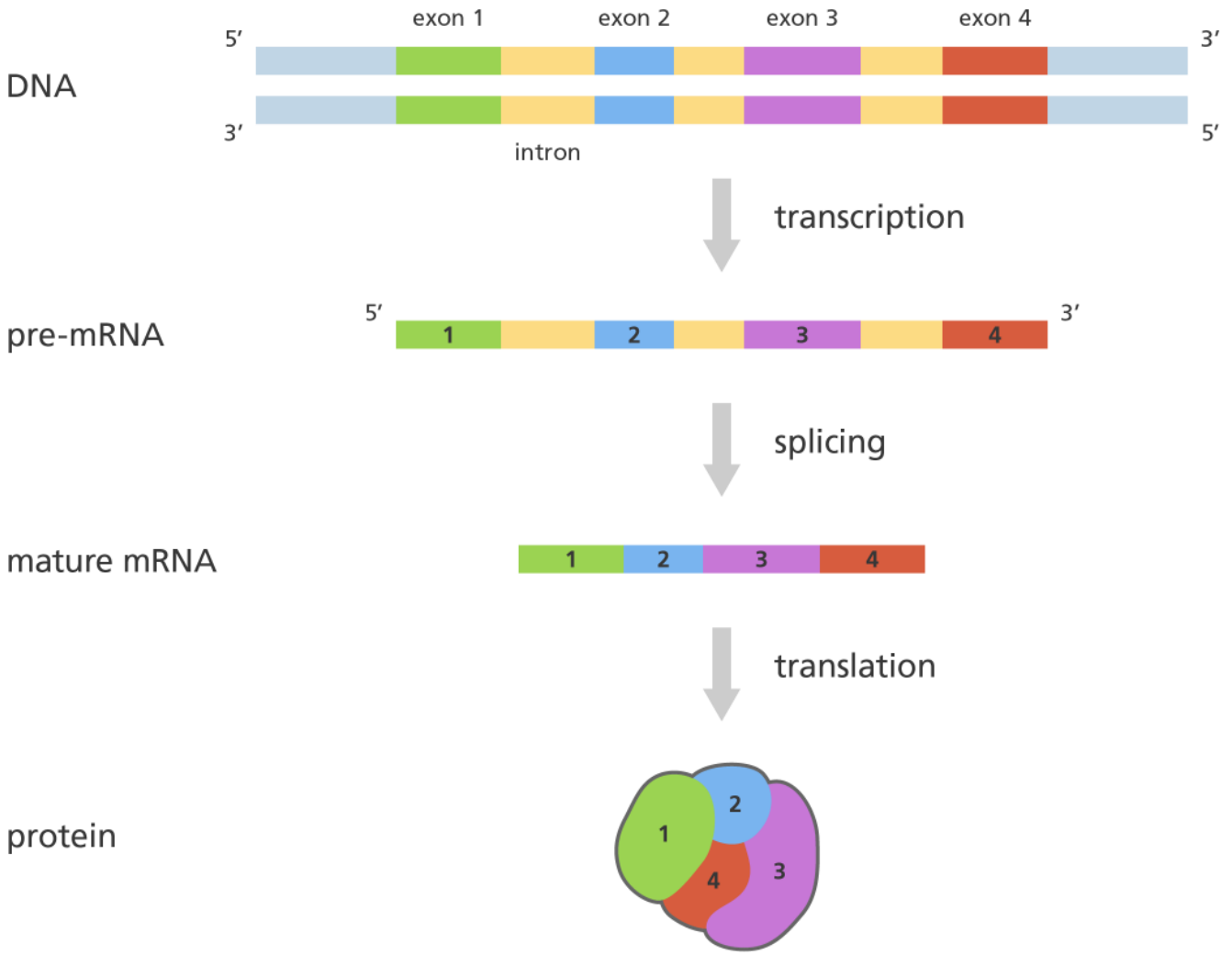


https://www.nature.com/scitable/topicpage/gene-expression-14121669/

RNA-Splicing

For those eukaryotic genes that contain introns, splicing is usually needed to create an mRNA molecule that can be translated into protein.

Removing all the introns (non-coding regions of RNA) and splicing back together exons (coding regions) a messenger RNA (pre-mRNA) transcript is transformed into a mature messenger RNA (mRNA).



https://www.yourgenome.org/facts/what-is-rna-splicing/

RNA-Splicing Background

In 1993, Richard J. Roberts and Phillip Allen Sharp received the Nobel Prize in Physiology or Medicine for their discovery of "split genes".

The split gene theory is a theory of the origin of introns, long non-coding sequences in eukaryotic genes between the exons.

Discovered splicing, the fact that pre-mRNA is processed into mRNA once introns were removed from the RNA segment.

They also discovered that the splicing of the messenger RNA can occur in different ways.

Photo from the Nobel Foundation archive.
Richard J. Roberts

Photo from the Nobel Foundation archive.
Phillip A. Sharp

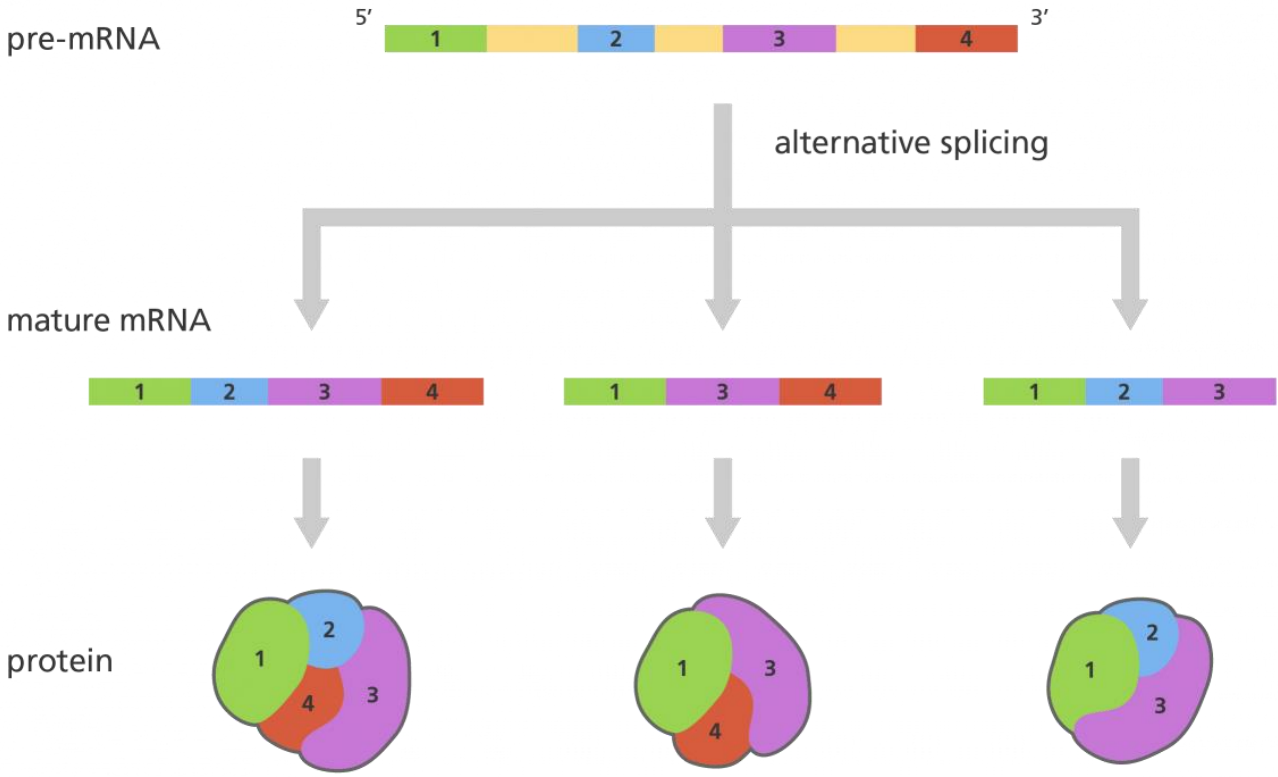
https://www.nobelprize.org/prizes/medicine/1993/summary/

Alternative splicing

Alternative splicing allows during gene expression a single gene to code for multiple proteins.

Exons are joined in different combinations, leading to different mRNA strands.

proteins translated from alternatively spliced mRNAs will contain differences **in** their amino acid sequence and, often, in their biological functions.



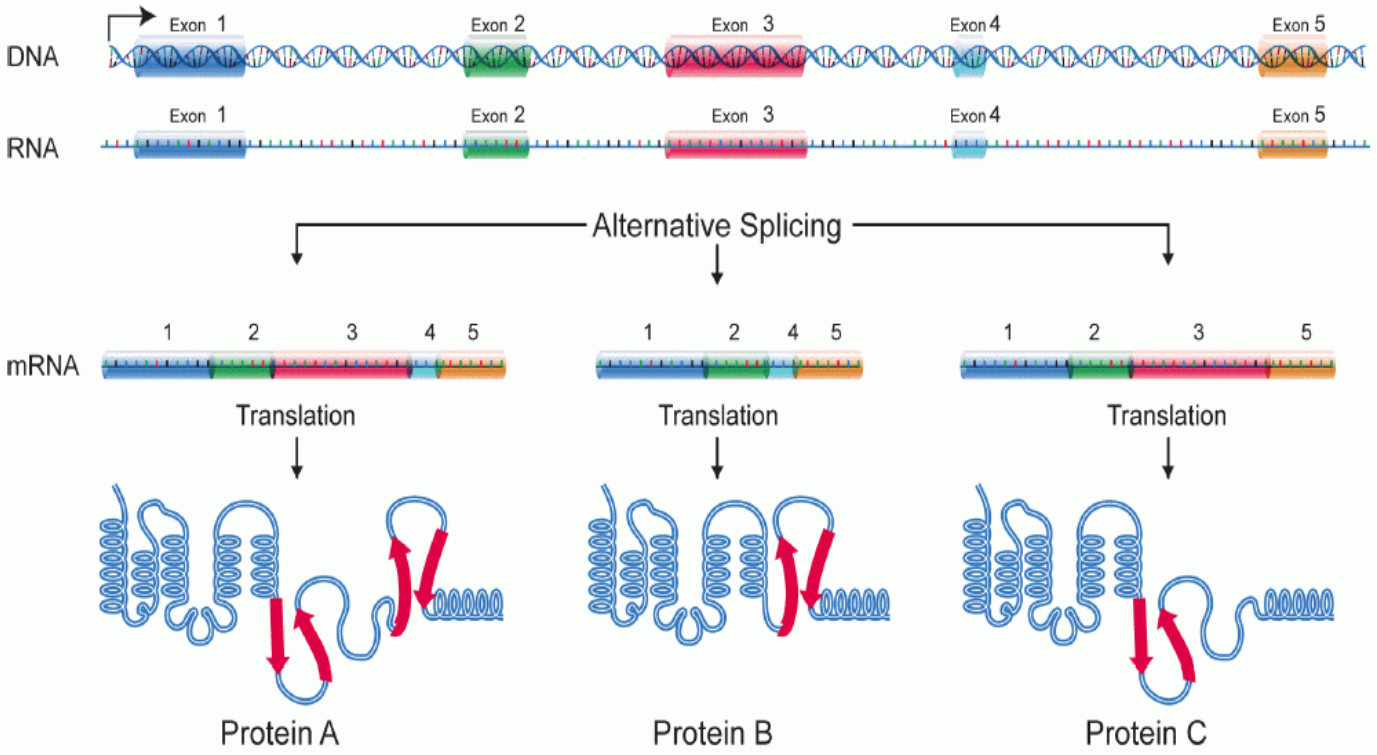
https://www.yourgenome.org/facts/what-is-rna-splicing/

Alternative splicing

Produces various mature mRNAs with different structures and functions.

Alternative splicing of pre-mRNA is a key mechanism for increasing the complexity of proteins in humans.

Causing a diversity of expression of transcriptomes and proteomes in a tissue-specific manner.



https://en.wikipedia.org/wiki/Alternative_splicing

Regulation of Alternative splicing

What makes splicing alternative?

Enhancers, silencers and regulatory proteins.

Splice sites can be strong **or** weak depending on how far their sequences diverge from the *consensus sequence.*

This effectively determines their affinities for cognate splicing factors**.**

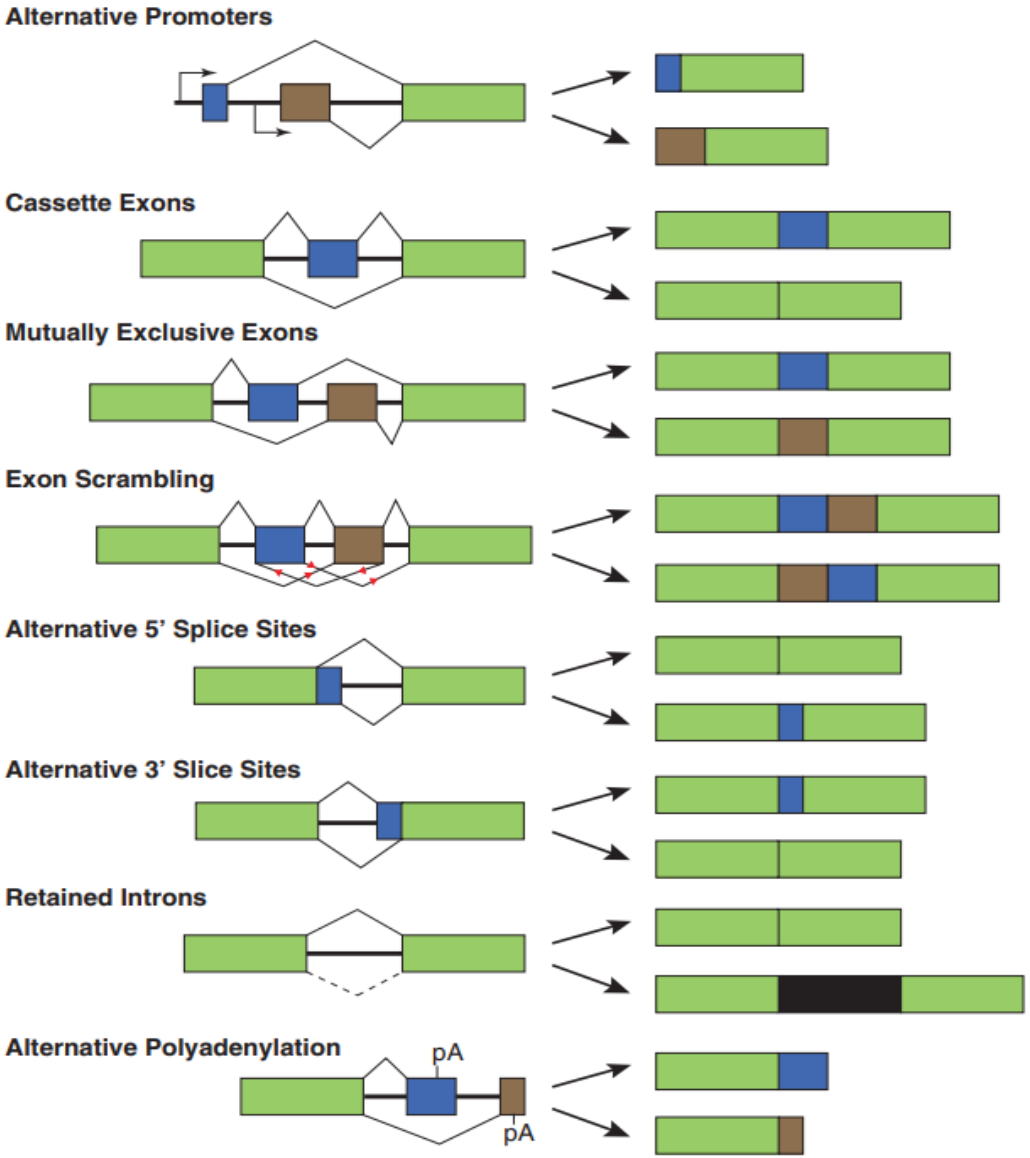
Strong splice sites lead to constitutive splicing and full usage of the site.

The relative positions of weak and strong sites give rise to the **different modes of alternative splicing including**

Types of alternative splicing

Common mechanisms of alternative splicing.

Retained introns occur with the absence of splicing, with the intervening intron (black) included in the final transcript [Chen J et al.,2015 , Scotti MM et al., 2016].



https://www.nature.com/articles/onc2013570

Regulation of alternative splicing

Enhancers, silencers and regulatory proteins

The degree to which weak sites are used is regulated by both cisregulatory sequences and transacting factors

Cisregulatory sequences

Include:

- exonic splicing enhancers (ESEs)
- exonic splicing silencers (ESSs)
- intronic splicing enhancers (ISEs)
- intronic splicing silencers (ISSs)

depending on their locations and on how they affect the usage of a splice site.

[Kornblihtt AR et al., 2013]

Trans-acting factors

function through binding to splicing enhancers and silencers and include members of well characterized Ser/Argrich and heterogeneous nuclear ribonucleoprotein (hnRNP) protein families as well as tissue specific factors such as nPTB and PTB15, NOVA16 and FOX17.

Some of these factors activate, whereas others inhibit the use of splice sites. Many of them can act in both ways depending on the sequence and position of the target site in the premRNA.

[Kornblihtt AR et al., 2013]

Regulation of alternative splicing

Trans-regulatory elements = DNA encoding transcription factors ⇒ modify or regulate the expression of distant genes.

Cis-regulatory elements (CRE) = non-protein-coding DNA that regulates transcription of neighboring genes**.**

Trans-acting factors interact with **cis-regulatory elements** to regulate gene expression.

Spliceosome

A spliceosome is a large ribonucleoprotein complex found primarily within the nucleus of eukaryotic cells.

The spliceosome is assembled from five small nuclear RNAs (snRNA) and numerous proteins.

The spliceosome removes introns from a transcribed pre-mRNA.

This process is generally referred to as splicing.

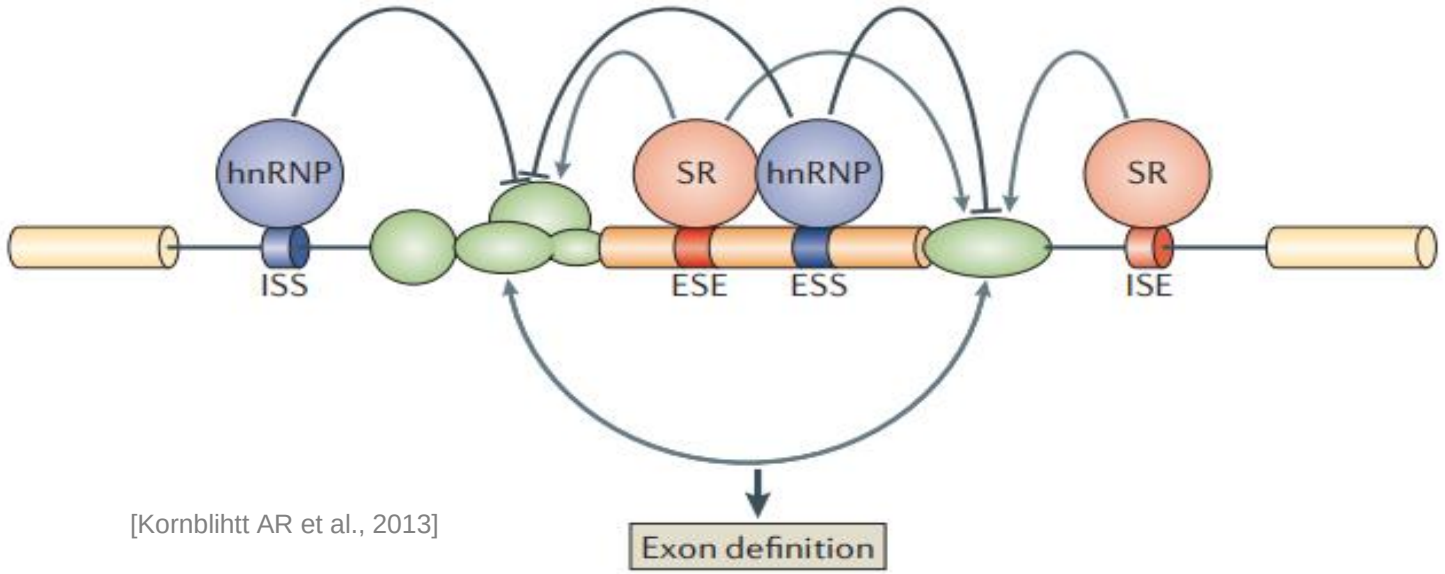
The spliceosome recruited by cis-acting elements and trans-acting factors plays a critical role in regulating alternative splicing procedure

Regulation of alternative splicing

Alternative splicing regulatory sequences and factors

Splicing is governed by *cis*-regulatory sequences in the pre-mRNA.
exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), intronic splicing enhancers (ISEs) and intronic splicing silencers (ISSs)) and two main families of alternative splicing regulatory proteins, Ser/Arg-rich proteins (SRs) and heterogeneous nuclear ribonucleoproteins (hnRNPs).

These regulatory proteins target components of the spliceosome (shown in green) that associate with both the 5‘ and the 3‘ splice sites flanking the alternative exon and can have either activating or inhibitory effects on the recognition and use of that site. [Kornblihtt AR et al., 2013] => REGULATION OF THIS SITES

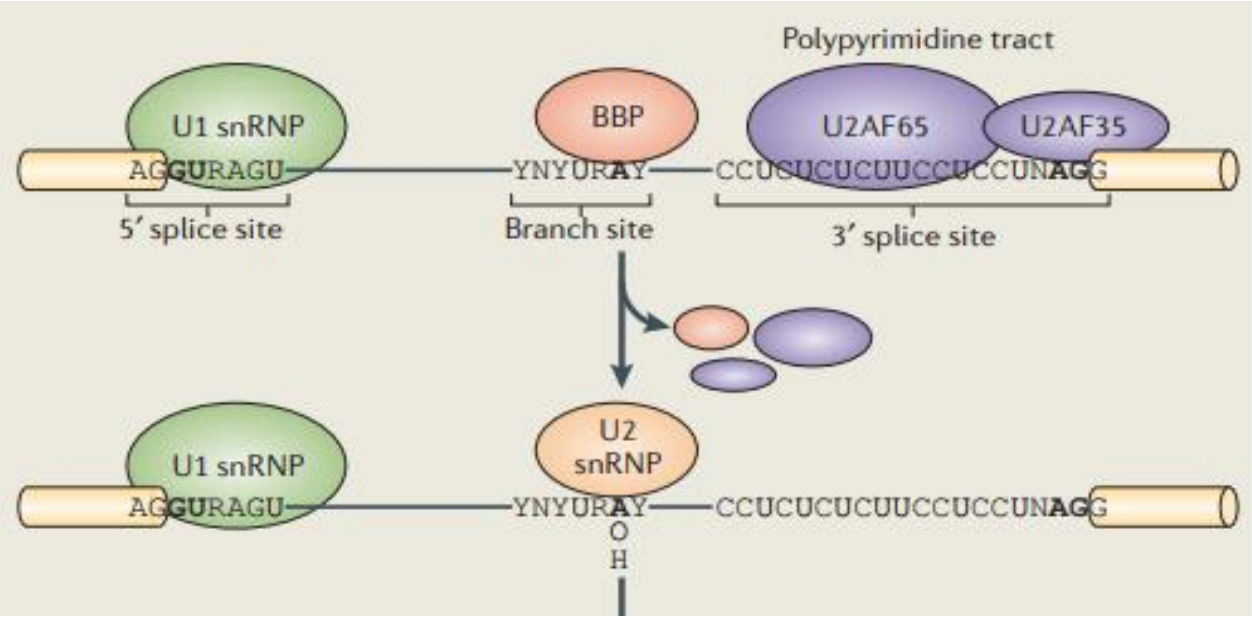


[Kornblihtt AR et al., 2013]

Spliceosome

The spliceosome mediates a two-step splicing reaction

1



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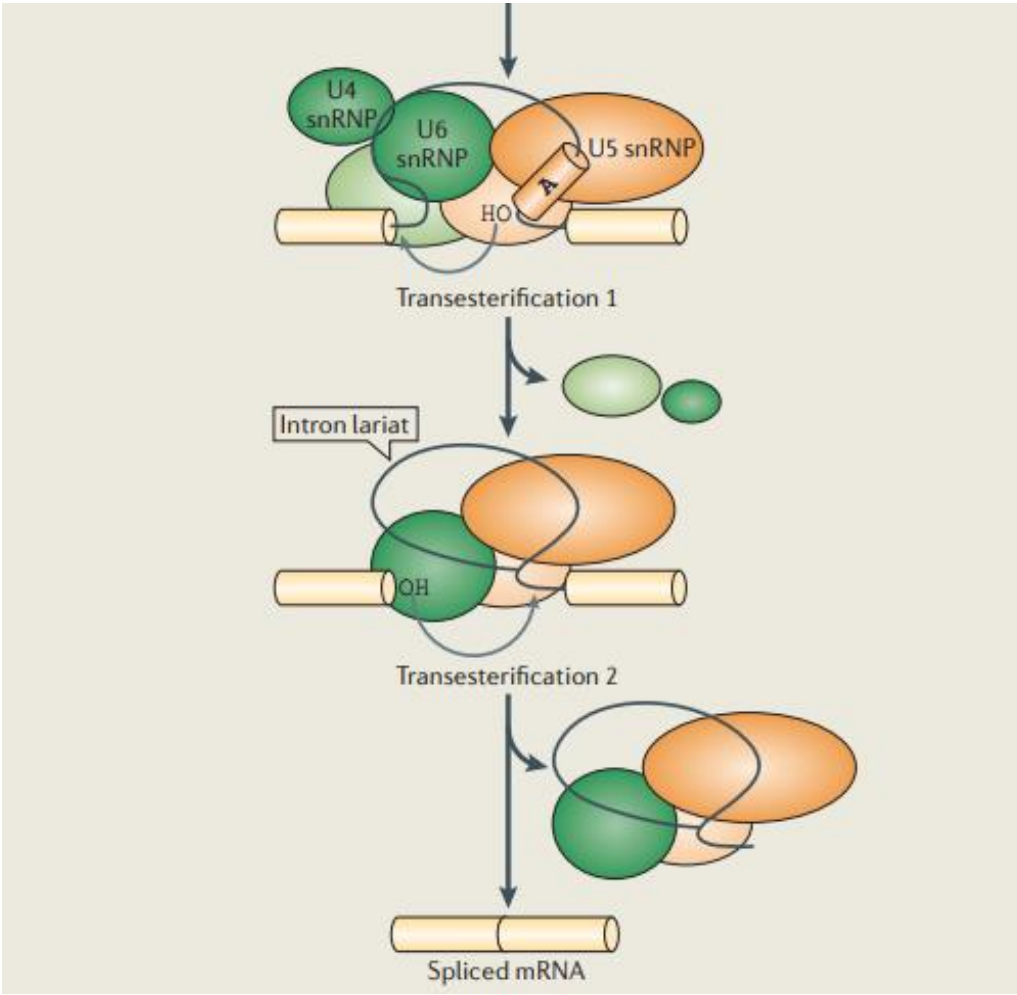
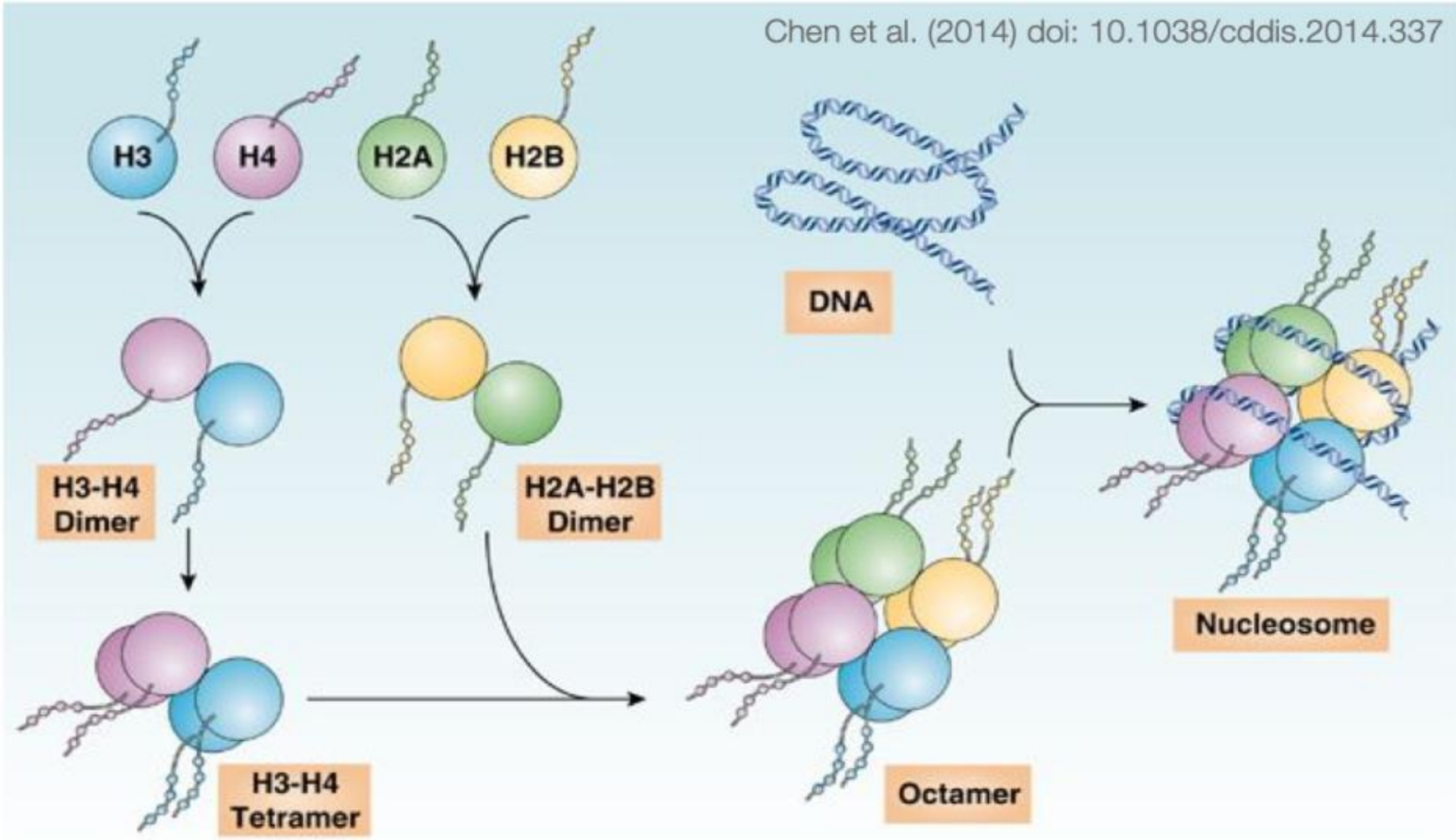


[Kornblihtt AR et al., 2013]

Histone Proteins

Histone proteins are small alkaline proteins around which the DNA molecule is wrapped.



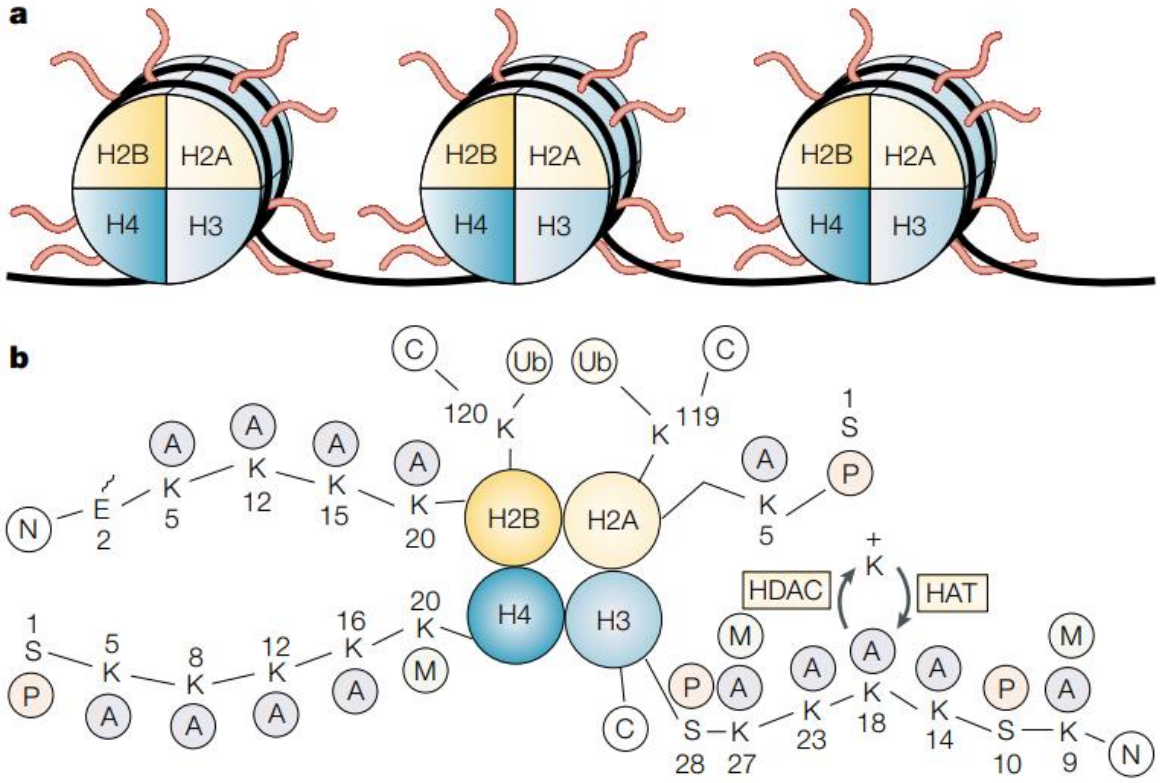
https://physiology.med.cornell.edu/faculty/skrabanek/lab/angsd/lecture_notes/13_lecture.pdf

Histone Proteins

Schematic of the structure of histones in nucleosomes

a. The core proteins of nucleosomes are designated *H2A (histone 2A), H2B (histone 2B), H3 (histone 3) and H4 (histone 4).*
Each histone is present in two copies, so the DNA (black) wraps around an octamer of histones — the core *nucleosome*.

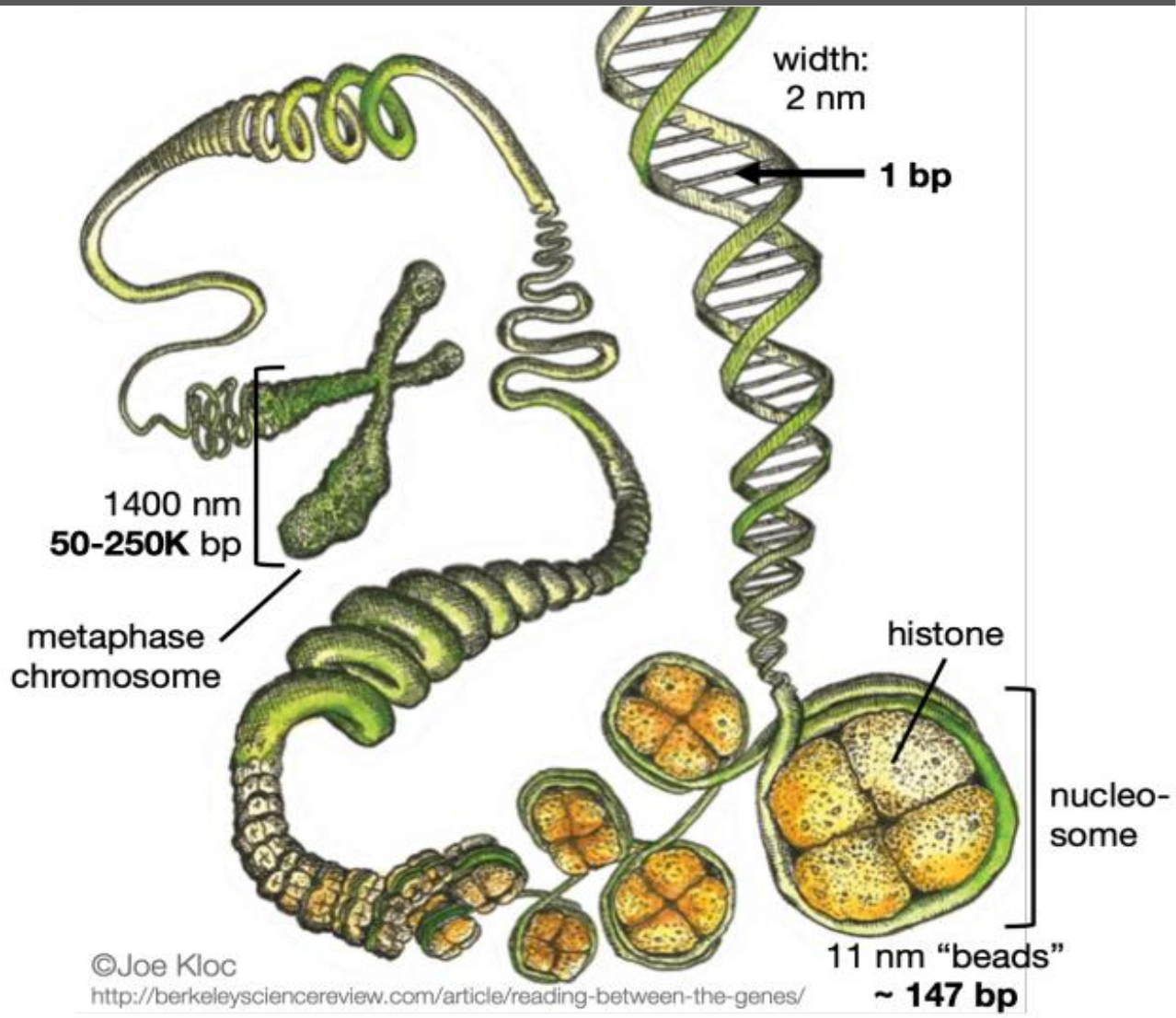
b. The amino-terminal **tails** of core histones.
Lysines (K) in the **amino-terminal tails** of histones H2A, H2B, H3 and H4 are potential **acetylation/deacetylation sites for histone** acetyltransferases (HATs) and histone deacetylases (HDACs).



https://pubmed.ncbi.nlm.nih.gov/11902574/

Chromatin

Chromatin = DNA + proteins + ncRNA
The most obvious function of chromatin is DNA compaction.



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Chromatin and alternative splicing

Chromatin has a key role in alternative splicing through the effects of Histone modifications.

Histone post-translational modifications are major regulators of alternative splicing.

These modifications may be divided into those associated with active transcription (for example, histone H3 Lys36 trimethylation (H3K36me3), H3K4me2, H3K4me3 and H3K9 acetylation (H3K9ac)) and those associated with transcriptional silencing (for example, H3K9me2, H3K9me3 and H3K27me3).

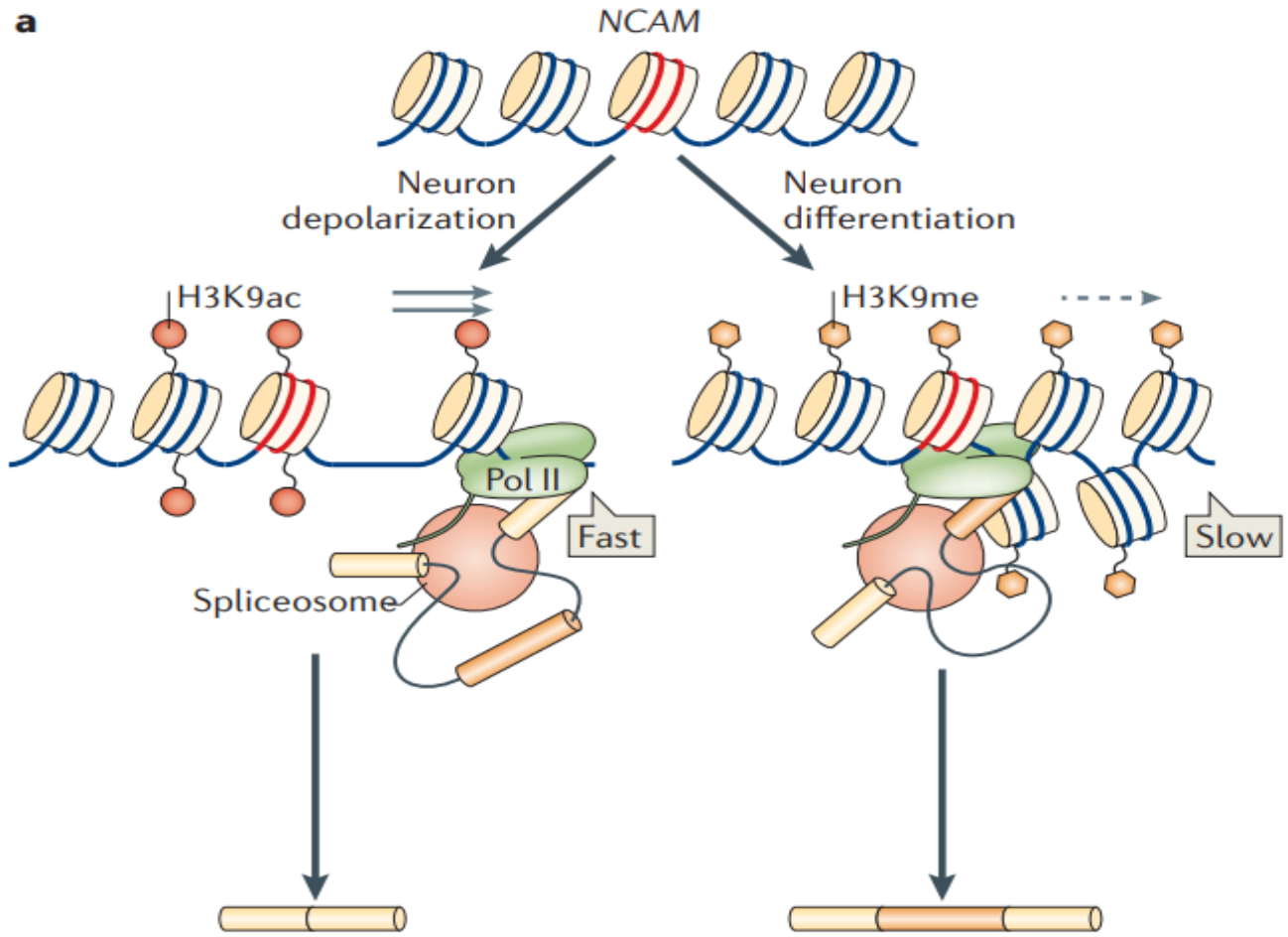
H3K36me3 is generally enriched at exons but less prominent at alternative exons.

This histone mark has been shown to regulate alternative splicing by recruiting the adaptor protein MRG15, as MRG15 association with the splicing factor PTB reduces the inclusion of a subset of alternative exons.

Regulation of alternative splicing

Two alternative mechanisms by which chromatin may influence alternative splicing.

Alternative splicing decisions are affected by the nature of histone marks that are deposited on the chromatin around a gene in response to external stimuli or to the differentiation state of the cell.



[Kornblihtt AR et al., 2013]

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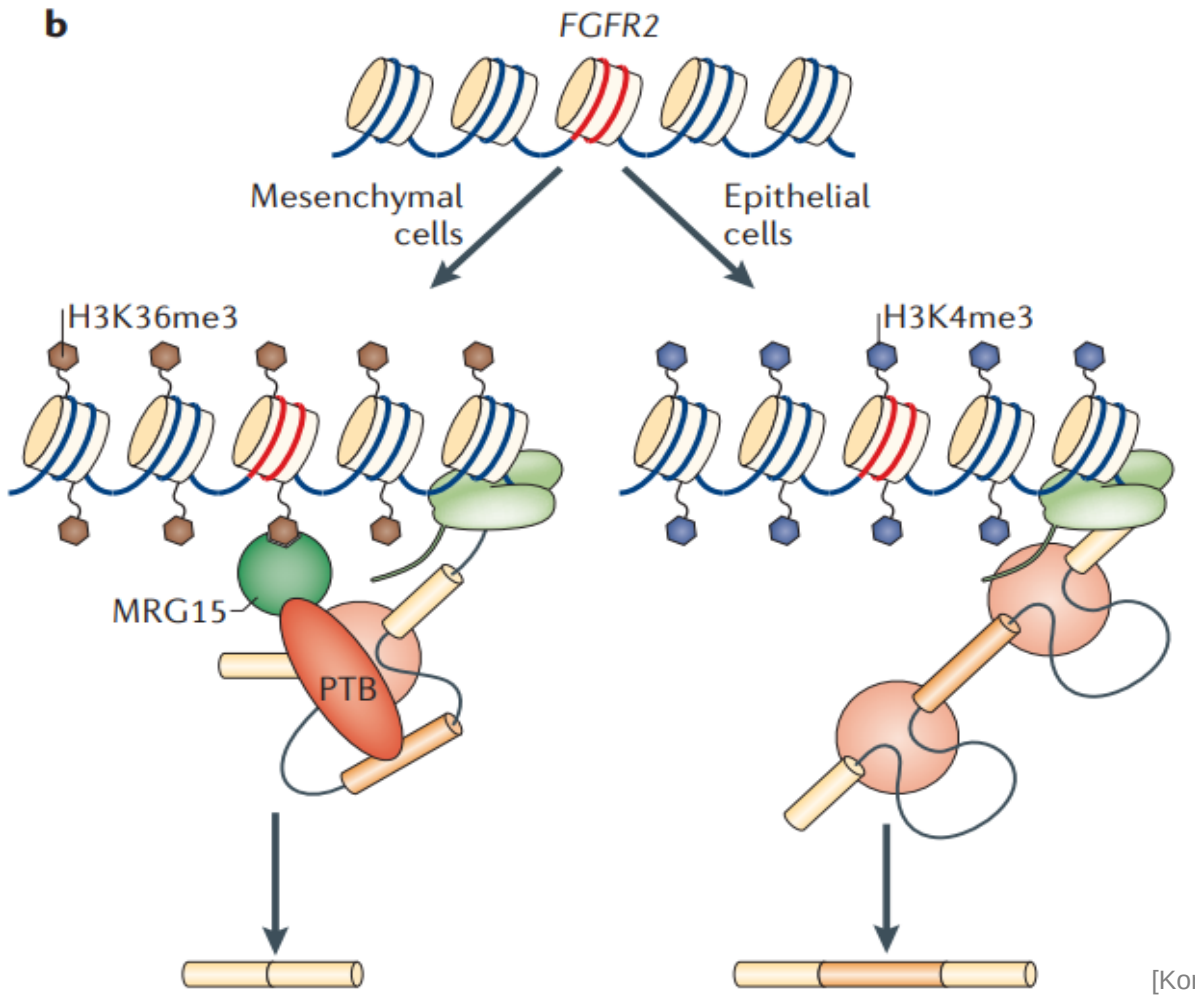


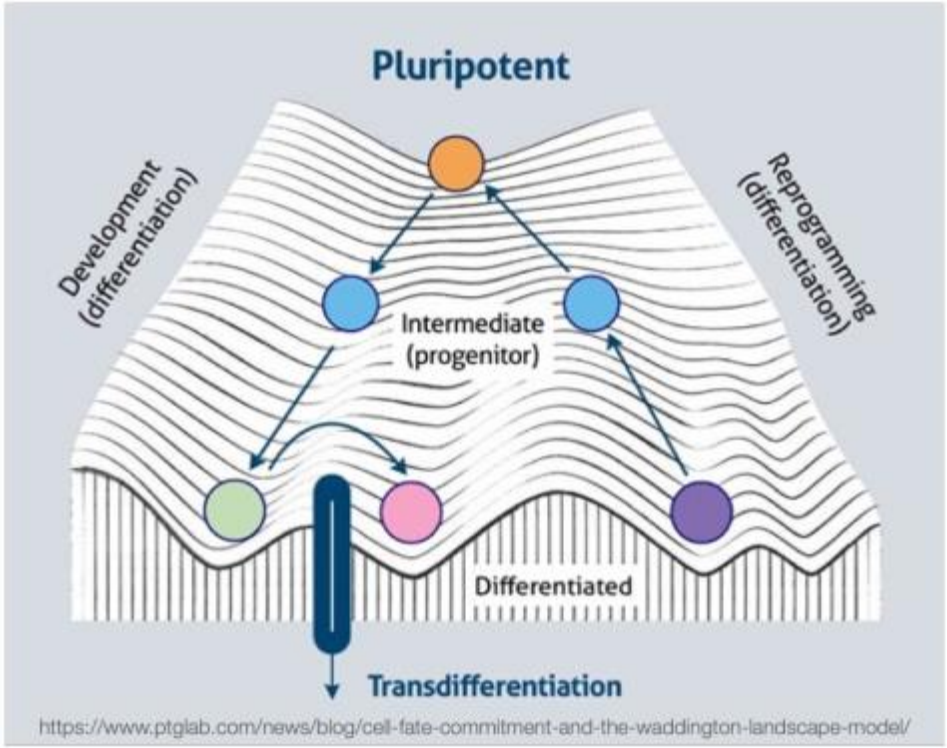
[Kornblihtt AR et al., 2013]

Chromatin and alternative splicing

The importance of being an alternative exon: *Alternative exon, flexible protein*

Pluripotent stem cells can divide into most, or all, cell types in an organism, but cannot develop into an entire organism on their own.

These intriguing results greatly strengthen the idea that regulation of alternative splicing is at least as important to cell differentiation as regulation of transcription [Kornblihtt AR et al., 2013].



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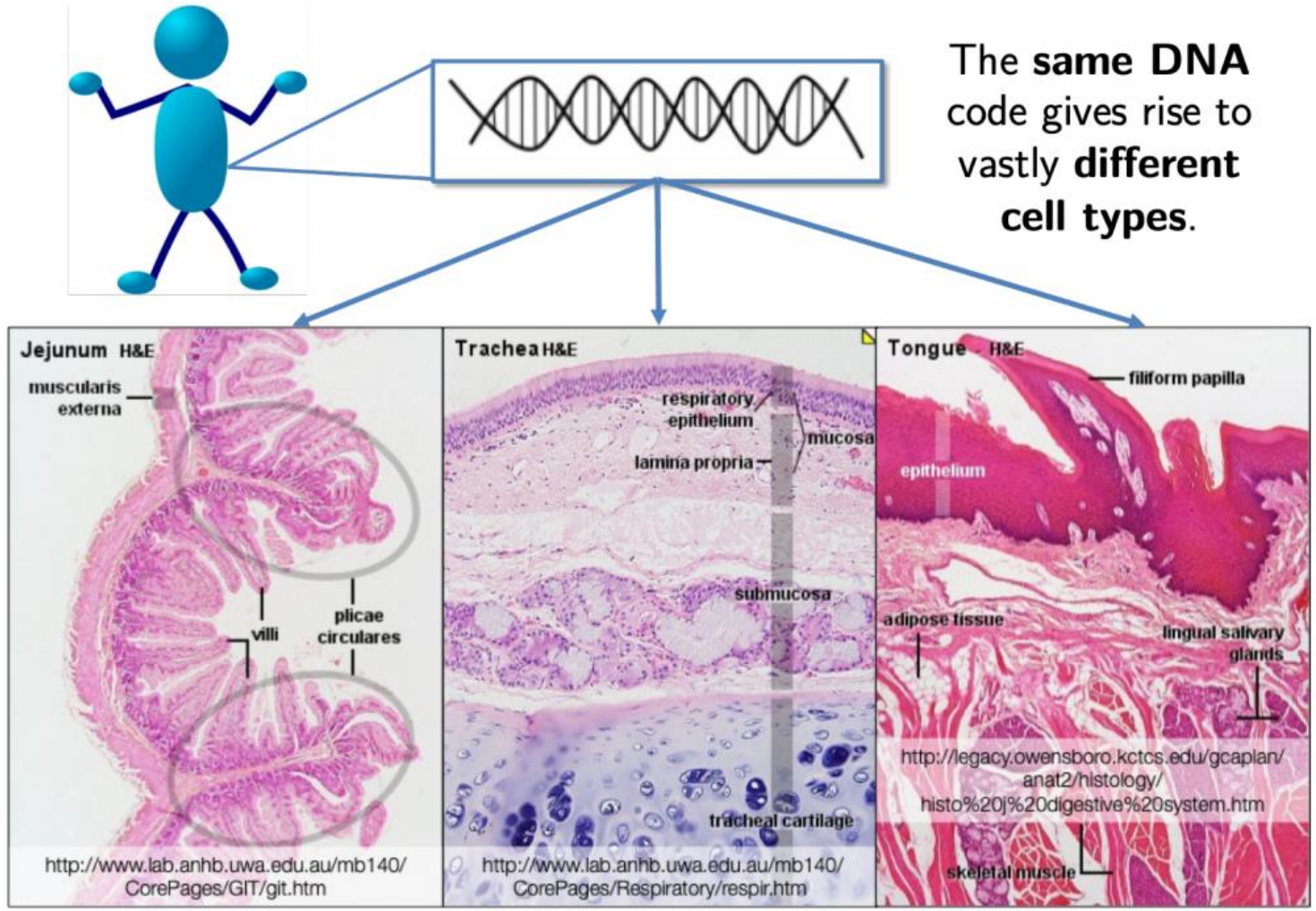
Chromatin and alternative splicing

The distribution of alternative splicing factors is tissue-specific, which is the reason for the diversity of cell differentiation and tissue specificity.

It has been indicated that more than 50% of genes express different alternative spliced isoforms among tissues.



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Evolution of Alternative splicing

The split organization of eukaryotic genes into exons and introns and the concomitant evolution of premRNA splicing seem to have had at least two advantages:

Alternative splicing allows a single gene to produce two or more mature mRNA variants that are similar but not identical, greatly expanding the coding capacity of eukaryotic genomes.

At the phylogenetic level, nondisruptive recombination events at intronic sequences allowed protein coding exons from different ancestor genes to be placed together to form new genes.

Relationship between Alternative Splicing and Diseases

Alternative splicing and Diseases

Mutations in regulatory sequences that affect alternative splicing are a widespread cause of human hereditary disease and cancer.

These mutations can disrupt existing splicing enhancers or silencers or create new ones, thereby altering the alternative exons that are included or even converting constitutive exons into alternative exons.

When these changes occur in the protein coding sequences of exons, their effects can be misinterpreted by considering only the putative effects they would have in the encoded protein.

Alternative splicing and Diseases

Gene expression profiles of tumor cells are different from normal samples.

Alternative splicing plays a key role in post-transcriptional regulation and controls the formation of spliced variants, the mutations and changed level of splice factors may contribute to tumorigenesis.

Abnormal expressions of specific splicing isoforms can cause specific cancers but also can serve as a biomarkers and therapeutic targets.

Understanding of alternative splicing regulation mediated by cellular factors is a prospective choice to develop specific drugs for targeting the dynamic RNA splicing process.

Alternative splicing and Diseases

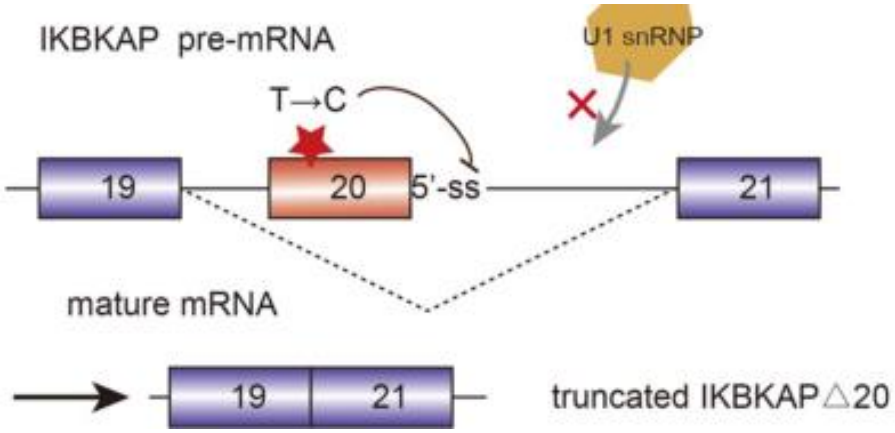
The Relationship between Alternative Splicing and Neurological Diseases

Neurodegenerative disease constitutes a variety of mental and neuromuscular disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), spinal muscular atrophy (SMA), and familial dysautonomia (FD) [Liu Q et al., 2022].

Alternative splicing and Diseases

Familial dysautonomia (FD) is a recessive disease mainly caused by the mutation of the i-kappa-B kinase complex associated protein (IKBKAP).

Mutated i-kappa-B kinase complex associated protein (IKBKAP) plays a role in the development of familial dysautonomia (FD).
The single nucleotide mutation from T to C disrupts the interaction between pre-mRNA and U1 snRNP and induces the exon 20 exclusion [Liu Q et al., 2022].



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Conclusion

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Alternative exon ➔ *flexible protein*

The distribution of alternative splicing factors is tissue-specific, which is the reason for the diversity of cell differentiation and tissue specificity.

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References

Kornblihtt AR, Schor IE, Alló M, Dujardin G, Petrillo E, Muñoz MJ. Alternative splicing: a pivotal step between eukaryotic transcription and translation. Nature reviews Molecular cell biology. 2013 Mar;14(3):153-65.

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Liu Q, Fang L, Wu C. Alternative Splicing and Isoforms: From Mechanisms to Diseases. Genes. 2022 Feb 24;13(3):401.

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