Genetics & molecular biology

◉ Sheet ○ Slide

Number:		10
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What is Posttranscriptional Processing:

1. Capping.
2. Splicing.
3. Polyadenylation (tailing).

❖ Capping

- occurs during transcription, when the first few nucleotides are made they will be capped by cap specialized enzymes.
- **Question**: what is the purpose of capping?
  The main purpose is to protect the single strand from nucleases, and help with attachment of m-RNA to ribosomes.
  (لازم يفوت عالرايبوسوم من جهة الكاب والا بتخربط التسلسل وبطلع بروتين ثاني).

- Cap synthesizing enzymes:

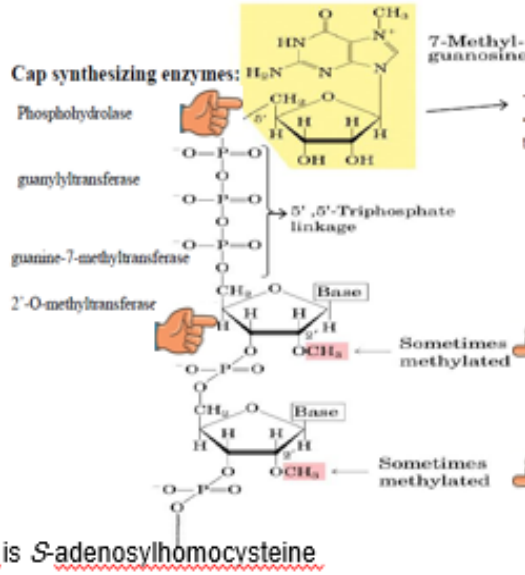
The cap is 1-methylguanosine (the methyl is for protection)

1) Phosphohydrolase: removing a phosphate group from the first nucleotide (it is triphosphate).
2) Guanyltransferase: transfers guanine to the first nucleotide and removes a di phosphate (Gppp becomes Gp to connect because of diphosphate removal.)
3) Guanine-7-methyltransferase: methylation for guanine on 7 n, methyl group taken from adenine methylase.
4) 2'-O-methyltransferase: sometimes (not always) sugar is methylated on carbon number two by this enzyme.

- After capping for more protection a CBC binding complex makes CTD loop on the cap.

Generation of the 5’ cap involves four to five separate steps

5’ cap of mRNA: unusual 5’,5’-triphosphate linkage..

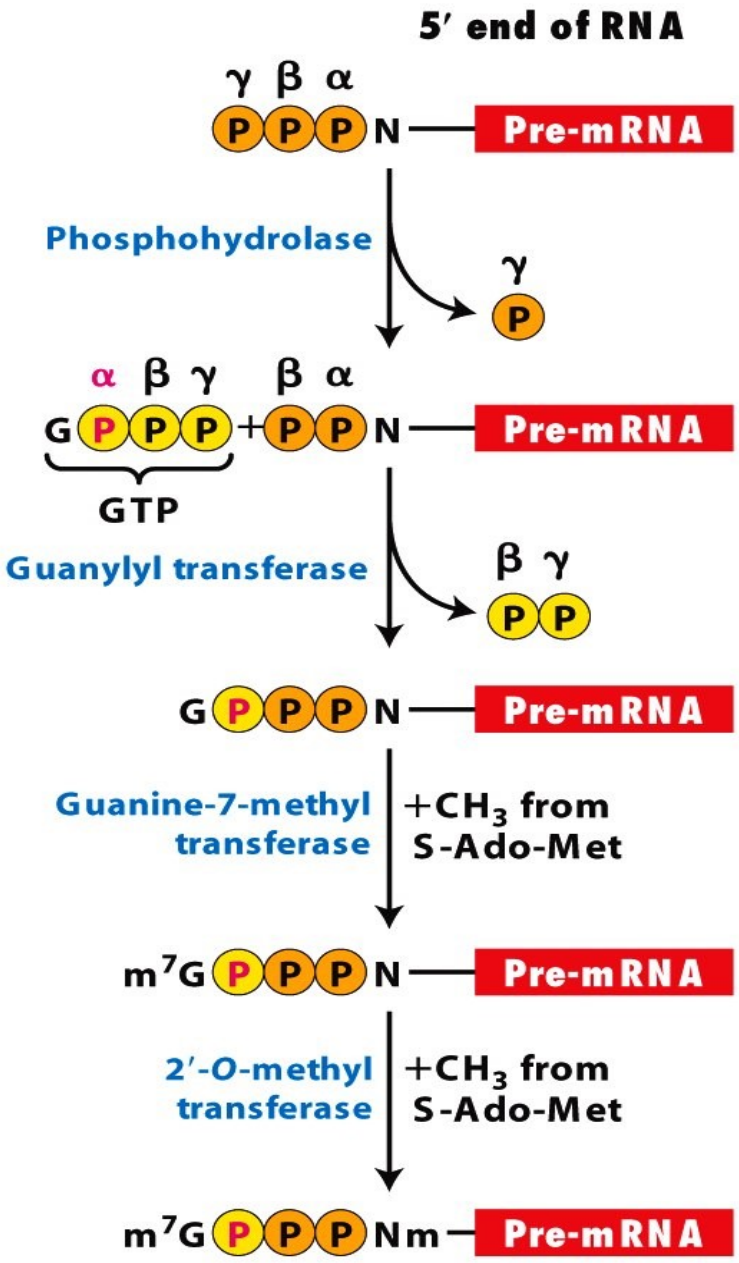


Cap functions

-Protects mRNA from ribonucleases.
-participates in binding of the mRNA to the ribosome to initiate translation

-Often methylated- Not in yeast

adoHcy is *S*-adenosylhomocysteine


5′ end of RNA
γ β α
P P P N
Pre-mRNA
Phosphohydrolase
γ
P
α β γ
G P P P
+
β α
P P N
Pre-mRNA
GTP
Guanylyl transferase
β γ
P P
G P P P N
Pre-mRNA
Guanine-7-methyl transferase
+CH3 from S-Ado-Met
m7G P P P N
Pre-mRNA
2′-O-methyl transferase
+CH3 from S-Ado-Met
m7G P P P Nm
Pre-mRNA

Splicing and Alternative Splicing:

Premature RNA has introns and exons, we make it mature through several processes which are splicing (removing the introns and joining the exons together) and capping.

- Splicing and alternative splicing are physiological (من الطبيعي والمهم حدوثهم)
- Missplicing (aberrant splicing) abnormal and usually associated with diseases.

- أي خربطة في عملية الsplicing بشكل غير طبيعي ممكن يتغير ال codeوبالتالي البروتين وبسبب العديد من الامراض.
- معظم الجينات المتواجدة في ال vertebrate عبارة عن introns باستثناء بروتين ال histone
- وجد حديثا ان قليل من البكتيريا تحتوي جيناتها على introns

What is the purpose of the presence of introns?

حتى تقدر تشكل الجين في اكثر من صورة وبذلك كل شكل للجين ينتج منه بروتين مختلف بوساطة ال alternative splicing. وهذا دليل على ما اكتشفه العلماء بعد ال human genome project ان جسم الانسان يحتوي فقط على 25000-35000 جين بخلاف التوقعات

ملاحظة: كودون البدء وكودون الايقاف لا يكونوا في طرف الاكسون لذلك ليس بالضرورة كل اكسون يكون حامل كودونات ال amino acid وهدول المناطق في الاكسون لا يحدث لها ترجمة التي تسمى ب (5'UTR & 3'UTR)

RNA Splicing:

There are 4 classes of splicing of introns; dependent on splicing mechanism:

Group 1 and 2: self-splicing which means that they don't need enzymes and ATP, in addition group 1 varies from group 2 in that it needs co-factors. They use guanosine (GMP, GTP, GDP) not as an energy source but as a splicing source, and it exists in all m-RNA, t-RNA, r- RNA and in mitochondria. They work as ribozymes (RNA enzymes). Whereas group two just exists in mitochondria m-RNA.

*both groups involve two transesterification reaction steps

Group 3: it is the major and largest class in nuclear m-RNA. These are called spliceosomal introns, catalyzed by a large protein complex called a spliceosome it contains tens of different proteins and different types of RNA (small nuclear RNA = snRNA). They use small nuclear ribonucleoproteins

(snRNPs) which are an association between snRNA and some proteins. it needs ATP for it to assemble.

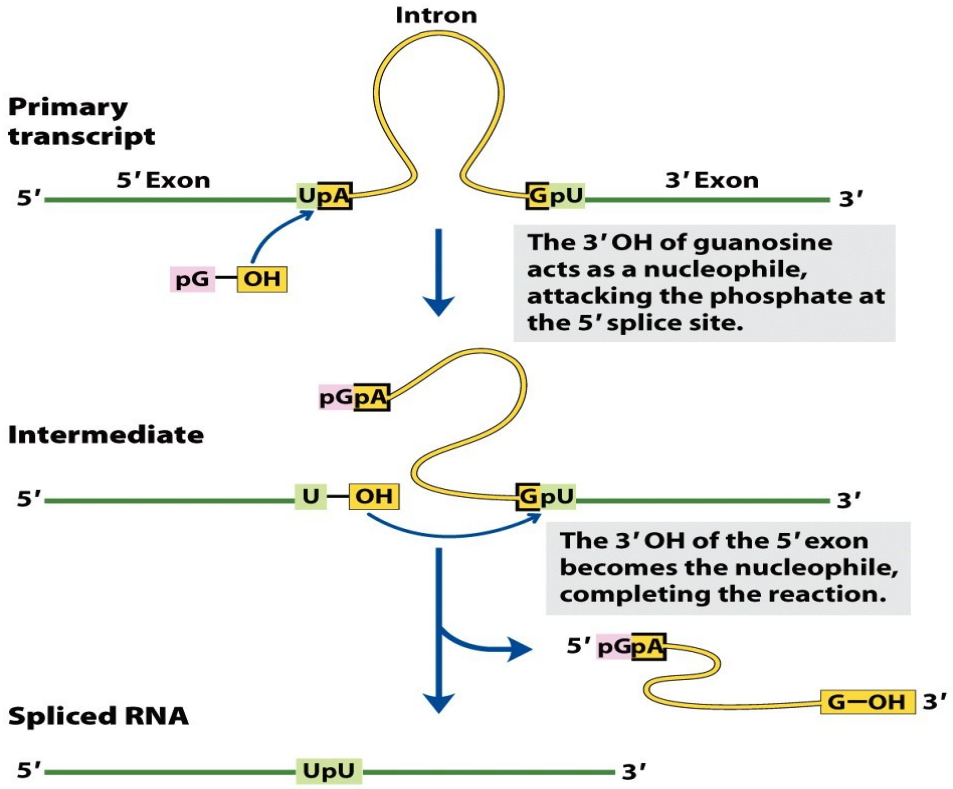
Group 4: just in genes that give t-RNA, it is removed by endonuclease enzyme and they consume ATP and are then rejoined by an enzyme similar to ligase.

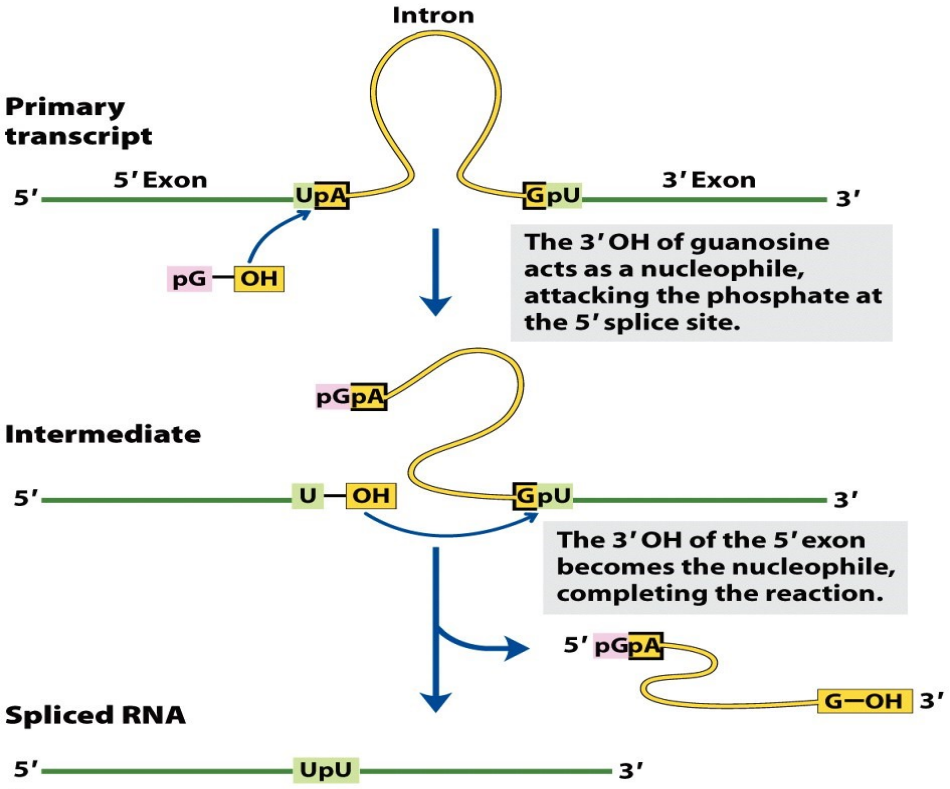
Group I introns:

Guanine nucleoside (guanine on sugar)
The transcribed chain (pre m-RNA) has introns located between A and G bases and exons begin with u bases.

عندي sequence محفوظ على الحدود بين الانترونات والاكسونات حتى تعرف أي مقطع لازم ينشال

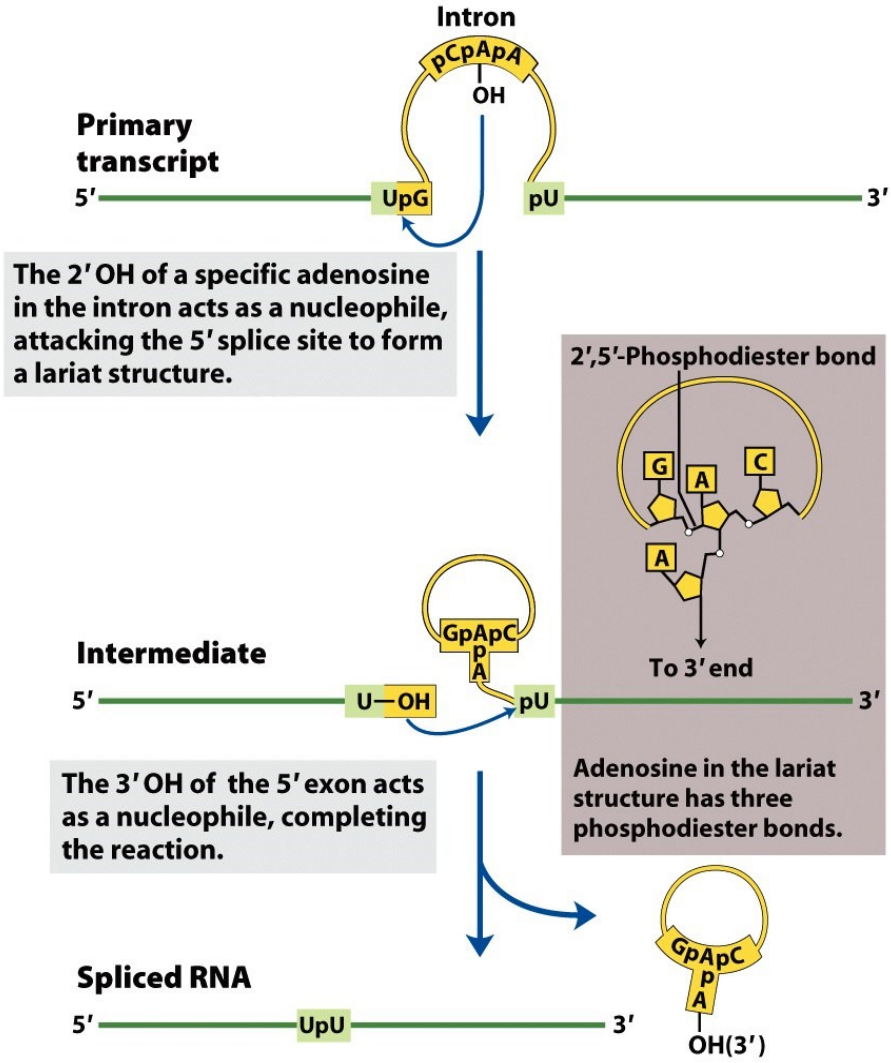
Transesterification reaction: the first step in the two-step splicing of group I introns. In this example, the 3′ OH of a guanosine molecule acts as a nucleophile, attacking the phosphodiester linkage between U and A residues at an exon-intron junction of an mRNA molecule using GTP, GMP and GDP. Then the 3′ OH of the 5' exon becomes the nucleophile completing the reaction. It is a process of exchanging the organic group of an ester with the organic group of an alcohol.





Group ll introns: The introns located between G base and uracil of exon.

- There are no co factors but there is a branch sequence with A that has an exposed OH which makes a nucleophilic attack on the G base making a lariat structure. This lariat separates from the chain when the free OH of uracil attacks the phosphate of the opposite exon.
- The chemistry is similar to that of group I intron splicing, except for the attacker.
- اتضح انه في الانترون عندي منطقة معينة اسمها branch site تحتوي على أدينين A الذي هو مصدر المهاجمة الأولى للطرف الأول وبعدين بكمل زي group ll



Group lll introns:

snRNPs (snRNA+ Protein) has a RNA complimentary to the transcript.

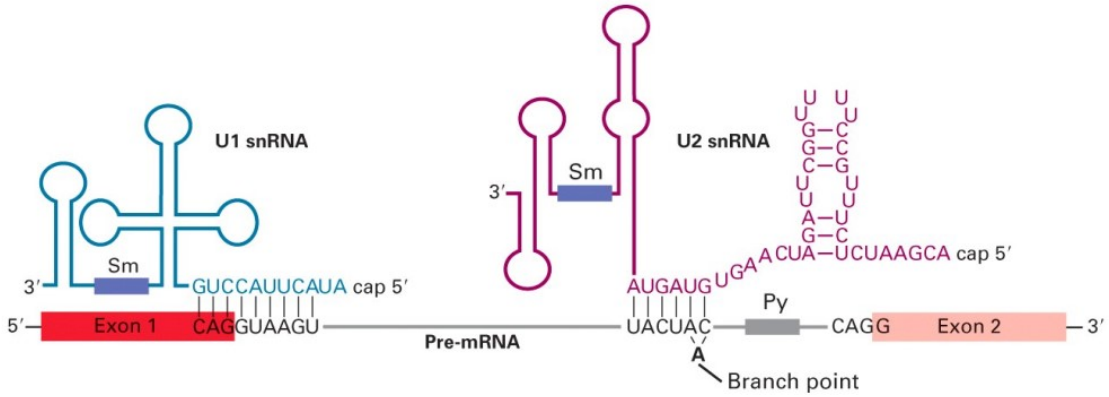
The U1 snRNA has a sequence near its 5′ complementary to the splice site at the 5′ end of the intron.

snRNA بشتغلوا في عملية ال splicing وعندي عدة أنواع منهم وترتبط عليهم البروتينات
تبدأ العملية في snRNA (U1) بعمل base pair وبعلم الطرف الاول (the exonuclease junction (AGG

U2 marked the branch site and makes a base pairing with it (u2 snRNP attaches to A sequence within the introns).

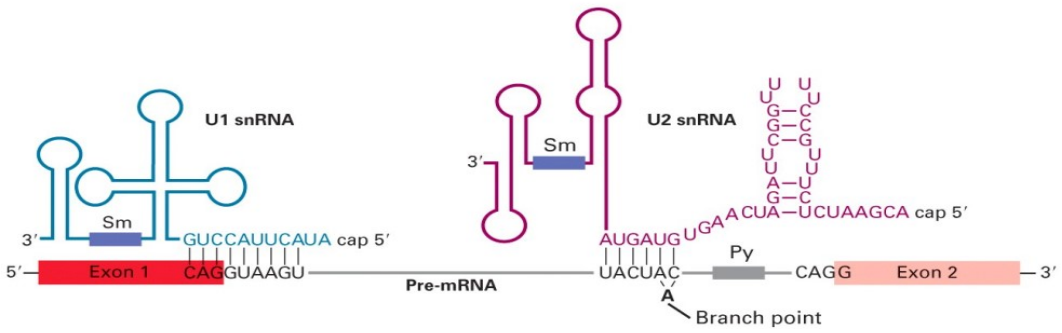
ولكن بضل عندي A ما في شي قبالها تعمل معه base pair فبتطلع من مكانها وبنصير مكشوفة وهيك بصير عندها مجال تعمل التفاعل

في منطقة تسمى Sm ربطوا عليها بروتينات بتتحول من snRNA ل snRNPs. بعد ارتباط U1 & U2 على هاي المنطقة بحفزوا ارتباط U4, U5, U6 وبصير conformation معين بقرب ال A لمكان الهجوم. وهاد ال complex بكون inactive وبتحول ل active بانه بخرج ال U1 & U4 وبصير تغيير في الشكل conformationبقرب ال A بشكل كبير. وبعد هيك ال A بتهاجم الطرف الاول وبتحط OH group وال OHبتهاجم الطرف الثاني وهيك بنكون تخلصنا من هاد ال introns.



Note: (A) base in introns group 3 sequence is similar to the branch point in introns group 2.

Note: RNA in snRNP has sodouracil bases



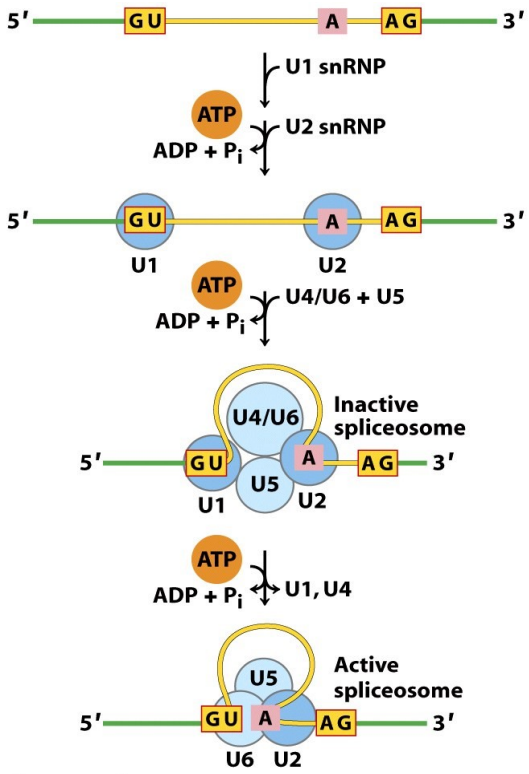


Figure 26-17b part 1
Lehninger Principles of Biochemistry, Fifth Edition
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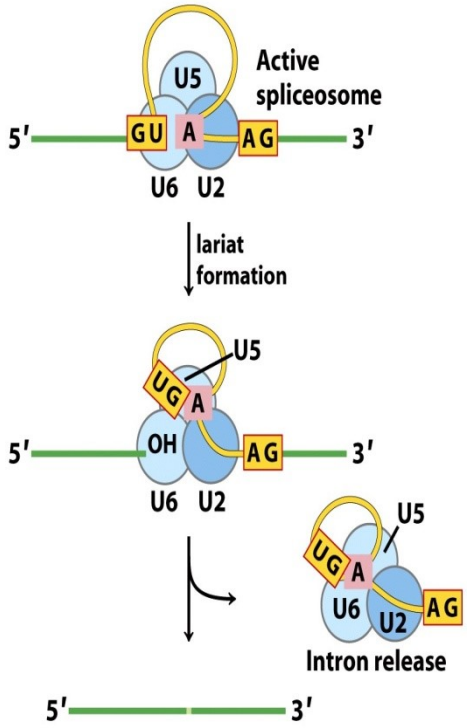

Figure 26-17b part 2
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Note about splicing: some groups of introns do undergo splicing after transcription process, EXCEPT introns class 3 it will never splice after transcription due to the very long gene it splices during transcription.

Alternative splicing

Changing regions are not always functional, meaning by functional is that it isn't translated.

In the immature m-RNA there is more than one cleavage site but they don't work together, if one of them is cut the other will be covered by processing factor protein.

The side which will be cut depends on the kind of cell.

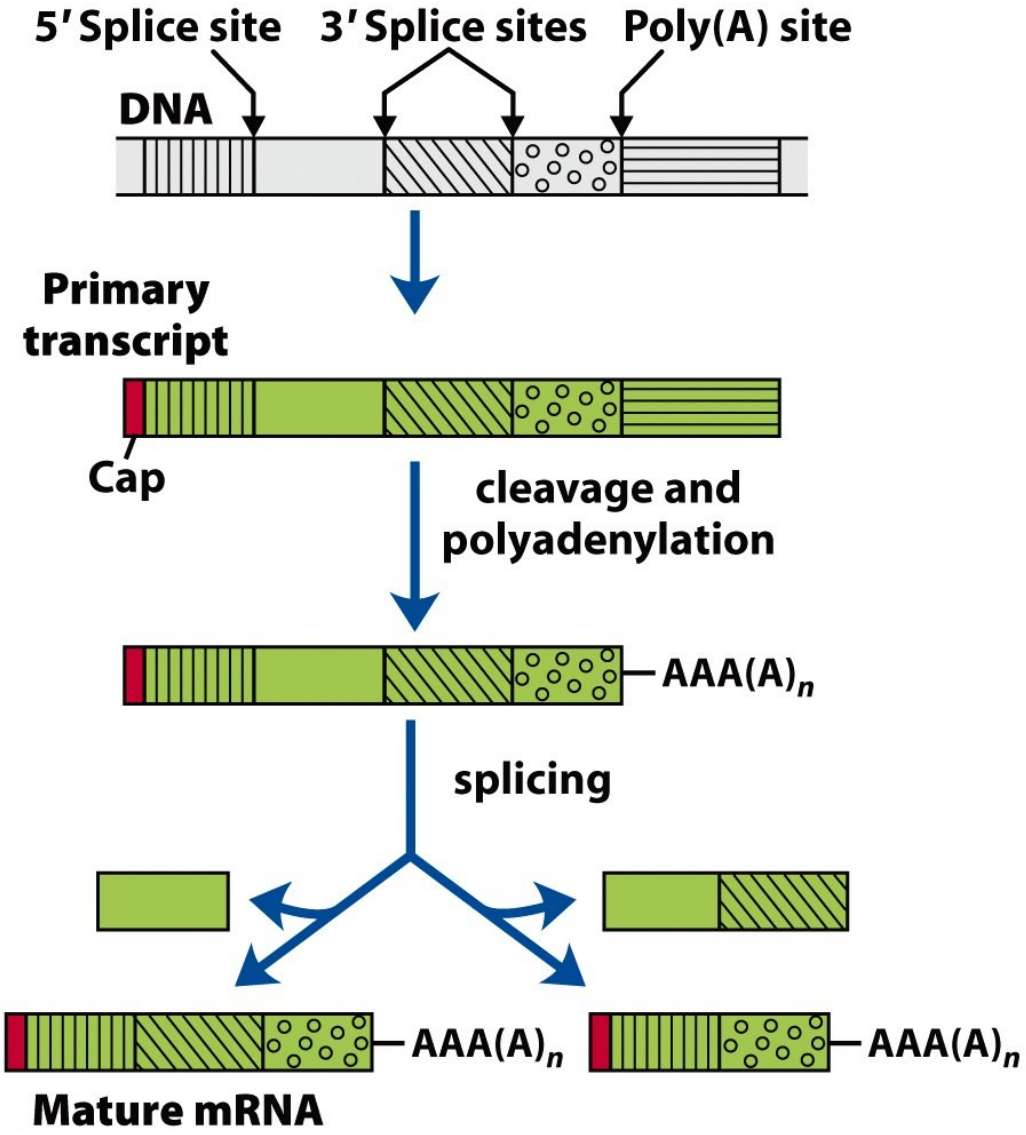
example: insulin is expressed only in the pancreas.

Mutations in alternative splicing **example**:

Thalassemia disease due to an error in splicing.

- So mutation happens because of losing part of the exons or entering of introns in the mature mRNA.

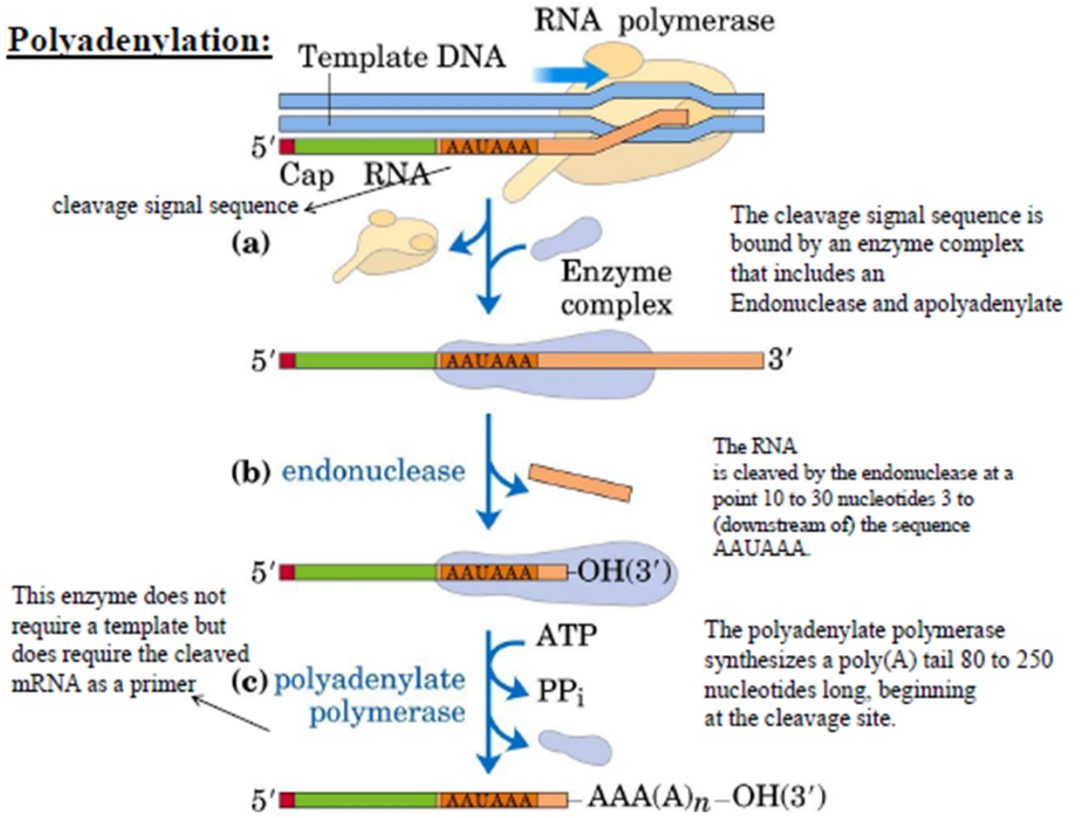
General note: protein after it is synthesized will undergo protease to become functional.



Termination (Tailing / Polyadenylation):

لما ال RNA polymerase يوصل للنهاية الموجودة في ال 3'UTR after splicing process بكون عندي sequence محفوظ (AAUAAA) في نهاية الجين و هوم بنضاف ال tail (poly A). بترتبط بعض البروتينات على هاد المكان منهم:

1. Endonuclease: cutting the site after AAUAAA sequence it will cut 10 to 30 nucleotide
2. Poly A polymerase (polyadenylate): adding a stretch of A on the cutting site (80-250 nucleotide) attached with phosphodiester bond depending on free OH of the cleavage site. So it doesn't need a primer or a template . ما بحتاج برايمر لانه بعتبر كل القطعة الي ضلت بعد القص هي البرايمر تبعه)



Alternative tailing:

Used in protein diversity dependent on the site where the endonuclease starts from.

Regulation of gene expression:

Genes divides into two classes:

1. **Housekeeping genes:** I need their products all the time. Such as: Histone.
2. **Inducible genes:** I need their products in special conditions. Like the genes I need in hypoxia.

In the promoter we have two regions (enhancers & silencers) which activate and repress transcription respectively. And they are far away from the promoter so we need to get them closer to the promoter by the conformation (looping) of the DNA. (هاي البروتينات هي عبارة عن منشطات ومثبطات لعملية النسخ . بس المسكلة انهن بعاد كثير عن البروموتر ..فكيف رح ياثرن عليه ويحفزن و يثبطن عملية النسخ؟ الحل: انه يصير DNA looping وهيك رح يقربن عالبروموتر.)

Promoters, enhancers, silencers etc.