

KAPITULLI 16 “ Sinteza e ARN-ve dhe Proteinave “

Paraqisni në mënyrë përmbledhëse dhe krahasuese të proceseve të transkriptimit e translatimit tek prokariotët dhe eukariotët.

= transkriptimi dhe translatimi ndodhin shpejt te prokariotet
= transkriptimi dhe translatimi ndodhin ne hapësirë kohore te eukariotet

= te prokariotet nuk ndodh procesing ARN-se
= te eukariotet ndodh procesingu ARN-se

= te prokariotet kemi ribozome 70S
= te eukariotet kemi ribozome 80S

= 1 ARN POLIMERAZE NE PROKARIOTE
= 3 LLOJ POLIMERAZASH NE EUKARIOTE

= Transkriptimi dhe translatimi ndodhin ne citoplazem te prokariotet
= Transkriptimi ndodh ne berthame ndersa translatimi ndodh ne citoplazem.

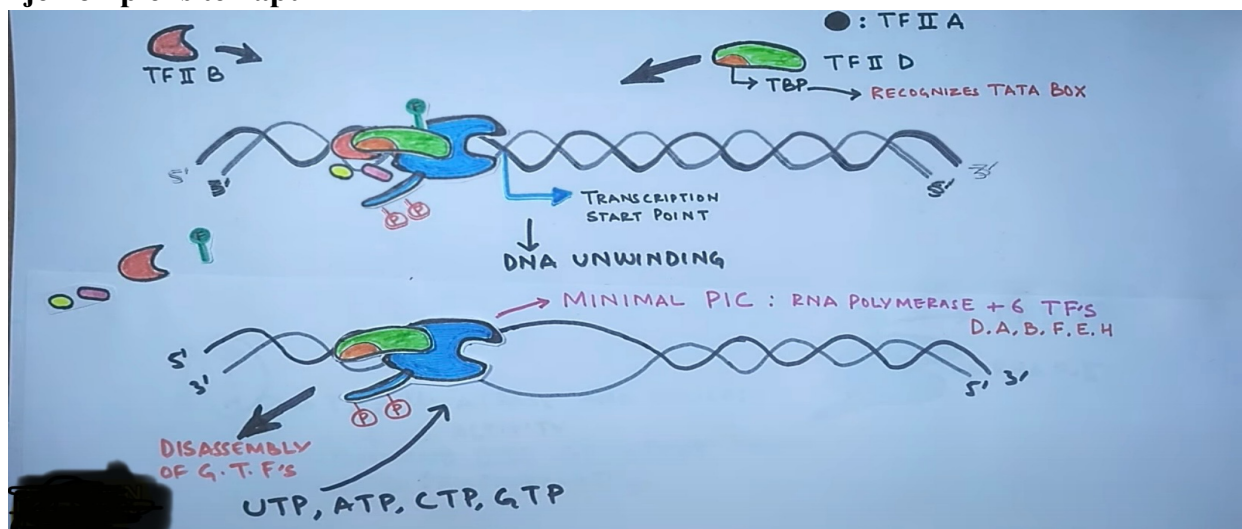
ARN POLIMERAZA I : e pandjeshme

ARN POLIMERAZA II : shume ndjeshme

ARN POLIMERAZA III : ndjeshmeri e moderuar

PROCESI I INICIMIT TE TRANSKRIPTIMIT TE EUKARIOTEVE

= ADN permban nje TATA BOX dhe nje Transcription Start Point
= Aty ndodhet TFIID qe ka nje pjese TBP i cili njeh TATA BOX-in , ky kooperim krijon nje tjeter faktor transkriptues TFIIA
= Pasi TFIID-TBP lidhet me TATA - box lind nje faktor transkriptues TFIIIB i cili i bashkohet kompleksin
= ARN polimeraza II ka nje faktor transkriptues TFIIIF dhe lidhet me kompleksin me siper
= dy faktore te tjere TFIIIE dhe TFIIH
= TFIIIE dhe TFIIH lidhen me kompleksin
= Faktori TFIIH eshte i rendesishem sepse permban nje ATP-ase e cila e kthen ADN ne nje kompleks te hapur



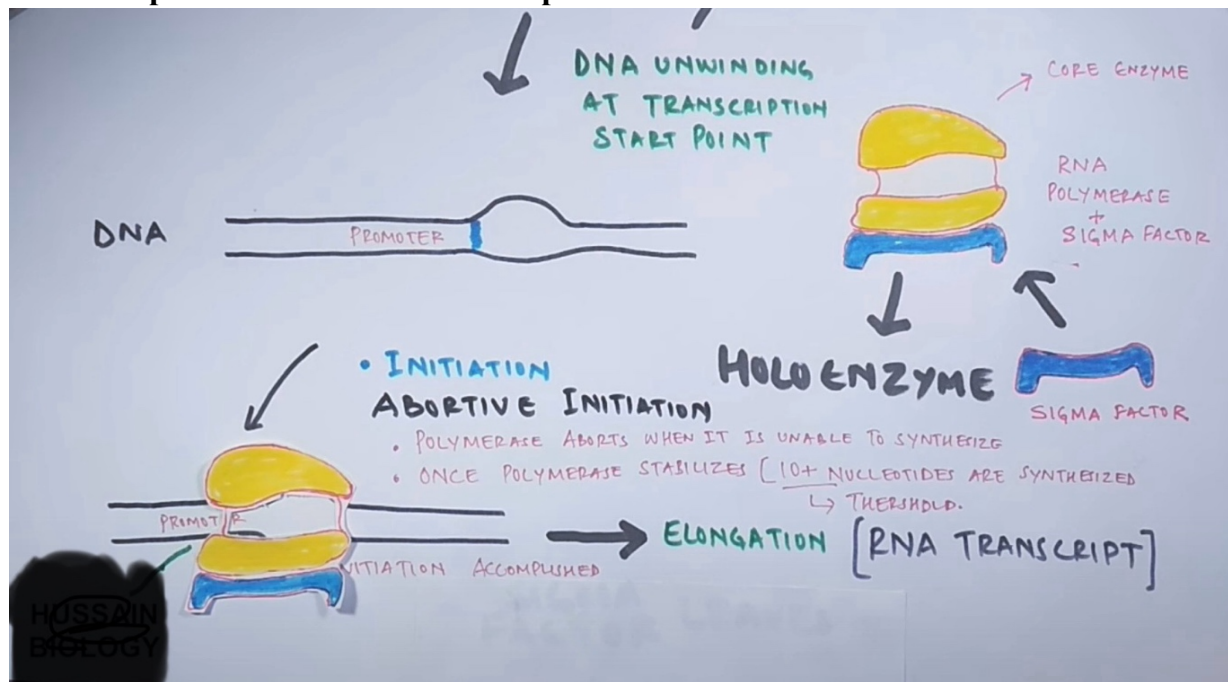
= Faktoret UTP , ATP , CTP DHE GTP zberthejne kompleksin dhe polimeraza nis transkriptimi

PROCESI I INICIMIT TE TRANSKRIPTIMIT TE PROKARIOTEVE

= ne ADN kemi nje nxites te inaktivizuar

= kur do nisi transkriptimi faktori sigma lidhet me ARN polimerazen duke aktivizuar dhe tashme merr emrin holoenzime

= holoenzima do lidhet me nxitesin dhe kjo do bej te mundur qe te formohet nje kompleks I hapur ne te cilin do niset dhe transkriptimi
 = transkriptimi nuk behet nese ka me pak se 10 nukleotide

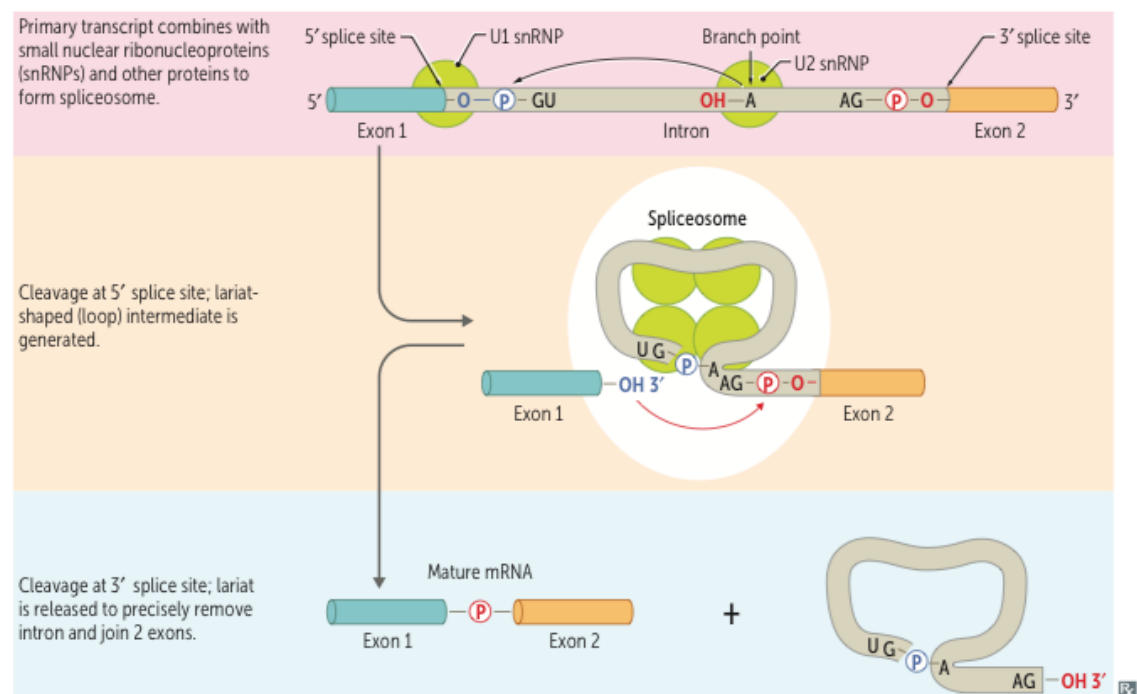


= nga e majta ne te djathte ARN polimeraza do transkriptoje zinxhirin e poshtem , nga e djathta ne te majte do transkriptoje zinxhirin e siperm

Splicing of pre-mRNA

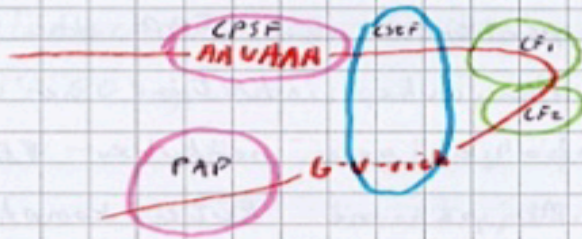
Part of process by which precursor mRNA (pre-mRNA) is transformed into mature mRNA. Introns typically begin with GU and end with AG. Alterations in snRNP assembly can cause clinical disease; eg, in spinal muscular atrophy, snRNP assembly is affected due to ↓ SMN protein → congenital degeneration of anterior horns of spinal cord → symmetric weakness (hypotonia, or “floppy baby syndrome”).

snRNPs are snRNA bound to proteins (eg, Smith [Sm]) to form a spliceosome that cleaves pre-mRNA. Anti-U1 snRNP antibodies are associated with SLE, mixed connective tissue disease, other rheumatic diseases.

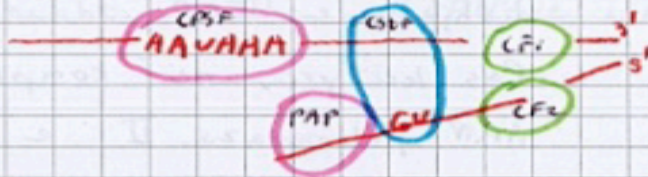


Formimi i bishtit Poli-A.

1- Ka vetem një kompleks të procesimit në skajin 5'.



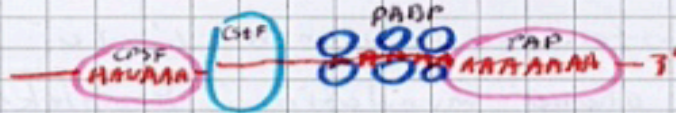
2- Faktori i keputjes krijon skajin 3'



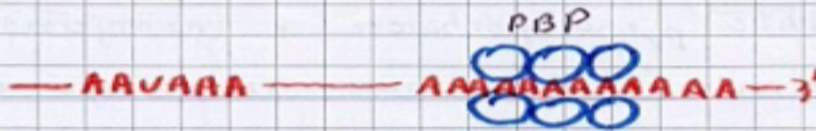
3- PAP shton radikale të A.



4- Proteina PABP lidhet në Poli-A



5- Kompleksi shpërbehët pas shtimit të 200 A.



FORMIMI I BOSHTIT TE POLIT A

Te gjithë mRNA ne qelizat eukariote janë të pajisura me bisht POLI A .

-> Kemi nukleotidet AAUAAA në vendin ku do formohet bishti që shërben si sinjal për poli A ndërsa më pas ndodhte një zonë GU .

-> Te sekuenca nukleotidike do lidhet nënjesia CPSF

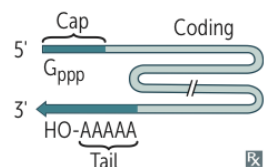
dhe pasohet nga CstF që lidhet me zonën GU ku stabilizohet kompleksi proteinik .

-> Më pas lidhen dhe CFII dhe CFI . Përpara se të ndodhë keputja lidhet poli-A-polimeraza e cila bën të mundur shtimin e radikaleve A .

-> Si fillim shtohen 12 nukleotide A dhe pasi

lidhet PABP në kompleks shtohen 200 NA , kështu formohet bishti poli A

RNA processing (eukaryotes)



Initial transcript is called heterogeneous nuclear RNA (hnRNA). hnRNA is then modified and becomes mRNA.

The following processes occur in the nucleus:

- Capping of 5' end (addition of 7-methylguanosine cap; cotranscriptional)
- Polyadenylation of 3' end (~200 A's → poly-A tail; posttranscriptional)
- Splicing out of introns (posttranscriptional)

Capped, tailed, and spliced transcript is called mRNA.

mRNA is transported out of nucleus to be translated in cytosol.

mRNA quality control occurs at cytoplasmic processing bodies (P-bodies), which contain exonucleases, decapping enzymes, and microRNAs; mRNAs may be degraded or stored in P-bodies for future translation. Poly-A polymerase does not require a template. AAUAAA = polyadenylation signal. Mutation in polyadenylation signal → early degradation prior to translation.

Kozak sequence—initiation site in most eukaryotic mRNA. Facilitates binding of small subunit of ribosome to mRNA. Mutations in sequence → impairment of initiation of translation → ↓ protein synthesis.

RNA polymerases

Eukaryotes

RNA polymerase I makes **r**RNA, the most common (**r**ampant) type; present only in nucleolus.

RNA polymerase II makes **m**RNA (**m**assive), **micro**RNA (**mi**RNA), and **s**mall **n**uclear RNA (**sn**RNA).

RNA polymerase III makes 5S rRNA, **t**RNA (**t**iny).

No proofreading function, but can initiate chains. RNA polymerase II opens DNA at promoter site.

I, II, and III are numbered in the same order that their products are used in protein synthesis: rRNA, mRNA, then tRNA.

α-amanitin, found in *Amanita phalloides* (death cap mushrooms), inhibits RNA polymerase II. Causes dysentery and severe hepatotoxicity if ingested.

Dactinomycin inhibits RNA polymerase in both prokaryotes and eukaryotes.

Prokaryotes

1 RNA polymerase (multisubunit complex) makes all 3 kinds of RNA.

Rifamycins (rifampin, rifabutin) inhibit DNA-dependent RNA polymerase in prokaryotes.

Introns vs exons

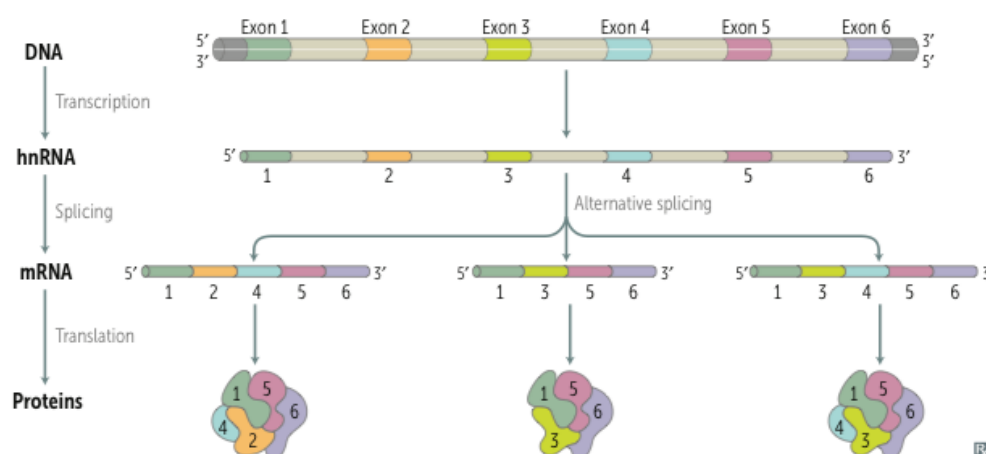
Exons contain the actual genetic information coding for protein or functional RNA.

Introns do not code for protein, but are important in regulation of gene expression.

Different exons are frequently combined by alternative splicing to produce a larger number of unique proteins.

Introns are **in**tervening sequences and stay **in** the nucleus, whereas **exons** **ex**it and are **ex**pressed.

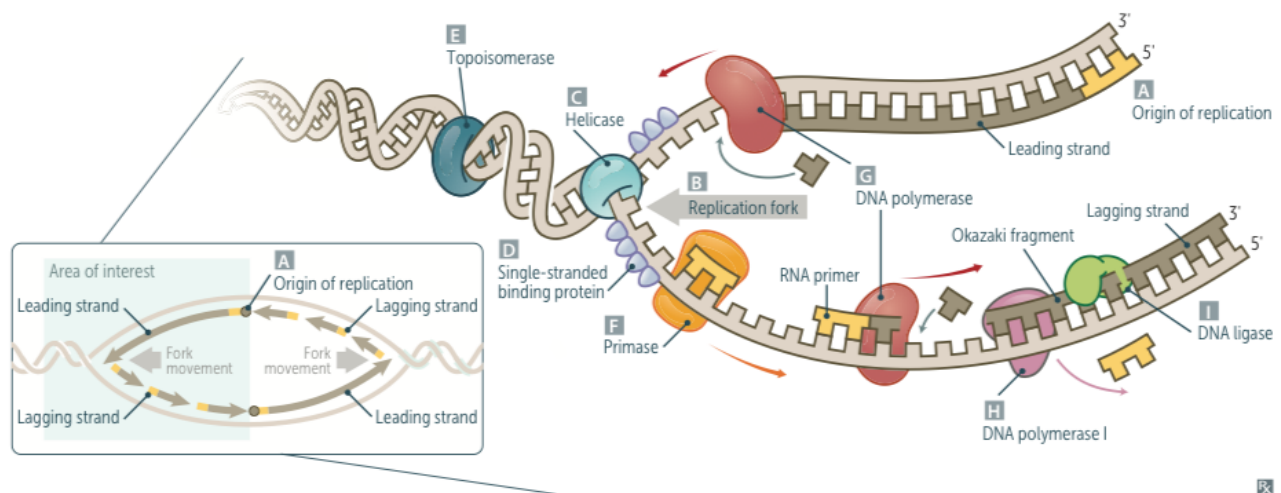
Alternative splicing—can produce a variety of protein products from a single hnRNA (heterogenous nuclear RNA) sequence (eg, transmembrane vs secreted Ig, tropomyosin variants in muscle, dopamine receptors in the brain, host defense evasion by tumor cells).



DNA replication

Occurs in $5' \rightarrow 3'$ direction (“**5**ynth**3**sis”) in continuous and discontinuous (Okazaki fragment) fashion. Semiconservative. More complex in eukaryotes than in prokaryotes, but shares analogous enzymes.

Origin of replication A	Particular consensus sequence in genome where DNA replication begins. May be single (prokaryotes) or multiple (eukaryotes).	AT-rich sequences (eg, TATA box regions) are found in promoters (often upstream) and origins of replication (ori).
Replication fork B	Y-shaped region along DNA template where leading and lagging strands are synthesized.	
Helicase C	Unwinds DNA template at replication fork.	Helicase h alves DNA. Deficient in B loom syndrome (BLM gene mutation).
Single-stranded binding proteins D	Prevent strands from reannealing or degradation by nucleases.	
DNA topoisomerases E	Creates a s ingle- (topoisomerase I) or d ouble- (topoisomerase II) stranded break in the helix to add or remove supercoils (as needed due to underwinding or overwinding of DNA).	In eukaryotes: irinotecan/topotecan inhibit topoisomerase (TOP) I, etoposide/teniposide inhibit TOP II. In prokaryotes: fluoroquinolones inhibit TOP II (DNA gyrase) and TOP IV.
Primase F	Makes RNA primer for DNA polymerase III to initiate replication.	
DNA polymerase III G	Prokaryotes only. Elongates leading strand by adding deoxynucleotides to the 3' end. Elongates lagging strand until it reaches primer of preceding fragment.	DNA polymerase III has $5' \rightarrow 3'$ synthesis and proofreads with $3' \rightarrow 5'$ exonuclease. Drugs blocking DNA replication often have a modified 3' OH, thereby preventing addition of the next nucleotide (“chain termination”).
DNA polymerase I H	Prokaryotes only. Degrades RNA primer; replaces it with DNA.	Same functions as DNA polymerase III, also excises RNA primer with $5' \rightarrow 3'$ exonuclease.
DNA ligase I	Catalyzes the formation of a phosphodiester bond within a strand of double-stranded DNA.	Joins Okazaki fragments. L igase links DNA.
Telomerase	Eukaryotes only. A reverse transcriptase (RNA-dependent DNA polymerase) that adds DNA (TTAGGG) to 3' ends of chromosomes to avoid loss of genetic material with every duplication.	Upregulated in progenitor cells and also often in cancer; downregulated in aging and progeria. T elomerase T AGs for G reatness and G lory.



PROCESI INICIMIT TE TRANSLATIMIT TE EUKARIOTET FORMIMI KOMPLEKSIT PREINICIAL :

= faktoret eIF1 , eIF1A dhe eIF3 lidhen me nenjesine 40S gjithashtu i atashohet dhe met-tARNimet

= faktori eIF5B lidhet me faktorin eIF1A

=eIF4A lidhet afer start te translatimit

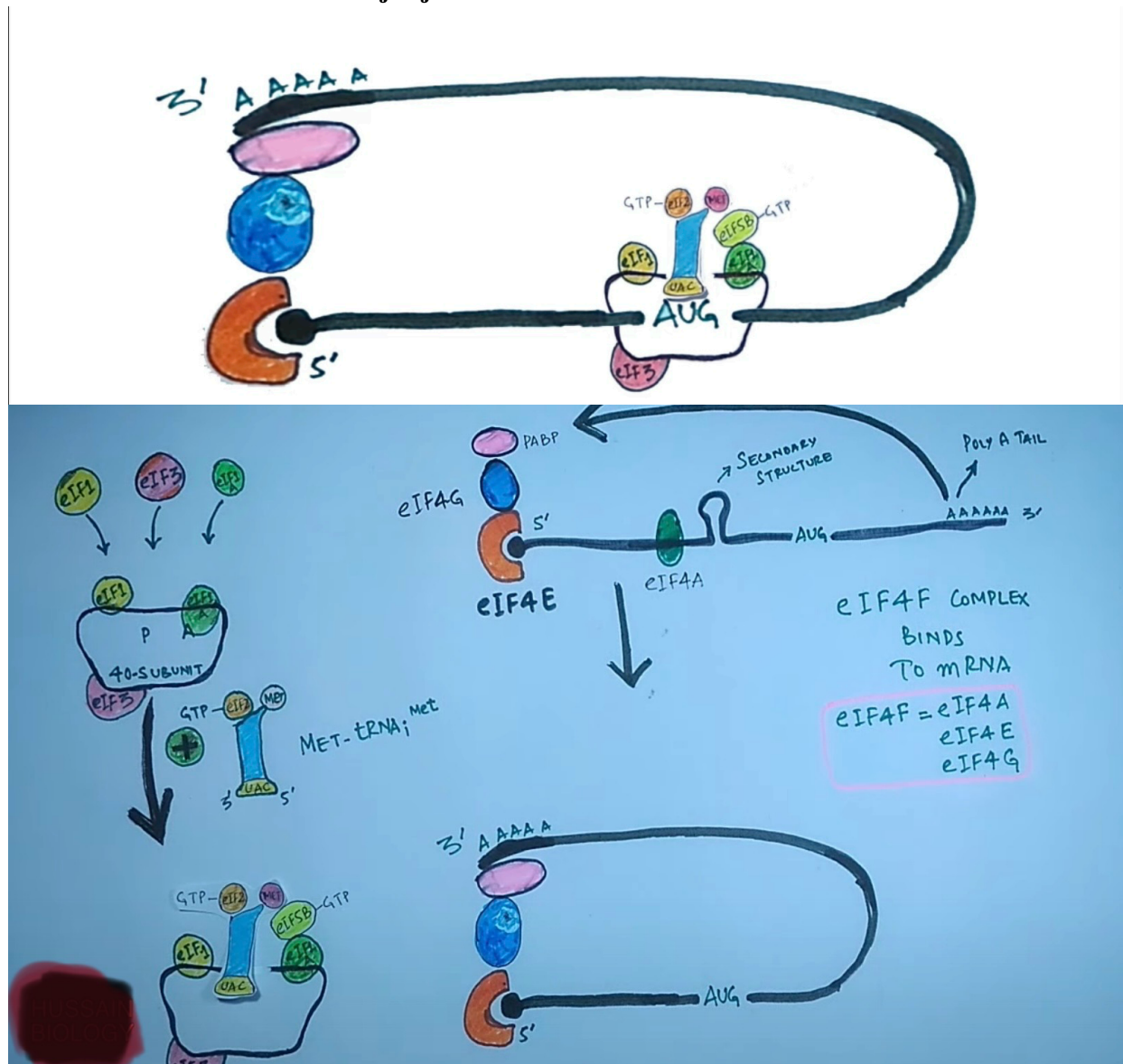
=eIF4E lidhet ne skajin 5' , siper tij vendoset eIF4G dhe me siper PABP

= formohet kompleksit preinicial 43 S , kur eIF2 cliron met-tARN ne sitin P te nenjesise se vogel 40S ribosomale

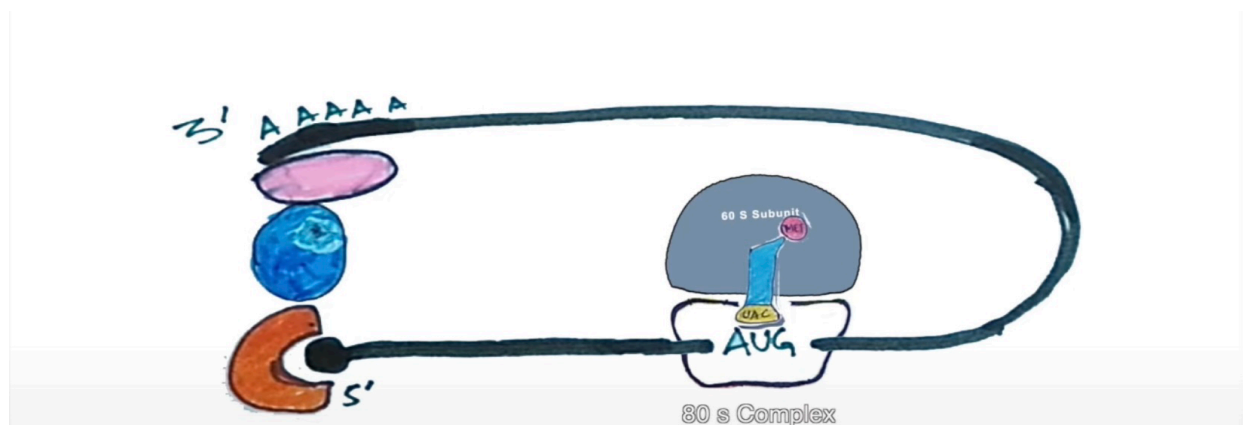
= rekrutimi me anen e faktoreve eIF3 dhe eIF4 , i kompleksit 43S ne skajin 5'cap te

mARN

= skanimi zones 5'UTR dhe njohja kodonit AUG

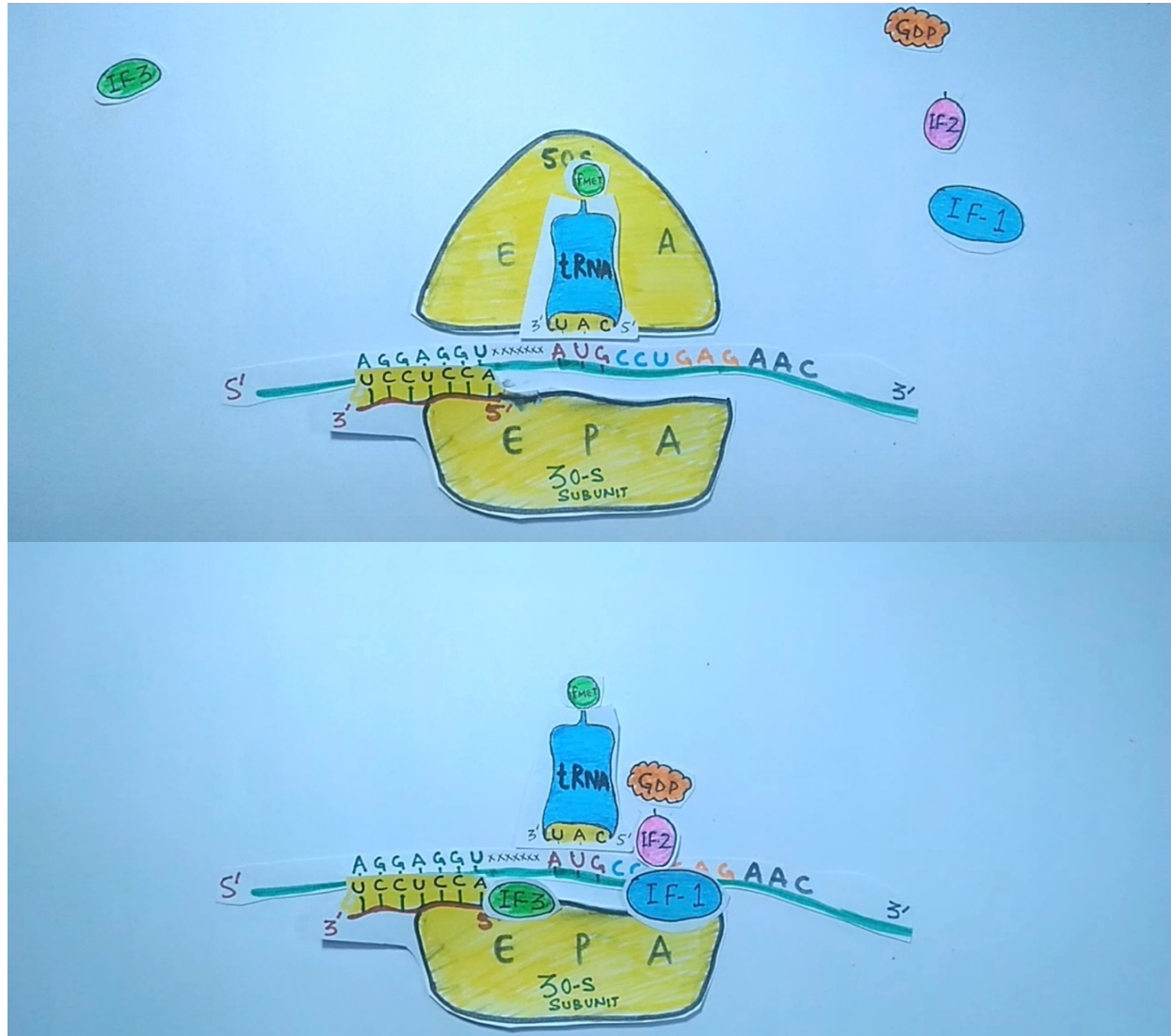


= bashkimi nenjesise se madhe ribozomale 60S



PROCESI INICIMIT TE TRANSLATIMIT TE PROKARIOTET:

- = Kemi nje nenjesi ribosomale 30S e cila ka tri site E P dhe A
- = gjithashtu aty gjendet nje zinxhire komplementar
- = IF3 dhe IF1 lidhen me nenjesine 30 s dhe zinxhiri mARN lidhet me ribozomin
- = IF2 lidhet me tARN e cila eshte nje antikodon per kodonin start
- = Kur GTP hidrolizohet ne GDP te gjithe faktoret largohen dhe behet e mundur qe nenjesia 50S ti bashkohet nenjesise tjeter



= keshtu nis translatimi