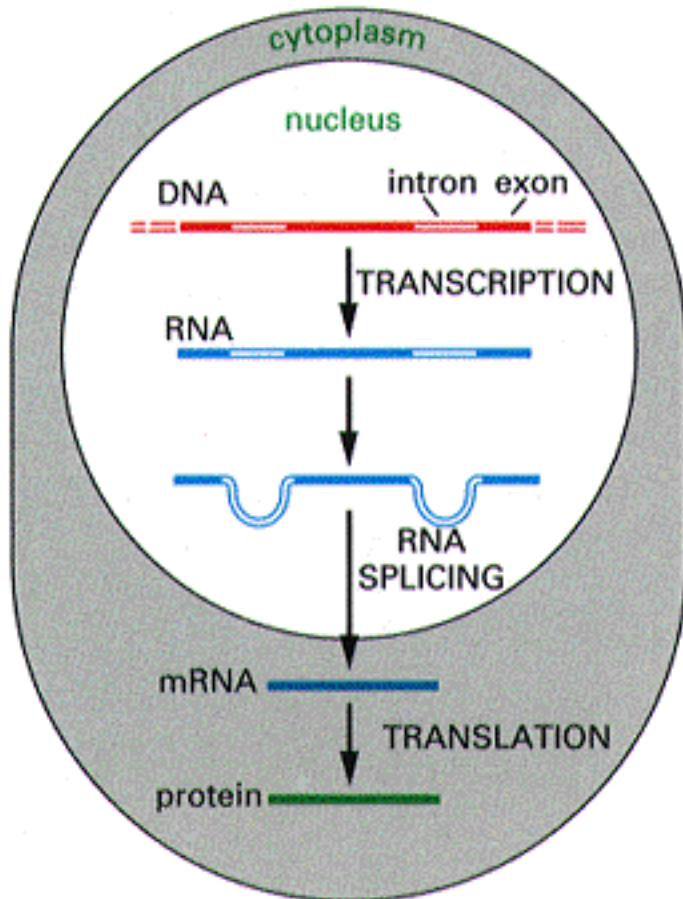


DNA, RNA in proteini

Prvo poglavje: Human Molecular
Genetics, 4. izdaja (Strachan in Read)

Pretok genetske informacije

EUCARYOTES



- Genetska informacija potuje iz **DNA** do **RNA** in nato v **protein**.
- **Geni** so osnovne informacijske enote DNA in proteini, ki jih geni kodirajo, so osnovne funkcijske enote v celici.
- V jedru se s procesom **transkripcije** prepíše predhodnik mRNA, **pre-mRNA**.
- Nato se pre-mRNA procesira do **mRNA**.
- mRNA gre nato iz jedra v citoplazmo, kjer se s procesom **translacije** prepíše v **protein**.

1962: Nobelova nagrada za fiziologijo in medicino

Watson, J.D. and F.H. Crick, "Molecular Structure of Nucleic Acids: A Structure for Deoxynucleic Acids". *Nature* 171 (1953), p. 738.



James D.
Watson



Francis H.
Crick



Maurice H. F.
Wilkins



2013: DNA - trdi disk prihodnosti

LETTER

doi:10.1038/nature11875

Towards practical, high-capacity, low-maintenance information storage in synthesized DNA

Nick Goldman¹, Paul Bertone¹, Siyuan Chen², Christophe Dessimoz¹, Emily M. LeProust², Botond Sipos¹ & Ewan Birney¹

Digital production, transmission and storage have revolutionized how we access and use information but have also made archiving an increasingly complex task that requires active, continuing maintenance (ASCII text), giving a total of 757,051 bytes or a Shannon information¹⁰ of 5.2×10^6 bits (see Supplementary Information and Supplementary Table 1 for full details).

- V DNA so zakodirali štiri različne oblike računalniških zapisov:
 - tekstovno (.txt) datoteko vseh 154 Shakespearejevih sonetov,
 - 26-sekundni zvočni (.mp3) zapis znamenitega govora Martina Luthra Kinga ml. I Have a Dream,
 - digitalni (.jpg) barvni posnetek njihovega laboratorija v Hinxtonu,
 - digitalni (.pdf) zapis znanstvene razprave Jamesa Watsona in Francisa Cricka iz leta 1953, v katerem sta »očeta sodobne molekularne biologije« prvič opisala strukturo DNK, ter
 - datoteko, ki pojasnjuje kodiranje.

Gradbeni elementi

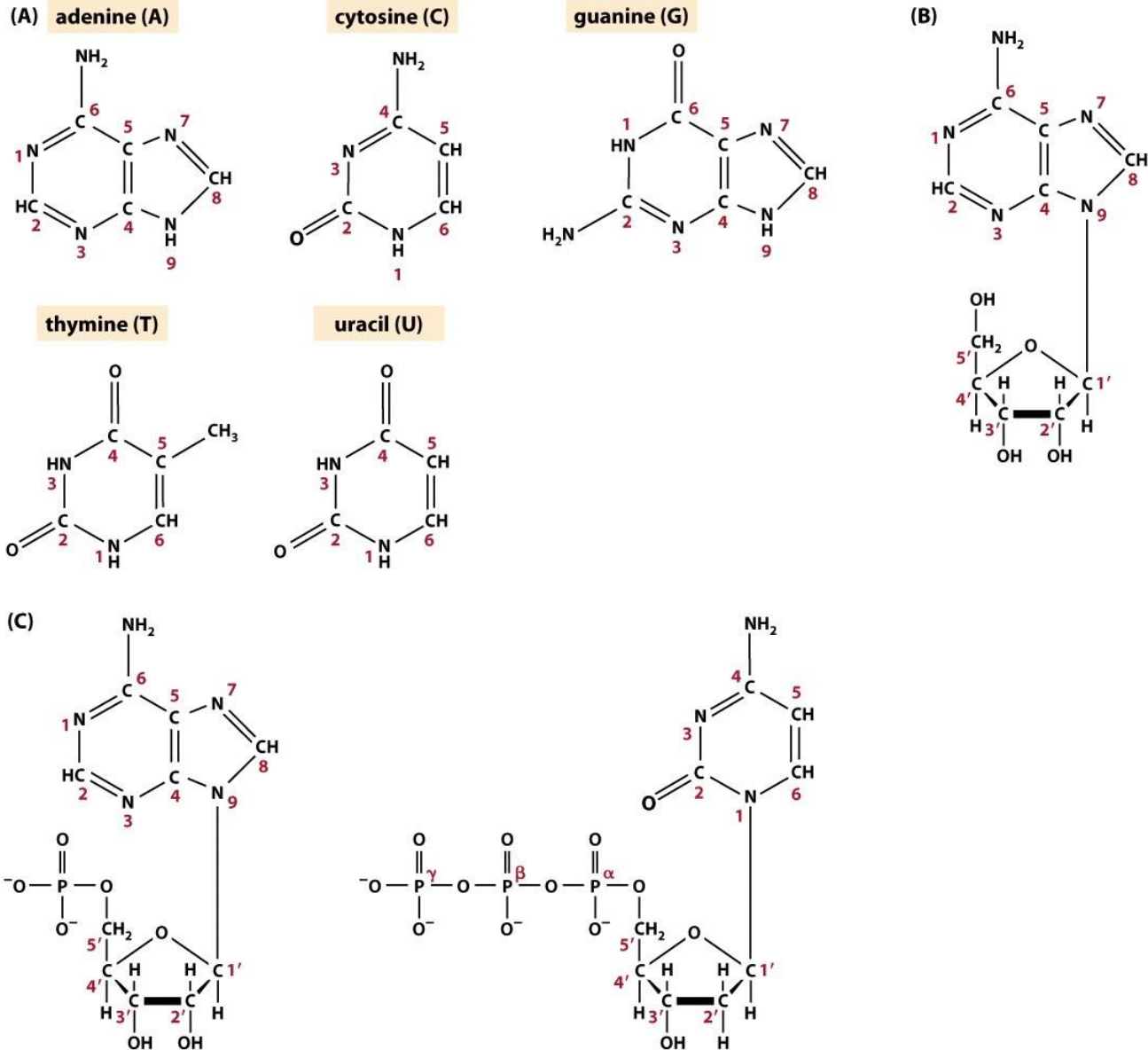
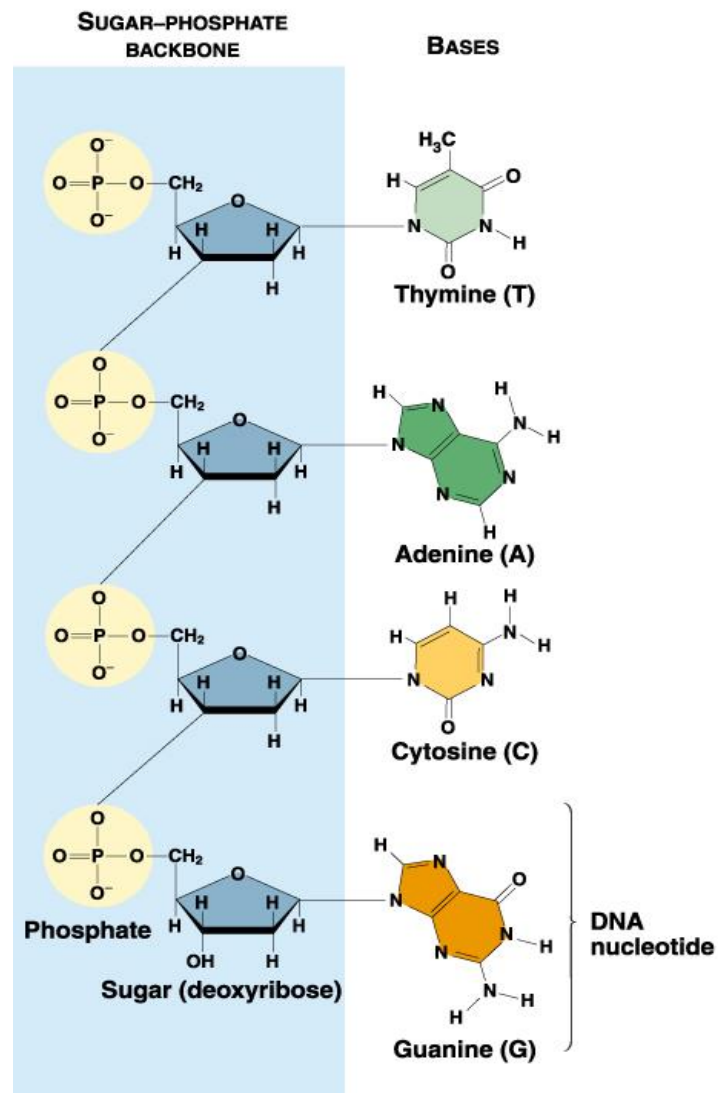


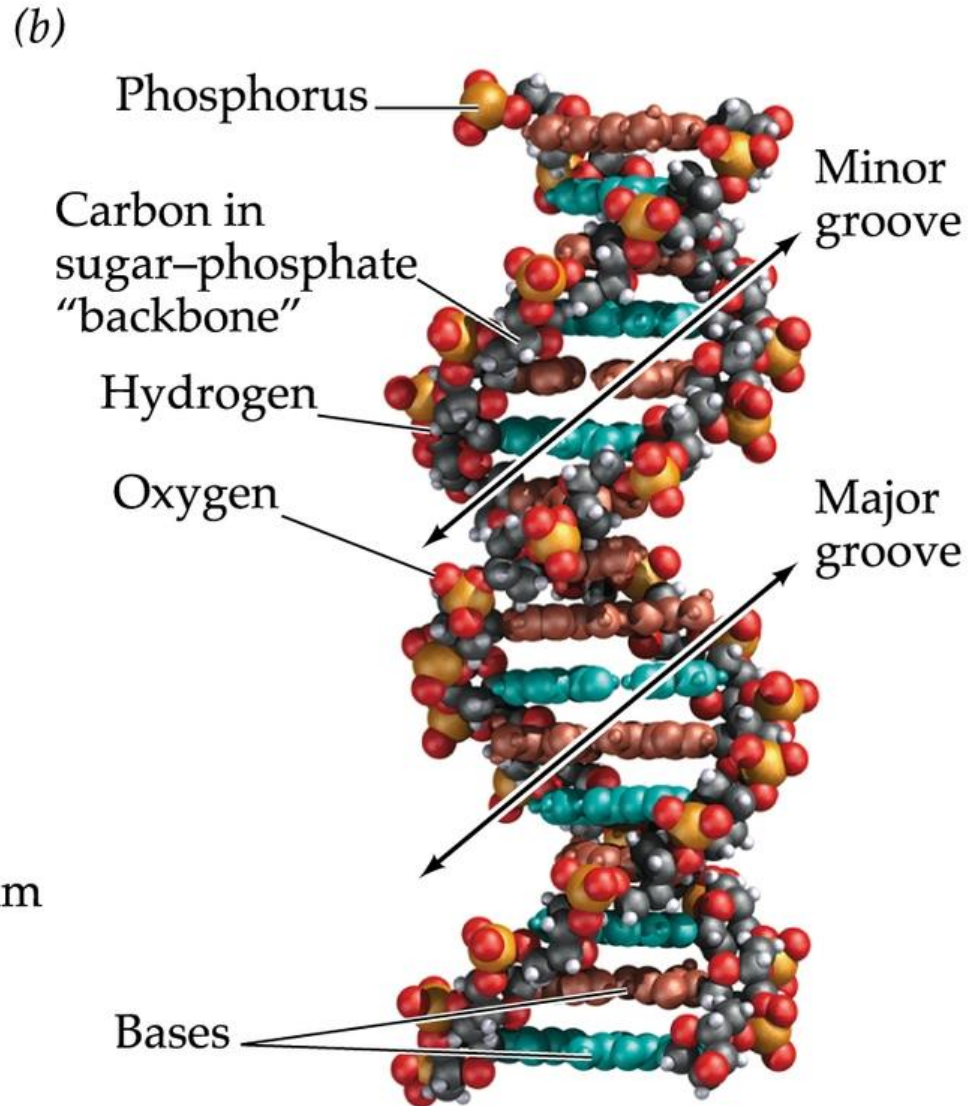
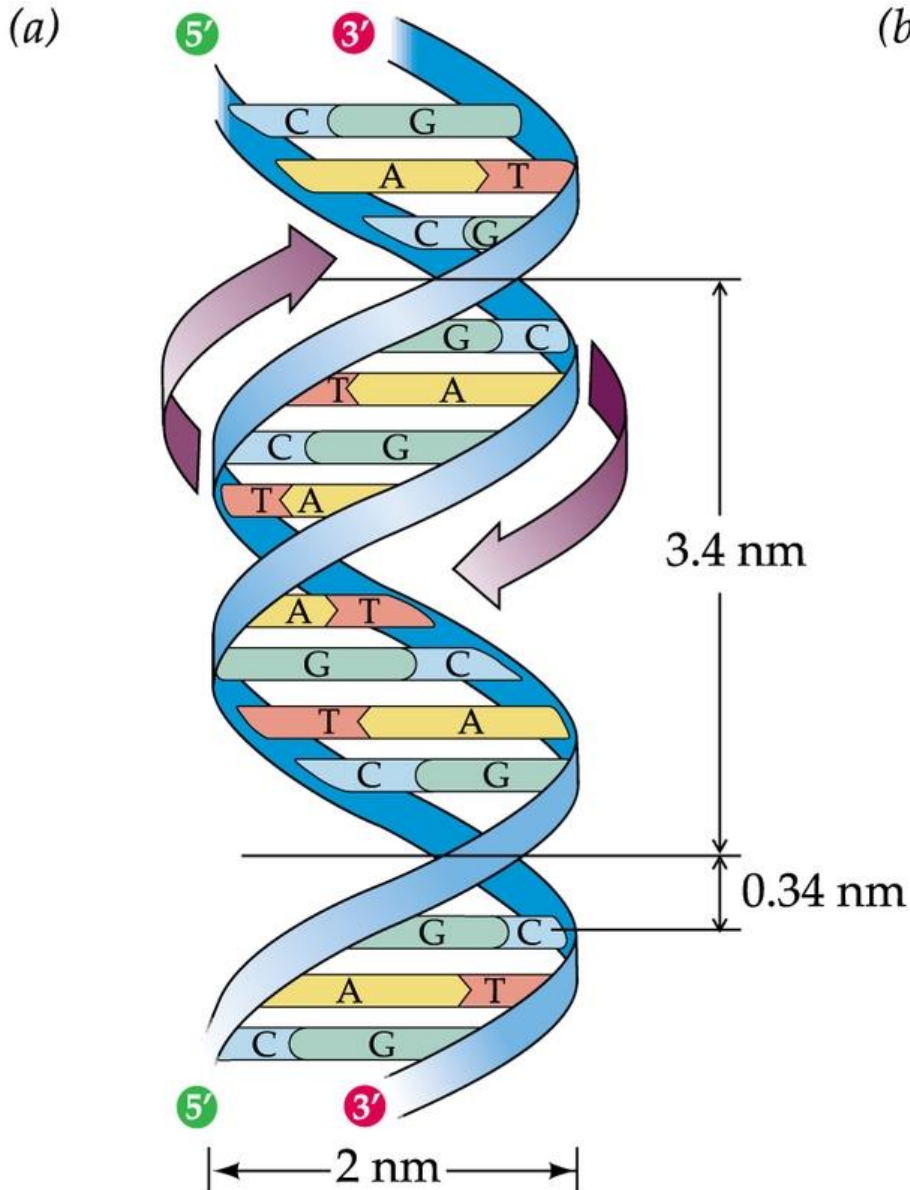
Figure 1.2 Human Molecular Genetics, 4ed. (© Garland Science)

Struktura DNA

- DNA je sestavljena iz štirih baz: adenina, citozina, timina, in gvanina.
- Število A = T, G = C, ter purinov = pirimidinov [Chargaffovo pravilo].
- DNA je dvovertična vijačnica z antiparalelnimi verigami [Watson in Crick].
- V vsaki verigi so baze med sabo povezane s 5'-3' fosfodiesterskimi vezmi.
- Baze na nasprotujočih se verigah so med sabo vezane z vodikovimi vezmi: A s T in G s C.

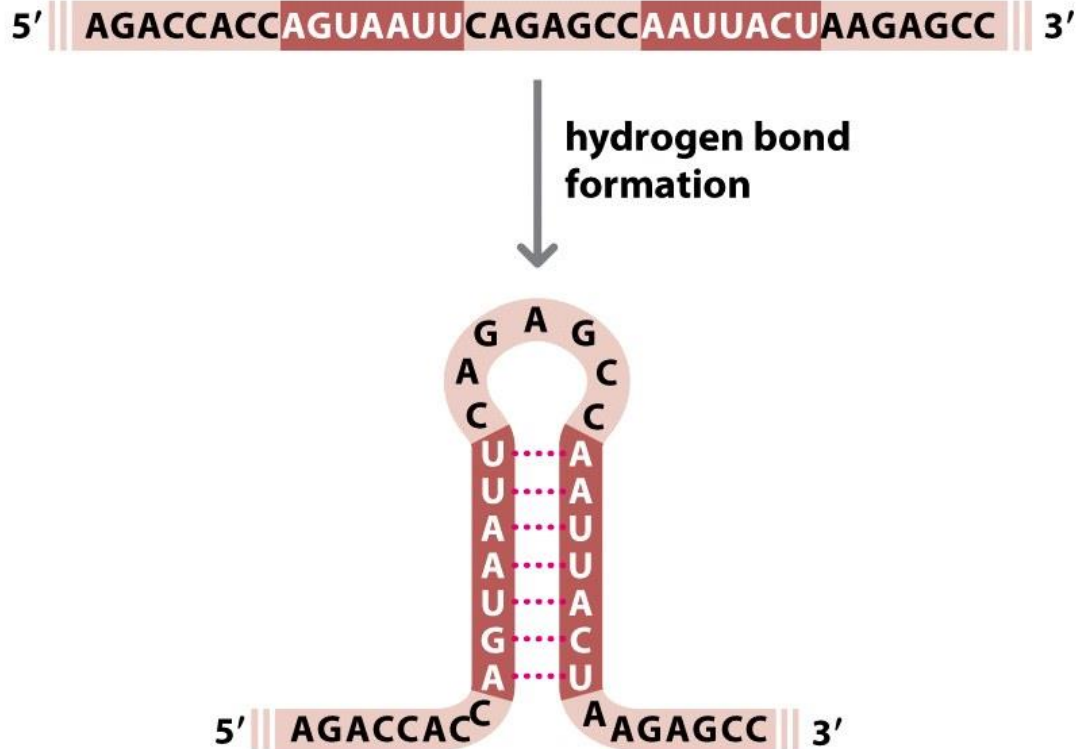


Strukturne lastnosti DNA



Parjenje baz v enojni verigi

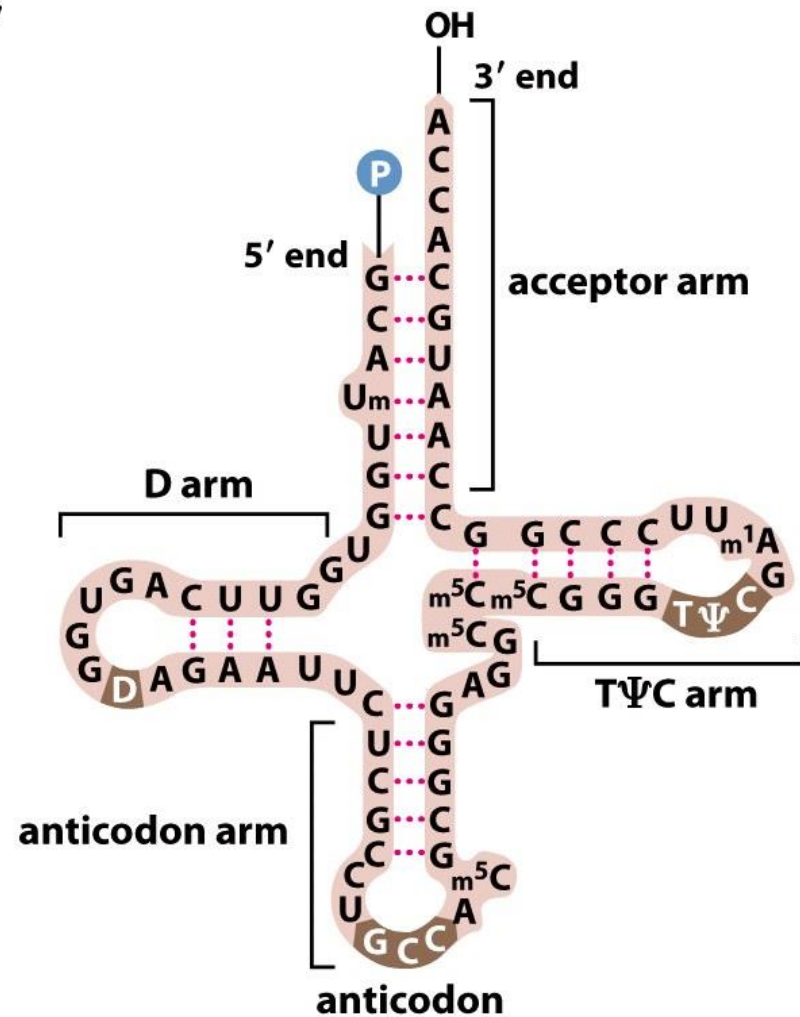
(A)



(A) Lasnična zanka s tvorbo vodikovih vezi znotraj verige - zanka z dvovijačnim stebrom

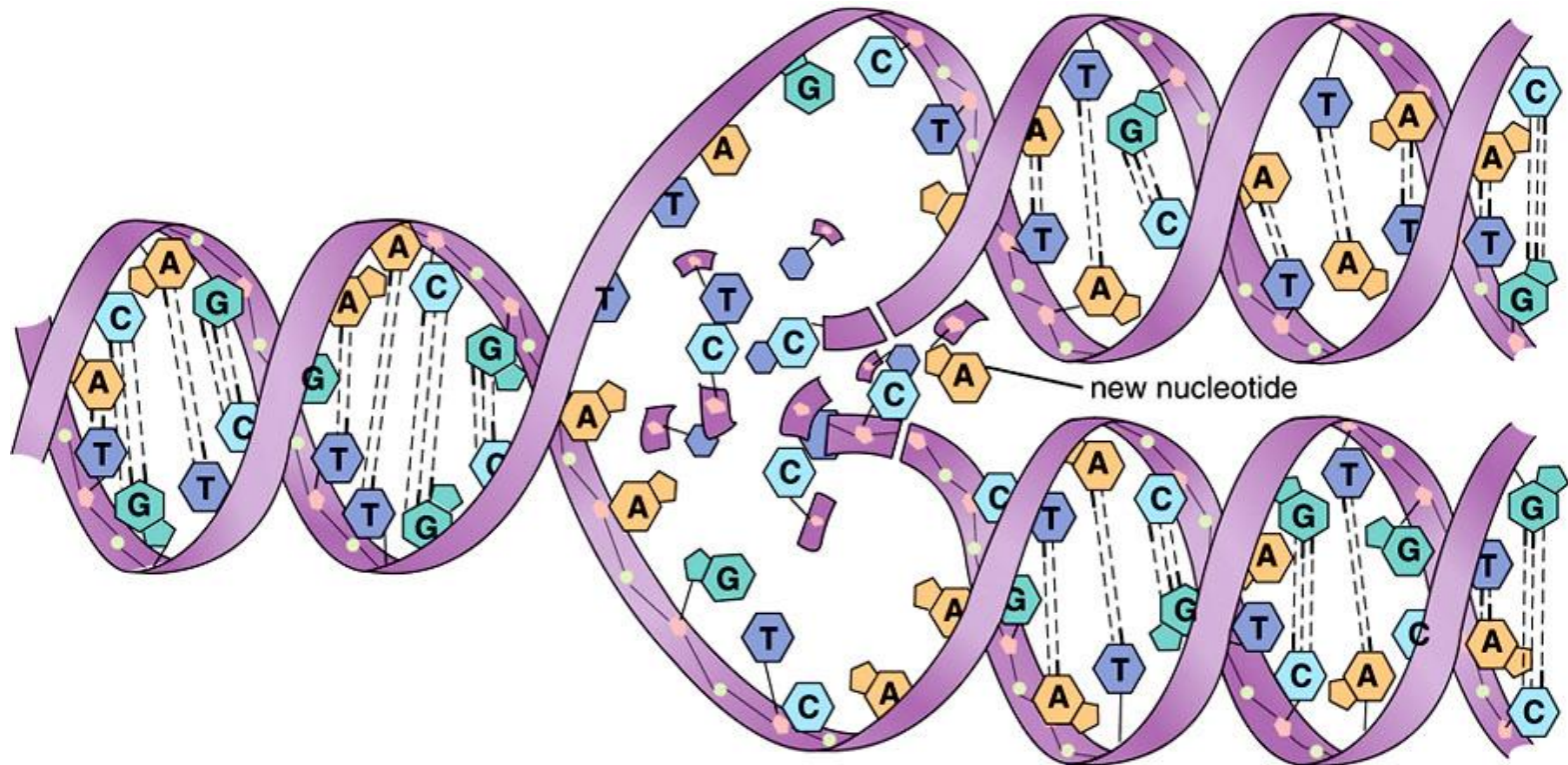
(B) Povečano parjenje znotraj verige omogoča bolj kompleksne strukture kot je tRNA.

(B)



Podvojevanje DNA

- Vsaj 5 bilijonov delitev celic je potrebnih, da se iz oplojenega jajčeca razvije odrasli človek. Pri tem nastane več kot 20 milijard metrov DNA!
- Podvojevanje DNA je izjemno hitro in natančno.
- Pred podvojevanjem je dvojno vijačnico potrebno odviti.
- Pri tem sodeluje veliko število encimov in drugih proteinov.



5 klasičnih DNA polimeraz

- Prikazani so katalitski (rdeče) in eksonukleazni (vijolčasto) motivi.
- Pomanjkanje Pol α lahko vodi v sindrom N (mentalna retardacija) in levkemijo T celic.
- Mutacije Pol δ so povezane z rakom debelega črevesja.
- Mutacije Pol β so ugotovljene pri približno 30% rakov.

a

Polymerase	Gene	Catalytic subunit	Accessory subunits (kDa)	3'→5' exonuclease	Fidelity	Primary function
Pol α	<i>POLA1</i>	1462 aa	49 <i>PRIM1</i> 58 <i>PRIM2A</i> 70 <i>PRIM2B</i>	No	10^{-4} – 10^{-5}	RNA and/or DNA primers
Pol β	<i>POLB</i>	335 aa	None	No	5×10^{-4}	Base-excision repair
Pol γ	<i>POLG1</i>	1239 aa	55 <i>POLG2</i>	Yes	10^{-5}	Mitochondrial DNA replication and repair
Pol δ	<i>POLD1</i>	1107 aa	50 <i>POLD2</i> 68 <i>POLD3</i> 12 <i>POLD4</i>	Yes	10^{-5} – 10^{-6}	Lagging-strand synthesis DNA repair
Pol ϵ	<i>POLE</i>	2286 aa	59 <i>POLE2</i> 12 <i>POLE4</i> 17 <i>POLE3</i>	Yes	10^{-6} – 10^{-7}	Leading-strand synthesis

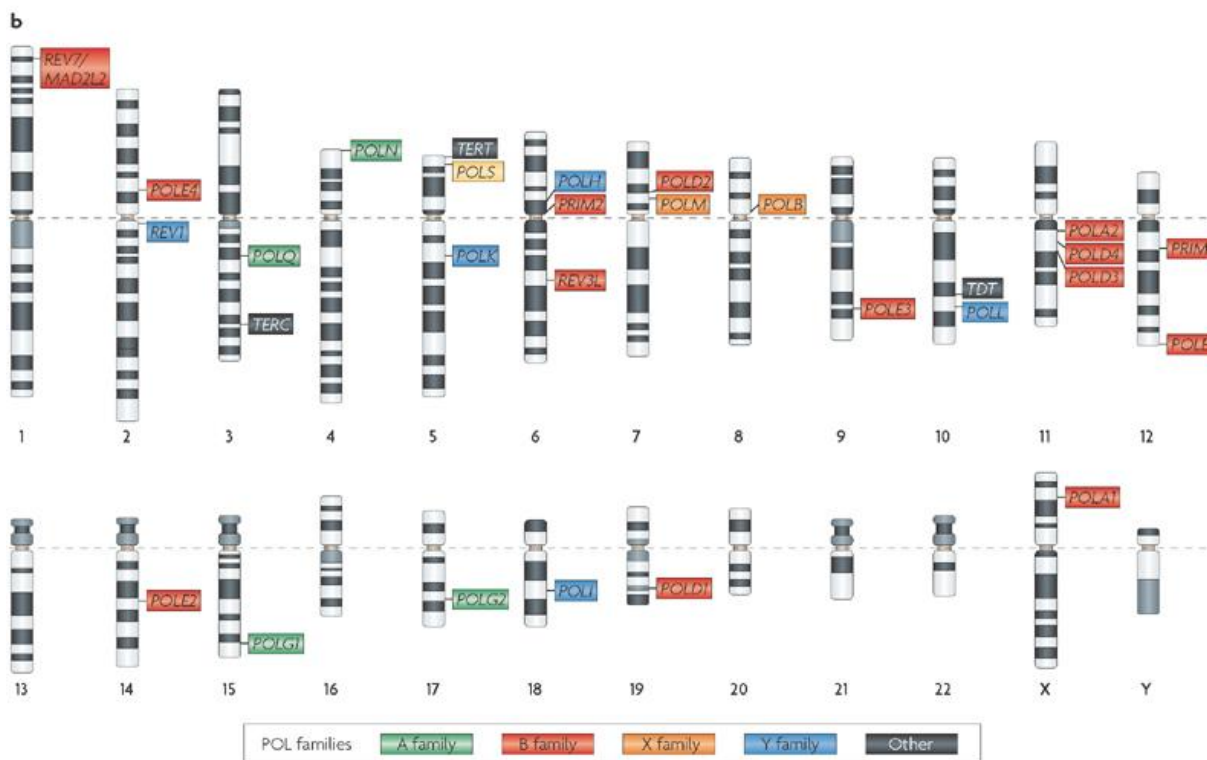


TABLE 1.3 MAMMALIAN DNA POLYMERASES

DNA-DIRECTED DNA POLYMERASES			
Polymerase	Family	Standard DNA replication	Additional or alternative roles in DNA repair, recombination, etc.
α (alpha)	B	initiates synthesis at replication origins and initiates synthesis of Okazaki fragments on lagging strand	
β (beta)	X		base excision repair ^b
γ (gamma)	A	mitochondrial DNA synthesis	mitochondrial DNA repair
δ (delta)	B	main polymerase that synthesizes lagging strand	multiple roles in DNA repair
ϵ (epsilon)	B	synthesizes leading strand	multiple roles in DNA repair
ζ (zeta)	B		translesion synthesis ^c
η (eta)	Y		translesion synthesis ^c
θ (theta)	A		possible role in interstrand crosslink repair ^d ; base excision repair ^b ; translesion synthesis ^c ; somatic hypermutation ^g
ι (iota)	Y		translesion synthesis ^c ; possible roles in base excision repair ^b and mismatch repair ^e
κ (kappa)	Y		translesion synthesis ^c ; nucleotide excision repair ^f
λ (lambda)	X		double-strand break repair; VDJ recombination ^g ; base excision repair ^b
μ (mu)	X		
ν (nu)	A		possible role in interstrand crosslink repair ^d
Rev1	Y		translesion synthesis ^c
TdT ^a	X		VDJ recombination ^g
RNA-DIRECTED DNA POLYMERASES (REVERSE TRANSCRIPTASES)			
Interspersed repeat reverse transcriptases (LINE-1 or endogenous retrovirus elements)		occasionally converts mRNA and other RNA into cDNA, which can integrate elsewhere into the genome	
Telomerase reverse transcriptase (Tert)		replicates DNA at the ends of linear chromosomes	

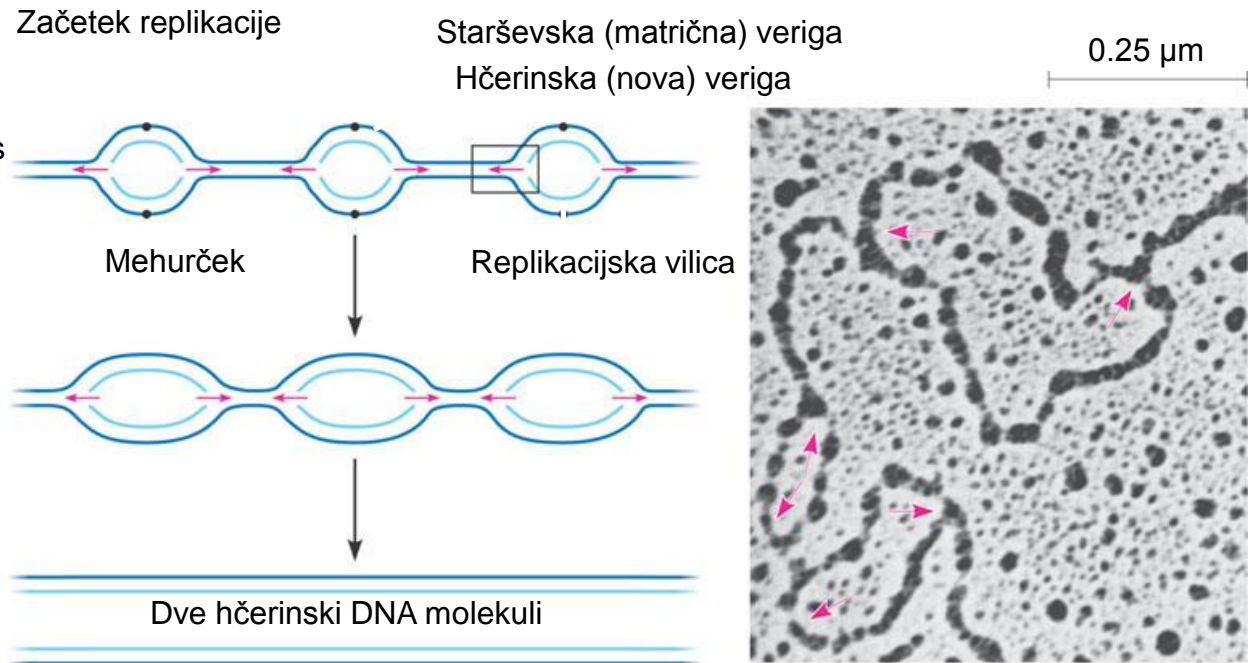
^aTerminal deoxynucleotide transferase. ^bBase excision repair identifies and removes inappropriate bases or inappropriately modified bases.

^cTranslesion synthesis involves the replication of DNA past damaged DNA (lesions) on the template strand. ^dInterstrand crosslink repair is the repair of highly cytotoxic lesions where covalent DNA bonds have been formed between the DNA strands. ^eMismatch repair is a form of DNA repair that corrects mistakes arising when noncomplementary nucleotides form a base pair. ^fNucleotide excision repair is used to fix helix-distorting lesions.

^gSomatic hypermutation and VDJ recombination are mechanisms used in B cells to diversify immunoglobulin sequences.

Začetki podvojevanja

- Podvojevanje DNA se začne na posebnih mestih, kjer se dve verigi ločita.
- Evkariontski kromosom ima lahko več sto do več tisoč začetkov podvojevanja.



1 Replication begins at specific sites where the two parental strands separate and form replication bubbles.

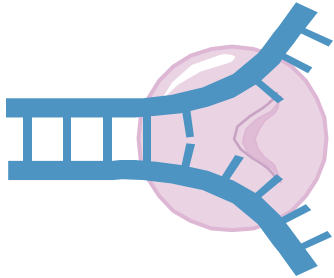
2 The bubbles expand laterally, as DNA replication proceeds in both directions.

3 Eventually, the replication bubbles fuse, and synthesis of the daughter strands is complete.

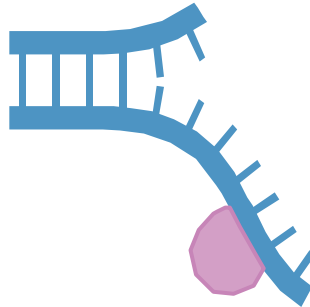
(a) In eukaryotes, DNA replication begins at many sites along the giant DNA molecule of each chromosome.

(b) In this micrograph, three replication bubbles are visible along the DNA of a cultured Chinese hamster cell (TEM).

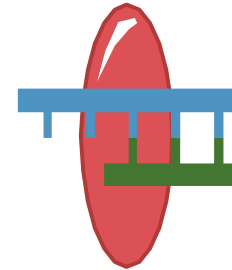
Encimi replikacije DNA



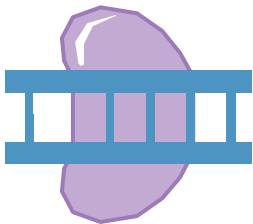
Helikaza razvije starševsko dvojno vijačnico.



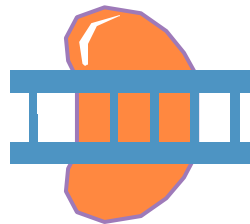
Vezavni proteini RPA stabilizirajo enojno verigo.



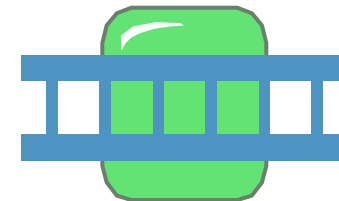
DNA pol α dodaja kratek začetnik na matrično verigo ter odstrani isti začetnik in zapolni z točnimi bazami.



DNA polimeraza δ tvori novo verigo na zastajajoči starševski verigi.

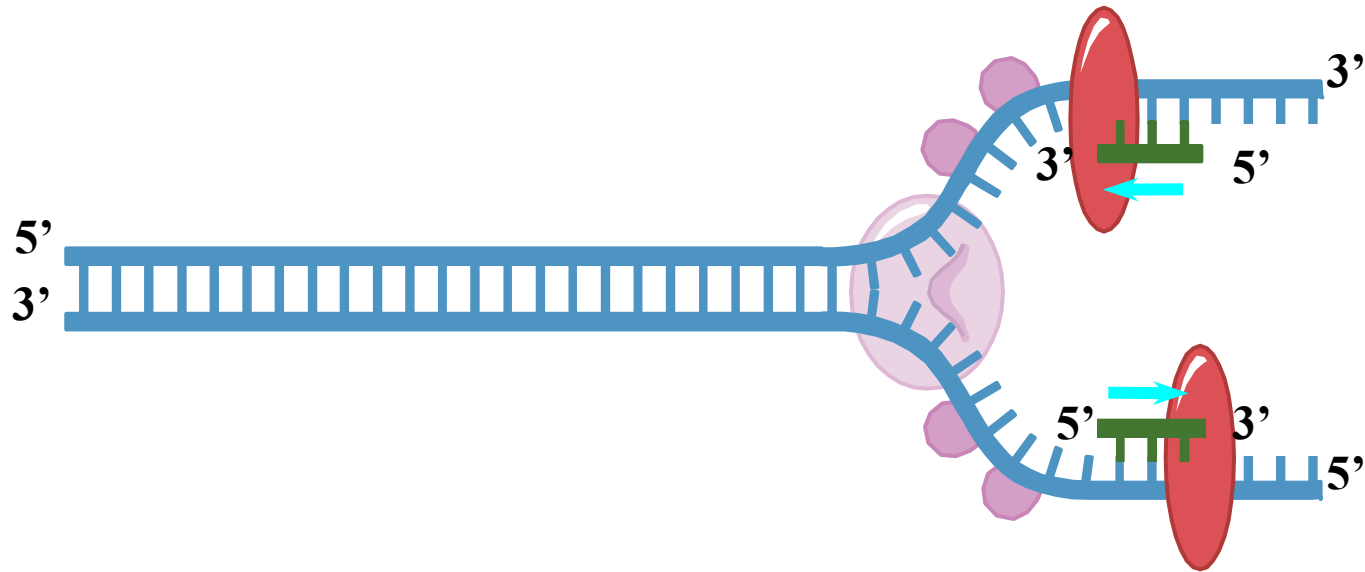


DNA polimeraza ϵ tvori novo verigo na vodilni starševski verigi.



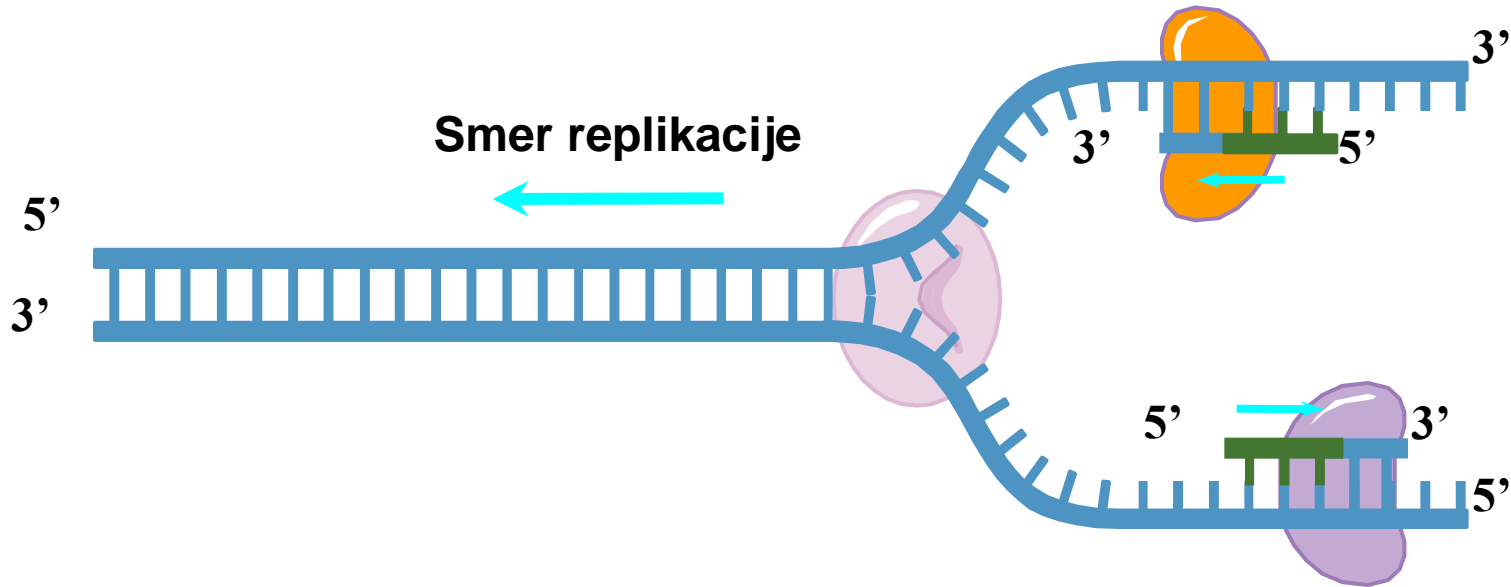
Ligaza združi Okazaki fragmente ter popravi ostale prekinitve.

Replikacija



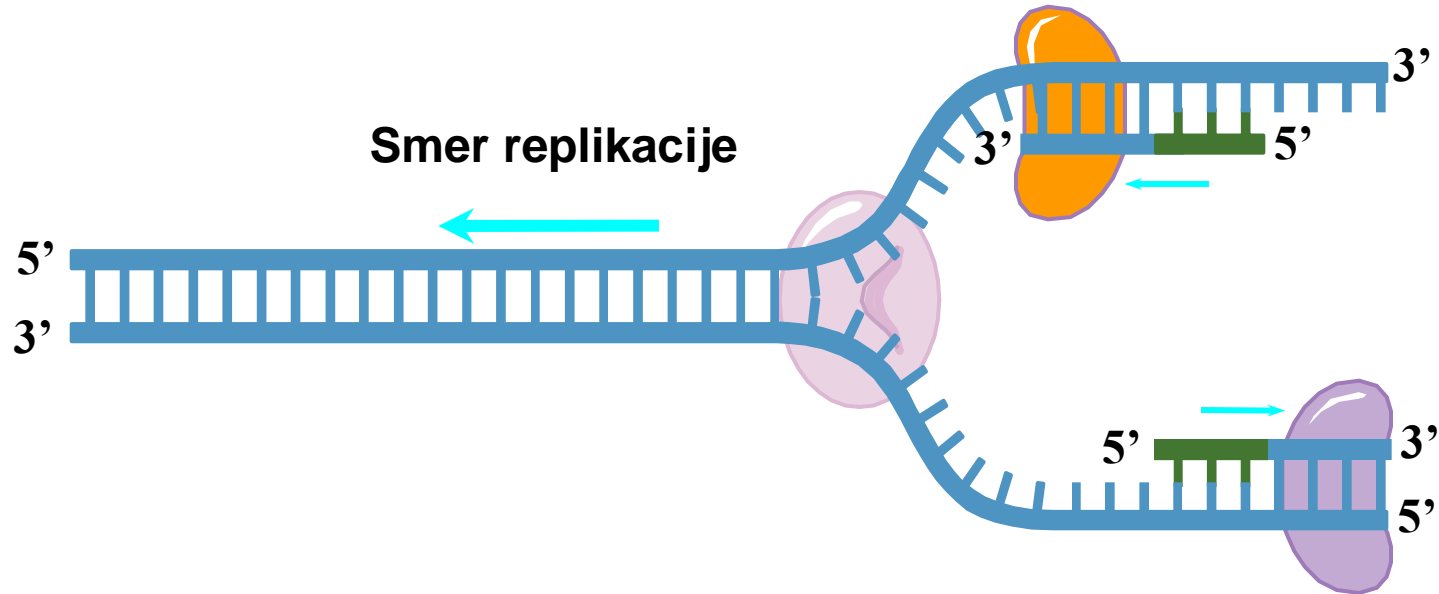
- Helikaza se veže na mesto začetka replikacije in prične razvijati starševsko DNA.
- RPA proteini stabilizirajo enojni verigi.
- DNA pol α naredi kratek komplementaren RNA oligonukleotid.

Replikacija



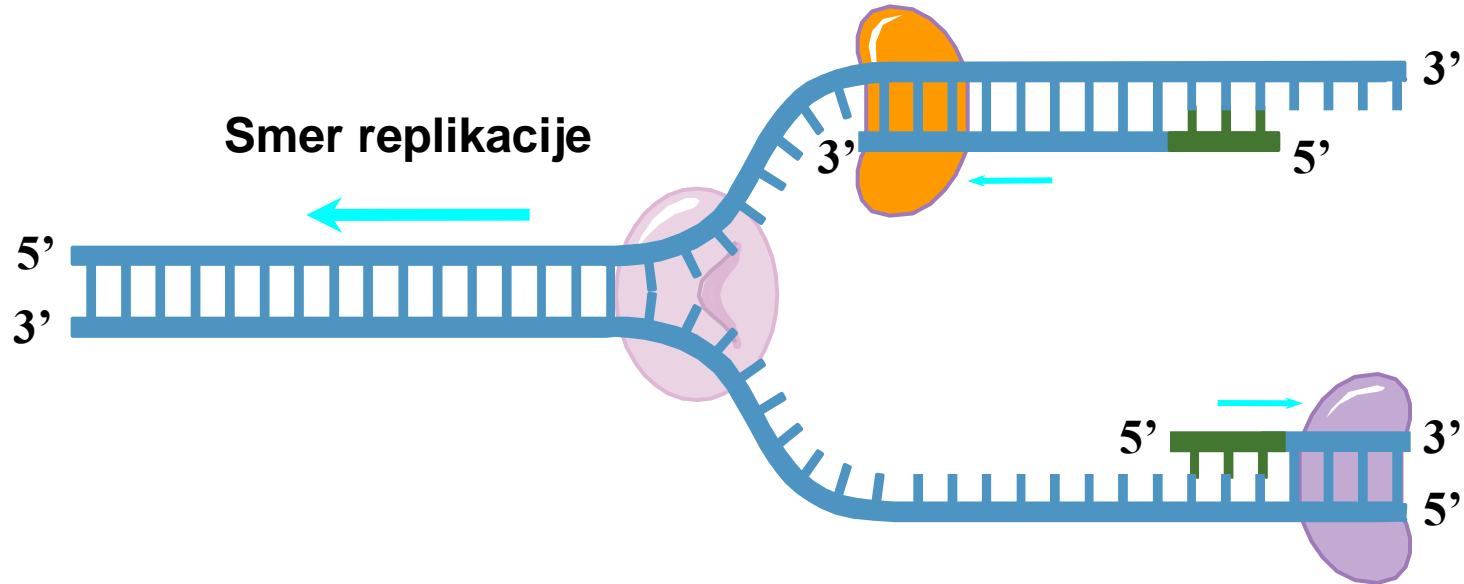
DNA polymerazi ϵ in δ dodata DNA nukleotide na RNA oligonukleotidni začetnik.

Replikacija



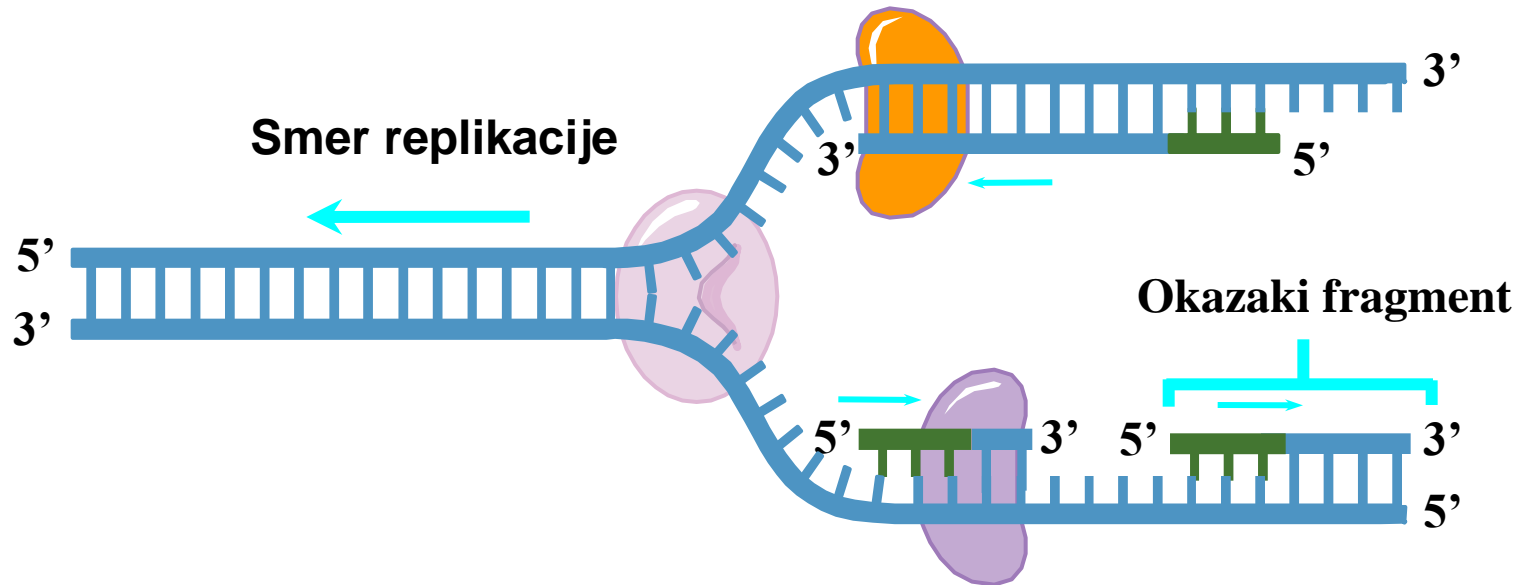
DNA polimerazi ϵ in δ preverita natančnost parjenja baz ter popravita možne napake.

Replikacija



Sinteza vodilne verige se nadaljuje v smeri 5' proti 3'.

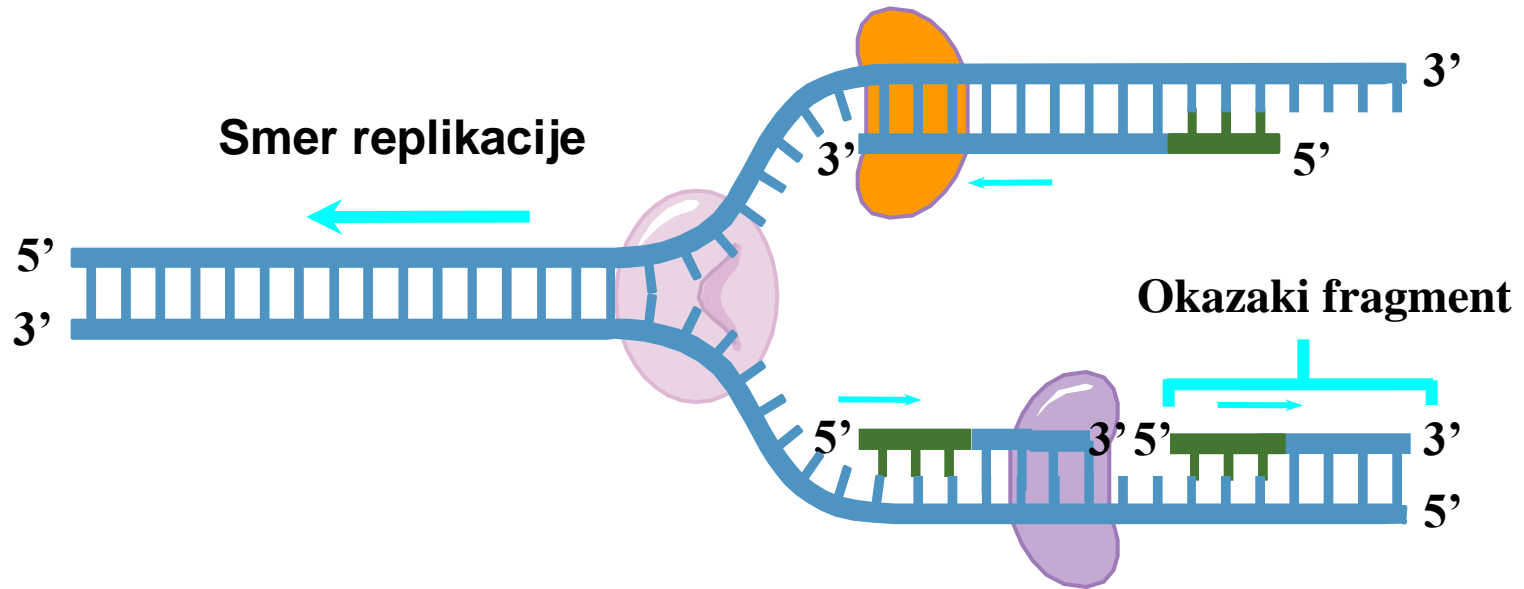
Replikacija



Sinteza vodilne verige se nadaljuje v smeri 5' proti 3'.

Na zastajajoči verigi se tvorijo Okazakijevi fragmenti.

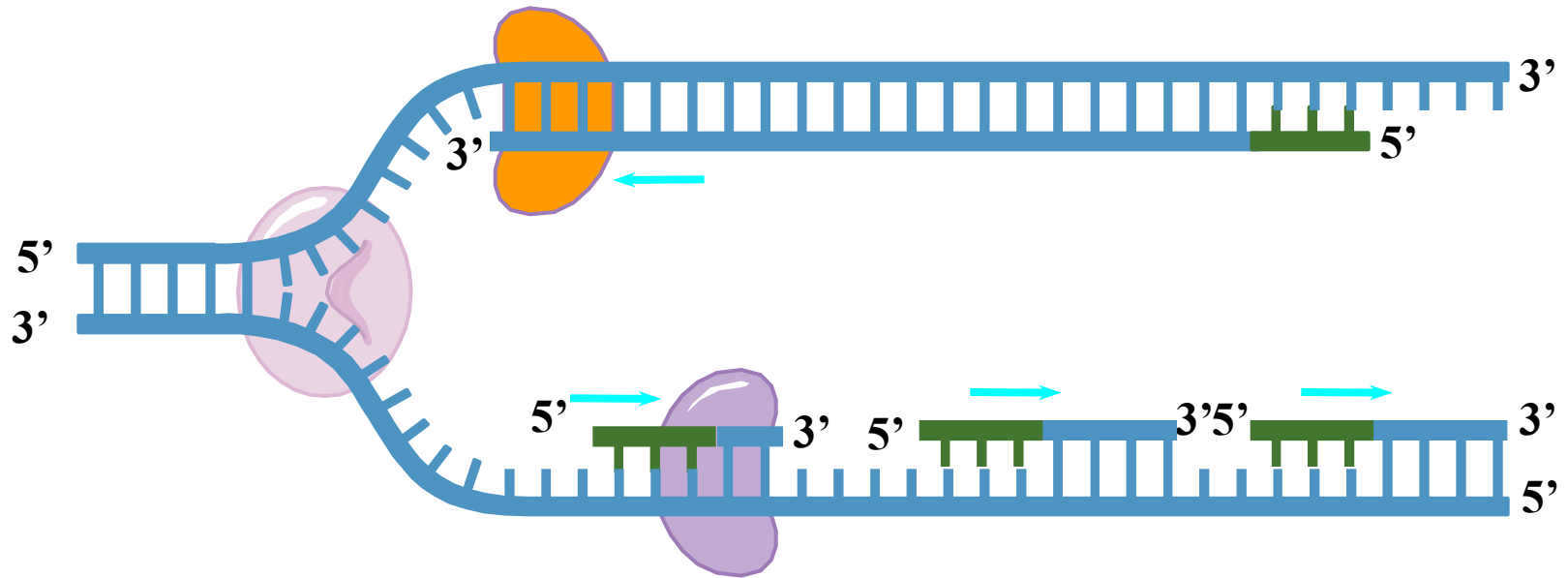
Replikacija



Sinteza vodilne verige se nadaljuje v smeri 5' proti 3'.

Na zastajajoči verigi se tvorijo Okazakijevi fragmenti.

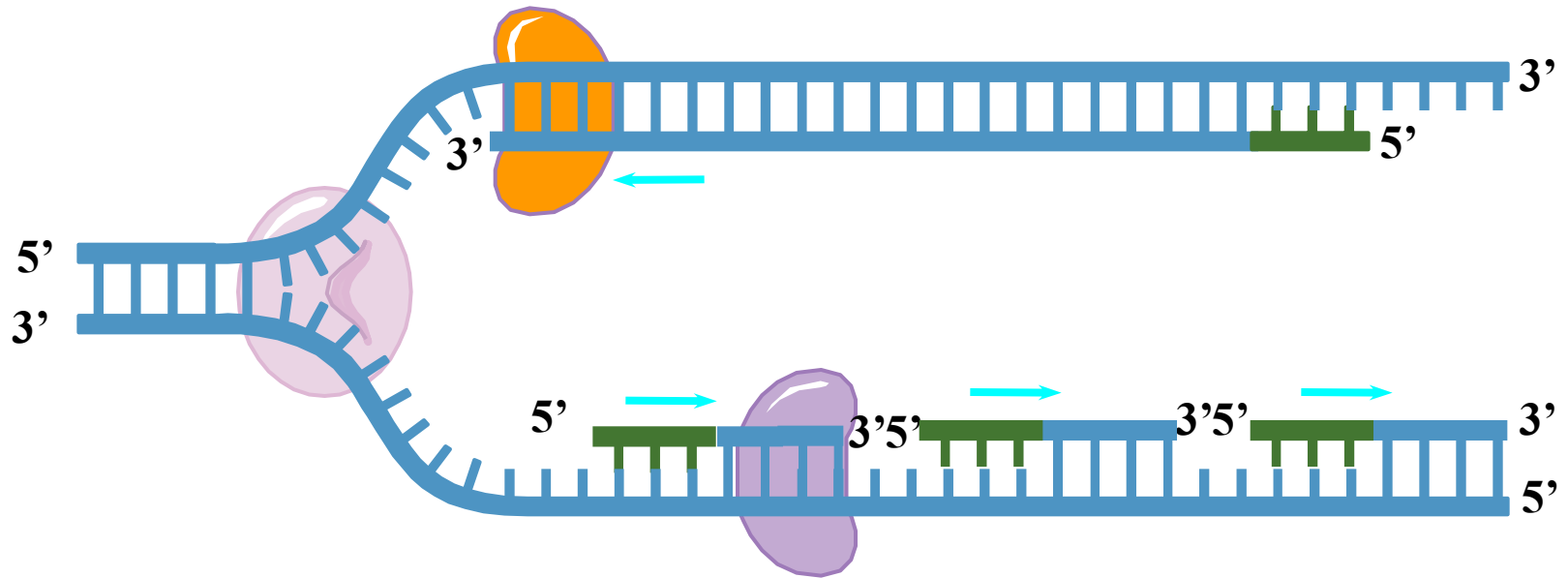
Replikacija



Sinteza vodilne verige se nadaljuje v smeri 5' proti 3'.

Na zastajajoči verigi se tvorijo Okazakijevi fragmenti.

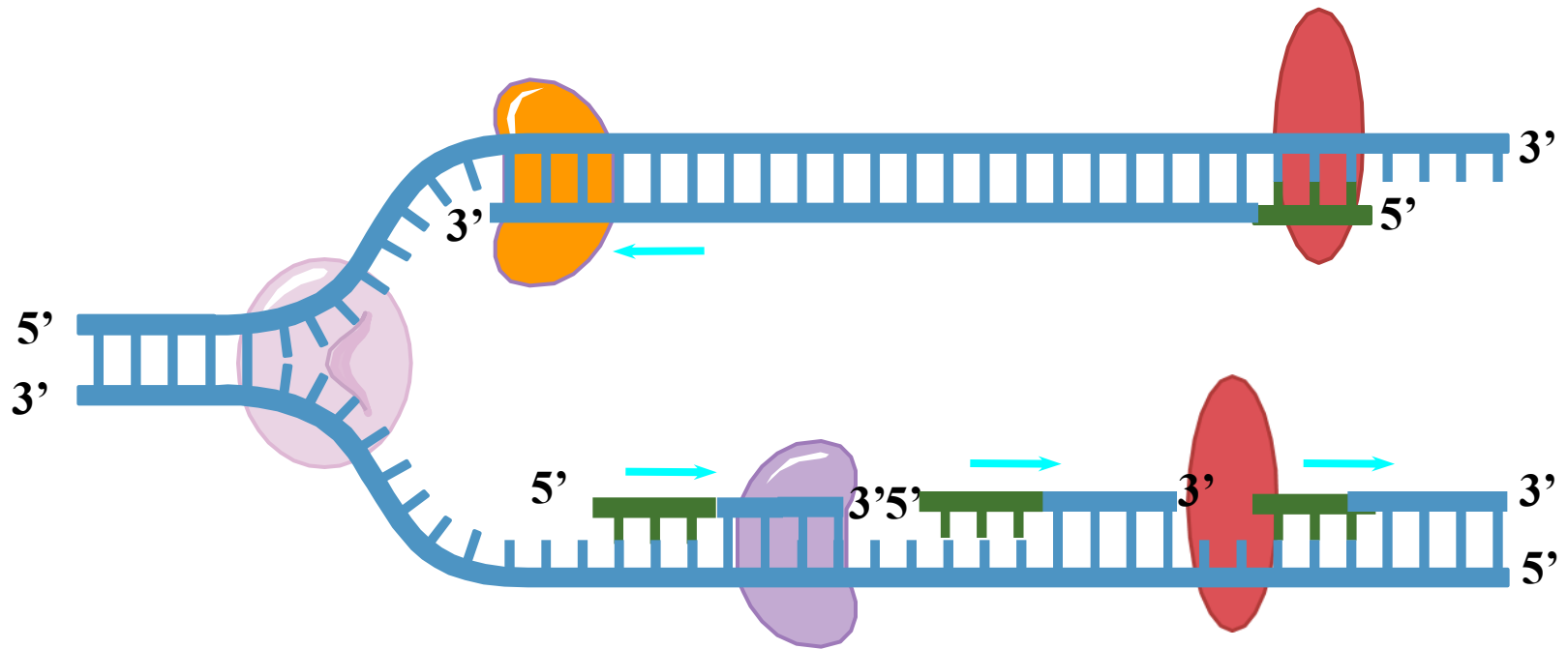
Replikacija



Sinteza vodilne verige se nadaljuje v smeri 5' proti 3'.

Na zaostajajoči verigi se tvorijo Okazakijevi fragmenti.

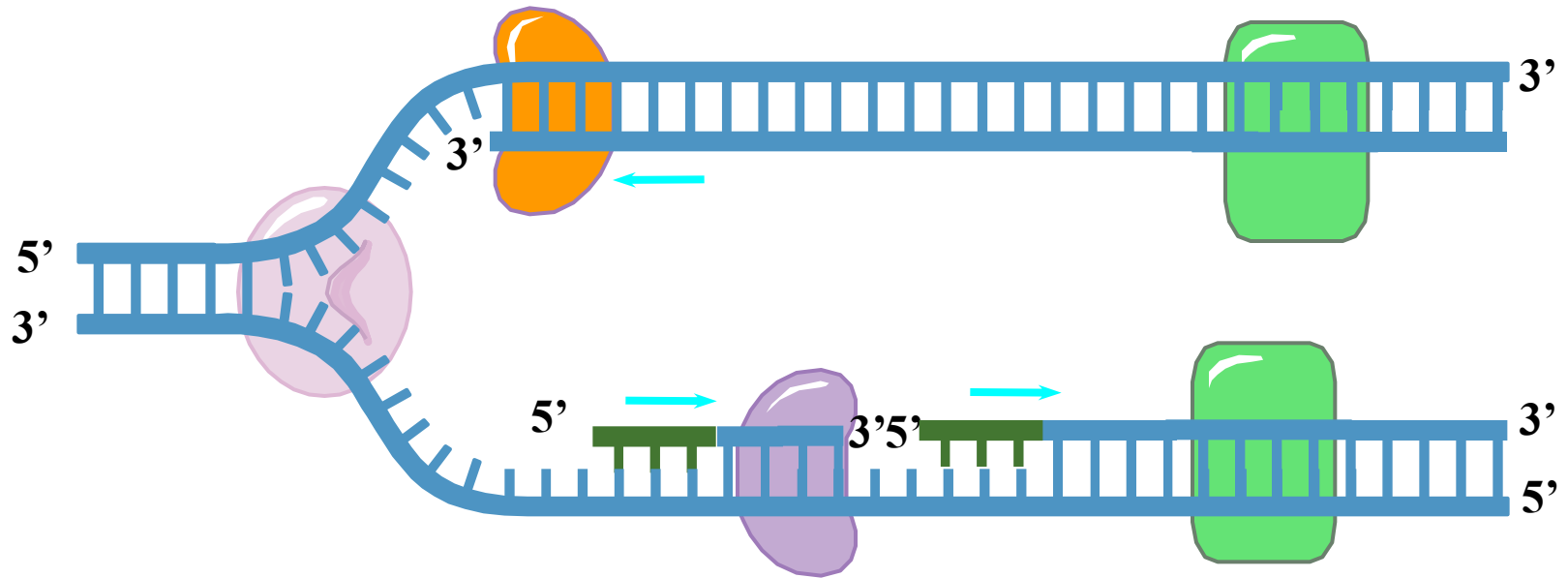
Replikacija



Eksonukleazno delovanje DNA polimeraze α odstrani RNA oligonukleotidni začetnik.

Polimerazno delovanje DNA polimeraze α zapolni nastale vrzeli.

Replikacija

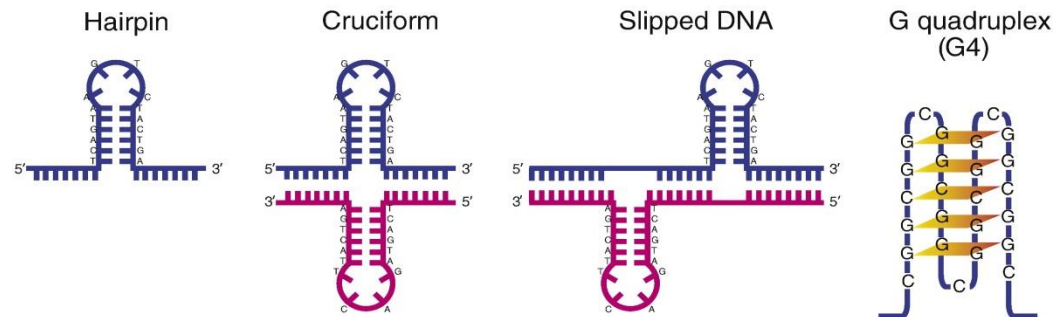
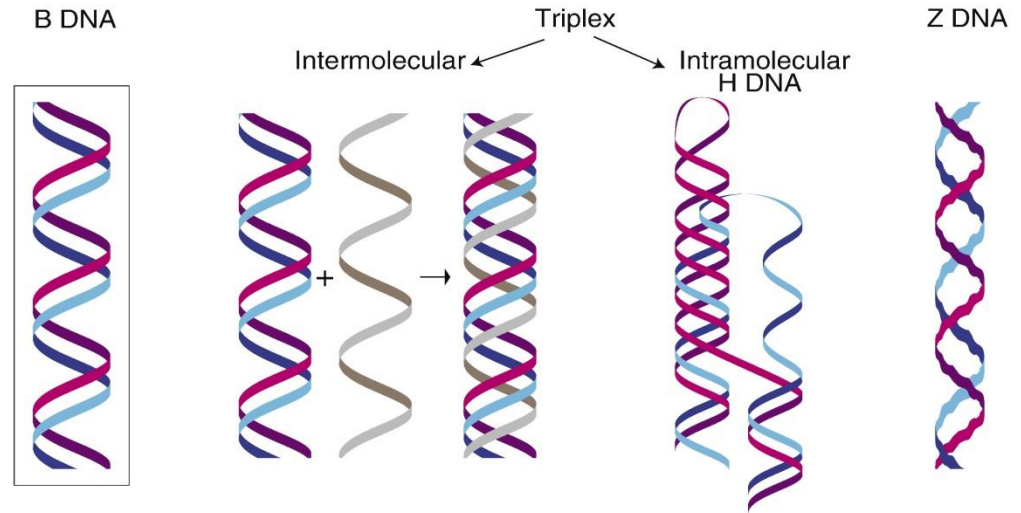


Ligaza združi Okazakijeve fragmente na zastajajoči verigi.

Pregled replikacije

Replikacija nestandardnih oblik DNA

- Poznamo preko deset alternativnih struktur DNA.
- Alternativne strukture pripeljejo do upočasnitve ali celo ustavljanja replikacijske vilice.



Replikacija nestandardnih oblik DNA

Table 1. DNA sequences associated with non-B DNA conformations

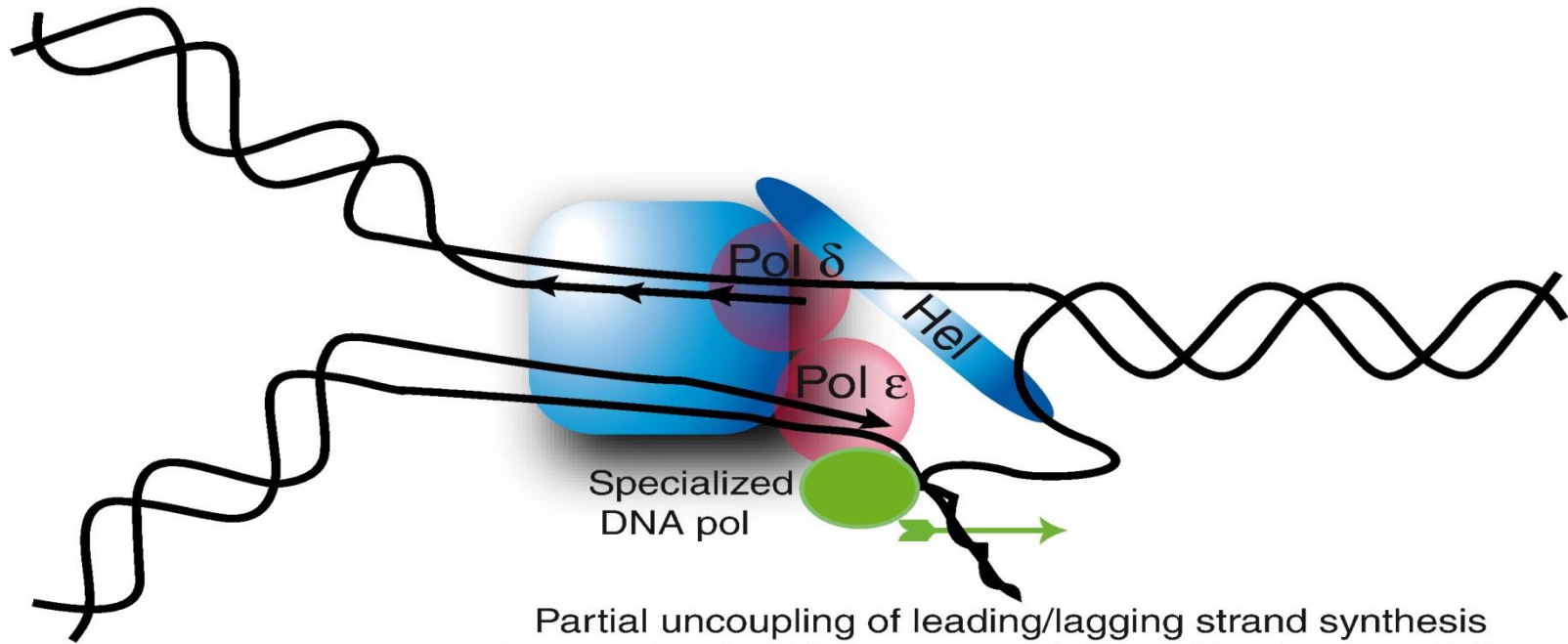
Sequence	Non-B DNA often formed	
AT rich	5' ATTTCACTTA 3' 3' TAAAGTGAAT 5'	Unwound DNA
Inverted repeats (IR)	5' TCGGTACCGA 3' 3' AGCCATGGCT 5'	Hairpins Cruciforms
Mirror repeats (MR)	5' GATCA ACTAG 3' 3' CTAGT TGATC 5'	Triplex: intramolecular (H DNA) Intermolecular
Direct repeats (DR)	(AT) _n , dinucleotide repeats (CAG) _n , trinucleotide repeats (<10 nt) _n , microsatellites, direct tandem repeats (>10 nt) _n , minisatellites	Z DNA Slipped DNA G-quadruplex (G4)

- Več kot 50% človeškega genoma vsebuje ponavljajoča se DNA zaporedja kot so obrnjene ponovitve, zrcalne ponovitve, direktne tandemske ponovitve ter mikro- in minisatelite.
- Zaporedja s ponavljajočo DNA so pomembna mesta genetske nestabilnosti.

Replikacija nestandardnih oblik DNA

- Fragilni (krhki, lomljivi) lokusi so lahko redki ali pogosti. RFS („rare fragile site“) so prisotna samo v 5 % populacije. CFS („common fragile site“) so prisotna pri vsakem osebkju na različnih lokacijah na skoraj vsakem kromosomu.
- Pri človeških limfocitih so mapirali 200 CFS.
- CFS so znana mesta kromosomskih prerazporeditev.
- RFS so povezana z mentalnimi retardacijami. Npr. CGG trinukleotidna ponovitev na FRAXA in FRAXE lokusih gena FRM1/2 je povezana s sindromom fragilnega X kromosoma.
- Več kot 40 nevrodegenerativnih bolezni je povezanih z trinukleotidnimi ponovitvami. Ponovitve so dokazane tudi pri rakah prostate in jajčnikov.
- Mikrosateliti so povezani s 30 dednimi boleznimi.

Replikacija nestandardnih oblik DNA



Partial uncoupling of leading/lagging strand synthesis
Intra-molecular folding on ssDNA, resulting in non-B DNA

- DNA helikaze sodelujejo tudi pri odvijanju nestandardnih oblik DNA.
- Helikaze družine RecQ so pomembne pri odvijanju G-kvadrupleksov. Mutacije WRN, BLM in RECQ4 so glavni vzrok za nekatere redke genetske bolezni (Wernerjev sindrom, Bloomov sindrom in Rothmund-Thomson/Rapadilino/Baller-Gerold sindrom).

Replikacija nestandardnih oblik DNA

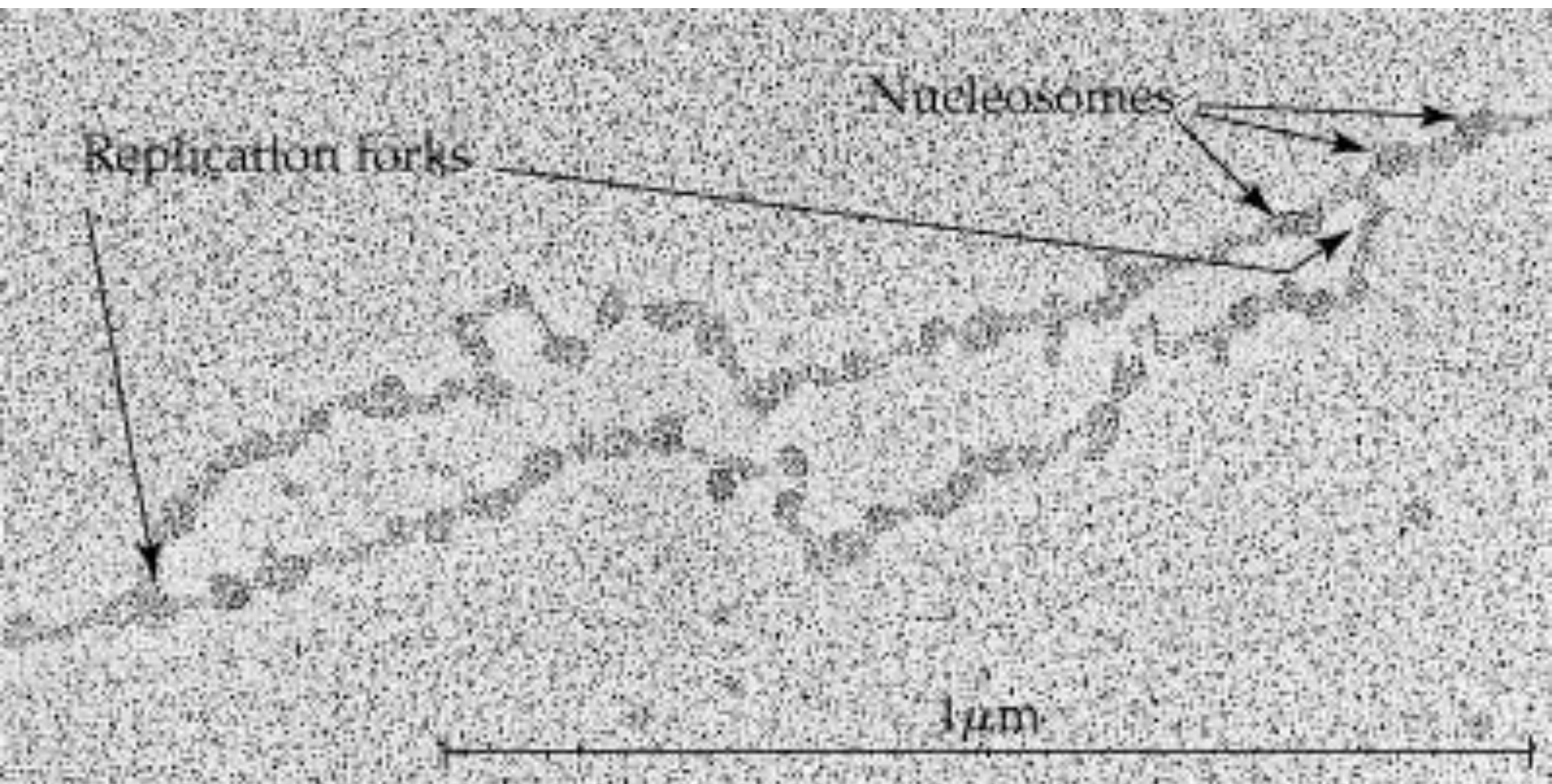
Table 2. Various specialized DNA polymerases and their putative role in non-B DNA replication

Human DNA polymerase (family, gene, protein size)	Known function	New function and putative role in non-B DNA replication
Pol η (Y family, POLH, 78 kDa)	TLS of DNA damage resulting from UV	CFS stability DNA synthesis of non-B DNA within CFS
Pol κ (Y family, POLK, 99 kDa)	TLS of bulky adducts	Prevents genomic instability on G4 Replication checkpoint
	Nucleotide excision repair	DNA synthesis of microsatellite Prevents genomic instability on G4
Rev1 (Y family, Rev1, 138 kDa)	Platform to recruit the TLS polymerases	Processes G4 motifs Preserves epigenetic marks
Pol ζ (B family, Rev3L, 353 kDa)	TLS, mutagenesis	CFS stability Replication of repeated sequences

- Novejše raziskave kažejo, da specializirane DNA polimeraze sodelujejo pri prepisovanju nestandardne DNA.

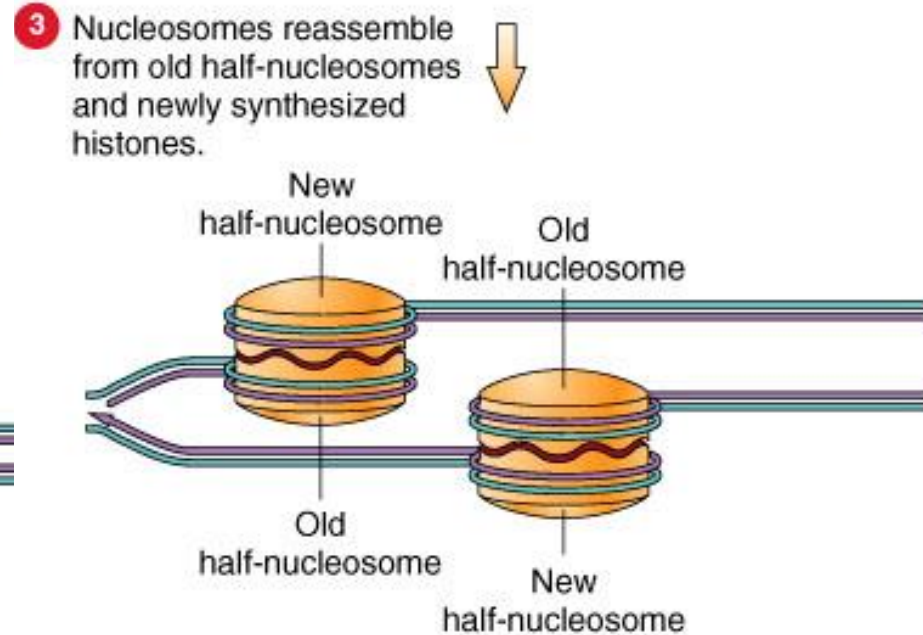
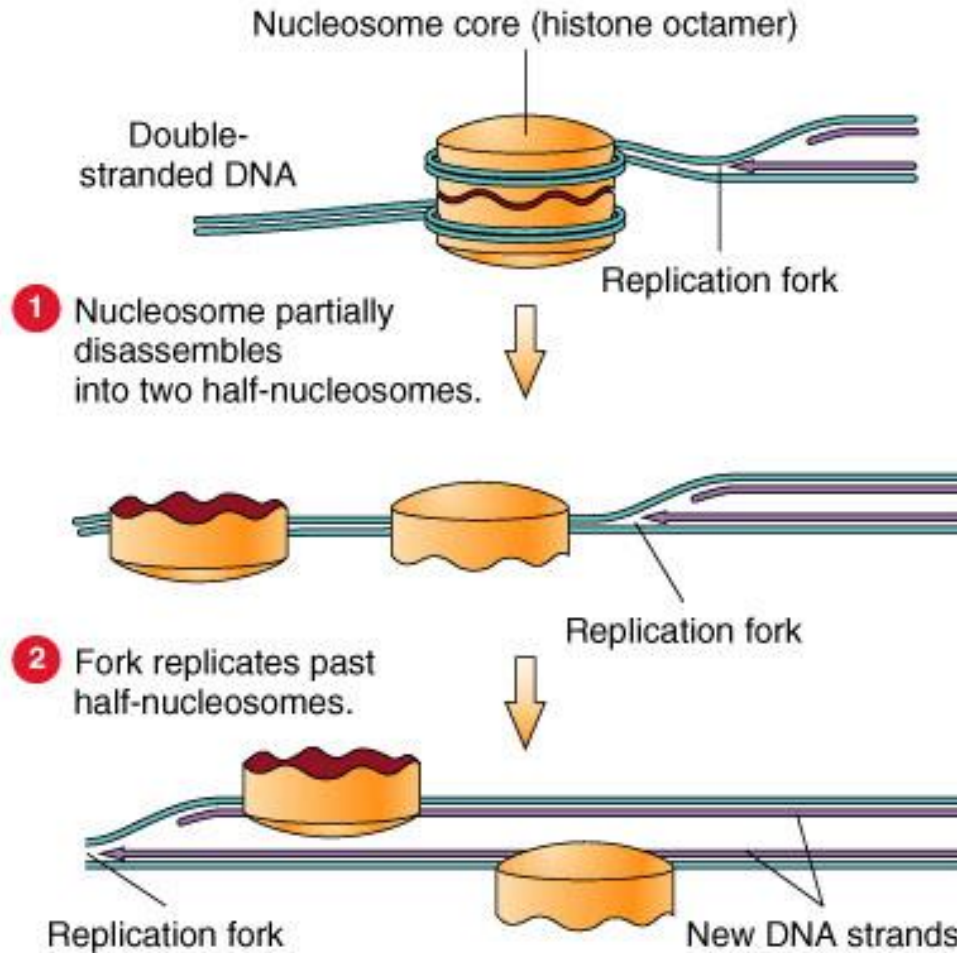
Replikacija nukleosomov

- Evkariontska DNA je navita okoli histonov in tvori nukleosome.
- Kaj se zgodi z nukleosomi, ko replikacijska vilica in replisom prideta blizu in odvijeta ter razdvojita dvojno vijačnico?
- Nukleosomi obdržijo pravilne zamike na obeh hčerinskih verigah tudi za replikacijsko vilico.



(a) Electron micrograph showing nucleosomes on both sides of each of two replication forks in *Drosophila*.

Model replikacije nukleosomov



Natančnost podvojevanja DNA

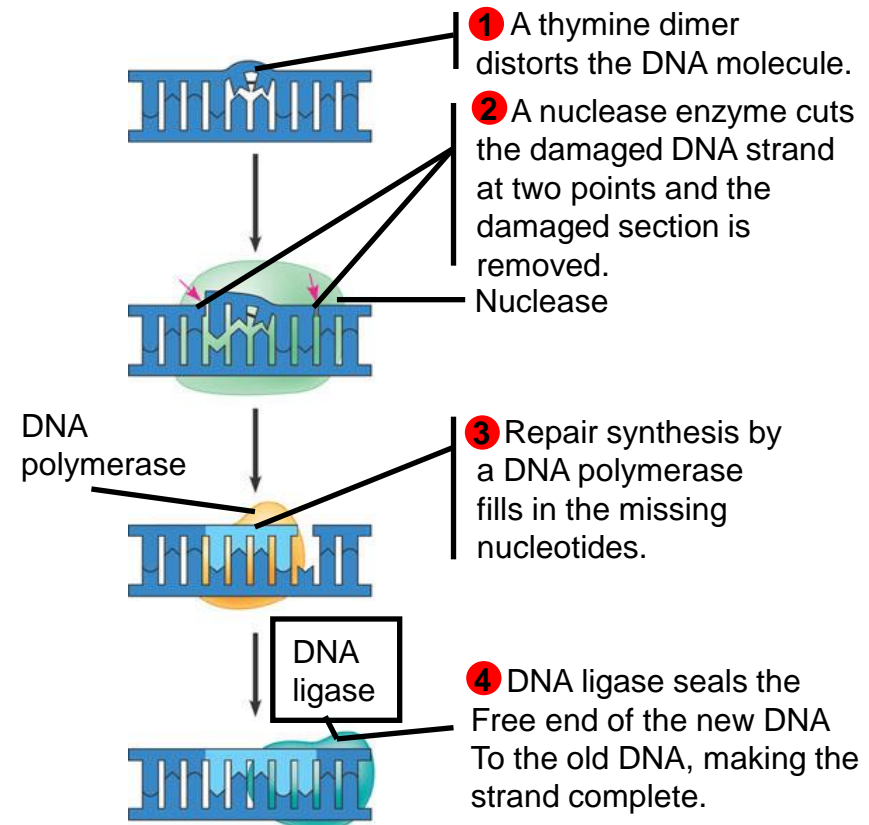
- DNA bakterije *E. coli* vsebuje okoli 5 milijonov (5×10^6) baznih parov.
 - Za podvojevanje celotne DNA potrebuje bakterija manj kot eno uro.
- 46 kromosomov človeka vsebuje 6 milijard (6×10^9) baznih parov DNA!!
 - Celice prekopirajo DNA v nekaj urah.
 - Zelo natančno – samo 10 napak na milijardo bp

Preverjanje natančnosti podvojevanja

- Podvojevanje DNA mora biti natančno, vendar vseeno prihaja do napak.
- DNA polimeraza (DNA pol) vstavi napačno bazo na vsakih 10.000 baz.
- DNA pol lahko preveri natančnost podvojevanja in popravi napake.
- Popravljanje na mestih nekomplementarnih baz – napačna baza se lahko zamenja.
- Popravljanje z izrezovanjem, ko je DNA poškodovana s kemikalijam, radijacio, ...

Preverjanje natančnosti in popravljanje DNA

- DNA polimeraze preverijo natančnost nove DNA in zamenjajo napačne nukleotide



Vzroki in razširjenost mutacij

- Vsaka človeška celica doživi čez 50.000 poškodb DNA na dan!
- Naravna replikacija DNA ima občasne napake, čeprav DNA polimeraza odstrani večino le teh.
- Imamo 6 milijard baz v DNA v vsaki celici, torej vsaka celica v našem telesu vsebuje nekaj mutacij.
- Večina mutacij je nevtralnih – nimajo posledic.
- Samo mutacije v spermijih in jajčnih celicah se prenašajo na naslednjo generacijo.
- Mutacije v drugih celicah (somatske mutacije) so lahko nevarne kot vzroki za bolezni npr. rak.

Mutacije so povezane s staranjem!

ARTICLE

doi:10.1038/nature11396

Rate of *de novo* mutations and the importance of father's age to disease risk

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Mutations generate sequence diversity and provide a substrate for selection. The rate of *de novo* mutations is therefore of major importance to evolution. Here we conduct a study of genome-wide mutation rates by sequencing the entire genomes of 78 Icelandic parent-offspring trios at high coverage. We show that in our samples, with an average father's age of 29.7, the average *de novo* mutation rate is 1.20×10^{-8} per nucleotide per generation. Most notably, the diversity in mutation rate of single nucleotide polymorphisms is dominated by the age of the father at conception of the child. The effect is an increase of about two mutations per year. An exponential model estimates paternal mutations doubling every 16.5 years. After accounting for random Poisson variation, father's age is estimated to explain nearly all of the remaining variation in the *de novo* mutation counts. These observations shed light on the importance of the father's age on the risk of diseases such as schizophrenia and autism.

Mutacije so povezane s staranjem!

- Otrok ima povprečno 60 novih mutacij.
- Pri pregledu 78 družin na Islandiji so ugotovili, da 20 letni oče prenese okrog 25 mutacij na svojega otroka, med tem ko jih 40 letni oče prenese okrog 65.
- Mame prenesejo povprečno 15 mutacij ne glede na starost. Raziskava zajema samo točkovne mutacije.
- Vsako leto po dvajsetem očetje pridobijo povprečno dve novi mutaciji.
- Možna razlaga za povečanje možnosti shizofrenije in avtizma pri otrocih starejših očetov.

Sinteza proteinov

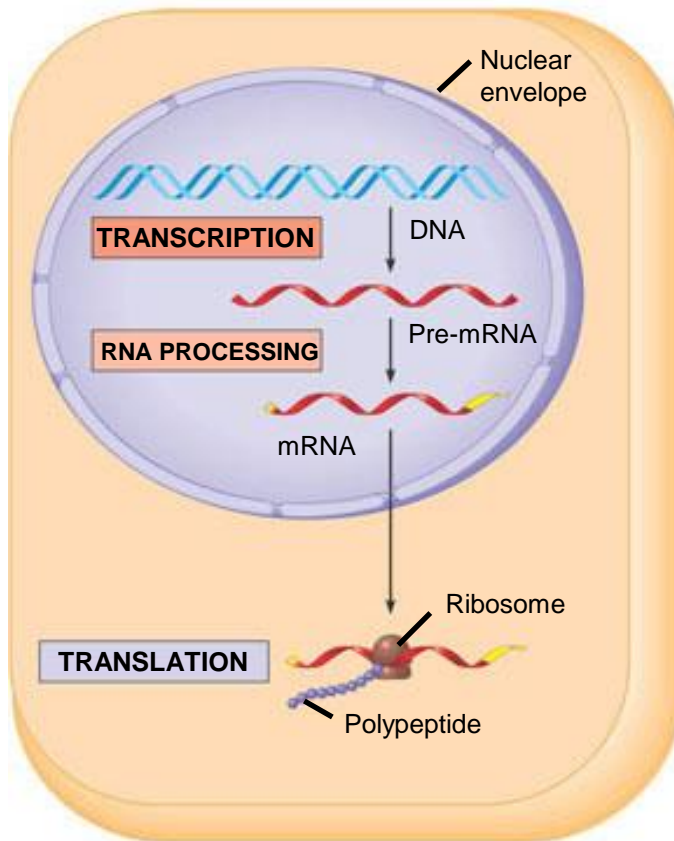
- DNA vsebuje informacijo v obliki specifičnega zaporedja nukleotidov na verigi DNA.
- Z narekovanjem sinteze proteinov podedovana DNA določa posebne lastnosti organizma.
- Sinteza proteinov oziroma izražanje genov poteka na dveh nivojih – transkripcija in translacija.

Transkripcija in translacija

- Pretok informacije DNA → RNA → protein je dogma molekularne biologije
- Transkripcija
 - Sinteza RNA, ki je pod kontrolo DNA
 - Tvorijo se informacijska RNA (messenger RNA - mRNA)
 - Prepišejo se tudi nekodirajoče RNA, ki ne nosijo zapisa za proteine, imajo pa druge pomembne vloge v celicah.
- Translacija
 - Dejanska sinteza polipeptida iz zapisa mRNA
 - Ribosomi urejajo prepis z RNA

Transkripcija in translacija

- V evkariontskih celicah jedrna membrana ločuje transkripcijo od translacije.
- V jedru pride do obsežnega procesiranja RNA.

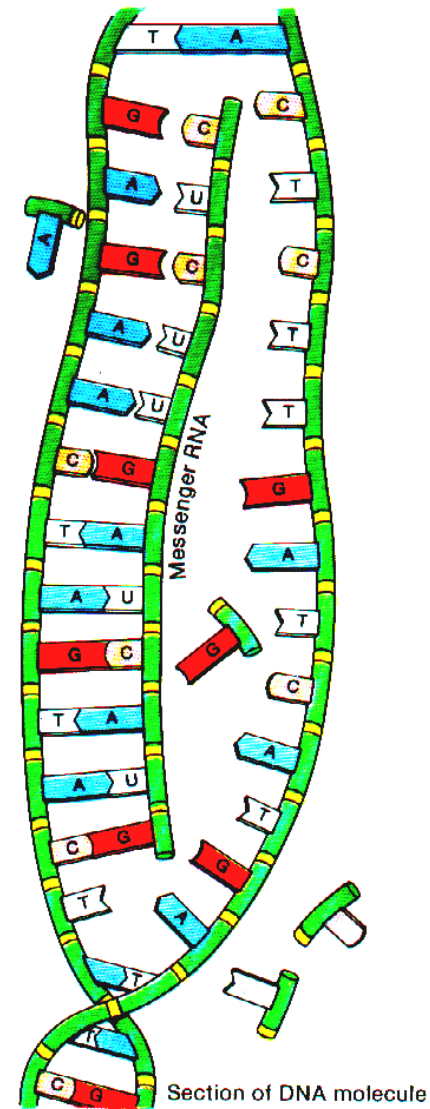


(b) Eukaryotic cell. The nucleus provides a separate compartment for transcription. The original RNA transcript, called pre-mRNA, is processed in various ways before leaving the nucleus as mRNA.

Transkripcija

- Sinteza RNA
- Katalizira jo RNA polimeraza, ki razpre DNA verigi in sintetizira komplementarno verigo ribonukleotidov – RNA.
- V uporabi so ista pravila parjenja baz kot pri DNA, razen, da je timin (T) zamenjan z uracilom (U).

KEY
T = thymine
C = cytosine
A = adenine
G = guanine



Trije razredi evkariontskih RNA polimeraz

TABLE 1.5 THE THREE CLASSES OF EUKARYOTIC RNA POLYMERASE

RNA polymerase	RNA synthesized	Notes
I	28S rRNA ^a , 18S rRNA ^a , 5.8S rRNA ^a	localized in the nucleolus; RNA polymerase I produces a single primary transcript (45S rRNA) that is cleaved to give the three rRNA classes listed here
II	mRNA ^b , miRNA ^c , most snRNAs ^d and snoRNAs ^e	RNA polymerase II transcripts are unique in being subject to capping and polyadenylation
III	5S rRNA ^a , tRNA ^f , U6 snRNA ^g , 7SL RNA ^h , various other small noncoding RNAs	the promoter for some genes transcribed by RNA polymerase III (e.g. 5S rRNA, tRNA, 7SL RNA) is internal to the gene; for others, it is located upstream of the gene (see Figure 1.15)

^aRibosomal RNA. ^bMessenger RNA. ^cMicroRNA. ^dSmall nuclear RNAs. ^eSmall nucleolar RNAs. ^fTransfer RNA. ^gU6 snRNA is a component of the spliceosome, an RNA–protein complex that removes unwanted noncoding sequences from newly formed RNA transcripts. ^h7SL RNA forms part of the signal recognition particle, which has an important role in the transport of newly synthesized proteins.

Table 1.5 Human Molecular Genetics, 4ed. (© Garland Science)

Raznolikost RNA

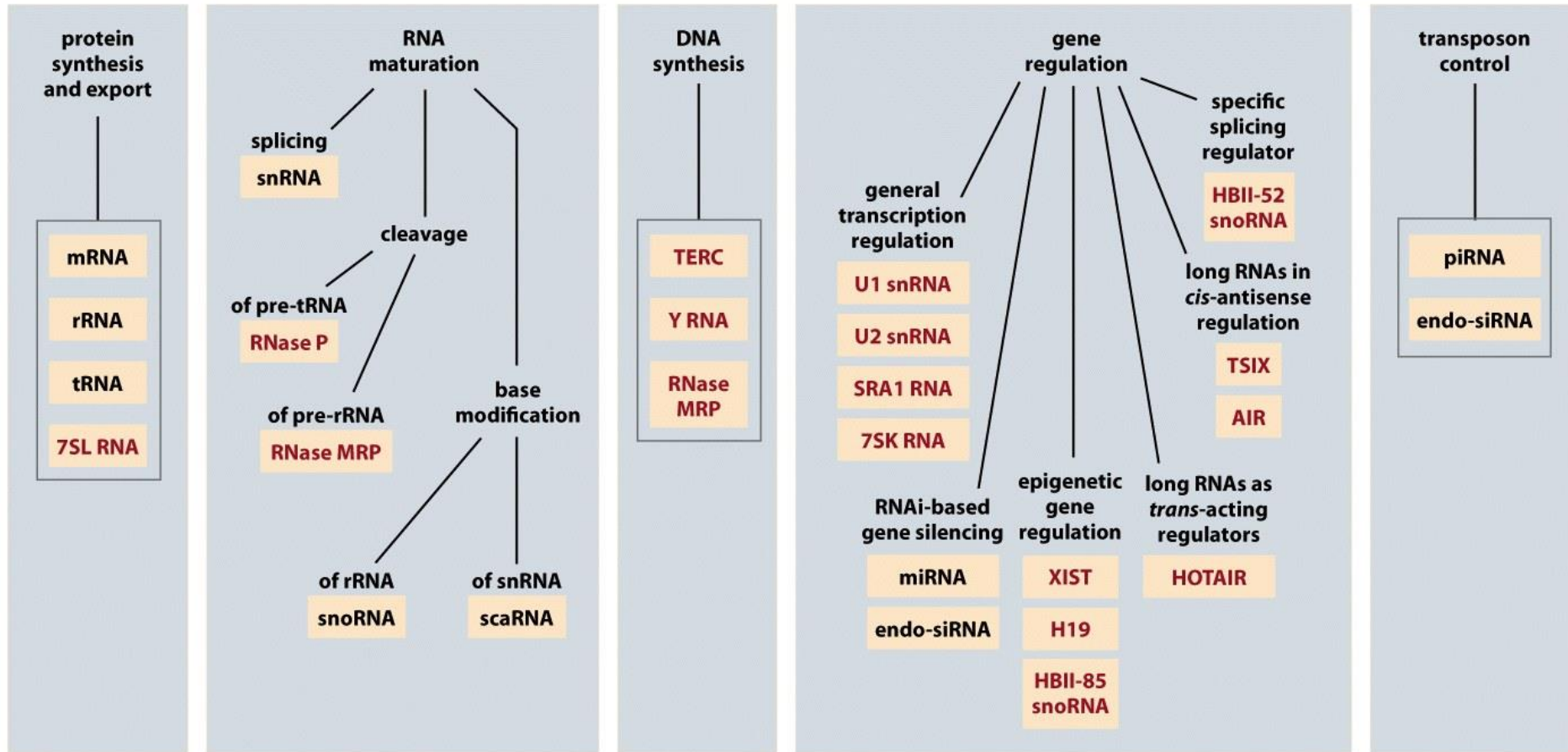


Figure 9.13 Human Molecular Genetics, 4ed. (© Garland Science)

TABLE 9.9 MAJOR CLASSES OF HUMAN NONCODING RNA

RNA class	Subclass or evolutionary/ functional subfamily	No. of different types	Function	Gene organization, biogenesis, etc.
Ribosomal RNA (rRNA), ~120–5000 nucleotides	12S rRNA, 16S rRNA	1 of each	components of mitochondrial ribosomes	cleaved from multigenic transcripts produced by H strand of mtDNA (Figure 9.3)
	5S rRNA, 5.8S rRNA, 18S rRNA, 28S rRNA	1 of each	components of cytoplasmic ribosomes	5S rRNA is encoded by multiple genes in various gene clusters; 5.8S, 18S, and 28S rRNA are cleaved from multigenic transcripts (Figure 1.22); the multigenic 5.8S–18S–28S transcription units are tandemly repeated on each of 13p, 14p, 15p, 21p, and 22p (= rDNA clusters)
Transfer RNA (tRNA), ~70–80 nucleotides	mitochondrial family	22	decode mitochondrial mRNA to make 13 proteins on mitochondrial ribosomes	single-copy genes. tRNAs are cleaved from multigenic mtDNA transcripts (Figure 9.3)
	cytoplasmic family	49	decode mRNA produced by nuclear genes (Figure 9.13)	700 tRNA genes and pseudogenes dispersed at multiple chromosomal locations with some large gene clusters
Small nuclear RNA (snRNA), ~60–360 nucleotides	spliceosomal family with subclasses Sm and Lsm (Table 9.10)	9	U1, U2, U4, U5, and U6 snRNAs process standard GU–AG introns (Figure 1.19); U4atac, U6atac, U11, and U12 snRNAs process rare AU–AC introns	about 200 spliceosomal snRNA genes are found at multiple locations but there are moderately large clusters of U1 and U2 snRNA genes; most are transcribed by RNA pol II
	non-spliceosomal snRNAs	several	U7 snRNA: 3' processing of histone mRNA; 7SK RNA: general transcription regulator; Y RNA family: involved in chromosomal DNA replication and regulators of cell proliferation	mostly single-copy functional genes

RNA class	Subclass or evolutionary/ functional subfamily	No. of different types	Function	Gene organization, biogenesis, etc.
Small nucleolar RNA (snoRNA), ~60–300 nucleotides	C/D box class (Figure 9.15A)	246	maturation of rRNA, mostly nucleotide site-specific 2'-O-ribose methylations	usually within introns of protein-coding genes; multiple chromosomal locations, but some genes are found in multiple copies in gene clusters (such as the HBII-52 and HBII-85 clusters—Figure 11.22)
	H/ACA class (Figure 9.15B)	94	maturation of rRNA by modifying uridines at specific positions to give pseudouridine	
Small Cajal body RNA (scaRNA)		25	maturation of certain snRNA classes in Cajal bodies (coiled bodies) in the nucleus	usually within introns of protein-coding genes
RNA ribonucleases, ~260–320 nucleotides		2	RNase P cleaves pre-tRNA in nucleus + mitochondria; RNase MRP cleaves rRNA in nucleolus and is involved in initiating mtDNA replication	single-copy genes
Miscellaneous small cytoplasmic RNAs, ~80–500 nucleotides	BC200	1	neural RNA that regulates dendritic protein biosynthesis; originated from Alu repeat	1 gene, <i>BCYRN1</i> , at 2p16
	7SL RNA	3	component of the signal recognition particle (SRP) that mediates insertion of secretory proteins into the lumen of the endoplasmic reticulum	three closely related genes clustered on 14q22
	TERC (telomerase RNA component)	1	component of telomerase, the ribonucleoprotein that synthesizes telomeric DNA, using TERC as a template (Figure 2.13)	single-copy gene at 3q26
	Vault RNA	3	components of cytoplasmic vault RNPs that have been thought to function in drug resistance	<i>VAULTRC1</i> , <i>VAULTRC2</i> , and <i>VAULTRC3</i> are clustered at 5q31 and share ~84% sequence identity
	Y RNA	4	components of the 60 kD Ro ribonucleoprotein, an important target of humoral autoimmune responses	<i>RNY1</i> , <i>RNY3</i> , <i>RNY4</i> , and <i>RNY5</i> are clustered at 7q36

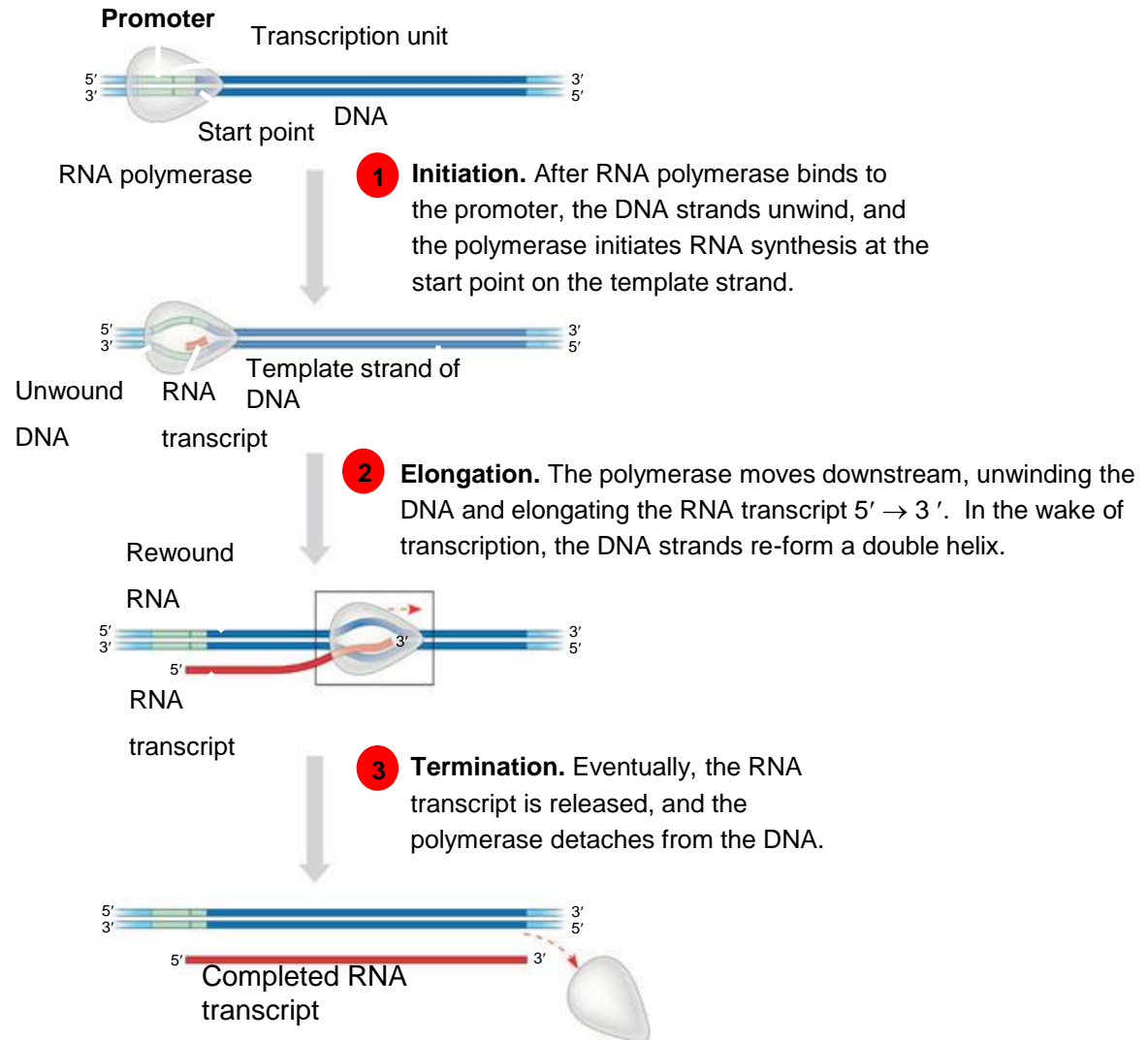
TABLE 9.9 (cont.) MAJOR CLASSES OF HUMAN NONCODING RNA

RNA class	Subclass or evolutionary/ functional subfamily	No. of different types	Function	Gene organization, biogenesis, etc.
MicroRNA (miRNA), ~22 nucleotides	> 70 families of related miRNAs	~1000	multiple important roles in gene regulation, notably in development, and implicated in some cancers	see Figure 9.17 for examples of genome organization, and Figure 9.16 for how they are synthesized
Piwi-binding RNA (piRNA), ~24–31 nucleotides	89 individual clusters	> 15,000	often derived from repeats; expressed only in germ-line cells, where they limit excess transposon activity	89 large clusters distributed across the genome; individual clusters span from 10 kb to 75 kb with an average of 170 piRNAs per cluster
Endogenous short interfering RNA (endo-siRNA), ~21–22 nucleotides	many	probably more than 10,000 ^a	often derived from pseudogenes, inverted repeats, etc.; involved in gene regulation in somatic cells and may also be involved in regulating some types of transposon	clusters at many locations in the genome
Long noncoding regulatory RNA, often > 1 kb	many	> 3000	involved in regulating gene expression; some are involved in monoallelic expression (X-inactivation, imprinting), and/or as antisense regulators (Table 9.11)	usually individual gene copies; transcripts often undergo capping, splicing, and polyadenylation but antisense regulatory RNAs are typically long transcripts that do not undergo splicing

^aBased on extrapolation of studies in mouse cells.

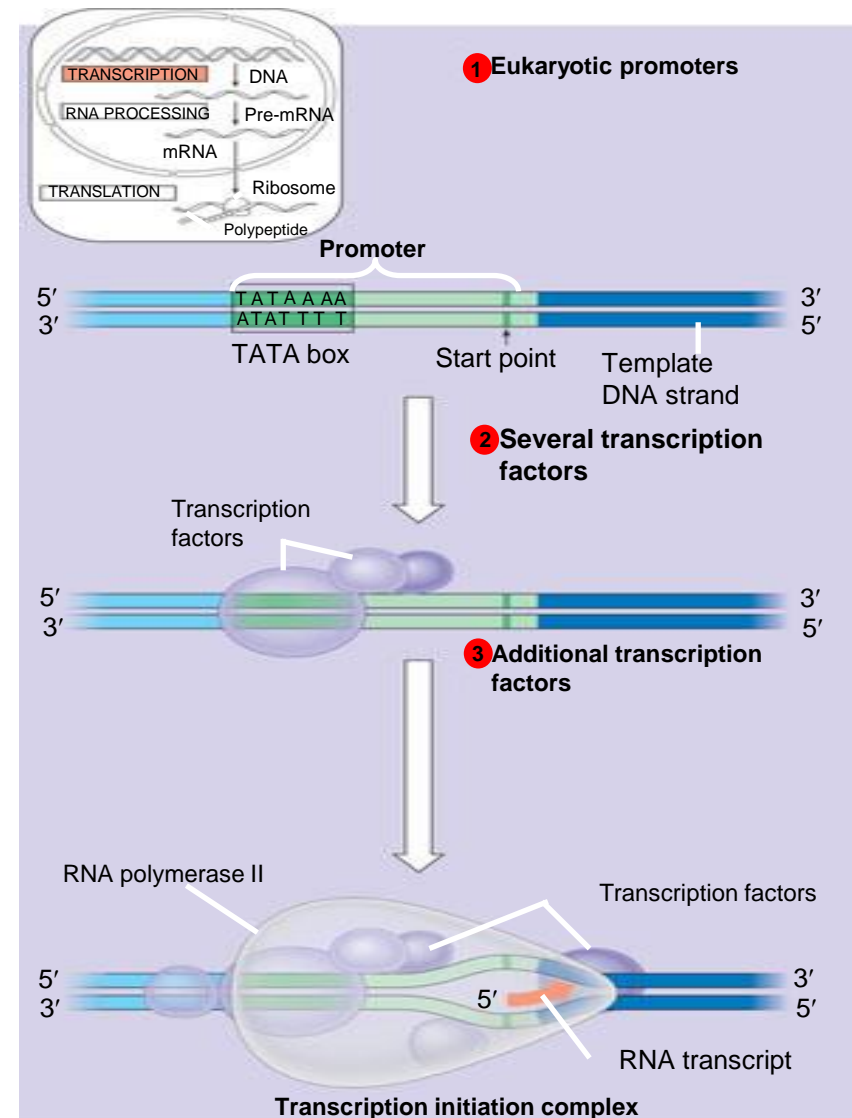
Sinteza RNA transkripta

- Trije nivoji sinteze
 - Inicijacija
 - Elongacija
 - Terminacija

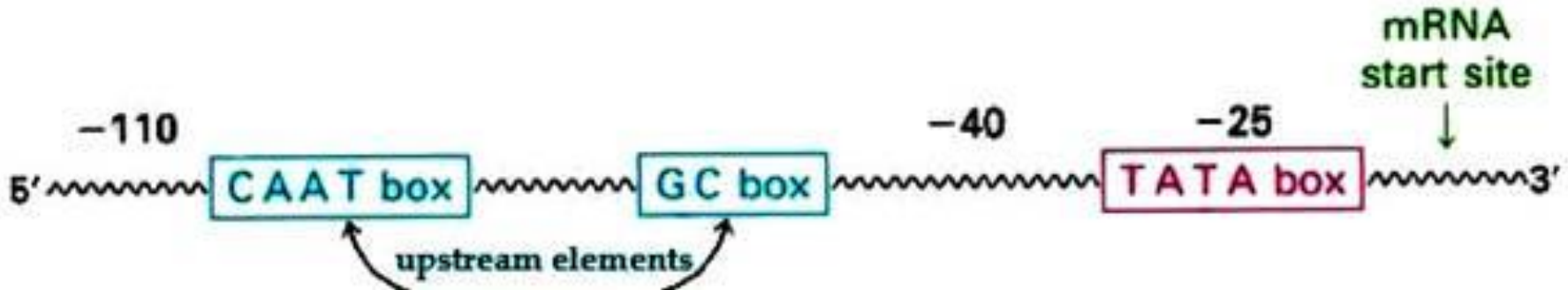


Sinteza RNA transkripta - iniciacija

- Promotorji označijo mesto pričetka sinteze RNA.
- Transkripcijski faktorji pomagajo evkariontskim RNA polimerazam pri prepoznavanju promotorskih zaporedij.



Promotorji pol II



- TATA box.
 - Običajno TATAA okrog mesta -25
 - Pogost pri genih, ki se močno prepisujejo s RNA pol II v določeni stopnji celičnega cikla ali v specifičnih celicah
 - Mutacije TATA povzročijo pričetek transkripcije na napačnem mestu
- GC box.
 - Najbolj pogosto GGGCGG
 - Pogost pri genih, ki nimajo TATA boxa in ki se izražajo v vseh celicah (DNA in RNA polimeraze, histoni, ribosomalni proteini)
 - Deluje v obe smeri
- CAAT box.
 - Pogosto na mestu -80
 - Najpomembnejša komponenta učinkovitosti promotorja
 - Deluje v obe smeri

Promotorji pol III

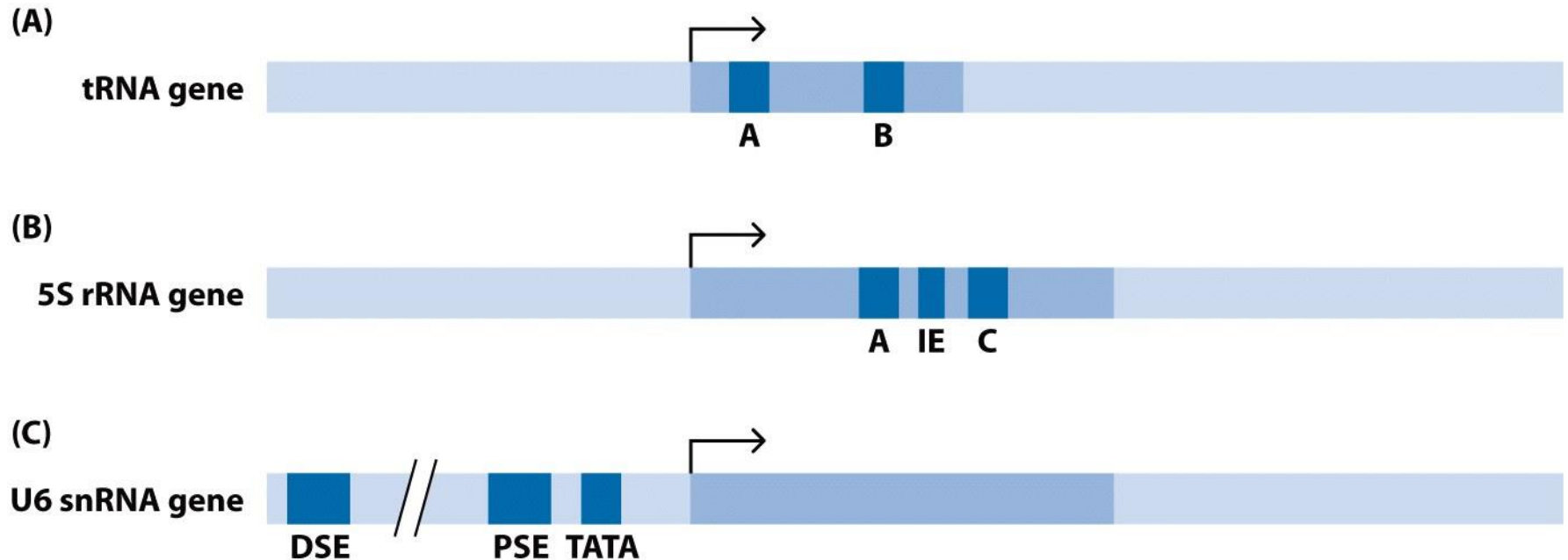
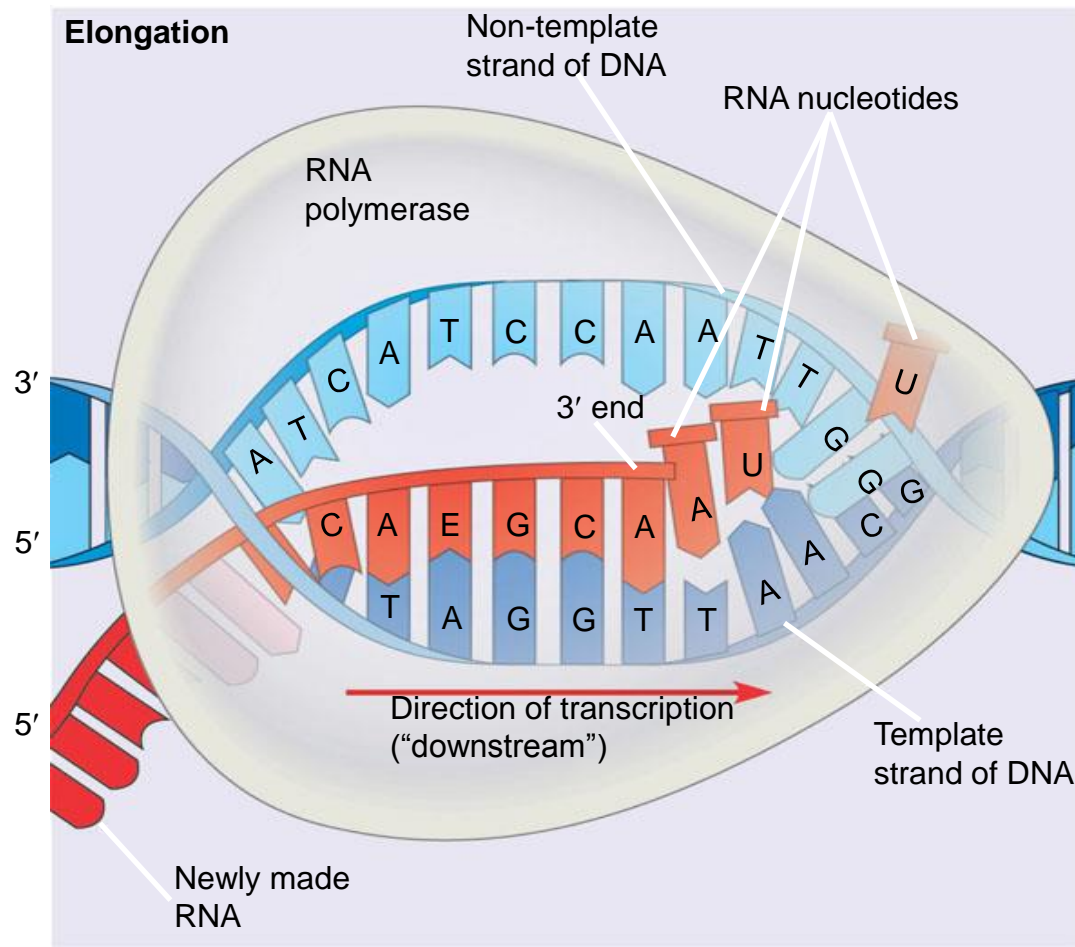


Figure 1.15 Human Molecular Genetics, 4ed. (© Garland Science)

- tRNA geni imajo interne promotorje
- 5S rRNA vsebuje A in C box ter vmesni (“intermediate”) element
- U6 snRNA vsebuje distalni in proksimalni sekvenčni element ter TATA box.

Sinteza RNA transkripta - elongacija

- RNA polimeraza sintetizira enoverižno RNA z branjem protismerne ('anti-sense') DNA v smeri 5' proti 3'.
- S premikanjem po DNA, RNA polimeraza odvija in odpira DNA. Transkripcijski mehurček je dolg 10 do 20 baz.



Sinteza RNA transkripta - terminacija

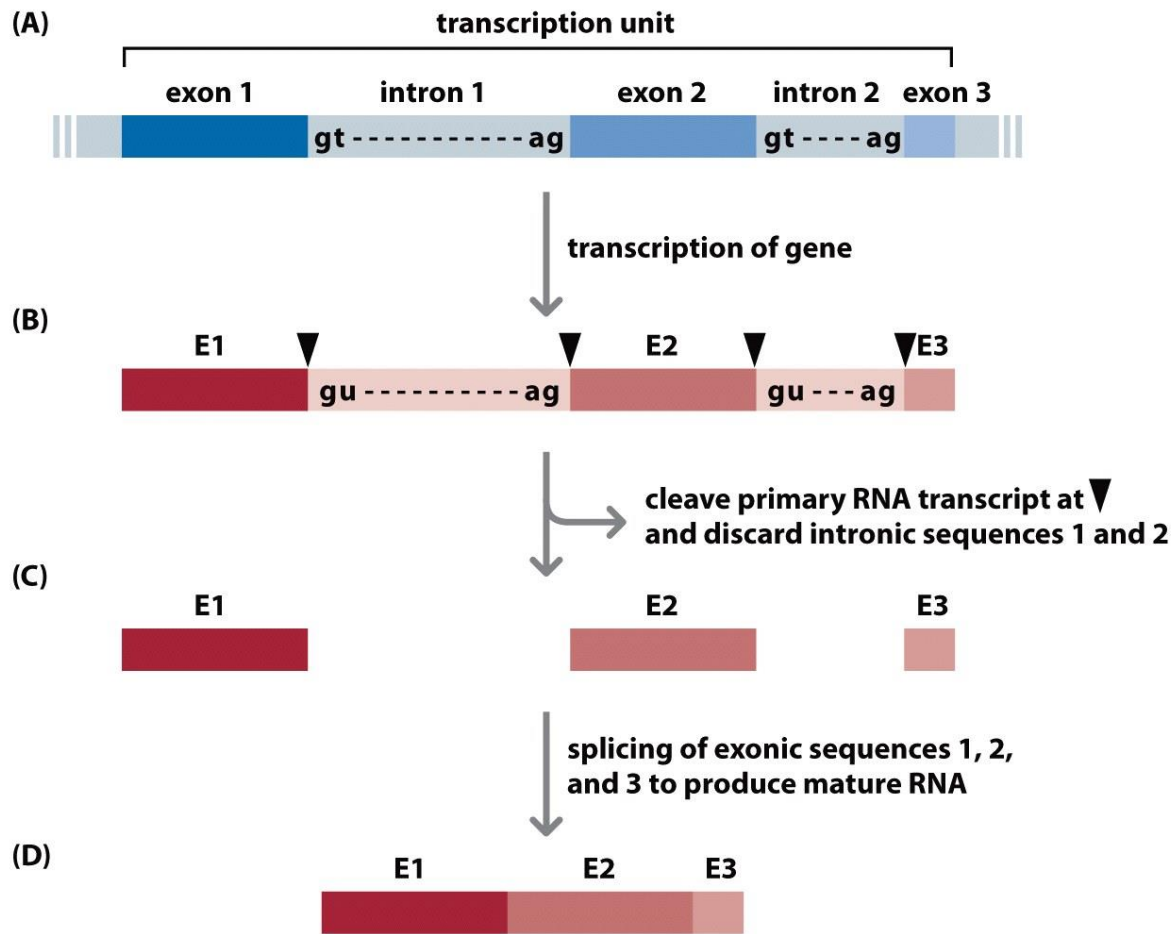
- Specifična zaporedja na DNA signalizirajo konec transkripcije.
- Ko RNA polimeraza pride do signala, sprosti RNA transkript in se odstrani z DNA, ki se nato zapre.

Pregled transkripcije

Procesiranje RNA

- Po transkripciji se mora večina evkariontskih mRNA še dodatno procesirati pred translacijo.
- Procesiranje mRNA poteka v jedru. Nato se mRNA prenese v citoplazmo.
- Večji del genov vsebuje nekodirajoče dele, ki jih je potrebno izrezati
 - Introni imajo lahko posebne kromosomske funkcije ali regulatorne funkcije
 - Introni so pomembni pri regulaciji alternativnega spajanja RNA
- Pri procesiranju se na 5' del doda kapa, na 3' del dolgi poli(A) rep. Oba ščitita mRNA pred razgradnjo.
- Pre-mRNA se torej procesira v mRNA z dvema procesoma:
 - Izrezovanjem intronov
 - Dodajanjem zaščitnih baz na koncih

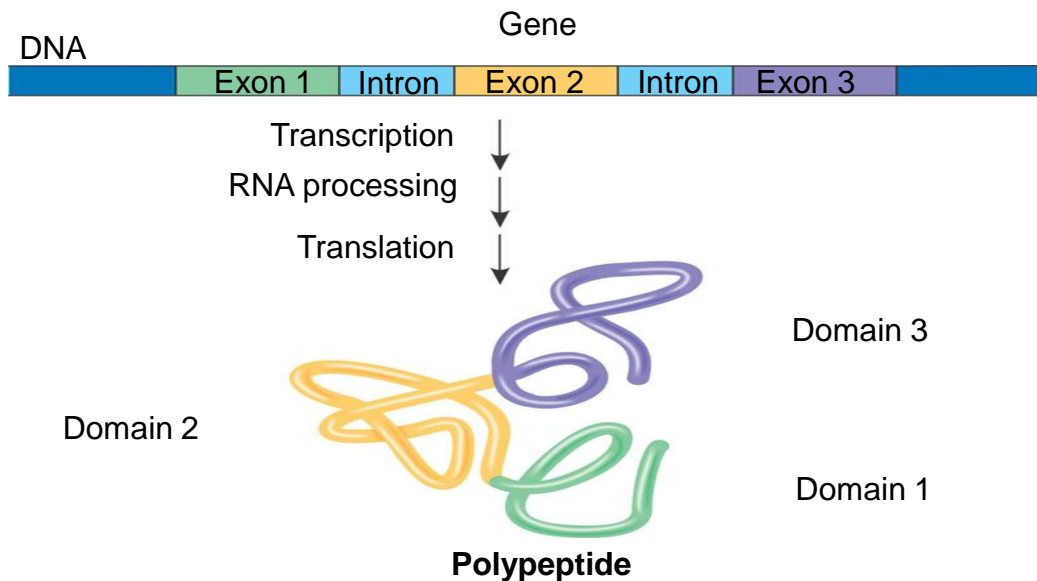
Izrezovanje in spajanje



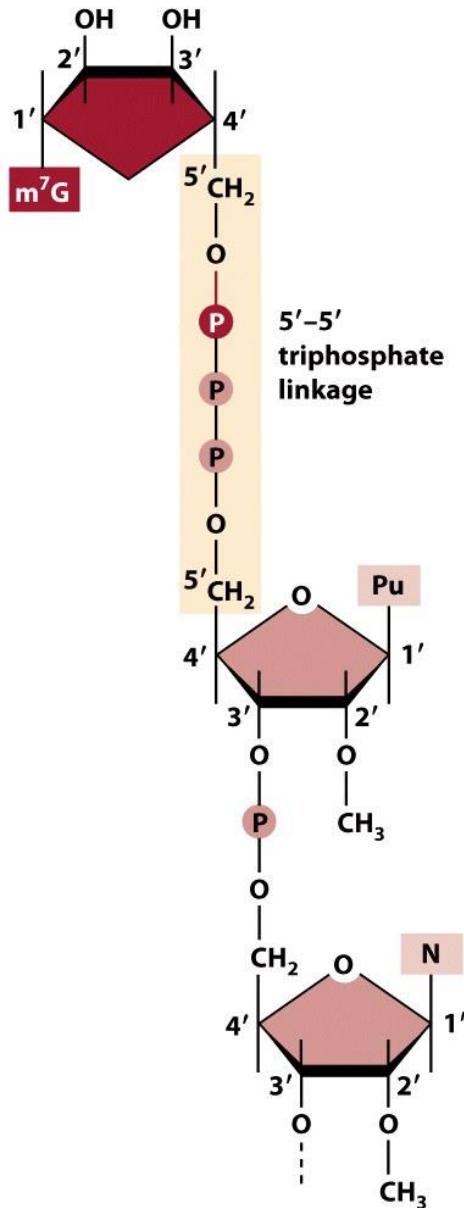
- V tem primeru, gen vsebuje 3 eksone in 2 introna.
- Pre-mRNA, ki je primarni transkript, vsebuje eksone in introne.
- Pre-mRNA se izreže na mejah med exoni in introni.
- Introni se odstranijo.
- Eksoni se spojijo v eno verigo.

Izrezovanje in spajanje

- Proteini imajo pogosto modularno strukturo – so sestavljeni iz ločenih strukturnih in funkcionalnih delov oziroma domen.
- V mnogih primerih različni eksoni kodirajo za različne domene v proteinu.



5' kapa evkariontske mRNA



- Takoj po inicijaciji transkripcije se na pre-mRNA doda metiliran nukleozid – 7-metilgvanozin, m⁷G.
- Veže se na 5' konec s fosfodiestersko vezjo.

Pomen 5' kape:

- Zaščita transkripta pred 5'→3' exonukleazno razgradnjo.
- Transport mRNA iz jedra v citoplazmo.
- Regulacija izrezovanja in spajanja RNA.
- Pomaga pri vezavi mRNA na 40S podenoto ribosoma.

Poliadenilacija

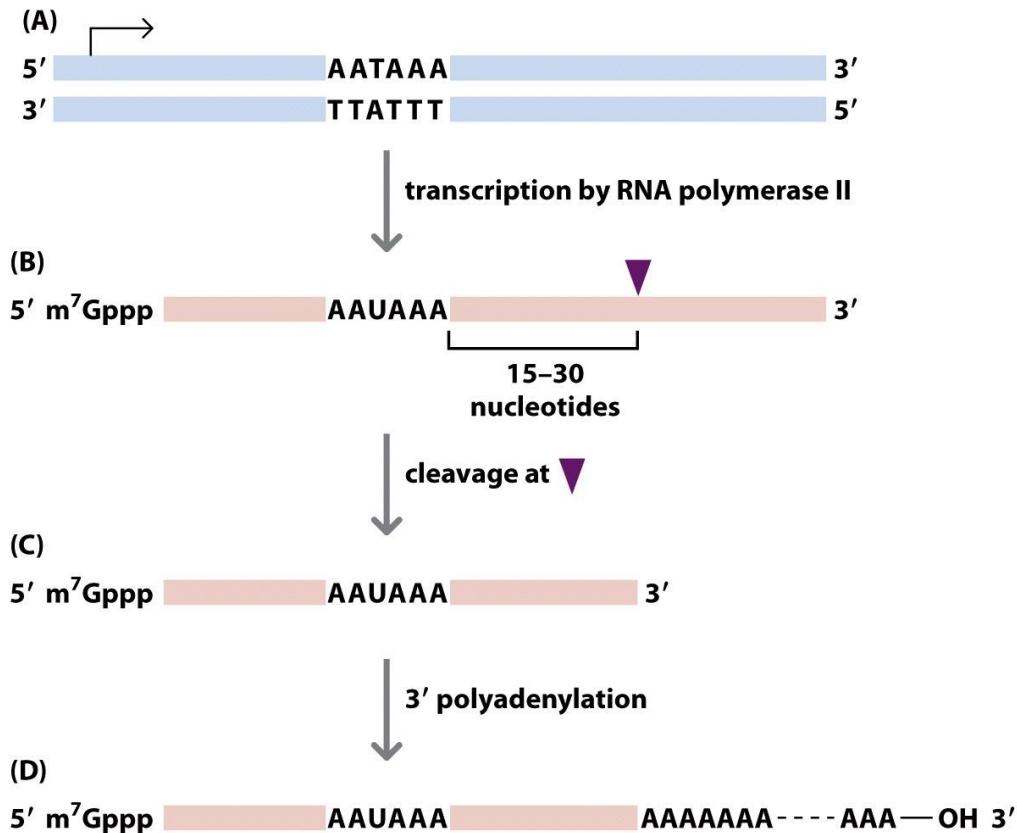


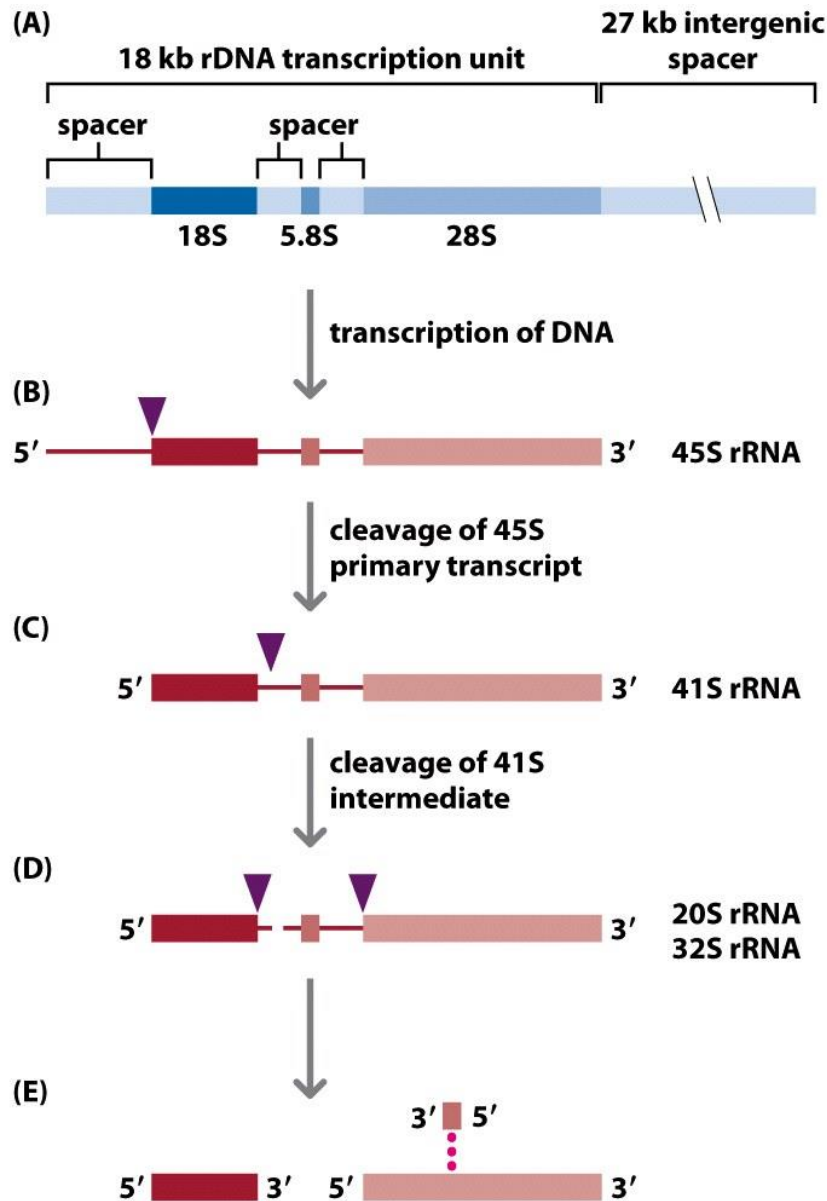
Figure 1.21 Human Molecular Genetics, 4ed. (© Garland Science)

Pomen poli(A) repa:

- Transport mRNA v citoplazmo.
- Stabilizacija nekaterih mRNA v citoplazmi.
- Ribosomi prepoznajo mRNA.

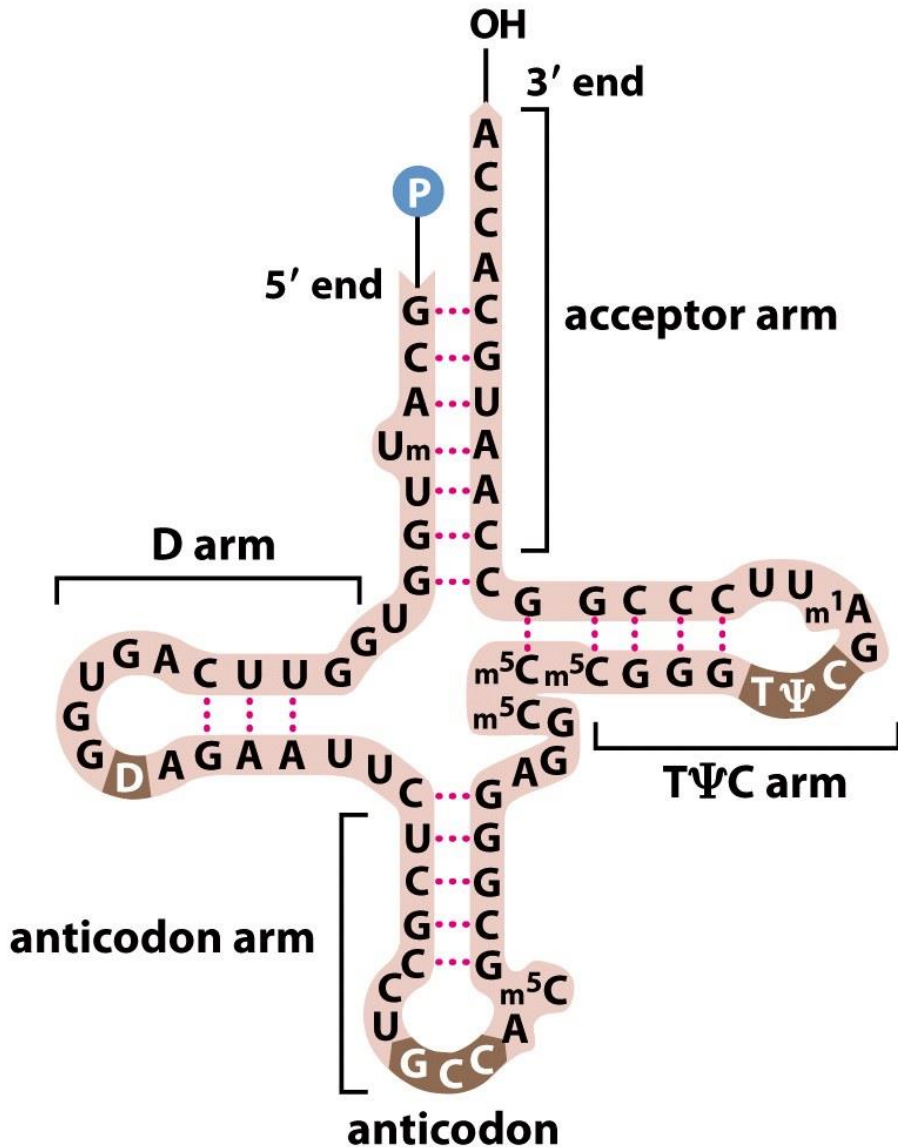
- RNA pol II vsebuje dva multiproteinska kompleksa:
 - CPSF – cleavage and polyadenylation specificity factor (faktor specifičnosti cepitve in poliadenilacije)
 - CStF – cleavage and stimulation factor (faktor cepitve in stimulacije)
- Prepoznata AAUAAA signal.
- Cepitev nastane 15-30nt navzdol.
- Poli(A) rep - Poli(A) polimeraza doda 200x A.

Procesiranje rRNA



- V človeškem genomu so 18S, 5,8S in 28S rRNA zapisana v enem, 13kb dolgem transkriptu.
- Skupaj s 27kb dolgim vmesnim fragmentom tvori tandemske ponovitve.
- Teh ponovitev je 30-40 in se nahajajo na krajši ročici kromosomov 13, 14, 15, 21 in 22. Regijam, ki so velike 1,5Mb, rečemo ribosomalna DNA (rDNA).
- Transkripcija 13kb gena poteka v jedrcu s RNA pol I.
- Primarni transkript se nato večkrat cepi.

Procesiranje tRNA

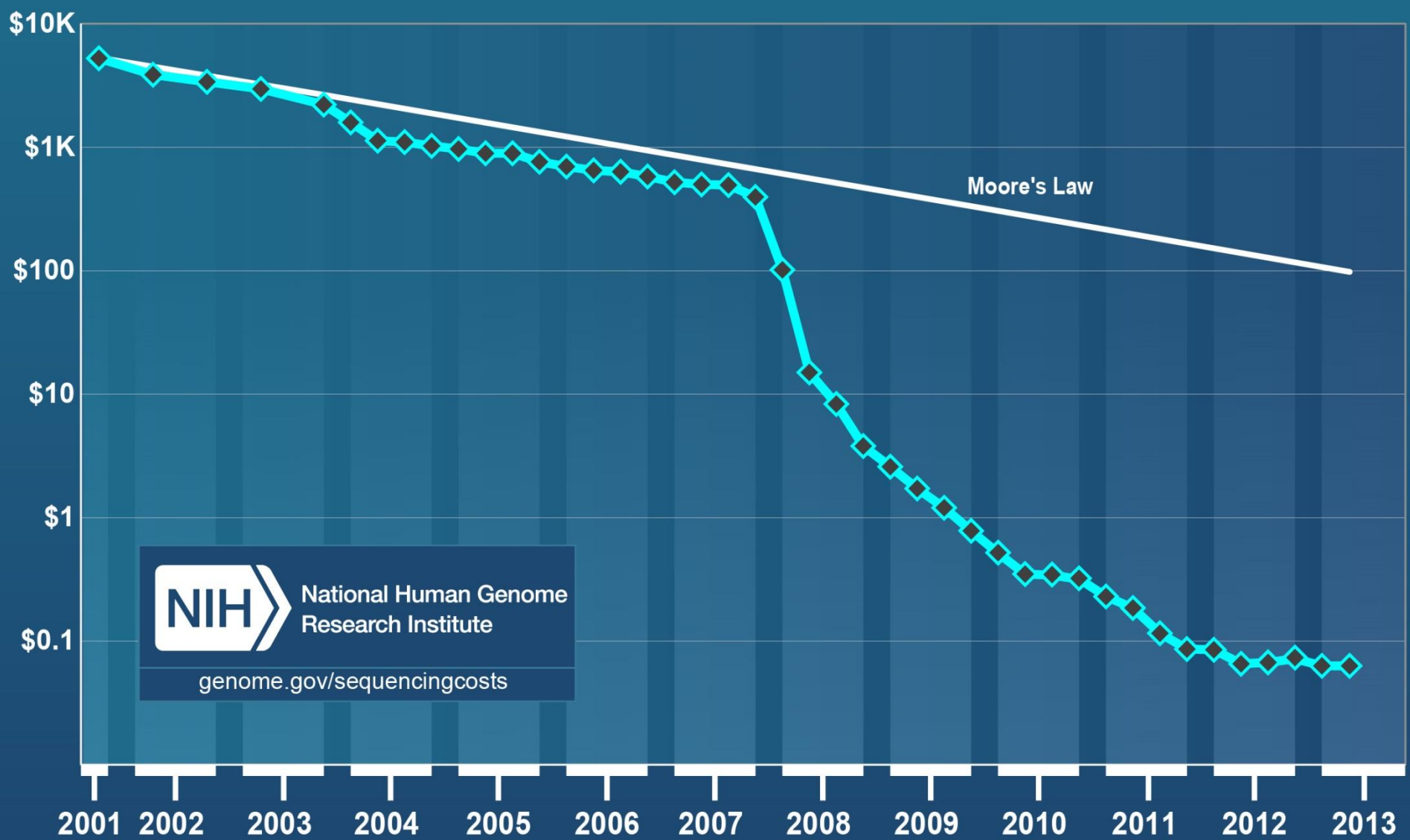


- Približno 10% baz na tRNA je modificiranih.
- Modifikacije potekajo v jedrcu pod taktirko snoRNA.
- Podobne modifikacije baz najdemo tudi na rRNA.

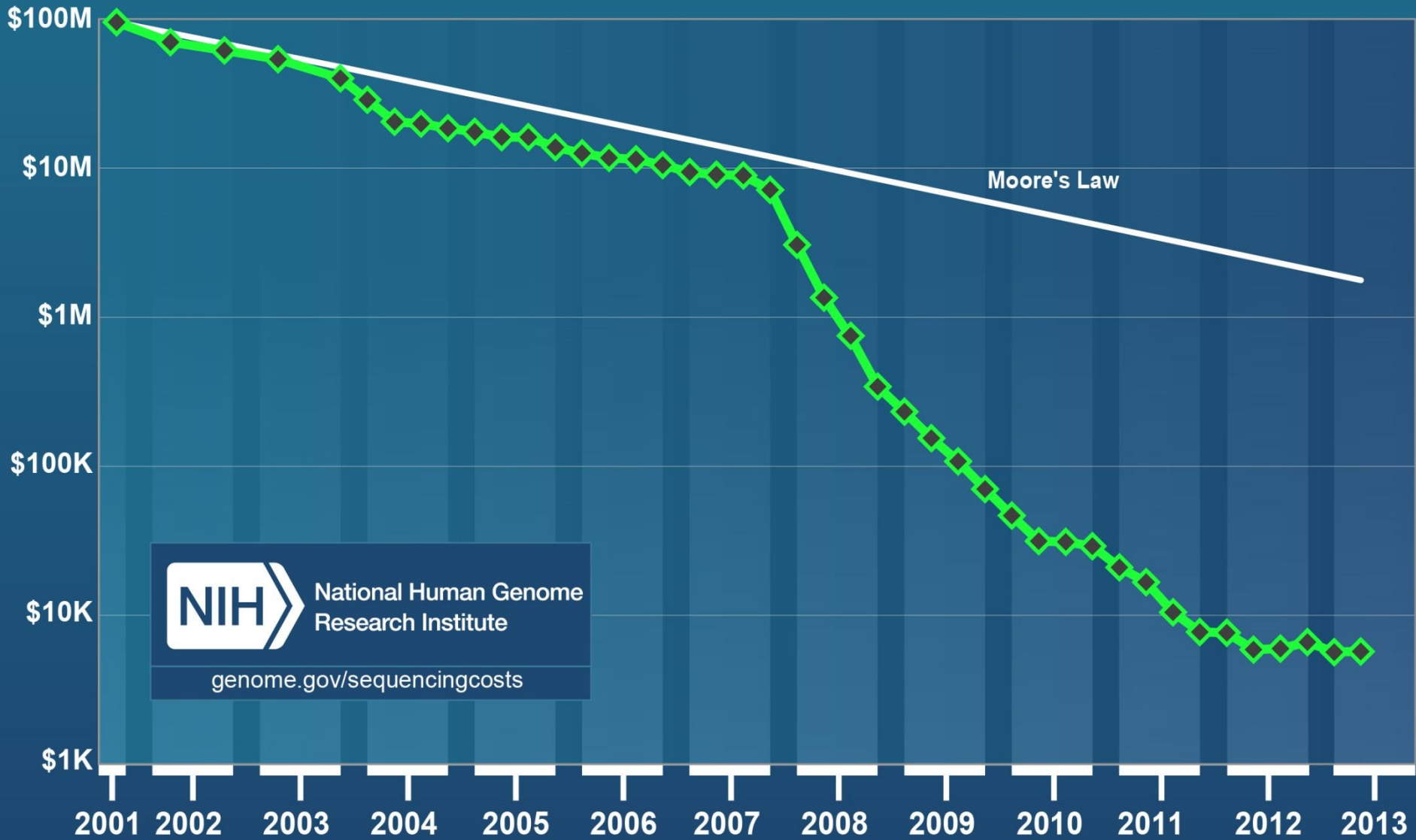
Določanje zaporedja DNA/RNA

- Izredna rast hitrosti sekveniranja.
- Razvoj novih tehnologij nižja cena.
- Zastoji pri obdelavi podatkov – bioinformatika.

Cost per Raw Megabase of DNA Sequence



Cost per Genome





Human
Genome
project
1990-2003
>2 billion \$



J. Watson's
genome
Jan-May, 2007
1 million \$



Personal genome
Week, Jan. 2012
3000\$



Personal genome
Day, End 2013
1000\$

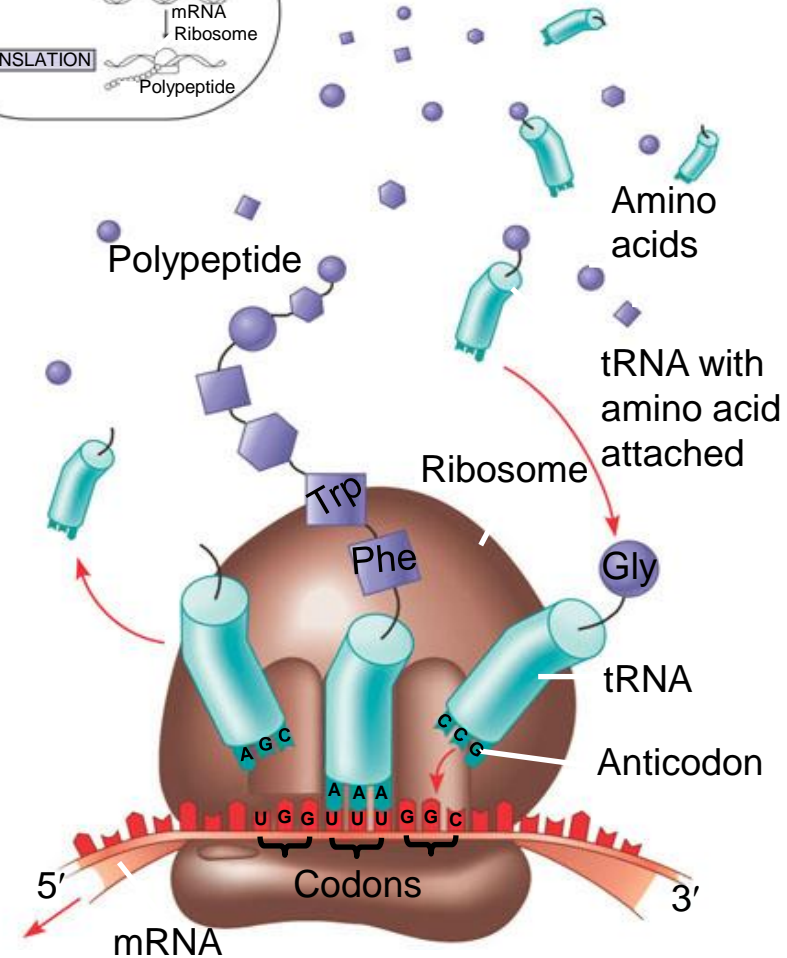
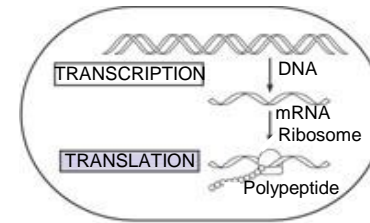


Personal genome
Hour, 2015?
100\$

Nanopore filmček

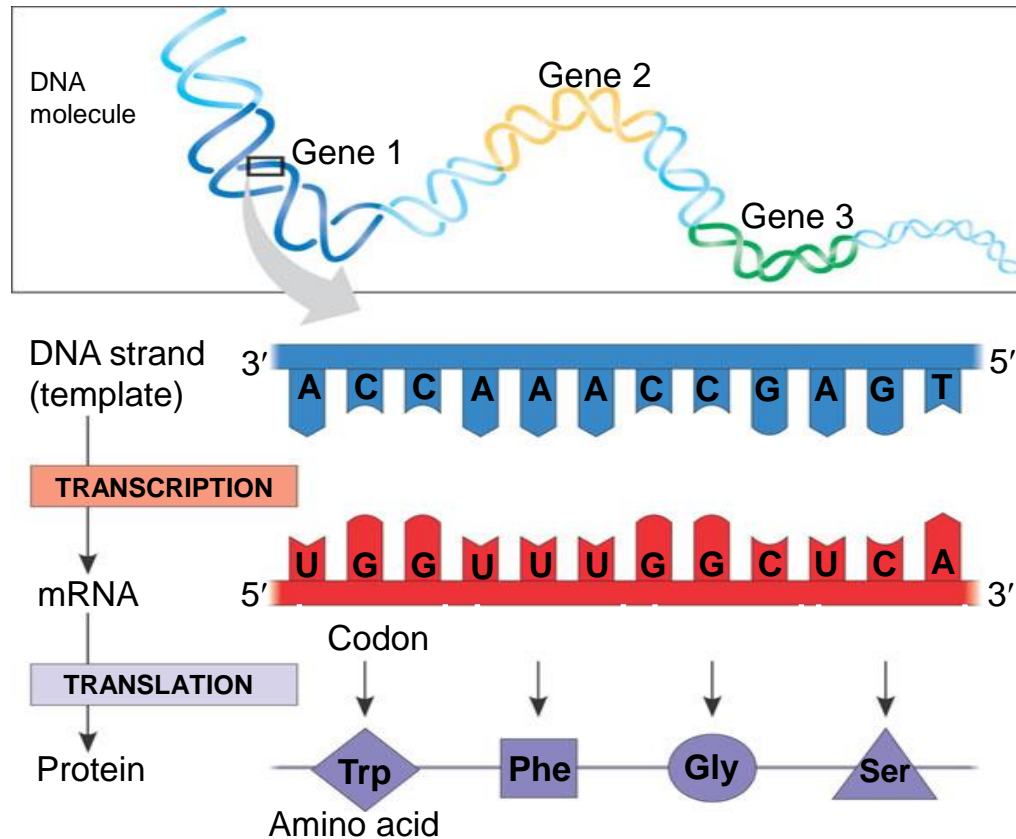
Translacija

- Translacija vsebuje
 - mRNA
 - Ribosome – rRNA+proteini
 - Transfer RNA
 - Genetski kod



Genetski kod

- Genetska informacija je kodirana v zaporedju tripletov baz ali kodonov.



Genetski kod

- Genetski kod je univerzalen – imajo ga vsi živi organizmi.
- Kodoni: koda treh baz za eno specifično amino kislino.
- Glede na to, da imamo 4 različne baze, ki lahko zasedajo 3 mesta, obstaja $4 \times 4 \times 4 = 64$ kodonov.
- 64 kodonov za samo 20 amino kislin pomeni, da imajo nekatere aminokislino več kodonov.
- 3 od 64 kodonov so STOP signali, ki so na koncu vsakega gena in označujejo konec proteina.
- En kodon, AUG, je začetni (START) signal, ki se nahaja na začetku vsakega proteina.

Genetski kod

- Mitohondrijska DNA ima za odtenek drugačen kod od jedrne DNA

mtDNA variants						mtDNA variant			
AAA	Lys	CAA	Gln	GAA	Glu	UAA	STOP		
AAG		CAG		GAG		UAG			
AAC	Asn	CAC	His	GAC	Asp	UAC	Tyr		
AAU		CAU		GAU		UAU			
ACA	Thr	CCA	Pro	GCA	Ala	UCA	Ser		
ACG		CCG		GCG		UCG			
ACC		CCC		GCC		UCC			
ACU		CCU		GCU		UCU			
STOP	AGA	Arg	CGA	Arg	GGA	Gly	UGA	STOP	Trp
	AGG	Arg	CGG	Arg	GGG	Gly	UGG	Trp	
	AGC	Ser	CGC	Arg	GGC	Gly	UGC	Cys	
	AGU	Ser	CGU	Arg	GGU	Gly	UGU	Cys	
Met	AUA	Ile	CUA	Leu	GUA	Val	UUA	Leu	
	AUG	Met	CUG	Leu	GUG	Val	UUG	Leu	
	AUC	Ile	CUC	Leu	GUC	Val	UUC	Phe	
	AUU	Ile	CUU	Leu	GUU	Val	UUU	Phe	

TABLE 1.6 RULES FOR BASE PAIRING CAN BE RELAXED (WOBBLE) AT POSITION 3 OF A CODON

Base at 5' end of tRNA anticodon	Base recognized at 3' end of mRNA codon
A	U only
C	G only
G (or I) ^a	C or U
U	A or G

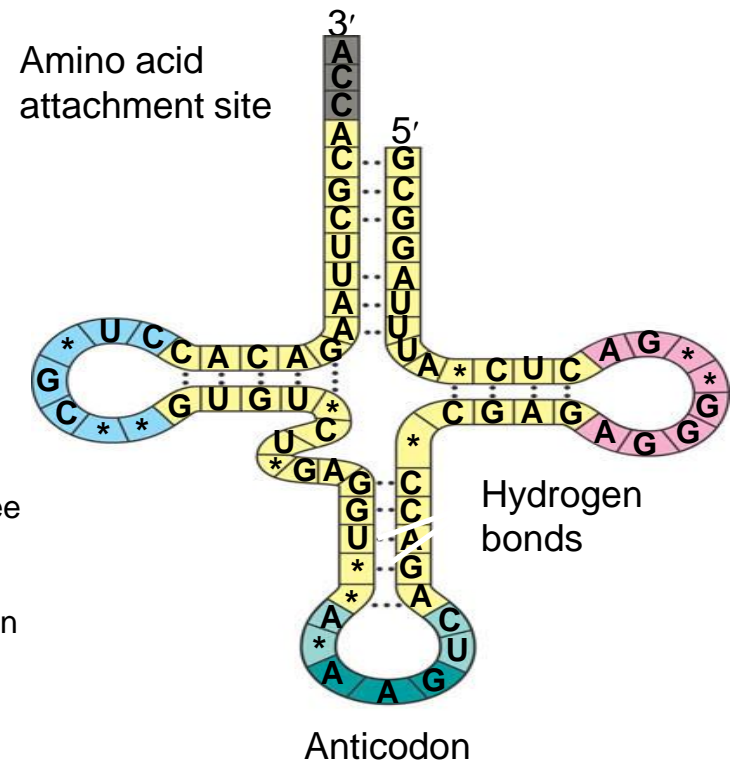
^aInosine (I) is a deaminated form of guanosine.

Transfer RNA

- Enoverižna RNA, ki je dolga približno 80 nukleotidov.
- Vsaka nosi specifično aminokislino na enem koncu ter antikodon na drugem.
- **Aminoacil tRNA sintetaze** vežejo pripadajočo aminokislino na tRNA
- tRNA prinese aminokislino na ribosom.

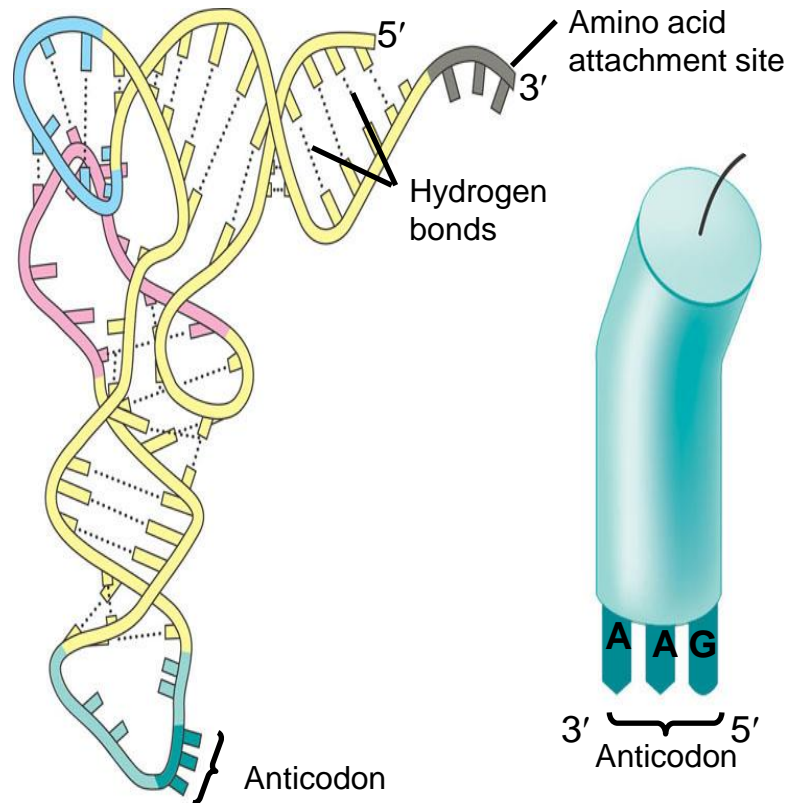
The “anticodon” is the 3 RNA bases that matches the 3 bases of the codon on the mRNA molecule

- (a) **Two-dimensional structure.** The four base-paired regions and three loops are characteristic of all tRNAs, as is the base sequence of the amino acid attachment site at the 3' end. The anticodon triplet is unique to each tRNA type. (The asterisks mark bases that have been chemically modified, a characteristic of tRNA.)



Transfer RNA

- 3 D struktura tRNA je v obliki črke "L"

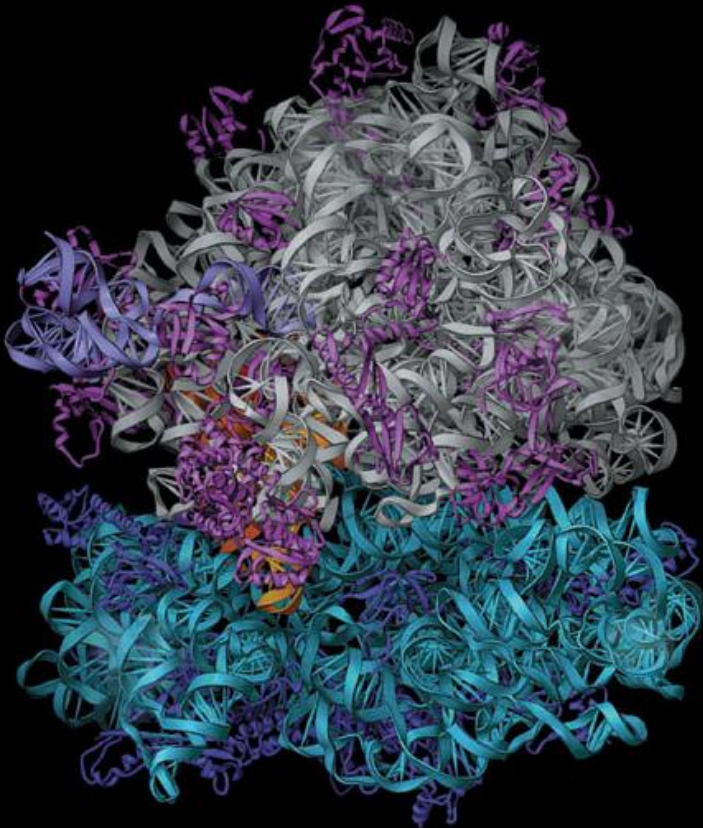


(b) Three-dimensional structure

(c) Symbol used in the book

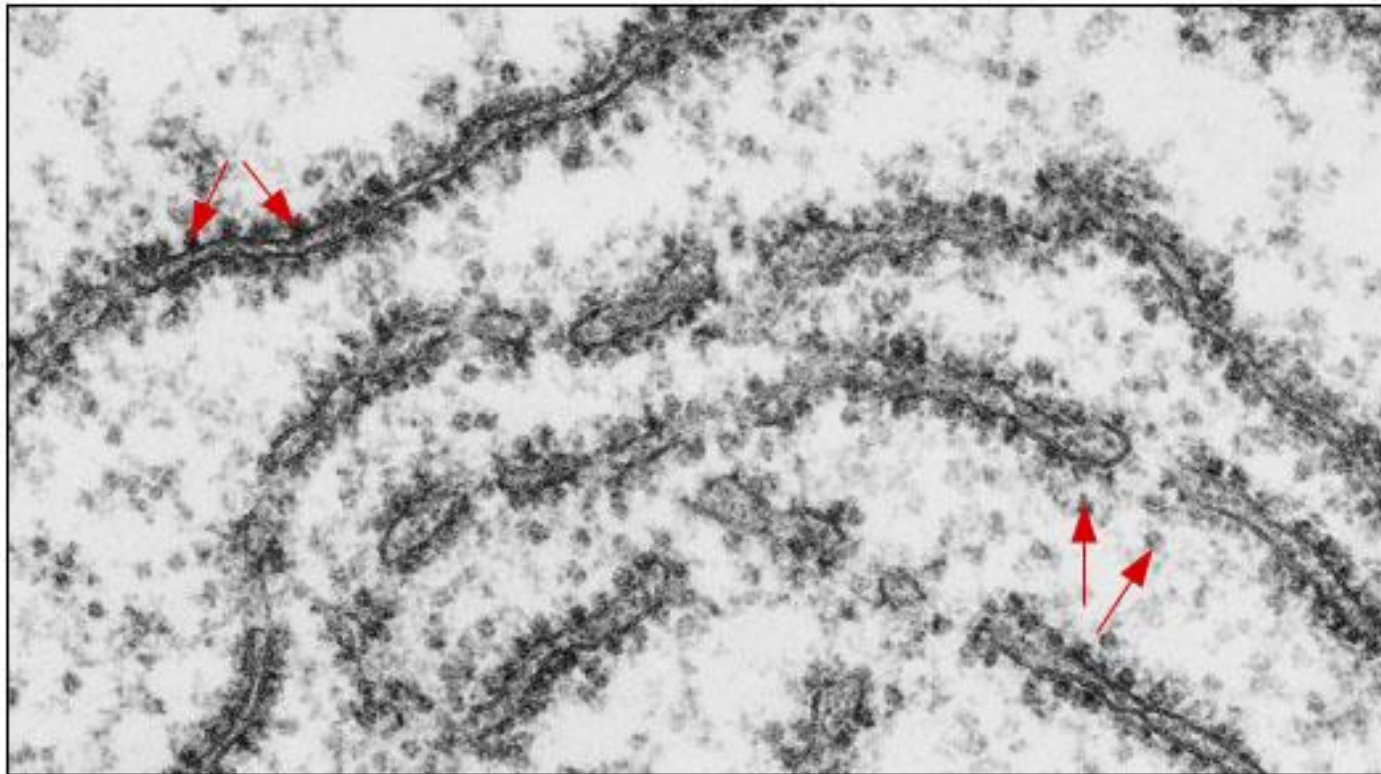
Ribosom

- Ribosomi omogočajo vezavo antikodona tRNA s kodonom na mRNA in tvorbo polipeptidne verige.
- Dve ribosomski podenoti sta sestavljeni iz rRNA in proteinov.



- Evkarionti imajo 80S ribosome.
- Majhna podenota (40S) vsebuje
 - 18S rRNA (1900 nukleotidov)
 - 33 proteinov
- Velika podenota (60S) vsebuje
 - 5S rRNA (120 nukleotidov),
 - 28S rRNA (4700 nukleotidov),
 - 5,8S rRNA (160 nukleotidov)
 - 46 proteinov.
- Mitochondriji imajo ribosome podobne prokariontskim – 70S (30S/50S)

Ribosomi v citoplazmi evkariontske celice



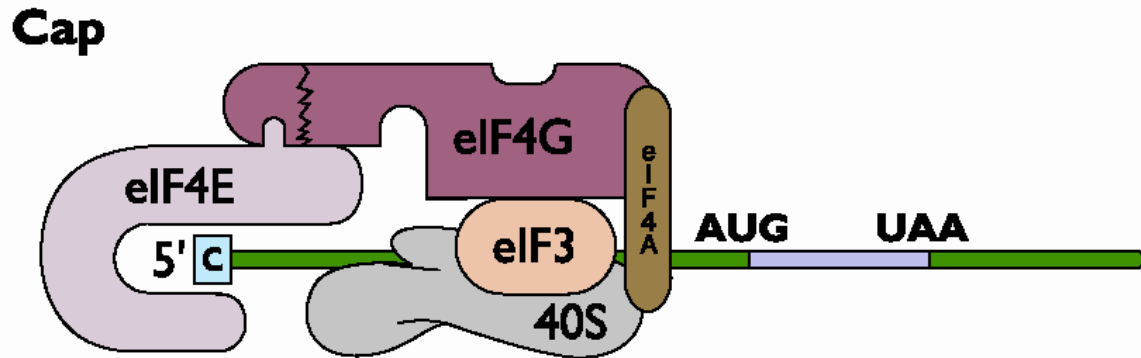
400 nm

Tri stopnje translacije

Iniciacija – začetna stopnja

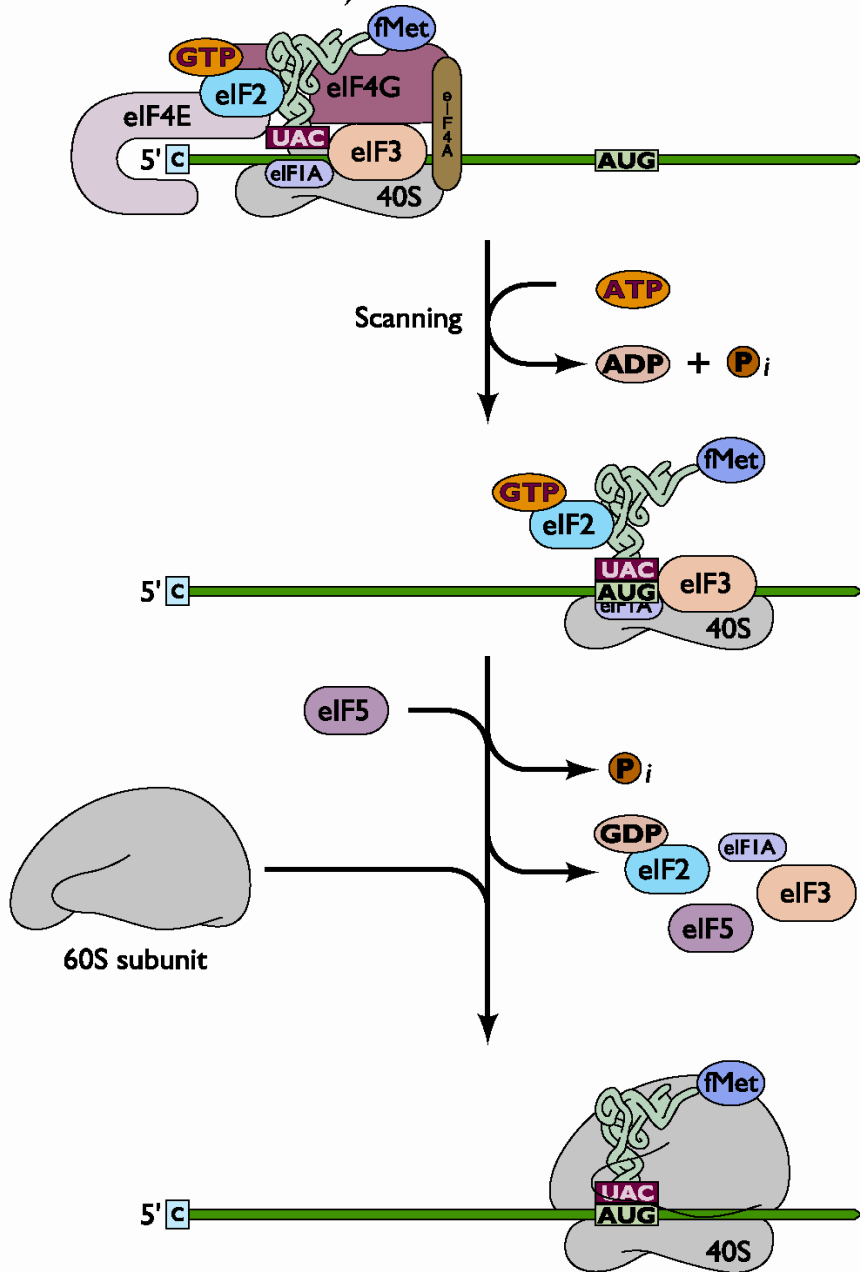
- Pogojuje hitrost translacije.
- Potrebuje hidrolizo ATP in GTP.
- Pride do tvorbe kompleksa, ki vsebuje mRNA, ribosom in začetno Met-tRNA.
- Iniciacijski kompleks se vež na 5' kapo in išče iniciacijski AUG.

Iniciacija povezana s 5' kapo



- V prvem koraku, eIF4F, ki vsebuje tri proteine eIF4E, eIF4G and eIF4A, prepozna 5' kapo.
- eIF4E se veže na kapo.
- N-terminus eIF4G veže eIF4E in C-terminus veže eIF4A.
- 40S podenota se veže na eIF4G s pomočjo eIF3.

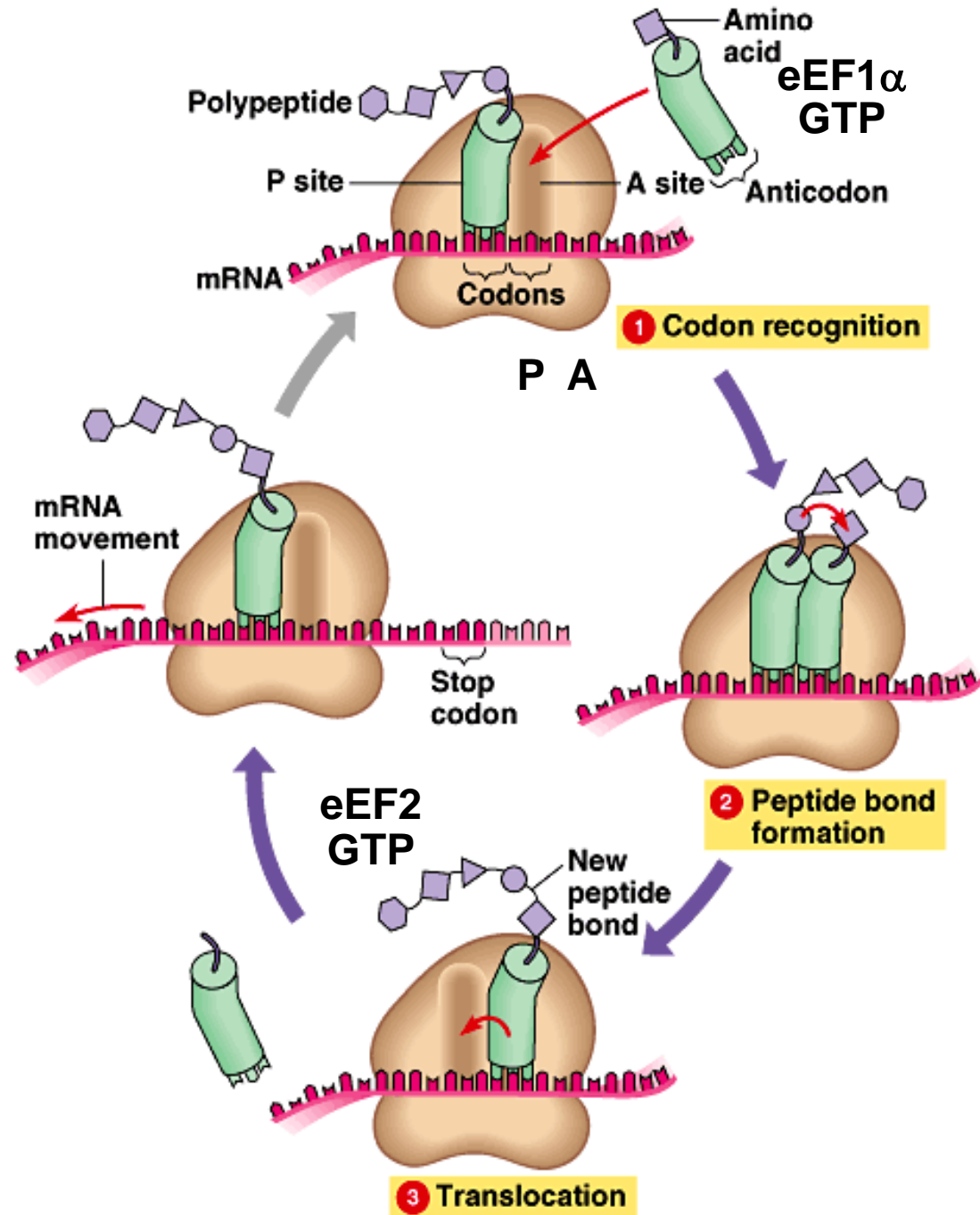
Iniciacija povezana s 5' kapo



- Na 5' kapi se tvori iniciacijski kompleks, sestavljen iz 40S podenote ribosoma in drugih translacijskih dejavnikov.
- Kompleks nato išče prvi AUG kodon v 5' UTR regiji.
- Hidroliza GTP z eIF5 sproži vezavo GDP na eIF2 in sprostitev iniciacijskih proteinov.
- Pride do vezave 60S podenote in začetka prepisovanja s kodirajočega zaporedja.

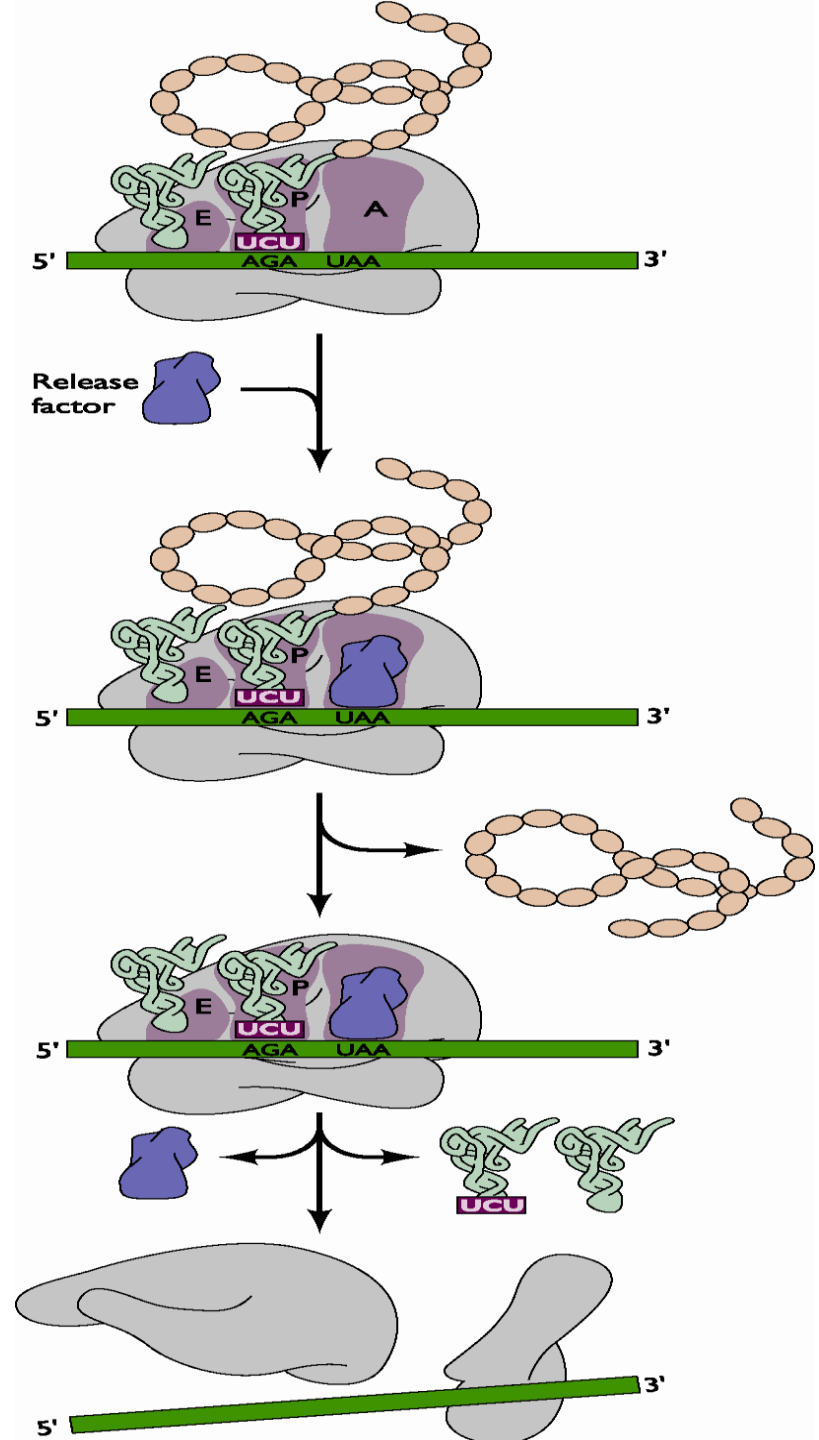
Elongacija

- Ribosom izbere aminoacil-tRNA.
- eEF1a in GTP sta vezana na aminoacil-tRNA.
- Ribosom katalizira tvorbo peptidne vezi.
- Translokacija je odvisna od eEF2 ter hidrolize GTP.
- Več ribosomov lahko hkrati prevaja z ene mRNAs.

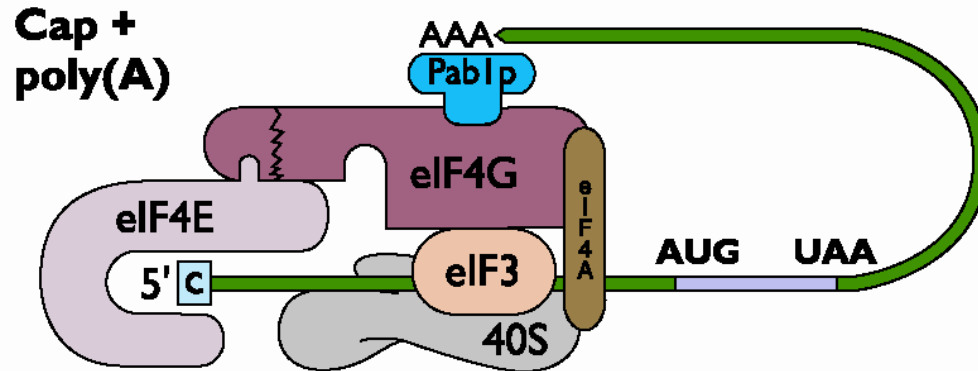


Terminacija

- Z enim od treh stop kodonov (UAA, UAG & UGA) pride do terminacije translacije.
- Sprostivni dejavnik prepozna stop kodon in se nanj veže.
- Peptidna veriga se sprosti in pride do razdruženja podenot ribosoma.



Model zaprte zanke

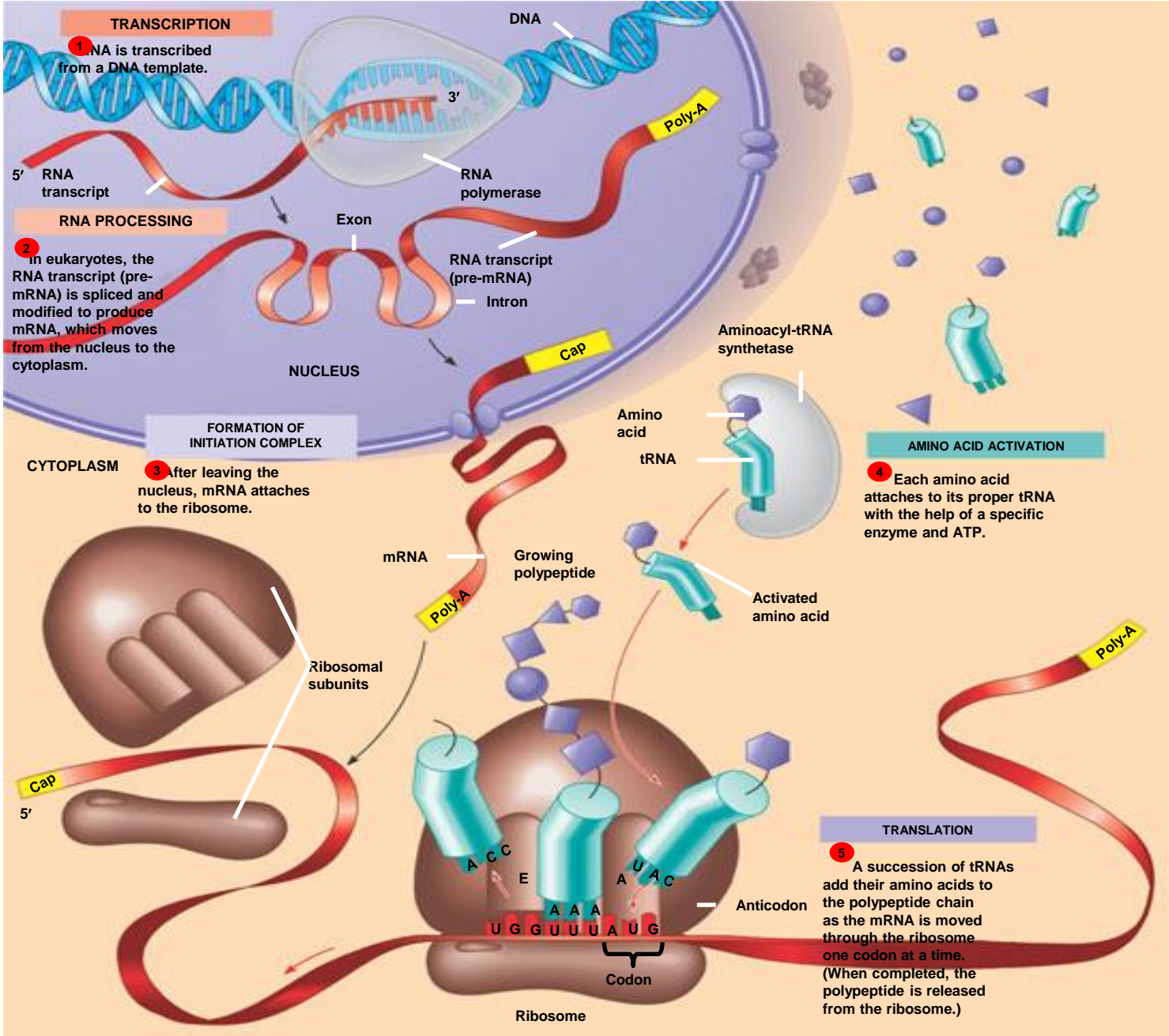


- Poly(A) vezavni protein, Pabp1p, interagira z eIF4G ter stimulira iniciacijo odvisno od 5' kape.
- mRNA se cirkularizacije, ki zagotavlja, da se prevede samo nepoškodovana mRNA.

Pregled translacije

HHMI

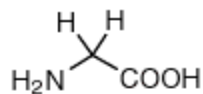
Povzetek transkripcije in translacije v evkariontski celici



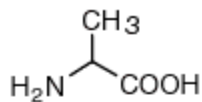
Aminokisljine – gradbeni elementi

- Proteini so verige aminokisljin.
- Aminokisljine
 - Gradbeni elementi proteinov.
 - Imajo različne kemične lastnosti.
 - Določajo zvijanje proteinov, strukturo in funkcijo.

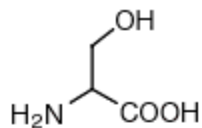
Small



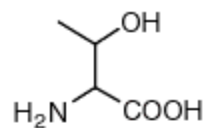
Glycine (Gly, G)
MW: 57.05



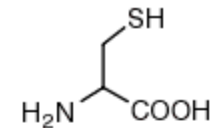
Alanine (Ala, A)
MW: 71.09



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

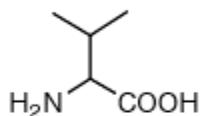


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

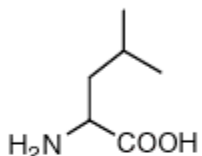


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

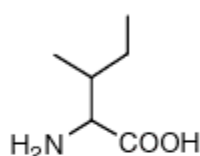
Hydrophobic



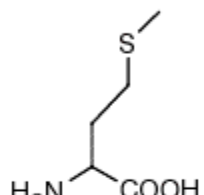
Valine (Val, V)
MW: 99.14



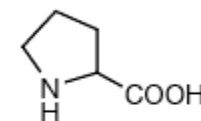
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

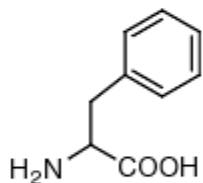


Methionine (Met, M)
MW: 131.19

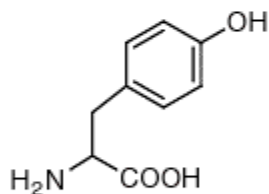


Proline (Pro, P)
MW: 97.12

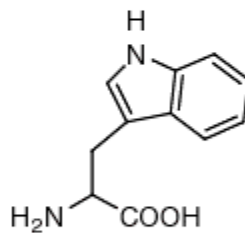
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

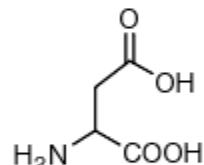


Tyrosine (Tyr, Y)
MW: 163.18

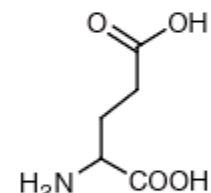


Tryptophan (Trp, W)
MW: 186.21

Acidic

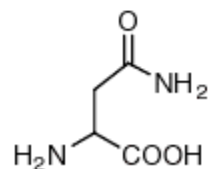


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

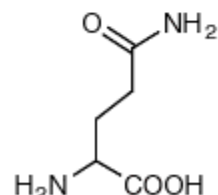


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

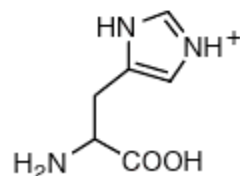


Asparagine (Asn, N)
MW: 114.11

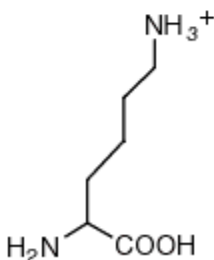


Glutamine (Gln, Q)
MW: 128.14

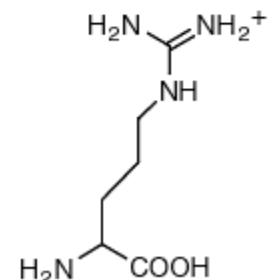
Basic



Histidine (His, H)
MW: 137.14, pK_a = 6.04



Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

Druge funkcije aminokislin

- Poleg tega, da so gradbeni elementi proteinov, imajo aminokislino druge biološko pomembne funkcije.
- Mnoge a.k. so prekurzorji drugih molekul:
 - **Triptofan** je prekurzor nevrottransmitterja **serotonina**
 - **Glicin** se uporablja pri sintezi porfirinov, npr. hema.
 - **Arginin** se uporablja pri sintezi hormona **nitričnega oksida**
- Uporabnost derivatov a.k.
 - **5-HTP** (5-hidroksitriptofan) se uporablja pri zdravljenju nevroloških težav povezanih s fenilketonurijo in depresijo.
 - **L-DOPA** (L-dihidroksifenilalanin) se uporablja pri zdravljenju Parkinsonove bolezni.
 - **Aspartam** (aspartil-fenilalanin-1-metil ester) je umetno sladilo.
 - **Natrijev glutamat** je dodatek hrani. Umami okus – peti okus.
- Nekatere a.k. in njihovi derivati imajo vlogo nevrottransmitterjev.

Nevrotransmitterji

- Neurotransmitter je katerakoli spojina, ki vpliva na nevronske impulze.

- Običajno jih proizvajajo nevroni.

- Večina se jih veže na receptorje na postsinaptični (dendritski) membrani, kjer

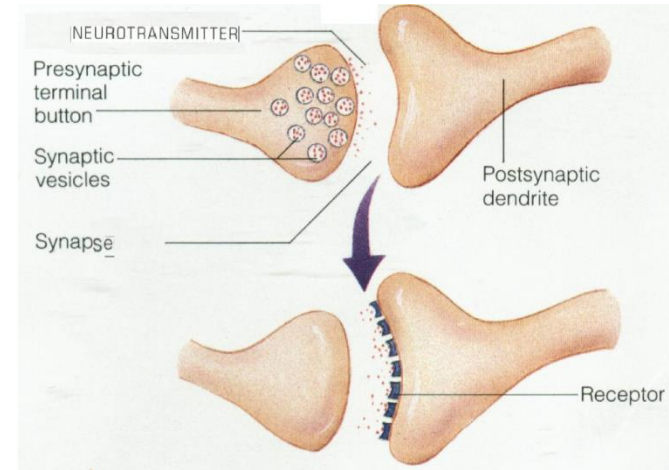
- Odpirajo kanalčke

- Povzročijo znotraj-celični odgovor (npr. vpliv na izražanje določenega proteina).

- Ko se vežejo na receptor, neurotransmitterji povzročajo EPSP ali IPSP (ekscitatorni ali inhibitorni postsinaptični potencial)

- Ali je odgovor nevrona EPSP ali IPSP ni odvisno of neurotransmitterja, vendar od receptorja.

- Receptor določa, kateri kanalček se odpre oz. katera signalna kaskada se sproži.



Aminokislinski nevrottransmiterji

•Glutamat

- Naravno prisoten v prehrani.
- Skoraj vsi nevroni proizvedejo EPSP po zaznavanju Glu.
- Glu je povezan z vsem oblikam vedenja.

•GABA (gama-amino butirat)

- V mnogih nevronih encim glutamat dekarboksilaza spremeni glutamat v GABA.
- Vezava GABA na receptor povzroča predvsem IPSP pri skoraj vseh nevronih.
- Izguba GABAergičnih nevronov vodi do epileptičnih napadov, ker nevroni niso inhibirani.
- Z vezavo na GABA receptorje, zdravila kot benzodiazepini in barbiturati olajšajo GABA signalizacijo.

•Glicin

- Pomemben inhibitorni nevrottransmiter v hrbtenjači.

Monoamini

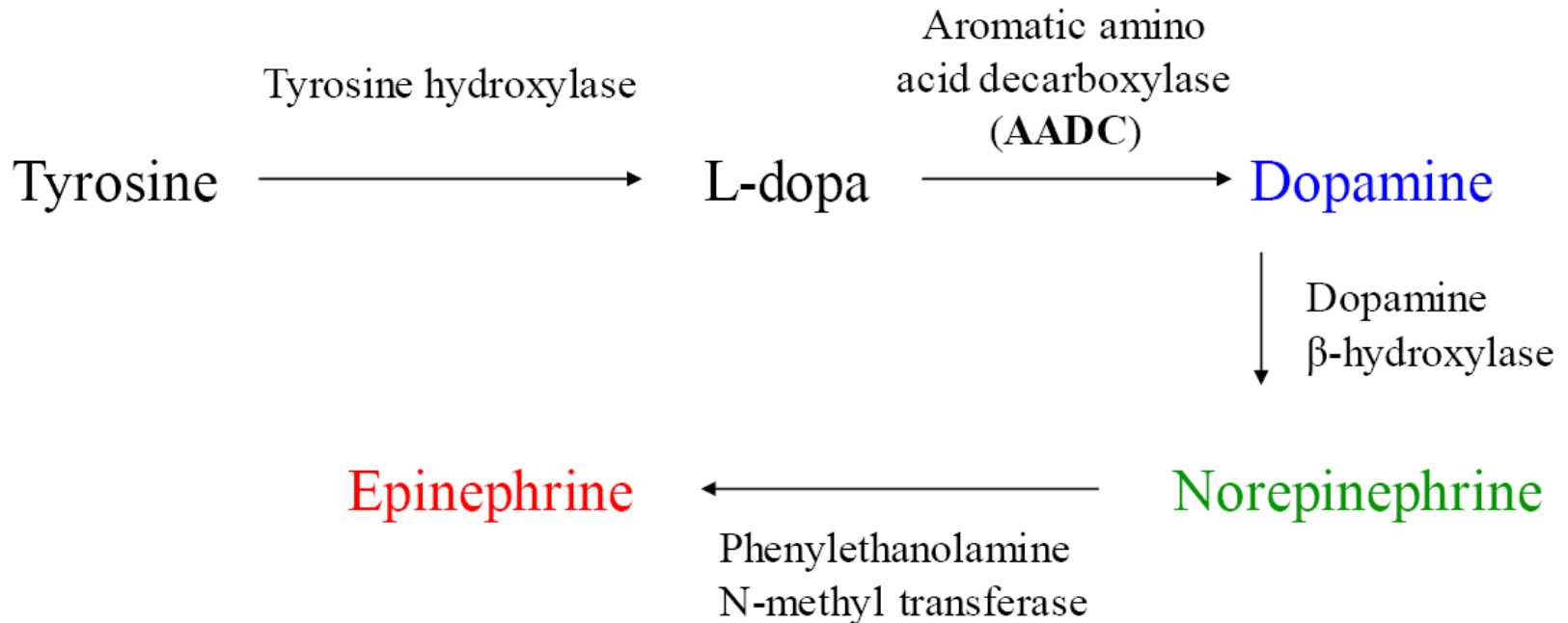
- Dva razreda
 - Kateholamini, katerih prekurzor je tirozin.
 - Indolamini, katerih prekurzor je triptofan.

Kateholamini

- **Dopamin (DA)**
 - povezan s pričetkom in nadgradnjo gibanja.
 - zavestna kontrola mišic (v povezavi s Parkinsonovo boleznijo).
 - povezan s kognitivnimi motnjami pri ADHD in shizofreniji.
- **Noradrenalin/adrenalin (NA/A)**
 - Funkcija v avtonomnem živčevju.
 - NA v locus coeruleus v centralnem živčevju, stresni hormon.
 - A v perifernem živčevju zvišuje hitrost in jakost srčnega utripa, širi dihalne poti.
 - Sinapse, ki uporabljajo A so adrenergične.

Biosinteza kateholaminov

- Adrenalin = epinefrin
- Noradrenalin = norepinefrin

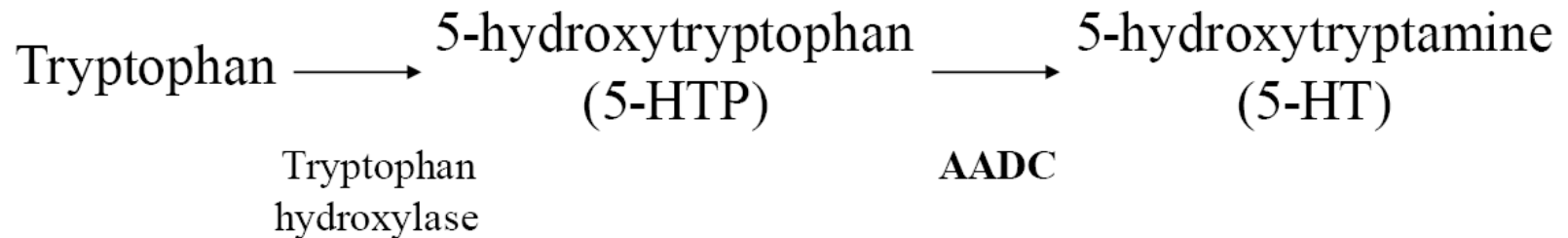


Indolamini

- **Serotonin (5-HT)**

- povezan s počutjem in agresijo

- Prozac in Ecstasy povečajo nivo 5-HT



Peptidni nevrottransmitterji

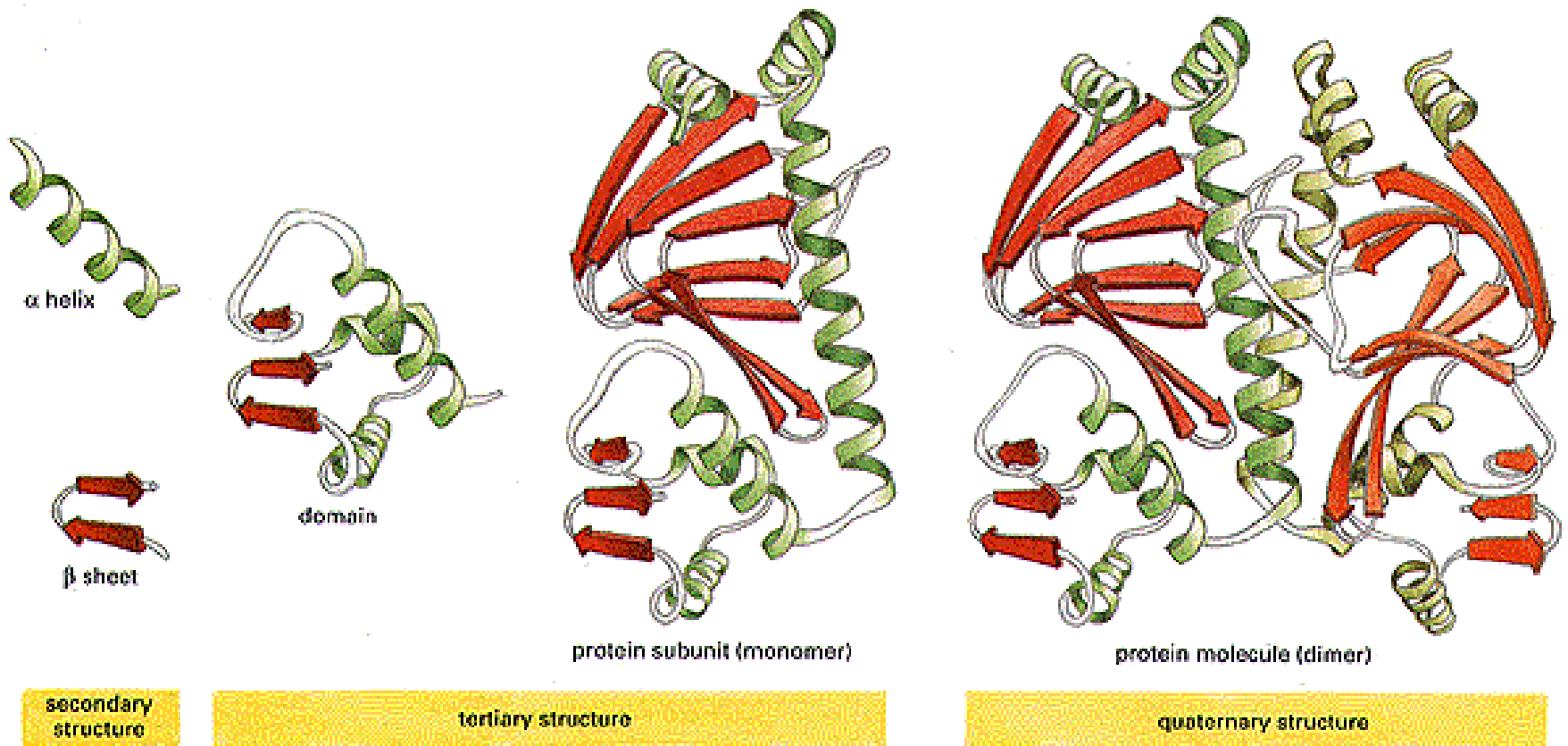
- Enkefalin (5 aminokislin) – povezan z ustavljanjem bolečine.
- Substanca P (11 aminokislin) – sodeluje v procesih vnetja in zaznavanja bolečine.

Po translaciji

- Nov polipeptid se nahaja v citoplazmi, če je sintetiziran na prostem ribosomu.
- Lahko pa je vstavljen v membrano ali pripravljen za prenos v medcelični prostor, če je translacija potekla na ribosomu, vezanem na endoplazmatični retikulum.
- Polipeptidi se zvijejo v aktivno konfiguracijo. Nekateri se povežejo z drugimi polipeptidi in tvorijo proteinske komplekse.
- S postranslacijskim modifikacijam se dodajo še druge molekule, ki uravnavajo funkcijo proteina.

Struktura proteinov

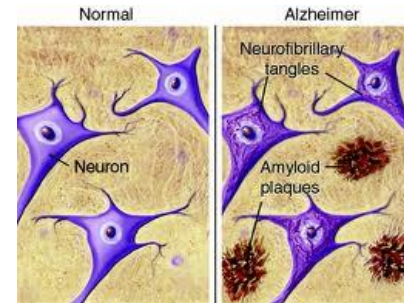
Primarna, sekundarna, terciarna in kvartarna struktura



Zvijanje proteinov in bolezni

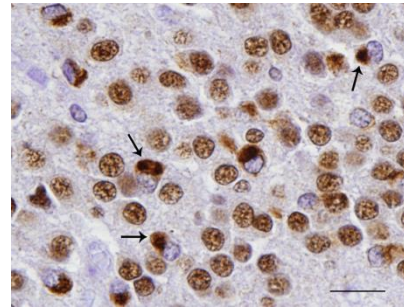
- Alzheimerjeva bolezen

- Tvorba amiloidnih plakov izven celic.
- Tvorba neurofibrilarnih pentelj znotraj celic.
- Mutacije v APP ali proteinih, ki procesirajo APP.



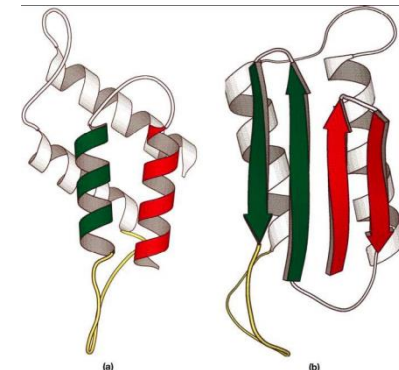
- ALS/FTD

- Agregati RNA-vezavnih proteinov.



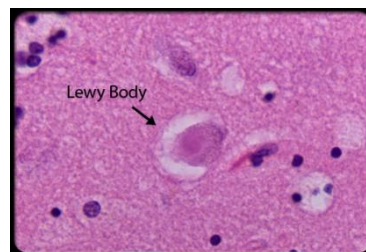
- BSE, CJD

- Normalni prionski protein je večinoma α -heliks.
- Bolezenski prionski protein je mešanica α in β struktur, ki tvorijo netopne agregate, ki poškodujejo nevrone.



- Parkinsonova bolezen

- Mutacije sinukleina in tvorba Lewyevih telesc.



Posttranslacijske modifikacije

- PTM se zgodijo po sintezi proteina.
- Spremenijo konformacijo proteina – spremenijo/uravnavajo aktivnost/funkcijo proteina.
- Reverzibilne modifikacije - omogočajo dodaten nivo regulacije funkcije proteinov brez potrebe po de novo sintezi.

Posttranslacijske modifikacije

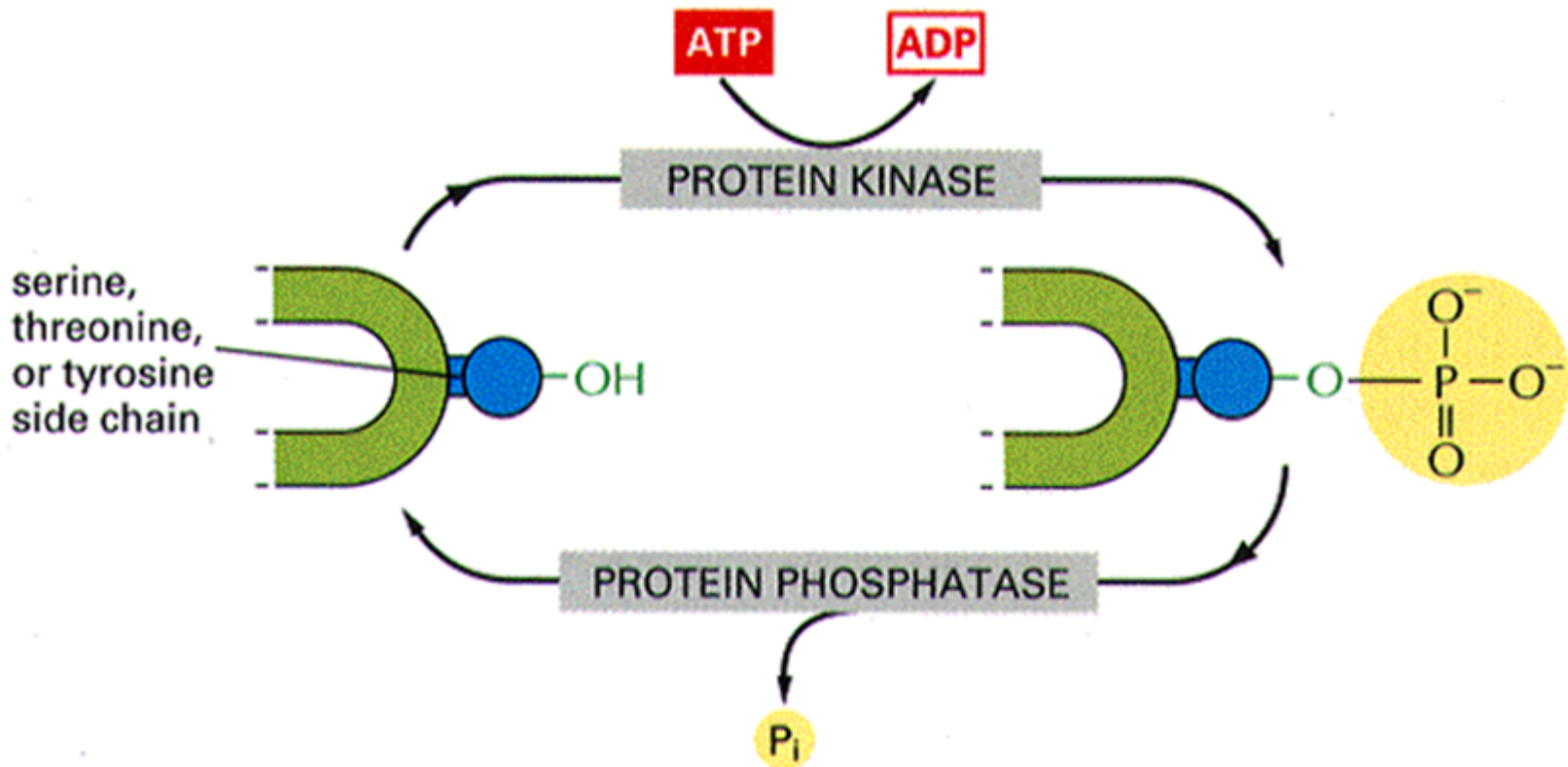
TABLE 1.7 MAJOR TYPES OF MODIFICATION OF POLYPEPTIDES		
Type of modification (group added)	Target amino acid(s)	Notes
Phosphorylation (PO ₄ ⁻)	Tyr, Ser, Thr	achieved by specific kinases; may be reversed by phosphatases
Methylation (CH ₃)	Lys	achieved by methylases; reversed by demethylases
Hydroxylation (OH)	Pro, Lys, Asp	hydroxyproline (Hyp) and hydroxylysine (Hyl) are particularly common in collagens
Acetylation (CH ₃ CO)	Lys	achieved by an acetylase; reversed by deacetylase
Carboxylation (COOH)	Glu	achieved by γ -carboxylase
<i>N</i> -glycosylation (complex carbohydrate)	Asn ^a	takes place initially in the endoplasmic reticulum, with later additional changes occurring in the Golgi apparatus
<i>O</i> -glycosylation (complex carbohydrate)	Ser, Thr, Hyl ^b	takes place in the Golgi apparatus; less common than <i>N</i> -glycosylation
Glycosylphosphatidylinositol (glycolipid)	Asp ^c	serves to anchor protein to outer layer of plasma membrane
Myristoylation (C ₁₄ fatty acyl group)	Gly ^d	serves as membrane anchor
Palmitoylation (C ₁₆ fatty acyl group)	Cys ^e	serves as membrane anchor
Farnesylation (C ₁₅ prenyl group)	Cys ^c	serves as membrane anchor
Geranylgeranylation (C ₂₀ prenyl group)	Cys ^c	serves as membrane anchor

^aThis is especially common when Asn is in the sequence: Asn-X-(Ser/Thr), where X is any amino acid other than Pro. ^bHydroxylysine. ^cAt C-terminus of polypeptide. ^dAt N-terminus of polypeptide. ^eTo form S-palmitoyl link.

Table 1.7 Human Molecular Genetics, 4ed. (© Garland Science)

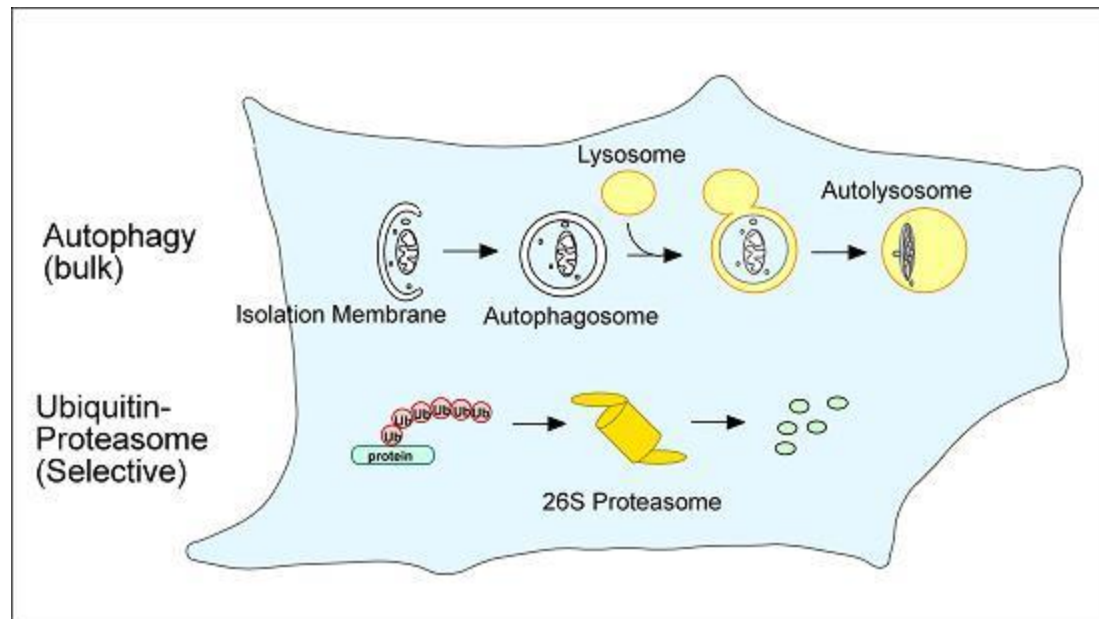
Fosforilacija proteinov

- Dominantna oblika PTM.
- Reverzibilna in z visoko stopnjo regulacije.
- Protein kinaze dodajo fosfatno skupino.
- Protein fosfataze odstranijo fosfatno skupino.



Razgradnja proteinov

- Evkarionti imajo dva pomembna sistema razgradnje proteinov.
 - **Autofagija**: izolacijska membrana zajame majhen volumen citoplazme in tvori autofagosom. Autofagosom se nato združi z lizosomom in pride do razgradnje.
 - **Ubikvitin-proteasomna pot**: poliubikvitinirane proteine razgradi 26S proteasom.



SCA1 - bolezen razgradnje

- Napake v ataksinu 1 (ATXN1) povzročajo spinocerebelarno ataksijo tip 1 (SCA1)
- Zaradi degeneracij malih možganov, bolniki imajo slabo koordinacijo hoje in gibanja rok, oči, govora.
- SCA1 nastane zaradi podaljšanja CAG ponovitev v kodirajoči regiji ATXN1. Daljše ponovitve prej pripeljejo do bolezni, ki je tudi bolj huda.

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