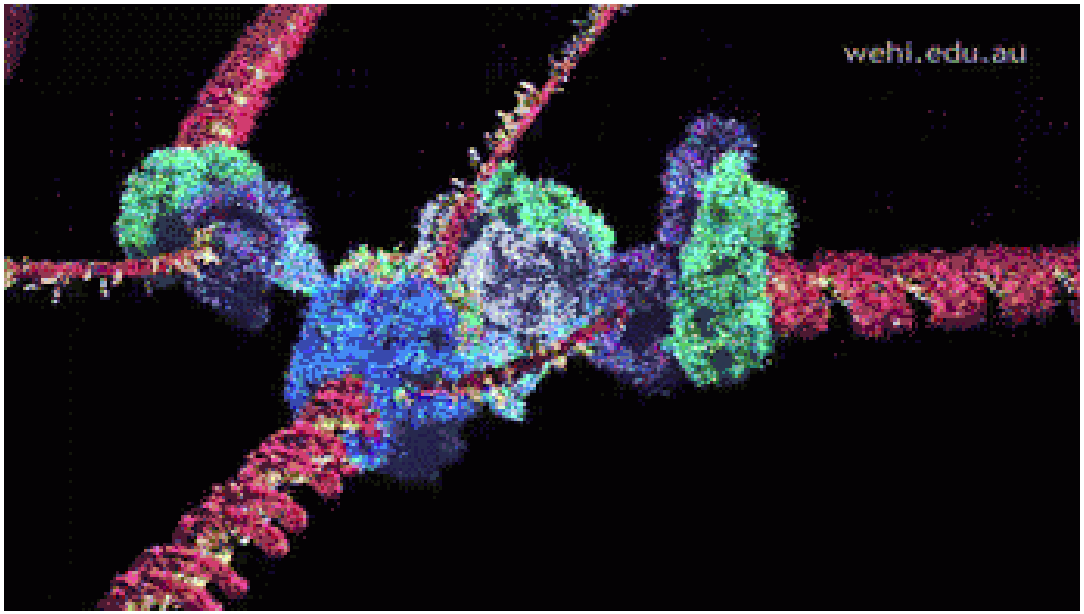
Sinteza proteinelor

Sinteza proteinelor este datorata translatiei informatiei genetice!

Translatie = "limbajul" secventei de nucleotide din molecula ARNm este tradus in "limbajul" unei secvente de aminoacizi.



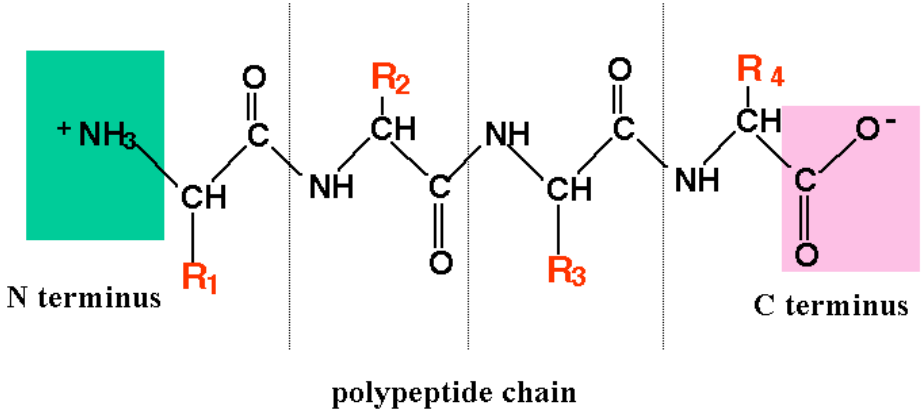
Actiunea unei enzime (proteina) in timpul replicarii ADN

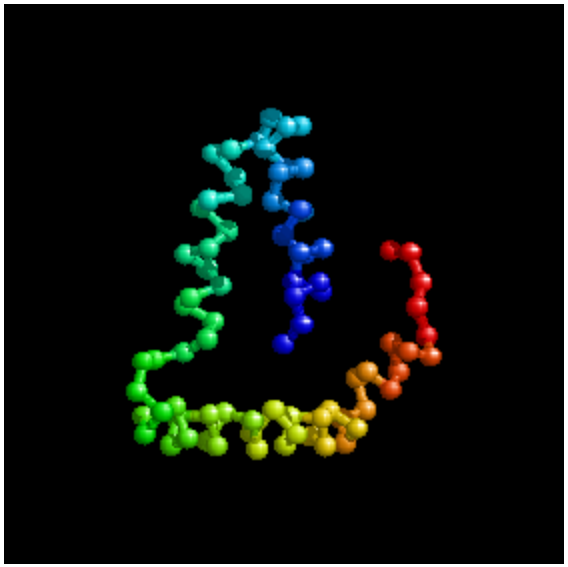
https://amit1b.wordpress.com/the-molecules-of-life/about/

Proteinele au roluri foarte diversificate: de la cataliza unor reactii chimice, la structurarea tuturor organismelor vii..

Informatia genetica inmagazinata in cromozomi:

- este transmisa celulelor fiice prin **replicarea** ADN;
- este exprimata in ARN prin **transcrierea** informatiei genetice;
- este folosita in **sinteza** proteinelor pornind de la ARNm (**translatie**)





proteinele = lanturi de aminoacizi

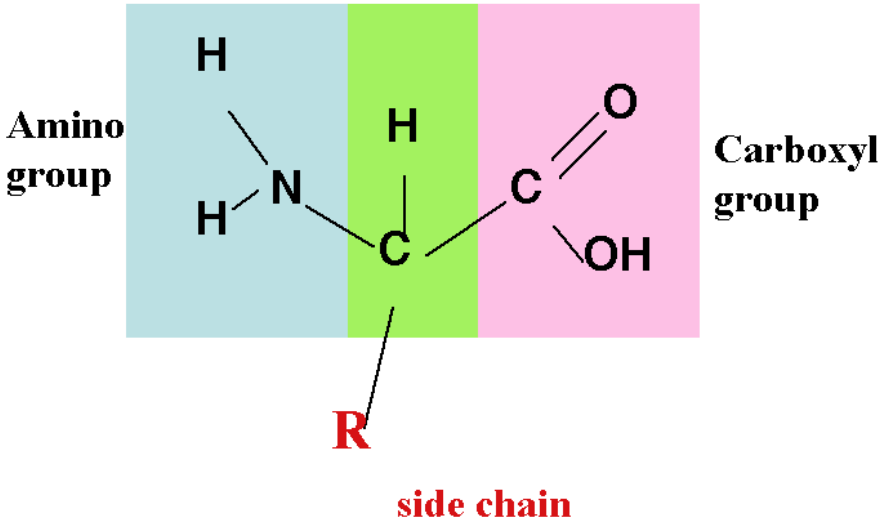
Orice modificare a secventei acizilor nucleici poate determina inserarea incorecta a unui aminoacid in lantul de proteine (mutatie - aparitia unor boli).

Dupa ce sunt sintetizate, numeroase proteine sunt modificate covalent. Astfel devin active, isi modifica actiunea sau sunt dirijate spre destinatia finala intracelulara sau extracelulara.

Proteinele sunt cele mai numeroase molecule din structura sistemelor vii, toate procesele din organism depinzand direct de proteine.

Desi proteinele indeplinesc functii foarte diferite in organism, toate au structuri primare similare: **polimeri liniari de aminoacizi!**

In structurile proteice animale s-au identificat **20 aminoacizi** din cei 300 existenti.



Toti **aminoacizii** ce intra in compozitia proteinelor au o grupare carboxil, o grupare amino primara si o catena laterala distincta, atasata carbonului α .

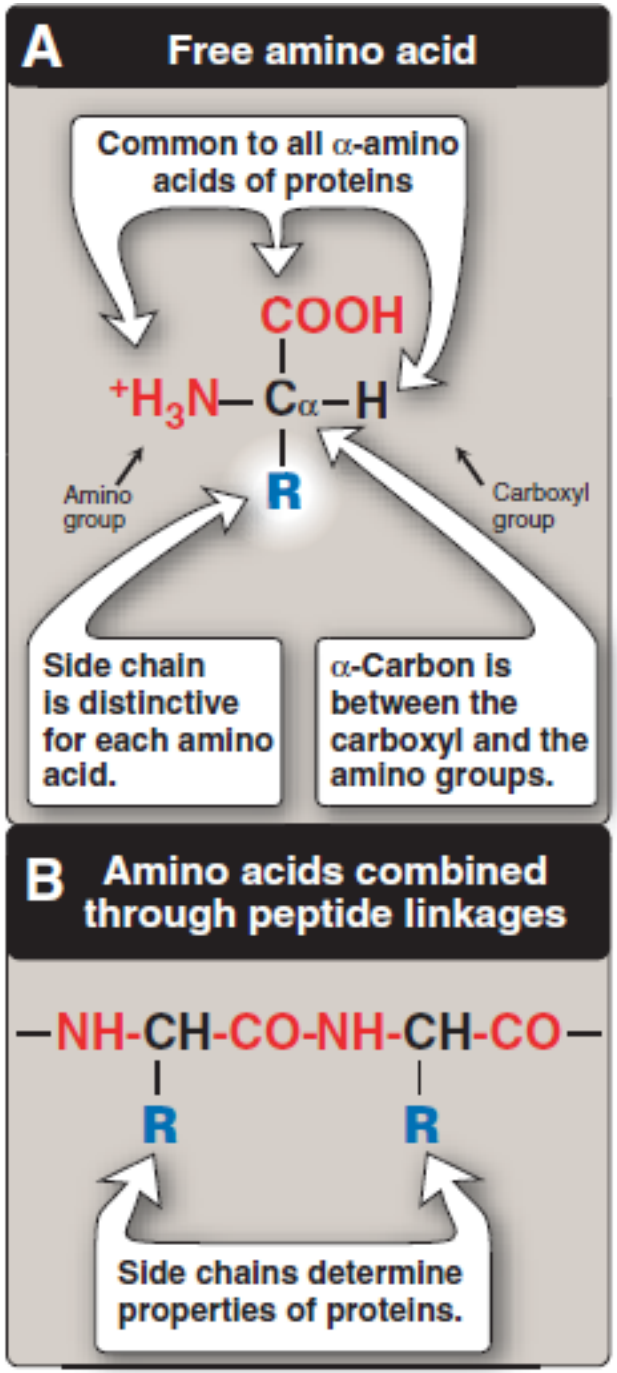
(exceptie: prolina contine o grupare amino secundara).

Aminoacizii

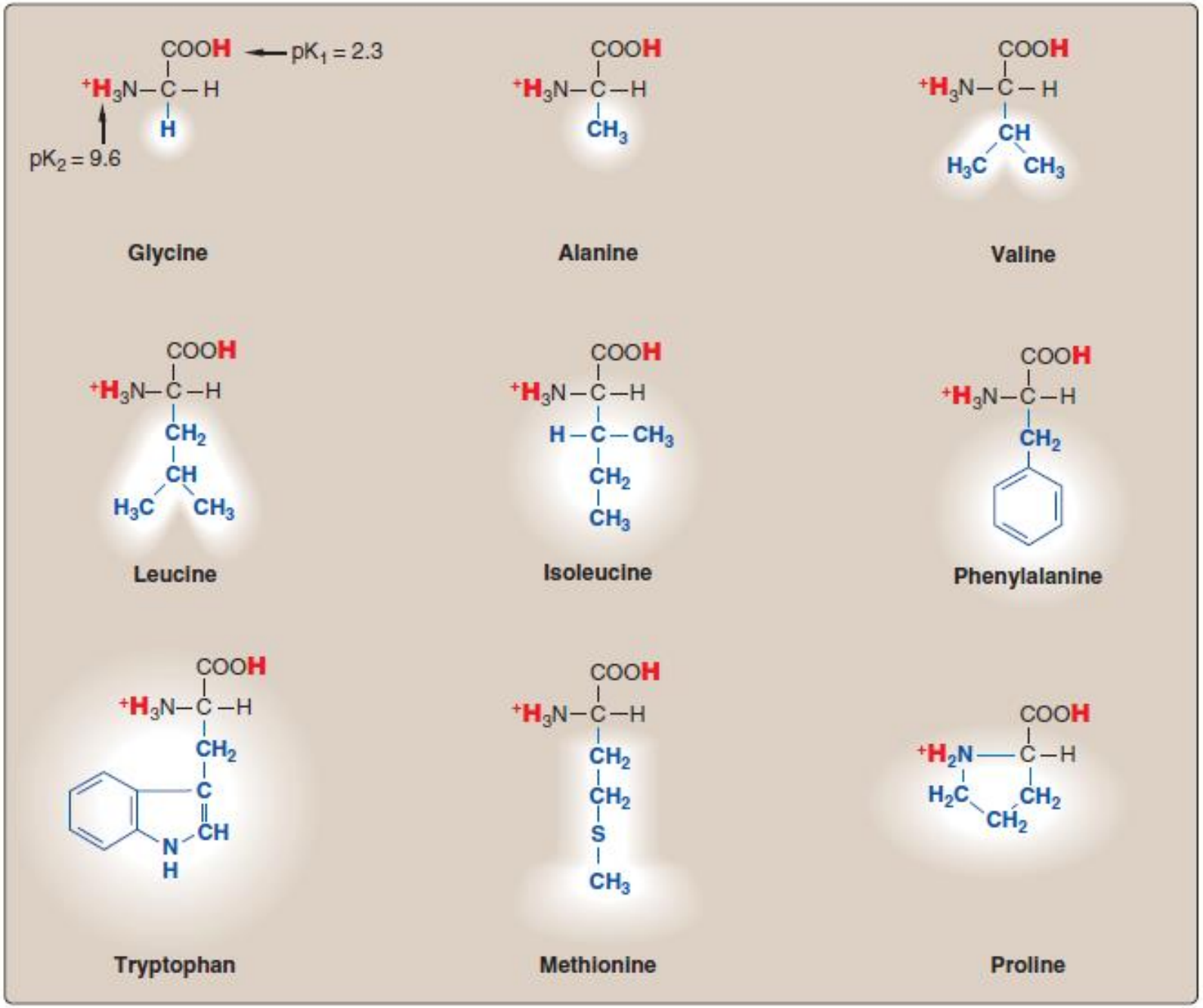
In conditii de pH fiziologic (7,4) gruparea carboxil este disociata (COO^-), iar gruparea amino este protonata ($NH3^+$).

In structura proteinelor cele mai multe grupari carboxil si amino sunt angrenate in **complexe peptidice** (prin legatura **C-N**) fiind capabile sa reactioneze chimic doar pentru a genera legaturi de hidrogen.

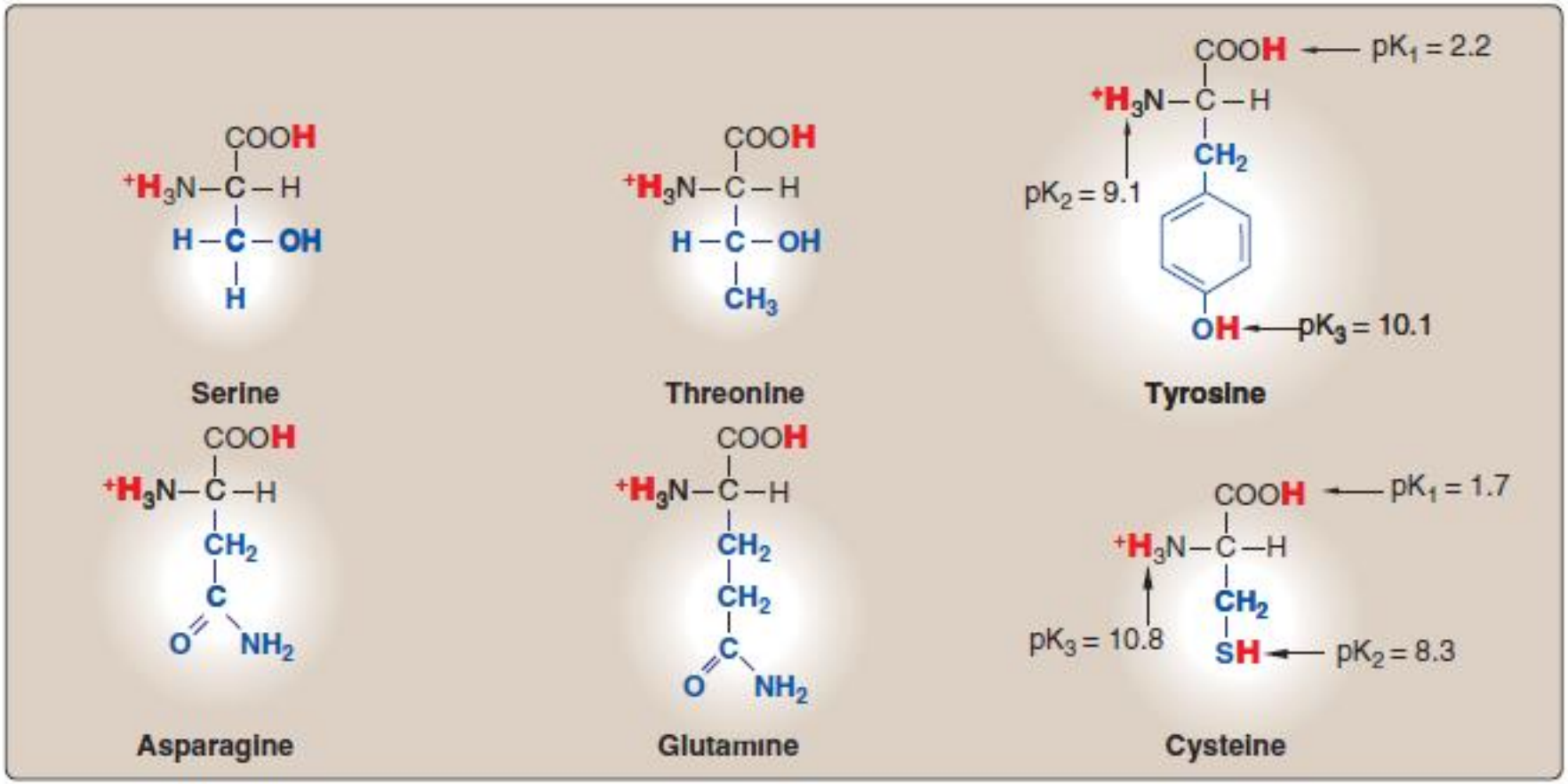
Catena laterala este principalul factor, din structura unui aminoacid, care determina proprietatile proteinei.



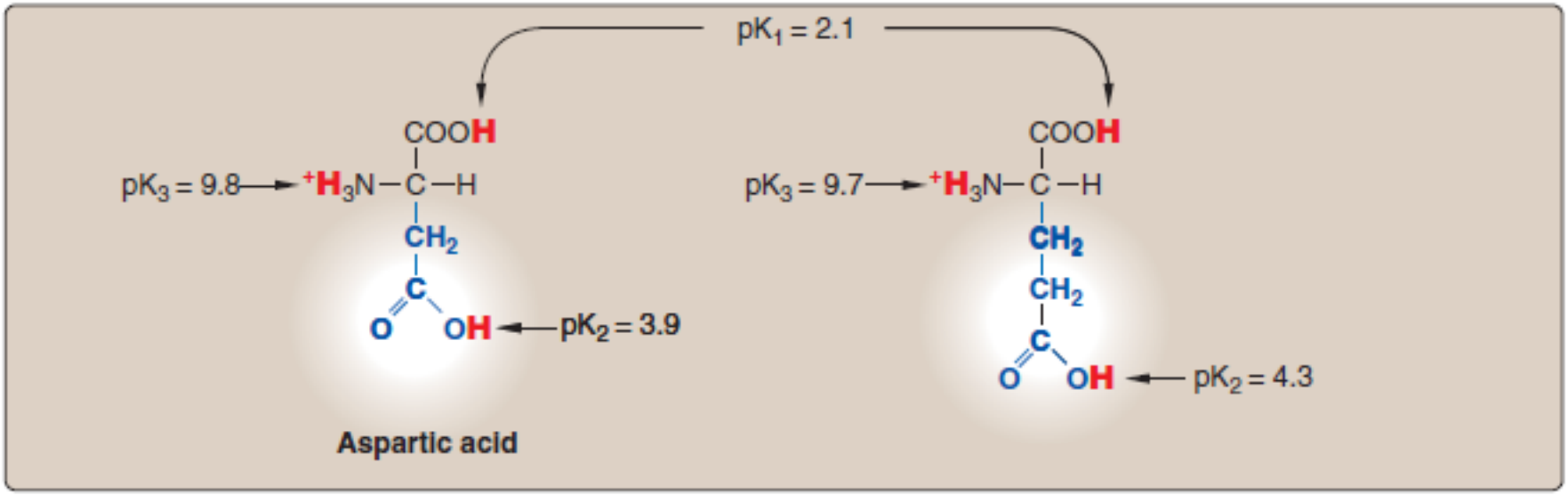
Aminoacizi cu catene laterale nepolare



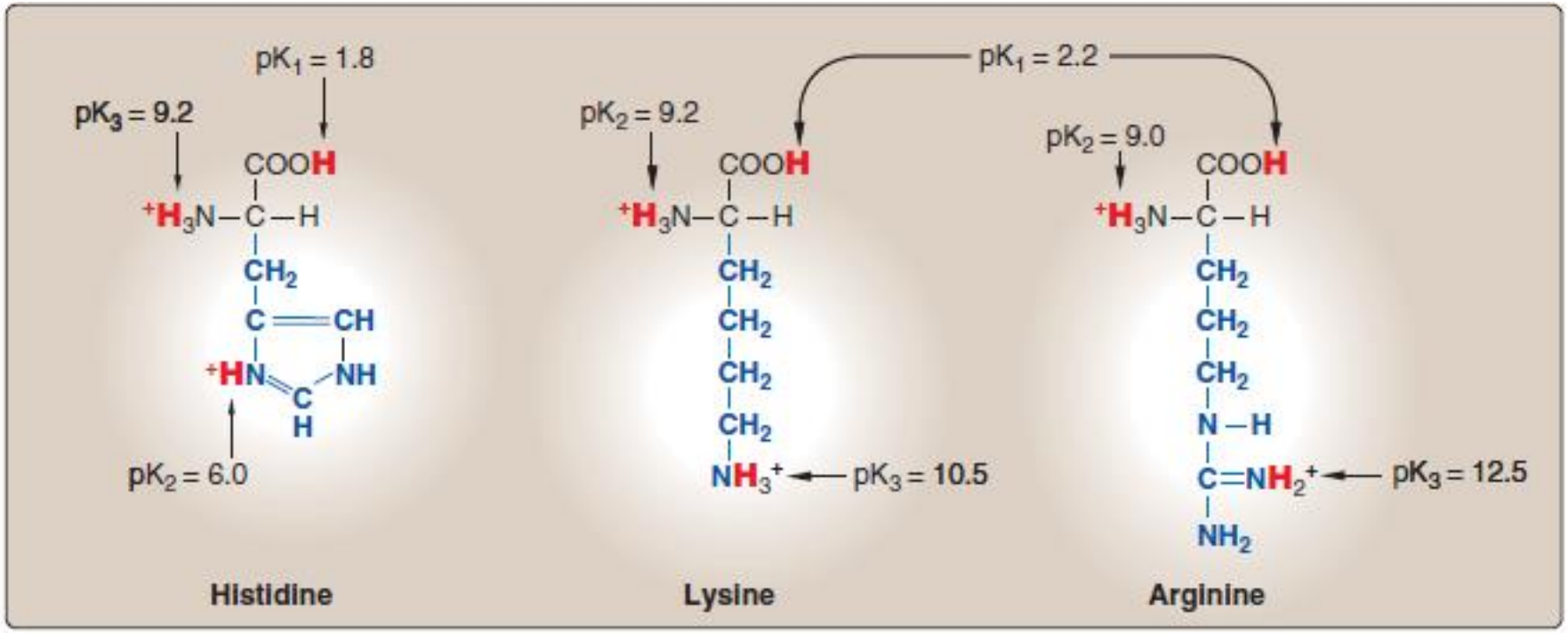
Aminoacizi cu catene laterale neutre



Aminoacizi cu catene laterale acide



Aminoacizi cu catene laterale bazice



Fiecarui aminoacid ii sunt asociate o **abreviere alcatuita din 3 litere** si un simbol reprezentat de o singura litera.

Simbolurile cu o litera se supun urmatoarelor reguli:

1 Unique first letter:

Cysteine	=	Cys	=	C
Histidine	=	His	=	H
Isoleucine	=	Ile	=	I
Methionine	=	Met	=	M
Serine	=	Ser	=	S
Valine	=	Val	=	V

2 Most commonly occurring amino acids have priority:

Alanine	=	Ala	=	A
Glycine	=	Gly	=	G
Leucine	=	Leu	=	L
Proline	=	Pro	=	P
Threonine	=	Thr	=	T

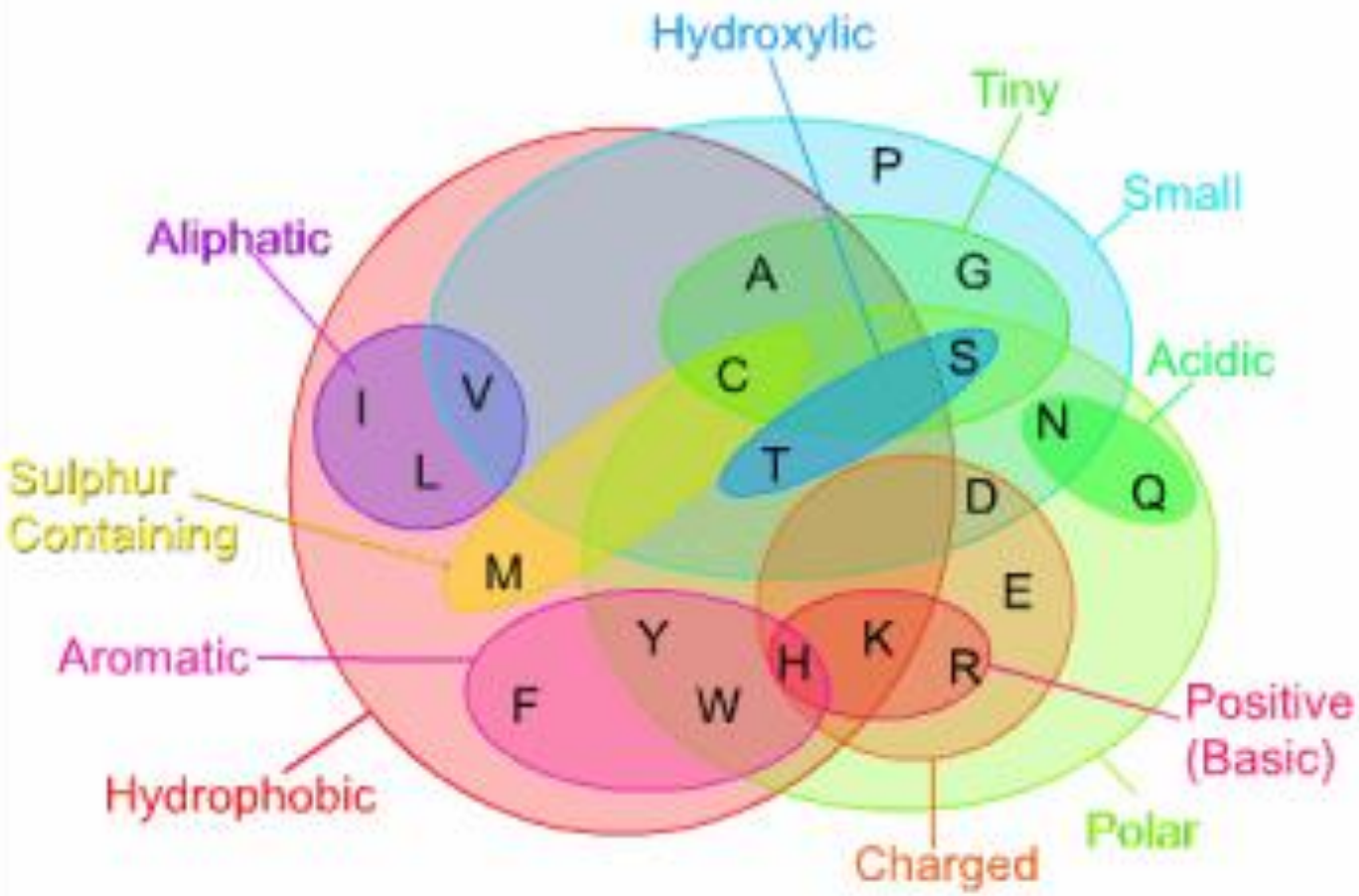
3 Similar sounding names:

Arginine	=	Arg	=	R	("aRginine")
Asparagine	=	Asn	=	N	(contains N)
Aspartate	=	Asp	=	D	("asparDic")
Glutamate	=	Glu	=	E	("glutEmate")
Glutamine	=	Gln	=	Q	("Q-tamine")
Phenylalanine	=	Phe	=	F	("Fenylalanine")
Tyrosine	=	Tyr	=	Y	("tYrosine")
Tryptophan	=	Trp	=	W	(double ring in the molecule)

4 Letter close to initial letter:

Aspartate or asparagine	=	Asx	=	B	(near A)
Glutamate or glutamine	=	Glx	=	Z	
Lysine	=	Lys	=	K	(near L)
Undetermined amino acid	=			X	

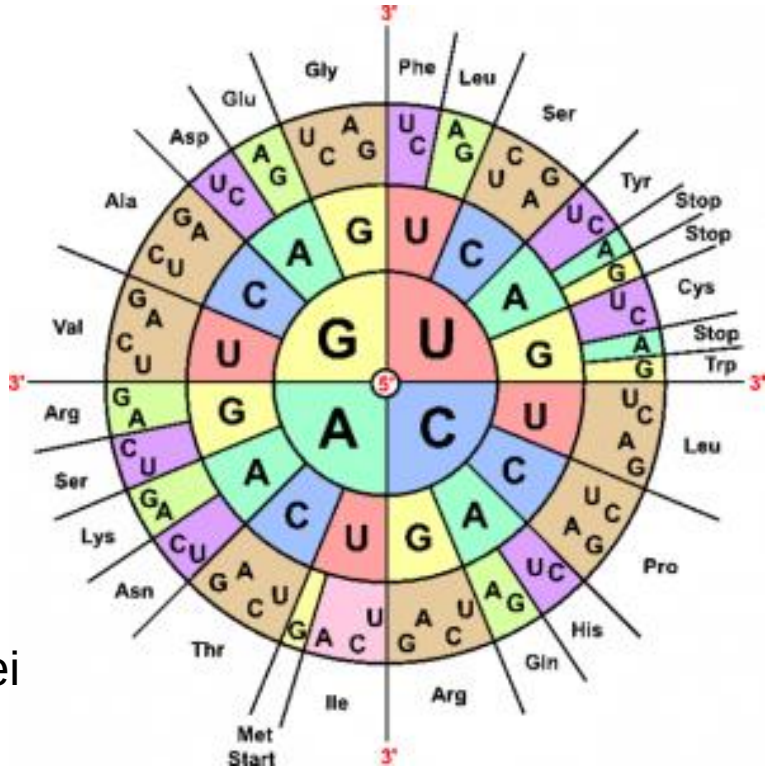
1. Unicitatea initialei
2. Frecventa aparitiei aminoacidului
3. Similitudini fonetice
4. Regula proximitatii alfabetice



Amino Acids

A alanine (ala)
R arginine (arg)
N asparagine (asn)
D aspartic acid (asp)
C cysteine (cys)
Q glutamine (gln)
E glutamic acid (glu)
G glycine (gly)
H histidine (his)
I isoleucine (ile)
L leucine (leu)
K lysine (lys)
M metioneine (met)
F phenyalanine (phe)
P proline (pro)
S serine (ser)
T threonine (thr)
W trytophan (trp)
Y tyrosine (tyr)

Codul genetic



Codul genetic este asemeni unui "dictionar" reprezentand un sistem de corespondenta intre o secventa de nucleotide si o secventa de aminoacizi.

Fiecare "**cuvant**" din acest cod este alcatuit din trei nucleotide (**codon).**

Exista 4 tipuri de nucleotide care intra in alcatuirea codonilor din molecula ARNm: adenina (A), guanina (G), citozina (C), uracil (U).

Secventele nucleotidice se scriu **de la capatul 5' catre capatul 3'.**

Exista 64 combinatii diferite de cate 3 nucleotide:

61 codifica cei 20 de aminoacizi esentiali,

3 (UAG, UGA, UAA) sunt codoni "STOP" - indica finalizarea sintezei proteice.

Fiecare proteina incepe cu metionina (AUG), deci codonul **AUG** este considerat codonul " **START**" .

ADN - proteina

ARN - proteina

second base in codon

first base in codon	T	C	A	G	third base in codon
T	TTT Phe	TCT Ser	TAT Tyr	TGT Cys	T
	TTC Phe	TCC Ser	TAC Tyr	TGC Cys	C
	TTA Leu	TCA Ser	TAA stop	TGA stop	A
	TTG Leu	TCG Ser	TAG stop	TGG Trp	G
C	CTT Leu	CCT Pro	CAT His	CGT Arg	T
	CTC Leu	CCC Pro	CAC His	CGC Arg	C
	CTA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CTG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	ATT Ile	ACT Thr	AAT Asn	AGT Ser	T
	ATC Ile	ACC Thr	AAC Asn	AGC Ser	C
	ATA Ile	ACA Thr	AAA Lys	AGA Arg	A
	ATG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GTT Val	GCT Ala	GAT Asp	GGT Gly	T
	GTC Val	GCC Ala	GAC Asp	GGC Gly	C
	GTA Val	GCA Ala	GAA Glu	GGA Gly	A
	GTG Val	GCG Ala	GAG Glu	GGG Gly	G

Second letter

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

ADN → ADN: A → T; T → A; C → G; G → C

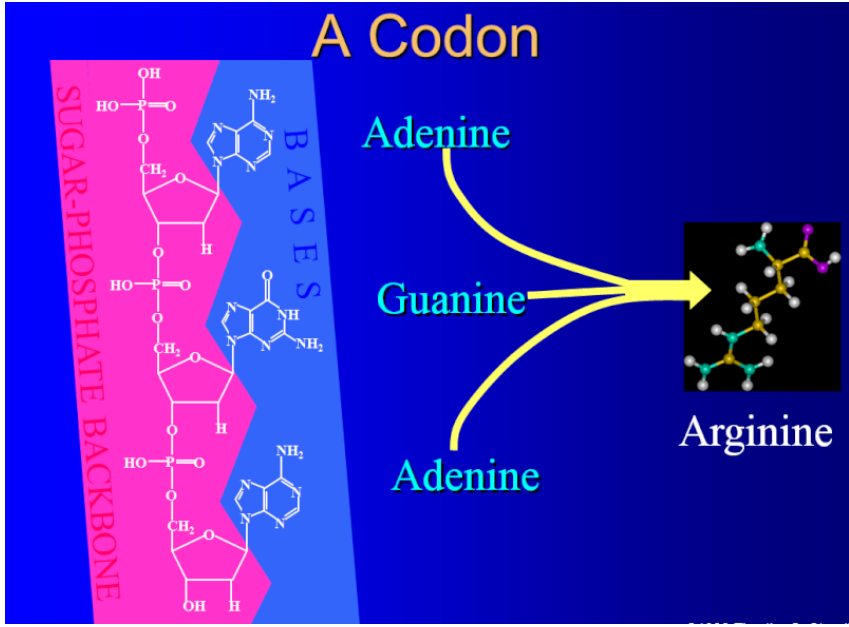
ADN → ARN: A → U; T → A; C → G; G → C

catena1 → catena 2 → ARN

Ex: Codonul start (Metionil): ADN → ADN → ARN

5 ATG 3 → 3 TAC 5 → 5 AUG 3

Caracteristicile codului genetic



- **Specificitatea:**

Un codon semnifica totdeauna acelasi aminoacid!

- **Universalitatea:**

Codul genetic este acelasi din cele mai timpurii stadii ale evolutiei

Exceptie: codul genetic mitocondrial

- **Degenerarea:**

Un anumit aminoacid poate fi codificat de mai multe triplete de nucleotide *(exemplu: arginina este codificata de 6 codoni diferiti;*

Exceptie: metionina!!!

- **Nesuprapunerea si lipsa punctuatiei:**

Codul este citit dintr-un punct fix, ca o secventa continua de baze in grupuri de cate 3 nucleotide care nu sunt separate prin virgule.

Consecintele modificarii secventelor de nucleotide

Modificarea unui singur nucleotid in lantul ARNm duce la:

- **Mutatie silentioasa:**

Codonul care contine un nucleotid modificat codeaza acelasi aminoacid

(exemplu: UCA → UCU serina → serina)

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

- **Mutatie cu sens gresit:**

Codonul care contine un nucleotid modificat codeaza un aminoacid diferit

(exemplu: UCA → CCA serina → prolina)

- **Mutatie nonsens:**

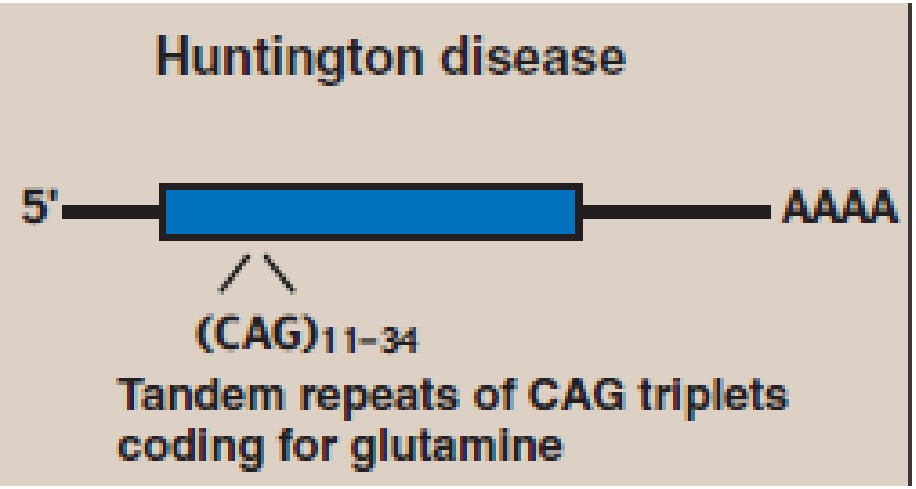
Codonul care contine un nucleotid modificat este un codon nonsens

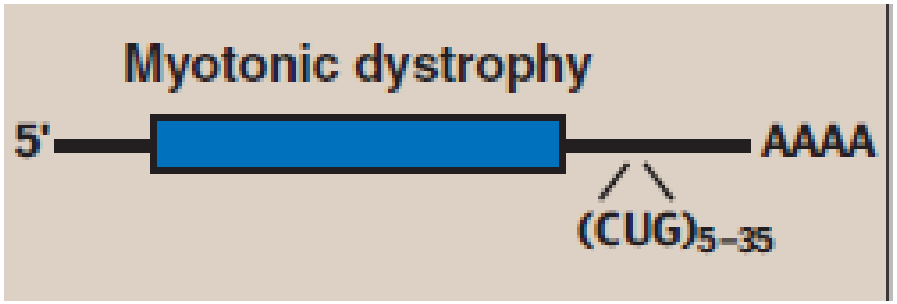
(exemplu: UCA → UAA serina → STOP)

Alte mutatii (cele mai grave!)

- **Amplificarea tripletelor repetitive**:

O secventa de 3 nucleotide care se repeta poate fi amplificata numeric (vor aparea prea multe copii ale tripletului respectiv).





- **Mutatiile de la nivelul sinusurilor de splicing**:

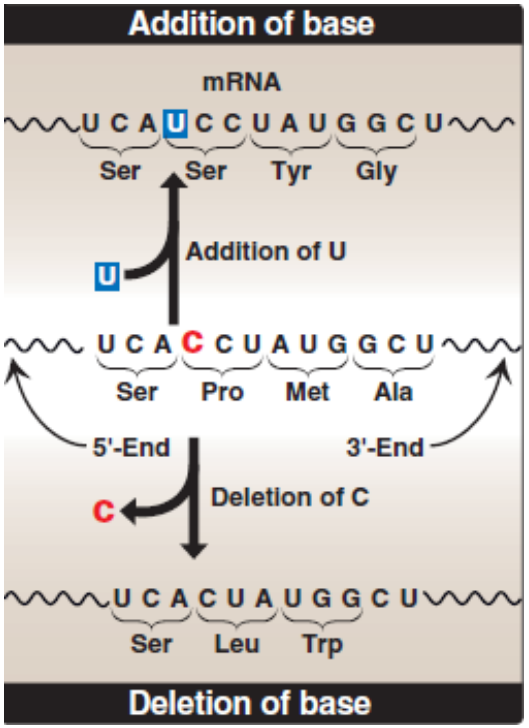
Se modifica modul in care intronii sunt indepartati, avand drept rezultat sinteza unor proteine aberante!

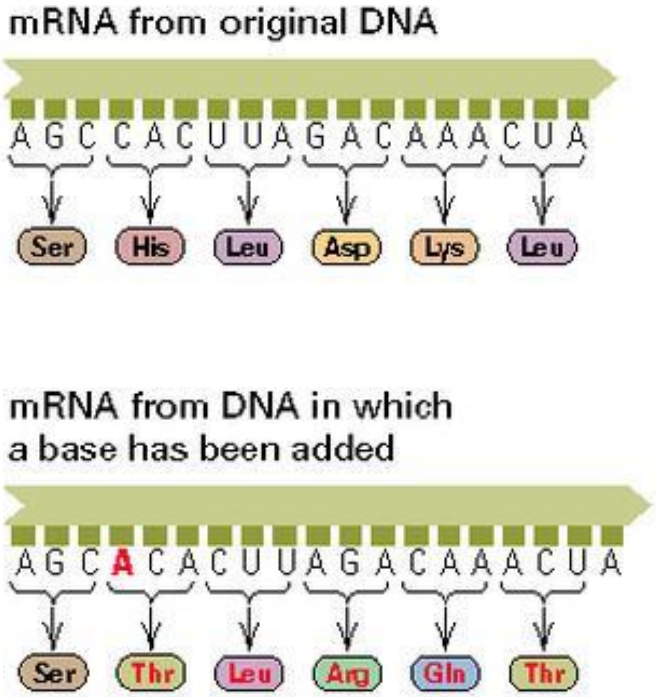
- **Mutatiile "cadrului de citire":**

Intr-o regiune codanta se suprima sau se insera unul sau doua nucleotide.

Rezulta o secventa de aminoacizi complet diferita, sau lantul peptidic va fi scurtat din cauza formarii unui codon terminal.

proteina normala →





Insertion

THE FAT CAT ATE THE RAT

THE FAT HCA TAT ETH ERA T

Deletion

THE FAT CAT ATE THE RAT

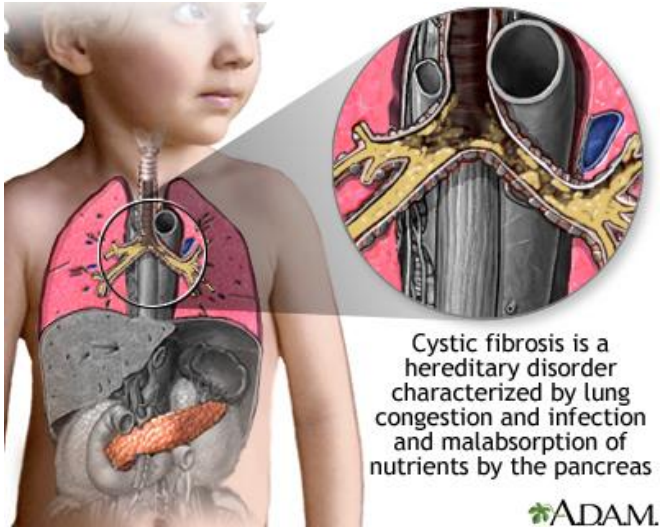
TEF ATC ATA TET GER AT

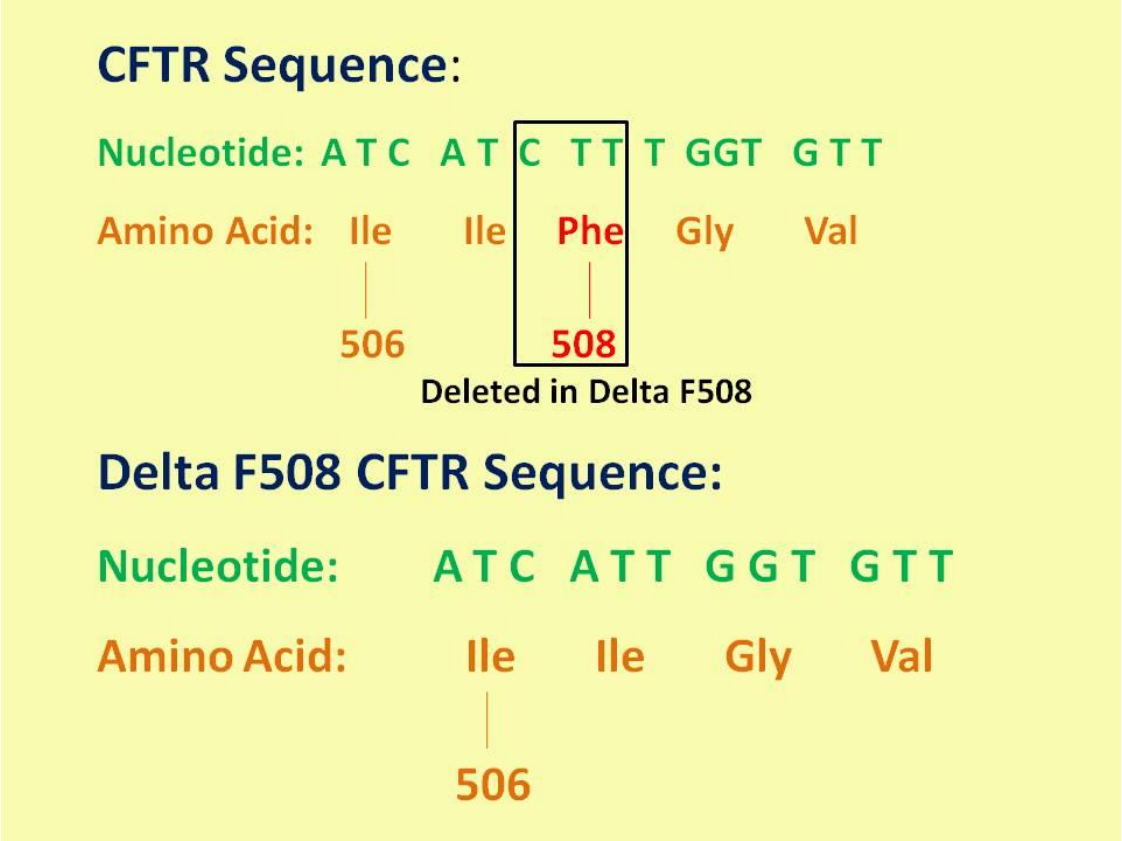
- **Aditia sau stergerea a 3 nucleotide:**

Desi se pastreaza cadrul de citire, introducerea/pierderea unui aminoacid poate duce la aparitia unor boli grave .

Ex: in fibroza chistica se pierde fenilalanina din pozitia 508.

Mutatia (ΔF508) impiedica plierea normala a proteinei, ceea ce determina distrugerea acesteia de proteazomi. Lipsa proteinei determina producerea de secretii vascoase in plamani si pancreas.





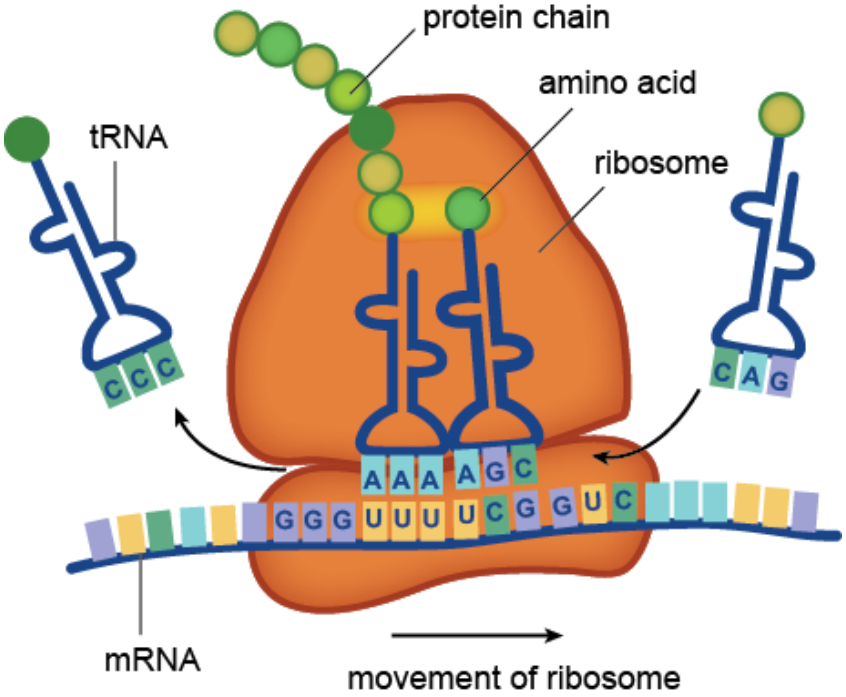
La **sinteza unei proteine** participa:

ARN mesager: molecula (purtatoare de informatie genetica) care are rol de matrita pentru structura proteinelor.

Aminoacizi: toti aminoacizii ce formeaza o proteina trebuie sa fie prezenti in celula in momentul sintezei proteice.

Daca un aminoacid lipseste (dieta deficitara) sinteza se opreste la codonul ce specifica aminoacidul respectiv!

Ribozomii: locul in care are loc sinteza proteica

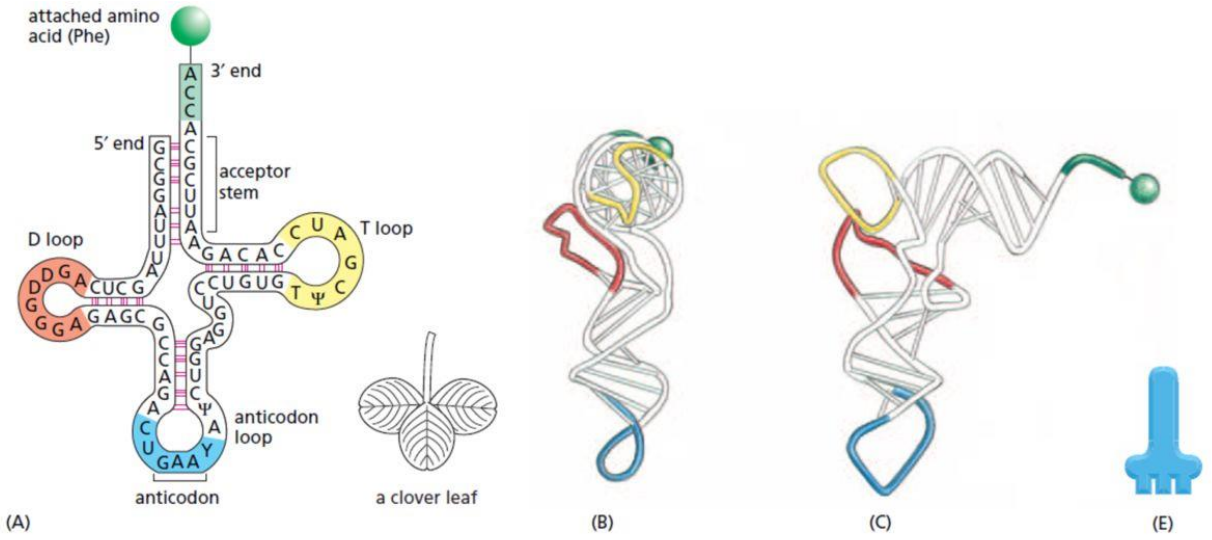


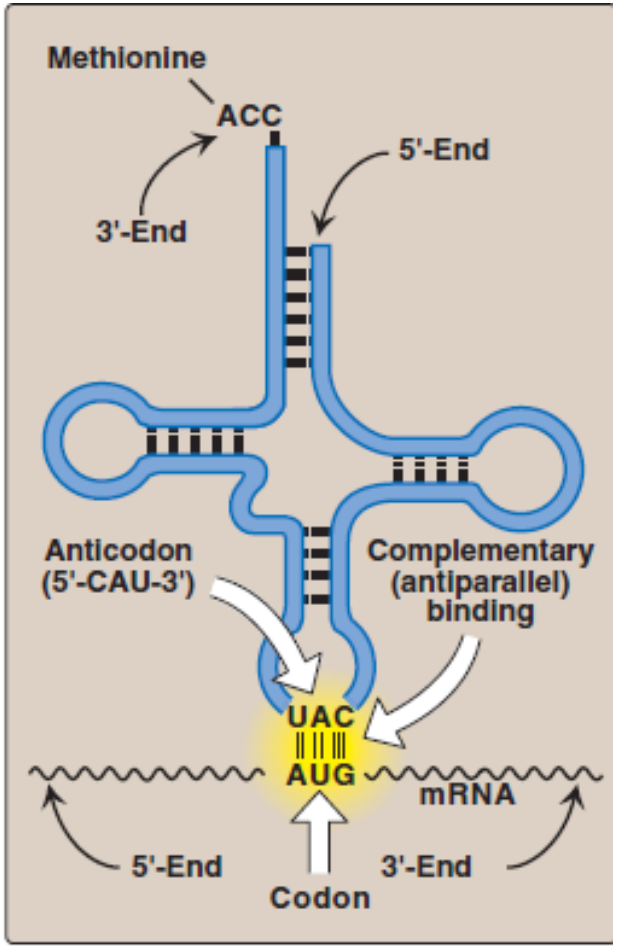
Factori proteici: cu rol de initiere, elongare si terminare. Unii au functie catalitica in timp ce altii stabilizeaza mecanismul de sinteza.

ARN de transfer: pentru fiecare aminoacid este necesar cel putin un tip specific de ARNt (in organismul uman exista 50 molecule ARNt diferite).

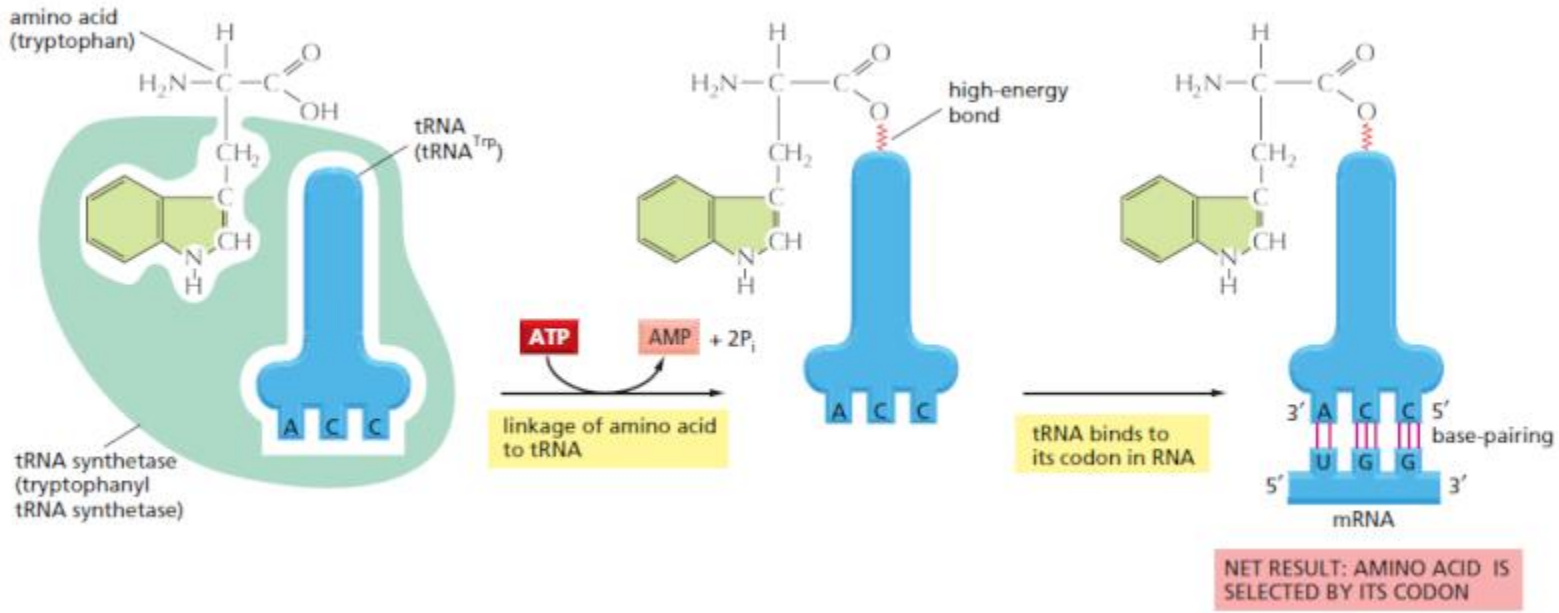
Fiecare molecula ARNt are un **situs de legare** la capatul 3', pentru aminoacidul specific (secventa -CCA). Aminoacidul care se leaga de molecula ARNt este considerat activat.

Fiecare molecula ARNt contine o secventa de 3 baze nucleotidice - **anticodonul** - care recunoaste un **codon specific de pe ARNm**. Acest codon specifica insertia aminoacidului transportat de ARNt la nivelul lantului peptidic.

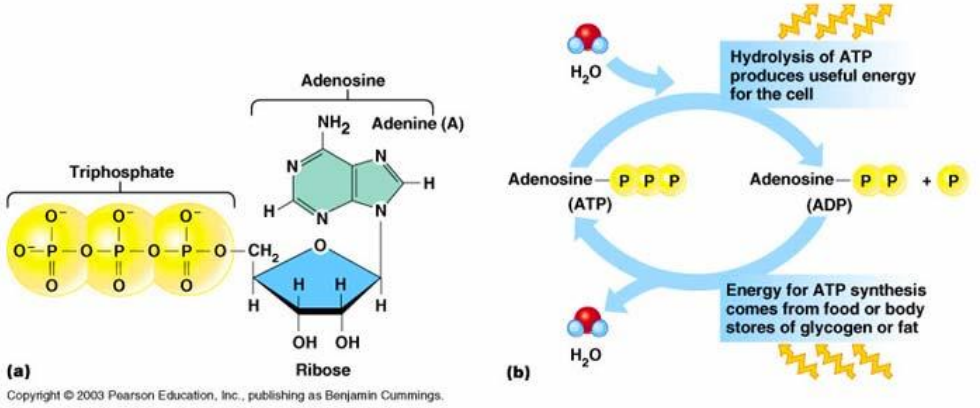


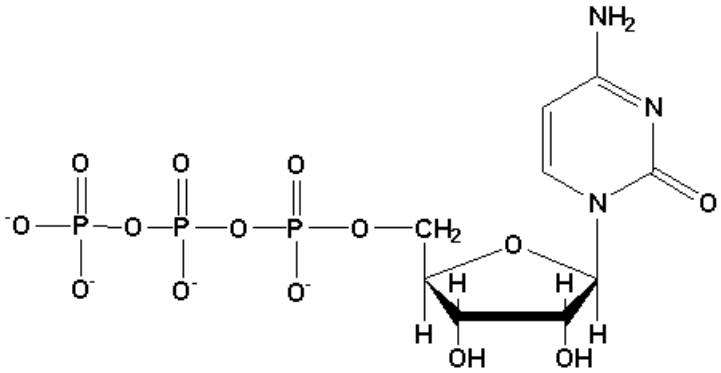


- **Aminoacil-ARNt-sintetazele:** o familie de enzime necesara pentru legarea aminoacizilor de moleculele ARNt corespunzatoare



ATP (adenozin trifosfat) **si GTP** (guanozin trifosfat) (molecule folosite ca sursa de energie!). In procesul de adaugare a unui aminoacid se consuma energia provenita din clivarea a 2 legaturi din molecula ATP si doua legaturi din molecula GTP.





Guanosine Triphosphate (GTP)

Cuplarea codon - anticodon

Cuplarea corecta a codonului din ARNm cu anticodonul din ARNt este esentiala pentru translatia corecta. Cuplarea codon-anticodon respecta principiile de **complementaritate** si **antiparalelism**.

Codonul din ARNm este "citit" in directia 5'-3', de un anticodon ce e desfasurat in directia inversa (3'-5')

Secventa de nucleotide (*atat a codonilor cat si a anticodonilor)* trebuie sa fie **TOTDEAUNA** specificata in directia 5'-3'.

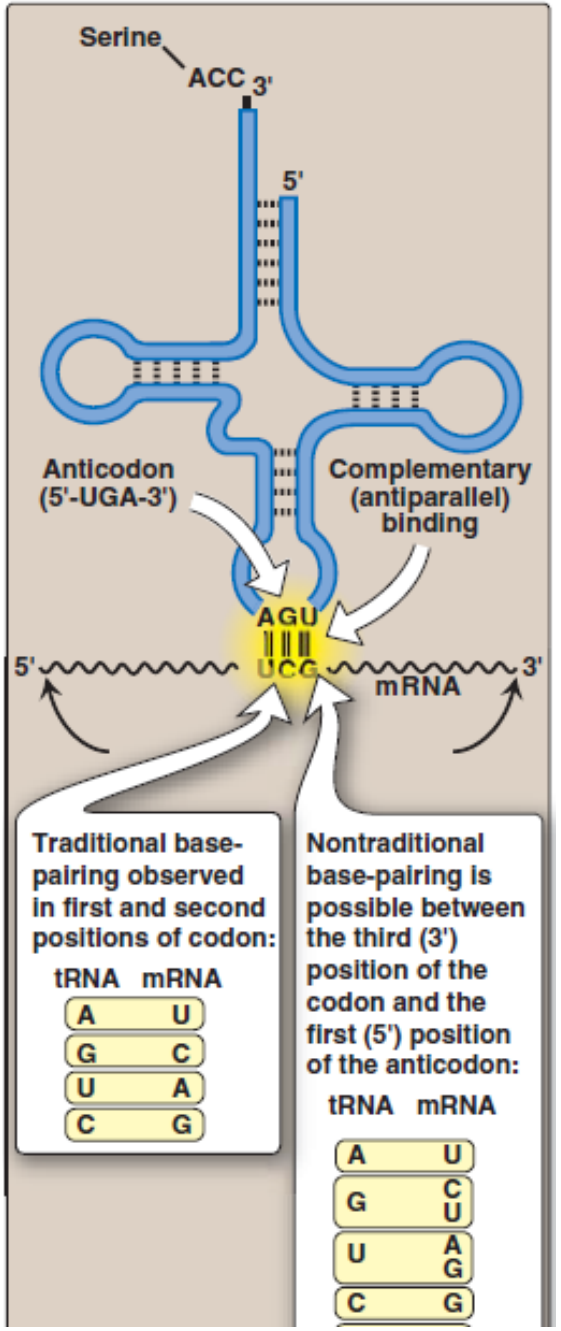
Cuplarea codon - anticodon traditionala: se respecta principiul complementaritatii

***Ipoteza pendulului**:* unele molecule ARNt recunosc mai mult de un codon pentru un anumit aminoacid.

Se presupune ca baza de la capatul 5' al anticodonului (prima) nu e foarte bine definita spatial, miscarea acesteia permitand imperecheri neobisnuite cu baza 3' a codonului (ultima).

Aceasta miscare (pendulara) permite ca un singur ARNt sa recunoasca mai mult de un codon.

Deci nu mai sunt necesare 61 de specii ARN pentru a citi cei 61 de codoni care codifica aminoacizii.



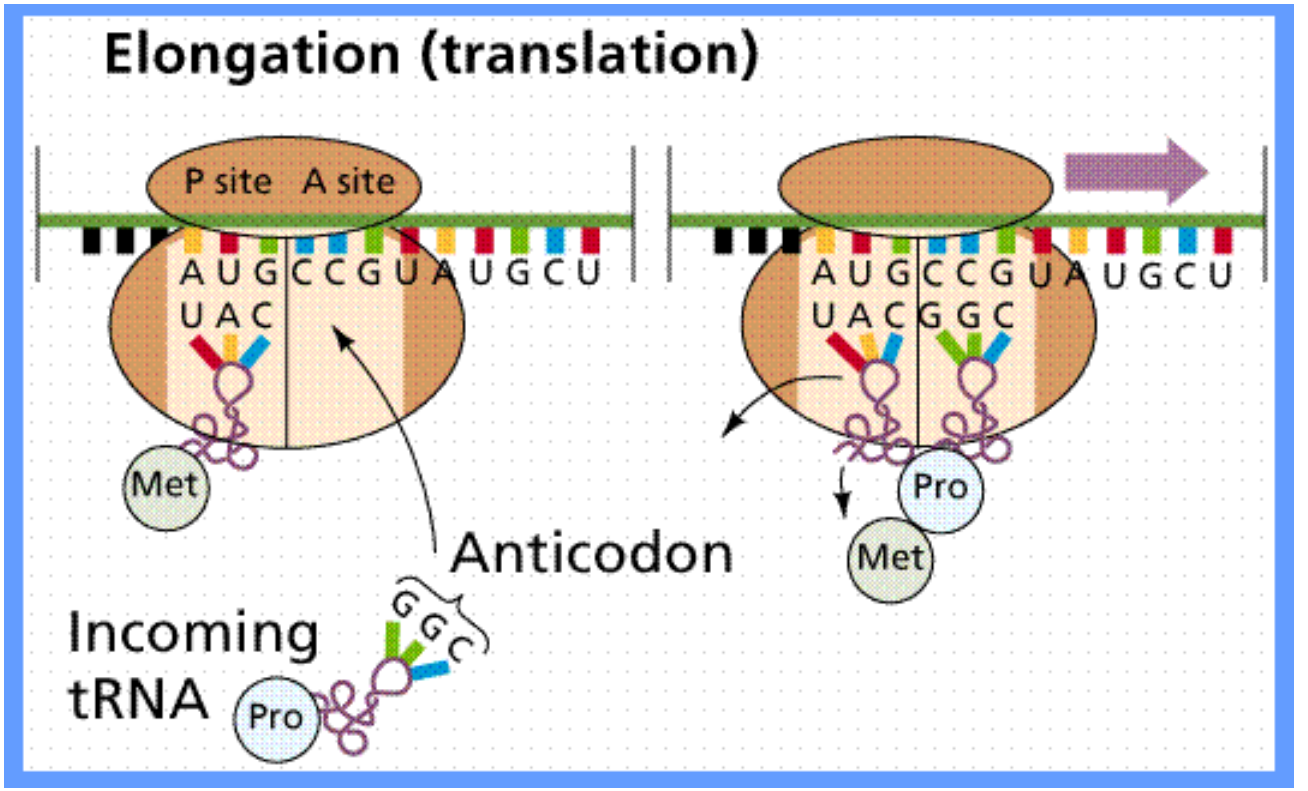
Etapele sintezei proteice: initiere

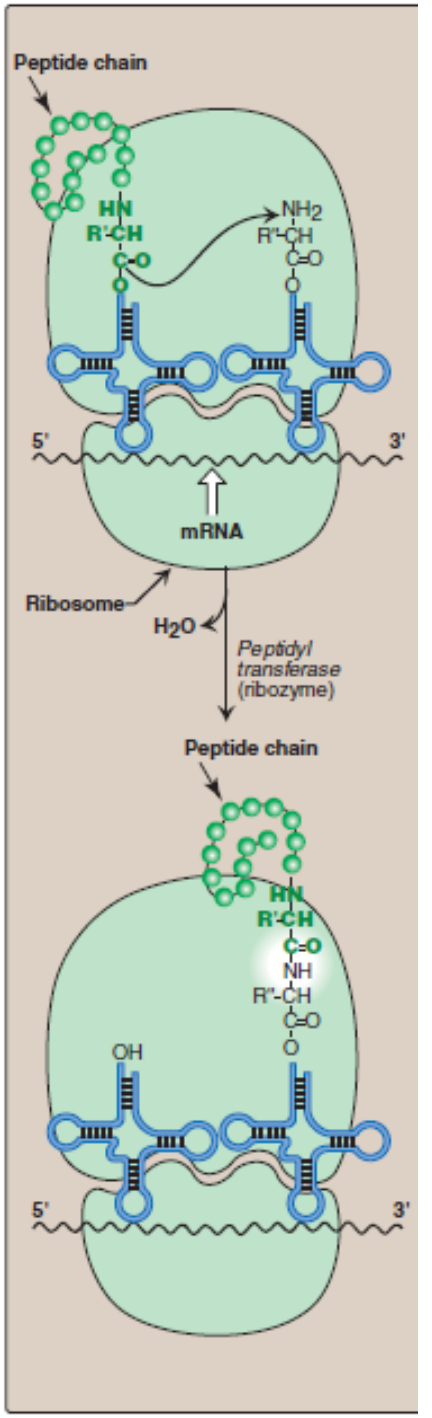
elongare

terminare

Initierea

Este dictata de **codonul initiator AUG** (metionina). Acesta este recunoscut de un **ARNt initiator** special.



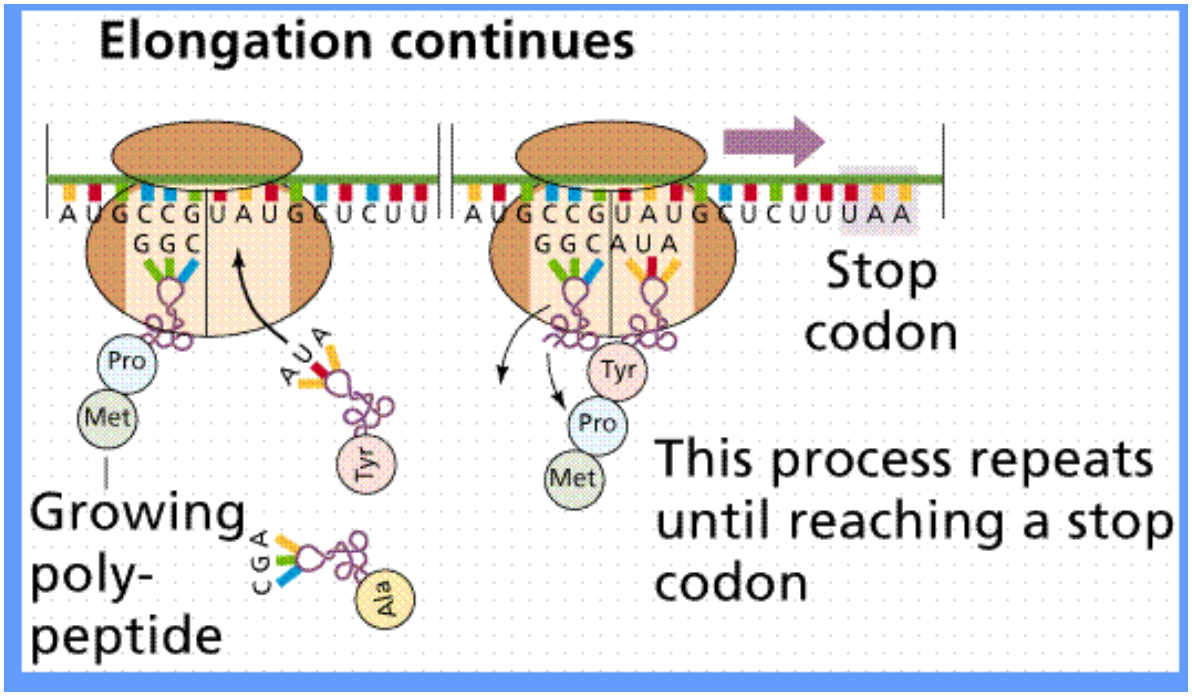


Elongarea

Implica adaugarea de aminoacizi la capatul carboxil al lantului aflat in formare.

In timpul elongarii ribozomii se deplaseaza de la capatul 5' spre capatul 3' al moleculei ARNm care este transcrisa.

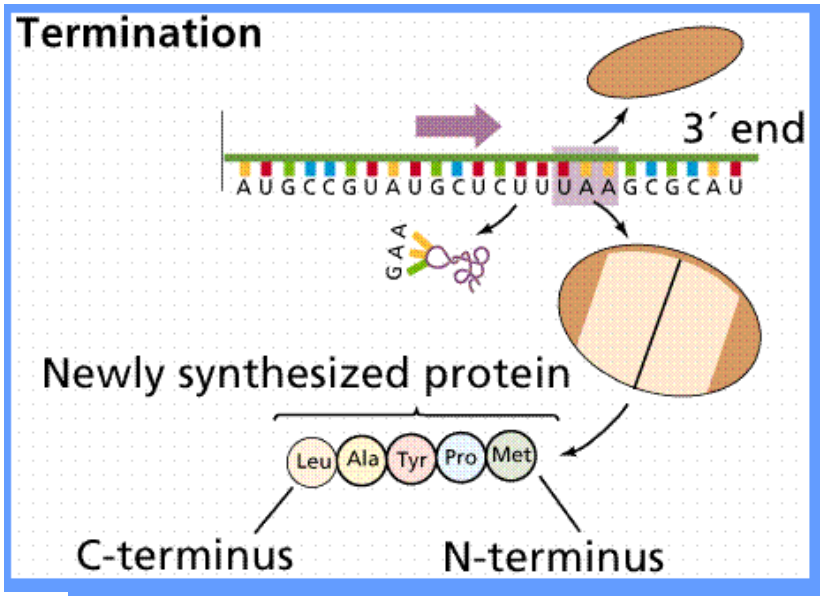
Dupa ce s-a format legatura peptidica (catalizata de enzima *peptidil-transferaza*), ribozomul avanseaza cu 3 nucleotide.

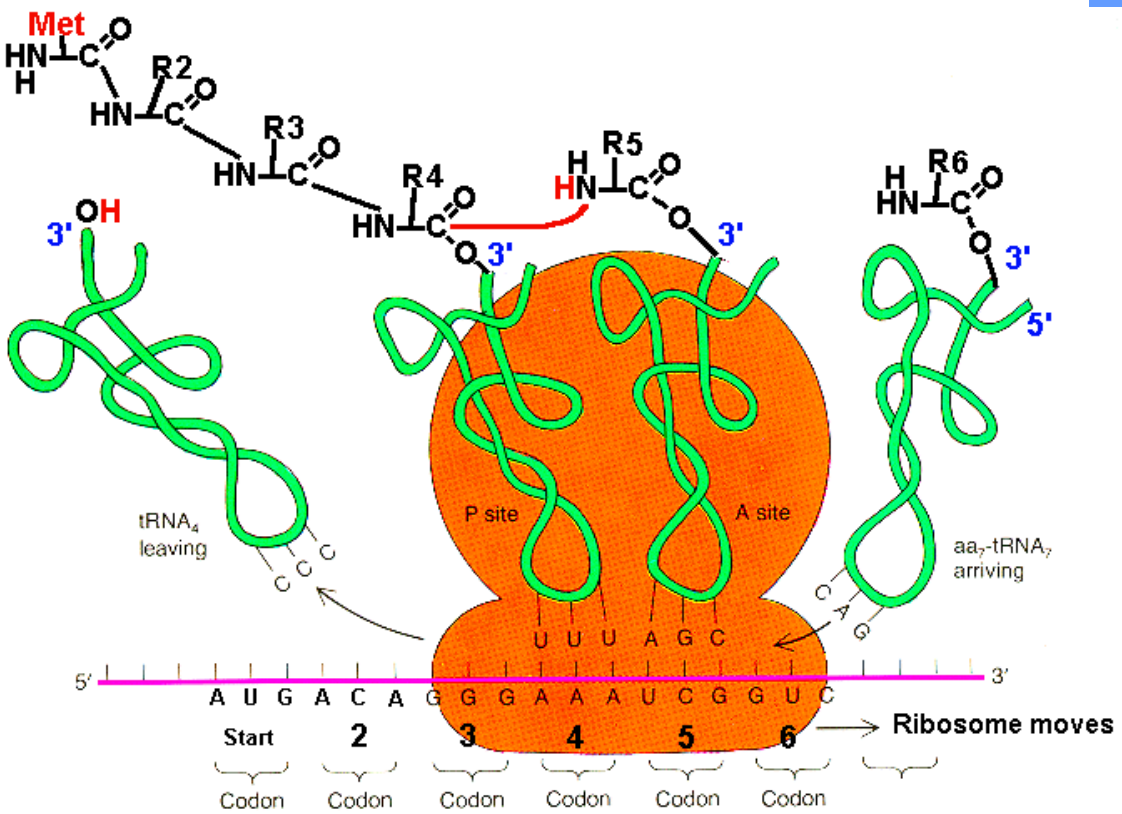


Terminarea

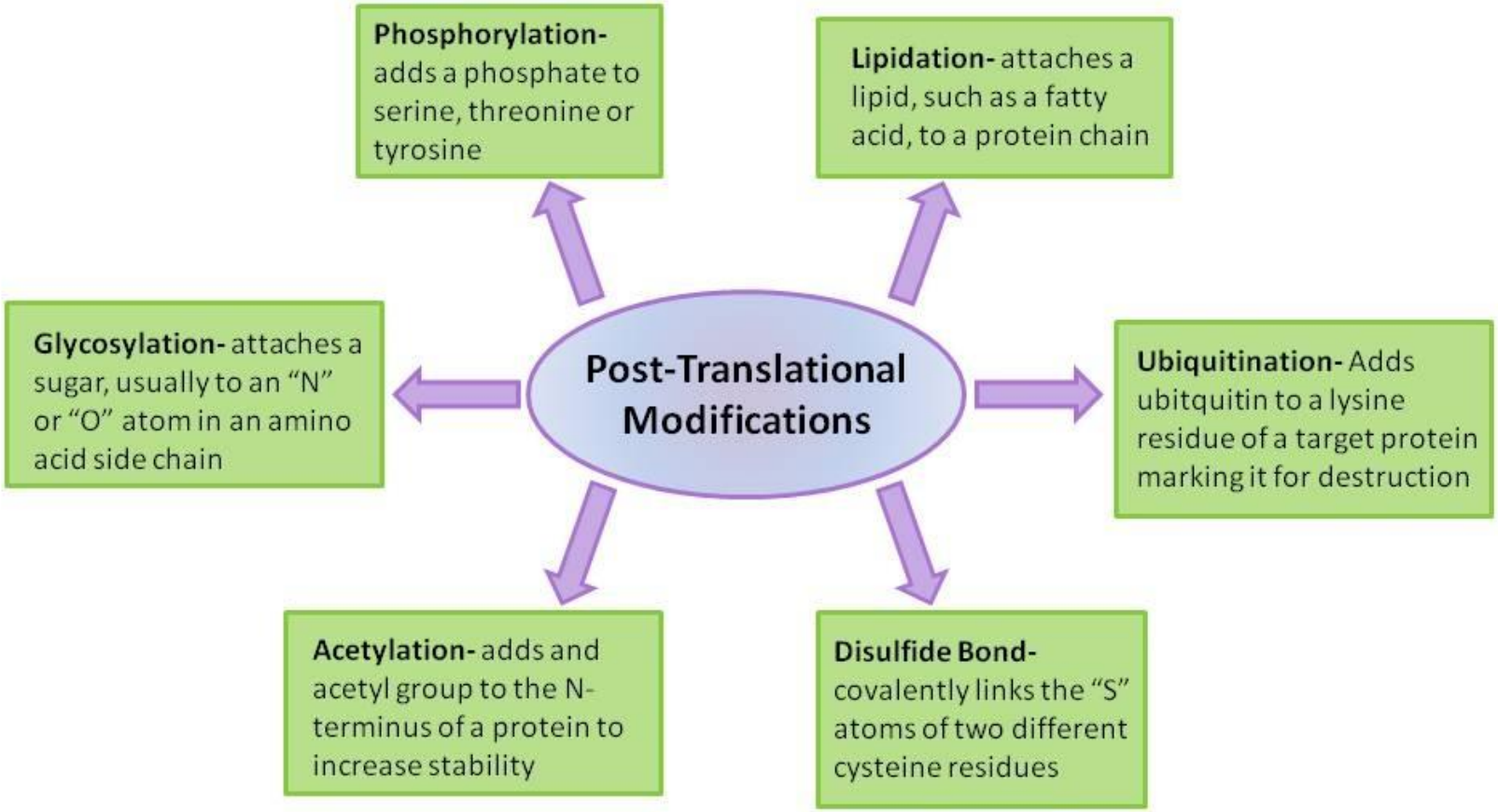
Are loc atunci cand unul din cei **3 codoni stop** ajunge in situsul ribozomal.

Codonul "stop" este recunoscut de un factor de eliberare (RF - *release factor*) care determina peptidil-transferaza sa elibereze proteina nou sintetizata.

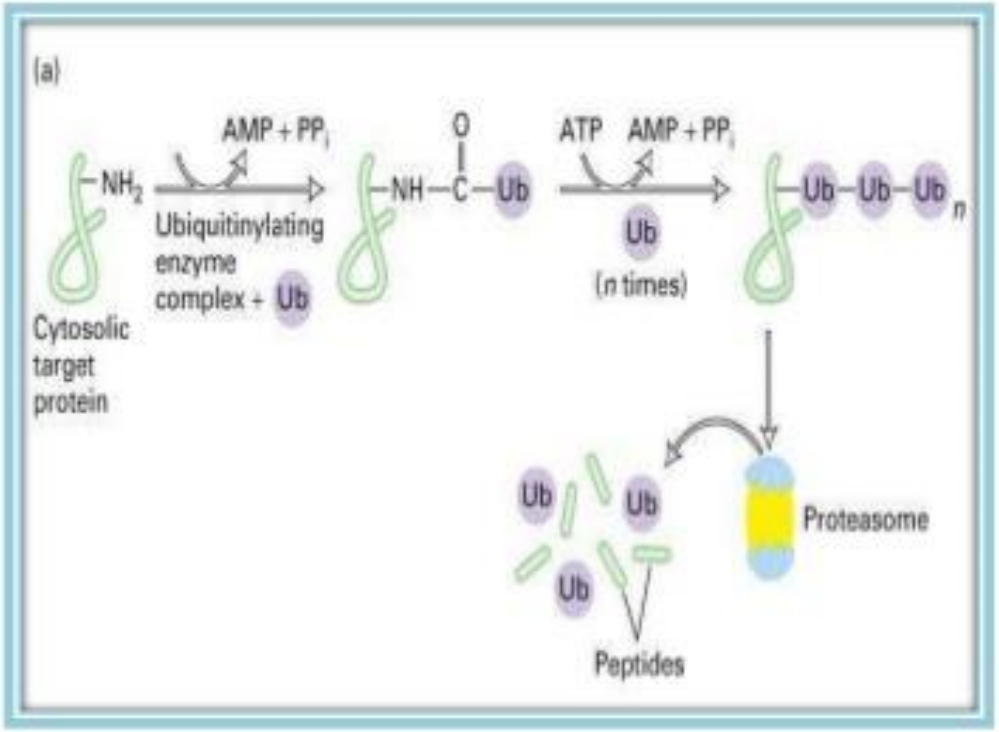


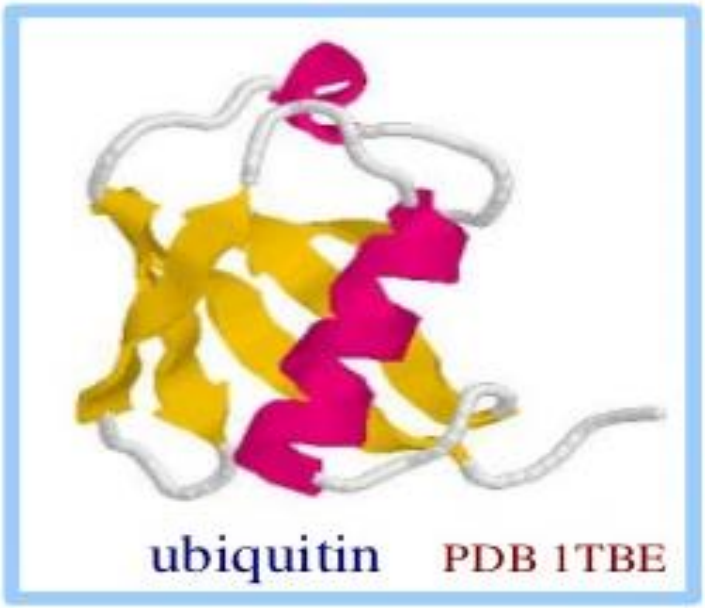


Lanturile polipeptidice pot sa sufere modificari post-translationale *(indepartarea unei parti a secventei transcrise, fosforilarea, glicolizarea, hidroxilarea, etc)*

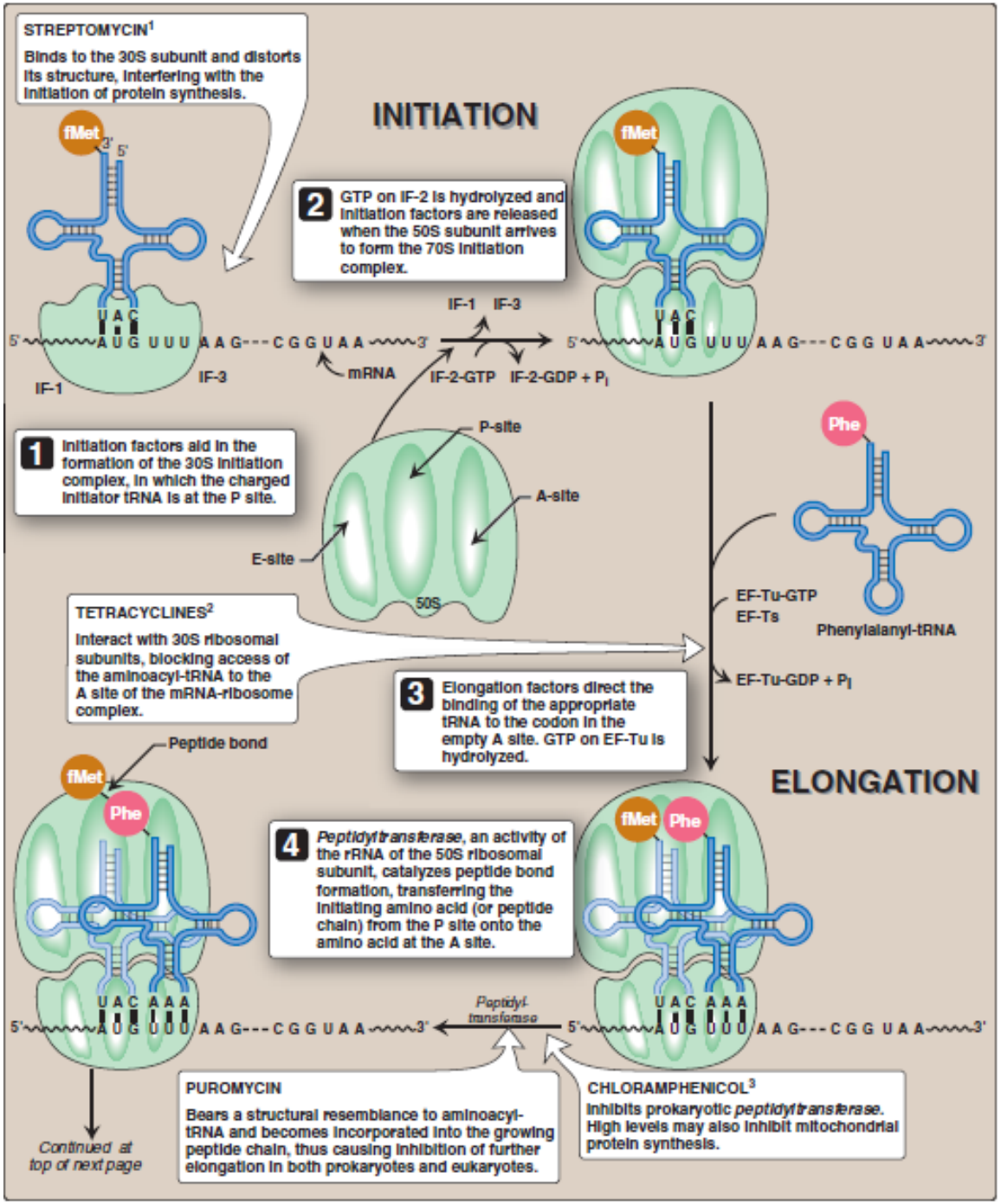


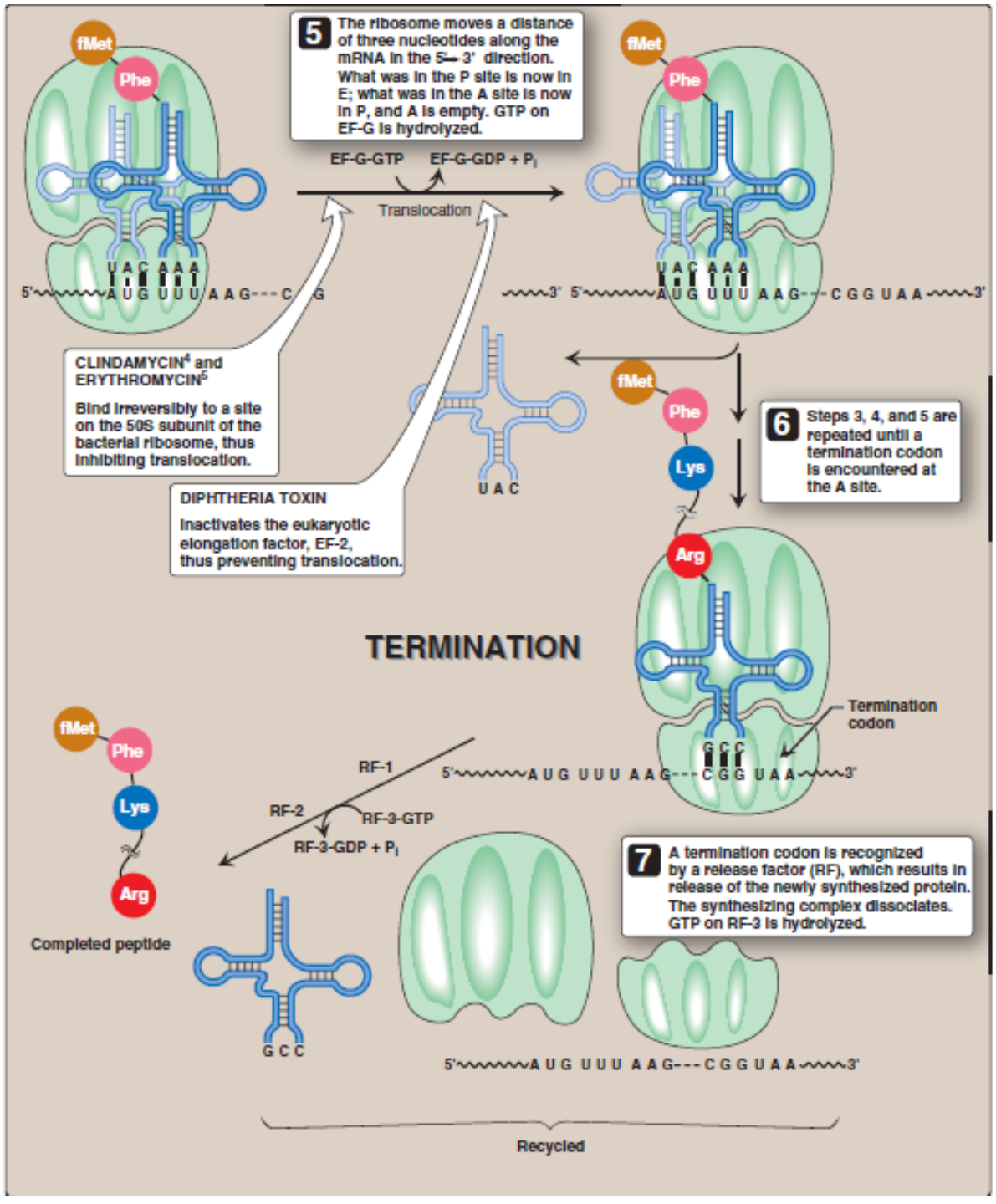
Proteinele defecte sunt marcate prin atasarea unei proteine mici numita ***ubiquitina***.



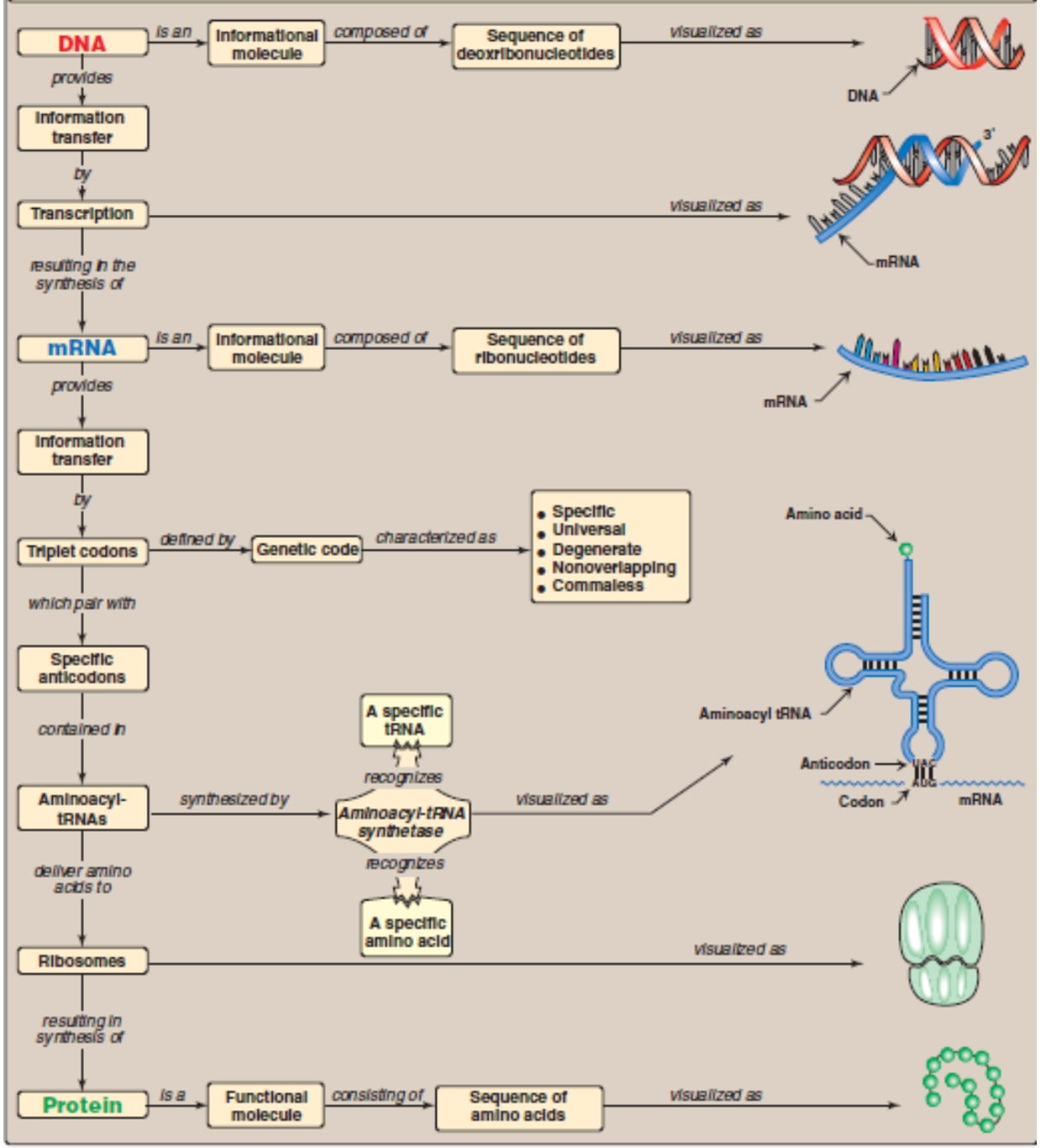


Proteinele astfel marcate sunt degradate rapid de catre "*proteazomi*" (organite complexe localizate in citosol).


STREPTOMYCIN[1]
Binds to the 30S subunit and distorts its structure, interfering with the initiation of protein synthesis.
INITIATION
fMet
3'
5'
U A C
5' A U G U U U A A G - - - C G G U A A 3'
IF-1
IF-3
2 GTP on IF-2 is hydrolyzed and initiation factors are released when the 50S subunit arrives to form the 70S initiation complex.
IF-1 IF-3
mRNA
IF-2-GTP IF-2-GDP + P_i
1 Initiation factors aid in the formation of the 30S initiation complex, in which the charged initiator tRNA is at the P site.
P-site
A-site
E-site
50S
Phe
EF-Tu-GTP
EF-Ts
Phenylalanyl-tRNA
EF-Tu-GDP + P_i
TETRACYCLINES[2]
Interact with 30S ribosomal subunits, blocking access of the aminoacyl-tRNA to the A site of the mRNA-ribosome complex.
3 Elongation factors direct the binding of the appropriate tRNA to the codon in the empty A site. GTP on EF-Tu is hydrolyzed.
Peptide bond
ELONGATION
4 *Peptidyltransferase*, an activity of the rRNA of the 50S ribosomal subunit, catalyzes peptide bond formation, transferring the initiating amino acid (or peptide chain) from the P site onto the amino acid at the A site.
U A C A A A
5' A U G U U U A A G - - - C G G U A A 3'
Peptidyl-transferase
PUROMYCIN
Bears a structural resemblance to aminoacyl-tRNA and becomes incorporated into the growing peptide chain, thus causing inhibition of further elongation in both prokaryotes and eukaryotes.
CHLORAMPHENICOL[3]
Inhibits prokaryotic *peptidyltransferase*. High levels may also inhibit mitochondrial protein synthesis.
Continued at top of next page


5 The ribosome moves a distance of three nucleotides along the mRNA in the 5′→3′ direction. What was in the P site is now in E; what was in the A site is now in P, and A is empty. GTP on EF-G is hydrolyzed.
fMet
Phe
EF-G-GTP
EF-G-GDP + P_i
Translocation
U A C A A A
5′ A U G U U U A A G - - - C G 3′
5′ A U G U U U A A G - - - C G G U A A 3′
CLINDAMYCIN[4] and ERYTHROMYCIN[5]
Bind irreversibly to a site on the 50S subunit of the bacterial ribosome, thus inhibiting translocation.
DIPHTHERIA TOXIN
Inactivates the eukaryotic elongation factor, EF-2, thus preventing translocation.
U A C
Lys
Arg
6 Steps 3, 4, and 5 are repeated until a termination codon is encountered at the A site.
TERMINATION
Termination codon
G C C
5′ A U G U U U A A G - - - C G G U A A 3′
RF-1
RF-2
RF-3-GTP
RF-3-GDP + P_i
Completed peptide
7 A termination codon is recognized by a release factor (RF), which results in release of the newly synthesized protein. The synthesizing complex dissociates. GTP on RF-3 is hydrolyzed.
G C C
5′ A U G U U U A A G - - - C G G U A A 3′
Recycled

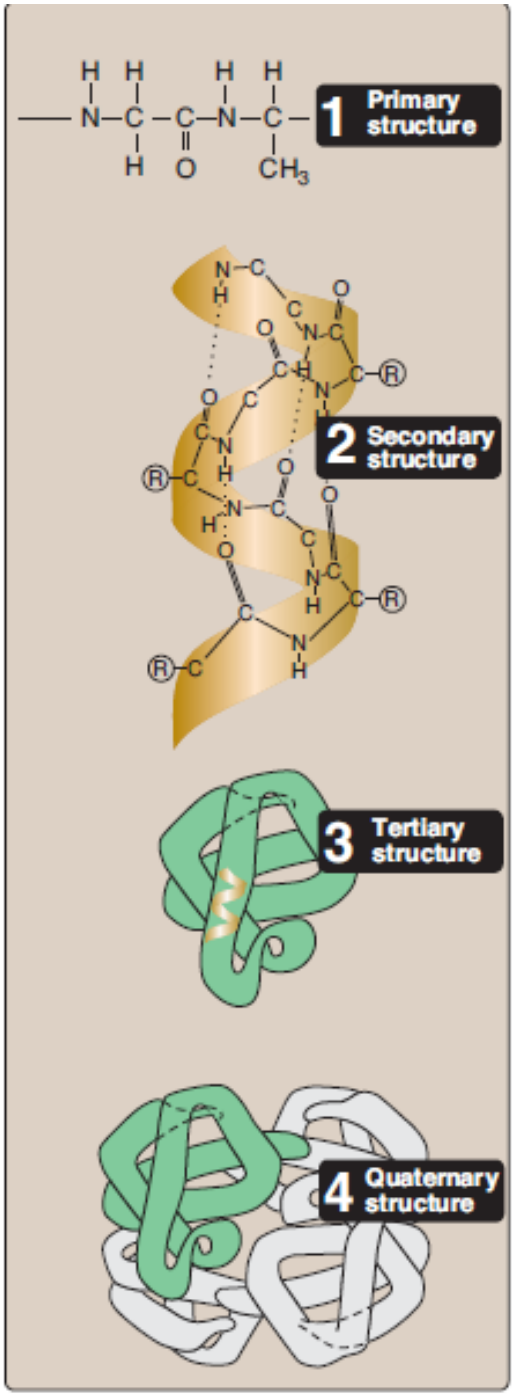
Flow of genetic information



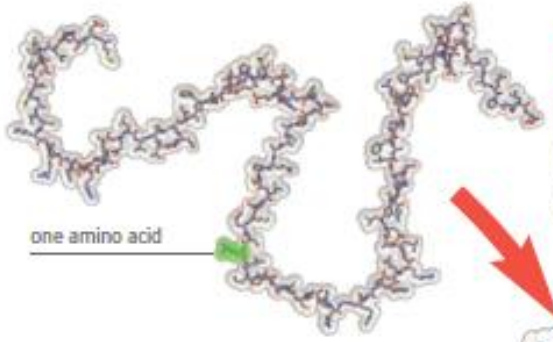
Structura proteinelor

Complexitatea structurala a proteinelor poate fi analizata prin evidentierea celor patru nivele de organizare:

- structura primara
- structura secundara
- structura tertiara
- structura cuaternara

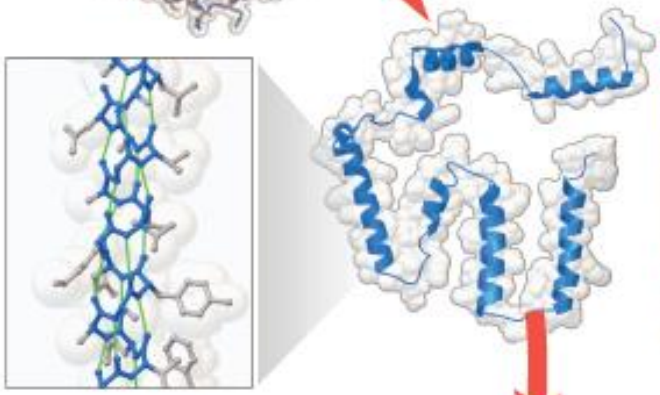


Anumite elemente structurale se repeta intr-o mare varietate de proteine → exista "reguli" generale referitoare la modul de pliere a moleculelor proteice.



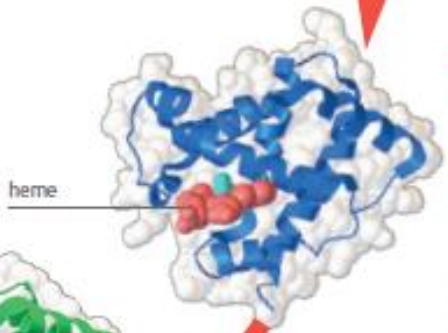
Primary Structure

Primary structure is the linear sequence of amino acids as encoded by the DNA. This sequence defines how the protein will fold and therefore also defines how it will function. A single change in the amino acid sequence of hemoglobin can cause the proteins to clump together, resulting in the disease sickle cell anemia.



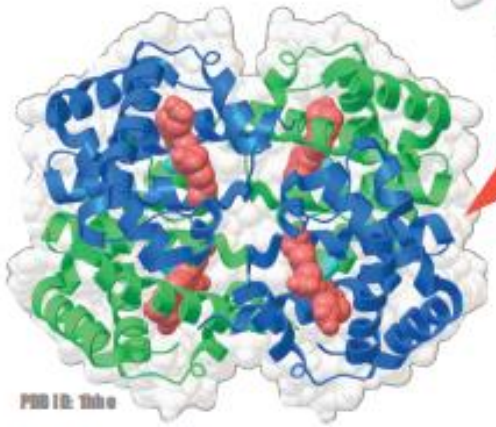
Secondary Structure

Hydrogen bonds between amino acids form two particularly stable structural elements in proteins: alpha helices and beta sheets. Alpha helices (shown in blue) are the basic structural elements found in hemoglobin, but many other proteins also include beta sheets. The inset highlights the pattern of hydrogen bonds (shown in green) that stabilizes alpha helices.



Tertiary Structure

Many functional proteins fold into a compact globular shape, with many carbon-rich amino acids sheltered inside away from the surrounding water. The folded structure of hemoglobin includes a pocket to hold heme, which is the molecule that carries oxygen as it is transported throughout the body.



Quaternary Structure

Two or more polypeptide chains can come together to form one functional molecule with several subunits. The four subunits of hemoglobin cooperate so that the complex picks up and delivers more oxygen than is possible with single subunits.

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PROTEIN DATA BANK
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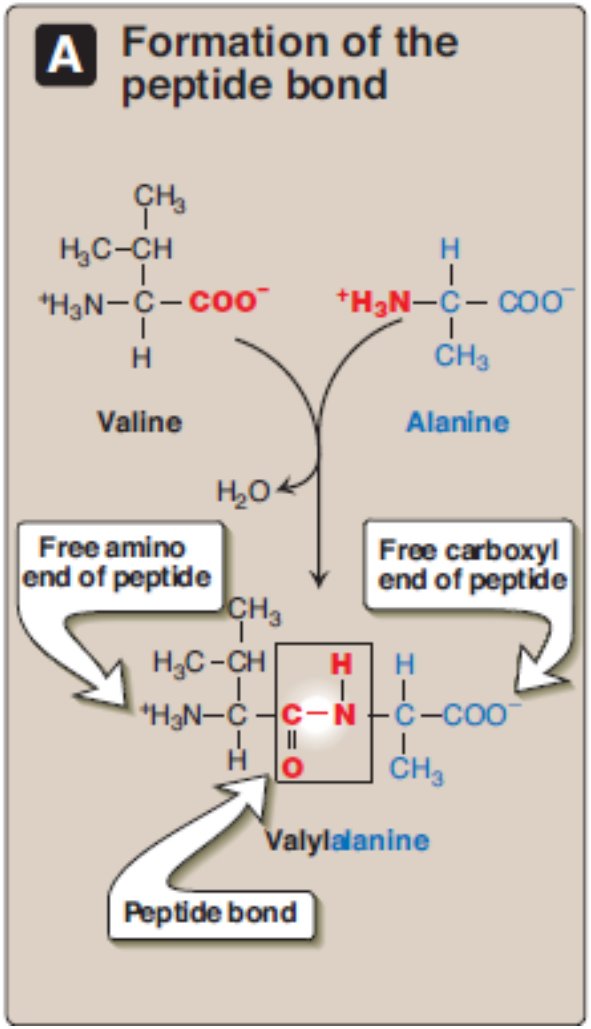
Structura primara a proteinelor este data de secventa liniara de aminoacizi ce formeaza polipeptidul. Aminoacizii sunt uniti prin legaturi peptidice de tip covalent

Legatura peptidica = punte amidica intre gruparea carboxil a unui aminoacid si gruparea amino a aminoacidului urmator.

Legaturile peptidice nu pot fi desfacute de factorii ce pot sa denatureze proteinele (caldura, concentratii crescute de uree). Pentru a desface aceste legaturi este necesata expunerea prelungita la un acid sau o baza tare si la temperatura crescuta.

Prin conventie, capatul amino liber al peptidului (capatul N-terminal) este scris totdeauna in stanga, iar capatul carboxil liber (capatul C-terminal) in dreapta.

Secventele de aminoacizi se citesc dinspre capatul N-terminal spre capatul C-terminal (de la stanga la dreapta!).



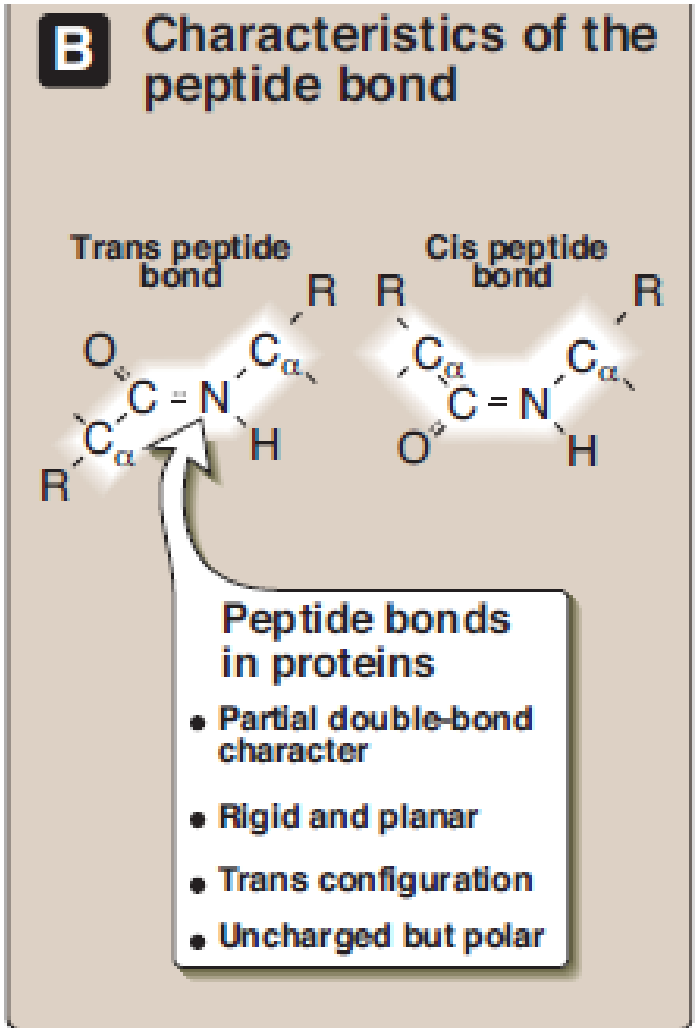
Pentru a denumi un polipeptid, se schimba sufixele resturilor de aminoacizi (-ina, -an, -ic sau -at) in -il (cu exceptia capatului terminal).

Legatura peptidica se comporta partial ca o legatura dubla (mai scurta ca o legatura simpla), este rigida si plana.

Rotatia libera in jurul legaturii peptidice este impiedicata datorita rigiditatii legaturii peptidice,

Legaturile din interiorul aminoacizilor (dintre atomii de carbon α si gruparile -amino sau -carboxil) se pot roti liber, ceea ce determina o mare varietate de configuratii posibile.

Legatura peptidica este in general o **legatura de tip trans** (nu cis!).



Gruparile -C=O si –N-H din legatura peptidica:

- sunt grupari **neutre**, deci nu accepta si nu cedeaza protoni.
- sunt grupari **polare**, capabile sa formeze legaturi de hidrogen (punti de hidrogen)

(exemplu: in cazul α helixurilor si a lanturilor β se formeaza punti de H).

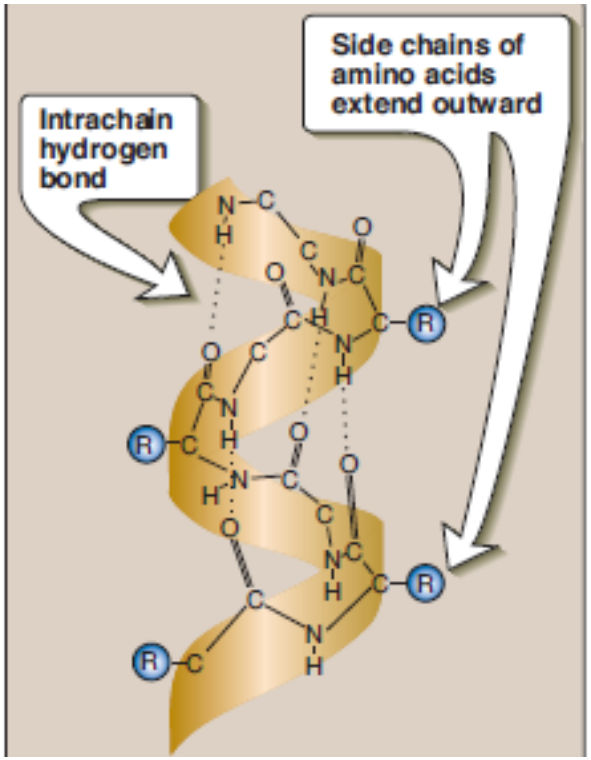
Structura secundara a proteinelor: reprezinta *structura tridimensionala*, data de aranjamentul regulat al aminoacizilor.

Structurile secundare cel mai des intalnite in constitutia proteinelor sunt: **α-helix** (elicea α), **β-sheet** (foile β) si **β-turn / β- bend** (pliurile β).

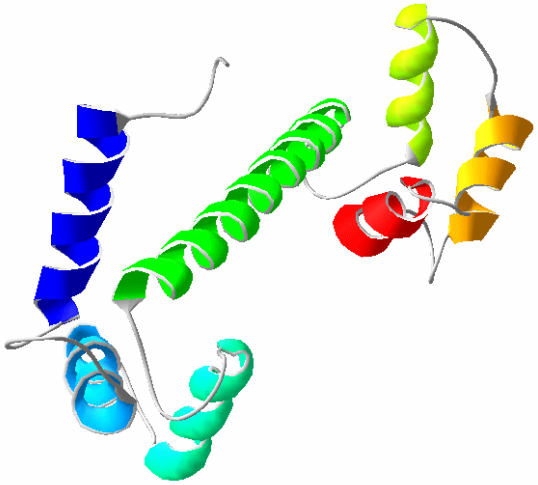
α-helix este o structura spiralata elicoidala, constituita dintr-un schelet polipeptidic central, strans impachetat, rasucit. Catenele laterale ale aminoacizilor sunt orientate in exteriorul axului central.

Stabilitatea structurii α-helix este data de numarul mare de l*egaturi de hidrogen* realizate intre atomul de oxigen al gruparii carbonil (C=O) si atomul de hidrogen din gruparea -NH al altei legaturi peptidice aflate la distanta de 3 sau 4 aminoacizi consecutivi.

Fiecare spira a unui α-helix contine un numar de 3,6 aminoacizi.



α-Helix showing peptide backbone.



β-sheet este o structura in care toate componentele legaturilor peptidice participa la formarea legaturilor de hidrogen.

Suprafata foilor β pare pliata.

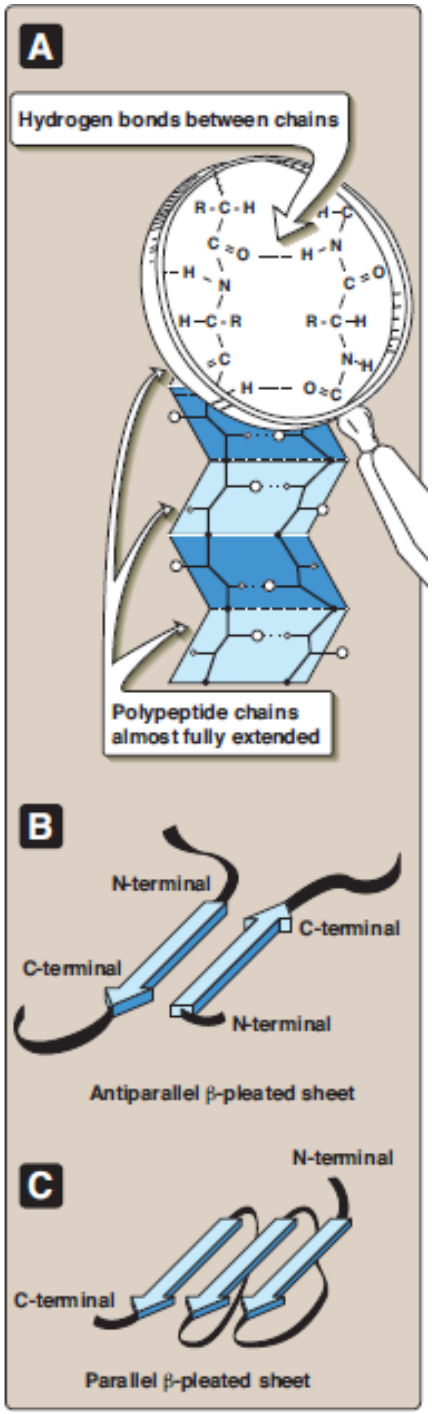
Pentru ilustrarea structurii proteinelor, foile β sunt reprezentate sub forma unor sageti groase.

Legaturile de hidrogen sunt perpendiculare pe scheletul polipeptidic.

Conformatia β-sheet este alcatuita din segmente de lanturi polipeptidice orientate fie paralel, fie antiparalel.

Legaturile de hidrogen formate intre scheletele axiale ale celor doua lanturi se numesc *legaturi intercatenare*.

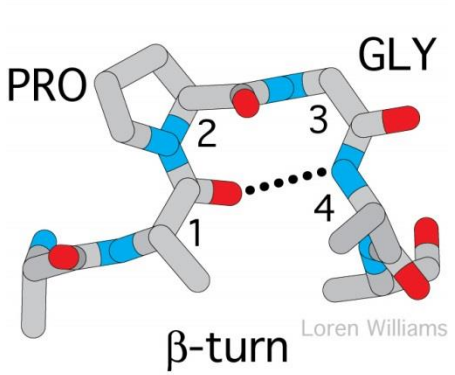
Conformatiile de tip α-helix si β-sheet permit realizarea unui numar maxim de legaturi de hidrogen intre componentele legaturilor peptidice din interiorul polipeptidelor.

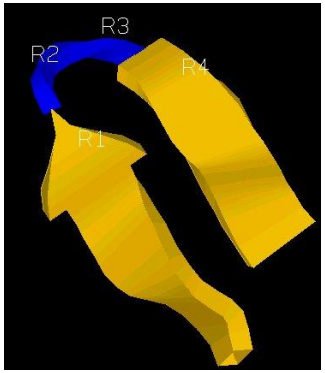


β-turn inverseaza directia de rotatie a unui lant polipeptidic, usurand adoptarea unei forme globulare compacte.

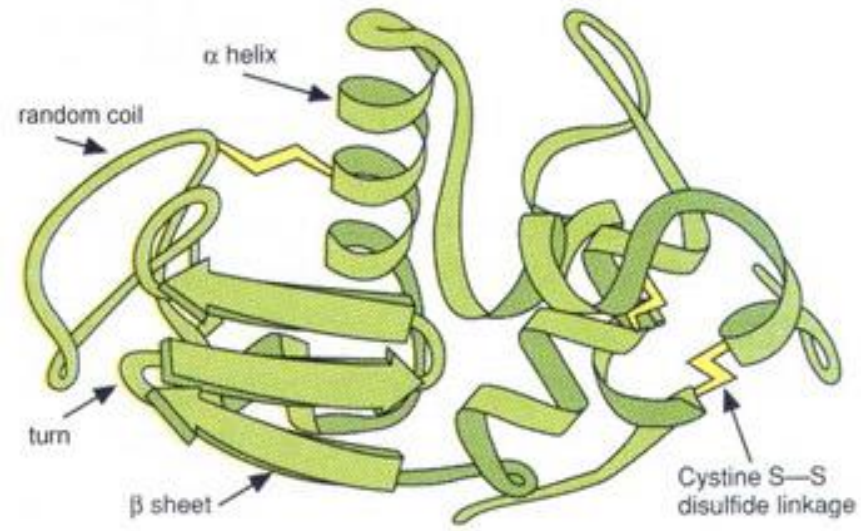
Structura β-turn e formata din 4 aminoacizi, dintre care unul e prolina (genereaza deformarea lantului polipeptidic).

Stabilizarea pliurilor β-turn se face prin legaturi de hidrogen si prin legaturi ionice.





Exista si zone ale proteinelor fara structura: “**random coil**” !



Structura tertiara a proteinelor globulare se refera la formarea domeniilor prin *plierea* in continuare a lantului peptidic (gruparile secundare) pana la obtinerea aranjamentul final.

Interactiunile dintre catenele laterale ale aminoacizilor influenteaza modul de **pliere** al lantului polipeptidic in forma tridimensionala specifica a proteinei functionale.

Plierea proteinelor se desfasoara in decurs de cateva secunde sau minute, in interiorul celulelor.

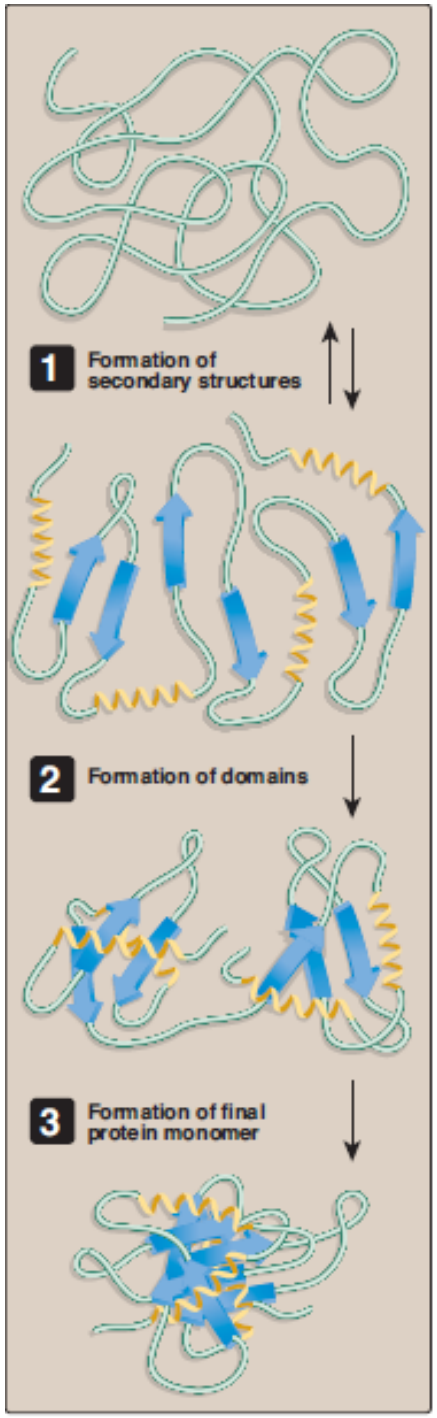
In procesul plierii, catenele laterale sunt atrase sau respinse in functie de proprietatile chimice ale acestora.

Procesul de pliere este influentat si de legaturile de hidrogen, interactiunile hidrofobe si legaturile disulfidice.

Proteina ajunge sa aiba acea structura in care procesele de atractie sunt mai puternice decat cele de respingere.

Se obtine o proteina cu incarcatura electrica redusa!

Denaturarea proteinelor determina deplierea si dezorganizarea structurilor, dar nu produce hidroliza legaturilor peptidice.



In solutie apoasa, structura proteinelor globulare este compacta, cu o mare densitate de atomi in centrul moleculei.

Catenele laterale hidrofobe sunt dispuse in interiorul moleculei, in timp ce gruparile hidrofile se gasesc, in general, pe suprafata moleculei.

Domeniile reprezinta unitati functionale si structurale tridimensionale fundamentale ale polipeptidelor.

Lanturile polipeptidice cu un numar mai mare de 200 de aminoacizi contin, de regula, doua sau mai multe domenii.

Fiecare domeniu are caracteristicile unei proteine globulare mici, compacte, care din punct de vedere structural este independenta de restul domeniilor.

Structura tertiara a proteinelor este stabilizata prin:

legaturi disulfidice,
interactiuni hidrofobe,
legaturi de hidrogen,
interactiuni ionice.

Stuctura cuaternara a proteinelor reprezinta aranjamentul spatial al subunitatilor (domeniilor) polipepditice

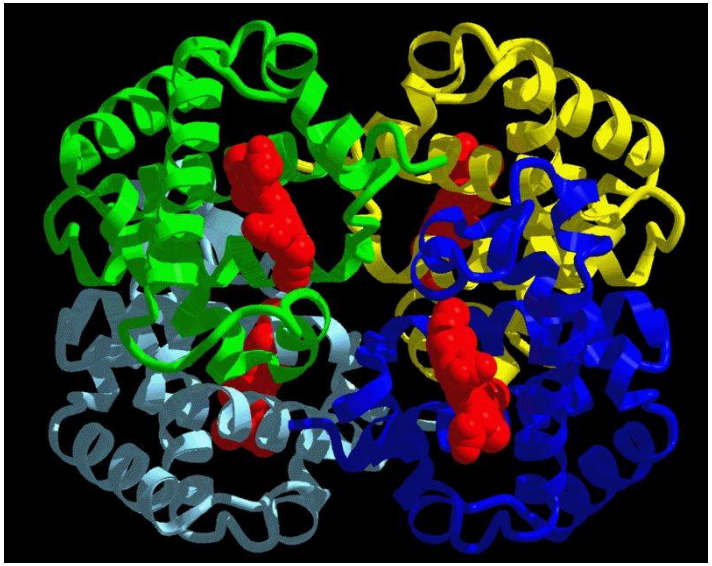
Subunitatile sunt legate prin legaturi necovalente (legaturi de hidrogen, legaturi ionice, interactiuni hidrofobe). Ele pot functiona independent unele de celelalte, sau pot coopera.

Proteine monomerice (cele mai numeroase): nu au structura cuaternara deoarece contin un singur lant polipeptidic

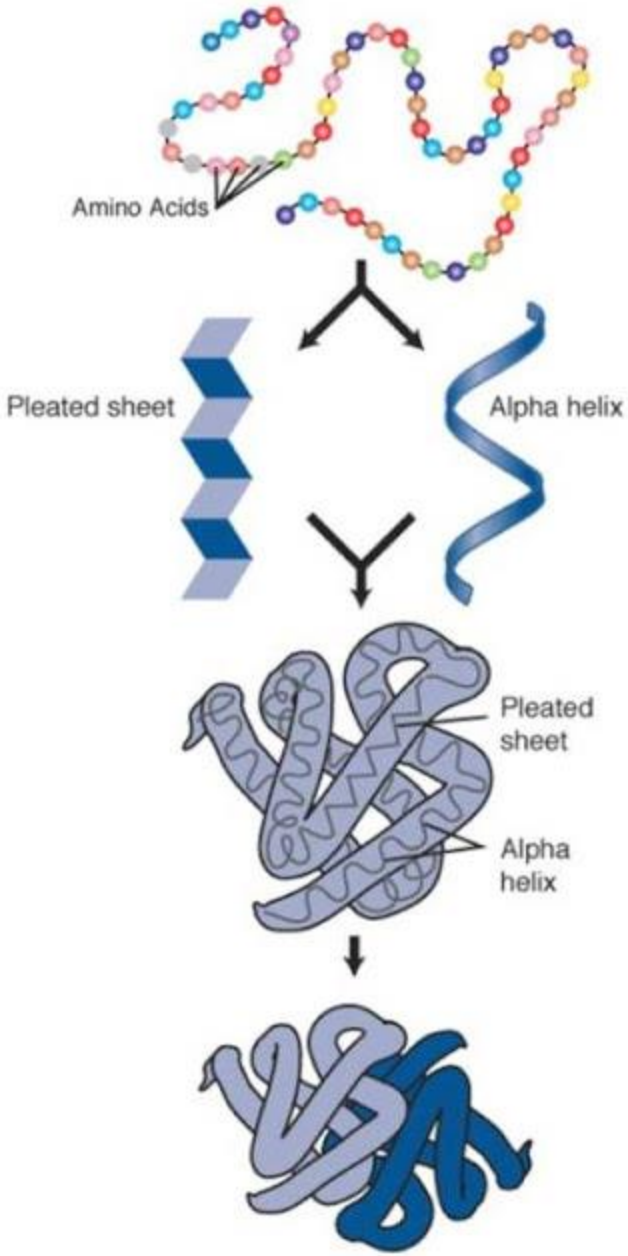
Proteine polimerice: sunt alcatuite din doua sau mai multe lanturi polipeptidice (identice din punct de vedere structural, sau complet diferite

Exemplu: hemoglobina e formata din patru subunitati.

Atasarea unui atom de oxigen la una din subunitatile tetramerului hemoglobinic, duce la cresterea afinitatii pentru oxigen a celorlalte subunitati.



Levels of protein organization

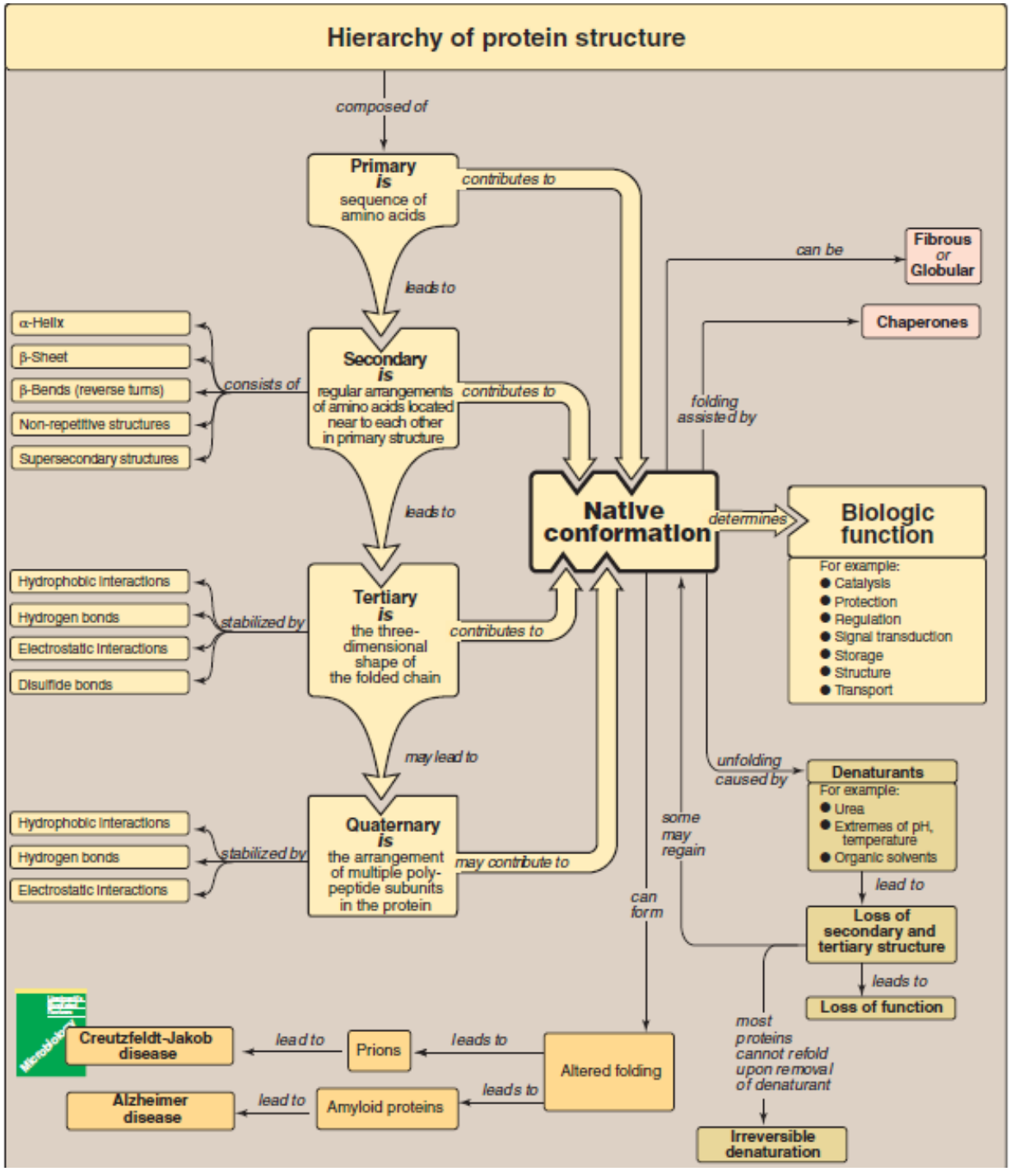


Primary protein structure
is sequence of a chain of amino acids

Secondary protein structure
occurs when the sequence of amino acids are linked by hydrogen bonds

Tertiary protein structure
occurs when certain attractions are present between alpha helices and pleated sheets.

Quaternary protein structure
is a protein consisting of more than one amino acid chain.


Hierarchy of protein structure
composed of
Primary
is
sequence of amino acids
contributes to
leads to
Secondary
is
regular arrangements of amino acids located near to each other in primary structure
consists of
α-Helix
β-Sheet
β-Bends (reverse turns)
Non-repetitive structures
Supersecondary structures
contributes to
leads to
Tertiary
is
the three-dimensional shape of the folded chain
stabilized by
Hydrophobic interactions
Hydrogen bonds
Electrostatic interactions
Disulfide bonds
contributes to
may lead to
Quaternary
is
the arrangement of multiple poly-peptide subunits in the protein
stabilized by
Hydrophobic interactions
Hydrogen bonds
Electrostatic interactions
may contribute to
Native conformation
can be
Fibrous
or
Globular
Chaperones
folding assisted by
determines
Biologic function
For example:
Catalysis
Protection
Regulation
Signal transduction
Storage
Structure
Transport
unfolding caused by
Denaturants
For example:
Urea
Extremes of pH, temperature
Organic solvents
lead to
Loss of secondary and tertiary structure
leads to
Loss of function
some may regain
can form
most proteins cannot refold upon removal of denaturant
Irreversible denaturation
Altered folding
leads to
Prions
lead to
Creutzfeldt-Jakob disease
leads to
Amyloid proteins
lead to
Alzheimer disease

Key point about protein!!

All proteins have specific shapes that are best suited for their function(s)

Strategic locations of amino acid residues, with different physical and chemical properties (side-chains), enable proteins to perform a variety of different functions in the cell.

Small globular proteins (made of one single polymer chain) present in the cytoplasm or secreted by the cell, have their hydrophobic amino acid residues tucked in the core of the protein.

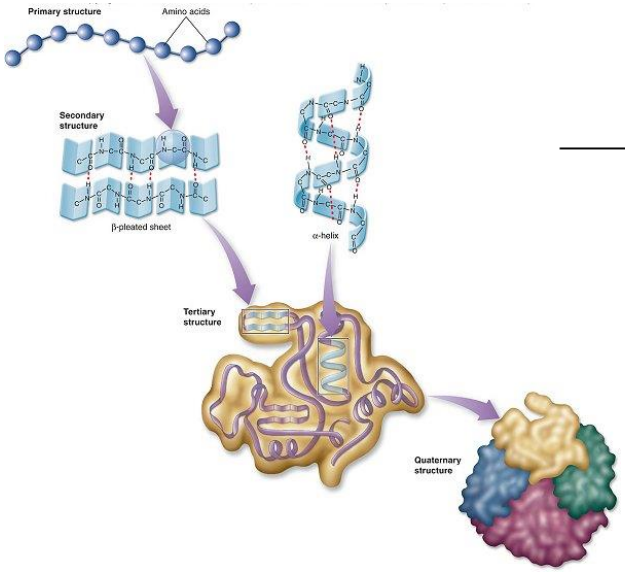
Larger protein complexes (formed from multiple copies of the same or different proteins) may have protein chains with interfaces that have hydrophobic amino acids. These proteins chains seek out and bind to partner proteins with complimentary interfaces and form functional assemblies.

In proteins that are composed of multiple domains, connected with flexible linker regions, scientists often selectively study regions or domains of the protein that are structurally stable and functionally important. The PDB archive may have multiple structures of one or more domains of the protein. To get a sense of how all these parts (domains) may work together, some of the domains of specific proteins have been shown next to each other in the Molecular Machinery interactive to represent the complete molecule. However, the actual structure of the complete protein may be slightly different from these composite presentations.

http://mm.rcsb.org/

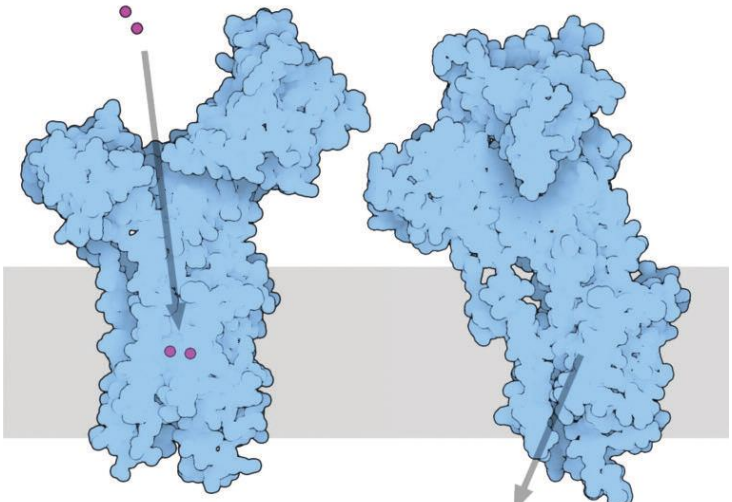
Protein Structure(Summary)

•Primary	The amino acid sequence	Glu-Arg-Phe-Gly
•Secondary	Characteristic structures that occur in many proteins (E.g. alpha helix , beta sheets)	alpha helix beta sheets
•Tertiary	Three dimensional structure of proteins	Y Z X
•Quaternary	Three dimensional structure of proteins composed of multiple subunits	

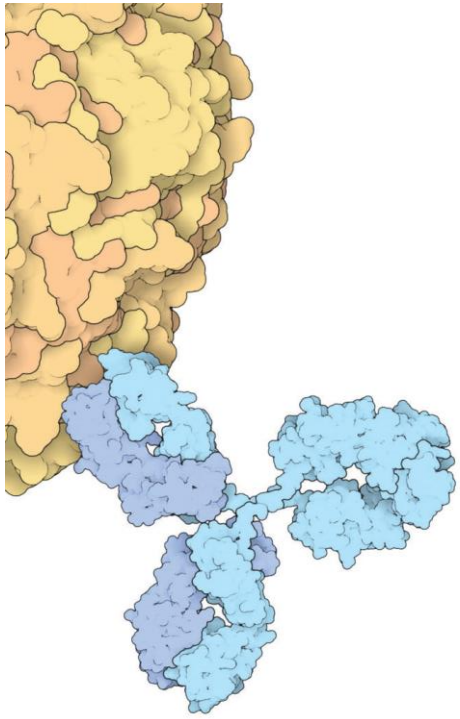


Protein types

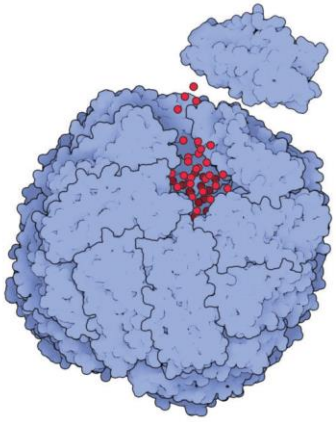
Type	Function	Examples
Structural	Give shape and structure to cell or organelles	Actin Tubulin
Enzymes	Catalyse biological reactions	Trypsin Adenylate cyclase
Receptors	Bind to other molecules and transmit signal	Glutamate R. Steroid R.
Other functional proteins	Have specific functions	Antibodies Nuclear factors Neuropeptides



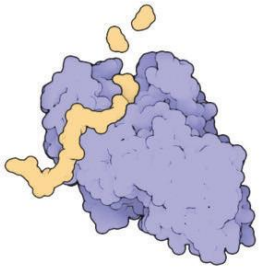
Transport (pompa de Ca)



Aparare (anticorp)

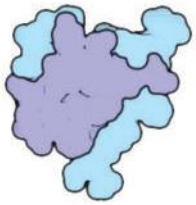


Depozitare (ferritin)



Enzima (amilaza)

Structura (collagen)



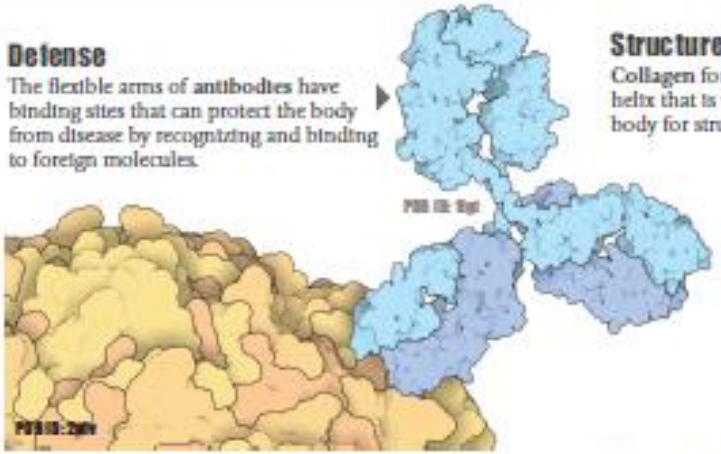
Comunicare (insulina)

Protein
Shape & Function

Specific amino acid sequences give proteins their distinct shapes and chemical characteristics. Protein shape is important because many proteins rely on the recognition of specific 3D molecular shapes to function correctly.

Defense

The flexible arms of **antibodies** have binding sites that can protect the body from disease by recognizing and binding to foreign molecules.



Structure

Collagen forms a strong and flexible triple helix that is widely used throughout the body for structural support.

Enzymes

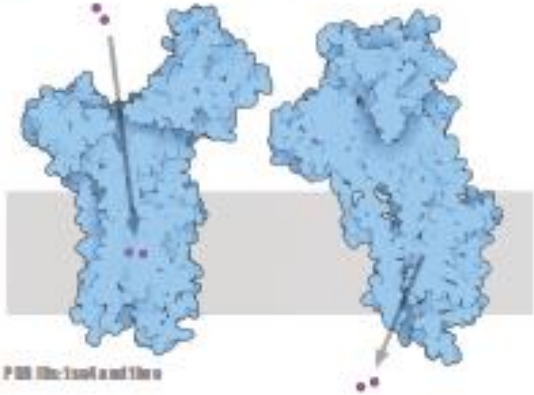
Alpha amylase is an enzyme with a specific catalytic site that begins the breakdown of carbohydrates in our saliva.

Communication

Insulin is a small, stable protein that can easily maintain its shape while traveling through the blood to regulate blood sugar levels.

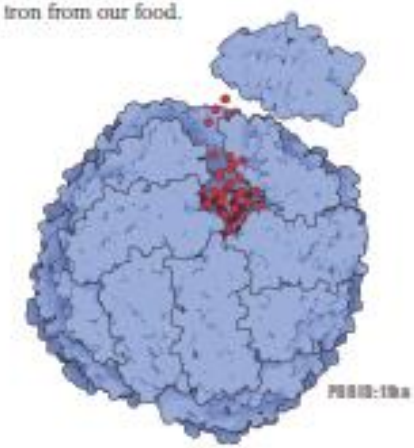
Transport

The **calcium pump** moves ions across cell membranes allowing the synchronized contraction of muscle cells.



Storage

Ferritin forms a hollow shell that stores iron from our food.



	U	C	A	G
U	UUU = phe UUC = phe UUA = leu UUG = leu	UCU = ser UCC = ser UCA = ser UCG = ser	UAU = tyr UAC = tyr UAA = stop UAG = stop	UGU = cys UGC = cys UGA = stop UGG = trp
C	CUU = leu CUC = leu CUA = leu CUG = leu	CCU = pro CCC = pro CCA = pro CCG = pro	CAU = his CAC = his CAA = gln CAG = gln	CGU = arg CGC = arg CGA = arg CGG = arg
A	AUU = ile AUC = ile AUA = ile AUG = met	ACU = thr ACC = thr ACA = thr ACG = thr	AAU = asn AAC = asn AAA = lys AAG = lys	AGU = ser AGC = ser AGA = arg AGG = arg
G	GUU = val GUC = val GUA = val GUG = val	GCU = ala GCC = ala GCA = ala GCG = ala	GAU = asp GAC = asp GAA = glu GAG = glu	GGU = gly GGC = gly GGA = gly GGG = gly

ARN → aminoacizi

P1.

Un barbat in varsta de 20 ani cu anemie, este diagnosticat cu un tip anormal de β-globina alcatuita din 172 de aminoacizi in loc de 141 cat contine poroteina normala.

Ce se intampla in fiecare din urmatoarele mutatii?

Care dintre urmatoarele mutatii punctiforme reflecta aceasta anomalie?

A. UAA → CAA

B. UAA → UAG

C. CGA → UGA

D. GAU → GAC

E. GCA → GAA

	U	C	A	G
U	UUU = phe UUC = phe UUA = leu UUG = leu	UCU = ser UCC = ser UCA = ser UCG = ser	UAU = tyr UAC = tyr UAA = stop UAG = stop	UGU = cys UGC = cys UGA = stop UGG = trp
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UAA → CAA
UAA → UAG
CGA → UGA
GAU → GAC
GCA → GAA

A. Mutatia codonului stop (UAA) in CAA determina insertia unei glutamine in acel punct. Astfel lantul proteic va continua sa se alungeasca pana cand va ajunge la urmatorul codon stop, determinand aparitia unei proteine anormal de lungi.

B. Modificarea de la UAA la UAG schimba un codon stop cu altul, deci nu afecteaza proteina.

C. Modificarea de la CGA (arginina) la UGA (stop) determina aparitia unei proteine prea scurte.

D. Mutatia GAU (arpartan) la GAC (aspartan) nu afecteaza proteina.

E. Mutatia GCA (alanina) la GAA (glutamat) modifica proteina, dar fara sa ii schimbe dimensiunea.

P2.

La un pacient cu fibroza chistica determinata de mutatia ΔF508, proteina reglatoare a conductantei transmembranare a fibrozei chistice (CFTR) se pliaza incorect, fiind o proteina mutanta. La nivel celular, aceasta proteina anormala este marcata prin atasarea moleculelor de ubiquitina. Care este soarta acestei proteine marcate?

A. Isi indeplineste functia normala, deoarece ubiquitina corecteaza in mare masura efectul mutatiei.

B. Este secretata din celula

C. Este depozitata in vezicule de secretie

D. Este degradata de proteazomi

E. Este reparata de enzimele celulare

Prin atasarea ubiquitinei sunt marcate proteinele imbatranite, defecte sau pliate gresit pentru a fi distruse de catre proteazomi.

Nu se cunoaste nici un mecanism celular pentru repararea proteinelor defecte!

P3.

Translatia unui poliribonucleotid sintetic ce contine secventa repetitiva CAA intr-un sistem de sinteza proteic in vitro produce 3 homopolipeptide: poliglutamina, poliaspargina si politreonina.

Tinand cont de faptul ca CAA si AAC sunt codonii pentru glutamina, respectiv asparagina, care dintre urmatoarele triplete reprezinta codonul pentru treonina?

A. AAC

B. CAA

C. CAC

D. CCA

E. ACA

Secventa polinucleotidica sintetica CAACAACAACAACAA ... ar putea fi citita incepand de la prima litera C, prima litera A sau a doua litera A.

In primul caz se obtine codonul CAA care codifica glutamina,

In cel de-al doilea se obtine codonul AAC care codifica asparagina

In ultimul caz se obtine codonul ACA care codifica treonina.

	U	C	A	G
U	UUU = phe UUC = phe UUA = leu UUG = leu	UCU = ser UCC = ser UCA = ser UCG = ser	UAU = tyr UAC = tyr UAA = stop UAG = stop	UGU = cys UGC = cys UGA = stop UGG = trp
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G	GUU = val GUC = val GUA = val GUG = val	GCU = ala GCC = ala GCA = ala GCG = ala	GAU = asp GAC = asp GAA = glu GAG = glu	GGU = gly GGC = gly GGA = gly GGG = gly

P4.

Ce aminoacid este transportat spre ribozom de catre molecula ARNt care are secventa anticodon:

a) 5' UUC 3'?

b) 5’-AAA-3’?

P5.

Stiind ca viteza de sinteza proteica este limitata de sinteza ARNm, iar ARNm este sintetizat cu o viteza de 50 nucleoni/sec, care este viteza maxima a sintezei proteice?

P6.

O molecula ARNm incepe cu urmatoarea secventa de nucleotide:

5' UGGGGUUAAUGUUGAUGGUAUGCAUU 3'

Care este secventa de aminoacizi a proteinei sintetizate?

(translatia ARNm incepe la primul codon AUG)

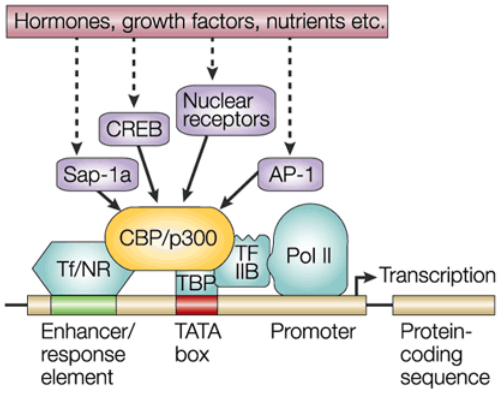
P7.

Mai jos gasiti secventa ADN (dublu-catenara) a genomului drojdiei. Transcriptia incepe la "Transcription Start Site" (TSS) dupa promoter (galben), si are loc in directia sagetii. Transcriptia se opreste la sfarsitul zonei "Transcription Terminator" (albastru). Catena ADN matrita este catena de jos.

a) Care este secventa ARNm produsa de aceasta gena? (notati capetele 5' si 3'.

b) Care este secventa de proteina produsa de ARNm de la punctul (b). (notati capetele N si C.

c) Presupunem ca a aparut o mutatie in cadrul careia imediat dupa perechea de baze T-A (bold) s-a adaugat inca o pereche de baze T-A (sus-jos). Determinati noua secventa ARNm obtinuta prin transcriptie si noua secventa de proteina obtinuta prin translatie?



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a) *5′ GAGCC**AUG**CAUUAUCUAGAUAGUAGGCUCUGAGAAUUUAUCUC 3′*

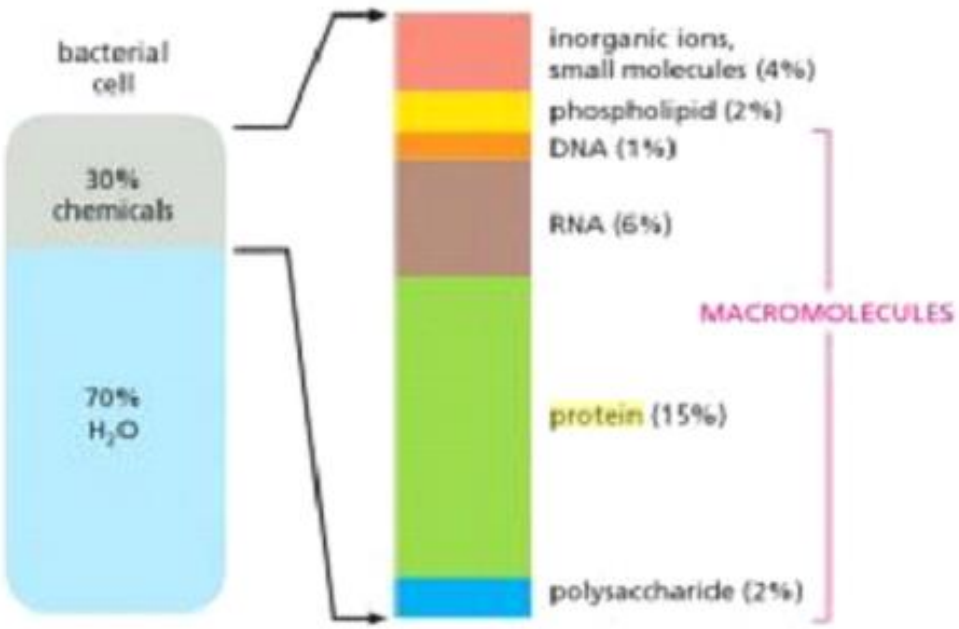
b) Codonul start este "AUG"

lantul de aminoacizi obtinut este: *Met-His-Tyr-Leu-Asp-Ser-Arg-Leu*

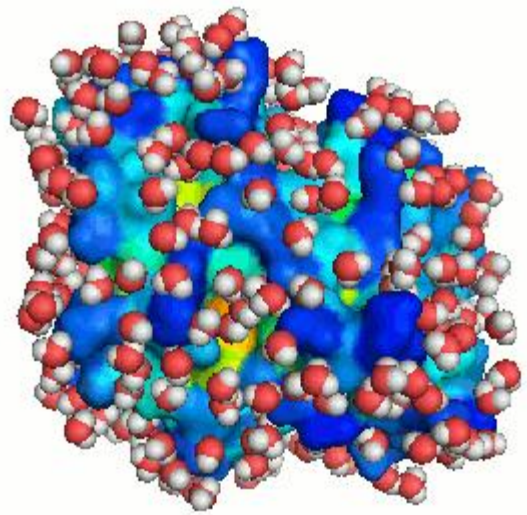
c) *5′ GAGCC**AUG**CAU**U**UAUCUAGAUAGUAGGCUCUGAGAAUUUAUCUC 3′*
Met-His-Leu-Ser-Arg

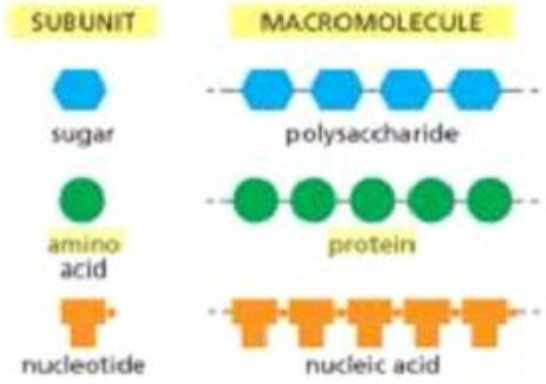
Compozitia unei celule

Component	% of Total Cellular Mass
Water	70-90%
Proteins	10-15%
Carbohydrates	3%
Lipids	2%
Nucleic Acids	5-7%
Ions	1%



Compozitia unei celule animale este asemanatoare cu cea a unei celule ce provine de la o bacterie.





Fiecare macromolecula este un polimer format din molecule mici (monomer) legate prin legaturi covalente:

structura proteinei:

http://www.youtube.com/watch?v=Ikq9AcBcohA

Interactiv:

http://www.wiley.com/college/boyer/0470003790/animations/translation/translation.htm

Quiz:

http://www.biology.arizona.edu/molecular_bio/molecular_bio.html

http://www.biology.arizona.edu/molecular_bio/problem_sets/nucleic_acids/nucleic_acids_1.html

http://www.biology.arizona.edu/molecular_bio/problem_sets/mol_genetics_of_eukaryotes/eukaryotes.html

Tutorial:

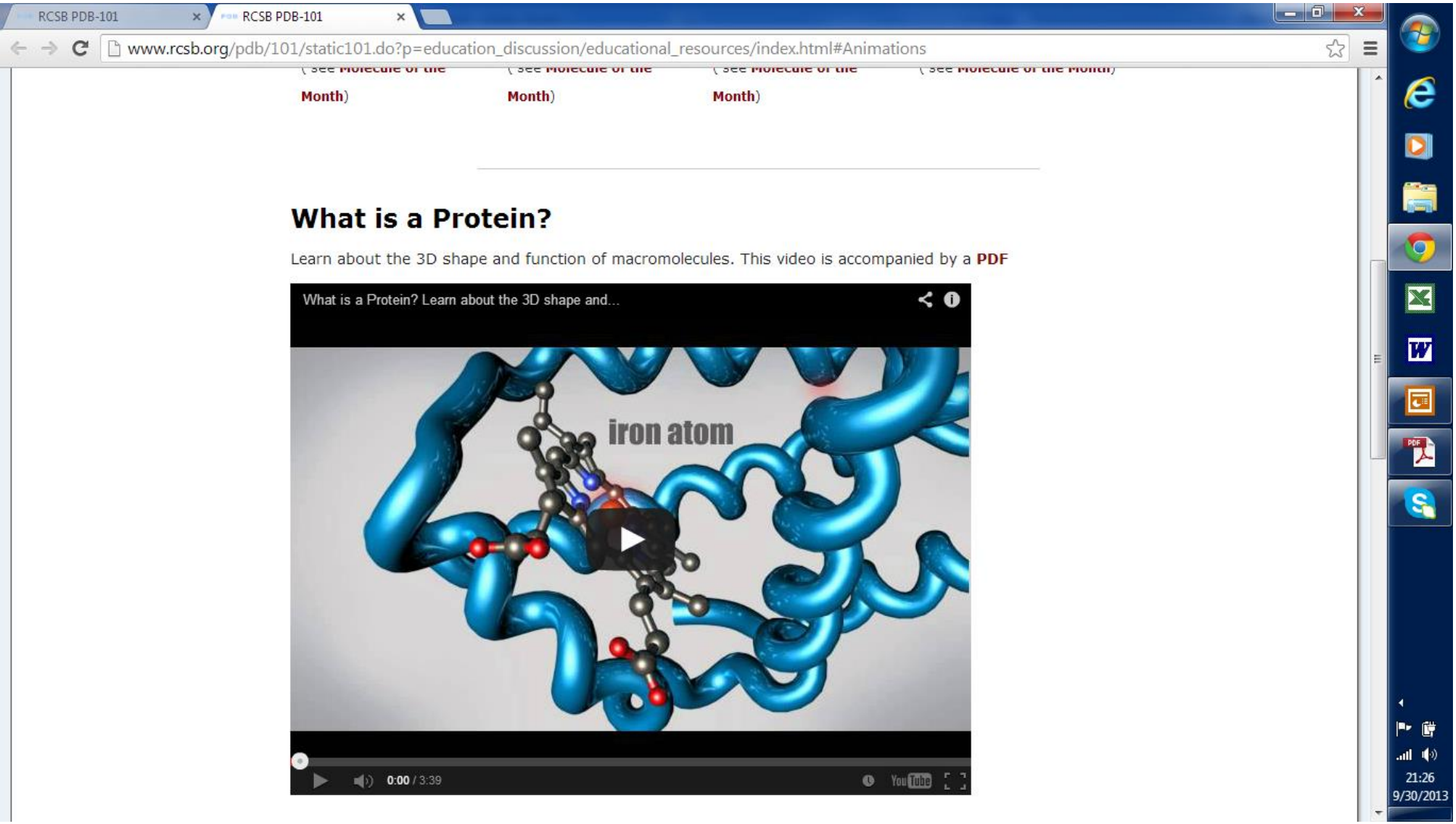
http://biology.kenyon.edu/courses/biol114/Chap05/Chapter05.html

Games:

http://www.yourgenome.org/dgg/detailed/cell/cell_3.shtml

http://learn.genetics.utah.edu/content/begin/dna/transcribe/

http://www.yourgenome.org/dgg/detailed/cell/cell_3.shtml



http://www.rcsb.org/pdb/101/static101.do?p=education_discussion/educational_resources/index.html#Posters-Exhibits