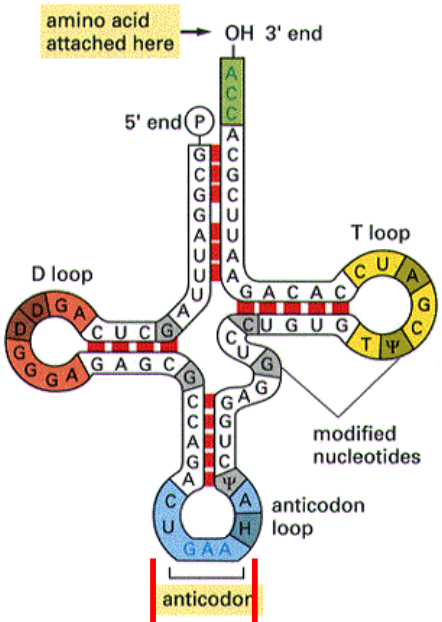
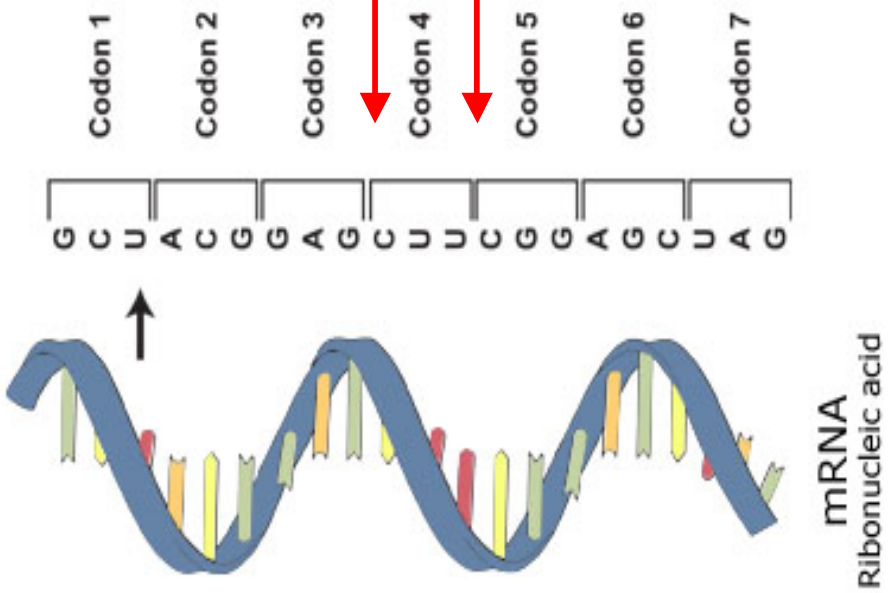
Traduzione e indirizzamento delle proteine

Traduzione



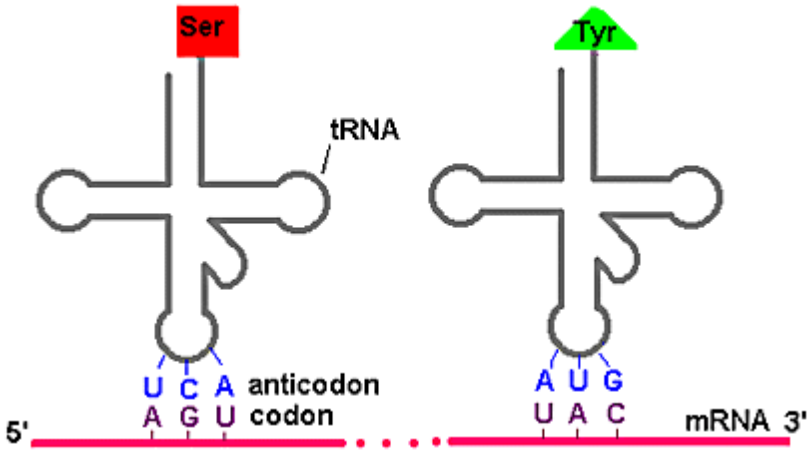
tRNA

Traduzione:
mRNA -------> proteine



mRNA

Genetic code



2nd base in codon

1st base in codon	U	C	A	G	3rd base in codon
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

codone di inizio = AUG =Metionina =Met

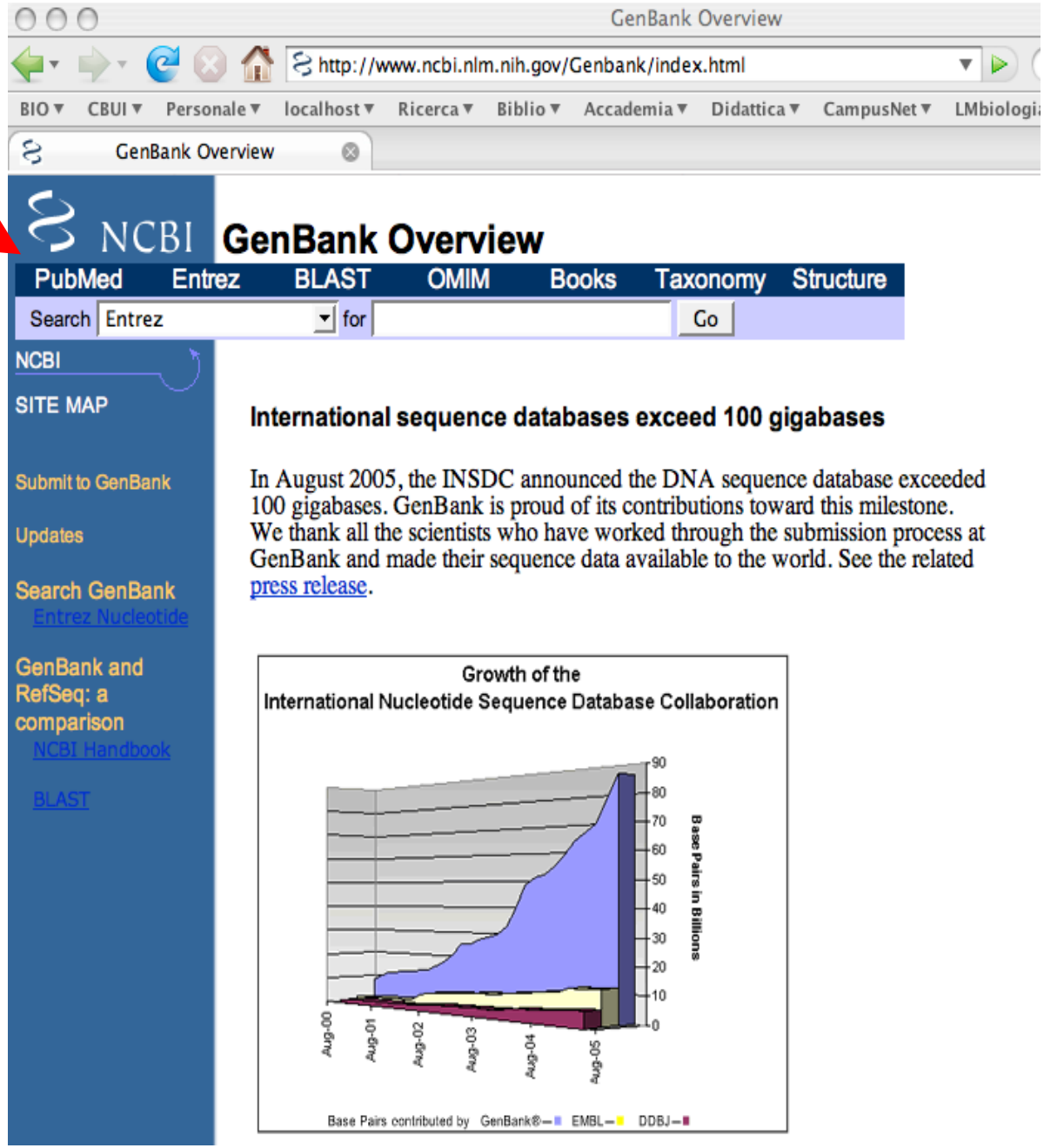
	T			C			A			G		
T	TTT	Phe	F	TCT	Ser	S	TAT	Tyr	Y	TGT	Cys	C
	TTC	Phe	F	TCC	Ser	S	TAC	Tyr	Y	TGC	Cys	C
	TTA	Leu	L	TCA	Ser	S	TAA	stop	*	TGA	stop	*
	TTG	Leu	L	TCG	Ser	S	TAG	stop	*	TGG	Trp	W
C	CTT	Leu	L	CCT	Pro	P	CAT	His	H	CGT	Arg	R
	CTC	Leu	L	CCC	Pro	P	CAC	His	H	CGC	Arg	R
	CTA	Leu	L	CCA	Pro	P	CAA	Gln	Q	CGA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

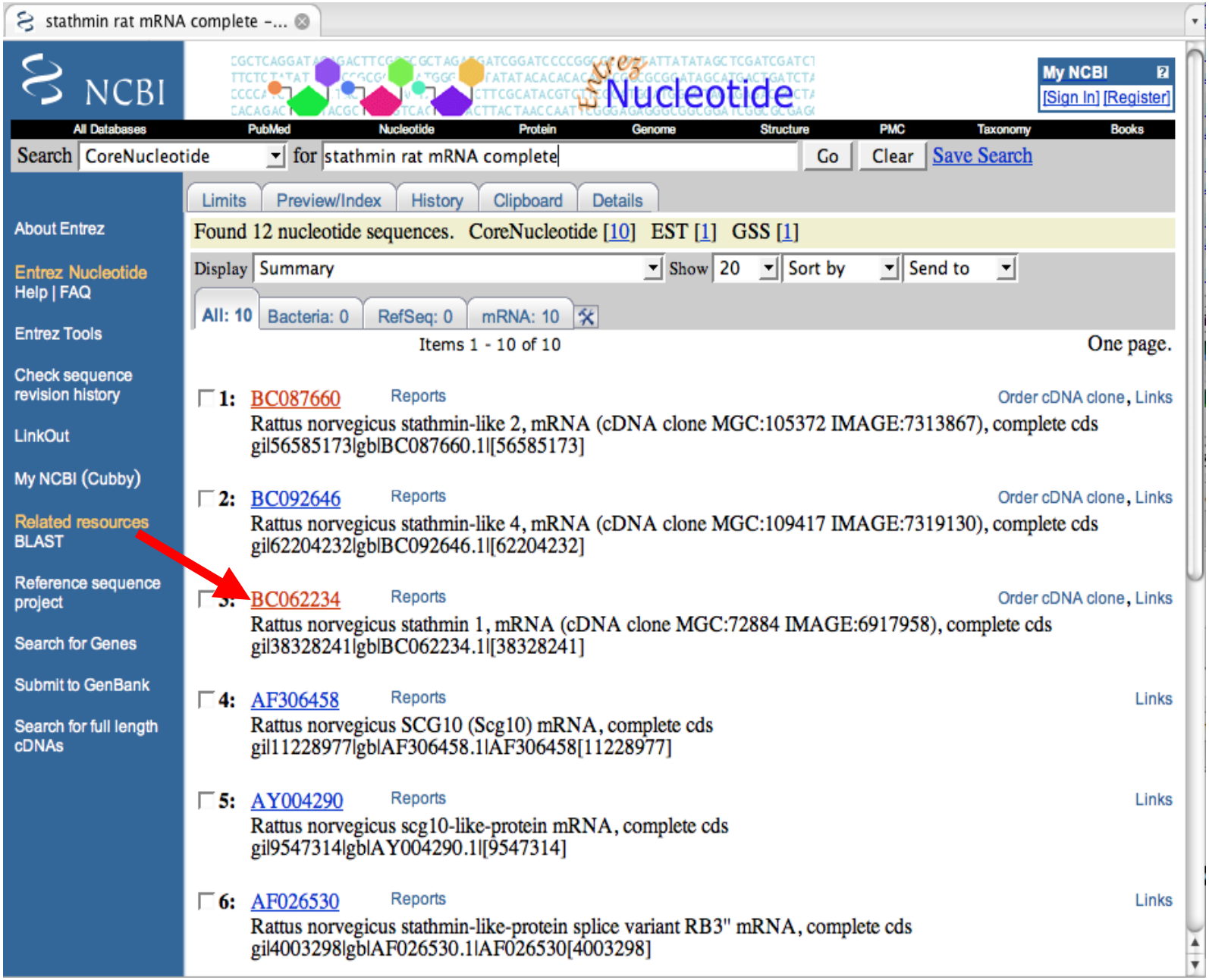
GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

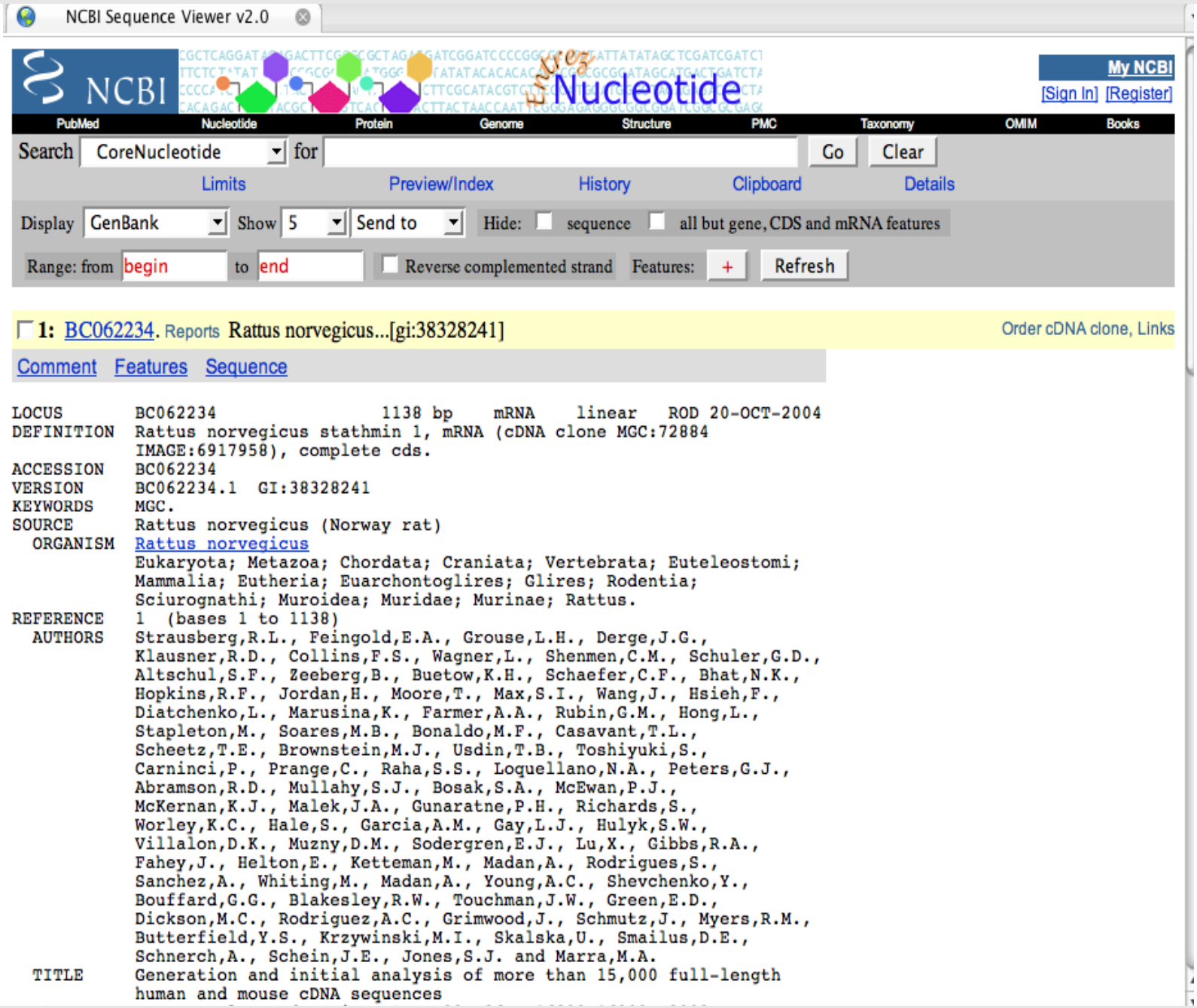
M A G L T A .....

```
   1 atggcgggtc tgacggcggc ggccccgcgg cccggagtcc tcctgctcct gctg
  61 ctccacccct ctcggcctgg aggggtccct ggggccattc ctggtggagt tcct
 121 gtcttttatc caggggctgg tctcggagcc cttggaggag gagcgctggg gcct
 181 aaacctctta agccagttcc cggagggctt gcgggtgctg gccttggggc aggg
 241 gccttccccg cagttacctt tccgggggct ctggtgcctg gtggagtggc tgac
 301 gcagcctata aagctgctaa ggctggcgct gggcttggtg gtgtcccagg agtt
 361 ttaggagtgt ctgcaggtgc ggtggttcct cagcctggag ccggagtgaa gcct
 421 gtgccgggtg tggggctgcc aggtgtatac ccaggtggcg tgctcccagg agct
 481 cccggtgtgg gggtgctccc tggagttccc actggagcag gagttaagcc caag
 541 ggtgtaggtg gagcttttgc tggaatccca ggagttggac cctttggggg accg
 601 ggagtcccac tggggtatcc catcaaggcc cccaagctgc ctggtggcta tgga
 661 tacaccacag ggaaactgcc ctatggctat gggcccggag gagtggctgg tgca
 721 aaggctggtt acccaacagg gacaggggtt ggcccccagg cagcagcagc agcg
 781 aaagcagcag caaagttcgg tgctggagca gccggagtcc tccctggtgt tgga
 841 ggtgttcctg gcgtgcctgg ggcaattcct ggaattggag gcatcgcagg cgtt
```

Come raggiungere le informazioni contenute nelle banche dati?







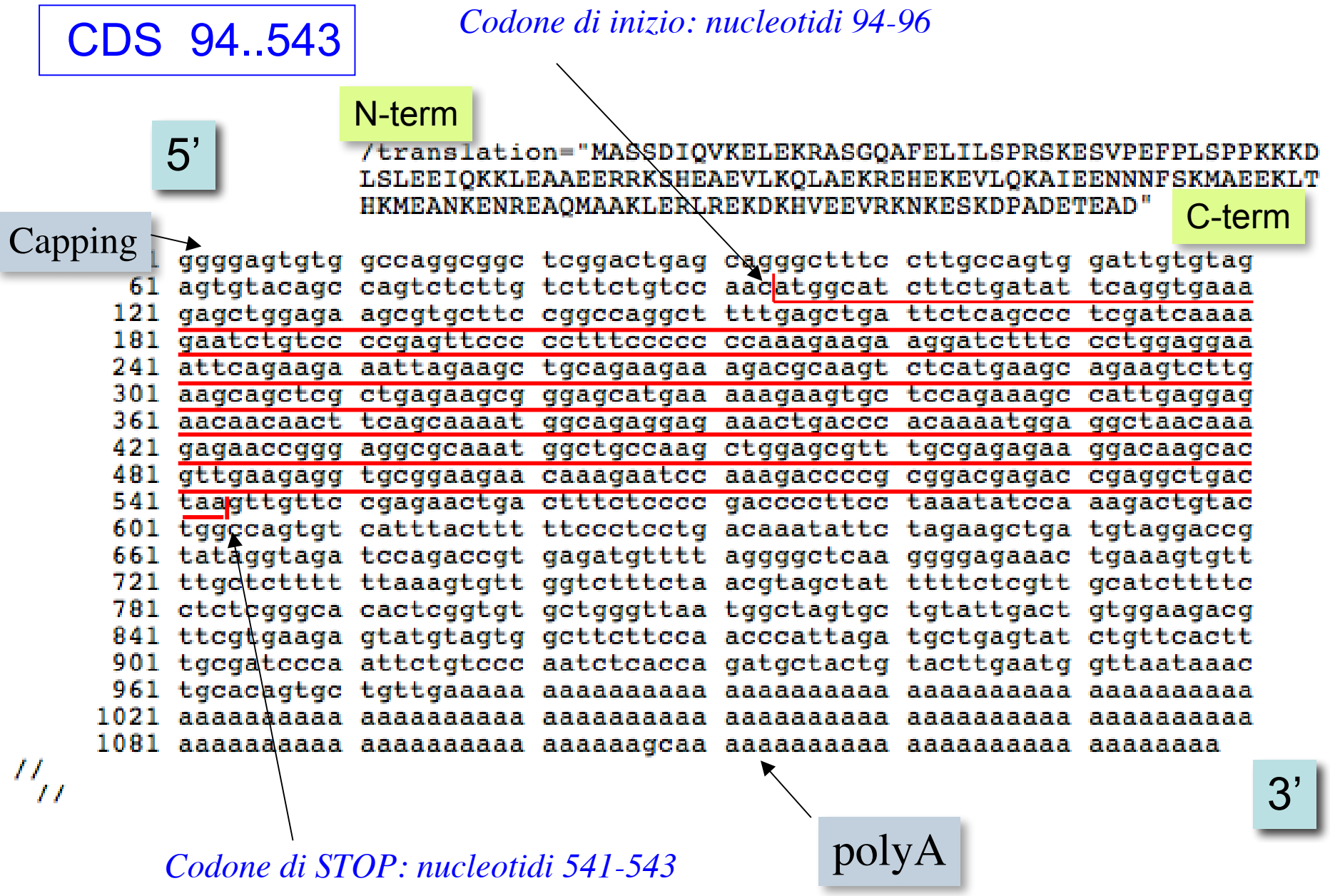
```
            human and mouse cDNA sequences
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 99 (26), 16899-16903 (2002)
   PUBMED   12477932
REFERENCE   2  (bases 1 to 1138)
  AUTHORS   Director MGC Project.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-NOV-2003) National Institutes of Health, Mammalian
            Gene Collection (MGC), Cancer Genomics Office, National Cancer
            Institute, 31 Center Drive, Room 11A03, Bethesda, MD 20892-2590,
            USA
  REMARK    NIH-MGC Project URL: http://mgc.nci.nih.gov
COMMENT     Contact: MGC help desk
            Email: cgapbs-r@mail.nih.gov
            Tissue Procurement: John C. Marshall, M.D., Ph.D
            cDNA Library Preparation: CLONTECH Laboratories, Inc.
            cDNA Library Arrayed by: The I.M.A.G.E. Consortium (LLNL)
            DNA Sequencing by: Genome Sequence Centre,
            BC Cancer Agency, Vancouver, BC, Canada
            info@bcgsc.bc.ca
            Steve Jones, Sarah Barber, Mabel Brown-John, Yaron Butterfield,
            Andy Chan, Steve S. Chand, William Chow, Alison Cloutier, Ruth
            Featherstone, Malachi Griffith, Obi Griffith, Ran Guin, Nancy Liao,
            Kim MacDonald, Amara Masson, Mike R. Mayo, Josh Moran, Ryan Morin,
            Teika Olson, Diana Palmquist, Anca Petrescu, Anna Liisa Prahbu,
            Parvaneh Saeedi, JR Santos, Angelique Schnerch, Ursula Skalska,
            Duane Smailus, Jeff Stott, Miranda Tsai, George Yang, Jacquie
            Schein, Asim Siddiqui, Rob Holt, Marco Marra.

            Clone distribution: MGC clone distribution information can be found
            through the I.M.A.G.E. Consortium/LLNL at: http://image.llnl.gov
            Series: IRAL Plate: 52 Row: h Column: 14
            This clone was selected for full length sequencing because it
            passed the following selection criteria: matched mRNA gi: 8393695.
FEATURES             Location/Qualifiers
     source          1..1138
                     /organism="Rattus norvegicus"
                     /mol_type="mRNA"
                     /db_xref="taxon:10116"
                     /clone="MGC:72884 IMAGE:6917958"
                     /tissue_type="Pituitary gland, anterior, rat"
                     /clone_lib="NICHD_Rr_Pit1"
                     /lab_host="DH10B"
                     /note="Vector: pDNR-LIB"
     gene            1..1138
                     /gene="Stmn1"
                     /note="synonyms: Lap18, OP18, prosolin, stathmin, PR22,
                     PP17, SMN, PP19"
                     /db_xref="GeneID:29332"
```

```
                     /gene="Stmn1"
                     /note="synonyms: Lap18, OP18, prosolin, stathmin, PR22,
                     PP17, SMN, PP19"
                     /db_xref="GeneID:29332"
                     /db_xref="RATMAP:44620"
                     /db_xref="RGD:2992"
     CDS             94..543
                     /gene="Stmn1"
                     /codon_start=1
                     /product="Stmn1 protein"
                     /protein_id="AAH62234.1"
                     /db_xref="GI:38328242"
                     /db_xref="GeneID:29332"
                     /db_xref="RATMAP:44620"
                     /db_xref="RGD:2992"
                     /translation="MASSDIQVKELEKRASGQAFELILSPRSKESVPEFPLSPPKKKD
                     LSLEEIQKKLEAAEERRKSHEAEVLKQLAEKREHEKEVLQKAIEENNNFSKMAEEKLT
                     HKMEANKENREAQMAAKLERLREKDKHVEEVRKNKESKDPADETEAD"
ORIGIN
        1 ggggagtgtg gccaggcggc tcggactgag cagggctttc cttgccagtg gattgtgtag
       61 agtgtacagc cagtctcttg tcttctgtcc aacatggcat cttctgatat tcaggtgaaa
      121 gagctggaga agcgtgcttc cggccaggct tttgagctga ttctcagccc tcgatcaaaa
      181 gaatctgtcc ccgagttccc cctttccccc ccaaagaaga aggatctttc cctggaggaa
      241 attcagaaga aattagaagc tgcagaagaa agacgcaagt ctcatgaagc agaagtcttg
      301 aagcagctcg ctgagaagcg ggagcatgaa aaagaagtgc tccagaaagc cattgaggag
      361 aacaacaact tcagcaaaat ggcagaggag aaactgaccc acaaaatgga ggctaacaaa
      421 gagaaccggg aggcgcaaat ggctgccaag ctggagcgtt tgcgagagaa ggacaagcac
      481 gttgaagagg tgcggaagaa caaagaatcc aaagaccccg cggacgagac cgaggctgac
      541 taagttgttc cgagaactga ctttctcccc gacccccttcc taaatatcca aagactgtac
      601 tggccagtgt catttacttt ttccctcctg acaaatattc tagaagctga tgtaggaccg
      661 tataggtaga tccagaccgt gagatgtttt aggggctcaa ggggagaaac tgaaagtgtt
      721 ttgctctttt ttaaagtgtt ggtctttcta acgtagctat ttttctcgtt gcatcttttc
      781 ctctcgggca cactcggtgt gctgggttaa tggctagtgc tgtattgact gtggaagacg
      841 ttcgtgaaga gtatgtagtg gcttcttcca acccattaga tgctgagtat ctgttcactt
      901 tgcgatccca attctgtccc aatctcacca gatgctactg tacttgaatg gttaataaac
      961 tgcacagtgc tgttgaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
     1021 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
     1081 aaaaaaaaaa aaaaaaaaaa aaaaaagcaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa
//
```

Sequenza aminoacidica

Sequenza nucleotidica



Ribosomi e sintesi proteica

The Nobel Prize in Chemistry 2009

"for studies of the structure and function of the ribosome"

Photo: MRC Laboratory of Molecular Biology

Venkatraman Ramakrishnan

1/3 of the prize

United Kingdom

MRC Laboratory of Molecular Biology
Cambridge, United Kingdom

Credits: Michael Marsland/Yale University

Thomas A. Steitz

1/3 of the prize

USA

Yale University
New Haven, CT, USA; Howard Hughes Medical Institute

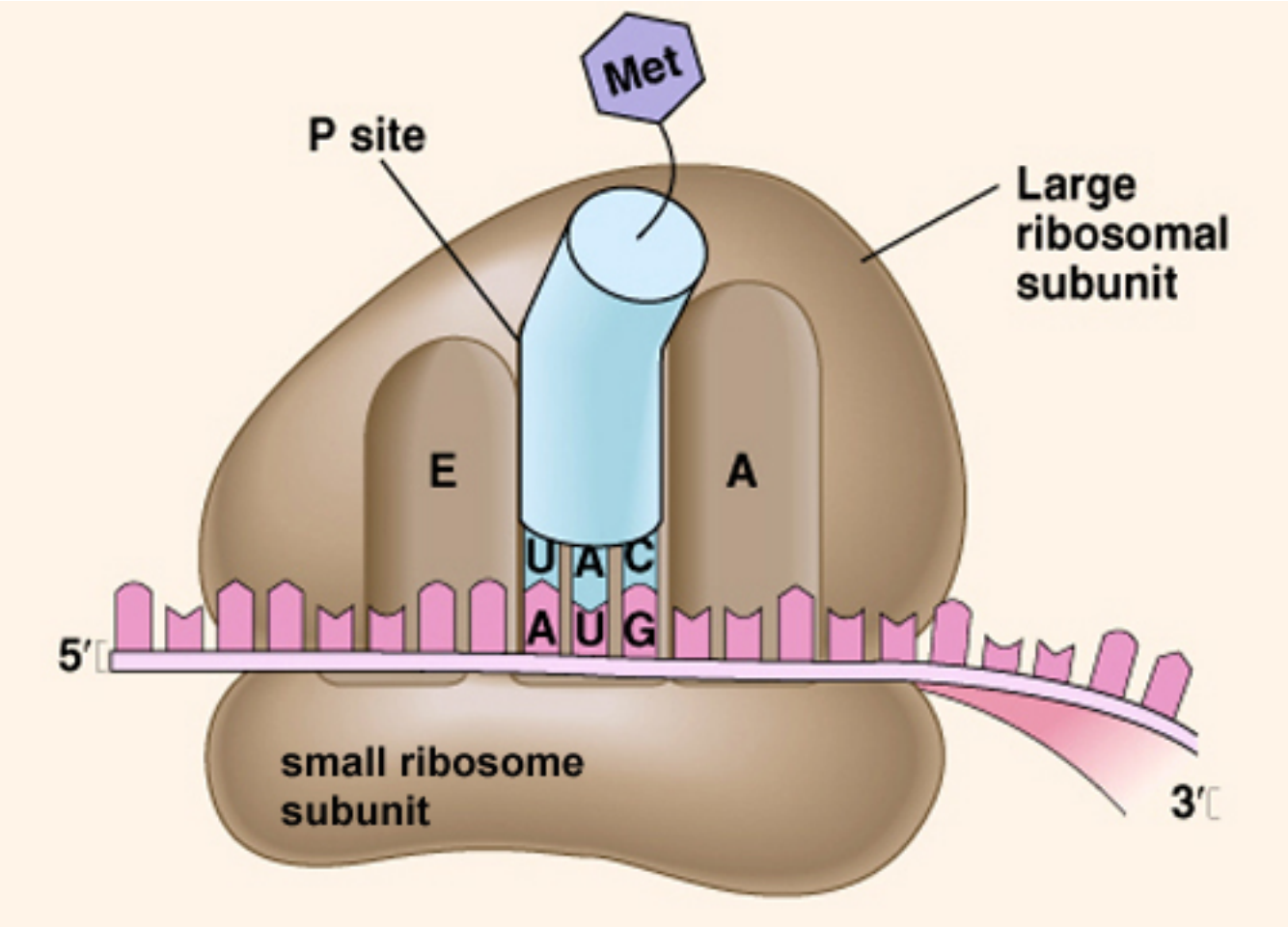
Credits: Micheline Pelletier/Corbis

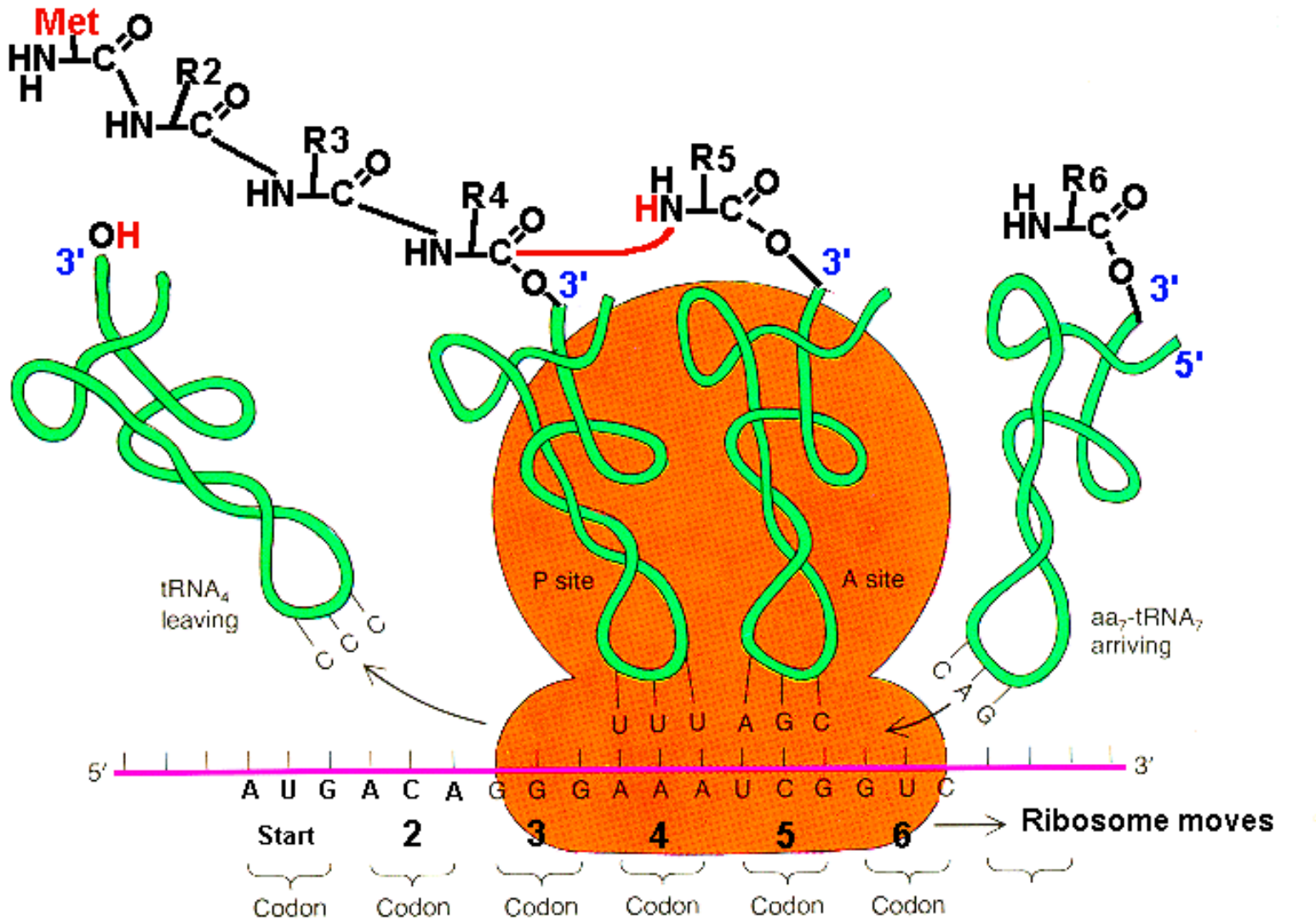
Ada E. Yonath

1/3 of the prize

Israel

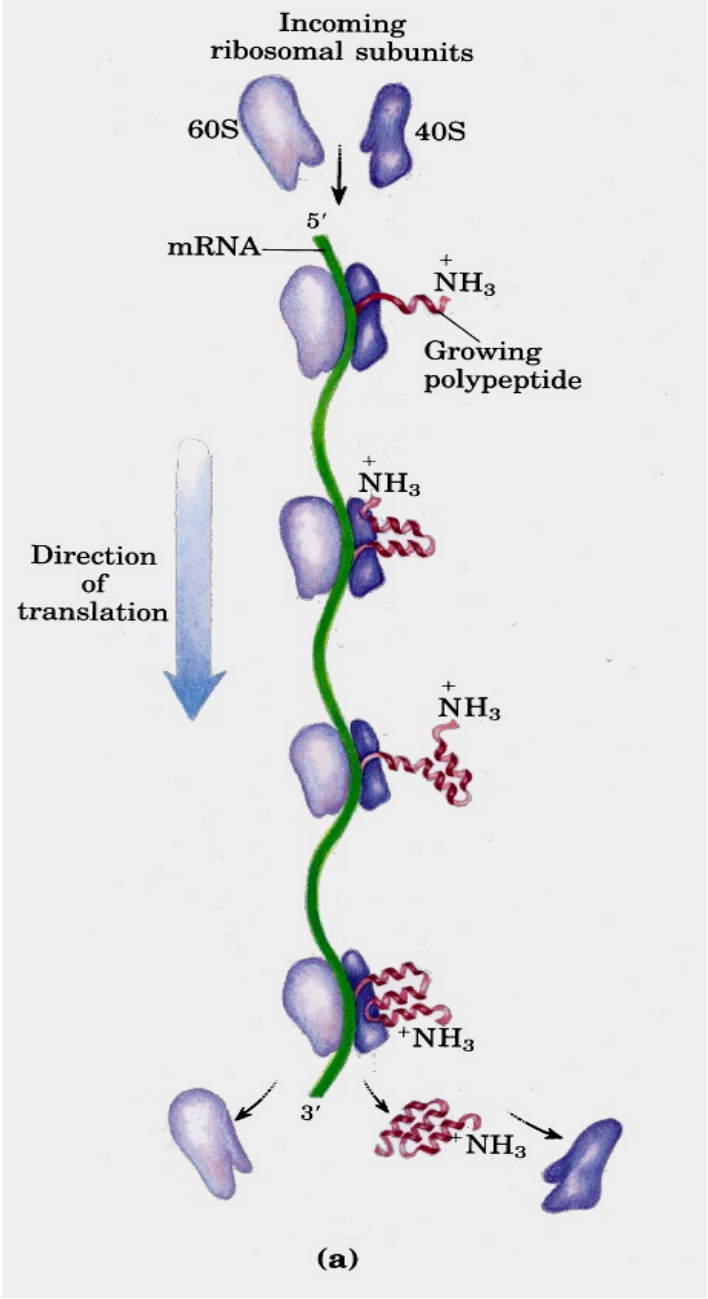
Weizmann Institute of Science
Rehovot, Israel





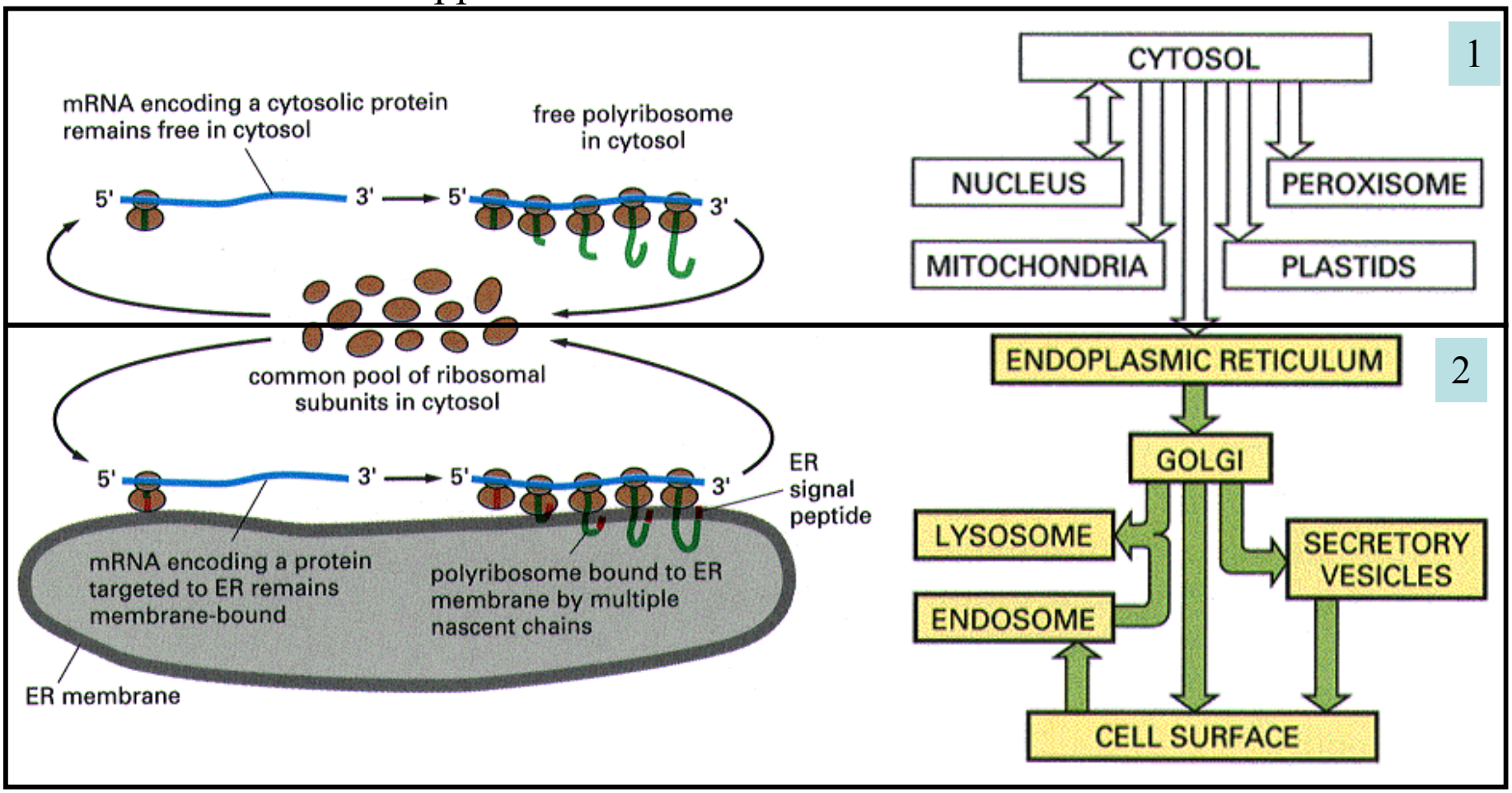
Modified from Griffiths et al., AN INTRODUCTION TO GENETIC ANALYSIS, 6th Ed., W.H. Freeman & Co., 1996.

La sintesi proteica inizia nel citoplasma con l'aggancio della piccola subunità ribosomale al estremità 5' dell'mRNA. In corrispondenza del codone di inizio "AUG" si aggancia la grande subunità ribosomale. Il primo a.a. al N-terminale è dunque una metionina (Met) e la sintesi proteica prosegue con la lettura dell'mRNA nella direzione 5'--->3'. A questo punto, se i primi a.a. sintetizzati corrispondono al "peptide segnale" allora la sintesi potrà proseguire soltanto in corrispondenza della membrana del RER.
Nelgi altri casi il ribosoma rimane "libero" e la sintesi prosegue fino allo stop codon.



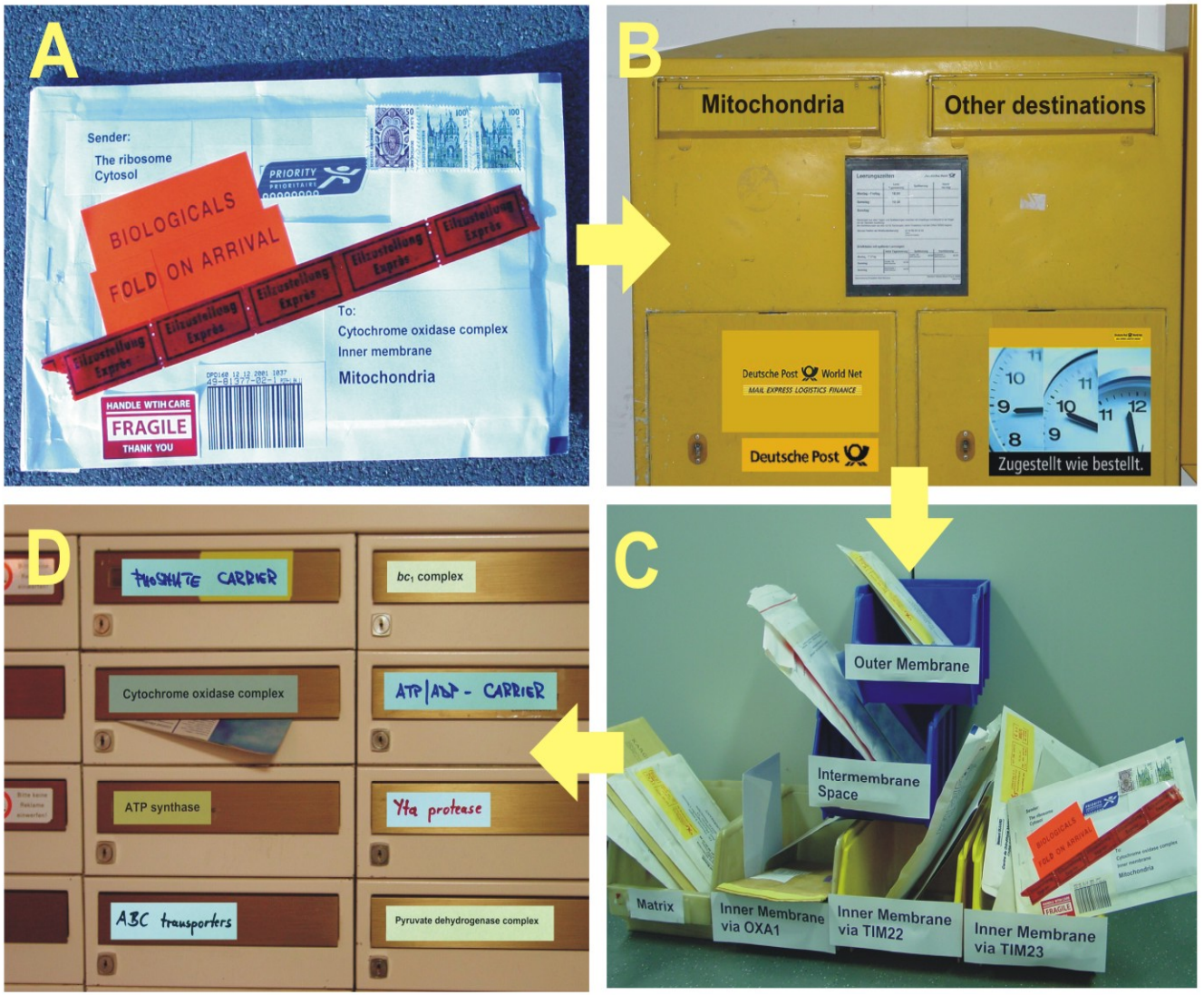
Indirizzamento delle proteine

E' la proteina nascente a determinare se il ribosoma che catalizza la sua sintesi deve rimanere libero oppure essere associato alla membrana del RER.



Proteine che hanno destinazione finale in un compartimento dell'elenco 1 sono sintetizzate da ribosomi liberi mentre proteine che hanno destinazione finale in un compartimento dell'elenco 2 guidano il ribosoma che le traduce verso il RER.

La sequenza delle proteine determina non soltanto le loro regolazioni e funzioni ma anche la loro localizzazione.



L'informazione sulla localizzazione finale delle proteine è contenuta nella loro sequenza amminoacidica

Esempi di sequenze di localizzazione:

Importazione nel nucleo (NLS) -Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-

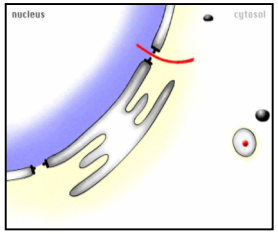
Exportazione dal nucleo (NES) -leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu_Asp_Ile-

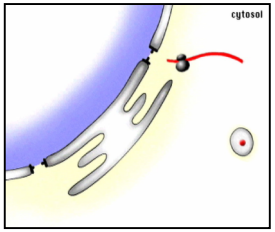
Importazione nel RER (peptide segnale): H2N-Met-Met-Ser-Phe-Val-Ser-Leu-
Leu-Leu-Val-Gly-Ile-Leu-Phe-
Trp-Ala-Thr-Glu-Ala-Glu-Gln-
Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-

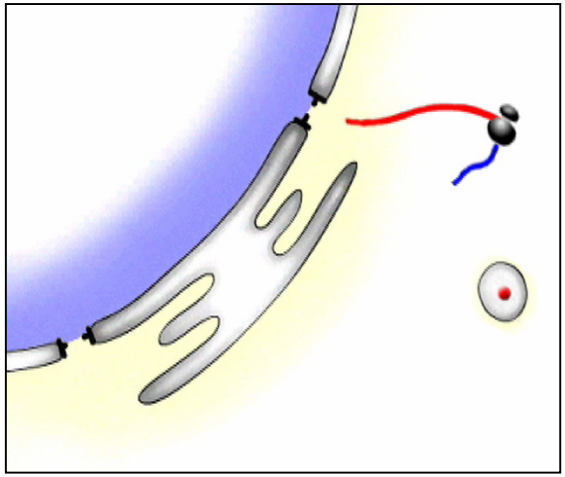
Ritorno al RER -Lys-Asp-Glu-Leu-COOH

Importazione nella matrice del mitochondrio H2N-Met-Leu-Ser-Leu-Arg-Gln-Ser-
Ile-Arg-Phe-Phe-Lys-Pro-Ala-
Thr-Arg-Thr-Leu-Cys-Ser-Ser-
Arg-Tyr-Leu-Leu-

Importazione nei perossisomi (PTS1) -Ser-Lys-Leu-COOH

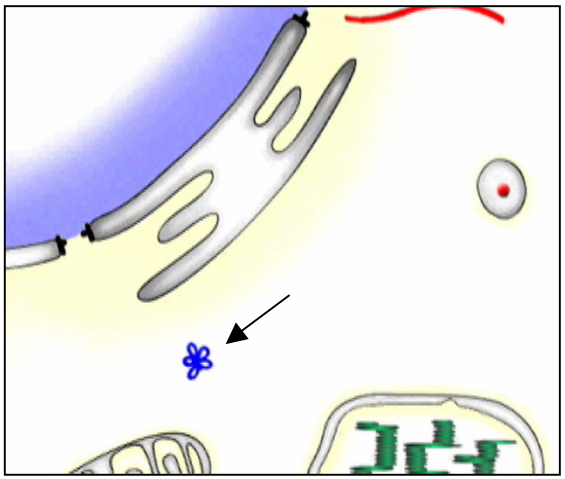


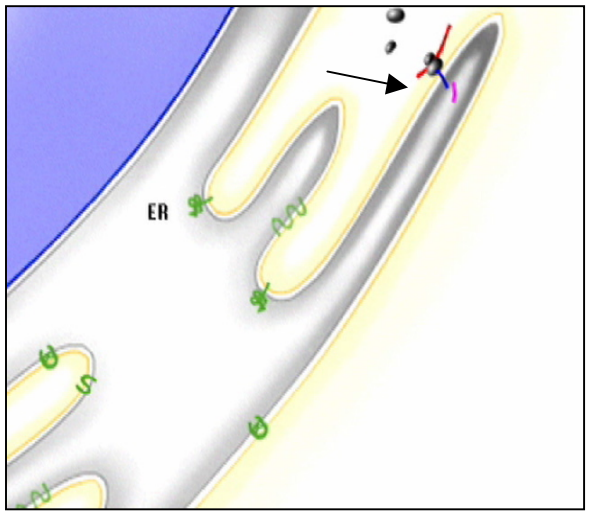


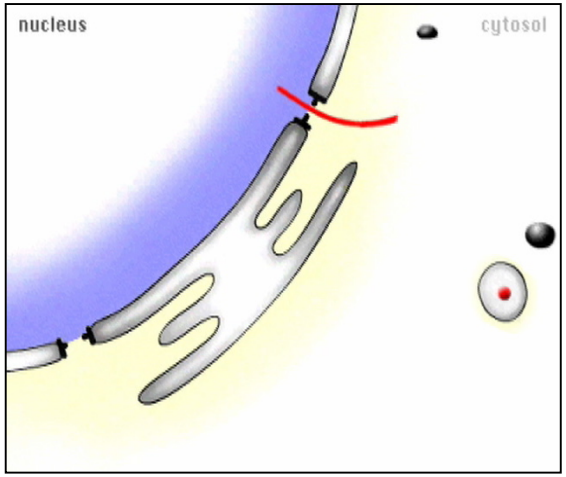


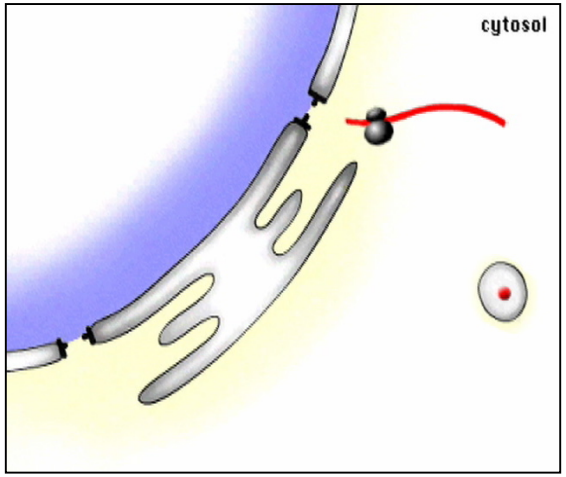
Assenza di peptide segnale
(traslocazione post-traduzionale)

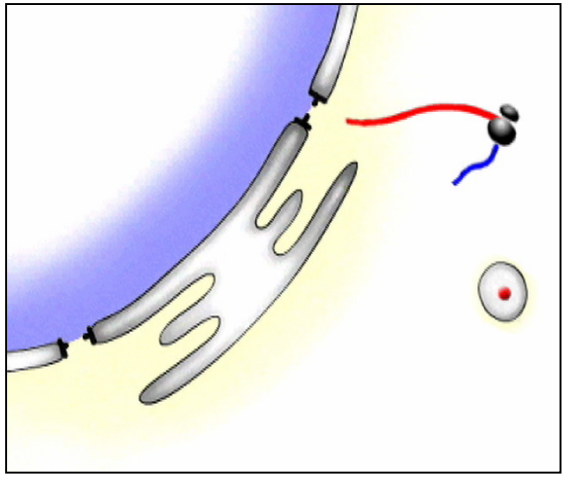
Peptide segnale ---> RER
(traslocazione co-traduzionale)

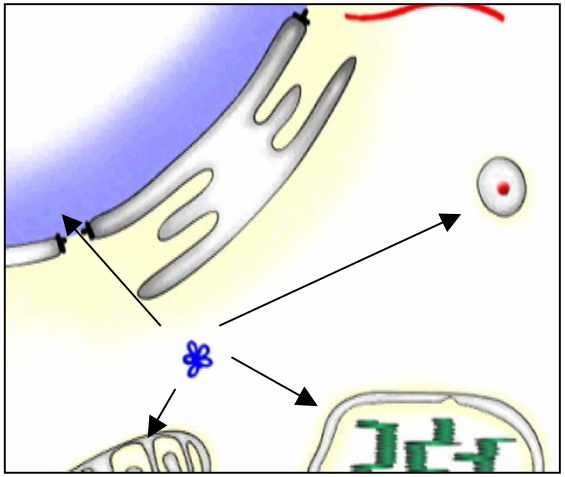




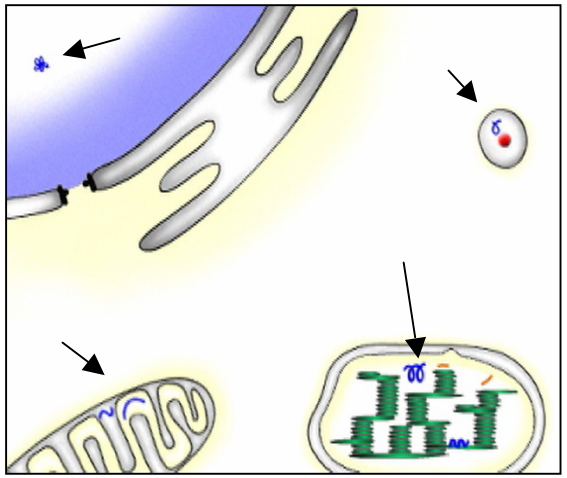




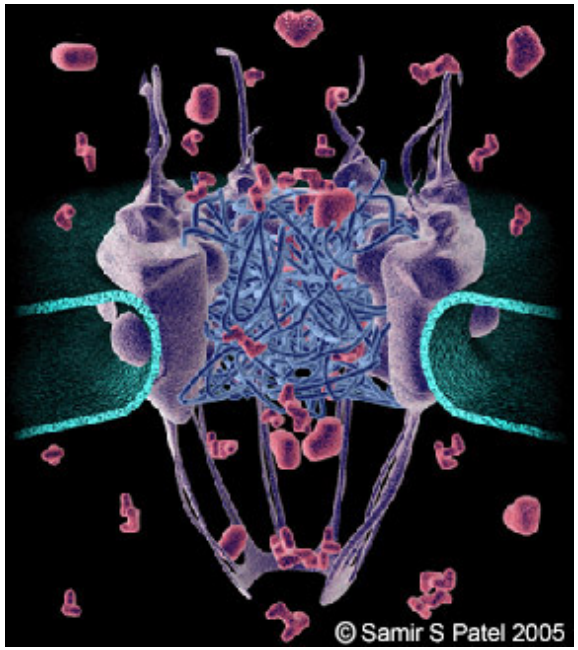




Proteine con sequenze di localizzazione nucleare oppure sequenze di localizzazione mitocondriali oppure sequenze di localizzazione perossisomiali oppure sequenze di localizzazione cloroplastica (cellule vegetali).

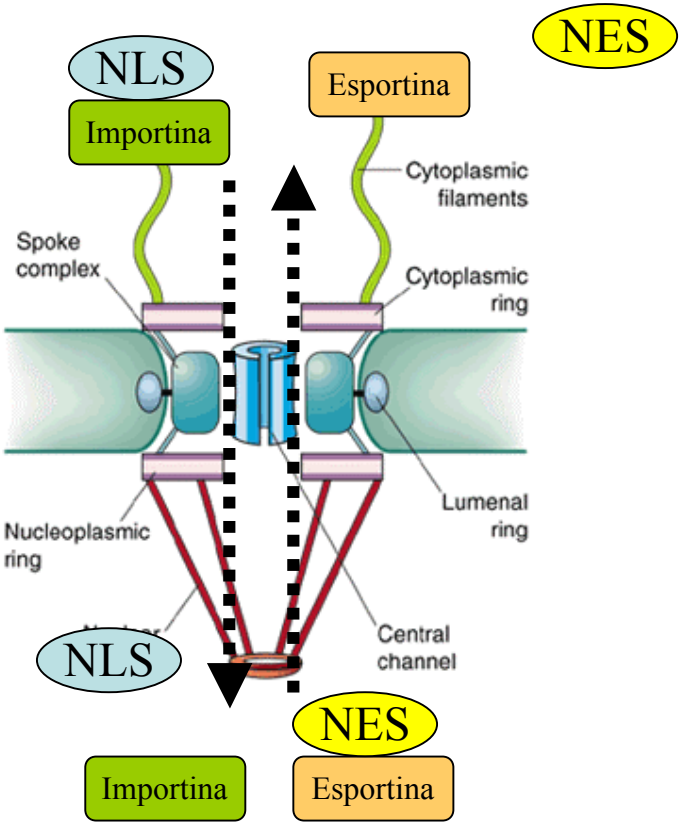


Sequenza di localizzazione nucleare (NLS):
Proteine di trasporto tipo “importine” accompagnano le proteine che possiedono una sequenza “NLS” nel passaggio dal citoplasma al nucleoplasma attraverso i pori nucleari.



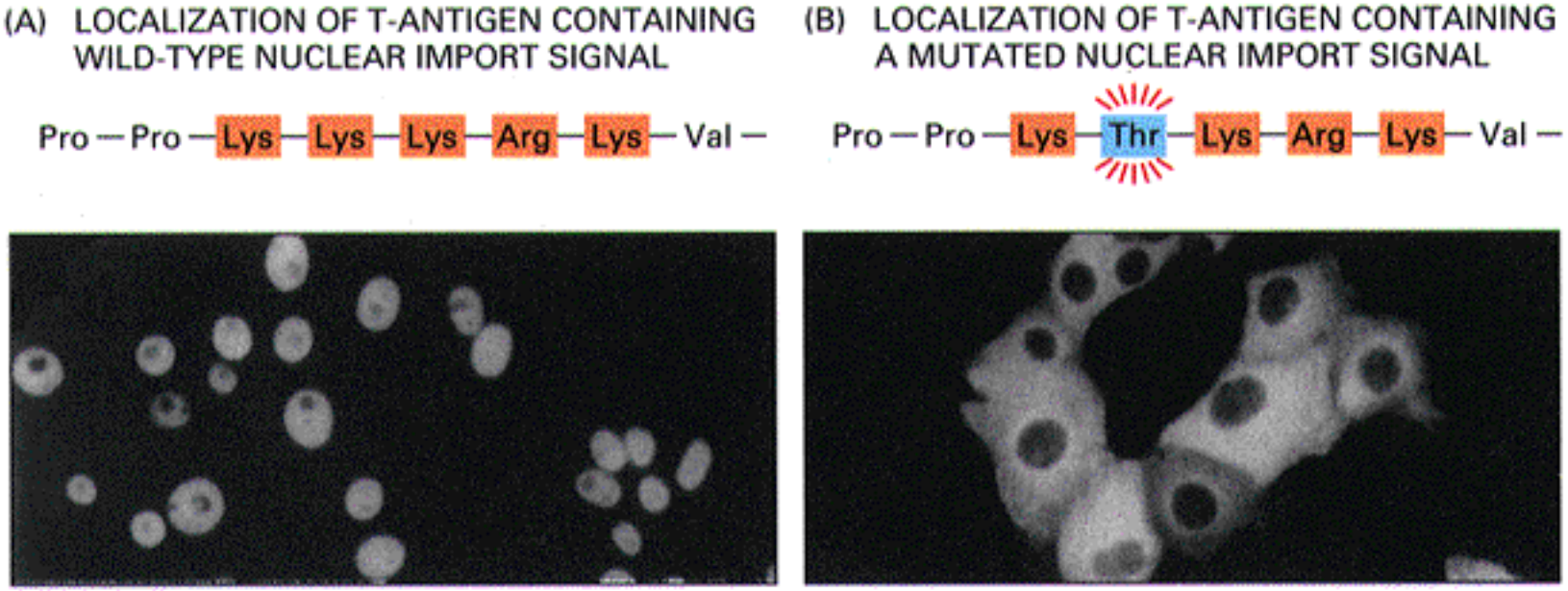
citosol

nucleo



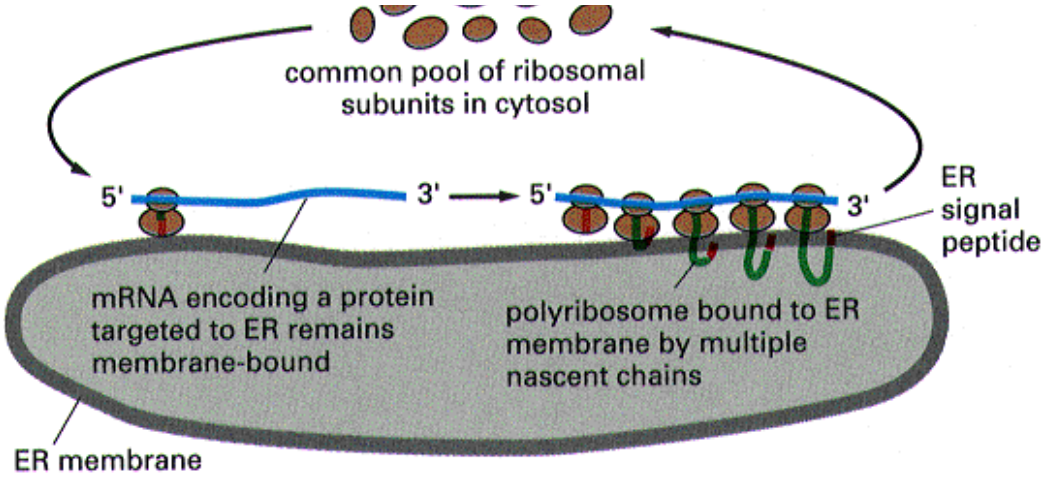
Se la sequenza NLS è mutata, la proteina non viene più trasportata dal citoplasma al nucleoplasma.

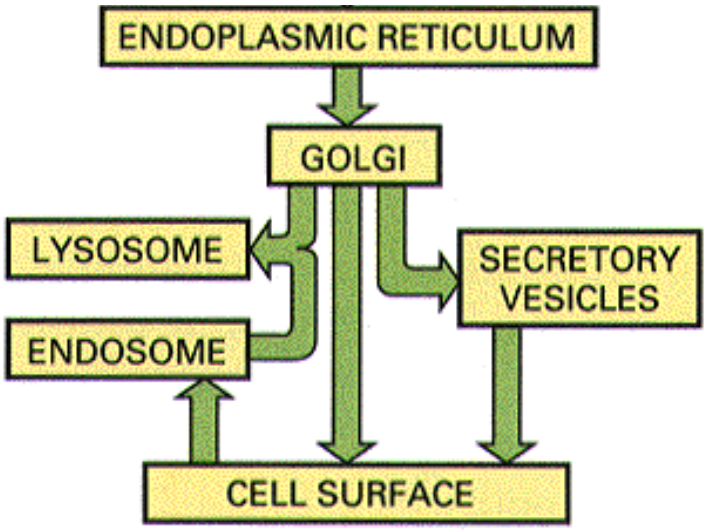
(A): sequenza NLS corretta: la proteina ha una localizzazione nucleare
(B): stessa proteina messa in evidenza in (A) ma con una mutazione puntiforma nella sequenza NLS (a.a. treonina in sostituzione della seconda lisina della sequenza NLS): la localizzazione è chiaramente citoplasmatica e non più nucleare perché la porteina mutata non è più riconosciuta dall'importina.



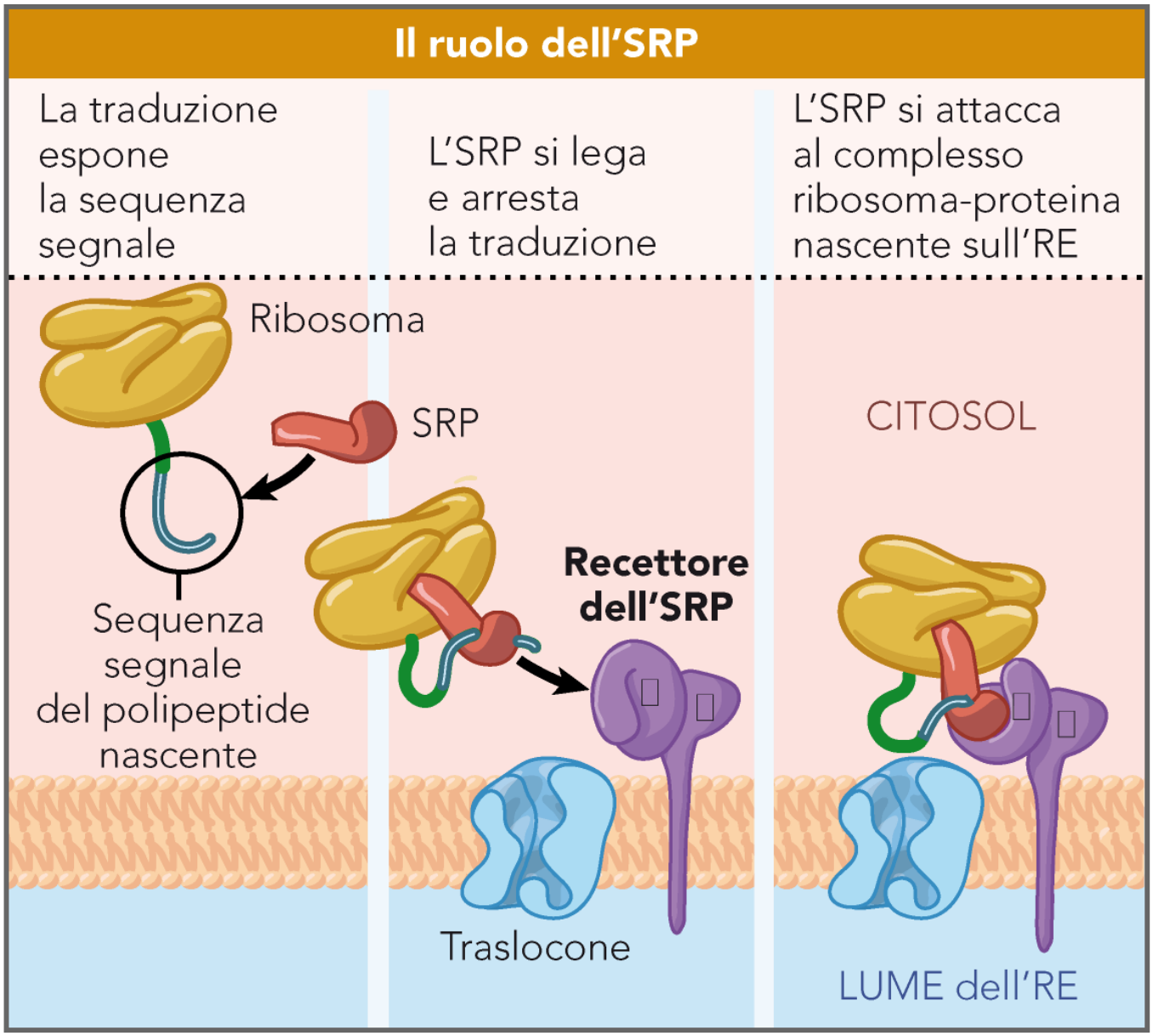
indirizzamento delle proteine della via secretoria

Con alcune eccezioni, le sequenze delle proteine della via “secretoria” iniziano con al N-terminale il peptide segnale





Nota: anche se chiamata genericamente via “secretoria”, proteine di questa via possono avere come localizzazione finale uno qualsiasi dei compartimenti elencati ed essere solubili, associate a membrane oppure transmembrana.



Function of Signal Peptide	Example of Signal Peptide
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-

1- La sintesi del polipeptide inizia su di un ribosoma libero nel citosol

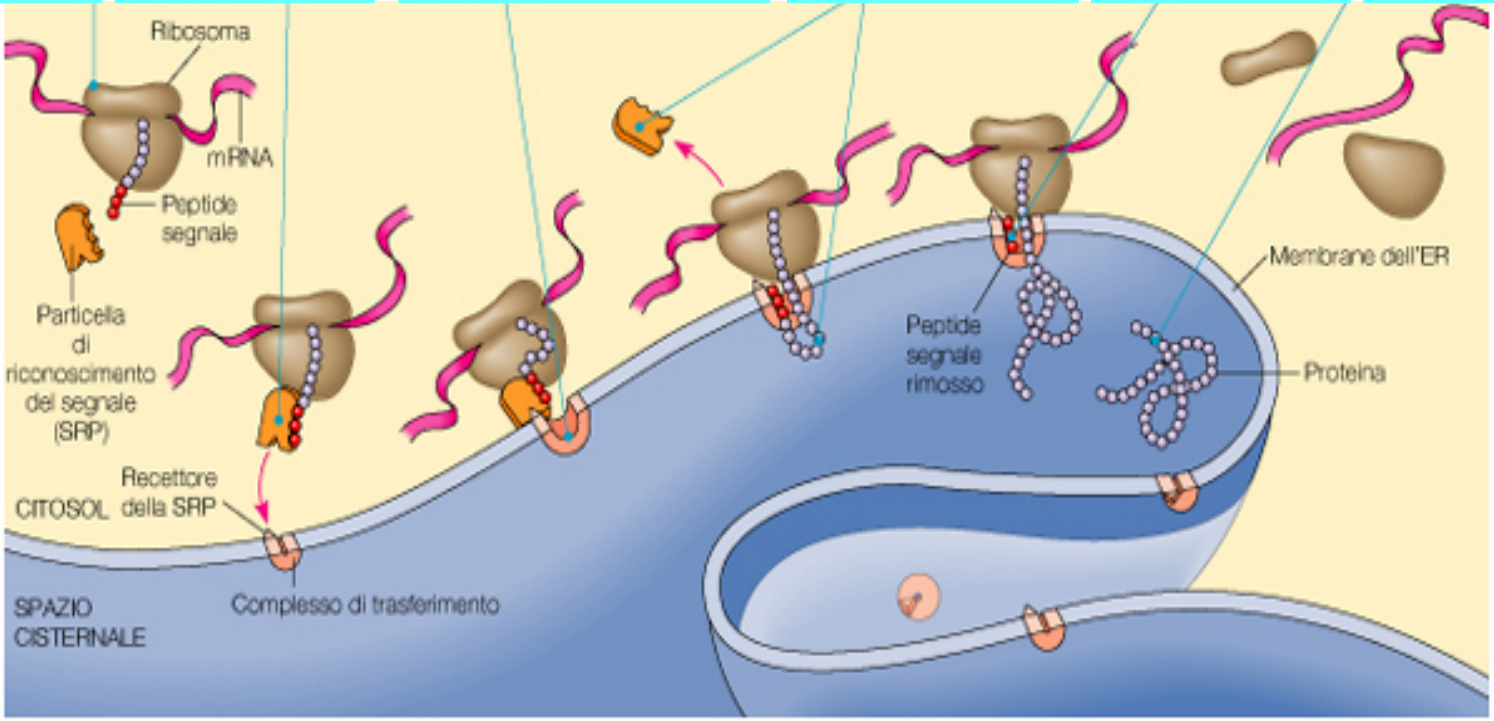
2- la proteina SRP si lega al peptide segnale e blocca temporaneamente la sintesi proteica

3- SRP si lega ad un recettore posto sulla membrana del RE. Tale recettore fa parte del complesso di trasferimento o traslocone che forma un poro sulla membrana del RE e lega il peptide segnale

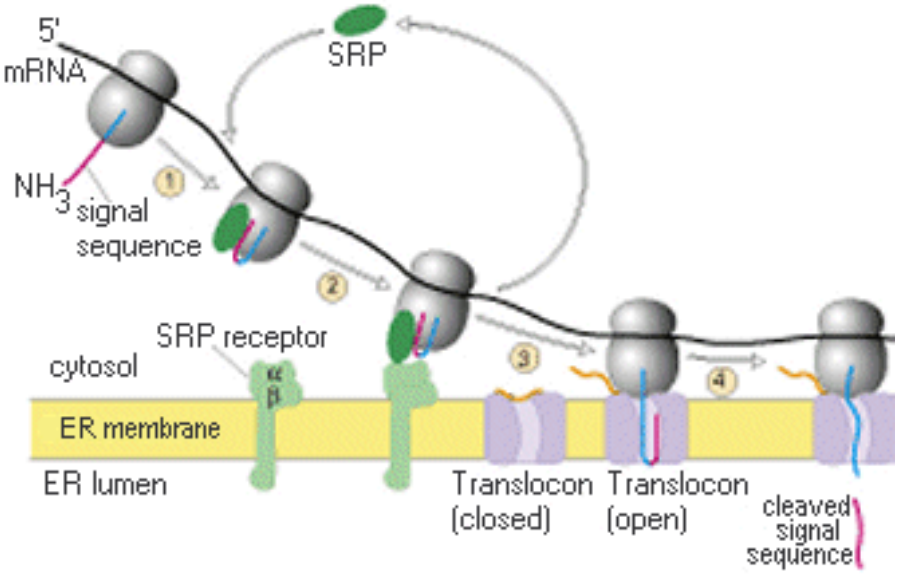
4- SRP abbandona il complesso, la sintesi proteica riprende. La proteina nascente attraversa contemporaneamente

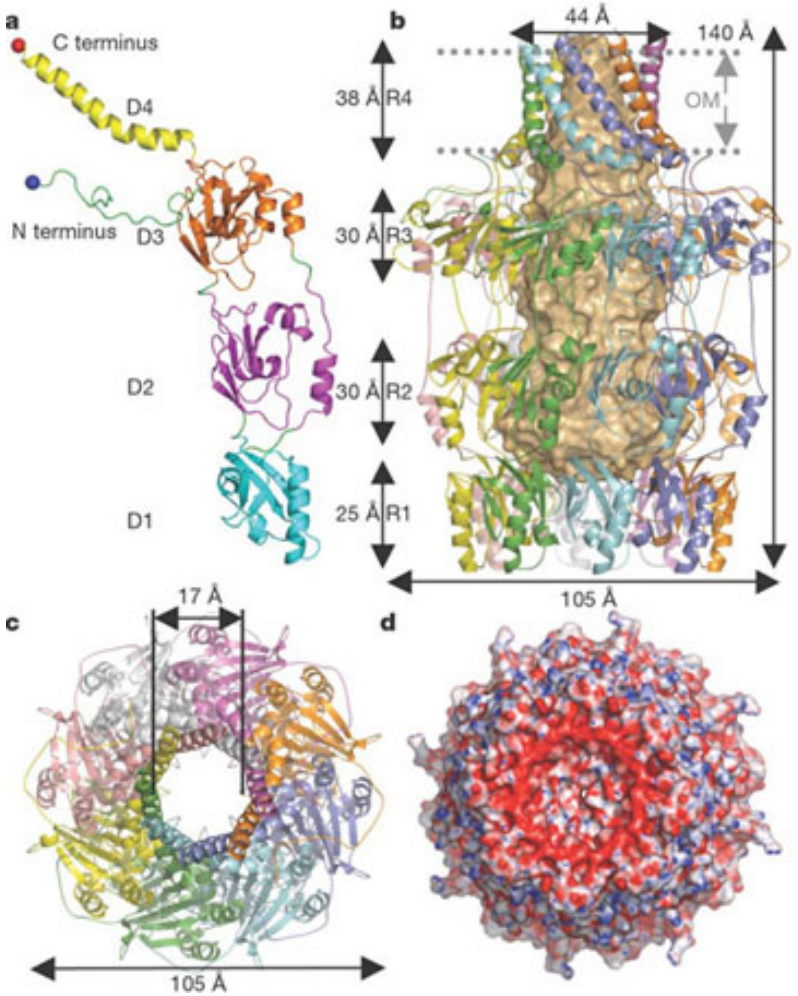
5- un enzima idrolitico rimuove il peptide segnale della proteina

6- In assenza di altro segnale, raggiunto il codone di stop, il ribosoma si sganccia dall'mRNA e la proteina è localizzata nel lume del RER



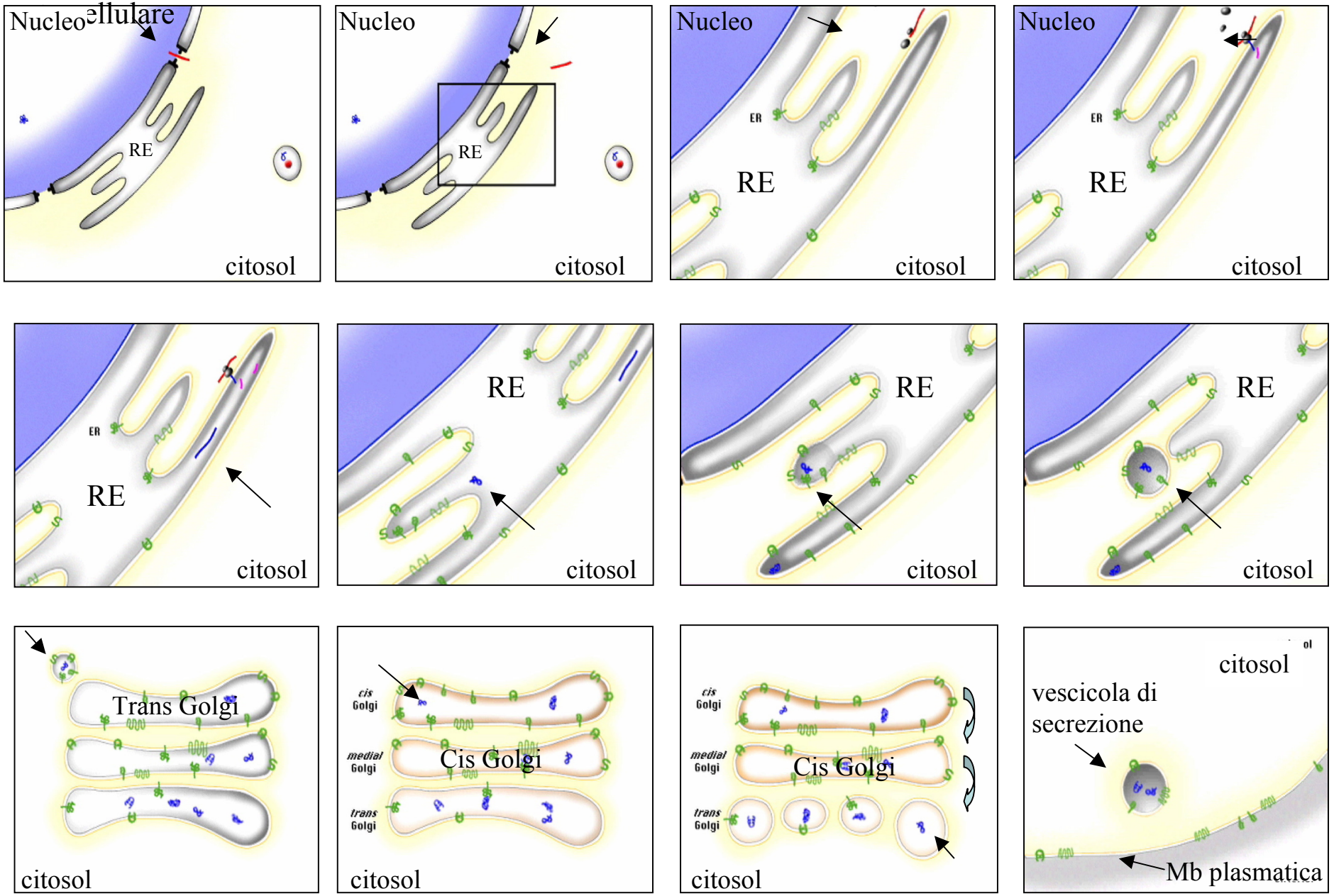
Le proteine SRP sono riciclate



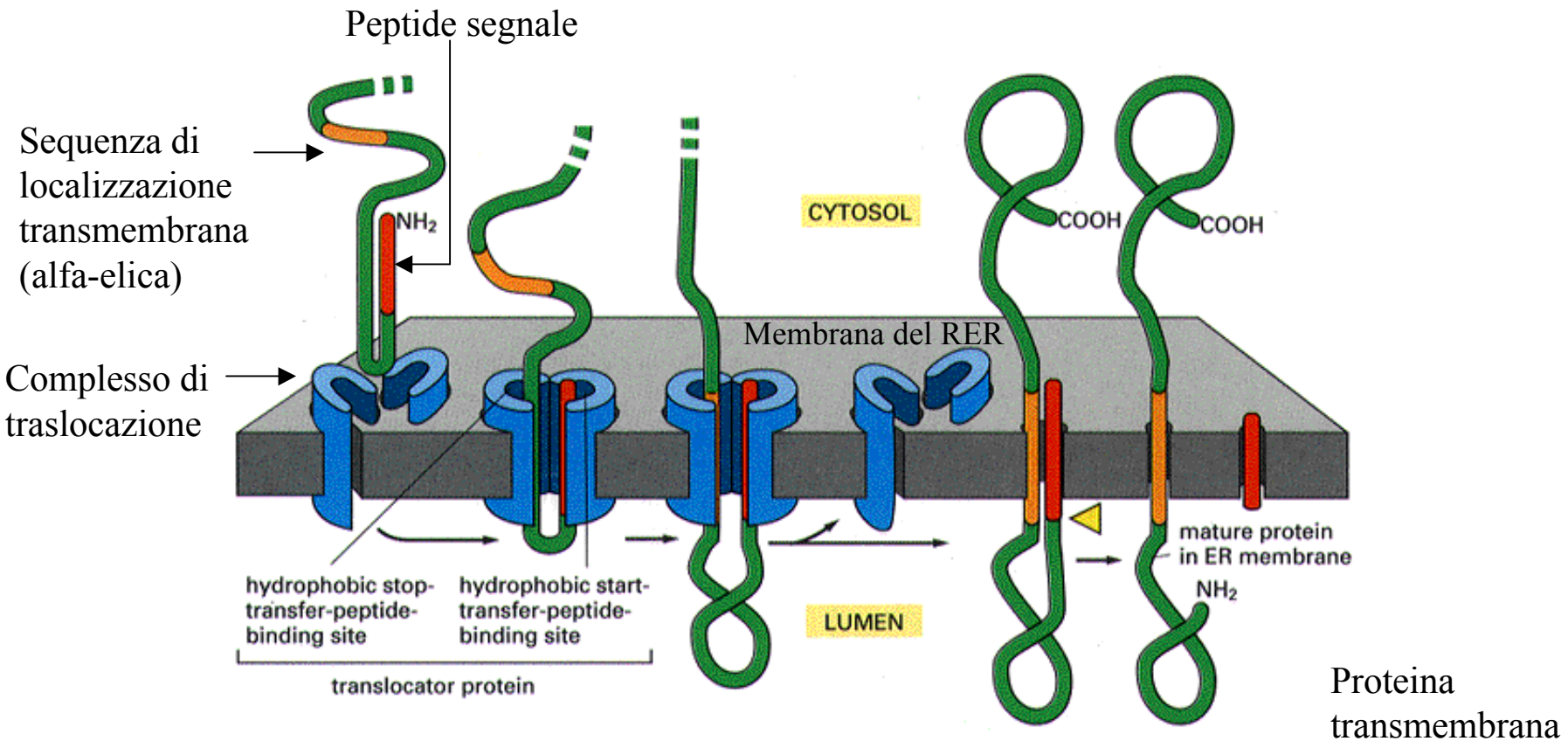


Il traslocone è un poro proteico tappato sul versante del lume del RE che si apre soltanto dopo interazione con un ribosoma.

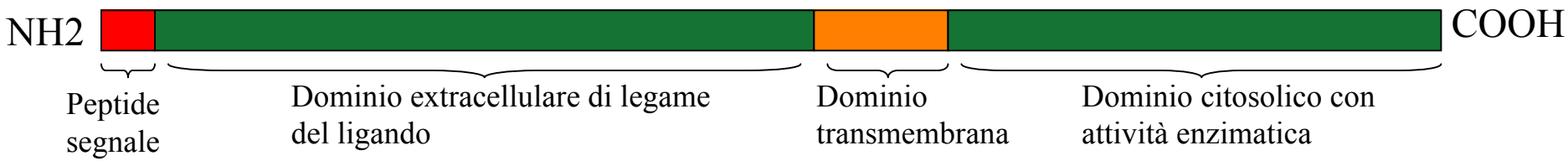
indirizzamento di una proteina di secrezione: RE --> Golgi --> vescicola di secrezione --> spazio



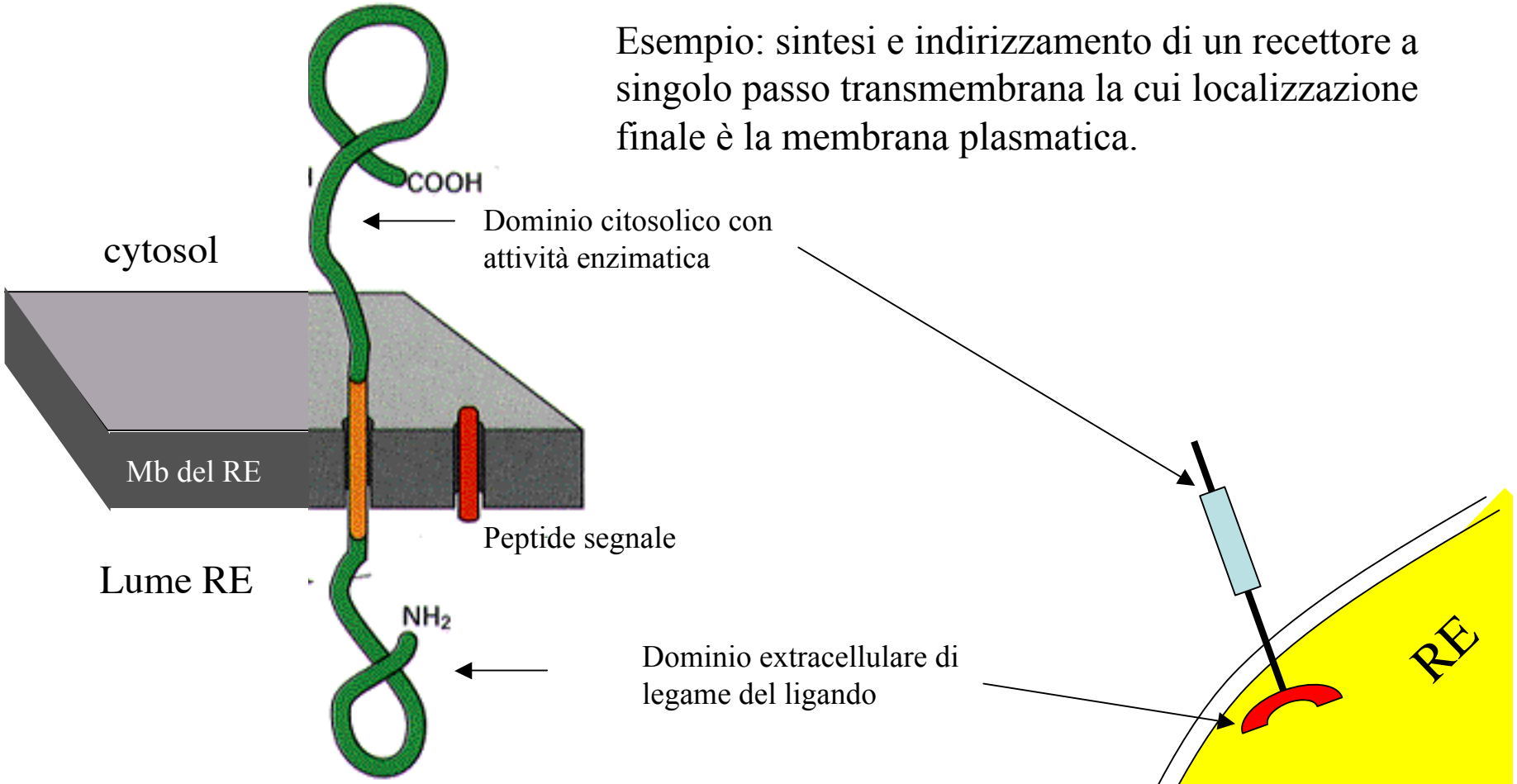
Proteine transmembrana: Inserimento co-traduzionale nel doppio strato fosfolipidico

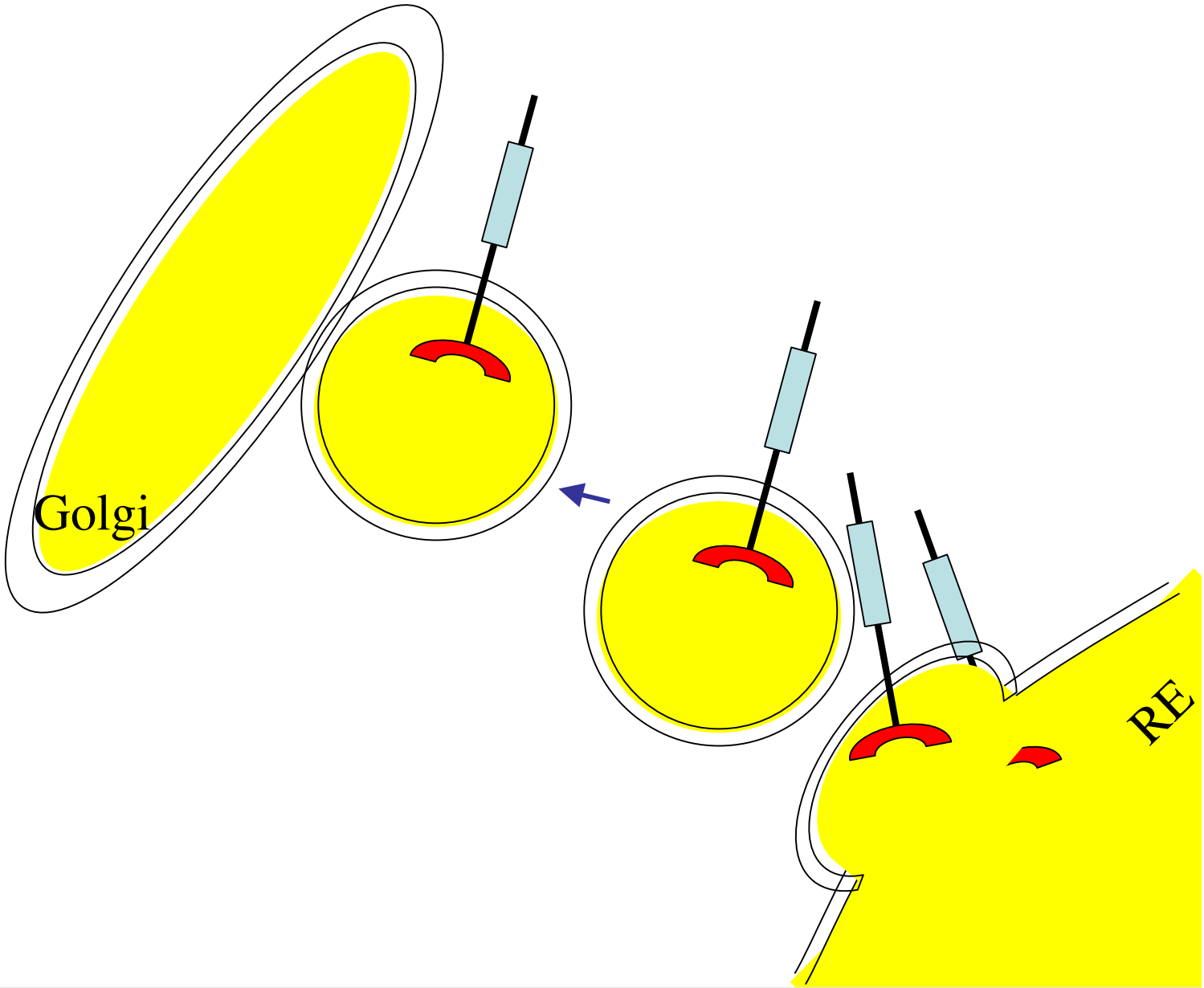


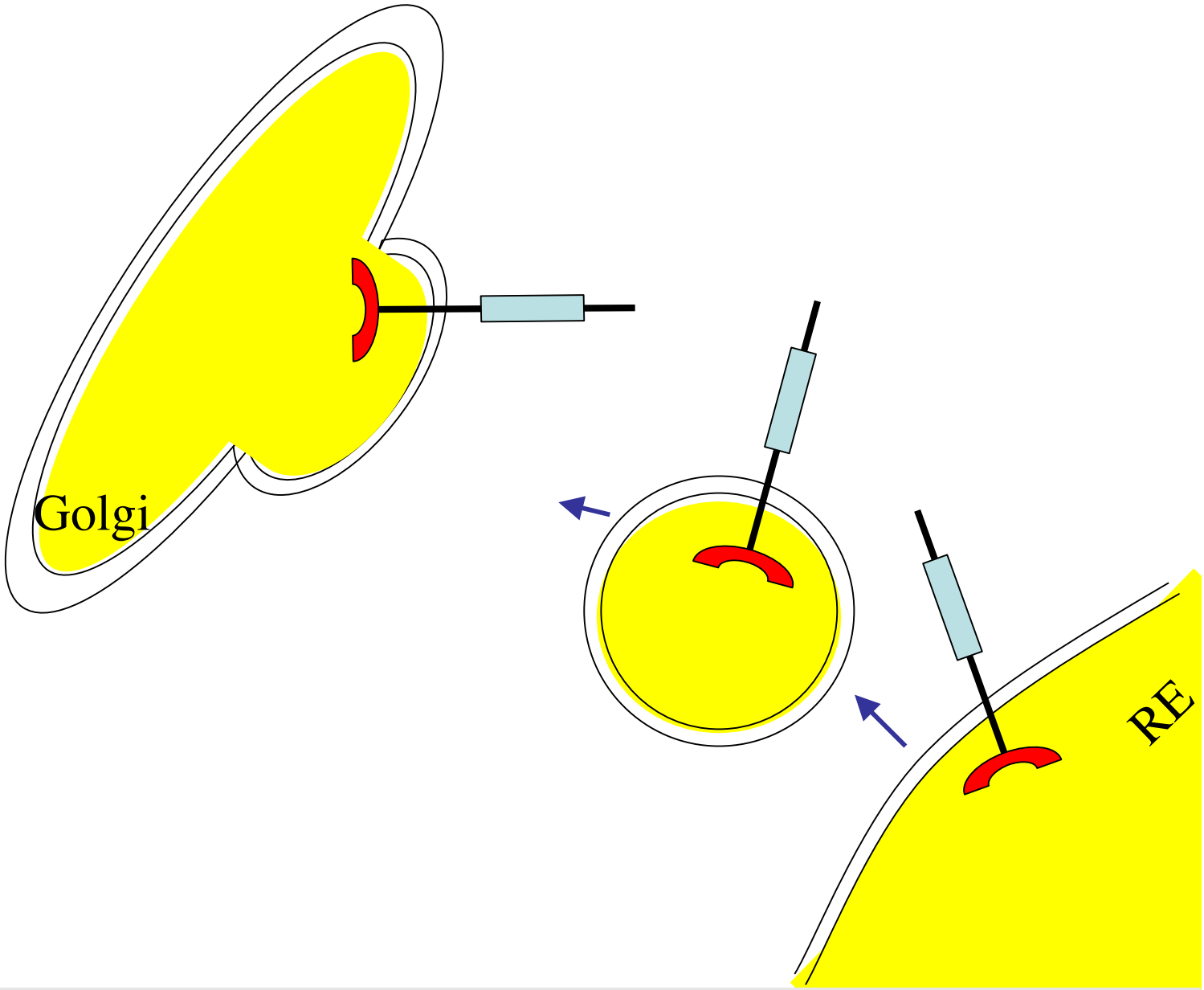
Nota: in questo schema per semplicità non sono più stati rappresentati mRNA e ribosoma.

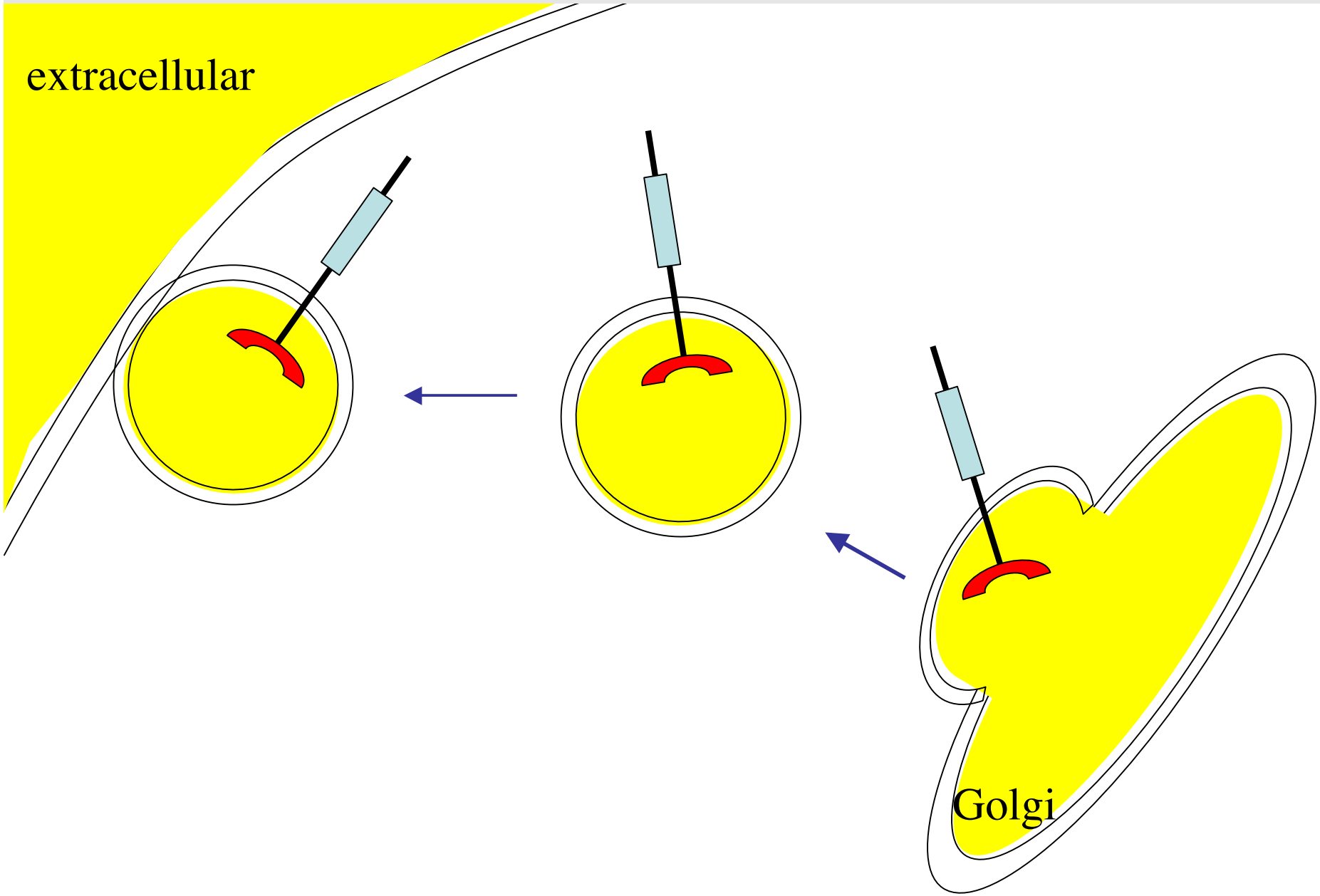


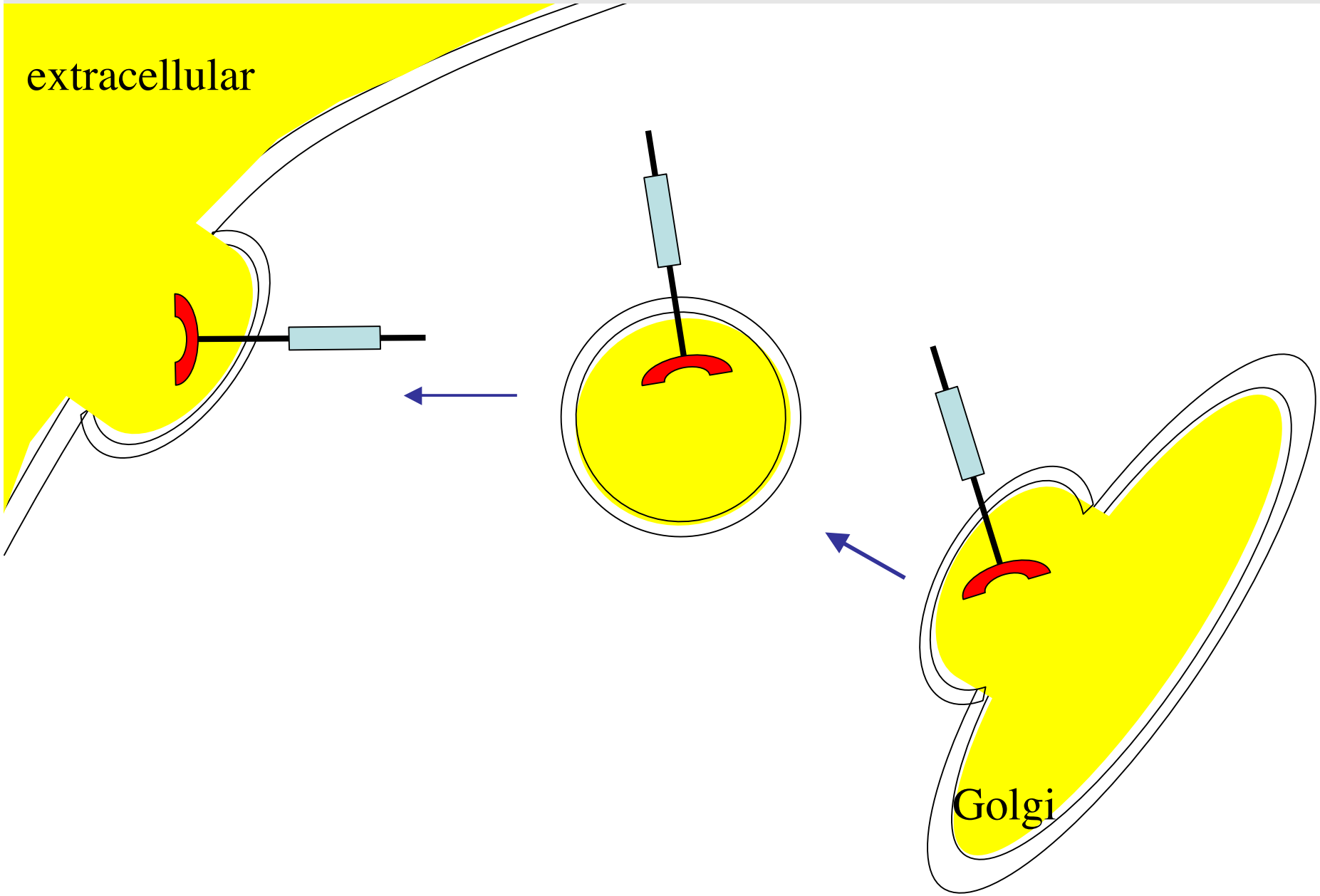
Esempio: sintesi e indirizzamento di un recettore a singolo passo transmembrana la cui localizzazione finale è la membrana plasmatica.

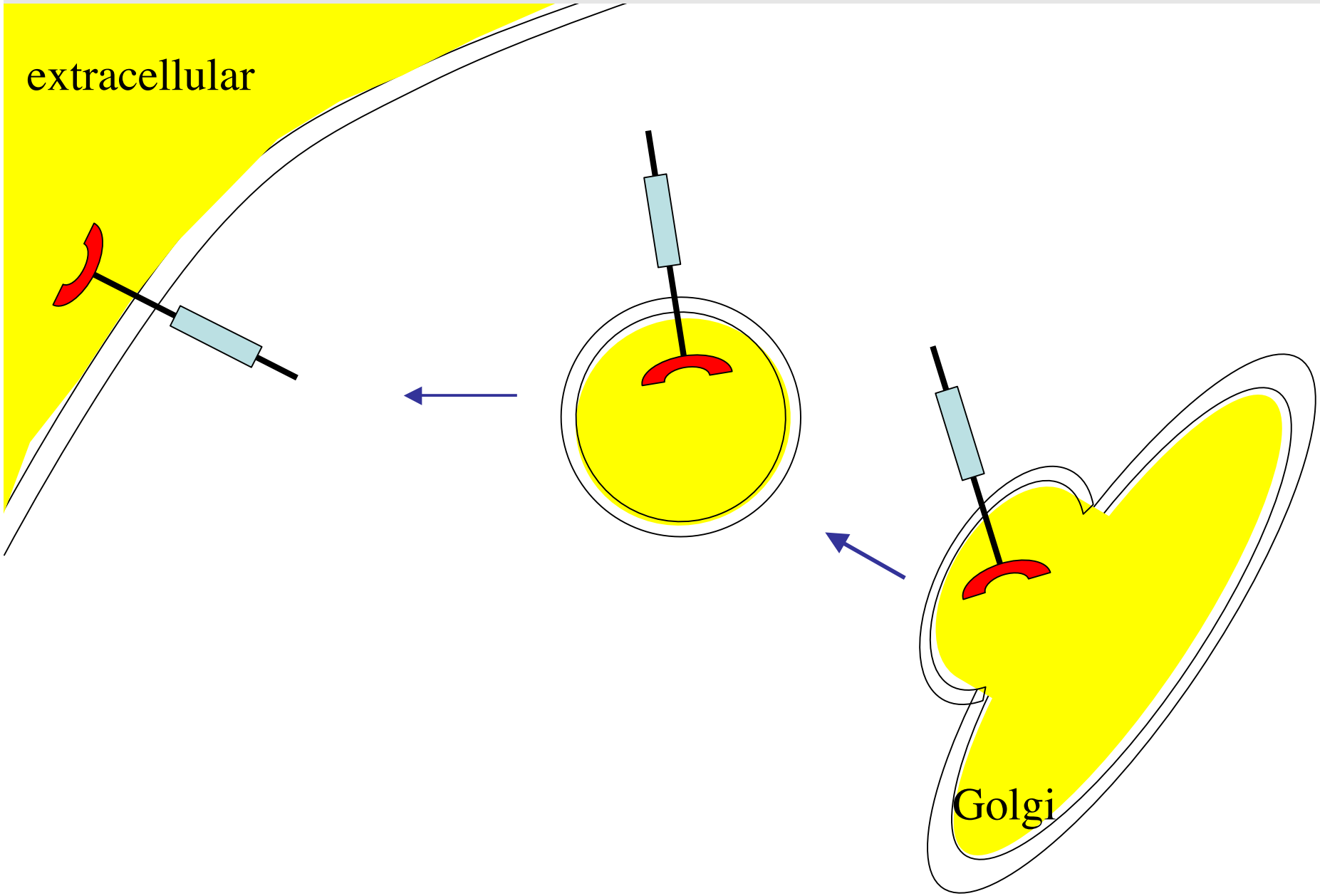












Spiegare la sintesi e lo indirizzamento del recettore metabotropico al glutamato?

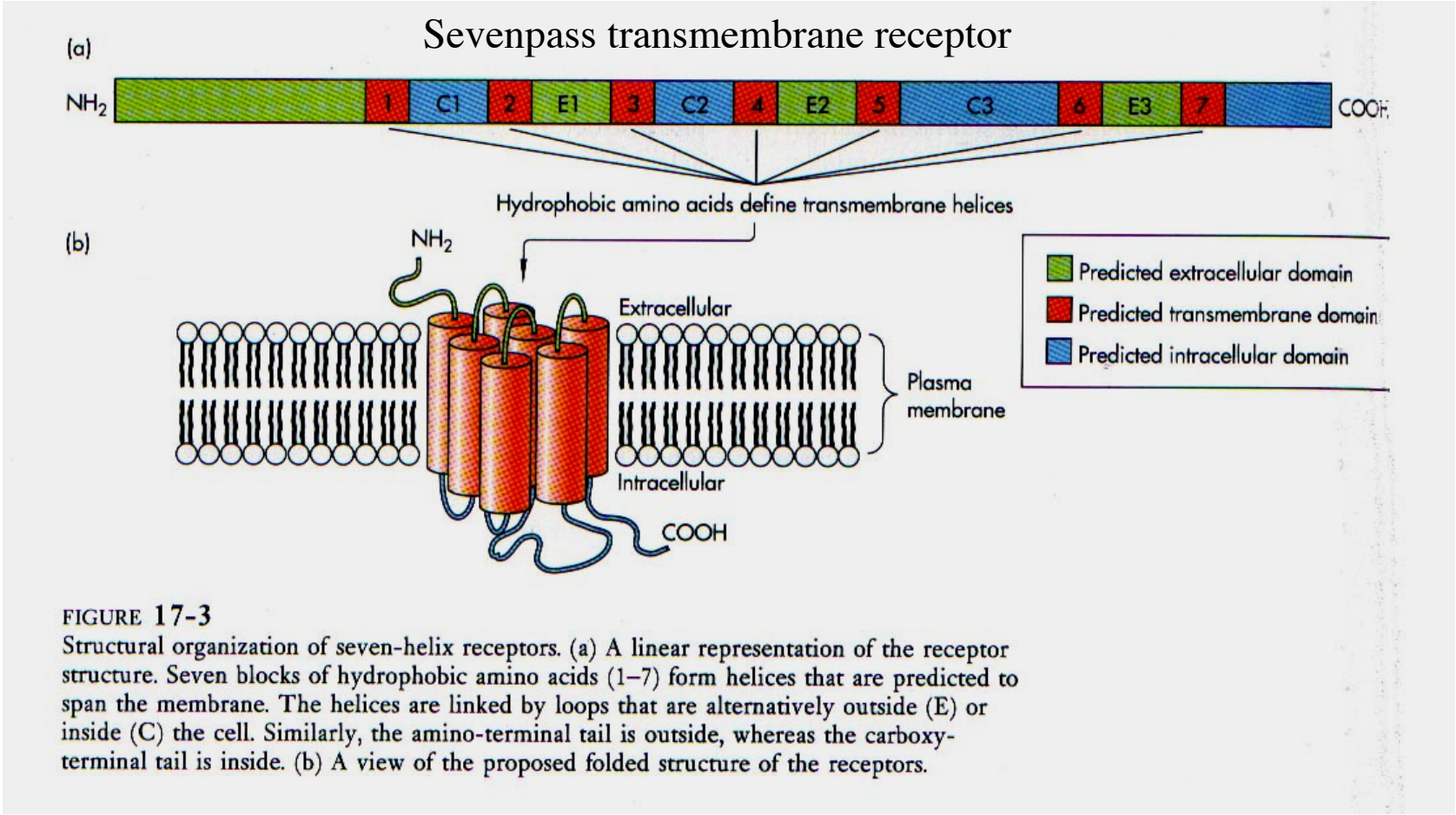
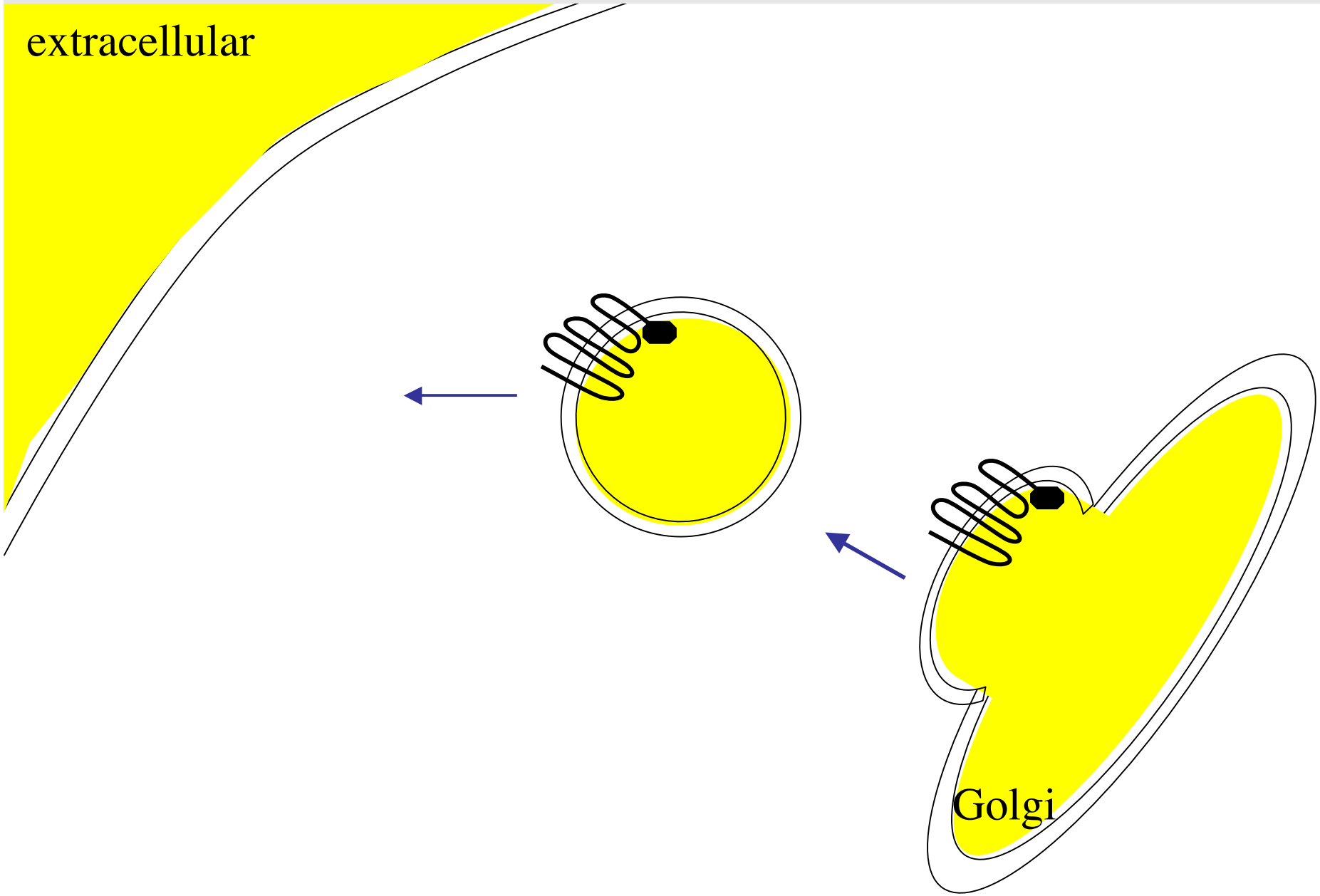
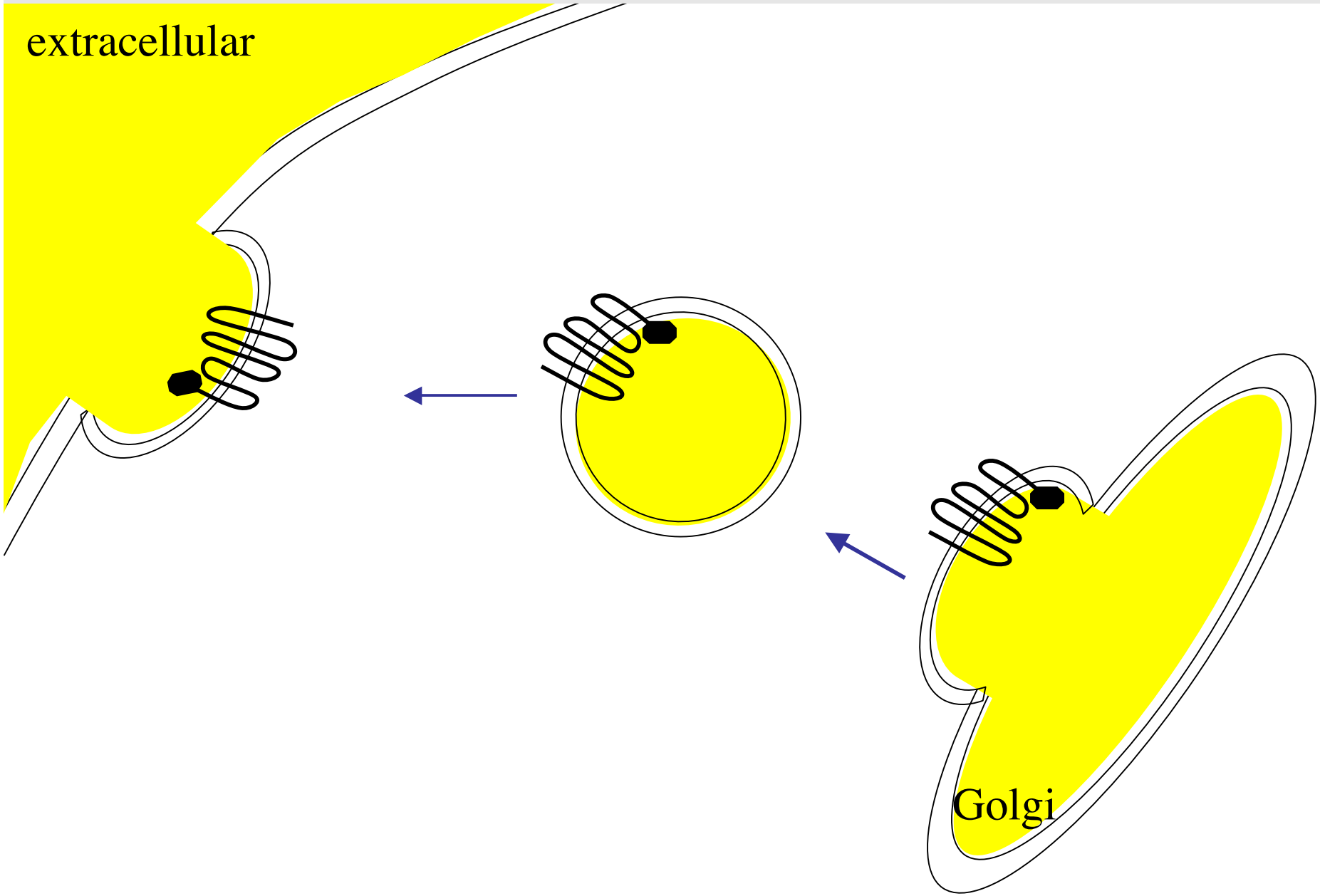
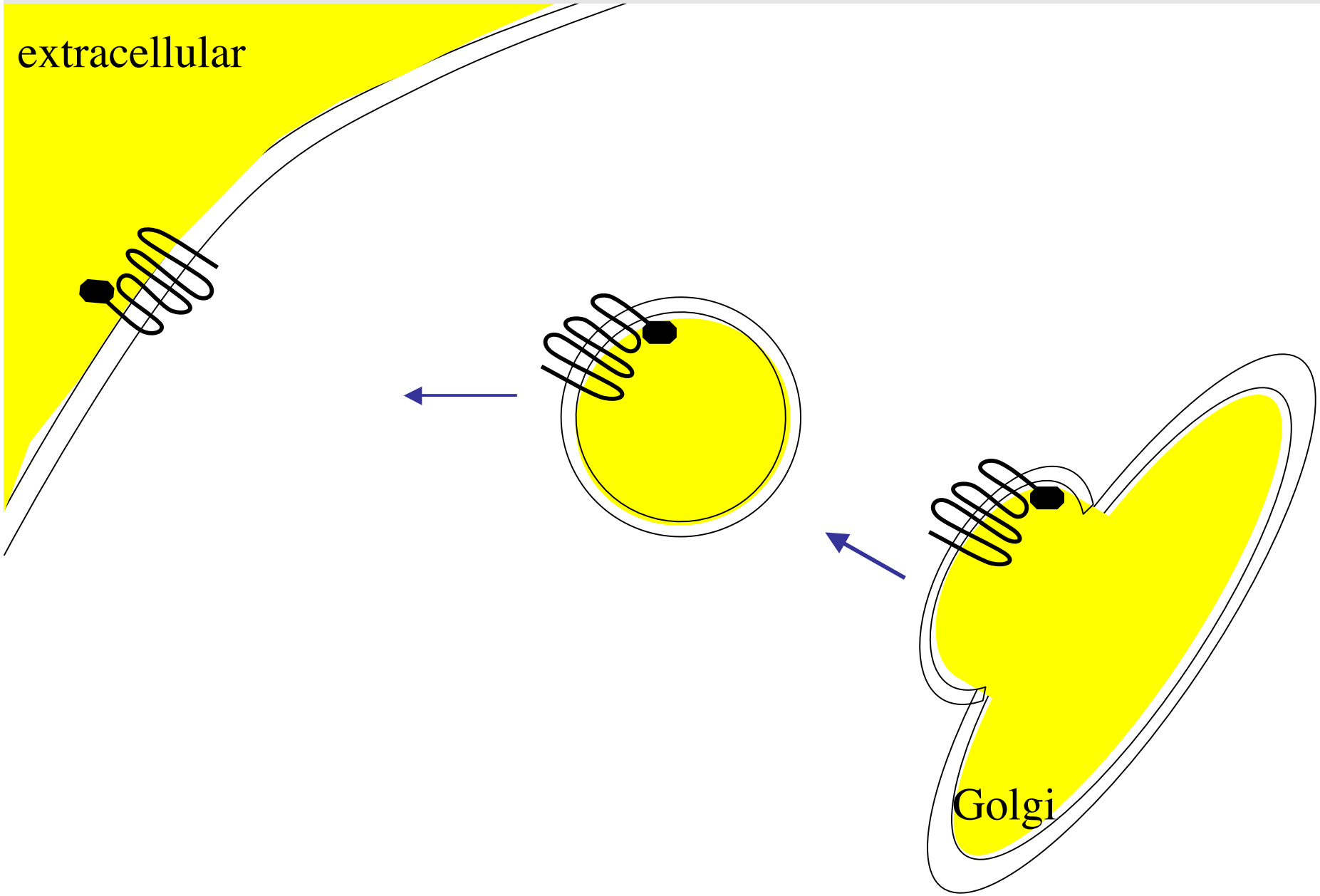


FIGURE 17-3
Structural organization of seven-helix receptors. (a) A linear representation of the receptor structure. Seven blocks of hydrophobic amino acids (1–7) form helices that are predicted to span the membrane. The helices are linked by loops that are alternatively outside (E) or inside (C) the cell. Similarly, the amino-terminal tail is outside, whereas the carboxy-terminal tail is inside. (b) A view of the proposed folded structure of the receptors.

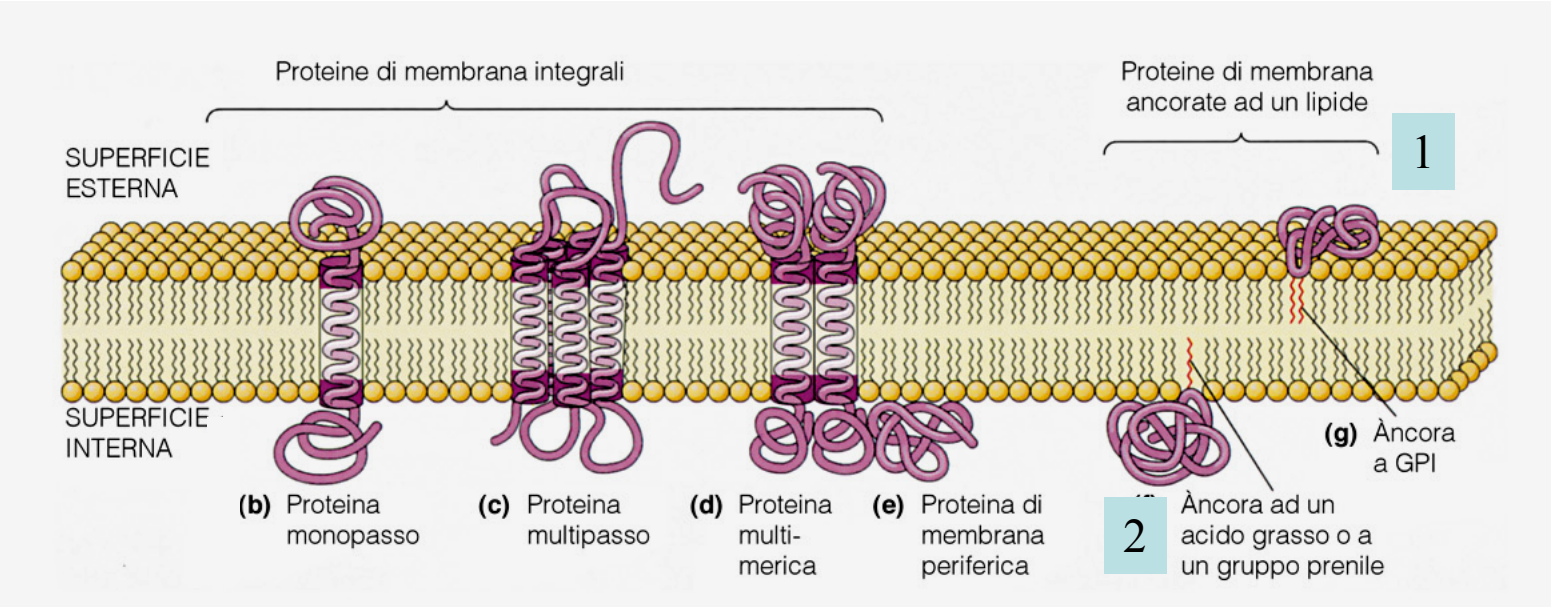






Proteine di membrana (ma non transmembrana) ancorate a lipidi

1 Proteine extracellulari ancorate a lipidi sono sintetizzate da ribosomi associati al RE, traslocate in modo co-traduzionale nel lume del RER, agganciate a GPI, trasportate da vescicole attraverso il Golgi fino alla membrana plasmatica



2 Proteine intracellulari (citoplasmatiche) ancorate a lipidi sono sintetizzate da ribosomi liberi, ancorate alla membrana del RE sul lato citoplasmatico e trasportate fino alla membrana plasmatica associate a vescicole (vedi diapo successiva)