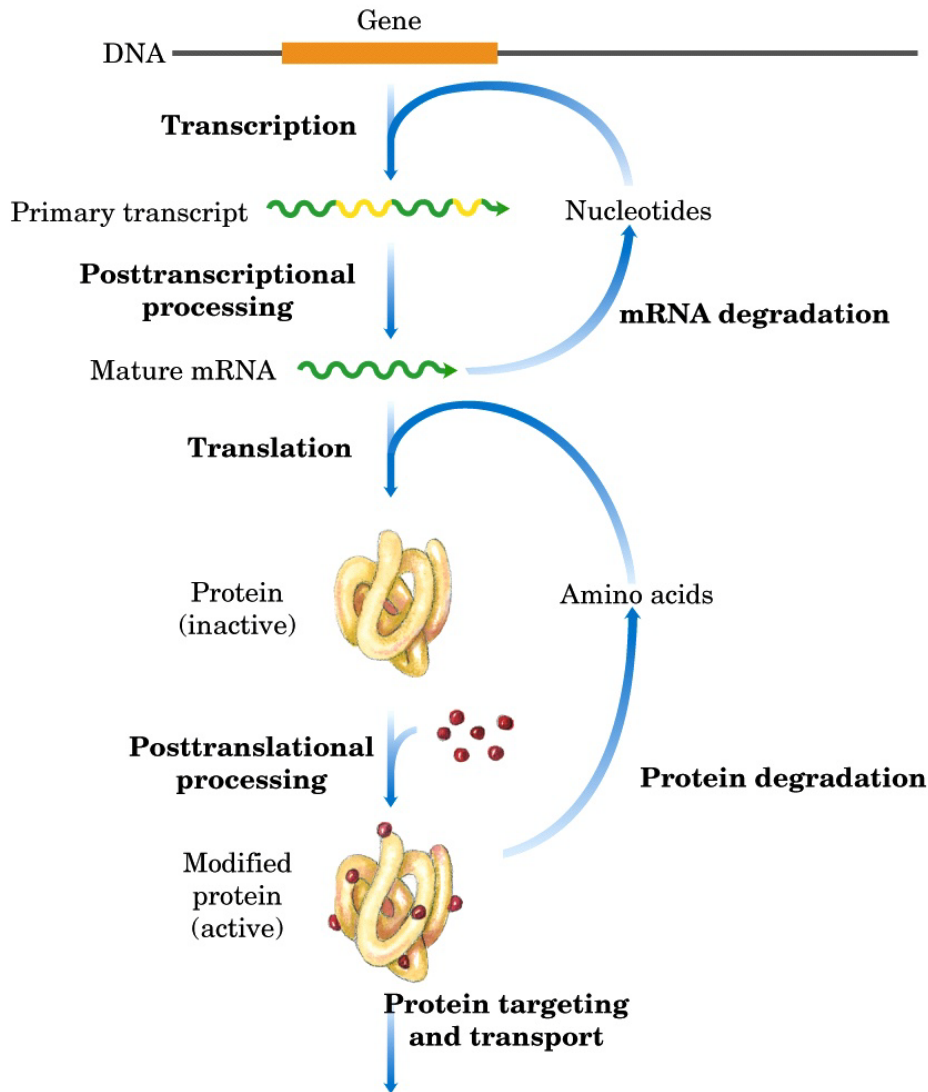


Stufen der Genexpression



Wie wird die Basensequenz der RNA in die Aminosäuresequenz der Proteine umgewandelt ?

Posttranslationale Prozesse

Sobald das Polypeptid aus dem Ribosom austritt steht es vor 2 wichtigen Entscheidungen:

Wie erreicht es die korrekte Faltung ?

Wie kommt es zu seinem Bestimmungsort in der Zelle ?

Ausgangstunnel des Ribosoms erlaubt noch keine Faltung (1,5 nm)-
Faltung beginnt aber noch bevor Translation abgeschlossen ist.

Allgemein: Proteinkern hydrophob, Proteinaussenschicht hydrophil.

Einnahme der nativen Konformation durch
Wasserstoffbrückenbildung, Van der Waals Kräfte, Ionische
Bindungen, hydrophobe Interaktionen.

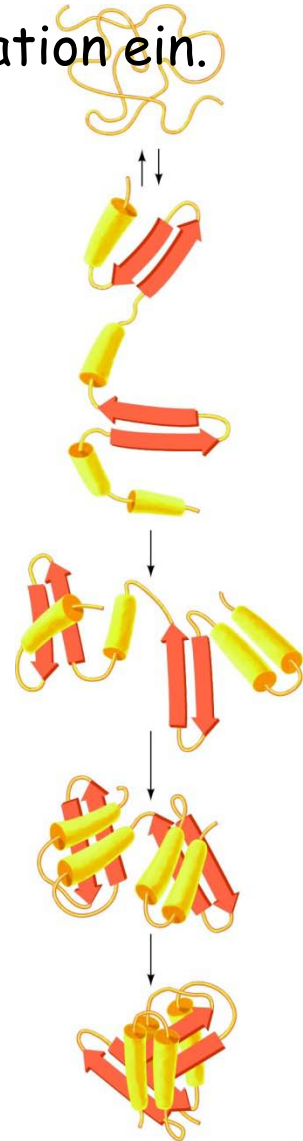
Proteinfaltung

Proteine nehmen innerhalb weniger Sekunden native Konformation ein. Gerichteter Mechanismus und nicht wahlloses Ausprobieren. Stabilität steigt an. Beginnt mit Faltung v. α -helices + β -Faltblatt (ms).

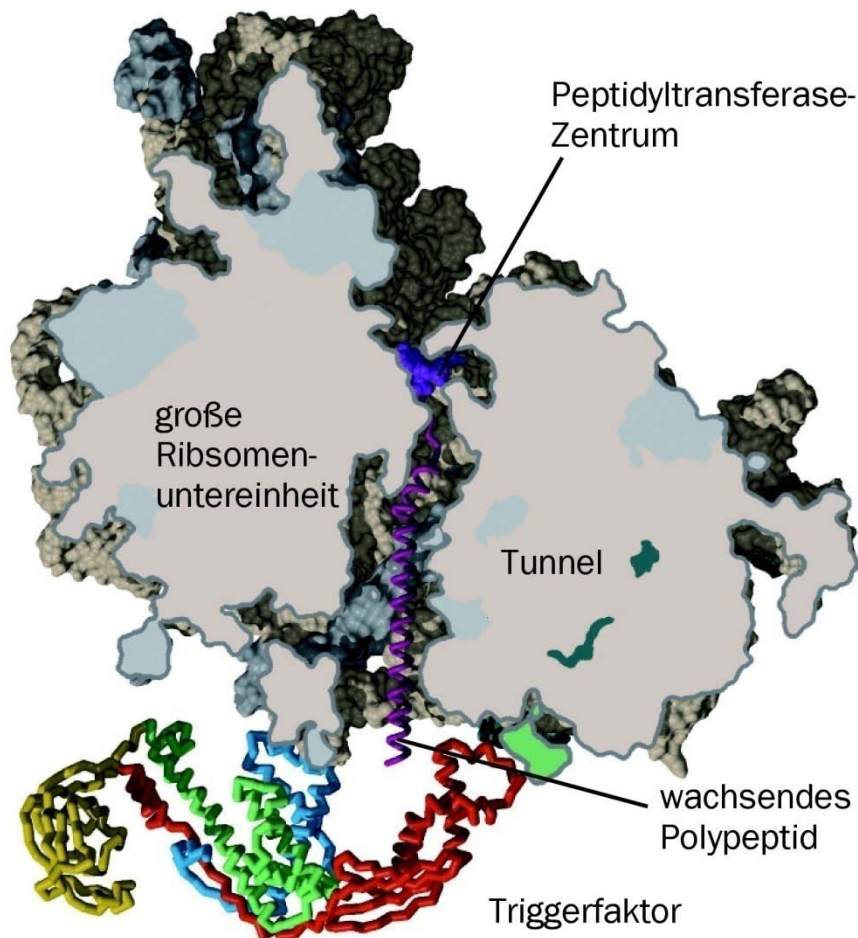
Foldasen: geschwindigkeitslimitierende Schritt.
kovalente Veränderungen:
Disulfidbrückenbildung und
Prolinisomerisierung.

Bsp.: Proteindisulfidisomerase (Sekretionsweg)

Molekulare Chaperones: assistierende Proteine.
binden kurzzeitig bzw vorübergehend, nicht
kovalent an das nicht-native Protein, verhindern
nicht produktive Proteininteraktionen
exponierter hydrophober Segmente und
ermöglichen so korrektes Falten, ohne dass es
zu Aggregationen oder Proteinausfällung kommt.



Ribosomenassoziierte Chaperones



© 2010 Wiley-VCH, Weinheim
Voet - Lehrbuch der Biochemie
ISBN: 978-3-527-32667-9 Fig-27-38

Triggerfactor (Prok.)
assoziiert mit L23, das am
Ausgang des Peptidtunnels
sitzt.

Erkennt kurze hydrophobe
Proteinabschnitte mit
eigener hydrophober
konkaven Seite.

DnaK (Hsp70 Chaperon)
DnaJ (Hsp40 Cochaperon)

GroEL, GroES: Faltung nach
Beendigung der
Translation.

Ziel: Abschirmung der hydrophoben Abschnitte, damit sie keine
Fehlfaltung bzw Aggregation verursachen.

Chaperones

Viele molekulare Chaperones wurden ursprünglich als Hitzeschockproteine beschrieben (Heat shock proteins, Hsp), da ihre Synthese bei erhöhter Temp oft zunimmt.

Hsp70 Proteine (helfen auch in der Entfaltung, Sekretionsweg)

Chaperonine

Hsp90 Proteine

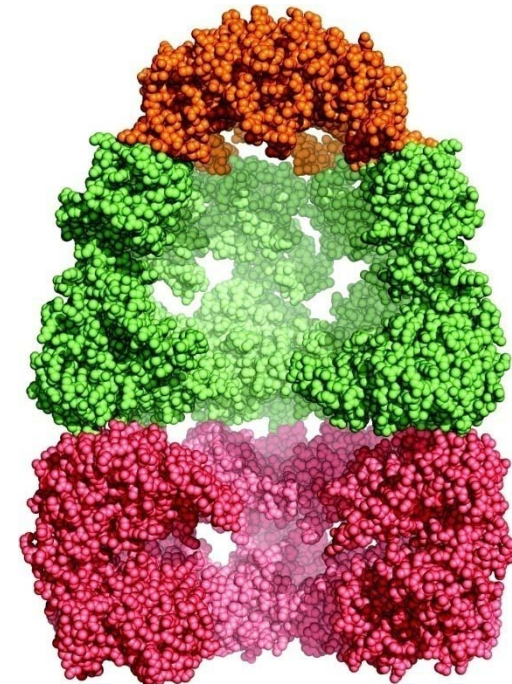
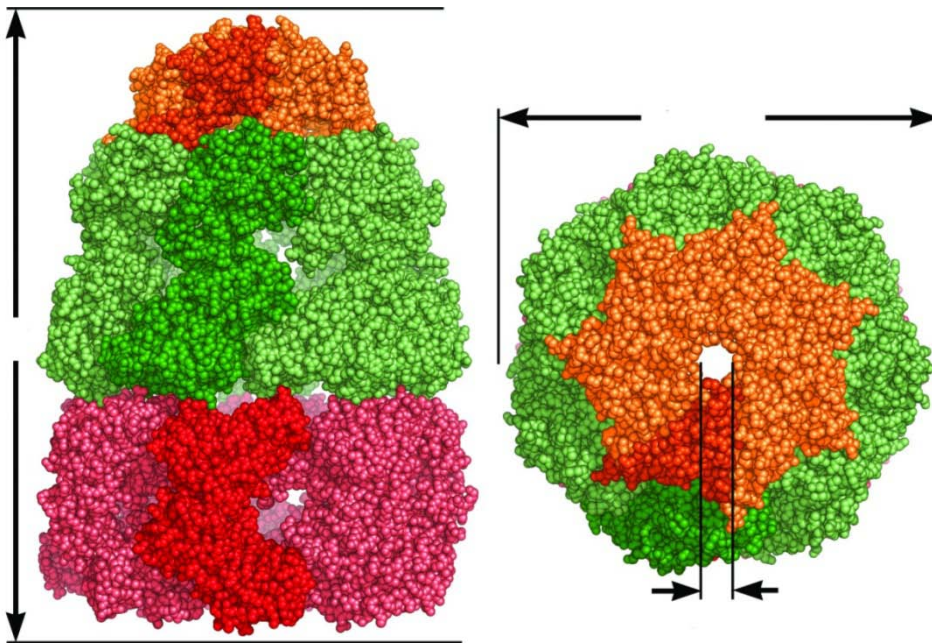
Triggerfaktor.

Sind oft ATPasen, Abnahme der freien Enthalpie liefert Energie für Bindungs- und Freisetzyklus der Chaperones.

E. coli GroEL/ES Chaperonin

Chaperonine bestehen aus 2 versch. UE, GroEL (grün und rot) und GroES (orange), pro Ring/Kappe aus je 7 UE.

Hohlraum von 4.5 nm, kann durch ATP Hydrolyse vergrößert werden und max. ein 70 kDa Protein aufnehmen.



GroEL/ES Chaperonin Reaktionszyklus

GroEL Ring (ATP gebunden)
nimmt ungefaltetes Protein
auf (nur hydrophobe)

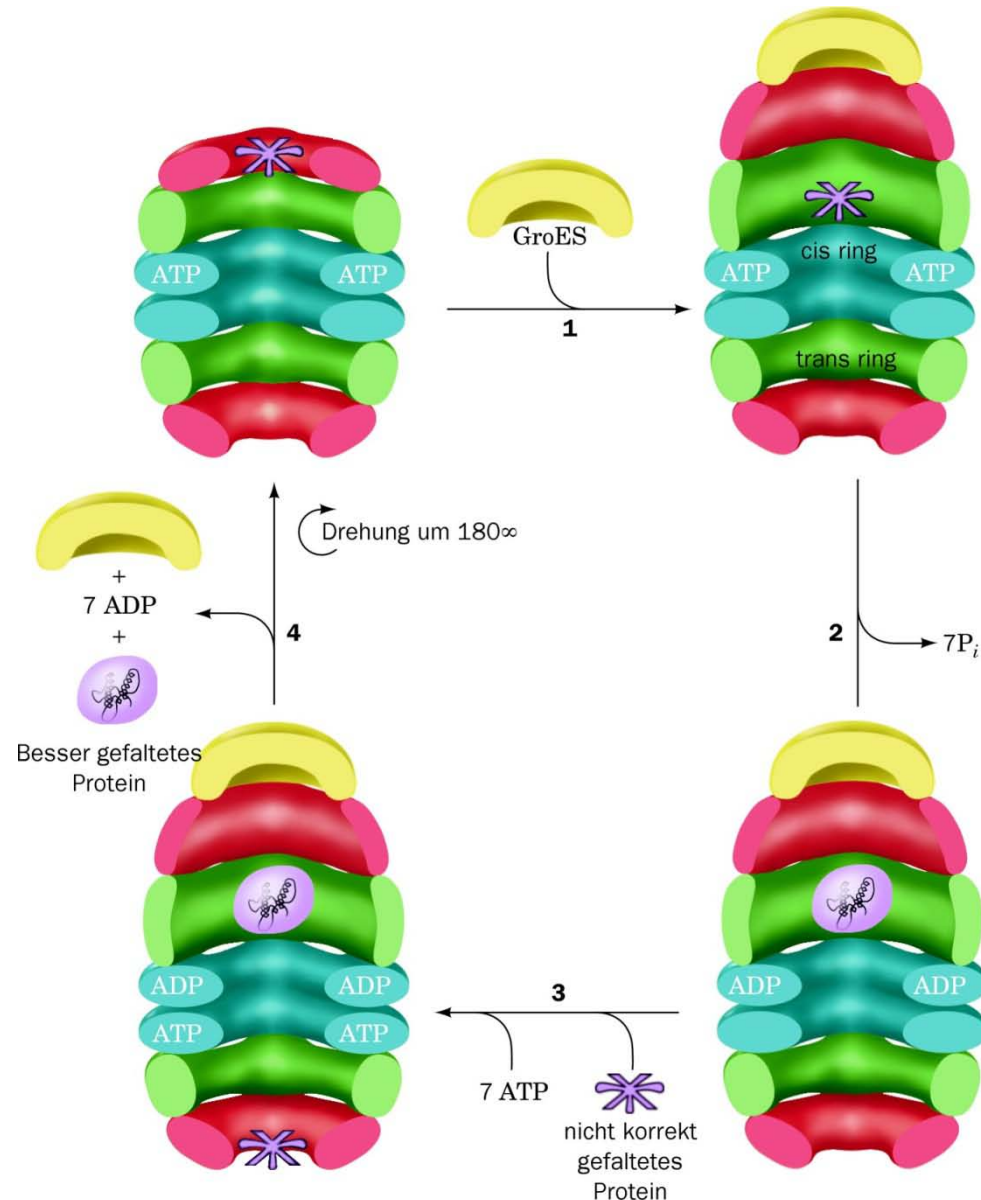
GroES Kappe verschließt,
führt zur ATP Hydrolyse,
Konformationsänderung,
hydrophobe Stellen werden
maskiert.

Protein wird freigesetzt um
sich zu falten, Hohlraum
ist nun hydrophil, Dehnung
des Proteins, ca 10 sec
dauert ATP Hydrolyse

2tes Protein bindet an trans
Ring.

Durchschnitt: 24 Faltungs-
cyclen, 168 ATPs

(Euk: Chaperonin TRiC)



Post-translationale Modifikationen

Table 1.7 Types of post-translational modifications that polypeptides may undergo. Refer to text for additional details

Modification	Example
Proteolytic processing	Various proteins become biologically active only upon their proteolytic cleavage (e.g. some blood factors)
Glycosylation	For some proteins glycosylation can increase solubility, influence biological half life and/or biological activity
Phosphorylation	Influences/regulates biological activity of various polypeptide hormones
Acetylation	Function unclear
Acylation	May help some polypeptides interact with/anchor in biological membranes
Amidation	Influences biological activity/stability of some polypeptides
Sulfation	Influences biological activity of some neuropeptides and the proteolytic processing of some polypeptides
Hydroxylation	Important to the structural assembly of certain proteins
γ -Carboxyglutamate formation	Important in allowing some blood proteins bind calcium
ADP-ribosylation	Regulates biological activity of various proteins
Disulfide bond formation	Helps stabilize conformation of some proteins

Ubiquitinierung:
76 AA
Ubiquitinrest
Abbau
Proteasome

Posttranslationale Prozesse: Faltung und Prozessierung

Amino- und Carboxy-terminale Modifikationen:

N-Formylmethionin (Prok), Methionin (Euk) werden oft abgespalten + zusätzliche AA an beiden Enden.

N-Acetylierung des N-Terminus in 50% aller eukaryontischen Proteine: N-Acetyltransferase

Prostethische Gruppen: Biotinmolekül der Acetyl-CoA Carboxylase.

Häm Gruppe des Cytochrom c.

Disulfidbrückenbildung: zw Cysteinen. Inter oder intramolekular. V.a. bei extrazellulären Proteinen

Proteolytische Prozessierung

Inaktive Vorstufen:
Präproproteine.

Proteine mit Signalpeptid
Prä(pro)proteine für
Export aus
Cytoplasma/Zelle.

Zusätzliche Proteolyse
während der Reifung.

Bsp.: Insulin

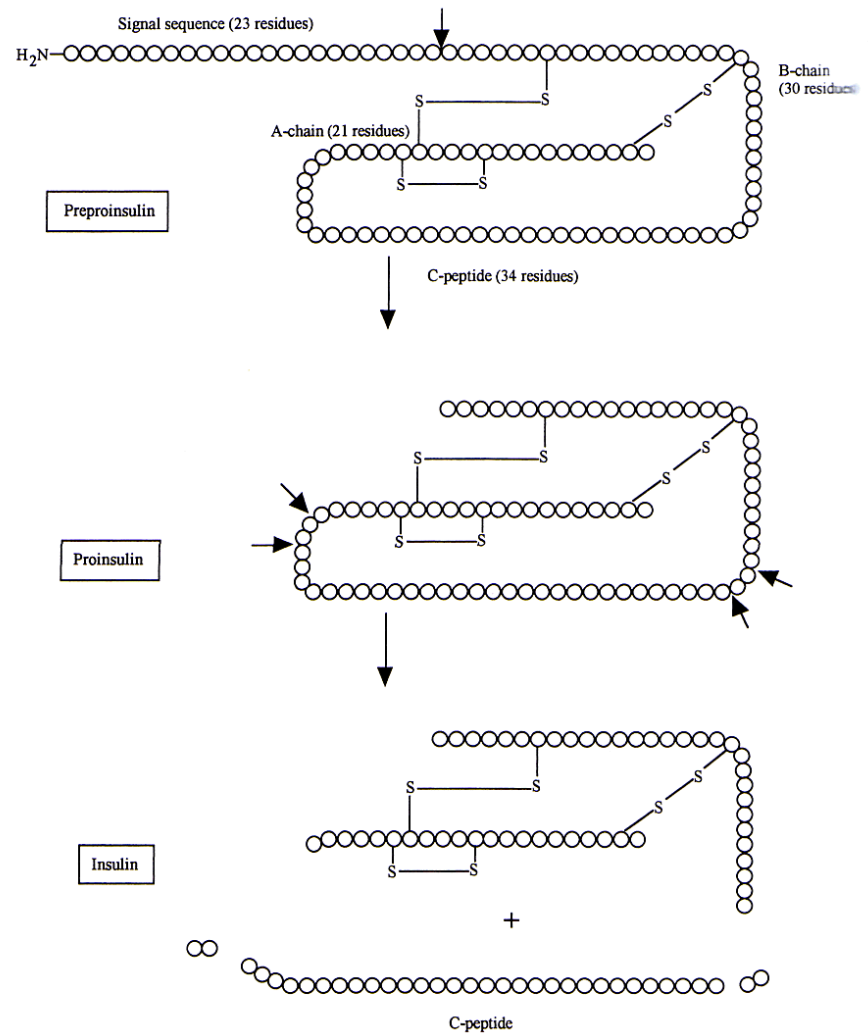
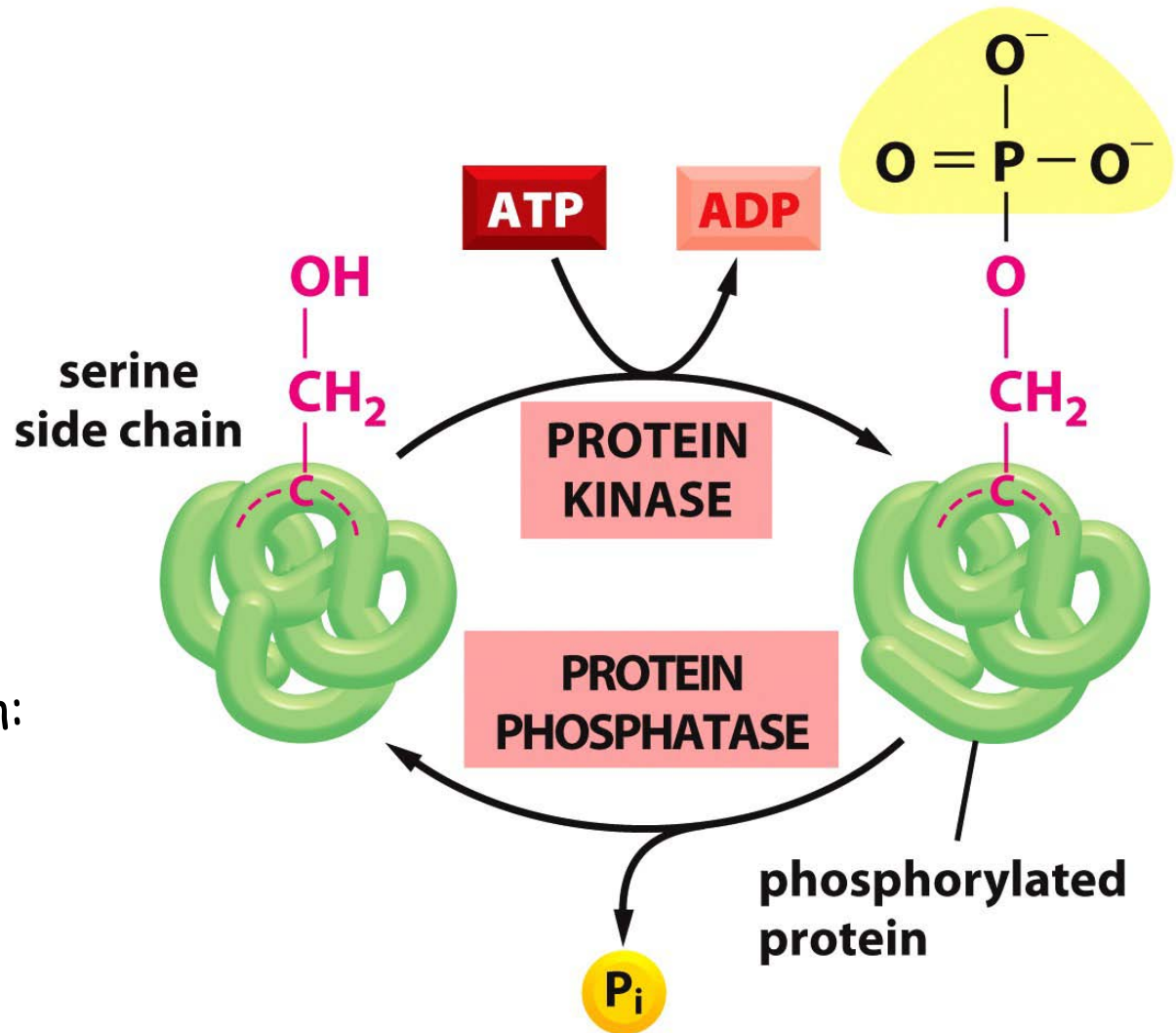


Figure 7.1 Synthesis of human insulin from preproinsulin. The initial proteolytic event involves removal of a 23 amino acid signal sequence from the amino terminal end of preproinsulin, thus yielding proinsulin. Proinsulin is converted into insulin by additional proteolytic events, resulting in the generation not only of insulin but also a 30 amino acid sequence (termed C or connecting peptide) and two dipeptide moieties.

Phosphorylierung

Proteinphosphorylierung
kann die Enzym
bzw Proteinaktivität
regulieren.
1000de Proteine in Euk
betroffen

Phosphorylierungsketten:
Ermöglichen schnelles
Umschalten



Phosphorylierung: Ein/Ausschalten

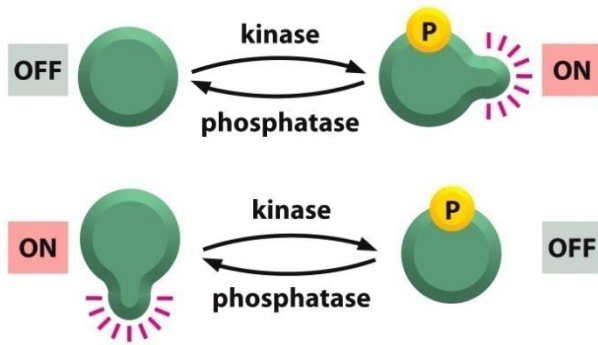
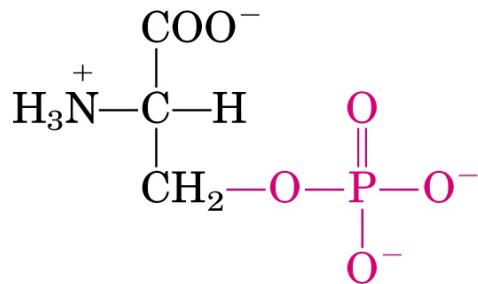
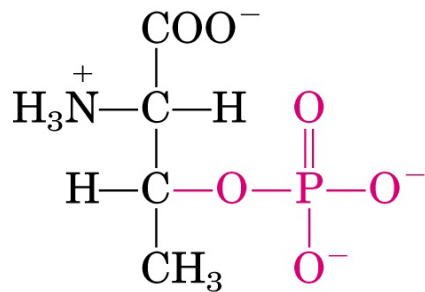


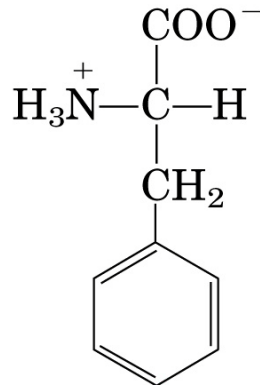
Figure 4-38b
*Essential Cell
Biology* (© Garland
Science 2010)



Phosphoserine



Phosphothreonine



Phosphotyrosine

(a)

Visualisierung des Phosphorylierungs- zustandes in 2 D Gel Analyse

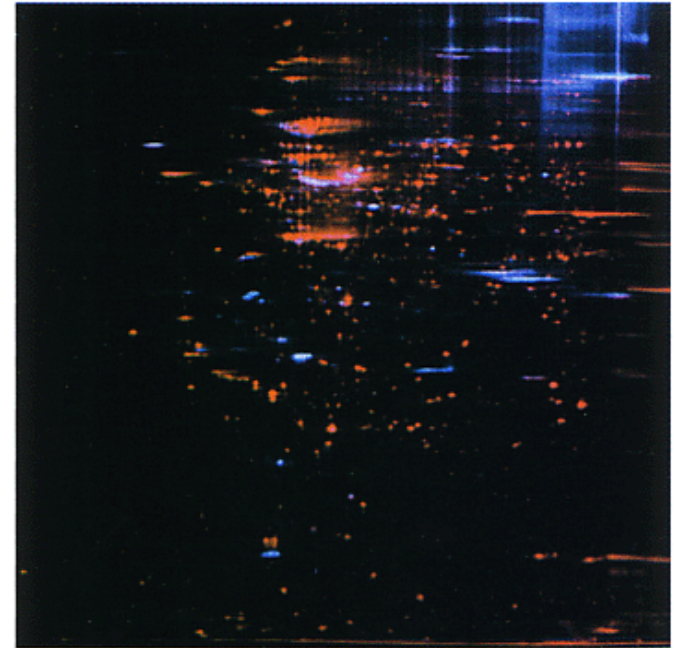
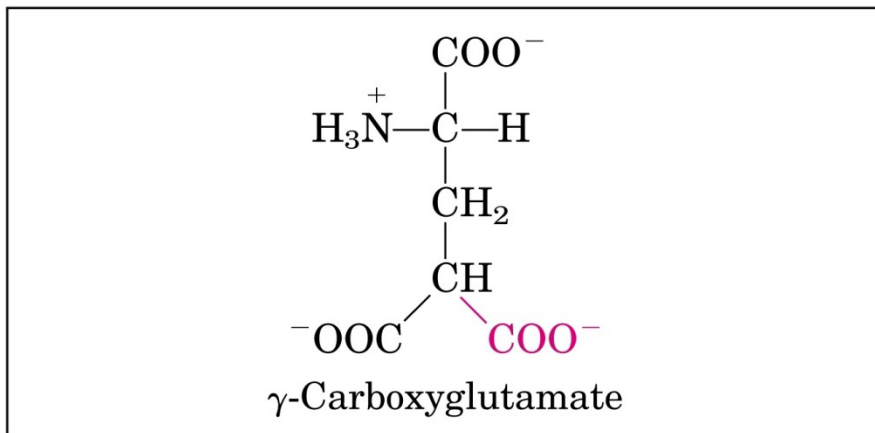


Figure 4. Visualization of total protein and phosphoprotein in a 2-D gel. Proteins from a Jurkat T-cell lymphoma line cell lysate were separated by 2-D gel electrophoresis and stained with Pro-Q Diamond phosphoprotein gel stain (P-33300, P-33301, P-33302, blue) followed by SYPRO Ruby protein gel stain (S-12000, S-12001, S-21900, red). After each dye staining, the gel was imaged on an FLA-3000 laser-based scanner (Fuji). The digital images were acquired using Z3 software (Compugen), then pseudocolored and overlaid.

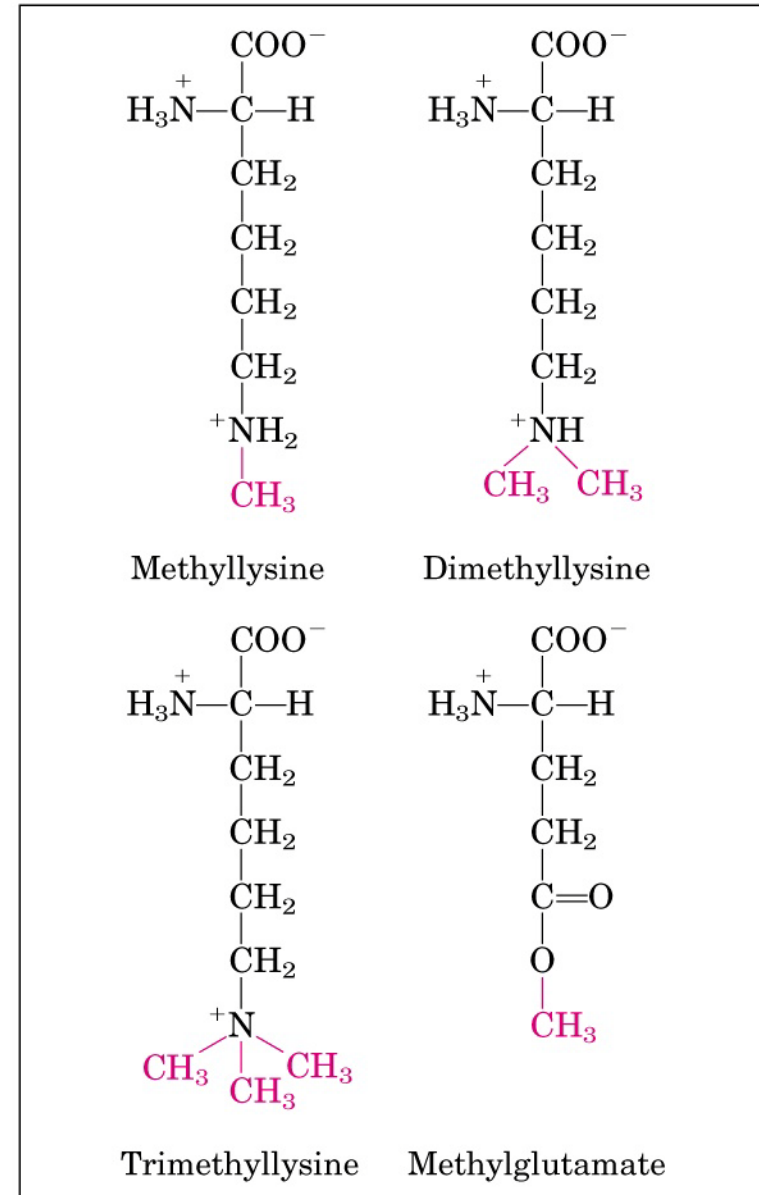
Anheftung von Carboxy und Methylgruppen

Prothrombin: Carboxylgruppen an Glu
zu Ca^{+2} Bindung

Methylierungen:
in einigen Muskelproteine u Cytochrom c
Calmodulin: Trimethyllysine
Glu: um neg. Ladung zu entfernen



(b)



(c)

Glykosylierung

N-Glykosylierung: an Asparagin: Erkennungssequenz: Asn-X-Thr/Ser/Cys; X alles außer Prolin

O-Glykosylierung: Ser/Thr

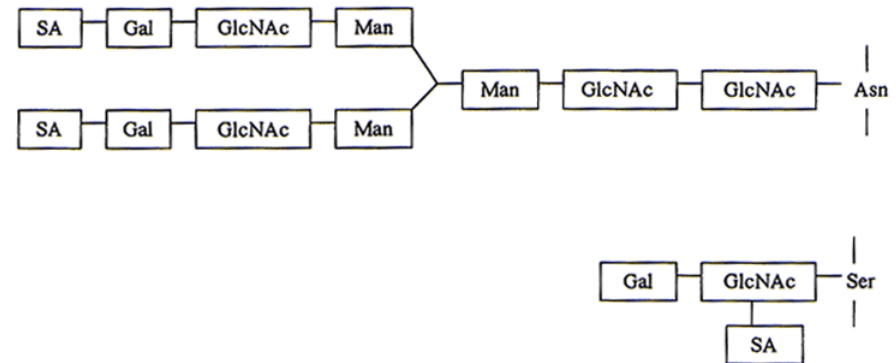
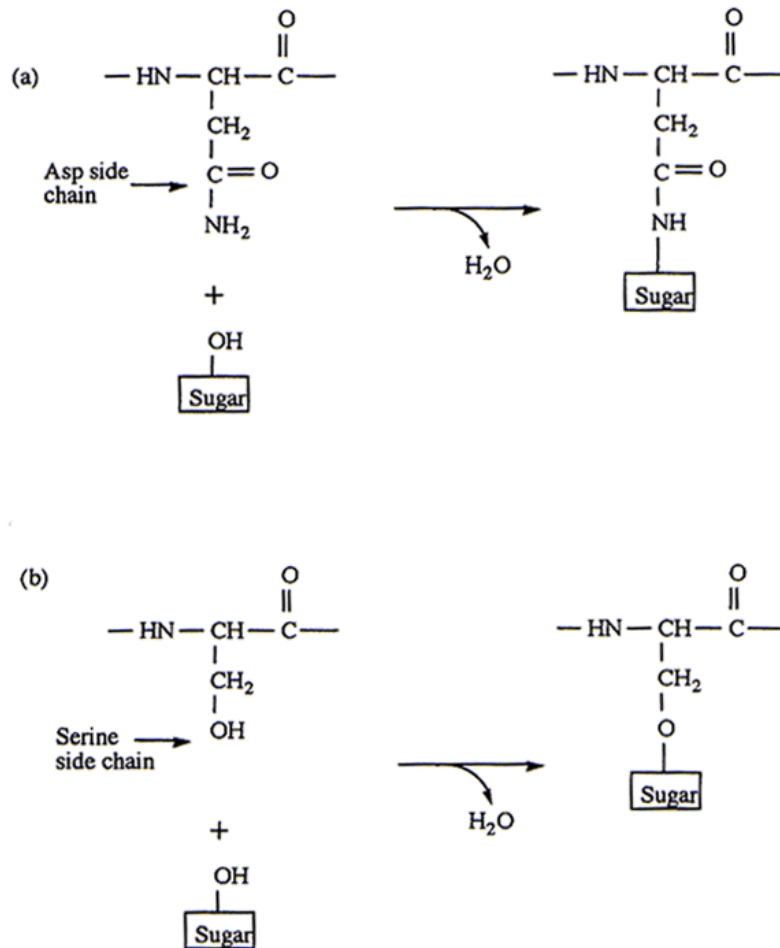


Figure 1.16 Structure of two sample oligosaccharide side chains (one *N*-linked the other *O*-linked) found in glycoproteins. Man, mannose; Gal, galactose; SA, sialic acid; GlcNAc, *N*-acetyl glucosamine; GalNAc, *N*-acetyl galactosamine; Ser, Serine; Asn, asparagine

Figure 1.15 *N*-linked (a) versus *O*-linked (b) glycosylation. 'Sugar' represents an oligosaccharide chain, an example of which is provided in Figure 1.16

Glykosylierung

Erfolgt während oder nach der Proteinsynthese v.a. bei extrazellulären Proteinen in Eukaryonten.

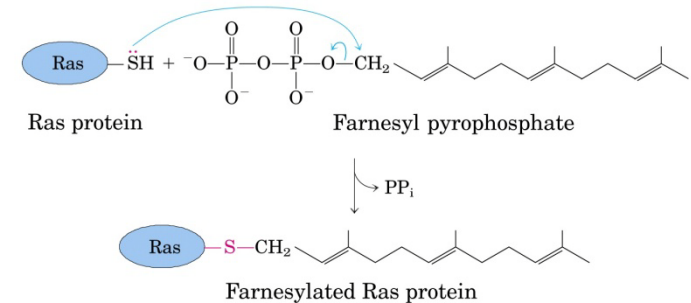
- Targeting/Zielsteuerung einzelner Proteine zu spezifischen zellulären Orten: Lysosomale Enzyme (Phosphorylierte Mannose)
- Recognition/Erkennung: können eine direkte Rolle in der Bindung eines Glykoproteins zu seinem Ligand (zB Cytokin Rezeptoren) spielen.
- Zelloberflächenproteine, die glykosyliert sind spielen auch eine große Rolle in der Zell-Zelladhäsion und in der gegenseitigen Erkennung.
- Konformationsstabilisierung
- Schutz vor Proteolyse
- Löslichkeit
- Veränderung der biologische Halbwertszeit
- Qualitätskontrolle (Sekretionsweg)

Acylierung

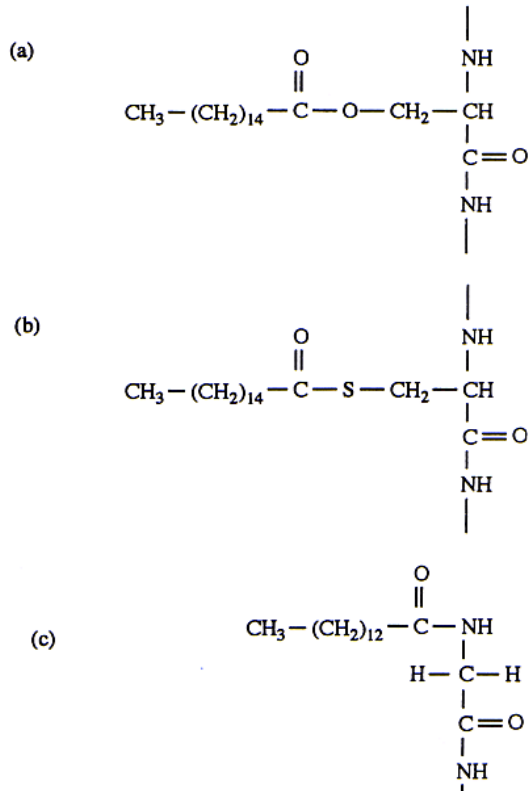
Table 1.8 Representative examples of some acylated proteins. The fatty acid moiety attached as well as the locations of eucaryotic-derived acylated proteins is also listed

Protein	Cellular location	Fatty acid
cAMP-dependent protein kinase	Cytoplasm	Myristic acid
Cytochrome b ₅ reductase	ER and mitochondria	Myristic acid
G _i and G _o α-subunits	Plasma membrane	Myristic acid
Insulin receptor	Plasma membrane	Palmitic acid
Interleukin-1 receptor	Plasma membrane	Palmitic acid
Transferrin receptor	Plasma membrane	Palmitic acid
Rhodopsin	Disc membranes in retina	Palmitic acid
P55 & P28	HIV virus	Myristic acid
VP4	Picorna viruses	Myristic acid
P19 (gag)	HTLVI	Myristic acid
HA	Influenza virus	Palmitic acid
gE	Herpes simplex virus	Palmitic acid

HIV, human immunodeficiency virus; HTLVI, human T lymphotropic virus-1.



Acylierung



Key:

$\text{CH}_3-(\text{CH}_2)_{14}-\text{COOH}$ = Palmitic acid

$\text{CH}_3-(\text{CH}_2)_{12}-\text{COOH}$ = Myristic acid

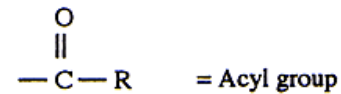
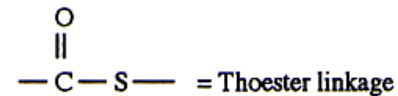
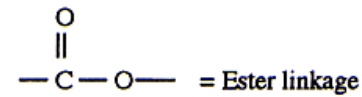
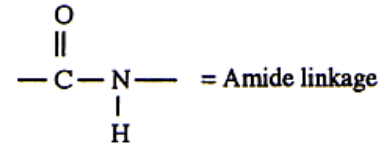


Figure 1.19 Linkage of (a) palmitic acid via an ester bond to a serine residue; (b) palmitic acid via a thioester bond to a cysteine residue; and (c) myristic acid to an N-terminal glycine residue via an amide bond

Protein Targeting

Euk. Zelle bestehen aus versch.
Kompartimenten und Organellen.
Funktionelle Kompartimentierung
durch membranumgebene
Organellen.
Sekretionswege,
Transportprozesse

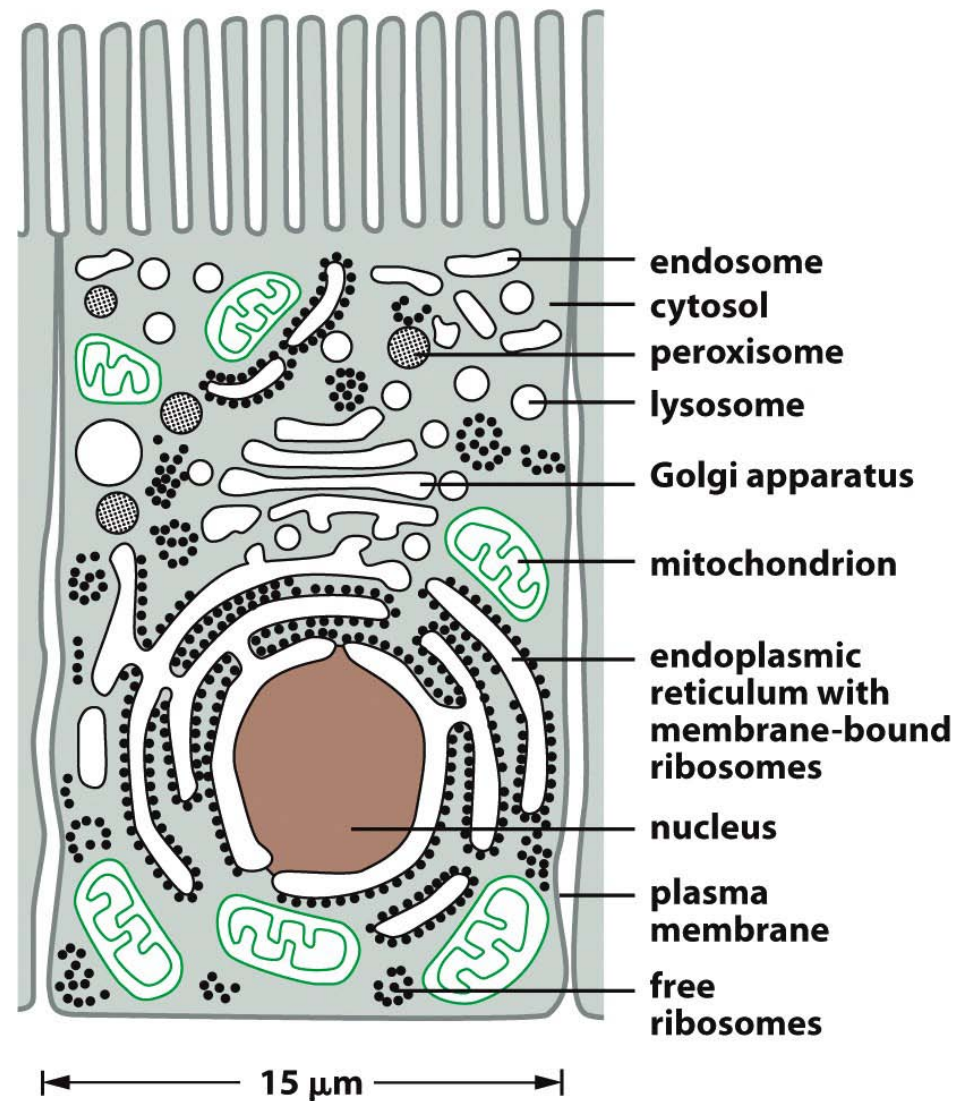


Figure 15-2 *Essential Cell Biology* (© Garland Science 2010)

Hauptfunktionen der membranumgebenen Kompartimente

TABLE 15–1 THE MAIN FUNCTIONS OF THE MEMBRANE-ENCLOSED COMPARTMENTS OF A EUKARYOTIC CELL

COMPARTMENT	MAIN FUNCTION
Cytosol	contains many metabolic pathways (Chapters 3 and 13); protein synthesis (Chapter 7)
Nucleus	contains main genome (Chapter 5); DNA and RNA synthesis (Chapters 6 and 7)
Endoplasmic reticulum (ER)	synthesis of most lipids (Chapter 11); synthesis of proteins for distribution to many organelles and to the plasma membrane (this chapter)
Golgi apparatus	modification, sorting, and packaging of proteins and lipids for either secretion or delivery to another organelle (this chapter)
Lysosomes	intracellular degradation (this chapter)
Endosomes	sorting of endocytosed material (this chapter)
Mitochondria	ATP synthesis by oxidative phosphorylation (Chapter 14)
Chloroplasts (in plant cells)	ATP synthesis and carbon fixation by photosynthesis (Chapter 14)
Peroxisomes	oxidation of toxic molecules

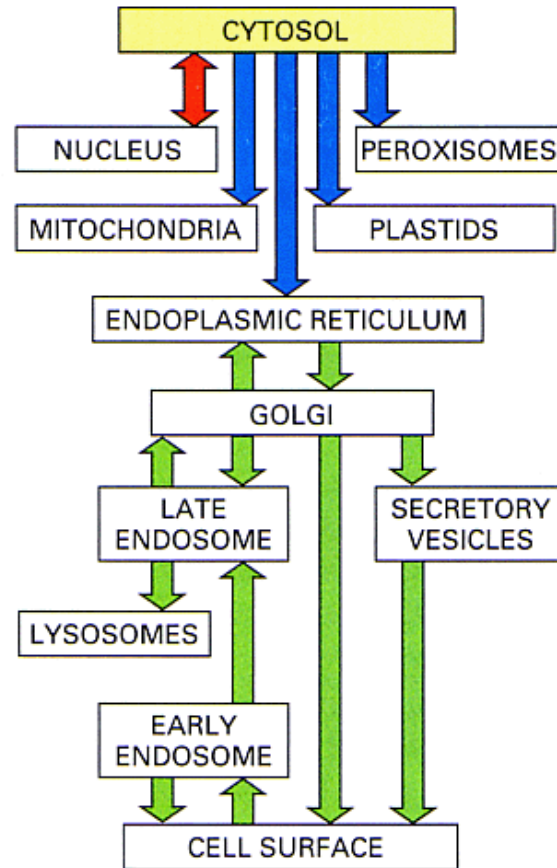
Wege des Proteintransports, Proteinsortierung

3 Möglichkeiten:

Gated Transport

Transmembran
transport

Vesikeltransport



KEY: █ = gated transport
█ = transmembrane transport
█ = vesicular transport

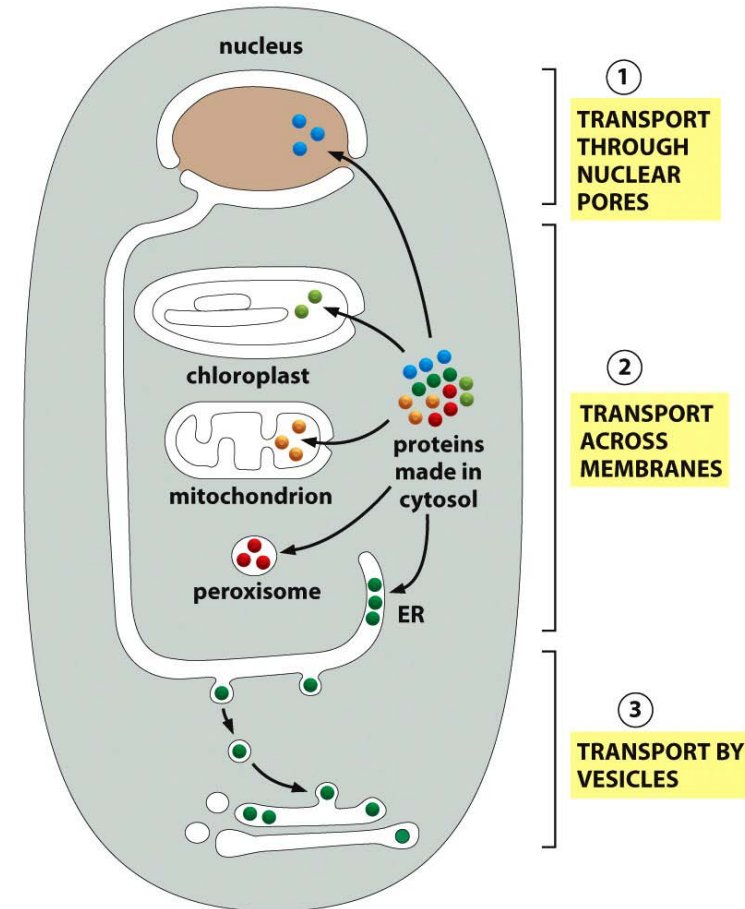
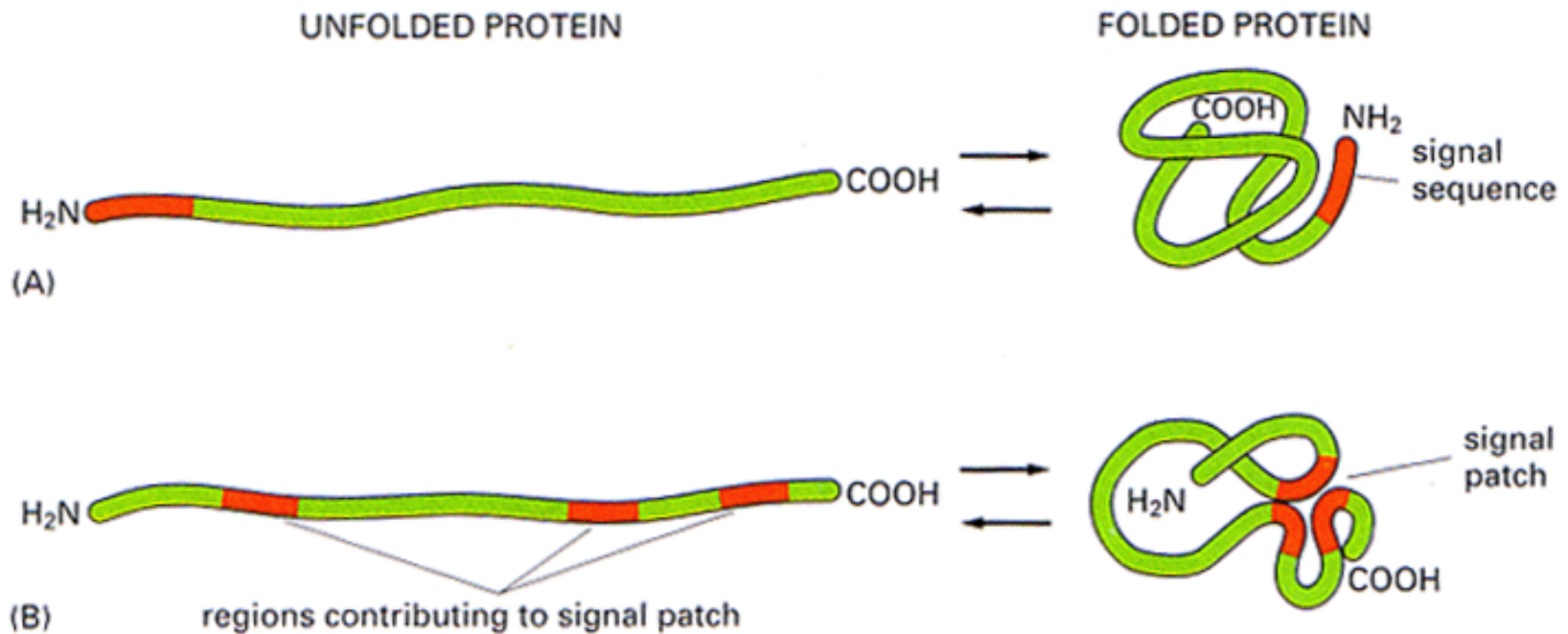


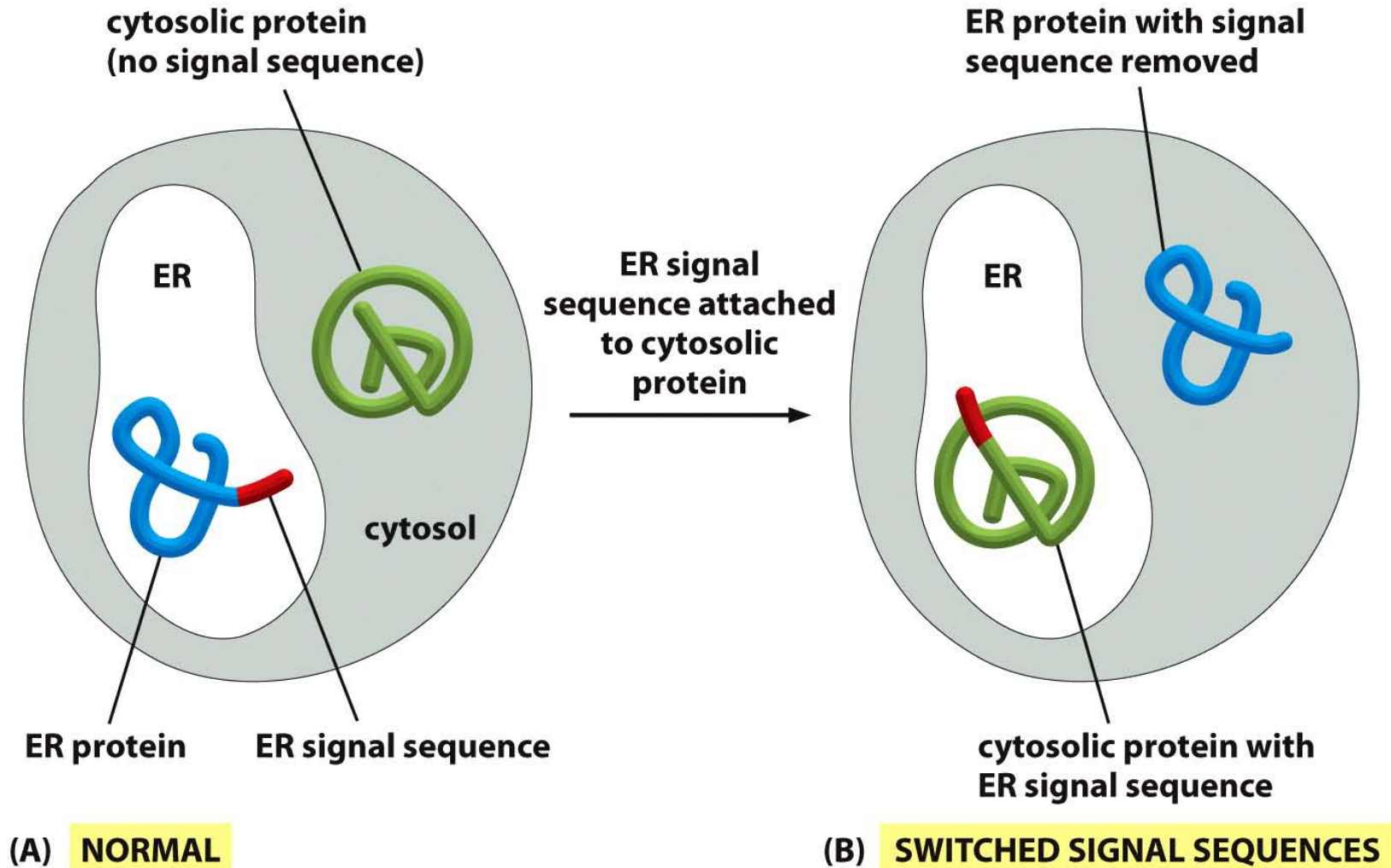
Figure 15-5 *Essential Cell Biology* (© Garland Science 2010)

Signalsequenzen dienen als Lieferadresse

Signalsequenz ist dafür verantwortlich, dass ein Protein zu seiner richtigen Stelle in der Zelle gebracht wird. Befindet sich meist am N-Terminus. Bei Anheftung an cytosolisches Protein erfolgt Targeting z.B. ins ER.



Mit Signalsequenzen kann der Bestimmungsort verändert werden



Signalsequenzen

Viele Proteine sind z.B. für die Translokation ins ER gekennzeichnet durch eine N-terminale Sequenz. Wird durch eine Signalpeptidase abgespalten. Besteht aus einer oder mehreren pos. geladenen AA, 10-15 hydrophoben AA und am C-Terminus polare AA mit kurzen Ketten (Ala).

TABLE 15-3 SOME TYPICAL SIGNAL SEQUENCES

FUNCTION OF SIGNAL	EXAMPLE OF SIGNAL SEQUENCE
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-
Retention in lumen of ER	-Lys-Asp-Glu-Leu-COO $^-$
Import into mitochondria	^+H_3N -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-
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Import into peroxisomes	-Ser-Lys-Leu-

Positively charged amino acids are shown in **red**, and negatively charged amino acids in **blue**. An extended block of hydrophobic amino acids is shown in **green**. ^+H_3N indicates the N-terminus of a protein; COO $^-$ indicates the C-terminus. The ER retention signal is commonly referred to by its single-letter amino acid abbreviation, KDEL.

Der Sekretionsweg

Ribosomen können frei im Cytoplasma vorkommen oder ans ER gebunden sein (rauhes ER).

Freie Ribosomen: lösliche Proteine, Cytoplasten oder Mitochondrien.

Gebundene Ribosomen: Membranproteine, Sekretorische Proteine
Verwendung im ER oder Lysosomen.

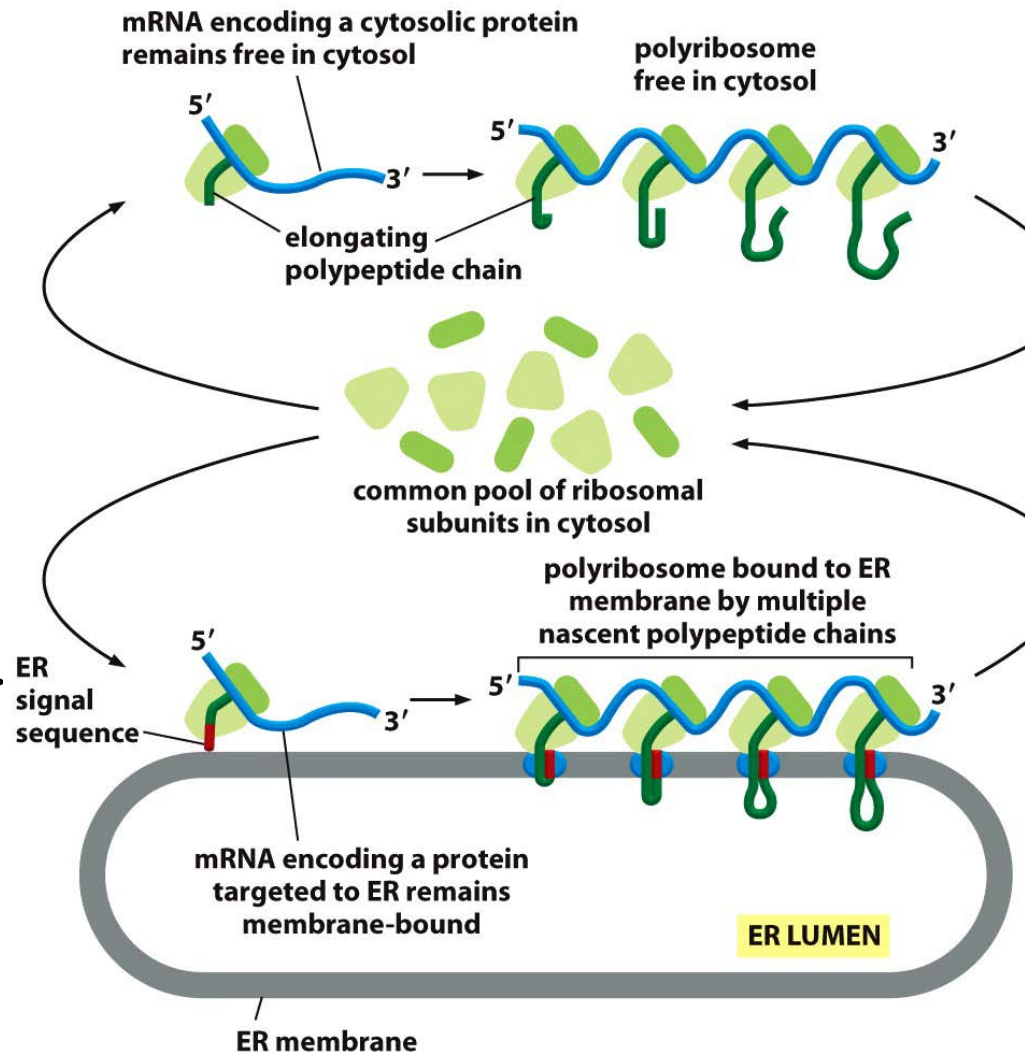
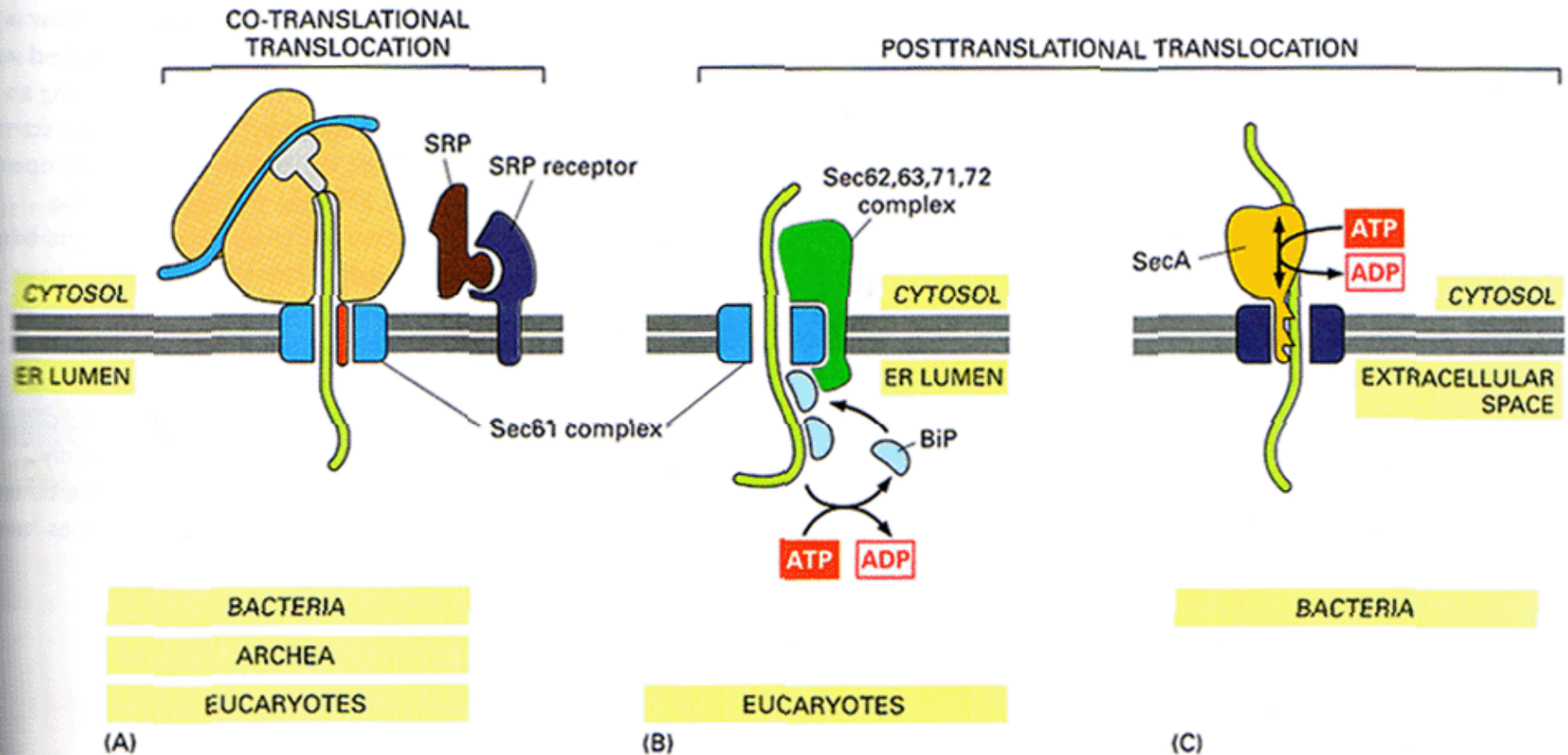


Figure 15-13 *Essential Cell Biology* (© Garland Science 2010)

Co- und Posttranslational Proteintranslokation



Cotranslational Translocation

Targeting into ER: Signal Hypothesis

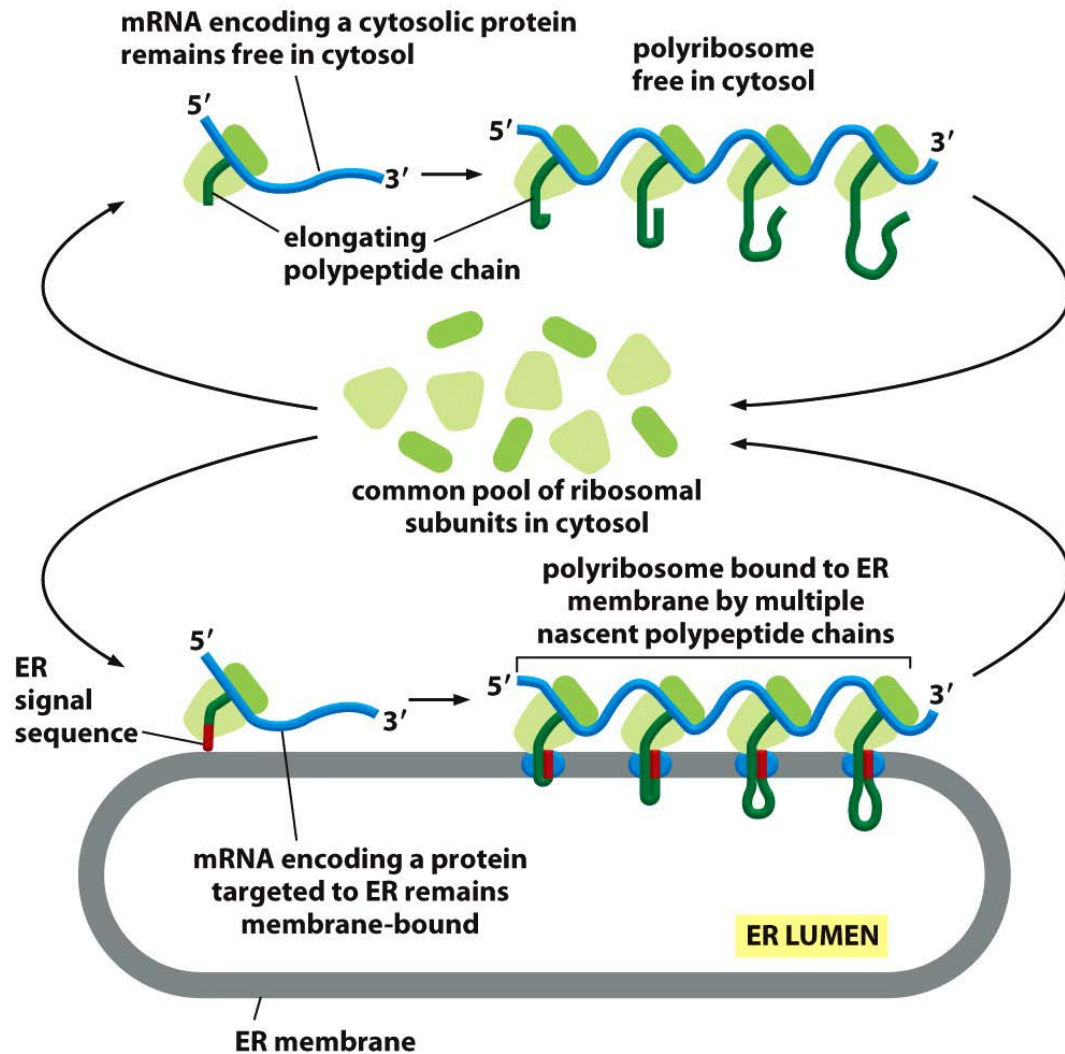
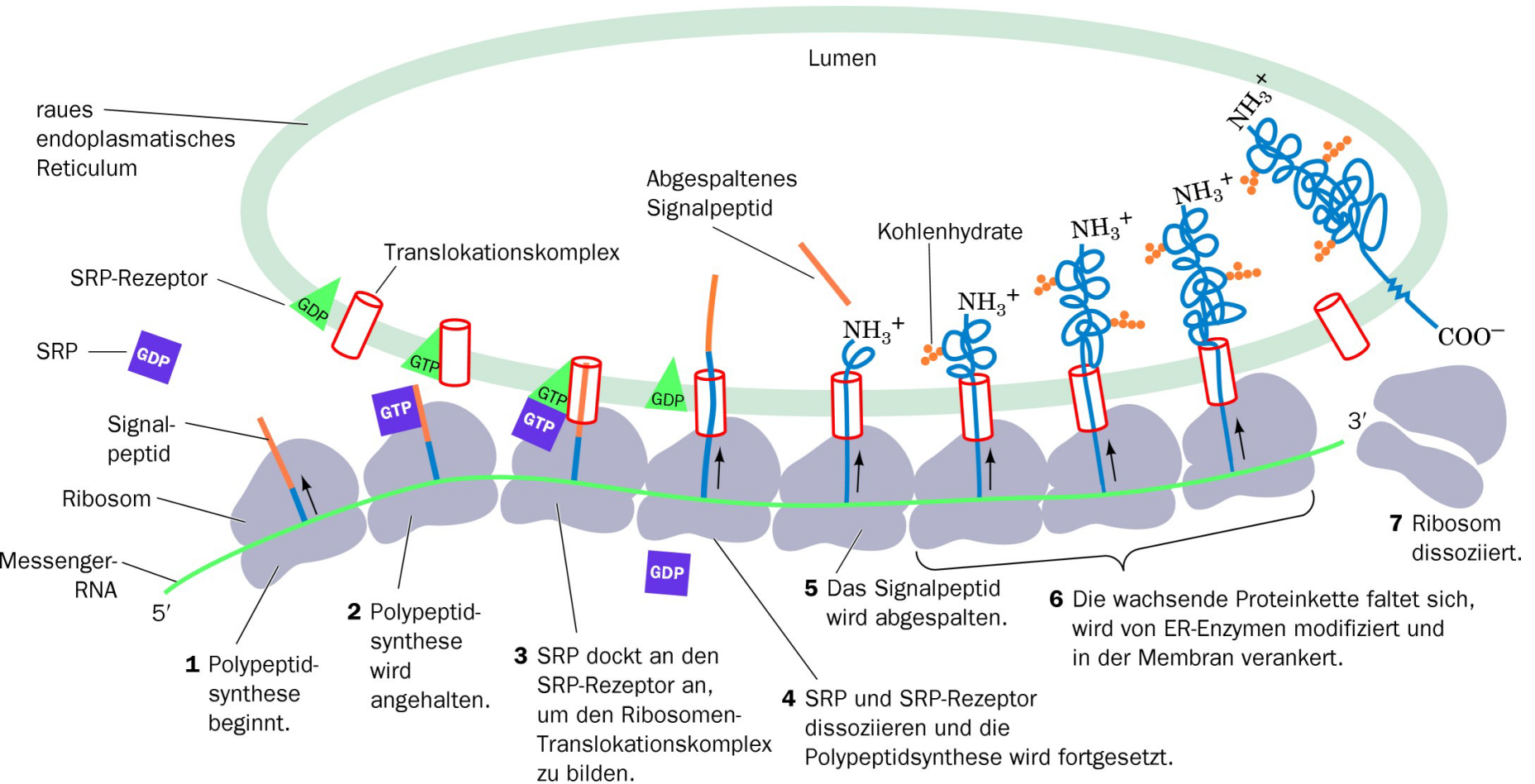


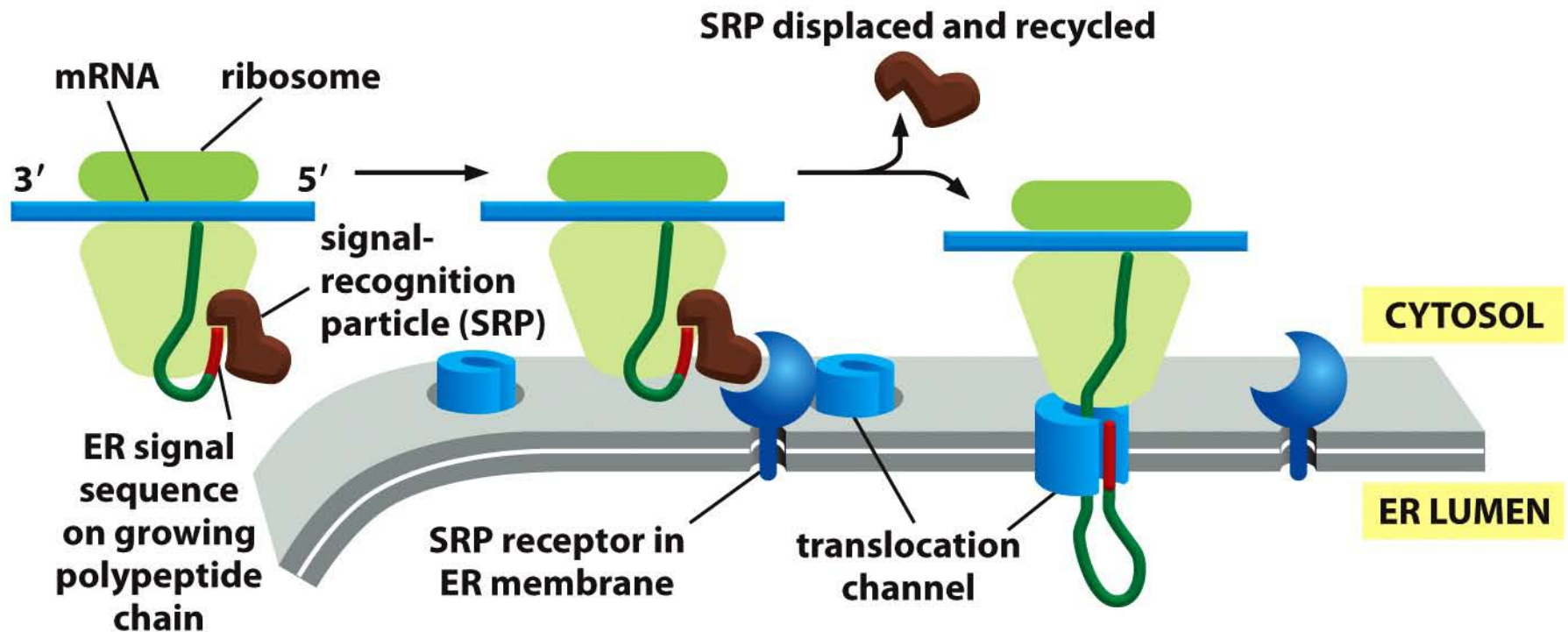
Figure 15-13 *Essential Cell Biology* (© Garland Science 2010)

Initiale Stufen des Sekretionsweg: Cotranslational



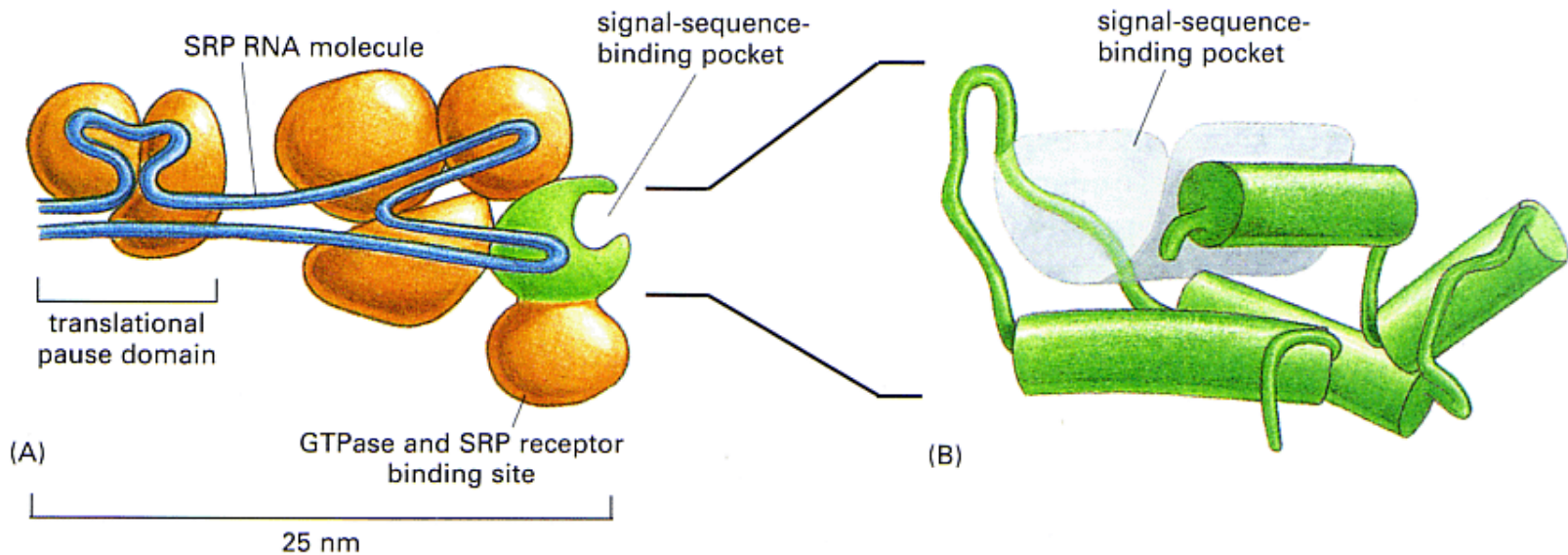
Targeting ins ER: Signalthypothese

Cotranslationale Translokation



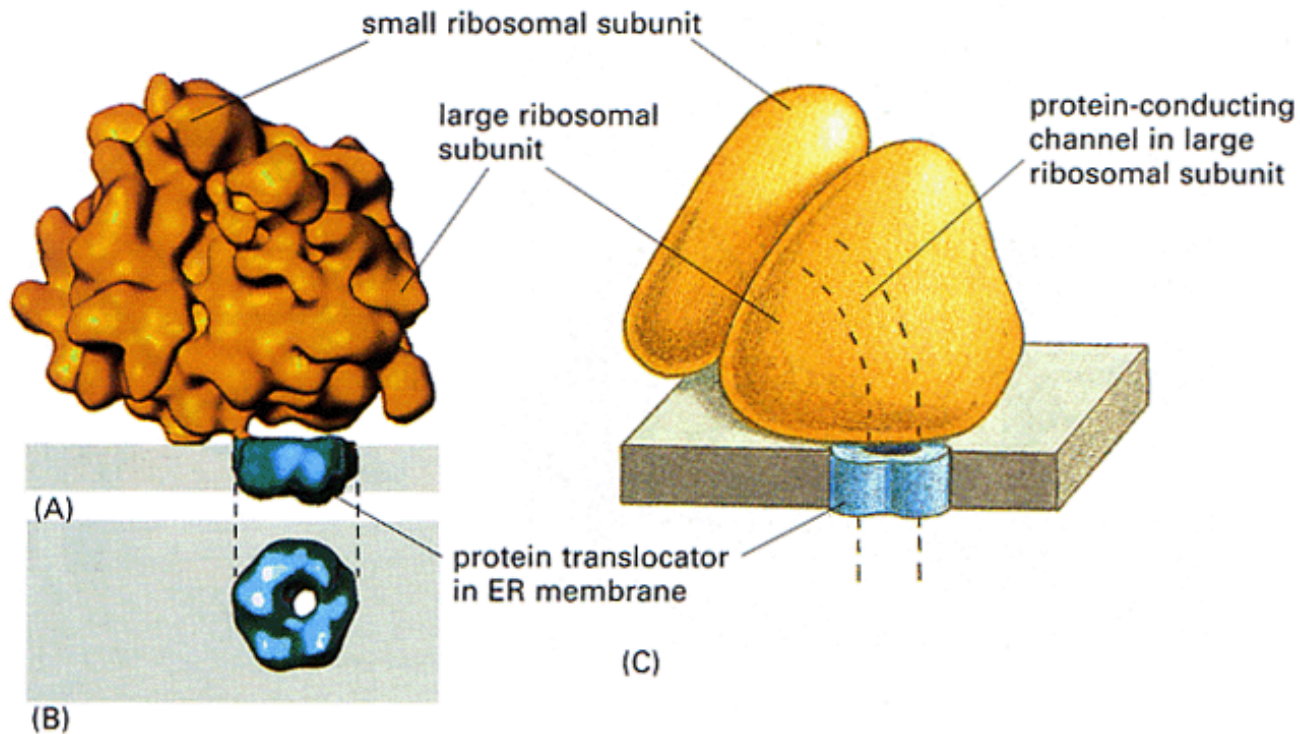
SRP stoppt die Elongation der Polypeptidkette

Aufbau des Signal Recognition Particle (SRP)



Methioninreiche Signalsequenz
Bindungstasche: ermöglicht Bindung
unterschiedlicher Signalsequenzen.

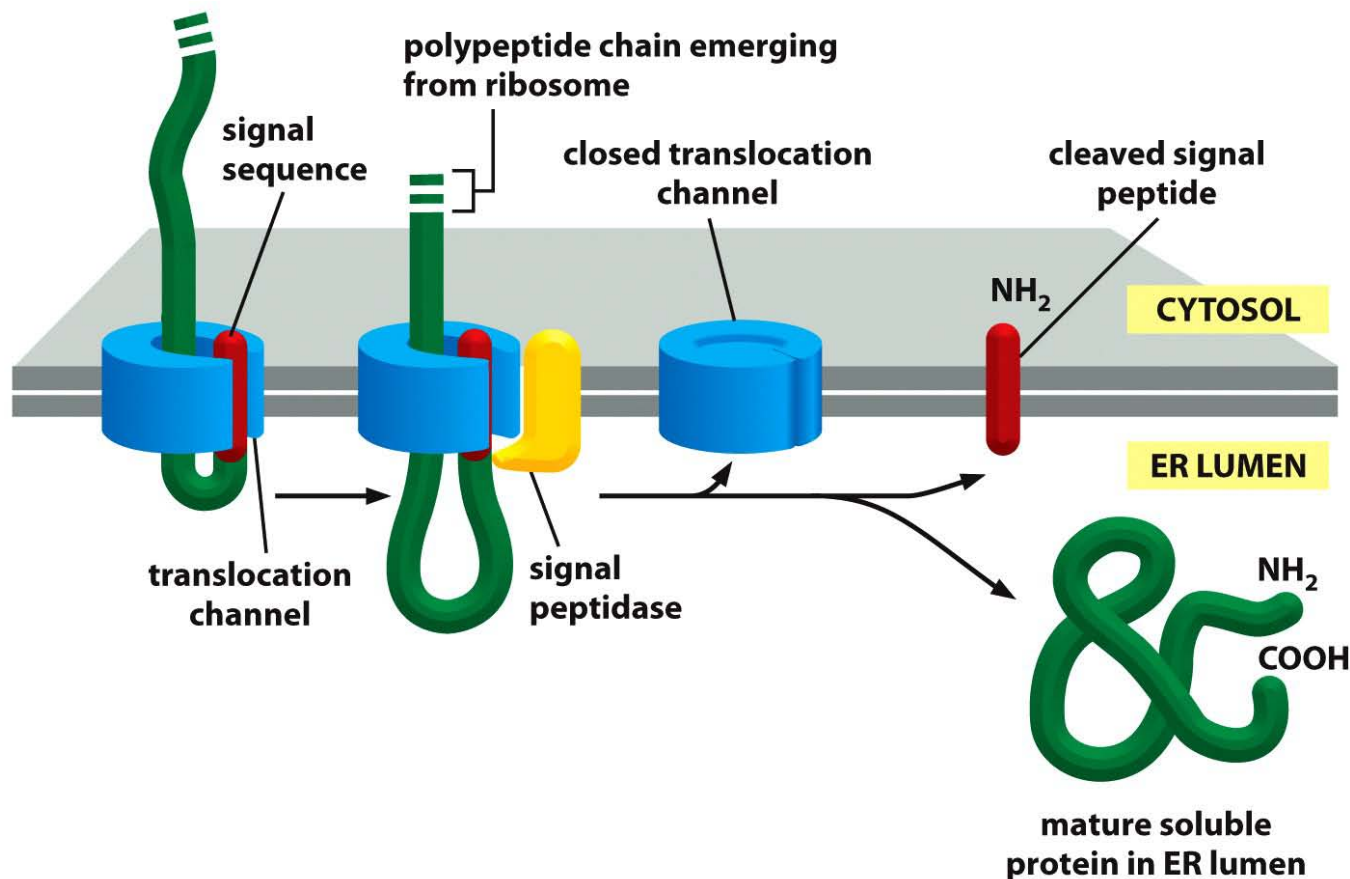
Ribosom und Sec61 Proteintranslokator



Es darf zu keinem Austausch Cytosol/ER kommen. Fest verschlossen!

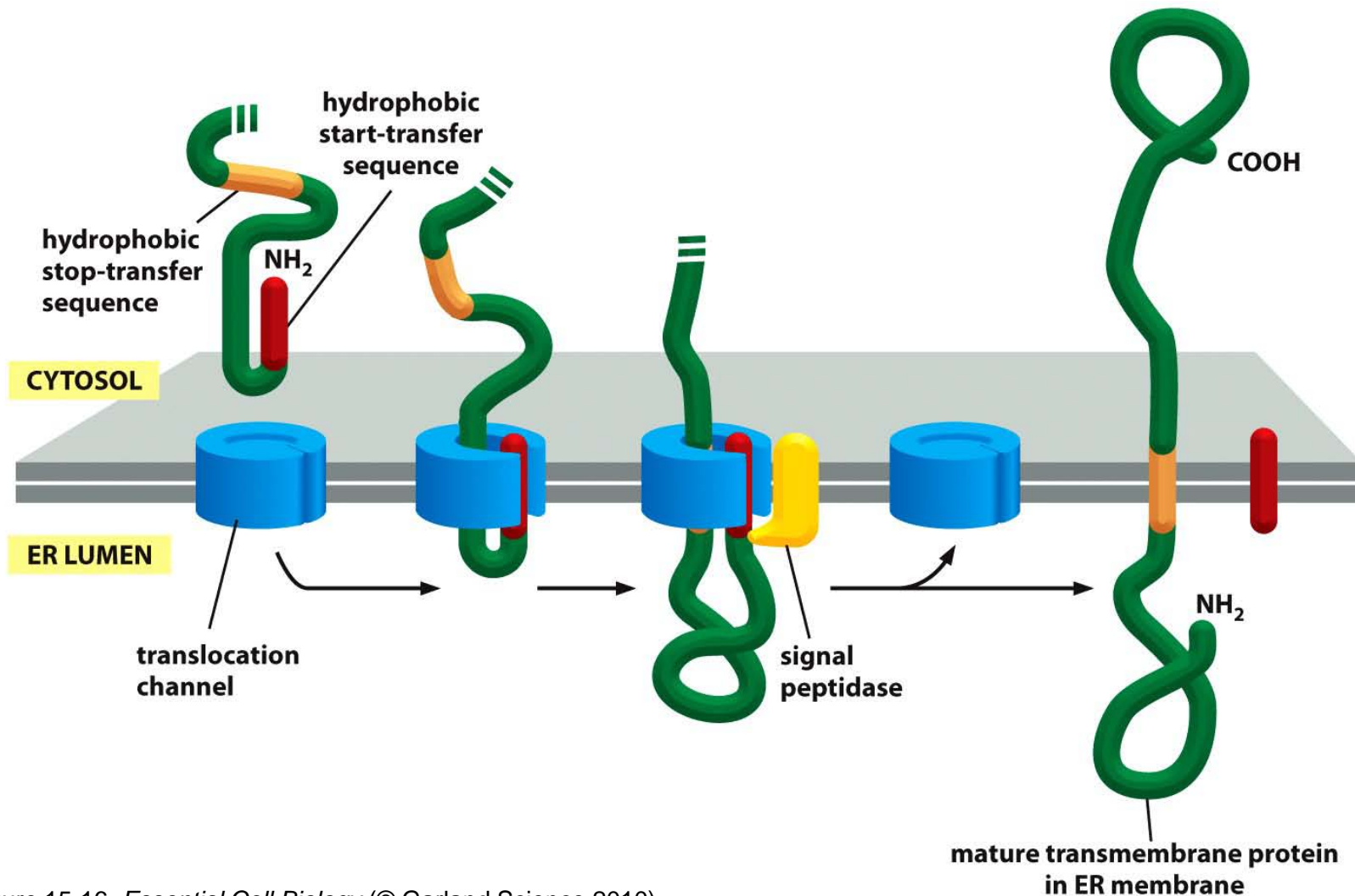
Translokation eines löslichen Proteins

SRP unabhängiger Weg, posttranslationale Translokation
Niedere Eukaryonten (z.B. Hefe), Chloroplasten, Mitochondrien,
Peroxisome



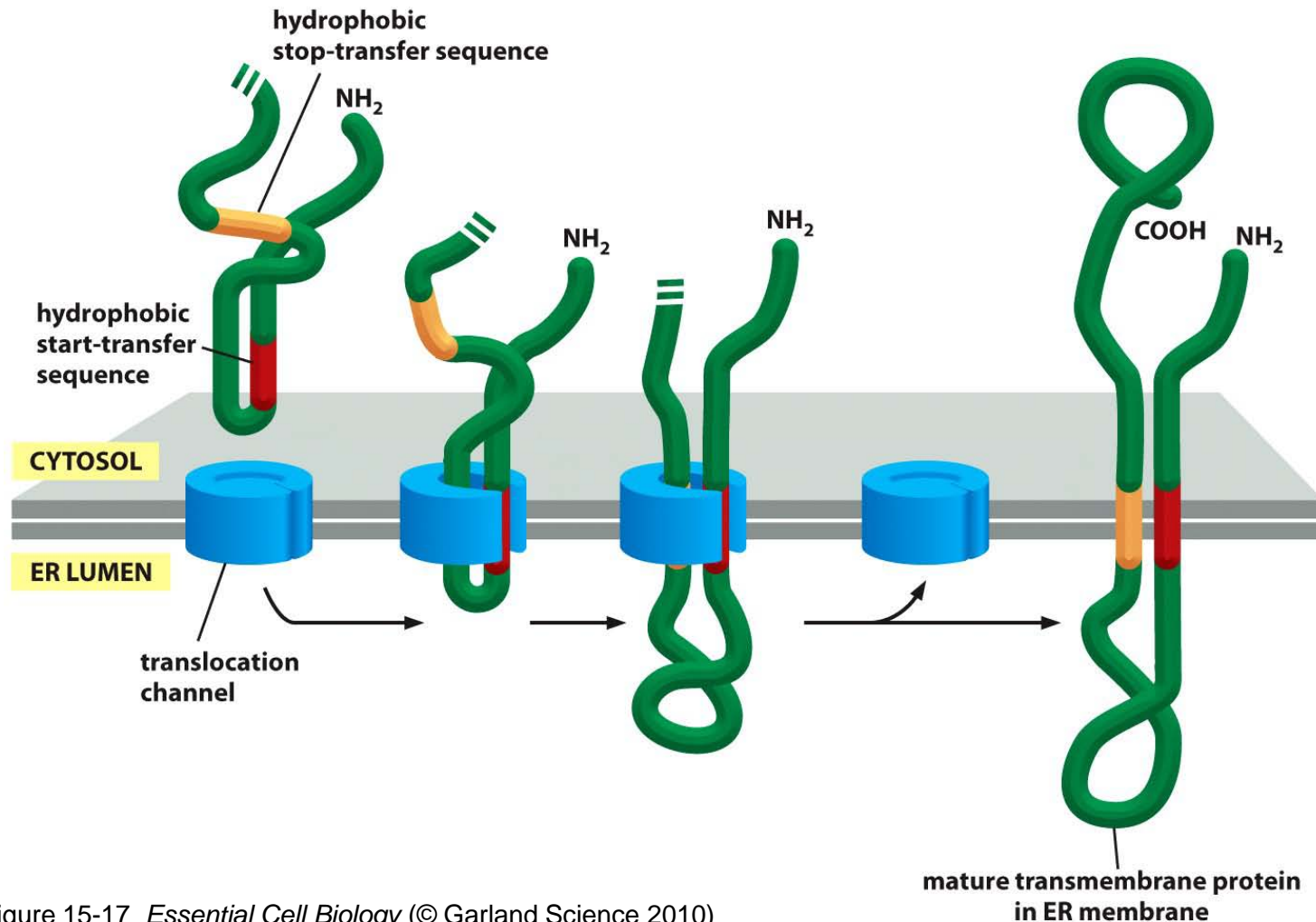
Translokation eines Einpfad-Transmembranproteins

Hydrophobe Transfer Stoppsequenz sorgt für Anhalten, wird vom Translokationskanal freigegeben. Ausrichtung wird beibehalten.

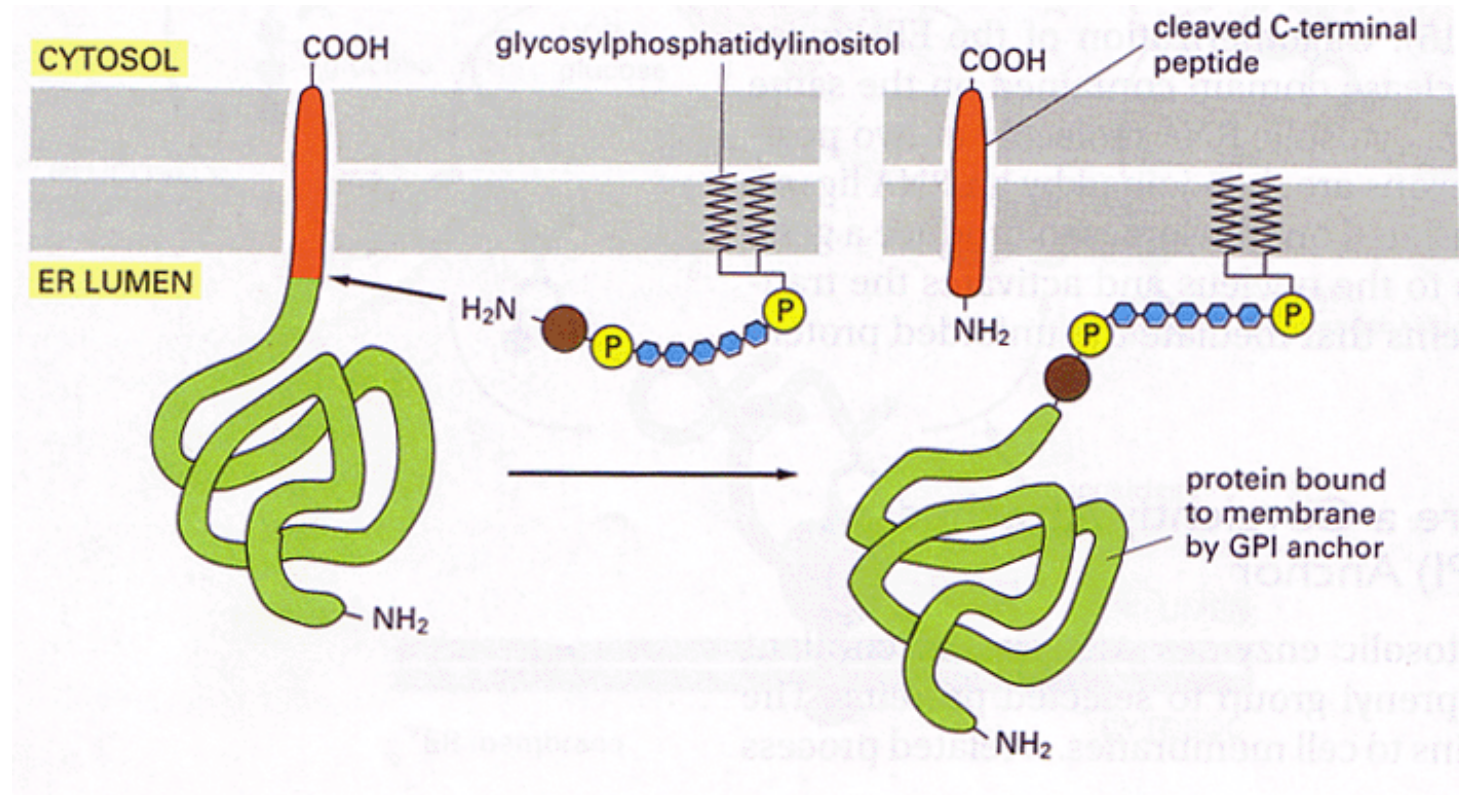


Translokation eines Doppelpfad-Transmembranproteins

Interne Start-Transfer Sequenz, wird nicht entfernt, bis zur hydrophoben Stopp-Transfer Sequenz. Bei Mehrpfadproteinen zusätzliche Paare.



Anheftung eines Membranankers



Phosphatidylinositol, Glucosamin, Mannose(n), Ethanolamin

N-Glykosylierung im ER

1. Synthese eines Oligosaccharidrestes (14) gebunden an den Lipid-carrier Dolichol-Phosphat.
2. Transfer des Oligosaccharids auf Asn durch eine Oligosaccharidtransferase
3. Prozessierung dieser Oligosaccharide während des Transportes

KEY:



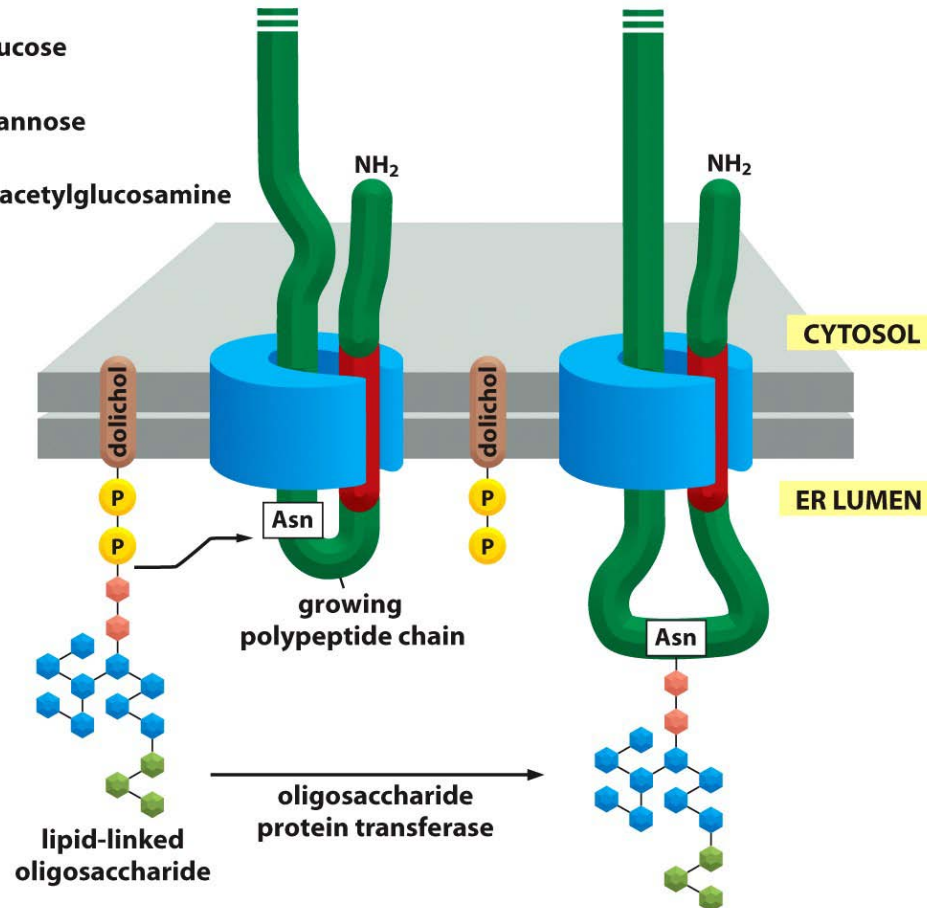
= glucose



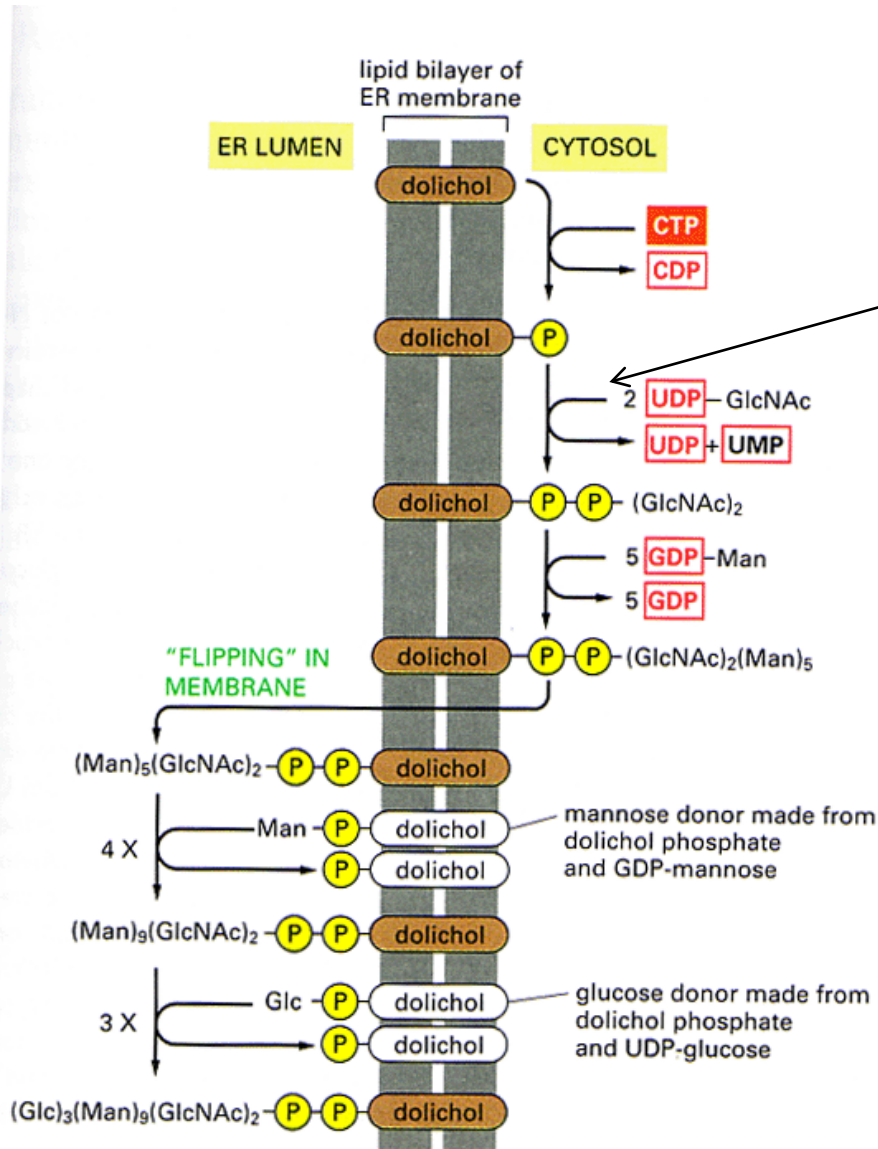
= mannose



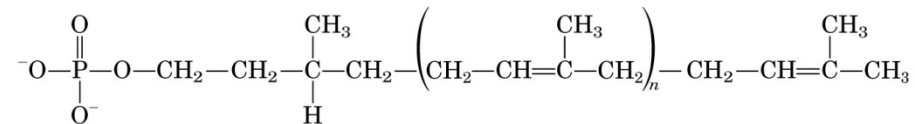
= N-acetylglucosamine



Synthese der Oligosaccharide



Tunicamycin



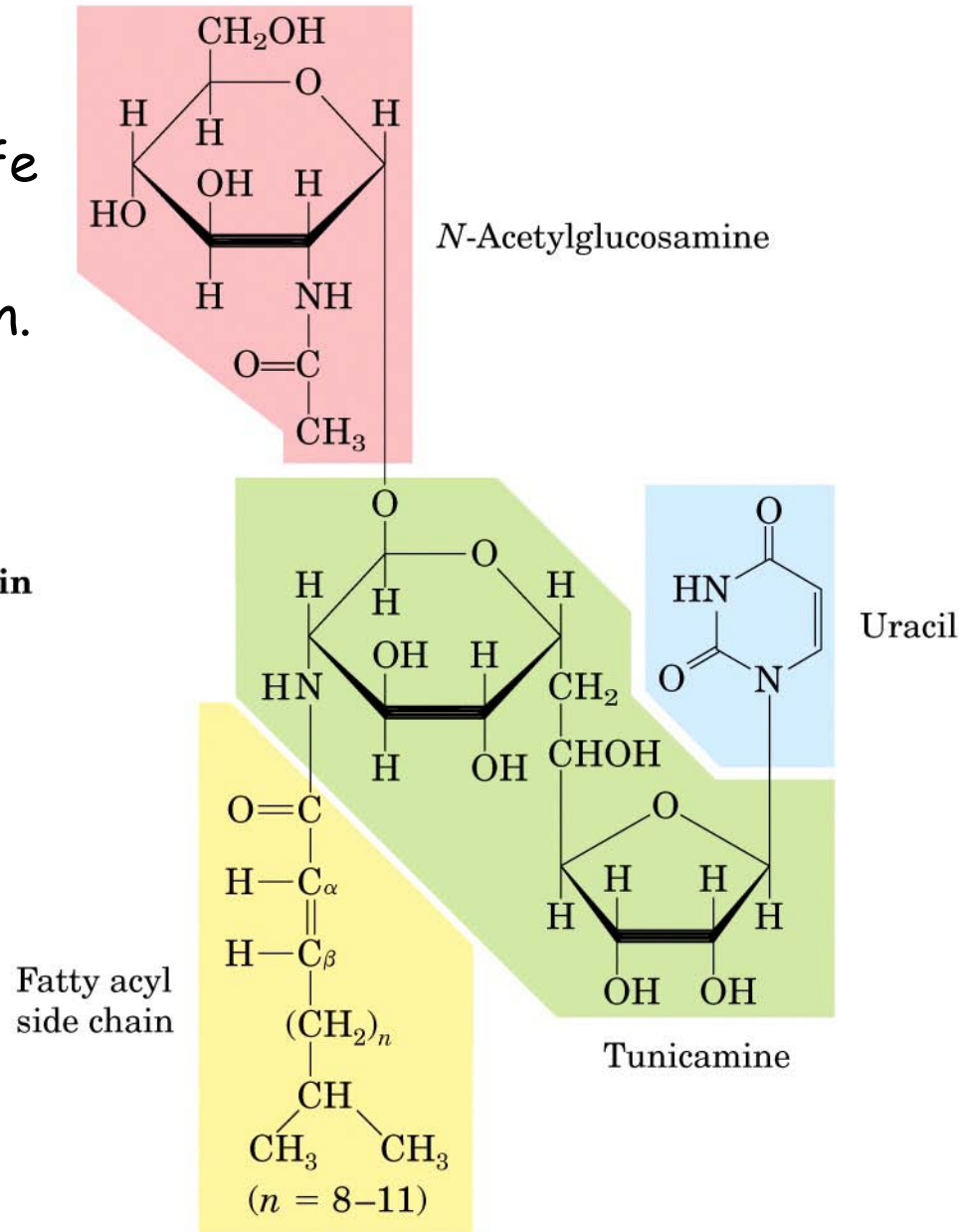
Dolichol phosphate
($n = 9-22$)

Tunicamycin

Tunicamycin blockiert die erste Stufe der N-Glykosylierung. Aufbau ist ähnlich dem UDP-*N*-Acetylglucosamin.

Ergebnis: nicht glykosylierte Proteine.

Tunicamycin



N-Glykosylierung

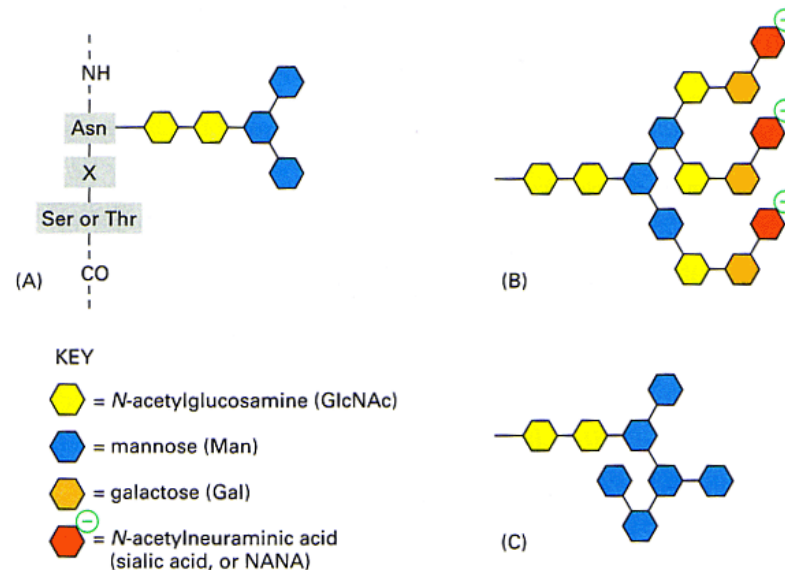


Figure 13-25 The two main classes of asparagine-linked (N-linked) oligosaccharides found in mature glycoproteins.

(A) Both complex oligosaccharides and high-mannose oligosaccharides share a common *core region* derived from the original N-linked oligosaccharide added in the ER and typically containing two N-acetylglucosamines (GlcNAc) and three mannoses (Man). (B) Each complex oligosaccharide consists of a *core region*, together with a *terminal region* that contains a variable number of copies of a special trisaccharide unit (N-acetylglucosamine–galactose–sialic acid) linked to the core mannoses. Frequently, the terminal region is truncated and contains only GlcNAc and galactose (Gal) or just GlcNAc. In addition, a fucose residue may be added, usually to the core GlcNAc attached to the asparagine (Asn). Thus, although the steps of processing and subsequent sugar addition are rigidly ordered, complex oligosaccharides can be heterogeneous. Moreover, although the complex oligosaccharide shown has three terminal branches, two and four branches are also common, depending on the glycoprotein and the cell in which it is made. (C) High-mannose oligosaccharides are not trimmed back all the way to the core region and contain additional mannose residues. Hybrid oligosaccharides (not shown) with one Man branch and one GlcNAc and Gal branch are also found.

The three amino acids indicated in (A) constitute the sequence recognized by the oligosaccharyl transferase enzyme that adds the initial oligosaccharide to the protein. Ser = serine; Thr = threonine; X = any amino acid.

Oligosaccharid Prozessierung

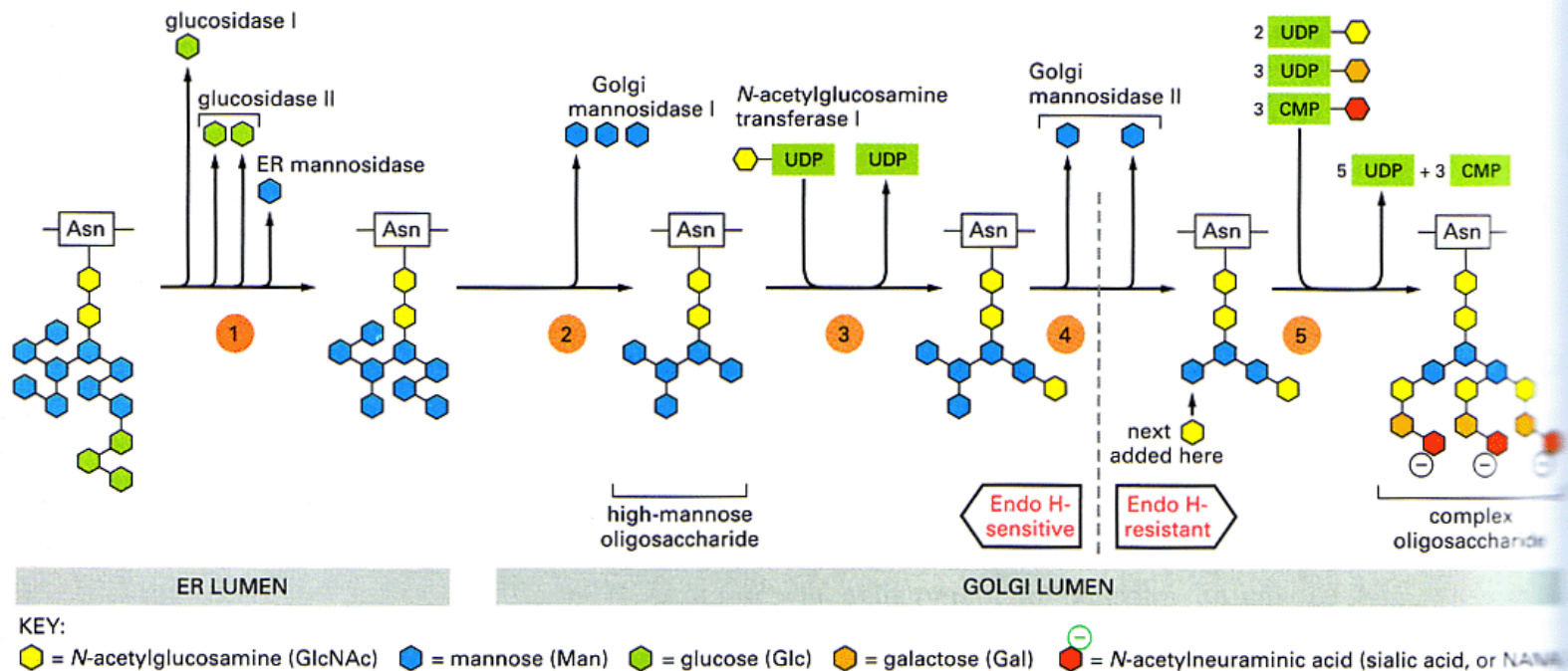


Figure 13–26 Oligosaccharide processing in the ER and the Golgi apparatus. The processing pathway is highly ordered, so that each step shown is dependent on the previous one. Processing begins in the ER with the removal of the glucoses from the oligosaccharide initially transferred to the protein. Then a mannosidase in the ER membrane removes a specific mannose. The remaining steps occur in the Golgi stack, where Golgi mannosidase I first removes three more mannoses and N-acetylglucosamine transferase I then adds an N-acetylglucosamine, which enables mannosidase II to remove two additional mannoses. This yields the final core of three mannoses that is present in a complex oligosaccharide. At this stage, the bond between the two N-acetylglucosamines in the core becomes resistant to attack by a highly specific endoglycosidase (*Endo H*). Since all later structures in the pathway are also *Endo H*-resistant, treatment with this enzyme is widely used to distinguish complex from high-mannose oligosaccharides. Finally, as shown in Figure 13–24, additional N-acetylglucosamines, galactoses, and sialic acids are added. These final steps in the synthesis of a complex oligosaccharide occur in the cisternal compartments of the Golgi apparatus. Three types of glycosyl transferase enzymes act sequentially, using sugar substrates that have been activated by linkage to the indicated nucleotide. The membranes of the Golgi cisternae contain specific carrier proteins that allow each sugar nucleotide to enter in exchange for the nucleoside phosphates that are released after the sugar is attached to the protein on the luminal face.