Einführung in die Molekularbiologie
Prof. Dr. Martin Hagemann

Vorlesung 11

Proteinbiosynthese

Proteinexport
Proteinmodifikation
Proteinabbau am Proteasom
Innere Uhr

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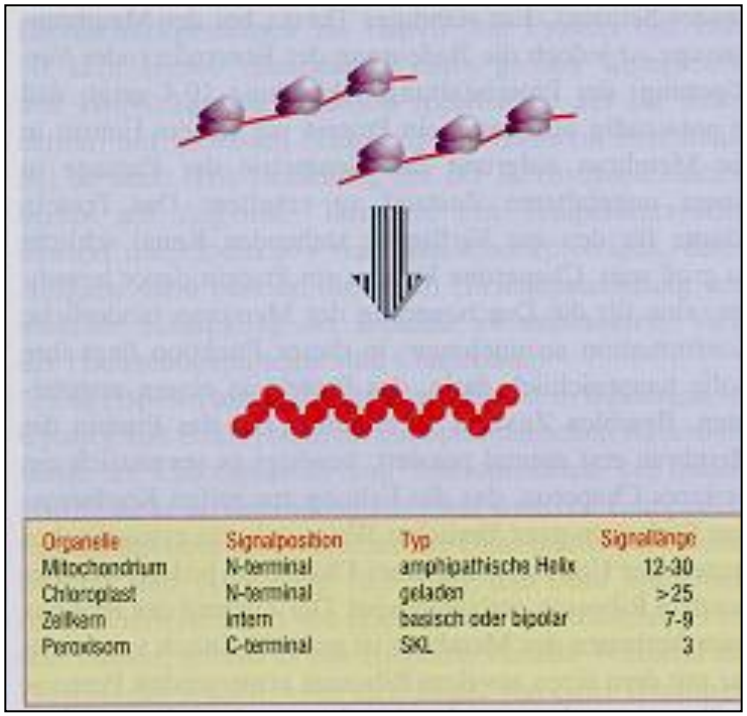
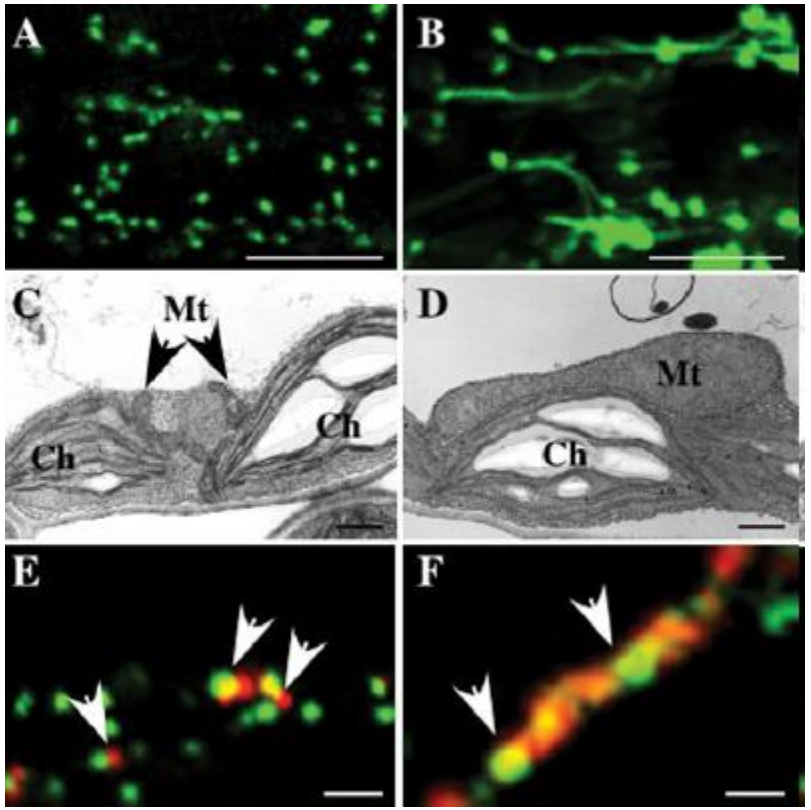
Synthese von Proteinen für Zellkompartimente

Posttranslationaler Transport hängt von spezifischen Targetsequenzen ab
Wichtiger Hinweis auf zelluläre Lokalisation von Proteinen!
Sollte durch Experimente bestätigt werden – z.B. GFP-Fusion!

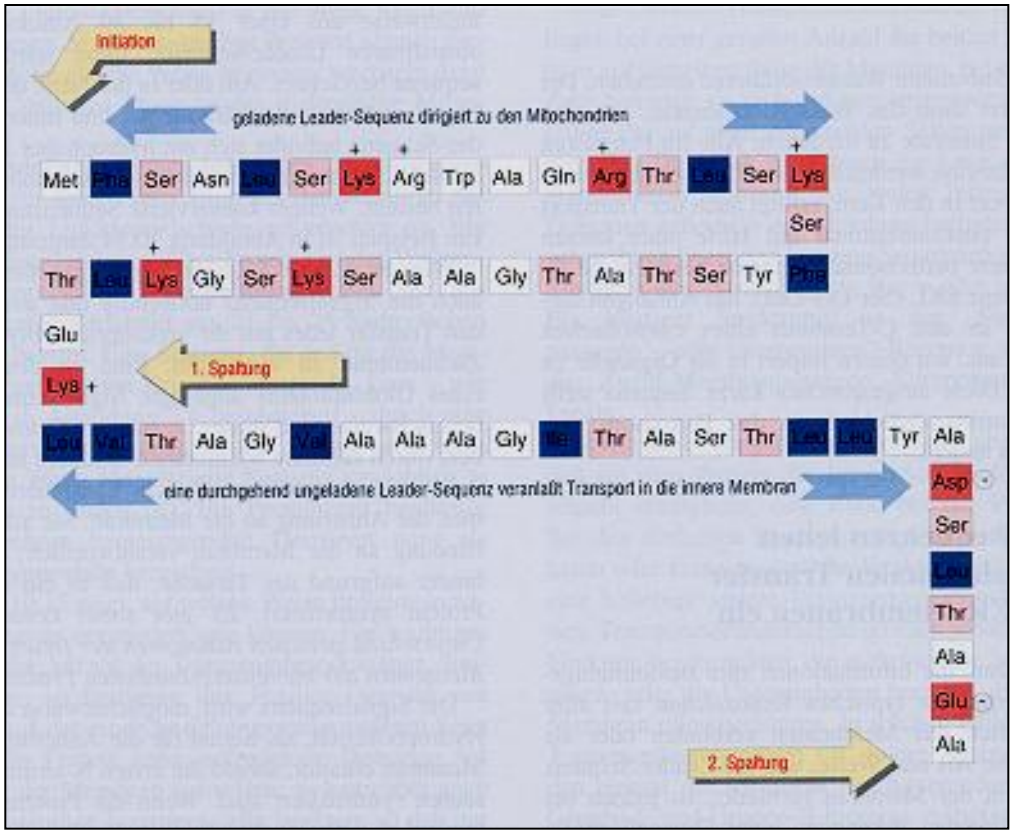


Organelle	Signalposition	Typ	Signallänge
Mitochondrium	N-terminal	amphipathische Helix	12-30
Chloroplast	N-terminal	geladen	>25
Zellkern	intern	basisch oder bipolar	7-9
Peroxisom	C-terminal	SKL	3



Synthese von Proteinen für Zellkompartimente

Einige Proteine haben kombinierte Targetsequenzen
z.B. Cytochrom c1 in Hefe



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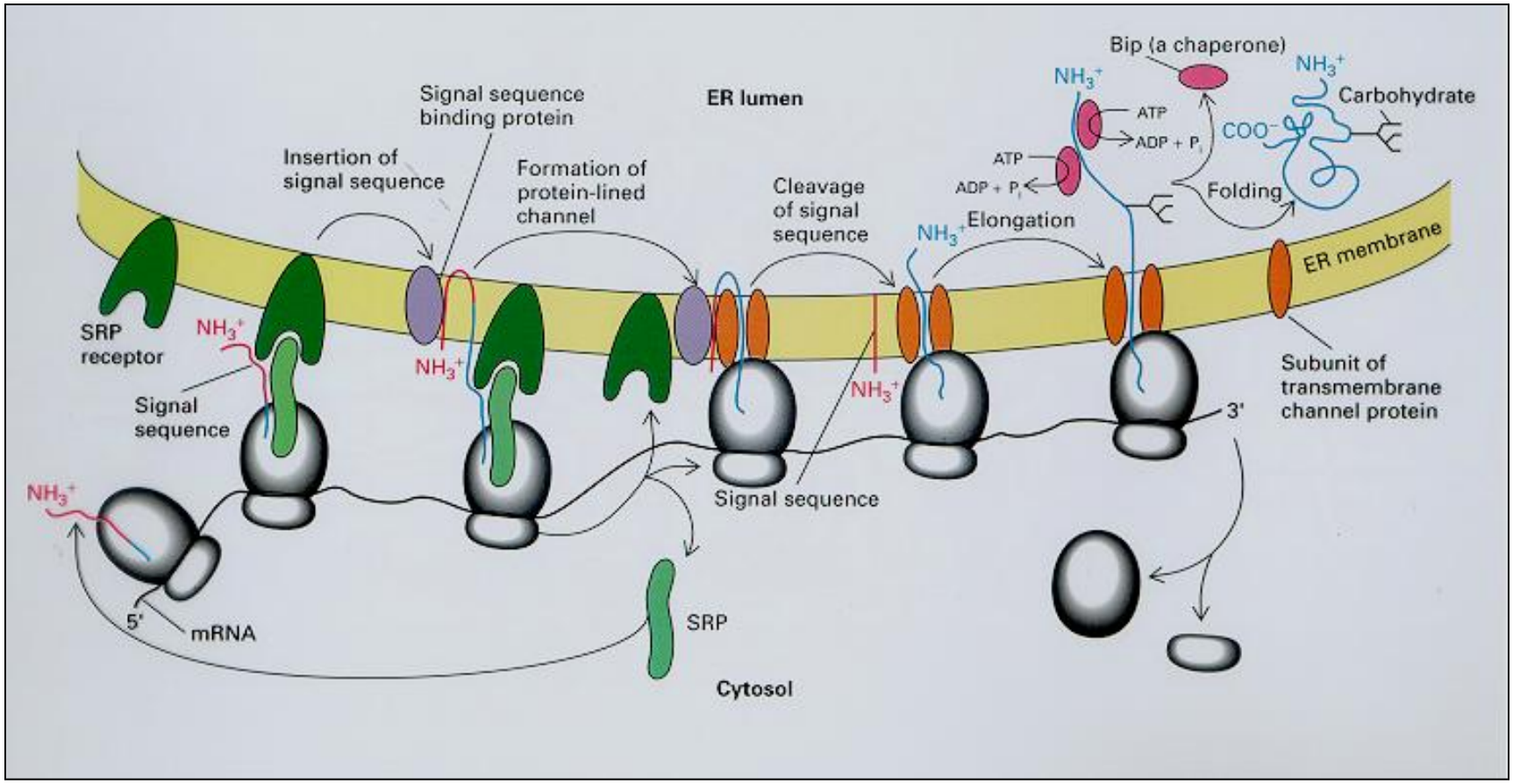
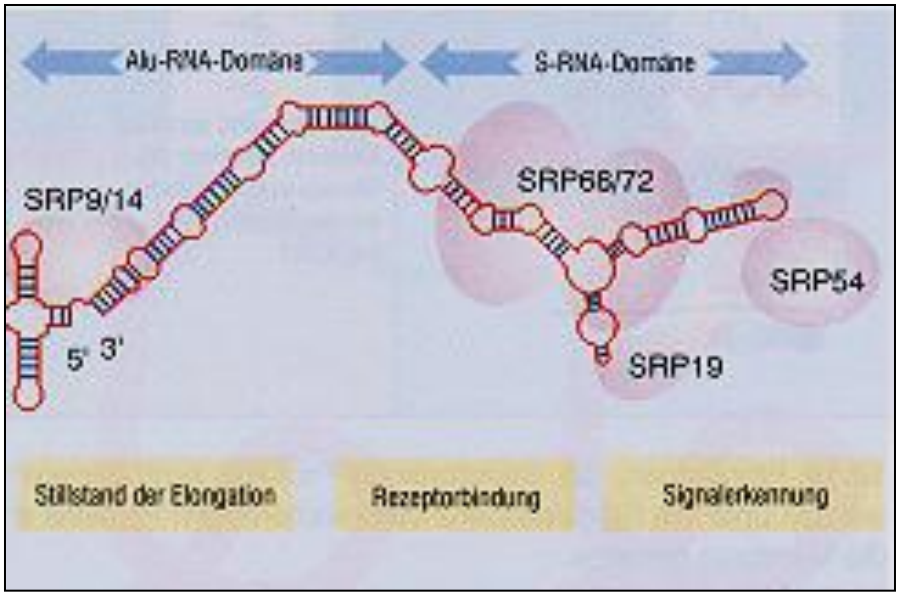
Synthese von Proteinen für Zellkompartimente

Modell für den cotranslationalen Transport



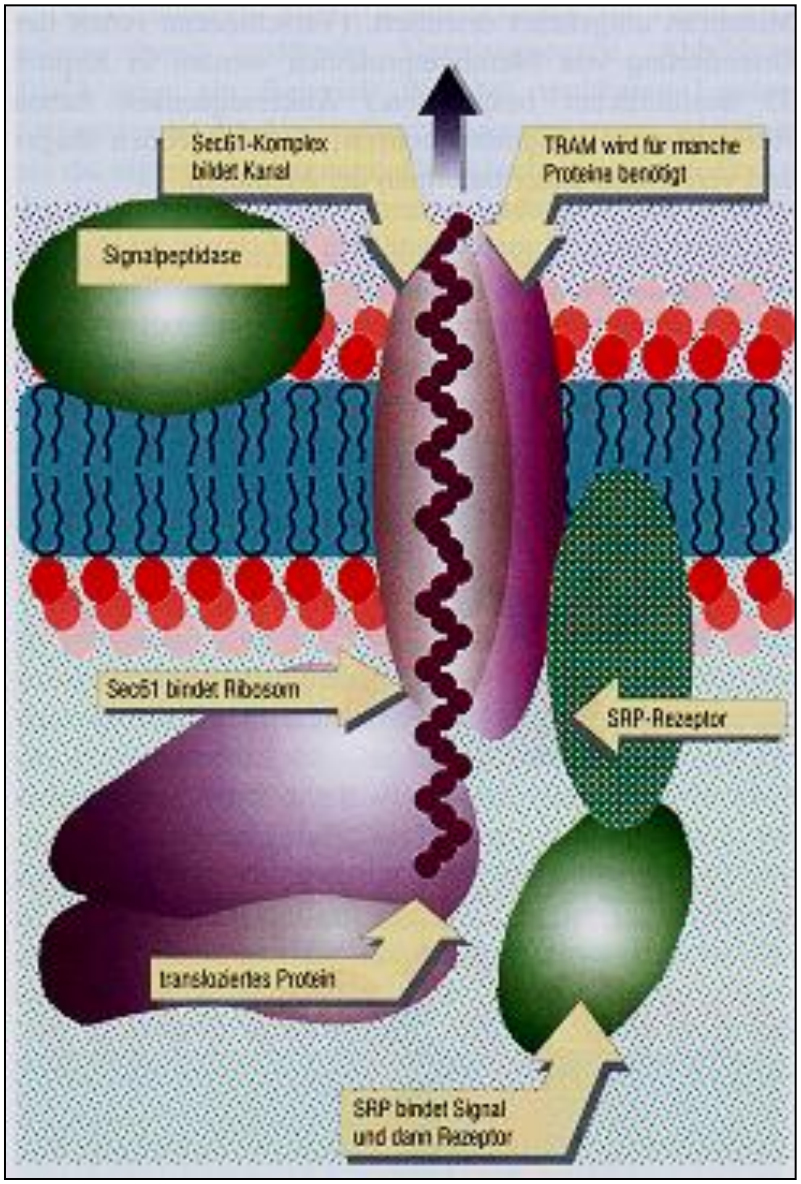
Synthese von Proteinen für Zellkompartimente

SRP – signal recognition particle
Ribonucleoproteinkomplex mit mehreren funktionellen Teilen



Synthese von Proteinen für Zellkompartimente

Modell des Translokationsapparates Sec (Tat)



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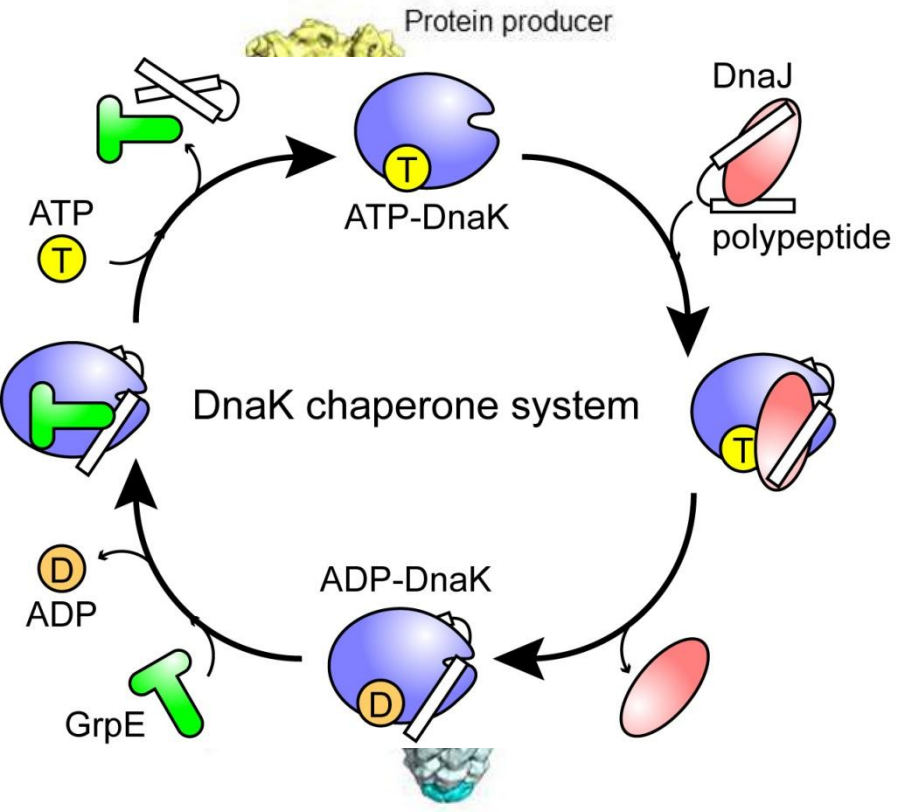
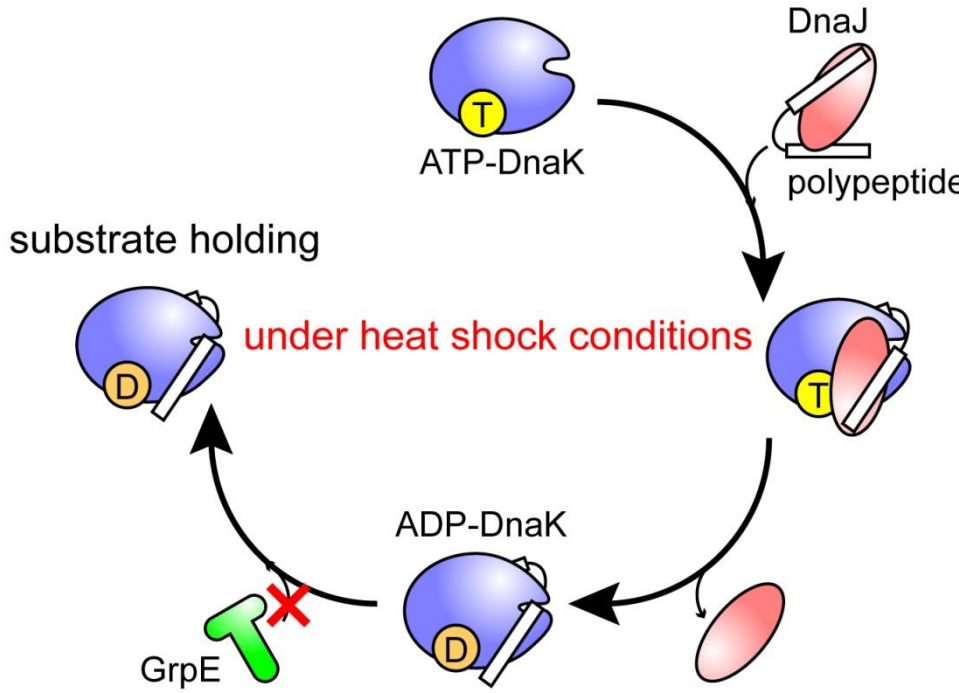
Faltung von Proteinen durch Chaperone

DnaK/GroE (Hsp70/Hsp60)-Chaperonfamilien kooperieren
DnaK bindet frisch translatiertes Protein und
faltet mit anderen Chaperonen Proteine in definierte 3D-Strukturen





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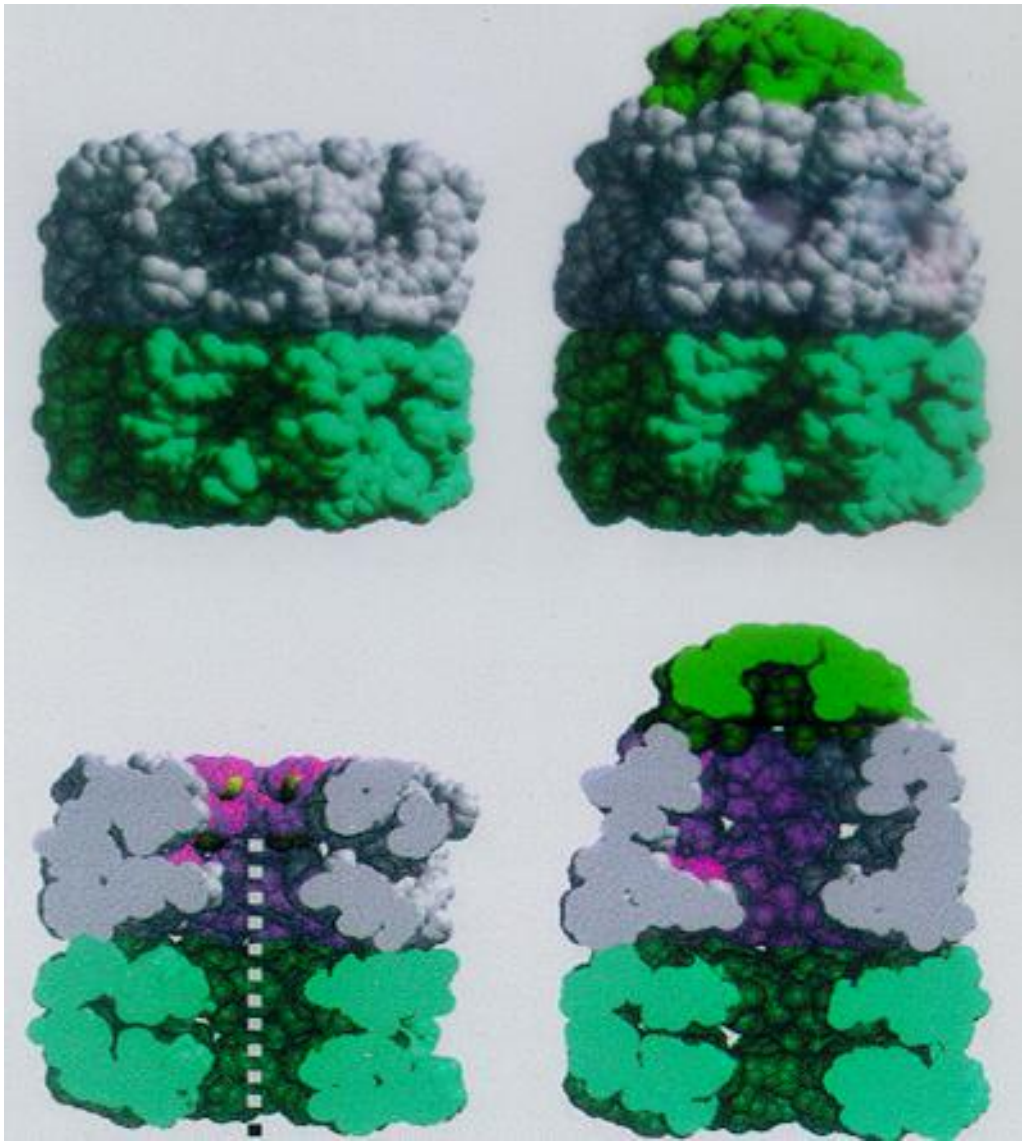
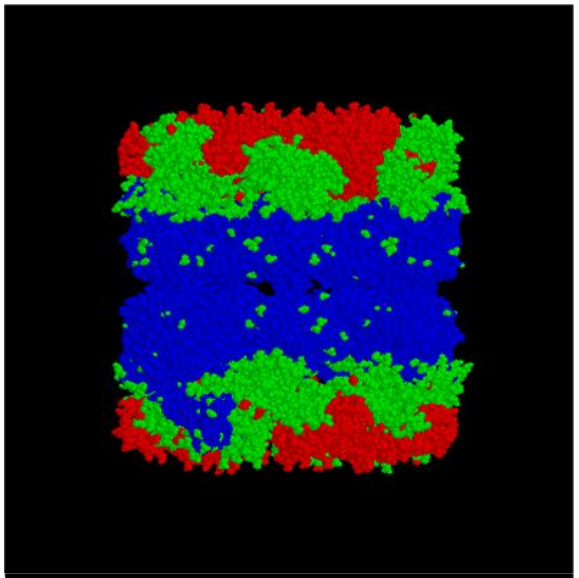
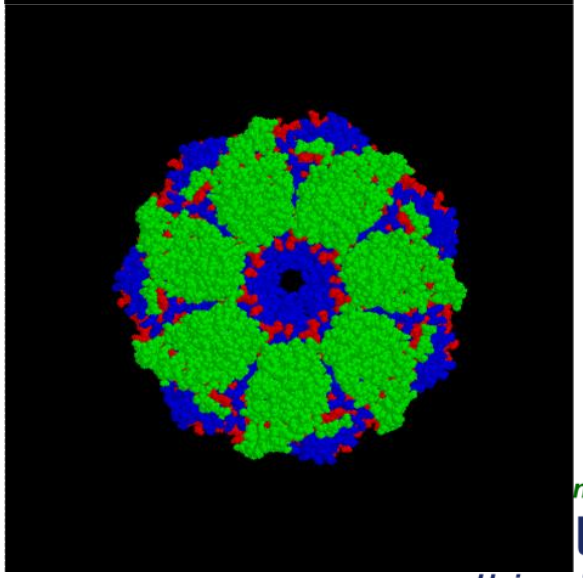
Faltung von Proteinen durch Chaperone

Hsp60/Hsp10-Chaperonfamilie – Bakterien GroELS, 7 GroEL pro Ring

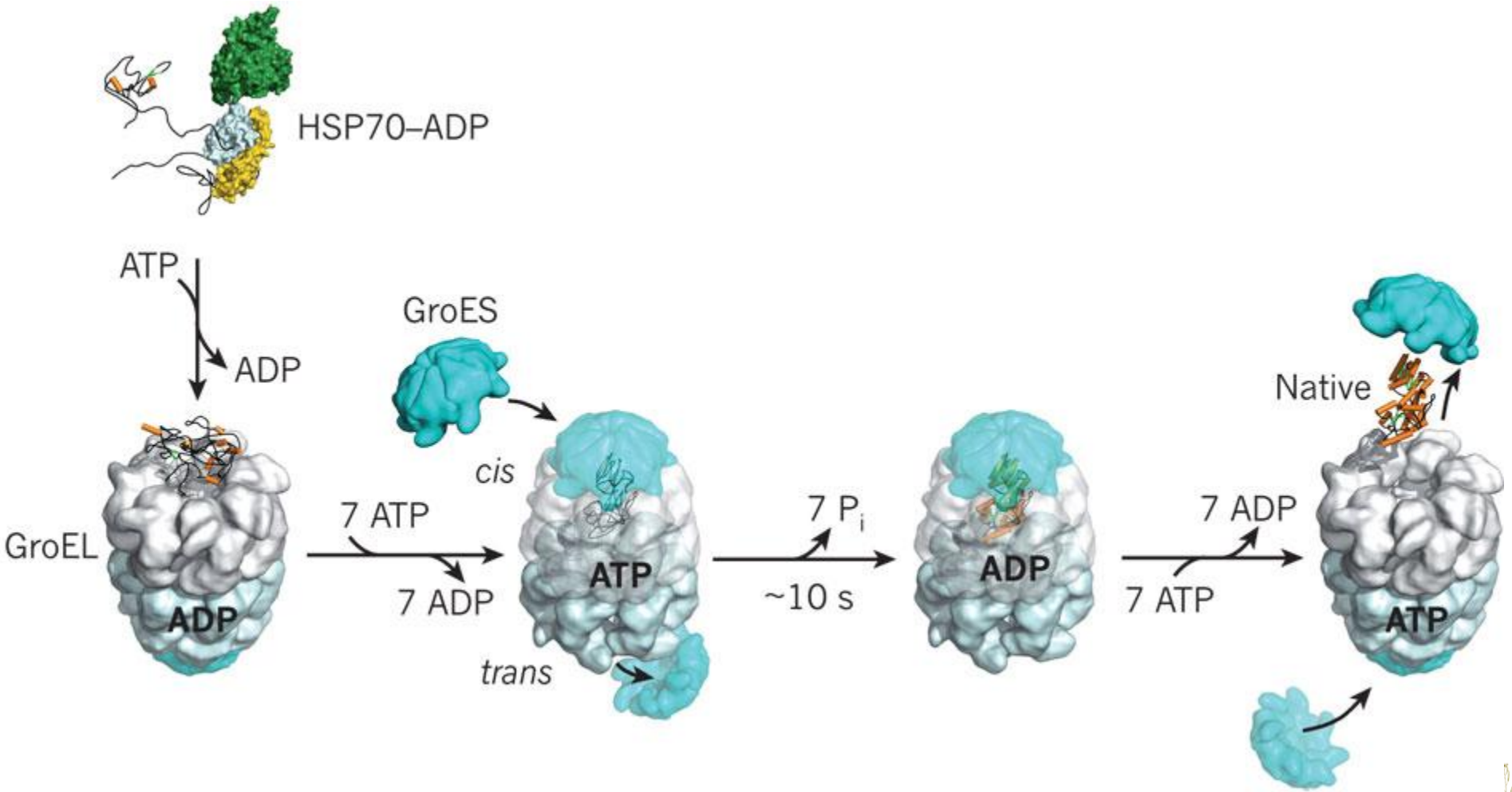






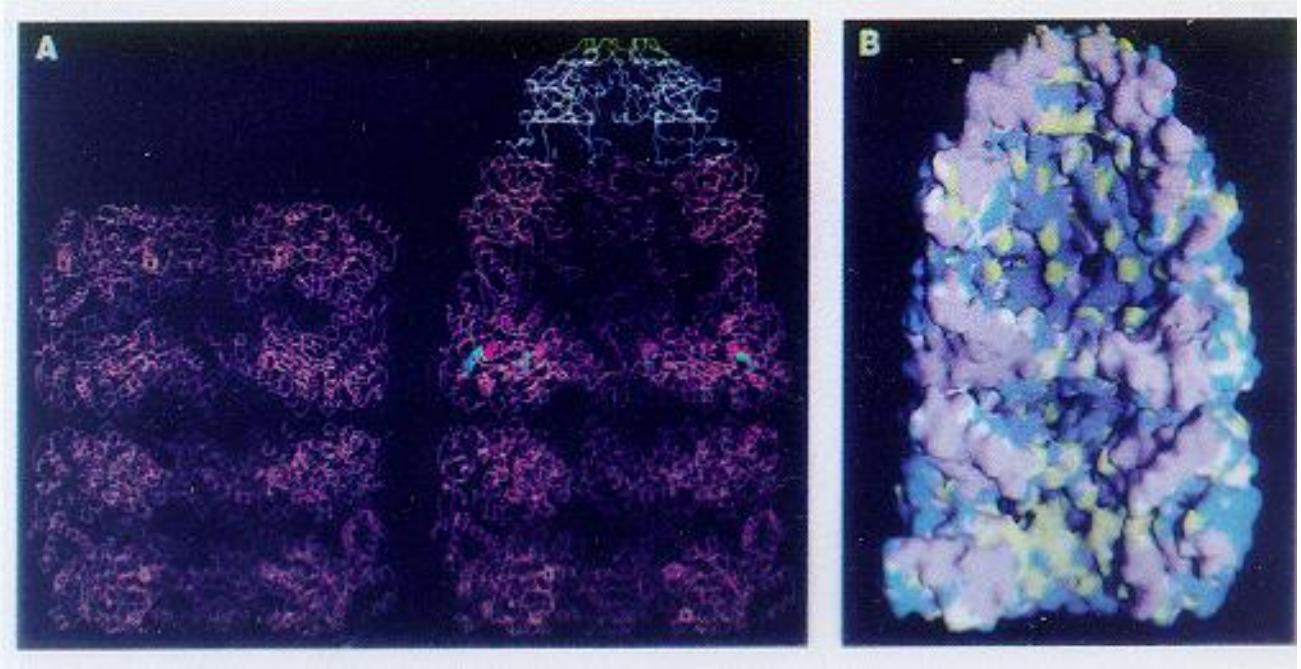
Faltung von Proteinen durch Chaperone

Biochemie des Reaktionscyclus von GroELS



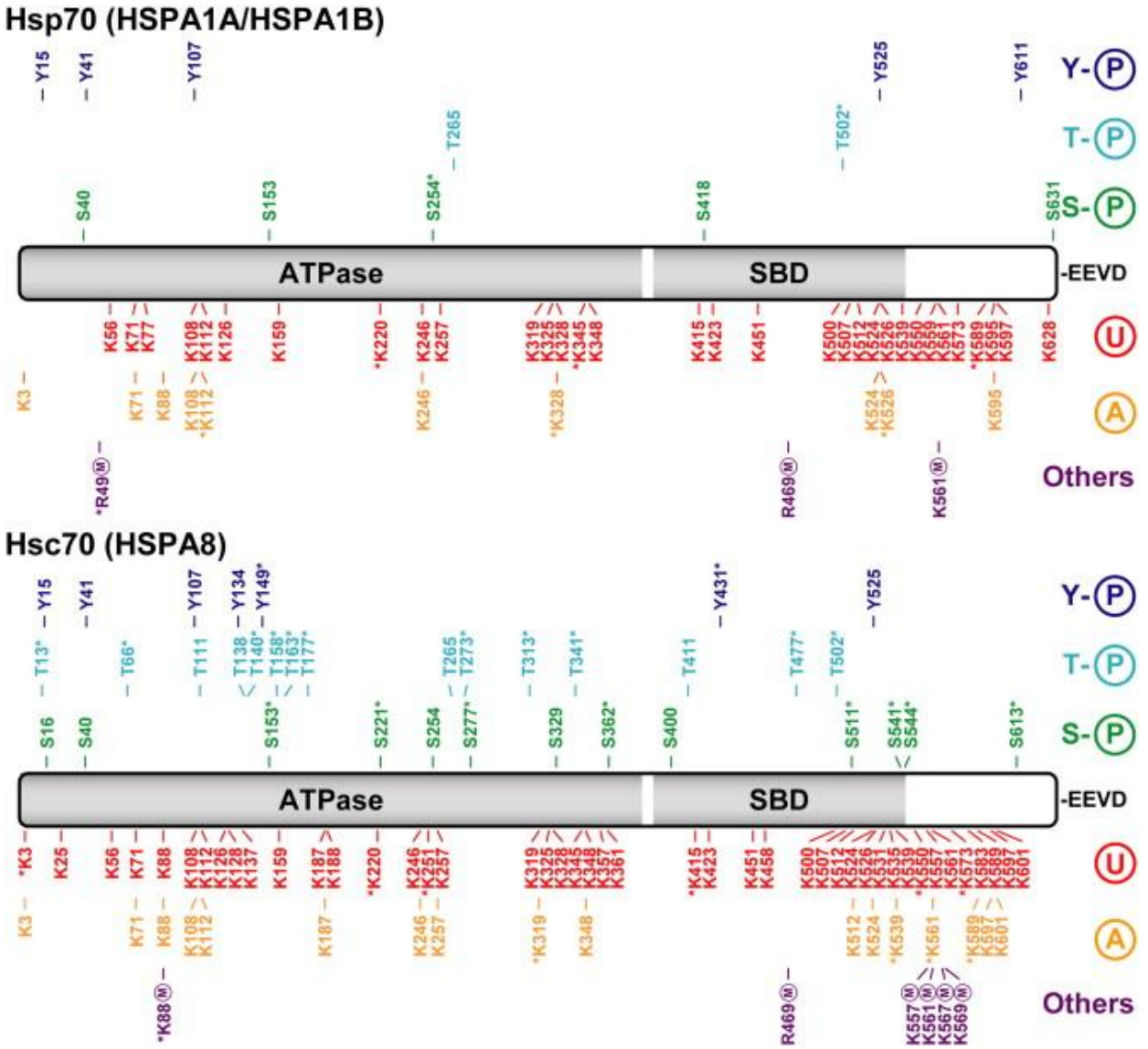
Faltung von Proteinen durch Chaperone

Biochemie des Reaktionszyklus von GroELS
Innere Oberfläche von GroEL variiert zwischen hydrophob/hydrophil nach Bindung des GroES Deckels.



Faltung von Proteinen durch Chaperone

In Eukaryoten gibt es Chaperonfamilien, die stark modifiziert sind – Spezifität!?



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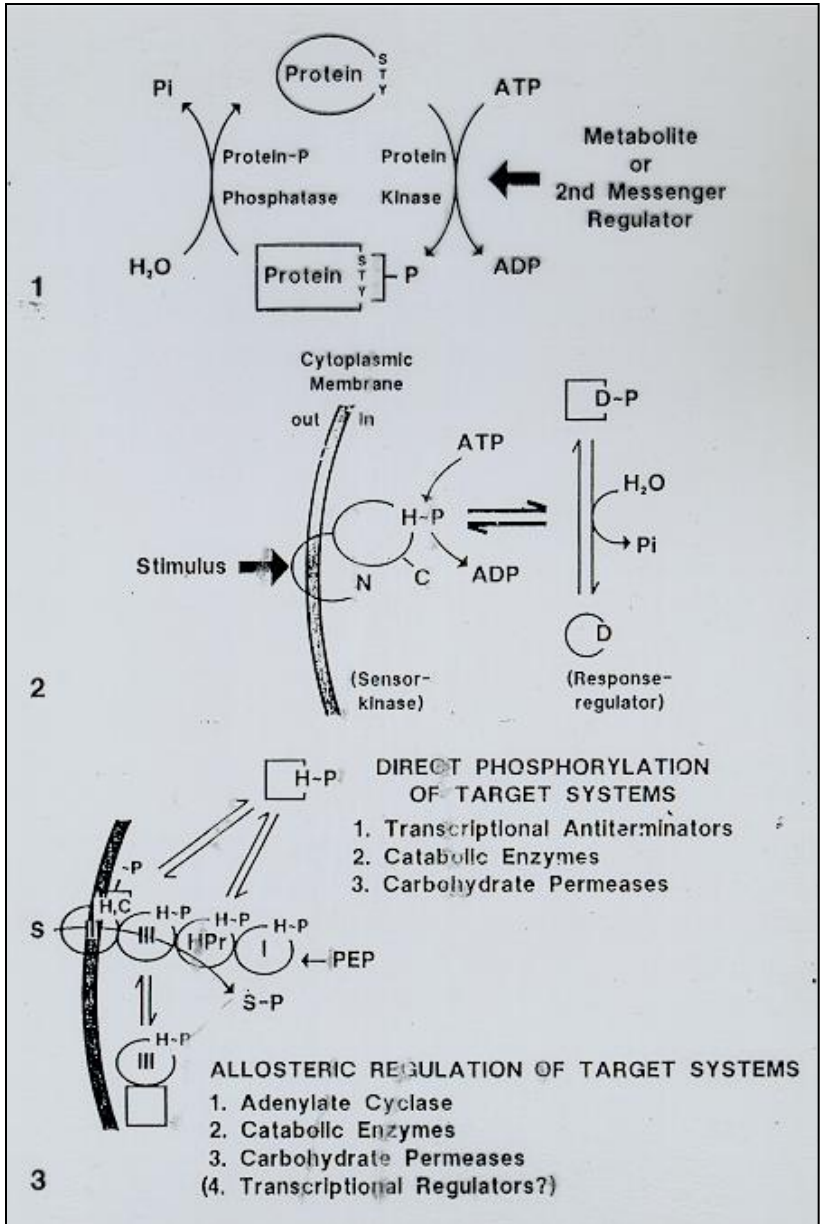
Posttranslationale Proteinmodifikationen

Proteinphosphorylierungen – regulatorische/metabolische (uvam.)



Metabolische Phosphorylierungen
Reversibel an Ser/Thr/Tyr-Resten
Kinasen mit ATP
z.B. Glycogensynthese
z.B. MAP-Kinasen

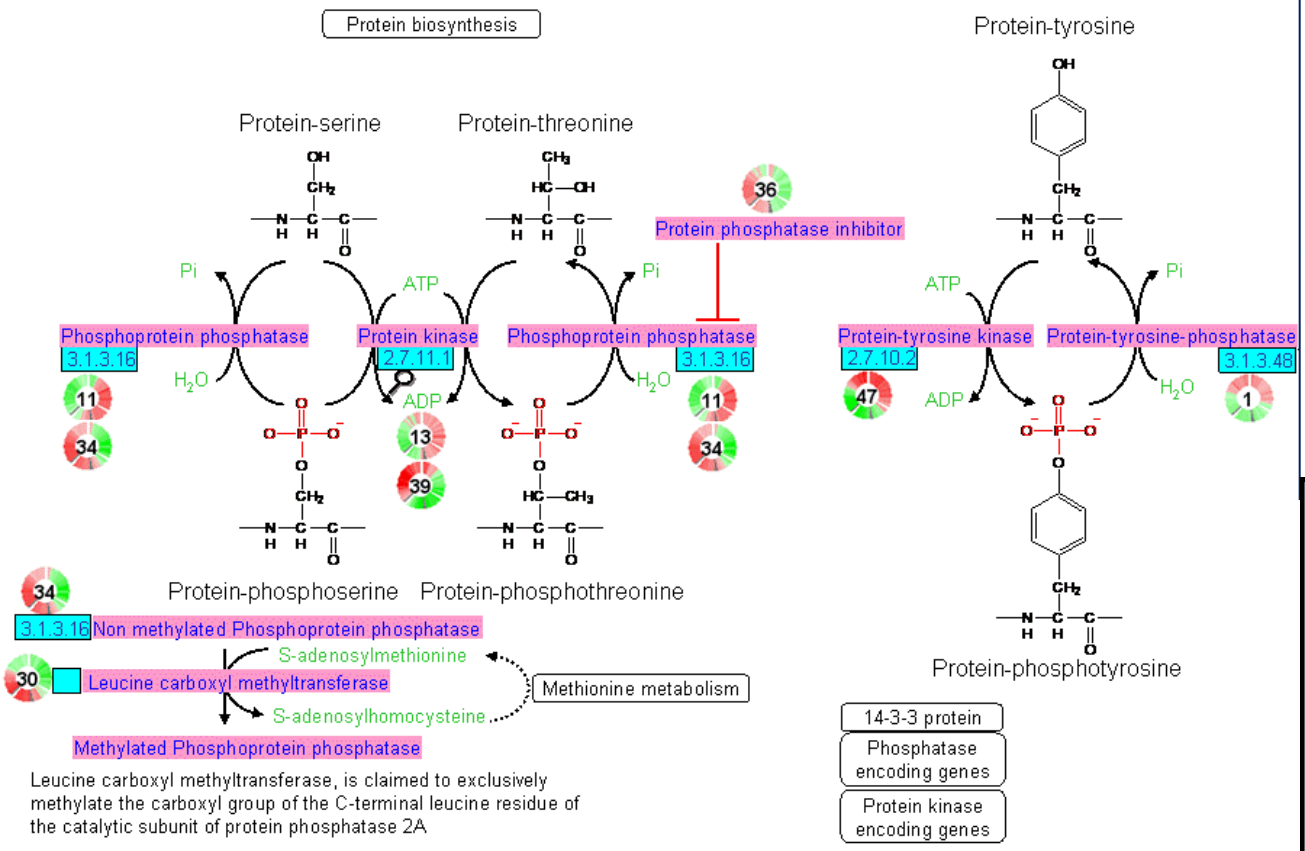
His/Asp in 2-Komponenetensystemen
Autophosphorylierung und Phosphorely

PTS-System durch PEP

Posttranslationale Proteinmodifikationen

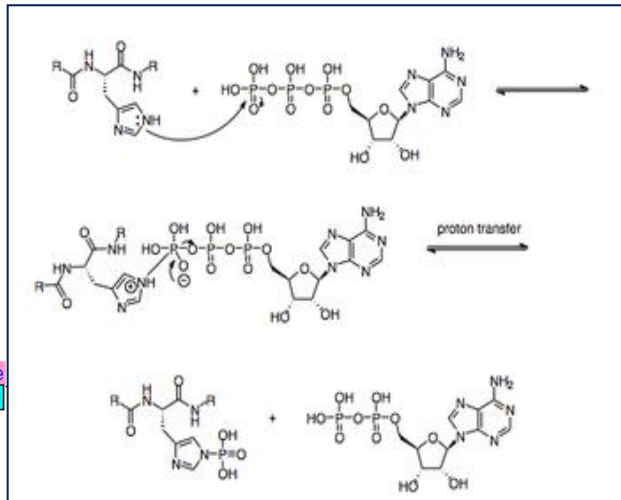
Proteinphosphorylierungen – Phosphoester an Ser/Thr/Tyr-Seitenketten

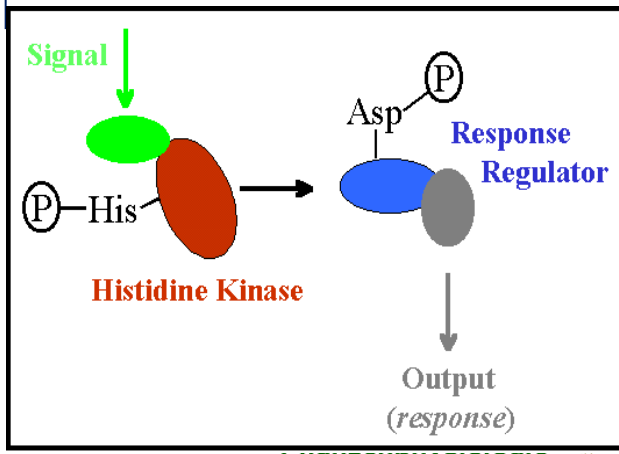
Protein phosphorylation



Leucine carboxyl methyltransferase, is claimed to exclusively methylate the carboxyl group of the C-terminal leucine residue of the catalytic subunit of protein phosphatase 2A

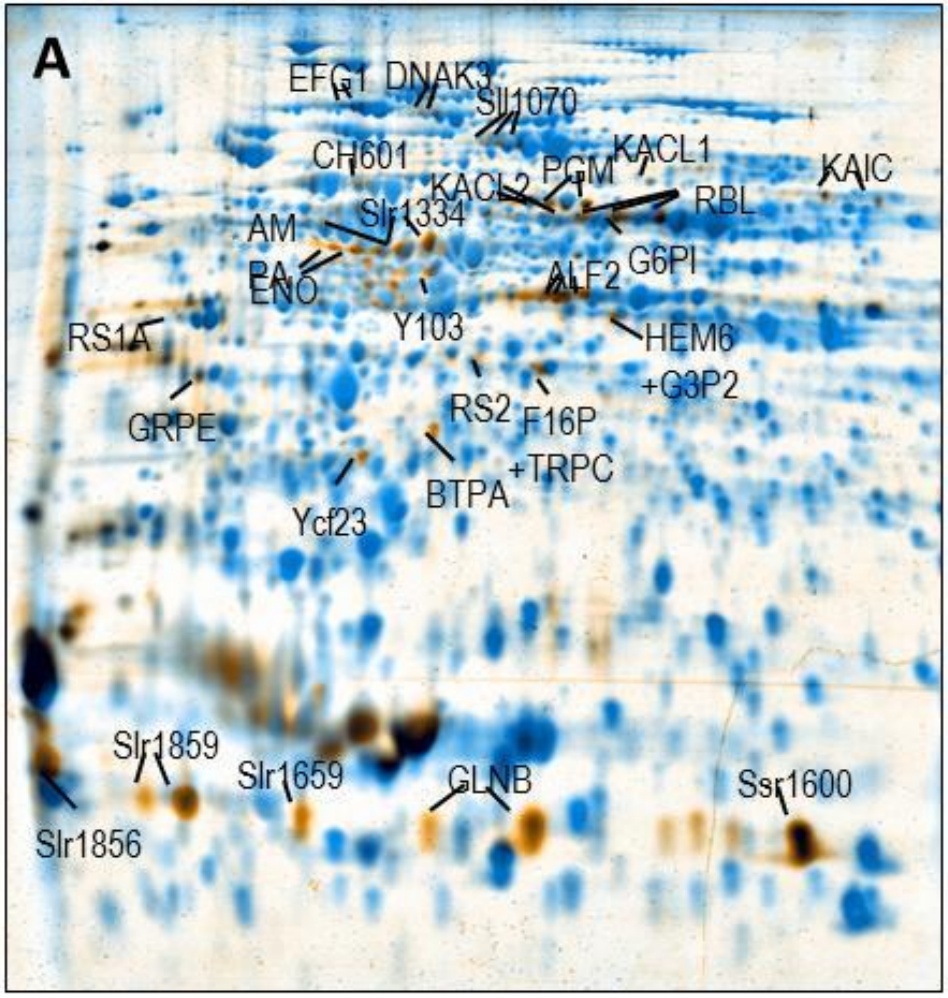
Histidin-Phosphorylierung

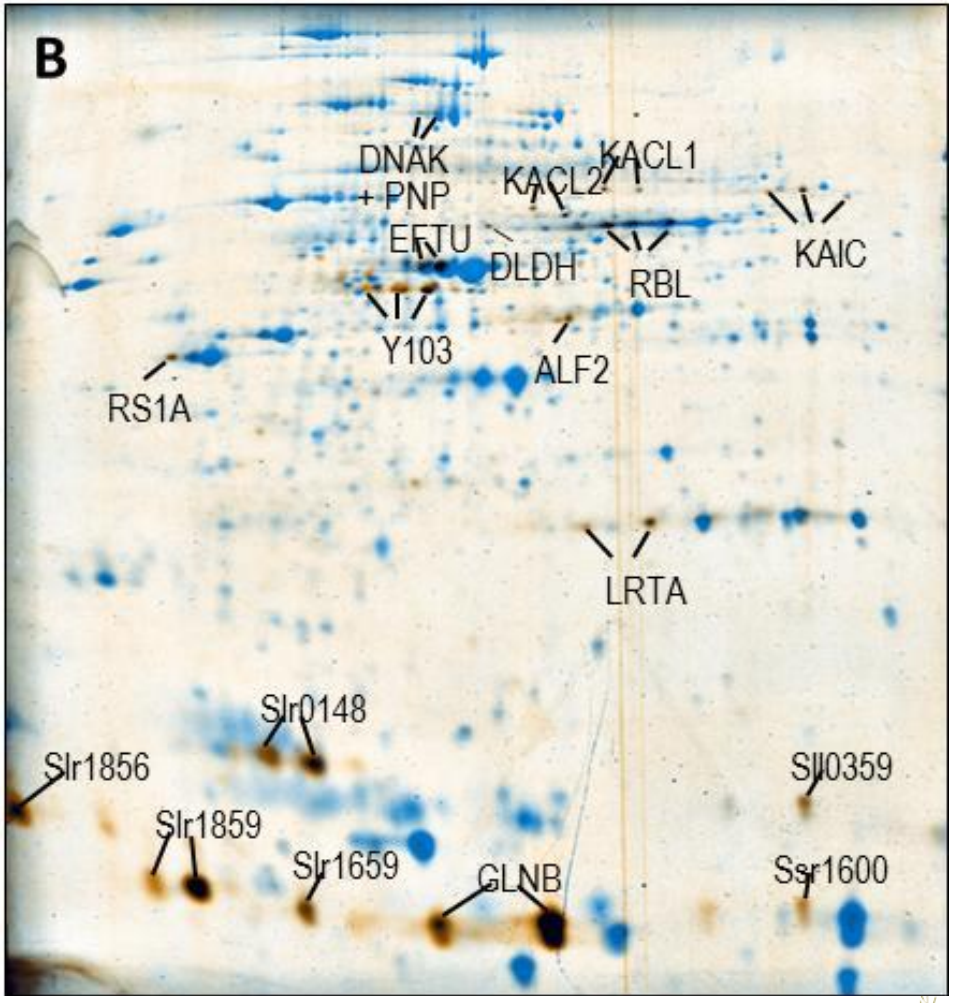




Posttranslationale Proteinmodifikationen

Proteinphosphorylierungsmuster in *Synechocystis* 6803





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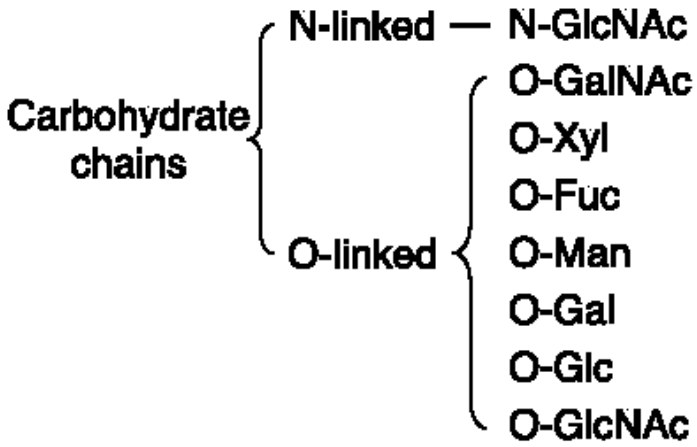
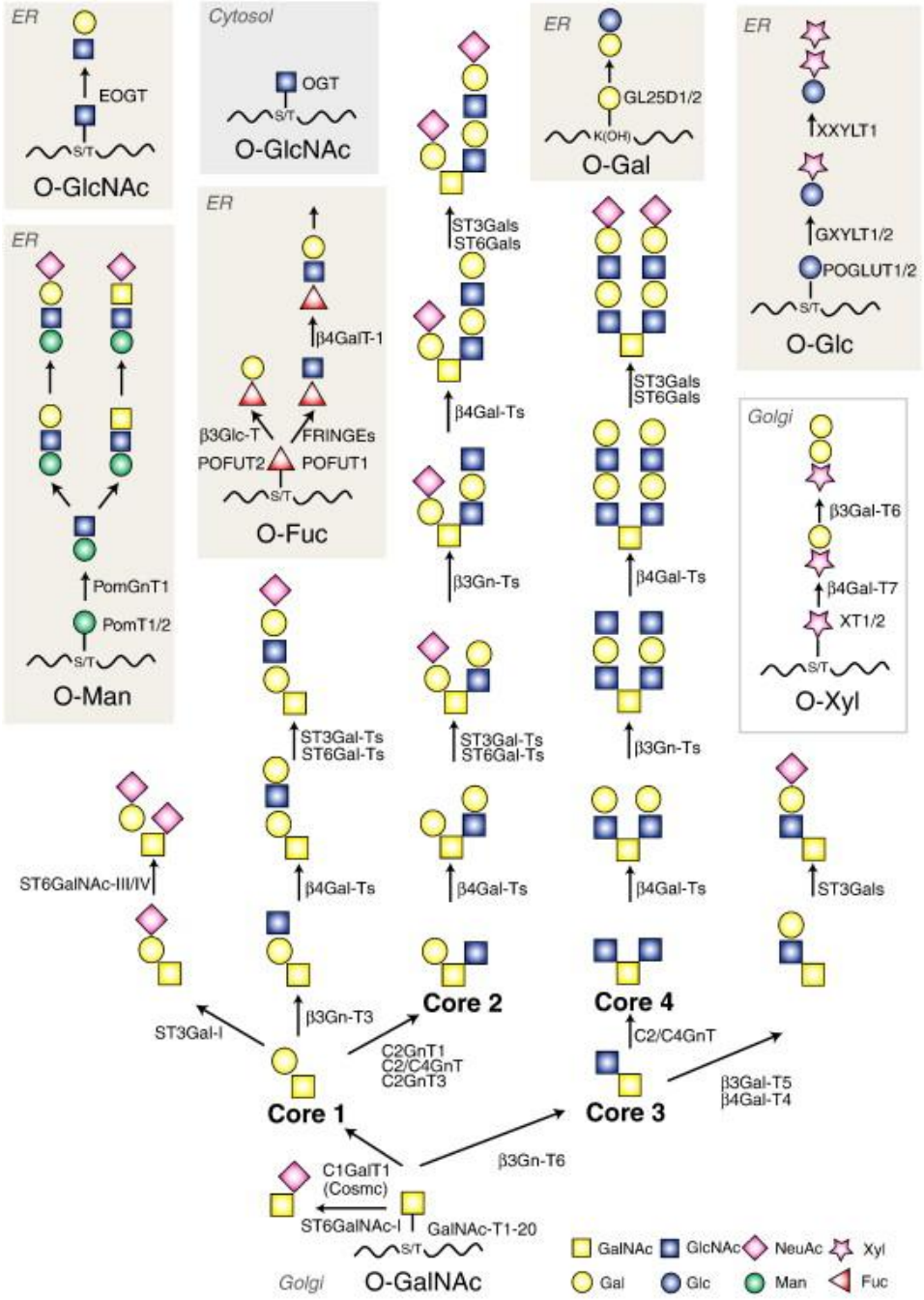
Posttranslationale Proteinmodifikationen

Proteinglykosylierung – meist via ER
Unterschiedliche Biochemie
Komplexe Funktion
Faltung, Stabilität, Zell/Zell-Kontakte





Posttranslationale Proteinmodifikationen

Bakterien – Protein-Deformylase (Formyl-Met zu Met)
Methionin-Aminopeptidase
Proteolyse – Pro/Präenzyme

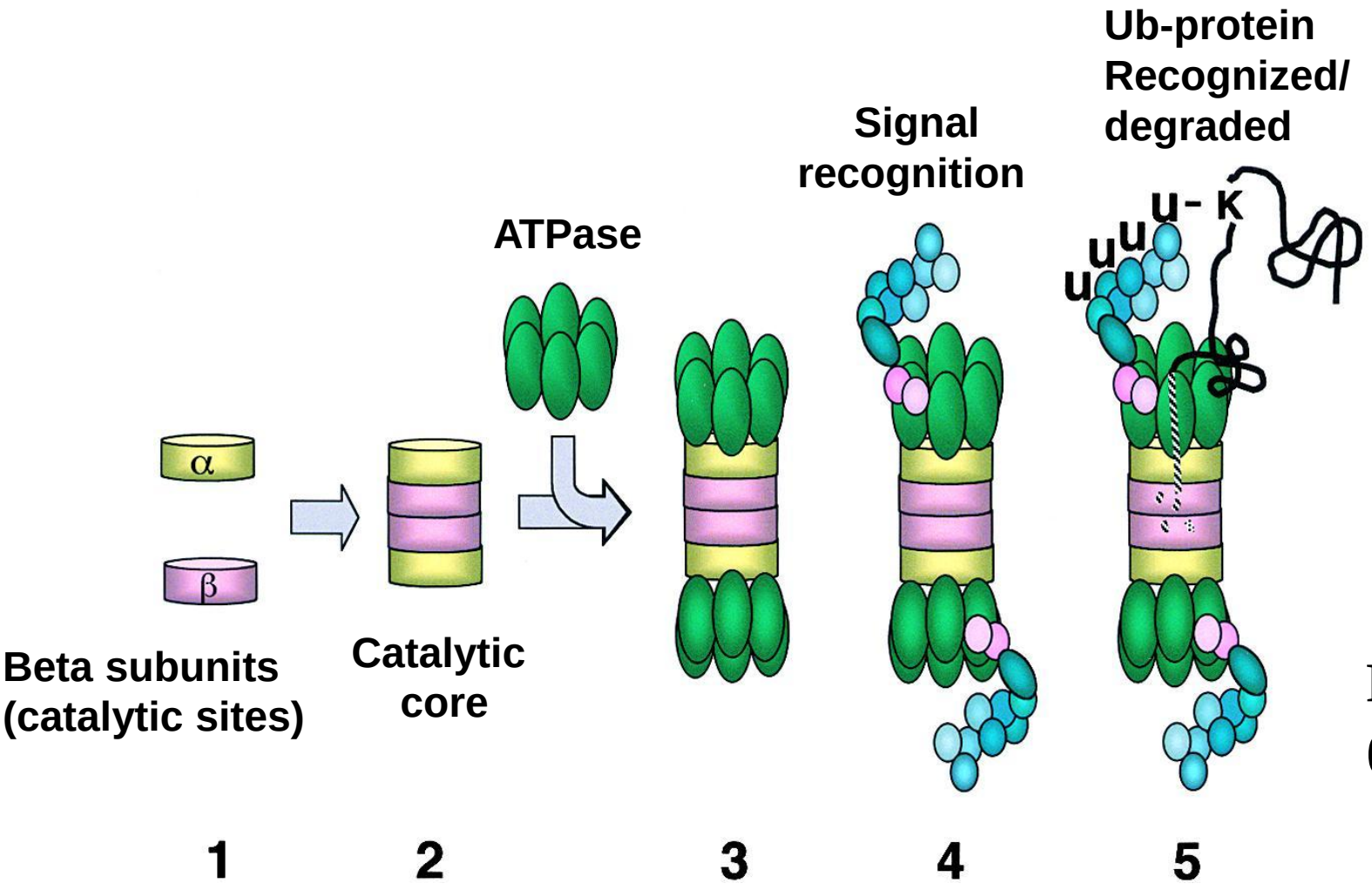
Tab. 15.3 N-terminale Aminosäuren

Amino-säure	Häufigkeit absolut, (Prozent)	N-terminale Acetyl-Gruppe (Anzahl der Proteine)
Methionin	25 (30%)	13
Serin	25 (30%)	16
Alanin	21 (25%)	15
Glycin	9 (11%)	4
Valin	6 (7%)	0
andere	2	1

Untersuchungen entstammen einer Kollektion von 84 Wirbeltier-Proteinen [aus 2].

Proteinabbau

Proteine werden häufig am so-genannten Proteasom abgebaut



Verma and Deshaies (2000) Cell 101, 341-344

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Proteinabbau

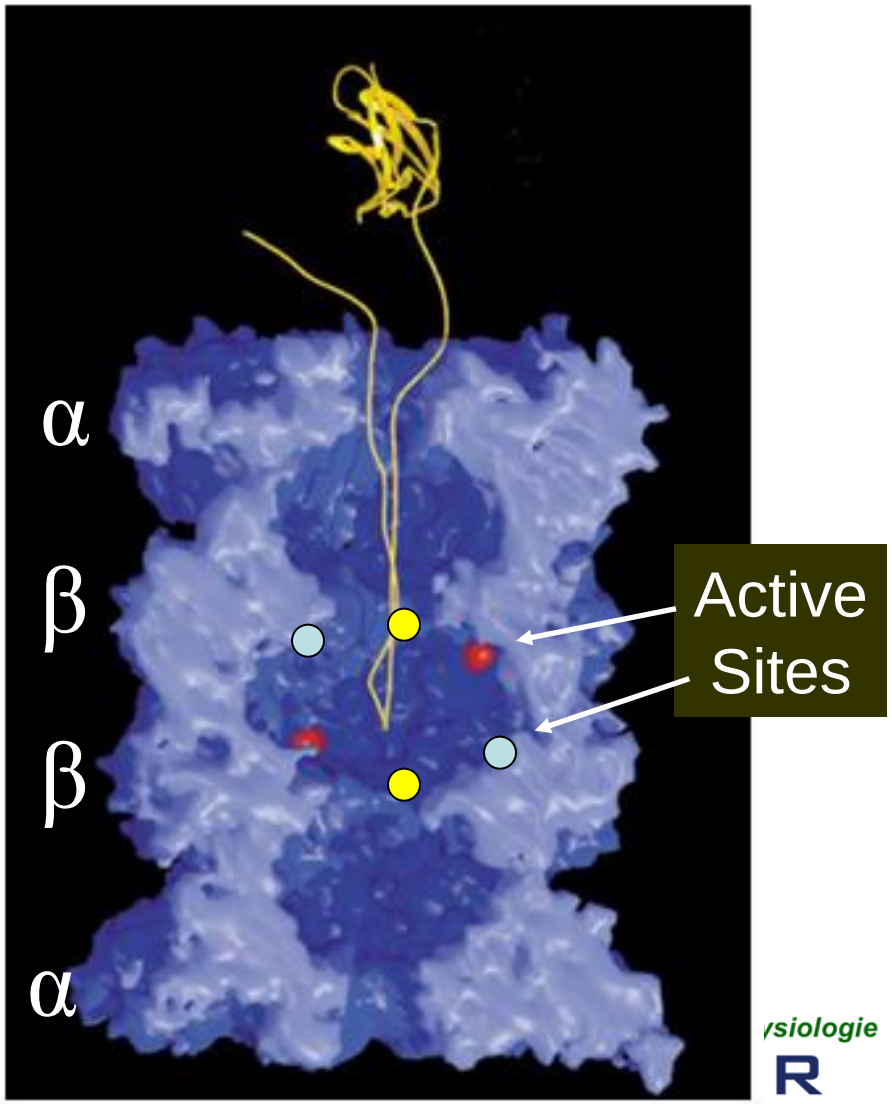
Proteinabbau erfolgt an entfalteten Proteinen in der 20S UE

Controlled Proteolysis by the Proteasome:

*3 pair of active sites sequestered in the interior chamber

*Each active site performs different cleavage

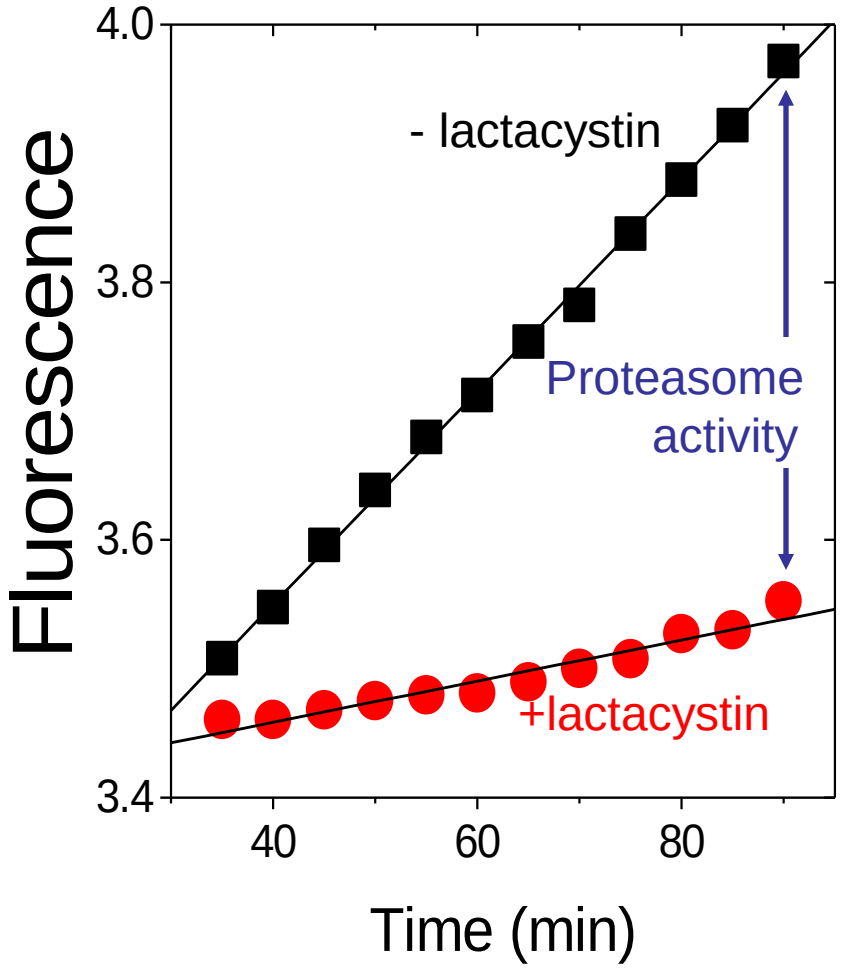
*Proteins must be unfolded to gain access to the active sites



Rape and Jentsch (2002) *Nature Cell Biol 4*

Proteinabbau

Proteinabbau erfolgt mit unterschiedlichen Spezifitäten



Activity	Peptide	Subunit
Chymotrypsin-like	LLVY-AMC	
	AAF-AMC	β5
Trypsin-like	LSTR-AMC	β2
Peptidylglutamyl hydrolyzing	LLE-AMC	β1

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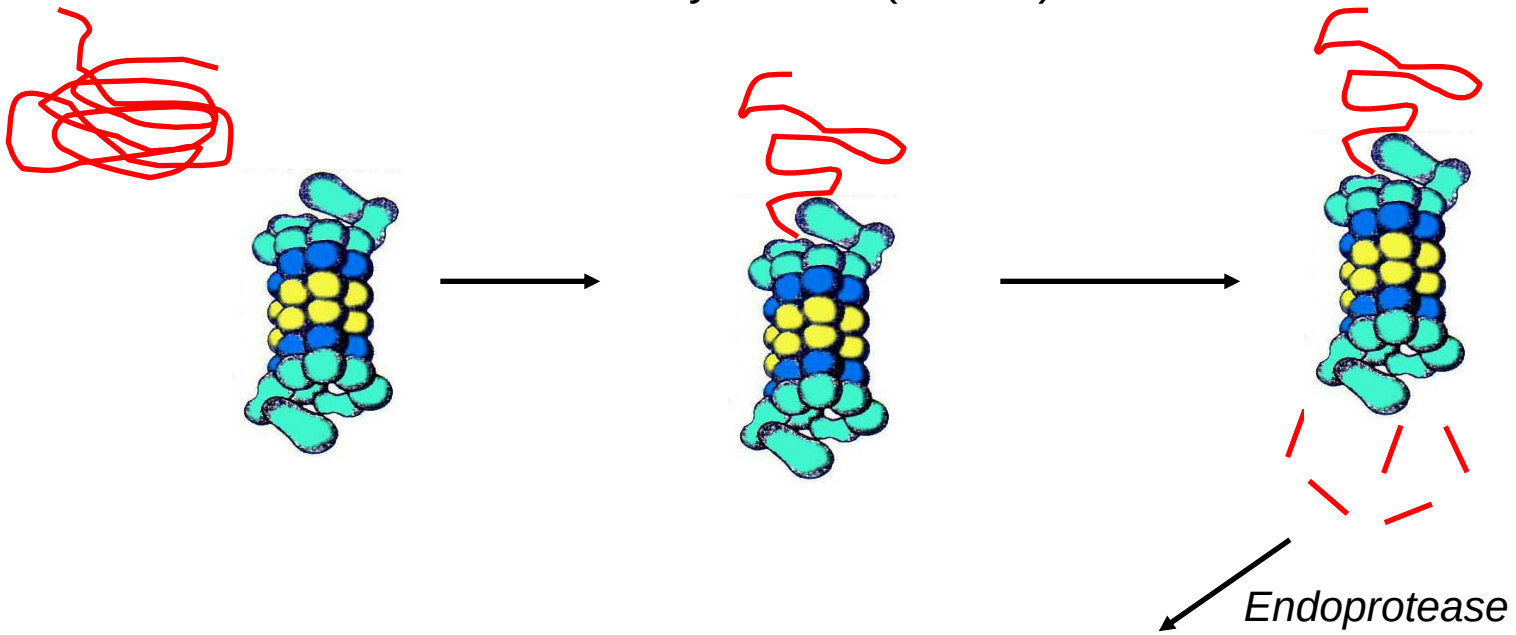
Proteinabbau

Proteinabbau erfolgt zu Peptiden, die anschließend weiter abgebaut werden

1. Protein targeted to the proteasome for degradation

2. Protein **unfolded** and fed into the catalytic core (PA700)

3. Protein degraded to short peptides



4. Endoproteases degrade peptides to amino acids

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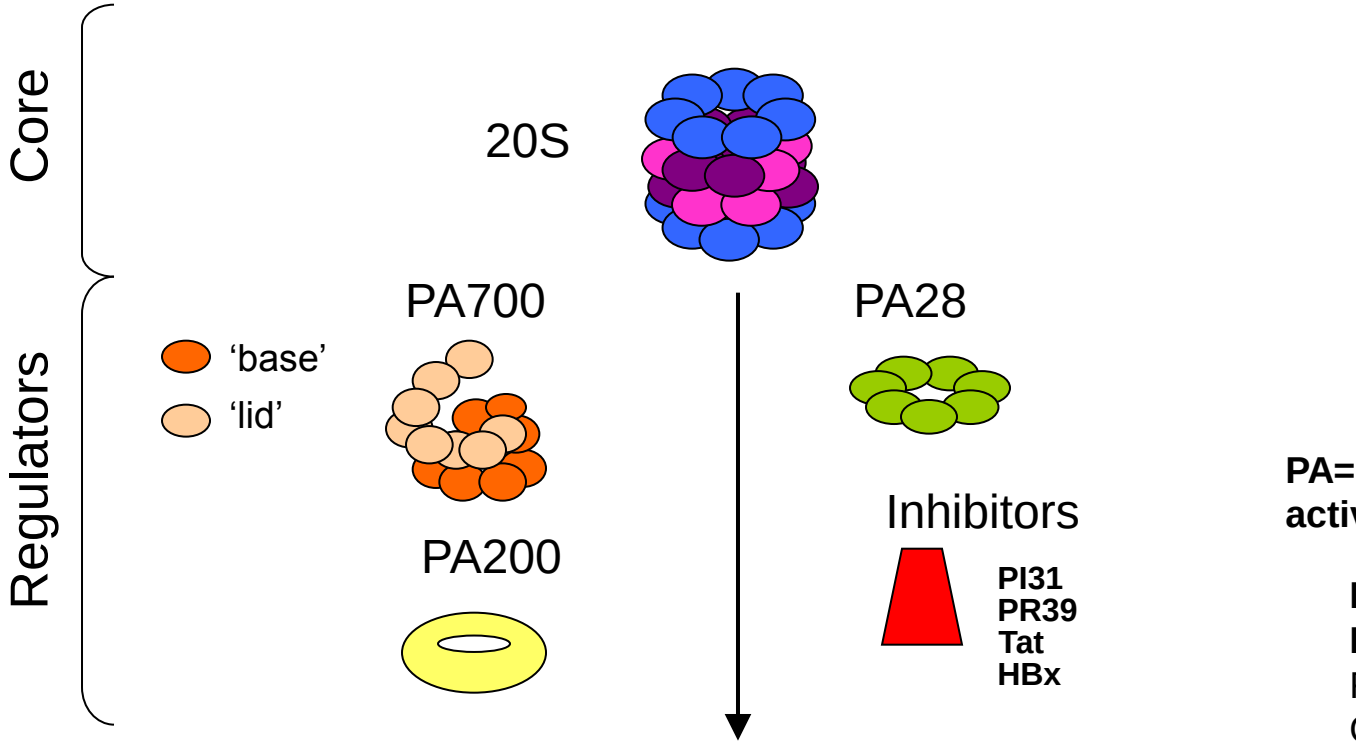
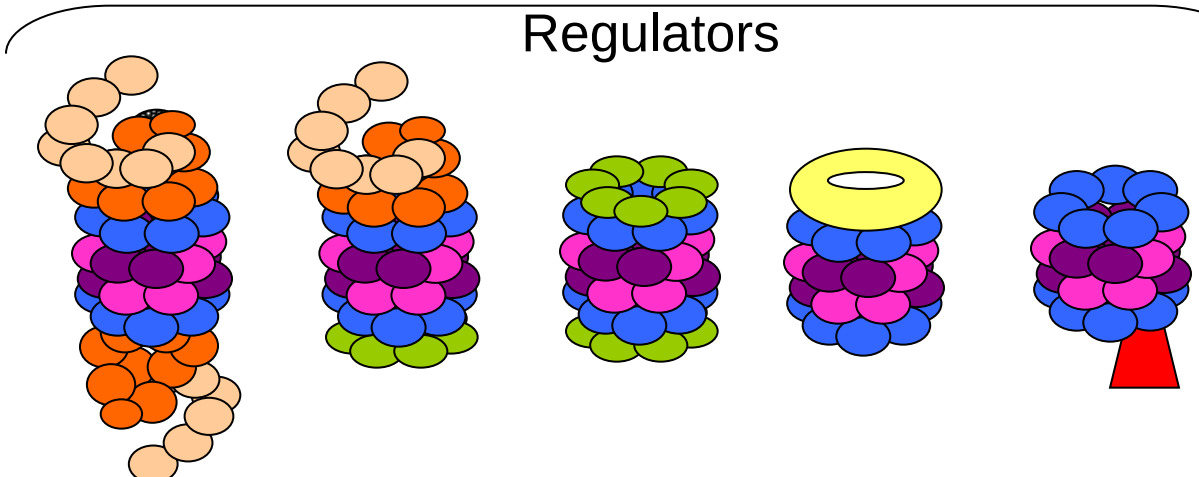
Regulation der Aktivität des Proteasome: Assoziation mit regulatorischen Proteinen (Phosphoryl. u.a.)



PA= proteasome activators

Micromolecular Regulators:
Fatty acids
Oxidative modif
Glutathione



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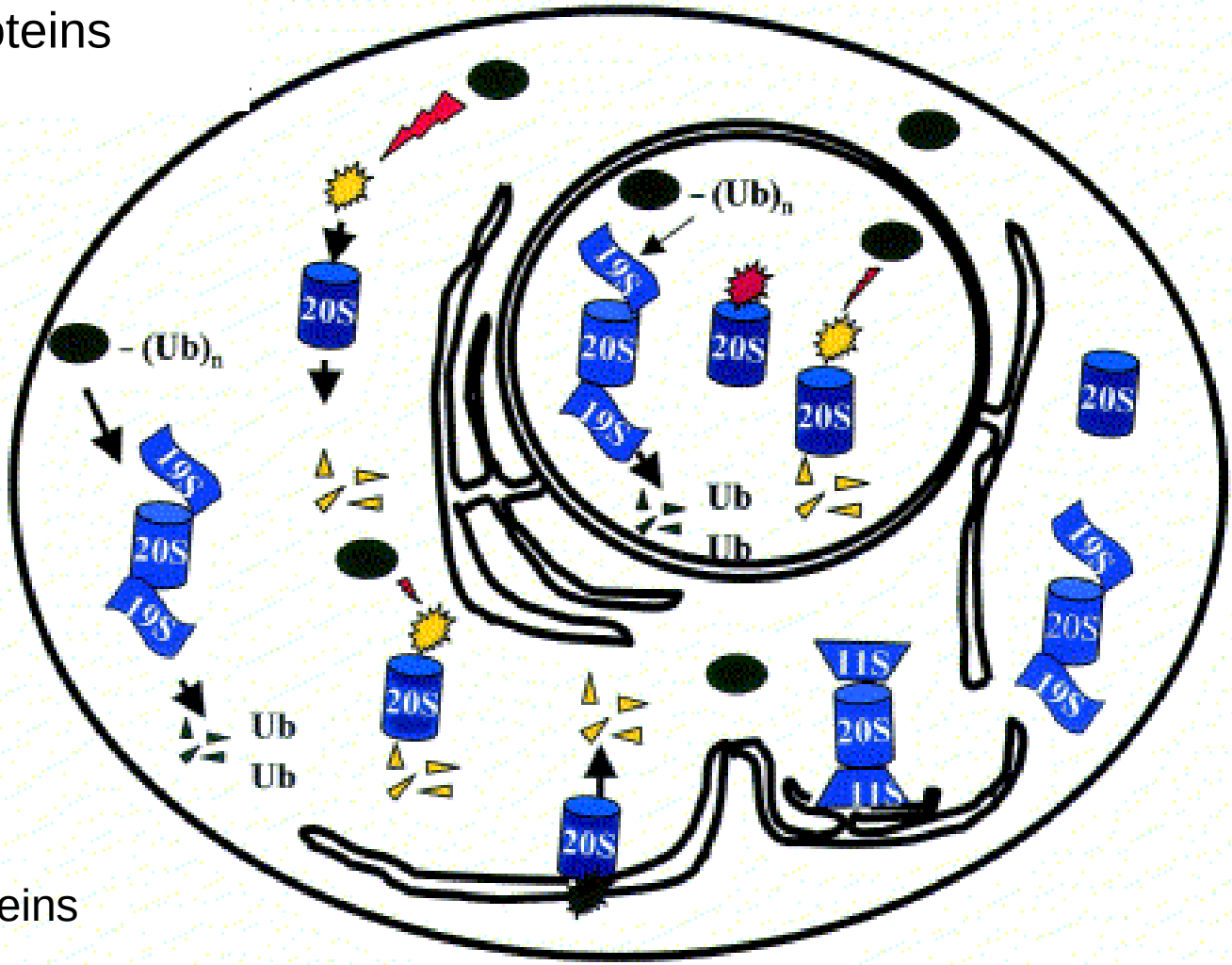
Zelluläre Lokalisation des Proteasoms

~30,000 / cell

Cytoplasm:
Ub and oxidized proteins

ER (outside):
Misfolded proteins
(newly synthesized)
ERAD

Nucleus:
Transcription
factors
Histones



Peptides

(-Ub) Normal proteins

Moderately oxidized proteins

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Spezifität des Proteinabbaus am Proteasom

What "signals" in protein substrates are recognized by the proteasome and initiate their destruction?

DEGRON- An element that is necessary and sufficient for substrate recognition.

1. Specific amino acid sequence (motif)
(ex., PEST, destruction boxes- KEN or RXXL)

2. Structural motifs (ex., Oxidative modification, exposure of hydrophobic amino acids)

3. Covalent attachment of ubiquitin

**Phosphorylation of the substrate is often an early event in each signal (*links Ub with other cellular events*).

Spezifität des Proteinabbaus am Proteasom

1. Specific amino acid sequence (motif)

Protein turnover determined by the amino acid sequence.

Cryptic *degrons*- the protein structure must be altered for the signal to be exposed.

PEST proline, glutamic acid, serine or threonine, flanked by charged Amino acids

Fos KVEQLSPEEEEK

ODC HGFPPEVEEQDDGTLPMSCAQESGMDR

Destruction boxes-

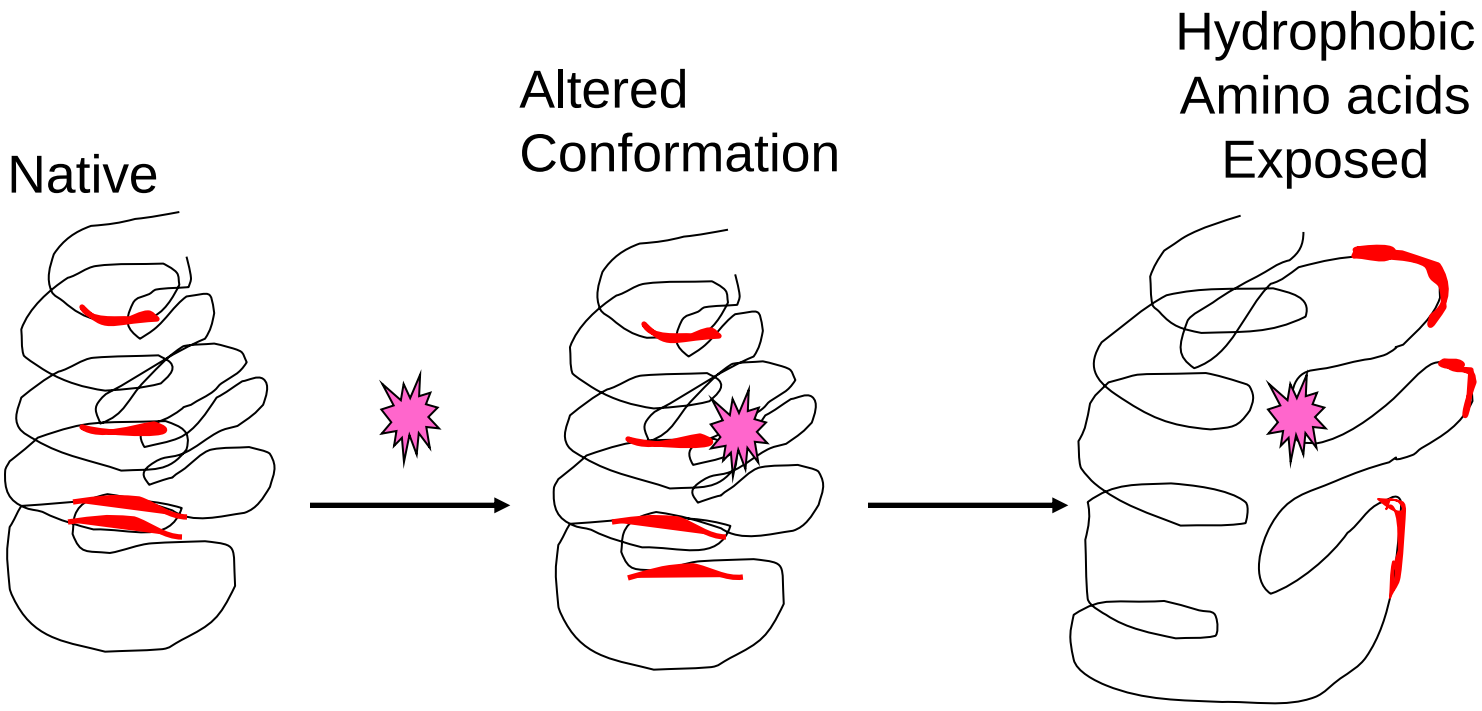
KEN KENXXXN/D

RXXL

} In the N or C terminus of a protein

Spezifität des Proteinabbaus am Proteasom

2. Structural motifs (ex., Oxidative modification, exposure of hydrophobic amino acids)

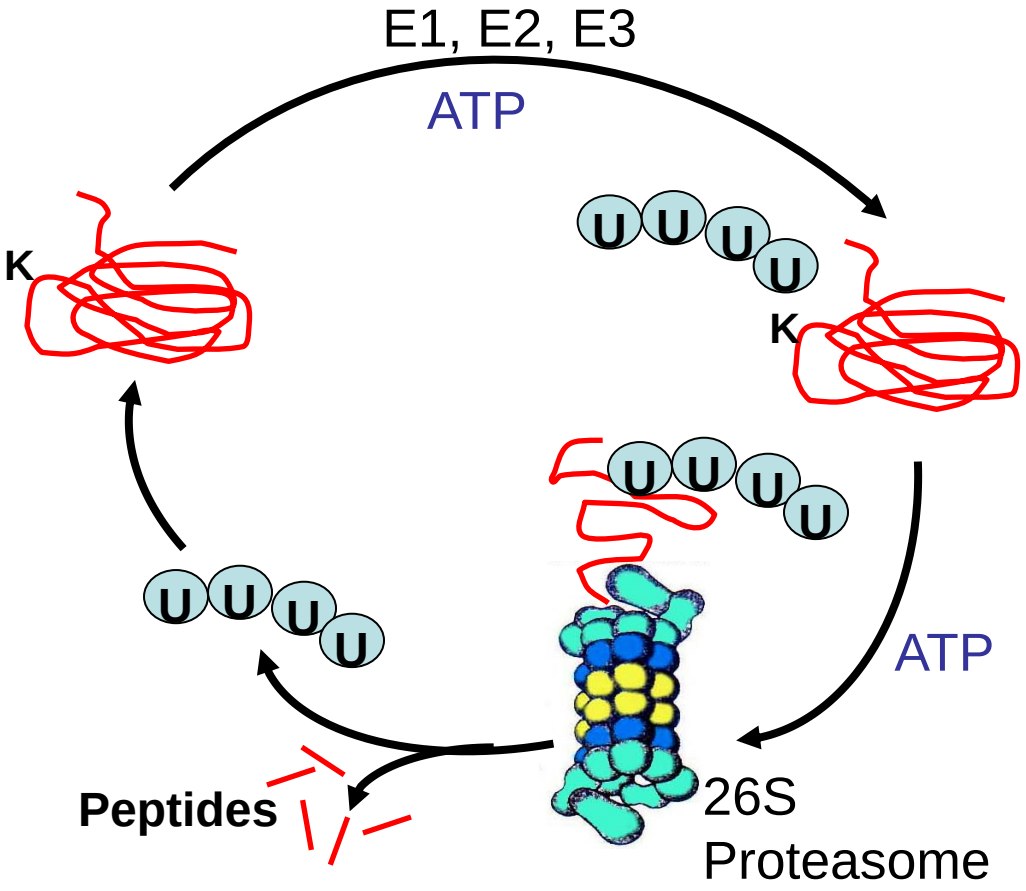


* Hydrophobic patches are recognized by the proteasome.

Spezifität des Proteinabbaus am Proteasom

3. Covalent attachment of ubiquitin

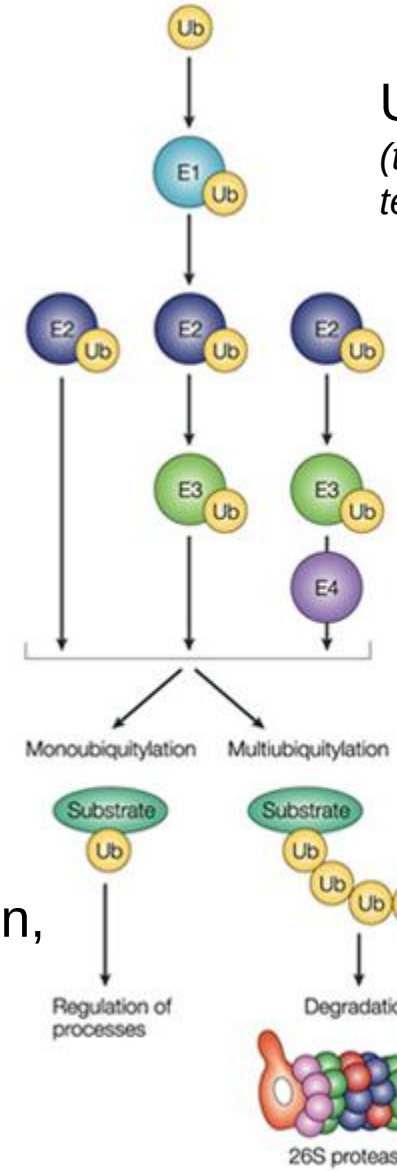
Ubiquitin (Ub) 76 amino acids (8.5 Kda)



Ubiquitin is recycled: Ub removed from the protein substrate by deubiquitinating enzymes.

Anheften der Ubiquitinreste an das Zielprotein

Ubiquitin conjugation to a Lys of proteins requires the sequential reaction of multiple enzymes.



Ub-activating Enzyme
(thio-ester linkage between E1 Cys and C-terminal Gly of Ub)

Ub-conjugating Enzyme
(transfer of Ub to E2 Cys)

Ub ligase (***Recognizes substrates***)
(Ub transferred to substrate- linkage C-terminal Gly (Ub) to E-amino Lys (substrate))

Ub chain-assembly factor
(Ub linkage C-terminal Gly to Lys)

Monoubiquitination can regulate protein activity (calmodulin), transcription, or target a protein for DNA repair or Endocytosis.

Four Ub (tetraubiquitin) is the minimum Ub required for proteasome proteolysis.

Jesenberger and Jentsch (2002) Nature Rev/Mol Cell Biol 3

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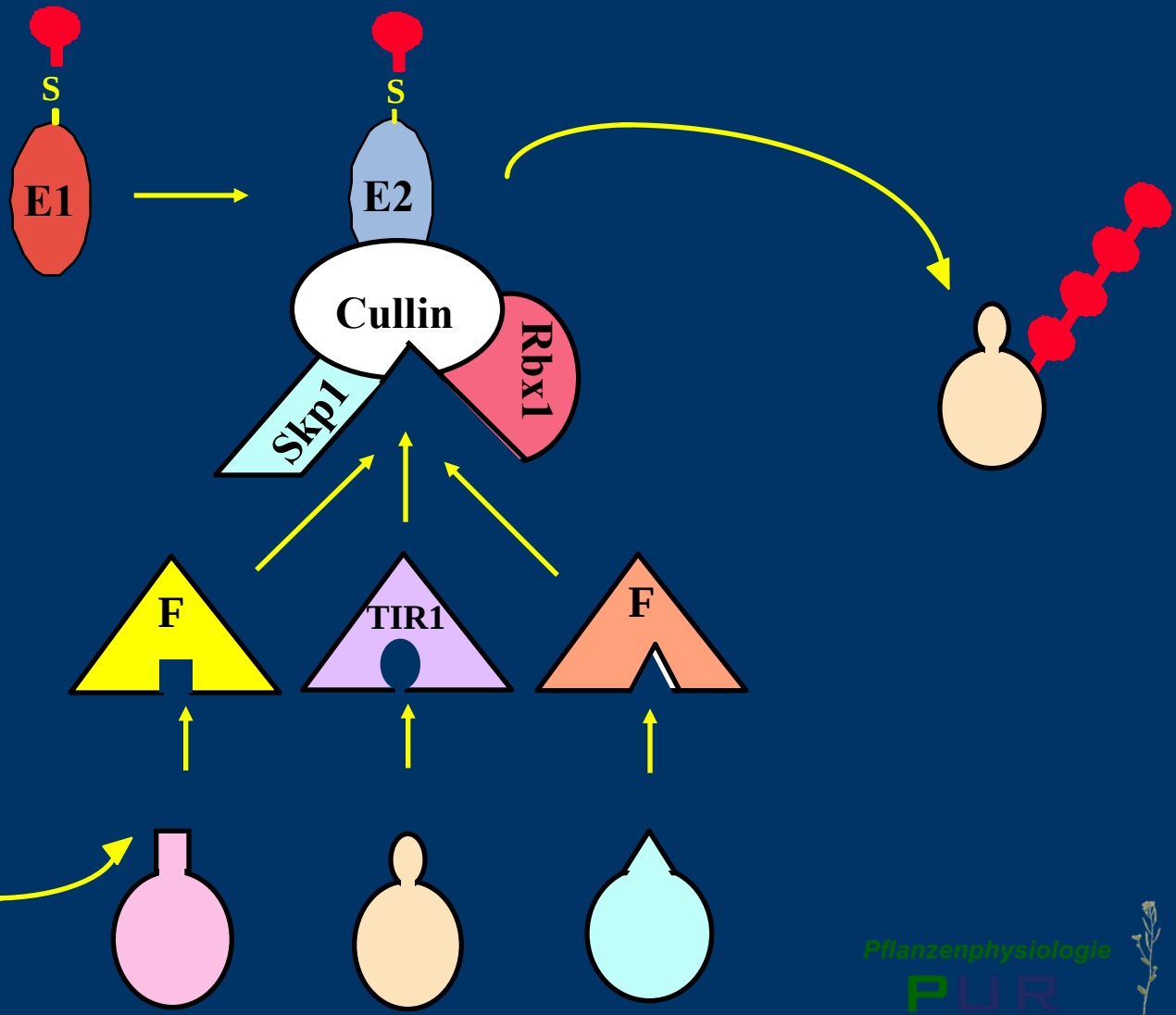
The SCF ubiquitin ligase model

E1- one
E2- many
E3- many more!

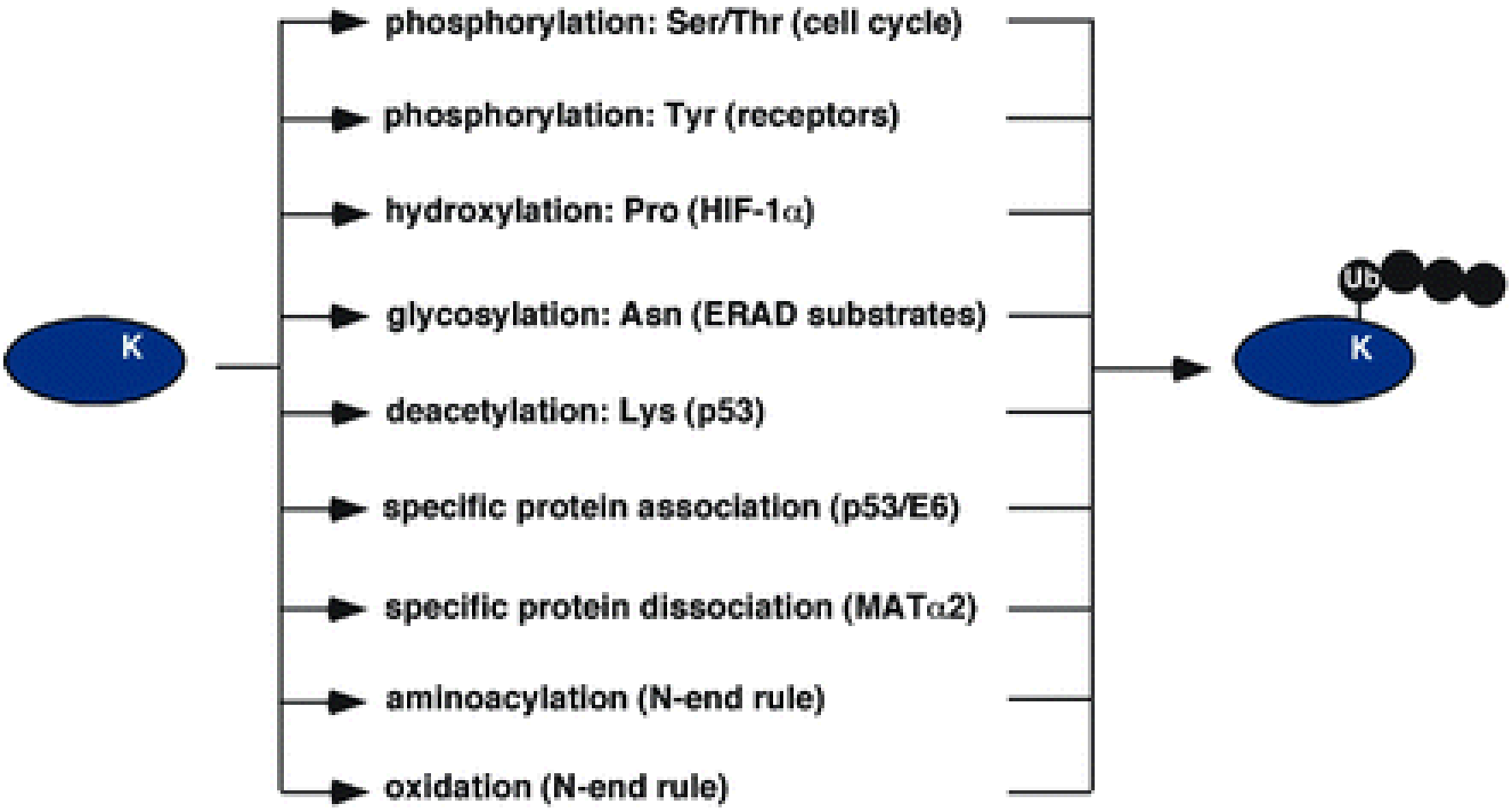


Modular construction of RING-domain E3s allows flexibility in substrate recognition.

SCF=Skp/cullin/F-box

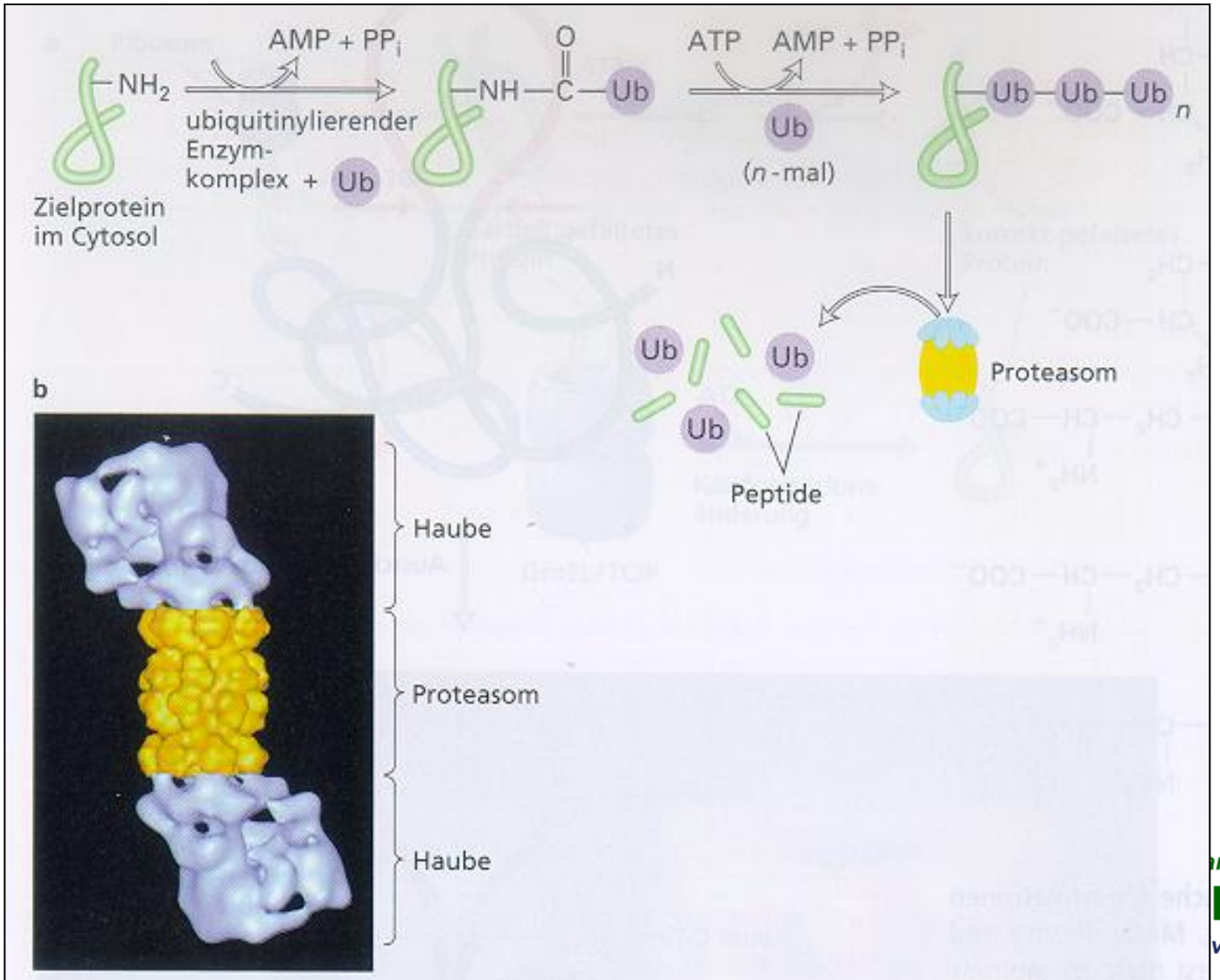
Proteinabbau

Mechanismen für Modulierung des Substraterkennung durch die E3-UE



Zusammenfassung Proteinabbau

Proteine werden häufig durch Bindung des Proteins Ubiquitin markiert
Versch. Konjugierungskomplexe mit versch. Erkennungssequenzen
(z.B. PEST – Prolin, Gutaminsäure, Serin, Threonin)



Innere Uhr – molekulare Mechanismen in Cyanobakterien

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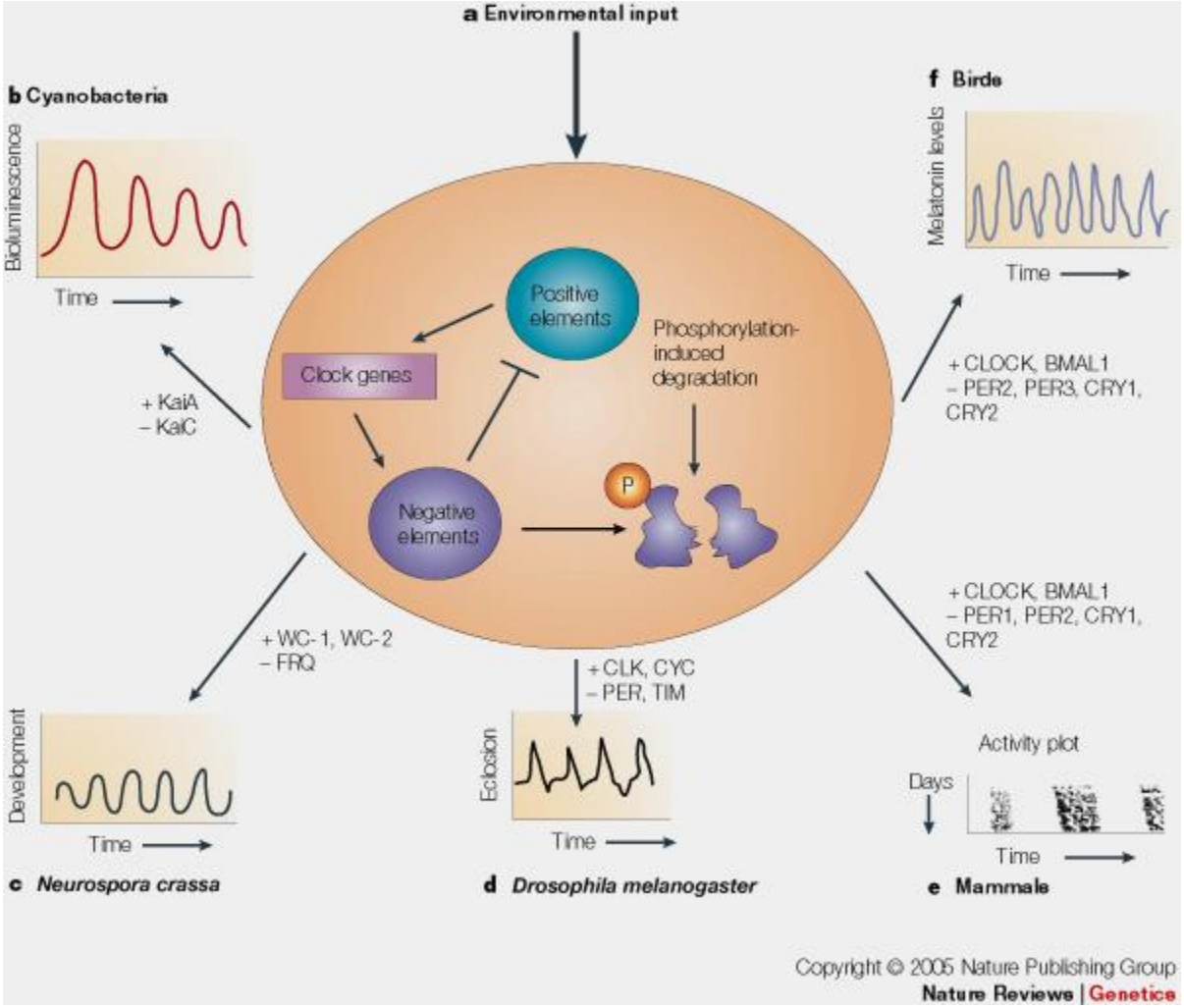
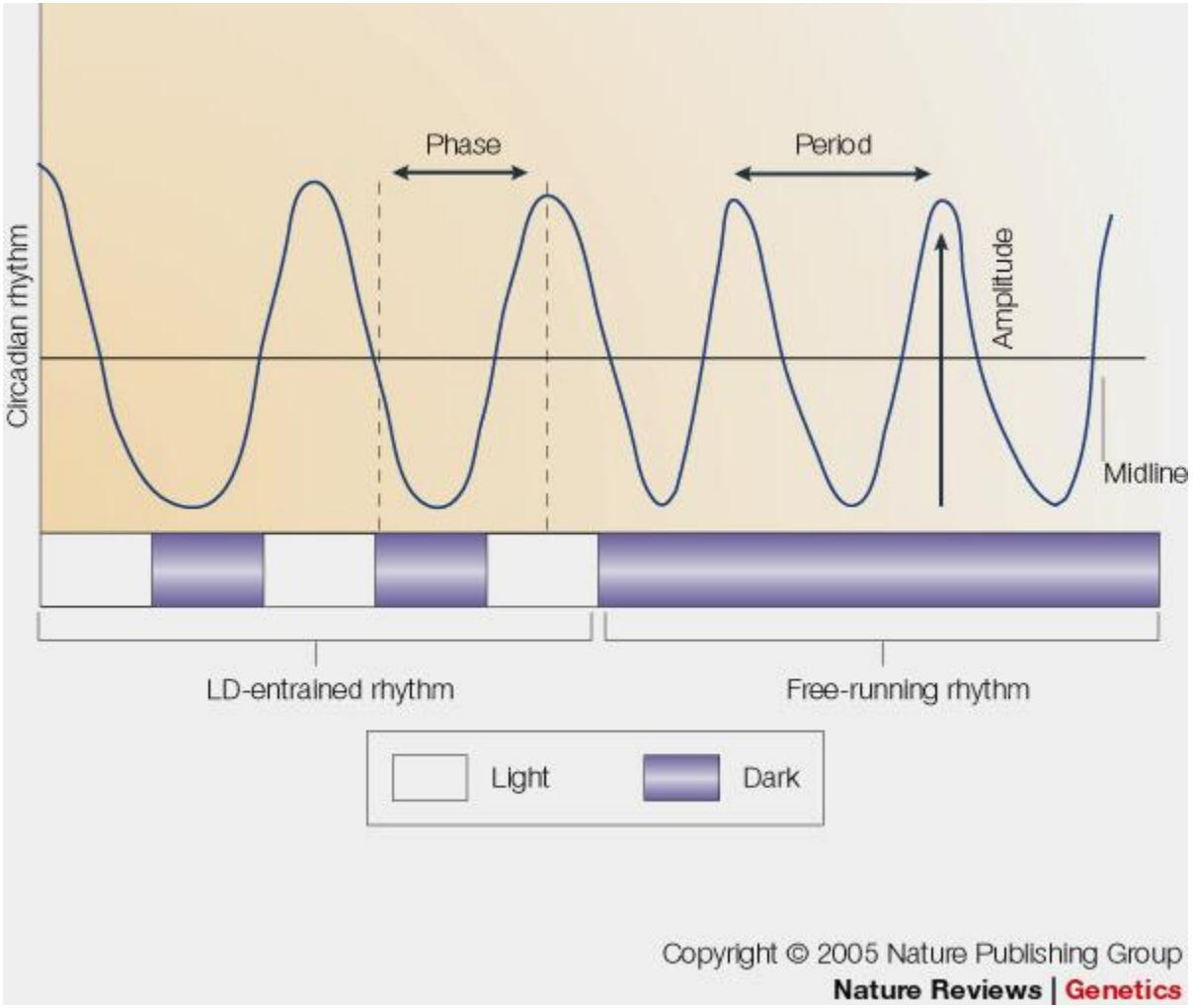
Innere Uhren gibt es in allen Zellen von Eukaryoten und Cyanobakterien
Modelle umfassen Genexpression, Proteininteraktion, Proteinstabilität, Proteinmodifikation



Merkmale der Inneren Uhr
Verstellbar, Temperaturkompensiert!!



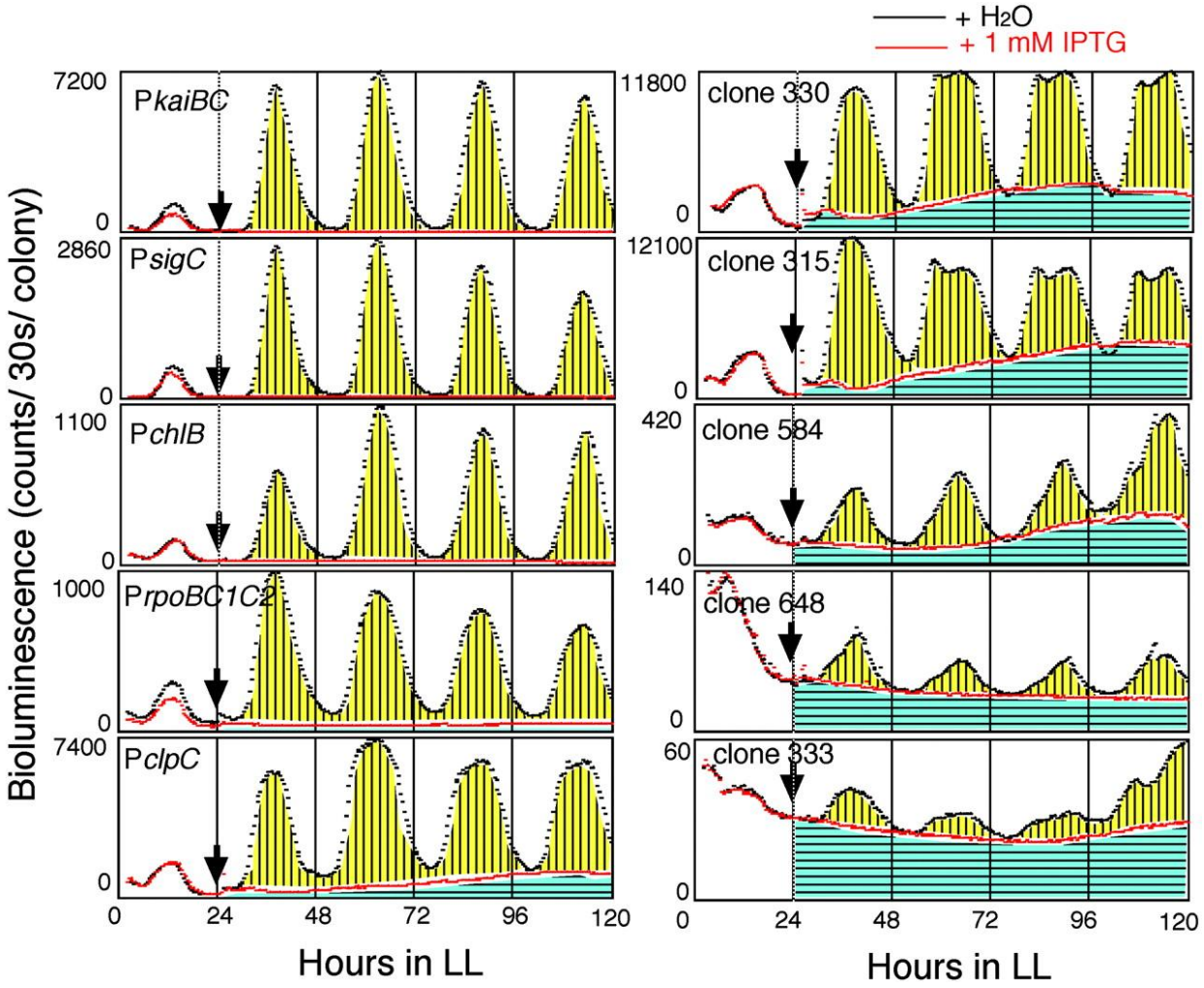
Ursprünglich – einzellige marine Cyanobakterien, die im Tag/Nacht-Rhythmus Photosynthese und N_2-Fixierung regulieren

Im Cyanobakterium *Synechococcus* sp. Stamm PCC 7942 ist die innere Uhr gut untersucht.
Innere Uhr ist langsamer als Generationszeit!!!

Kondo, Nagoya; Golden, Texas, Johnson, Tennessee
Mutanten – viele Gene identifiziert, KaiABC, entscheidend
Automatische Verfolgung der Genexpression
Genomsequenzierung
Proteinkristallisierung
Etc.

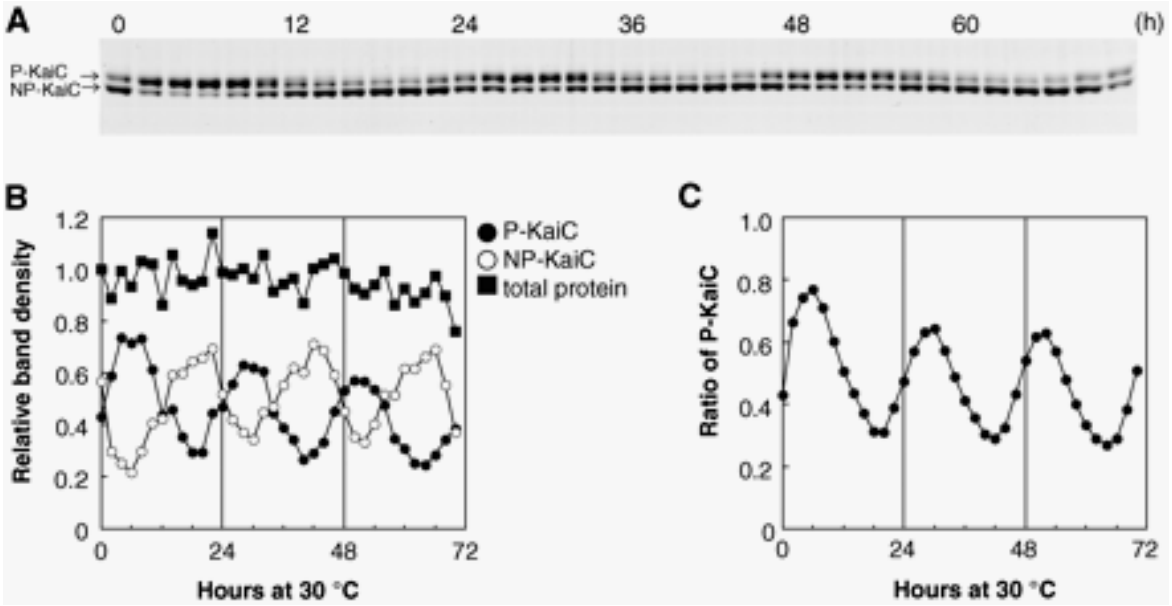
Vorteil der inneren Uhr – nur im Konkurrenzexperiment

Kai-proteins repress circadian rhythms of promoter activities



Nakahira, Yoichi et al. (2004) Proc. Natl. Acad. Sci. USA 101, 881-885

Eine Mixtur aus KaiC, KaiA, KaiB und ATP zeigt im Reagenzglas eine rhythmische KaiC-Phosphorylierung/Dephosphorylierun – Oscillator!!!



Nakayima et al., Kondo, Science 2005

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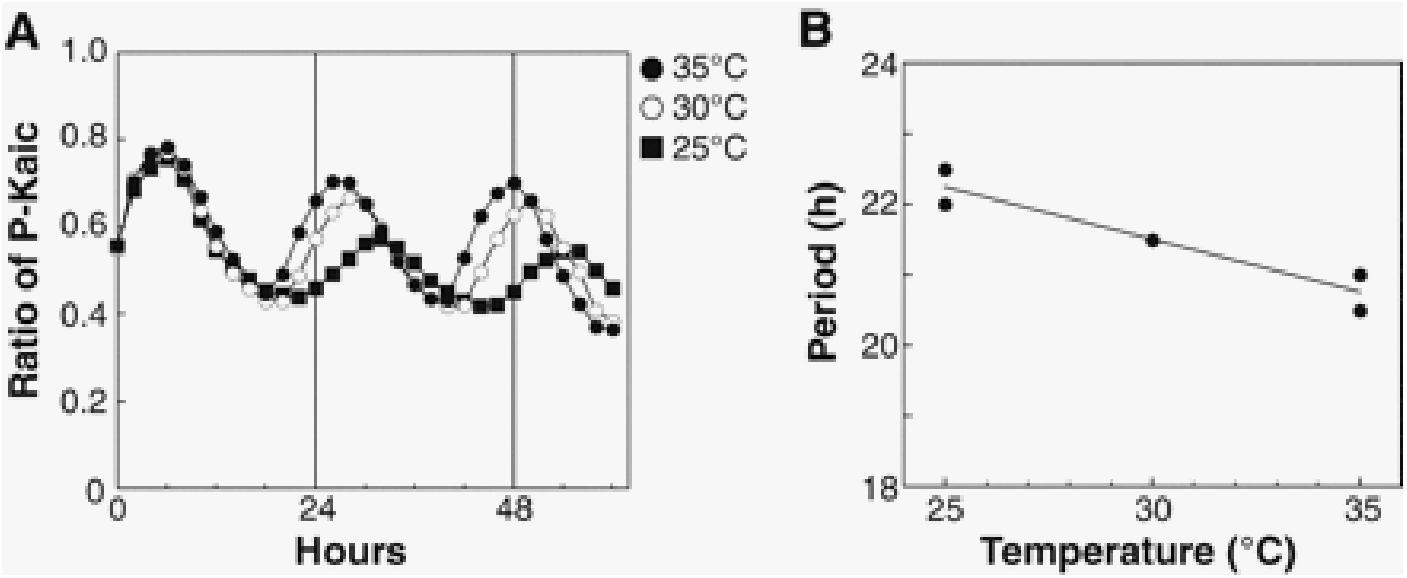
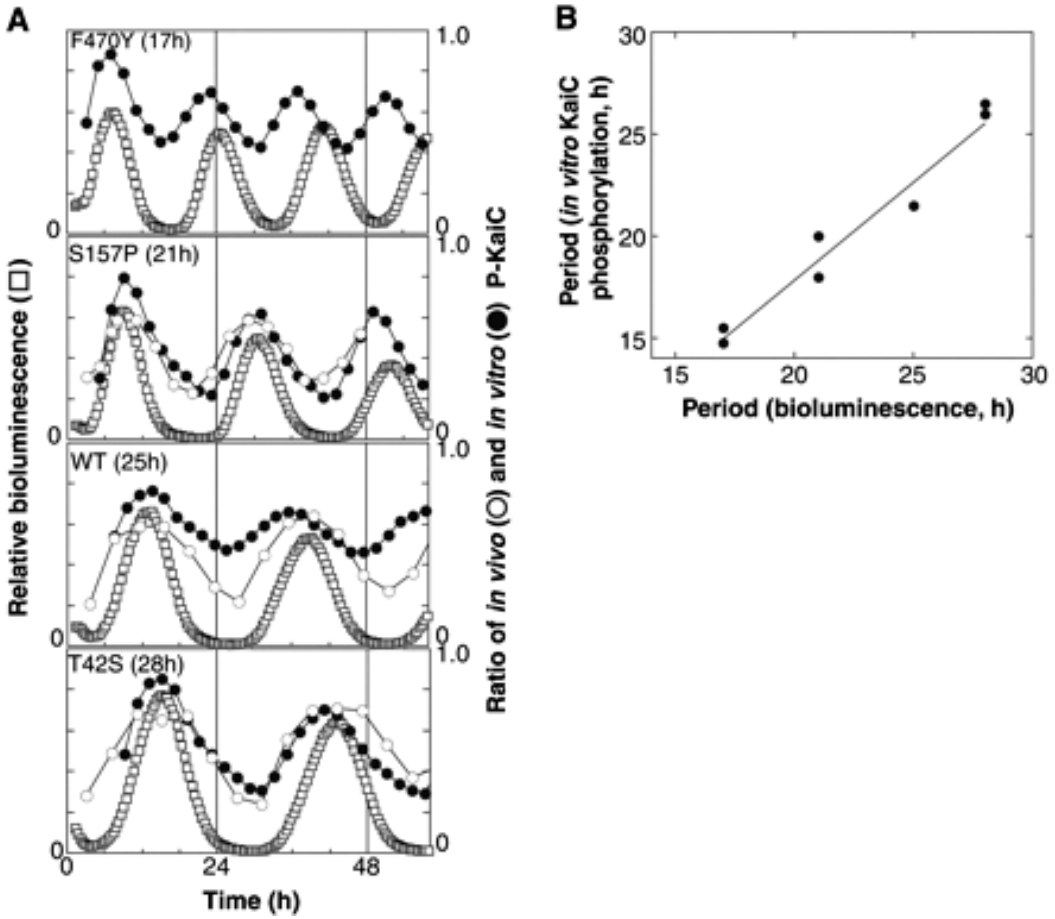
Die im Reagenzglas beobachtete rhythmische KaiC-Phosphorylierung/Dephosphorylierung ist Temperatur-kompensiert!!!



Nakayima et al., Kondo, Science 2005

Die im Reagenzglas beobachtete rhythmische KaiC-Phosphorylierung/Dephosphorylierun ist in Mutantenproteinen verändert!!!



Nakayima et al., Kondo, Science 2005

Modell der cyanobakteriellen inneren Uhr
Neben KaiABC sind input- und output-Proteinen beteiligt