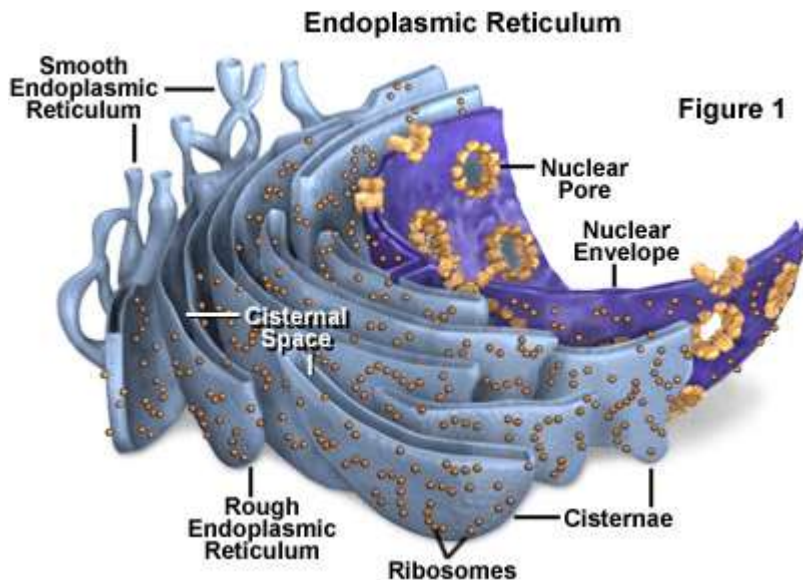




DMAX

Il reticolo endoplasmatico



Il reticolo endoplasmatico **liscio**

- Produce acidi grassi e fosfolipidi
- Contiene enzimi detossificanti e coniugativi
- Metabolismo del Glicogeno

Il reticolo endoplasmatico **ruvido**

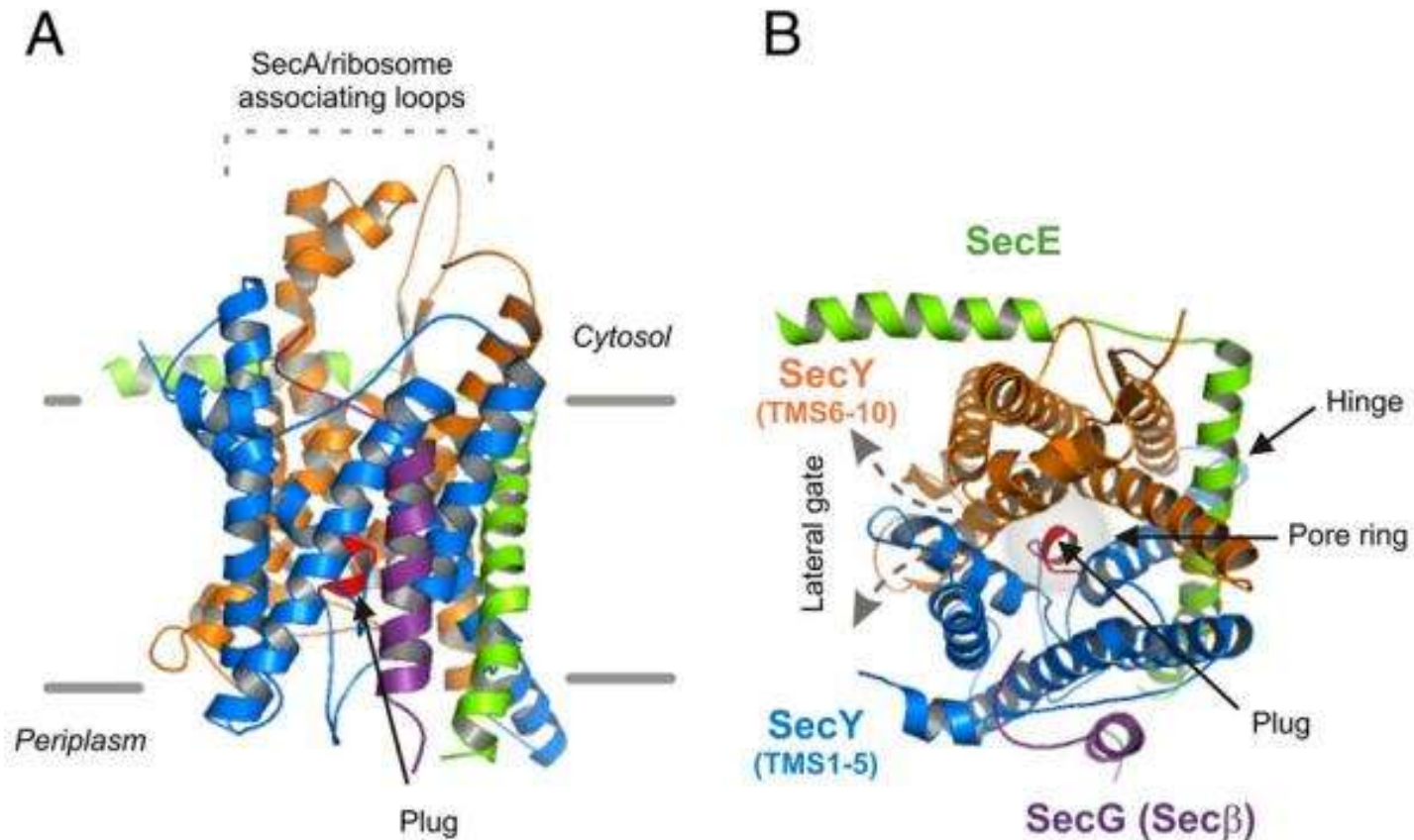
- Produce proteine di secrezione, lisosomiche, di membrana e multimeriche (30% del totale)
- Garantisce l'orientamento e la produzione corretta delle proteine transmembrana (**canali, recettori, proteine associate a lipidi**)
- Garantisce il folding corretto delle proteine
- Produce la prima glicosilazione
- Crea i ponti disolfuro essenziali per molte proteine**

Il Traslocone

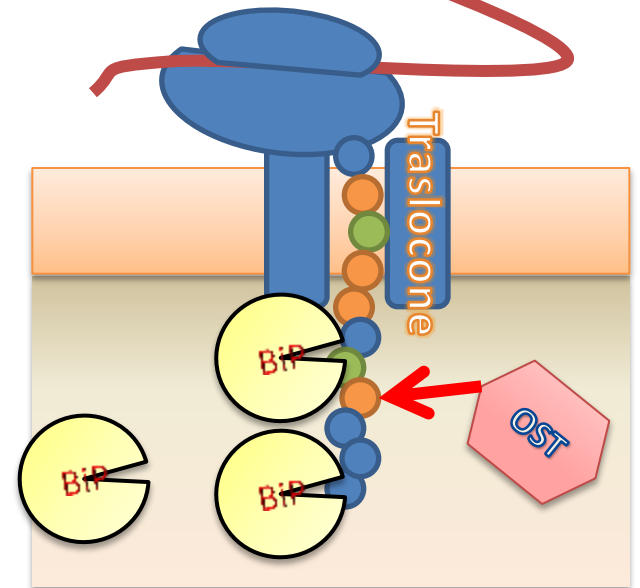
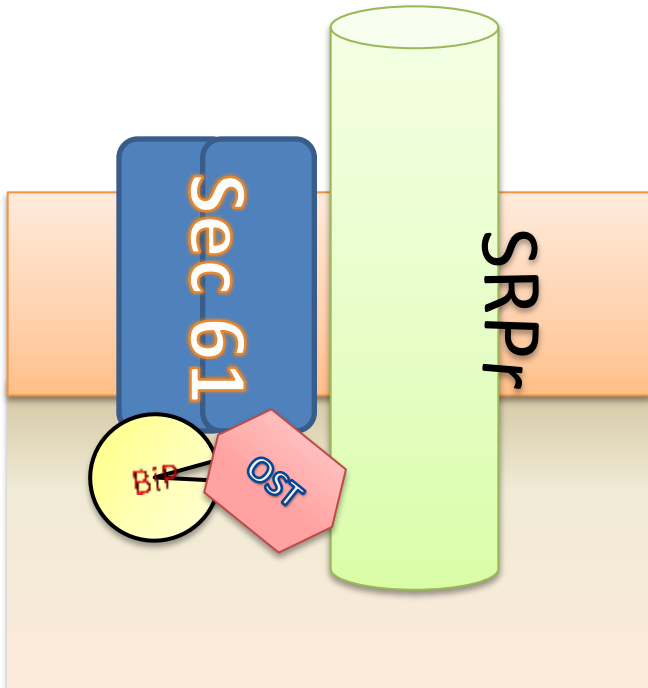
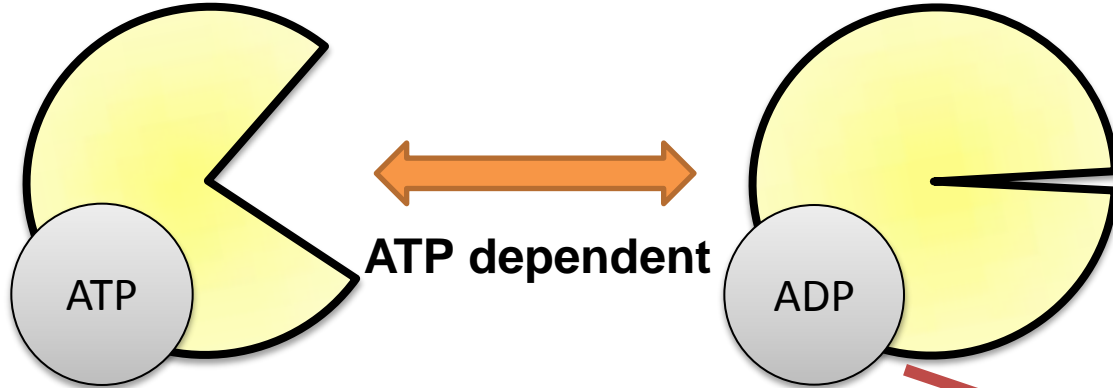
Il Traslocone è un complesso macromolecolare essenziale per la corretta sintesi proteica compartimentata, è conservato in tutti e tre i regni

Eterotrimerico

SecYEG (batteri) Sec61abc (eucarioti)



Hsp70s



Chaperone

Hsp70s

- ER: BiP - translocon gate, folding assistance, UPR transducer.
- Cyt: Hsp70s (stress inducible) Hsc70s (constitutive)
Transmembrane protein ERAD



Hsp40s

- Hsp70s Cochaperones,
- Help BiP during translocation,
- Aromatic/hydrophobic affinity
- J-domains: 4 α -helices (specificity and Hsp70s ATP hydrolysis)

Hsp90s

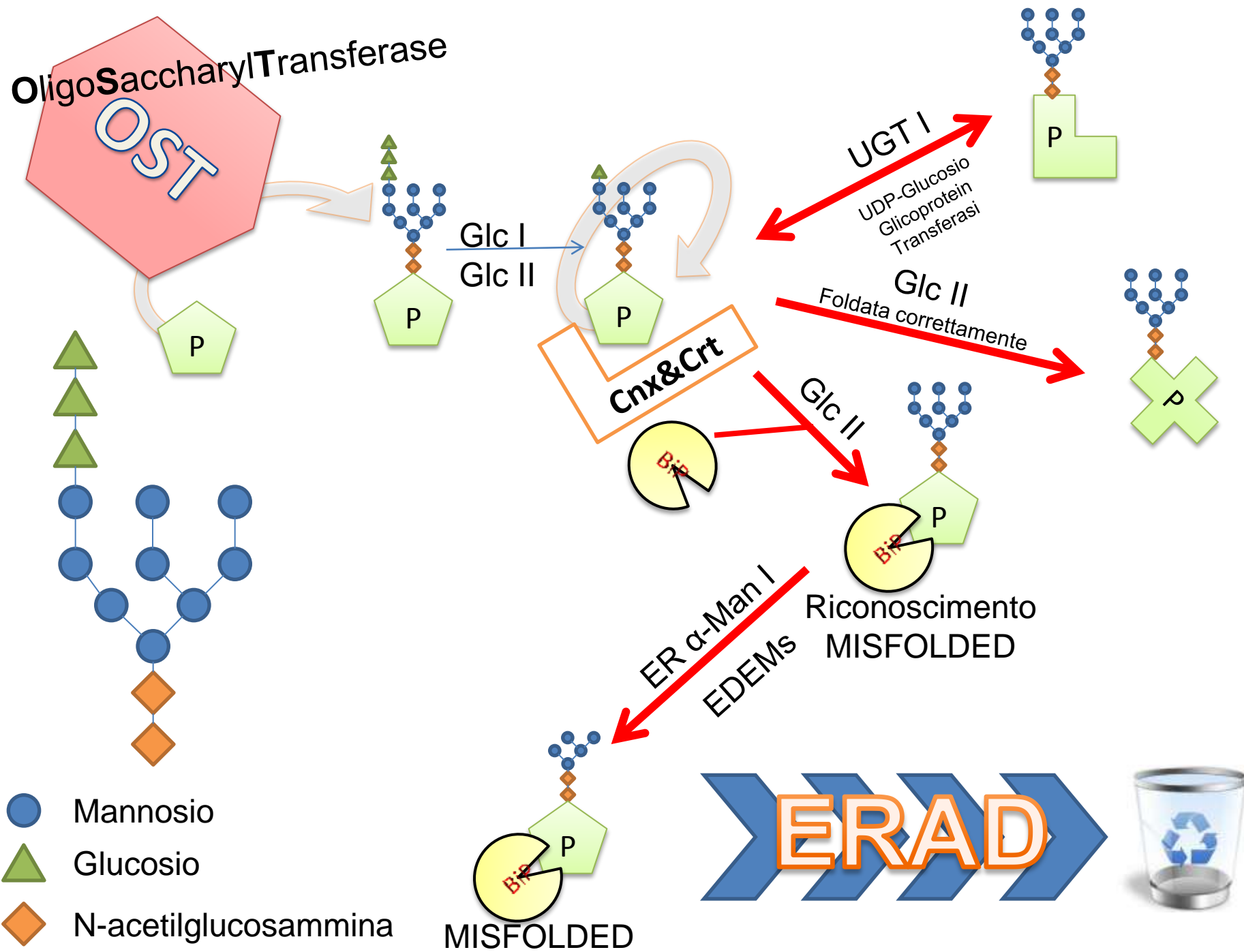
- Folding of specific set of protein (HR, PK, TF)
- hold protein until the interaction with required partner,
- ATPase activity requires dimerization,
- 2 cytoplasmic forms (α , β)

Chaperone

PDI	<ul style="list-style-type: none">• 5+ Protein Disulfide Isomerase (Thiol Oxidoreductase)• BiP co-worker or Stand-Alone	<ul style="list-style-type: none">• CxxC catalytic Motif, “U” shape• Ero1α (Hypoxia induced, ERAD linked), Ero1β (UPR induced)
AAA ATPase	<ul style="list-style-type: none">• Homohexameric• Transcription Factors, Apoptosis, ERAD related• Bind both Ubiquitinated proteins and Proteasome	<ul style="list-style-type: none">• 2 ATPase Domain, 1 Zn⁺², Denaturation-collar
Lectine - Like	<ul style="list-style-type: none">• Reconizes Oligosaccharyl-appended N-glycan• Facilitate folding and ERAD• in this family we find Calnexin (Cnx) and Calreticulin (Crt)	
NEFs	<ul style="list-style-type: none">• Nucleotide Exchange Factor; Enhance ADP from Hsp70s,• BAG-1 works also with Bcl-2• NEFs : Hsp70s Ratio varies folding efficiency	

OligoSaccharylTransferase

OST



UGT I
UDP-Glucosio
Glicoprotein
Transferasi

Glc II
Foldata correttamente

Glc II

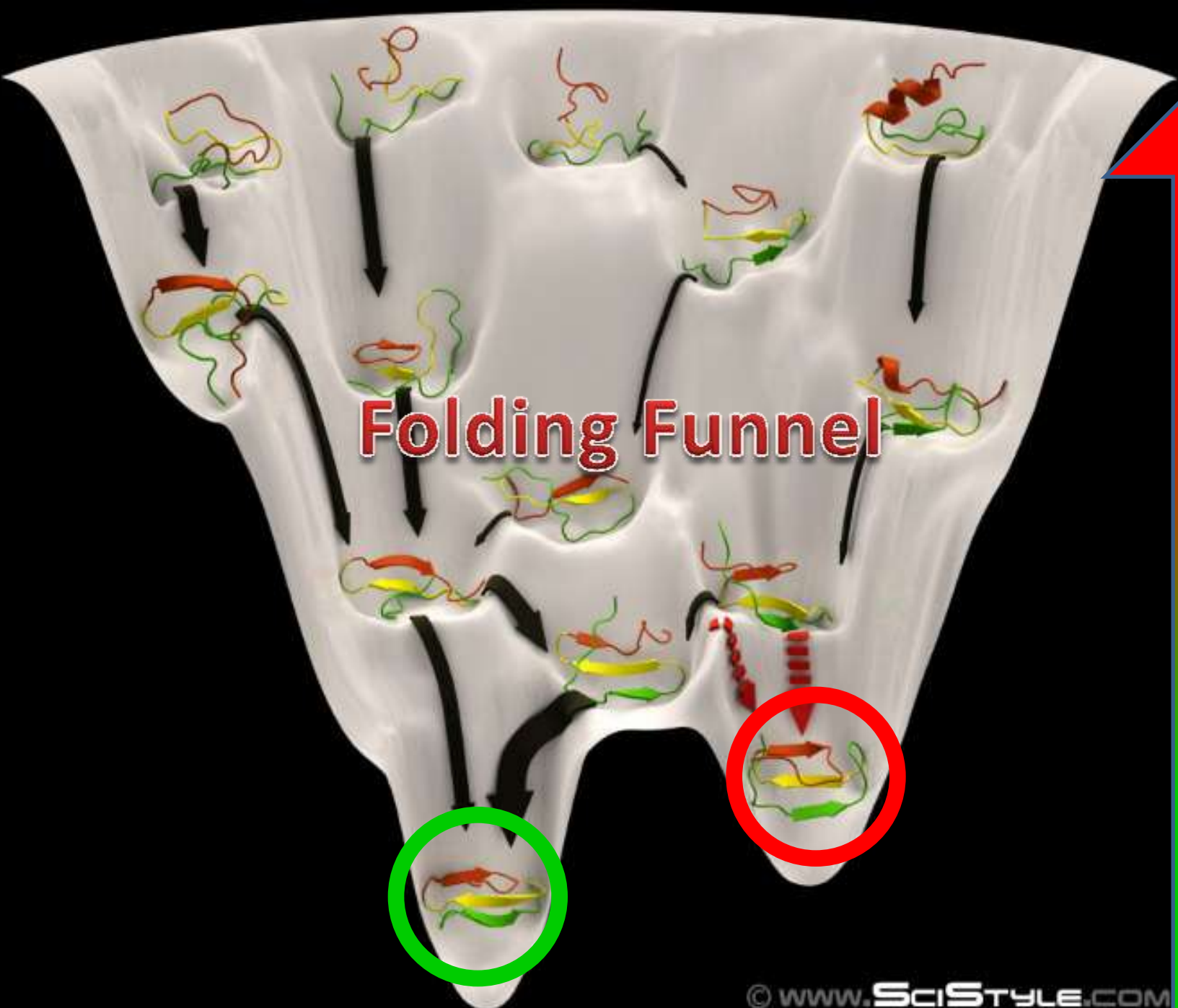
ER α-Man I
EDEMs

Riconoscimento
MISFOLDED

ERAD

- Mannosio
- ▲ Glucosio
- ◆ N-acetilglucosammina

MISFOLDED

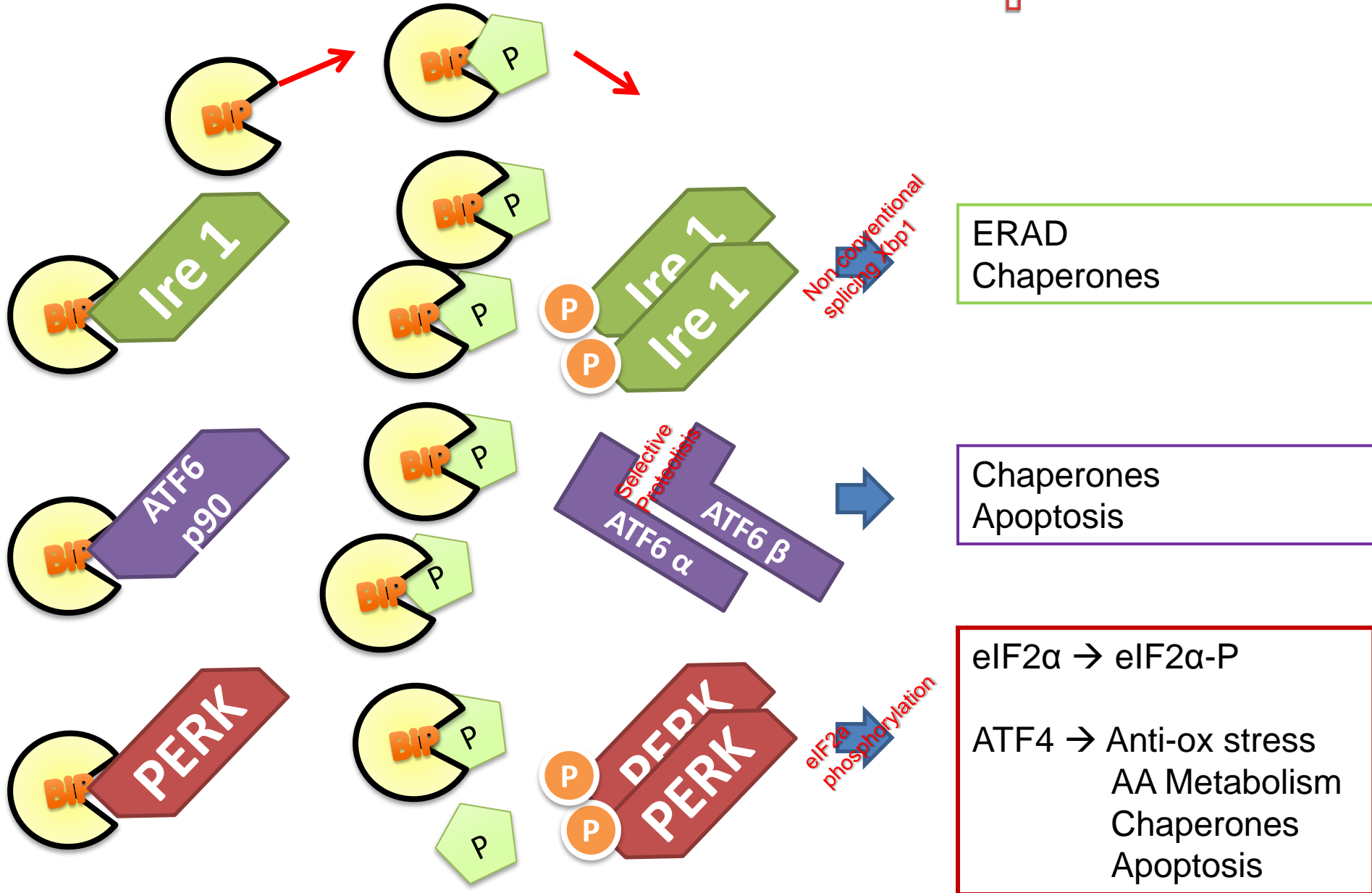


Folding Funnel



Energia

Unfolded Protein Response

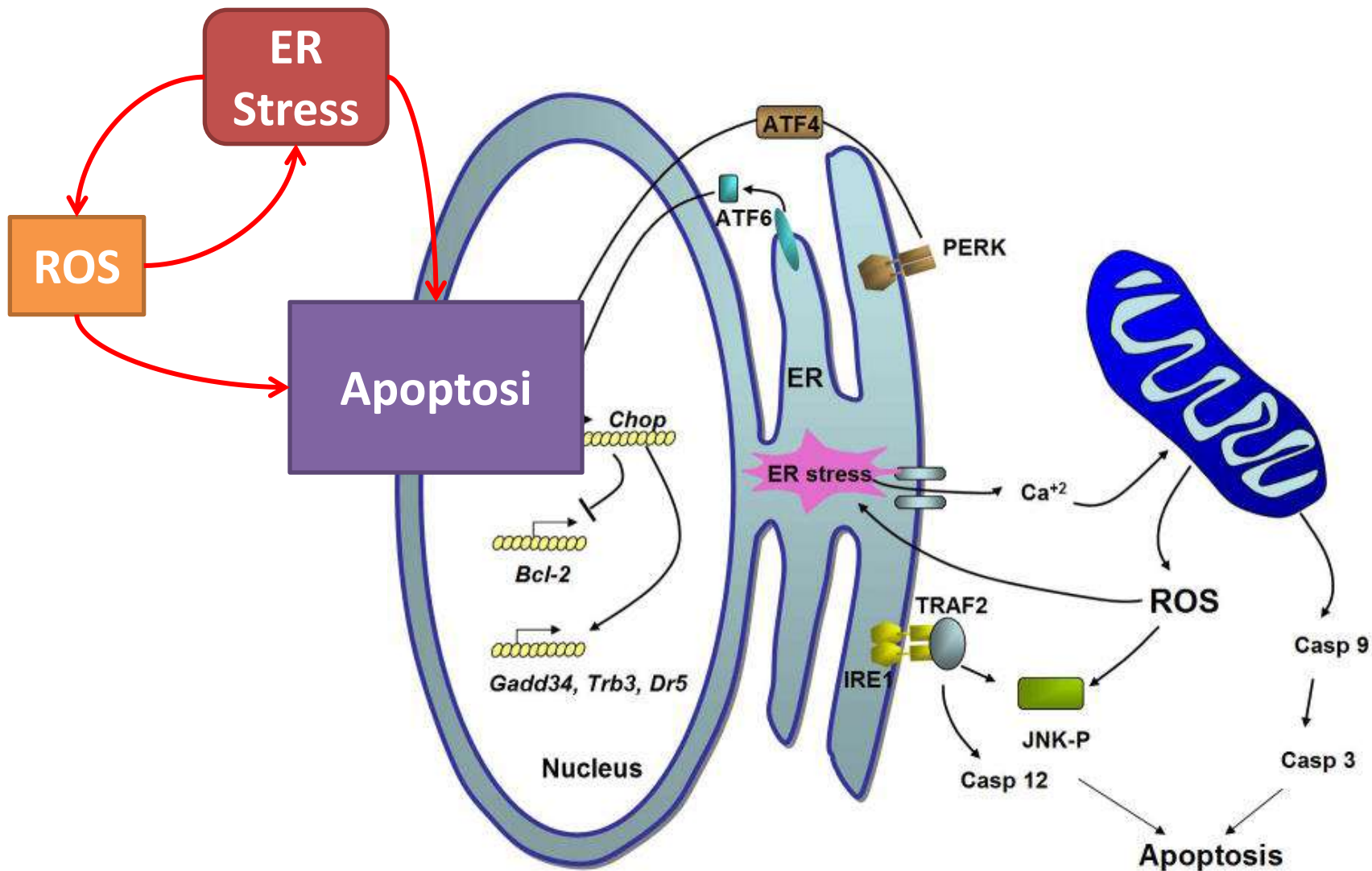


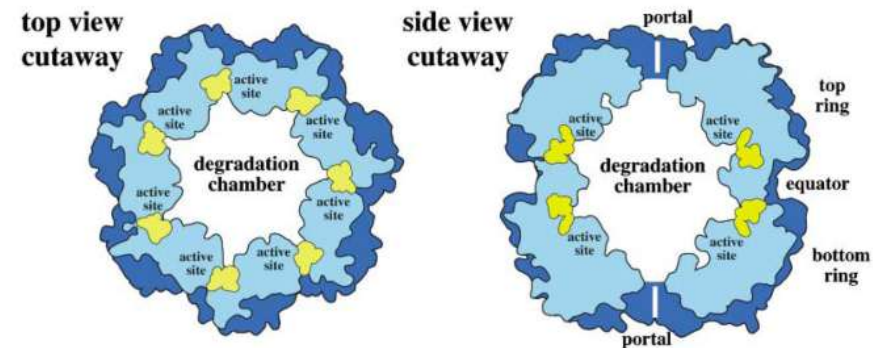
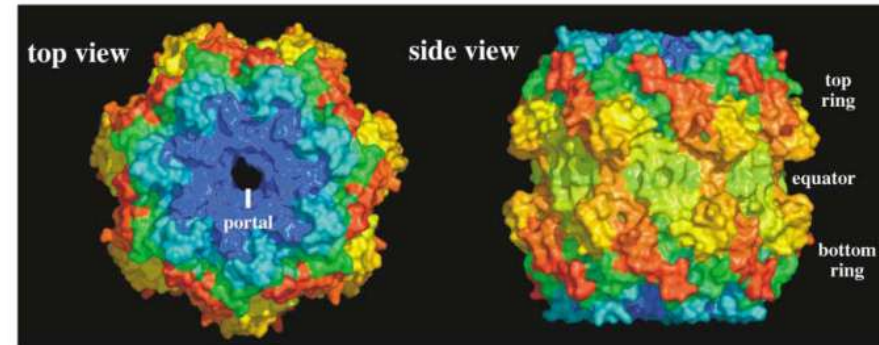
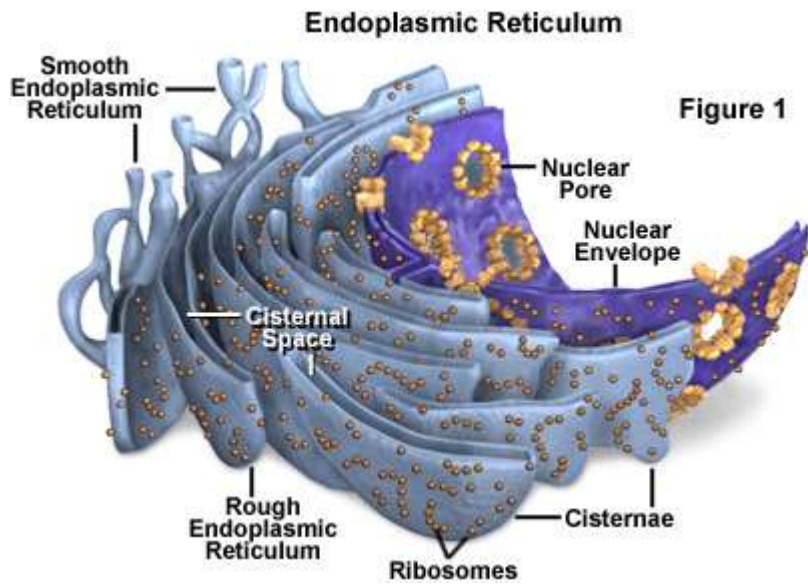
ERAD
Chaperones

Chaperones
Apoptosis

eIF2α → eIF2α-P
ATF4 → Anti-ox stress
AA Metabolism
Chaperones
Apoptosis

Unfolded Protein Response





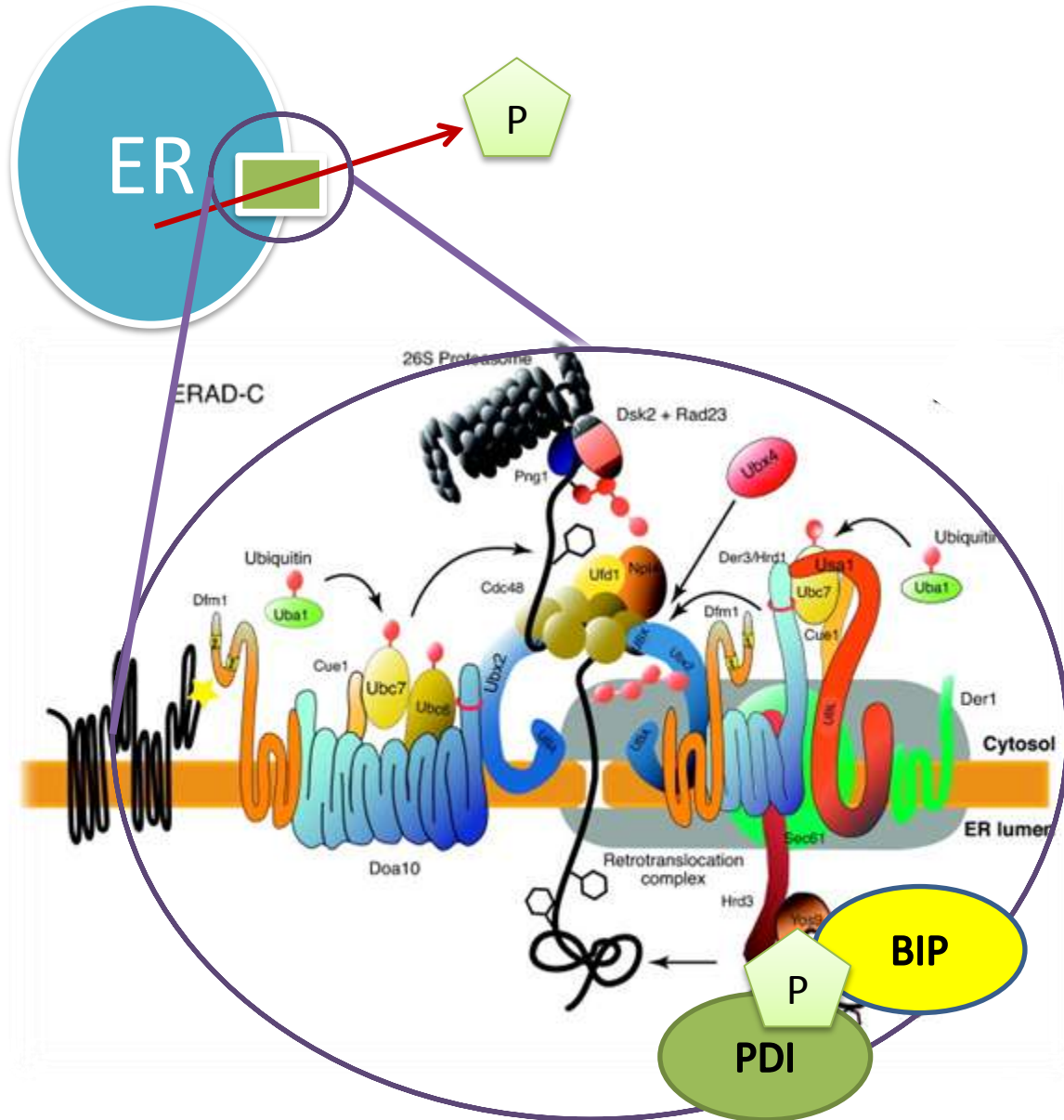


3,793 HP

Consumo medio 246 Litri/ora

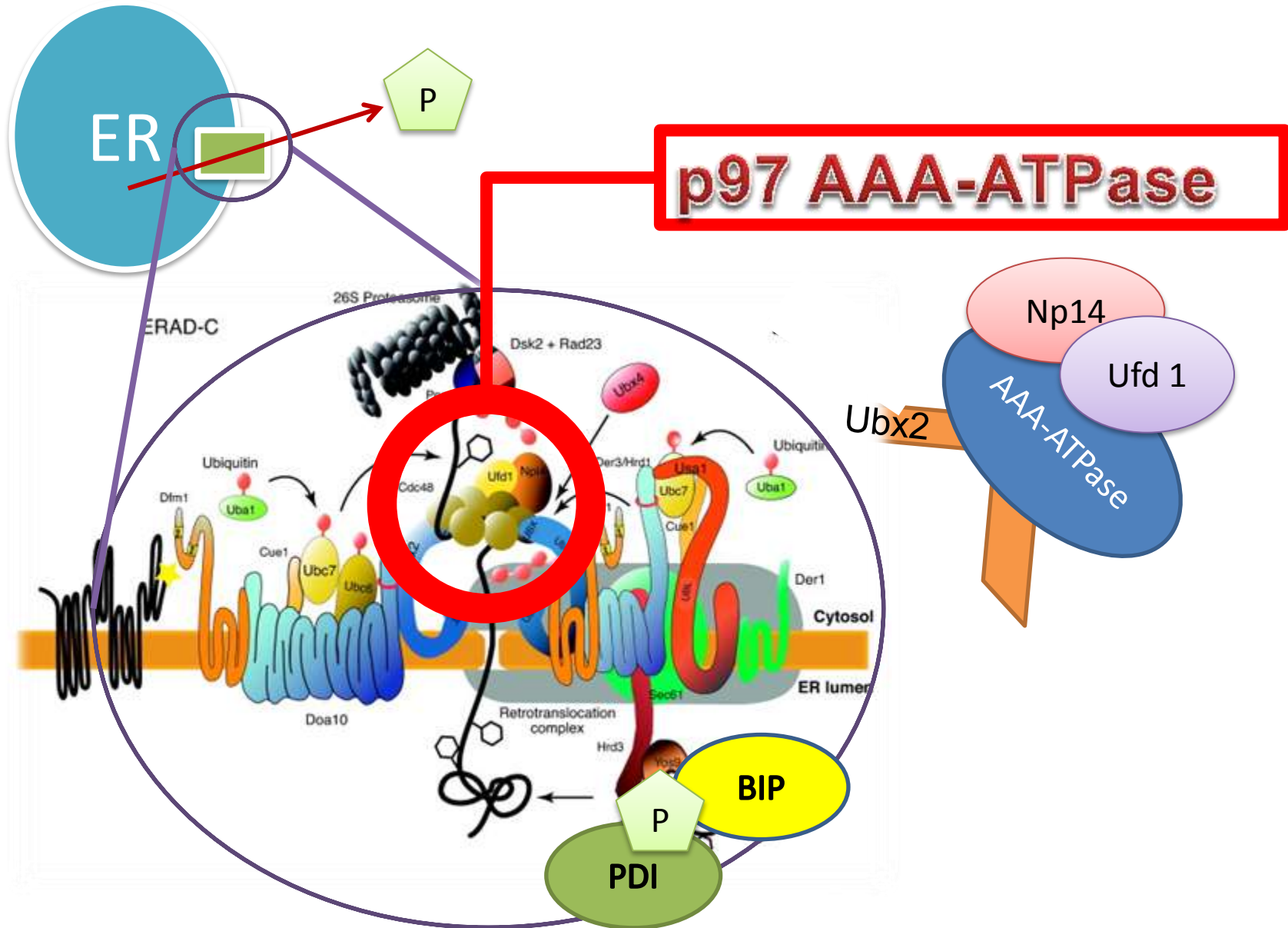
ERAD

ER-Associated Degradation

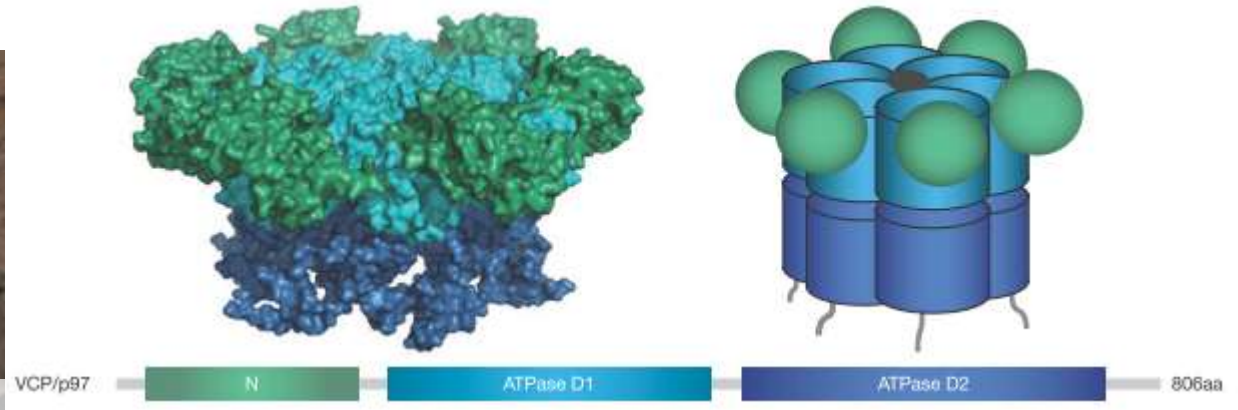


ERAD

ER-Associated Degradation

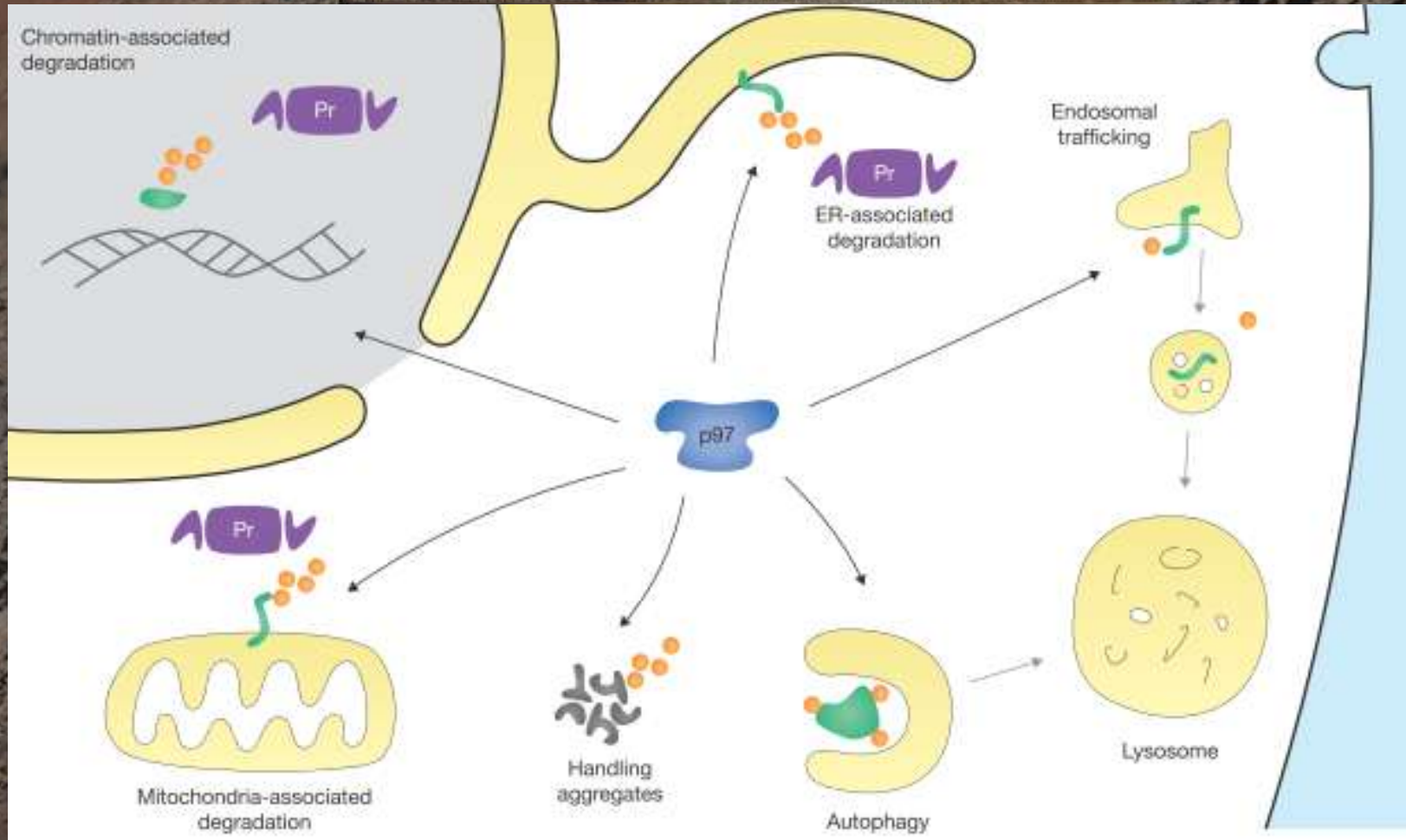


Cdc48 (yeast) / p97 AAA-ATPase (human)



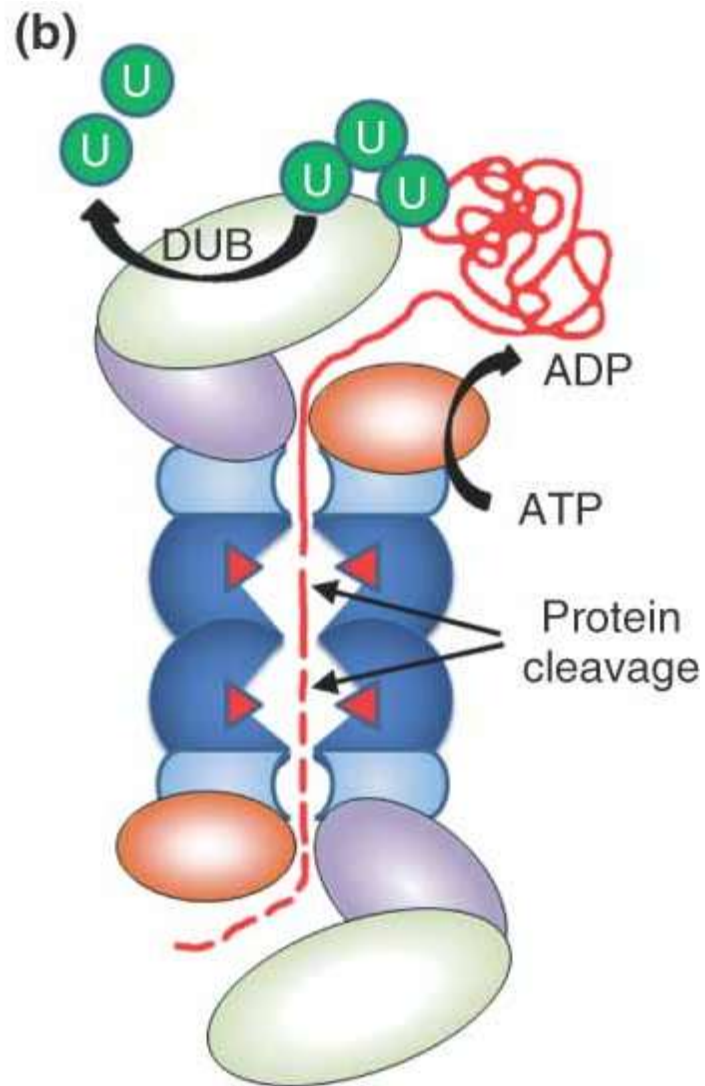
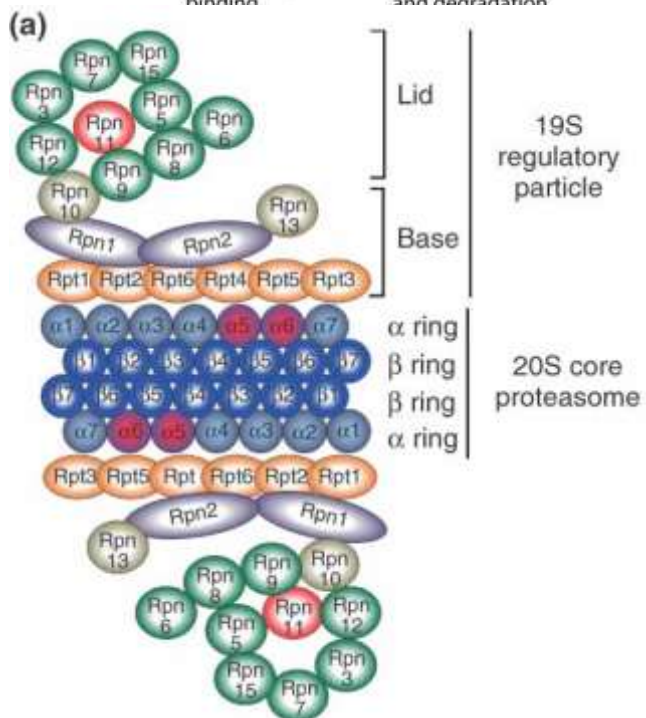
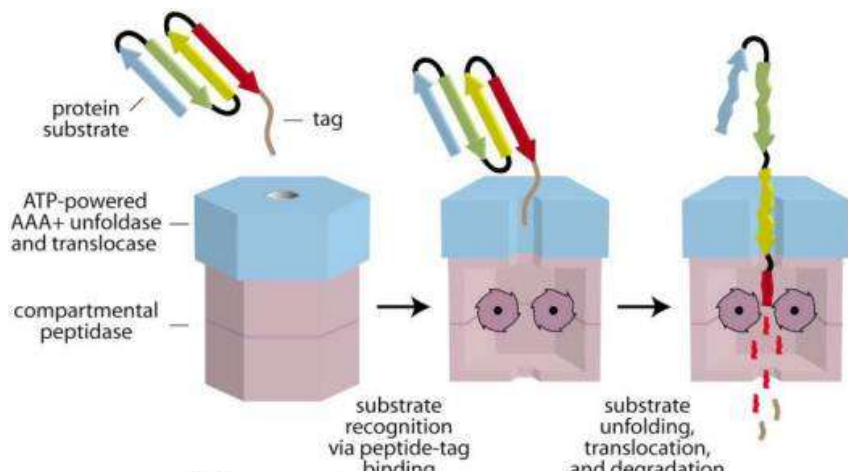
- **1%** della massa proteica della cellula
- Omoesamero
- Zn^{+2} coordinato all'interno del «barile»
- **ATP Dipendente ed ad altissimo consumo [30-80 ATP per 100 residui]**
 - **Può arrivare a consumare più di quanto è speso per la sintesi della proteina che degrada**
- Link fra L-ERAD ed il C-ERAD
- Motore del disassemblaggio
- Attività denaturante
- Essenziale per l'attività del proteosoma

Cdc48 (yeast) / p97 AAA-ATPase (human)



ERAD

Proteosoma



Studio del pathway UBXD7 - p97 - HIF1 α

Separazione per gel filtrazione

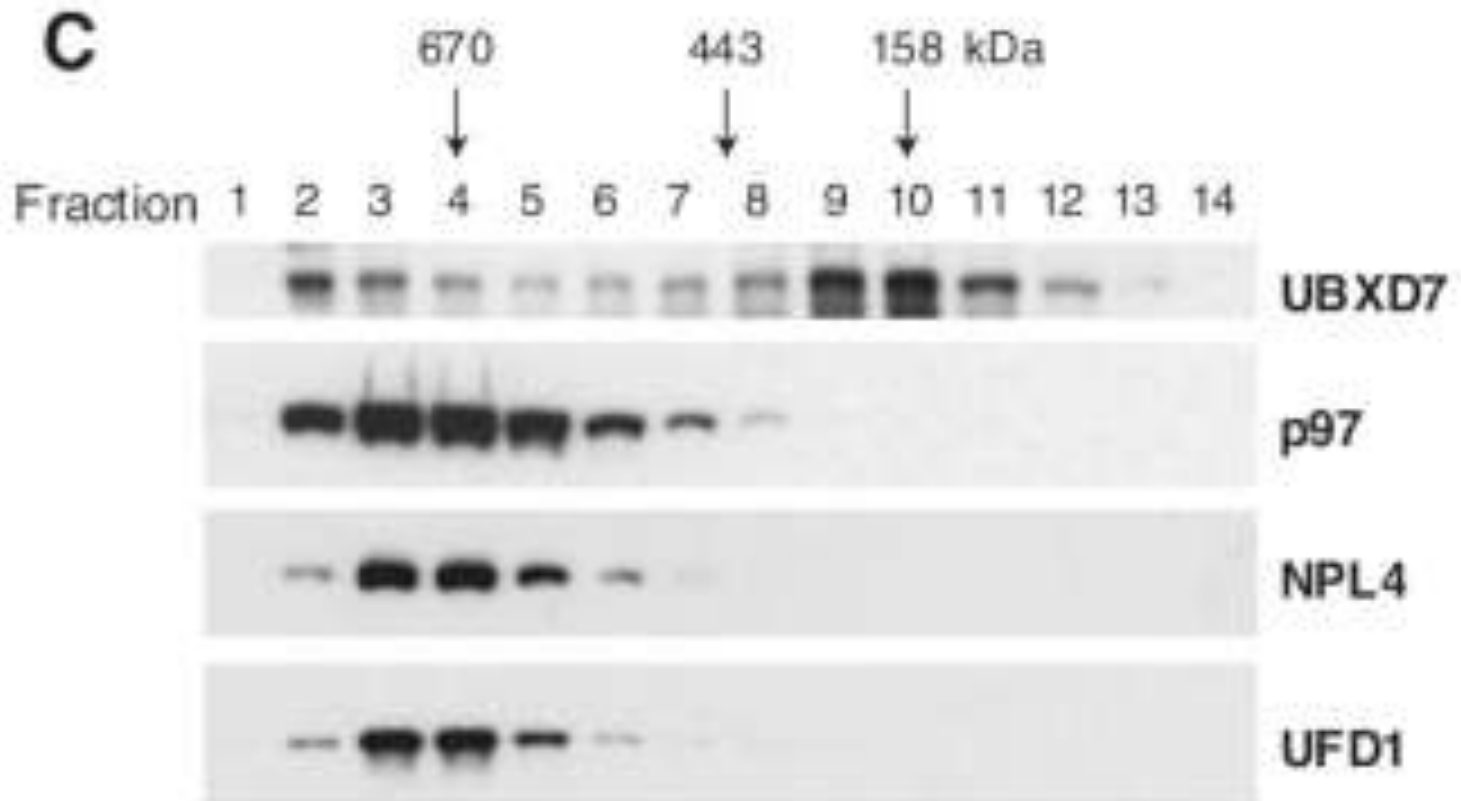
Proteina – FLAG

Immunoprecipitazione

Western Blotting

Chi lega in condizioni normali p97??

- Gel filtrazione di lisati cellulari (nativa)
- WB vs le singole proteine



Cosa lega UBXD7?

HIF1 α Ossigenazione normale \rightarrow ubiquitinata e degradata

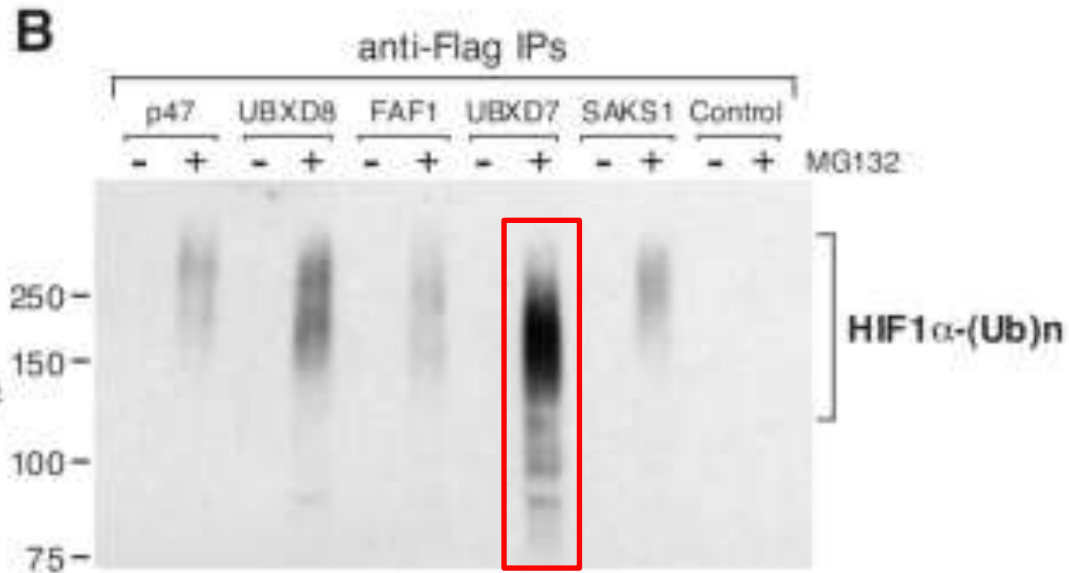
- Necessario bloccare la degradazione: MG132 (inibitore proteosoma)

- Immunoprecipitati per FLAG-UBA-UBX
- WB vs HIF1 α

Ma
IP

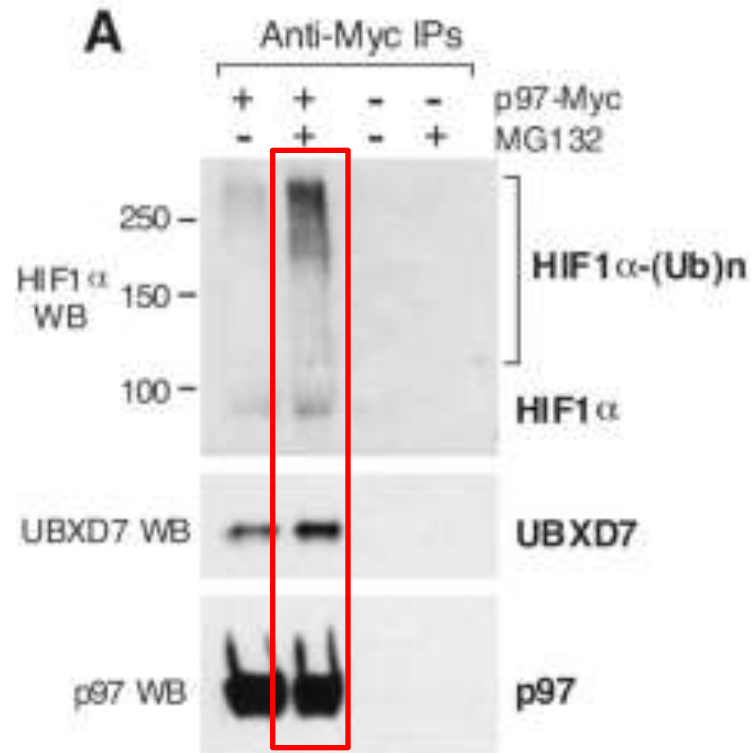
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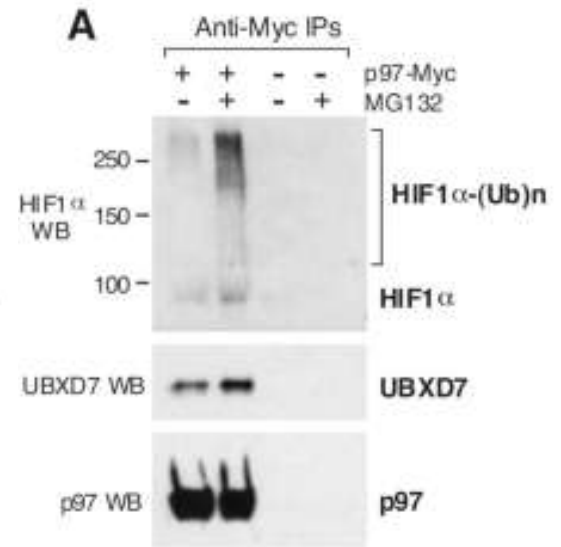
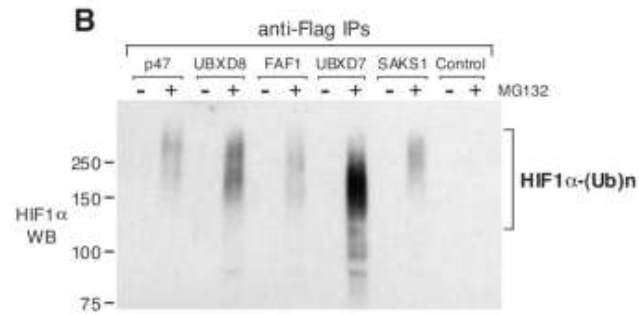
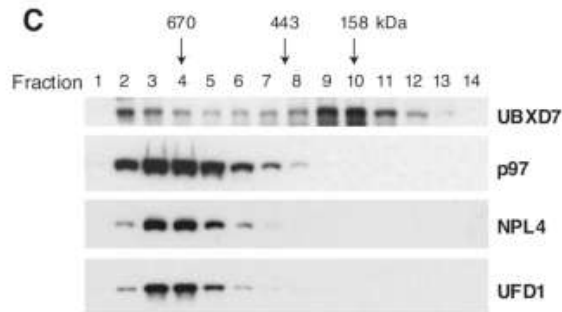
ide
VE
SS
LFA
SP
TT
ILA
TIIL
NLI



In che forma viene legata HIF1 α ?

- Immunoprecipitazione per p97-Myc
 - WB vs HIF1 α , UBXD7 , p97





- HIF1 α è normalmente degradata, degradazione che può essere inibita inibendo il proteosoma quindi la degradazione è mediata dal proteosoma,
- UBXD7 lega spontaneamente HIF1 α , e la lega ad alta affinità nella sua forma ubiquitinata,
- p97 è normalmente associata con gli adattatori NPL4/UFD1, e lega UBXD7 solo all'esigenza come risposte a stimoli o interazione con il substrato,
- p97 non lega spontaneamente HIF1 α , questo legame deve essere mediato da UBXD7.

