

07/12/2013

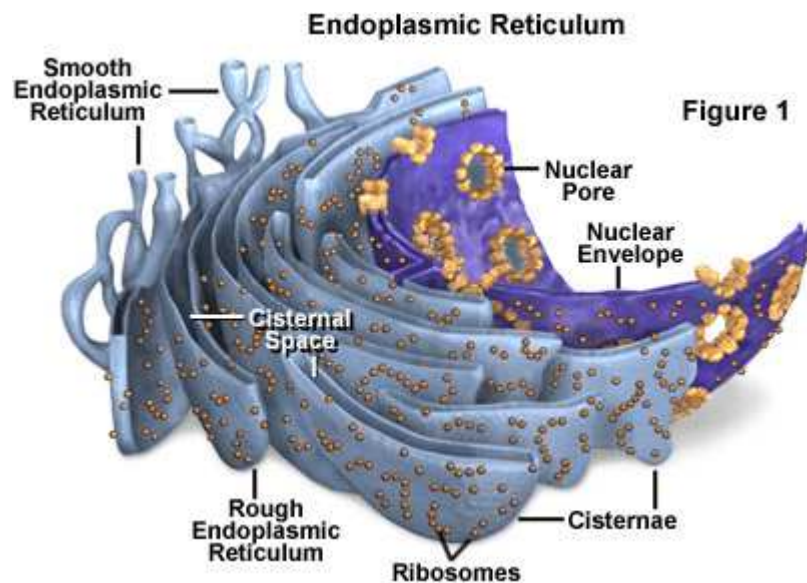
# DIRTY JOBS

with Mike Rowe



**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\*\*

Slide di RIPASSO

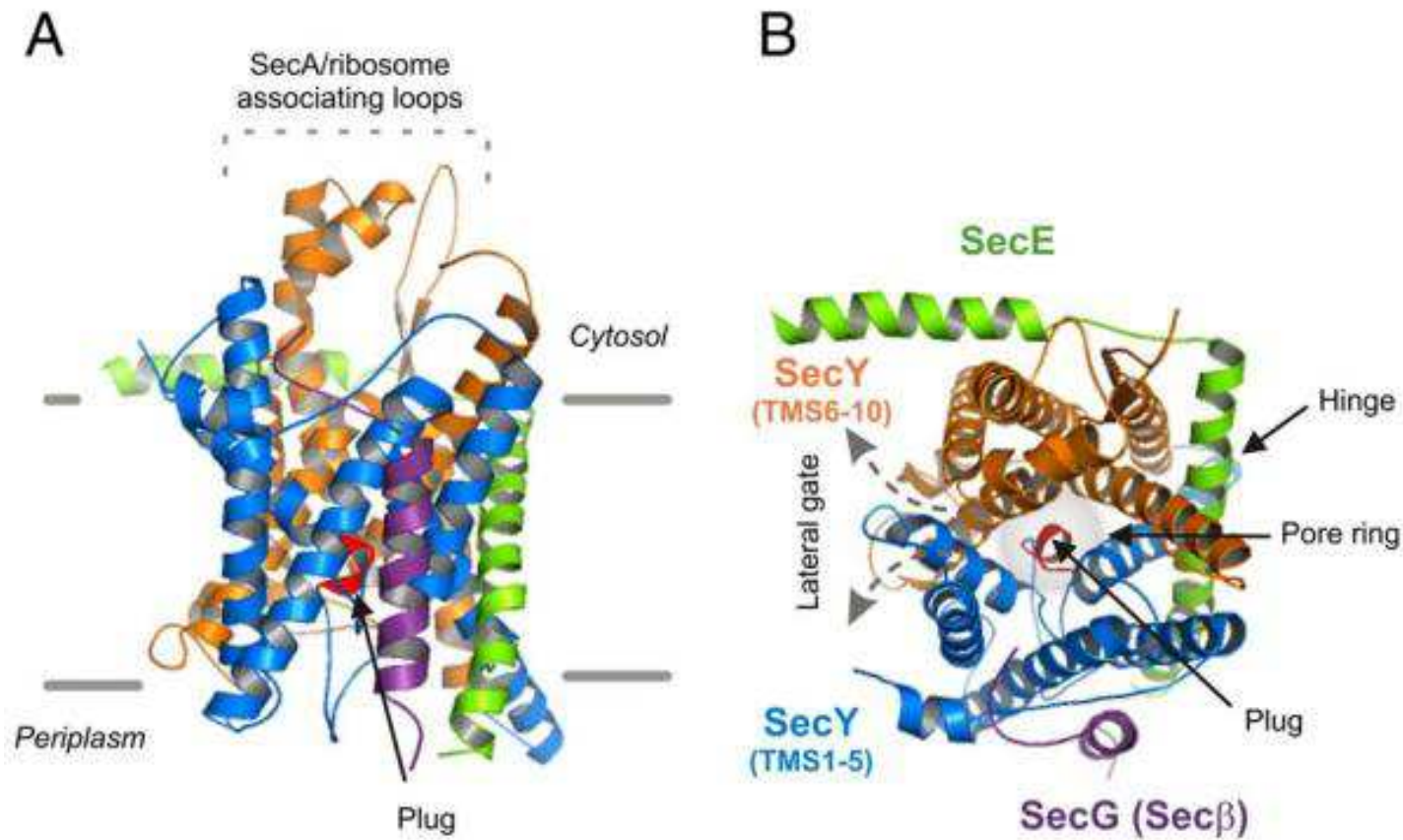
\*\* : anticorpi

# Il Traslocone


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

Eterotrimero

SecYEG (batteri) Sec61abc (eucarioti)



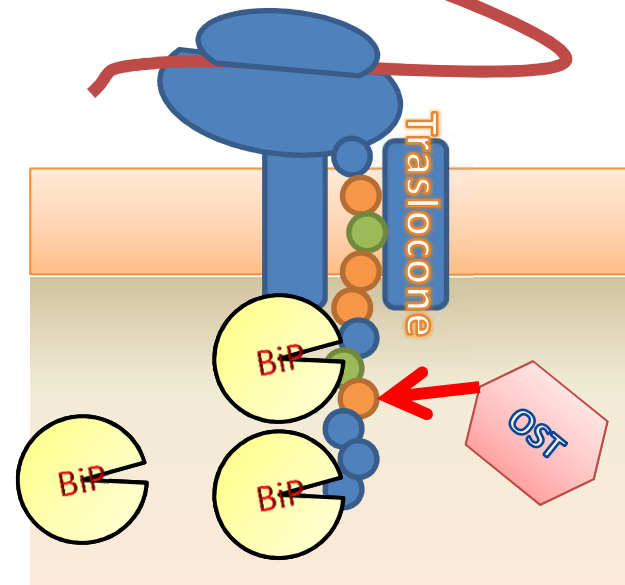
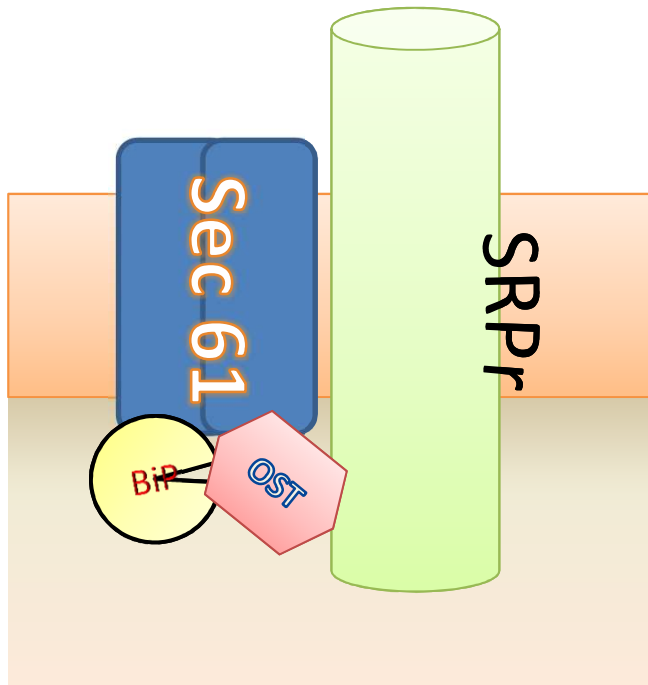
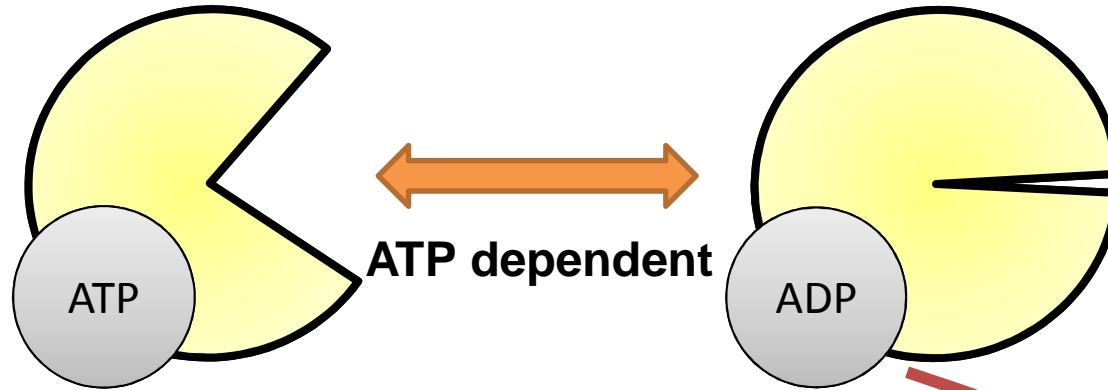
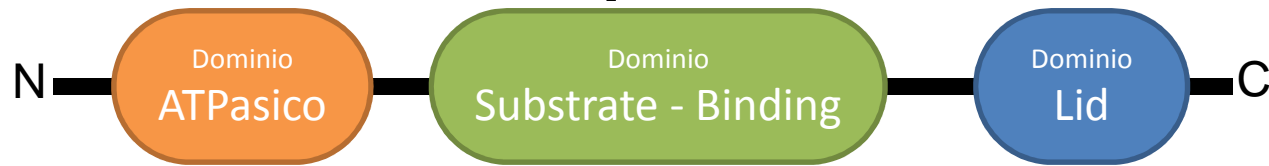
# Chaperone

<h2>Hsp70s</h2>	<ul style="list-style-type: none"> <li>• ER: BiP - translocon gate, folding assistance, UPR transducer.</li> <li>• Cyt: Hsp70s (stress inducible) Hsc70s (constitutive) Transmembrane protein ERAD</li> </ul>		
<h2>Hsp40s</h2>	<ul style="list-style-type: none"> <li>• Hsp70s Cochaperones,</li> <li>• Help BiP during translocation,</li> </ul>	<ul style="list-style-type: none"> <li>• Aromatic/hydrophobic affinity</li> <li>• J-domains: 4 <math>\alpha</math>-helices (specificity and Hsp70s ATP hydrolysis)</li> </ul>	
<h2>Hsp90s</h2>	<ul style="list-style-type: none"> <li>• Folding of specific set of protein (HR, PK, TF)</li> <li>• hold proten until the interction with requiried partner,</li> </ul>	<ul style="list-style-type: none"> <li>• ATPase activity requires dimerization,</li> <li>• 2 cytoplasmic forms (<math>\alpha</math>, <math>\beta</math>)</li> </ul>	

# Chaperone

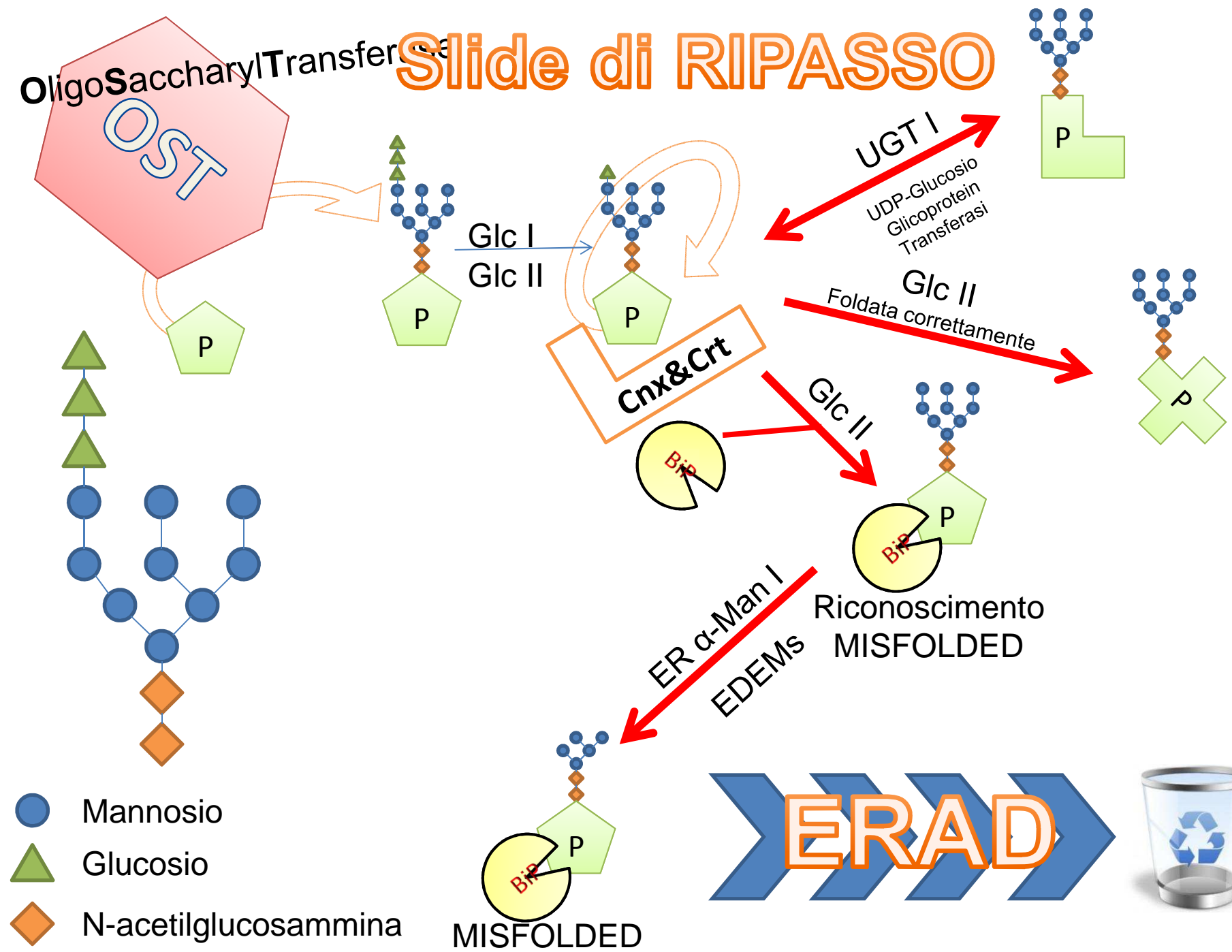
<p><b>PDI</b></p>	<ul style="list-style-type: none"> <li>• 5+ <b>P</b>rotein <b>D</b>isulfide <b>I</b>somerase (Thiol Oxidoreductase)</li> <li>• BiP co-worker or Stand-Alone</li> </ul>	<ul style="list-style-type: none"> <li>• CxxC catalytic Motif, “U” shape</li> <li>• Ero1<math>\alpha</math> (Hypoxia induced, ERAD linked), Ero1<math>\beta</math> (UPR induced)</li> </ul>
<p><b>AAA ATPase</b></p>	<ul style="list-style-type: none"> <li>• Homohexameric</li> <li>• Transcription Factors, Apoptosis, ERAD related</li> <li>• Bind both Ubiquitinated proteins and Proteasome</li> </ul>	<ul style="list-style-type: none"> <li>• 2 ATPase Domain, 1 Zn<sup>2+</sup>, Denaturation-collar</li> </ul>
<p><b>Lectine - Like</b></p>	<ul style="list-style-type: none"> <li>• Reconizes Oligosaccharyl-appended N-glycan</li> <li>• Facilitate folding and ERAD</li> <li>• in this family we find Calnexin (<b>Cnx</b>) and Calreticulin (<b>Crt</b>)</li> </ul>	
<p><b>NEFs</b></p>	<ul style="list-style-type: none"> <li>• Nucleotide Exchange Factor; Enhance ADP from Hsp70s,</li> <li>• BAG-1 works also with Bcl-2</li> <li>• NEFs : Hsp70s Ratio varies folding efficiency</li> </ul>	

# Hsp70s



OligoSaccharylTransferase OST

# Slide di RIPASSO



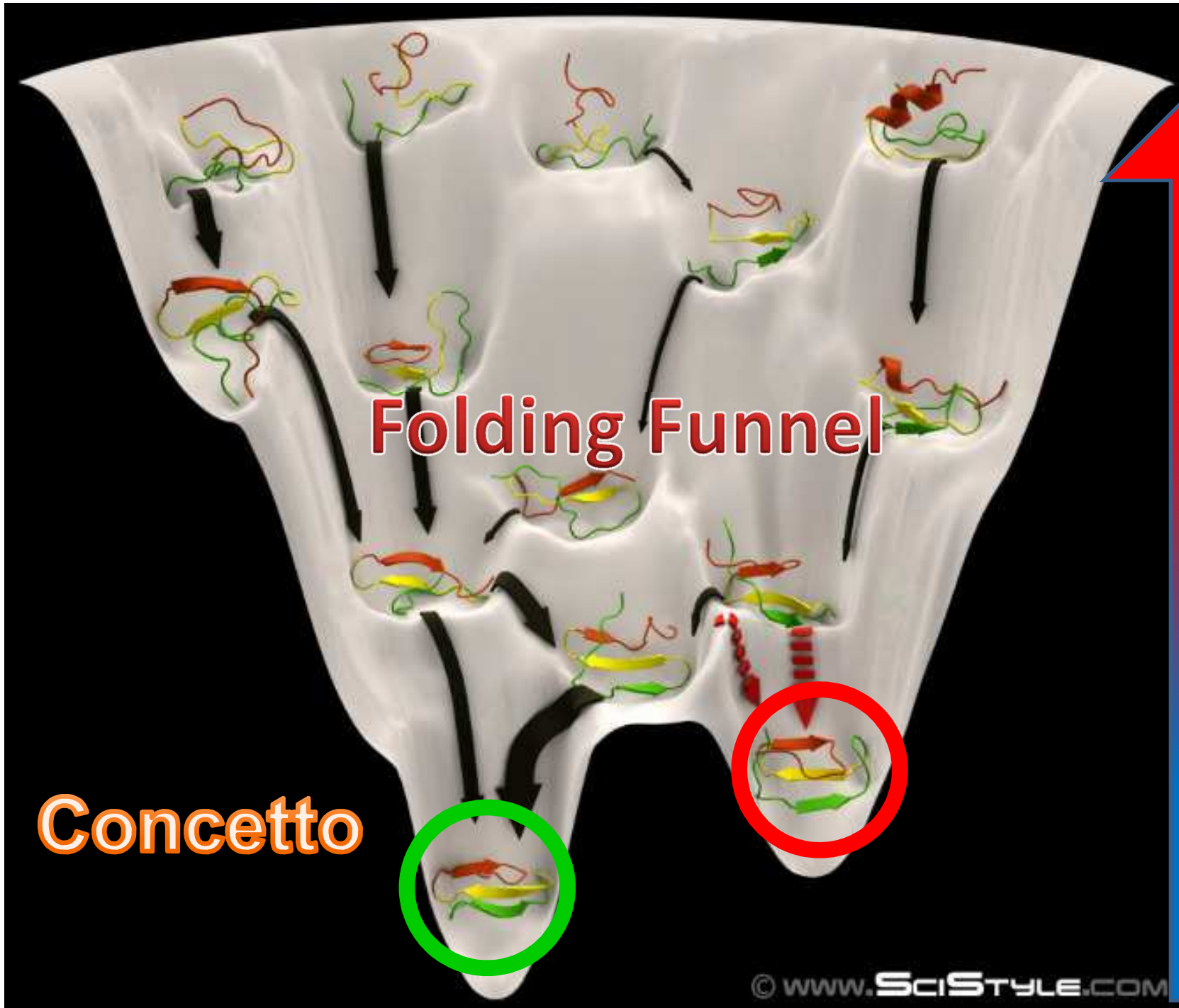
- Mannosio
- ▲ Glucosio
- ◆ N-acetilglucosammina

MISFOLDED

# ERAD







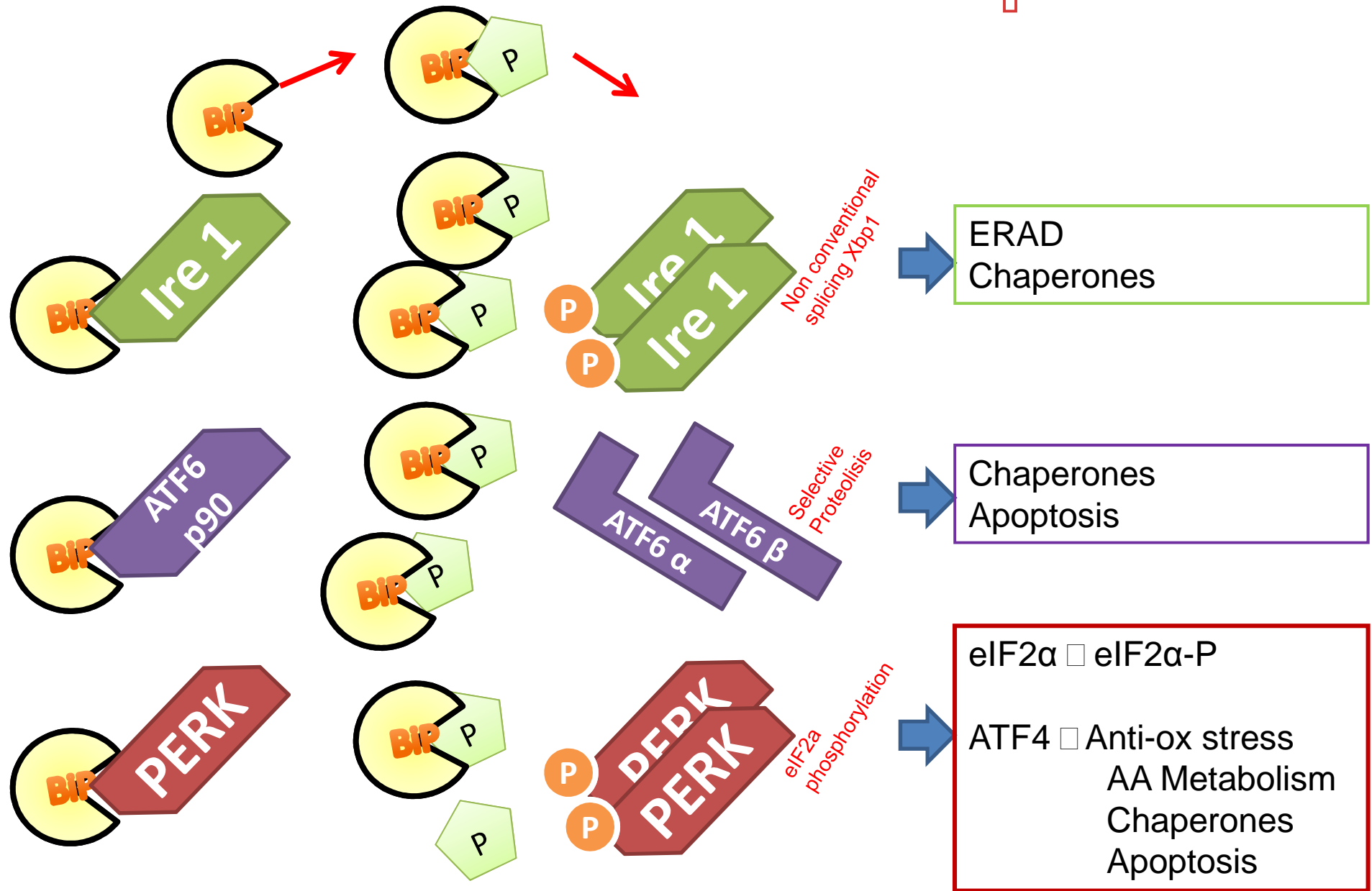
Folding Funnel

Energia

Concetto

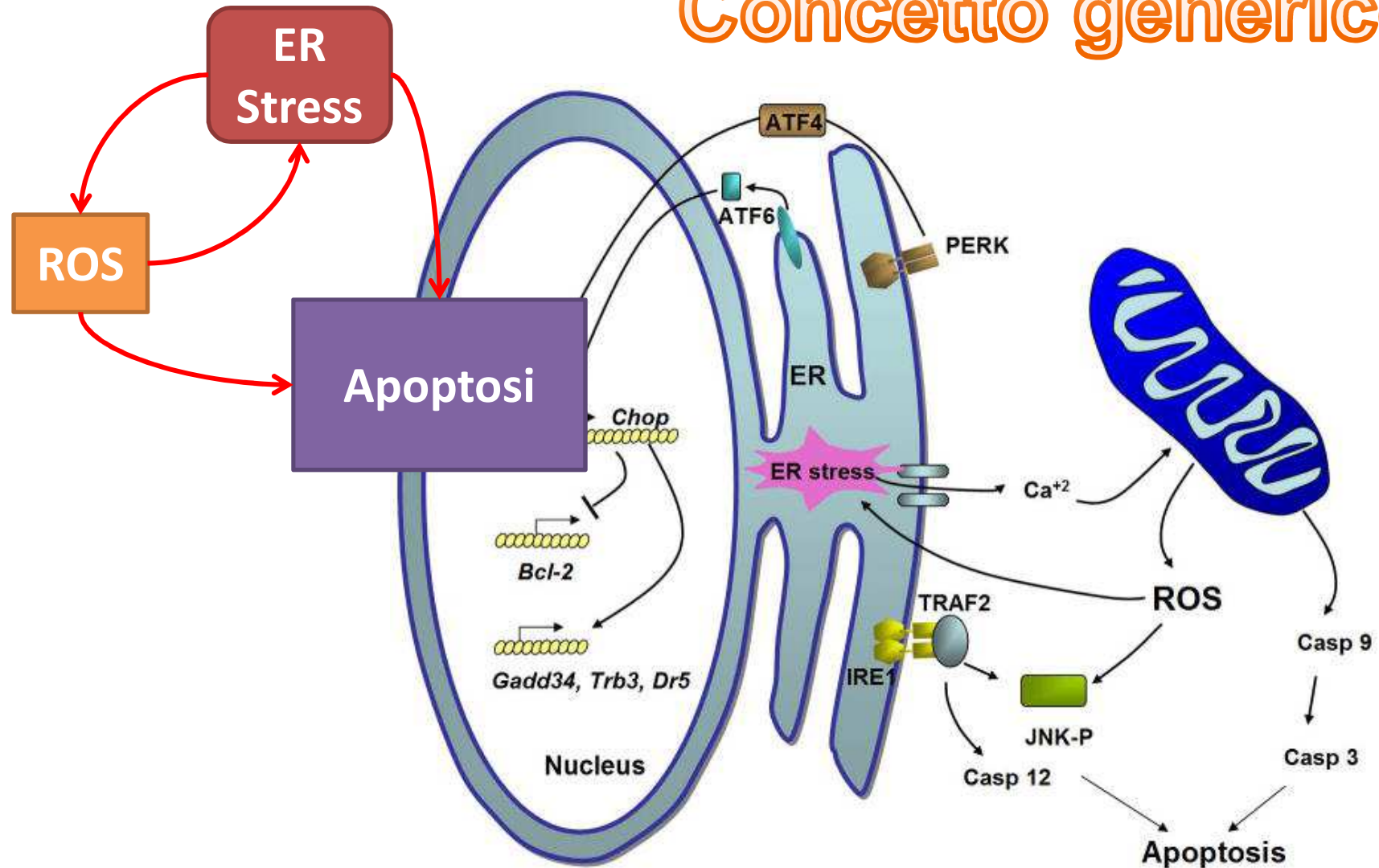


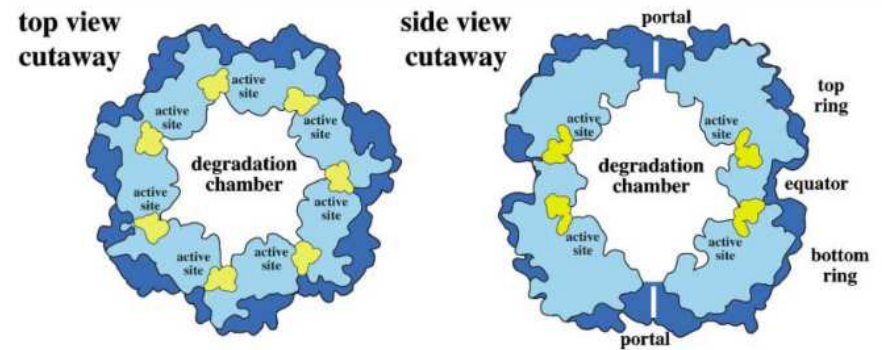
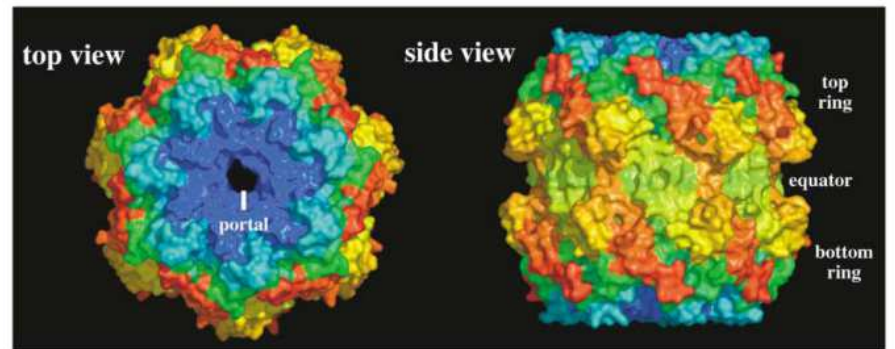
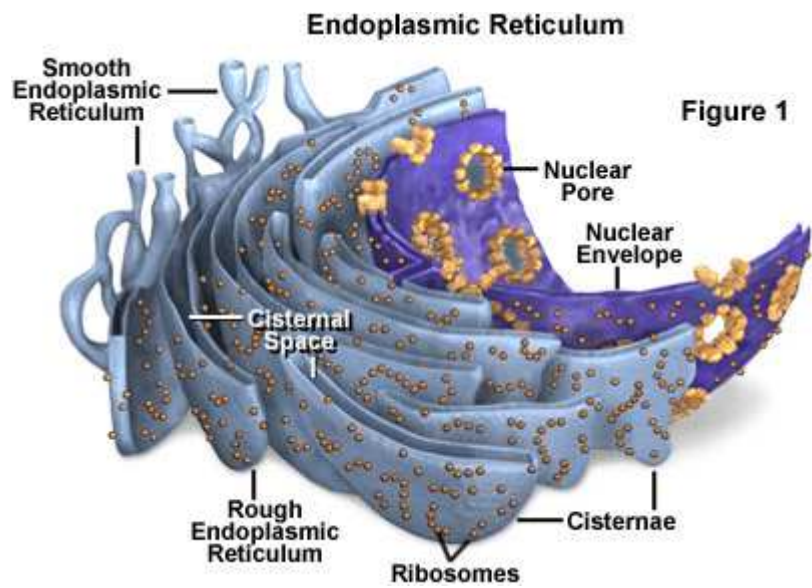
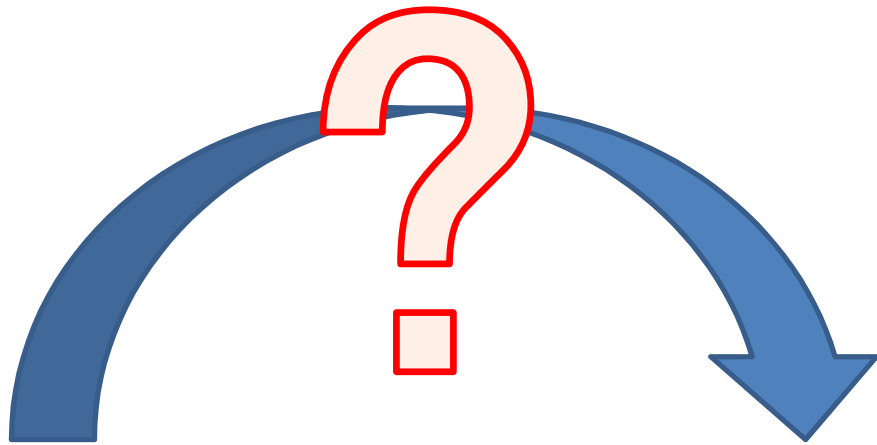
# Unfolded Protein Response



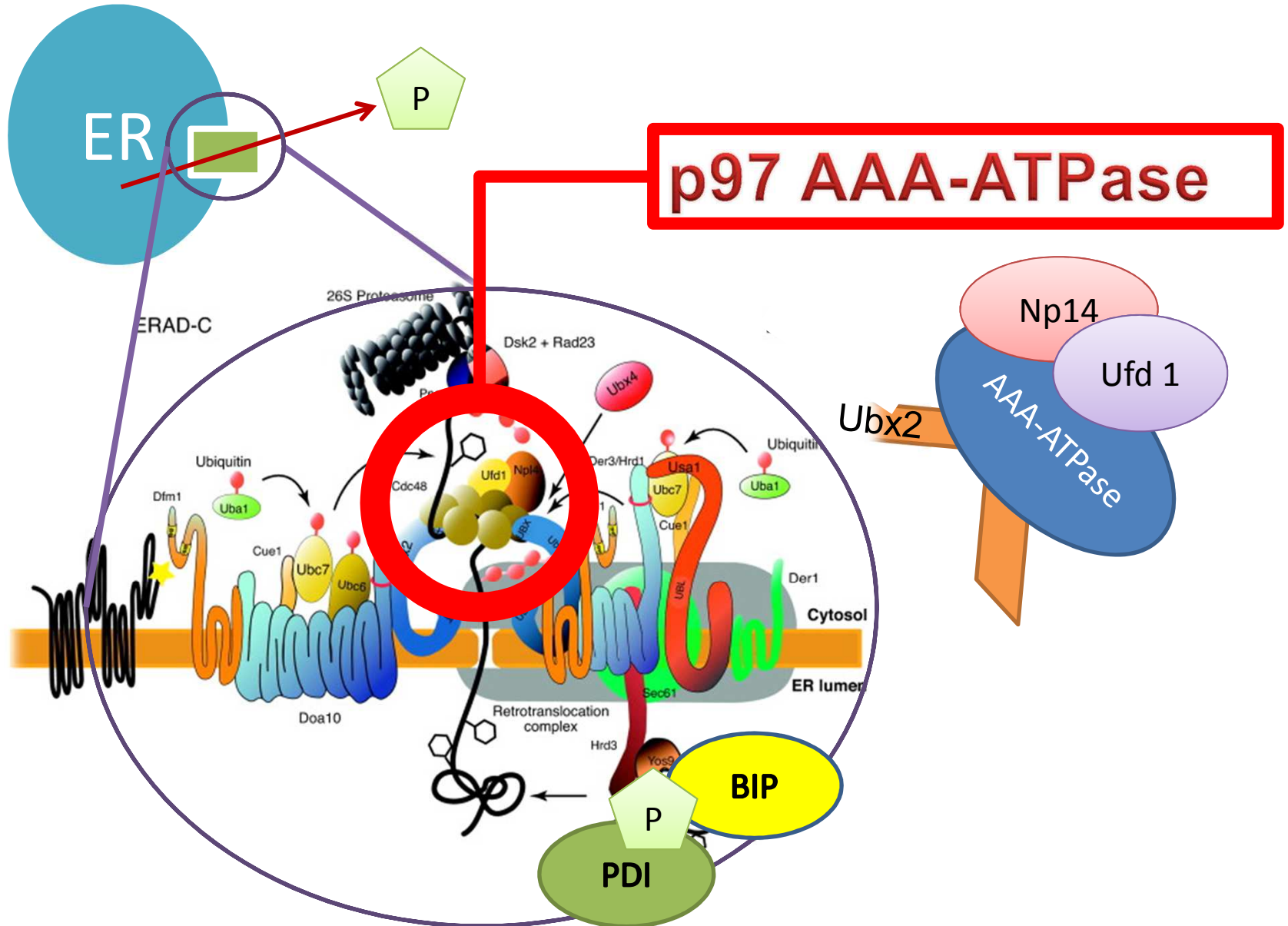
# Unfolded Protein Response

Concetto generico



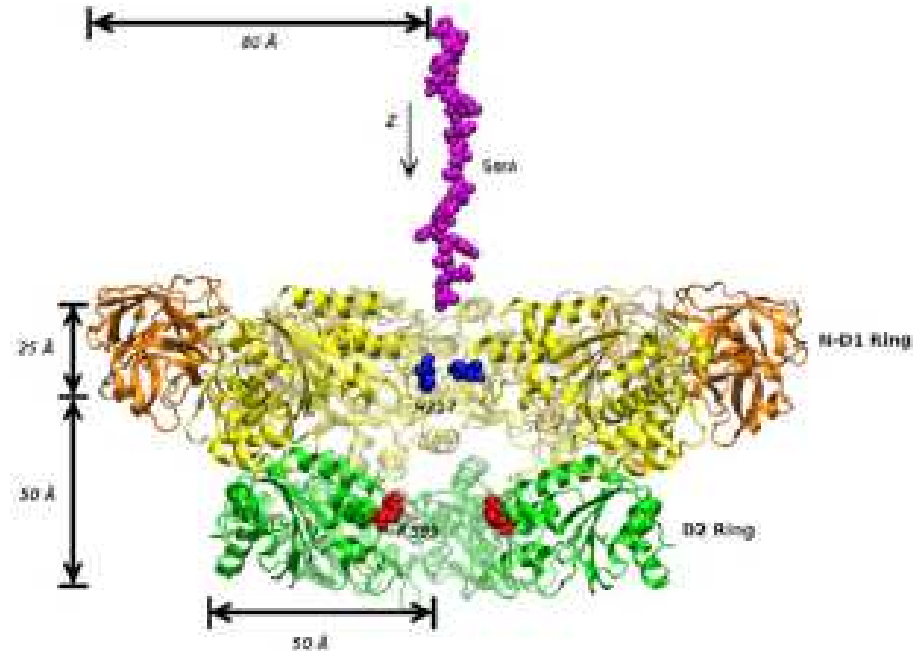
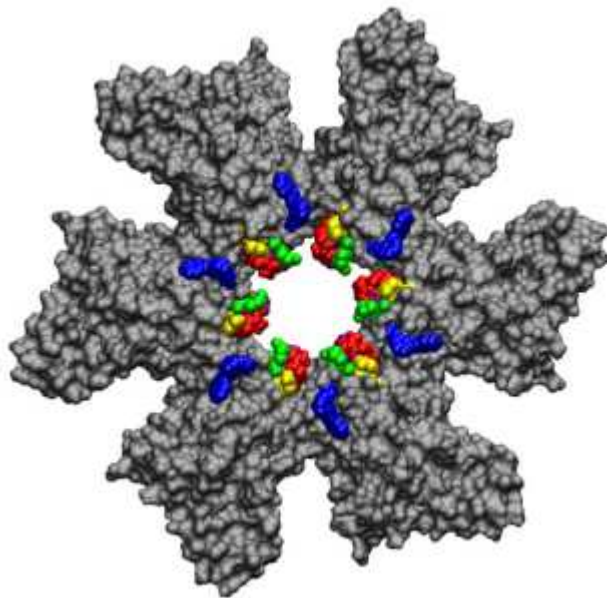
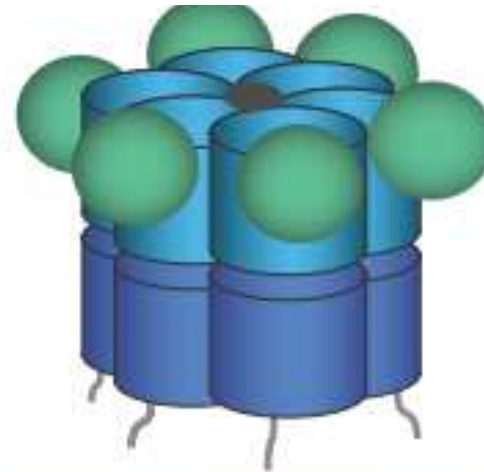
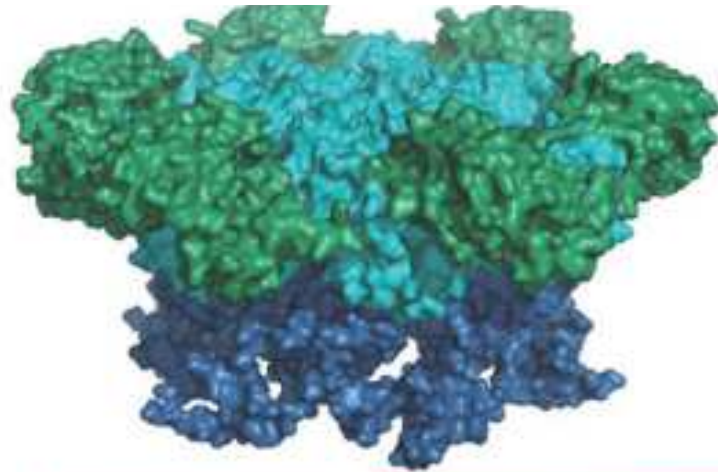


# ERAD ER-Associated Degradation

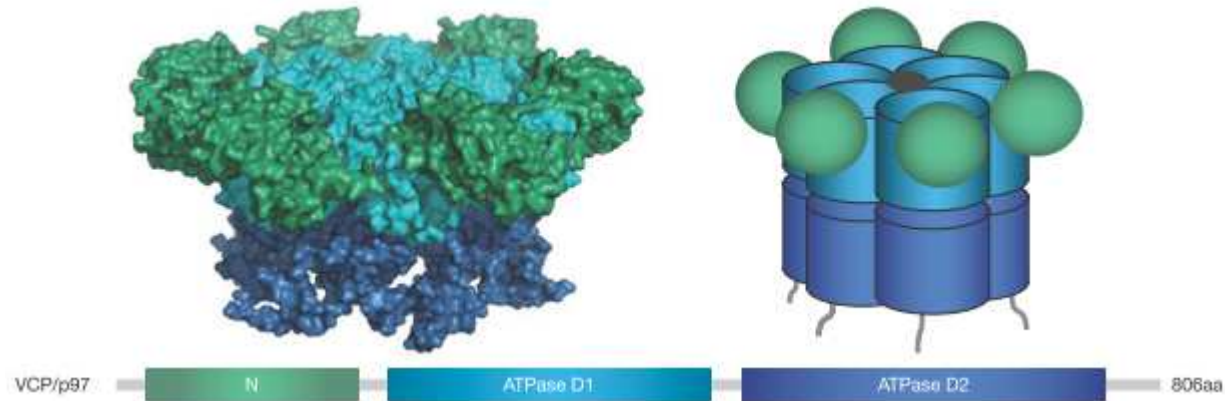




# Cdc48 (yeast) / p97 AAA-ATPase (human)



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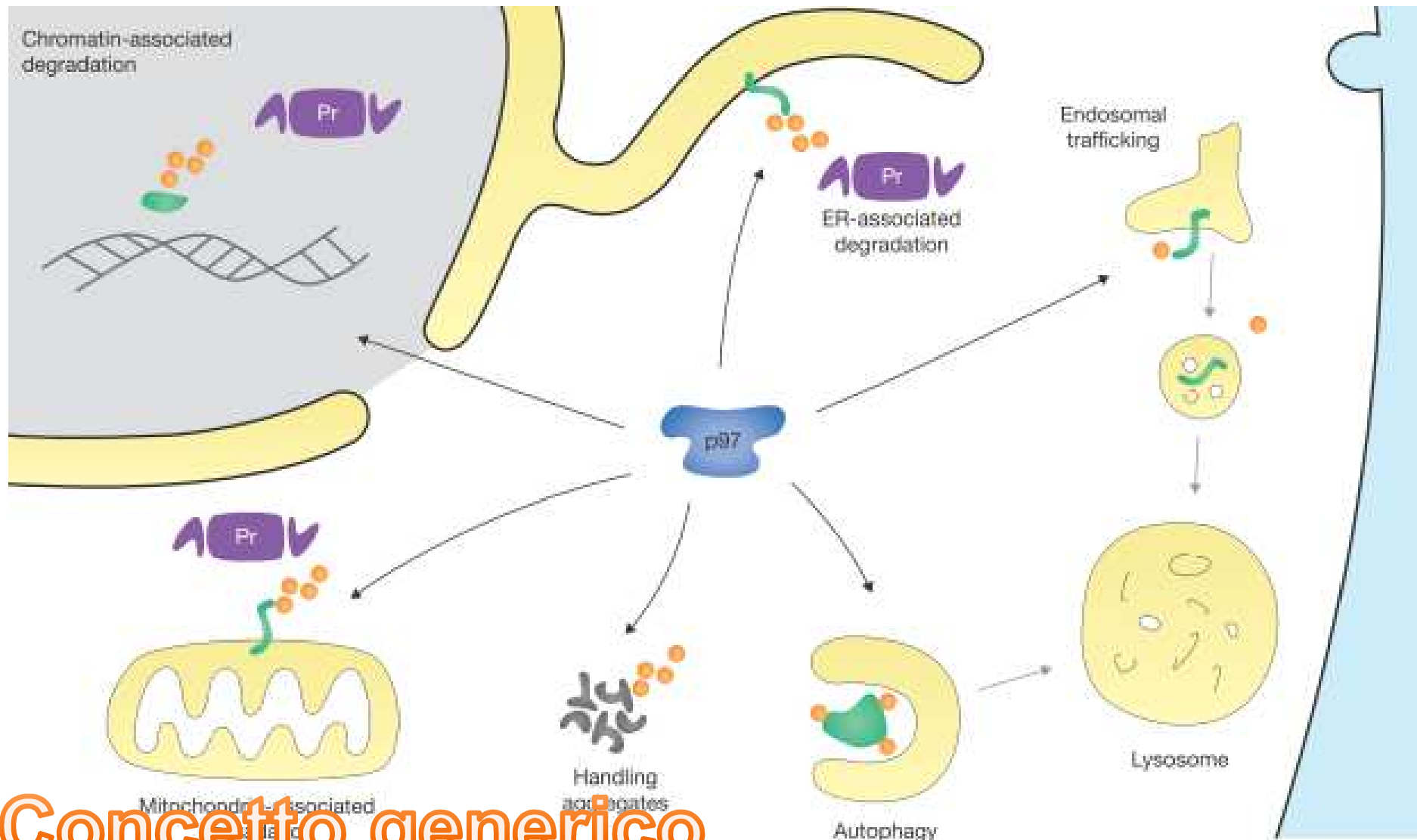
- **1%** della massa proteica della cellula
- Omoesamero
- Zn<sup>2+</sup> coordinato all'interno del «barile»
- **ATP Dipendente ed ad altissimo consumo [30-80 ATP per 100 residui]**

**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



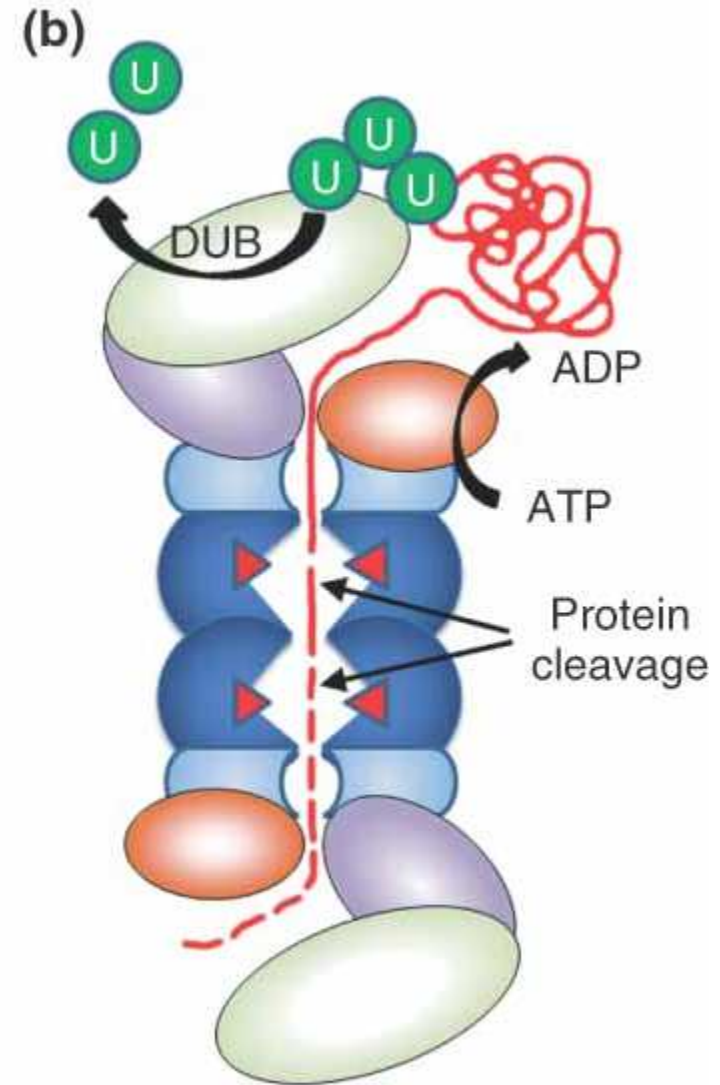
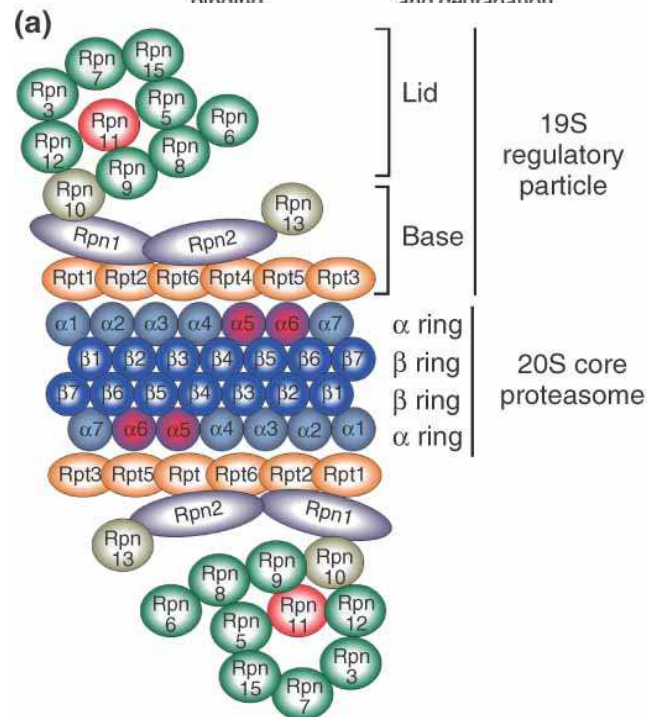
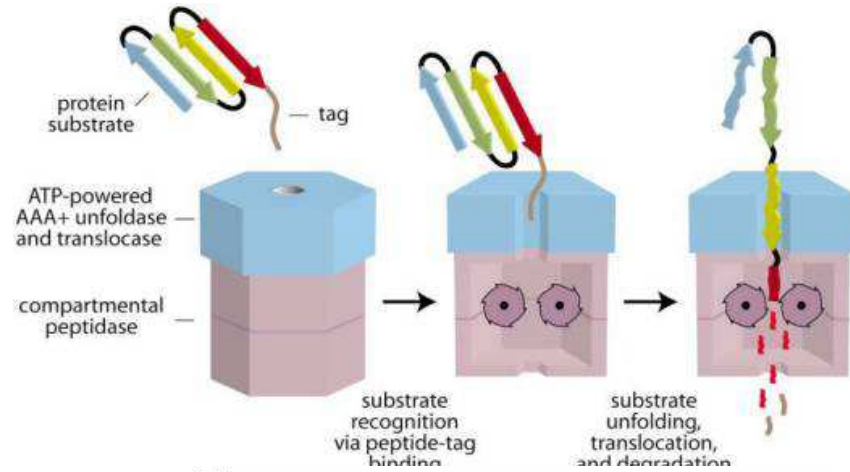
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Concetto generico

# ERAD

# Proteosoma



Studio del pathway  
UBXD7 – p97 – HIF1 $\alpha$

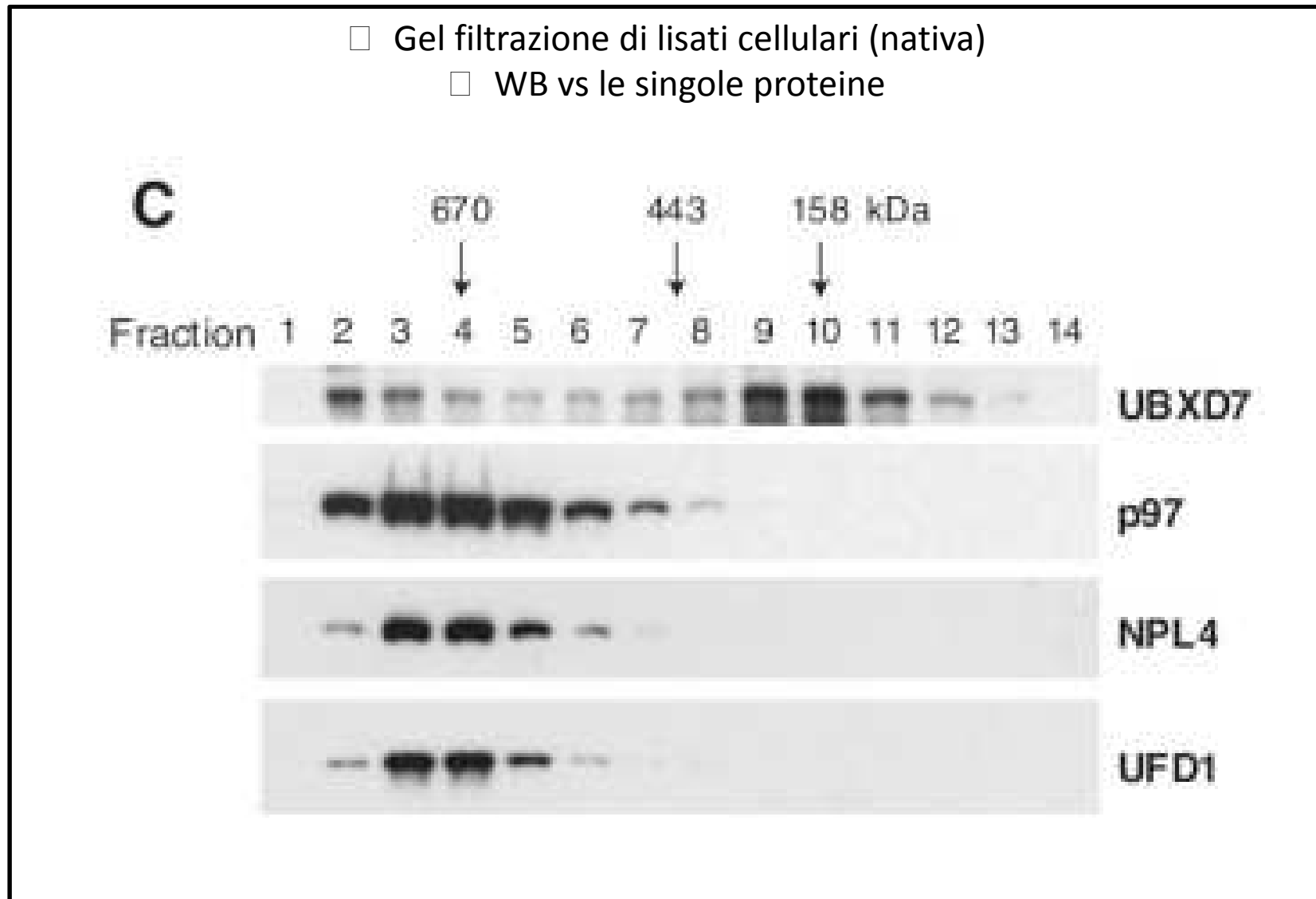
**Separazione per gel filtrazione**

**Proteina – FLAG**

**Immunoprecipitazione**

**Western Blotting**

# Chi lega in condizioni normali p97??



# Cosa lega UBXD7?

- HIF1 $\alpha$  Ossigenazione normale □ ubiquitinata e degradata
- Necessario bloccare la degradazione: MG132 (inibitore proteosoma)

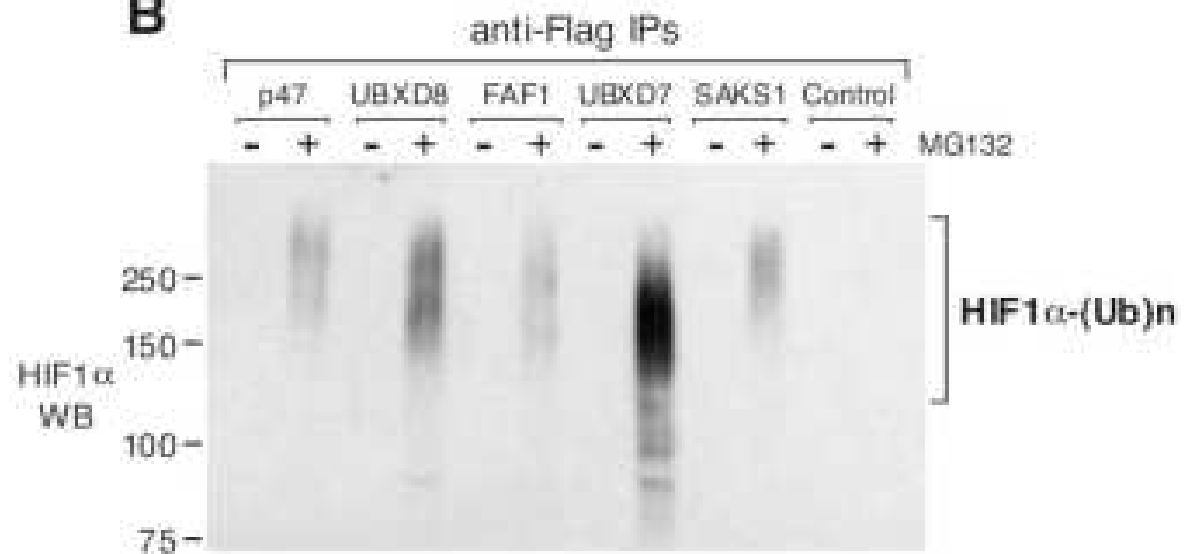
## Mass spectrometry IP by Flag-UBXD7

A

HIF1 $\alpha$ Peptides identified in UBXD7 IPs	Copy count
VESE DTSSLFDK	1
SSAD PALNOEV ALK	1
LFAEDTEAK	1
SPNVLSV ALS QR	1
TTVP EEE LNP K	1
ILALQN AQR	1
TIILIPSDL ACR	1
NLLOGEELLR	1

- Immunoprecipitati per FLAG-(UBA-UBX)
- WB vs HIF1 $\alpha$

B





# Cosa lega UBXD7?

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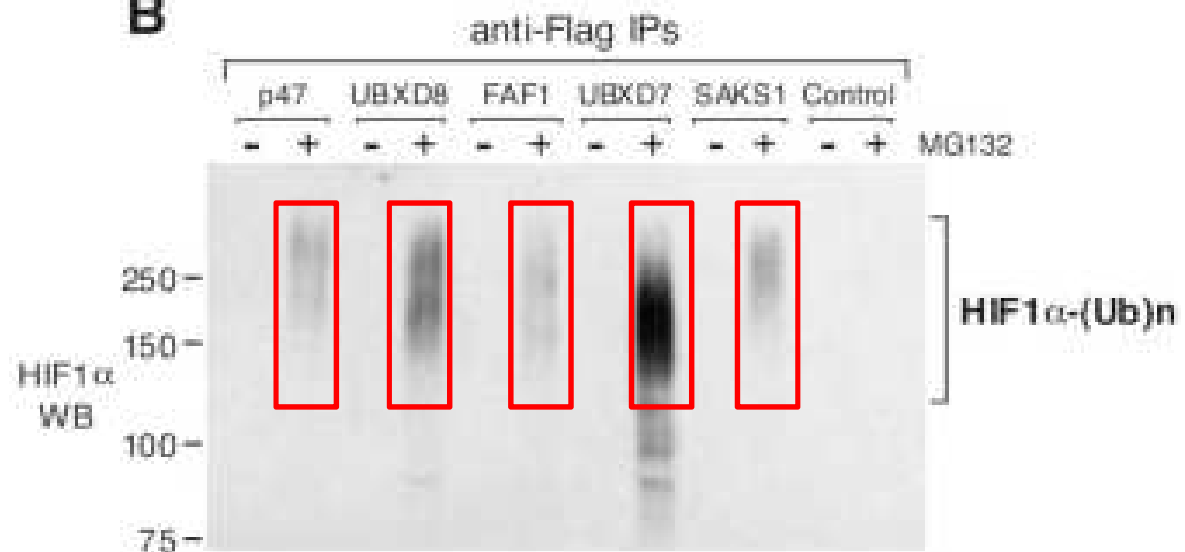
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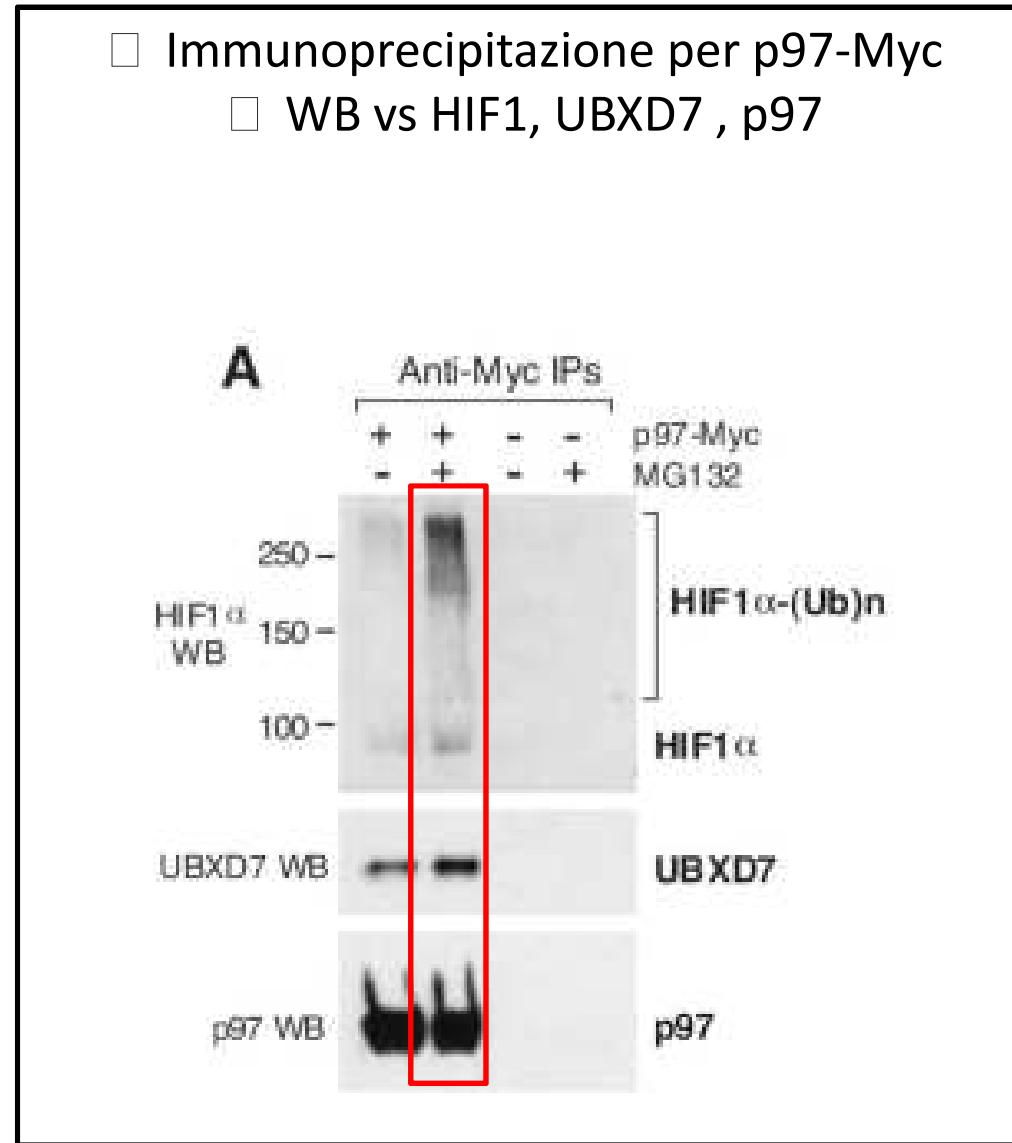
- Immunoprecipitati per FLAG-UBA-UBX
- WB vs HIF1 $\alpha$

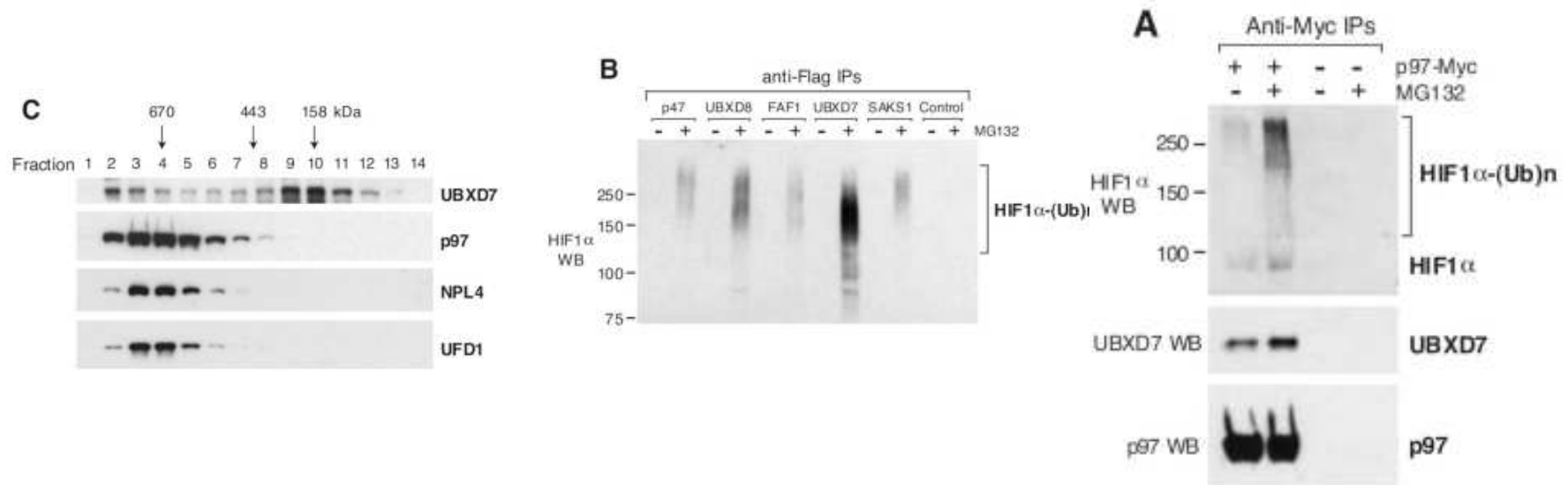
B



# Immunoprecipitation and Western Blotting

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





- HIF1 è normalmente degradata, degradazione che può essere inibita inibendo il proteosoma □ degradazione mediata dal proteosoma,
- UBXD7 lega spontaneamente HIF1, 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, questo legame deve essere mediato da UBXD7.