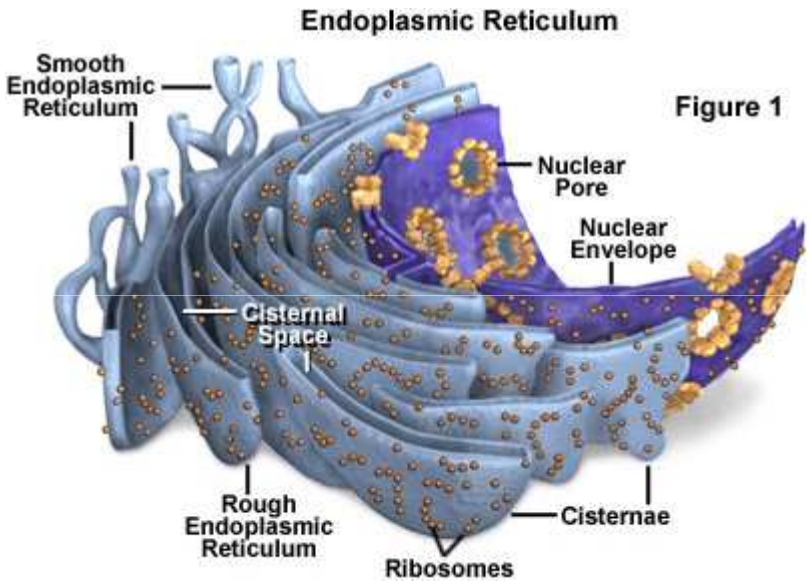
Il Reticolo Endoplasmatico

Il Controllo Qualità

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'orientemento e la produzione corretta delle proteine transmembrana (canali, recettori, proteine associate a lipidi)

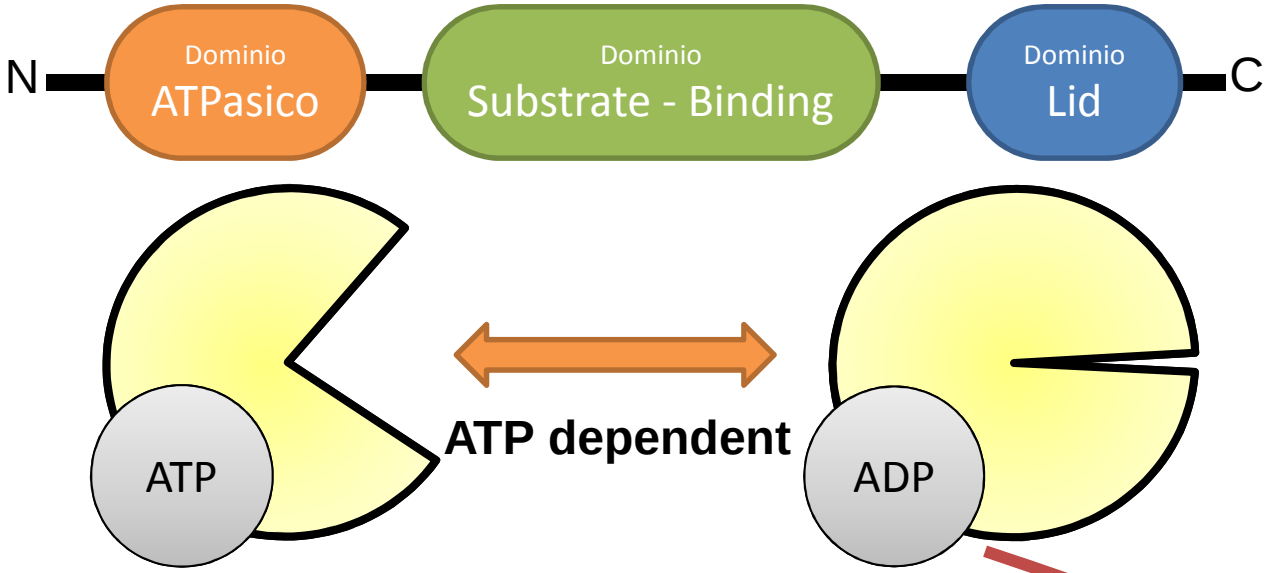
• Garantisce il folding corretto delle proteine

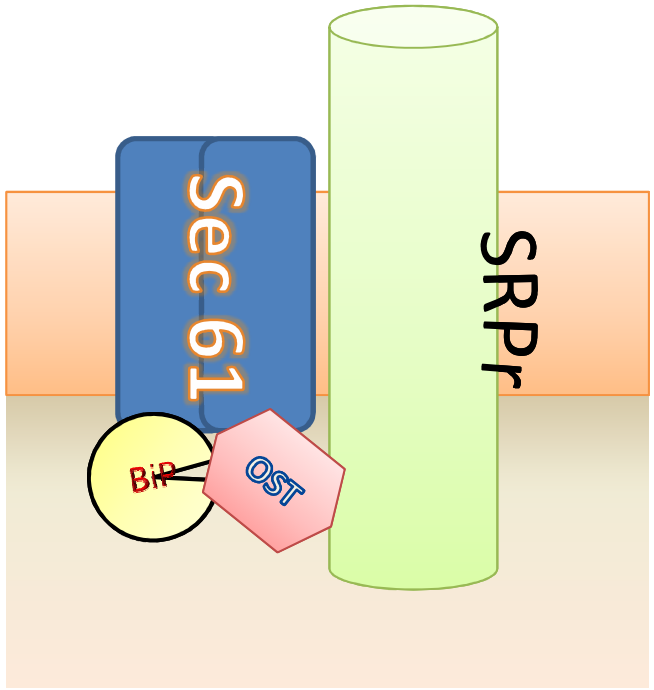
• Produce la prima glicosilazione

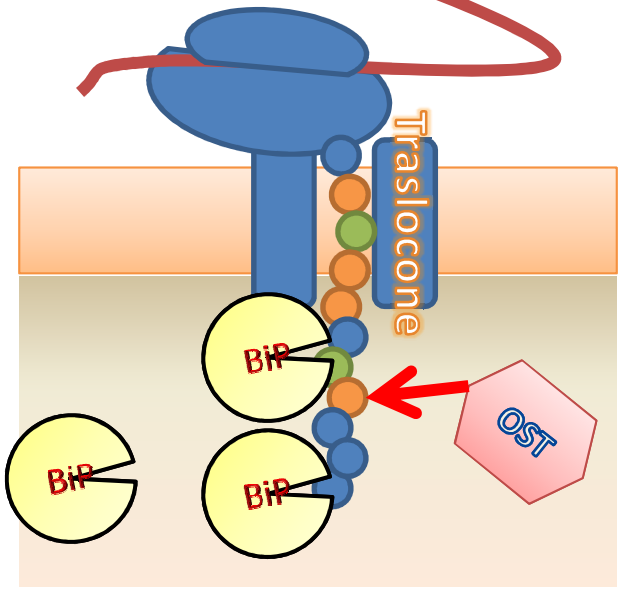
• Crea i ponti disolfuro essenziali per molte proteine**

**:anticorpi

Hsp70s


N
Dominio
ATPasico
Dominio
Substrate - Binding
Dominio
Lid
C
ATP
ATP dependent
ADP


Sec 61
SRPr
BiP
OST

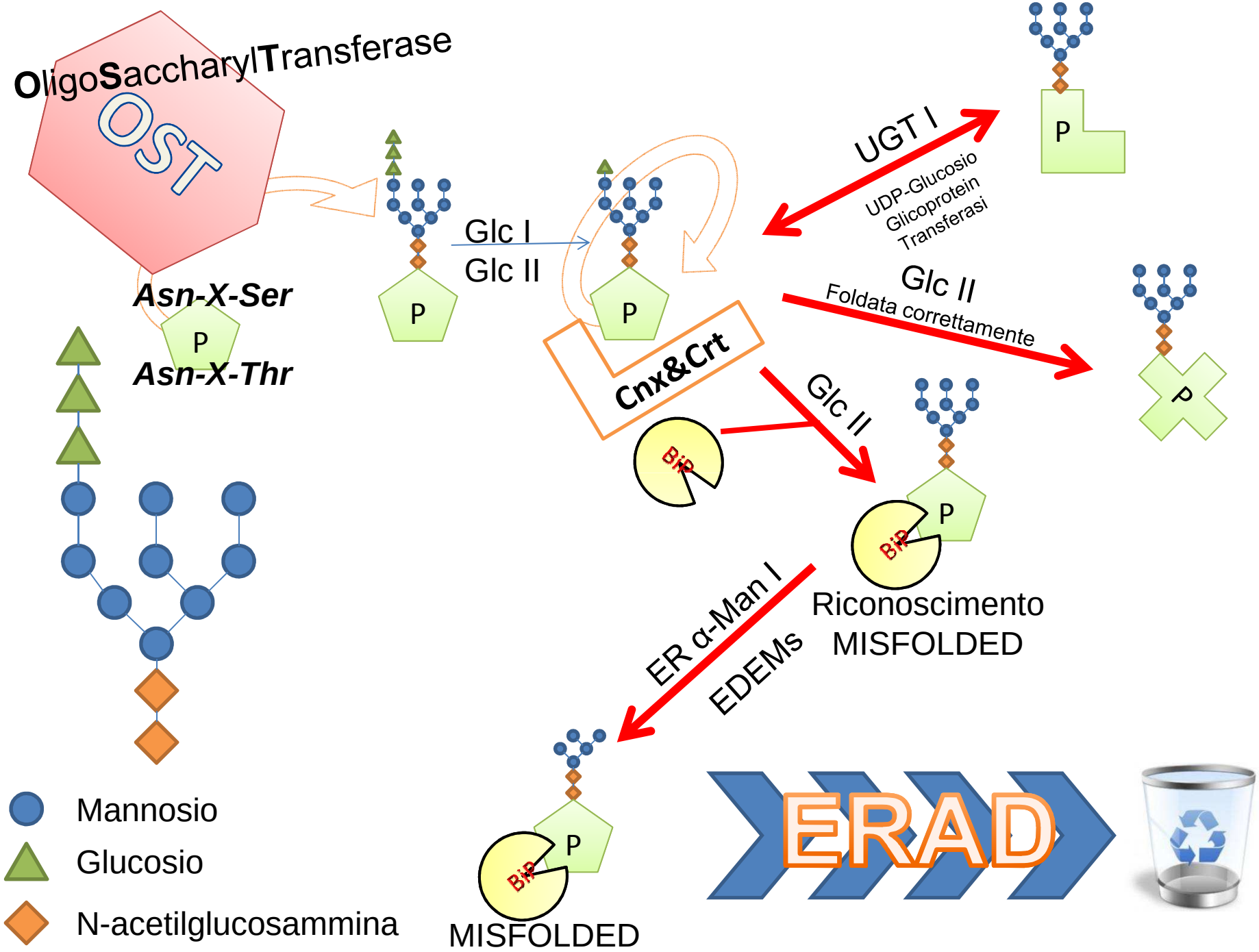

Traslocone
BiP
BiP
BiP
OST

Chaperone

Hsp70s	• ER: BiP - translocon gate, folding assistence, 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 proten until the interction with requiried partner,	• ATPase activity requires dimerization, • 2 cytoplasmic forms (α, β)

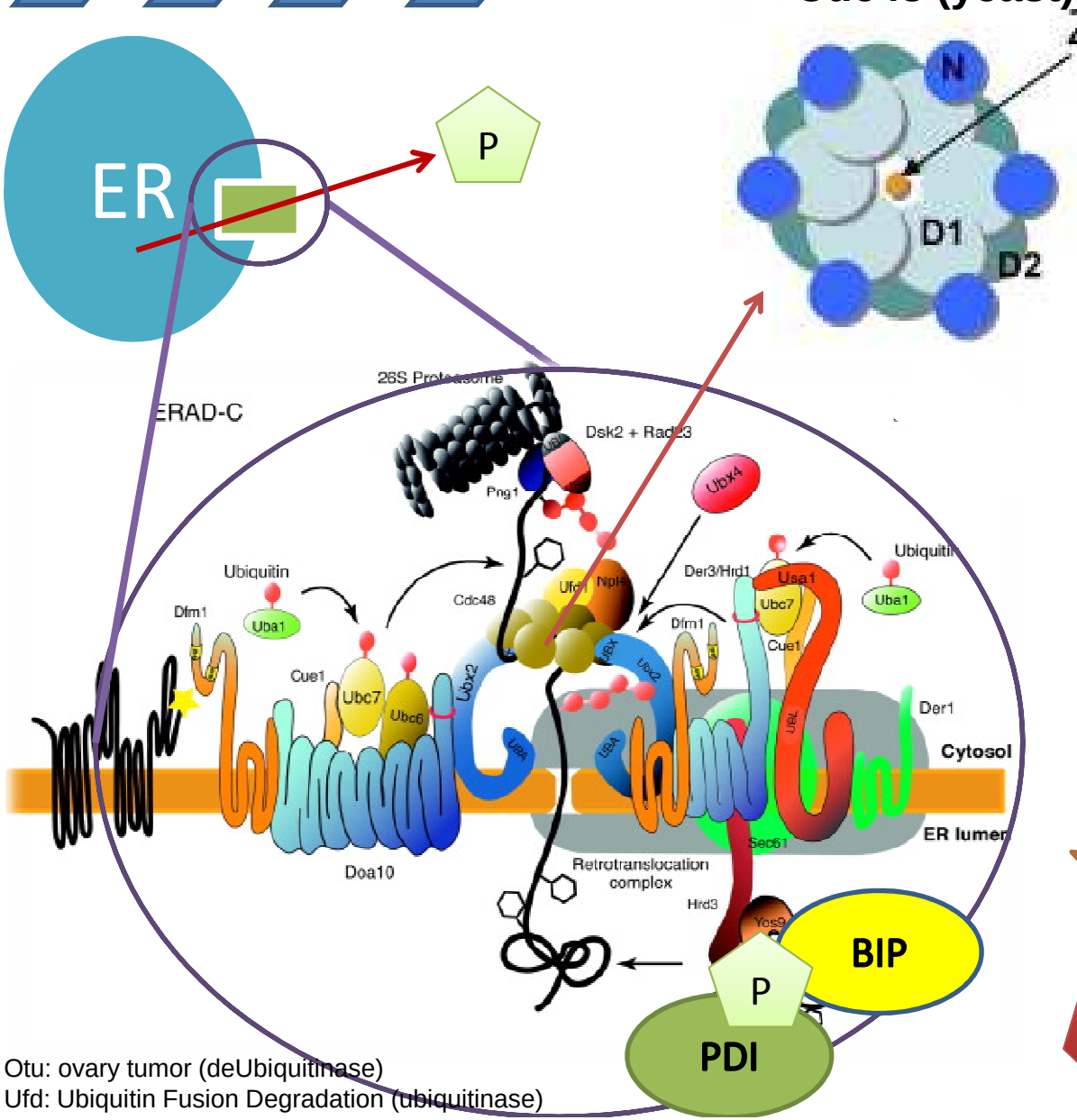
Chaperone

PDI	• 5+ **P**rotein **D**isulfide **I**somerase (Thiol Oxidoreductase) • BiP co-worker or Stand-Alone	• CxxC catalitic Motif, "**U**" shape • Ero1α (Hypoxia induced, ERAD linked), Ero1β (UPR induced)
AAA ATPase	•**A**TPase **A**ssociated with various cellular **A**ctivities • Homohexameric • Correlated to: Trascription Factors, Apoptosis, ERAD related • Bind both Ubiquitinated proteins and Proteasome	
Lectine - Like	• Reconizes Oligosaccharyl-appended N-glycan • Facilitate folding and ERAD • in this family we find Calnexin (**Cnx**) and Calreticulin (**Crt**)	
NEFs	• Nucleotide Exchange Factor; Enhance ADP from Hsp70s, • BAG-1 works also with Bcl-2 • NEFs : Hsp70s Ratio varies folding efficiency	


OligoSaccharylTransferase
OST
Asn-X-Ser
Asn-X-Thr
P
Glc I
Glc II
Cnx&Crt
BiP
UGT I
UDP-Glucosio
Glicoprotein
Transferasi
Glc II
Foldata correttamente
Glc II
ER α-Man I
EDEMs
Riconoscimento
MISFOLDED
MISFOLDED
ERAD
Mannosio
Glucosio
N-acetilglucosammina

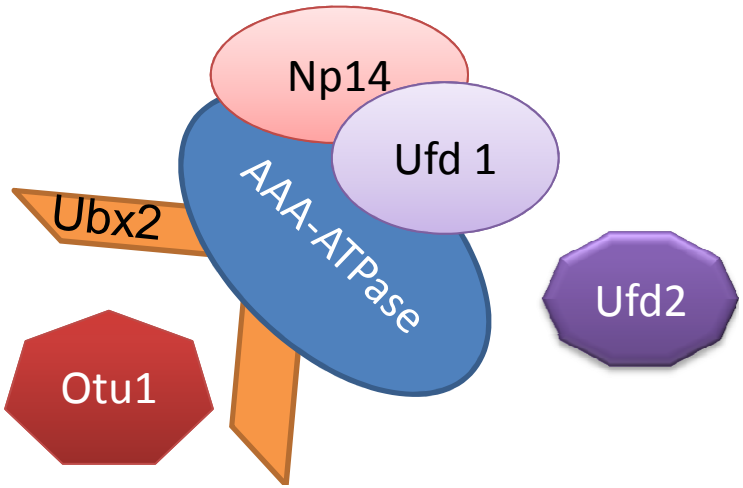
ERAD ER-Associated Degradation

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



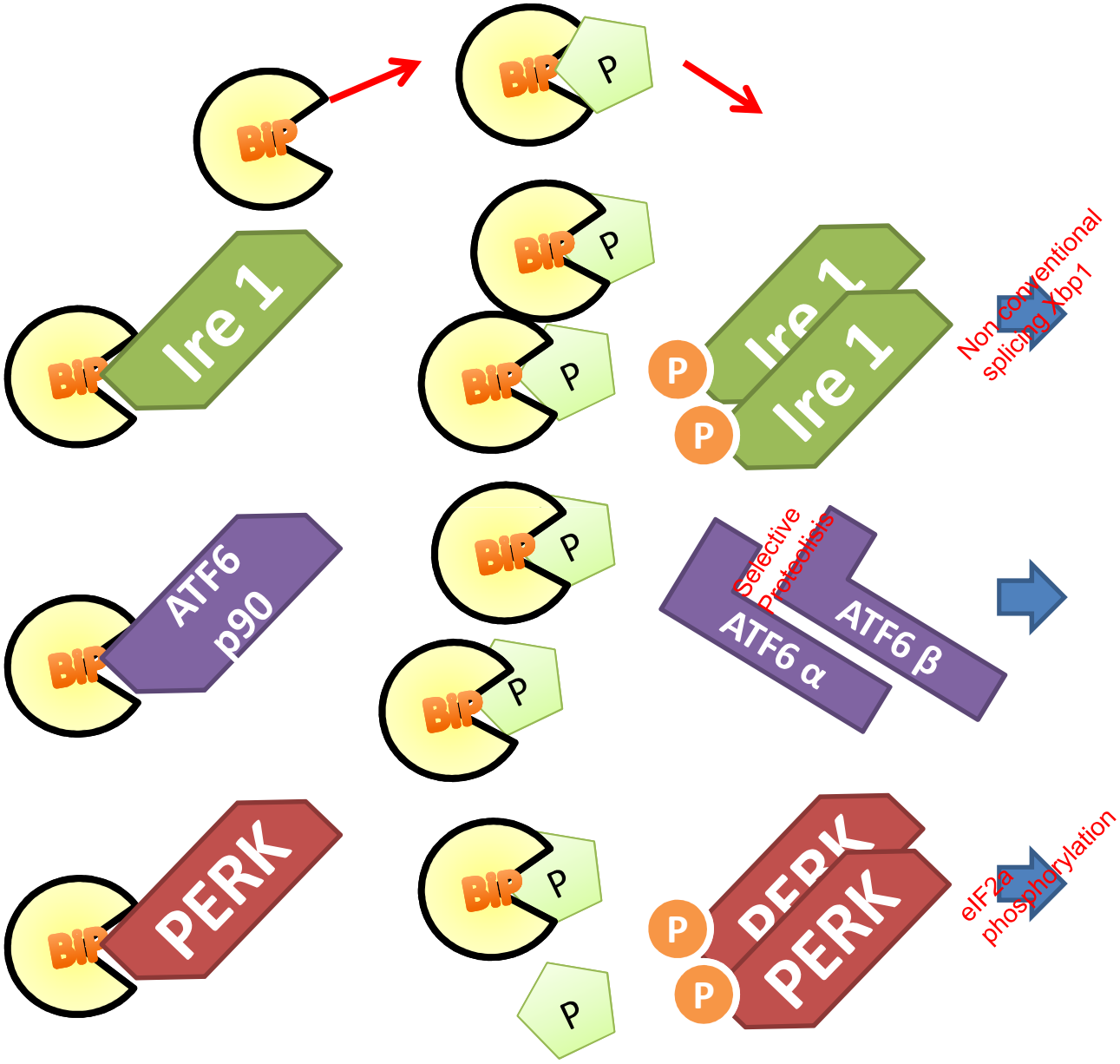
- Homohexmeric
- Coordinated Zn^{+2} into the "barrel"
- ATP dependent
- 12 active sites
- Active from 30° to 60℃

- Link L-ERAD and C-ERAD
- "Disassembly Engine"
- Denaturation-like activity
- Interact and help the proteasome



Otu: ovary tumor (deUbiquitinase)
Ufd: Ubiquitin Fusion Degradation (ubiquitinase)

Unfolded Protein Response



ERAD
Chaperones

Chaperones
Apoptosis

eIF2α → eIF2α-P

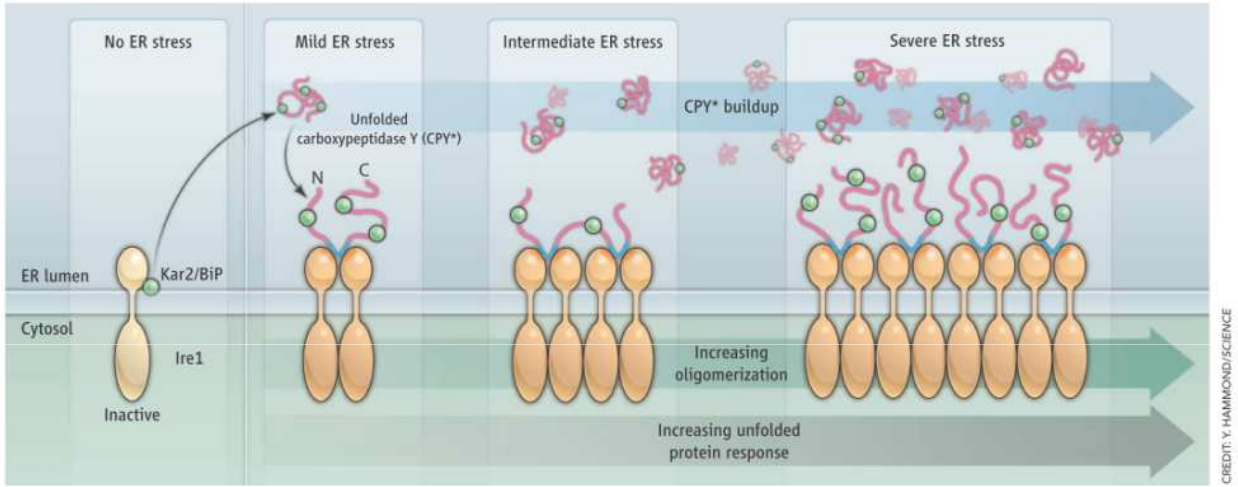
ATF4 → Anti-ox stress
AA Metabolism
Chaperones
Apoptosis

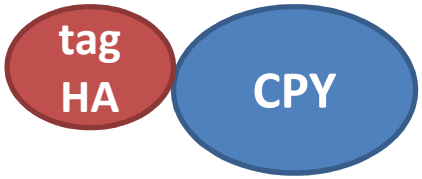
IRE1 E LO STUDIO DELL'UPR (YEAST)

IRE1 E LO STUDIO DELL'UPR

(YEAST)

Ire 1 FLAG tagged

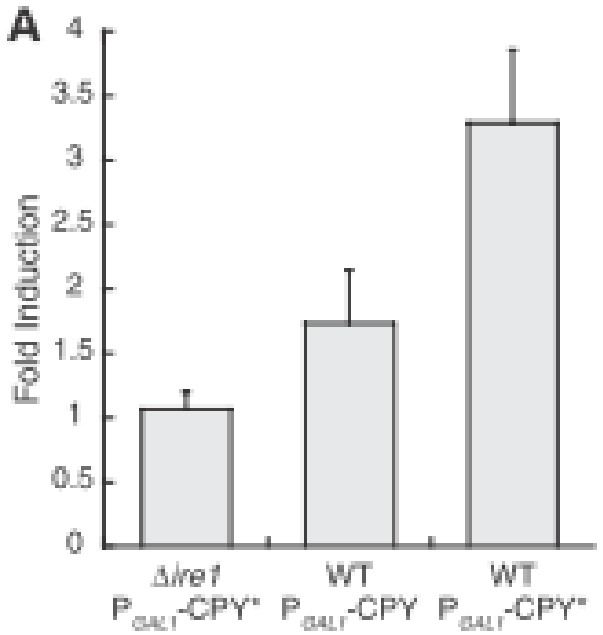




Carboxypeptidase Y hemagglutinin tagged

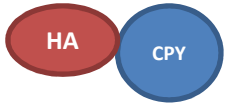
Domanda:
Ire1 induce ERAD se stimolata da CPY* (missfolded)?

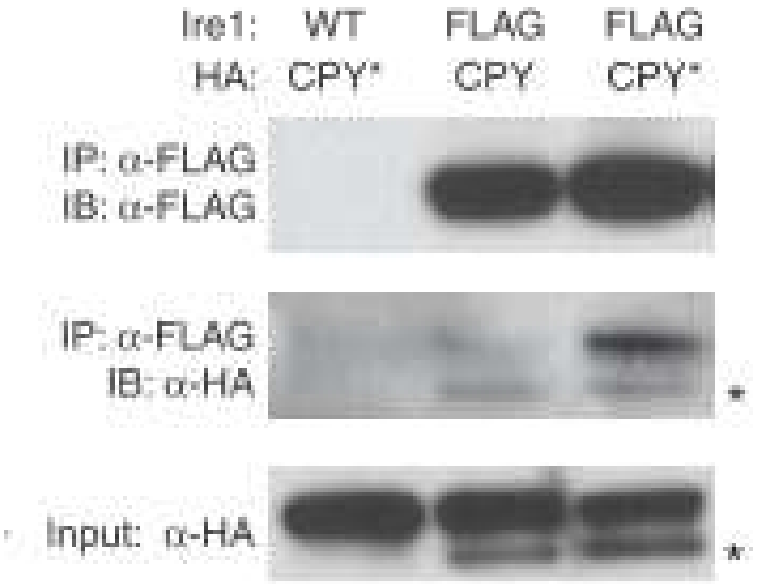
Induzione UPR
Misurata con GPF a valle di elementi responsivi promotori all'UPR



Domanda:
Ire1 lega selettivamente CPY*(missfolded)?

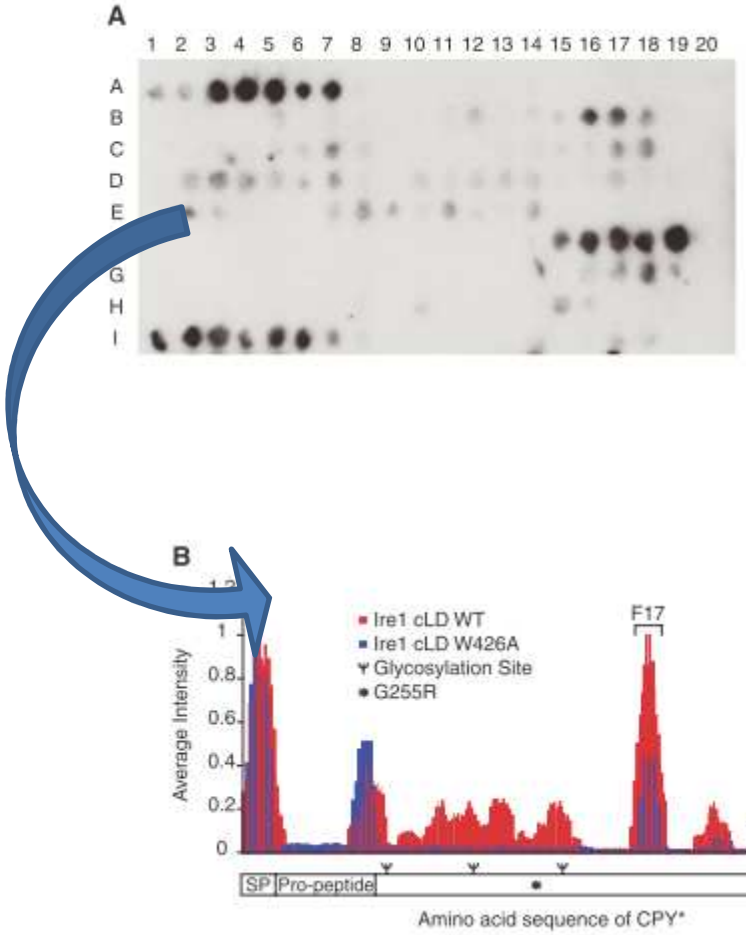
Western Blotting e Immunoprecipitazione





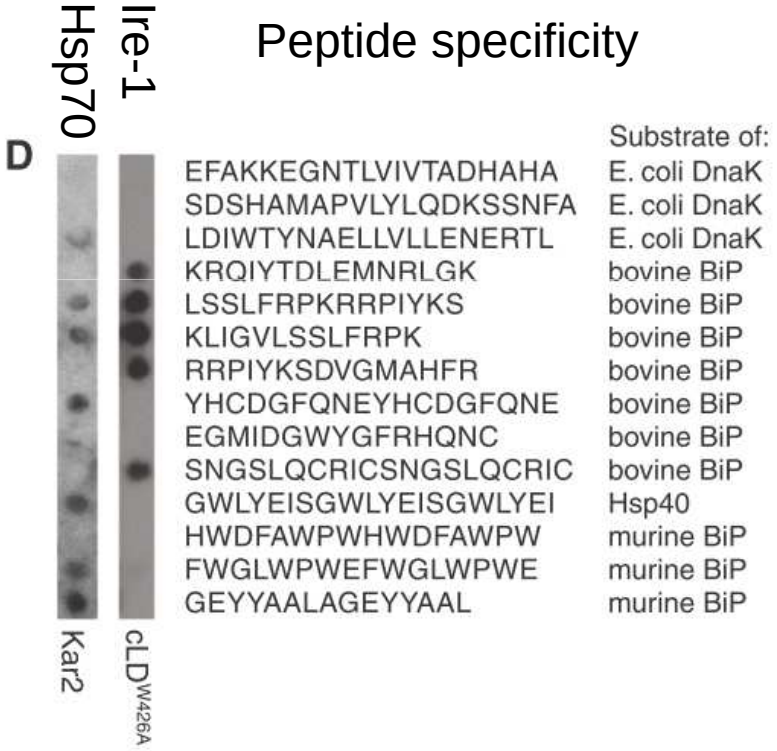
Domanda:
Che peptidi di CPY* lega Ire1?

Peptide Array



Domanda:
Ire1 lega peptidi riconosciuti anche da altre proteine coinvolte nel folding?

Peptide specificity



IRE1 E LO STUDIO DELL'UPR (YEAST)

- Ire 1 induce UPR
- Induce UPR legando direttamente la proteina missfoldata
- Ire 1 lega particolari sequenze della proteina, non è un binding aspecifico o generalizzato
- Ire 1 lega peptidi riconosciuti anche da altre proteine ma sostanzialmente questi peptidi sono diversi da quelli legati da Kar2 (omologo in lievito di Bip)

DIZIONARIO

EDEMs: ER Degradation Enhancing α-Mannosidase like proteins
ERAD: ER Associated Degradation
UPR: Unfolded Protein Response
Cnx: Calnexin (calnexina)
Crt: Calreticulin (calreticulina)
Glc I e II: Glucosidase I e II
Ire1: Inositol Requiring Enzime 1
AFT6: Amp dependent Trascription Factor 6
PERK: Precursor Eukaryotic translation initiation factor 2-alpha Kinase
PDI: Protein Disulfide Isomerase