

Formazione delle strutture amiloidi_1

- ✓ Gli aggregati possono essere amorfi

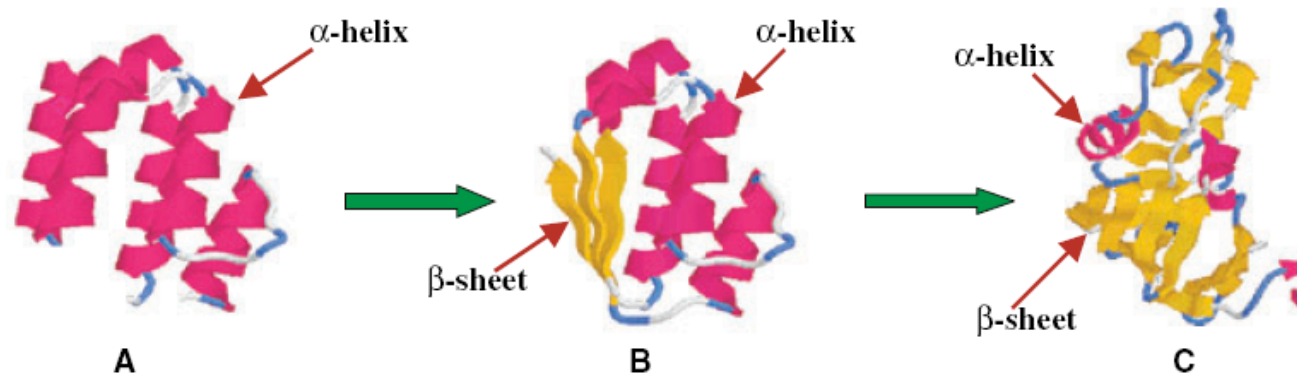


Fig. 1. During amyloid formation most of the α -helical structures in the polypeptide chain of a native protein are converted into β -pleated sheets. (A) Native polypeptide chain composed of mainly α -helical secondary structure. (B) Misfolding causes conversion of α -helical structure to β -pleated sheets and (C) final misfolded structure of polypeptide chain contains mostly β -pleated sheets.

Formazione delle strutture amiloidi_2

- ✓ Gli aggregati possono essere strutturati

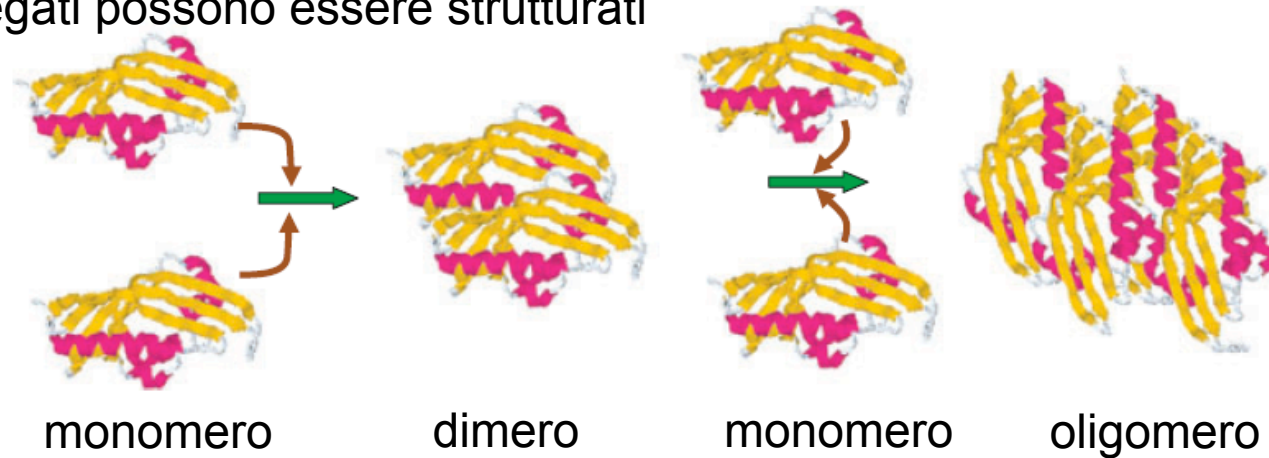


Fig. 2. Protein oligomerization. Misfolded monomers forming aggregate through intermolecular hydrogen bonding interaction leading to β -sheet formation.

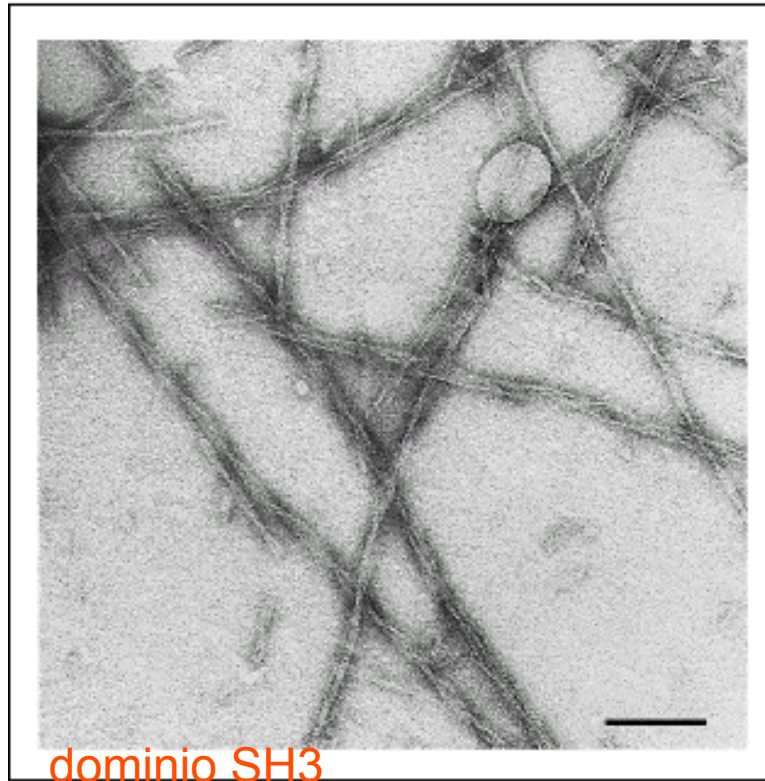
Strutture amiloidi

- ✓ Specifiche caratteristiche di colorazione (Congo Red, TioflavinaS)
- ✓ Specifiche proprietà biofisiche

dipendono dalla presenza di una struttura centrale comune costituita da

foglietti β altamente organizzati

Formazione delle strutture amiloidi_3



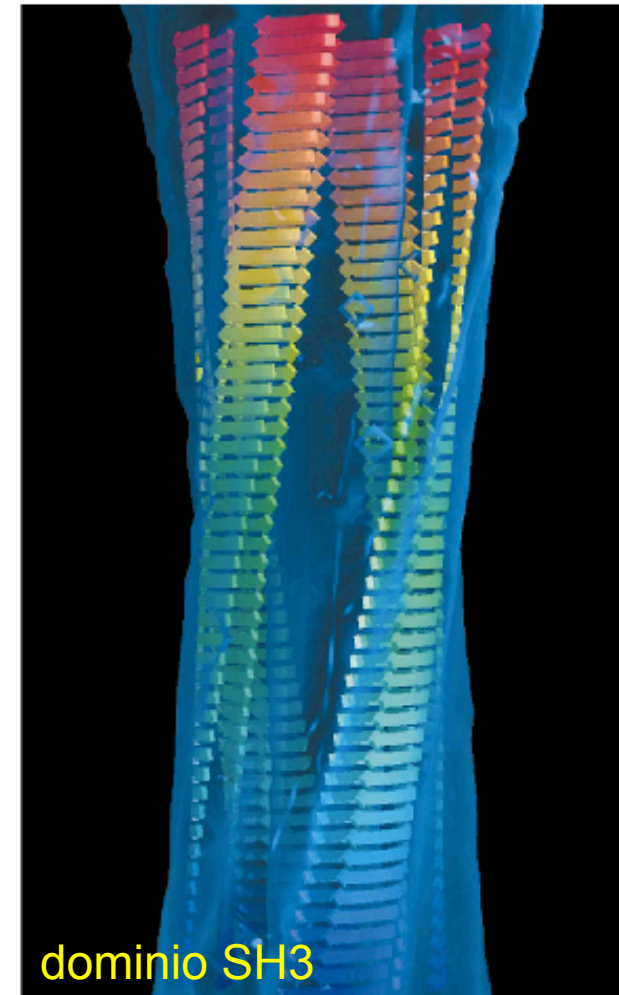
Fibrilla amiloide

6-12 nm diametro

2-6 protofilamenti

Avvolti uno sull'altro

Foglietti β perpendicolari all'asse



Formazione delle strutture amiloidi_4

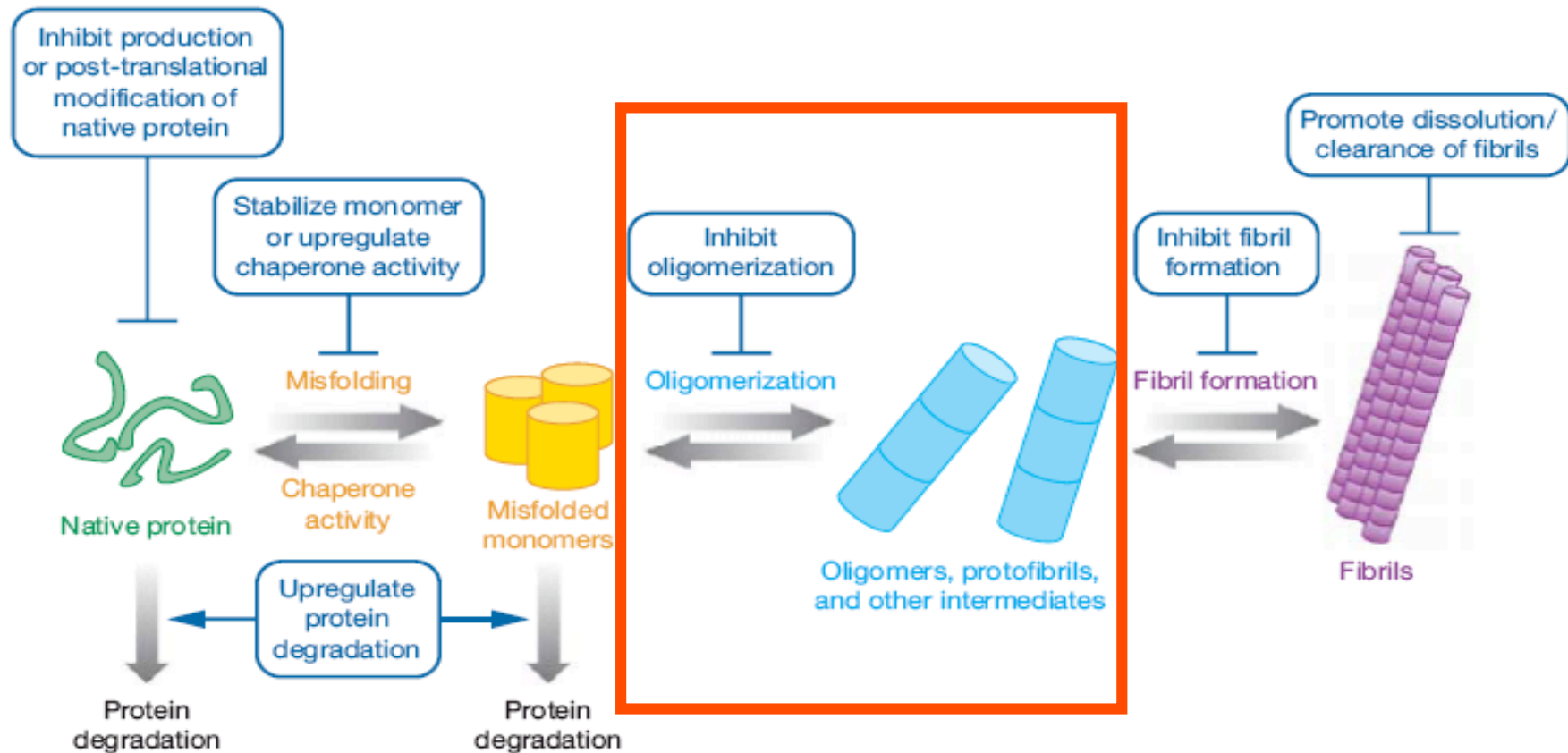
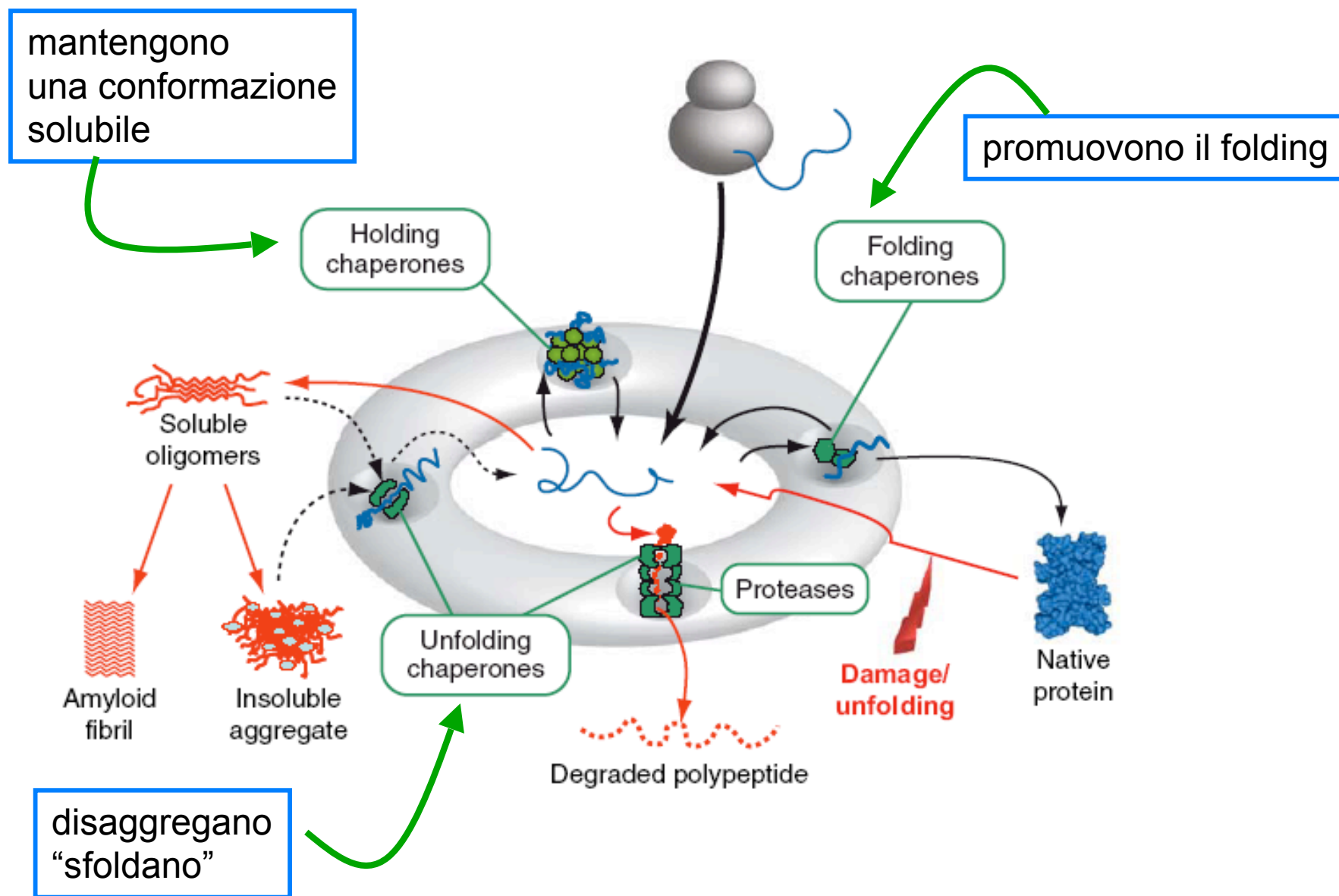


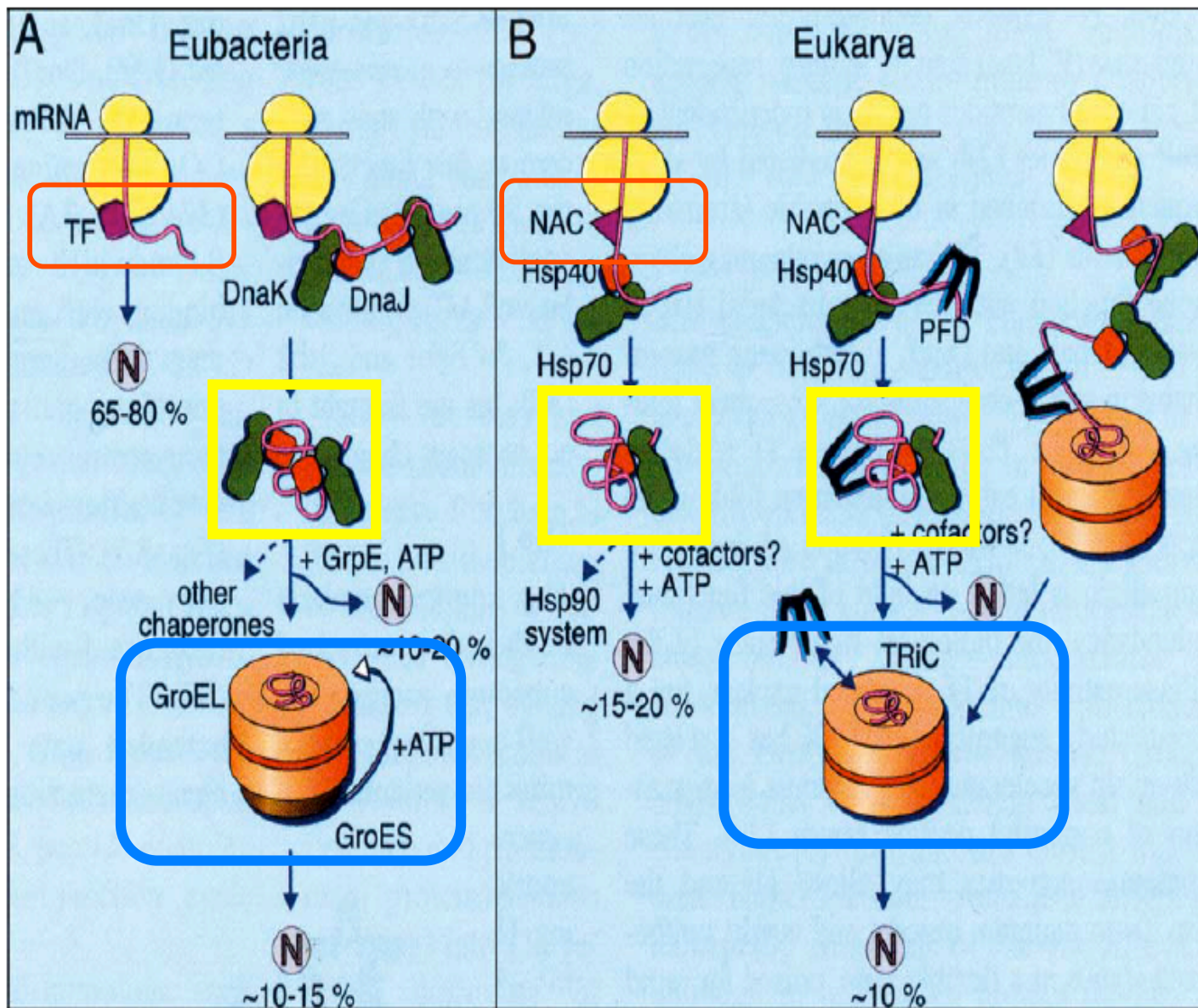
Figure 1

Model for protein misfolding and fibrillization. Soluble native protein is misfolded and associates in the form of oligomers and other intermediates that eventually give rise to fibrils. Potential opportunities for therapeutic intervention are shown in blue boxes.

Controllo di qualità delle proteine_1



Controllo di qualità delle proteine_2



catena nascente

DnaK
DnaJ
Hsp

GroEL
GroES
TRiC

CHAPERONI

Controllo di qualità delle proteine_3

Rimozione degli aggregati:

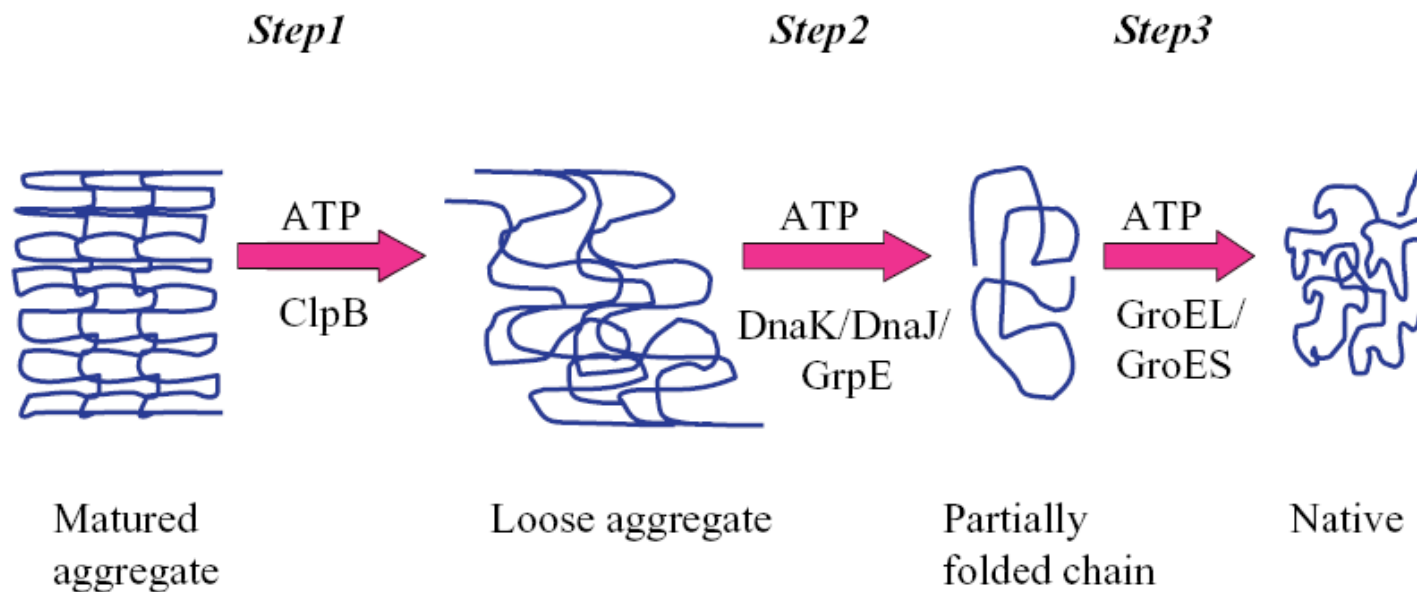


Fig. 5. Protein disaggregation process in *E.coli* by chaperones [121]. *Step1*: ClpB chaperone preprocesses the aggregate and produces a loose structure having more hydrophobic surfaces (HS) exposed to the solvent. *Step2*: DnaK binds to those newly exposed HS along with co-chaperones DnaJ and GrpE, disaggregates the loose aggregates and refolds them partially. *Step3*: GroEL and GroES assist final refolding from monomeric partially folded form to native state.

Evidenze del coinvolgimento degli chaperoni nelle neurodegenerazioni

- ✓ Alterazioni della risposta heat-shock in modelli di ND
- ✓ Inclusioni proteiche contengono chaperoni:
 - ✓ Hsp70 inclusioni di huntingtina e corpi di Lewy
 - ✓ Hsp70 e Hsp16 beta-amiloide
- ✓ Espressione di chaperoni attenua i danni cellulari in modelli di ND da aggregati

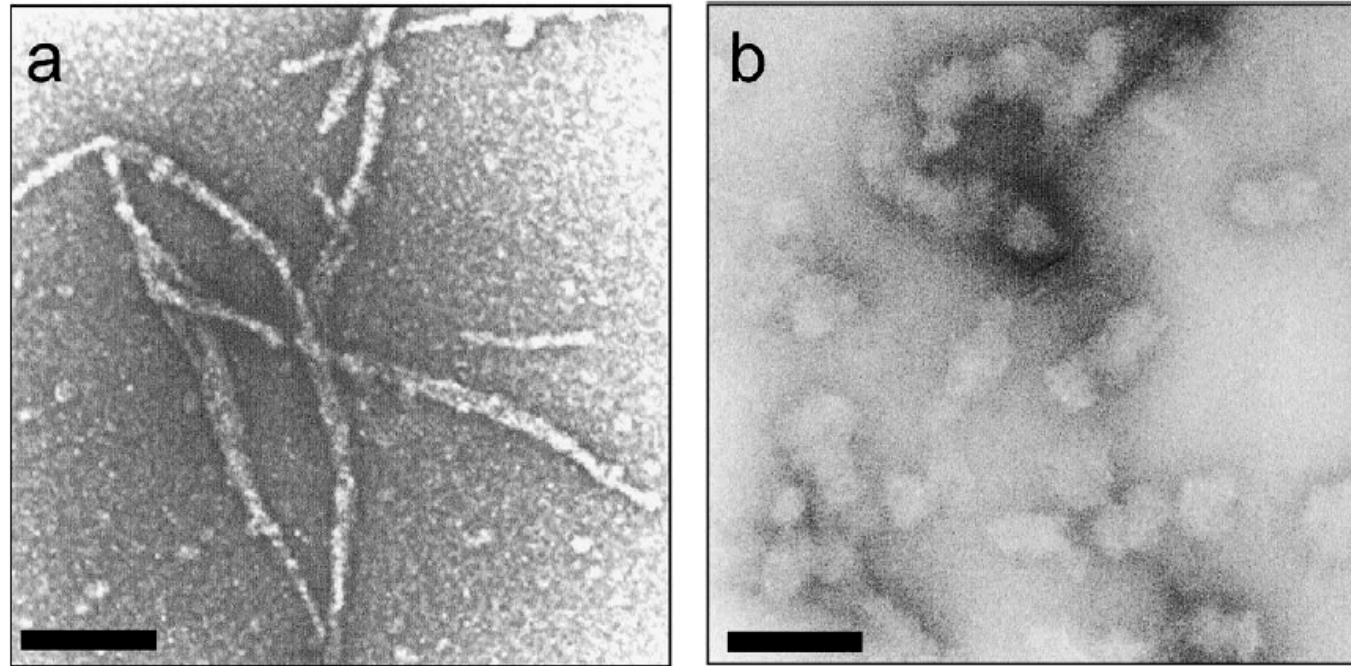
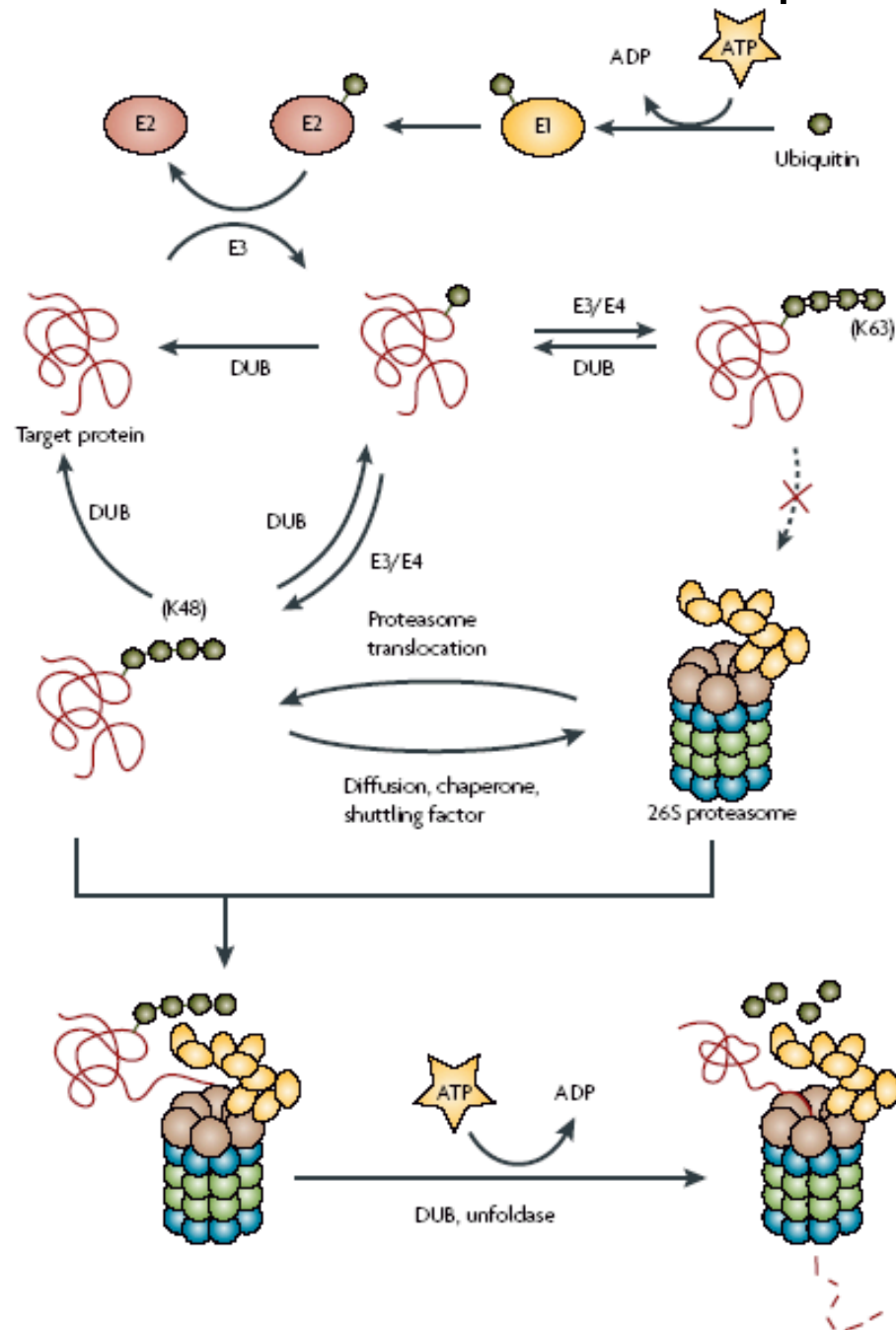


Fig. 3. Suppression of huntingtin fibril formation by molecular chaperones. Polyglutamine-expanded huntingtin was incubated in vitro in the absence of chaperones (a) or the presence of both Hsp40 and Hsp70 (b) (see [104]). Fibrillar aggregates (a) are converted to amorphous aggregates by the action of Hsp40 and Hsp70 (b) (Bar = 100 nm).

Sistema Ubiquitina/Proteasoma_1



Ubiquitina:

76aa

Lisina sulle proteine target

Lisina 48 sull Ub

4 classi di enzimi

- | | |
|----|-----------------------|
| E1 | attiva l'Ub (ATP) |
| E2 | Ub da E1 |
| E3 | Ub da E2 al substrato |
| E4 | ramificazione |

Evoluzione delle proteine ubiquitinanti

Class	<i>Homo sapiens</i>	<i>Mus musculus</i>	<i>Drosophila melanogaster</i>	<i>Saccharomyces cerevisiae</i>
E1	16	16	11	8
E2	53	61	32	15
E3	527	442	189	68
DUB	74	78	27	20

DUB, deubiquitylating enzyme. Data from REF. 66.

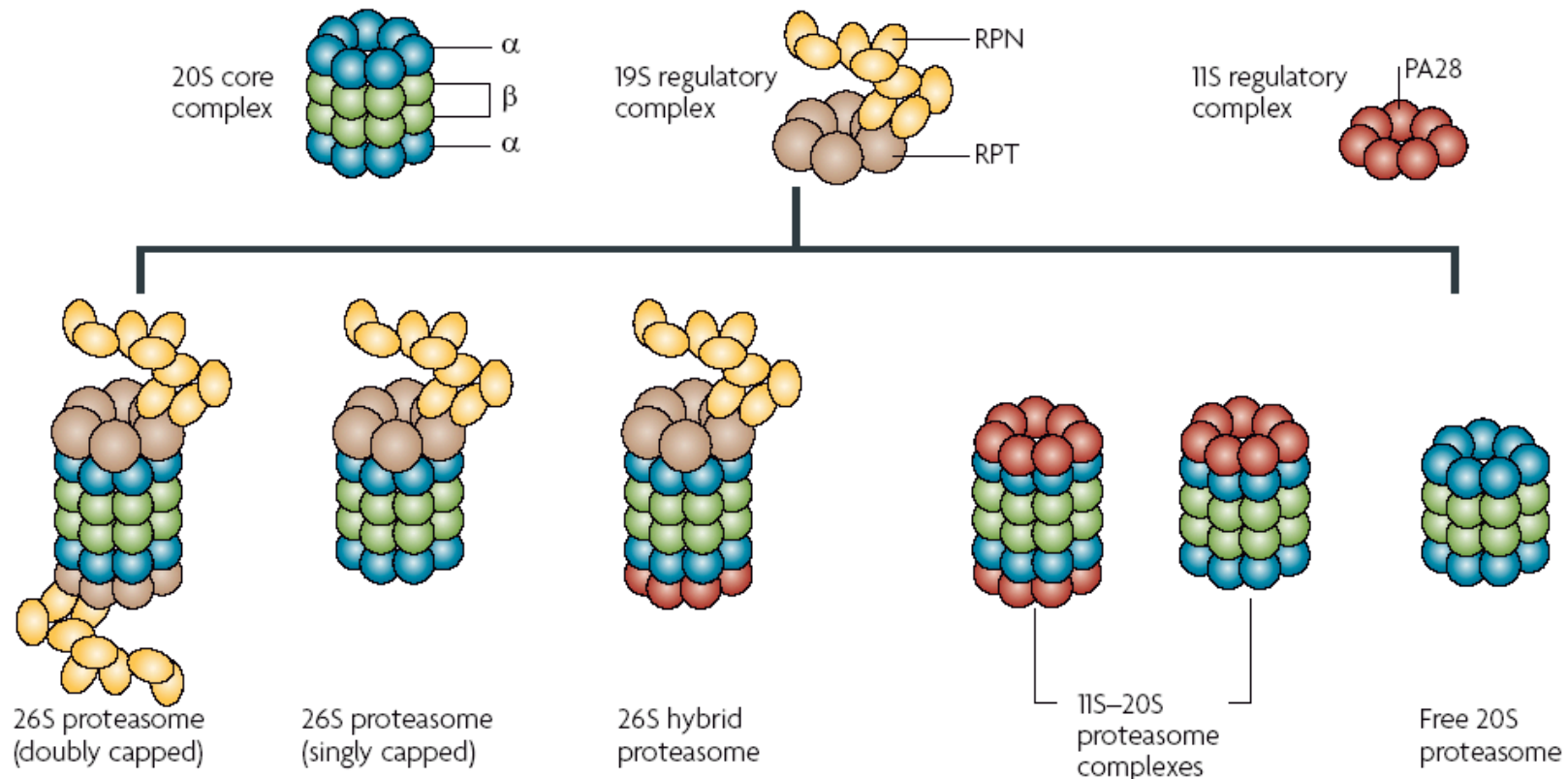
Sistema Ubiquitina/Proteasoma_2

20S: contiene le tre attività enzimatiche

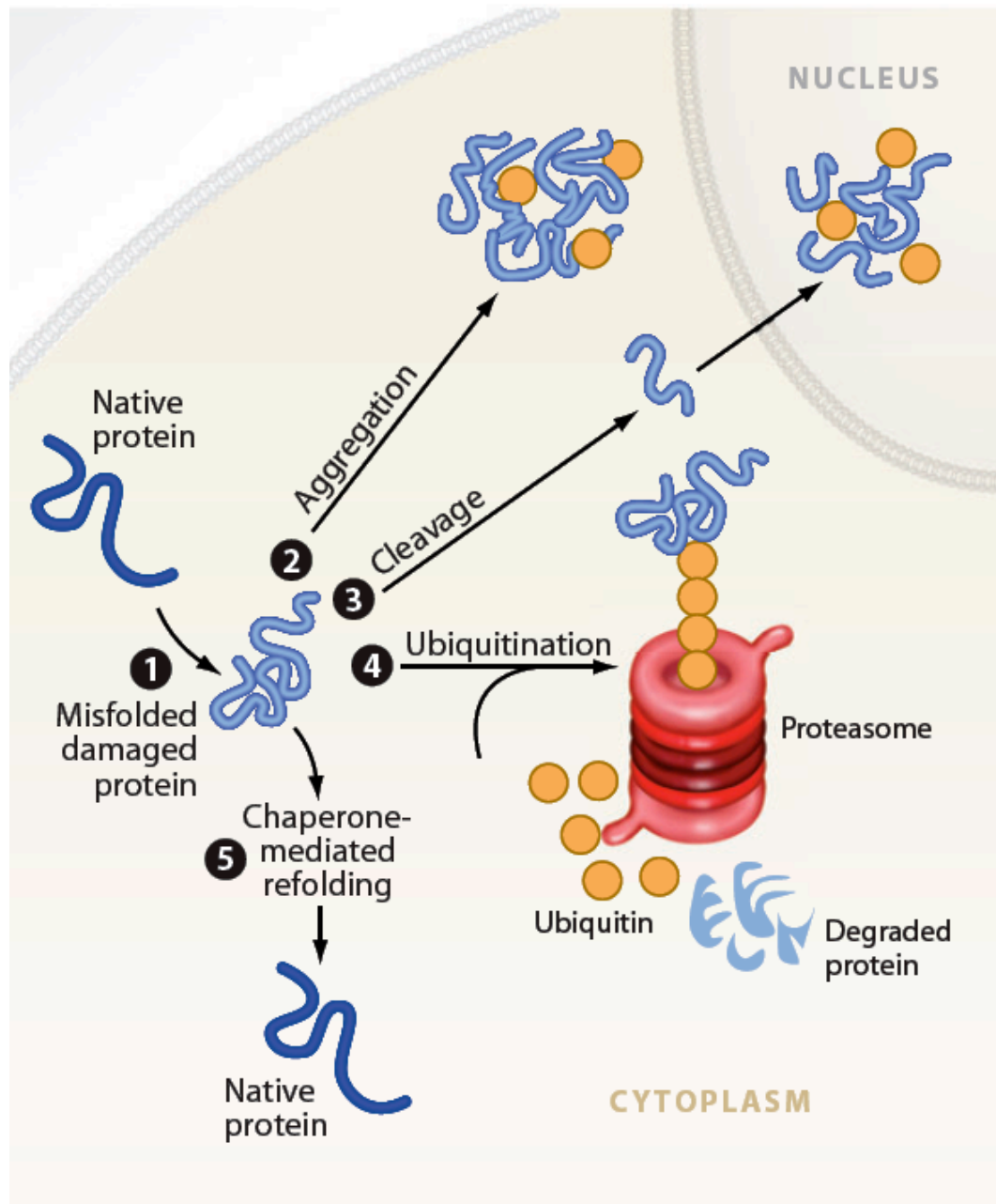
19S: regolatorio:

riconosce le catene poliUb
rimuove l'Ub
sfolda la proteina per
l'accesso all'interno del 20S

11S: attiva il 20S allargando
il poro



Sistema Ubiquitina/Proteasoma_2



Resultant cellular pathologies

Parkinson's disease



Lewy body

Alzheimer's disease



Plaque



Tangle

Polyglutamine disorders



Inclusion

PROTEASOMA

Evidenze del coinvolgimento del sistema ubiquitina/proteasoma nelle neurodegenerazioni

- ✓ Inclusioni proteiche contengono proteine poliubiquitinate
- ✓ Proteine mutate nelle ND sono substrati del sistema Ub/Prot
- ✓ Inibizione del proteasoma nei ratti induce sindromi parkinsoniane
- ✓ Mutazioni di geni per proteine correlate al sistema Ub/Prot:

Parkin (PD)

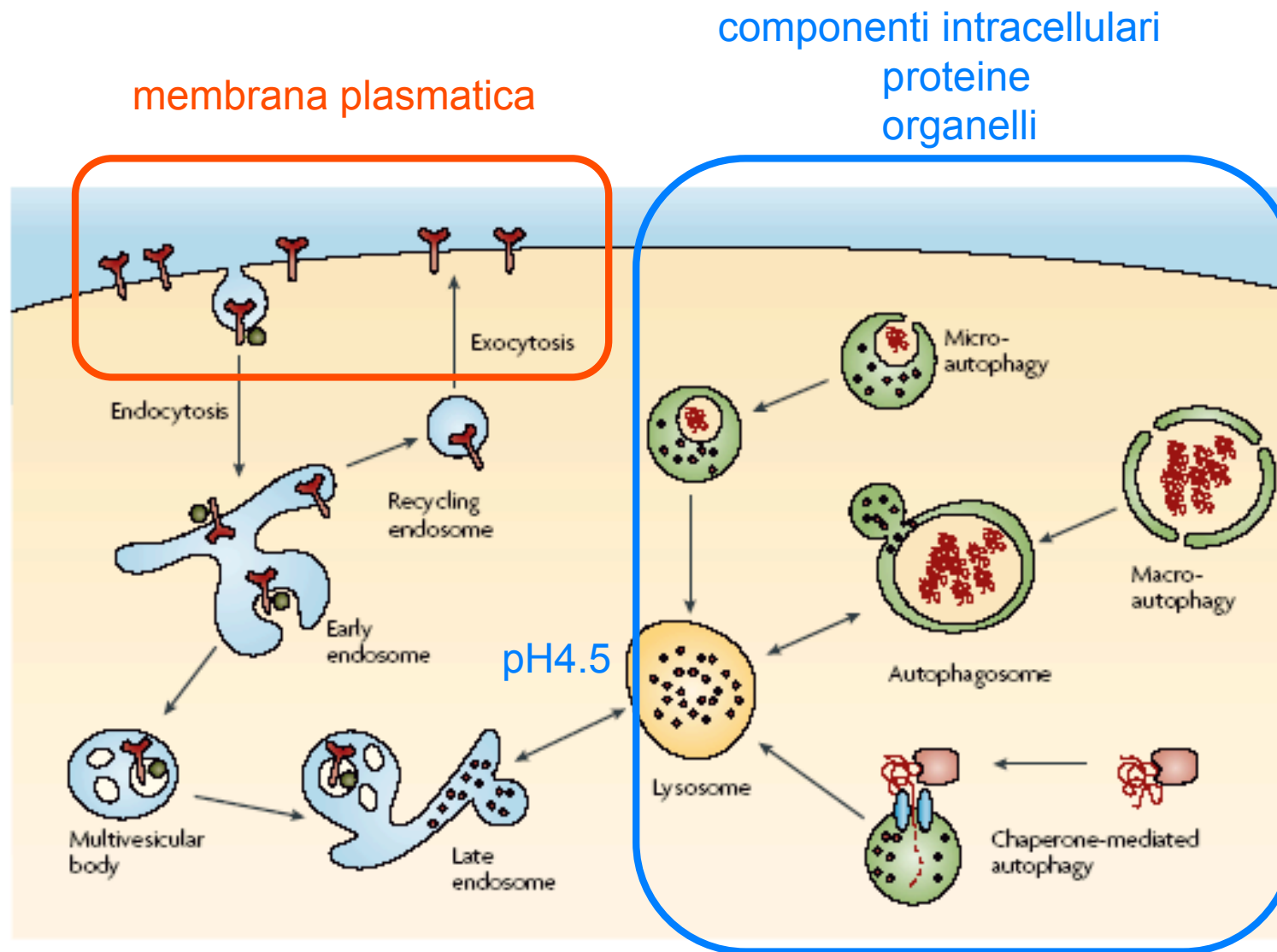
E3-ubiquitina ligasi

UCHL1 (AD,PD)

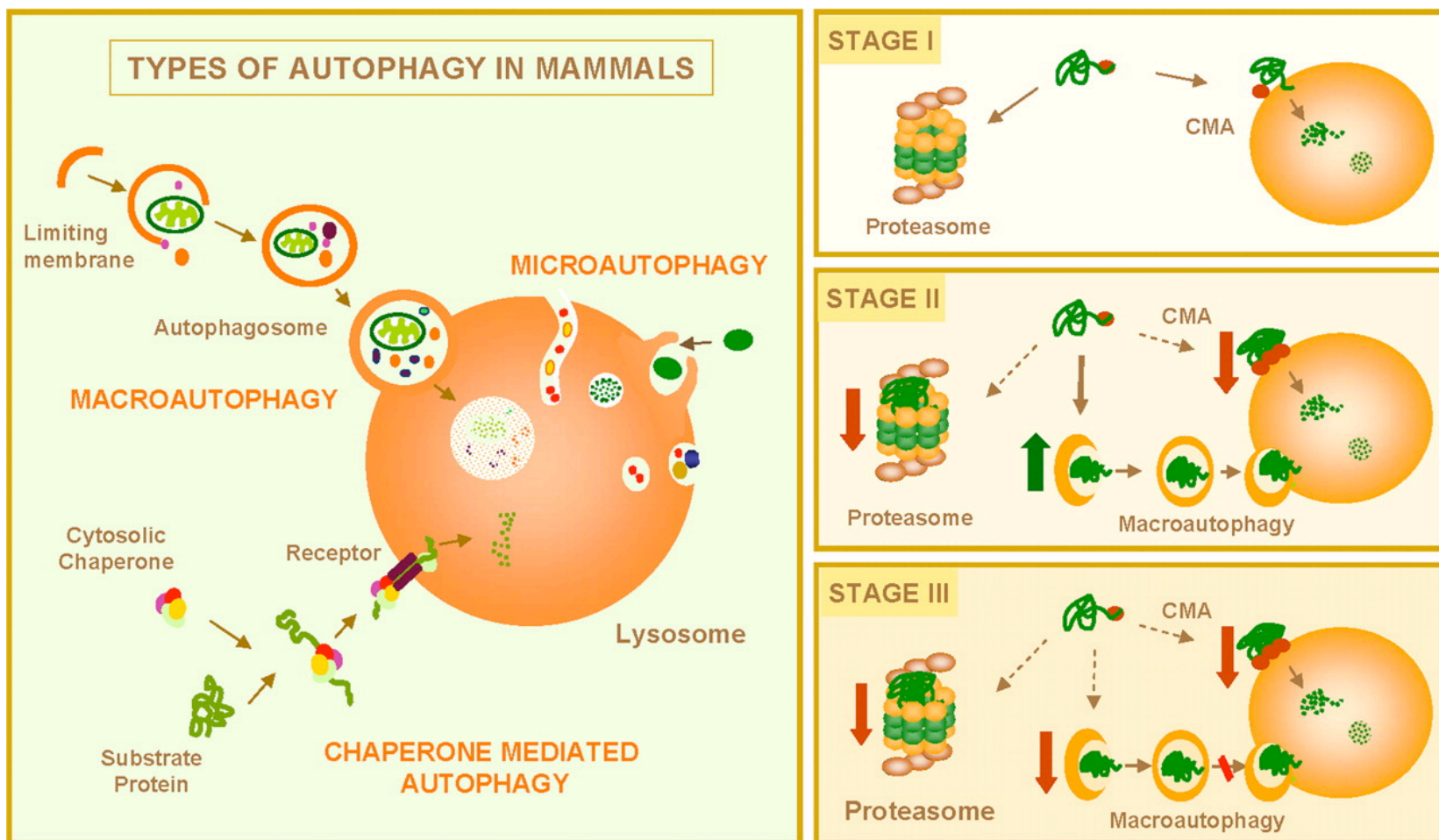
ubiquitina idrolasi

CDC48/p97 (**VCP**; FTD) proteina cargo per il Prot

Vie di degradazione lisosomale_1



Autofagia



Macro: organelli

Micro: proteine

Mediata dagli cahaperoni: proteine

Evidenze del coinvolgimento del sistema autofagico nelle neurodegenerazioni

- ✓ Animali knock-out per i geni autofagici nel sistema nervoso sviluppano aggregati poliubiquitinati e neurodegenerazione
- ✓ Mutazioni nel gene della dineina (coinvolta nel trasporto delle vescicole autofagiche) sono associate a degenerazioni dei motoneuroni nelle MND (ALS)
- ✓ α -synucleina è degradata da autofagia; a-syn mutata (PD) blocca la propria degradazione per autofagia

Controllo di qualità delle proteine_3

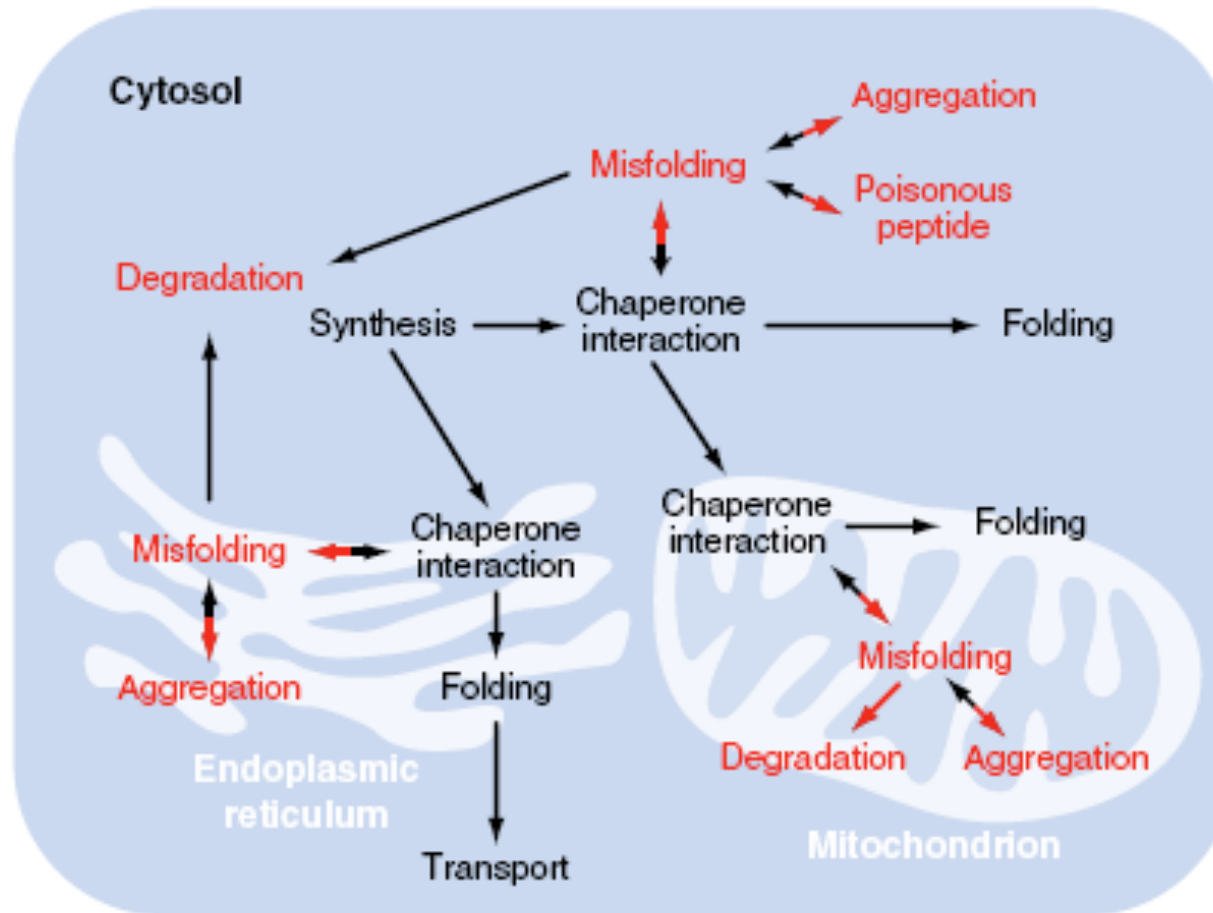


Figure 3

Schematic representation of the protein quality control systems in the cytosol, endoplasmic reticulum, and mitochondria.

Conseguenze fisiologiche dell'aggregazione proteica_1

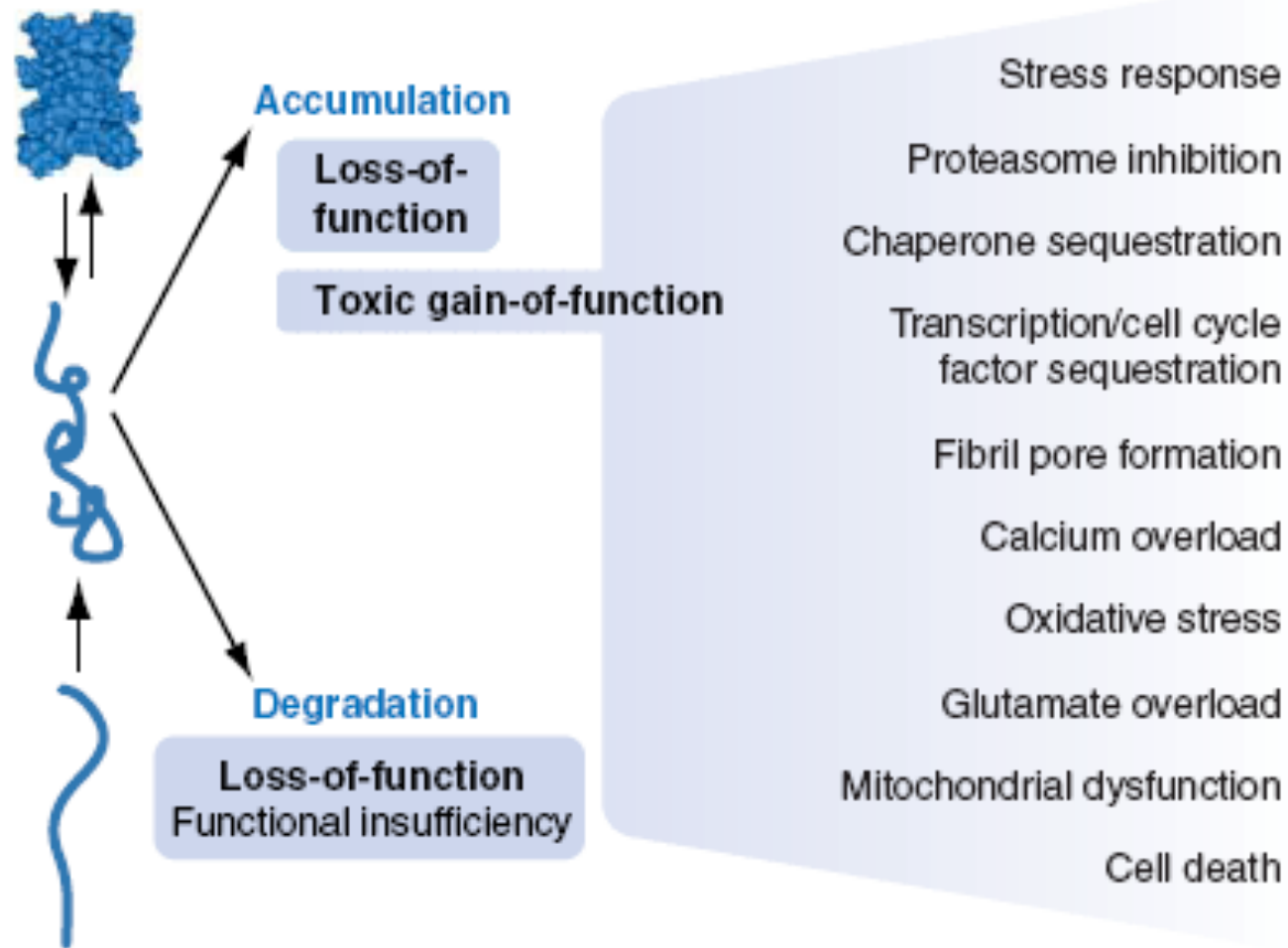


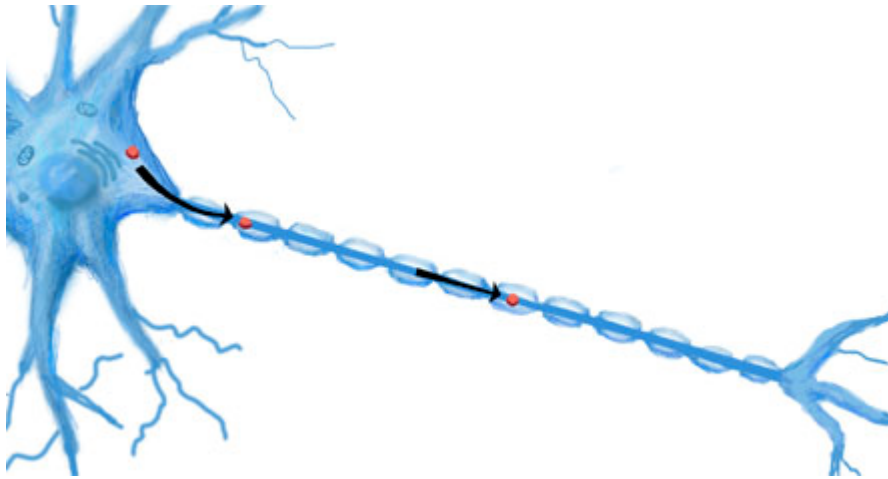
Figure 4

The fate of misfolded proteins and some important cellular consequences of accumulated proteins.

Conseguenze fisiologiche dell'aggregazione proteica_2

Malattie Conformazionali

- Perdita di funzione per misfolding o instabilità da misfolding
- Perdita di funzione per alterata localizzazione dovuta a misfolding
- Aggregazione da misfolding → formazione di strutture amiloidi
- Nelle malattie neurodegenerative le normali funzioni cellulari sembrano alterate dall'interazione delle proteine aggregate con varie componenti cellulari



Trasporto assonale_1

Microtubuli: polarizzati (+ e -)

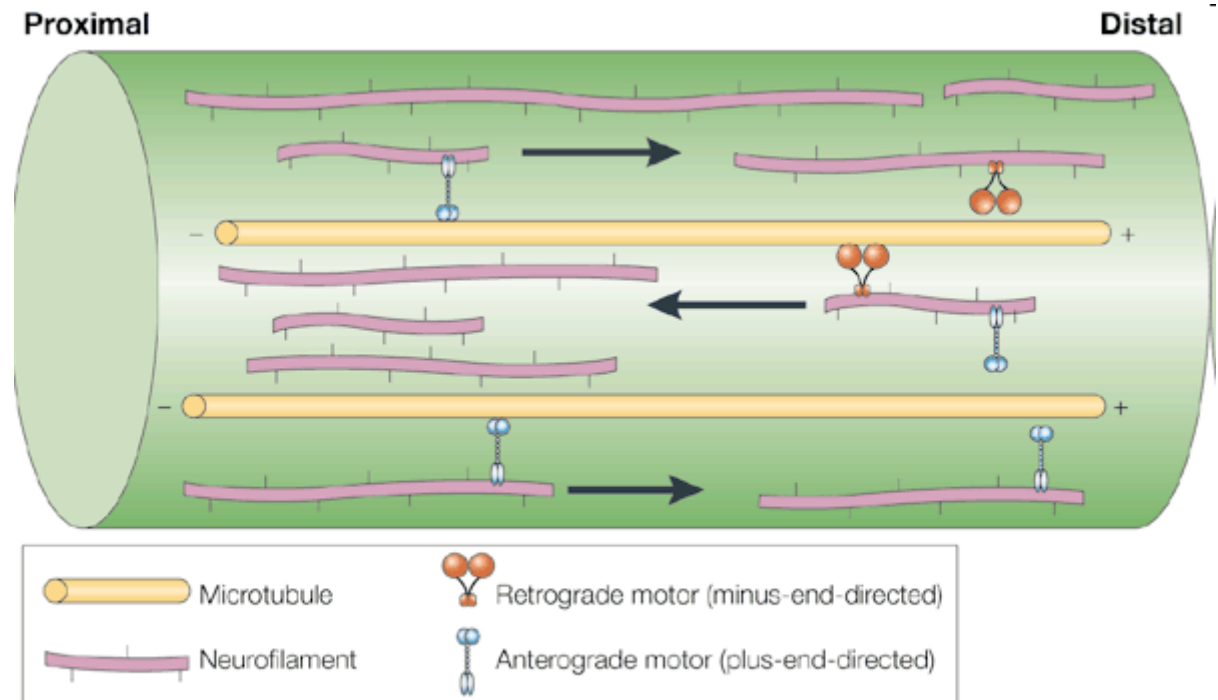
Motori molecolari

✓ kinesina (+)

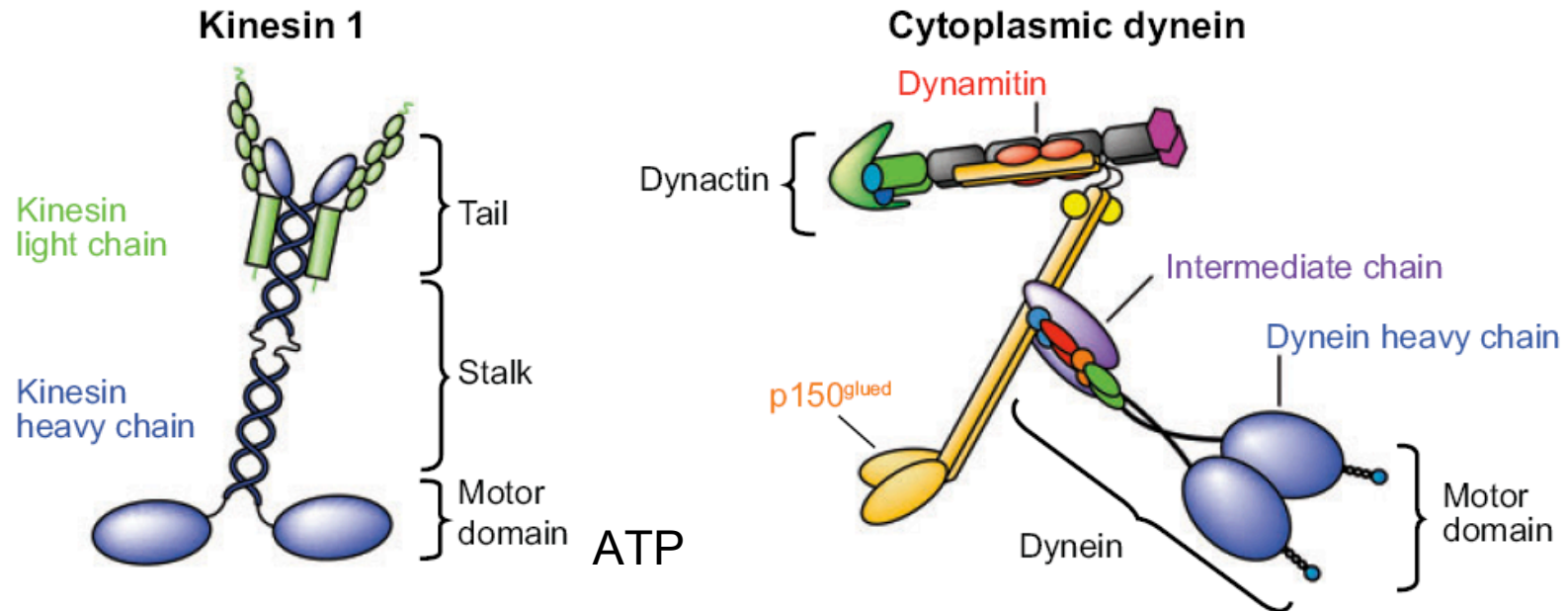
✓ dyneina (-)

Lento (1mm/giorno)

Veloce (1 μ m/sec)



Trasporto assonale_2



Distruzione dei meccanismi di trasporto:

danni al motore

danni alle rotaie

danni ai cargo

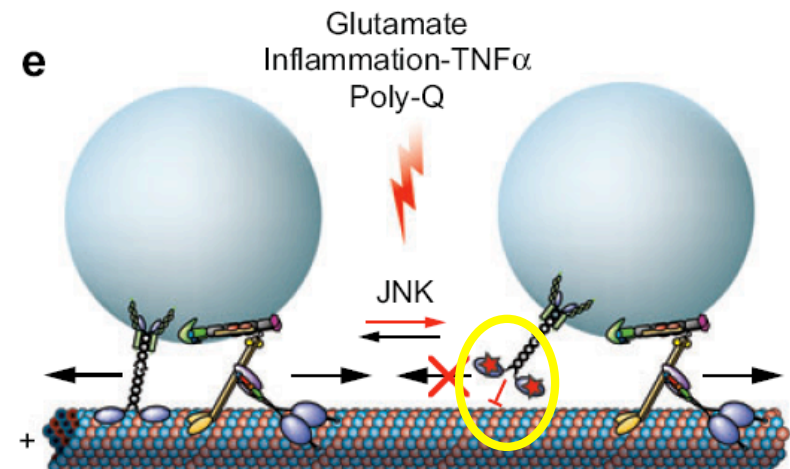
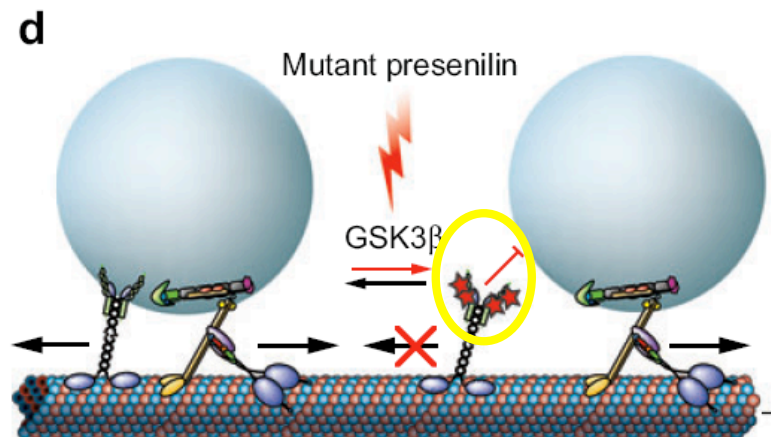
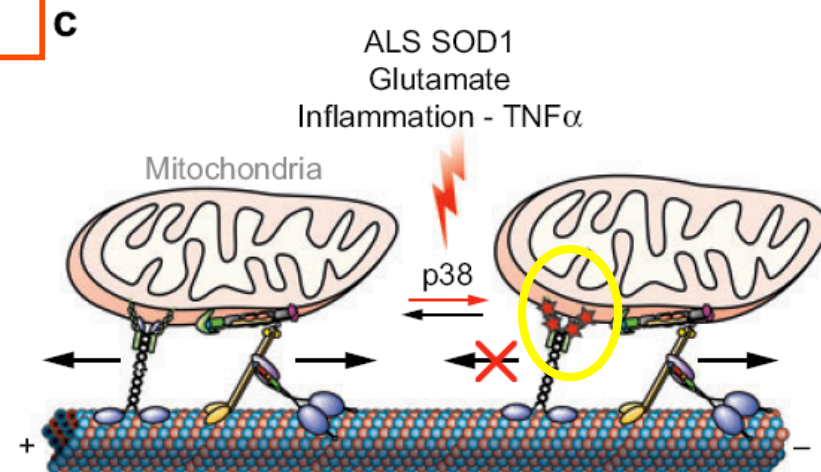
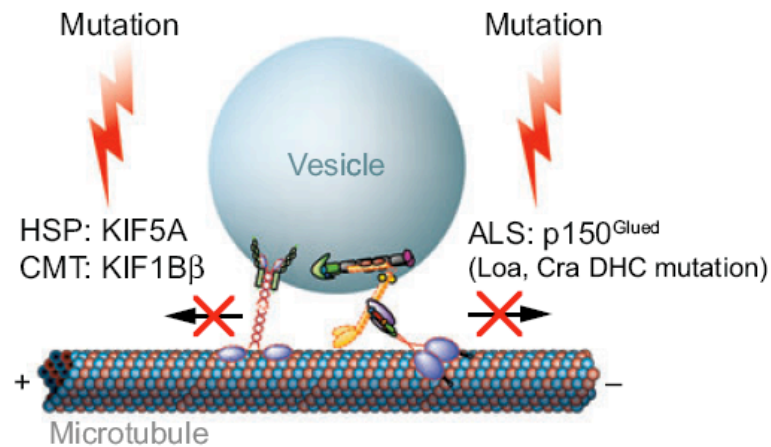
danni al rifornimento di ATP (mitocondri)

danno ai motori

mutazioni
Kinesine:
Paraplegia spastica
ereditaria
Charcot-Marie-Tooth

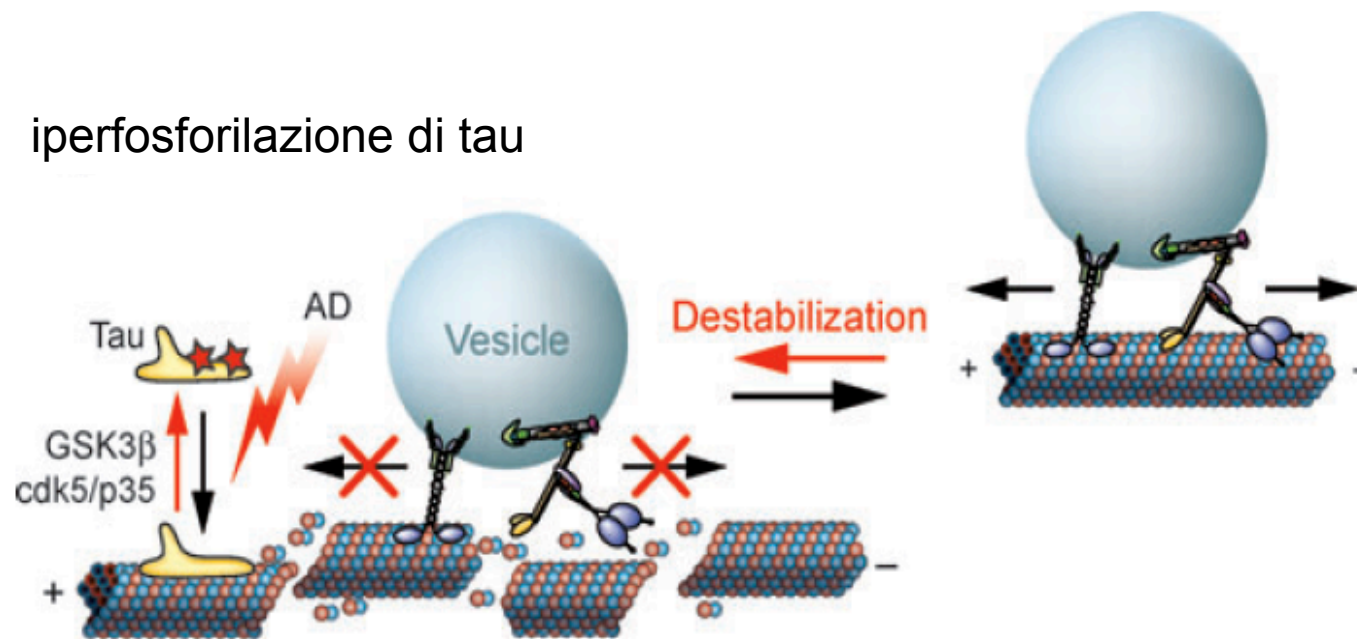
mutazioni
dyneine-dynactine:
SLA
MND (loa, cra)

fosforilazione
kinesin light chain



danno ai microtubuli

iperfosforilazione di tau



danno ai mitocondri

