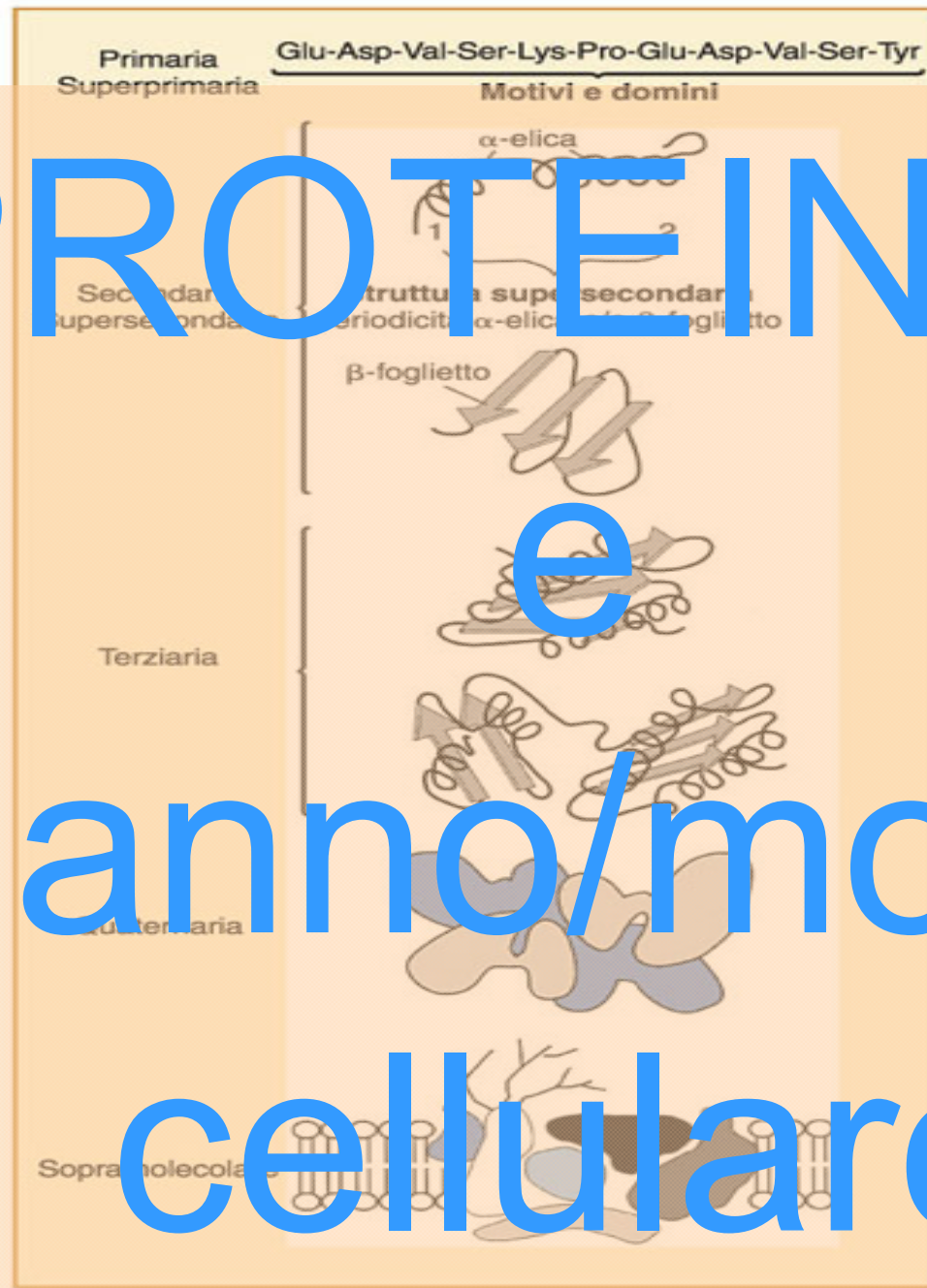


PROTEINE e danno/morte cellulare



■ Figura 2.5 - Vari livelli nella descrizione della struttura delle proteine.

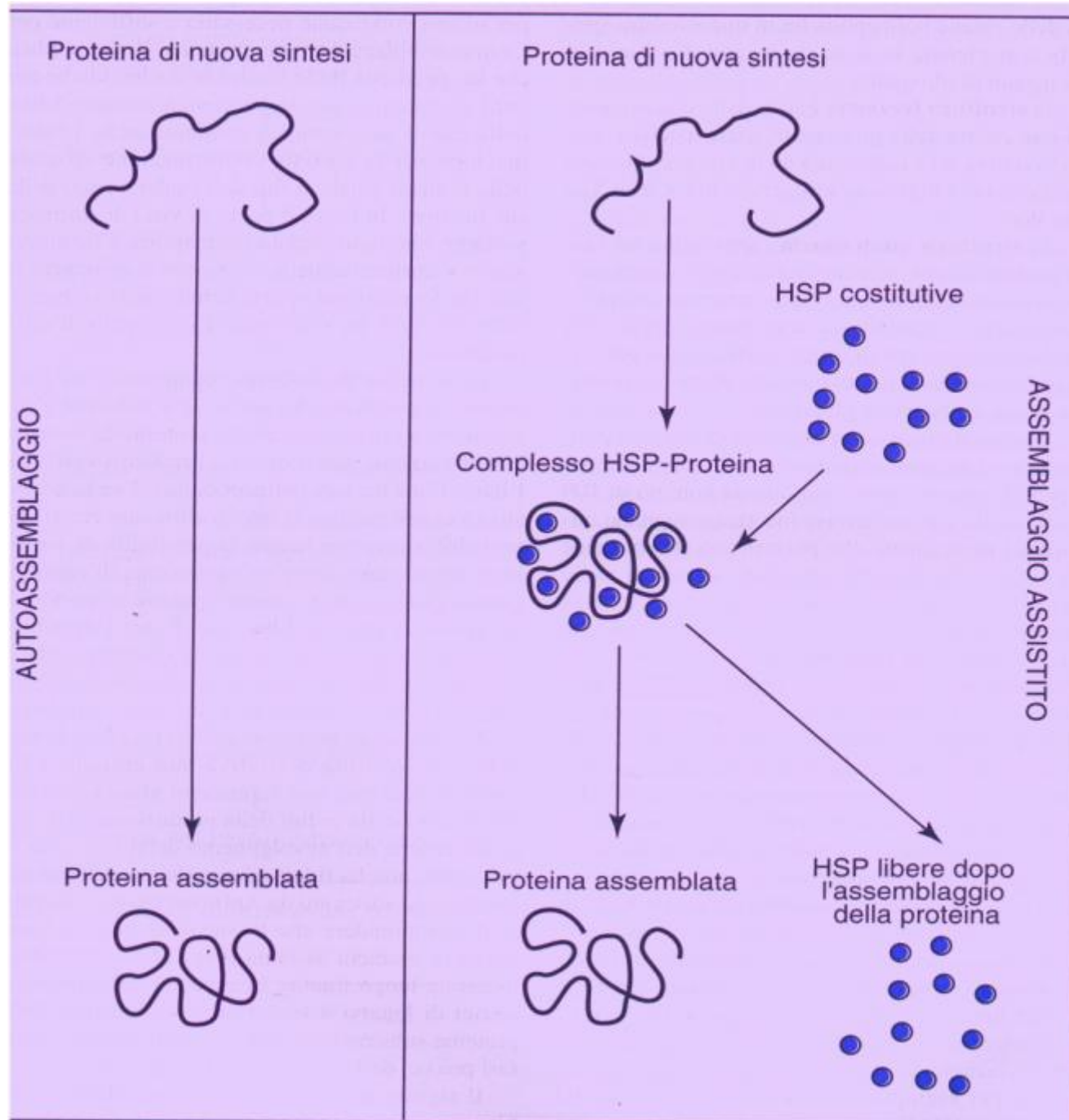


Fig. 12.5 - Autoassemblaggio ed assemblaggio assistito (da proteine da stress costitutive) di una proteina di nuova sintesi.

AGENTI STRESSANTI AMBIENTALI

- Shock termico
- Metalli pesanti
- Inibitori del metabolismo energetico
- Analoghi di aminoacidi
- Chemioterapici

CONDIZIONI PATOLOGICHE

- Processi flogistici
- Febbre
- Neoplasie
- Infezioni batteriche, virali, parassitarie
- Ischemia
- Ipertrofia
- Danno ossidativo

DENATURAZIONE
DI PROTEINE

Protein folding: complications

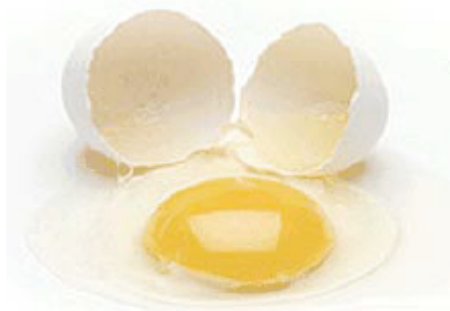


Fig. 12.2 - Eterogeneità degli agenti stressanti che inducendo nella cellula denaturazione di proteine provocano come risposta univoca la biosintesi adattiva di proteine da stress (HSF = Heat Shock Factor; HSP = Heat Shock Proteins).



Fig. 12.3 - Sequenza di eventi che portano alla trascrizione di proteine da stress.

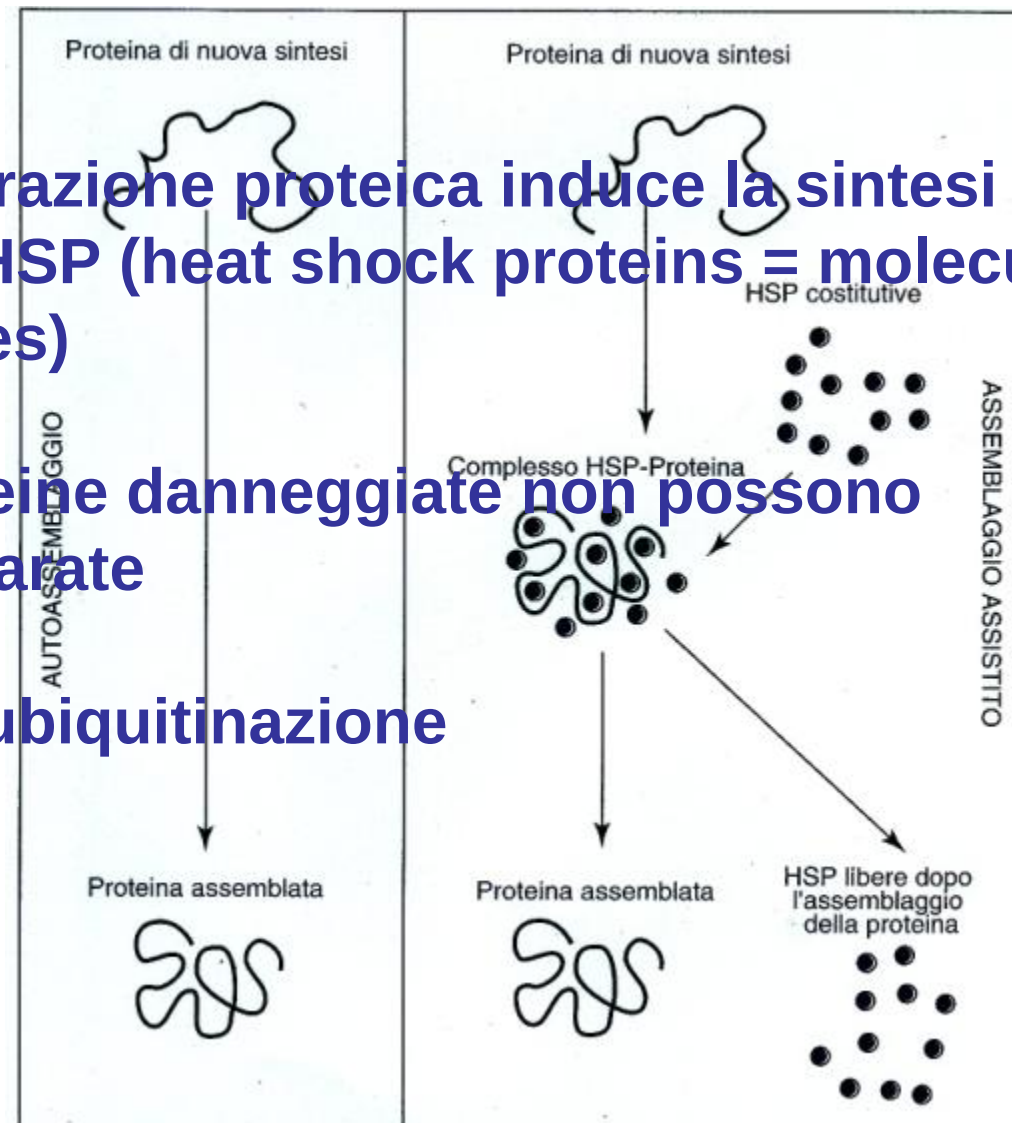
■ **Tabella 12.2 - Breve sommario della nomenclatura, localizzazioni negli eucarioti e possibili funzioni particolari delle HSP in aggiunta a quelle proprie dei chaperon molecolari.**

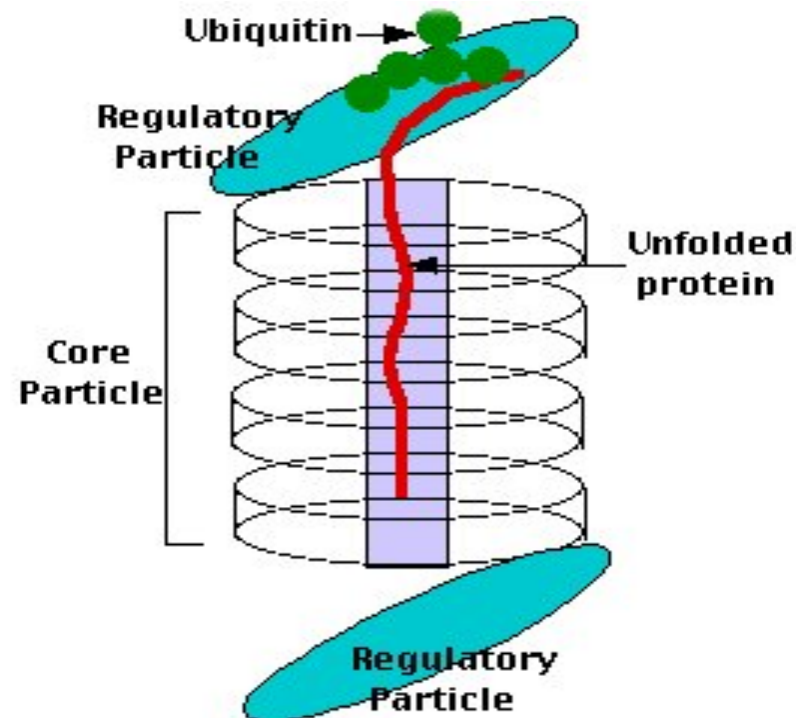
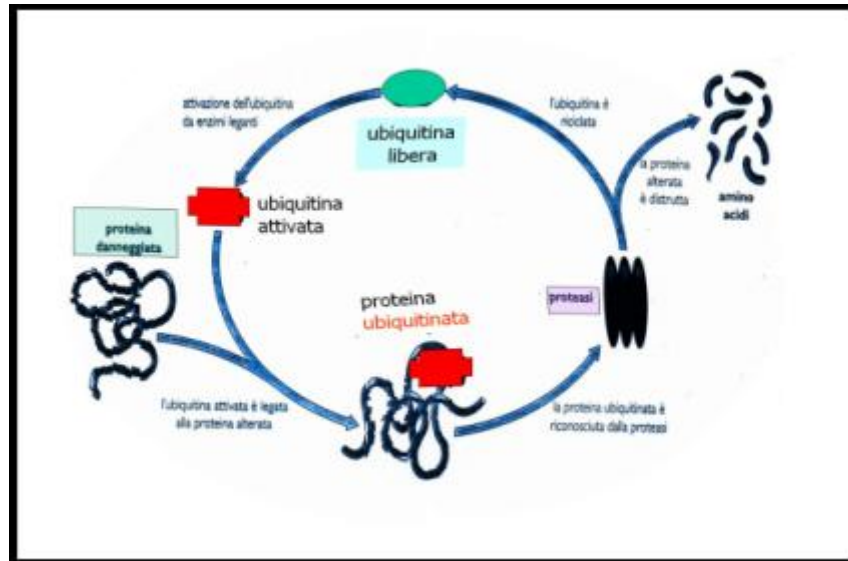
HSP 100	Citosol	Tolleranza allo stress
HSP 90 GRP 94	Citosol/nucleo RE	Legame con molecole di trasduzione del segnale: – recettori degli steroidi – ruolo nel ciclo cellulare e nella proliferazione
HSP 70 HSC 70 GRP 78 MHSP 70	Citosol/nucleo Citosol/nucleo RE Mitocondri	Antiapoptotica: citoprotettiva: termotolleranza: legame a oncogeni e antioncogeni. Trasporto proteine fra compartimenti cellulari Presentazione dell'antigene (con molecole di classe II del MHC)
HSP 60	Mitocondri	Facilita degradazione proteica. Trasporto intracellulare di proteine.
HSP 32	Citosol/nucleo	Antiossidante
HSP 27	Citosol/nucleo	Antiapoptotica; termotolleranza; stabilizzante microfilamenti.
Ubiquitina	Citosol/nucleo	Degradazione proteica

La denaturazione proteica induce la sintesi di nuove HSP (heat shock proteins = molecular chaperones)

Se le proteine danneggiate non possono essere riparate

-----> ubiquitinazione





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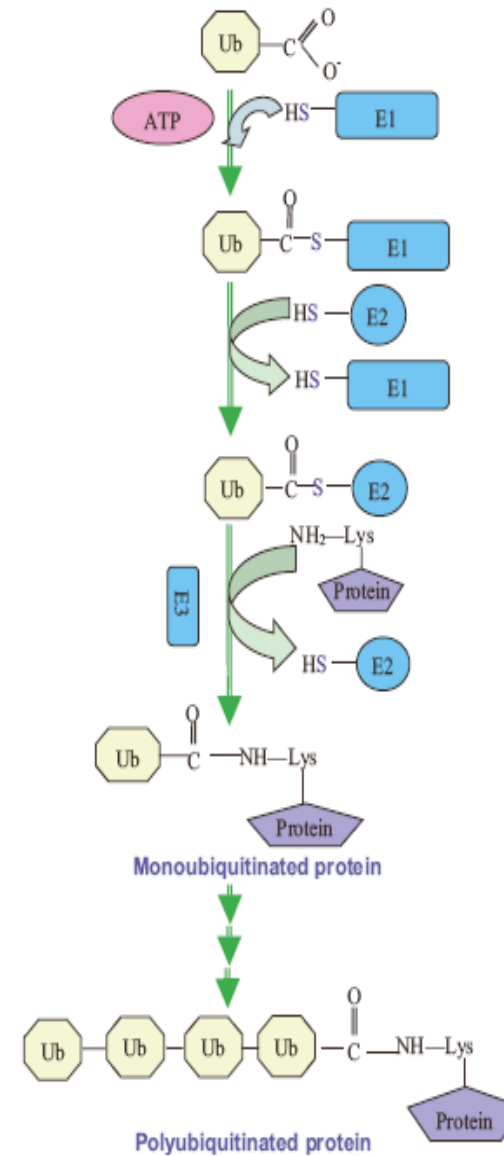


Figure 2. The protein ubiquitination pathway. Ubiquitin (Ub) is activated by E1 and transferred to the E2 enzyme and is, finally, conjugated to substrate proteins with a specific E3 ligase. Further polyubiquitination is required to target proteins for degradation.

Table 1. Abnormal Proteins Rapidly Degraded by the Ubiquitin-Proteasome Pathway

- Incomplete proteins
 - Misfolded proteins (from mutations and biosynthetic errors)
 - Denatured proteins
 - Free subunits of multimeric complexes
 - Proteins that fail to bind cofactors
 - Oxidant-damaged proteins
 - Membrane or secretory proteins that fail to fold in the ER
 - Misdirected proteins (in incorrect compartments)
-

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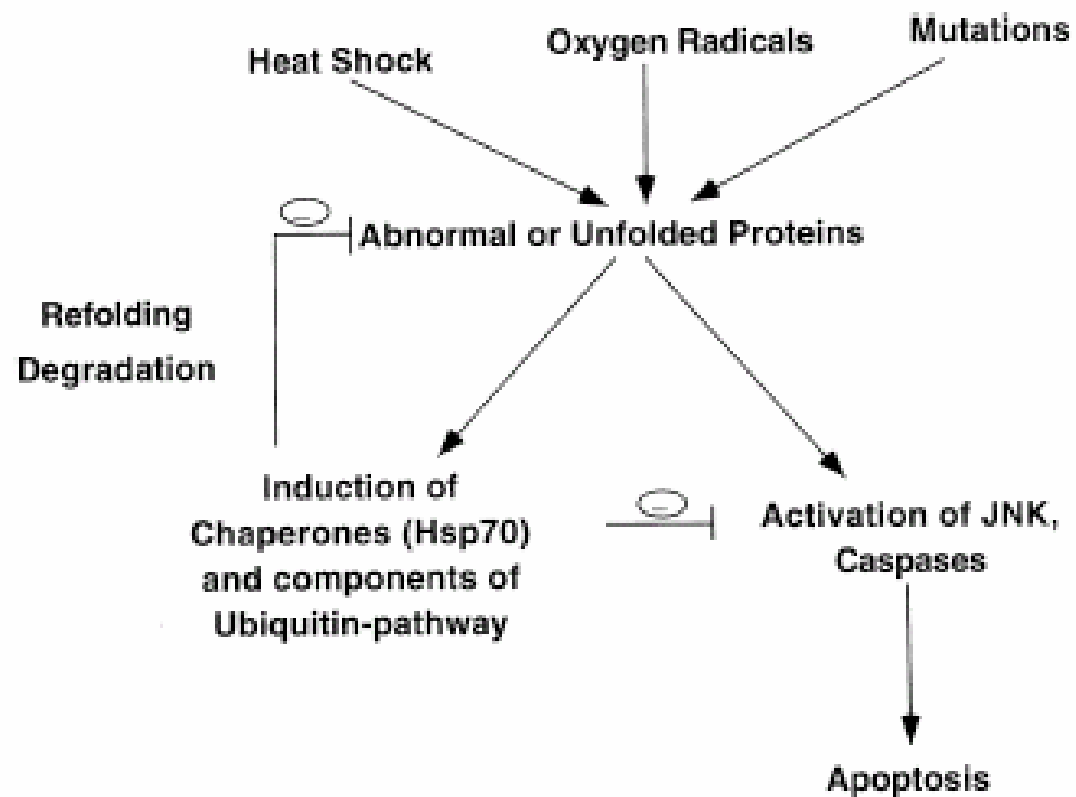
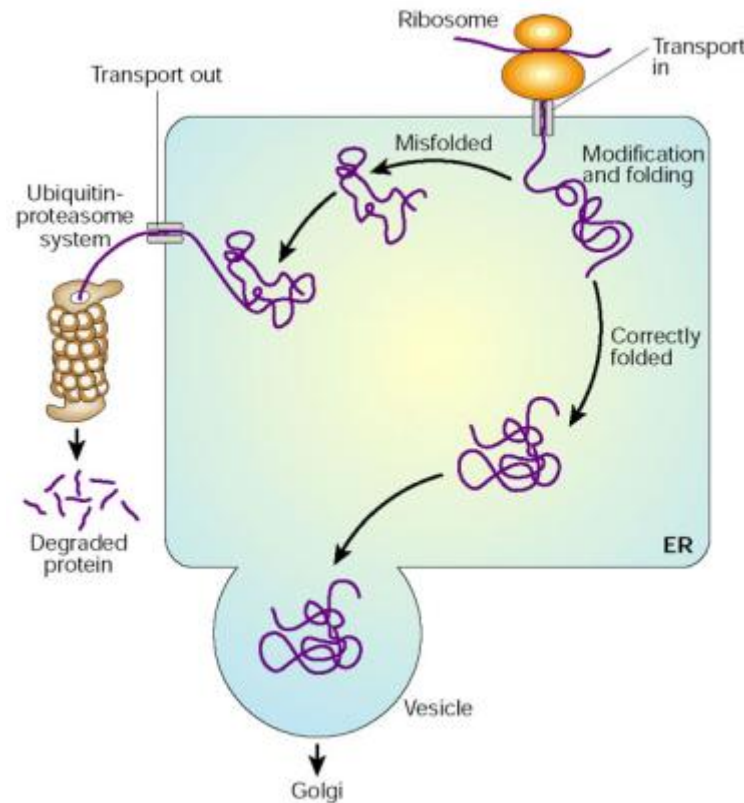


Figure 2. Induction of Protective and Apoptotic Programs by Abnormal Proteins

MY Sherman and AL Goldberg, "Cellular Defenses against Unfolded Proteins: A Cell Biologist Thinks about Neurodegenerative Diseases", Neuron, Vol. 29, 15–32, January, 2001,

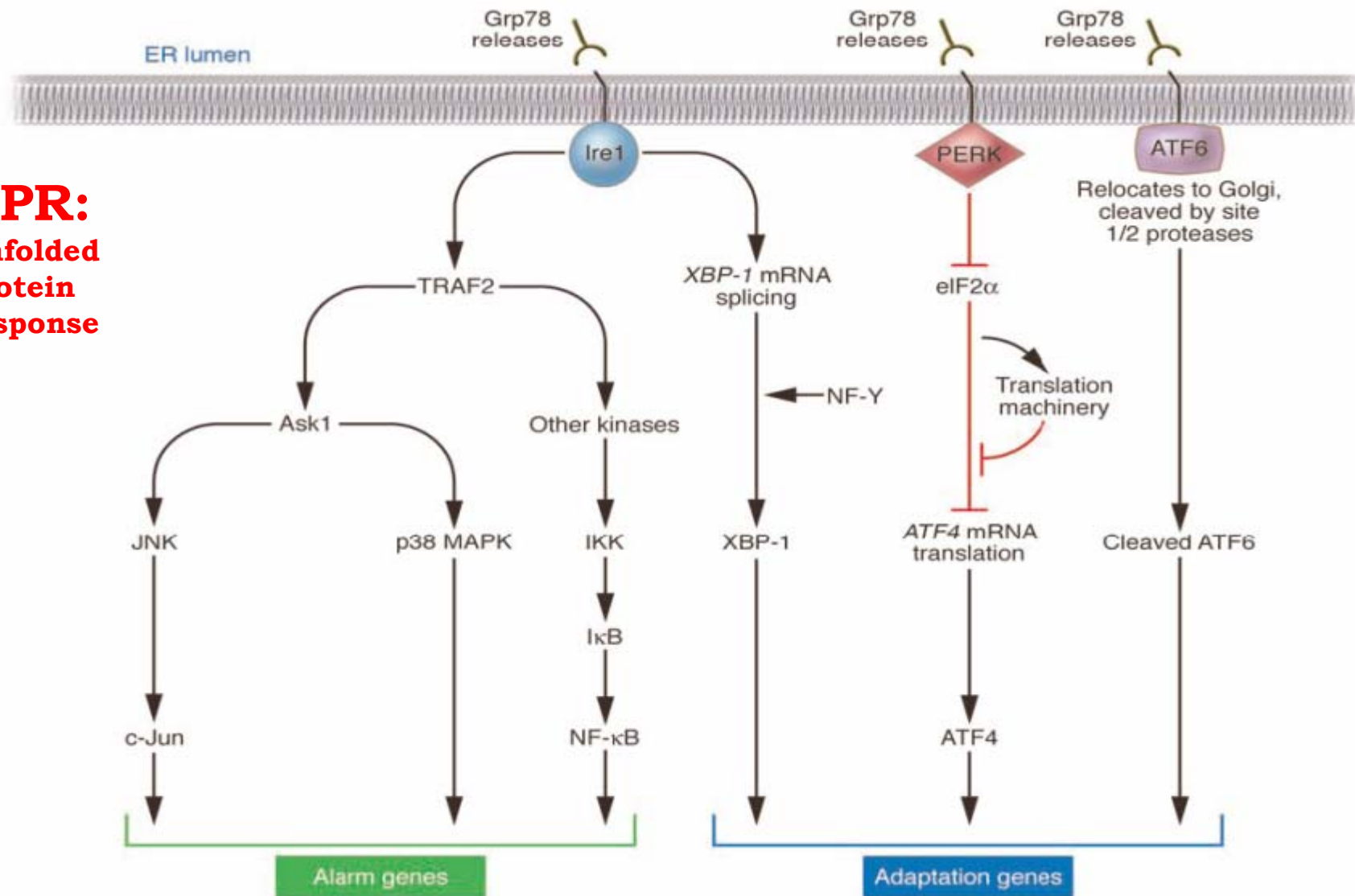
Misfolded proteins are normally detected and cleared from cell (or stored in aggresomes)



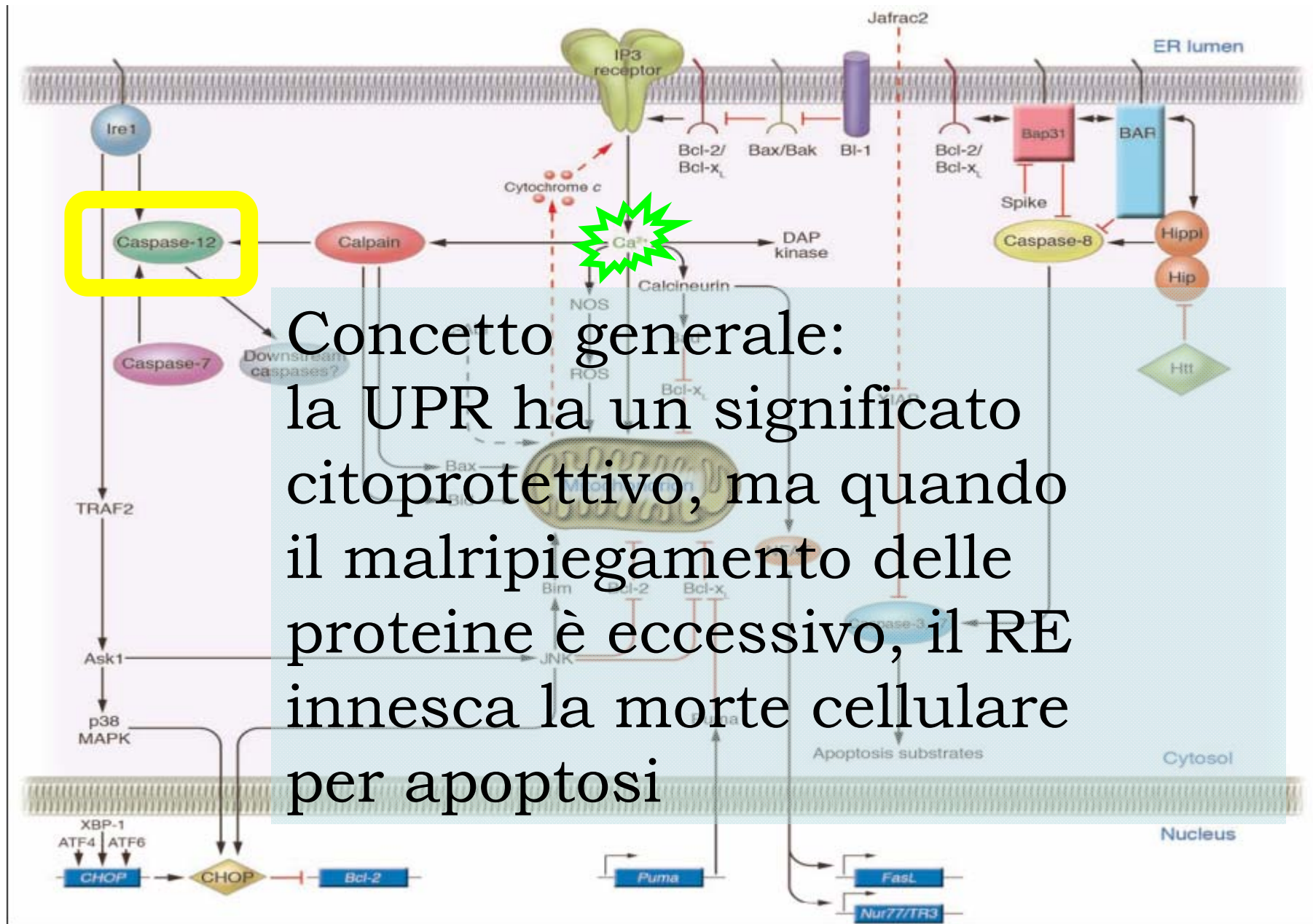
Regolazione del folding proteico nel RE. Molte proteine neosintetizzate sono traslocate nel RE, dove si ripiegano nelle loro strutture tridimensionali aidate da una serie di chaperons . Le proteine ripiegate correttamente sono poi trasportate al complesso del Golgi e poi inviate nell'ambiente extracellulare. Tuttavia, le proteine malripiegate sono individuate da un meccanismo di **controllo della qualità** e inviate verso un altro pathway (**UPR**) nel quale esse sono ubiquitinate e poi degradate nel citoplasma dai proteasomi

CM Dobson, "Protein folding and misfolding", *Nature*, **426**, 884-890 (2003)

UPR: Unfolded protein response



Signal transduction events associated with ER stress. Chaperone Grp78 binds the N-termini of Ire1, PERK, and ATF6, preventing their activation. Unfolded proteins in the ER cause Grp78 to release Ire1, PERK, and ATF6. Upon Grp78 release, Ire1 and PERK oligomerize in ER membranes. Oligomerized Ire1 binds TRAF2, signaling downstream kinases that activate NF-κB and c-Jun (AP-1), causing expression of genes associated with host defense (alarm). The intrinsic ribonuclease activity of Ire1 also results in production of XBP-1, a transcription factor that induces expression of genes involved in restoring protein folding or degrading unfolded proteins. Oligomerization of PERK activates its intrinsic kinase activity, resulting in phosphorylation of eIF2α and suppression of mRNA translation. Under these conditions, only selected mRNAs, including ATF4, are translated. ATF4 induces expression of genes involved in restoring ER homeostasis. Release of Grp78 from ATF6 allows this protein to translocate to the Golgi apparatus for proteolytic processing to release active ATF6, which controls expression of UPR genes.



Cell death mechanisms induced by ER stress. Several of the proposed pathways linking ER stress to cell death are depicted. Dashed lines indicate protein translocation events (c-Abl, Jafrac2). The mitochondrial permeability transition pore complex, which is Ca^{2+} -sensitive, is not shown in the diagram. See the text for additional details. (Xu et al., J Clin Invest. 115, 2656-64 (2005))

Patologia molecolare delle proteine

Deficit nel ripiegamento conformazionale :

alterazioni del trasporto intracellulare e di proteine critiche

es: deficit di alfa1-antitripsina, fibrosi cistica, ipercolesterolemia familiare

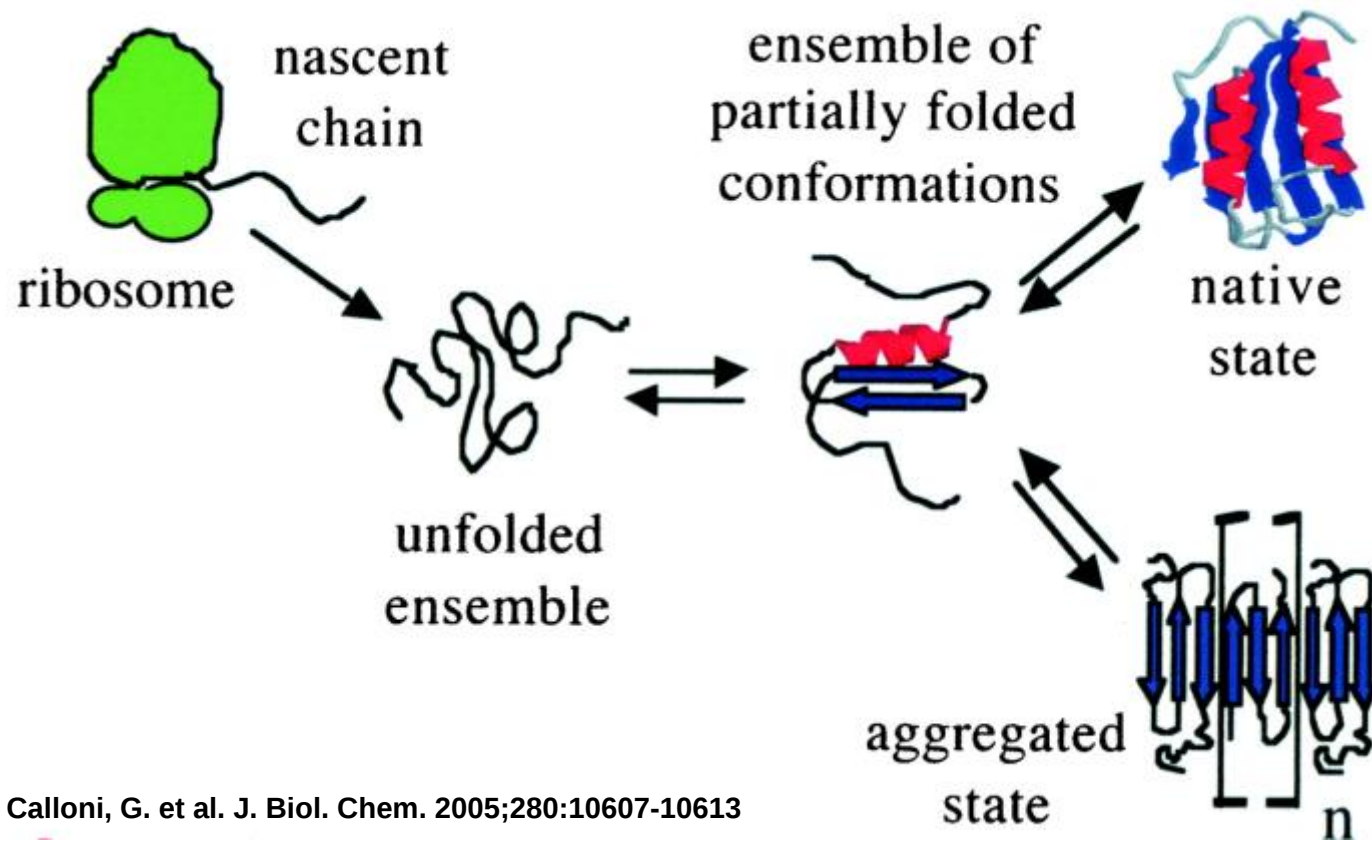
le proteine non ripiegate o malripiegate inducono uno stress sul

reticolo endoplasmatico (UPR): dapprima risposta citoprotettiva, poi attivazione apoptosi (caspasi 12)

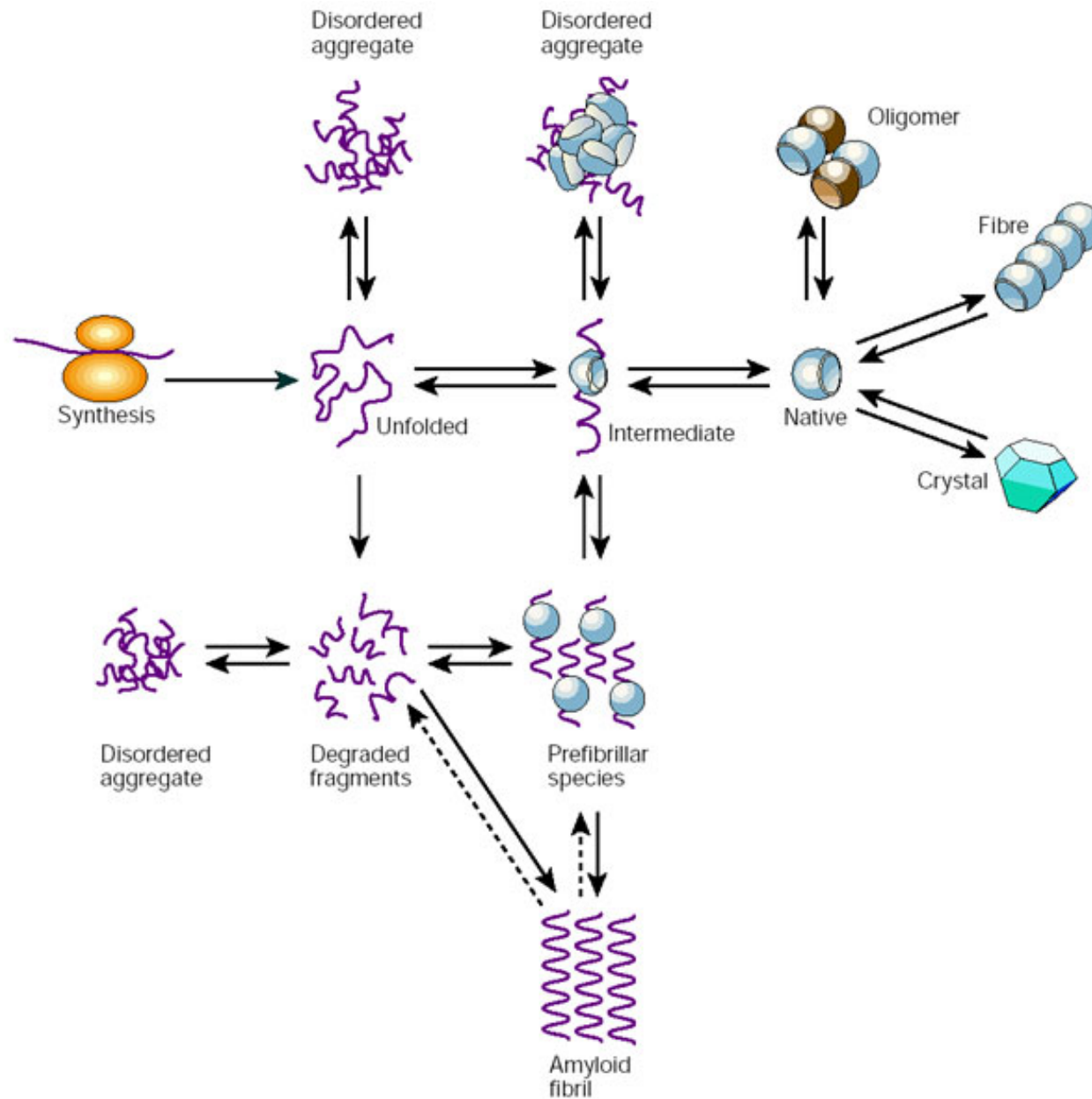
ruolo in alcune m. neurodegenerative, diabete tipo II ecc.

Aggregazione di proteine anomale: alcune forme di amiloidosi, (depositi intracellulari ed extracellulari)

Schematic representation of the equilibria existing between different conformational states of a protein in a cell

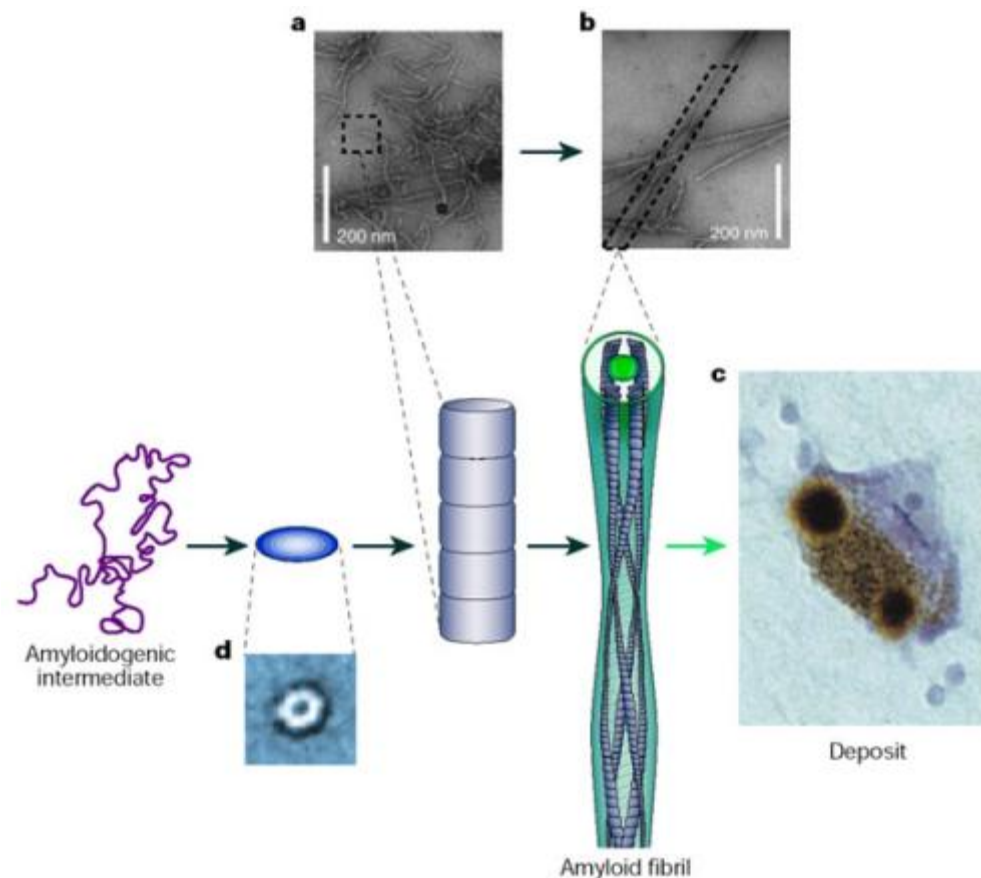


Calloni, G. et al. J. Biol. Chem. 2005;280:10607-10613



CM Dobson, "Protein folding and misfolding", *Nature*, **426**, 884-890 (2003)

General mechanism of aggregation to form amyloid fibrils



Unfolded or partially unfolded proteins associate with each other to form small, soluble aggregates that undergo further assembly into protofibrils or protofilaments (a) and then mature fibrils (b). The fibrils often accumulate in plaques or other structures such as the Lewy bodies associated with Parkinson's disease (c). Some of the early aggregates seem to be amorphous or micellar in nature, although others form ring-shaped species with diameters of approximately 10 nm (d).

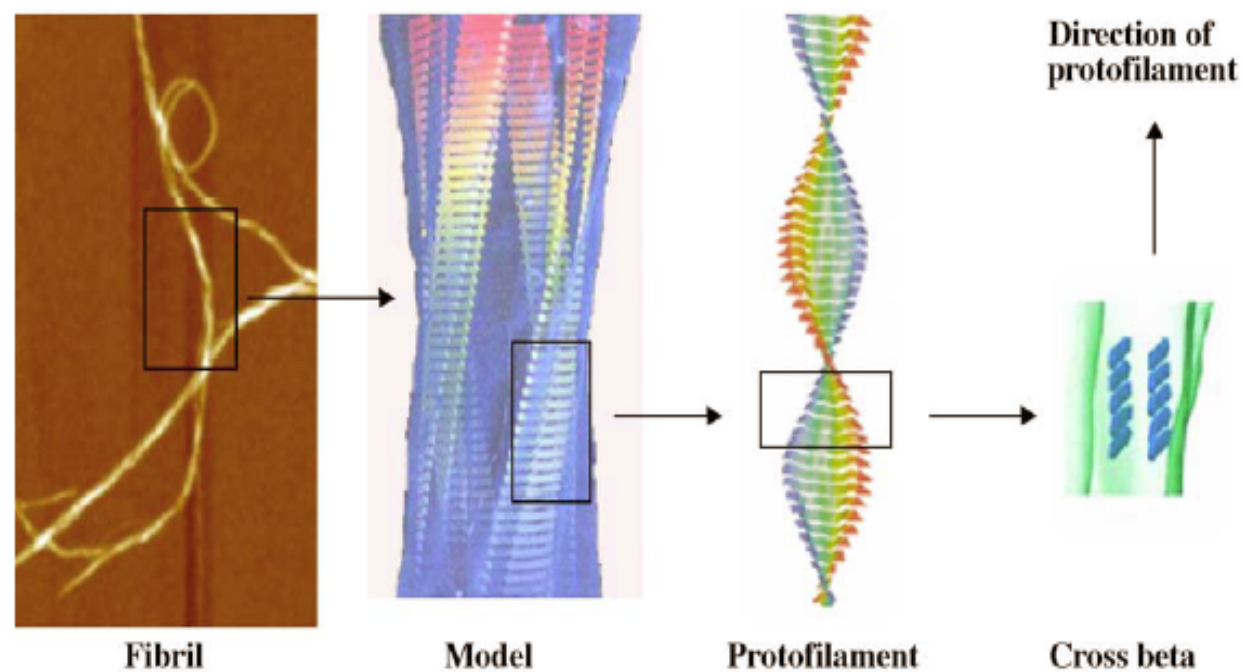


Fig. 3. Close-up view of the structural organization of an amyloid fibril. The four protofilaments are wound around each other and their core structure is a row of β -sheets where each strand runs perpendicular to the fibril axis (from. Refs. [28,163]).

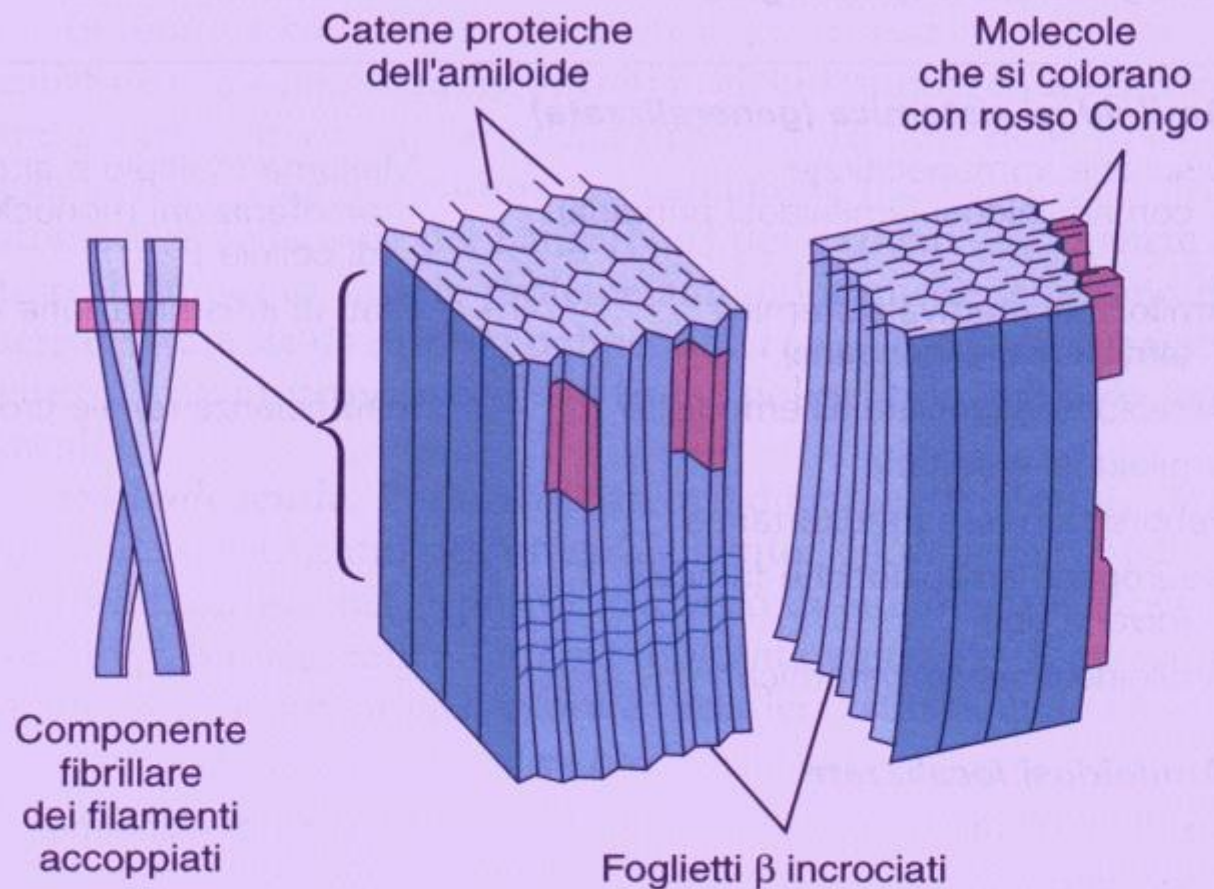
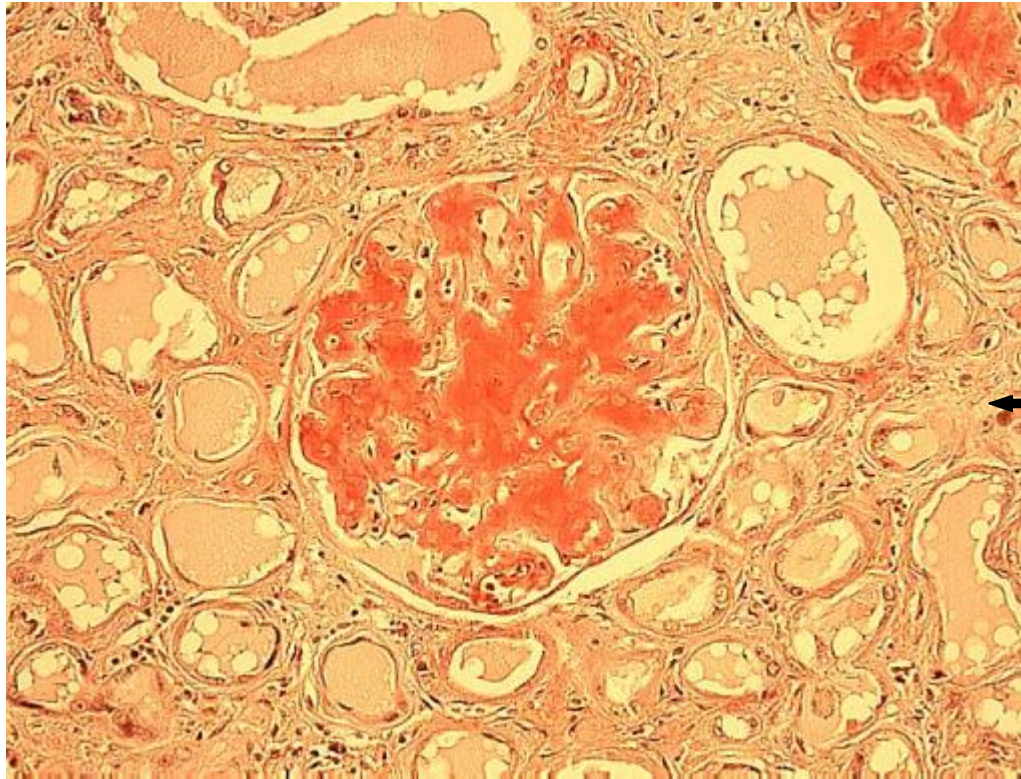


FIGURA 6-53 Struttura di una fibrilla di amiloide che mostra la struttura a β -foglietti ripiegati e i siti di legame per il colorante rosso Congo, utilizzato per la diagnosi di amiloidosi. (Modificata da Glenner GG: Amyloid deposit and amyloidosis. The β -fibrilloses. N Engl J Med 52:148, 1980. Con l'autorizzazione di The New England Journal of Medicine.)



Osservazione in
luce normale

Rosso Congo

Osservazione in
luce polarizzata

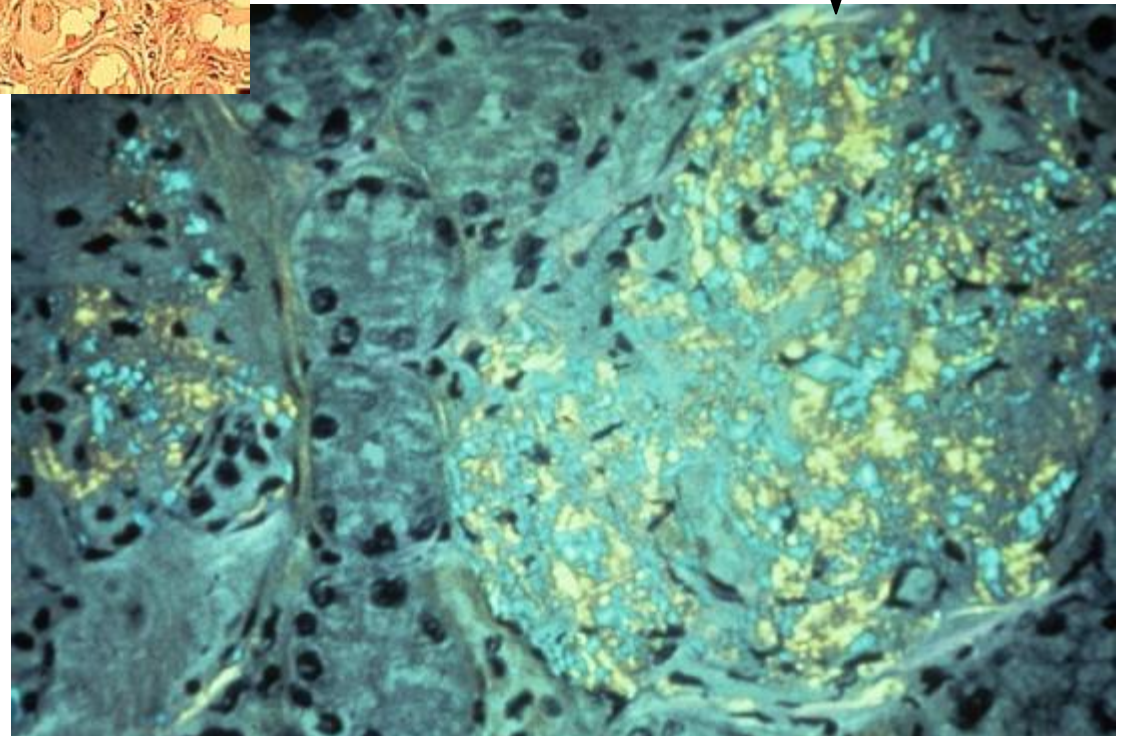


TABELLA 6-15 Classificazione dell'amiloidosi

Categoria clinicopatologica	Malattie associate	Principali proteine amiloidi	Precursore proteico chimicamente correlato
<i>Amiloidosi sistemica (generalizzata)</i>			
Discrasie immunocitiche con amiloidosi (amiloidosi primaria)	Mieloma multiplo e altre proliferazioni monoclonali di cellule B	AL	Catene leggere delle immunoglobuline, soprattutto tipo λ
Amiloidosi reattiva sistemica (amiloidosi secondaria)	Stati di infiammazione cronica	AA	SAA
Amiloidosi associata all'emodialisi	Insufficienza renale cronica	A β_2 m	β_2 -microglobulina
Amiloidosi ereditaria			
Febbre familiare mediterranea	—	AA	SAA
Neuropatie amiloidotiche familiari (diversi tipi)	—	ATTR	Transtiretina
Amiloidosi senile sistemica	—	ATTR	Transtiretina
<i>Amiloidosi localizzate</i>			
Senile cerebrale	Malattia di Alzheimer	A β	APP
Endocrine			
Carcinoma midollare della tiroide	—	A Cal	Calcitonina
Isole del Langerhans	Diabete tipo 2	AIAPP	Peptide dell'amiloide insulare
Amiloidosi isolata atriale	—	AANF	Fattore natriuretico atriale
Malattie da prioni	Diverse malattie da prioni del SNC	Proteina prionica mal assemblata (PrP ^{Sc})	Proteina prionica normale PrP

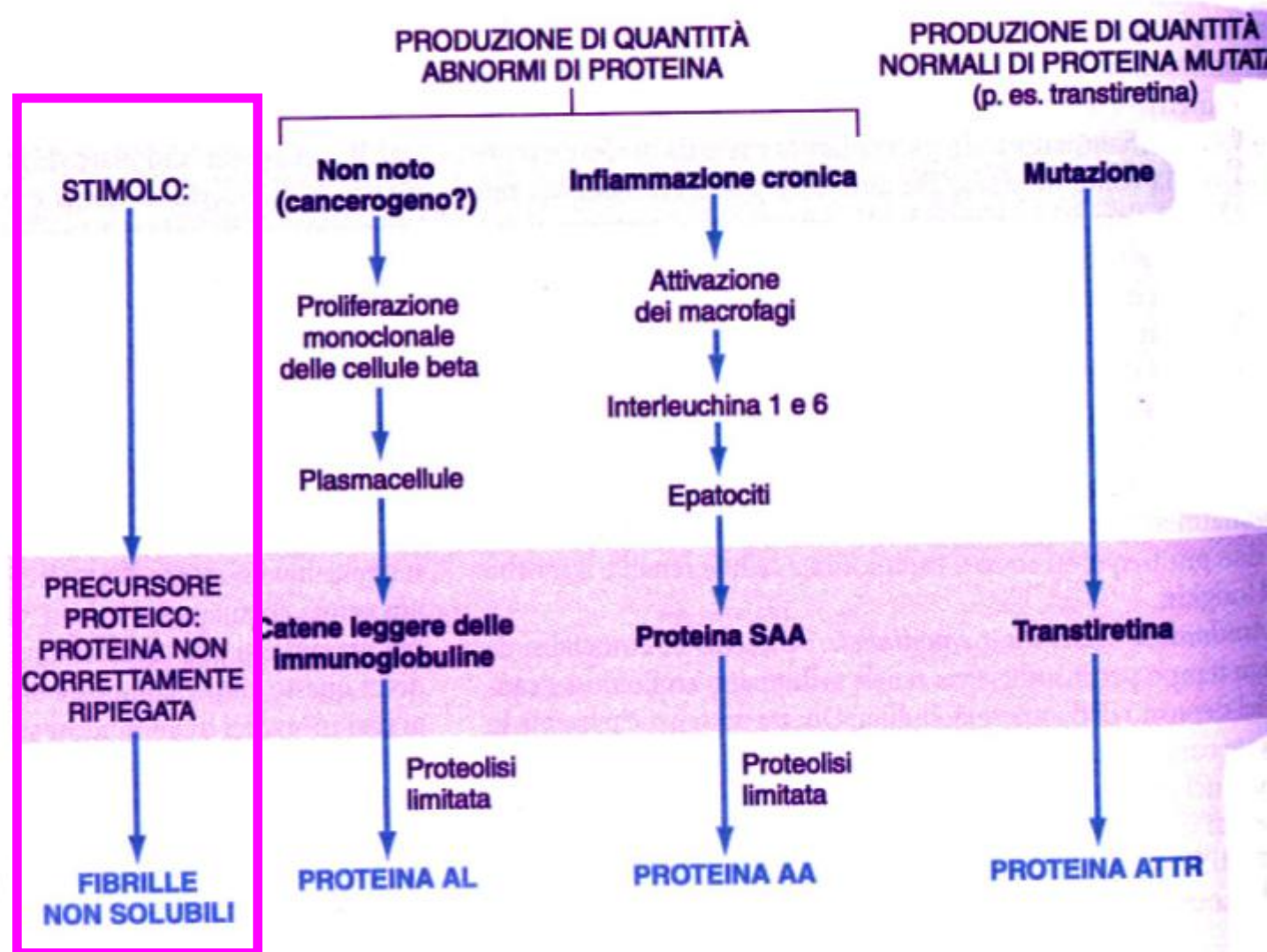


FIGURA 6-54 Schema della possibile patogenesi delle forme principali di fibrille di amiloide.

Protein Misfolding Diseases

- Una proteina specifica può essere incapace di svolgere la sua normale funzione perché non è correttamente ripiegata, oppure perché il mal ripiegamento produce una sua instabilità
- Una proteina può essere incapace di svolgere la sua normale funzione perché il malripiegamento impedisce la sua corretta collocazione
- Una proteina può non ripiegarsi correttamente o non conservare il corretto ripiegamento: la conseguenza è l'aggregazione (spesso con altre componenti (amyloid diseases). (il termine amiloidosi si riferisce strettamente alle malattie con depositi extracellulari, ma i termini "amyloid diseases" o "protein aggregation diseases" sono attualmente usati per malattie in cui i depositi sono sia intra che extracellulari)
- Alcuni dei segni clinici delle amiloidosi non-neurologiche sembra essere dovuto all'accumulo di grandi depositi di proteine aggregate in organi vitali
- Nelle malattie neurodegenerative la funzione cellulare appare inibita dalla interazione delle proteine aggregate con i componenti cellulari. Questo impedimento è associato con evidenza di elevato stress ossidativo (meccanismo non noto)

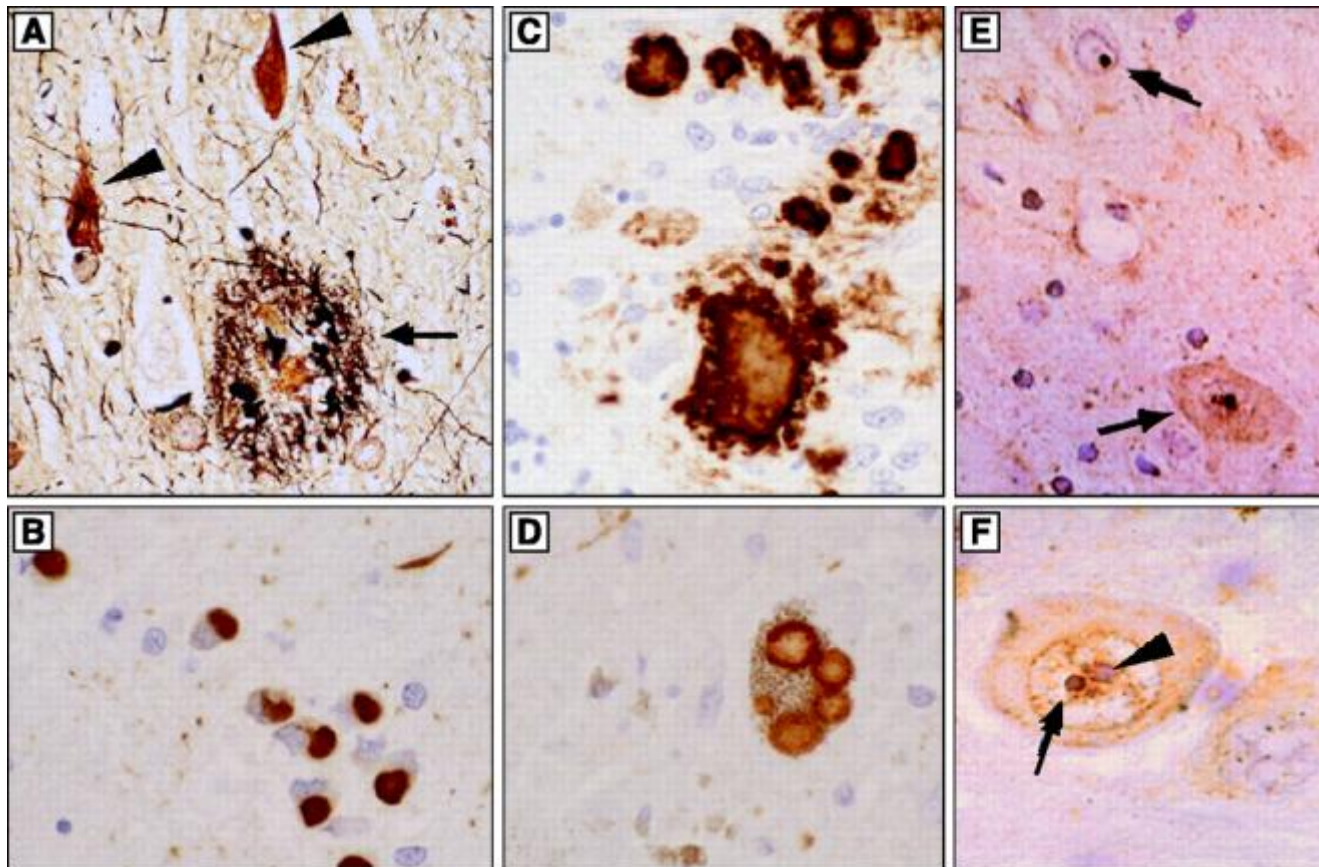
Aggregation of Proteins in Neurodegenerative Diseases

Table 1. Features of neurodegenerative disorders characterized by aggregation and deposition of abnormal protein.

Disease	Protein deposits	Toxic protein	Disease genes	Risk factor
Alzheimer's disease	Extracellular plaques	A β	APP* Presenilin 1† Presenilin 2†	apoE4 allele
Parkinson's disease	Intracellular tangles Lewy bodies	tau α -Synuclein	α -Synuclein* Parkin† UCHL1†	tau linkage
Prion disease	Prion plaque	PrP ^{Sc}	PRNP*	Homozygosity at prion codon 129
Polyglutamine disease	Nuclear and cytoplasmic inclusions	Polyglutamine-containing proteins	9 different genes with CAG repeat expansion*	
Tauopathy	Cytoplasmic tangles	tau	tau*	tau linkage
Familial amyotrophic lateral sclerosis	Bunina bodies	SOD1	SOD1*	

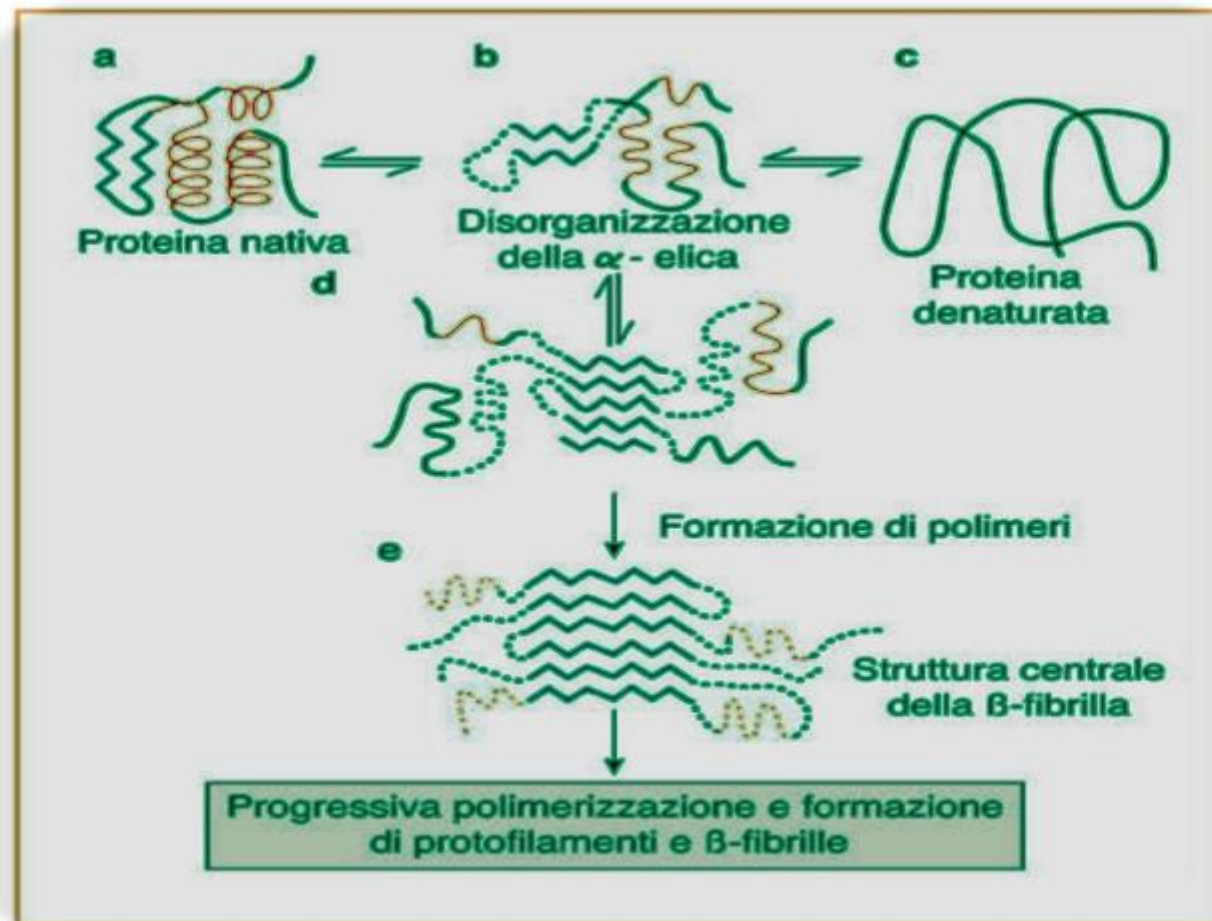
*Pathogenic mutations are associated with a toxic gain of function.
 †Pathogenic mutations are associated with a loss of function.

Taylor JP, Hardy J, Fischbeck KH. (2002) **Science**



Aggregation of misfolded proteins in microscopically visible inclusions or plaques in various neurodegenerative diseases. (A) Alzheimer's disease. Arrowhead, intracellular neurofibrillary tangles; arrow, extracellular amyloid plaque. (B) Fibrillar tau inclusions in Pick's disease. (C) PrPSc amyloid deposition in prion disease. (D) Multiple Lewy bodies in a nigral neuron in Parkinson's disease. (E) Neuronal intranuclear inclusions of mutant ataxin-3 in Machado-Joseph's disease. (F) Higher power micrograph of nuclear inclusion of mutant ataxin-3, demonstrating that it is distinct from the nucleolus. Magnification, $\times 40$.

"Toxic Proteins in Neurodegenerative Disease", JP Taylor et al., Science, **296**, 1991-1995, 2002



■ Figura 2.10 - Meccanismo generale per la formazione di β -fibrille intra- ed extracellulari.

I precursori delle fibrille di amiloide possono essere tossici per le cellule

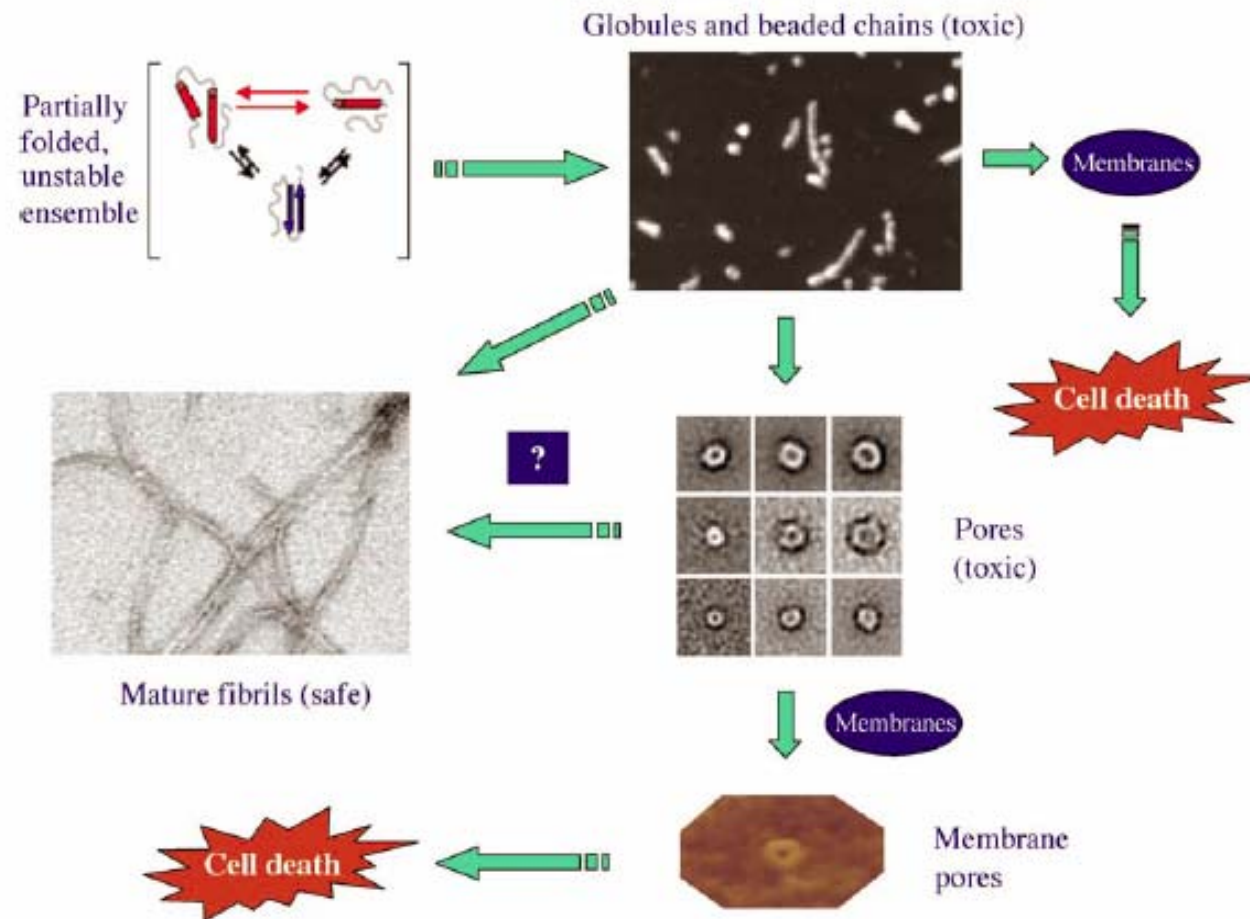


Fig. 4. The heterogeneous population of pre-fibrillar aggregates comprises globular assemblies further organising into beaded chains and doughnut-shaped entities currently associated with cytotoxicity due to their ability to interact with cell membranes. In particular, the pore-like assemblies may impair membrane permeability altering metal ion distribution between intracellular and extracellular media as well as among intracellular compartments triggering cell apoptosis. The question mark indicates that it is not known whether amyloid pores (when formed) are on path or dead end intermediates of fibril formation (modified from Ref. [5]). The electron micrographs are from Lashuel et al. [164] and from Harper et al. [165]. The AFM image is from Ding et al. [166].