INTRA AND EXTRACELLULAR PROTEIN ACCUMULATION

ENODPLASMIC RETICULUM (ER) STRESS

- PROTEIN FOLDING

- ABOUT 30% NEWLY SYNTHESIZED PROTEINS DEGRADED BY PROTASOME DUE TO FOLDING ERRORS

- ER ENDOWED WITH QUALITY CONTROL SYSTEMS

ER STRESS

- hyperstimulation of protein synthesis (plasmacells, β cells)
- viral infections
- ischemia, hypoxia, hypoglycemia, redox stress, altered Ca^{2+} homeostasis
- conformational defects

ACCUMULATION OF ALTERED PROTEIN IN THE ER LEADS TO

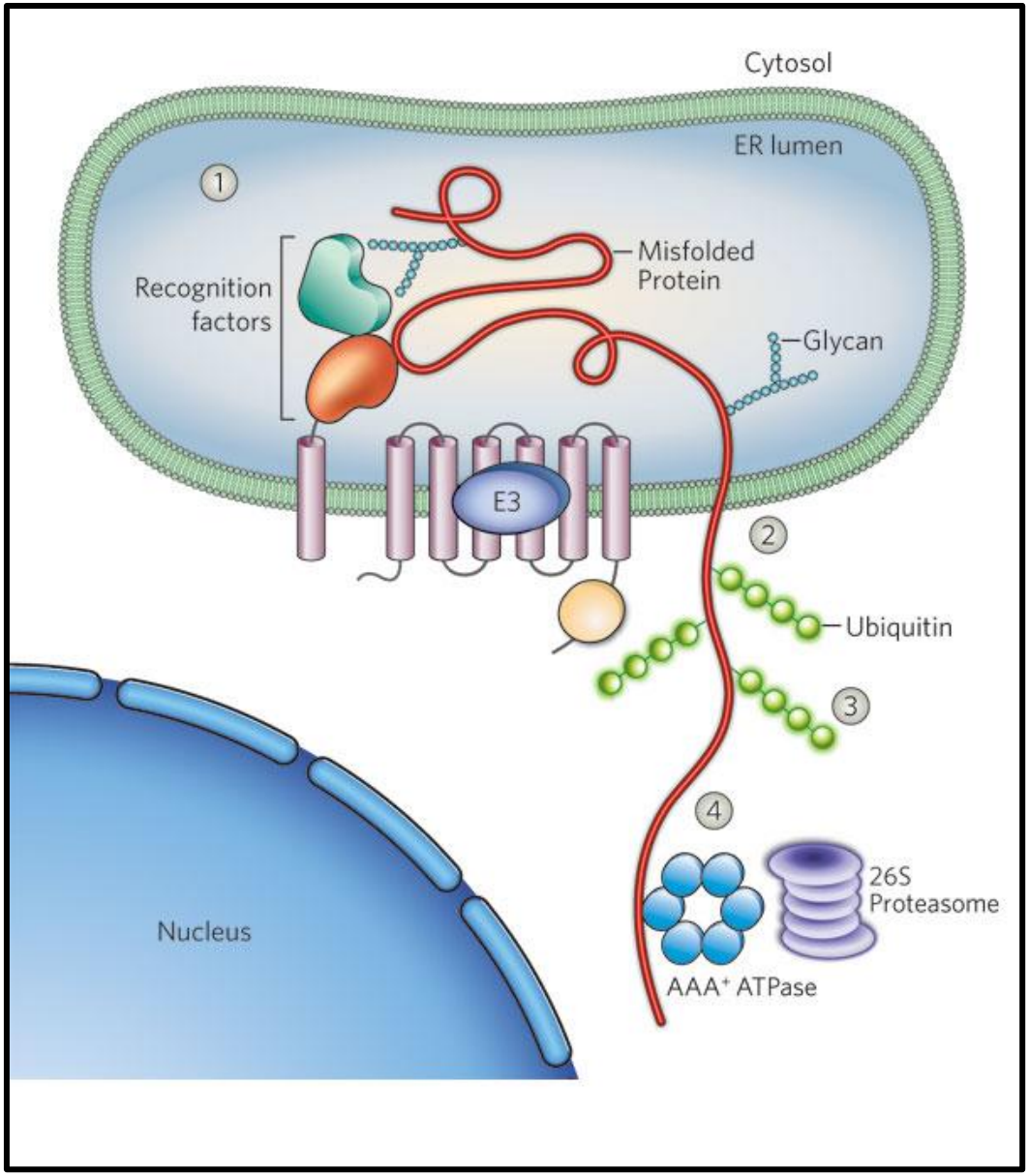
- **Unfolded Protein Response (UPR;** reduced protein synthesis, increased chaperonine levels**)**

- **Endoplasmic reticulum associated protein degradation (ERAD;** degradation of misfolded proteins **)**

Regulation of protein folding in the ER

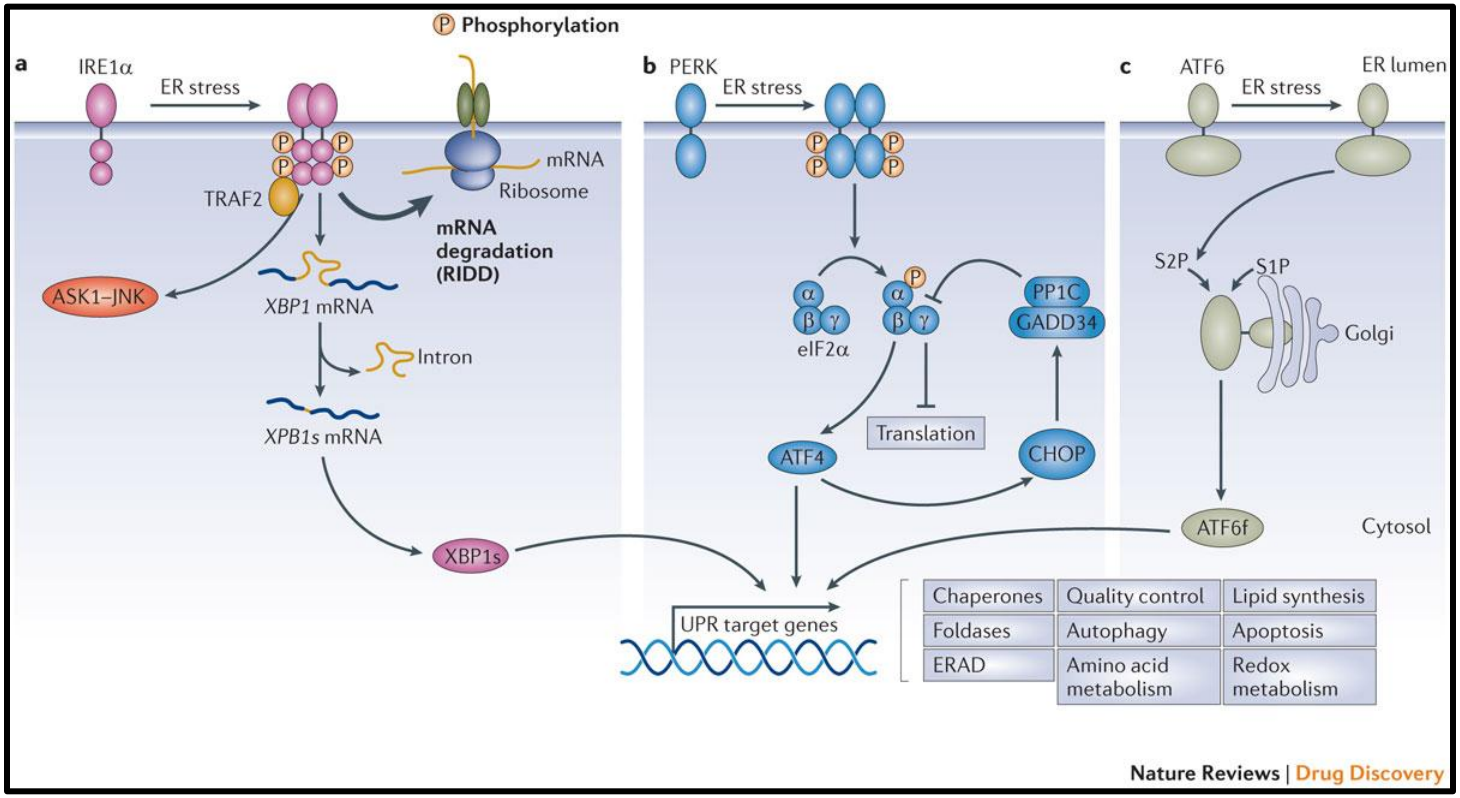
ER misfolded proteins

1. recognition (multichaperon complex; HSP40 and HSP70)
2. cytosol export
3. ubiquitylation and proteasome-dependent degradation



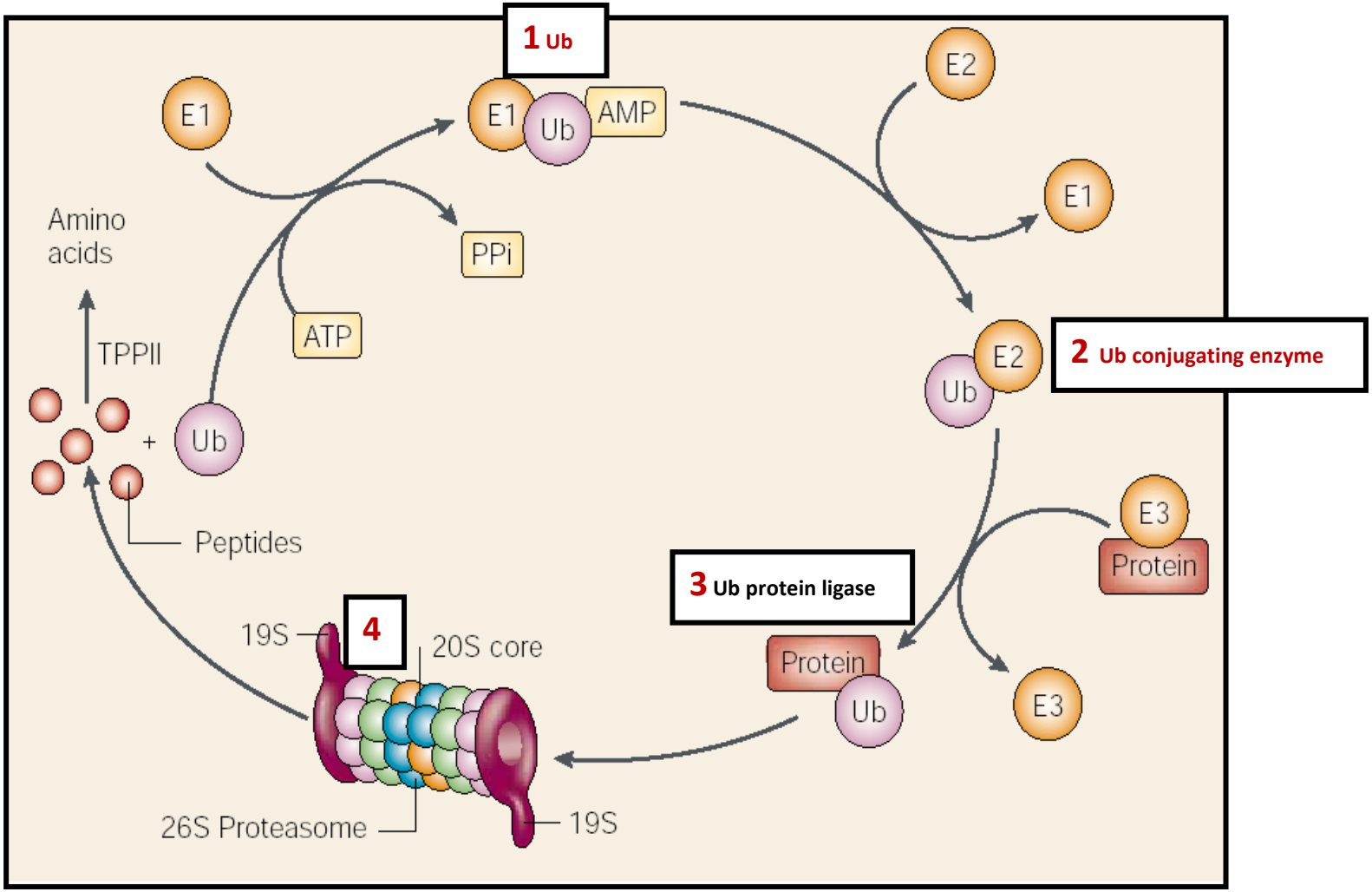
Hirsch et al., 2009

UPR

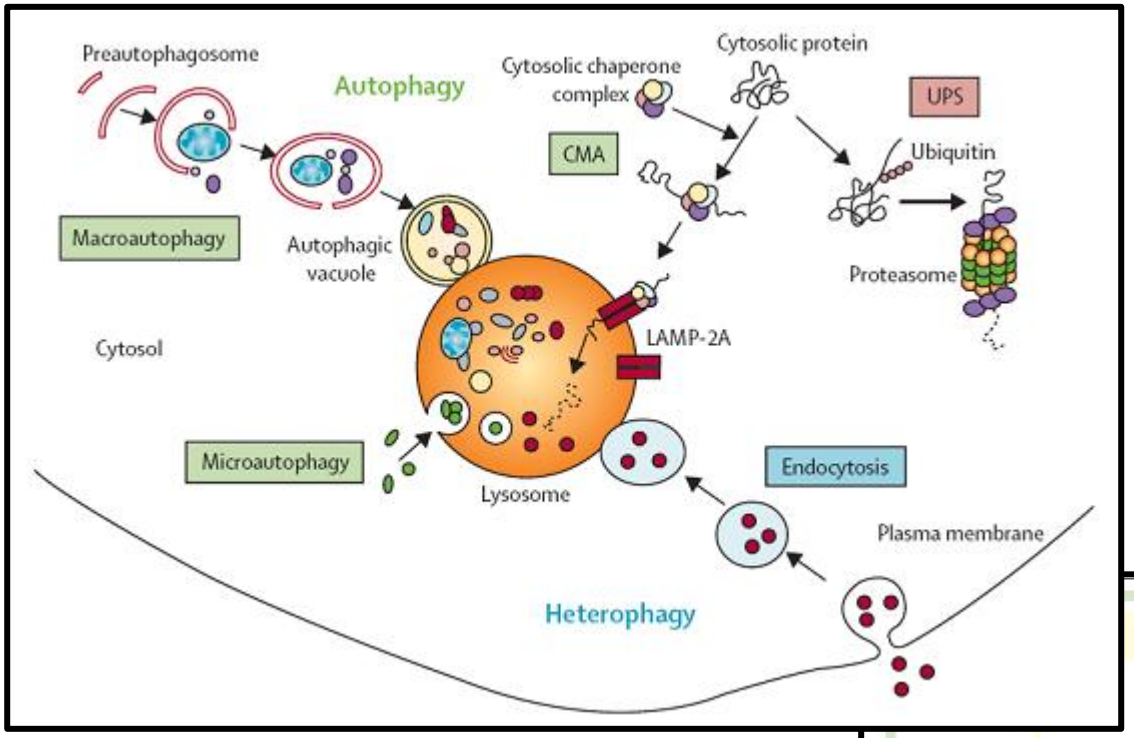


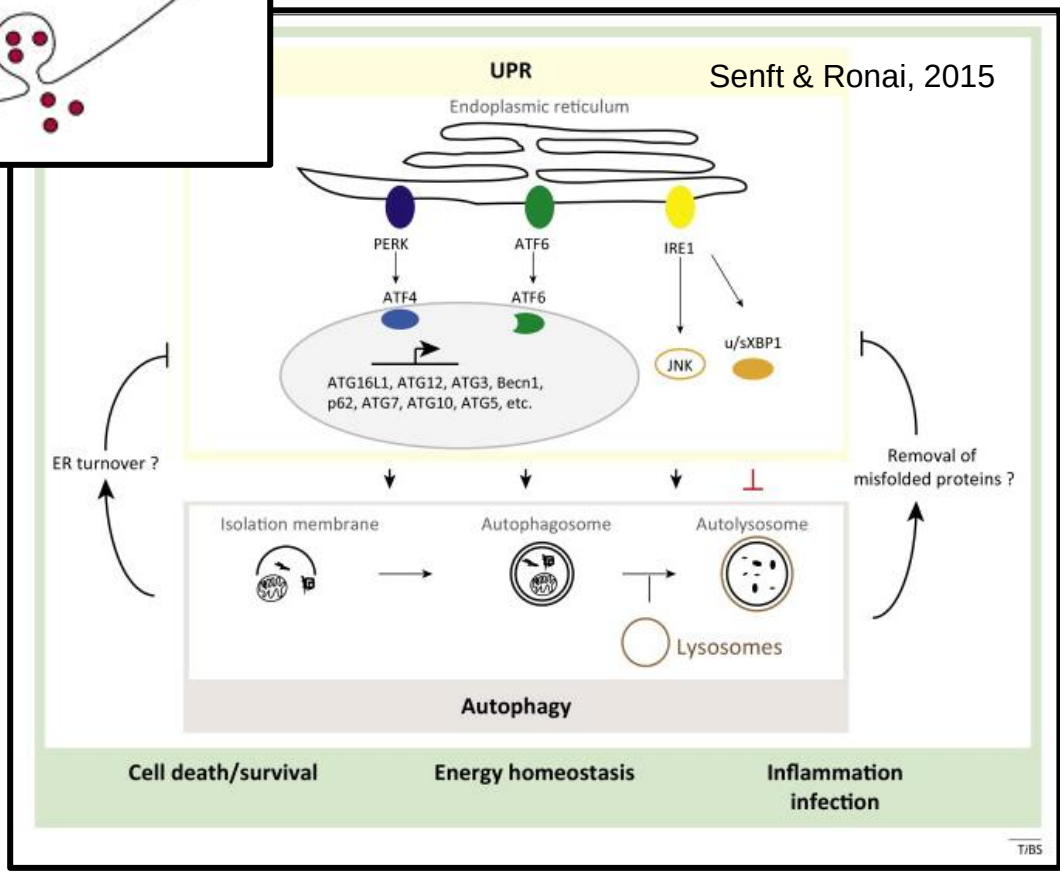
Hetz et al., 2013

ERAD: ubiquitin-proteasome pathway



ERAD: autophagy





Senft & Ronai, 2015

MISFOLDED PROTEIN FATE

- **Proteasomal/autophagic degradation → restored cell homeostasis**
- **Undegradable aggregates**

  cannot enter the proteasome

  proteasome ‘poisoning’

  inhibition of autophagy

Human diseases linked to ER stress - 1

Human diseases linked to ER stress

Disease		Protein	Function	Major pathology
Wolcott-Rallison Syndrome		PERK	UPR sensor	Infantile diabetes
Wolfram Syndrome		WFS1	Involved in ERAD?	Diabetes insipidus, neurodegeneration
Hereditary tyrosinemia type I		FAH	Tyrosine metabolism	Liver and renal dysfunction; accumulation of a metabolic intermediate causes ER stress
Familial hypercholesterolemia	*	LDLR	Low density lipoprotein receptor	Hypercholesterolemia; accumulation of mutant protein causes ER stress
Z α1-antitrypsin deficiency	*	A1AT (α1-antitrypsin)	Protease inhibitor	Early onset liver disease; accumulation of mutant protein causes ER stress
Inclusion body myopathy (IBMPFD)		p97/VCP/CDC 48	ERAD	Early onset Paget disease and frontal temporal dementia, muscular dystrophy, motor neuron degeneration
Parkinson's disease	*	Parkin Others	E3 ubiquitin ligase	Tremor, bradykinesia; loss of inclusion-containing dopaminergic neurons in the substantia nigra; Mutation of Parkin causes ER stress
Familial Alzheimer's disease	*	PS1 Others	γ-secretase complex	Memory loss, dementia; loss of neurons from frontal cortex, hippocampus, basal forebrains, formation of extracellular plaques and intracellular neurofibrilary tangles; ER stress?
Familial Amyotrophic Lateral Sclerosis	*	SOD1	Cu/Zn superoxide dismutase	Degeneration of motorneurons in spinal cord, cortex and brain stem; caspase 12 is activated and mutant SOD1 forms aggregates in ER in transgenic mice
Marinesco-Sjögren syndrome		SIL1/BAP/ SLS1	BiP adenine nucleotide exchange factor	Cerebellar ataxia, myopathy, cataracts; ER stress and UPR activation are observed in a mouse model
GM1 gangliosidosis		β-galactosidase	Carbohydrate/ lipid metabolism	Severe cerebral neurodegeneration; Accumulation of gangliosides; ER stress is observed in a mouse model

Human diseases linked to ER stress - 2

Pelizaeus-Merzbacher disease		PLP1	Proteolipid protein 1, myelin component	Spastic quadriplegia, ataxia; dysmyelination; ER accumulation of PLP causes ER stress
Batten disease/Infantile neuronal ceroid lipofuscinosis		PPT1	Palmitoyl-protein thioesterase-1	Seizures, mental deterioration, blindness; accumulation of toxic fatty-acylated proteins in neurons; may involve ER stress and activation of caspase 4
Bipolar disease		XBP1?	UPR sensor	Manic/depressive psychosis; *Xbp1* gene polymorphism?
Transmissible Spongiform Encephalopathy	*	PrP		Neuronal loss due to accumulation of the misfolded prion protein; enhanced calcium release and ER stress may be involved
Spinocerebellar ataxia 3/Machado-Joseph disease		SCA3		Ataxia, abnormal ocular movement, spasticity; activation of the IRE1 and PERK branches of UPR
Huntington's disease	*	Huntingtin		Neurodegeneration, motor dysfunction, abnormal cognition; mutant huntingtin changes ER calcium homeostasis
Sporadic inclusion body myositis	*	APP		Muscle degeneration with vacuolated muscle fibers; inclusions containing either β–amyloid or phosphorylated tau induces ER stress
Cerebral ischemia				Paresis, memory disturbance; neuron death; ER stress and CHOP activation
Atherosclerosis	*			Cholesterol deposition on the artery wall; hyperhomocysteinaemia and accumulation of unesterified cholesterol cause UPR induction
Solid tumors				UPR activation can protect tumor cells from hypoxia-induced apoptosis
Viral infection				UPR activation upon viral infection
Inflammation				Utilizes a specific UPR sensor, CREB-H
Fluoride tooth				Fluoride causes ER stress in ameloblast, resulting in dental enamel formation

AMYLOIDOSIS

EXTRACELLULAR AMORPHOUS DEPOSITION OF INSOLUBLE FIBRILS (amyloid substance; Rudolf Virchow, 1854)

RESULT: ALTERED CELL FUNCTION

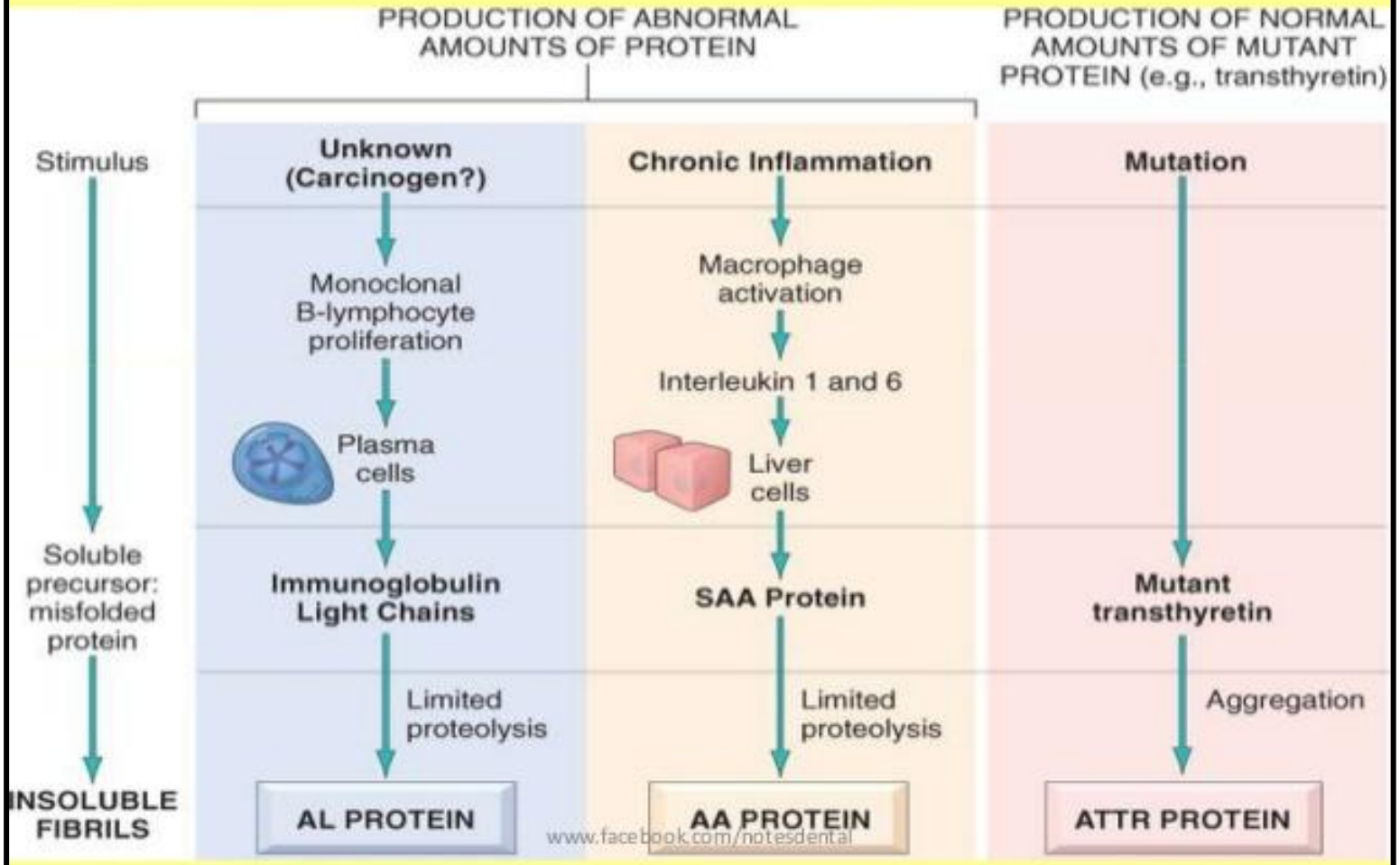
DUE TO ABNORMAL PROTEIN FOLDING WITH SPECIFIC CHARACTERISTICS:

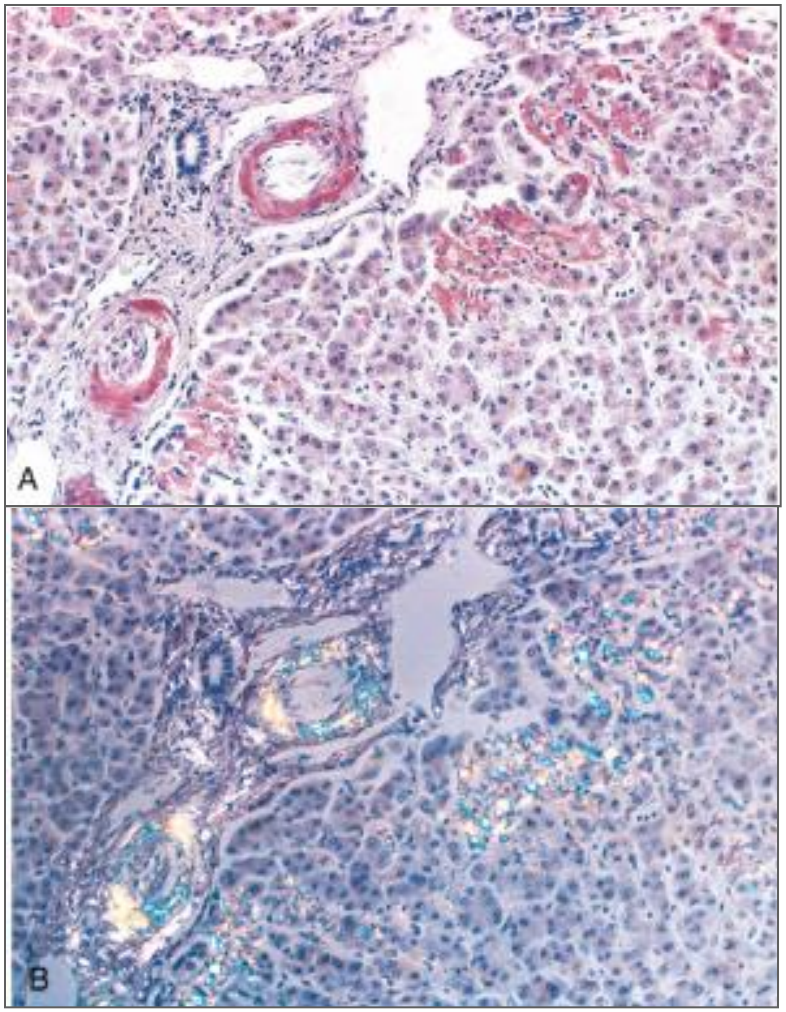
- eosinophilic/Congo red positive β-pleated sheet structure
- non-branching fibrils (7.5-10 nm)
- undegradable

Amyloid composition

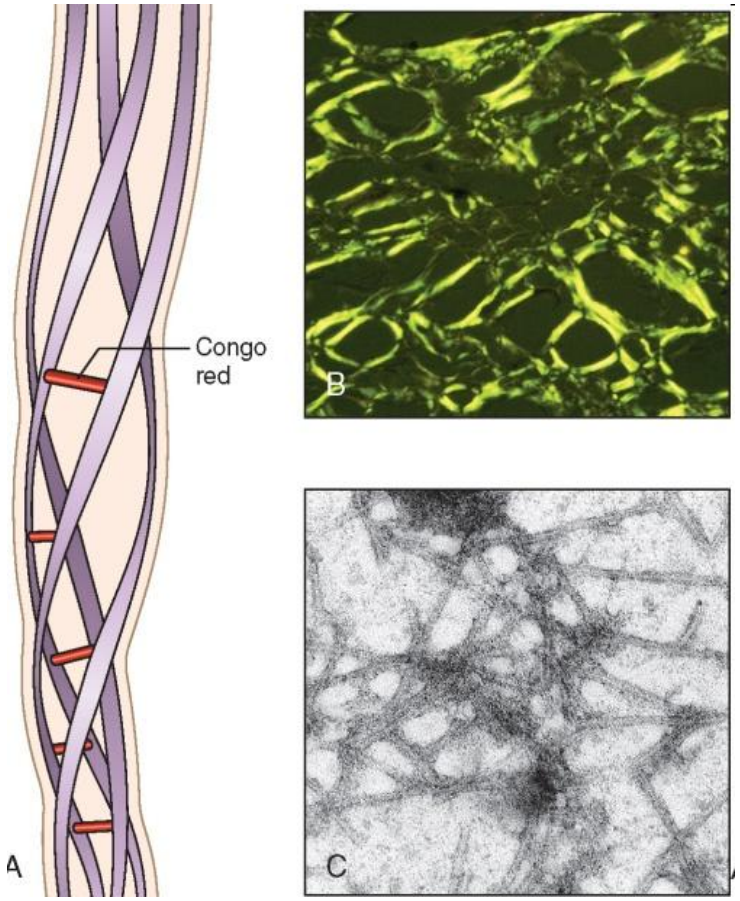
- Specific amyloid protein ~ 90%
- P component of SAP ~ 15%
- Glycosaminoglycans

PATHOGENESIS OF AMYLOIDOSIS



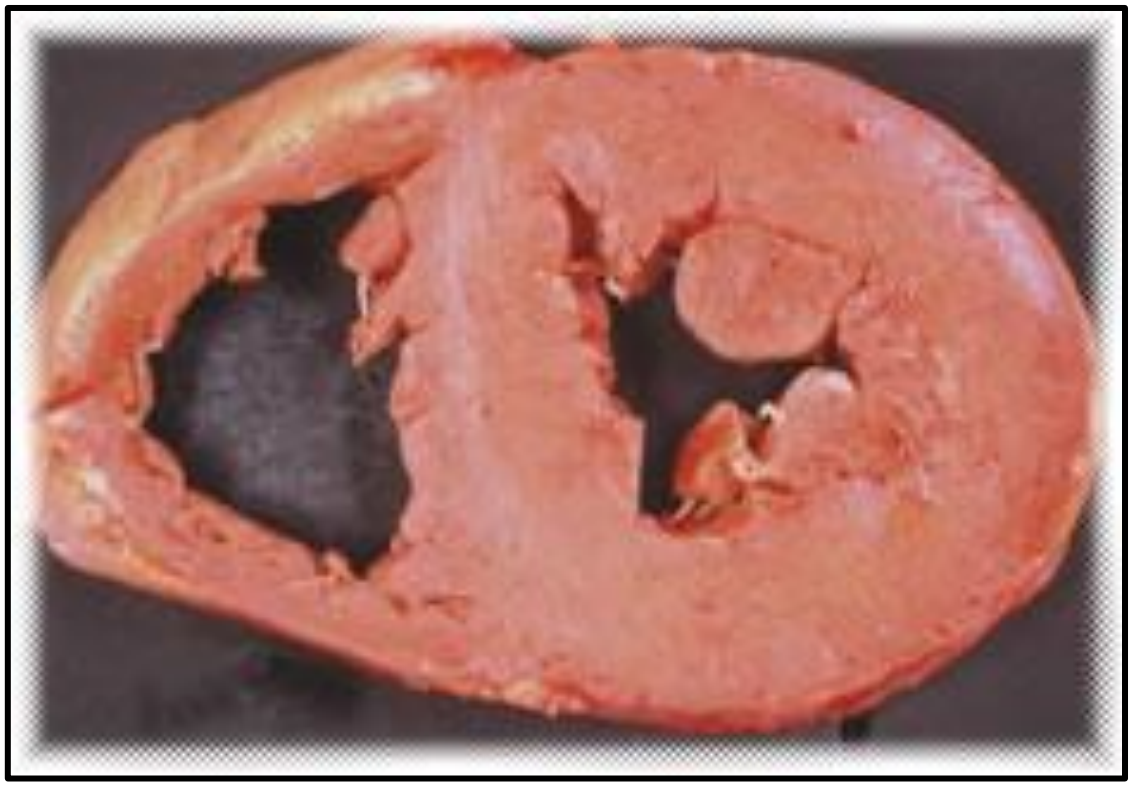


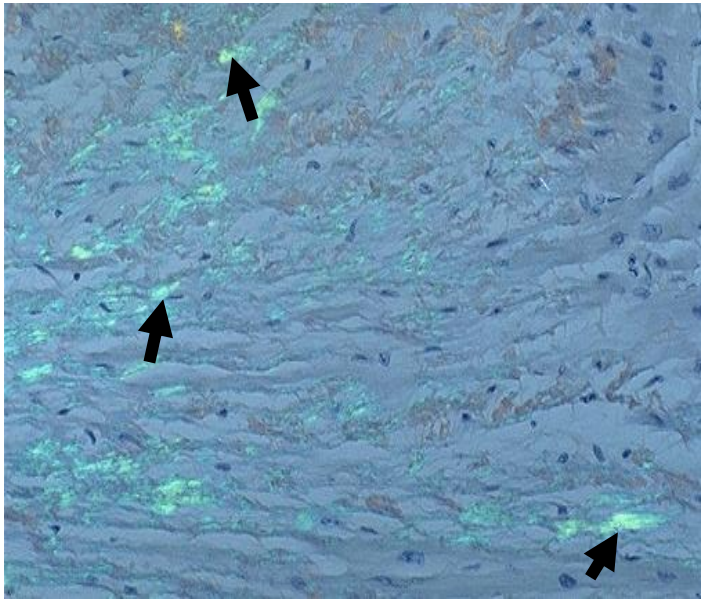
Amyloidosis liver
A, A section stained with **Congo red** reveals pink-red deposits of amyloid in the walls of blood vessels and along sinusoids.
B, Note the birefringence of the deposits when observed by polarizing microscope (Robbins, 2010)

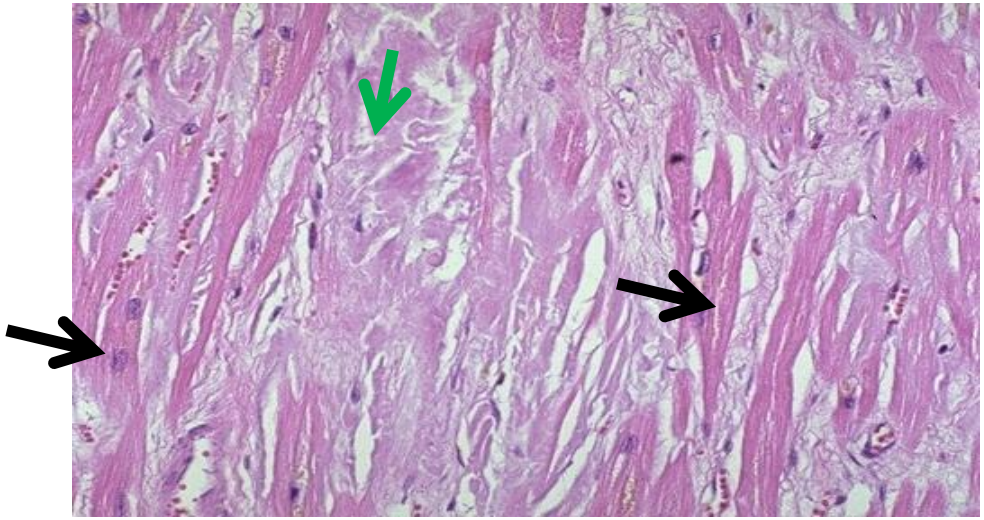


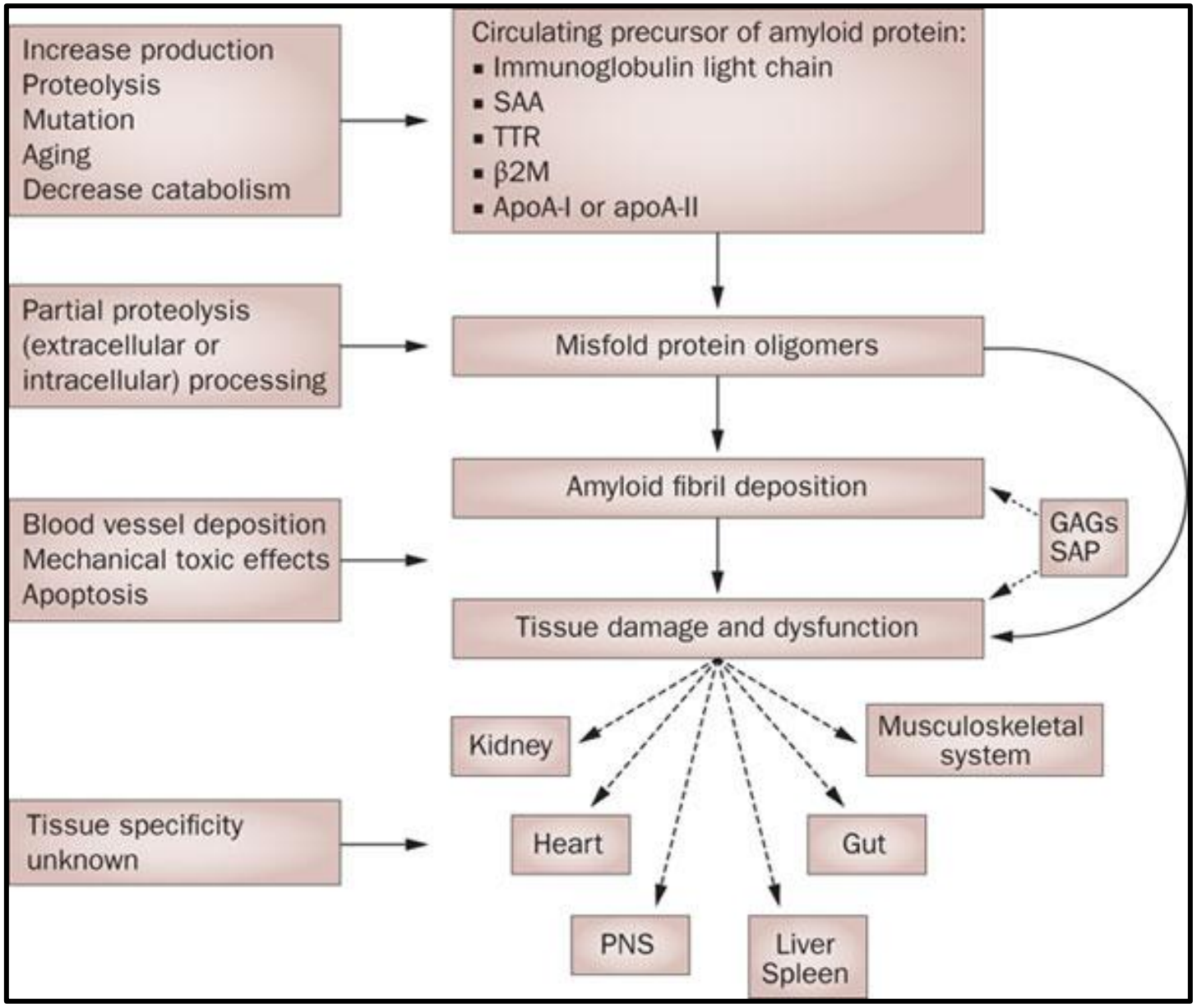
Structure of amyloid. A, An amyloid fiber schematically showing four fibrils (there can be as many as six in each fiber) wound around one another with regularly spaced binding of the Congo red dye. **B**, Congo red staining shows apple-green birefringence under polarized light, a diagnostic feature of amyloid. **C**, Electron micrograph of 7.5- to 10-nm amyloid fibrils (Robbins, 2010)

AMYLOIDOSIS: HEART

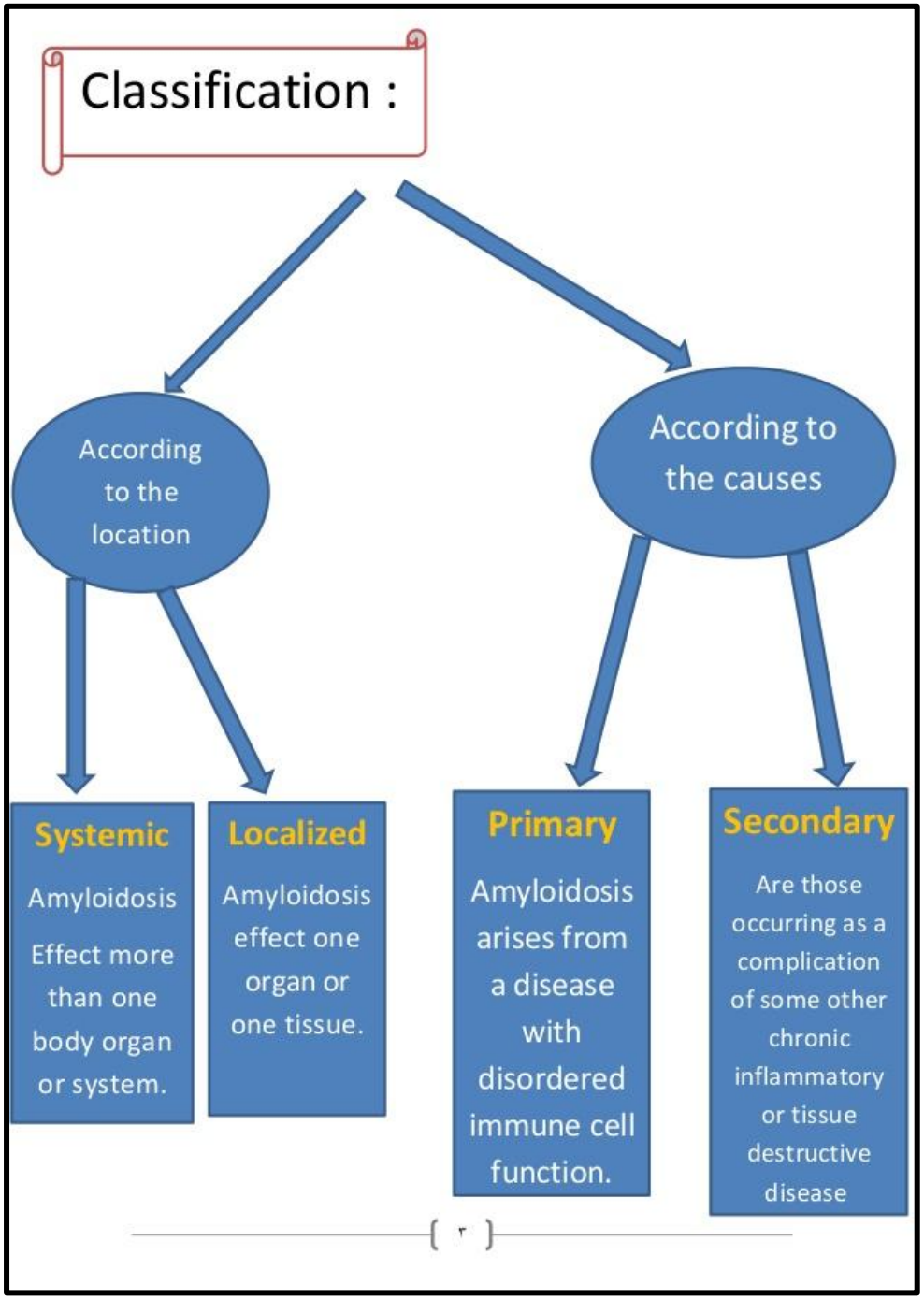








Perfetto et al., 2010


Classification :
According to the location
According to the causes
Systemic
Amyloidosis
Effect more than one body organ or system.
Localized
Amyloidosis effect one organ or one tissue.
Primary
Amyloidosis arises from a disease with disordered immune cell function.
Secondary
Are those occurring as a complication of some other chronic inflammatory or tissue destructive disease

AMYLOIDOSIS: MODERN CLASSIFICATION

OFFICIAL ABBREVIATION	AMYLOID TYPE/GENE	DESCRIPTION
AL	**Amyloid light chain (λ)**	**AL amyloidosis/ Multiple myeloma**
AA	**Serum amyloid A protein**	**AA amyloidosis**
Aβ	**β amyloid**	**Alzheimer's disease**
Aβ2M	**β_2 amyloid microglobulin**	**Hemodialysis related amyloid**
ALect2	LECT2 protein	LECT2 amyloidosis
ATTR	**Transthyretin**	**Familial amyloid polyneuropathies, Wild type transthyretin amyloidosis, leptomeningeal amyloidosis**
AIAPP	**amylin**	**Type II diabetes**
APrP	prion protein	CJD, BSE
ACys	**CST3**	**Cerebral Amyloid Angiopathy**
AGel	GSN	Finnish type amyloidosis
AApoA1[1]/ Afib[2]/ Alys[3]	APOA1[1]/ FGA[2]/LYZ[3]	Familial visceral amyloidosis
APro	prolactin	Prolactinoma
AKer	keratoepithilin	Familial corneal amyloidosis
AANF	Atrial natriuretic factor	Senile amyloid of the heart
ACal	Calcitonin	Medullary carcinoma of the thyroid

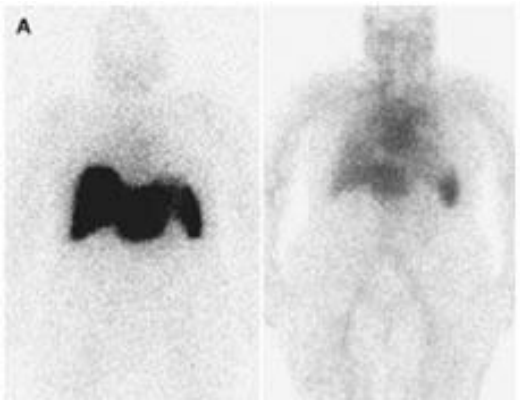
B. P Component:

- makes up about 10% of the amyloid's weight
- found on all amyloid fibrils, except those associated with Alzheimer's senile plaques.
- stabilizes fibril amyloid protein and decreases their clearance
- plays a role in some of the other special stains that can demonstrate amyloid (TFT)

Serum amyloid P component scintigraphy

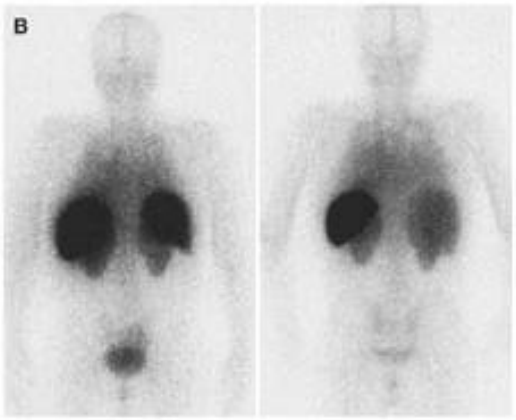
Multiple myeloma

(**A**) **Serial anterior whole-body scintigraphs** in a **patient with AL amyloidosis (multiple myeloma) treated with oral melphalan and corticosteroids**:
- left: baseline scan showed extensive amyloid deposits in the liver and spleen obscuring the signal from the kidneys;
follow-up scintigraphy 6 months later (**right)** showed complete regression of amyloid from the liver and marked regression from the spleen with a reciprocal increase in the blood-pool signal.



Rheumatoid arthritis

(**B**) **Serial posterior whole-body** ^{123}I-labeled serum amyloid P component scintigraphy in a 19-year-old man with **sustained remission from juvenile rheumatoid arthritis**.
Median serum amyloid A values were <10 mg/l during this 2-year interval.
Left: baseline scan showed extensive amyloid deposits in liver, spleen, and kidneys that had regressed very prominently in the liver at follow-up **(right).**
The patient had not voided before the baseline scan, which shows radioactive degradation products in the urinary bladder.



Gillmore JD and Hawkins PN (2006) Drug Insight: emerging therapies for amyloidosis
Nat Clin Pract Neprol **2:** 263–270 doi:10.1038/ncpneph0169

nature CLINICAL PRACTICE NEPHROLOGY