PROTEIN FOLDING and MISFOLDING, DISEASES ASSOCIATED WITH PROTEIN MISFOLDING & AGGREGATION

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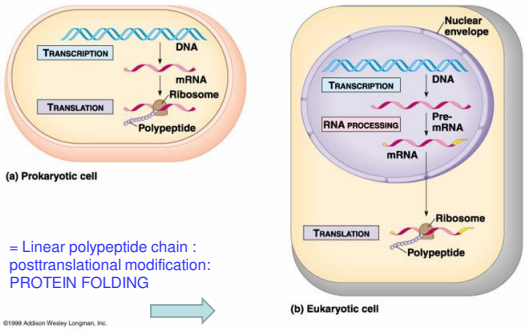
CENTRAL DOGMA IN MOLECULAR BIOLOGY

GENE EXPRESSION pathway



= Linear polypeptide chain : posttranslational modification: PROTEIN FOLDING

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Four levels of protein structure – Three levels of protein conformational folding

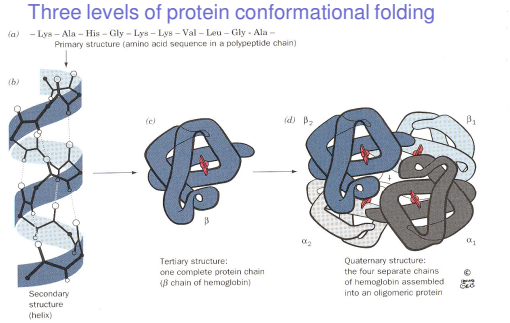

FIGURE 7-1 **The structural hierarchy in proteins.** (*a*) Primary structure, (*b*) secondary structure, (*c*) tertiary structure, and (*d*) quaternary structure. [Illustration, Irving Geis/Geis Archives Trust. Copyright Howard Hughes Medical Institute. Reproduced with permission.]

S. str.: regions of the polypept. chain with defined conformation stabilized by hydrogen bonds. **T. str.:** 3D conformation of a **protein** composed from elements of second. structure and unstructured regions. **Q. str.:** complexes from **protein oligomers**

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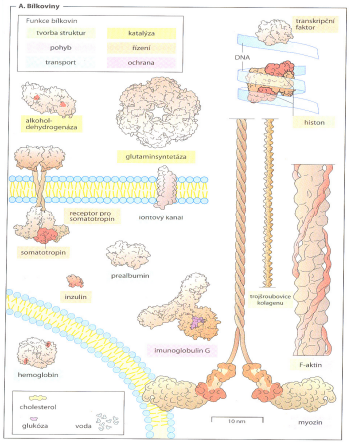
VARIOUS CONFORMATIONS OF PROTEINS



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HOW DOES THE PROTEIN REACH ITS FINAL CONFORMATION?

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Under physiological conditions the folding of proteins into their native conformation is a spontaneous, self-assembling process, with no need of external templates to guide their formation. Ch. Anfinsen (1957).

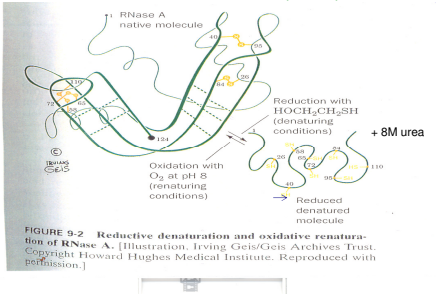

FIGURE 9-2 **Reductive denaturation and oxidative renaturation of RNase A.** [Illustration, Irving Geis/Geis Archives Trust. Copyright Howard Hughes Medical Institute. Reproduced with Permission.]

Today: the help of protein folding facilitating enzymes is required. Mainly to prevent protein aggregation and increase the speed of correct protein folding.

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POLYPEPTIDE CHAIN FOLDING

1) WHY?

THERMODYNAMICS

2) HOW?

STRUCTURE BUILDING RULES and SPACE RESTRICTIONS; NONCOVALENT INTERACTION STABILIZATION

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WHY is the folding taking place?

- Spontaneous = driven thermodynamically; the folding obeys laws of thermodynamics: to achieve the lowest energetic state for the given primary structure of the polypeptide chain under present (physiological) conditions and in an acceptable time period (seconds) => is accompanied by the reduction of free energy and/or increase in entropy:

  A + B =>>> AB + energy (heat, entropy) until the native state is reached. The folding/conformation is thus determined by the sum of interatomic interactions within given **AMINO ACID SEQUENCE**
- To attain the shape evolved by natural selection for the particular biological function

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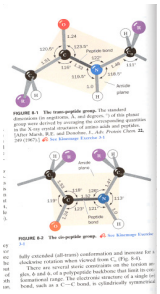
HOW do proteins reach their native 3D conformation from their primary structure?

Starting points:

A) PEPTIDE BOND



Two conformations:

(i) TRANS-

Peptide bonds (groups), with few exceptions, assume the trans-conformation: that, in which successive Cα atoms are on opposite sides of the peptide bond joining them.

(ii) CIS-

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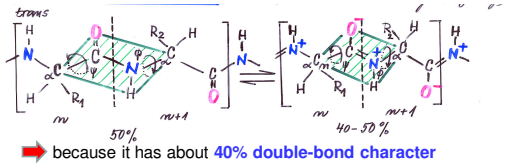
PEPTIDE BOND assumes a rigid, planar structure



➡ because it has about **40% double-bond character**
(exists in two resonance isomeric states)

Length: 13.3 nm (C-N ~ 14.5 nm, C=N ~ 12.5 nm; RTG)

No rotation around the C-N bond, all atoms linked to C or N are in **one plane** (in green)

However: the backbone N-Cα single bonds can rotate (ϕ torsion angels)
the backbone C-Cα single bonds can rotate (ψ torsion angels)

The rotations are not unlimited, they are greatly restricted by collision of molecular orbitals of neighbouring atoms of the polypeptide chain
– Ramachandran diagrams: sterically allowed values of ϕ and ψ

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What folding shapes/structures may a polypeptide chain attain that would be optimally compatible with the sterically allowed values of ϕ and ψ ?

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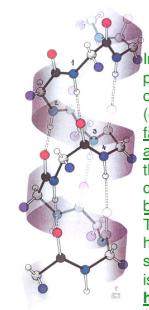
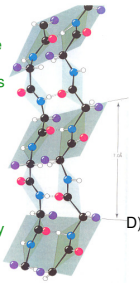
Optimal (and typical) folding shapes of secondary structural elements of proteins



A) **α–helical**
(right-handed, α_R)

In order to exist, the polypeptide chain conformation angles (ϕ, ψ) must not only fall within the allowed regions but the particular conformation must be also **stabilized**. The "glue" that holds the secondary structural elements is, in part, **hydrogen bonds**: 1NH….4CO, intra-chain



B) A two stranded anti-parallel pleated **β–sheet**. Hydrogen bonding NH….CO between neighboring polypeptide chains

C) **Turns** (coil or loop conformation)

D) **Disordered regions**

Helices and sheets constitute ~ 60% of the average protein.

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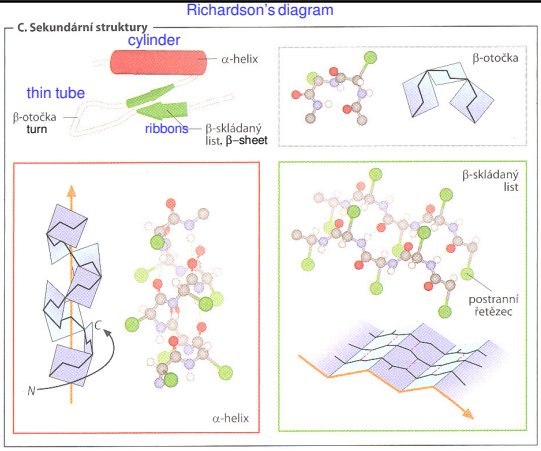
Richardson's diagram



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SUPERSECONDARY PROTEIN STRUCTURES

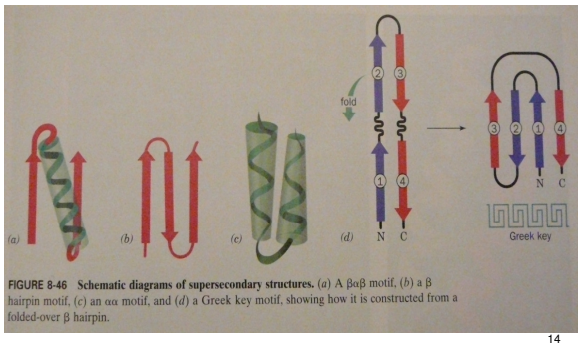

FIGURE 8-46 **Schematic diagrams of supersecondary structures.** (*a*) A βαβ motif, (*b*) a β hairpin motif, (*c*) an αα motif, and (*d*) a Greek key motif, showing how it is constructed from a folded-over β hairpin.

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Noncovalent forces leading to protein folding into secondary structure elements and 3D conformation and contributing to protein's stability

NONCOVALENT ("WEAK") INTERACTIONS: 4-29 kJ/mol

1) **Electrostatic forces**

a) ionic, ionic or salt linkages, 4-25 kJ/mol. Between charged groups. Even though comparatively strong, their contribution to the stability of proteins in aqueous solutions is low due to a high dielectric constant (D=78,5) of water. Those on the surface of a protein do not usually interact with other charged groups of the protein but instead are stabilized by hydrogen bonding and polar interactions with water molecules.

Asp-COO^- ……$^+NH_3$-Lys *etc*

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Strength of electrostatic interactions

$$U = \frac{kq_1q_2}{Dr}$$

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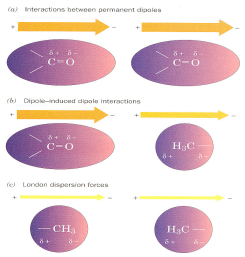

FIGURE 8-57 Dipole–dipole interactions. The strength of each dipole is represented by the thickness of the accompanying arrow. (*a*) Interactions between permanent dipoles. These interactions, here represented by carbonyl groups lined up head to tail, may be attractive, as shown, or repulsive, depending on the relative orientations of the dipoles. (*b*) Dipole–induced dipole interactions. A permanent dipole (here shown as a carbonyl group) induces a dipole in a nearby group (here represented by a methyl group) by electrostatically distorting its electron distribution (*shading*). This always results in an attractive interaction. (*c*) London dispersion forces. The instantaneous charge imbalance (*shading*) resulting from the motions of the electrons in a molecule (*left*) induces a dipole in a nearby group (*right*); that is, the motions of the electrons in neighboring groups are correlated. This always results in an attractive interaction.

Electrostatic forces (cont.)

b) dipole-dipole interactions, van der Waals forces. <4kJ/mol. Between electrically neutral molecules, arise from electrostatic interactions among permanent and/or induced dipoles. They may be attractive, as shown, or repulsive, dependent on the relative orientations of the dipoles. They maximally act at short distinct distances, which are the sum of van der Waals radii (electron orbitals) for two interacting atoms. However, as the atoms come even closer the repulsion, steric hindrance, results.
Although the weakest of the noncovalent forces they may be numerous and in the low dielectric constant core of the protein, dipole-dipole interactions significantly influence protein folding and determine its conformation.

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Electrostatic forces (cont.)
c) hydrogen bonds, H bonds. 12-40 kJ/mol, (4-29 kJ/mol), with ~10% covalent character. Between a weakly acidic donor group (**D-H**) and an acceptor (**:A**) that bears a lone pair of electrons.
D-H…….:A; **H (~proton)** is "shared" by the two atoms
~0.27-0.31 nm

In biology donors "D": weakly acidic **O**-H, **N**-H; **S**-H; rel. acidic $\mathbf{C_\alpha}$-H
acceptors "A": highly electronegative **:O**, **:N**; **:S**;
E.g. H bonds in α-helices and antiparallel β pleated sheets in proteins:
N-H…:O=C (atoms of peptide bonds!)
(in ideal α-helices between D and A at residues *n* and *n-4, (n-3),* respectively)

Many of the H bonds in proteins are members of **networks** in which each donor D is H bonded to two acceptors (a bifurcated hydrogen bond) and each acceptor A is H bonded to two donors.

On average, 68% of the H bonds in proteins are between backbone atoms: α-helices (1/3), 3^{10} helices (1/3), β pleated sheets (1/3). Only ~5% of the H bonds between backbone atoms are not wholly within a helix, sheet or turn.

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Electrostatic forces (cont.)
c) hydrogen bonds, H bonds (cont).

The strength of a hydrogen bond depends on the distance between donor and acceptor atoms (the highest is at a distance between 0.27 and 0.31 nm).
Although the H bonds contribute to thermodynamic stability of a protein's conformation, their formation may not be a major driving force for folding. This is because peptide bonds and other hydrogen-bonding groups form H-bonds to the water solvent (water is a strong H bonding donor and acceptor) from the protein before its folding (in a denatured state) and the energy required to brake them must be subtracted from that gained from formation of new H-bonds in the protein. As the H-bonds are electrostatic they are likely to be stronger in the low polarity protein interior than on the high polarity aqueous surface.

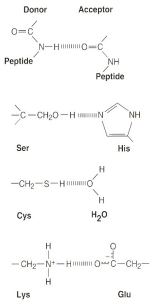

FIGURE 3.48
Some common hydrogen bonds found in proteins.

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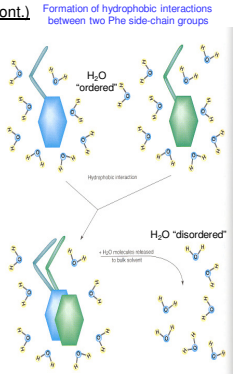
NONCOVALENT ("WEAK") INTERACTIONS (cont.)
2) Hydrophobic forces. 8/12,5 kJ/mol.
The hydrophobic effect causes nonpolar substances to minimize their contact with water. It is not due to any intrinsic attraction between nonpolar groups. Rather:
When two nonpolar side chains come together, the surface area exposed to solvent is reduced and some of the highly **ordered** water molecules in the solvation shell are **released** to bulk solvent. The entropy of the system (i.e. net disorder of water molecules in the system) is Increased. The INCREASE IN ENTROPY is thermodynamically favorable and IS THE DRIVING FORCE CAUSING NONPOLAR MOIETIES TO COME TOGETHER in aqueous solvent. A favourable Gibbs free energy change ΔG~-8.5 kJ/mol for association of two Phe side chains in water is due to this gain in entropy (Fig.)

Formation of hydrophobic interactions between two Phe side-chain groups



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2) Hydrophobic forces (cont.)
Individual amino acids can be scaled according to the hydropathies (hydropathic and hydrophilic tendencies) of their side chains; they are good predictors of which portions of the polypeptide chain are inside of the protein, and which outside in contact with the aqueous solvent.

W. Kauzmann (1958):

HYDROPHOBIC FORCES ARE A MAJOR INFLUENCE IN CAUSING PROTEINS TO FOLD INTO THEIR NATIVE 3D-CONFORMATION

Protein folding is directed mainly by internal residues.

TABLE 8-6 **Hydropathy Scale for Amino Acid Side Chains**

Side Chain	Hydropathy
Ile	4.5
Val	4.2
Leu	3.8
Phe	2.8
Cys	2.5
Met	1.9
Ala	1.8
Gly	−0.4
Thr	−0.7
Ser	−0.8
Trp	−0.9
Tyr	−1.3
Pro	−1.6
His	−3.2
Glu	−3.5
Gln	−3.5
Asp	−3.5
Asn	−3.5
Lys	−3.9
Arg	−4.5

Source: Kyte, J. and Doolitle, R.F., *J. Mol. Biol.* **157,** 110 (1982).

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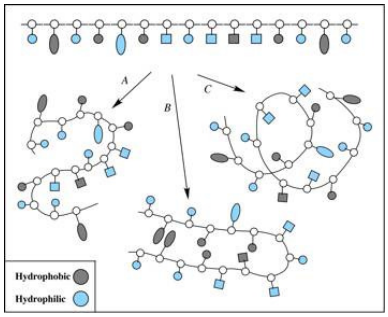

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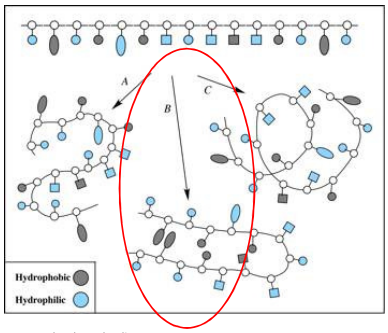

http://www.indiana.edu/~oso/lessons/prot/folding1.htm

PROTEIN STABILIZATION - **DISULFIDE BONDS**

Formation of disulfide bonds Cys-S – S-Cys is not efficient in the cytoplasm due its relatively reducing character. They are formed in ER, which has an oxidizing environment, as a protein folds into its native conformation. Disulfide bonds give **additional 3D structural stability** to proteins destined primarily for secretion into more oxidized, "hostile", extracellular destinations with e.g. uncontrolled temperatures or pH and before further protein processing/maturation, e.g. removal of a prosequence structure (Fig.)

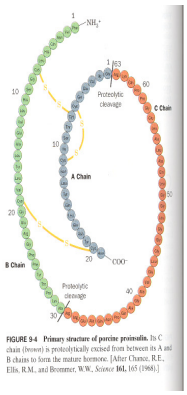

FIGURE 9-4 Primary structure of porcine proinsulin. Its C chain (brown) is proteolytically excised from between its A and B chains to form the mature hormone. [After Chance, R.E., Ellis, R.M., and Brommer, W.W., Science 161, 165 (1968).]

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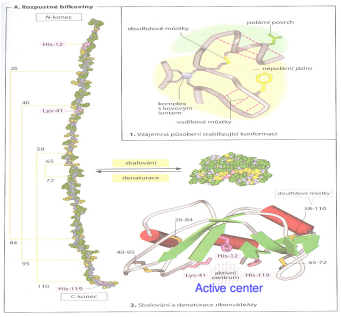
RNase A Folding and Denaturation



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Summary – protein folding and stabilization

The process of transformation of a randomly ordered (denatured) polypeptide chain into a regular **secondary structure** (α-helix, β-sheet) results in the **release of ~1/3 of the solvation shell water into the bulk solvent**. This represents the release of ~2-4 kJ/mol/aa residue.
The additional 1/3 of **water molecules is released** from the polypeptide solvation shell when the native **3D conformation** is achieved. By this way the native conformation of a protein reaches the one of the lowest Gibbs free energy accessible to its sequence within a physiological time frame.
Thus folding is under thermodynamic and kinetic control.

Despite that all, the conformational stabilities of native proteins are low making them easily susceptible to denaturation by altering the balance of weak stabilizing forces (e.g. by heat, chemicals-detergents, pH). This marginal stability of most proteins under physiol. conditions (~1.7 kJ/mol/aa res.) is an essential property that has arisen through evolution of function – to be flexible enough to carry out their physiological functions. [Average kinetic energy of thermal movement is ~-2.5 kJ/mol at 37°C. Association energy of the covalent bond ~>-250 kJ/mol]

Some proteins may have two (more?) different stable conformations (prions). Their certain regions (**"chameleon r."**) may adapt either helical or sheet structure dependent on the context of the surrounding. See later.

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Folding pathways -Levinthal paradox

What is the way a protein takes to fold to its native conformation?
A protein randomly explores all of the available, "allowed", conformations to it until it eventually "stumbles" onto its native conformation?
Calculations of C. Leventhal: **this cannot be the case!**

Assume that the 2*n* backbone torsional angles, ϕ and ψ, of an *n*-residue protein each have (only) three stable conformations. This yields $3^{2n} \sim 10^n$ possible conformations for the protein, (which is a gross underestimate, if only because the side chains are ignored). If a protein can explore new conformations at the rate at which single bonds can reorient, it can find ~ 10^{13} conformations per second (an overestimate).

For a small protein of $n = 100$ residues the time t, in seconds, required to explore all the conformations available to it is:

$t = 10^{100} : 10^{13} . sec^{-1} = 10^{87}$ **s !!!!**

This is immensely more than the apparent age of the Universe (~15 billion years = $4.5 . 10^{17}$ s) !

Proteins must fold in an ordered manner rather than via a random search of all their possible conformations. Each protein species appears to have evolved a conserved and simplified pathway of folding.

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"PROTEIN FOLDING PATHWAY"

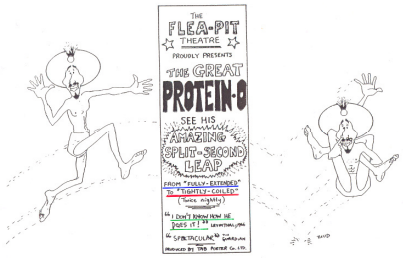

FIGURE 9-1 [Drawing by T.A. Bramley, *in* Robson, B., *Trends Biochem. Sci.* **1**, 50 (1976).

= Non-random + highly ordered +
+ ALL or NOTHING

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A similar paradox also exists concerning the formation of correct disulfide bridges in the protein.
E.g. the overall probability of RNase A reforming its four native disulfide linkes at random is

$$\frac{1}{7} \times \frac{1}{5} \times \frac{1}{3} \times \frac{1}{1} = \frac{1}{105}$$

=> the RNase is only about 1% enzymatically active. It can be made fully active by exposing it to a "trace" of 2-mercaptoethanol, the reducing agent. However, a 10-h period is required in contrast to the native environment when it takes only minutes. Here, additional proteins, enzymes, e.g. protein disulfide isomerase (PDI), that catalyze and accelerate disulfide interchange reaction to attain thermodynamically more favourable conformations, are active (Fig.).

Clearly, the disulfide bonds from RNAse A do not reform at random.
Protein disulfide isomerases.

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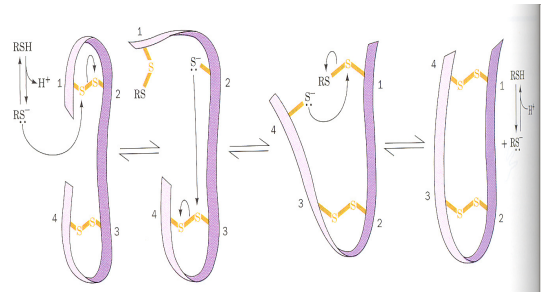

FIGURE 9-3 Plausible mechanism for the thiol- or enzyme-catalyzed disulfide interchange reaction in a protein. The purple ribbon represents the polypeptide backbone of the protein. The attacking thiol group must be in its ionized thiolate form.

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3D-FOLDING PATHWAYS (very complex, still not fully understood)

1) **"Hydrophobic collapse"** – the protein's hydrophobic groups coalesce so to expel most of their surrounding water molecules.
~ 5 ms. This initial state is known as the molten globule.
+ formation of local secondary structure elements
2) Intermediate folding events. The native-like elements are thought to take the form of subdomains, etc. 5 – 1000 msec. Cooperativity.
3) Final folding events. Complex. Several seconds.

The folding follows an **energy surface or landscape-folding funnel** that represents the energy states available to a polypeptide under the prevailing conditions. Polypeptides fold via a series of conformational adjustments that reduce their free energy and entropy until the native state is reached (Fig.).

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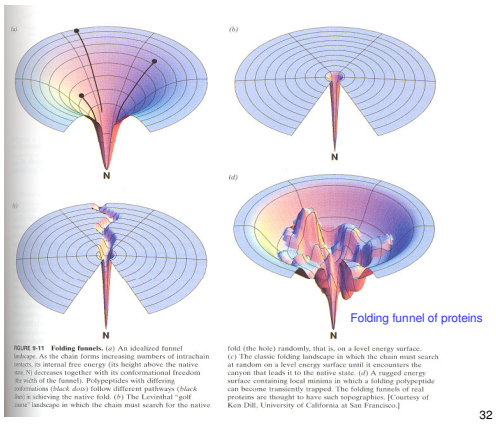

Folding funnel of proteins

FIGURE 8-11 Folding funnels. (*a*) An idealized funnel landscape. As the chain forms increasing numbers of intrachain contacts, its internal free energy (its height above the native state, N) decreases together with its conformational freedom (its width of the funnel). Polypeptides with differing conformations (*black dots*) follow different pathways (*black lines*) in achieving the native fold. (*b*) The Levinthal "golf course" landscape in which the chain must search for the native fold (the hole) randomly, that is, on a level energy surface. (*c*) The classic folding landscape in which the chain must search at random on a level energy surface until it encounters the canyon that leads it to the native state. (*d*) A rugged energy surface containing local minima in which a folding polypeptide can become transiently trapped. The folding funnels of real proteins are thought to have such topographies. [Courtesy of Ken Dill, University of California at San Francisco.]

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ENZYMES OF THE CELL NATURALLY FACILITATING CORRECT PROTEIN FOLDING

A)Protein disulfide isomerases: S-S bridges

B)Peptidyl prolyl cis-trans isomerases: Xaa-Pro peptide bond conformation.
Almost all the bonds are in the *trans* conformation; ~10% in the *cis*

C) Molecular chaperones: function to **prevent or reverse improper associations/aggregations of polypeptide chain regions**, particularly in multidomain and multisubunit proteins. They do so by binding to unfolded or aggregated polypeptide's **solvent-exposed hydrophobic surfaces** and subsequently releasing them, in a manner that **facilitates their proper folding and/or 4D assembly**. Pathological associations *in vivo* are promoted by the fact that the folding takes place in the presence of extremely high concentrations of other macromolecules (~300g/L, which occupy ~25% of the available volume). Consequently, unfolded proteins *in vivo* have a great tendency to form both intramolecular and intermolecular aggregates. This condition is very carefully monitored by the organism and, if it gets out of control, it may trigger an overall shock reaction called UNFOLDED PROTEIN RESPONSE (UPR, Fig.).
Many molecular chaperones are **ATPases**, requiring ATP for its function.
Heat shock proteins, Hsp, chaperonines, etc. Energy-dependent process.

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Folding into a compact conformation seems to be the main selective pressure against misfolding

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Cell's apparatus for handling of unfolded or misfolded proteins:

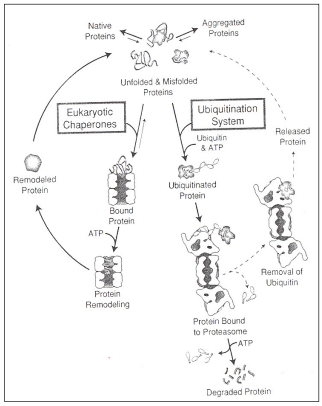
CHAPERONES and PROTEASOMES:

to catalyze removal of unfolded proteins either by

- renaturation/remodeling

or by

- degradation



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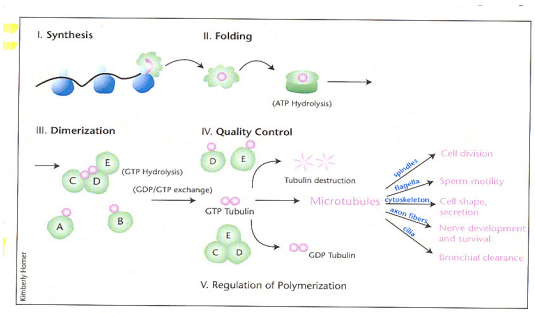

Fig. 1 Tubulin chaperones. The chaperone proteins prefoldin and chaperonin assist in the folding of newly synthesized tubulin subunits. The tubulin-specific chaperone proteins then act to dimerize α- and β-tubulin subunits, and, possibly regulate their polymerization into microtubules and degrade non-native or unneeded tubulins. Chaperone E is encoded by *TBCE*; mutations in *TBCE* give rise to HRD/Sanjad–Sakati syndrome in humans and progressive motor neuropathy in mice.

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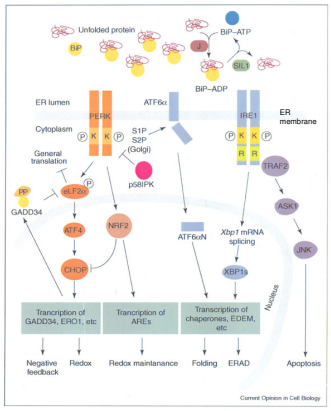
Pathology: UNFOLDED PROTEIN RESPONSE:

The capacity of the cell's unfolded protein control systems was exceeded:

GENE SIGNALLING PATHWAYS ACTIVATED BY UNFOLDED PROTEIN ACCUMULATION

= to cope with this risky condition by activating an effective response

BiP = chaperone
ERAD = endoplasmic reticulum-associated degradation



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ABNORMALITIES IN PROTEIN FOLDING AND ASSEMBLY MAY BE IMPORTANT MECHANISMS OF DISEASES – CONFORMATIONAL DISEASES

A) *Protein **degradation** due to **incorrect folding** caused **by mutation***

CYSTIC FIBROSIS - the most common recessive genetic disease in the Caucasian population (1:2000)

Defective CFTR (**c**ystic **f**ibrosis **t**ransmembrane **r**egulator): glycoprotein, 1480 aa, Cl^- membrane transporter in epithelial cells; DEFECT: deletion of Phe 508 (70%)

Clinic: highly viscous mucus that blocks the small airways in the lungs – persistent infections, death at ~30y. (Meconium ileus in newborns!).

Pathogenesis: deletion of Phe 508 results in an improper posttranslational processing of oligosaccharide chains in ER => improper conformation of CFTR => degradation by proteolytic surveillance system (proteasomes). DESPITE the fact that the MUTATED CFTR retains almost FULL biological activity!

(Treatment? Indian spice turmeric (curcumin) – mouse cell culture)

38

Uncontrolled protein aggregation due to protein's conformational change is a constant challenge in all compartments of living organisms. The failure of a peptide or protein to remain soluble may result in pathology.

So far, more than **40 human diseases, often fatal,** have been associated with **aggregation** of certain peptides/proteins, first into still soluble protein oligomers and then into insoluble **amyloid** fibrils. Fig. Amyloid fibrils form deposits extracellularly (amyloid plaques) and /or intracellularly (tangles). Figs.

- **Amyloid:** protein fibrils exhibiting a cross-β-core structure and specific staining characteristics with diagnostic dyes (thioflavins S and T, Congo red)

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B) *Protein/peptide **aggregation** into **protein oligomers** and **amyloid fibrils***
*(i) due to **mutation***
*(ii) due to **increased production** of amyloidogenic peptides*
*(iii) due to intrinsic **susceptibility to alternative folding (prions)***

"Aggregation" diseases include
- **amyloidoses** – deposition of fibrilar form of often mutant proteins in heart, liver, or kidney interferes with cellular function, resulting in cell death or organ failure (type 2 diabetes)
- **neurodegenerative diseases** - Alzheimer d., Huntington d., Parkinson d., transmissible spongiform encephalopathies (TSEs).

The exact pathogenesis is not fully understood, but it now appears that in at least neurodegenerative d., **the steps/protein forms *= soluble protein oligomers*, before their deposition into fibril plaques,** induce toxic effects.

Various types of amyloidogenic proteins are unrelated and their native forms have widely different folds but their **amyloid forms-fibrils have remarkably similar core structures:** Each consists of an array of self-assembled filaments, 0.1-10μm long and 10 nm wide, formed from limited portions of proteins/peptides in the β-sheet forms with extensive hydrophobic interactions, their β-strand chains running perpendicular to the long axis of the fibril: the **cross-β structure, cross-β spine.**

40

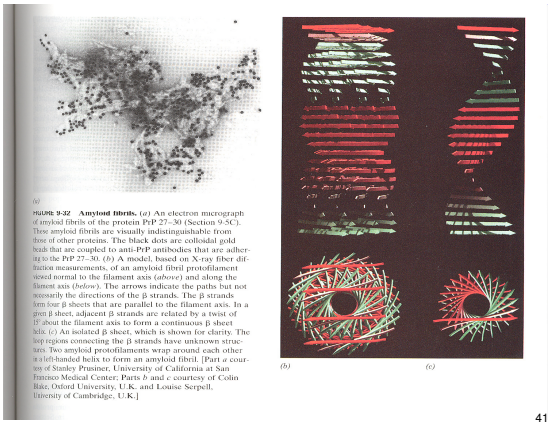

FIGURE 9-32 **Amyloid fibrils.** (*a*) An electron micrograph of amyloid fibrils of the protein PrP 27–30 (Section 9-5C). These amyloid fibrils are visually indistinguishable from those of other proteins. The black dots are colloidal gold beads that are coupled to anti-PrP antibodies that are adhering to the PrP 27–30. (*b*) A model, based on X-ray fiber diffraction measurements, of an amyloid fibril protofilament viewed normal to the filament axis (*above*) and along the filament axis (*below*). The arrows indicate the paths but not necessarily the directions of the β strands. The β strands form four β sheets that are parallel to the filament axis. In a given β sheet, adjacent β strands are related by a twist of 180° about the filament axis to form a continuous β sheet helix. (*c*) An isolated β sheet, which is shown for clarity. The loop regions connecting the β strands have unknown structures. Two amyloid protofilaments wrap around each other in a left-handed helix to form an amyloid fibril. [Part *a* courtesy of Stanley Prusiner, University of California at San Francisco Medical Center; Parts *b* and *c* courtesy of Colin Blake, Oxford University, U.K. and Louise Serpell, University of Cambridge, U.K.]

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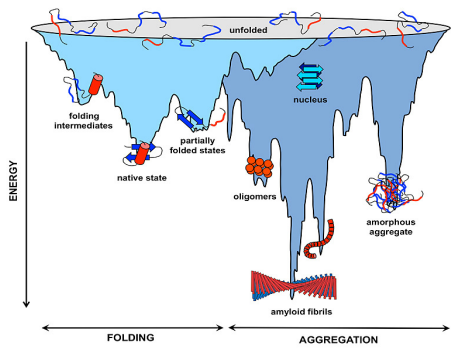
Folding funnel of proteins and their aggregated forms



42

Ad B (i): AMYLOIDOSES
Aggregation due to protein mutation

Many aggregation-prone/amyloidogenic proteins are mutant forms of normally occurring proteins. These include e.g.
lysozyme in the disease *familial visceral amyloidosis*,
transthyretin in *familial amyloid polyneuropathy*,
fibrinogen in *hereditary renal amyloidosis*,
immunoglobulin light chain AL in *primary myeloma*,
cystathion β–synthase in *homocysteinuria*, etc.

Most such diseases do not become symptomatic until the 3rd to 7th decades of life and typically progress over 5 to 15 years ending in death.

FIGURE 9-33 Superpositions of wild-type human lysozyme and its D67H mutant. Wild-type lysozyme is gray and its D76H mutant is color-ramped in rainbow order from blue at its N-terminus to red and back to blue at its C-terminus. The white arrows indicate the conformational shifts of residues 45 to 54 and 67 to 75 in the D67H mutant relative to those in the wild-type protein. The four disulfide bonds present in both the wild-type and mutant protein are shown in yellow. The positions of residues 56 and 67 are indicated. [Courtesy of Colin Blake, Oxford University, U.K., and Louise Serpell, University of Cambridge, U.K.]

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Ad B (ii): ALZHEIMER DISEASE (AD)
Aggregation due to an increased production? of an amyloidogenic peptide

Clinic: mainly elderly: 10% over 65y, 50% over 85y; progressing dementia, death after 4 – 8 years after diagnosis. The *most prevalent neurodegenerative disease.*

Pathology: brain hippocampus, cortex, entorhinal cortex (primary site of AD manifestation)
Extracellularly: amyloid plaques in the brain surrounded by dead and dying neurons. A. plaques consist of amyloid fibrils of a hydrophobic 40-42-res. long peptide called **amyloid-β protein/peptide (Aβ)**. Aβ is pathologically excised from a big precursor, 695/770-res. long transmembrane amyloid precursor protein (APP) by the action of β- and γ- secretases (instead of physiological α– and γ- secretases). Figs. APP appears to be neuroprotective.
Intracellularly: neurofibrilary tangles consisting of paired helical filaments containing the microtubule associated hyperphosphorylated protein Tau.

(Treatment: to block the secretases?)

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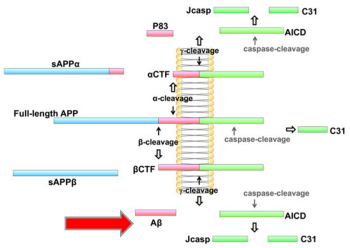
Schematic diagram of APP processing



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Pathogenesis: **neurotoxic agents in AD are most likely soluble prefibrillar aggregates/oligomers of Aβ** before their deposition in amyloid plaques (microinjection exp.- rhesus monkey).

- Aβ oligomers bind to neuronal synapses.
- Aβ oligomers stimulate Tau phosphorylation => collapse of the microtubule network.

Whereas Aβ plaques do not correlate with cognitive decay, the tau neurofibrilary tangles do. Memory deficit is usually diagnosed only after the plaques had already reached their plateau!

Aβ has a vastly different organization in oligomers than in plaques.

Enhanced expression of α-1-antichymotrypsin promotes Aβ polymerization.

Early onset of AD:
Mutants of APP - accelerated production of Aβ
Down sy - accelerated production of Aβ
apoE4 variant of apolipoprotein E (carrier of cholesterol). The main risk factor.

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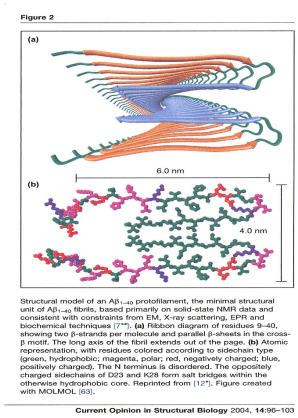

Structural model of an $A\beta_{1-40}$ protofilament, the minimal structural unit of $A\beta_{1-40}$ fibrils, based primarily on solid-state NMR data and consistent with constraints from EM, X-ray scattering, EPR and biochemical techniques [7**]. **(a)** Ribbon diagram of residues 9–40, showing two β-strands per molecule and parallel β-sheets in the cross-β motif. The long axis of the fibril extends out of the page. **(b)** Atomic representation, with residues colored according to sidechain type (green, hydrophobic; magenta, polar; red, negatively charged; blue, positively charged). The N terminus is disordered. The oppositely charged sidechains of D23 and K28 form salt bridges within the otherwise hydrophobic core. Reprinted from [12*]. Figure created with MOLMOL [63].

Current Opinion in Structural Biology 2004, 14:96–103

Green = hydrophobic residues

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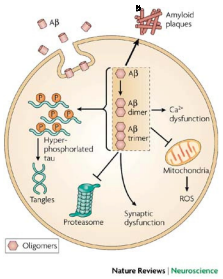

Nature Reviews | Neuroscience

Intracellular amyloid-beta in Alzheimer's disease

Amyloid-beta (Aβ), produced intracellularly or taken up from extracellular sources, has various pathological effects on cell and organelle function. Intracellular A-beta can exist as a monomeric form that further aggregates into oligomers, and it may be any of these species that mediate pathological events in vivo, particularly within a dysfunctional neuron. Evidence suggests that intracellular A-beta may contribute to pathology by facilitating tau hyperphosphorylation, disrupting proteasome and mitochondria function, and triggering calcium and synaptic dysfunction. ROS, reactive oxygen species.

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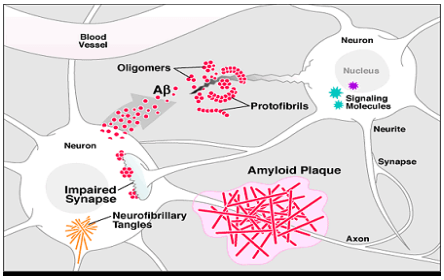
Alzheimer disease pathogenesis



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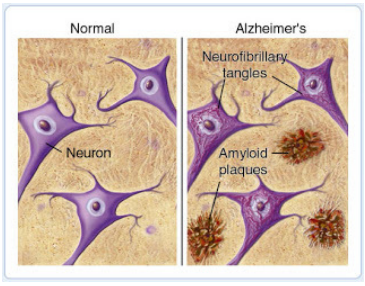
Alzheimer disease brain pathology



http://biol1020-2012-2.blogspot.cz/2012/09/d-melanogaster-in-alzheimers-disease.html

50

2013: Amyloid beta: Friend or Foe?

- L. Steinman: "Amyloid Aβ is highly therapeutic in a mouse model of *multiple sclerosis*, in models of stroke, brain trauma, optic nerve ischemia, myocardial infarction and brain trauma. Injections of **amyloid made semi-paralyzed multiple sclerosis mice walk**."
- "Low amyloid correlates with early cognitive decline?"
- S. Soriano: "Amyloid as a protective molecule against a variety of stresses? A. helps counteract the impact of cholesterol dysregulation."
- Mouse models: Fibril-forming six-amino-acid portions of several amyloid-forming proteins incl. amyloid beta appear to be working to remove dangerous chemicals involved in inflammation and immune response from the vicinity of injury.
- XXXXXX
- Three big anti-amyloid trials examining therapeutic effect of drugs = complete antibodies targeting amyloid beta = failed or were halted due to side effects.
- However: injection in mouse models of Alzheimer disease of ***antibody fragments*** consisting of active parts (trapping the etiological agent = amyloid beta oligomers) of one recombinant antibody, called bapineuzumab: 1) cleared from the mouse cerebral context the A-beta oligomers, 2) learning and memory deficits were ameliorated, 3) the levels of certain lipoproteins suspected to be the natural removers of A/beta peptide aggregates were recovered.

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Scientists reveal how beta-amyloid may cause Alzheimer's ? (2013):

- *Carla Shatz*: **Beta-amyloid Aβ binds to mouse PirB (and, the researchers proved, to its human analog, LilrB2), boosting cofilin activity and busting synapses' structural integrity.**
  Cofilin works by **breaking down actin**, a building-block protein essential to maintaining **synaptic structure**.

  "No actin, no synapse," Shatz said.

PirB: a nerve cell surface protein ordinarily situated very close to synapses. PirB is a high-affinity receptor for beta-amyloid in its "soluble cluster" form, meaning that soluble beta-amyloid clusters stick to PirB quite powerfully.
The PirB-lacking Alzheimer's mice were protected from the beta-amyloid-generating consequences: resistant to the memory breakdown and synapse loss.

Aβ also binds to RAGE (receptor for AGE) which activates inflammation processes.

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Deposition of Aβ is not limited to AD. Aβ plaques have been observed in vascular dementias, Parkinson disease with dementia, Levy body dementia as well as in the brains of aged individuals without any cognitive deficit.

Neuronal proteins such as tau, CE-B1-synuclein, and polyglutamine aggregates,
which are causally implicated in the neurodegenerative disorders Alzheimer's disease, Parkinson's disease, and Huntington's disease,respectively, can be released from donor cells and taken up by neighboring acceptor cells.

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Ad B (iii): PRION DISEASES - infectious, transmissible

origin of the protein misfolding and aggregation is its ***intrinsic susceptibility to alternative folding***

a) **Scrapie** – 1732, sheep, goats; ataxia; transmission by ingestion of the feed from the diseased sheep
b) **Creutzfeld-Jacob disease** (**CJD**), people
- **sporadic** – 1920, 85%, (1:1 million/y: 5-10 cases/y in the Czech rep.), progressive, loss of memory & skill, depression, gait stability problems, speech problems, paralysis, + 0,5-(5y)
- **familial** – 10-15%, hereditary mutation, "Oravian kuru"; Fatal familiar insomnia; Gerstmann-Sträussler-Schinkler sy
- **iatrogenic** – cornea transplantation, surgery instruments, growth hormone, gonadotropin therapy (~100 cases)

c) **Kuru** – Papua, New Guinea – people (cannibalism, brain); weakness, cerebellar ataxia, loss of coordination, trembles. Daniel Carleton Gajdusek:

1966 – **infectious disease!** (**long incubation period, death in 3–9 months**)
exp. transmission to chimpanzees
1976 Nobel Prize

d) **BSE** – 1986, bovine spongiform encephalopathy, GB, mad cow disease, >180 000; transmission through feeding cattle by meat & bone meal from scrapie sheep?
e) **A new variant of CJD** – GB, 1955, young people (+ 20 years), due to **ingestion** of (nerve) tissues from BSE cows (> 30 months old) = Zoonotic. Loss of communication.

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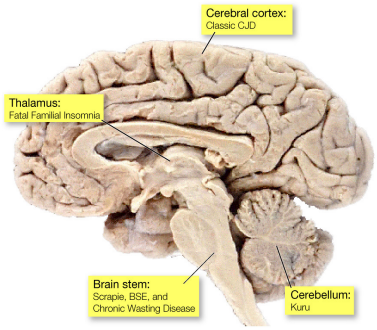

55

ALL of the diseases are fatal, (have similar symptoms), there is no cure.

Neurons develop large vacuoles that gives brain tissue a spongelike microscopic appearance:

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSE).

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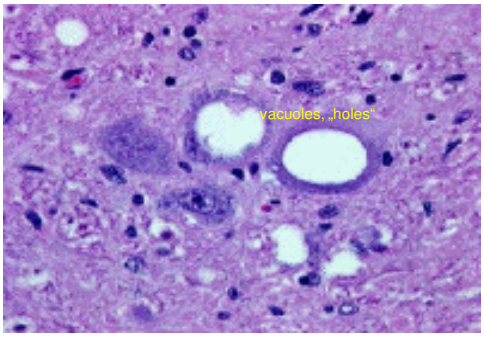
Spongiform encephalopathy



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1984: Stanley Prusiner-isolated the infectious particle of scrapie disease and called it **prion** (for *pr*oteinaceous *in*fectious particle): **PrP^{Sc}**

Nobel Prize 1997

The scrapie particle is a **new kind of infectious agent, one that does not need a nucleic acid to replicate. It is a single species of**

MISFOLDED BODY PROTEIN PrP^{c} !

NEW PARADIGM IN MOLECULAR BIOLOGY!

(Now we know that we can die from an infection by a misfolded protein only, no genes are required !?)

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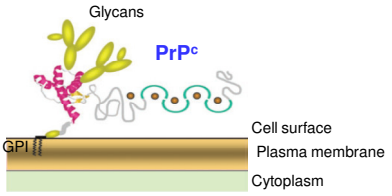

Synthesized glycoprotein PrP^{c} is attached to the plasma membrane through the glycosylphosphatidylinositol (GPI) anchor

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- **PrP^{C}** is a normal protein (prion protein) found on the membranes of cells. It is an expression product of a *prp^{c}* gene. PrP^{C} has 208/209 amino acids (in humans) -after posttranslational modification (from 253 aa), one disulfide bond, a molecular mass of 35–36 kDa and a mainly alpha-helical structure. PrP^{C} is readily digested by proteases (incl.proteinase K) and can be degraded into amino acids.
  It can also be liberated from the cell surface *in vitro* by the enzyme phospho-inositide phospholipase C (PI-PLC), which cleaves the glycophosphatidylinositol (GPI) glycolipid anchor.
- PrP^{C} function is not fully known: roles in cell-cell adhesion and intracellular signaling *in vivo?*, cell-cell communication in the brain?, biological clock?, memory?
- PrP^{C} is highly conserved through mammals and expressed in all vertebrates as well as invertebrates.

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- **Prions = PrP^{Sc}** are the infectious isoform of the prion protein PrP^{C}. PrP^{Sc} are not considered living organisms but are misfolded protein molecules which may propagate by transmitting a misfolded protein state.
- PrP^{Sc} has a different secondary and tertiary structure from PrP^{C} but identical primary sequence. The exact 3D structure of PrP^{Sc} is not known but it has a higher proportion of β-sheet structure in place of the normal α-helix structure (43%:30%) than PrP^{C} (3%:43%). Fig.
- Aggregations of these abnormal isoforms form highly structured amyloid fibers, which accumulate to form plaques. These aggregates are considered to be the cause of cell damage.
- Prions are species specific (have different strains) with different stable conformations.

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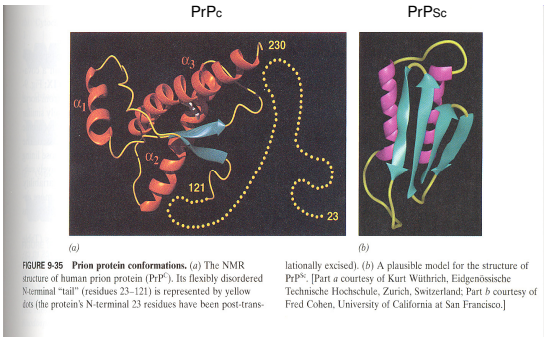
Two stable conformational variants of the prion protein

PrPc PrPSc



(a) (b)

FIGURE 9-35 Prion protein conformations. (*a*) The NMR structure of human prion protein (PrP^{C}). Its flexibly disordered N-terminal "tail" (residues 23–121) is represented by yellow dots (the protein's N-terminal 23 residues have been post-translationally excised). (*b*) A plausible model for the structure of PrP^{Sc}. [Part *a* courtesy of Kurt Wüthrich, Eidgenössische Technische Hochschule, Zurich, Switzerland; Part *b* courtesy of Fred Cohen, University of California at San Francisco.]

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Prions PrP^{Sc}

are (due to different space configuration):

resistant to

proteases (proteinase K, **body** proteases) – only partial hydrolysis, heat, boiling, UV radiation, H_2O_2, I_2, ethanol, lysol, peracetic acid and formalin treatments.

degraded by

concentrated bases (NaOH, KOH), NaClO (SAVO), water steam (for 20 min., 3 bar, 135°C)

keratinase

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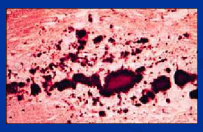
Prion detection

- Reaction of prion-containing tissue digested with proteinase (K) with an antibody against PrP^{Sc} (immuno-elfo)
- Histological examination of the brain
- Biological tests on mice, (+genetically modified mice). Syrian hamster. PMCA



Tests: with blood, urine, brain, meat and lymphatic tissues

Prion Propagation

Current research suggests that the primary method of infection in animals is **through ingestion**. It is thought that prions may be deposited in the environment through the remains of dead animals and via urine, saliva, and other body fluids. They may then linger in the soil by binding to clay and other minerals.

Movement of prion proteins from ER (the site of their origin), via Golgi and cytosol to the cell membrane is mediated by vesicular transport. This also applies to infectious prion particles or, it can be carried out by endocytic vesicles for prions formed in the cell membrane. Transport of prions from the cell is apparently carried out by an extracellular form of the vesicles called exosomes. Viruses employ the same system of vesicles to reconstitute their particles and to get out from the cell and infect new cells. Viruses are in exosomes protected against the attacks of immune system. It was shown that they can also accept prions and further promote their propagation from cell to cell.

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HYPOTHETICAL PATHOGENESIS OF PRION DISEASES – PRION HYPOTHESIS

1) **Prion protein PrP^c** converts into **infection prion PrP^{Sc}** due to spontaneous? change of the PrP^c conformation. (Under normal conditions the spontaneously converted forms of PrP^c are apparently eliminated by the protein folding quality control system of the cell).

2) **The PrP^c** --> **PrP^{Sc}** conversion becomes highly accelerated by exogenous **PrP^{Sc}** ("chain reaction", Figs). **PrP^{Sc}** is a **stable conformational variant** of normal **PrP^c** (Fig.).

3) To develop into a disease the expression of the corresponding normal **PrP^c prion protein is required.** Animals that do not express the normal form of the prion protein can neither develop nor transmit the disease.

4) All known prions induce the formation of an amyloid fold, in which the protein polymerises into an aggregate consisting of tightly packed beta sheets. Amyloid aggregates are fibrils, growing at their ends, and replicating when breakage causes two growing ends to become four growing ends. This altered structure is extremely stable and accumulates in infected tissue, causing tissue damage and cell death.

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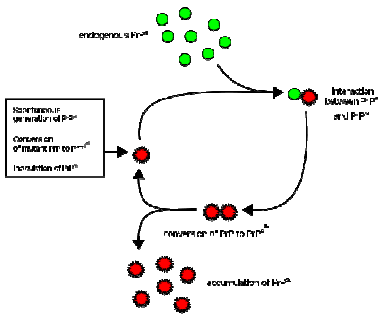
Heterodimer model of prion propagation



Wikipedia

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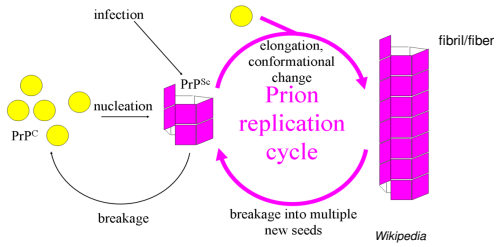
Fibril model of prion propagation.



Wikipedia

The end of each fiber acts as a template onto which free protein molecules may attach, allowing the fiber to grow. Under most circumstances, *only PrP^{c} molecules with an identical amino acid sequence to the infectious PrP^{Sc} are incorporated* into the growing fiber. However, rare cross-species transmission is also possible (see the text).

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The most infectious prion appears to be a particle composed of 14-24 PrP monomers (~300-600 kDa). Brain tissue from CJD victims contains a protein that cross-react with Ab raised against PrP^{Sc} .

The inherited prion diseases can be diagnosed by finding mutation in the prion protein gene. No such pathogenic mutations are present in sporadic and acquired prion disease.
A common PrP polymorphism at residue 129, where either methionine or valine can be encoded, is a key determinant of genetic susceptibility to acquired and sporadic prion diseases, the large majority of which occur in homozygous individuals.

Typically, no signs of inflammation or fever are present in CJD.

GPI anchor-less form of the infectious prion (as a special *PrP^{Sc}* strain) is typical for the Gerstmann-Sträussler-Schinkler sy. Brains of genetically modified mouse models of this disease are free of vacuoles (no sponge-like appearance) but display a picture of a typical cerebral amyloid angiopathy, similar to AD brain situation. The mice further suffer from clotting of lymphatic veins by freely floating GPI-free prions.

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Treatment?

- **Heterologous PrP molecules**, which differed by as little as one residue, **interfere** with the generation of PrP^{Sc} in scrapie-infected mouse cells (Priola et al. 1994)
  => interaction between dissimilar PrP^{Sc} and PrP^{c} molecules (= have different aa sequence) might slow the aggregation and accumulation of PrP^{Sc} by interfering with the interaction of similar PrP monomers.
- To design a molecule that binds to prion protein and stabilizes its normal shape, thereby preventing propagation of the disease.

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Amyloid Diseases		Prion Diseases		Aggregation Diseases	
Disease	Protein	Disease	Protein	Disease	Protein
Alzheimer's	Aβ	$[Psi^+]_{yeast}$	Sup35	Huntington	Huntingtin
Alzheimer's and tauopathies	Tau	[Ure2]	Ure3	Parkinson's	α-synuclein
Diabetes II	Amylin	CJD	PrP	ALS (Lou Gehrig's)	Superoxide dismutase
Injection amyloidosis	Insulin	BSE (mad cow)	PrP		
Dialysis amyloidosis	β2-micro-globulin				
Senile amyloidosis	Trans-thyretin				
Hereditary cystatin C amyloid angiopathy	Human Cystatin C				

Repeats of Gln

Table 1. Amyloid, prion and aggregation diseases. The table lists some of the amyloid-associated diseases and the protein that aberrantly aggregates in each of them. The amyloid deposits and aggregates possess some or all of the following properties: long, unbranched fibrils, the ability to bind the dyes Congo Red and Thioflavin T and the cross-β diffraction pattern.

Alpha-synuclein (A.S.) can exist in two different forms, one form can serve as a template to damage not only normal A.S. to form fibrils and clumps but also influence the development of other pathologies, e.g. tau tangles (2013). (Treatment: manitol?)

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SUMMARY

Prions **PrP^{Sc}** are infectious, species specific strains of proteins, that are responsible for a number of fatal mammalian diseases. They are highly protease-resistant and thus easily transmissible by ingestion. **PrP^{Sc}** are toxic to neurons and as such causative agents of transmissible spongiform encephalopathies (TSEs).
TSEs have been described in a number of species such as mink (TME), cat (FSE), elk (CWD) and of course sheep (scrapie), cattle (BSE) and humans (CJD).
Normally, interspecies transmission of different **PrP^{Sc} strains** doesn't induce the disease except the sheep-cattle (BSE) and recent cattle-human (new variant CJD) transmission events.
To develop into a disease the spontaneously generated/ingested/inoculated **PrP^{Sc}** molecules must increase in number. They propagate themselves by converting cellular **PrP^{c}** into **PrP^{Sc}**. The conversion mechanism has not been elucidated.
Infectious **PrP^{Sc}** molecules originate from native, naturally occurring non-infectious cellular protein **PrP^{c}** by a **spontaneous change in its conformation or induced change** by an introduced **PrP^{Sc}**. Clearly, infectious **PrP^{Sc}** **is a misfolded β-sheet-rich form of** **PrP^{c}**. This conformational conversion of some regions of **PrP^{c}** gives the protein a **fibrillogenic/amyloidogenic character leading to its aggregation into insoluble plaques**. It appears that just the plaques are the toxic agents causing the pathology. **PrP^{Sc}** can be transmitted from cell to cell.

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SUMMARY (cont.)
Genetically well-defined prion proteins were also found **in yeast**. Critical for establishment and propagation of yeast prion aggregates are the prion-forming domains (PrD), which are rich in glutamines (Q) and asparagines (N). *In vitro*, the individual PrDs are able to spontaneously undergo conformational rearrangement (**"chameleon" regions**) in the absence of any other proteins or nucleic acids, to generate highly stable amyloid fibrils. One such fibril forming motif in the N-terminal domain of the best documented yeast prion, Sup 35 (translation termination factor) is a seven residue peptide, GNNQQNY, (G=Gly; Q=Gln; Y=Tyr). It forms amyloid-like fibrils and also closely related microcrystals. The X-ray diffraction analysis of the microcrystals allowed to determine the first fully objective atomic structure of the cross-β spine. The structure consists of the peptide molecules, each an extended strand stacking on each other to form parallel, in register sheets. The strands within the sheet run perpendicular to the long axis of the microcrystals (fibrils). Each strand hydrogen bonds with the strands above and below it in its sheet, using both main chain and side-chain hydrogen bonds. A pair of such sheets mates across interfaces formed by N- and Q- side-chains to form a steric zipper with strong van der Waals contacts and, less firmly, at Y residue by bridging water molecules (Fig.). In the Aβ model protein (from AD) the strong interaction at the sheet interfaces is provided by hydrophobic amino acid residues (Fig.). The basic building block of the fibril is a pair of β-sheets. Yeast's prions do not appear to cause disease in their hosts. Investigations of the yeast prion system suggest that **molecular chaperones** may be involved in prion propagation.

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In conclusion:

C. Dobson: "Peptides or proteins convert under some conditions from their soluble forms into highly ordered fibrilar aggregates. The ability to form the amyloid structures is an inherent or generic property of polypeptide chain although the propensity to do so varies dramatically. Such transitions can give rise to pathological conditions ranging from neurodegenerative disorders to systematic amyloidosis".

"Living organisms can take advantage of this ability to generate novel and diverse biological functions "(e.g. a domain of the human Pmel17 protein: it forms, inside melanosomes, fibrous striations upon which melanin granules form; yeast's Sup 35 enables the survival under changing conditions, simply by protein conformational change = the quick adaptation-epigenetic mechanism, before change in DNA).

"In light of the fact that cells have evolved protein folding quality control systems (chaperons and proteasomes), how prions managed to survive and replicate *in vivo* is puzzling".

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