

# **CHAPTER 4**

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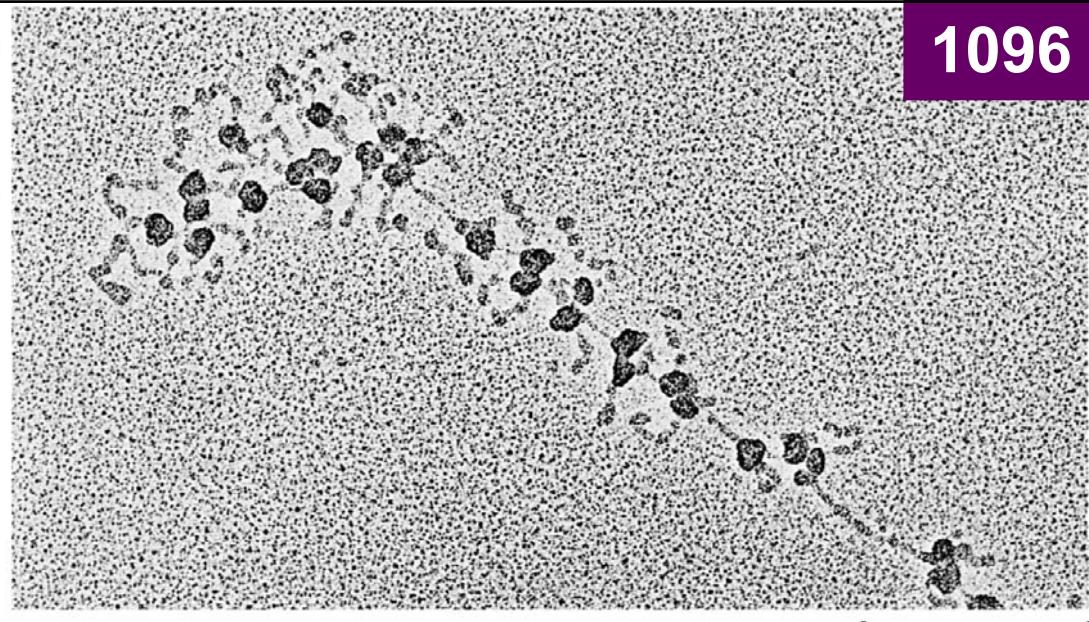
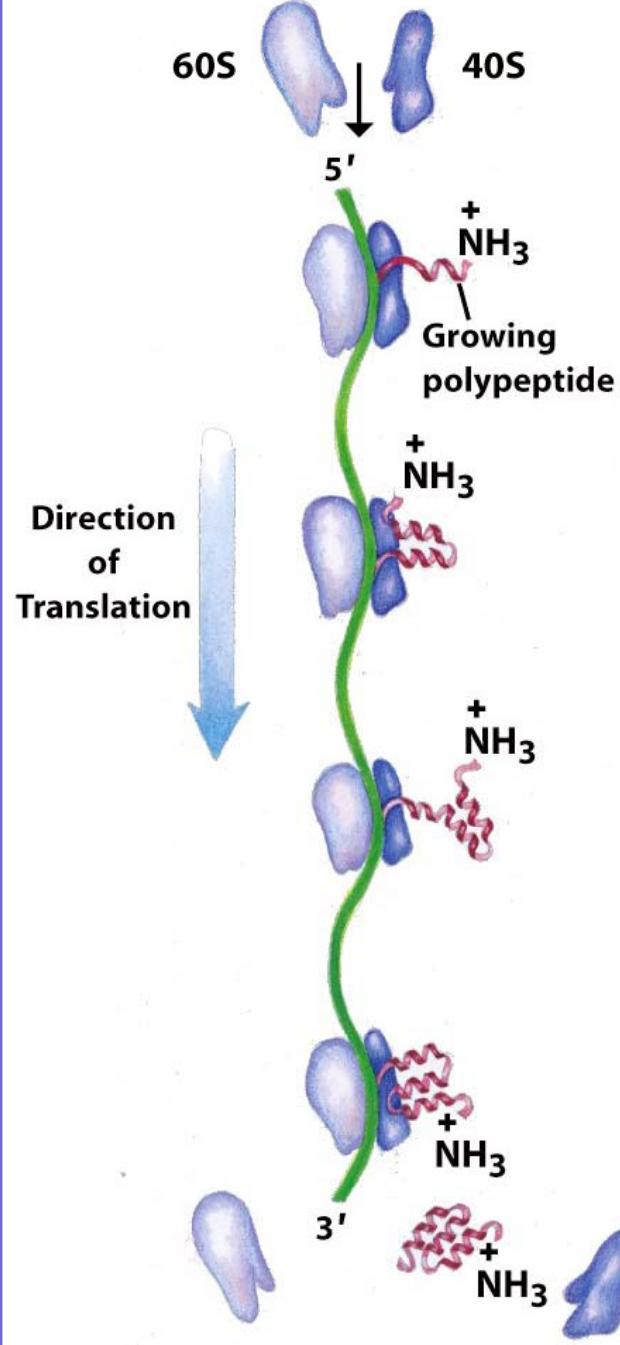
# **THE THREE- DIMENSIONAL STRUCTURE OF PROTEINS**

# **PROTEIN DENATURATION AND FOLDING**

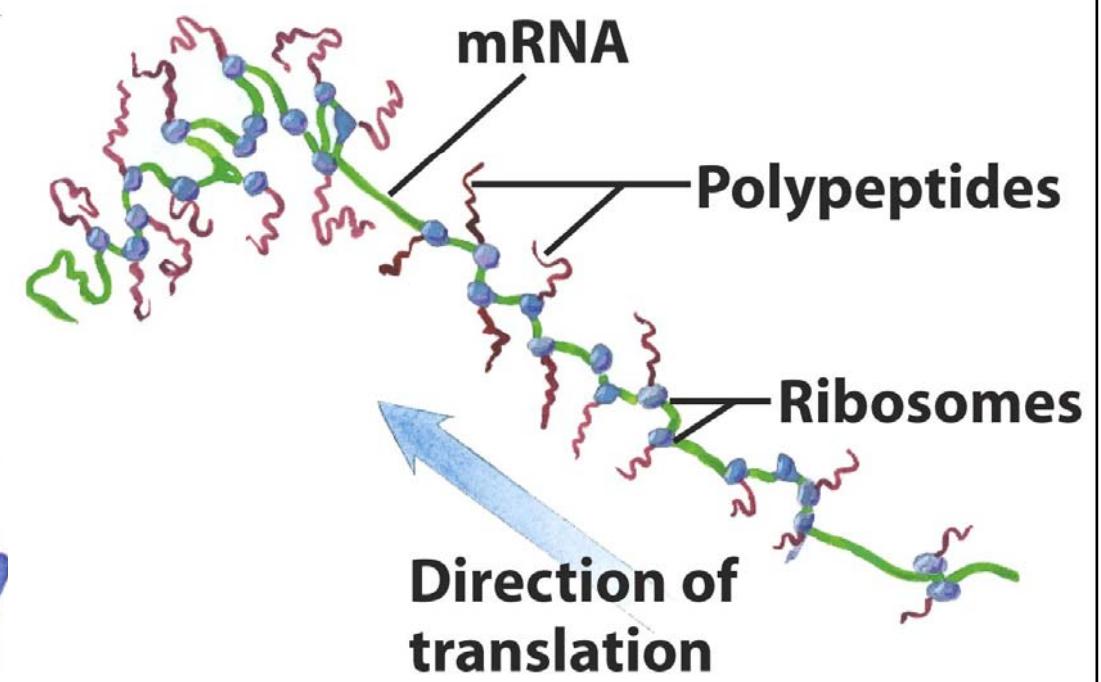


# Ribosomes synthesizing a polypeptide from N to C terminus

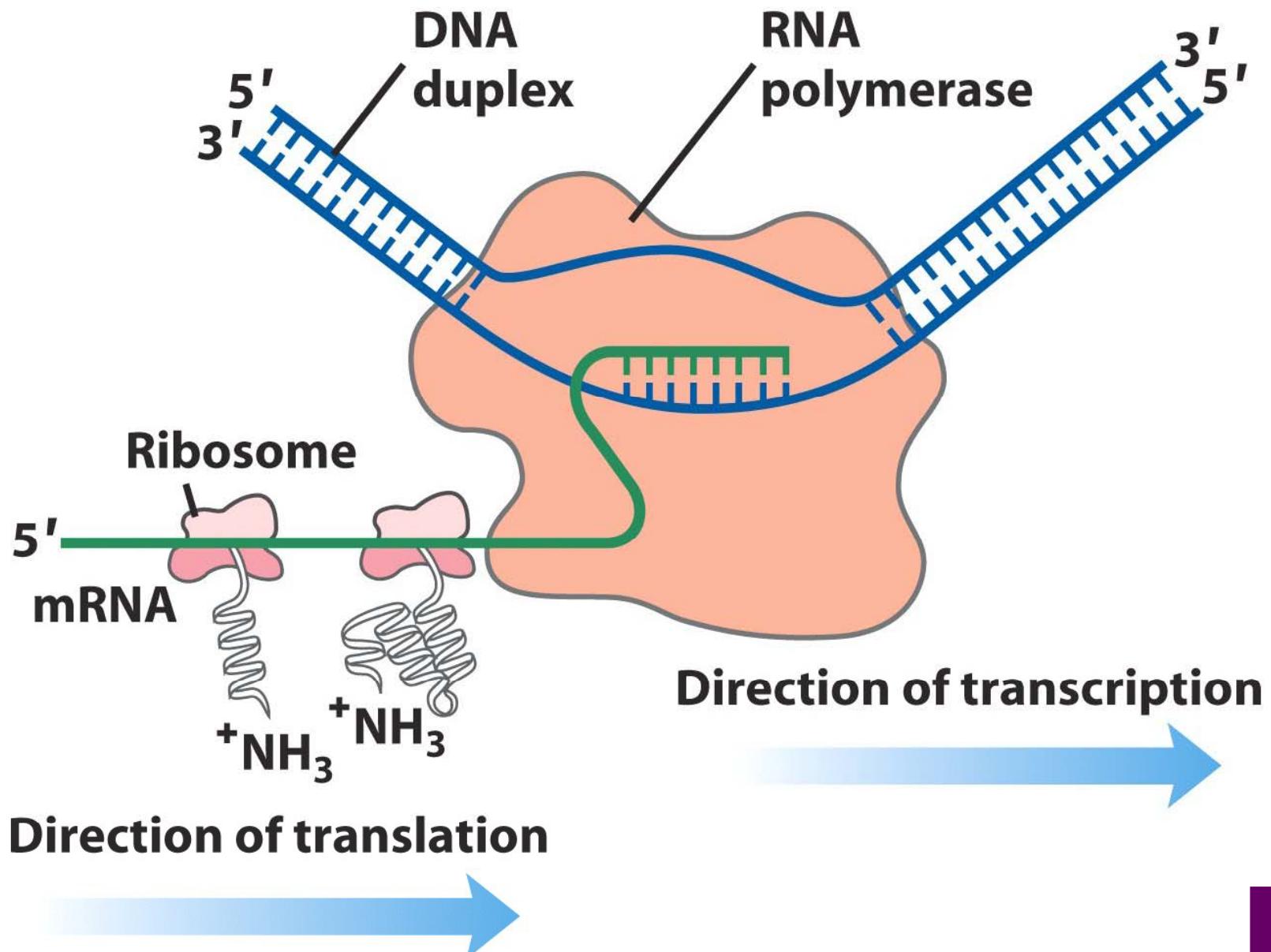
Incoming ribosomal subunits



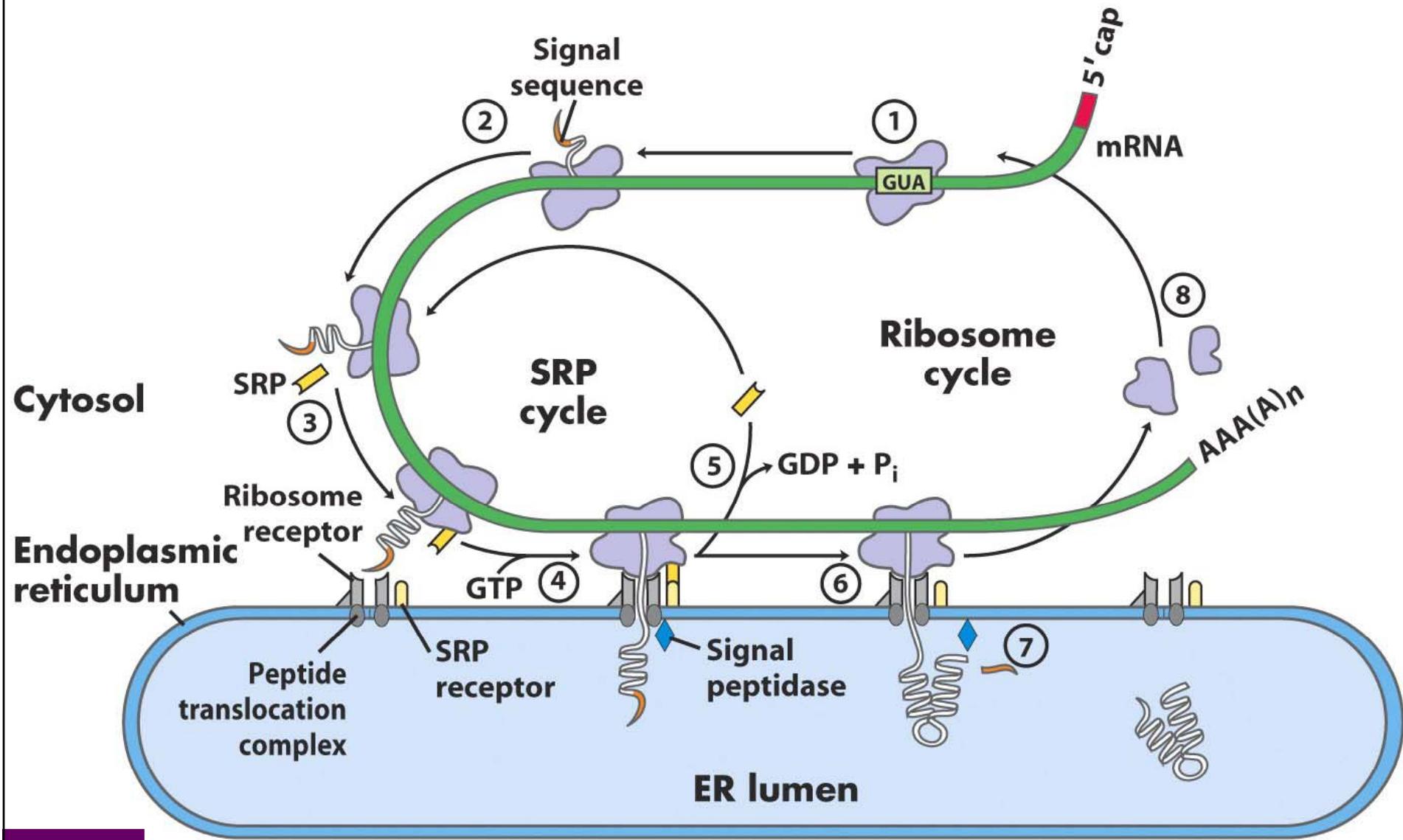
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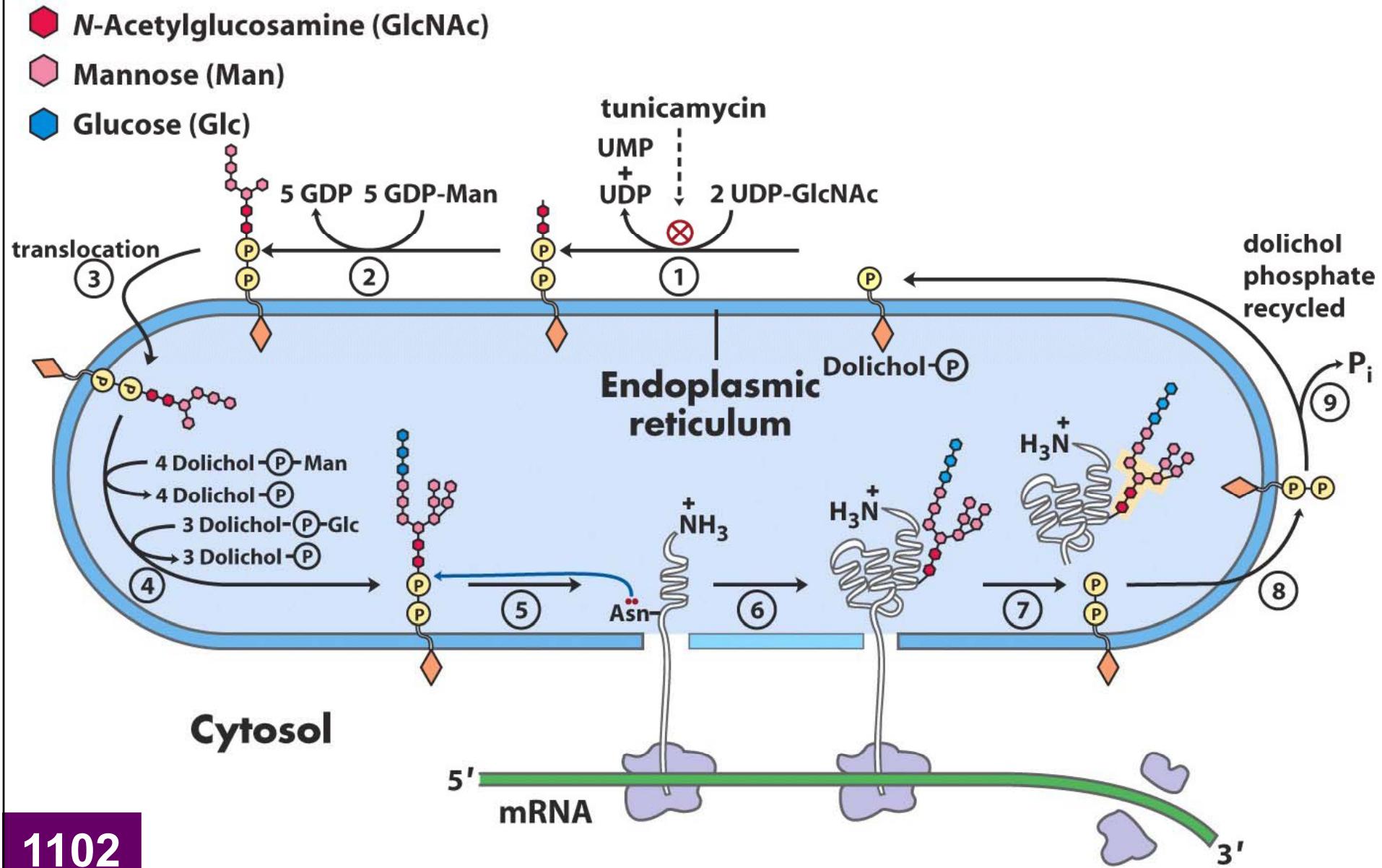
# Coupling of transcription and translation in bacteria



# Directing eukaryotic proteins with the appropriate signals to the ER



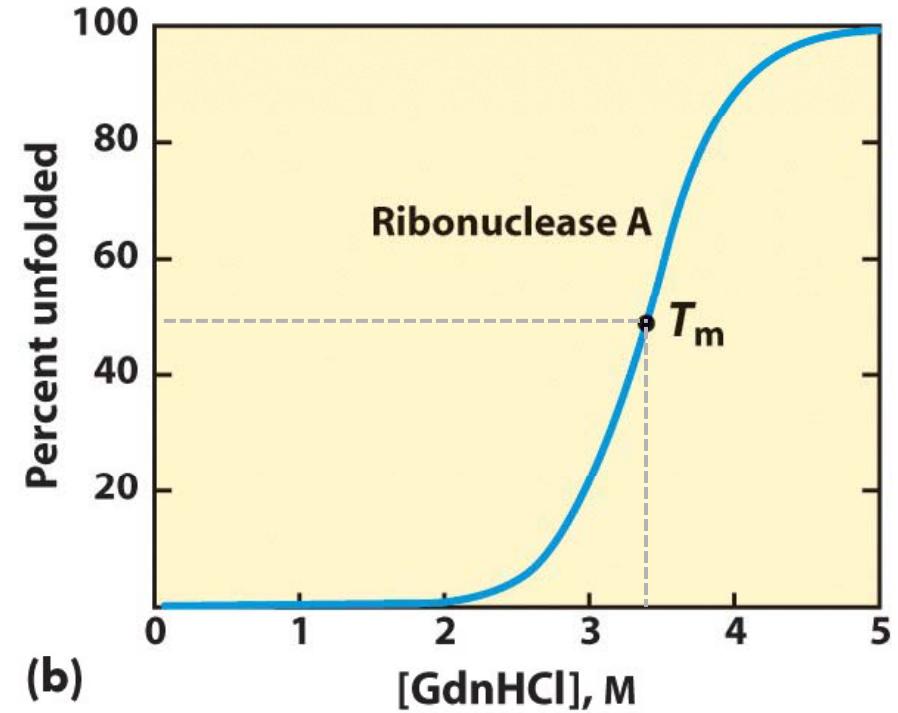
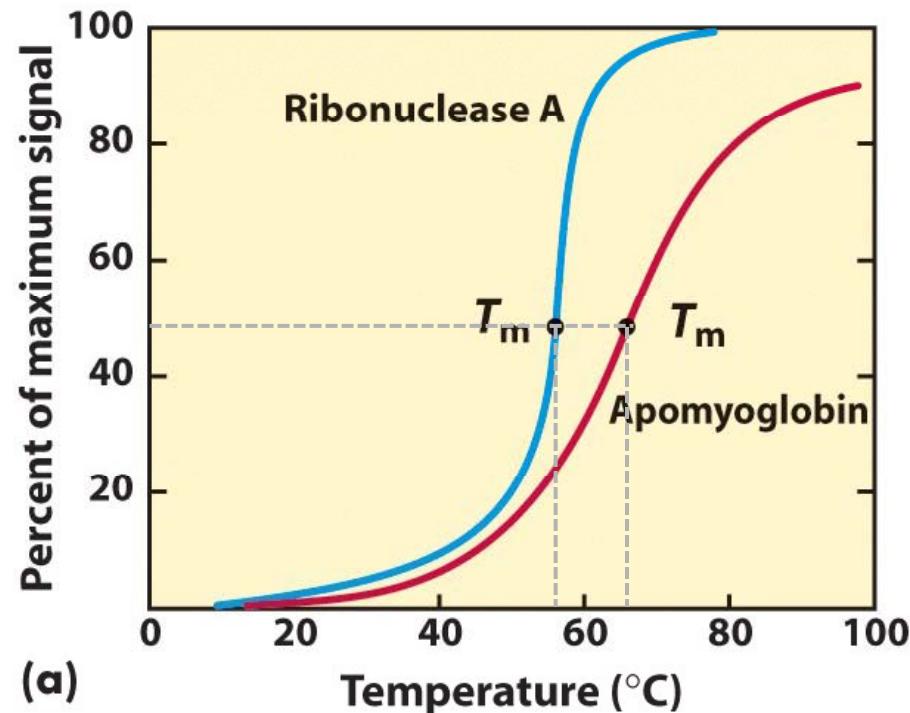
# Synthesis of the core oligosaccharide of glycoproteins in the ER



# Protein denaturation

If a solution containing a protein is heated, it will reach a temperature at which properties such as viscosity or the absorption of ultraviolet (UV) light will change abruptly. This temperature is called the melting temperature of the protein (because the measurement is analogous to that made for the melting of a solid). The melting temperature varies for different proteins.

$T_m$ : The mid-point of the temperature range over which denaturation occurs



A loss of three-dimensional structure sufficient to cause loss of function is called **denaturation**

- Apomyoglobin: myoglobin without the heme prosthetic group

# Protein denaturation method

No covalent bonds in the polypeptide chain are broken

- **Heating**

Unfolding is a cooperative process: loss of structure in one part of the protein destabilizes other parts

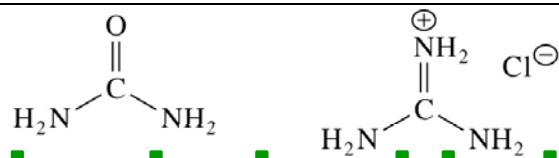
- **Extremes of pH**

Altering the net charge, causing electrostatic repulsion, and disrupting some H-bonds

- **Miscible organic solvents such as alcohol or acetone**

Disrupting the hydrophobic interactions that make up the stable core of globular proteins

- **Urea, guanidine hydrochloride**



Disrupting the hydrophobic interactions that make up the stable core of globular proteins

- **Detergent**

Disrupting the hydrophobic interactions that make up the stable core of globular proteins

# Denaturation and renaturation of ribonuclease A

Demonstrated by Anfinsen in 1950s

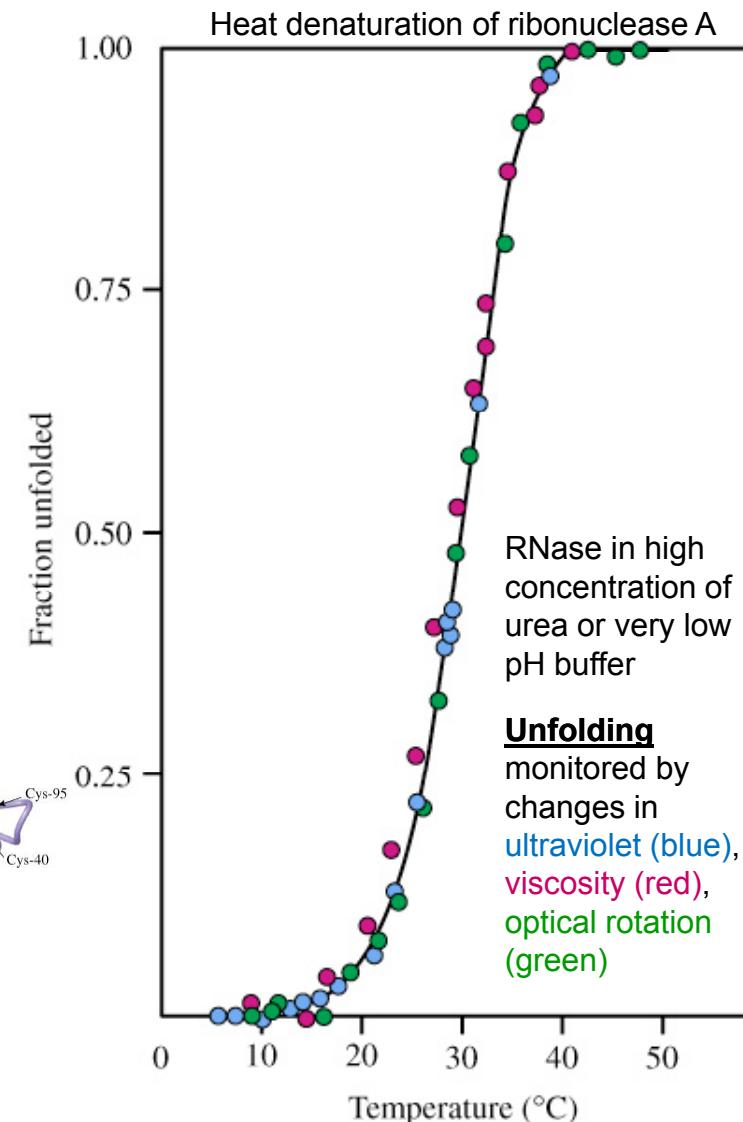
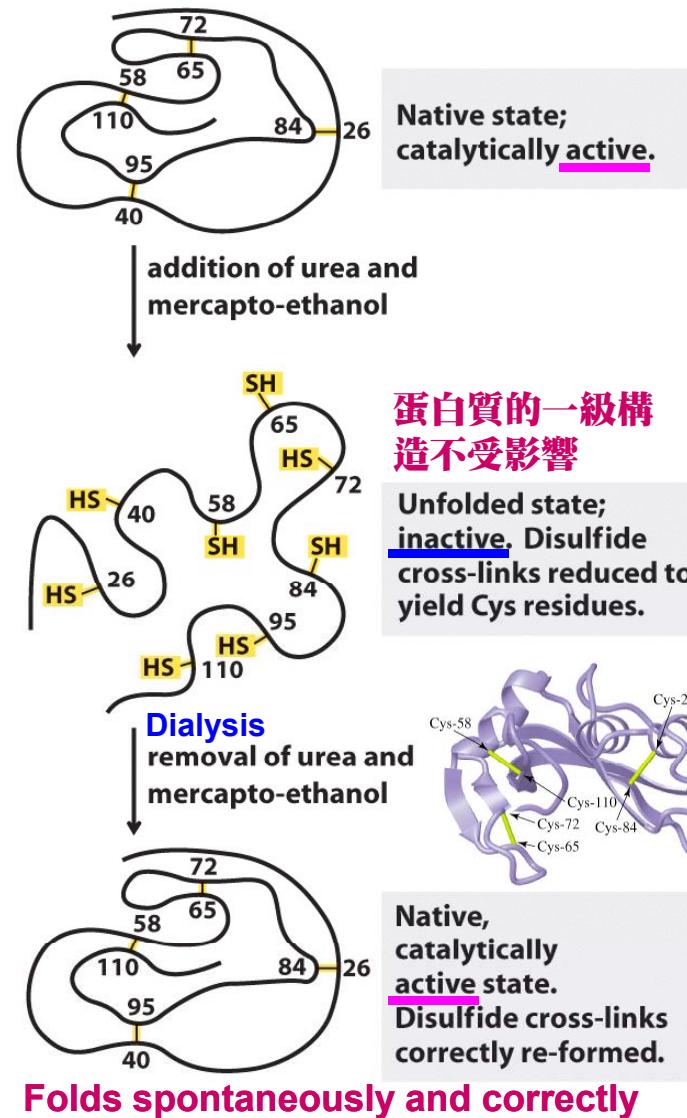
提出論點：

- 蛋白質的一級構造決定蛋白質特定的立體構形
- 蛋白質的功能與其特有的構形有關

事實上

雖然蛋白質可以自行折疊為原態的構形，但是很多的蛋白質還是需要其它的協助才可以完成。能夠自行折疊的多為小分子且本身構形穩定的蛋白質，如RNase A。

*The amino acid sequence of a polypeptide chain contains all the information required to fold the chain into its “native”, three-dimensional structure*





Christian B. Anfinsen



Stanford Moore



William H. Stein

The Nobel Prize in Chemistry 1972 was divided, one half awarded to Christian B. Anfinsen "for his work on ribonuclease, especially concerning the connection between the amino acid sequence and the biologically active conformation", the other half jointly to Stanford Moore and William H. Stein "for their contribution to the understanding of the connection between chemical structure and catalytic activity of the active centre of the ribonuclease molecule".

*The amino acid sequence of a polypeptide chain contains all the information required to fold the chain into its “native”, three-dimensional structure*





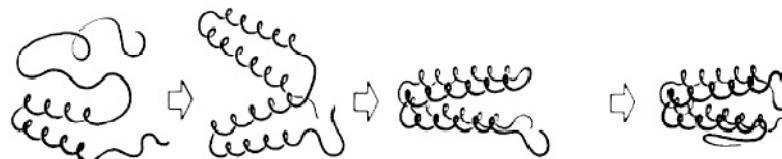
Christian B. Anfinsen at desk in Copenhagen, Denmark

Christian B. Anfinsen, 1916-1995

# The folding pathway of a large polypeptide chain

## Model 1: The folding process is hierarchical

- Local secondary structures form first.
- Assembly of local structures with two secondary structures to form motifs.
- The process continues until complete domains form and then the entire polypeptide is folded.

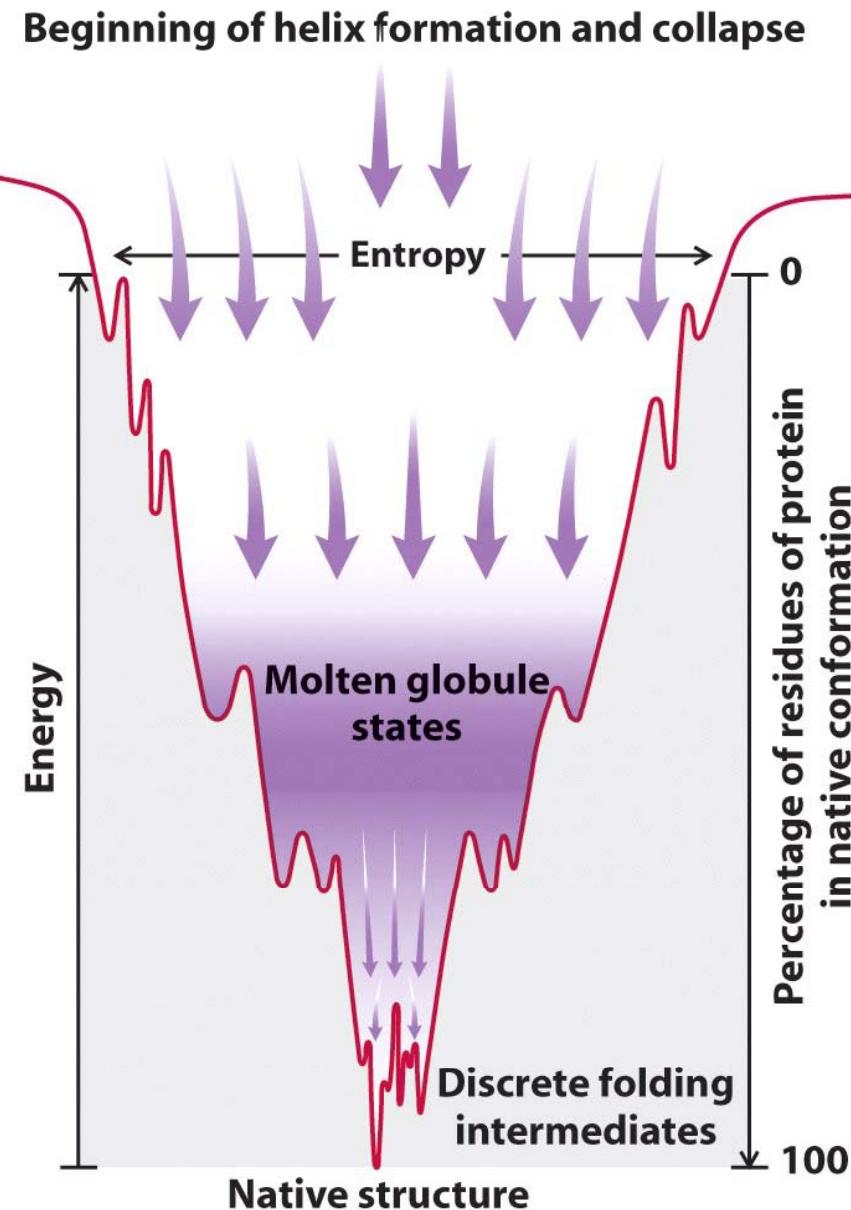


## Model 2: Hydrophobic collapse and molten globule model

- Folding is initiated by a spontaneous collapse of polypeptide into a compact state, mediated by hydrophobic interactions among nonpolar residues.
- posits that hydrophobic collapse is relatively early event in the folding pathway, occurring before the formation of many secondary structures.
- The collapsed intermediate is often referred to as a molten globule and corresponds to a partially folded state whose energy is lower than that of the denatured state but higher than that of the native state.

# Hydrophobic Collapse Model 疏水摺疊模型

- It was first found in cytochrome c, which conserves a native-like secondary structure content but without the tightly packed protein interior, under low pH and high salt concentration.
- Molten globule states are “thermodynamic states” or “partially folded states” clearly different both from the native and the denatured states.
- Molten globule states suggest the existence of the intermediate states during polypeptide folding



# Molten-globule state

We would here like to present supporting evidence for the existence of a new type of structural state of the globular protein, which has first been proposed in [3]. In this state the polypeptide chain is supposed to be compactly packed just as in the native state, while intramolecular motions of atoms are extensively released. The state may be called the 'molten-globule state' (the name was contrived during the discussion of Drs O.B. Ptitsyn and C. Crane-Robinson at the International Symposium on Peptides, Polypeptides and Proteins at Galzignano, Padova, Italy, June 20–26, 1982). We describe experimental evidence which proves the existence of this state in cytochrome c under the acid perturbation, present a diagram, and discuss

## 'Molten-globule state': a compact form of globular proteins with mobile side-chains

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Abstract not received

<i>Cytochrome c</i>	<i>Protein denaturation</i>	<i>Compact denaturation</i>	<i>Side chain mobility of protein</i>
	<i>NMR spectroscopy</i>	<i>Viscosity</i>	

### 1. INTRODUCTION

The polypeptide chain which composes a globular protein molecule varies its tertiary structure under different solvent conditions. Among these structural states, the native state and the fully denatured state have been well investigated. In a native state the polypeptide chain folds to a uniquely defined rigid and compact structure, while in a fully denatured state it unfolds into a flexible, swollen, and randomly coiled chain which has very little intramolecular contact and a high degree of internal freedom of motion [1,2].

We would here like to present supporting evidence for the existence of a new type of structural state of the globular protein, which has first been proposed in [3]. In this state the polypeptide chain is supposed to be compactly packed just as in the native state, while intramolecular motions of atoms are extensively released. The state may be called the 'molten-globule state' (the name was contrived during the discussion of Drs O.B. Ptitsyn and C. Crane-Robinson at the International Symposium on Peptides, Polypeptides and Proteins at Galzignano, Padova, Italy, June 20–26, 1982). We describe experimental evidence which proves the existence of this state in cytochrome c under the acid perturbation, present a diagram, and discuss

its physical and biochemical significance.

### 2. MATERIALS AND METHODS

Horse cytochrome c (Type VI, Sigma) was used throughout this work. For viscosity measurement, cytochrome c was dissolved in an aqueous solution without purification. For absorbance and NMR measurements cytochrome c was oxidized with ferricyanide. Ferricyanide was then removed using an ion-exchange column (Shatman CM 52) or Amicon membrane filter (UM 10).

The viscosity of the protein solution and the solvent ( $\eta_{\text{solv}}$  and  $\eta_{\text{red}}$ ) were measured with a rotary viscometer [4]. The reduced viscosity was calculated as  $\eta_{\text{red}} = (\eta_{\text{solv}} - \eta_{\text{red}})/c$ , where the typical protein concentration used ( $c$ ) was 2.0 g/100 ml.

$^1\text{H}$ -NMR spectra were measured with a Bruker 360 MHz FT NMR spectrometer. Oxidized and lyophilized ferricytochrome c was dissolved into 99.7%  $\text{D}_2\text{O}$ . Indicated values of  $pD$  are direct readings of the pH meter.

The diffusion coefficient,  $D$ , of the protein molecule was measured with the quasielastic light scattering method [5]. The correlation function of scattered light intensity of 0.5% cytochrome c solution was of single exponential form, which shows the monodisperse nature of the protein solu-

# Protein Folding and Stability

- Some proteins can fold spontaneously to the low-energy conformation.
- Proteins are thought to fold cooperatively. The first few interactions assist subsequent alignment and folding
- During folding, nonpolar side chains associate with each other causing a polypeptide chain to collapse to a molten globule.
- The intermediate “molten globule” forms with elements of secondary structure.
- The backbone is rearranged to achieve a stable native conformation.
- Folded proteins occupy a low-energy well that makes the native structure most stable
- The driving force for protein folding is the large increase in entropy from water released to bulk solvent.
- Folding is extremely rapid, the native conformation is generally reached < 1 second.

# Some proteins undergo assisted folding

## **Molecular chaperone 分子伴護蛋白，如 Hsp70, Hsp40**

- 結合疏水性及unfolded區域，或newly synthesized but not yet folded peptide chains，以保護其不被降解或防止不當的聚集與沈澱。
- 維持某些蛋白質於unfolded型態，以利於穿膜運輸
- 加速蛋白質四級結構的形成

## **Chaperonin (Cpn) 伴護蛋白，如 Hsp60, Hsp10, Cpn60, Cpn10**

- 直接促進蛋白質的摺疊

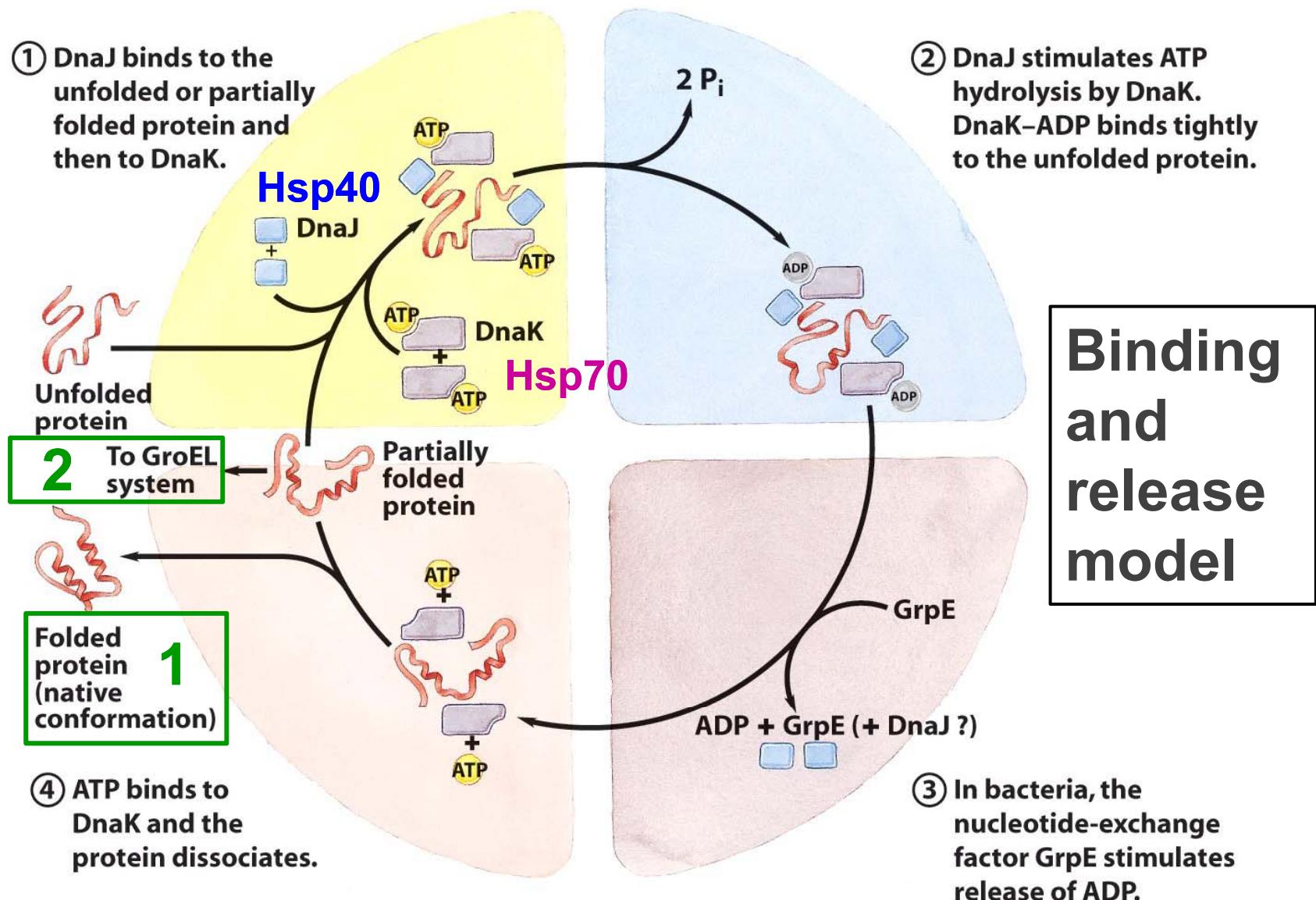
## **Protein disulfide isomerase (PDI)**

- Catalyzes the interchange, or shuffling, of disulfide bonds until the bonds of native conformation are formed.
- Catalyzes the elimination of folding intermediates with inappropriate disulfide crosslinks.

## **Peptide prolyl cis-trans isomerase (PPI)**

- Interconversion of cis and trans isomers of Pro residue peptide bonds can be a slow step in the folding of proteins that contain some Pro peptide bonds in the cis conformation.

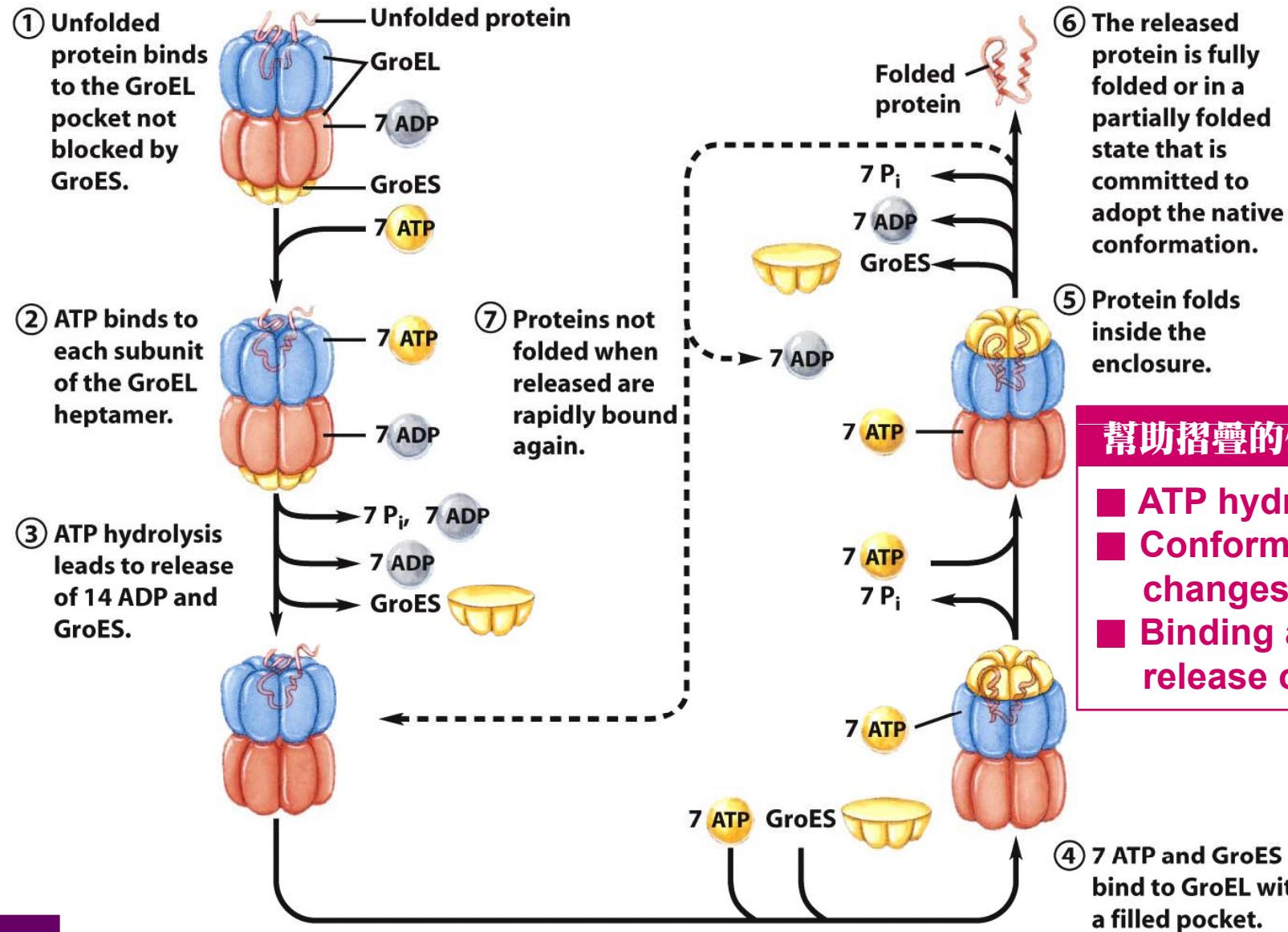
# Chaperones in protein folding

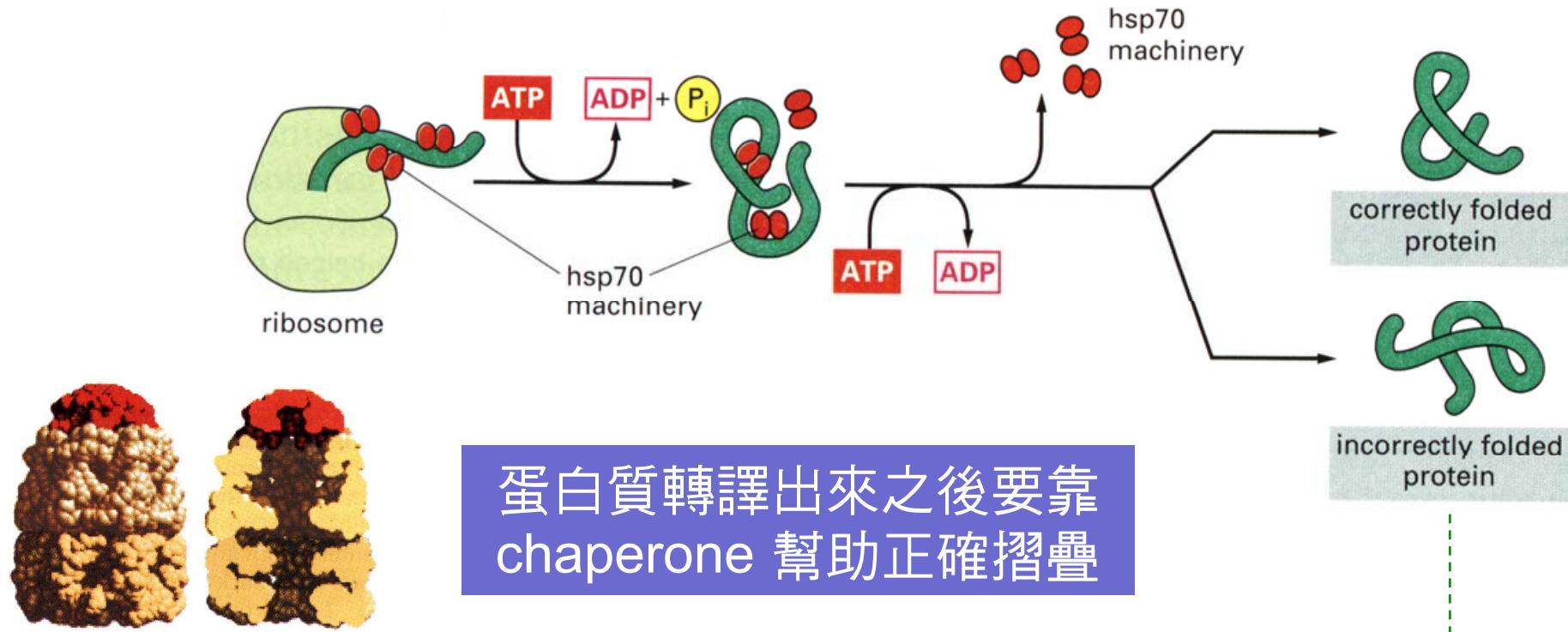


**DnaK and DnaJ in *E. coli*:** first identified as proteins required for *in vitro* replication of certain viral **DNA** molecules

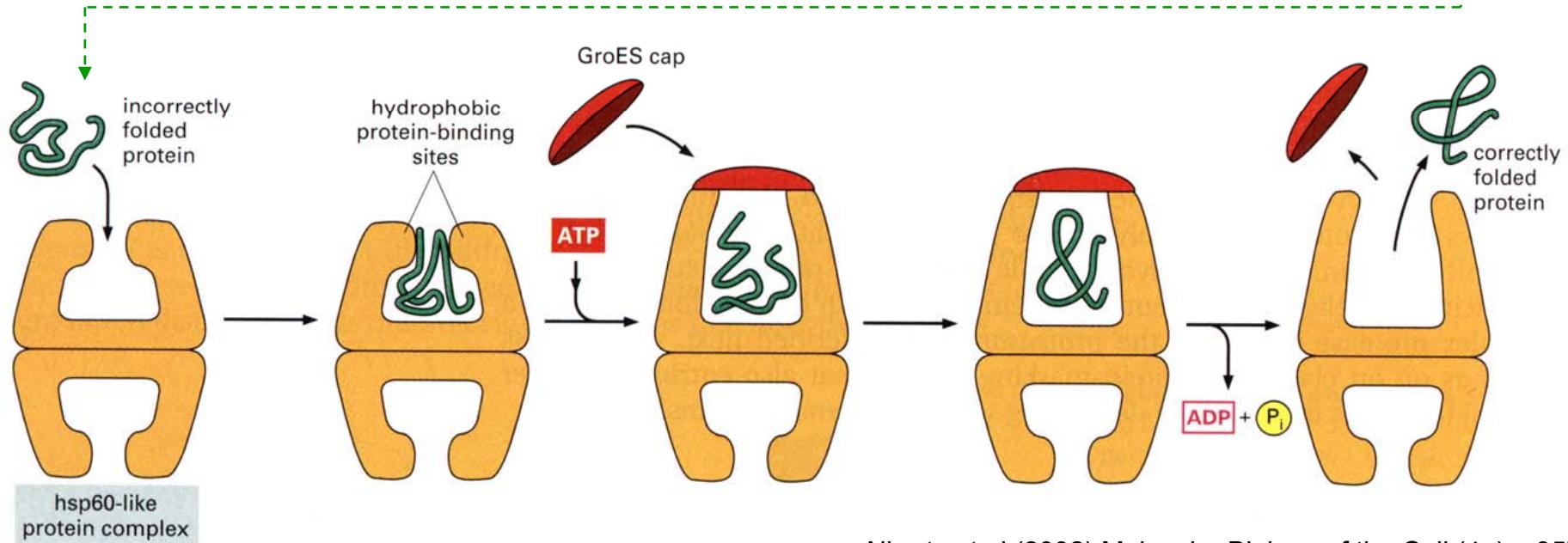
**Hsp:** heat shock protein

# Chaperonins in protein folding





蛋白質轉譯出來之後要靠  
chaperone 幫助正確摺疊



# Surface and cut-away images of the GroEL/GroES complex

7

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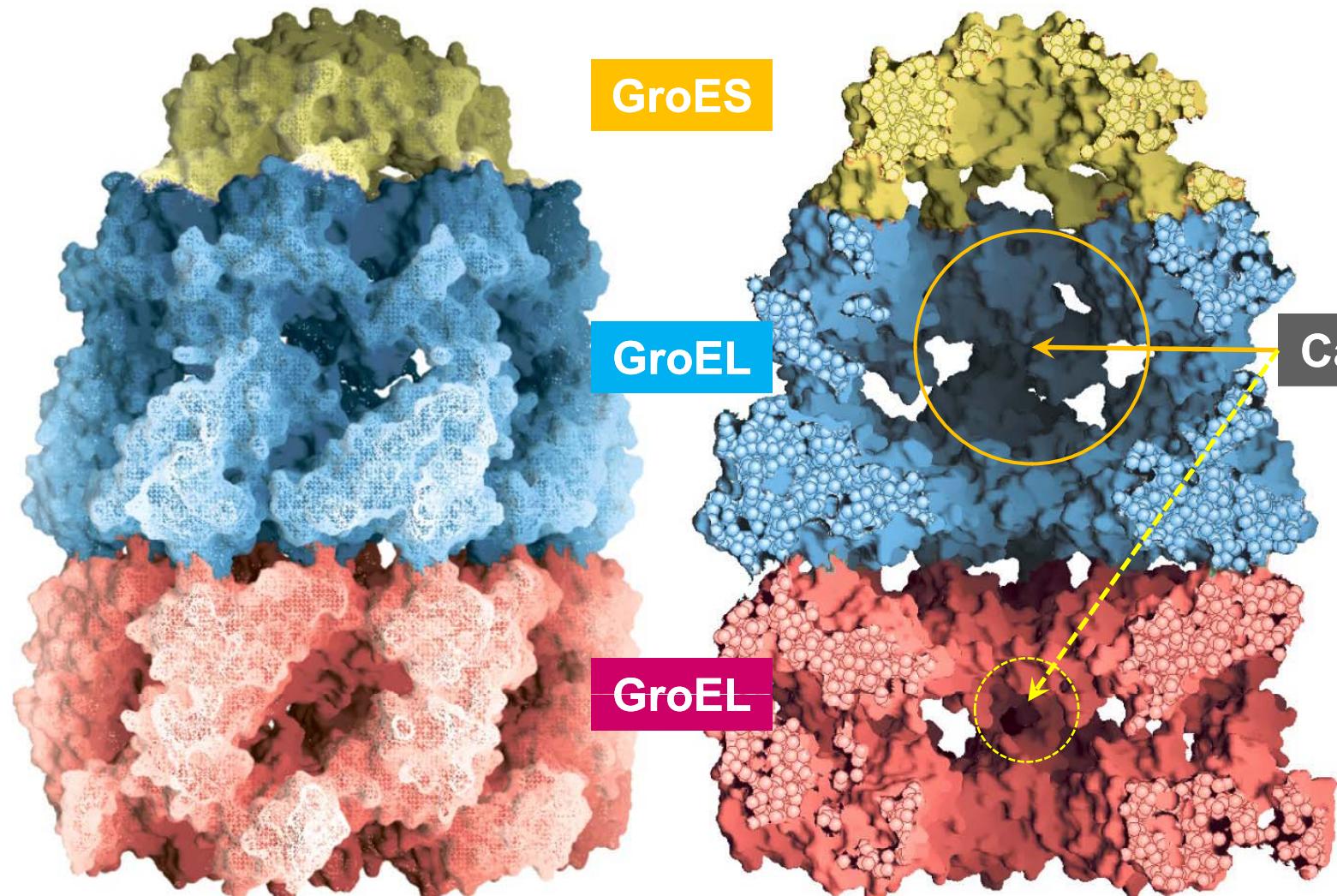
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GroES

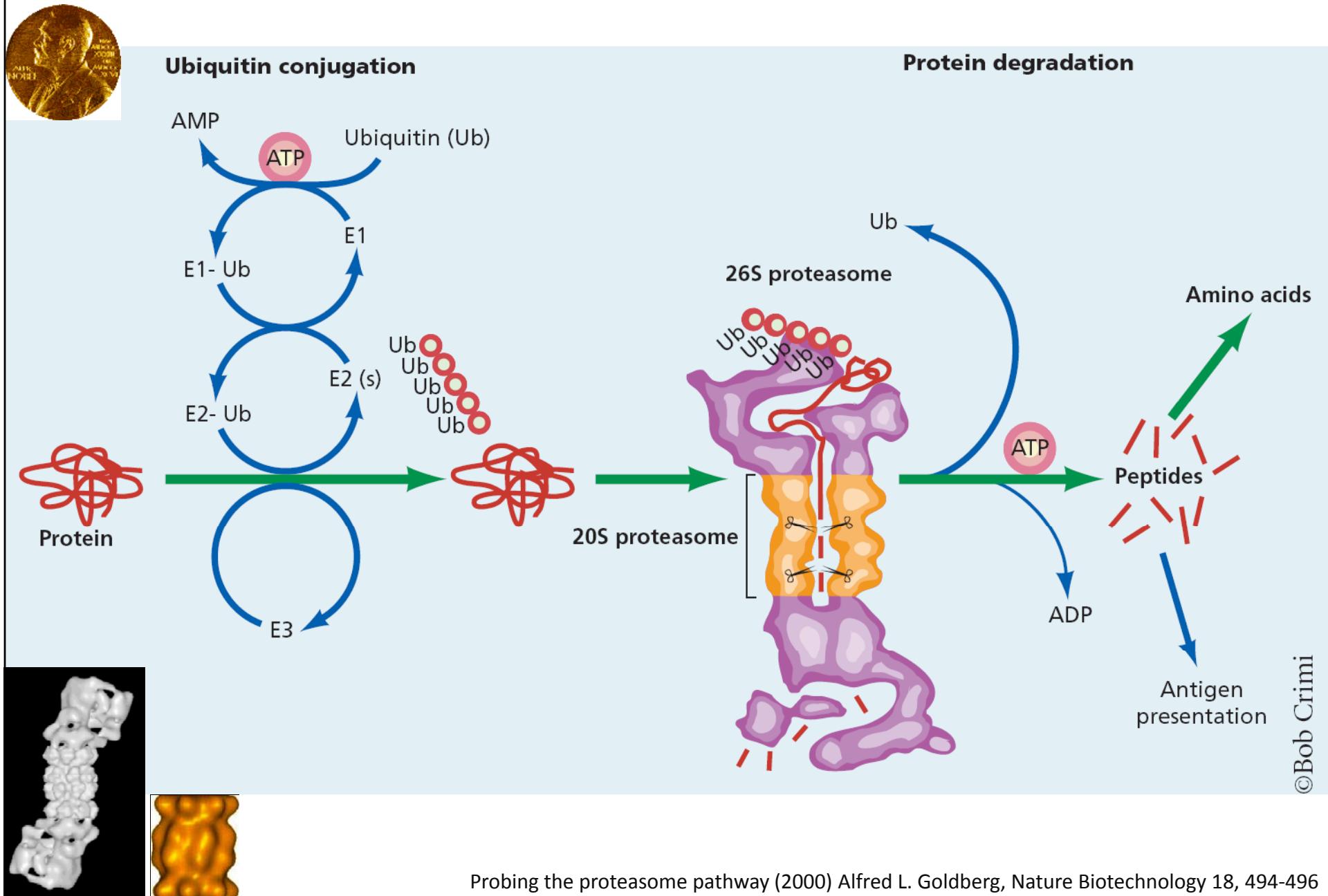
GroEL

GroEL

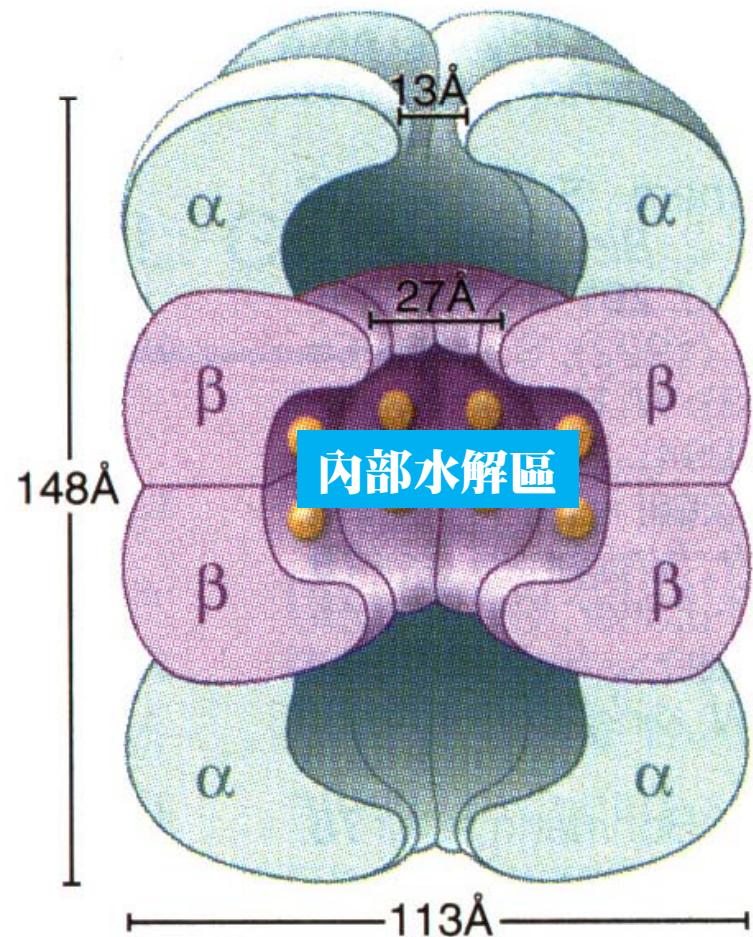
Cavity



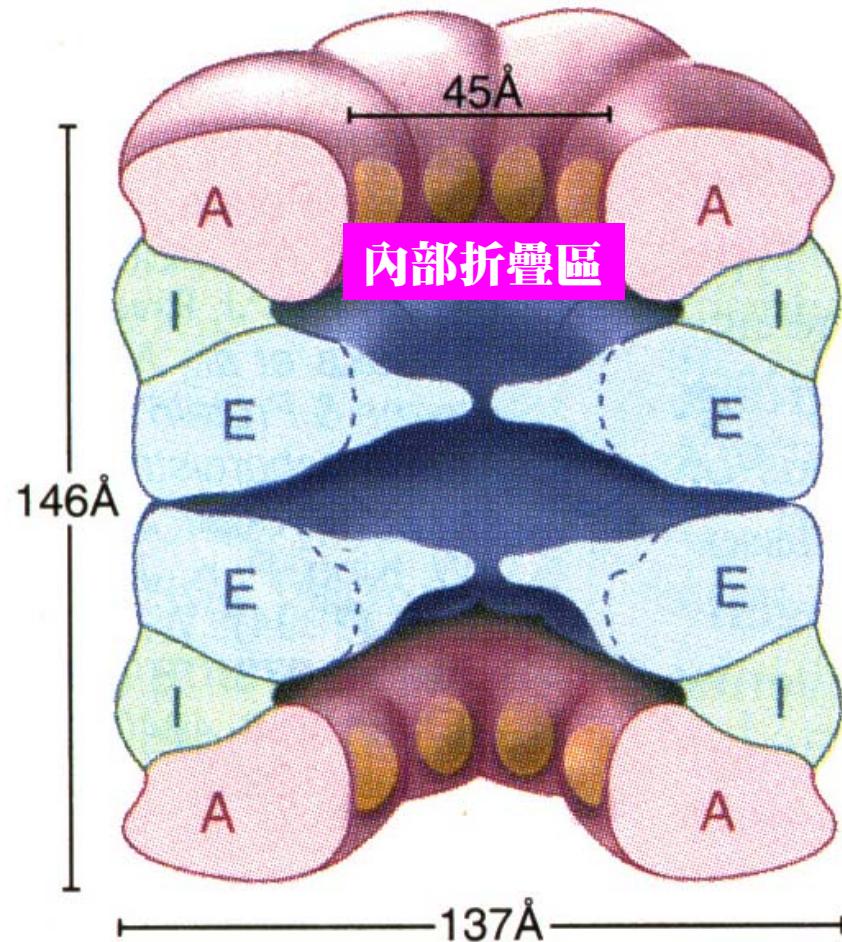
# 摺疊不良的蛋白質會被送到蛋白酶體降解系統



# 相似的桶狀巨分子卻有相反的功能



**Proteasome (20S)**



**Chaperonin (GroEL)**

# 與蛋白質摺疊缺失有關的疾病

- A soluble protein is secreted in a misfolded state and converted into an insoluble extracellular amyloid fiber.

- The diseases are collectively referred to as amyloidoses.

◆ Amyloid 類澱粉蛋白/澱粉樣蛋白 ◆ Amyloidosis 類澱粉變性症/澱粉樣變性病 ◆ Amyloid plaque 澱粉樣蛋白斑

## Type II diabetes

Amyloid deposition near the **pancreatic islet  $\beta$  cells**

## Alzheimer's disease

Extracellular amyloid deposition by neuron **amyloid  $\beta$ -peptide** derived from amyloid  $\beta$ -peptide precursor protein or APP

- Intracellular aggregation of misfolded proteins

## Parkinson's disease

$\alpha$ -synuclein aggregates into Lewy bodies

## Huntington's disease

Huntingtin with a **long polyglutamine repeat**

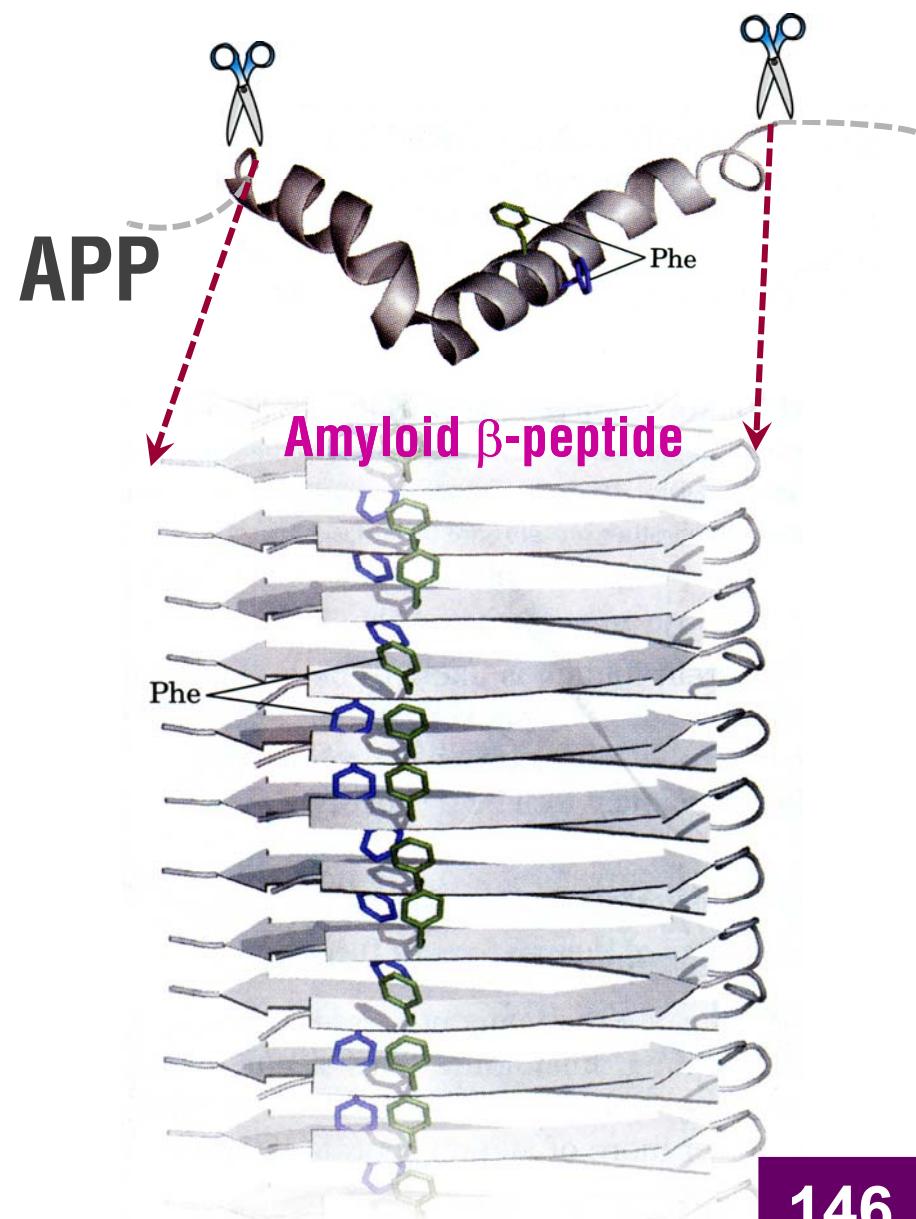
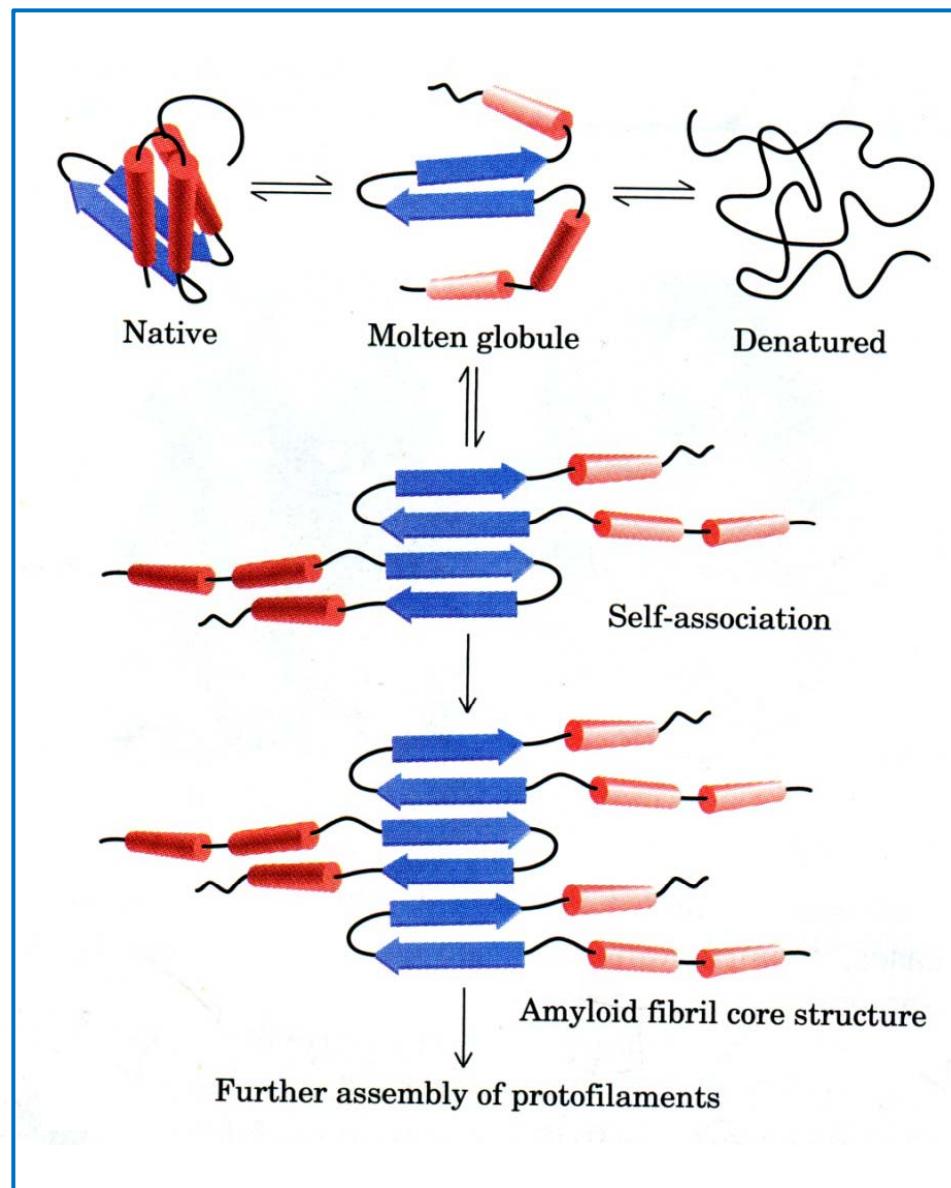
- Misfolded leads to degradation and loss of function

## Cystic fibrosis 囊性纖維化病

Defects in a membrane-bound protein called **CFTR**

**CFTR:** Cystic fibrosis transmembrane conductance regulator, which act as a channel for chloride ions

# Formation of disease-causing amyloid fibrils



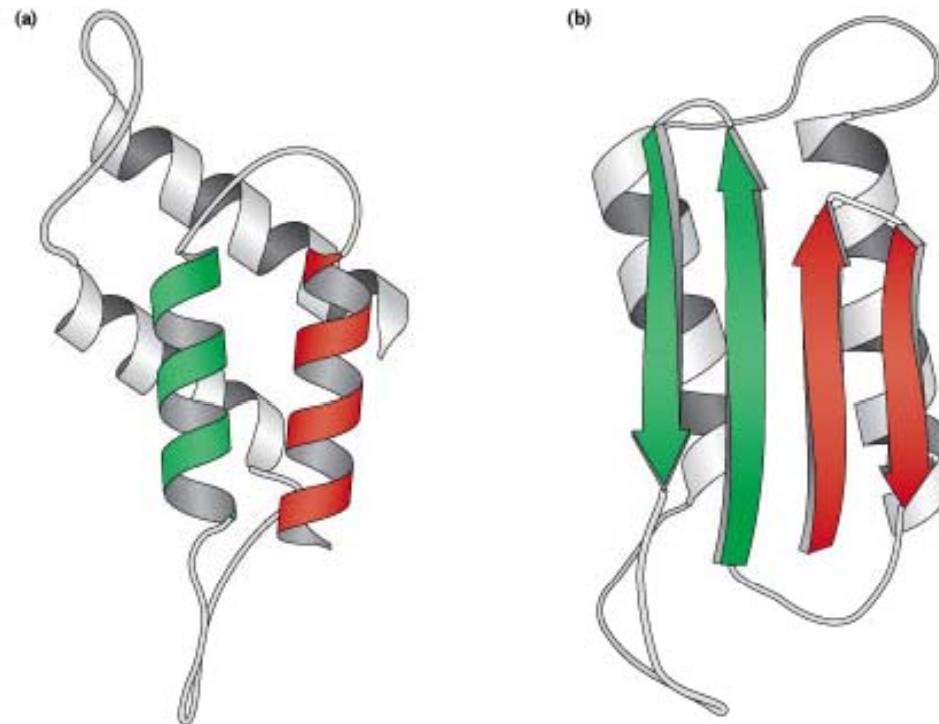
# The prion (**proteinaceous infectious particle**) disease

coined in 1982 by Dr. Stanley B. Prusiner

Tikvah Alper suggested the hypothesis in 1960s that some transmissible spongiform encephalopathies are caused by an infectious agent consisting solely of proteins.



Stanley B. Prusiner



左邊為**正常的  $\text{PrP}^c$** 蛋白質結構，多由  $\alpha$  helices 組成

右邊則是呈現  $\beta$  sheets 構型的**致病性  $\text{PrP}^{sc}$**  蛋白質結構

The Nobel Prize in Physiology or Medicine 1997 was awarded to Stanley B. Prusiner "for his discovery of Prions - a new biological principle of infection".

**PrP**, the **prion protein**, comes in various forms, such as  **$\text{PrP}^c$** , the normal **cellular prion protein**, and  **$\text{PrP}^{sc}$** , the **scrapie form**

# Prion

## Proteinaceous infectious particles

**Summary.** After infection and a prolonged incubation period, the scrapie agent causes a degenerative disease of the central nervous system in sheep and goats. Six lines of evidence including sensitivity to proteases demonstrate that this agent contains a protein that is required for infectivity. Although the scrapie agent is irreversibly inactivated by alkali, five procedures with more specificity for modifying nucleic acids failed to cause inactivation. The agent shows heterogeneity with respect to size, apparently a result of its hydrophobicity; the smallest form may have a molecular weight of 50,000 or less. Because the novel properties of the scrapie agent distinguish it from viruses, plasmids, and viroids, a new term "prion" is proposed to denote a small proteinaceous infectious particle which is resistant to inactivation by most procedures that modify nucleic acids. Knowledge of the scrapie agent structure may have significance for understanding the causes of several degenerative diseases.

### Scrapie Agent Contains Protein

Six separate and distinct lines of evidence show that the scrapie agent contains a protein that is required for infectivity: (i) inactivation as a result of digestion with proteinase K, (ii) inactivation by chemical modification with diethyl pyrocarbonate, (iii) inactivation by SDS, (iv) inactivation by chaotropic salts such as guanidinium thiocyanate, (v) inactivation by phenol, and (vi) inactivation by urea (60). The cumulative evidence for a protein within the scrapie agent appears to be compelling (Table 1).

### Novel Proteinaceous Infectious Particles Cause Scrapie

Stanley B. Prusiner

A major, unanswered question in mo- senile dementia, was shown by Gibbs, ek, and co-workers to be caused insmissible agent (6, 7).

cent study suggests that there may larities between the agents caus-  
ipie and CJD (8). Goats inoculat-

brain tissue from demented pa-  
lying of CJD developed a neuro-  
disorder 3 to 4 years after inocula-  
g. 1). Five out of ten CJD inocula  
duced disease in goats (9). Ex-  
tural CJD in goats is indistinguish-  
clinically and neuropatholog-  
rom natural scrapie. Monkeys

bation period, the scrapie agent us system in sheep and goats. Six es demonstrate that this agent . Although the scrapie agent is ith more specificity for modifying shows heterogeneity with respect ; the smallest form may have a vel properties of the scrapie agent i new term "prion" is proposed to hich is resistant to inactivation by dge of the scrapie agent structure ses of several degenerative dis-

animals were vaccinated against louping ill virus with a formalin-treated suspension of ovine brain and spleen that, as was shown subsequently, had been contaminated with the scrapie agent (2). Two years later, 1500 sheep developed scrapie. Subsequently, studies on CNS diseases (including scrapie) of sheep provided the foundation for Sigurdsson's concept of slow infections (3). In 1959, Hadlow suggested that kuru, a CNS degenerative disease of New Guinea highlanders, might be similar to scrapie because the pathologies of these disorders share many features (4). The transmission of kuru to chimpanzees in 1965 by Gajdusek, Gibbs, and Alpers forced a major reconsideration of the etiology of all degenerative disorders and made scrapie a subject of intense medical interest (5). Subsequently, Creutzfeldt-Jakob disease (CJD), a progressive, pre-

have been used as a common experimental host for scrapie and CJD; curiously, chimpanzees are susceptible to CJD but not scrapie (10). Numerous attempts to link scrapie epidemiologically to CJD have been unsuccessful (11). At present, there is no direct evidence that the scrapie agent causes disease in humans.

In contrast to CJD which occurs worldwide, kuru is found only in a small mountainous region of Papua New Guinea. Epidemiological studies of kuru provide evidence for incubation periods of 20 to 30 years (12, 13). Although considerable evidence implicates cannibalism in the spread of kuru, no direct observations of cannibalistic acts in the "endemic" region have been recorded. Attempts to transmit kuru by feeding infected brain tissue to chimpanzees have been unsuccessful although one monkey developed a kuru-like illness 36 months

after oral ingestion of the kuru agent (14). In contrast, goats fed scrapie-infected tissue frequently develop disease (15). Recently, we have taken advantage of the natural cannibalistic activities of hamsters to develop an experimental model of scrapie transmitted by cannibalism (16). Oral transmission of the scrapie agent appears to be extremely inefficient. Cannibalism requires a dose of agent  $10^9$  times greater than that needed to produce scrapie by intracerebral injection. These results provide compelling evidence for oral transmission of the scrapie agent and may offer new insights into the spread of kuru by cannibalism among the Fore people and their neighboring tribes.

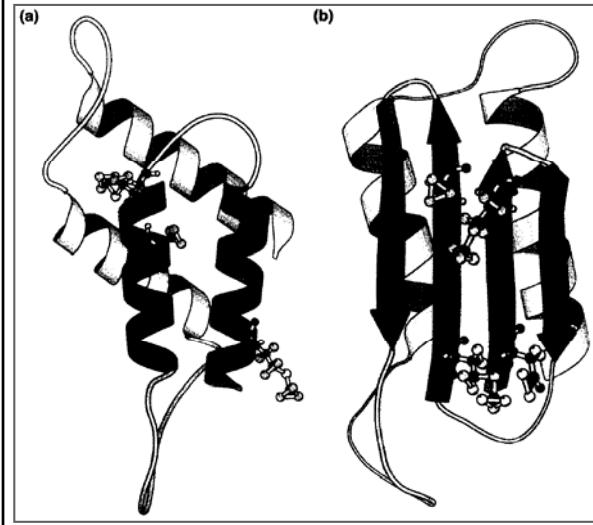
### Bioassay of the Scrapie Agent

Studies on the scrapie, kuru, and CJD agents have been greatly limited by the slow, tedious, and costly bioassays used to detect these agents. Since tissue culture systems are not available for the replication and assay of these agents and they appear to be nonantigenic in their native forms, animal bioassays must be used. For many years all assays for the scrapie agent were performed in sheep and goats (17). In 1961, transmission of the scrapie agent to mice transformed research (18), but the murine end-point titration assay was still heroic. Quantifying a single sample required eight to ten serial tenfold dilutions and injection of each dilution into six mice (19). Then 50 to 60 mice were held for 1 year and examined weekly for signs of scrapie. The number of animals developing scrapie at the highest dilution was used to calculate an end point. The time required for titration of a sample was reduced to 200 days when a more rapid form of the disease in hamsters was discovered (20, 21).

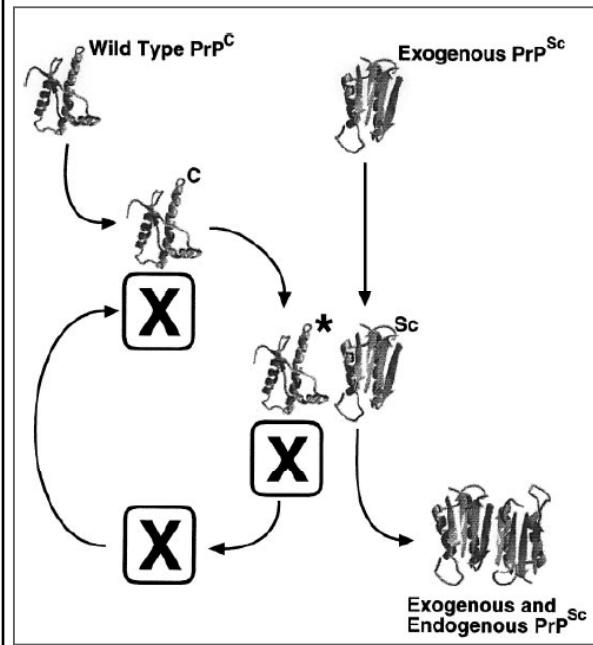
Several investigators have estimated scrapie titers by measuring the time interval from inoculation to onset of illness (incubation period) in mice (22, 23). Reluctance to refine such measurements has prevented its wide use in mice.

With hamsters, studies on the scrapie agent have been accelerated by development of a bioassay based on measurements of incubation time (24, 25). It is now possible to assay samples with the use of four animals in 60 to 70 days if the titers of the scrapie agent are high. As is shown in Fig. 2, the interval from inocu-

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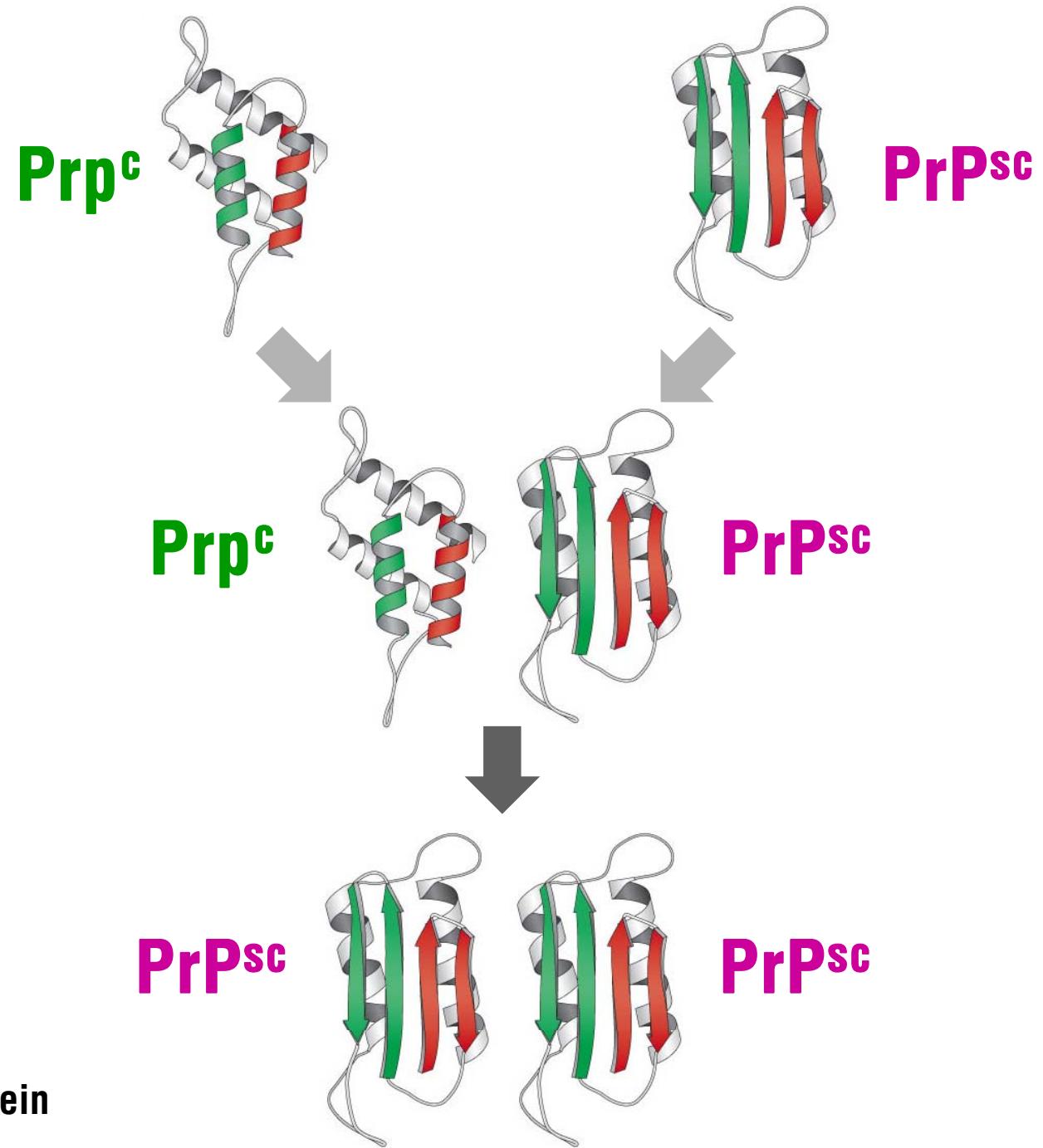


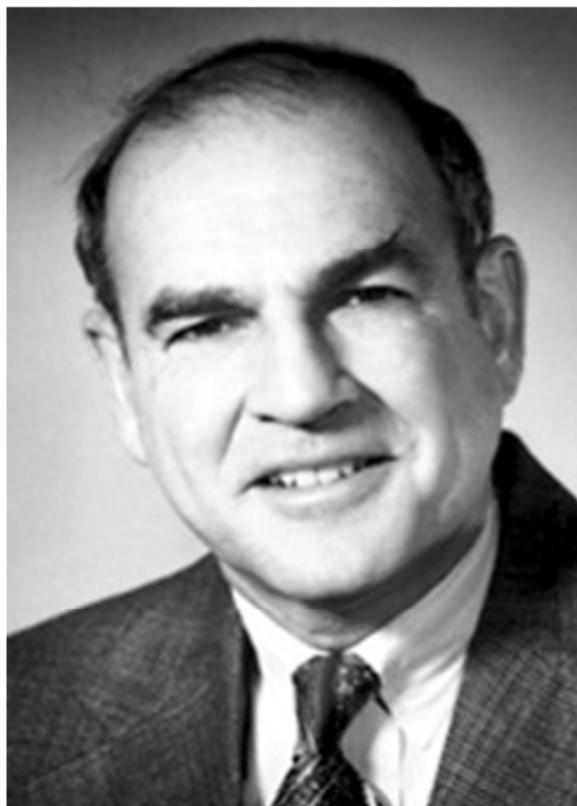
TIBS 21 - December 1996



Annu. Rev. Biochem. 1998. 67:793–819

$\text{PrP}^{\text{C}}$ , normal cellular prion protein  
 $\text{PrP}^{\text{Sc}}$ , scrapie form





Baruch S. Blumberg



D. Carleton Gajdusek

在研究狂牛病這個領域上已經產生兩位諾貝爾醫學暨生理學獎得主(1976及1997)。第一位是 Gajdusek，他於巴布亞新幾內亞的食人族部落發現庫魯(kuru)症，並推測病源可能是一種慢性作用病毒(slow-acting virus)。Prusiner 則是第二位研究該領域獲獎的科學家，他發現造成羊搔癢症的病原並不是病毒，它是一種不含 DNA 或 RNA 的物質，而是一種變異的蛋白質，Prusiner 並將此具感染力病原命名為 Prion。

The Nobel Prize in Physiology or Medicine 1976 was awarded jointly to Baruch S. Blumberg and D. Carleton Gajdusek "for their discoveries concerning new mechanisms for the origin and dissemination of infectious diseases"

B型肝炎表面抗原

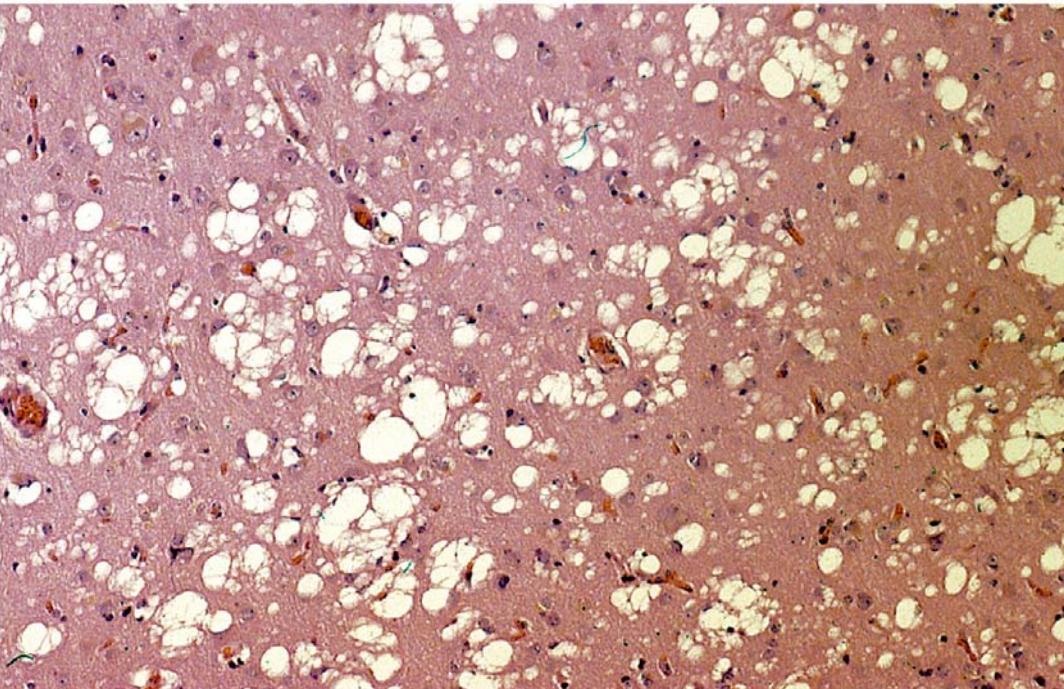
Photos: Copyright © The Nobel Foundation

Kuru



# The prion-related diseases

- Creutzfeldt-Jakob Disease and kuru in human
- Bovine spongiform encephalopathy, BSE (Mad Cow Disease) 牛海綿狀腦病
- Scrapie in sheep
- Chronic Wasting Disease in deer and elk



■ Stained section of **cerebral cortex** from autopsy of a patient with **CJD** shows **spongiform degeneration**, the most characteristic neurohistological feature



John B. Fenn



Koichi Tanaka



Kurt Wüthrich

The Nobel Prize in Chemistry 2002 was awarded "*for the development of methods for identification and structure analyses of biological macromolecules*" with one half jointly to John B. Fenn and Koichi Tanaka "*for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules*" and the other half to Kurt Wüthrich "*for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution*".

Determined the prion structure by NMR

