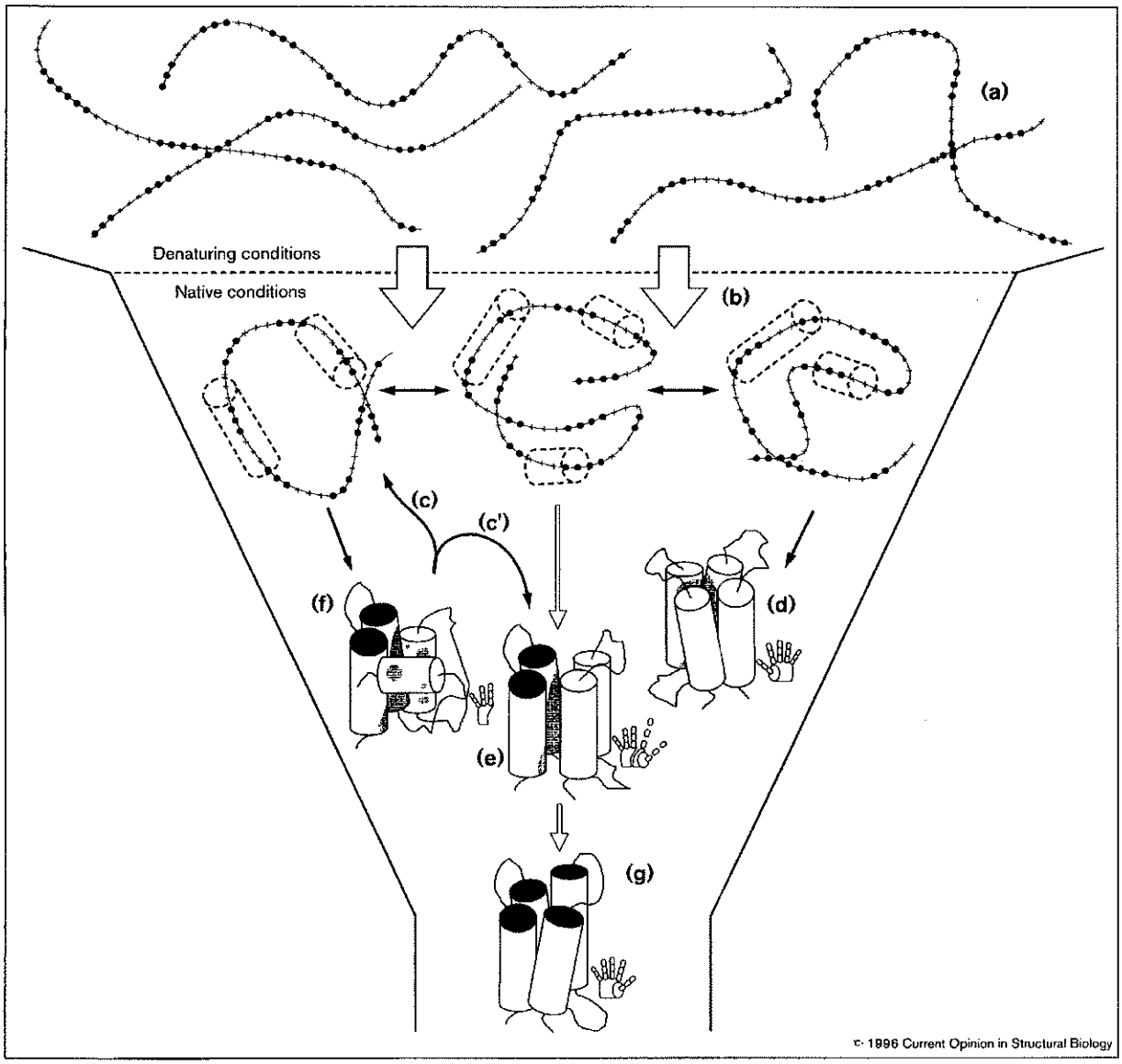
**Figure 1**




Schematic representation of the folding of a simple protein. **(a)** In the unfolded state of a protein under highly denaturing conditions, a large number of conformational states are accessible and local structural preferences are limited primarily by steric restrictions and excluded volume effects. **(b)** When placed under refolding conditions, local interactions result in nascent secondary structure and/or nucleation sites, the distribution of which will be dependent on refolding conditions. The fraction of native contacts at this point is small, as is the degree of global cooperativity. The process of collapse is linked with the formation of largely native-like secondary structure. These events are principally driven by the intrinsic tendency of a heteropolymer in aqueous solution to form a hydrophobic core. The pattern of residue types (polar residues are represented as crosses and hydrophobic residues are represented as dots) is crucial to stabilizing secondary structure and dictating the overall architecture of the collapsed state. **(g)** The native protein in this example is represented as a right-handed up-down-up-down four-helix bundle. Within the collapsed state, various structural families might exist. Some of these may have incorrect topologies, for example, left-handed rather than right-handed twist **(d)**, or incorrect connectivity **(f)**. These families may not be very different in energy or in native contacts, but the pattern of residues will destabilize the incorrect folds relative to the native-like fold. Only molecules with the correct fold, **(e)**, can progress down the 'folding funnel'. Molecular chaperones and other auxiliary factors may help to avoid aggregation of the states with exposed hydrophobic residues *in vivo* and may help effect the rearrangement process. The folding process might involve substantial unfolding of non-productive intermediates **(c)** or local reorganization of productive intermediates **(c')**. Some individual subdomains (cylinders with shaded caps) may reach their native-like states before others. Whether or not intermediates accumulate, or whether parallel paths are seen, ultimately depends on the energetics affecting the relative stabilities and rates of formation of these species compared with the final consolidation of the native fold. The shaded areas of the helices represent hydrophobic residues.

## PROTEIN FOLDING IN VIVO IS CATALYZED BY ISOMERASES AND CHAPERONE PROTEINS

The refolding of many proteins in vitro is much slower and less efficient than in vivo. The reason for this difference is that *protein folding in vivo is assisted by catalysts.*

1. The formation of correct disulfide pairings in nascent proteins is catalyzed by *protein disulfide isomerase (PDI).* PDI binds the polypeptide backbone of protein substrates. The enzyme preferentially interacts with peptides that contain cysteine residues but is otherwise undiscriminating. The broad substrate specificity of PDI enables it to speed the folding of diverse disulfide-containing proteins. By shuffling disulfide bonds, PDI enables proteins to quickly find the thermodynamically most stable pairings amongst those that are accessible. What is the catalytic mechanism of this enzyme? PDI contains two -Cys-Gly-His-Cys- sequences. The thiols of these cysteines are highly reactive at physiologic pH because they have a lower pK (7.3) than do most thiols in proteins (8.5). A disulfide bond of the protein substrate is attacked by an $RS^-$ group of the enzyme to form a covalent enzyme-substrate intermediate. The liberated thiol of the substrate is now free to attack another disulfide bond to form a different pairing (Figure 16-22). *PDI is especially important in accelerating disulfide interchange in kinetically trapped folding intermediates,* such as the BPTI intermediate containing [5-55] and [14-38] disulfides (see Figure 16-20). The isomerase accelerates disulfide shuffling 6000-fold in this intermediate.

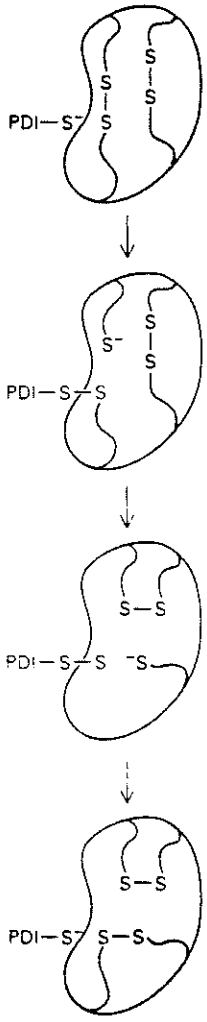



**Figure 16-22**
Protein disulfide isomerase (PDI) catalyzes disulfide interchange. A thiolate anion of the enzyme plays a key role in catalysis.

2. Peptide bonds in proteins are nearly always in the trans configuration. The exceptions are X-Pro peptide bonds (X denotes any residue), of which 6% are cis (Figure 16-23; also see Figure 16-7). Prolyl isomerization is the rate-limiting step in the folding of many proteins in vitro.

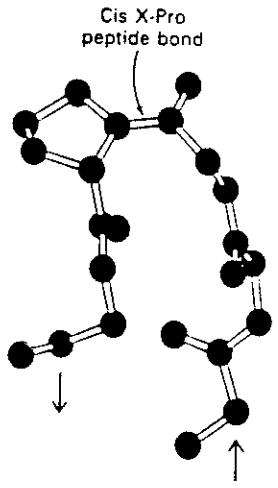



**Figure 16-23**
*Cis*-proline is well suited to making hairpin turns in polypeptide chains. [Drawn from 2rns.pdb. E.E. Kim, R. Varadarajan, H.W. Wyckoff, and F.M. Richards, *Biochemistry* 31(1992):12304.]

Spontaneous isomerization is slow because the bond between the carbonyl carbon and the amide nitrogen has partial double bond character (p. 27). *Peptidyl prolyl isomerases (PPIases) accelerate cis-trans isomerization more than 300-fold by twisting the peptide bond so that the C, O, and N atoms are no longer planar.* In this transition state, the C–N bond has more single bond character because resonance is minimized. Hence, the activation barrier for isomerization is lowered.

3. Unfolded and partially folded proteins tend to aggregate when present at high concentration. The refolding of denatured proteins in vitro is usually carried out in dilute solution to minimize intermolecular contact and aggregation. Protein folding in vivo, however, effectively occurs in a very crowded milieu. A nascent protein in the cytosol or endoplasmic reticulum is surrounded by many potential molecular distractions. How are improper intermolecular interactions prevented in vivo? The answer is that several classes of proteins, picturesquely termed *molecular chaperones,* assist protein folding by inhibiting improper attachments and prying apart illegitimate liaisons. *Chaperones reversibly bind unfolded segments of polypeptide that might otherwise serve as centers of aggregation or diversion.* The hydrolysis of ATP powers the untangling of trapped intermediates. We will return to these fascinating proteins in a later chapter (p. 919).

# GroEL/ES chaperon

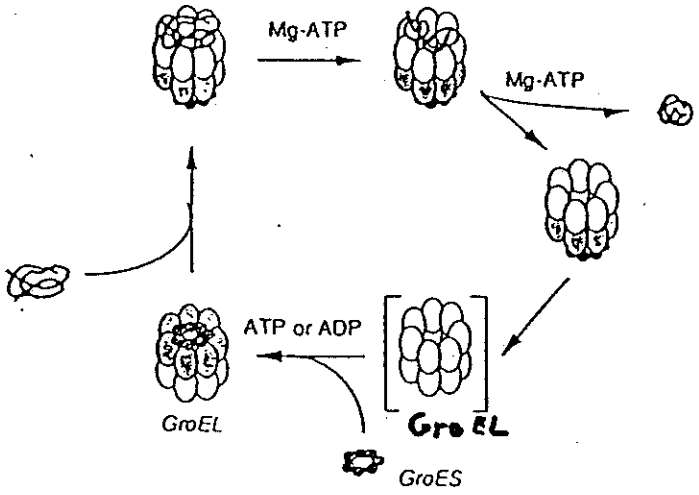
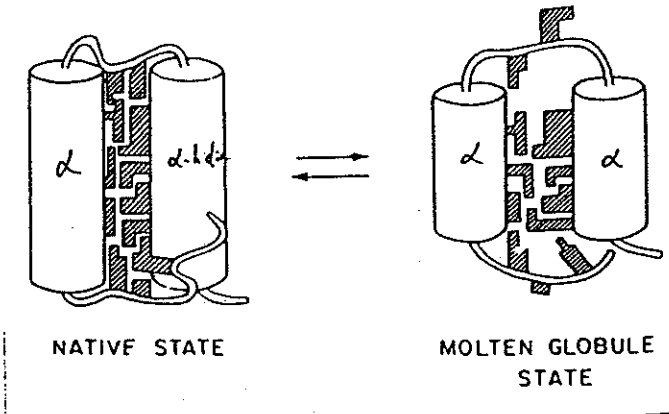



FIGURE 6-6. *Schematic representation of the native and the molten globule states of protein molecules. Nonpolar side chains are hatched.*




**Molten Globule**: 1. Nästan intakt sekundär struktur (α-helix + β-fält)
2. Tertiära interaktioner brutna
3. Något större volym
4. Vissa hydrofoba ytor exponerade