STRUCTURAL BIOLOGY

Protein self-assembly intermediates

Proteins can self-assemble into functional states, or they can end up as aberrant and sometimes toxic aggregates. Metastable intermediate states are often detected in these processes, and their structural characterization provides vital information about the balance between functional and pathological behavior in living systems.

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It has long been recognized that intermediate states are ubiquitous in protein self-assembly[1–3]. On their way to such states, protein molecules tend to hop between transiently populated partially folded states, thus spending most of their traveling time in a few metastable minima on their free energy landscapes. This behavior can be attributed to the fact that protein self-assembly is generally driven initially by nonspecific forces, such as hydrophobic interactions, and then fine-tuned by more specific interactions, such as hydrogen bonding. From the point of view of structural biology, the challenge is to describe in detail the conformations of such intermediates, because their intrinsically transient nature makes them recalcitrant to standard experimental methods. By exploiting recent advances in NMR spectroscopy, however, it is becoming possible to identify the structural properties of these states at atomic resolution[4,5]. Indeed, in this issue, Jaremko *et al.*[6] provide a remarkably detailed description of a well-defined intermediate that exists during the process by which two proteins assemble into a functional complex.

Understanding the nature of such reaction intermediates and determining their structures is a crucial step in describing not only the processes of folding and of complex formation but also those of misfolding and aggregation—aberrant behavior that can result in fatal conditions, such as Alzheimer's disease and type 2 diabetes. The folding process generally involves one or more intermediates between the unfolded and folded states (**Fig. 1**). Self-assembly may then proceed to larger functional assemblies via encounter complexes in which proteins populate assembly intermediates. Furthermore, although many proteins are normally very resistant to aggregation as components of large complexes, they are prone to misfolding if they dissociate into intermediate states, where they can undergo larger thermal fluctuations[3,7]. It is also possible for proteins to misfold and associate from normal folding intermediates[5] through encounter complexes that lead to transient oligomeric assemblies and ultimately to stable species such as amyloid fibrils[3,7,8].

As reported in this issue, Jaremko *et al.*[6] have obtained a glimpse of an assembly intermediate. By applying a 'cold denaturation' approach, they studied CylR2, a homodimeric regulatory bacterial protein, finding that the monomer-to-dimer intermediate differs from the native state simply in the way that certain side chains at the dimer interface are oriented. To obtain this result, Jaremko *et al.*[6] mapped the intermediate state by taking snapshots of the dissociation of the dimer upon lowering the temperature in steps and by using diffusion NMR experiments to locate the midpoint temperature of dissociation. By projecting a metastable state at room temperature onto its stable counterparts at decreasing temperatures, this study has provided detailed NOE-derived interatomic distance information about a series of states in which the population of the homodimeric state is progressively reduced[6].

An important open question concerns the extent to which the variety of different intermediates accessible to a protein resemble each other. Because the fundamental forces at play in protein self-assembly are essentially the same regardless of whether the endpoint is the functional or a dysfunctional state of the

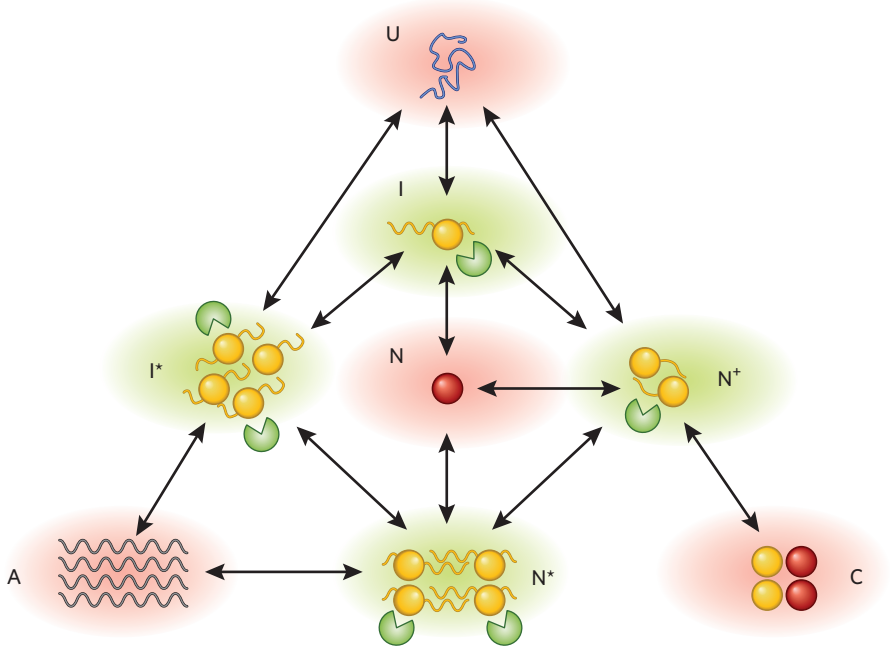

Figure 1 | Schematic view of the various metastable and stable states in protein self-assembly. Intermediate states (I) are commonly present between the unfolded (U) and native (N) states[2,4–6]. Similarly, intermediate states of the type characterized in this issue by Jaremko *et al.*[6] (N⁺) tend to appear during the association into functional complexes (C) and other intermediate states (N* and I*) in the process of amyloid fibril (A) formation[3,5,7,8]. Molecular chaperones (green) are essential in regulating the transitions between stable and metastable states as well as the interconversions between the metastable states themselves.

protein, intermediates along the pathways of folding and complex formation may well resemble those populated during misfolding and aggregation. Indeed, it is increasingly evident that intermediates tend to share similar, and often highly native-like, topologies[4–7]. Thus, determining the detailed structures of intermediates of the type described by Jaremko *et al.*[6] is a crucial step in defining the multiplicity of states shown schematically in **Figure 1**.

Given the apparent similarities between the precursors of normal and aberrant assemblies, the regulation of the processes that involve the different states of proteins is of crucial importance. Thus, just as enzymes control chemical processes in living systems, molecular chaperones and other regulatory processes control the conformational states of proteins. The similarities between intermediate states and the intricacies of their transitions have led to the evolution of a highly sophisticated means of ensuring that the populations and rates of interconversion are carefully managed in properly functioning biological systems.

As shown by Jaremko *et al.*[6] and other recent studies[4,5], NMR spectroscopy is particularly effective in providing detailed experimental data concerning self-assembly intermediates. Although such information is often of a sparse nature, its combination with molecular simulations[9,10] offers opportunities for generating atomic-resolution structures of the intermediates even when they are otherwise highly elusive[4–6]. These advances are providing a fundamental and vital understanding of the specific molecular events that lead to the self-assembly of proteins into functional macromolecular structures or that, in the event of failure, result in the formation of potentially cytotoxic protein deposits. Such understanding undoubtedly provides the framework for the development of rational strategies for the design of new functional assemblies and of therapeutic compounds able to influence specific steps in the complex self-association processes so as to reduce the risk of misfolding events.

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References

1. Kim, P.S. & Baldwin, R.L. *Annu. Rev. Biochem.* **59**, 631–660 (1990).
2. Radford, S.E., Dobson, C.M. & Evans, P.A. *Nature* **358**, 302–307 (1992).
3. Kelly, J.W. *Curr. Opin. Struct. Biol.* **8**, 101–106 (1998).
4. Korzhnev, D.M., Religa, T.L., Banachewicz, W., Fersht, A.R. & Kay, L.E. *Science* **329**, 1312–1316 (2010).
5. Neudecker, P. *et al. Science* **336**, 362–366 (2012).
6. Jaremko, M. *et al. Nat. Chem. Biol.* **9**, 264–270 (2013).
7. Chiti, F. & Dobson, C.M. *Nat. Chem. Biol.* **5**, 15–22 (2009).
8. Cremades, N. *et al. Cell* **149**, 1048–1059 (2012).
9. Lindorff-Larsen, K., Best, R.B., DePristo, M.A., Dobson, C.M. & Vendruscolo, M. *Nature* **433**, 128–132 (2005).
10. Cavalli, A., Salvatella, X., Dobson, C.M. & Vendruscolo, M. *Proc. Natl. Acad. Sci. USA* **104**, 9615–9620 (2007).

Competing financial interests

The authors declare no competing financial interests.

SIGNALING

Making a leaner Hedgehog

Secreted Hedgehog (Hh) proteins are essential in development, and their aberrant activity contributes to certain cancers. Chemically targeting a lipid modification of Hh proteins results in loss of their cellular activity, revealing new strategies for cancer intervention and elucidation of the role of lipids in signal transduction.

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The secreted Hh proteins are dually lipid-modified cell-cell communication molecules with cholesterol and palmitate covalently attached at their C and N termini, respectively[1] (**Fig. 1a**). The activation of the Hh pathway entails binding of Hh to its receptor Patched (Ptch), thereby relinquishing the suppressive action of Ptch on Smoothened (Smo), a seven-transmembrane effector protein (reviewed in ref. 2). Hh-induced accumulation of Smo in the primary cilium, an antenna-like organelle found in most cells, initiates a series of biochemical events that culminate in the activation of the Gli family of DNA-binding proteins. First established as a developmental signaling system, the Hh pathway is now recognized as a platform used in cancerous cells for achieving deviant cell growth[3]. Using an *in vitro* screening strategy, Petrova *et al.*[4] identify small molecules, including RU-SKI 43, that disable the palmitoylation of Hh, thus introducing chemical probes that can be applied to understand the role of Hh lipidation in signal transduction and defining a new chemical strategy for targeting Hh signaling in disease.

The cholesterol modification in Hh is an autocatalytic event mediated by the C-terminal domain. The addition of the palmitoyl adduct to the thiol group in the N-terminal cysteine residue is catalyzed by an enzyme known as Hedgehog acyltransferase (Hhat)[1]. A chemical rearrangement transfers the fatty-acyl adduct onto the N terminus, thereby yielding a free thiol side chain. Hhat belongs to a superfamily of multitransmembrane proteins termed membrane-bound O-acyl transferases (MBOATs) that link fatty acids to membrane-embedded targets, including lipids and proteins[4]. In addition to Hh, two other protein substrates for MBOATs have been identified: the secreted Wnt signaling molecules and the orexigenic ghrelin hormone (**Fig. 1b**). Many studies have been devoted to reconciling how the hydrophobic nature of dually lipidated Hh protein can be compatible with their ability to engage cells distant from the cells that produce them. These studies have provided evidence that the palmitoyl adduct facilitates the membrane release of Hh by Dispatched, a protein similar to Ptch, and engages the extracellular chaperone molecule Scube (**Fig. 1a**). Although the lipid modification status of Hh is not thought to influence its binding to Ptch, Hh proteins that have atypical fatty-acyl adducts or lack an N-terminal cysteine show altered activity, suggesting that this post-translational modification may affect its interaction with one or more Hh co-receptors[2,5].

Basal cell carcinoma, medulloblastoma and rhabdomyosarcoma are cancers that can be driven by either inactivating or activating mutations in Ptch and Smo, respectively. So far, disabling Smo seems to be the most chemically tractable approach to crippling these tumors. One compound (vismodegib) received US Food and Drug Administration