have alternately high and low affinities for partially folded polypeptides; the cycle continues until the polypeptide reaches its native conformation. This work shed no light on the involvement of GroES, however, because the discharge of the particular polypeptide used is only slightly stimulated by GroES *in vitro*. This lack of an absolute requirement for GroES has been reported for the GroEL-assisted refolding of several polypeptides *in vitro*[11]. But GroES is both present *in vivo* and essential for cell viability, so it is likely to be involved with GroEL in protein folding in the cell. What, then, is the exact role of GroES?

## Reaction cycle

Hartl and colleagues[1,2] have carried out ingenious *in vitro* experiments that lead to the conclusion that GroEL and GroES are involved in a reaction cycle that continues until the discharged polypeptide has folded to the point where it no longer binds to GroEL; the cycle is driven by the binding and hydrolysis of ATP. The essential feature of this model is the opposing effects of GroES and the partially folded polypeptide on the properties of GroEL; that is, the binding of partially folded polypeptide reduces binding of GroES and vice versa. The authors took advantage of the observation that GroES protects the subunits of only one of the two GroEL rings from removal by proteinase K of 16 carboxy-terminal residues. This truncation does not affect the function of GroEL but does enable the two rings to be distinguished from each other by, for example, crosslinking to labelled nucleotides. These new results, together with other data, are consistent in my view with the following model.

*In vivo*, with the continual presence of Mg–ATP and GroES, the predominant state is likely to be the asymmetric complex of GroEL with GroES stabilized by ADP bound to the GroEL, as previously suggested[7]. Entry of polypeptide triggers the release of both ADP and GroES to produce a binary complex of GroEL and bound polypeptide. In the absence of ATP this complex is stable, and is the form observed in the discovery of the plastid chaperonin[12]. But, *in vivo*, the binding of ATP weakens the interaction between GroEL and bound polypeptide, and induces the binding of GroES to the other ring to produce a ternary complex. Co-operative ATP hydrolysis by both rings then causes release of all parts of the bound polypeptide more or less simultaneously into the central cavity where it folds. If folding is incomplete after ATP hydrolysis, the polypeptide rebinds to GroEL now containing bound ADP and GroES; the cycle repeats until the GroEL-binding motifs in the polypeptide are no longer accessible — that is, until the polypeptide is correctly folded.

So GroES does three things. First, it stabilizes one ring of GroEL in a high-affinity state for binding ADP, this state accepting partially folded polypeptide; the inhibition of ATPase activity in this ring also reduces wasteful hydrolysis of ATP when protein folding is not occurring. Second, it rebinds to GroEL upon exchange of ADP with ATP and increases the cooperativity of subsequent ATP hydrolysis; the discovery that GroES, as well as GroEL, binds ATP[2] may indicate that GroES donates ATP directly to GroEL as part of its job of increasing the cooperativity of ATP binding. Third, GroES rebinds to the GroEL ring containing bound polypeptide, and so prevents the polypeptide being released into the medium before folding is complete.

Why should ATP binding and hydrolysis be cooperative? Probably it is because the polypeptide is bound in the GroEL cavity at seven or more sites, so preventing premature folding of any part of the chain. But for correct folding it is advantageous for all parts of the chain to be discharged at much the same time, so that the entire primary structure is available for folding at the same instant. Thus the oligomeric structure of GroEL provides not only a sequestered environment for partially folded proteins but also a simultaneous-discharge machinery. Polypeptides whose discharge does not require GroES *in vitro* may bind to GroEL less strongly and/or at fewer sites than those that do. Determination of the crystal structure of GroEL–polypeptide complexes should resolve this point, as well as others such as the connectedness of the two cavities.

This model should appeal to those working on protein refolding who have been discomfited by the discovery of the chaperonins. It suggests that proteins do indeed fold inside cells as they do in experiments *in vitro*, but that they do so in the protected environment of an Anfinsen cage provided by the chaperonin molecular machine. □

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1. Martin, J., Mayhew, M., Langer, T. & Hartl, F.-U. *Nature* **366**, 228–242 (1993).
2. Martin, J., Geromanos, S., Tempst, P. & Hartl, F.-U. *Nature* **366**, 279–282 (1993).
3. Gething, M.-J. & Sambrook, J. *Nature* **355**, 33–45 (1992).
4. Hendrick, J. P. & Hartl, F.-U. *A. Rev. Biochem.* **62**, 349–384 (1993).
5. Ellis, R. J. *Curr. Opin. Struct. Biol.* (in the press).
6. Horwich, A. L. *et al. Cell* **74**, 909–917 (1993).
7. Jackson, G. S. *et al. Biochemistry* **32**, 2554–2563 (1993).
8. Anfinsen, C. B. *Science* **181**, 223–230 (1973).
9. Saibil, H. R. *et al. Curr. Biol.* **3**, 265–273 (1993).
10. Gray, T. E. & Fersht, A. R. *FEBS Lett.* **292**, 254–258 (1991).
11. Lorimer, G. H., Todd, M. J. & Viitanen, P. V. *Phil. Trans. R. Soc.* B**339**, 297–304 (1993).
12. Barraclough, R. & Ellis, R. J. *Biochim. biophys. Acta* **608**, 19–31 (1980).

DAEDALUS

# The magic bullet

**THE first antibiotic, Ehrlich's 'Salvarsan', was essentially a poisonous bacterial stain. Like any selective dye, it was taken up specifically by its target cells. It then killed them. This strategy has been recently refined. The coloured drug Photofrin, when injected, spreads throughout the body. But it can be activated locally by aiming a laser at the disease site, generating radicals which attack the local problem without causing trouble elsewhere.**

**Daedalus now wants to work the same trick with radiation therapy, which at the moment is a woefully indiscriminate weapon. He recalls that heavy elements absorb X-rays strongly — hence the unappetizing 'barium meal' given to a patient to outline his intestines for X-ray examination. A barium-based 'X-ray dye', taken up only by the target cells, could make them selectively sensitive to radiation. A derivative of unstable barium azide, for example, would be broken up into its elements by an incident X-ray photon. The resulting metallic barium would wreck any cell.**

**DREADCO's chemists are extending this idea. They point out that most elements, when excited by electron impact, emit specific X-ray fluorescence frequencies. They argue that an element should absorb its own fluorescence frequency extremely strongly, by resonant absorption. So they are devising heavy-element dyes containing more convenient elements than barium, with well-placed X-ray fluorescence frequencies. The end product should be a range of X-ray dyes which, injected into the body, are specifically taken up by the target cells. An intense blast of fluorescence-frequency X-rays, instead of being absorbed evenly by the patient, will dump nearly all its energy into these cells. The ensuing chemical mayhem should kill them instantly.**

**This trick may work well on parasites, like those of malaria and other tropical diseases, which resist drug therapy. But Daedalus is dreaming up quite a different application. He recalls that high explosive needs a heavy-metal detonator to set it off: typically lead azide. So his new airport-security X-ray scanner works on the lead fluorescence frequency. A detonator will absorb this radiation strongly. Not only will it show up in powerful contrast: the absorbed photons will decompose the unstable azide. An intense, focused blast of the X-rays might even fire the detonator. Passengers could then simply be invited to walk with their luggage through the armoured X-ray scanning tunnel. The survivors could safely be allowed to board their plane.**

David Jones