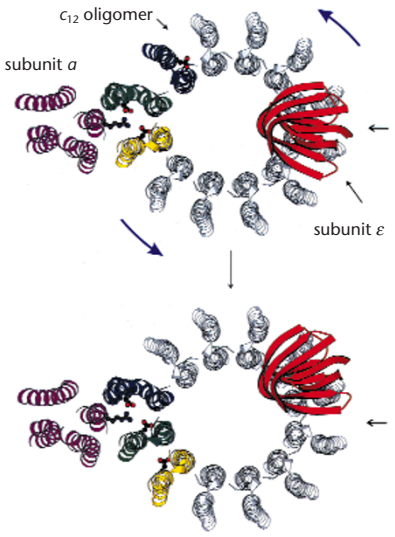
picture story

How proton pumps make ATP

If ATP is the universal currency of free energy in biological systems then the F_1F_0 ATP synthase is the mint. This enzyme can synthesize ATP using a transmembrane proton gradient. The ATP synthase is a multisubunit complex with a water-soluble F_1 domain, the crystal structure of which has been solved, and a transmembrane F_0 domain about which there is very little structural information. The F_0 domain is made up of three types of subunits, in an $a_1b_2c_{12}$ stoichiometry. Low-resolution images and biochemical experiments suggest that the 12 *c* subunits are in a cylindrical arrangement with the *a* (purple) and *b* (not shown) subunits on the periphery. While it is clear that the F_1 core subunit γ rotates during catalysis, the mechanism by which proton translocation through F_0 is coupled to F_1 subunit rotation is unknown.

Several models have been proposed in which ATP synthesis in the F_1 domain is coupled to proton movement through F_0 via movements of the *c* subunits. To explore this possibility, Rastogi and Girvin examined the structural changes induced by deprotonating a specific aspartic acid (Asp 61) on the *c* subunit known to be essential for proton translocation (*Nature*; **in the press**). They combined their studies of the NMR structures of the deprotonated and protonated forms of the *c* subunit with distance constraints from crosslinking experiments to come up with a model for the c_{12} oligomer. The *a* subunit was modeled using biochemical data and then positioned with respect to the c_{12} oligomer using crosslinking data. Interestingly, this model places Asp 61 (shown in red and black) in a position to interact with Arg 210 (shown in blue and gray) in subunit *a* that is also essential for proton translocation.



The model proposes the following scenario. The flow of protons down the gradient (towards the F_1 face) would drive protonation of Asp 61. The C-terminal helix of this newly protonated monomer (green) would rotate. As a result subunit *a* would then be in a position to interact with the next (blue) monomer. This local rotation within subunit *c* would drive larger scale rotations of the c_{12} ring as a whole. Translocation of a single proton would lead to rotation of the c_{12} oligomer by 30° with respect to the static elements (for example, *a* in F_0). Because the F_1 core, which includes the ε subunit (red) is linked to the c_{12} ring it would rotate in concert with the ring. Four steps would result in the observed 120° rotation of the F_1 core that has been shown to drive the catalytic conformational changes in the active sites of F_1 that result in ATP synthesis.

Boyana Konforti

history

The discovery of chaperonins

Both the editorial in this issue of *Nature Structural Biology* and the paper on page 1132 focus on protein folding research. Here we note that our understanding of the general properties of folding reactions *in vivo* took a major step forward in the late 1980s, when research from several disparate fields revealed the widespread function of the GroEL (hsp60) related proteins, members of the class of chaperones known as the chaperonins.

GroEL is required for the correct assembly of the oligomeric structure that connects the head to the tail of λ phage. It was identified in 1972–1973 by genetic approaches, in the search for factors involved in the replication of bacteriophages[1,2] and its name reflects this history: 'Gro' stands for phage growth; 'E' indicates that the growth defect can be overcome by a mutation in the phage head gene E; and 'L' stands for 'large subunit'.

In the next decade, several lines of research converged. Research on the assembly of the multisubunit ribulose-bisphosphate carboxylase-oxygenase (Rubisco) enzyme in chloroplasts led to the discovery of the Rubisco binding protein, which assists assembly but is not part of the final structure[3]. Sequencing of the gene for this protein[4] revealed high homology (~50% identity) with GroEL and, to distinguish these as a family of proteins, they were named the chaperonins. In addition, the study of mitochondrial protein import uncovered a temperature sensitive lethal mutation in the hsp60 gene of yeast (named for 'heat shock protein' and its approximate molecular weight). These mutants could transport the test protein ornithine transcarbamoylase into mitochondria but were unable to assemble the active trimer[5]. Sequencing of the hsp60 gene[6] revealed homology to both GroEL and the Rubisco binding protein. Later, a distinct but similar family of chaperonin proteins were found in archaebacteria and eukaryotes[7], thus demonstrating the ubiquitous requirement for *in vivo* chaperonin function.

Around the same time, *in vivo* studies of mitochondrial import and subsequent folding of dihydrofolate reductase (DHFR) showed a requirement for hsp60[8]. This was particularly interesting since DHFR is a monomeric protein that can fold spontaneously without chaperonin assistance *in vitro*. Thus, this work clearly indicated that protein folding reactions *in vitro* and *in vivo* can have different characteristics, a point that is of major interest today as researchers attempt to reconcile the large amount of *in vitro* and *in vivo* folding data, much of which has accumulated in the decade since the discovery of the chaperonins.

Tracy Smith

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