Community Reference Laboratory on Marine Biotoxins, Ministerio de Sanidad y Consumo, Estación Marítima sn, 36271 Vigo, Spain
José Franco
Instituto de Investigaciones Marinas de Vigo, Apdo 1552, 36200 Vigo, Spain
Victoria López-Rodas, Eduardo Costas
Department of Genetics, Veterinary Faculty, Complutense University, 28040 Madrid, Spain

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ATP synthase's second stalk comes into focus

Almost all the adenosine triphosphate (ATP, the predominant energy-transfer mediator in living organisms) used by cells is made by ATP synthases, which have two parts known as F_1 and F_O. These enzymes are found in bacterial plasma membranes, chloroplast thylakoid membranes and mitochondrial inner membranes; they function as rotary motors, coupling nucleotide binding in the F_1 part to proton translocation by the transmembrane F_O part[1,2]. Here we present cryo-electron microscopy images showing details of the connection between the F_1 and F_O parts of the enzyme.

The bacterium *Escherichia coli* contains the simplest form of the enzyme, EC F_1F_O. It comprises eight different subunits, five in the F_1 part (α_3, β_3, γ, δ, ε) and three in the F_O part (a_1, b_2, c_{9-12}) (ref. 3). The proton pore is formed at the interface between the single-copy 'a' subunit and the 'c' subunits[4], which are arranged as a ring[5].

The γ subunit sits within a ring of alternating and hexagonally arranged α and β subunits in the F_1 part[6]. It extends from F_1 and, in association with the ε subunit, forms the 40–45-Å-long stalk that has been seen by electron microscopy to link the F_1 and F_O parts[7]. Both the γ and ε subunits bind to the c subunit ring, and the emerging mechanism of energy coupling[1,8] involves the γ, ε and c subunit ring rotating as a unit relative to the fixed $\alpha_3\beta_3$ domain of the F_1 and ab_2 domain of F_O.

Biochemical evidence supports the idea of a second connection[9] between F_1 and F_O, but this feature had not been observed directly. Previous electron microscopy studies used membranous EC F_1F_O embedded in a thin layer of ice. These studies could have missed a narrow second stalk, because the density difference between protein and solvent water is low and consequently the images are noisy, so many must be averaged to see any features clearly. Also, the membranous nature of the sample often resulted in superimposition of individual complexes in projection, making interpretation of weak features more difficult.

We used detergent-solubilized EC F_1F_O, prepared and examined after negative staining to enhance the contrast between protein and solvent. Staining with heavy metals had previously been avoided because this treatment led to release of F_1 from F_O; however, we found that reaction of the enzyme with dicyclohexylcarbodiimide helped to prevent this release.

We observed two stalks linking the F_1 to the F_O (Fig. 1) in about 40% of all images obtained. The fatter, more central stalk, which had been observed previously, contains the γ and ε subunits. The second connector is at the side of the molecule, clearly evident extending down from the F_1, and a corresponding density rises up from the F_O. This second stalk probably includes both the δ and the b subunits. Density in the middle of the stalk is weak, as would be expected if this region derives from one or two α-helices from each of the b subunits.

At the top of the F_1 is a cap that extends in the direction of the more asymmetrically placed stalk. Such a feature is lacking in the X-ray structure reported for bovine F_1 (ref. 6). It is probable that this cap is formed by the amino-terminal 30 or so residues of the α subunits — which were unresolved in the X-ray experiments — together with a part of the δ subunit.

There is also a clear asymmetry of the F_O part, consistent with a substructure in which the c subunits form a ring with the a and b subunits outside[5,10]. This asymmetry has been observed in enzyme solubilized with amphipol (Fig. 1), as well as in lysolecithin and laurylmaltoside (results not shown). So, although detergent binding to the F_O part may contribute to the effect, it is likely that the observed asymmetry is a feature of the protein.

The presence of the second stalk — which is also evident in electron micrographs of the less well-defined V_1V_O-type ATPase from another bacterium, *Clostridium fervidus*[11] — has important functional implications. It could provide the stator against which the γ–ε subunit crankshaft rotates[1]. Thus, ATP hydrolysis in one direction and ATP synthesis in the other would rotate the γ–ε–c subunit domain, thereby mechanically coupling nucleotide binding in catalytic sites with proton translocation.

Stephan Wilkens*, Roderick A. Capaldi
Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403-1229, USA
e-mail: rcapaldi@oregon.uoregon.edu
**Present address: Department of Biochemistry, University of California at Riverside, Riverside, California 92507, USA*

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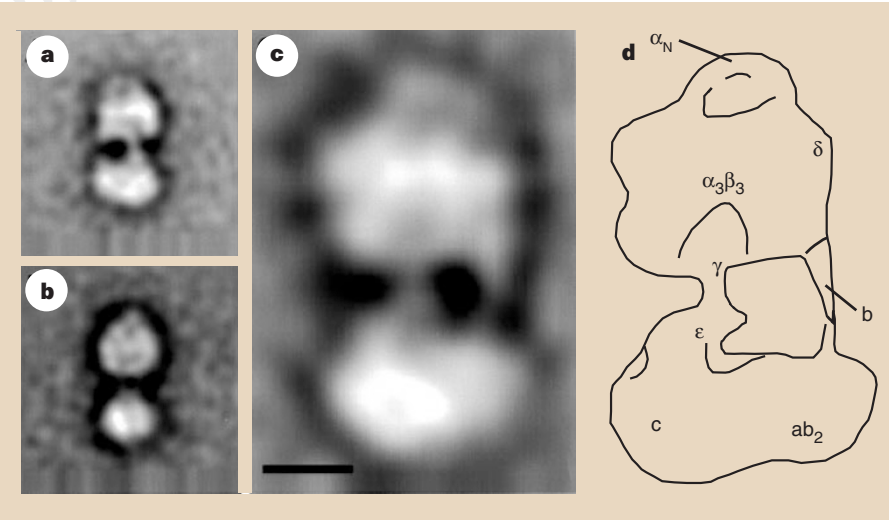

Figure 1 Electron microscopy images of EC F_1F_O in side view. **a–c,** Different views based on image analysis and classification of a data set of 139 single molecules; **a** is a mirror image of **c**; **b** appears to be a projection at around 60° to that in **a** and **c**. **d,** Our interpretation of the arrangement and composition of the second stalk, and the extra density on top of the F_1 molecule.