Stalking the mitochondrial ATP synthase: Ina found guilty by association

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In this issue, the groups of Peter Rehling and Martin van der Laan report the identification of a new protein complex that is involved in the assembly of the peripheral stalk of the yeast mitochondrial F_1F_o -ATPase (Lytovchenko *et al*, 2014). Their work sheds new light onto the biogenesis of this fascinating and important machine.

See also: O Lytovchenko *et al* (August 2014)

Mitochondrial oxidative phosphorylation produces the vast bulk of ATP in aerobic cells. The F_1F_o -ATPase (or mitochondrial ATP synthase; complex V) is a large multisubunit machine of the mitochondrial inner membrane (Fig 1). ATP is produced by the F_1F_o -ATPase by utilizing the proton gradient formed by the electron transport chain. It is comprised of two major structural regions—an F_1 -ATPase matrix module that harbors a central stalk which connects to an F_o membrane rotor domain. A peripheral stalk lies at the side of the complex and connects the F_o domain to the F_1 segment and functions as a “stator” as it is the stationary part of the motor (Devenish *et al*, 2008). The fully assembled complex is approximately 600 kDa in size and harbors 13 different subunits in yeast (Fig 1). Additional subunits are involved in the F_1F_o -ATPase assembling into higher-ordered, dimeric and ribbon-like structures at the ends of membrane invaginations found in mitochondrial cristae (Strauss *et al*, 2008). How the F_1F_o -ATPase assembles has been under investigation for some years. Owing to the genetic malleability and ability to undergo anaerobic respiration, the model yeast *Saccharomyces cerevisiae* has been used most extensively for this analysis.

Here, Lytovchenko *et al* (2014) performed functional genomic analysis to investigate novel proteins involved in maintenance of oxidative phosphorylation. It had previously been established that Ina22 (formerly YIR024c) is found in yeast mitochondria, while knockout of its gene leads to a respiration-deficient phenotype. Ina22 is imported into mitochondria where it integrates into the inner membrane via its single transmembrane anchor. Mitochondria lacking Ina22 show defects in the assembly of the ATP synthase and accumulate the F_1 matrix module. To investigate binding partners, the authors performed co-immunoprecipitation and quantitative mass spectrometry analysis, revealing that Ina22 interacts with subunits of the peripheral stalk and F_1 module while subunits of the F_o module or those involved in dimerization were not present. Interestingly, the authors also found that Ina22 associated with proteins involved in assembly of complex III. While further work is required to establish this connection, it is possible that assembly factors of various complexes form assembly factories at discrete regions within the mitochondrial inner membrane as is seen for other membrane complexes (Wiedemann *et al*, 2007).

The pull-down approach also led the authors to identify an additional uncharacterized protein that interacts with Ina22. Like Ina22, Ina17 (formerly Aim43) also contains a single transmembrane anchor and assembles at the mitochondrial inner membrane following its import into the organelle. Interestingly, both Ina17 and Ina22 contain significant regions exposed to the intermembrane space which include a predicted coiled-coil motif that is important for their interaction. Yeast cells lacking Ina17 also are respiratory deficient, and mitochondria exhibit defects

in F_1F_o -ATPase assembly. Ina22–Ina17 (collectively termed the inner membrane assembly complex, or INAC) did not associate with newly synthesized subunits of the F_o membrane module. Given that cells lacking Ina22 or Ina17 led to increased levels of the unattached F_1 module, the authors investigated whether INAC is involved in assembly of the peripheral stalk of the complex. In this case, Lytovchenko and colleagues performed *in vitro* import and assembly assays of representative F_1F_o -ATPase subunits into mitochondria isolated from Ina22 or Ina17 knockout cells. Strong assembly defects were observed for subunits of the peripheral stalk including Atp4 and Atp5. Assembly of subunits not found in the peripheral stalk were largely unaffected although the assembly of the central stalk subunits Atp3 and Atp16 were somewhat reduced.

As Ina17 and Ina22 are of low abundance relative to ATP synthase subunits and are not part of the final assembled complex, they appear to represent novel assembly factors for the peripheral stalk. The additional interaction of Ina17 and Ina22 with F_1 subunits led Lytovchenko and colleagues to propose a new model for ATP synthase assembly where the peripheral stalk and the F_1 module are brought together by INAC (Fig 1) before attaching to the F_o rotor. This model requires further work to validate as a recent study proposed that mtDNA-encoded membrane subunits of F_o assemble with subunits of the peripheral stalk and this comes together with a preassembled F_1 module (Rak *et al*, 2011). As INAC is not essential for assembly of the ATP synthase, separate assembly processes may be in operation.

Finally, it should be noted that many assembly factors of yeast F_1F_o -ATPase have not been identified in mammals and Ina17

Figure 1. Lytovchenko and colleagues propose a model for F₁–F₀ ATP synthase assembly where INAC is involved in attachment of the F₁ and peripheral stalk components to the F₀ membrane module of the ATP synthase.

Inset: Summary of yeast F₁–F₀ ATP synthase subunits with their human homologs. ^{nuc}F₁, nuclear-encoded F₁ subunits; ^{mt}F₀, F₀ subunits encoded by mitochondrial DNA; ^{nuc}F₀, nuclear-encoded F₀ subunits.

and Ina22 also appear to lack obvious homologs. These differences may stem from the ability of yeast to undergo switching from aerobic respiration to anaerobic respiration and also may be due to the fact that the c-subunit that forms the central oligomeric ring of the F₀ module is encoded by mtDNA in yeast, while in mammals, the c-subunit is encoded by a nuclear gene. Thus, assembly pathways are likely to differ in other ways. Nevertheless, as the peripheral stalk is common to the F₁F₀-ATPase, it is expected that assembly factors equivalent to INAC exist in other organisms.

Conflict of interest

The authors declare that they have no conflict of interest.

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