Have you seen?

Stalking the mitochondrial ATP synthase: Ina found guilty by association

David A Stroud & Michael T Ryan

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$_1$F$_o$-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$_1$F$_o$-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$_1$F$_o$-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$_1$F$_o$-ATPase assembly 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$_1$F$_o$-ATPase assemblies have 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 assemblies 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$_1$F$_o$-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$_1$F$_o$-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 Atp6 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 reassembled 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$_1$F$_o$-ATPase have not been identified in mammals and Ina17...
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 Fo 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 F1Fo-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.

**References**


