Cell Metabolism
Previews

Assembling Complex I with ACAD9

Immo E. Scheffler[1,*]
[1]Division of Biology, Molecular Biology Section, University of California, San Diego, La Jolla, CA 92093-0322, USA
*Correspondence: ischeffler@ucsd.edu
DOI 10.1016/j.cmet.2010.08.008

Acyl-Co dehydrogenase 9 (ACAD9) was thought to play a role in fatty acid oxidation. Nouws et al. (2010) reveal a novel and essential role for this enzyme in mitochondrial complex I assembly. A mutation in ACAD9 causes an isolated complex I deficiency in a subset of patients with mitochondrial disease.

Mitochondrial diseases due to partial deficiencies in the mitochondrial oxidative phosphorylation system have been recognized for more than 20 years, and they constitute a substantial fraction of metabolic defects responsible for neuropathies, myopathies, and related dysfunctions (Wallace et al., 2010). Among patients, those with "isolated complex I deficiencies" have presented a particular challenge because many did not carry mutations in any of the known complex I structural genes. The function of complex I, or NADH-ubiquinone oxidoreductase, is to reoxidize NADH produced from the Krebs cycle, and it also uses the free energy released from this oxidation to pump protons out of the mitochondrial matrix to establish the proton gradient that drives ATP synthesis. The mammalian complex I contains 45 subunits, of which 38 are encoded by nuclear genes and 7 by mitochondrialDNA (mtDNA). The analysis of these structural genes in a substantial number of patients with isolated complex I deficiencies has not revealed any obvious mutations, strongly suggesting that factors encoded by other genes, commonly referred to as assembly factors (or chaperones), must be involved in the biogenesis and assembly of a fully functional complex I.

In this issue, Nouws et al. (2010) identify Acyl-Co dehydrogenase 9 (ACAD9) as a novel assembly factor critical for complex I biogenesis and establish that a defect in this factor is responsible for the exercise intolerance, cardiomyopathy, lactic acidosis, and related symptoms in two unrelated patients with isolated complex I deficiency. ACAD9 had previously been cloned and identified as a member of the acyl-CoA dehydrogenase family, exhibiting such enzymatic activity in vitro (Zhang et al., 2002). One prior publication on patients with ACAD9 mutations had described a new genetic disorder in mitochondrial fatty acid oxidation (He et al., 2007). Noews et al. found that, in vivo, however, ACAD9 does not have significant β oxidation activity or function and instead plays an essential role in the assembly of functional complex I.

The initial attention on ACAD9 was the result of a successful "fishing expedition" using the known assembly factors NDUFAF1 and Ecsit as "baits" in coprecipitation experiments (Vogel et al., 2007a). The proposed interactions were confirmed by mitochondrial membrane fractionations and colocalization of this protein with assembly intermediates (Vogel et al., 2007a, 2007b). Knockdown by RNA interference showed not only the role of these proteins in complex I assembly, but also a role for ACAD9 in affecting NDUFAF1 and Ecsit protein levels, with NNDUFAF1 knockdown affecting ACAD9 levels. Complex formation between these three proteins, perhaps in combination with complex I subunits, may therefore be required for their stabilization and accumulation.

Though the participation of these assembly factors in complex I biogenesis is becoming well established, and their association with characterized assembly intermediates has been demonstrated, much remains to be learned. Although they are essential for assembly, they are not present in the final active complex. Thus, there must be a mechanism for their release and recycling. This raises another question, as the mammalian complex I contains 31 subunits that are referred to as "supernumerary" or "accessory," but no definite function has been assigned to any of them. They have been speculated to function in assembly, in stabilization of complex I, in its incorporation into supercomplexes, in its activity, or perhaps in its involvement with other metabolic pathways. It should be kept in mind that the distinction between an assembly factor and a subunit may be artificially created by the conditions used for solubilizing the complex from intact mitochondria.

Unexpectedly, ACAD9 is closely related to the well-characterized very long-chain fatty acid dehydrogenase enzyme (VLCAD), with both being membrane associated and localized to the mitochondria. Extensive sequence homology with VLCAD and a phylogenetic analysis suggest that ACAD9 arose from a gene duplication of the ACADVL gene (encoding VLCAD) at the time of the origin of vertebrates. From molecular modeling and a comparison with the known crystal structure of VLCAD, it is suggested that sequence changes in ACAD9 are associated with two external α helices in the ACAD9 dimer that might represent new interaction domains important for its recently acquired role in complex I assembly. Further investigation should examine whether ACAD9 has any role at all in fatty acid metabolism in vivo.

The crystal structure of the prokaryotic complex I, including its peripheral membrane and integral membrane domains, has recently been published, and it is fully functional with the 14 subunits that constitute the "core" of the mammalian complex I (Efremov et al., 2010). Describing a detailed function for the assembly factors and for the other 35 subunits remains a future challenge. The fact that they are essential has been demonstrated in several cases by studies with mutants in tissue culture (Scheffler et al., 2004) and by the existence of patients suffering isolated complex I deficiencies (Potluri et al., 2009; Fernandez-Moreira et al., 2007). The studies with fibroblasts from patients and cell culture studies clearly establish ACAD9's function as a

complex I assembly factor in vertebrates; it should be noted, however, that similar multisubunit complex I structures exist in most eukaryotic mitochondria, and ACAD9 is absent in all lineages below vertebrates.

More than 50 years ago at the dawn of molecular biology, Salvador Luria stated that "it is always better to have a mutant." In our time, patients with genetic diseases pose such a challenge. The successful molecular genetic characterization of such patients nowadays facilitates a definitive diagnosis, offers the possibility of a cure or amelioration, and provides a solid foundation for genetic counseling. The study by Nouws and colleagues (2010) is also a splendid illustration of how the analysis of patients/mutants can provide fundamental new insights into very basic phenomena in biology, specifically in the context of mitochondrial disease.

REFERENCES

Efremov, R.G., Baradaran, R., and Sazanov, L.A. (2010). Nature *465*, 441–445.

Fernandez-Moreira, D., Ugalde, C., Smeets, R., Rodenburg, R.J., Lopez-Laso, E., Ruiz-Falco, M.L., Briones, P., Martin, M.A., Smeitink, J.A., and Arenas, J. (2007). Ann. Neurol. *61*, 73–83.

He, M., Rutledge, S.L., Kelly, D.R., Palmer, C.A., Murdoch, G., Majumder, N., Nicholls, R.D., Pei, Z., Watkins, P.A., and Vockley, J. (2007). Am. J. Hum. Genet. *81*, 87–103.

Nouws, J., Nijtmans, L., Houten, S., van den Brand, M., Huynen, M., Venselaar, H., Hoefs, S., Gloerich, J., Kronick, J., Hutchin, T., et al. (2010). Cell Metab. *12*, this issue, 283–294.

Potluri, P., Davila, A., Ruiz-Pesini, E., Mishmar, D., O'Hearn, S., Hancock, S., Simon, M., Scheffler, I.E., Wallace, D.C., and Procaccio, V. (2009). Mol. Genet. Metab. *96*, 189–195.

Scheffler, I.E., Yadava, N., and Potluri, P. (2004). Biochim. Biophys. Acta *1659*, 160–171.

Vogel, R.O., Janssen, R.J., van den Brand, M.A., Dieteren, C.E., Verkaart, S., Koopman, W.J., Willems, P.H., Pluk, W., van den Heuvel, L.P., Smeitink, J.A., and Nijtmans, L.G. (2007a). Genes Dev. *21*, 615–624.

Vogel, R.O., Smeitink, J.A., and Nijtmans, L.G. (2007b). Biochim. Biophys. Acta *1767*, 1215–1227.

Wallace, D.C., Fan, W., and Procaccio, V. (2010). Annu. Rev. Pathol. *5*, 297–348.

Zhang, J., Zhang, W., Zou, D., Chen, G., Wan, T., Zhang, M., and Cao, X. (2002). Biochem. Biophys. Res. Commun. *297*, 1033–1042.