Published in final edited form as:

Science. 2017 October 20; 358(6361): 307–308. doi:10.1126/science.aap9299.

A biochemical sulfur delivery service:

A steady supply of iron-sulfur clusters sustains biosynthesis of the lipoyl cofactor

Amy C. Rosenzweig

Departments of Molecular Biosciences and Chemistry, Northwestern University, Evanston, IL 60208, USA

A number of metabolically important enzyme complexes depend on lipoic acid as an essential cofactor. Lipoic acid consists of a chain of eight carbon atoms, terminated at one end by a carboxylate group and at the other by two sulfur atoms appended to the carbons in the sixth and eighth positions. The sulfur atoms can exist as a disulfide-bonded ring or as free thiols. Once attached to a lipoyl carrier protein in an enzyme complex (see the figure), the lipoyl cofactor “arm” roves around, exploiting the redox activity of the sulfurs to facilitate decarboxylation reactions (1). Deficiencies in the two-step process by which cells make this lipoyl cofactor have been linked to severe and often fatal genetic diseases (2). On page 373 of this issue, McCarthy and Booker now report insights into lipoyl cofactor biosynthesis that resolve a major conundrum (3).

In the first step of lipoyl cofactor biosynthesis, the enzyme octanoyltransferase, or LipB, transfers the eight-carbon *n*-octanoyl group from an acyl carrier protein to a lipoyl carrier protein. In the second step, another protein, lipoyl synthase or LipA, attaches the two sulfur atoms (1, 4). LipA is a radical *S*-adenosylmethionine (SAM) enzyme, an enzyme family that uses a [4Fe-4S] cluster to generate 5'-deoxyadenosyl radicals. Two such radicals in LipA abstract hydrogen atoms from C6 and C8 of the *n*-octanoyl group for sulfur insertion (5). LipA also contains a second, or auxiliary, [4Fe-4S] cluster that is believed to provide the two sulfur atoms and fall apart during the reaction (6, 7).

This model is supported by experimental evidence. It has remained unclear, however, how LipA can be an enzyme if it is destroyed in a single reaction. McCarthy and Booker now show that another protein, NfuA, delivers a new [4Fe-4S] cluster to LipA and does so fast enough as to not hinder the rate of lipoyl cofactor biosynthesis.

NfuA is a dimeric protein that contains a single [4Fe-4S] cluster (8). It belongs to an alphabet soup of proteins implicated in assembly of Fe-S clusters in vivo. Fe-S clusters can be synthesized readily in vitro, but cellular biosynthesis requires their preparation on scaffold proteins followed by transfer to target proteins and enzymes (9). McCarthy and Booker zeroed in on NfuA because mutations in its human homolog, NFU1, cause severe mitochondrial dysfunction (2), and deletion of the yeast homolog leads to lowered lipoic acid loading of metabolic enzymes, suggesting a role in LipA cluster repair (10). In a typical LipA reaction, the amount of lipoyl product formed is equal to the amount of LipA used (single turnover) because the auxiliary [4Fe-4S] cluster in LipA is sacrificed to provide sulfur for the product (6, 11, 12). However, when the authors added NfuA to the reaction,

much more product was formed, and the reaction became catalytic. Thus, it appears that NfuA can restore the [4Fe-4S] cluster in LipA.

McCarthy and Booker were able to track the pathway of sulfur transfer from NfuA to lipoyl product by using NfuA in which the sulfurs in its [4Fe-4S] cluster were isotopically labeled. These data are consistent with cannibalization of the LipA cluster, followed by [4Fe-4S] cluster transfer from NfuA to LipA for formation of additional lipoyl product. The results suggest that all four LipA sulfurs in the auxiliary cluster, not just two as proposed previously (5), can be used to generate lipoyl product.

The results from two additional experiments indicate that the sulfur is directly transferred from NfuA to LipA in the form of an Fe-S cluster, rather than released into solution and then assembled into the LipA cluster. First, addition of citrate, which chelates iron in solution, did not affect LipA activity. Second, labeled sulfide added to the reaction did not end up in the product, consistent with direct transfer. The results of these experiments, combined with the observation that LipA and NfuA can form a protein-protein complex, albeit between monomers rather than with the NfuA [4Fe-4S]-containing dimer (3), support direct cluster transfer between the two proteins.

NfuA cannot be solely responsible for restoring LipA in *Escherichia coli*. In humans, mutations in NFU1 are fatal (2), but McCarthy and Booker found that *E. coli* grow normally without NfuA. Therefore, there must be some other way to rebuild the cluster. Angelini *et al.* have previously shown that combined disruption of both the NfuA gene and the gene encoding the Fe-S cluster assembly scaffold protein IscU diminishes growth (8). McCarthy and Booker investigated whether IscU can fill in for NfuA. Using sulfur-labeling studies, they show that IscU containing a [4Fe-4S] cluster can also enhance the LipA reaction. It remains to be shown how the efficiencies of transfer compare for the two proteins.

The demonstration that NfuA and IscU can reinstall the LipA Fe-S cluster after each turnover explains how Fe-S clusters can serve as a sulfur source in catalytic reactions and dispels controversy surrounding this mechanism (13). It is remarkable that an Fe-S cluster can be destroyed and repaired rapidly enough as to not impede catalysis. This strategy is likely applicable to related radical SAM enzymes that contain auxiliary clusters, including biotin synthase, for which a similar mechanism has been proposed (14).

A number of questions remain unresolved, however. Further verification that NfuA transfers an Fe-S cluster to LipA, rather than directly providing sulfur for the lipoyl product, is desirable. In addition, it is unclear how NfuA and IscU recognize LipA or other targets that they supply with Fe-S clusters. It may be that in vivo, chaperones, which did not enhance the reaction studied here (3), play a role in guiding these processes (12). Finally, a major question for this system, and for Fe-S assembly proteins in general, is how the clusters are moved from scaffold to target protein. The molecular details of how the cluster dissociates from one protein and is transferred to another remain to be elucidated.

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Figure. Rapid restoration of an iron-sulfur cluster

Biosynthesis of the lipoyl cofactor by the enzyme LipA involves insertion of two sulfur atoms into an *n*-octanoyl group attached to a lipoyl carrier protein (LCP).