A missed Fe-S cluster handoff causes a metabolic shakeup

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The general framework of pathways by which iron–sulfur (Fe-S) clusters are assembled in cells is well-known, but the cellular consequences of disruptions to that framework are not fully understood. Crooks *et al.* report a novel cellular system that creates an acute Fe-S cluster deficiency, using mutants of ISCU, the main scaffold protein for Fe-S cluster assembly. Surprisingly, the resultant metabolic reprogramming leads to the accumulation of lipid droplets, a situation encountered in many poorly understood pathological conditions, highlighting unanticipated links between Fe-S assembly machinery and human disease.

Since their discovery more than 50 years ago, iron–sulfur (Fe-S) clusters have been shown to play essential functions in a vast array of proteins, reflecting their ancient origin and functional versatility (1). Fe-S clusters in proteins exist as various combinations of iron and sulfur atoms, including cubane-type [4Fe-4S] clusters, [2Fe-2S] and [3Fe-4S], as well as the [8Fe-7S] clusters found in nitrogenases from *Azotobacter vinelandii*. Fe-S clusters are typically bound by cysteinyl ligands, although other amino acid residues have been reported to be involved in Fe-S cluster coordination or stabilization (2). In cells, Fe-S clusters serve as conduits for electron transport in photosynthetic and respiratory complexes, sensors for iron and oxygen (3), storage sites for iron and sulfur, and as catalytic centers in radical SAM and other enzymes (4, 5).

Because of these essential functions, efforts to study the consequences of disruption to the Fe-S cluster assembly pathways have been stymied. A new study by Crooks *et al.* (6) helps to solve this problem with the report of an inducible transgenic cell line, which led to the surprising discovery that impairment of Fe-S cluster assembly provoked a major metabolic rerouting, resulting, notably, in the accumulation of lipid droplets in cells.

Fe-S clusters can spontaneously assemble in solution, but Fe-S cluster assembly in cells, although not fully understood, is a tightly controlled and well-orchestrated process. In prokaryotes, several Fe-S cluster biogenesis machineries, each involving multiple proteins, have been identified, including nitrogen fixation (NIF), sulfur utilization factor (SUF), and iron–sulfur cluster (ISC)[2] systems (7). A functionally related ISC system exists in yeast and mammals, but the presence of organelles complicates the situation (3). For instance, in addition to the mitochondrial ISC system, maturation of cytosolic and nuclear Fe-S proteins requires the cytosolic Fe-S protein assembly (CIA) machinery (7) and other components for Fe-S cluster trafficking. Furthermore, some evidence suggests the possible *de novo* synthesis of Fe-S clusters in the cytosolic and nuclear compartments (3).

One key step in the biogenesis of Fe-S clusters, in both prokaryotes and eukaryotes, is their assembly onto an intermediate scaffold protein called iron–sulfur cluster assembly enzyme (ISCU) prior to their ATP-dependent transfer to recipient proteins. Thus, ISCU is a key target for scientists seeking to understand the outcomes of Fe–S cluster assembly disorders. However, in agreement with its central function, complete loss of ISCU activity leads to acute Fe-S cluster deficiency, a condition that is lethal for all organisms (8). How then can we investigate the effects of ISCU deficiency and acute loss of Fe-S proteins on cellular physiology?

To address this tricky question, Crooks *et al.* (6) devised a clever approach inspired by pioneer studies on ISCU from *A. vinelandii*, a model organism for [Fe-S] cluster biogenesis (1). Early studies have shown that a conserved aspartate residue (Asp-37 in *A. vinelandii*) adjacent to one of the cysteine residues ligating the Fe-S cluster is responsible for releasing ISCU's nascent Fe-S cluster to recipient proteins (2). The authors thus changed the corresponding residue (Asp-71) in the human ISCU homolog to obtain a mutant scaffold with a more stably bound Fe-S cluster that should not be transferable. In addition, they constructed another mutant in which one of the coordinating cysteine residues (Cys-69) was mutated, with the aim of preventing Fe-S cluster formation on ISCU. These two mutants were each integrated into a human cell line (HEK293 Flp-In™ cells) under a doxycycline-controlled promoter, which tightly repressed their expression.

When doxycycline was added to the cell medium, growth was halted, but no significant cell death was measured, suggesting a cytostatic effect of the mutations. Immunoblot and immunoprecipitation analyses showed that the expression levels and binding of NFS1, ISD11, and frataxin, three well-known binding partners of ISCU, were not changed compared with the WT ISCU, demonstrating that the mutants have not lost their interaction properties. However, EM revealed dramatic morphological alterations in mitochondria, and two reporter systems indicated that the cells were experiencing iron starvation. One of these reporter systems reads out the activity of aconitase, which interconverts citrate and isocitrate dependent on the correct insertion of an Fe-S cluster; this readout clearly showed a loss

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[2] The abbreviations used are: ISC, iron–sulfur cluster; LIAS, lipoic acid synthase.

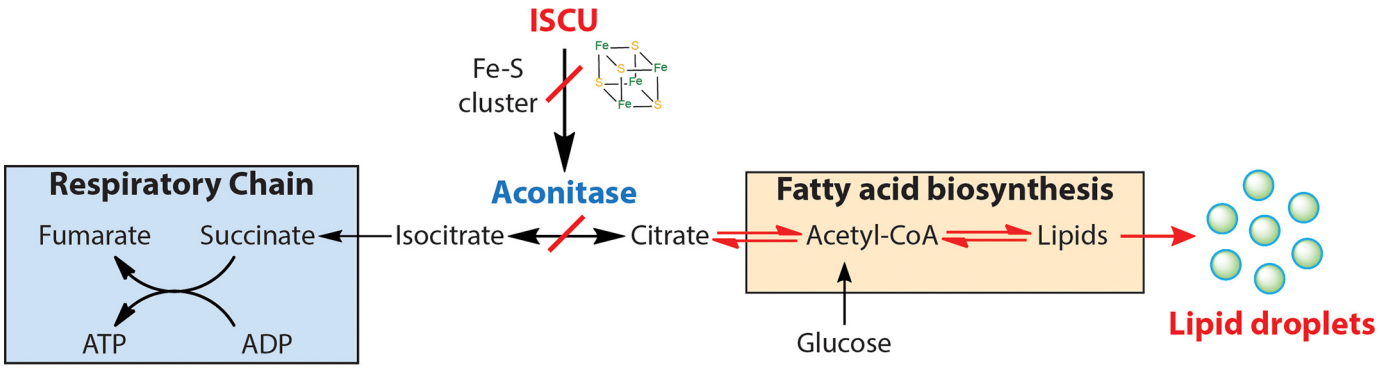

Figure 1. ISCU deficiency leads to major reprogramming of cellular metabolism. Crooks *et al.* (6) discover that ISCU-induced Fe-S cluster deficiency diverts cellular carbon flux toward fatty acid biosynthesis, a condition leading to lipid droplet accumulation (pathway highlighted in *red*) as encountered in several human pathologies.

of both mitochondrial and cytosolic aconitase activities. A broader analysis including the radical SAM enzyme lipoic acid synthase (LIAS) (9) revealed a decrease in LIAS abundance upon expression of the ISCU mutants. Because lipoate, the product of LIAS, is crucial for several mitochondrial enzymes, Crooks *et al.* (6) also analyzed the content of lipoylated lysine residues in mitochondrial proteins. Consistent with the decrease in LIAS expression, the authors measured a decrease in lipoylation but not necessarily a reduced expression of known lipoylated proteins, similar to what is observed in some human pathologies linked to a defect in Fe-S cluster assembly (3).

Given that citrate is a central metabolite in several biochemical pathways, the authors performed metabolic analysis to gauge the impact of these profound changes in protein expression and enzyme activity. They observed major consequences: an 11-fold increase in levels of citrate, the inhibition of glycolysis (likely through citrate-mediated inhibition of the kinase PFK-1), a decrease in total cellular AMP and ADP levels, and the shunting of glucose 6-phosphate into the pentose phosphate pathway, a route that does not require Fe-S cluster–dependent enzymes. Another metabolic pathway that is independent of iron or Fe-S clusters is the fatty acid biosynthesis pathway, which can also use the now abundant citrate as a substrate. As the authors elegantly showed, *de novo* fatty acid biosynthesis was indeed induced, and, as visible consequence, cytosolic lipid droplets accumulated (Fig. 1) (10).

Collectively, the study by Crooks *et al.* (6) strongly supports that ISCU is crucial for the function of Fe-S proteins in all cellular compartments. It also illustrates how acute Fe-S cluster deficiency impacts metabolism, leading to profound metabolic reprogramming with dramatic cellular consequences. Unexpectedly, one of the major findings was that the carbon flux is diverted to iron-independent pathways with the notable induction of *de novo* fatty acid biosynthesis. More broadly, these findings hold significance for the biochemical and medical communities because the cellular phenotype induced mirrors pathological conditions encountered, for example, in nonadipose tissues such as heart and liver with possible connections to Friedreich's ataxia, nonalcoholic fatty liver disease, and nonalcoholic steatohepatitis. Further research at the intersection between Fe-S cluster biogenesis and cellular metabolism is thus more than likely to bring unexpected insights into the pathogenesis of poorly understood diseases.

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