

Metabolic regulation of citrate and iron by aconitases: role of iron–sulfur cluster biogenesis

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Abstract Iron and citrate are essential for the metabolism of most organisms, and regulation of iron and citrate biology at both the cellular and systemic levels is critical for normal physiology and survival. Mitochondrial and cytosolic aconitases catalyze the interconversion of citrate and isocitrate, and aconitase activities are affected by iron levels, oxidative stress and by the status of the Fe–S cluster biogenesis apparatus. Assembly and disassembly of Fe–S clusters is a key process not only in regulating the enzymatic activity of mitochondrial aconitase in the citric acid cycle, but also in controlling the iron sensing and RNA binding activities of cytosolic aconitase (also known as iron regulatory protein IRP1). This review discusses the central role of aconitases in intermediary metabolism and explores how iron homeostasis and Fe–S cluster biogenesis regulate the Fe–S cluster switch and modulate intracellular citrate flux.

Keywords Iron–sulfur cluster biogenesis · Aconitase · Citrate metabolism · Iron metabolism

Introduction

Iron–sulfur (Fe–S) proteins constitute one of the most ubiquitous and functionally versatile classes of metalloproteins (Beinert et al. 1997; Johnson et al. 2005). More than 120 distinct types of proteins contain Fe–S clusters and many Fe–S proteins are involved in fundamental processes such as respiration, photosynthesis, intermediary metabolism and nitrogen fixation. The citric acid cycle enzyme aconitase contains a [4Fe–4S] cluster and the enzymatic reaction catalyzed by aconitase involves substrate coordination to a specific iron atom in this cluster (Beinert et al. 1996). Two aconitase isozymes are present in mammalian cells: the mitochondrial enzyme (m-aconitase) that functions in the citric acid cycle, and the bifunctional cytosolic enzyme (c-aconitase/IRP1) which also plays a role in the regulation of iron metabolism. Moreover, recent studies in yeast suggested that mitochondrial aconitase is also essential for mtDNA maintenance and this activity is independent of its enzymatic activity (Chen et al. 2005). Thus, modulation of the aconitases can impact a wide spectrum of cellular activities under both normal and pathophysiological conditions. Here, we will present an overview of the physiological roles of mitochondrial aconitase and cytosolic aconitase/IRP1, and review recent findings related to the biogenesis/repair of Fe–S clusters that have shed

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new lights on the regulation of aconitase functions and emphasized the intricate links between iron and citrate metabolism.

Cellular and systemic citrate metabolism

Citrate in energy metabolism pathways

Aconitase is best known for its function in catalyzing the reversible isomerization of tricarboxylic acids—citrate, cis-aconitate, and isocitrate (Breusch 1937). Citrate is a key intermediate in several major pathways of energy and intermediary metabolism (Fig. 1). In the mitochondria, citrate is an intermediate in the citric acid cycle, which converts acetyl-CoA to two molecules of CO_2 with concomitant generation of NADH and FADH_2 . Reoxidation of NADH and FADH_2 via the electron transport chain yields ATP. Citric acid cycle flux is in part controlled by the activity of isocitrate dehydrogenase through

allosteric inhibition by ATP and product inhibition by NADH (Lawlis and Roche 1980). Thus, when the need for ATP synthesis is low, citrate accumulates and can be transported across the inner mitochondrial membrane via the tricarboxylate carrier (Bisaccia et al. 1989). In the cytosol, citrate is the substrate for ATP-citrate lyase, which generates acetyl-coenzyme A (acetyl-CoA), the building block for cholesterol and fatty acid biosynthesis in liver and adipose tissue. Citrate is also metabolized via cytosolic aconitase and cytosolic NADP^+ -dependent isocitrate dehydrogenase to generate α -ketoglutarate. This process reduces NADP^+ to NADPH, which is an essential cofactor for many enzymatic reactions involved in glutathione metabolism and lipid and cholesterol biosynthesis (Koh et al. 2004; Minard and McAlister-Henn 2005).

In addition to being an intermediate, citrate also has regulatory roles in glycolysis, fatty acid

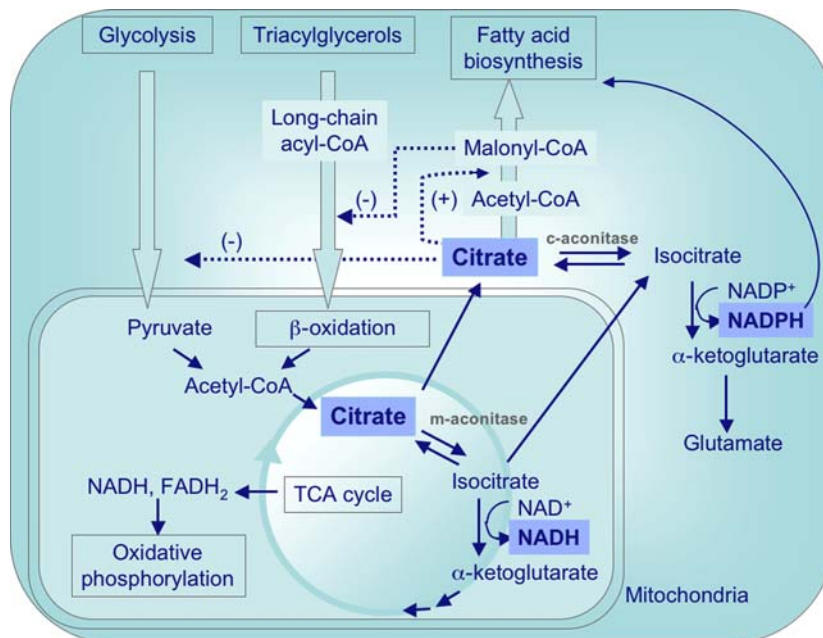


Fig. 1 Overview of the roles of citrate and aconitases in the major energy metabolism pathways. Citrate is a key intermediate that interconnects the metabolic pathways of the citric acid cycle and oxidative phosphorylation in the mitochondria to glycolysis and fatty acid synthesis in the cytosol. When the need for ATP synthesis is high, citrate is metabolized through the citric acid cycle to generate NADH and FADH_2 , the reducing equivalents needed for ATP production. When the need for ATP synthesis is low, citrate is exported into the cytosol and channeled into fatty

acid biosynthesis for energy storage. Inhibition of aconitases due to iron deficiency or oxidative damage to the Fe-S cluster could decrease ATP production, promote fat accumulation, decrease glycolysis, and decrease fatty acid oxidation. In addition, decreased citrate flux through c-aconitase may decrease production of cytosolic NADPH, a major source of reducing equivalents for fatty acid synthesis and an important defense against cytosolic oxidative stress

synthesis and oxidation. Citrate is a negative regulator of the glycolytic enzyme phosphofructokinase (Denton and Randle 1966; Randle 1998). Since citrate efflux from mitochondria occurs when ATP and NADH are high, glycolysis would be repressed. Citrate is also an allosteric activator of acetyl-CoA carboxylase, the enzyme that generates malonyl-CoA (Munday 2002). In turns, malonyl-CoA is a potent allosteric inhibitor of carnitine palmitoyltransferase-1 (McGarry et al. 1977), which controls the transfer of long chain acyl-CoA into mitochondria, the site of fatty acid oxidation (Saha and Ruderman 2003). An increase in malonyl-CoA concentration inhibits fatty acid β -oxidation and diverts acetyl-CoA into lipogenesis for energy storage or membrane assembly. Conversely, a decrease in malonyl-CoA level directs long chain acyl-CoA into the β -oxidation pathway in the mitochondria. Thus, through its complex biological effects on malonyl-CoA production, glucose utilization, fatty acid synthesis and oxidation, citrate biology can affect the pathophysiology of obesity, insulin resistance and diabetes (Belfiore and Iannello 1998; Wolfgang and Lane 2006).

In multicellular organisms, the complex network of processes involved in energy metabolism are distributed among different subcellular sites as well as in different organs of the body. Thus, the mediated membrane transport and systemic trafficking of citrate also plays an essential metabolic role. The mitochondrial tricarboxylate carrier activity that mediates citrate export is low in heart and high in liver, reflecting the high ATP demand in heart and the cytosolic location of fatty acid biogenesis in liver (Sluse et al. 1971; LaNoue and Schoolwerth 1979). Furthermore, the mitochondrial tricarboxylate carrier is repressed in type I diabetes (Kaplan et al. 1990) and during starvation (Siculella et al. 2002), and enhanced during hyperthyroidism (Paradies and Ruggiero 1990). Serum contains significant levels of citrate (~0.1 mM), and the serum levels of citrate varies appreciably between feedings and fastings (Hodgkinson 1963). Recent studies in *Drosophila* showed that decreased expression of a plasma membrane citrate transporter *Indy* resulted in decreased lipid content and increased life span (Rogina et al. 2000; Knauf et al. 2006). Functional

knockdown of *ceNAC-2*, the *Indy* orthologue in *C. elegans*, also led to decreased body size, decreased fat content, and a significant increase in life span (Fei et al. 2004). The presence of the mammalian *orthologue* NaC2 (formerly know as NaCT) in liver and in brain is consistent with the uptake of serum citrate for the synthesis of fatty acid acids and cholesterol in liver, and for energy synthesis in liver and brain (Inoue et al. 2002). Taken together, these studies indicated that systemic citrate homeostasis is of great importance for an organism's health and survival.

Citrate in specialized cells

In cholinergic neurons, citrate can be used to generate acetyl-CoA for acetylcholine synthesis. In the central nervous system and the retina, aconitase is important for the metabolic pathway that generates glutamate, the major excitatory neurotransmitter (McGahan et al. 2005). It is also reported that, due to the absence of pyruvate carboxylase, neurons lack the capacity to perform de novo synthesis of glutamate from glucose and are therefore dependent on a supply of glutamate and citric acid cycle intermediates synthesized in astrocytes (Hertz and Zielke 2004). Studies in primary cultures indicated that citrate is synthesized and released from astrocytes (Sonnewald et al. 1991), and plasma membrane Na^+ -coupled citrate transporters have been shown to be expressed in the brain (Inoue et al. 2002; Wada et al. 2006).

In the kidney, citrate is an important inhibitor of urinary stone formation (Caudarella et al. 2003), and regulation of renal cortical m-aconitase has been shown to affect urinary citrate excretion (Melnick et al. 1998). Citrate secretion requires that the rate of citrate synthesis exceed the rate of citrate oxidation via the citric acid cycle. Thus, in normal citrate-secreting prostate epithelial cells, m-aconitase expression is hormonally suppressed (Costello and Franklin 2002). Elevated zinc levels can also inhibit m-aconitase (Costello et al. 1997) and may be responsible for the impaired citrate oxidation observed in normal prostate epithelial cells (Dakubo et al. 2006). Conversely, in prostate cancer, normal citrate-secreting epithelial cells are metabolically transformed to malignant citrate-

oxidizing cells, and the depletion of zinc in malignant cells is thought to be an important factor in this metabolic transformation (Singh et al. 2006).

Citrate as a chelator of divalent metal ions

Citrate can chelate divalent cations such as Fe^{2+} , Ca^{2+} , and Zn^{2+} , and various studies have indicated that citrate has complex functions in the homeostasis of these divalent metal ions. Serum citrate is thought to be one of the carriers of non-transferrin-bound iron (Grootveld et al. 1989) that can contribute to hepatic iron loading in hemochromatosis (Chua et al. 2004). Although it has been suggested that cytoplasmic citrate might be needed for transport of iron into mitochondria (Melefors and Hentze 1993), in vitro experiments and yeast genetic studies have shown that citrate–iron complexes (Martin 1986; Pierre and Gautier-Luneau 2000) can promote autooxidation of ferrous iron and may contribute to iron-dependent toxicity (Chen et al. 2002). Serum citrate can also effect anticoagulation by binding Ca^{2+} and rendering Ca^{2+} unavailable to the clotting cascade (Weinstein 2001). Systemic hypocalcemia due to chelation of Ca^{2+} by citrate can depress cardiac function by causing hypotension, decreased cardiac output, cardiac arrest and muscle tetany (Bosakowski and Levin 1986; Dzik and Kirkley 1988). In addition, the relatively high concentration (0.4 mM) of citrate in the cerebrospinal fluid (Bell et al. 1987) and the ability of citrate to chelate Zn^{2+} , Ca^{2+} and Mg^{2+} are thought to play a role in modulating the activity of *N*-methyl-D-aspartate (NMDA) receptors and neuronal excitability (Westergaard et al. 1995).

Cytosolic aconitase in the regulation of iron metabolism

Biochemical and phylogenetic studies have suggested that during the evolution of the aconitase family, an early gene duplication allowed cytosolic aconitase to evolve independently from mitochondrial aconitase, and a second duplication of the cytosolic aconitase produced two cytosolic homologues in animals, which subsequently acquired the ability to bind to RNAs containing

the iron responsive element (IRE) (Gruer et al. 1997). Hence, unicellular eukaryotes such as *Saccharomyces cerevisiae* (Regev-Rudzki et al. 2005) and protozoan parasites such as *Trypanosoma brucei* (Saas et al. 2000) contain a single aconitase gene which encodes isozymes that function in the cytosol and in the mitochondria, whereas multicellular eukaryotes have separate genes that encode m- and c-aconitases (Kennedy et al. 1992; Muckenthaler et al. 1998). In the case of *C. elegans*, the c-aconitase has no RNA-binding activities (Gourley et al. 2003), whereas in *Drosophila*, one of the two c-aconitases also functions as an RNA-binding, iron regulatory protein (Lind et al. 2006). In mammalia, IRP1 switches between aconitase and RNA-binding functions, whereas IRP2 has no aconitase activity and functions solely as an RNA-binding protein (Fig. 2) (Pantopoulos 2004).

Iron is a vital micronutrient, but excess free iron is cytotoxic and misregulation of iron homeostasis can contribute to a number of hematological, metabolic and neurodegenerative diseases (Pietrangelo 2003; Hentze et al. 2004; Napier et al. 2005). In mammalian cells, intracellular iron homeostasis is largely achieved by the iron-dependent regulation of transferrin receptor (TfR) and iron storage protein ferritin. The mRNAs of TfR and ferritin contain IREs in their untranslated regions. In iron-depleted cells, binding of the IRP1 and IRP2 to the IREs leads to stabilization of the TfR mRNA and translational inhibition of ferritin mRNAs, resulting in increased TfR-dependent iron uptake and decreased iron sequestration into ferritin. Conversely, decreased IRP/IRE interactions in iron-replete cells results in TfR mRNA degradation and ferritin translation. The list of IRE-containing transcripts also includes the iron transporter ferroportin (Abboud and Haile 2000; Donovan et al. 2000; McKie et al. 2000) and one of the spliced forms of the divalent metal transporter DMT1 (Gunshin et al. 1997), although the exact role for IRPs in the regulation of these proteins is still under investigation. In addition to proteins involved in iron homeostasis, the identification and characterization of IREs in the transcripts of erythrocyte-specific aminolevulinate synthase (Cox et al. 1991; Zoller et al. 2002; Cooperman

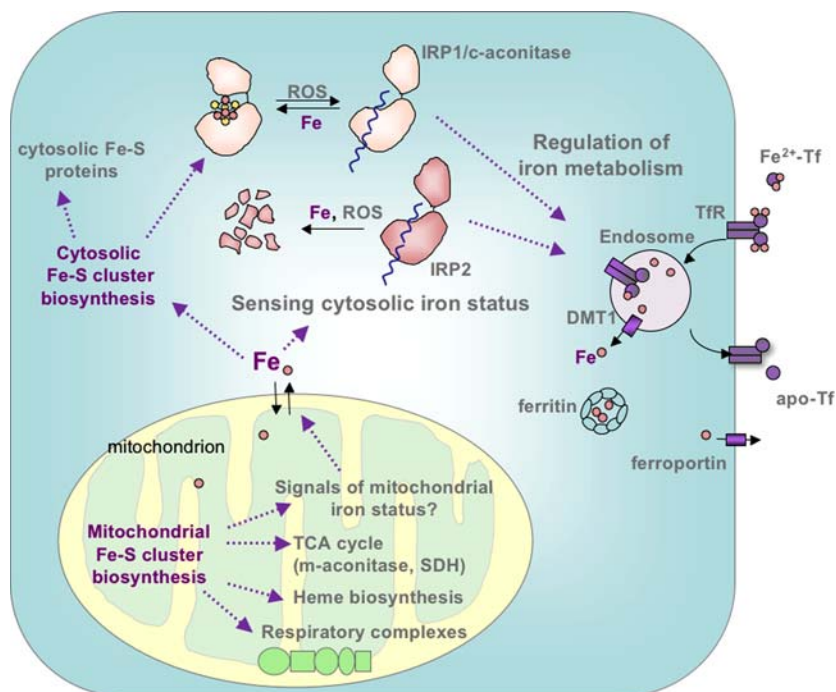


Fig. 2 Regulation of intermediary metabolism and intracellular iron metabolism by iron and Fe–S cluster biogenesis in the cytosol and in the mitochondria of mammalian cells. In the mitochondria, the citric acid cycle, respiration complexes, and heme biosynthesis are all dependent on iron availability and Fe–S cluster biogenesis. In addition, Fe–S cluster biogenesis is a key factor in the regulation of iron homeostasis in both the cytosol and the mitochondria. In most tissues, due to low oxygen concentration and efficient Fe–S cluster assembly in IRP1, IRP2 assumes a greater role in regulating intracellular iron homeostasis, although the substantial pool of latent IRE-

binding activity of IRP1 may be activated by oxidative stress or decreased Fe–S cluster biogenesis. In the mitochondria, decreased Fe–S cluster biogenesis results in mitochondrial iron accumulation and cytosolic iron depletion, leading to activation of IRE-binding by IRP1 and IRP2, which can result in altered expressions of TfR, ferritin, DMT1 and iron exporter ferroportin. In addition to facilitating compartmentalization of iron homeostasis, the spatial separation of Fe–S cluster assembly in m- and c-aconitase may also allow cells to perform anabolic and catabolic processes in a finely coordinated and efficient fashion (see Fig. 1)

et al. 2005), mammalian mitochondrial aconitase (Kim et al. 1996; Ross and Eisenstein 2002) and insect succinate dehydrogenase (SDH) (Kohler et al. 1995) indicated that the function of IRP/IRE regulatory system extends to the control of heme biosynthesis and citric acid cycle.

Although IRP1 and IRP2 are highly homologous, they sense cytosolic iron levels by different mechanisms. IRP2 is regulated through protein degradation in the presence of iron and oxygen. Various studies have suggested that iron might be involved in targeting IRP2 for degradation by directly binding to IRP2 or indirectly through a mechanism that involves heme or Fe(II) α -ketoglutarate-dependent oxygenases (Hanson et al. 2003; Pantopoulos 2004; Ishikawa et al. 2005).

In contrast, IRP1 registers cytosolic iron and oxidative stress through its labile Fe–S cluster (Pantopoulos 2004). In the absence of the Fe–S cluster, IRP1 loses aconitase activity and binds to IREs with high affinity. When iron is abundant, the Fe–S cluster is efficiently repaired/regenerated, and IRP1 functions mainly as an aconitase. In vitro experiments showed that IRP1 and IRP2 bind with similar affinities to IRE-containing transcripts (Kim et al. 1995), and studies in rats showed that dietary iron intake can modulate the activity of both IRP1 and IRP2 in rat liver (Chen et al. 1997). However, IRP1 $-/-$ mice developed no overt phenotype, whereas IRP2 $-/-$ mice developed microcytic anemia, hyperferritinemia and erythropoietic protoporphyria (Cooperman et al.

2005; Galy et al. 2005) as well as adult-onset neurodegeneration (LaVaute et al. 2001). A major reason that IRP2 assumes a much greater regulatory role than IRP1 in the mouse models is that IRP2 is stable and active at normal tissue oxygen concentrations (3–6%) (Meyron-Holtz et al. 2004b). In contrast, under the same conditions, the Fe–S cluster in IRP1 is stable, and IRP1 functions mainly as an active aconitase. Furthermore, iron diet studies in mice showed that IRP2 is more responsive to dietary iron changes than IRP1 (Meyron-Holtz et al. 2004a). Although IRP1 is not required for the regulation of iron metabolism in wild type mice, IRP2^{−/−} animals that also lack one copy of IRP1 developed more severe anemia and neurodegeneration (Smith et al. 2004), and IRP1^{−/−} IRP2^{−/−} embryos die at the blastocyst stage (Smith et al. 2006), indicating that, in the absence of IRP2, retention of at least one allele of IRP1 is critical for survival through embryonic development.

As mentioned above, the presence of an IRE in the mRNA of mammalian m-aconitase makes it a potential target for regulation by IRPs. Indeed, studies have shown that iron deficiency decreased the abundance of m-aconitase (Kim et al. 1996) and enhanced citrate utilization in rat liver, although no increase in liver lipogenesis was observed during the course of the study (Ross and Eisenstein 2002). Several interesting hypotheses have been proposed for the reason why the proteins involved in cellular energy production, such as m-aconitase and SDH, should be coupled to iron availability. First, it has been suggested that cytosolic citrate might be needed for transport of iron into mitochondria and down-regulation of aconitases in iron-depleted cells would maintain a certain level of citrate needed for this transport (Melefors and Hentze 1993). Second, some repression of these Fe–S proteins may be advantageous in iron-depleted cells, since lack of sufficient iron could lead to synthesis of non-functional apoproteins (Kim et al. 1996). The possibility that intermediary metabolism may be regulated by the reactive oxygen species (ROS) generated in mitochondrial respiration forms the basis of a third hypothesis, in which oxidative stress not only inactivates m-aconitase directly through chemical modification of the Fe–S clus-

ter, but also activates the IRE-binding activity of IRP1, resulting in repression of m-aconitase synthesis (Gray et al. 1996). Decreased m-aconitase activity would decrease NADH production and electron flux through the respiratory complexes, thereby creating a feedback loop that decreases respiration-generated ROS (Armstrong et al. 2004). Last but not least, recent studies in yeast have demonstrated that, in response to iron deprivation, the RNA-binding protein Cth2 down-regulates several Fe-dependent pathways to limit iron expenditure (Puig et al. 2005). From this prospective, the IRP-dependent suppression of mammalian m-aconitase and insect SDH may serve to limit the utilization of iron in iron deficient cells. Interestingly, iron deprivation also decreased the expression of the mammalian Fe–S cluster assembly proteins ISCU (Tong and Rouault 2006) and ISCS (Liew and Shaw 2005), further suggesting that selective suppression of Fe-dependent pathways is a general cellular response to iron deficiency in mammalian cells.

Biochemical and physiological features of aconitase inhibition

Although m-aconitase is not generally considered to be the rate-limiting step (Williamson and Cooper 1980), in cardiac myocytes, decreased aconitase activity led to decreases in citric acid cycle flux (Janero and Hreniuk 1996). Mitochondrial aconitase is also a major target of oxidative damage during aging (Delaval et al. 2004; Yarian et al. 2006), and oxidative inactivation of aconitase has been associated with decreased life span in *Drosophila* (Yan et al. 1997). Cellular inactivation of aconitase is largely attributable to the enzyme's sensitivity to oxidative stress and iron availability (Fig. 3) (Chen et al. 1997; Gardner 1997; Tong and Rouault 2006), although phosphorylation may also affect the stabilities of the Fe–S cluster and the protein (Fillebeen et al. 2005; Clarke et al. 2006). The Fe–S clusters in both m-aconitase and c-aconitase can be disassembled upon exposure to oxidants including O₂[−], H₂O₂, NO, and ONOO[−] (Pantopoulos and Hentze 1995; Gardner 1997; Bouton and Drapiers 2003; Han et al. 2005), and the liberation of reactive iron may further induce oxidative damage of cellular

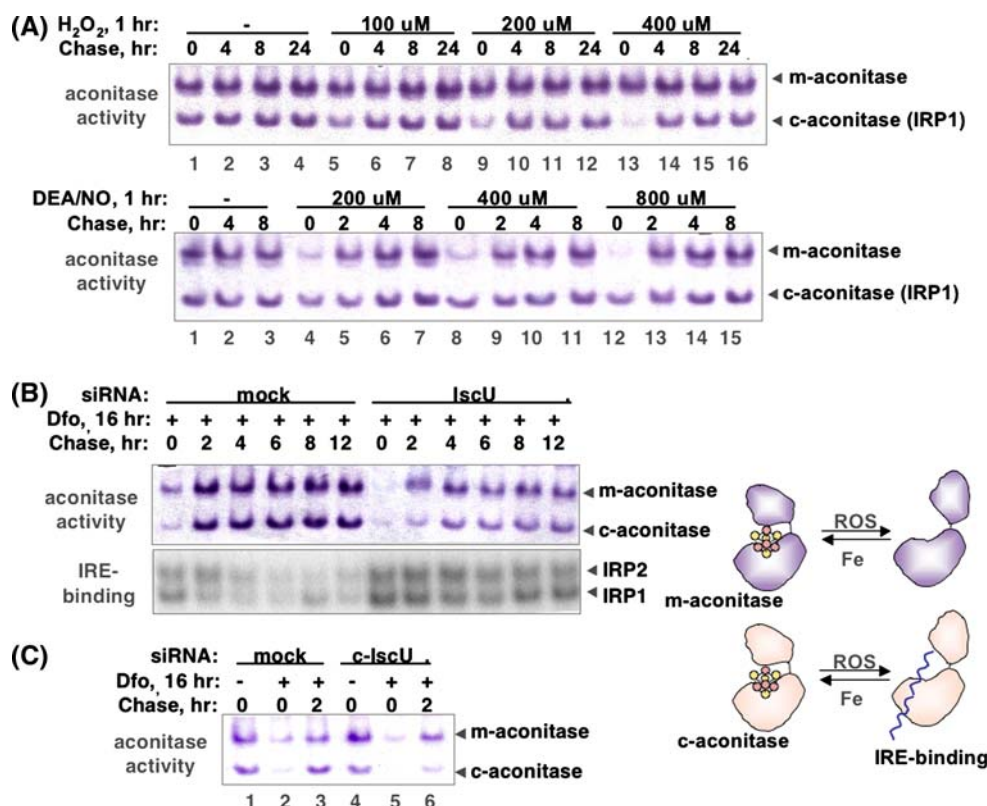


Fig. 3 Aconitase activities are affected by iron levels, oxidative stress and Fe–S cluster assembly activity. **(A)** Mitochondrial and cytosolic aconitases can be selectively inactivated by different inducers of oxidative stress. HeLa S3 cells were treated for 1 h with H₂O₂ or DEA/NO, then washed and incubated in normal growth medium (chase). Treatment with H₂O₂ (lanes 1, 5, 9,13) led to dose-dependent inactivation of c-aconitase (Tong and Rouault 2006), whereas treatment with DEA/NO (lanes 1, 4, 8,12) resulted in inactivation of m-aconitase (Tong et al. unpublished data). Subsequent withdrawal of either oxidant resulted in reactivation of aconitase activity, indicating robust repair/regeneration of the [4Fe–4S] cluster in both the mitochondrial and the cytosolic compartments. **(B)** Iron deficiency, induced by treatment

with the iron chelator Desferal (Dfo), lowers both m- and c-aconitase activities and activates the IRE-binding activities of both IRP1 and IRP2. Reactivation of aconitase activities and deactivation of IRE-binding activities depends on the presence of the Fe–S cluster assembly protein ISCU. **(C)** Isoform-specific depletion of cytosolic ISCU (c-ISCU) impairs Fe–S cluster assembly in c-aconitase only (compare lane 3 and 6). In comparison, Fe–S cluster assembly in m-aconitase were unaffected due to the presence of a distinct mitochondrial ISCU. Reprinted from Cell Metabolism, v.3, Tong and Rouault, “Functions of mitochondrial ISCU and cytosolic ISCU in mammalian iron-sulfur cluster biogenesis and iron homeostasis”, pp. 199–210, 2006, with permission from Elsevier

constituents (Lipinski et al. 2005). Oxidative damage can also increase the proteolytic susceptibility of m-aconitase and IRP1, and severe oxidation can cause aggregation of the aconitase protein (Das et al. 2001; Bota and Davies 2002; Starzynski et al. 2005). Since citrate can be channeled into cholesterol and lipid biosynthesis if the citric acid cycle is inhibited, it has been suggested that the massive lipid accumulation observed in the livers of MnSOD-deficient mice results from oxidative

damage to the Fe–S clusters in mitochondrial aconitase and SDH (Li et al. 1995; Armstrong et al. 2004). Consistent with this hypothesis, recent studies in *Drosophila* and *C. elegans* have shown that decreased expression of the plasma membrane citrate transporters resulted in decreased lipid content and increased life span (Rogina et al. 2000; Fei et al. 2004), further underscoring the pivotal role of citrate flux in energy homeostasis.

In addition to affecting citrate metabolism, it has been suggested that oxidative or nitrosative modulation of IRPs may cause changes in iron metabolism during inflammation and anaemia of chronic disease (Cairo et al. 2002a). The response of IRPs to oxidative stress is very complex. Various studies in different cell culture systems have shown that different oxidizing species can activate or inactivate IRP1 and IRP2 (Cairo et al. 2002b; Bouton and Drapiers 2003; Hanson et al. 2003; Kim and Ponka 2003; Pantopoulos 2004). Likewise, studies in tissue or animal models indicated that, while the IRE-binding activity of IRP1 increased in rat liver perfused with a H_2O_2 -generating system that mimicked a physiological inflammatory response (Muller et al. 2001), mice that lack the cytosolic superoxide dismutase (SOD) display a marked decrease in IRP1 aconitase activity and protein levels, but a normal iron metabolism phenotype (Starzynski et al. 2005).

Citric acid cycle genetic defects in humans are rare, and the most severe are likely embryonic lethal events (Rustin et al. 1997). Yeast with mutations in *AcoI*, the single gene that encodes both m- and c-aconitase, exhibit glutamate auxotrophy, defects in respiratory and cytosolic glyoxylate pathways, and are unable to grow on non-fermentable carbon source (Gangloff et al. 1990; Regev-Rudski et al. 2005). While a genetic model for the ablation of mitochondrial aconitase in vertebrates has not been reported, IRP1 $^{-/-}$ IRP2 $^{-/-}$ mice are not viable past the blastocyst stage (Smith et al. 2006). Surprisingly, IRP1 $^{-/-}$ mice have no obvious defects (Meyron-Holtz et al. 2004a). In situ hybridization and activity assays indicated that IRP1 levels are highest in the kidney, liver and brown fat (Mullner et al. 1992; Meyron-Holtz et al. 2004a), and aconitase activity assays indicated that c-aconitase accounts for ~50% of the total aconitase activities in liver and kidney (Konstantinova and Russanov 1996; Huang et al. 2002). However, there is no evidence for compromised renal function, abnormal blood chemistry, tissue pathology, or difference in body weight or brown fat in IRP1 $^{-/-}$ mice (Meyron-Holtz et al. 2004a), indicating that the presence of mitochondrial aconitase and IRP2 can substitute for the lack of IRP1 under basal metabolic conditions.

The complex roles of aconitases in normal physiology were also revealed by extensive toxicological studies of fluoroacetate (Goncharov et al. 2006). Fluoroacetate undergoes a series of metabolic conversions to yield the extremely toxic compound, fluorocitrate, a potent mechanism-based inhibitor of aconitase. Acute fluoroacetate poisoning in humans mainly affects the central nervous system, cardiovascular system and kidney, and the long list of biochemical effects include TCA cycle blockage, respiratory failure, excessive citrate accumulation in heart, kidney and spleen, deviation in carbohydrate metabolism regulation, metabolic acidosis, increased serum citrate, decreased plasma Ca^{2+} , and lactate accumulation in cerebrospinal fluid.

Aconitase activities in mammalian cells are modulated by the mitochondrial and cytosolic Fe–S cluster assembly machineries

In recent years, Fe–S cluster biogenesis has received increasing attention, not only for its role in supporting the functions of many metabolic pathways, but also for its involvement in the sensing of cytosolic and mitochondrial iron status, and the communication of iron needs in the different subcellular compartments (Fig. 2). In the cytosol, Fe–S cluster assembly and disassembly controls the function of one of the iron regulatory proteins IRP1. In the mitochondria, disruption in Fe–S cluster biogenesis results in mitochondrial iron overload, cytosolic iron depletion, activation of cellular iron uptake and increased oxidative stress (Babcock et al. 1997; Knight et al. 1998; Garland et al. 1999; Foury and Talibi 2001; Yang et al. 2006). In patients with the neurodegenerative disease, Friedreich ataxia, decreased frataxin levels results in reduced heme and Fe–S cluster biogenesis, defective mitochondrial respiration, mitochondrial iron overload, and increased oxidative damage (Rotig et al. 1997; Pandolfo 2003; Seznec et al. 2004; Napoli et al. 2006).

The complexity and importance of the process of Fe–S cluster biogenesis is underscored by the number of genes involved and the lethal phenotypes associated with disruption of many such genes (Fig. 4) (Zheng et al. 1998; Li et al. 1999; Schilke et al. 1999; Cossee et al. 2000; Seidler

et al. 2001; Ponderre et al. 2006). Excellent summaries of the current understanding of Fe–S cluster biogenesis in bacteria, yeast, plant and animal are available in several recent reviews (Johnson et al. 2005; Lill and Muhlenhoff 2005; Rouault and Tong 2005; Pilon et al. 2006). *E. coli* and *S. cerevisiae* have the most extensively characterized Fe–S cluster assembly systems. More than a dozen genes have been implicated in Fe–S cluster biogenesis, and homologues of some of them have been characterized in mammalian systems. Briefly, cysteine desulphurases cleave cysteine to yield sulfur in the form of an enzyme-bound persulfide (Zheng et al. 1993), whereas frataxin may function as the iron donor (Gerber et al. 2003; Yoon and Cowan 2003; Bulteau et al. 2004) (Fig. 4A). Sulfur transfer (Smith et al. 2001; Urbina et al. 2001) and iron acquisition leads to assembly of [2Fe–2S] and [4Fe–4S] clusters on the scaffold proteins (Agar et al. 2000). Chaperone proteins are thought to facilitate cluster transfer to target proteins (Silberg et al. 2004; Dutkiewicz et al. 2006), and redox proteins (Lange et al. 2000; Wingert et al. 2005) provide the reducing equivalents that are necessary for cluster assembly and cluster transfer. Genetic and biochemical studies have indicated that many other proteins are also involved in Fe–S cluster biogenesis, although their exact functions are still under active investigation (Fig. 4B) (Balk et al. 2005; Johnson et al. 2005; Lill and Muhlenhoff 2005; Tury et al. 2005; Adam et al. 2006; Balasubramanian et al. 2006; Molina-Navarro et al. 2006).

In yeast and animal cells, most of the known Fe–S proteins are found in the mitochondrial matrix and the respiratory chain of the mitochondrial inner membrane, and mitochondria constitute the major subcellular site of Fe–S cluster assembly (Lill and Muhlenhoff 2005; Rouault and Tong 2005). In human, multiple tissue Northern blot analyses have indicated that mitochondrial-rich tissues such as heart and skeletal muscle have the highest levels of ABC7, ISCS, ISCU and ISCA transcripts, reflecting the high demand for Fe–S cluster biogenesis in these tissues (Allikmets et al. 1999; Tong and Rouault 2000). Frataxin is also abundant in the adult heart and the developing brain, which correlates with the hypertrophic

cardiomyopathy and neuropathy observed in Friedreich ataxia patients (Campuzano et al. 1996; Koutnikova et al. 1997).

In yeast, many of the Fe–S cluster assembly proteins have thus far been detected only in the mitochondria (Fig. 4B), leading to the proposal that Fe–S cluster assembly occurs exclusively in the mitochondria, and that preformed Fe–S clusters are exported through the ABC transporter Atm1 into the cytosol, where several auxiliary factors facilitate the incorporation of these clusters into cytosolic proteins (Lill and Muhlenhoff 2005). However, Fe–S cluster biogenesis is not confined to the mitochondrial matrix in plants and animals. In plants, genomic and biochemical analysis have revealed that isoforms of several Fe–S cluster assembly proteins exist in both the chloroplast and the mitochondria (Pilon et al. 2006). In mammalian cells, a small but significant extramitochondrial fraction of Fe–S cluster assembly proteins including ISCS (Land and Rouault 1998), ISCU (Tong and Rouault 2000; Acquaviva et al. 2005), NFU (also known as HIRIP5) (Lorain et al. 2001; Ganesh et al. 2003; Tong et al. 2003) and frataxin (Acquaviva et al. 2005; Condo et al. 2006) have been identified (Fig. 4B). More recently, three studies have provided further evidence for extramitochondrial Fe–S cluster biogenesis. First, the mitochondrial and cytosolic ISCU proteins can be selectively suppressed by isoform-specific siRNA, and silencing of both m- and c-ISCU impaired Fe–S cluster assembly in both m- and c-aconitase (Fig. 3B) (Tong and Rouault 2006). Interestingly, Fe–S cluster assembly in c-aconitase was specifically affected by silencing of cytosolic ISCU (Fig. 3C) whereas m-aconitase was more sensitive to the depletion of m-ISCU, in keeping with the presence of a functional Fe–S cluster assembly machinery in the cytosol (Tong and Rouault 2006). Second, in vitro studies confirmed that recombinant human cytosolic ISCS and cytosolic ISCU can promote Fe–S cluster assembly in c-aconitase/IRP1 (Li et al. 2006). Third, expression of extramitochondrial frataxin can promote survival of frataxin-deficient cells from a Friedreich ataxia patient (Condo et al. 2006).

Compartmentalization is a valuable tool for creating functional diversity, regulatory flexibility,

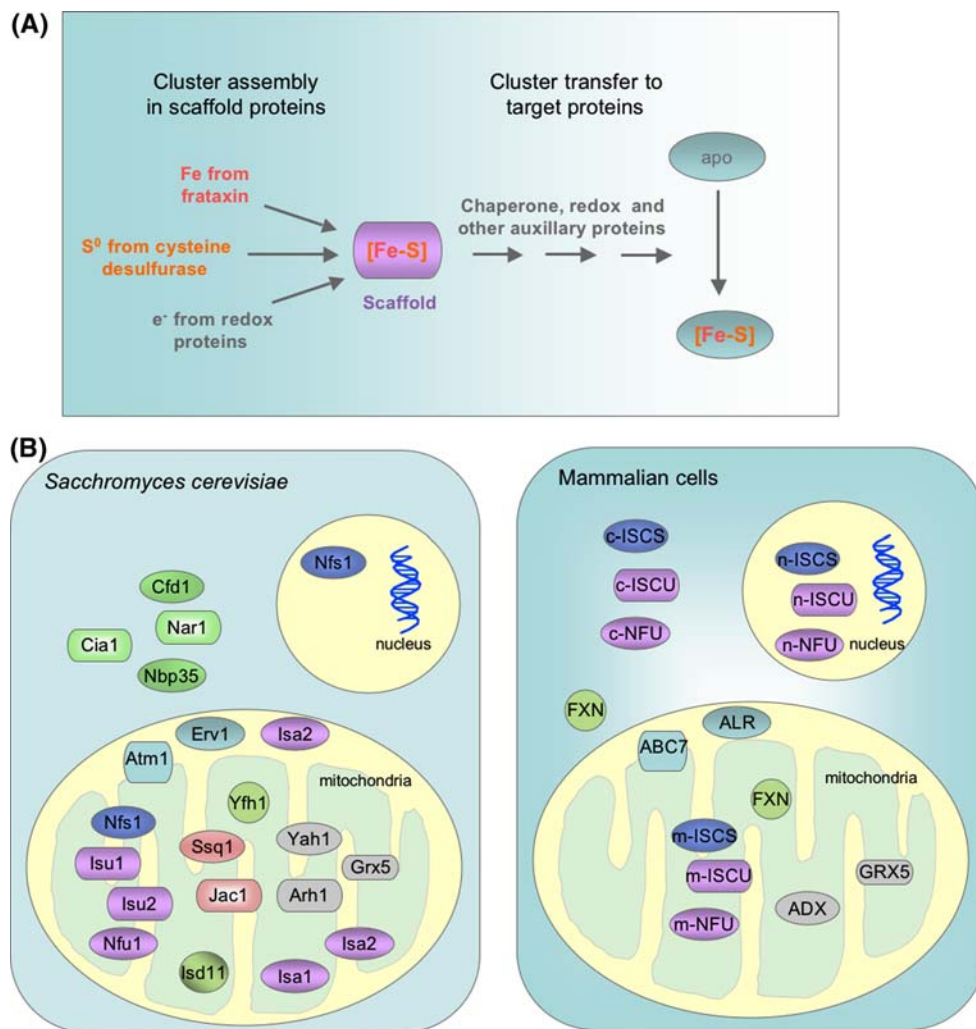


Fig. 4 Fe-S cluster assembly proteins and their subcellular localization in *S. cerevisiae* and in mammalian cells. **(A)** Formation of intracellular Fe-S cluster involves a complex biosynthetic machinery that assembles rudimentary Fe-S clusters in scaffold proteins, and subsequently transfers these clusters to target proteins. **(B)** Up to 19 different proteins have been proposed to be involved in Fe-S cluster biogenesis in *S. cerevisiae*. A number of the human homologues that have been characterized biochemically and cytologically are shown here, and putative homologues of many others were revealed by sequence comparison. In yeast, many components of the Fe-S cluster assembly machinery, including the yeast homolog of frataxin, Yfh1, the scaffold proteins Isu1, Isu2, and Nfu, the chaperones Ssq1 and Jac1, the

redox proteins Yah1, Arh1, and Grx5, and other proteins such as Isa1, Isa2 and Isd11 have been detected in the mitochondria. Isa2 is also in the intermembrane space and the cysteine desulfurase Nfs1 is present in both the mitochondria and the nucleus. The ABC type transporter Atm1 is in the mitochondrial inner membrane, and the sulfhydryl oxidase Erv1 is in the intermembrane space. In yeast cytosol, Cfd1, Nar1, Cia1 and Nbp35 were proposed to assist in maturation of Fe-S protein. In contrast to yeast, small but significant amounts of the cysteine desulfurase ISCS, scaffold proteins ISCU and NFU, and frataxin have been identified in the cytosol of mammalian cells, and recent functional studies have confirmed that Fe-S cluster biogenesis occurs in the cytosol as well as in the mitochondria of mammalian cells

and specificity in signaling. Spatial separation of the Fe-S cluster assembly apparatus into different subcellular compartments provides the cell increased capacity to respond to changing metabolic

needs (Figs. 1 and 2). The redox environment also varies markedly in the different subcellular compartments (Hansen et al. 2006), and mitochondrial and cytosolic aconitases can be selectively

inactivated by different inducers of oxidative stress (Fig. 3A). Studies in *Drosophila* and *S. cerevisiae* have shown that the mitochondrial and cytosolic SOD provide independent protection to Fe–S proteins against damage by O₂ generated within those compartments (Missirlis et al. 2003; Wallace et al. 2004), whereas compartment-specific Fe–S cluster assembly would facilitate robust repair/regeneration of Fe–S clusters in the different locations (Tong and Rouault 2006). Furthermore, separate Fe–S cluster assembly machineries allow independent sensing of iron status in the cytosol and in the mitochondria in mammalian cells (Fig. 2). In the cytosol, Fe–S cluster biogenesis is important for the switch between cytosolic aconitase and IRE-binding activities of IRP1. In the mitochondria, Fe–S cluster biogenesis is needed not only to support respiratory functions and heme biosynthesis, but also for sensing and regulation of mitochondrial iron homeostasis (Rouault and Tong 2005). When the need for Fe–S cluster-dependent activities in the mitochondria is high, the cell may respond by increasing delivery of iron to the mitochondria and decreasing mitochondrial iron export. Activation of IRE-binding activities as a result of cytosolic iron depletion and inadequate Fe–S cluster biogenesis (Fosset et al. 2006; Tong and Rouault 2006) would lead to increased TfR-dependent iron uptake and decreased iron sequestration by ferritin (Anderson et al. 2005; Fosset et al. 2006). Since expression of m-ISCU and m-ISCS are down-regulated by iron depletion (Liew and Shaw 2005; Tong and Rouault 2006), iron deficiency would suppress Fe–S proteins both by decreasing availability of a basic component of the Fe–S cluster, and by decreasing the Fe–S cluster assembly protein levels. Taken together, these results place Fe–S cluster biogenesis at the forefront of iron sensing and reprogramming of intracellular iron trafficking in response to changing iron needs.

Concluding remarks

Iron deficiency is the most common and devastating nutritional disorder in the world (Lewis 2005), and much effort has been directed to understanding the metabolic consequences of

iron deficiency (Beard and Connor 2003; Umbreit 2005). We provided here one example of how iron deficiency can affect many aspects of cellular metabolism through its effects on aconitase activities. Iron deficiency can decrease aconitase protein levels and limit the assembly of the Fe–S clusters required for their enzymatic activities. As a result, iron deficiency can potentially affect the citric acid cycle, lipid biosynthesis, carbohydrate metabolism and many other biological pathways that involve citrate. Similarly, iron deficiency and suppression of Fe–S cluster biogenesis may hamper the functions of many other Fe–S proteins in intermediary metabolism, respiratory complexes, heme biosynthesis and DNA repair. The selective suppression of iron-containing proteins in the mitochondria can also lead to decoupling of the electron transport chain and increased oxidative stress. Although tremendous progress has been made recently in understanding the impact of Fe–S cluster biogenesis on cellular iron metabolism, the mechanism by which this system communicates information about mitochondrial iron status to modulate cellular iron trafficking remains to be elucidated. Lessons from this new and exciting area of research should provide new insights into the mechanisms of mitochondrial signaling and the interrelationship between iron and energy metabolism.

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