Loss of cardiolipin leads to perturbation of mitochondrial and cellular iron homeostasis

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THE DISORDER

Barth syndrome – a life-threatening X-linked disorder

- **cardioskeletal myopathy**
- **growth retardation**
- **neutropenia**

Barth et al., 1983. An X-linked mitochondrial disease affecting cardiac muscle, skeletal muscle and neutrophil leucocytes. *J. Neurol. Sci.* 62:327-355.

THE GENE

G4.5 (tafazzin) is responsible for Barth syndrome

Bione et al., 1996. A novel X-linked gene, G4.5, is responsible for Barth syndrome. *Nat. Genet.* **12:**385-389.

THE BIOCHEMICAL DEFECT

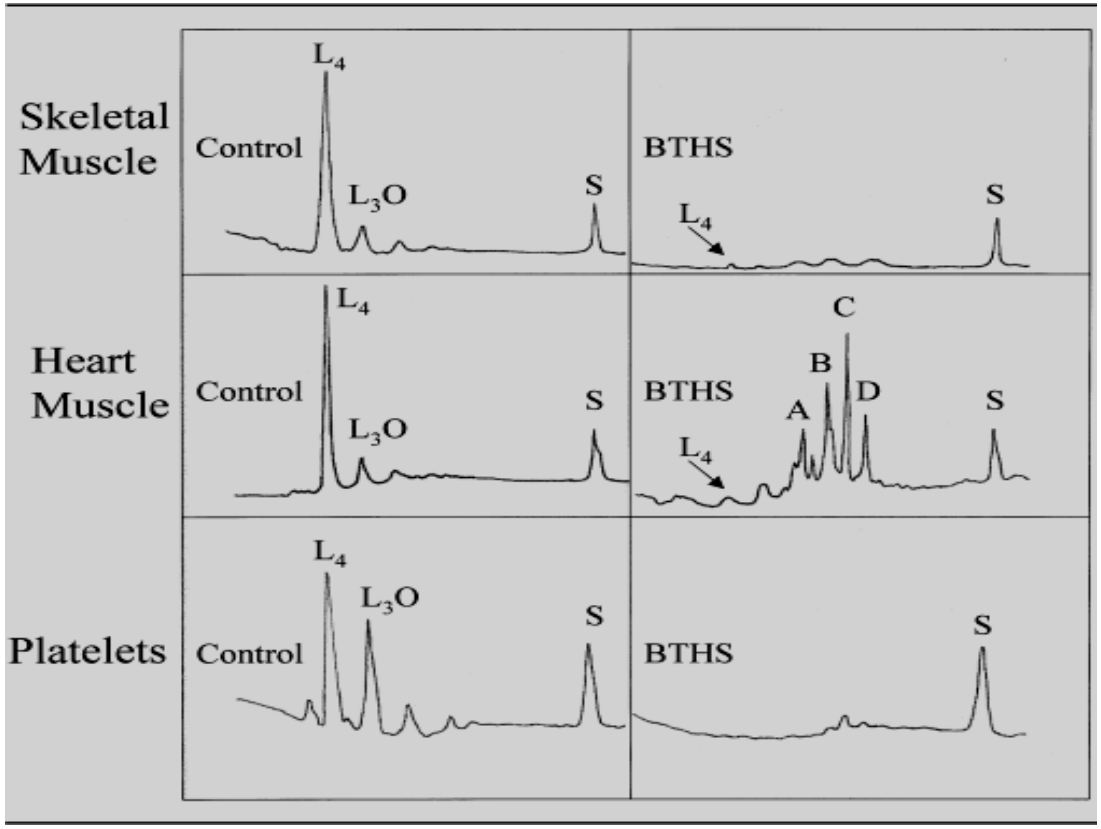
Barth syndrome is a disorder of cardiolipin remodeling

- **Decreased CL**
- **Increased MLCL (monolyso-CL)**
- **Decreased unsaturated fatty acyl CL species**

Vreken et al., 2000. Defective remodeling of cardiolipin and phosphatidylglycerol in Barth syndrome. *Biochem. Biophys. Res. Comm.* **279:**378-382.

THE BIOCHEMICAL DEFECT

L4-CL deficiency in Barth syndrome



Schlame et al. 2002. Deficiency of tetralinoleoyl-cardiolipin in Barth syndrome. *Ann. Neurol.* **51**: 634-7

MOLECULAR BASIS OF THE PATHOLOGY?
RELATIONSHIP OF GENOTYPE TO PHENOTYPE?

Clinical phenotypes vary widely in Barth syndrome

TAZ

asymptomatic

death in infancy

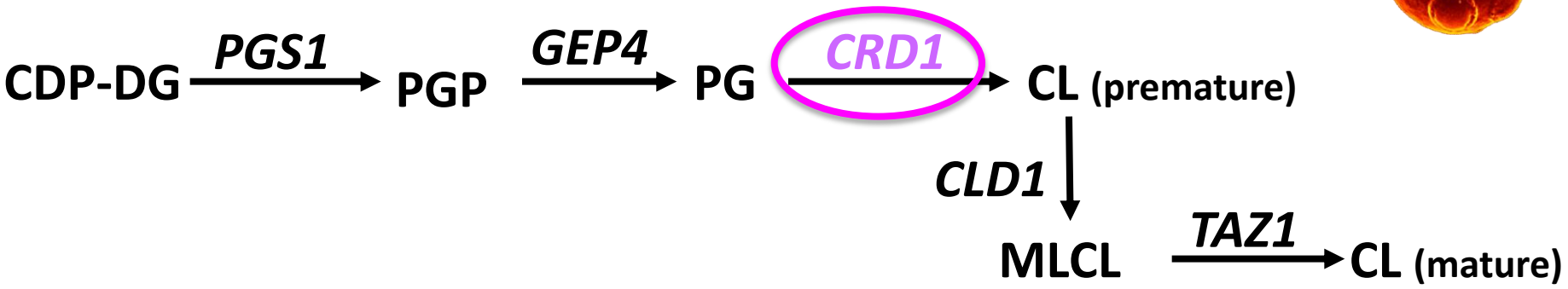
Molecular mechanisms underlying monogenic diseases

- "There are major problems associated with dissecting the molecular basis of even simple monogenic diseases caused by mutations in a single gene.
- Principal among these are the modifying effects of other genes… This results in marked variations in the symptoms of patients with the same disease.
- Of the 1500 or so monogenic diseases for which the mutated gene has been identified, there are only a few where the effects of other genes on disease pathogenesis have been studied."

Peltonen and McKusick *Science* 2001 **291**:1224-1229

Functions of cardiolipin may elucidate mechanisms underlying the pathology and identify physiological modifiers

Advantages of the yeast model



Yeast mutants are available for all yeast genes, including every step of CL synthesis.

The pathway is conserved.

Yeast are amenable to genetic and genomic analyses.

THE BIOCHEMICAL DEFECT

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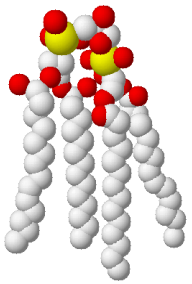
Functions of CL may elucidate mechanisms underlying pathology and identify physiological modifiers

iron homeostasis

mitochondrial bioenergetics

mitochondrial protein import

PKC signaling



mitochondrial fusion

life span/stress response

vacuolar function

cell integrity

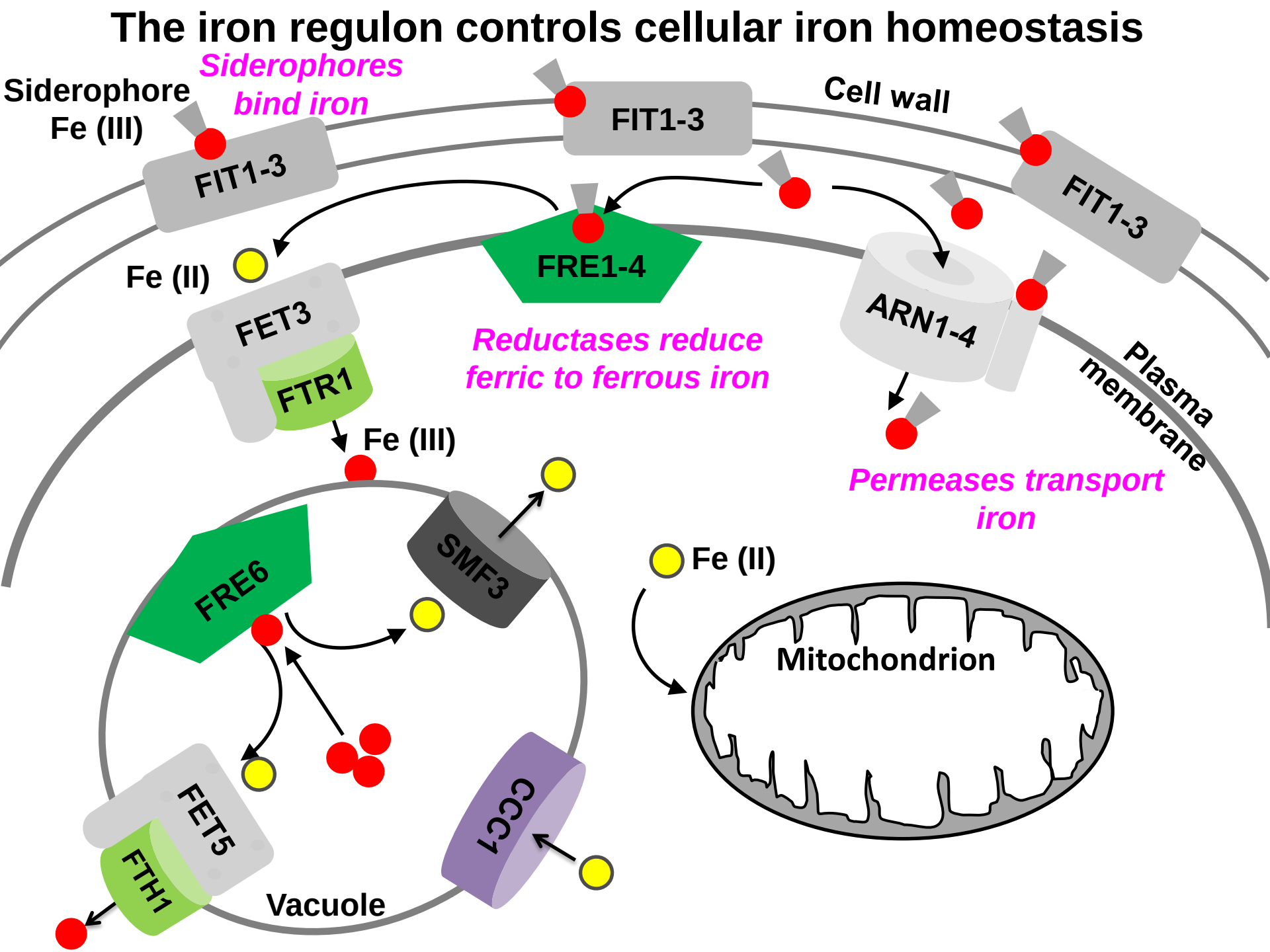
cell division

Vinay Patil

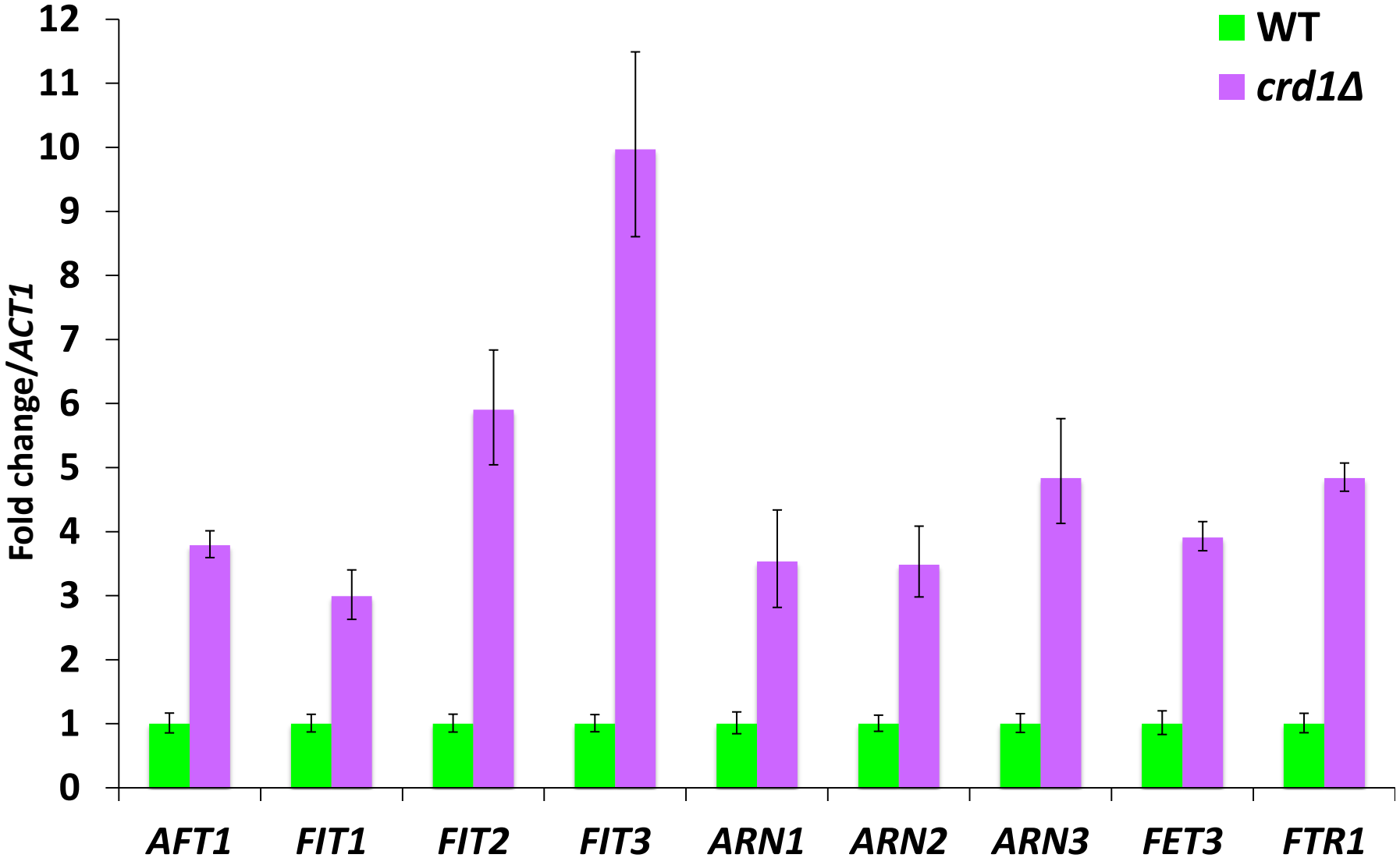
Vishal Gohil

Microarray analysis: Increased expression of iron regulon genes in *crd1Δ*

Gene	*crd1Δ*	Cellular function
FIT1	3.07	Retention of siderophore-iron
FIT2	17.57	Retention of siderophore-iron
FIT3	33.84	Retention of siderophore-iron
FET3	6.94	High-affinity iron uptake
FTR1	2.50	High affinity iron permease
ARN1	2.72	Transporter of siderophore-iron chelates
ARN4	2.29	Ferric enterobactin transporter
TIS11	2.95	Degradation of mRNA upon iron starvation


The iron regulon controls cellular iron homeostasis
Siderophores bind iron
Siderophore Fe (III)
FIT1-3
FIT1-3
Cell wall
FIT1-3
FRE1-4
Fe (II)
FET3
FTR1
Reductases reduce ferric to ferrous iron
ARN1-4
Plasma membrane
Fe (III)
Permeases transport iron
FRE6
SMF3
Fe (II)
Mitochondrion
FET5
FTH1
CCC1
Vacuole

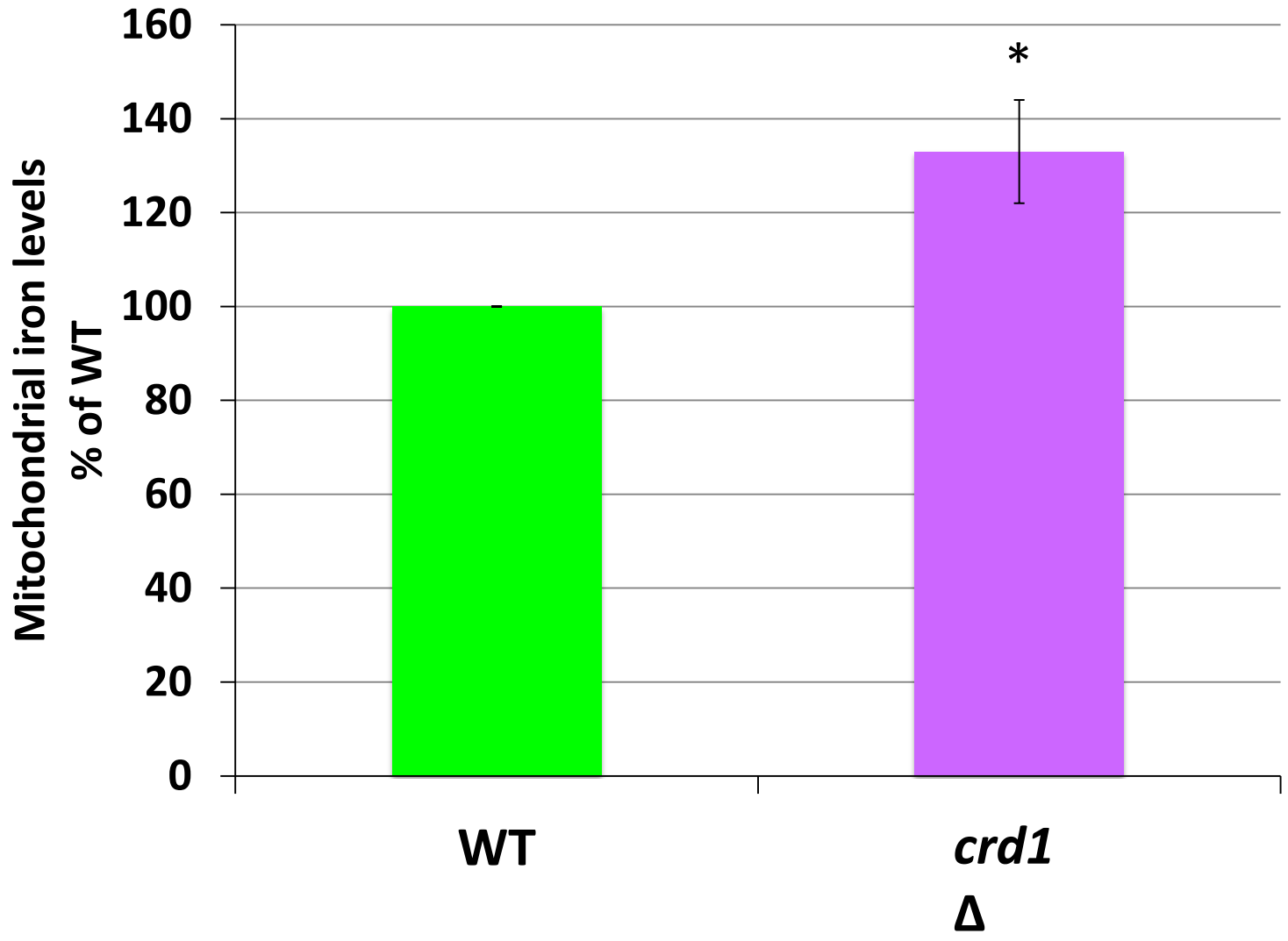
AFT1 regulated iron regulon is up-regulated in *crd1Δ*



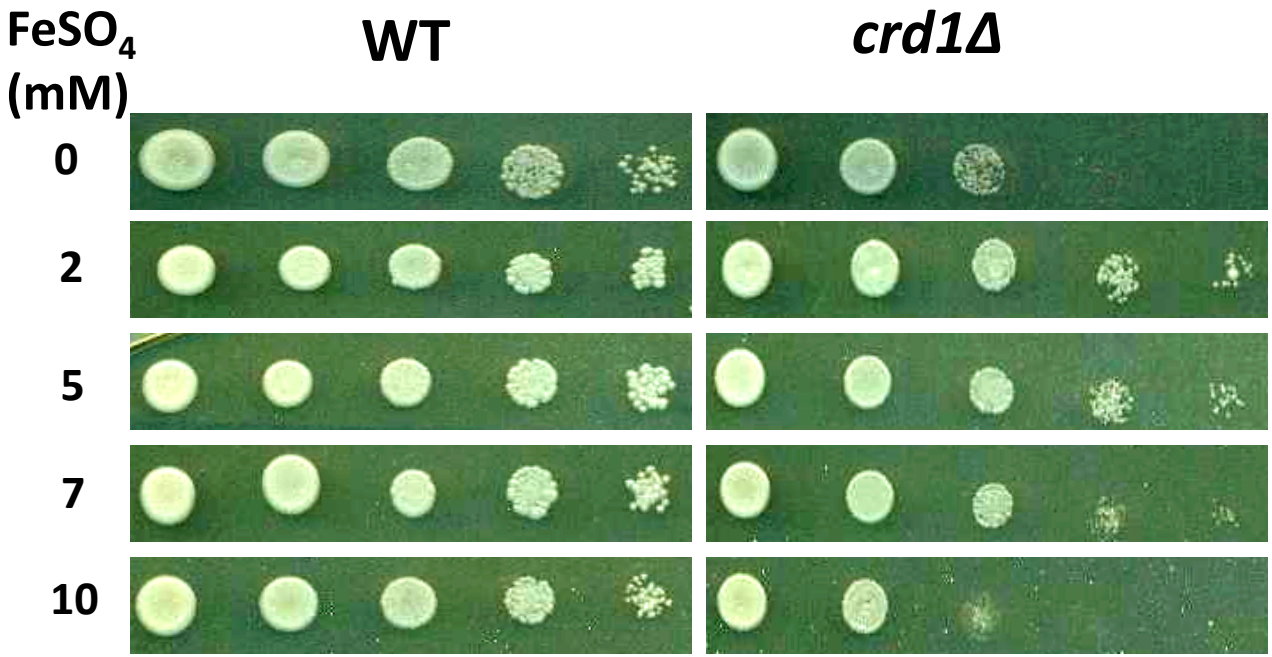
Does loss of CL lead to defective iron homeostasis?

- Perturbation of mitochondrial iron levels?
- Decreased growth on media with high iron?
- Sensitivity to ROS inducing agents?

*crd1*Δ has increased mitochondrial iron levels

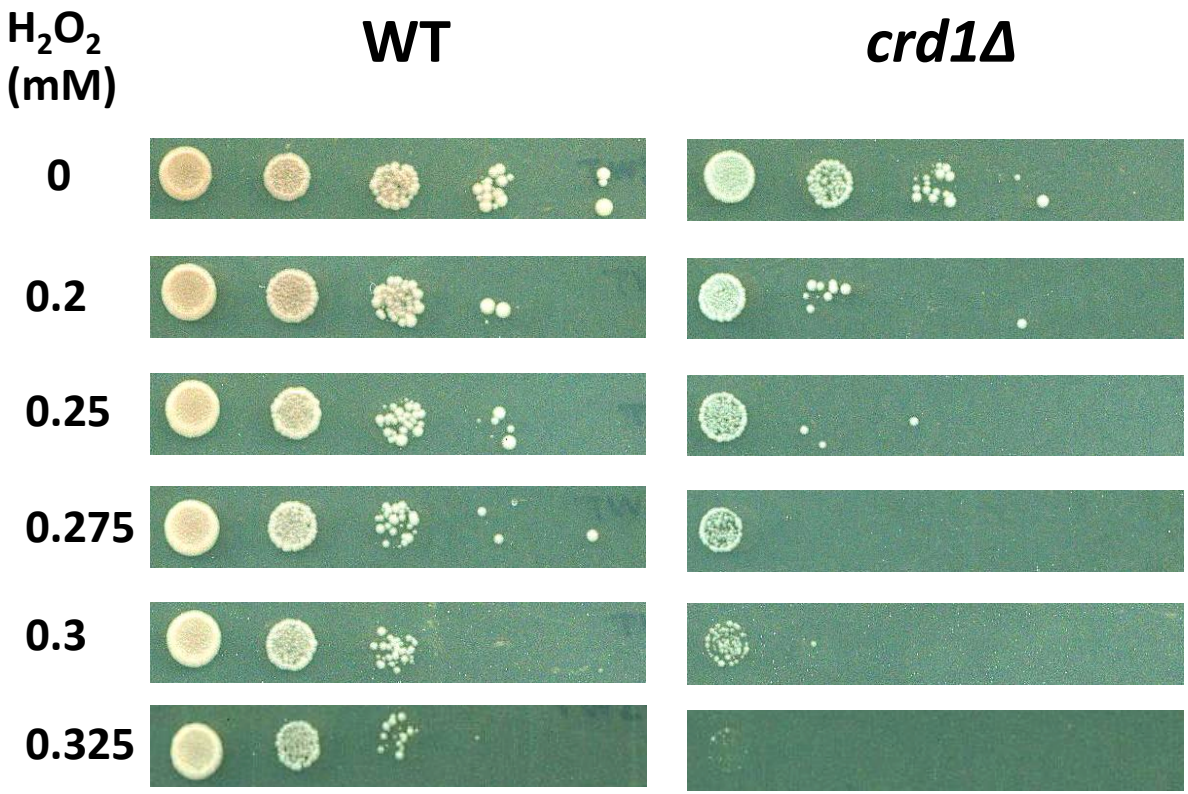


crd1Δ is sensitive to iron



glycerol/ethanol 30˚C

crd1Δ is sensitive to hydrogen peroxide



Loss of CL leads to defective iron homeostasis

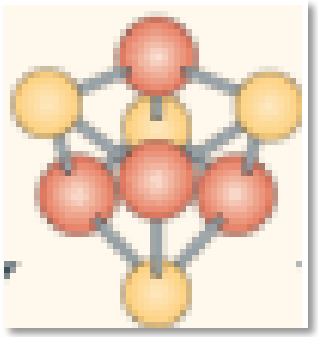
- Perturbation of mitochondrial metal levels
- Decreased growth on media with high iron
- Sensitivity to ROS inducing agents

What is the mechanism linking loss of CL to defective iron homeostasis?

Factors that cause increased expression of the iron regulon

- **Iron starvation**
- **Iron-sulfur (Fe-S) biogenesis defects**

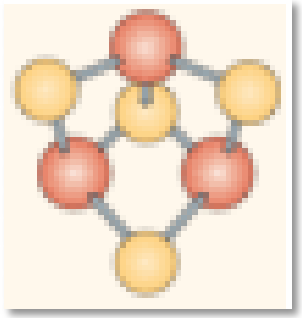
Iron-sulfur (Fe-S) clusters



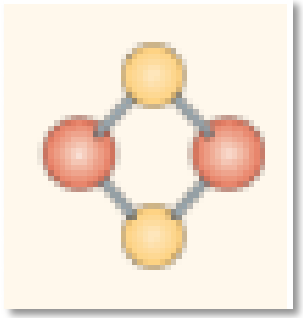
[4Fe-4S]

Cluster disassembly

Cluster interconversion



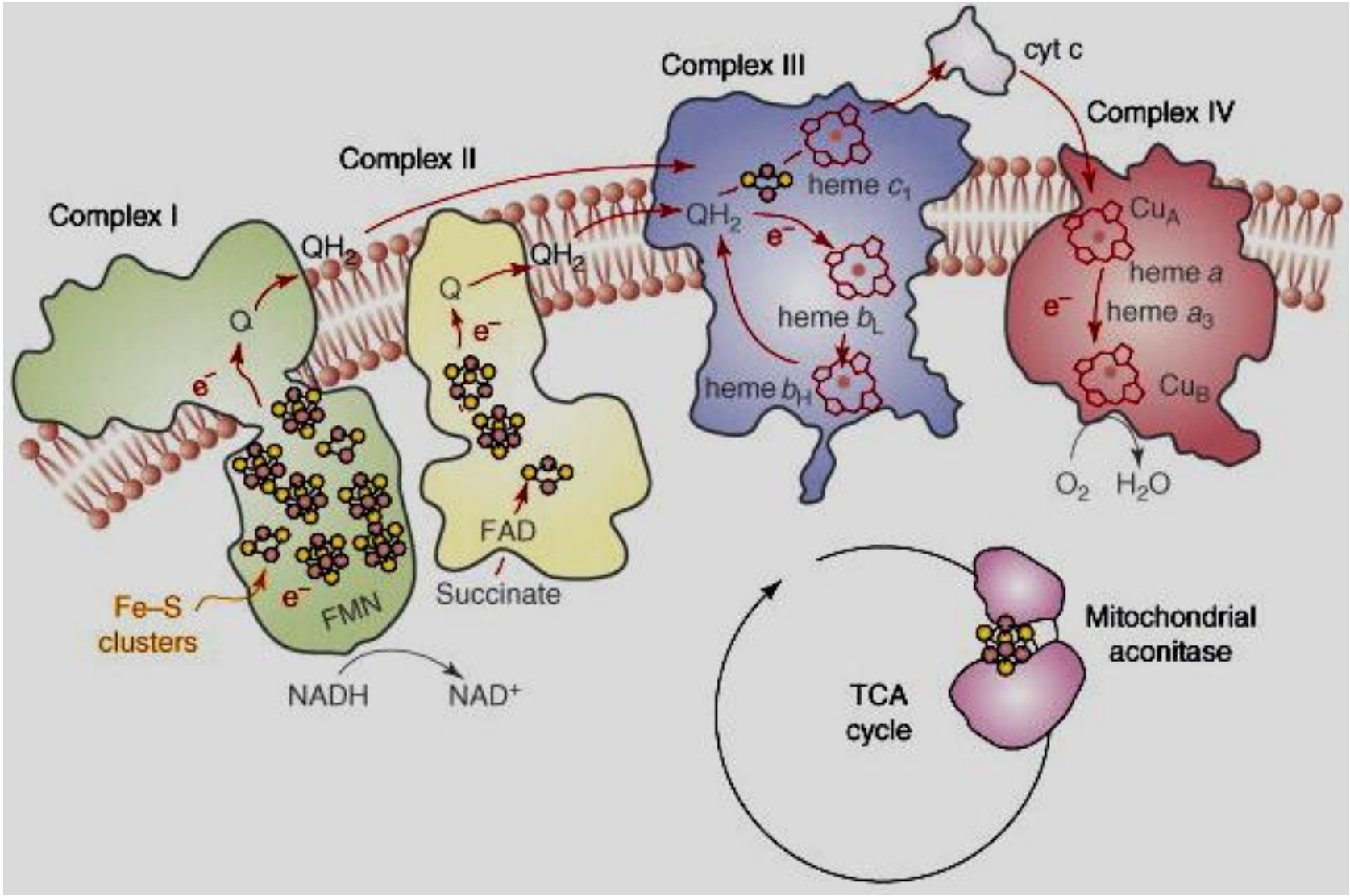
[3Fe-4S]



[2Fe-2S]

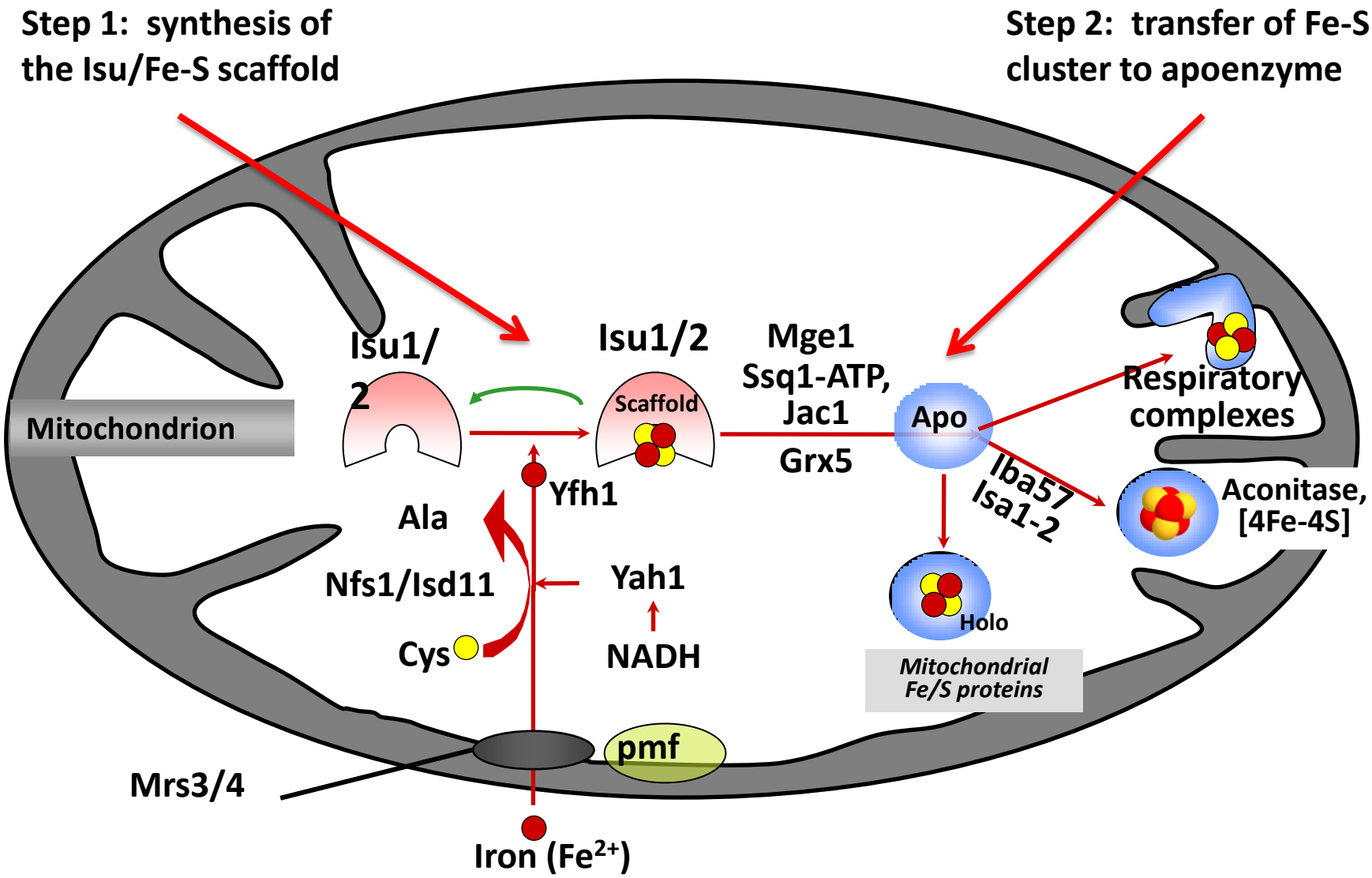
Rouault *Cell* 2005

Fe-S clusters are essential co-factors in energy metabolism



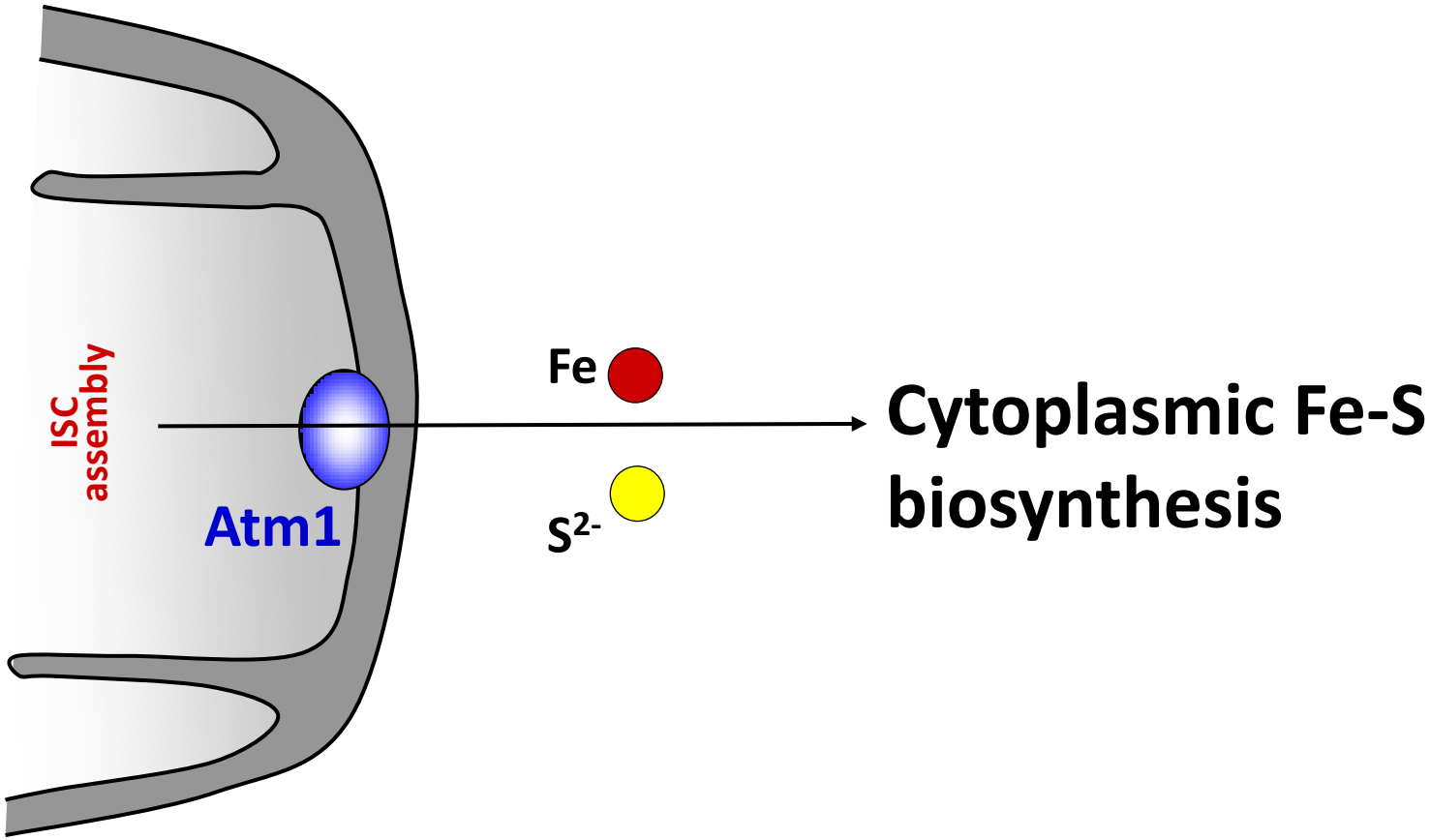
Rouault *Cell* 2008

Fe-S cluster biogenesis



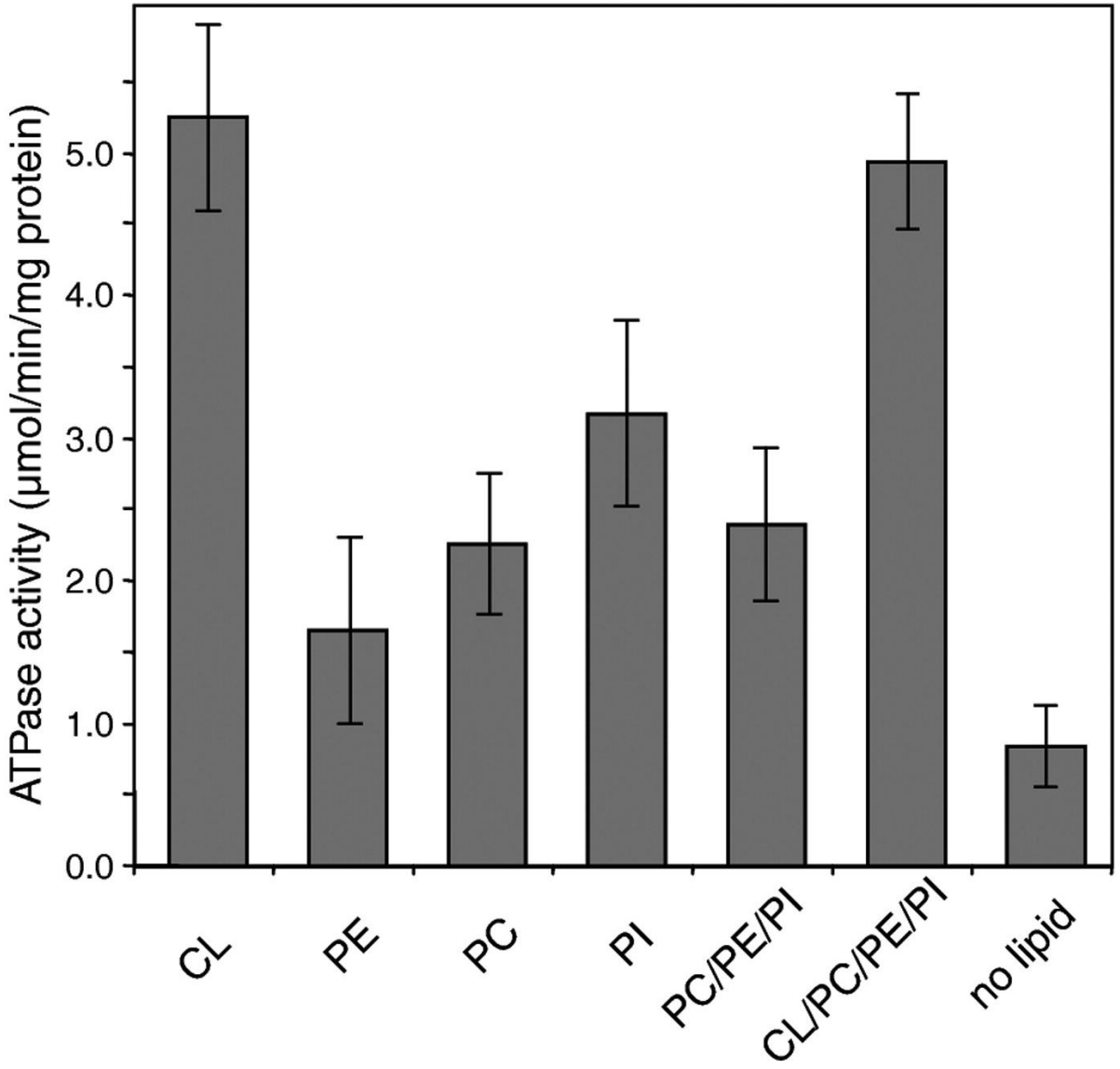
From Roland Lill

Atm1 mediates the export of Fe-S co-factors for cytoplasmic Fe-S biosynthesis



From Roland Lill

Cardiolipin stimulates ATPase activity of Atm1



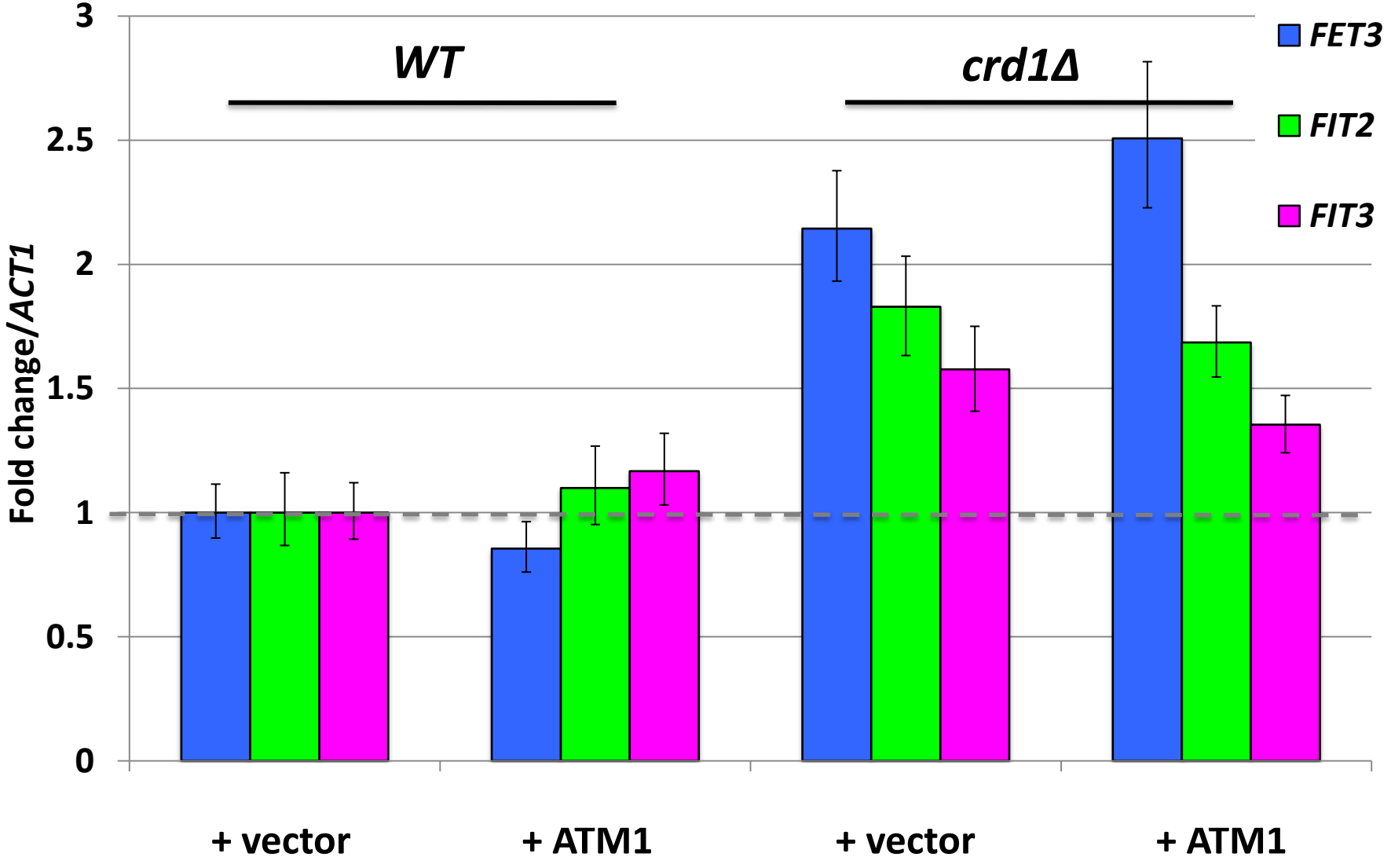
Kunhke *Mol Memb Biol* 2006

Is perturbation of iron homeostasis in *crd1Δ* due to decreased Atm1 activity?

Prediction:

- **Overexpression of Atm1 rescues the iron-associated defects in *crd1Δ***

Overexpression of *ATM1* does NOT restore WT iron regulon levels to *crd1Δ*



Is perturbation of iron homeostasis in *crd1Δ* due to defects in mitochondrial Fe-S cluster biogenesis?

Predictions:

- ***crd1Δ*** **exhibits decreased activity of Fe-S enzymes**
- ***crd1Δ*** **is sensitive to further perturbation of Fe-S synthesis**
- ***crd1Δ*** **exhibits up-regulation of Fe-S scaffold (characteristic of Fe-S perturbation)**

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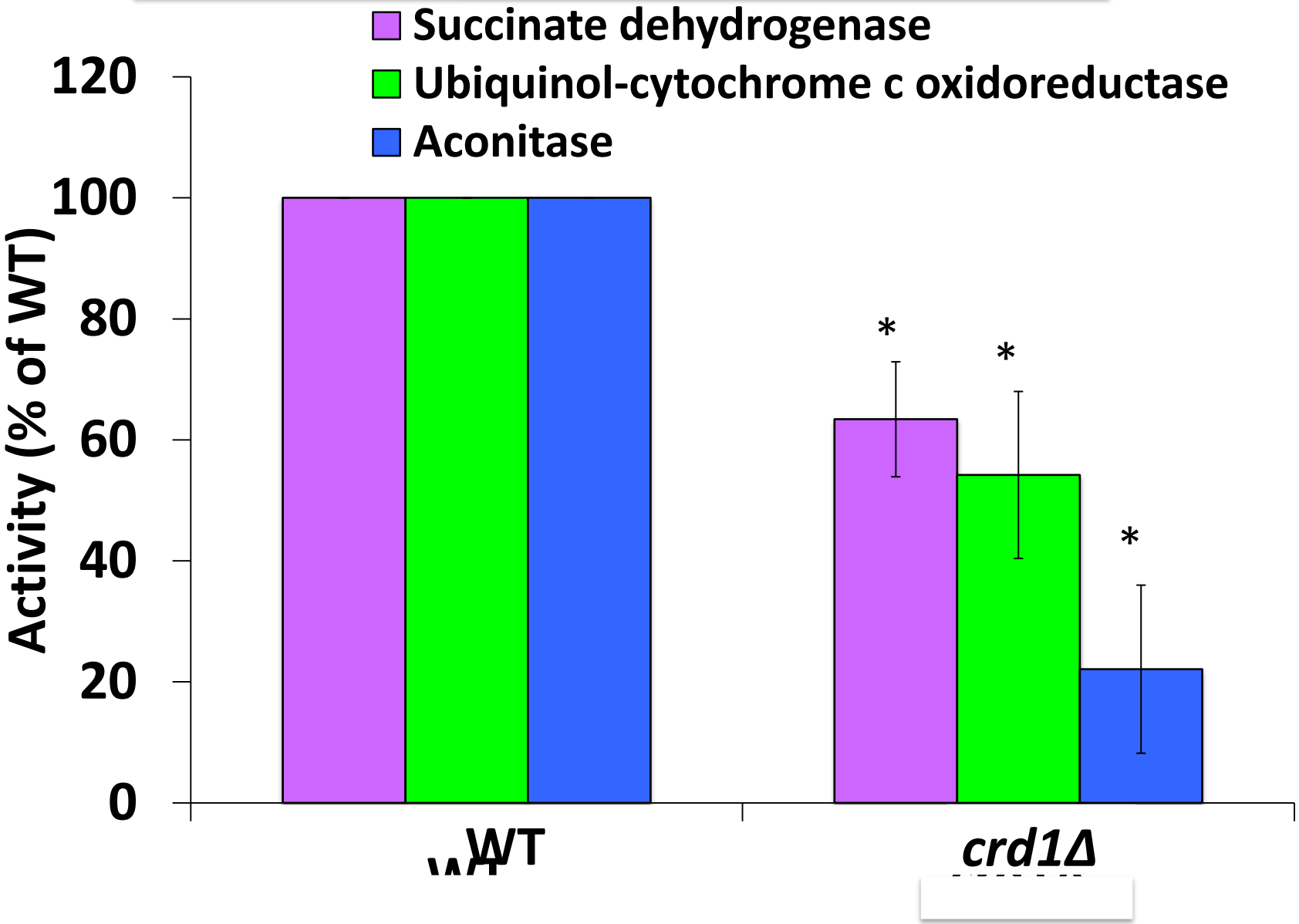
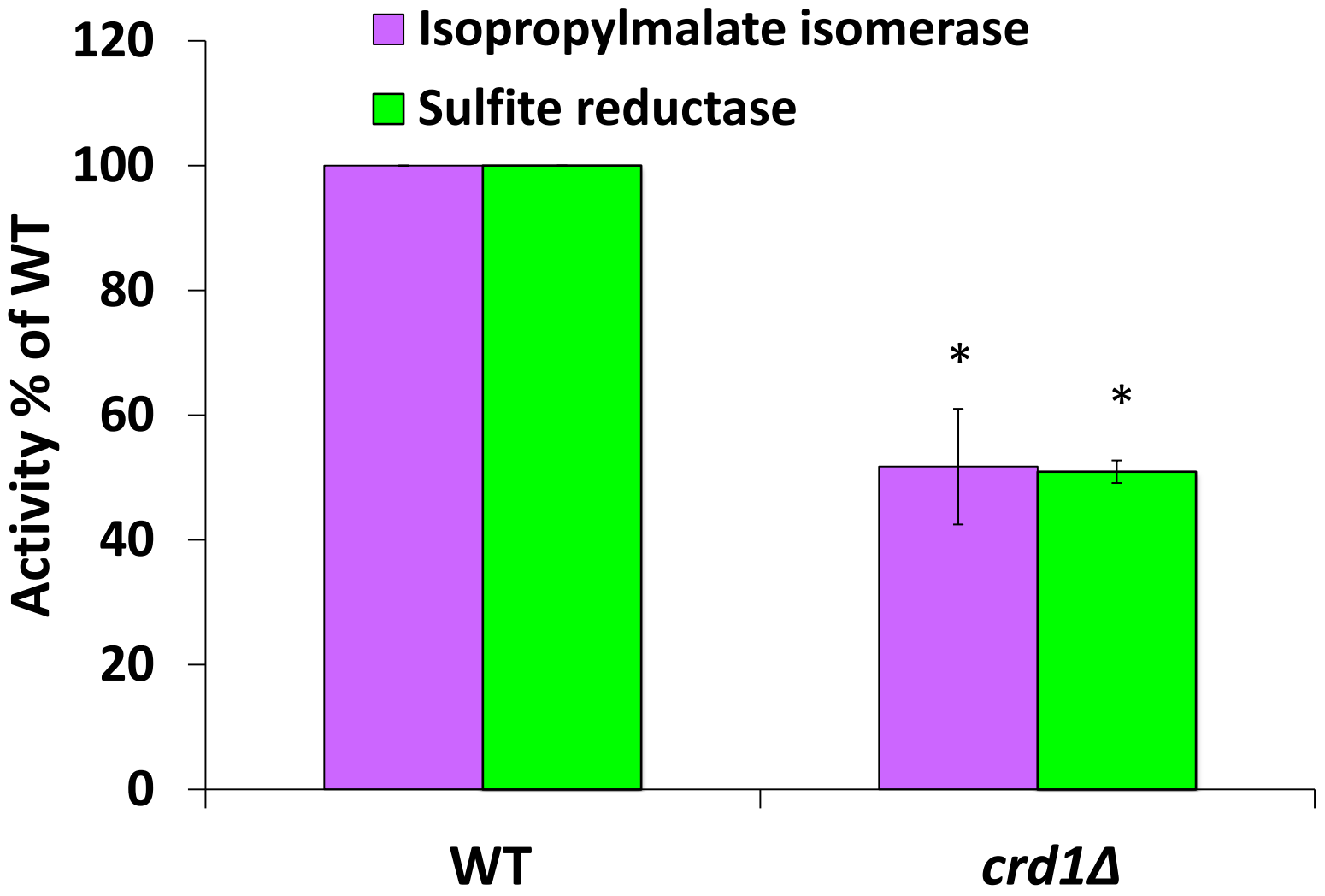
crd1Δ has decreased activities of *mitochondrial* Fe-S enzymes



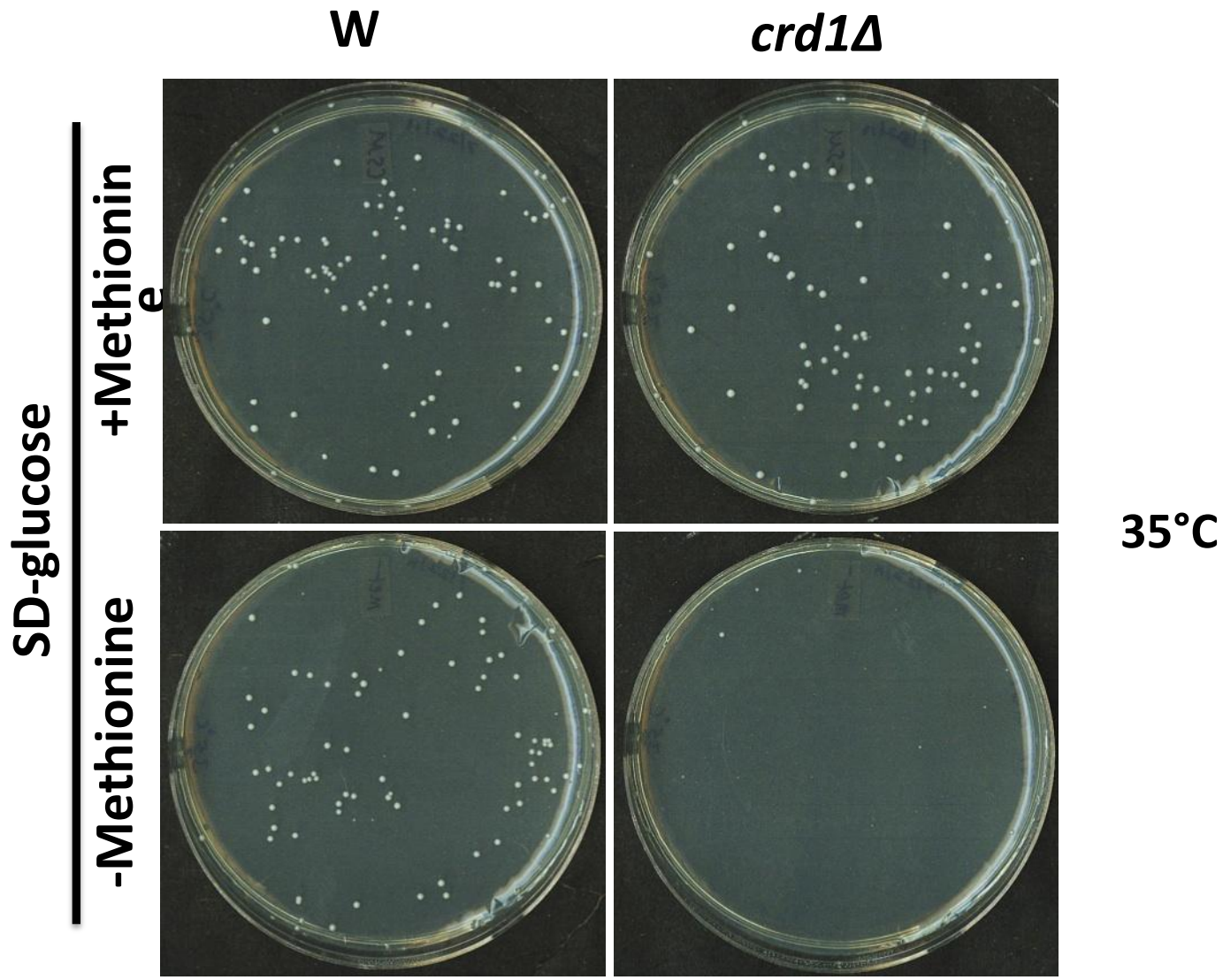
crd1Δ has decreased activities of *cytoplasmic* Fe-S enzymes



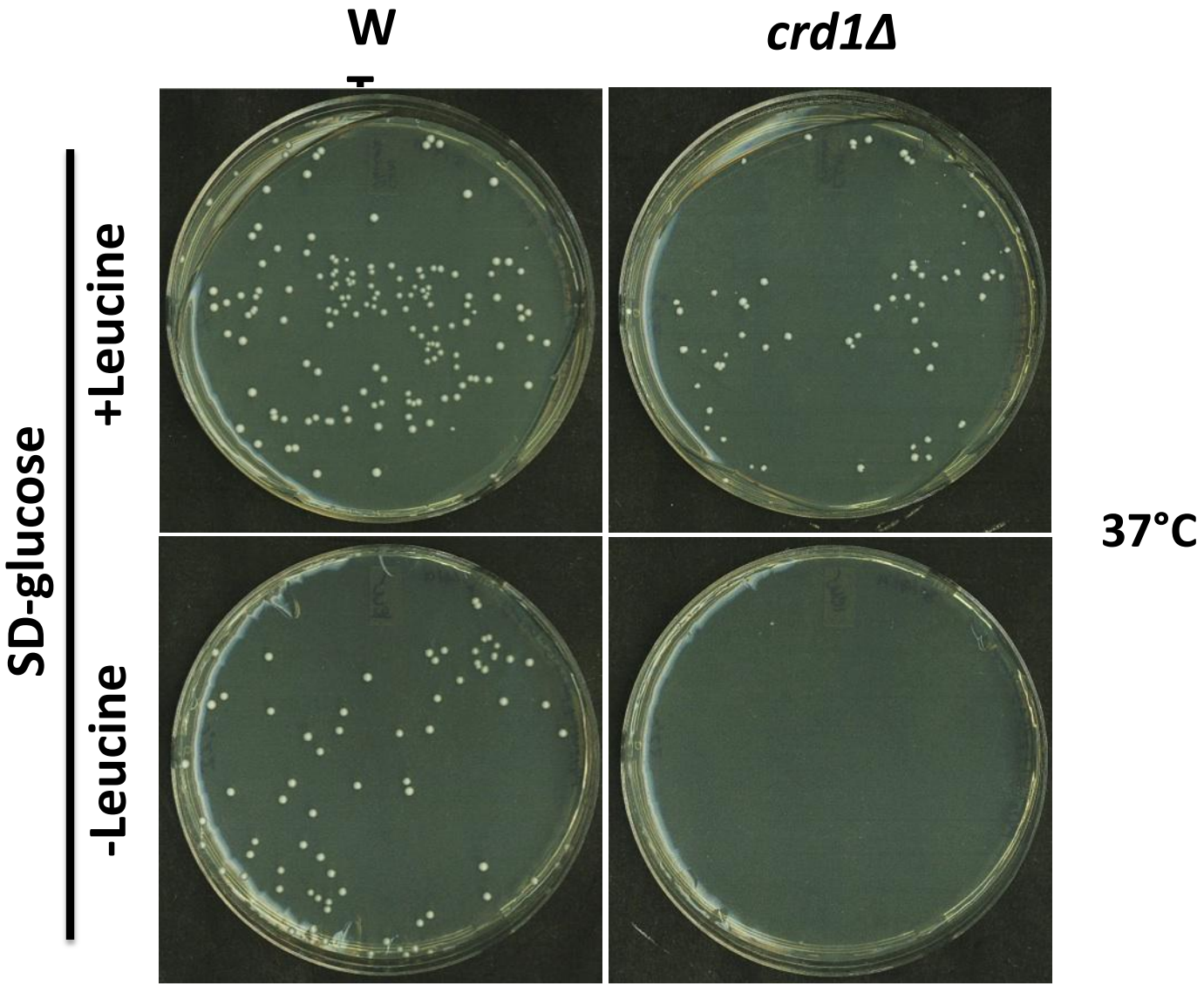
Decreased cytoplasmic Fe-S enzyme activities lead to amino acid auxotrophies in *crd1Δ*

- **sulfite reductase (Met5, Met10) - methionine synthesis**
- **isopropylmalate isomerase (Leu1) - leucine synthesis**

crd1Δ shows methionine auxotrophy at elevated temperature



crd1Δ shows leucine auxotrophy at elevated temperature

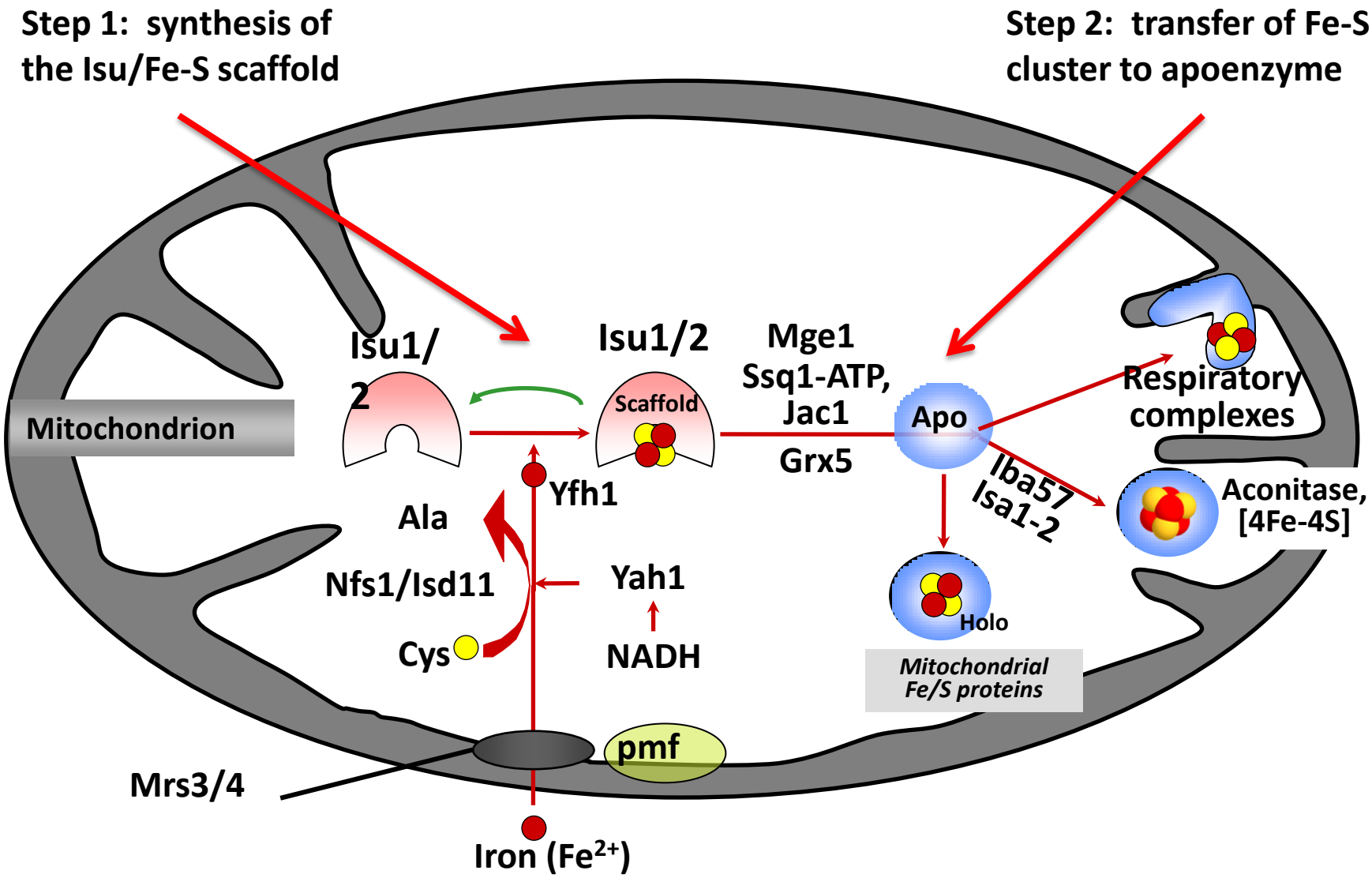


Is perturbation of iron homeostasis in *crd1Δ* due to defects in mitochondrial Fe-S cluster biogenesis?

Predictions:

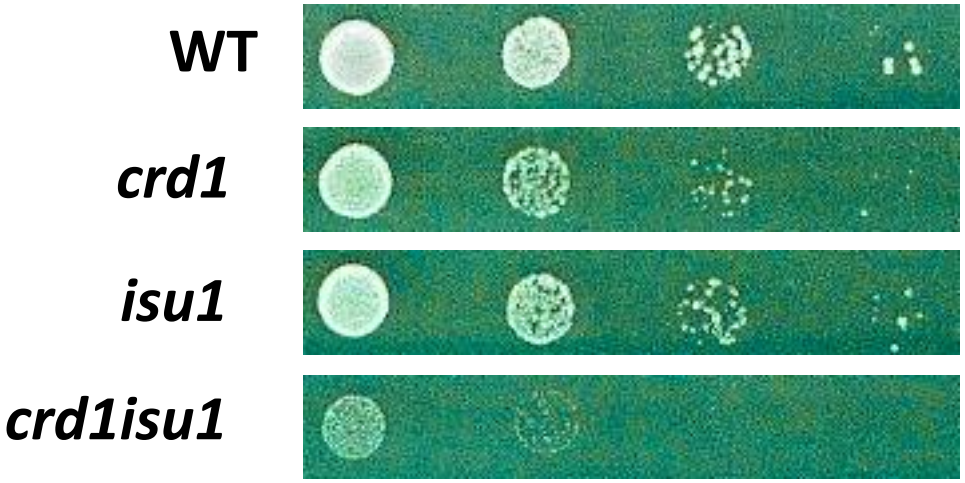
- ***crd1Δ*** **DOES exhibit decreased activity of Fe-S enzymes**
- ***crd1Δ*** **is sensitive to further perturbation of Fe-S synthesis – synthetic genetic interaction with Isu**
- ***crd1Δ*** **exhibits up-regulation of Fe-S scaffold (characteristic of Fe-S perturbation)**

Fe-S (iron sulfur) cluster biogenesis



From Roland Lill

The *isu1* mutant exacerbates the growth defect of *crd1Δ*



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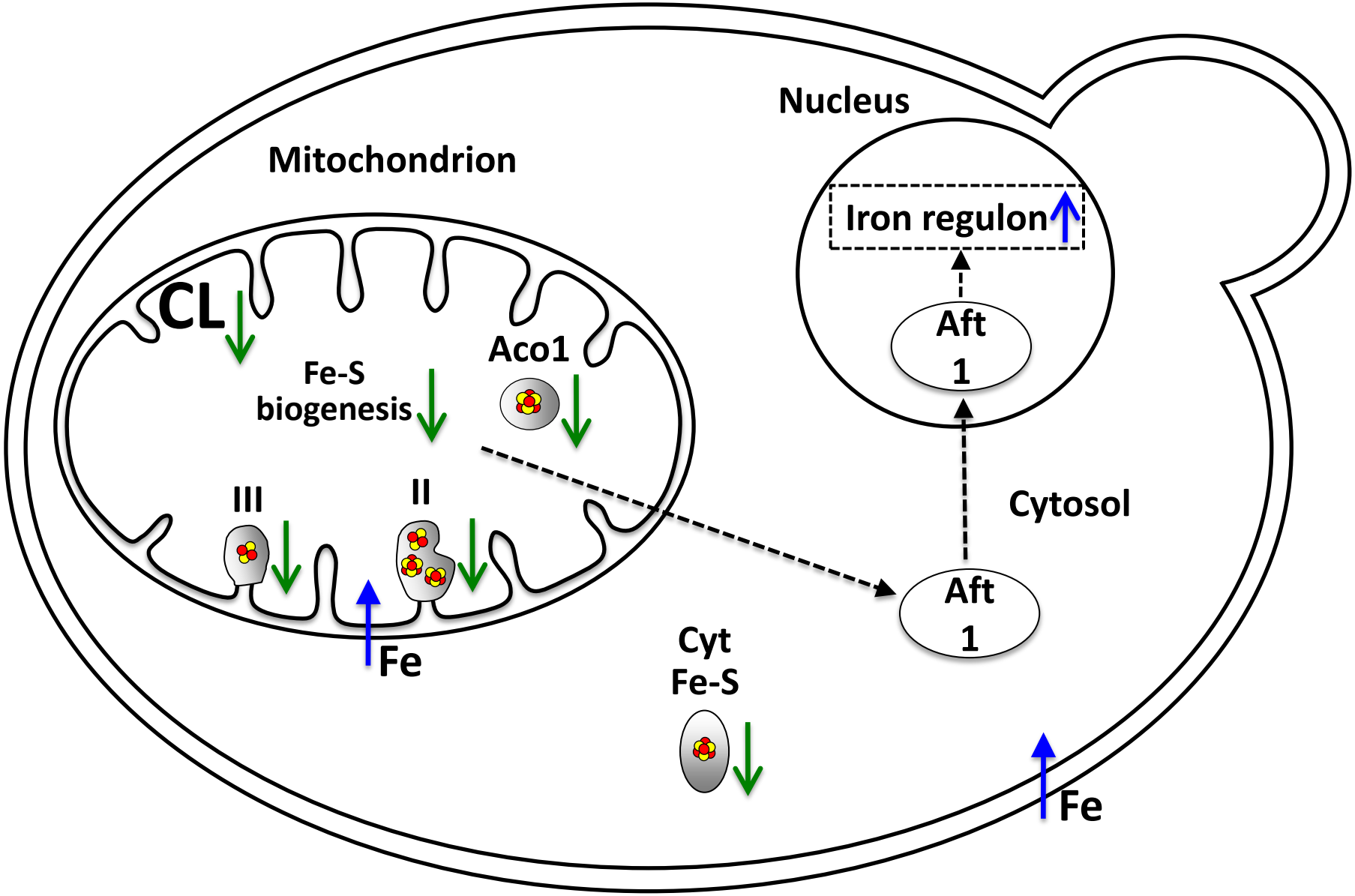
- ***crd1Δ*** **DOES exhibit up-regulation of Fe-S scaffold (characteristic of Fe-S perturbation)**

Summary: Loss of CL leads to iron homeostasis defects and perturbation of Fe-S biogenesis

- Phenotypes associated with altered Fe levels
  - Increased mitochondrial Fe
  - Sensitivity to iron
  - Sensitivity to peroxide
- Fe-S associated defects
  - Decreased activities of mitochondrial and cytoplasmic Fe-S enzymes
  - Synthetic interaction with Fe/S scaffold mutant *Isu1*
  - Phenotypes associated with Fe-S defects (increased expression of Isu1)

Proposed model: Loss of CL leads to perturbation of mitochondrial and cellular iron homeostasis



Cardiolipin is required for optimal mitochondrial protein import

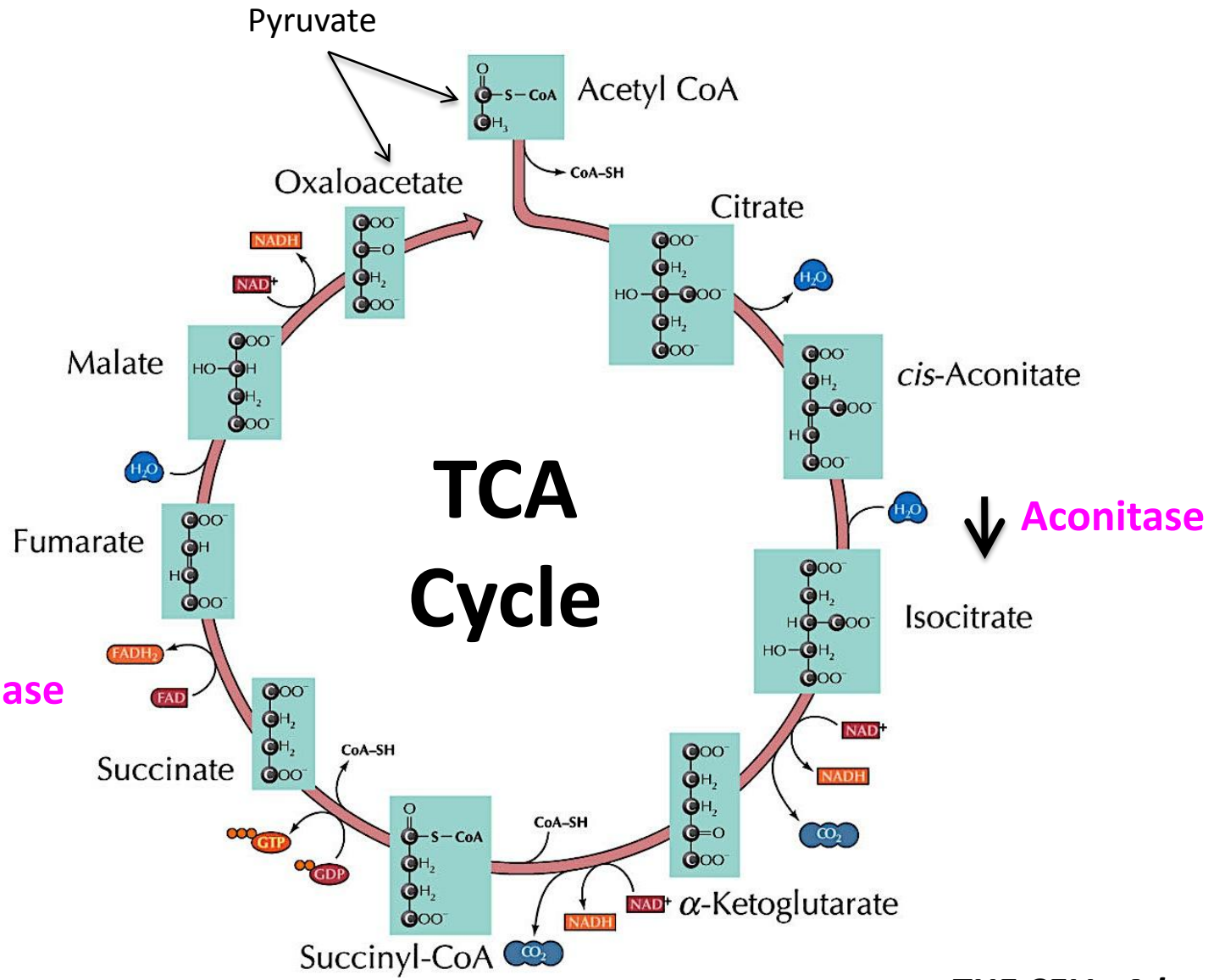
Jiang et al. 2000. Absence of Cardiolipin in the *crd1* Null Mutant Results in Decreased Mitochondrial Membrane Potential and Reduced Mitochondrial Function. *J. Biol. Chem.*, **275,** 22387-22394.

Gebert et al., 2009. Mitochondrial Cardiolipin Involved in Outer Membrane Protein Biogenesis: Implications for Barth Syndrome. *Current Biol.* **19,** 2133-2139.

Possible mechanism linking cardiolipin to defective iron homeostasis - defective import of proteins involved in Fe-S biogenesis

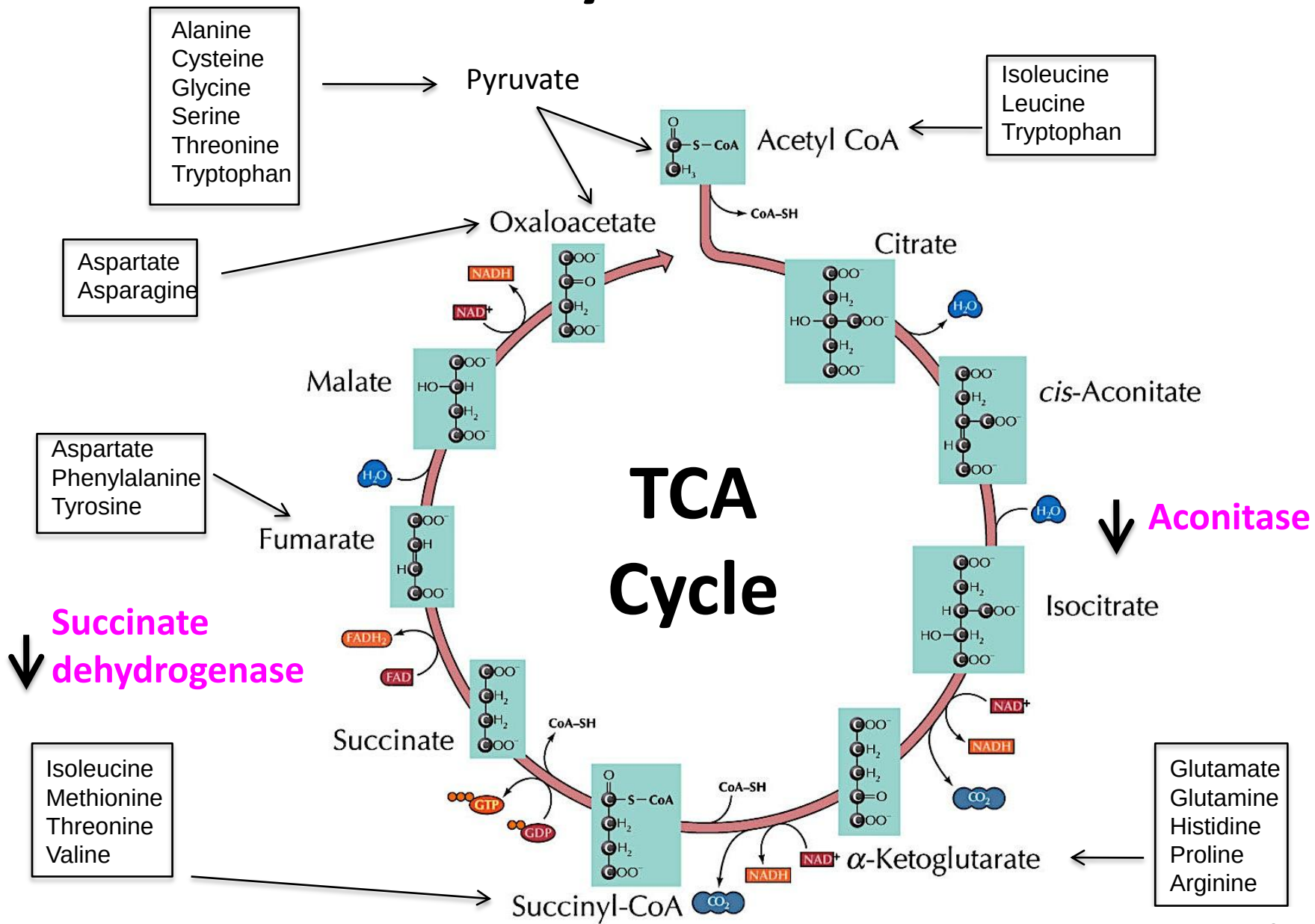
What are the consequences of Fe-S defects?

Perturbation of the TCA cycle



THE CELL, 4th edition

Secondary metabolic effects



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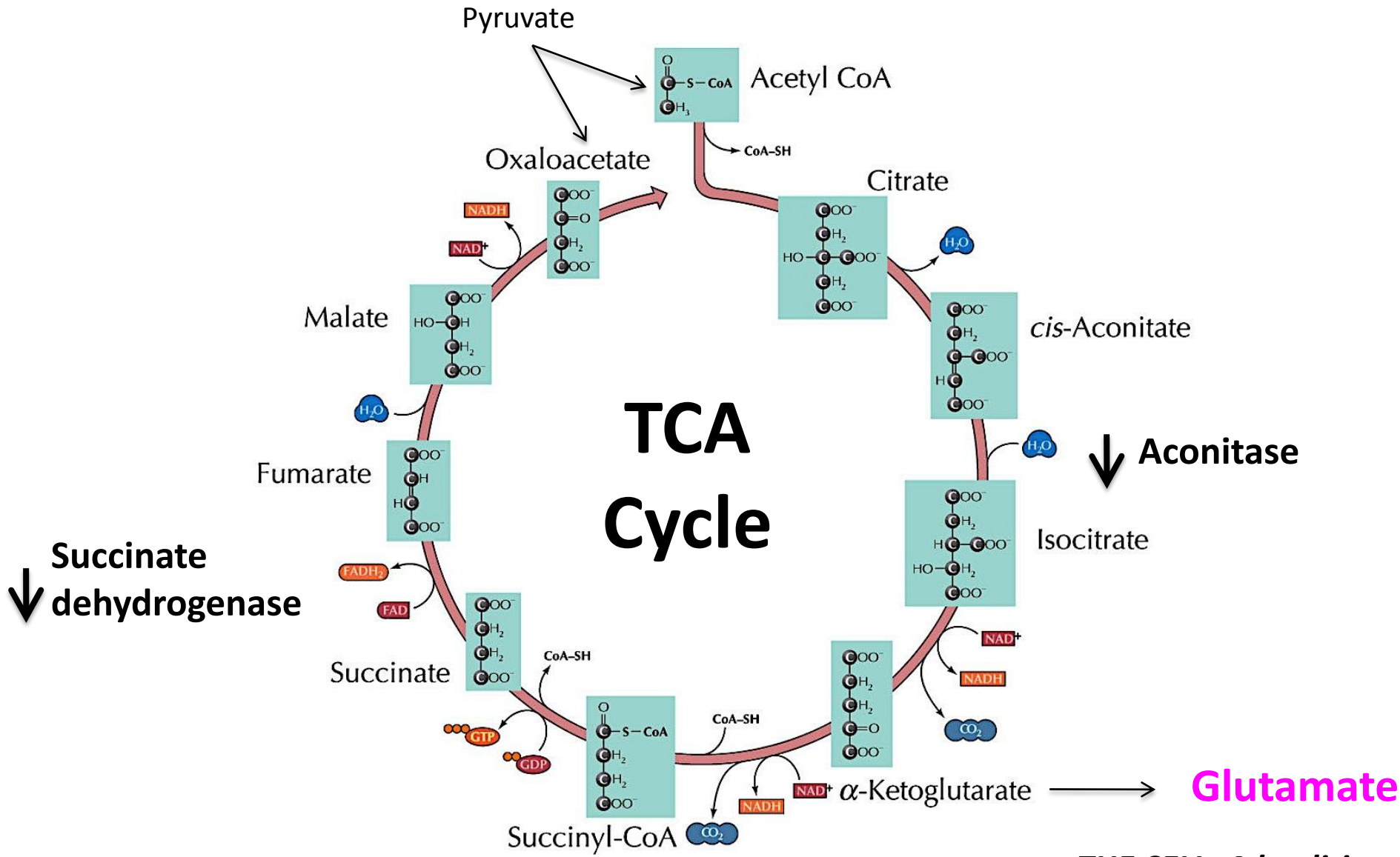

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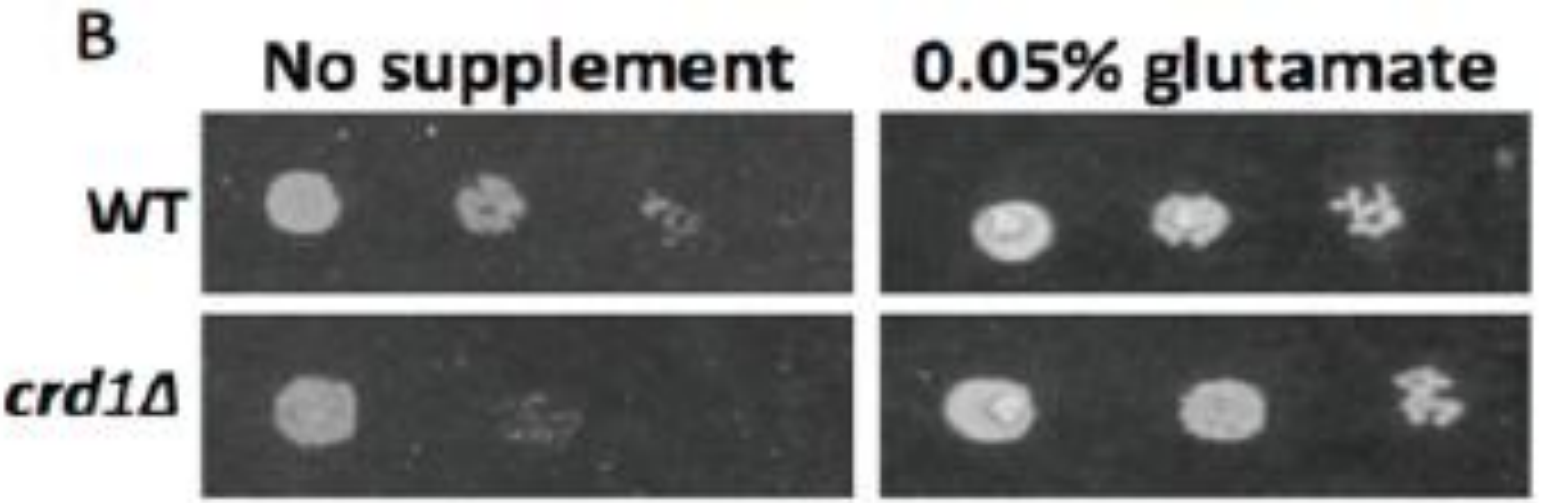
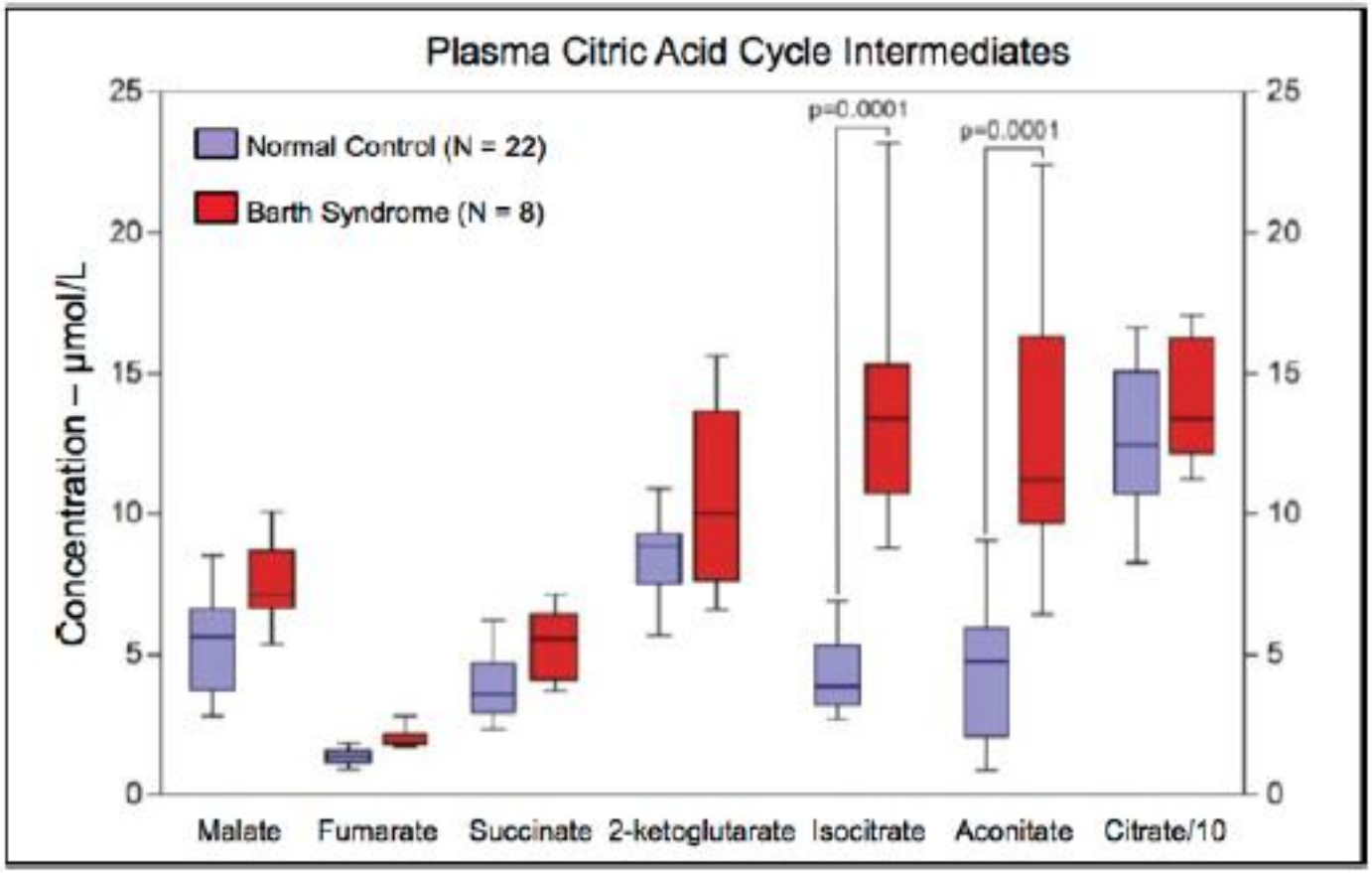
Supplementation of glutamate restores growth of *crd1Δ* at elevated temperature

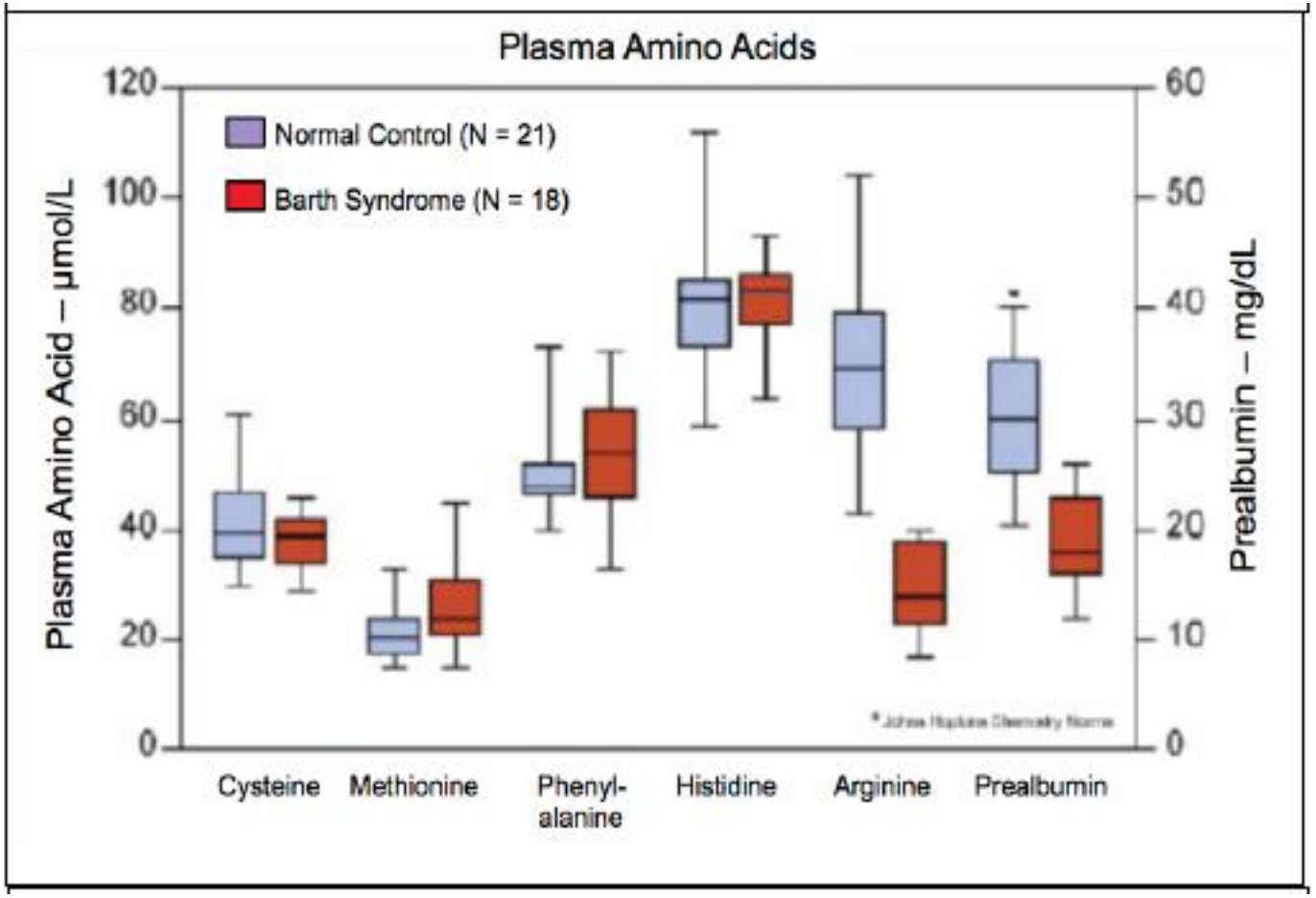


Aberrant plasma TCA cycle intermediates in BTHS patients



Data from Richard Kelley

Aberrant plasma amino acids in BTHS patients



Data from Richard Kelley

ISCU (iron sulfur cluster assembly protein) mutation in humans leads to myopathy

- **Clinical Manifestation and a New ISCU Mutation in Iron–Sulfur Cluster Deficiency Myopathy** (Kollberg *Brain* 2009).
- **Splice Mutation in the Iron-Sulfur Cluster Scaffold Protein ISCU Causes Myopathy with Exercise Intolerance** (Mochel *Amer J Hum Gen* 2008).
- **Myopathy with Lactic Acidosis is Linked to Chromosome 12q23.3–24.11 and Caused by an Intron Mutation in the ISCU Gene Resulting in a Splicing Defect** (Olsson *Human Molec Gen* 2008).
- **Reversal of Iron-Induced Dilated Cardiomyopathy During Therapy With Deferasirox in Beta-Thalassemia** (Trad *Pediat Blood Cancer* 2008).
- **Mitochondrial Myopathy with Succinate Dehydrogenase and Aconitase Deficiency Abnormalities of Several Iron-Sulfur Proteins** (Hall *J Clin Inv* 1993).

MOLECULAR BASIS OF THE PATHOLOGY?
RELATIONSHIP OF GENOTYPE TO PHENOTYPE?

Loss of cardiolipin

↓

defective Fe-S biogenesis

↓

perturbation of the TCA cycle

↓

secondary metabolic dysfunction

Future directions

- **Elucidate mechanisms linking cardiolipin to Fe-S biogenesis**
- **Determine consequences of TCA cycle perturbation in cardiolipin deficient cells**
- **Characterize role of cardiolipin in acetyl CoA synthesis and β-oxidation**

Acknowledgements

Vinay Patil

Vishal Gohil

Collaborators:

Dennis R. Winge
Jennifer L. Fox

Support:

NIH

Barth Syndrome Foundation

Detroit

Wayne State University