SSIEM Meeting, Geneva, August 31st 2011

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Intracellular defects of cobalamin metabolism. CblI trafficking

Pr Brian Fowler

« Cobalamin is the most complex and the most fascinating vitamin »

Riboflavin

The Revenge !

Structure of vitamin B12

Structure of vitamin B2

Richard Kuhn
The Nobel Price in Chemistry
1938

Paul György

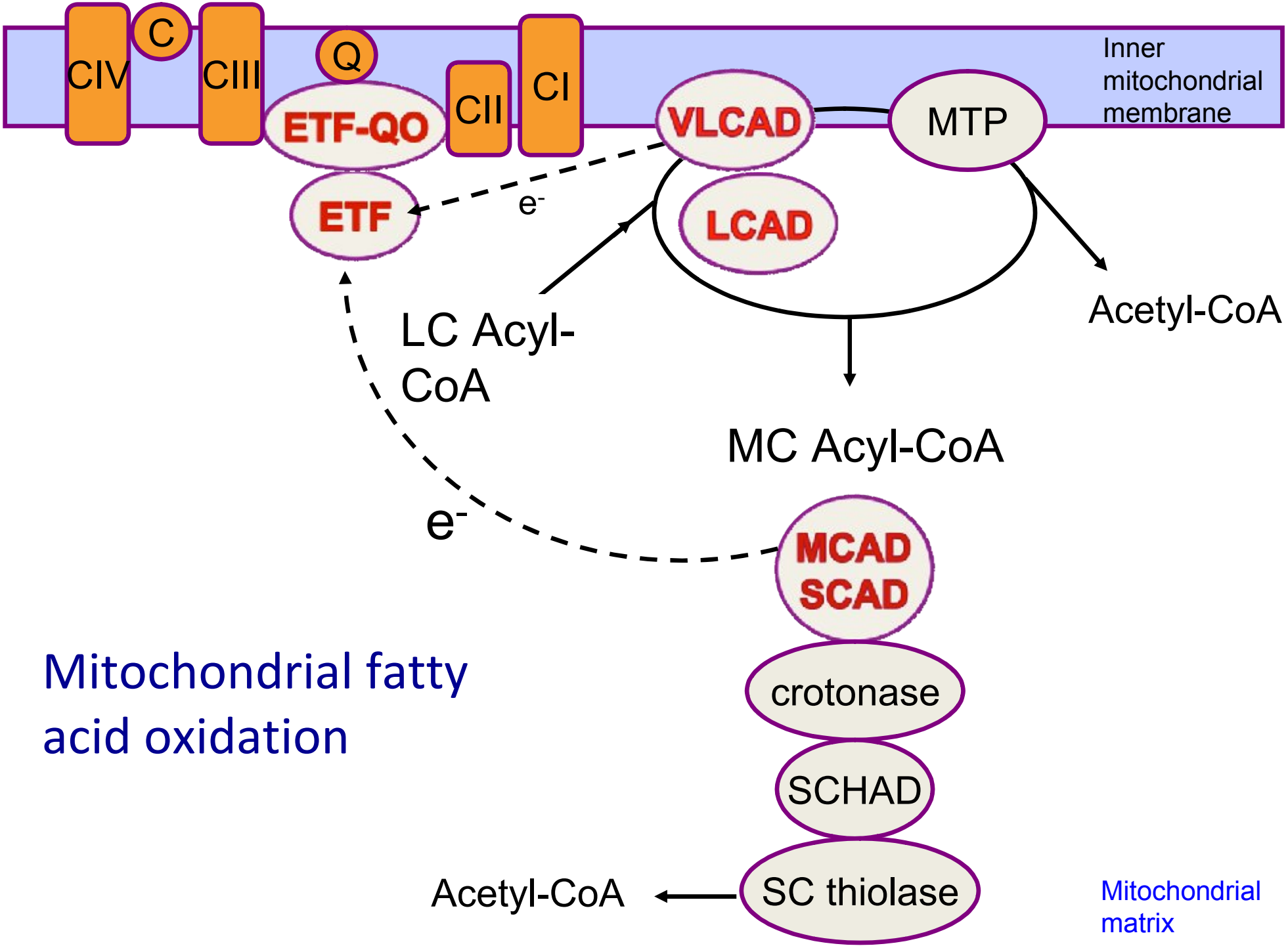
Paul Karrer
The Nobel Price in Chemistry
1937

Cobalamin (vitamin B12)

- Essential cofactor for 2 enzymes
  - Methionine synthase (methyl-Cbl)
  - Methylmalonyl-CoA mutase (adenosyl-Cbl)
- 2 metabolisms
  - Homocystein metabolism
  - Propionyl-CoA metabolism

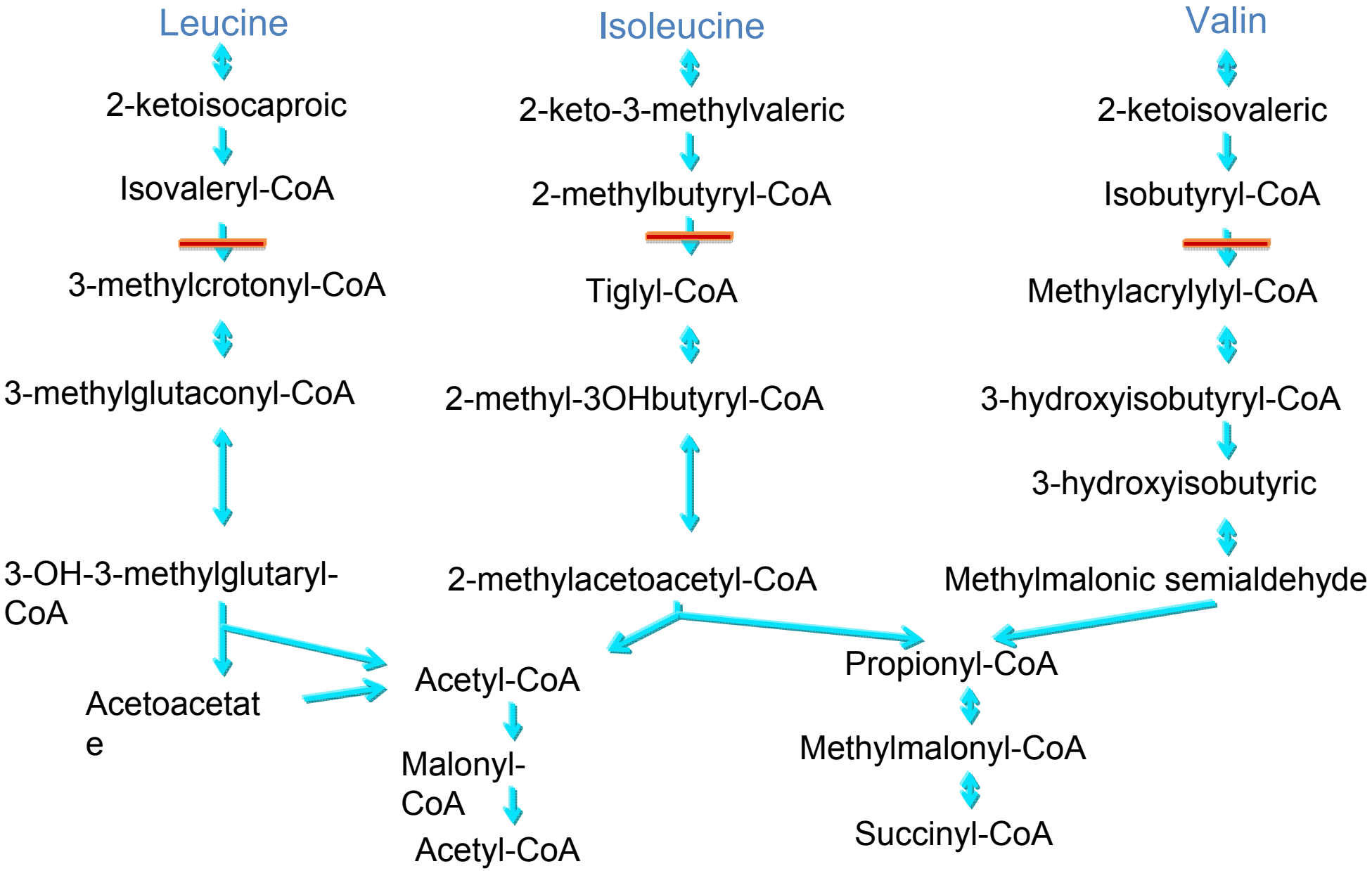
Riboflavin (vitamin B2)

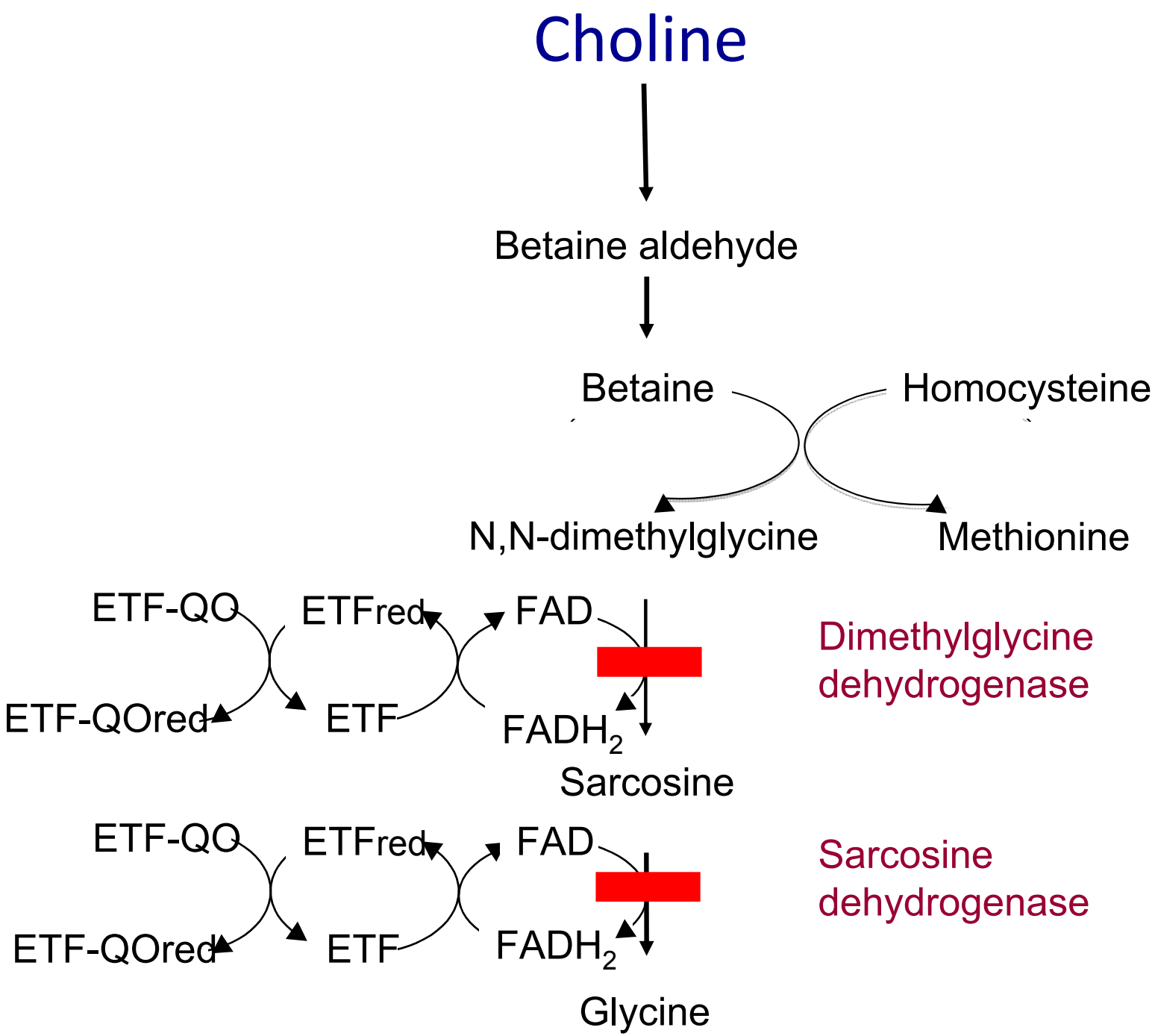
- Riboflavin is the precursor of FAD and FMN
- FAD is the essential cofactor for 11 ETF dependent dehydrogenases: 5 metabolisms
  - Fatty acid oxidation: VLCAD, LCAD, MCAD, SCAD
  - Branched-chain metabolism: Isovaleryl-CoA dehydrogenase, isobutyryl-CoA dehydrogenase, 2-methylbutyryl-CoA dehydrogenase
  - Choline metabolism: sarcosine dehydrogenase, dimethylglycine dehydrogenase
  - Lysine metabolism: glutaryl-CoA dehydrogenase
  - 2-hydroxyglutarate dehydrogenase
- FAD is the essential cofactor for the 2 electron transporters
  - Electron Transfer Flavoprotein (ETF)
  - Electron Tranfer Flavoprotein Ubiquinone Oxidoreductase (ETF-QO)

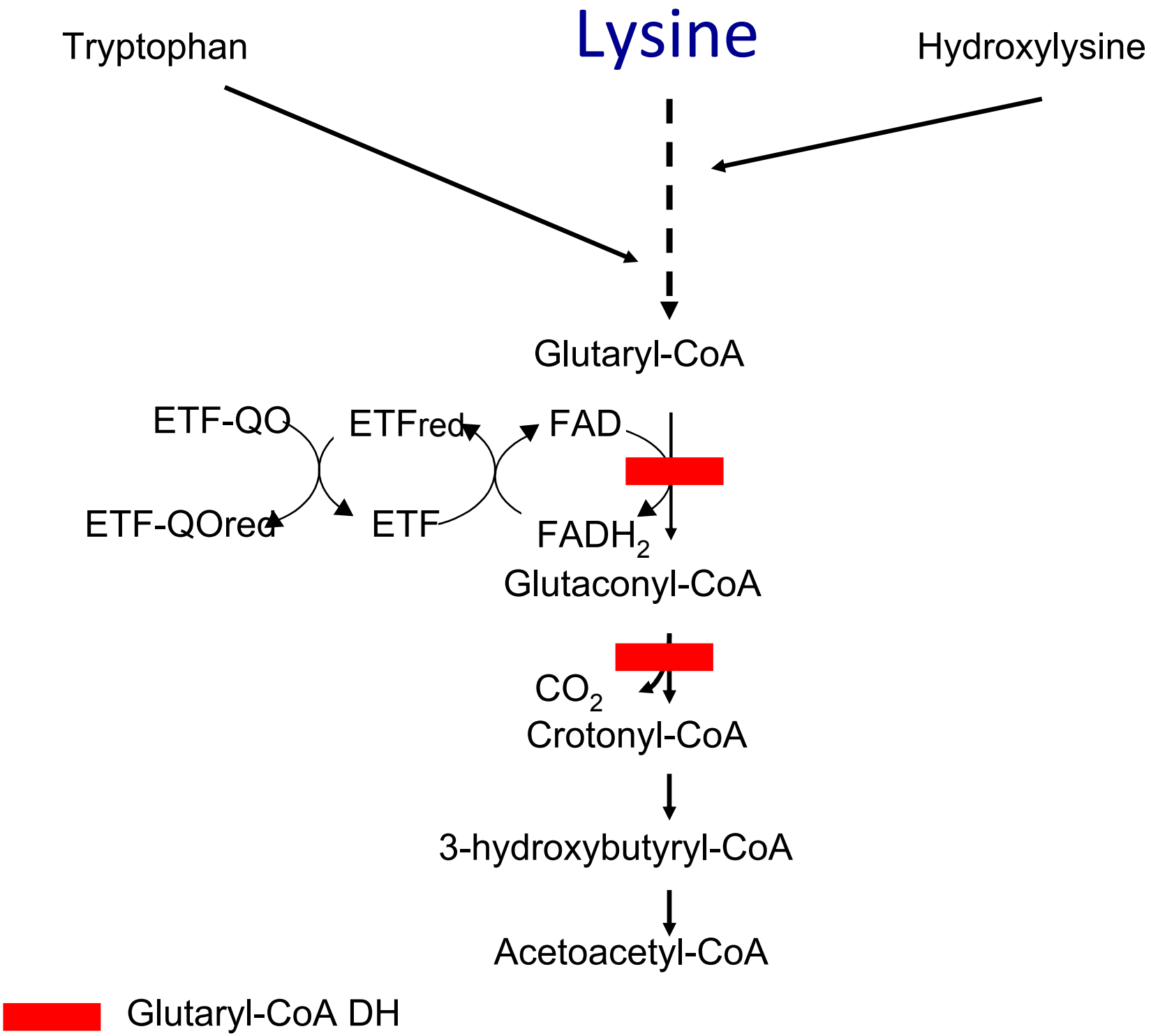


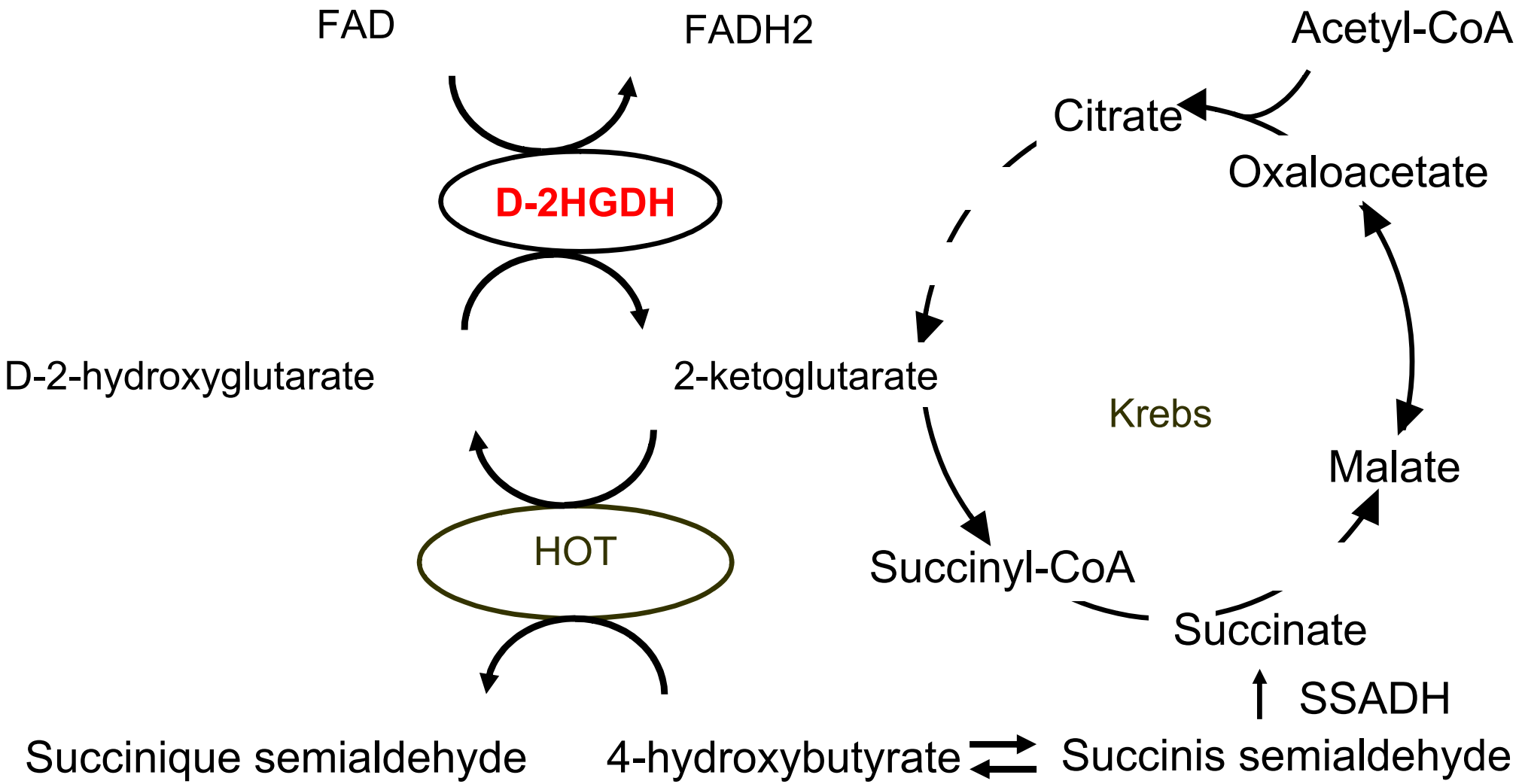
Mitochondrial fatty acid oxidation

Branched-chain amino acids




Choline
Betaine aldehyde
Betaine
Homocysteine
N,N-dimethylglycine
Methionine
ETF-QO
ETFred
FAD
ETF-QOred
ETF
FADH2
Sarcosine
Dimethylglycine dehydrogenase
ETF-QO
ETFred
FAD
ETF-QOred
ETF
FADH2
Glycine
Sarcosine dehydrogenase


Tryptophan
Lysine
Hydroxylysine
Glutaryl-CoA
ETF-QO
ETFred
FAD
ETF-QOred
ETF
FADH2
Glutaconyl-CoA
CO2
Crotonyl-CoA
3-hydroxybutyryl-CoA
Acetoacetyl-CoA
Glutaryl-CoA DH



D-2HGDH: D-2hydroxyglutarate dehydrogenase
SSADH : succinic semialdehyde dehydrogenase
HOT: 2-hydroxyacid transhydrogenase

Riboflavin (vitamin B2)

- FAD and FMN are the cofactors for many other enzymes called flavoproteins
- Most of these favoproteins are found in mitochondria
- Flavoproteins typically contain either FAD or FMN cofactor not bound covalently to the apoprotein
- For example
  - Succinate dehydrogenase (complex II)
  - Dihydrolipoamide dehydrogenase (E3): BCKDH, 2-ketoglutarate dehydrogenase, pyruvate dehydrogenase, glycine cleavage system
  - Glutathione reductase, involved in protection against oxidative stress
  - Methylene tetrahydrofolate reductase: interaction with cobalamin metabolism
  - ….

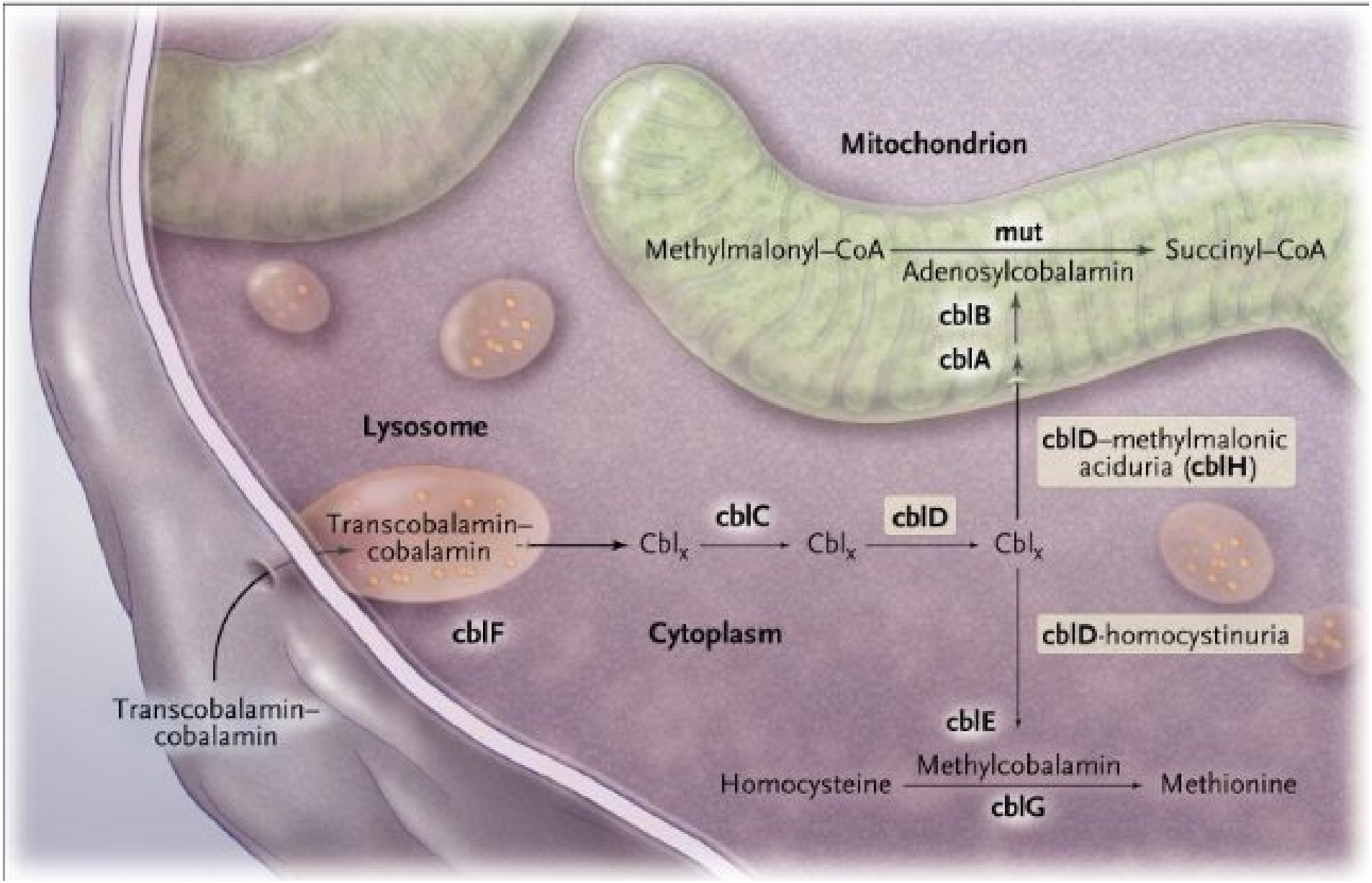
Cobalamin

- 2 enzymes
- 2 metabolisms

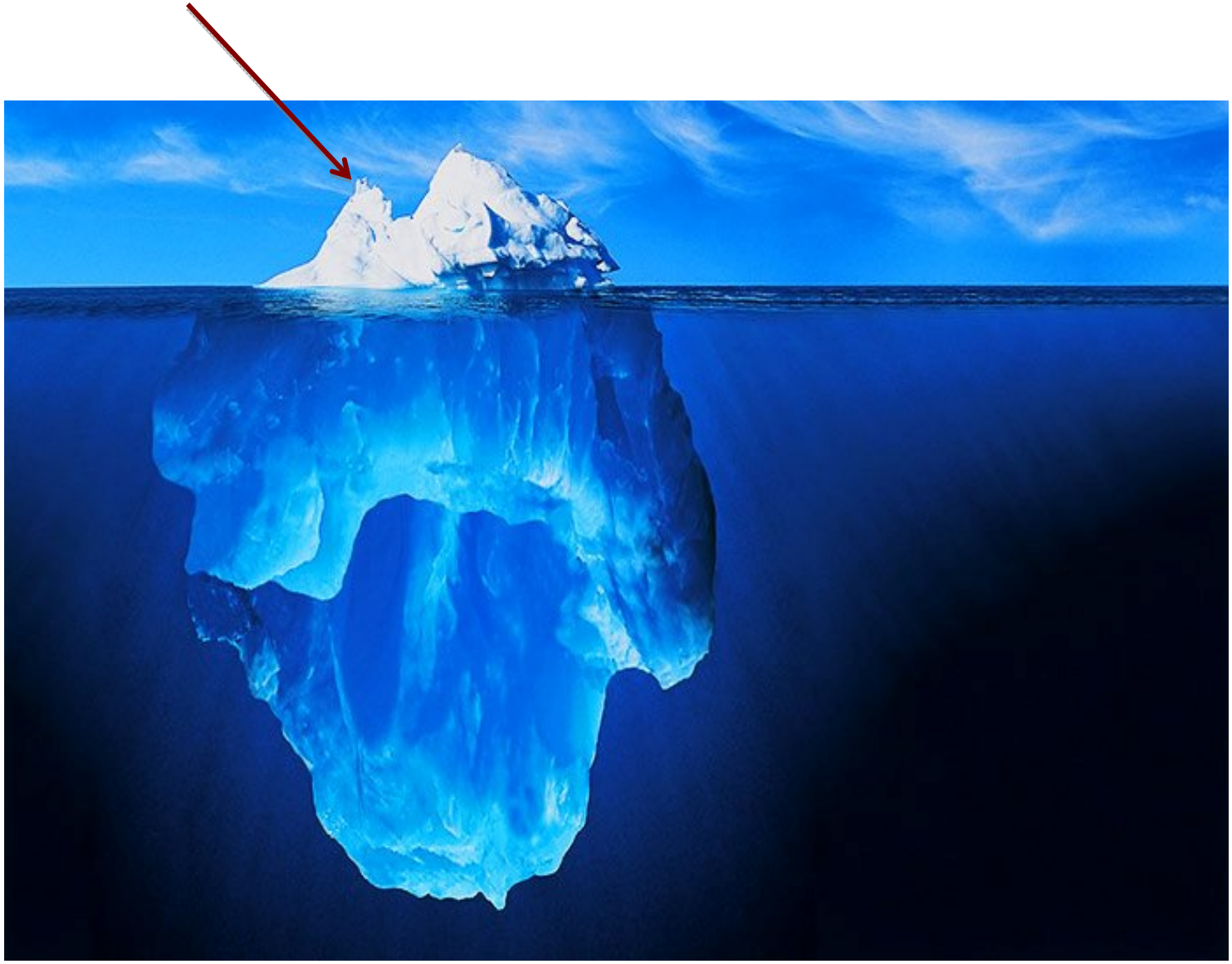
Riboflavin

- 11 ETF dependent enzymes (5 metabolisms)
- 2 electron transporters
- Many flavoproteins
- Many metabolisms

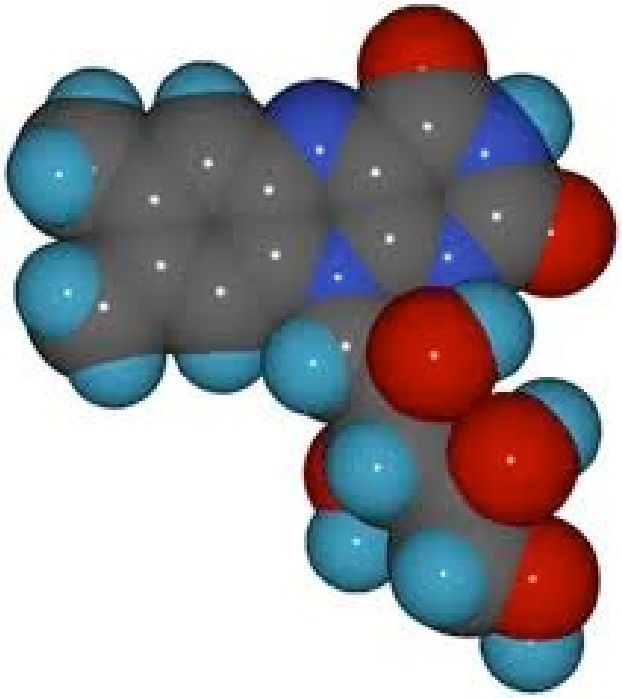
Metabolism of cobalamin

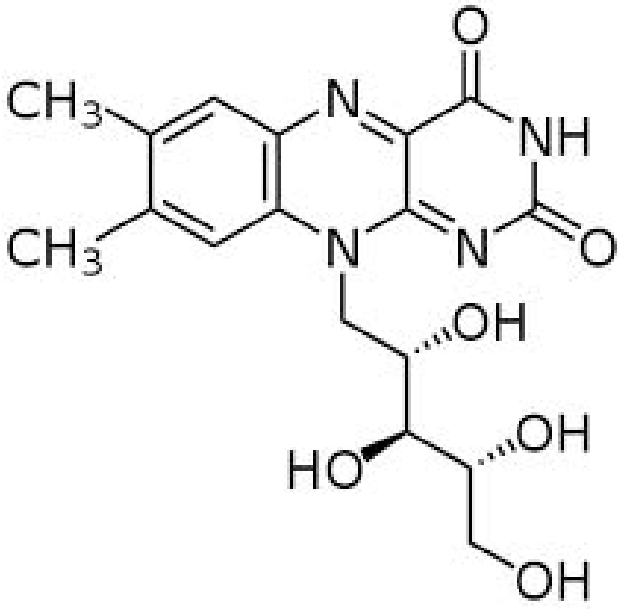


Metabolism of riboflavin



Riboflavin





Flavin

Ribitol

Vitamin B2 (riboflavin)

- Water soluble vitamin
- Sources of riboflavin
  - Milk and dairy products makes the greatest contribution of its intake in Western diets
  - Meat and fish are also good sources
  - Certain fruits and vegetables, especially dark-green vegetables, contain relatively high concentrations
  - Recommended intakes : range from 0.4 mg/day in infancy to 1.8 mg/day in adult females during lactation
- Riboflavin is degraded by light. It is relatively heat-stable
- Biochemical signs of depletion arises within only a few days of dietary deprivation
- Poor riboflavin status
  - Interferes with iron handling and contributes to anemia when iron intakes are low
  - Implicated as a risk factor for cancer, cardiovascular disease, and neurodegeneration

Riboflavin (vitamin B2)

- Bioavailibility
  - Small amount of riboflavin is present in foods as free riboflavin
  - Most is present as FAD, and a smaller amount occurs as FMN, both non-covalently bound to enzymes
  - Milk and eggs contains appreciable quantities of riboflavin bound to specific binding proteins
- Absorption
  - FMN and FAD has to be hydrolyzed to riboflavin by non specific phosphatases in the brush border membranes of enterocytes
  - Absorption takes place in the proximal small intestine and colon
  - The cellular uptake of riboflavin is saturable

Riboflavin transport

- Riboflavin transporter 1 (hRTF1) (*GPR172B* gene)
  - Identified in 2008
  - In the plasma membrane, mainly expressed in small intestine, placenta and kidney, but ubiquitous
  - Na+, potential and pH independent transport of riboflavin
  - High affinity transporter
  - Could play an important role in the homeostasis of riboflavin
- Riboflavin transporter 2 (hRFT2) (*C20orf54* gene)
  - Identified in 2009
  - Efficient in acidic conditions
  - Saturable
  - Inhibited by FMN and FAD
  - Highly expressed in jejunum and ileum
  - Plays a role in intestinal absorption of riboflavin
  - Located at the apical membrane domain

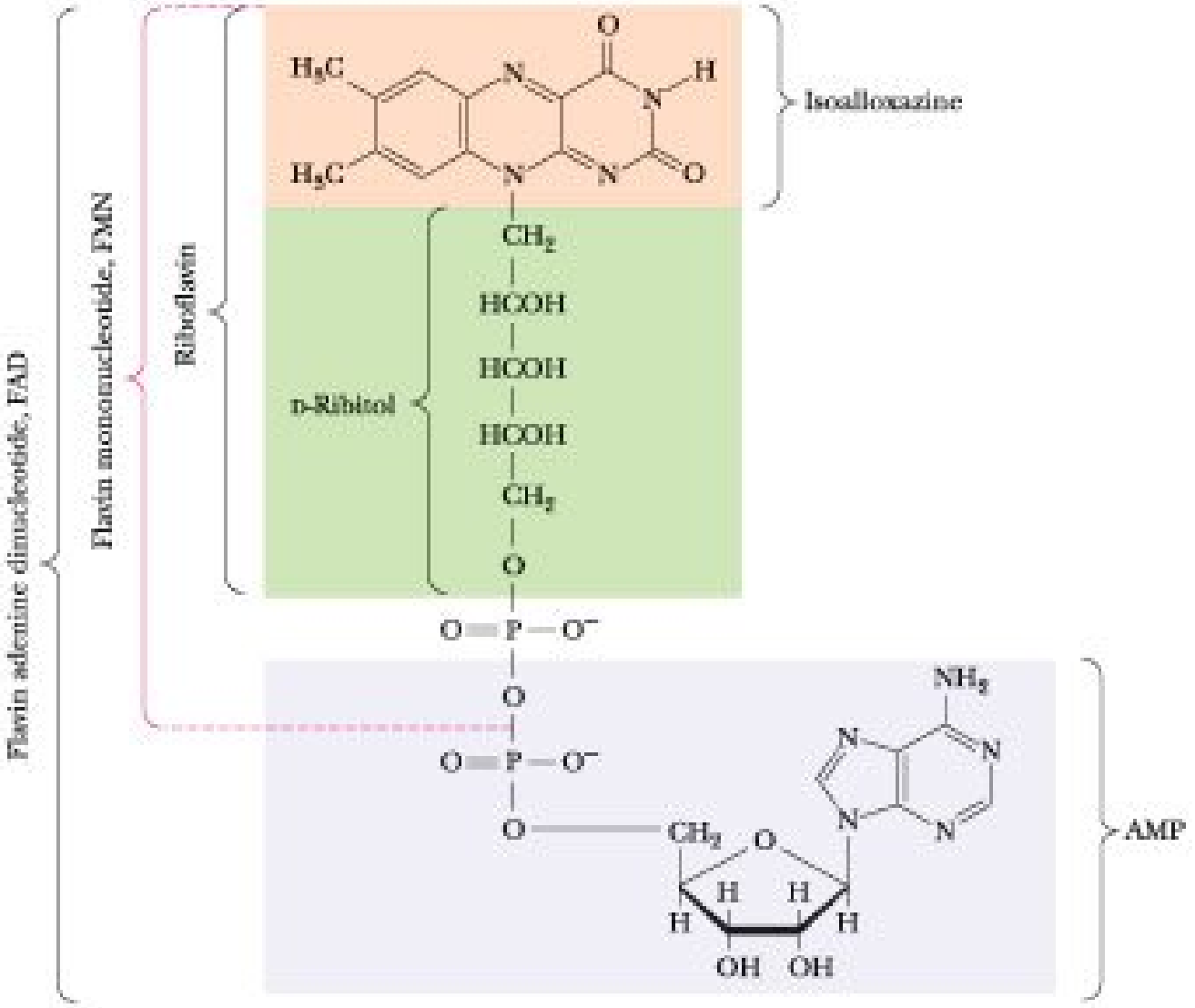
Riboflavin (vitamin B2)

- Riboflavin may enter the plasma as the free form or as FMN
- Free riboflavin is transported in the plasma bound to albumin and certain immunoglobulins
- Almost all riboflavin in tissues is enzyme bound, such as FAD bound to succinate dehydrogenase
- Excess of riboflavin is excreted in the urine as riboflavin or other metabolites such as 7-hydroxymethylriboflavine and lumiflavine

Intracellular metabolism of riboflavin

- Riboflavin kinase also called flavokinase (EC 2.7.1.26)
  - catalyses the phosphorylation of riboflavine to form flavin mononucleotide (FMN) in the presence of ATP and Mg(2+)
  - cytosolic
- FAD synthetase FADS (EC 2.7.7.2)
  - also called ATP-FMN adenylyl transferase
  - converts FMN to FAD in a ATP-dependent reaction
  - two different isoforms
    - hFADS1: localised in mitochondria
    - hFADS2: a soluble isoform; binds FAD very tightly, although non covalently : FAD release may represent the rate-limiting step of the catalytic cycle leading to FAD synthesis and delivery of FAD to apo-flavoproteins

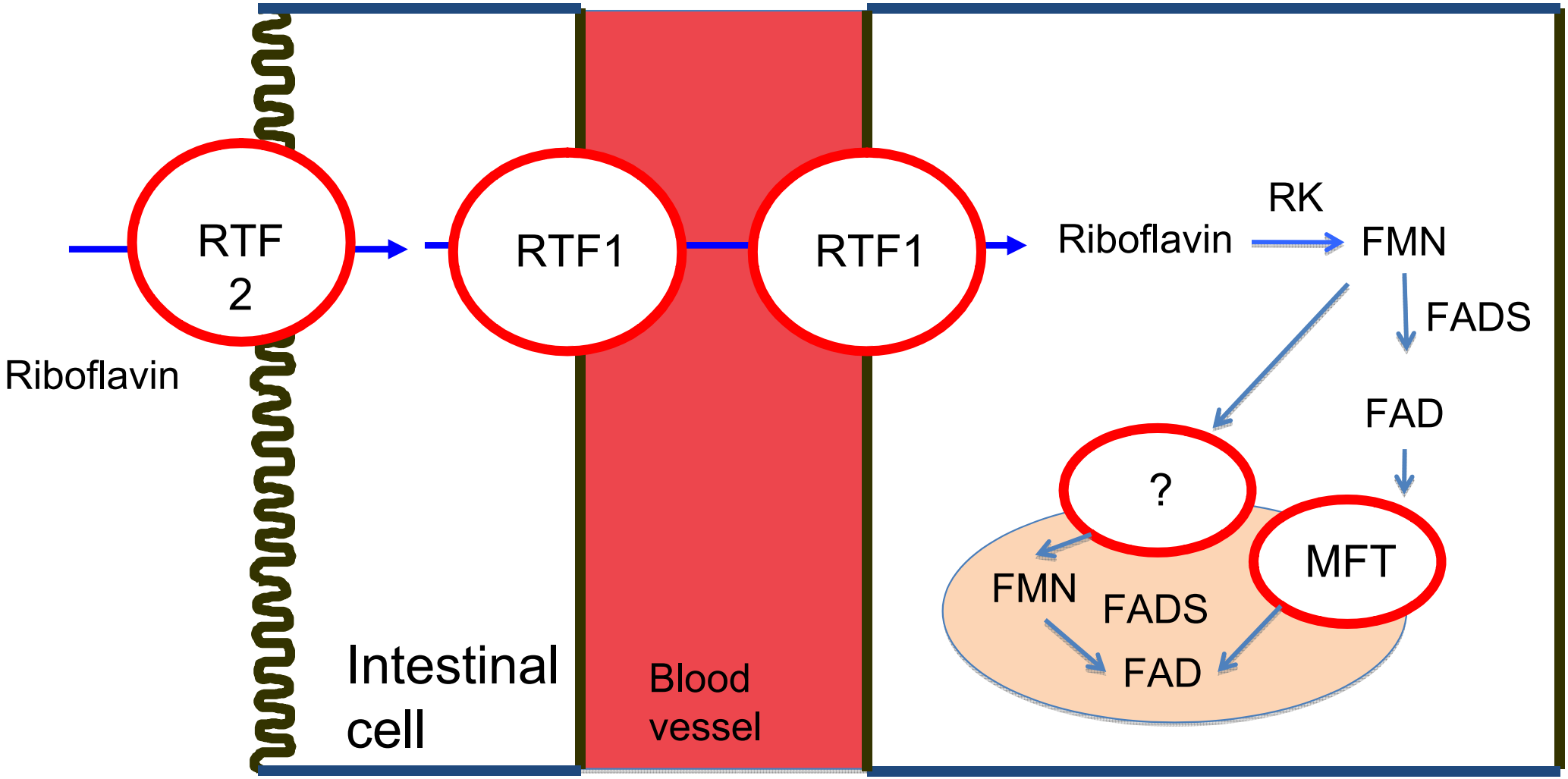
Molecular structure



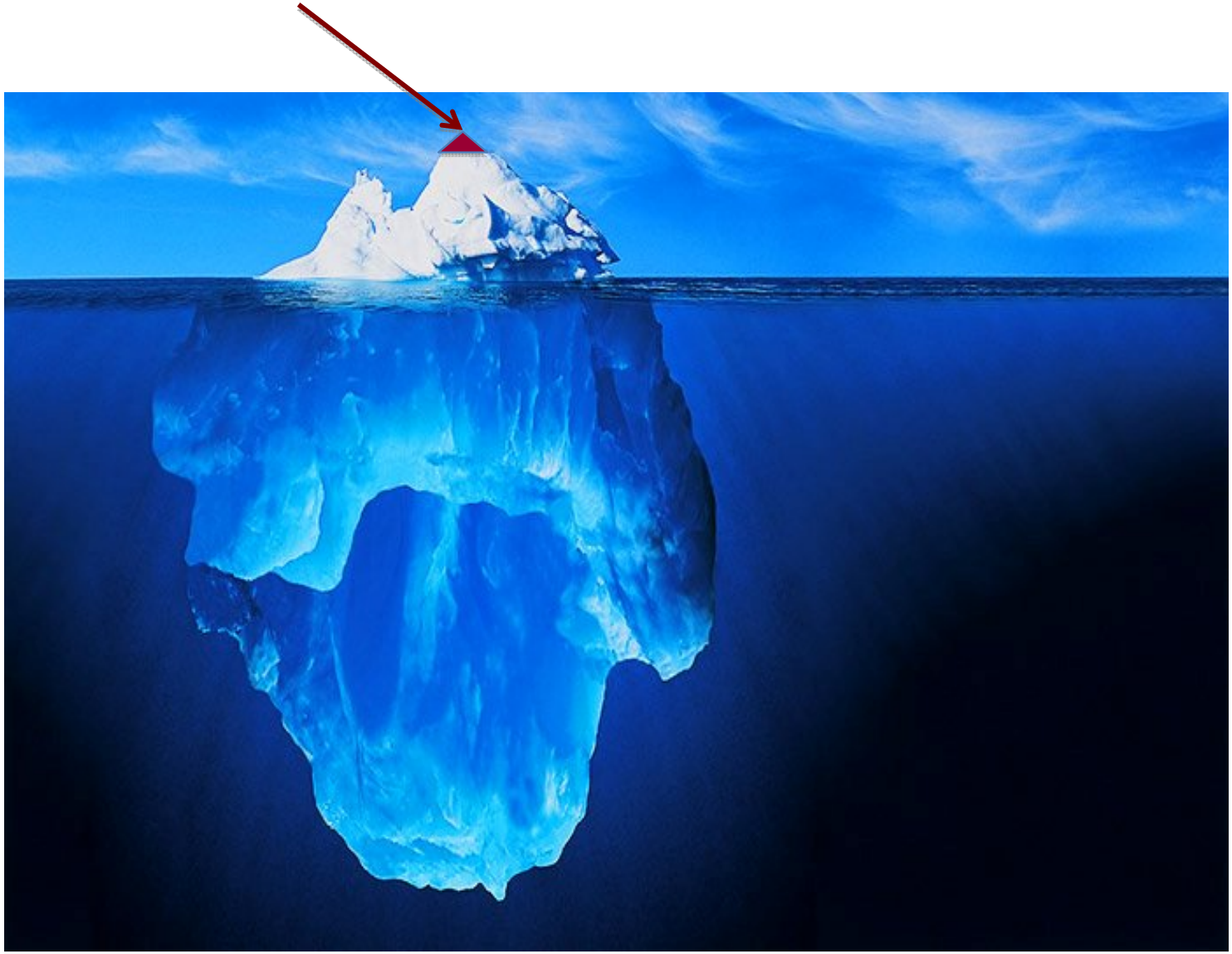
Intracellular metabolism of riboflavin

- Mitochondrial FAD transporter
  - In 2005, Spaan et al (Mol Genet Metab 86:441) identified the human orthologue of the yeast mitochondrial FAD transporter FLX1: MFT
  - MFT was previously identified as the human mitochondrial folate transporter
  - MFT facilitates the transport of FAD from the cytosol to the mitochondria or vice versa, therefore controlling the flavin homeostasis in the mitochondria

Metabolism of riboflavin



Inborn errors of riboflavin metabolism



Riboflavin transporter 1 deficiency

- RTF1: dominant disorder (Ho et al, Human Mutation 2010;32E1976)
- Heterozygous *de novo* microdeletion (1.9 kb, spanning exon 2 and 3) in the *GPR172B* gene in an asymptomatic adult patient
- The first child of this mother presented the clinical and biochemical features of multiple acyl-CoA dehydrogenase deficiency. Her clinical condition improved dramatically within 24 hours with riboflavin supplementation
- During the subsequent pregnancy, the mother has been on riboflavin supplementation: the second child was asymptomatic
- Manifestation of the MAD-like phenotype in only the infant can be attributed to the greater demand for nutrients in the growing foetus, as well as the high expression of RTF1 in placenta

Riboflavin transporter 2 deficiency

- RTF2: autosomic recessive disorder (Bosch et al, JIMD 2011;34:159)
- Point mutations in the *C20orf54* gene in 3 patients from 2 families with clinical phenotype of Brown-Vialetto-Van Laere syndrome or Fazio Londe disease
- Family 1, 2 patients: from the age of 6 months, hypotonia, progressive muscle weakness, respiratory insufficiency due to diaphragmatic paralysis, normal cognitive development at 4 years
- Family 2, 1 patient: from the age of 5 months, muscle weakness, diaphragmatic paralysis, sensorineural hearing loss, normal cognitive development
- Acylcarnitine and urinary organic acid profile suggestive of mild MADD (variable profile, no glutarate)
- Decreased plasma concentrations of riboflavin (except patient 3), FMN and FAD (HPLC) despite a normal riboflavin intake
- Riboflavin supplementation improved muscle tone and normalized the metabolic profile

Riboflavin transporter 2 deficiency

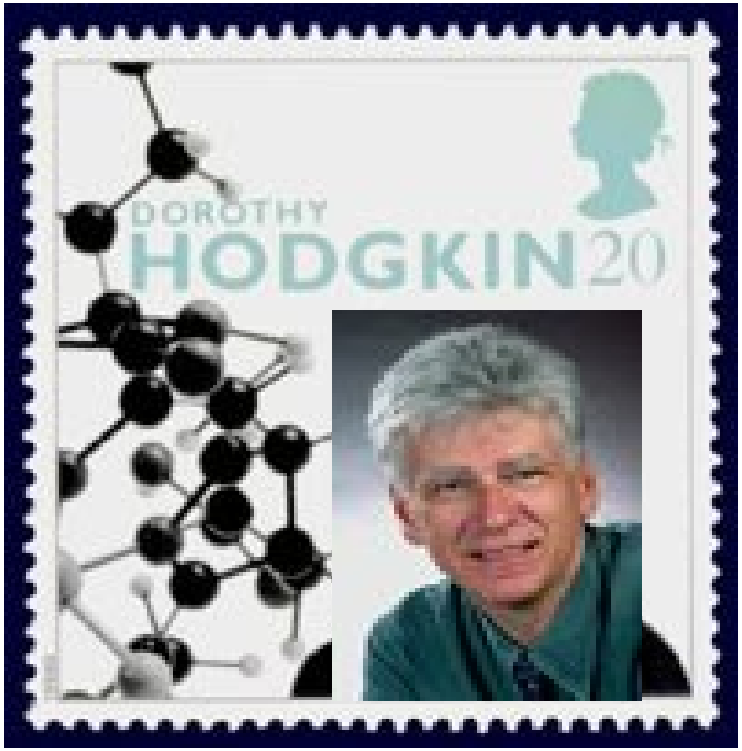
- At the same time, Green et al (Am J Hum Genet 2010; 86:485) reported the identification of mutations in the *C20orf54* gene as the cause of the Brown-Vialetto-Van Laere syndrome or Fazio Londe disease by autozygosity mapping in a large consanguineous family
- They subsequently identified mutations of *C20orf54* gene in 9 patients from 7 families
- No metabolic investigation, no therapeutic attempt
- A few months later, Johnson et al (Am J Hum Genet 2010; 87:567) identified mutations in the same gene by whole-exome sequencing in 2 families with Brown-Vialetto-Van Laere syndrome. No metabolic investigation

Inborn errors of intracellular riboflavin metabolism

- FAD synthetase and riboflavin kinase
  - No reported inborn deficiency : predicted to affect all flavoproteins and therefore expected to be lethal
  - Amyotrophic lateral sclerosis with IgA gammapathy : decreased mRNA expression levels of FAD synthetase and riboflavin kinase (Lin et al, J Neurol 2009; 256:774)
- Mitochondrial FAD transporter
  - No reported inborn deficiency
  - Has to be searched in all patients with a clinical and biochemical suspicion of MADD deficiency but without mutation in the *ETFA, ATFB* and *ETFDH* genes

Conclusion

If cobalamin has found his scientist



Riboflavin looks for its one !