‘Rotor free-wheeling’ in impaired F1FO-ATPase induces congenital hypermetabolism

"Rotor free-wheeling" in impaired F_1F_O-ATPase causes congenital hypermetabolism

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Abstract

A *de novo* heterozygous variant in the catalytic subunit of mitochondrial F_1F_O-ATPase arises as the main cause of an autosomal dominant syndrome of hypermetabolism associated with defective ATP production as recently discovered by Ganetzky *et al*. We describe how the "rotor free-wheeling" causes this F_1F_O-ATPase dysfunction in primary congenital hypothyroidism.

Main text

Two monozygotic twin boys with a new type of congenital euthyroid hypermetabolism characterized by caloric intake, inability to gain weight, and sporadic hyperthermia were recently described by Ganetzky *et al*. [1]. The Authors discovered a *de novo* heterozygous variant with c.1004 T→C transition in the nuclear *ATP5F1B* gene of mitochondrial F_1F_O-ATPase. Accordingly, the mutation p.Leu_{335}Pro influences the catalytic β subunit of the enzyme (Fig. 1A). The clinical features might be associated with the molecular evidence of pathological conditions that characterize the mitochondrial uncoupling syndromes. Indeed, the dominant-negative effect on bioenergetic parameters at the mitochondrial level highlighted a defective chemiosmotic coupling of oxidative phosphorylation (OXPHOS), which is typical of depolarized mitochondria in the presence of increased oxygen consumption rate without ATP production [1].

F_1F_O-ATPase is embedded in the inner mitochondrial membrane (IMM) working as a unique bifunctional enzyme in nature. Indeed, the electrochemical gradient of proton (H^+) or protonmotive force (Δp) through the IMM, built during the substrate oxidation by the mitochondrial respiratory complexes, is exploited by F_1F_O-ATPase to synthetizes ATP. However, under phato(physio)logical conditions the enzyme in the absence of oxygen working in reverse as H^+ pump and hydrolyzing ATP can polarize the IMM [2]. A hydrophilic F_1 domain directed into the mitochondria matrix and a hydrophobic membrane-localized F_O domain form the F_1F_O-ATPase. The former is responsible for ATP catalysis, *i.e.* ATP synthesis/hydrolysis, whereas the latter is involved in the H^+ translocation through the IMM [3]. The two domains are functionally coupled by the central stalk, which penetrates inside the F_1 inducing a mechanical conformational change in the catalytic sites by its

rotation, and a peripheral stalk connecting laterally F_1 with membrane subunits of F_O that avoids the rotation driven by the rotor [4].

In the F_O domain of the enzyme, an asymmetric half-channels system of *a* subunit dissipates the Δ*p* by H^+ translocation. The H^+ flow across the IMM consists of a mechanism of protonation and deprotonation of the H^+ binding site of *c* subunits that drives the *c*-ring rotation. The torsion generated by H^+ translocating down the Δ*p* is transmitted via the central stalk to power the mechanism of binding change conformation of catalytic sites into F_1 domain. Therefore, in energized mitochondria, the F_1F_O-ATPase can perform ATP synthesis as "a splendid molecular machine" [5]. The chemomechanical mechanism of F_1 domain produces three ATP molecules with one complete revolution of the rotor accordingly to the three-fold symmetric structure of the F_1 domain. Indeed, three catalytic β subunits and three non-catalytic α subunits are arranged in alternation to form a hexagonal structure $(\alpha\beta)_3$ with the cavity filled with the asymmetric shape of the central stalk γ subunit [6]. The $(\alpha\beta)_3$ hexamer has three adenine nucleotide (AN) binding sites placed on the three catalytic subunits at the interfaces with the homologous non-catalytic subunit that in response to the curvature of the γ subunit during the rotor rotation, adopt a different conformation with different nucleotide-binding and catalytic properties. These are identified in the order of transformation as empty β_E structure, β_{DP}, which contains bound ADP plus Pi and, in the last conformation of the cycle, as β_{TP} that binds ATP. Each binding change, which drives the simultaneous interchange of the $\beta_E \rightarrow \beta_{DP} \rightarrow \beta_{TP}$ forms, requires a 120° rotation of the γ subunit with respect to β subunits and triggers the synthesis of an ATP molecule [7] (Fig. 1B).

The new Leu_{335}Pro variant affects the mitochondrial bioenergetics causing an increase in basal oxygen consumption rate almost reaching maximal cell respiration level. This means that the low level of ATP production and depolarized IMM are inconsistent with the stimulated activity of substrate oxidation in mitochondria. The impaired chemiosmotic mechanism of OXPHOS dwelling in the failing energy transduction phenomenon is due to an intrinsic uncoupling of the F_1F_O-ATPase. In other terms, the enzyme depletes the Δ*p* without a mutual increase in ATP synthesis. The inhibition of H^+ translocation by an inhibitor of F_O H^+ transport, *i.e.* oligomycin, can therefore block the uncontrolled H^+ flow increasing the membrane potential and the oxygen consumption rate stopping the passive H^+ slip of F_1F_O-ATPase, whereas protonophore molecules maximize mitochondrial respiration. The results of Ganetzky *et al*. [1] found in mutated mitochondria highlight a synergistic action in dropping the mitochondrial membrane potential in the absence of ATP synthesis and in polarizing the IMM inhibiting the F_O domain. We may conclude that the mitochondrial dysfunction lies in the chemo-mechanical activity of F_1 domain. The damage in the nuclear *ATP5F1B* gene can modify the affinity of catalytic sites for adenine nucleotides and/or may give rise to a γ subunit "rotor free-wheeling" in the catalytic hexamer without causing the simultaneous interchange of the β subunits (Fig 1B).

The asymmetric bulge of the *γ* subunit has three communication points with the catalytic sites on the β subunits. Starting from the apex of the F_1 domain going towards the F_O domain, a hydrophobic loop sleeve is near the tip of the coiled-coil α-helices of the γ subunit. In the middle of the cavity of $(\alpha\beta)_3$ structure lies the Leu_{335}Pro mutation on a loop connecting a hydrophobic portion supporting the interaction with rotor rotation. Finally, in the C-terminal domain of the β subunit a critical helix-turn-helix motif known as "DELSSEED" is presumably involved in the coupling between catalysis and rotation.

Cis- trans-isomerization of the peptide bond is a feature of a secondary amine group, called imine, of Pro residue. Relevance on Leu_{335}Pro mutation might be the *cis-trans* isomerization during protein folding by torsional peptide bond that could displace the hydrophobic communication points gearing inside the $(\alpha\beta)_3$ from γ subunit. Accordingly, the torque generation of the central stalk required for the mechanochemical power of the F_1-ATPase domain [8] is obtained through a "free-wheeling of the rotor" (Fig 1B).

In general, the non-ohmic H^+ conductance of IMM is stimulated by Δ*p* and uncoupling protein 1 and ADP/ATP carrier have the physiological role of dissipation of the excessive membrane potential by H^+ leak [9]. The

mechanochemical destruction of F_1 energy transduction is the result of intrinsic F_1F_O-ATPase uncoupling [10]. During the H^+ translocation, the enzyme misses the ATP synthesis dropping the membrane potential and depicting the biochemical phenomenon of congenital hypermetabolism symptoms. On balance, the H^+ slip of F_1F_O-ATPase characterizes several primary mitochondrial disorders stemming from pathological "rotor free-wheeling" (Fig. 1B).

Declaration of Interests

All authors declare no competing interests.

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Figure

Figure 1. Mechanochemistry dysfunction of F_1F_O-ATPase caused by Leu_{335}Pro mutation. A) Transition T→C (red square), as well as the Leu_{335}Pro mutation highlighted during the transcription and translation of *ATP5F1B* gene. B) Representation of F_1F_O-ATPase drawn as ribbon representations obtained from modified Protein Data Bank identifier 6TT7. The differently coloured letters identify the subunits, drawn in the same colour as the letter. On the left is the *wild-type* enzyme during ATP synthesis and in the insert the mechanochemical coupling of the F_1 domain. On the right is F_1F_O-ATPase with mutated β subunit missing the coupling of H^+ translocation generation of rotor rotation to the binding chance mechanism of ATP production. Leu_{335}Pro mutation induces a rotor free-wheeling in the energy transduction mechanism of the F_1F_O-ATPase. The three dotted squares on the β subunits show in the space-filling model the three mutated amino acid residues, which are coloured in red. This figure was created using BioRender (https://biorender.com/).

Figure 1

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