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INSIGHT

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H+-slip correlated to rotor free-wheeling as cause of F_1F_O-ATPase dysfunction in primary mitochondrial disorders

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Abstract

Inborn errors of metabolism are related to mitochondrial disorders caused by dysfunction of the oxidative phosphorylation (OXPHOS) system. Congenital hypermetabolism in the infant is a rare disease belonging to Luft syndrome, nonthyroidal hypermetabolism, arising from a singular example of a defect in OXPHOS. The mitochondria lose coupling of mitochondrial substrates oxidation from the ADP phosphorylation. Since Luft syndrome is due to uncoupled cell respiration responsible for deficient in ATP production that originates in the respiratory complexes, 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 dysfunction in ATP production, which does not involve the respiratory complexes. The F_1F_O-ATPase works as an embedded molecular machine with a rotary action using two different motor engines. The F_O, which is an integral domain in the membrane, dissipates the chemical potential difference for H^+, a proton motive force (Δp), across the inner membrane to generate a torsion. The F_1 domain—the hydrophilic portion responsible for ATP turnover—is powered by the molecular rotary action to synthesize ATP. The structural and functional coupling of

F_1 and F_O domains support the energy transduction for ATP synthesis. The dissipation of Δp by means of an H^+ slip correlated to rotor free-wheeling of the F_1F_O-ATPase has been discovered to cause enzyme dysfunction in primary mitochondrial disorders. In this insight, we try to offer commentary and analysis of the molecular mechanism in these impaired mitochondria.

KEYWORDS

ATP synthase, bioenergetic failure, congenital hypermetabolism, de novo mutation, mitochondria

The numerous harmful mutations in genes that encode structural subunits or supplementary factors of oxidative phosphorylation (OXPHOS) complexes are one of the most common inborn disorders of energy metabolism, which are caused by cell bioenergetics dysfunction.[1,2] Defects of oxidation-phosphorylation coupling in OXPHOS occur in inborn metabolic defects and Luft disease is the most well-known example.[3] Mitochondrial uncoupling abnormalities, such as Luft syndrome, are distinguished by enhanced mitochondrial respiration that is unrelated to mitochondrial ATP production.[4] However, uncoupled respiration that originates outside the F_1F_O-ATPase, the last complex of the OXPHOS system that is able to ATP synthesis, is characterized by a notable decrease of ATP production and respiratory rate in the presence of ADP and nicotine adenine dinucleotide linked substrates or with a flavoprotein-linked substrate.

A case of congenital euthyroid hypermetabolism in two monozygotic twin boys has recently been investigated highlighting a high caloric intake, inability to gain weight, and sporadic hyperthermia.[5] The patients presented detailed molecular evidence that such a disorder could be classified as a mitochondrial uncoupling syndrome with a dominant-negative influence on the OXPHOS chemiosmotic coupling. However, the bioenergetic feature of the disease highlights an increased mitochondrial respiration without ATP synthesis resulting in a loss of mitochondrial membrane potential that is independent of cell respiration activity of respiratory complexes, but related to the F_1F_O-ATPase dysfunction.

On the β subunit of F_1F_O-ATPase, the c.1004 T → C transition in the nuclear *ATP5F1B* gene is a *de novo* heterozygous mutation causing the change of $p.Leu_{335}Pro$ residue in the catalytic site of the enzyme (Figure 1). It is known that F_1F_O-ATPase is a bi-functional enzyme that synthesizes ATP by exploiting the proton motive force (Δp) generated with the mitochondrial respiratory complexes activity driven by substrate oxidation. Conversely, in anaerobiosis pathological or physiological conditions, the F_1F_O-ATPase hydrolyzes ATP to polarize the inner mitochondrial membrane (IMM) working as proton (H^+) pump.[6] Structurally the F_1F_O-ATPase is composed of a hydrophilic F_1 domain, responsible for the ATP catalysis, and a membrane-inserted F_O domain, involved in the H^+ transport across the IMM.[7] The two domains are functionally and structurally joined by a central stalk rotating inside the F_1, which mechanically causes a conformational change in the catalytic sites of adenine nucleotides, and a lateral stalk that anchors the F_1 to the stationary membrane-embedded subunits of the F_O to avoid the torsion driven by the rotor.[8,9]

During ATP synthesis, the H^+ flow through the IMM is driven by Δp exploiting in F_O domain an asymmetric half-channels structure of *a* subunit inducing the *c*-ring rotation by a protonation/deprotonation mechanism of the H^+ binding site of *c* subunits.[10] The torsion generated by H^+ translocation is conveyed through the central rotor to power the binding change conformation of F_1 domain catalytic sites according to the binding change mechanism

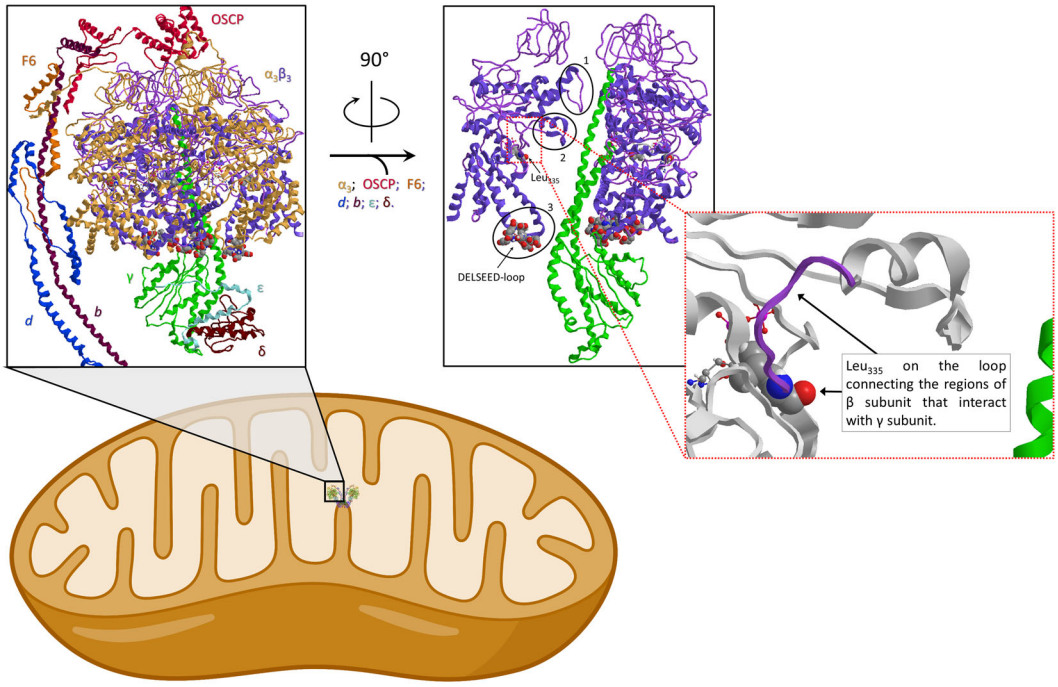

FIGURE 1 Molecular organization of the F_1 domain. The F_1 subunits are drawn as ribbon representations obtained from modified Protein Data Bank ID code: 6ZPO. The differently colored letters identify the subunits, drawn in the same color as the letter. The circles in the box on the right highlight the three sites of contact between β and γ subunits. Site 1, Site 2, and Site 3 are composed of the following sequence $_{273}$Gly-Arg-Ile-Pro-Ser-Ala-Val-Gly-Tyr-Gln-Pro$_{283}$, $_{313}$Pro-Ala-Asp-Asp-Leu-Thr-Asp$_{319}$, and $_{394}$Asp-Glu-Leu-Ser-Glu-Glu-Asp$_{400}$, known as DELSSED loop, respectively. The position of βLeu$_{335}$ is shown in the dotted red square. The conserved DELSEED motifs and the Leu$_{335}$ of the β subunit are shown in the space-filling model. [Color figure can be viewed at wileyonlinelibrary.com]

(BCM).[7] Since the F_1 domain is three-fold symmetric, three ATP molecules are produced with one complete rotor rotation, that is, one molecule every 120°. A hexagonal structure of three catalytic β subunits and three noncatalytic α subunits are arranged in alternation around the asymmetric structure of the central stalk γ subunit, which lies along the central cavity of the $\alpha_3\beta_3$ hexamer.[11] The Mg^{2+}-ADP and Mg^{2+}-ATP binding sites are placed on the three β subunits at the interfaces with the homologous noncatalytic subunit, the α subunits.[12] The rotation of the central stalk orients the "γ-bulge" obligating each of the β subunits to adopt the interconversion of different catalytic properties and conformational states designed as β_{TP}, β_{DP}, which contain bound ATP or ADP, and the empty β_E structure during the rotor rotation[13] following the BCM model. Therefore, the γ shaft has the role to communicate its rotational position to each β subunit and trigger the catalysis.

The Leu$_{335}$Pro mutation is bioenergetically characterized by increasing the basal oxygen consumption rate approximately to the maximum level of cell respiration. Consequently, *ATP5B* L335P cells have a drop in mitochondrial membrane potential associated with a low level of ATP production. The inefficiency of the chemiosmotic mechanism of OXPHOS is due to an intrinsic uncoupling of F_1F_O-ATPase energy transduction that causes the Δp dissipation in the absence of a mutual rise in ATP synthesis. Indeed, oligomycin, which is a specific inhibitor of H^+ translocation in the F_O domain and blocks the ATP turnover,[14] restores the IMM polarization decreasing the oxygen consumption rate. Moreover, in *wild-type* and mutated cells, the protonophore molecules (FCCP or CCCP) maximize mitochondrial respiration.[5] Inhibition with oligomycin indicates that the enzyme dysfunction lies in the chemo-mechanical F_1 domain activity, while the protonophore effect and the mutation have

a synergistic action in depolarizing the IMM without synthesizing ATP. The damage on β subunits, therefore, suggests a shift in the γ subunit rotation in the catalytic hexamer without inducing the conformational change of BCM stimulating cellular respiration. Consistently, we could also suggest a decrease in the affinity of the catalytic site to adenine nucleotides. Indeed, by adding saturating ADP substrate in fibroblasts of patient the increased oxygen consumption was normalized.[5]

However, the $Leu_{335}Pro$ mutation on β subunit is placed on the loop connecting regions of the subunit interacting with the γ subunit as the rotor is rotating (Figure 1). Two regions on the β subunit are noticeable for the presence of hydrophobic loop sleeves where the interactions with the γ subunit take place. These are located near the tip of the coiled-coil α-helices of the central stalk, primarily near the C-term of the γ subunit. Two "catch interactions" formed by H-bonds, one in the nucleotide-binding domain of β subunit adopting the empty conformation and the other comprises a critical helix-turn-helix motif arranged in the C-terminal domain of the closed conformation of β subunit ($_{394}$Asp-Glu-Leu-Ser-Glu-Glu-Asp$_{400}$) termed "DELSEED," which is supposedly involved in coupling between catalysis and rotation (Figure 1).[15] The DELSEED-loop of each of the three β subunits makes contact with the γ subunit playing an important function in coupling between rotor rotation and catalysis of ATP synthesis. Both switches operate as bridges between the catalytic sites of the β subunits and the rotor. Pro is the unique amino acid with *cis- trans*-isomerization property in the peptide bond involving its imino nitrogen.[16] We might consider that the torsional peptide bond by isomerization on the mutate Pro residue could displace the

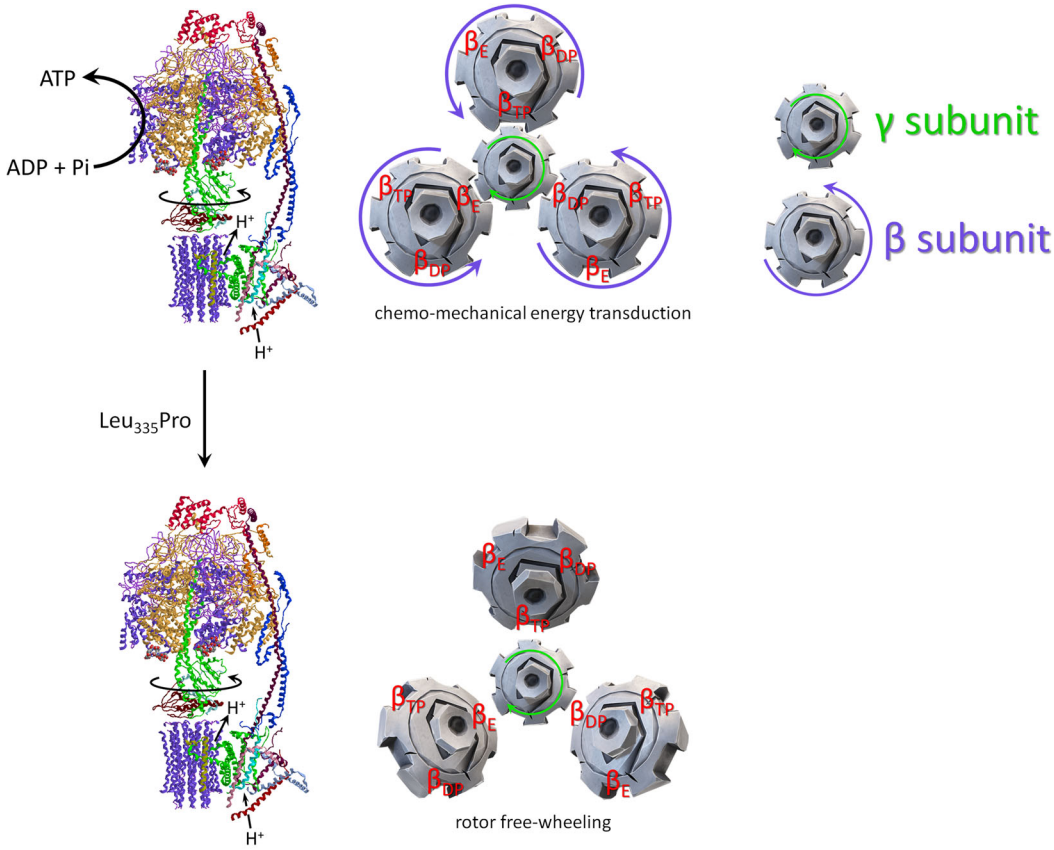

FIGURE 2 Structural representation of the mechanochemistry of ATP production. F_1F_O-ATPase during ATP synthesis coupled to H^+ translocation (above) and intrinsic uncoupling of the enzyme caused by $Leu_{335}Pro$ mutations that induce a rotor free-wheeling in the energy transduction mechanism of F_1F_O-ATPase (below). [Color figure can be viewed at wileyonlinelibrary.com]

hydrophobic portion bearing in the $\alpha_3\beta_3$ hexamer holding spaced the tip of γ shaft. Consequently, the torque generated by the rotor rotation that drives the mechanochemical power of the F_1-ATPase domain[17] displays a free-wheeling of the central stalk bypassing interactions of the γ subunit with the $\alpha_3\beta_3$ subcomplex (Figure 2).

The result of intrinsic F_1F_O-ATPase uncoupling means that the enzyme complex itself cannot match ATP production to H^+ channeling, caused by molecular defects. While mitochondrial uncoupling has a recognized physiological role sustaining the H^+ leak by uncoupling proteins (UCPs) and ADP/ATP carrier,[18,19] as far as we know the impaired chemo-mechanical energy transduction in F_1F_O-ATPase only represents a basic biochemical symptom of pathologies.[2] The enzyme could provide a broad spectrum of therapeutic strategies to counteract at the molecular level diseases whose treatment is still inadequate.[20] In conclusion, the H^+ slip correlated to rotor free-wheeling can be considered as the cause of F_1F_O-ATPase dysfunction in primary mitochondrial disorders.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Giovanni Romeo is a medical geneticist with wide international research experience in different Institutions, namely in Genova (Istituto G. Gaslini), Lyon (International Agency for Research on Cancer), and Bologna University. Major research interests: Hirschsprung disease, RET protooncogene, consanguinity studies and genetic epidemiology, mtDNA mutations in cancer. Director of the European School of Genetic Medicine (www.eurogene.org) started in 1988 in collaboration with the late Prof. Victor McKusick. He developed during the past 20 years several research and advanced teaching projects in Middle East countries.

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