

Complex V (ATP Synthase) Mutations: Diverse Effects on Supercomplex Formation and Oxidative Phosphorylation Function [P05.129]

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Background

Oxidative phosphorylation (OXPHOS) is critical to cellular function as the primary source for energy (ATP) in most cell types, the control point for cellular redox, and as a control point for essential metabolic and signaling pathways that range from the synthesis of pyrimidines to the regulation of apoptosis. Optimal OXPHOS function requires aggregation of individual OXPHOS enzymes into supercomplexes which allows efficient and rapid transport of electrons. Supercomplexes allow efficient formation of an electrochemical (proton) gradient created by Complexes I, III, and IV that is then used by Complex V to synthesize ATP. In many classes of mitochondrial disease, impairment of the monomeric enzymes (Complexes I-V) and supercomplex assembly occurs. Functional supercomplexes contain a single Complex I enzyme, two Complex III enzymes, and variable numbers of Complex IV enzymes (Complexes I+III₂+IV) plus the mobile electron carriers CoQ10 and cytochrome c. Complex II also can be associated with the Complex I+III₂+IV structure. During isolation of supercomplexes, other classes of supercomplexes are observed: (1) Complexes I+III₂+V; (2) Complexes I+III₂+IV; (3) Complexes III+IV. The role of these other supercomplex classes, particularly those lacking Complex IV are unknown, but they may be intermediate structures involved in functional supercomplex assembly. Whereas essentially all of Complex I is associated with supercomplexes, Complex II, Complex III, Complex IV, and Complex V can also be stand alone enzymes (i.e. exist as isolated monomeric enzymes). Complex V (F₁ - F₀ ATPase) is present primarily as monomeric and dimeric forms and to a lesser extent is associated with Complex I.

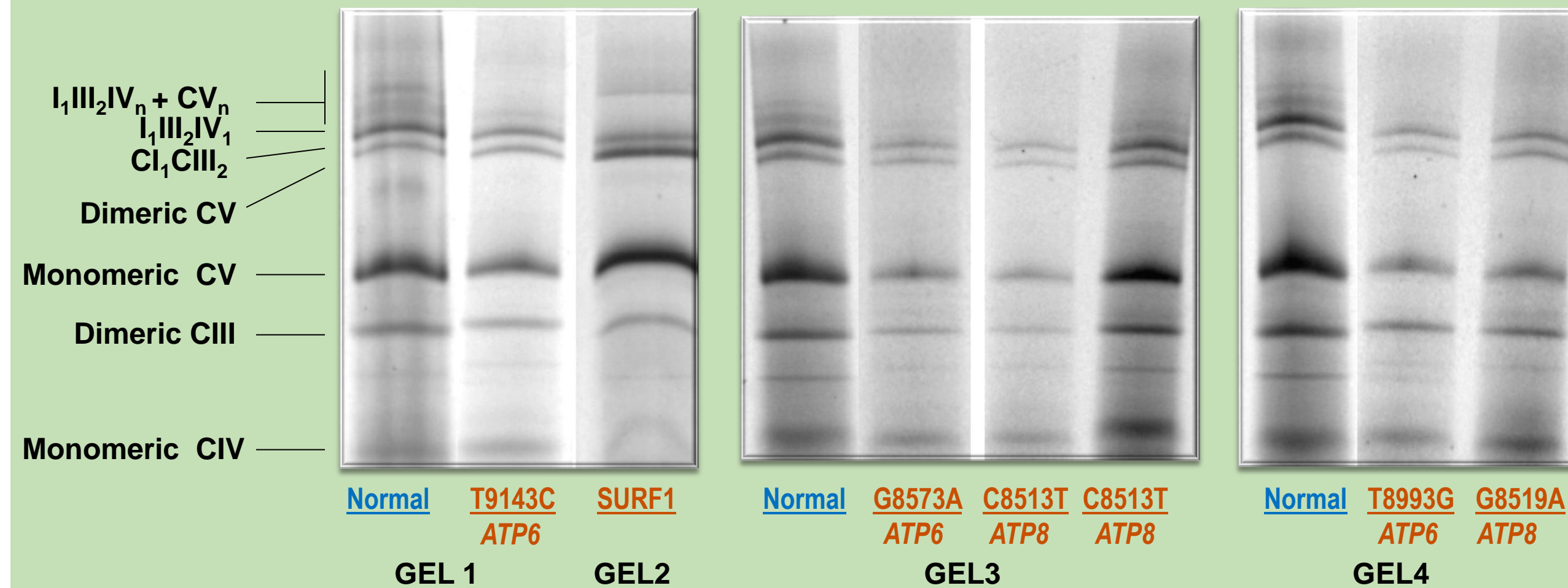
The effects of gene mutations involving Complex V (ATP synthase) on supercomplex formation has been investigated in a few cases. (1-3) Assessment of patients with Complex V mutations demonstrates diverse effects on OXPHOS function.

Complex V Mutations

MUTATIONS	MTDNA GENE
G8573A Glycine > Aspartate	ATP6
T9143C Valine (GTC) > Alanine (GCC)	ATP6
T8993G Leucine > Arginine	ATP6
C8513T Proline > Serine	ATP8
C8513T Proline > Serine	ATP8
G8519A Glutamate > Lysine	ATP8

Blue Native Gel

OXPHOS supercomplex formation and monomeric enzyme assembly was investigated in MUSCLE mitochondria by Blue Native Gel electrophoresis. Clear Native Gel electrophoresis provides higher resolution of Complex IV (cytochrome c oxidase) assembly.



Blue Native Gel Electrophoresis (2 different gels)

CI = Complex I; CIII = Complex III; CIV = Complex IV; CV = Complex V

- Decreased supercomplex formation (C₁C_{III2}; I₁III₂IV₁; I₁III₂IV_n) is identified in patients harboring Complex V mutations in their mtDNA
- Dimeric Complex V appears preserved in all samples.
- Complex V oligomers appear decreased. Comparison is made with a patient who harbors a SURF1 mutation. This mutation significantly impairs supercomplex formation but allows good visibility of Complex V oligomers.

Complex V In-Gel Enzymology (Clear Native Gel)

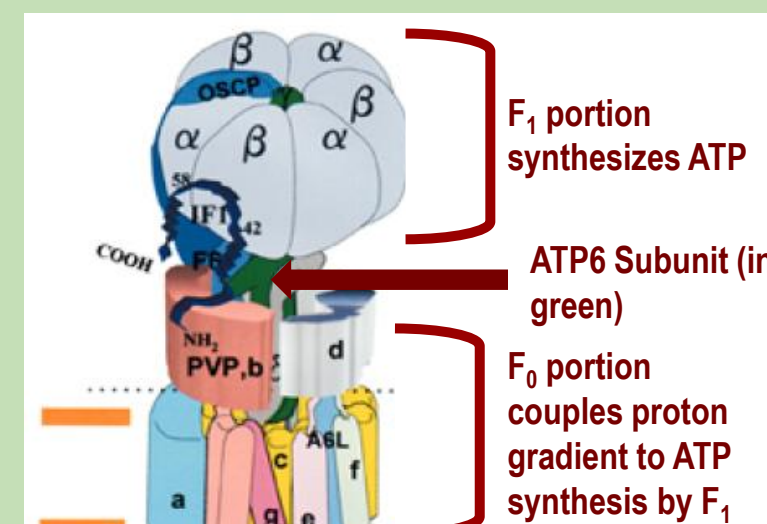
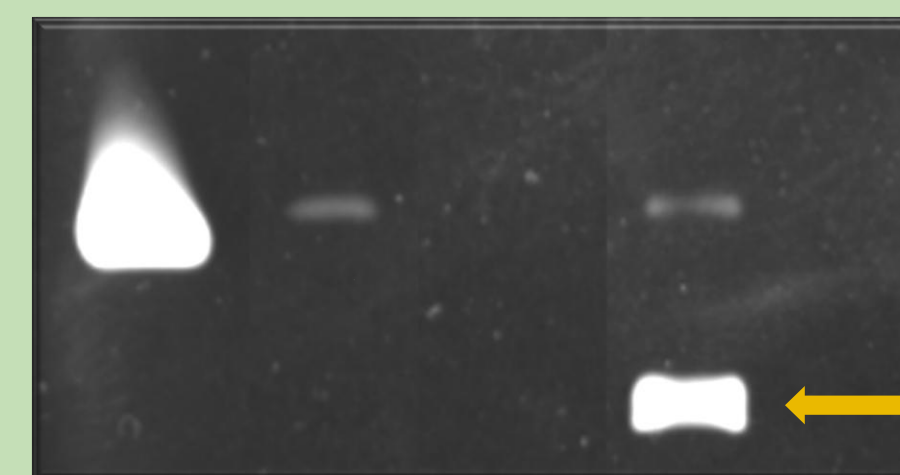


Figure from J Bioenerg Biomembr 2000, 32:401-411



Normal G8573A C8513T T8993G G8519A

The F₁ and F₀ portions of Complex V are not assembled correctly. This produces a smaller band composed of the F₁ portion (F₁ subcomplex). F₁ subcomplex has ATPase activity and can degrade cellular ATP, thus contributing to the patient's symptoms. (Am J Med Genet A. 2006 Apr 15;140(8):863-8)

Complex V in-gel ATPase activity: ATP hydrolysis causes formation of lead nitrate (white precipitate)

Conclusions

- Complex V (ATP synthase) mutations impair OXPHOS function through a variety of mechanisms.
- OXPHOS supercomplex analysis and monomeric enzyme analysis showed the following features:
 - Impaired formation of Complex V oligomers. Dimeric Complex V appeared intact.
 - Decreased supercomplex (C₁C_{III2}; I₁III₂IV₁; I₁III₂IV_n) formation can occur.
 - Instability in Complex V causes dissociation of the F₁ and F₀ portions of Complex V in some mutations (e.g. T8993G (NARP) mutation). This instability is difficult to detect with Blue Native gel analysis.
- Isolation of intact Complex V using Clear Native gel electrophoresis allows assessment of the ATPase function of Complex V. The ATP synthase function cannot be assessed since there is no proton gradient.
 - ATPase activity is localized to the F₁ portion of Complex V.
 - When the Complex V enzyme is intact, ATPase activity was decreased even though the mutations are in mtDNA coded subunits (ATP6, ATP8) that reside in the F₀ portion of the enzyme.
 - The NARP mutation (T8993G) is unique in that it produces instability of Complex V and the F₁ portion of the enzyme separates from the F₀ portion. Once the F₁ subcomplex is formed, ATPase activity returns to normal.
 - When the proton gradient is impaired, Complex V can function as an ATPase. This may contribute to the severe symptoms experienced by many patients who harbor Complex V mutations.

Selected References

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