Poster Presentation

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Transhydrogenase coupling proton translocation and hydride transfer

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Nicotinamide nucleotide transhydrogenase (TH) is a homodimeric 200 kDa membrane protein that is associated with glucose homeostasis in diabetes: mice with mutations or deletions in the TH-encoding gene exhibit glucose intolerance and impaired secretion of insulin [1-2]. TH couples hydride transfer between nicotinamide nucleotides to proton translocation between the matrix (in) and the intermembrane space (out) of mitochondria (or between the cytosol and the periplasm in prokaryotes) [3]: NADH + NADP + H^+(out) ⇌ NAD + NADPH + H^+(in). Each TH monomer contains three domains: a soluble 40 kDa NAD(H)-binding domain (domain I), a 40kDa membrane-intercalated proton channel (domain II), and a soluble 20 kDa NADP(H)-binding domain (domain III), which is connected to domain II. Hydride transfer between nucleotides occurs between domain I and domain III; and proton translocation is carried out in domain II [3]. The mechanism of TH is unknown due to the lack of structures of the transmembrane domain and the intact enzyme. We have solved three crystal structures of the membrane-intercalated domain II of Thermus thermophilus TH at 2.8-3.0 Å using selenomethionine derivatives and mercury derivatives of crystals obtained in the lipidic cubic phase. Four crystal structures of the soluble domains have also been obtained at 1.8-2.4 Å. Using the higher resolution structures of the subunits, we have determined the structure of the entire TH complex at 6.9 Å by crystallography, and 18 Å by single-particle cryogenic electron microscopy. The intact TH structure reveals that domain III subunits violate the local 2-fold symmetry: one has its NADP(H) binding site 'face-up' to interact with domain I for hydride transfer; the other 'face-down' to interact with domain II for proton translocation. An alternating mechanism of the NADP(H) binding domains provides insights into how TH couples hydride transfer to proton motive force.

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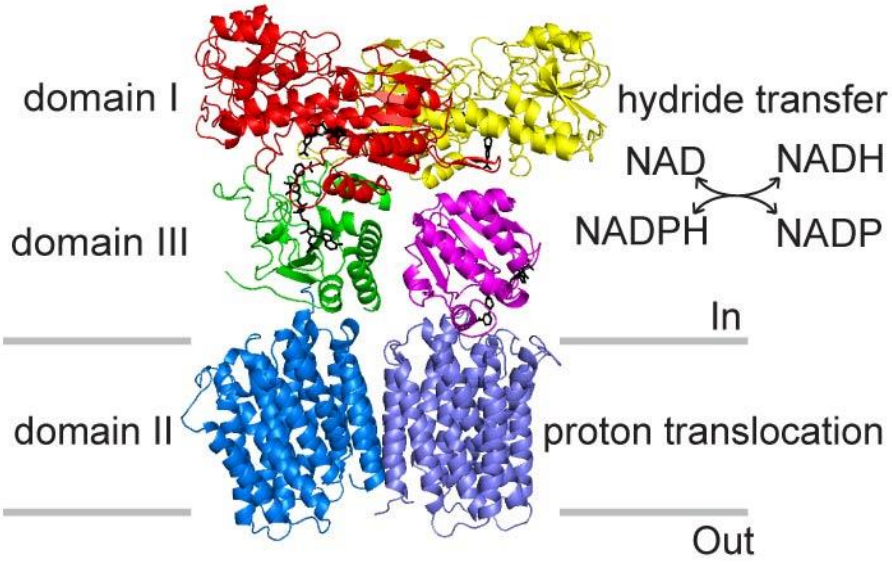

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