by shRNA [1], leads to decreased content of ATPase, which is functionaly and structuraly normal. Accumulation of aggregates of subunit c in the inner mitochondrial membrane suggests the role of the ε subunit in assembly of enzyme domains.

In the present study we examined the role of the stalk subunits γ and δ, using shRNA knockdown in the human HEK293 cell line. The protein levels of subunits γ and δ were decreased by the knockdown to 30 % and 10 % of non-silencing control levels, respectively. Other ATPase subunits as well as the content of the holoenzyme were decreased to similar levels as were the silenced subunits. Only steady-state levels of the subunit c were unchanged, and its aggregates/assemblies were detected by 2D BN/SDS PAGE. Similar phenotype as in case of the ε subunit deficiency suggests uniform position of the central stalk subunits in the assembly sequence of mammalian ATPase. Functional impact of the central stalk subunits knockdown was studied by high-resolution respirometry and mitochondrial membrane potential measurements using TPP^+- sensitive electrode. Both cell lines silenced for either γ or δ subunits displayed typical features of decreased ATPase functional capacity: i) decreased rate of the ADP-stimulated respiration, ii) two-fold increased sensitivity of respiration to ATPase inhibitor oligomycin, and iii) impaired utilization of mitochondrial membrane potential by ADP phosphorylation.

In summary, our results suggest the involvement of the central stalk subunits in c-oligomer attachment during ATPase assembly.

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Reference

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1P33

The H^+/ATP ratios of H^+-ATPsynthases from yeast mitochondria, spinach chloroplasts and *E.coli*

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The H^+/ATP ratio is an important parameter for the energy balance in cells as it defines the gearing between proton transport and proton coupled ATP synthesis. Based on the mechanism of rotational catalysis and the currently available structural information, it can be assumed that the H^+/ATP ratio is equal to the ratio of c-subunits (H^+ binding sites) to the number of β-subunits (ATP binding sites). The observed c-subunit numbers in different species imply the H^+/ATP ratios be 3.3 for the mitochondrial ATPsynthase from yeast, 4.7 for the chloroplast ATPsynthase, and between 3.3 and 4.0 for the *E.coli* enzyme. We reconstituted the isolated F_0F_1s into liposomes and measured ATP synthesis or ATP hydrolysis as a function of pH at several constant stoichiometric products $Q = [ATP]/([ADP][P_i])$. During reconstitution the internal phase of the liposomes was equilibrated with the acidic medium, so that the internal pH could be accurately measured with a glass electrode. An acid-base transition was carried out and the initial rate of ATP/ADP turnover measured for several pH at constant Q. The equilibrium pH_{eq} for a given Q was obtained by interpolation, and each H^+/ATP ratio was subsequently determined from the dependence of pH_{eq} on the stoichiometric product Q.

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Engineering rotor ring stoichiometries in the ATP synthase

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The F_1F_o-ATP synthase uses the energy stored in a transmembrane ion gradient to power a unique rotational mechanism that converts its mechanical energy into the chemical energy stored in the form of ATP. Two structurally separate molecular motor complexes, F_1 and F_o, operate in concert to translocate ions across the membrane in F_o to synthesize ATP from ADP and inorganic phosphate in F_1. The F_o rotor consist of a ring of c-subunits (c-ring); each subunit provides one ion binding site and contributes to the ion (H^+ or Na^+) specificity of the enzyme. The number of c-subunits (n) per c_n ring appears to be fixed for a given species but the c-ring stoichiometry ranges from $n = 8$ to $n = 15$ across different species. As the F_1 complex invariably harbors three β-subunits, which catalyze three ADP-to-ATP conversions upon full 360° turn of the rotor, and because F_1 and F_o motors are tightly coupled, the c-ring stoichiometry (n) determines the theoretical 'ion-to-ATP ratio' ($n/3$) of the enzyme. In our study we addressed the question how the c-ring stoichiometry could be manipulated for a given species. We investigate the importance of c/c-subunit contacts by site-directed mutagenesis of a conserved stretch of glycines (GxGxGxGxG) in the N-terminal helix of c-subunit in the c_{11} ring from *Ilyobacter tartaricus*. Structural and biochemical studies show a direct influence of mutations on the c-subunit stoichiometry, revealing $c_{9\text{-}16}$ rings. Molecular dynamics (MD) simulations of wild-type and mutant c-rings suggest that energetic and geometric perturbations in the c-subunit interface underlie the emergence of alternative c-ring stoichiometries. A protein interaction study by the surface plasmon resonance technique demonstrates that the assembly of the F_1F_o rotor complex is independent of the c-ring size from c_{11} to c_{15}. Real-time ATP synthesis experiments in proteoliposomes show the mutant enzyme, harboring the larger c_{12} instead of c_{11} ring, produce ATP at the lower ion motive force threshold – that is, at higher ion-to-ATP ratio. The high degree of compliance in the architecture of the ATP synthase rotor offers a rationale for the natural diversity of c-ring stoichiometries, which likely reflect adaptations to specific bioenergetic demands. These results provide the basis for bioengineering ATP synthases with customized ion-to-ATP ratios, by sequence modifications.

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