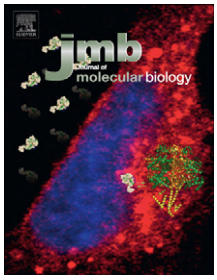

A Passive Function of Mitochondrial ATP Synthase: Target for Tumor Killer HAMLET

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Introduction

The main job of F_1F_0-ATP synthase is to provide organisms from bacteria to humans with ATP molecules that can be used in all kinds of energy-consuming reactions, of which muscle contraction is a clear example. In an average human, the production of ATP is at a rate of around 9×10^{20} molecules per second, equivalent to a turnover of ATP of 65 kg per day [10]. This value varies depending on activity but nevertheless is an impressive number. Because of its importance, F_1F_0-ATP synthase is one of the best studied proteins.

Early work showed that ATP synthases can be dived in two main components. A large F_1 headpiece of about 400 kDa is mainly formed of three copies of α and β subunits with a single γ subunit inside. The γ subunit connects the F_1 headpiece to the smaller membrane-bound F_0 part. In bacteria, a single copy of the small subunit ε binds to the lower part of the γ subunit, forming the stalk that connects F_1 to the membrane. The main component of F_0 is a hydrophobic ring of multiple copies of subunit *c*. It is connected to subunit *a* and two copies of subunit *b* (Fig. 1). In humans, the overall composition is pretty much the same, but instead of two *b* subunits, some other structurally homologous subunits are present. Besides, there is one extra stalk subunit, named δ, which has nothing to do with the bacterial subunit δ on top of F_1. The human counterpart of the latter is named OSCP.

A high-resolution structure of the complete set of F_1F_0-ATP synthase subunits is still lacking, but a large number of F_1 structures have become available since the first structure was obtained by John Walker and colleagues [1]. They present a wealth of information about different conformations and cofactor compositions. In fact, only details about the hydrophobic *a* subunit plus its interaction with the *c*-subunit ring are missing. Ideas on how the complete structure of the ATP synthase structure might be looking have been presented on several places, for instance, as "Molecule of the month of the Protein Data Bank"[†]. In the simplified scheme of Fig. 1, we can see the essentials of this intricate molecular motor protein. The rotating components are in green colors, whereas the fixed components are reddish. The latter include the stator structure, or second stalk on the outside, which prevents futile rotation of the F_1 headpiece.

Besides remaining open questions about the function of monomeric ATP synthase, some other aspects need further attention. One is the exact structure of the dimeric ATP synthase conformation, which is a special feature of this enzyme in mitochondria, and another one is the unusual shape of ATP synthase in some primitive unicellular organisms [3].

HAMLET

Even a thoroughly studied enzyme such as ATP synthase can sometimes disclose an unexpected feature. A common study by the groups of Gerhard Gruber (Singapore) and Catharina Svanborg (Sweden) shows that mitochondrial F-ATP synthase plays an important but passive role as a target for HAMLET [8]. HAMLET is an acronym for *h*uman *a*lpha-lactalbumin *m*ade *le*thal to *t*umor cells. In the 90s, the group of Svanborg discovered induction of apoptosis by a modified form of the 14-kDa milk protein α-lactalbumin [4]. The modified α-lactalbumin has a bound oleic acid as a cofactor, resulting in an extended conformation [6]. The extended

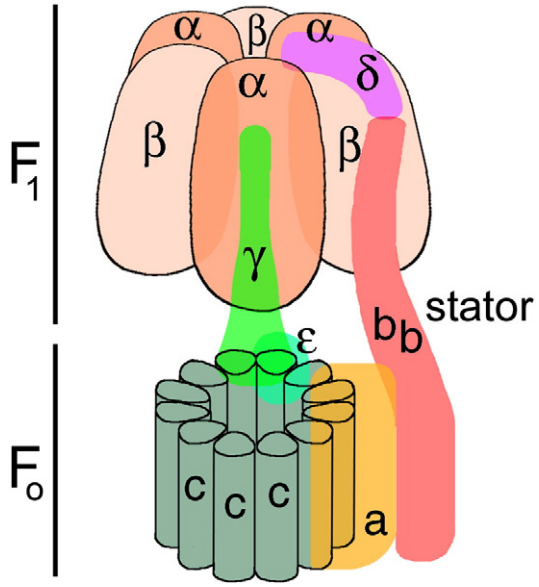

Fig. 1. Scheme of the ATP synthase motor protein, composed of a hydrophilic F_1 headpiece and a membrane-bound F_0 moiety. Most relevant is a division of rotating subunits (green to blue-green) in respect to rather static subunits (reddish to purple). Depicted is the bacterial subunit composition. The $\alpha_3\beta_3\gamma$ headpiece of a bacterium was used to show the effect of HAMLET on the rotation of γ within the $\alpha_3\beta_3$ structure [8].

conformation in HAMLET has been proposed to be required to form the tumoricidal active HAMLET complex.

HAMLET is the first member in a new family of protein–lipid complexes that kills tumor cells with high selectivity. The lipid cofactor serves the dual role as a stabilizer of the altered fold of the protein and a coactivator of specific steps in tumor cell death. HAMLET is broadly tumoricidal, suggesting that the complex identifies conserved death pathways suitable for targeting by novel therapies [7]. Cellular targets are located in the cytoplasmic membrane, mitochondria, lysosomes and nuclei, and specific signaling pathways are rapidly activated, first by interactions of HAMLET with the cell (and organelle) membrane and subsequently after HAMLET internalization. Therapeutic effects of HAMLET have been demonstrated in human skin papillomas and bladder cancers, and HAMLET limits the progression of human glioblastomas, with no evidence of toxicity for normal brain or bladder tissue. These findings open up new avenues for cancer therapy and the understanding of conserved death responses in tumor cells. Recently, HAMLET was tested on laboratory mice as potential agent for colon cancer prevention and treatment, especially for people carrying mutations of the *APC* gene, encoding a tumor suppressor, where colon cancer remains a leading cause of death [9]. These tests have proven successful in suppressing colon cancer cells. Remarkably, supplying HAMLET to the drinking water from the time of weaning also significantly prevented tumor development.

In the recent study, Ho and coworkers investigated if the diverse effects of HAMLET might be caused, in part, by a direct effect on the ATP synthase and a resulting reduction in cellular ATP levels. A dose-dependent reduction in cellular ATP levels was detected in lung carcinoma cells and HAMLET was found colocalizing with the nucleotide-binding subunit and the catalytic subunits α and β of the F_1F_0-ATP synthase [8]. As shown by fluorescence correlation spectroscopy, HAMLET binds to the F_1 domain with a dissociation constant of 20.5 μM. Increasing concentrations of the tumoricidal protein HAMLET added to the enzymatically active $\alpha_3\beta_3\gamma$ complex lowered its ATPase activity, demonstrating that HAMLET binding to the ATP synthase effects the activity of this molecular motor. Tumor cells are heavily dependent on glycolysis, the first steps of transforming sugars into energy by oxidative phosphorylation, of which ATP synthase catalyzes the last step. If this step becomes rate limiting, a reduced ATP synthase function caused by HAMLET is likely to impair glycolysis and thereby drives energy-deprived tumor cells to their death.

Conclusions

Single-molecule microscopy experiments can precisely monitor the rotation of the γ subunit within the α3β3γ headpiece of the ATP synthase. This was previously studied in detail by Japanese researchers [5]. The stable headpiece of the thermophilic *Bacillus* PS3 bacterium was used to show the effect of HAMLET on the counterclockwise direction of rotation. These findings indicate that HAMLET interacts with the F_1 headpiece. On a subcellular level, it is speculated that this might cause the release of headpieces from the membrane.

Further biochemical studies could reveal the exact mode of interaction of HAMLET with the ATP synthase needs to be investigated. It is unlikely that the interaction is next to the δ subunit at the top of F_1, which is according to the high-resolution structure mostly composed of rigid β structure [1]. Because HAMLET has an effect on the catalytic activity, it might associate with the γ subunit in a way reminiscent to the inhibitor protein [2]. Another tentative mode of interaction is an intercalation between α and β subunits, preventing movements in the headpiece that are responsible for synthesis/hydrolysis of ATP. Blocking flexibility in some way

or the other would stop the rotation of the γ subunit and decrease the catalytic activity in the headpiece.

†see http://dx.doi.org/10.2210/rcsb_pdb/mom_2005_12.

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