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Biochimica et Biophysica Acta 1555 (2002) 101–105

BIOCHIMICA ET BIOPHYSICA ACTA
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Atp11p and Atp12p are chaperones for F_1-ATPase biogenesis in mitochondria

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Received 28 January 2002; accepted 13 February 2002

Abstract

The bioenergetic needs of aerobic cells are met principally through the action of the F_1F_0 ATP synthase, which catalyzes ATP synthesis during oxidative phosphorylation. The catalytic unit of the enzyme (F_1) is a multimeric protein of the subunit composition $\alpha_3\beta_3\gamma\delta\varepsilon$. Our work, which employs the yeast *Saccharomyces cerevisiae* as a model system for studies of mitochondrial function, has provided evidence that assembly of the mitochondrial α and β subunits into the F_1 oligomer requires two molecular chaperone proteins called Atp11p and Atp12p. Comprehensive knowledge of Atp11p and Atp12p activities in mitochondria bears relevance to human physiology and disease as these chaperone actions are now known to exist in mitochondria of human cells.

Keywords: Mitochondria; F_1-ATPase; Atp11p; Atp12p; Chaperone; Biogenesis

1. Introduction

The ATP synthase of mitochondrial, chloroplast, and bacterial membranes catalyzes ATP synthesis coupled to respiration [1,2]. The catalytic unit of the enzyme (F_1 component) is an oligomer of five different subunits that are present in the stoichiometric ratio, $\alpha_3\beta_3\gamma\delta\varepsilon$. The subunit composition of the integral membrane component (F_0) of the ATP synthase ranges from the relatively simple form found in bacteria, in which there are only three types of subunits (called a, b, and c, see Ref. [3]), to the highly complex mitochondrial form, which may have as many as 10–12 different types of subunits [4,5].

The mitochondrial ATP synthase does not self-assemble in vivo. Work with respiratory deficient strains of *Saccharomyces cerevisiae* has identified three proteins that serve chaperone-type functions during assembly of the enzyme under normal growth conditions; Atp10p is required for F_0 assembly [6], while Atp11p and Atp12p both mediate formation of the F_1 oligomer [7]. Another yeast protein, Fmc1p, has been shown to be required for F_1 assembly in cells grown at elevated temperatures; interestingly, the deletion of Fmc1p function can be rescued by an additional copy of the gene for Atp12p [8]. The F_1F_0 assembly factors are not members of the major protein chaperone families (e.g. Hsp70, Hsp60) that service a broad range of substrates [9]. Instead, the actions of Atp10p, Atp11p, and Atp12p all appear to be limited to the biogenesis pathway of the ATP synthase. Of these proteins, Atp11p and Atp12p have been studied most extensively. In the absence of either Atp11p or Atp12p, both F_1 α and β subunits accumulate in aggregated form inside mitochondria [7]. Aggregation of the β subunit occurs also in strains that are simply deleted for the α subunit and the α subunit aggregates in a β subunit null strain [7]. In contrast, the solubility of the α and β subunits is not impaired significantly in γ [10], δ [11], or ε [12] disruption strains. The favored interpretation of these observations is that the non-productive side reaction of α and/or β subunit aggregation prevails only under conditions in which the $\alpha_3\beta_3$ hexamer cannot be formed, either because one of the partner subunits is missing, or because an assembly factor protein (i.e. Atp11p or Atp12p) is missing whose action is to protect the F_1 proteins from forming dead-end complexes. This article summarizes what is known about the chaperone actions of Atp11p and Atp12p in the biogenesis pathway of yeast mitochondrial F_1.

2. Domain structure of Atp11p and of Atp12p

Atp11p and Atp12p are nuclear gene products that are targeted to the mitochondrial matrix via leader sequences

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PII: S0005-2728(02)00262-1

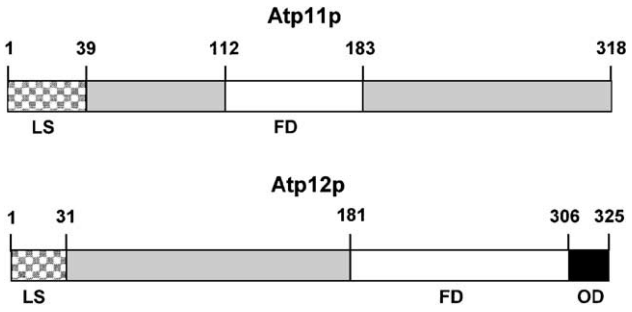

Fig. 1. Domain maps of Atp11p and Atp12p. LS, leader sequence; FD, functional domain; OD, oligomerization domain.

(Fig. 1, LS) present at the amino terminus of the initial translation products [13,14]. The estimated start site for mature Atp11p is Glu^{40} [15] and for mature Atp12p is Gly^{31} [16]. Deletion analysis has disclosed the largest spans of N-terminal or C-terminal sequences that can be removed from the mature Atp11p or Atp12p proteins without completely abolishing their action in F_1 assembly in vivo. Such work has revealed that the functional domain of Atp11p lies within the region between Asp^{112} and Arg^{183} (Fig. 1, FD, upper panel) [17]. In Atp12p, a minimal sequence that can be designated as the functional domain maps to the region between Asn^{181} and Val^{306} in the carboxyl half of the protein (Fig. 1, FD, lower panel), although the mutant protein that terminates with Val^{306} supports only a very low level of mitochondrial F_1 assembly in vivo, and is notably deficient for protein–protein interactions mitochondria [16]. Such observations suggest that the terminal 18 amino acids (Asp^{307}–Gln^{325}) constitute a separate oligomerization domain (Fig. 1, OD) that is dispensable, but not without significant consequences with respect to Atp12p activity.

3. Chaperone actions of Atp11p and Atp12p defined

The use of affinity resins to pull down artificially tagged forms of the F_1 chaperones, coupled with studies that employed the yeast two-hybrid assay as additional means to identify protein–protein interactions, has shown that Atp11p binds selectively to the β subunit of F_1 [18] and that Atp12p binds selectively to the α subunit of F_1 [19]. The two-hybrid screen further provided the information that the binding determinants for Atp11p in the β subunit, and those for Atp12p in the α subunit, are located in the adenine nucleotide binding domains (NBD) of the F_1 proteins. With respect to the NBDs of unassembled F_1 α and β subunits, the regions considered most likely to promote aggregation of the free form of the subunit are the hydrophobic sequences that will ultimately be sequestered at subunit interfaces in the $\alpha_3\beta_3$ structure, as nature did not intend such sequences to be exposed to aqueous solvent. On the basis of contributions made by amino acid residues of the chaperone binding domains in the α and β subunits to the interaction energies in F_1 of each α subunit with an adjacent β subunit, and of each β subunit with an adjacent α subunit, a model for Atp11p, Atp12p-mediated assembly of the F_1 oligomer has been proposed (Fig. 2, see also Ref. [19]). In this model, Atp12p binds to the unassembled F_1 α subunit at the pre-interface surface that will ultimately contribute to a non-catalytic site (NCS) with a neighboring β subunit, while Atp11p binds to the unassembled F_1 β subunit at the pre-interface surface that forms a catalytic site (CS) with an adjacent α subunit in the assembled enzyme.

The fact that Atp11p and Atp12p are each selective for binding only one of the two F_1 subunits raises the question of why both α and β subunits accumulate as aggregated proteins in yeast cells lacking only one of the two chaperones [7]. This issue can be resolved on the basis of protein stoichiometries. Atp11p and Atp12p are present in the mitochondria at a concentration that is at least 100-fold lower than the steady-state level of F_1 proteins [15,19], which is reasonable in view of the fact that the F_1 subunits are assembled into the oligomer as soon as they are imported from the cytoplasm; in other words, there are no pools of unassembled α and β subunits in the mitochondrial matrix [20]. When either Atp11p or Atp12p is absent, one of the two subunits aggregates as a direct consequence of the missing chaperone protein. However, under such conditions, the steady-state level of the "partner" F_1 subunit builds and eventually far exceeds the level of the remaining chaperone.

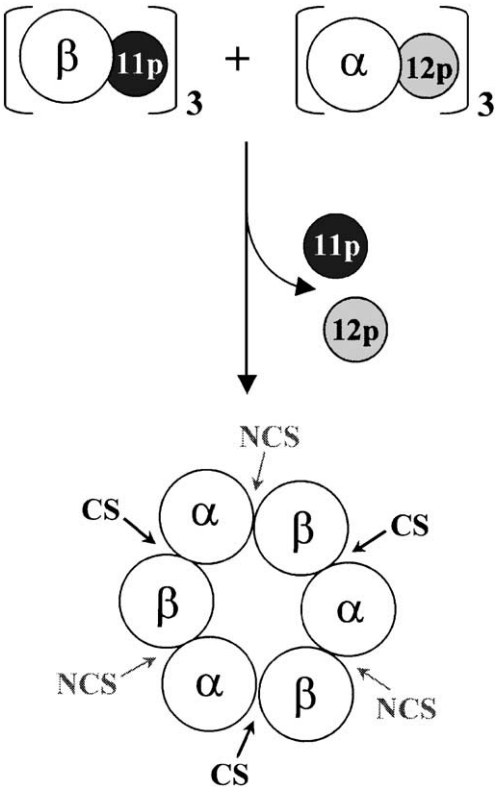

Fig. 2. Atp11p, Atp12p-mediated F_1 assembly. CS, catalytic site; NCS, noncatalytic site.

Hence, even though Atp11p and Atp12p only interact directly with one type of F_1 subunit, each chaperone is perceived to ultimately control the fate of both the α and the β subunits in mitochondria.

4. Evidence that hydrophobic residues are a key element of Atp11p chaperone action

Yeast Atp11p is overproduced as a soluble recombinant protein in bacteria and can be purified to homogeneity [15]. The marked insolubility of purified mitochondrial F_1 proteins has made it impossible to examine the action of Atp11p with its natural substrate in vitro (S. Ackerman and D. Sheluho, unpublished observations). Instead, we have employed insulin as a surrogate substrate for studies that analyze Atp11p chaperone action in vitro [21]. Aggregation of the insulin B chain occurs concomitant with reduction of the disulfide bonds in the parent protein [22]. The ability to induce this reaction under controlled conditions has been exploited to study the chaperone action of several proteins including α-crystallin [23], Hsp90 [24], and SecB [25]. At equimolar concentration to insulin, Atp11p provides 70% protection against aggregation of the B chain, while a control protein (ribonuclease A) has no effect [21].

The nonpolar character of the Atp11p functional domain noted in earlier work [17] suggested that the activity of this protein might be mediated through hydrophobic binding interactions. To investigate this issue, we employed the environment-sensitive fluorescent dye 1,1′-bis(4-anilino-5-napththalenesulfonic acid) (bis-ANS); bis-ANS has been used extensively to probe hydrophobic surfaces in a number of proteins [26–30]. Fluorescence from bis-ANS is barely detectable in aqueous buffer, while the inclusion of Atp11p in the mixture effects a several-fold increase in quantum yield and blue-shift of the emission that is highly diagnostic of bis-ANS binding to an accessible hydrophobic surface of the protein [21]. bis-ANS also quenches fluorescence from Atp11p tryptophans, giving further testimony to the fact that the dye forms a complex with the protein [21]. Titration studies indicate that ~ 3 moles of bis-ANS bind per mole of Atp11p at saturation [21]. Interestingly, the binding data are sigmoidal, indicative of positive cooperativity, which suggests that Atp11p may undergo a change in conformation upon binding bis-ANS in a manner similar to what has been observed when the dye binds to DnaK [26] and to the apical domain of GroEL [27]. While we do not know the nature of any putative conformational changes induced by bis-ANS, the spectral and solubility properties of Atp11p in the presence of stoichiometric amounts of the dye do not reveal a gross perturbation of the protein structure. However, it is clear that the protein function is greatly compromised by bis-ANS since at equimolar dye/protein concentration, the chaperone action of the Atp11p is virtually eliminated (Fig. 3) [21]. The inability of an Atp11p/bis-ANS complex to provide significant protection against aggregation of the reduced insulin B chain provides evidence that the hydrophobic surface in Atp11p to which the dye binds is important for the chaperone activity of the protein.

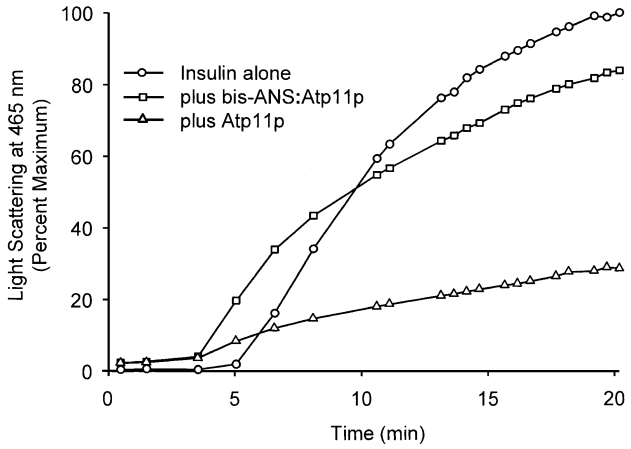

Fig. 3. Effect of bis-ANS binding on the chaperone action of Atp11p. Insulin was reduced with DTT and assayed for aggregation as described in Ref. [21]. *Circles*, insulin alone; *squares*, insulin plus Atp11p/bis-ANS complex (protein complex prepared by incubating Atp11p and bis-ANS at 1:1 molar ratio, see Ref. [21]); *triangles*, insulin plus unliganded Atp11p. Before addition to the assay, the Atp11p/bis-ANS sample and the unliganded Atp11p sample were each passed through a G25 centrifuge column. The concentrations of insulin, Atp11p, and bis-ANS were each 34 μM.

5. Atp11p and Atp12p actions are conserved from yeast to humans

In recent years, we have identified protein sequences from a number of eukaryotic organisms that are homologous with Atp11p and Atp12p of budding yeast [31,32]. The alignment of *S. cerevisiae* Atp11p with the homologous proteins of *Schizosaccharomyces pombe*, *Drosophila yakuba*, and *Homo sapiens* (Fig. 4A) indicates 23% overall similarity and 9% identity amongst the sequences. The alignment of six homologous Atp12p protein sequences (Fig. 4B) indicates 12.7% overall similarity and 4.6% identity. Pairwise analysis yields much higher values with at least 40% similarity and 20% identity in most cases. We isolated a full-length cDNA that codes for human Atp12p and showed that it complements a yeast Δ*atp12* disruption strain, and that the product of the cDNA interacts preferentially with human F_1 α subunit versus human F_1 β subunit in a two-hybrid screen [32]. A near-complete human *ATP11* cDNA that encodes a form of the protein deficient for only ~ 25 amino acids from the amino terminus does not complement a yeast Δ*atp11* deletion mutant; however, the product of this cDNA was shown to bind to the human β subunit, and not to the human α subunit, in a two-hybrid assay [32]. The substrate selectivity exhibited by human Atp11p and Atp12p suggests that these proteins function in a manner that is analogous to their yeast counterparts.

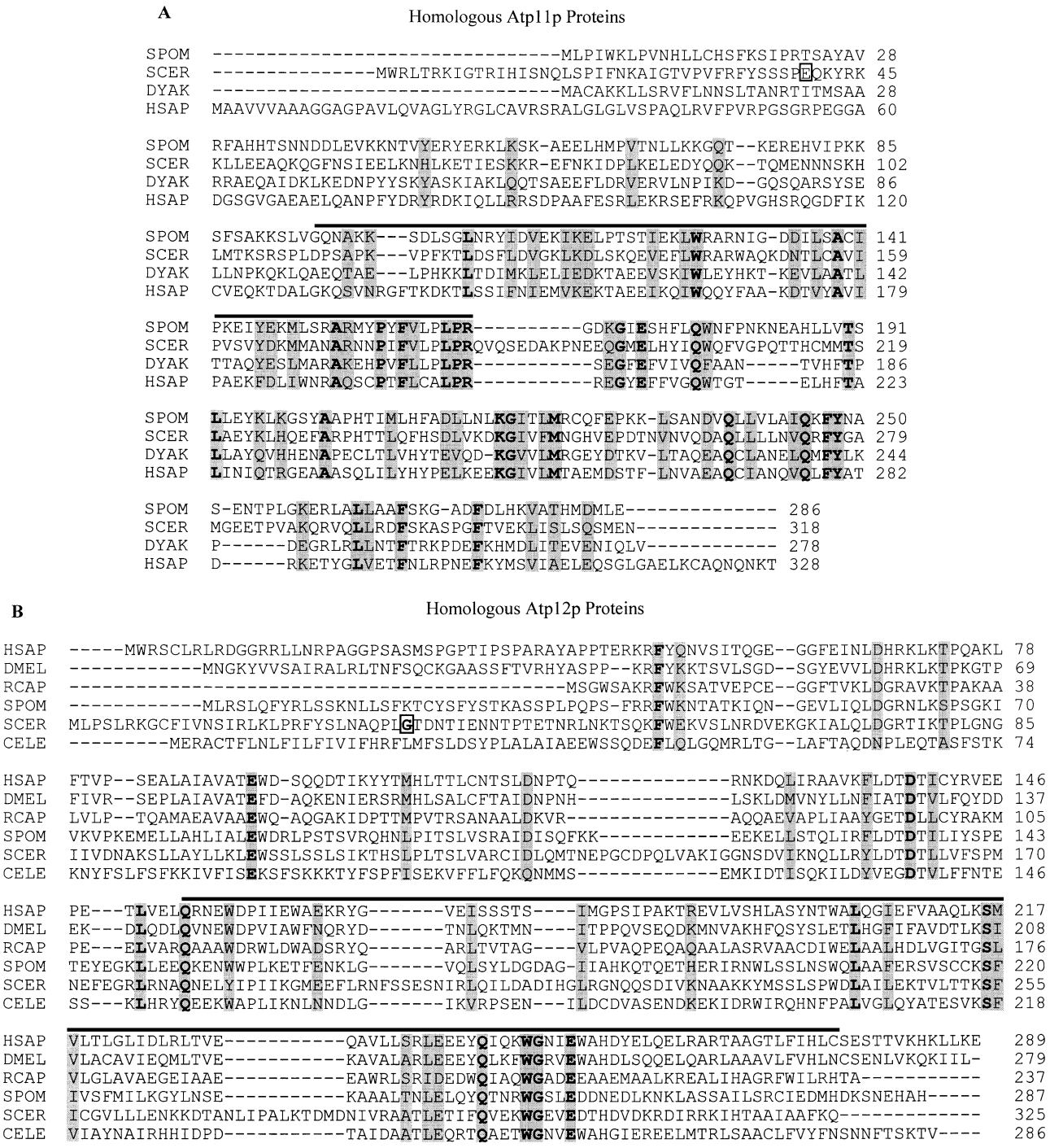

Fig. 4. Alignment of homologous Atp11p (A) and Atp12p (B) proteins. Sequences were aligned with CLUSTALW. SCER (*Saccharomyces cerevisiae*); SPOM (*Schizosaccharomyces pombe*); DYAK (*Drosophila yakuba*); DMEL (*Drosophila melanogaster*); HSAP (*Homo sapiens*); CELE (*Caenorhabditis elegans*); RCAP (*Rhodobacter capsulatus*). Shading indicates identical and physicochemically similar amino acids; identities are further highlighted in bold. The boxes indicate the estimated start of the *S. cerevisiae* proteins that are generated after cleavage of the targeting peptide. The bars indicate the span of sequence in Atp11p and in Atp12p that has been assigned as the functional domain in the protein (see Refs. [16,17]).

Human *ATP11* spans 24 kb in 9 exons and maps to 1p32.3–p33, while *ATP12* contains =8 exons and localizes to 17p11.2 [32]. Both genes are expressed in a wide range of tissues, which suggests that Atp11p and Atp12p are essential housekeeping proteins of human cells.

6. Conclusion

To date, our laboratory has provided information about primary sequence elements in Atp11p and Atp12p that are functionally relevant and we have identified the specific

binding interactions (Atp11p with F_1 β, Atp12p with F_1 α) that define the chaperone activities of these proteins. From the standpoint of mechanism, a key issue to be resolved is how Atp11p is exchanged for an α subunit and how Atp12p is exchanged for a β subunit to complete assembly of the F_1 $\alpha_3\beta_3$ oligomer. Of more physiological relevance is to fully characterize the phenotype that is associated with the loss of Atp11p or Atp12p function to better understand the type of disease that might be associated with a deficiency of these chaperones in human cells.

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