The Assembly Factor Atp11p Binds to the β-Subunit of the Mitochondrial F₁-ATPase*  

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Atp11p is a protein of Saccharomyces cerevisiae required for the assembly of the F₁ component of the mitochondrial FₐFₒ-ATP synthase. This study presents evidence that Atp11p binds selectively to the β-subunit of F₁. Under conditions in which avidin-Sepharose beads specifically adsorbed biotinylated Atp11p from yeast mitochondrial extracts, the F₁ β-subunit coprecipitated with the tagged Atp11p protein. Binding interactions between Atp11p and the entire β-subunit of F₁ or fragments of the β-subunit were also revealed by a yeast two-hybrid screen: Atp11p bound to a region of the nucleotide-binding domain of the β-subunit located between Gly¹¹⁴ and Leu¹⁸⁸. Certain elements of this sequence that would be accessible to Atp11p in the free β-subunit make contact with adjacent α-subunits in the assembled enzyme. This observation suggests that the α-subunits may exchange for bound Atp11p during the process of F₁ assembly.

The ATP synthase of mitochondria, chloroplasts, and bacteria catalyzes ATP synthesis during respiration (1, 2). The enzyme is a large protein complex composed of two oligomeric units: an integral membrane component (Fₒ) and a peripheral bound catalytic moiety (F₁). F₁ that is bound to the membrane sector catalyzes both ATP synthesis and ATP hydrolysis in reactions that are coupled to proton translocation through Fₒ (1, 2).

F₁ contains five different types of subunits in the stoichiometric ratio α₃β₃γδ6ε (1, 2). X-ray diffraction studies have revealed the three-dimensional structure of mitochondrial F₁ from bovine heart (3) and from rat liver (4). The α- and β-subunits alternate in a hexamer that surrounds a central helical structure composed of the N and C termini of the γ-subunit (3, 4). Three catalytic sites and three non-catalytic sites are located at the interfaces between α- and β-subunits (3, 4).

Previous work with respiratory-deficient strains of Saccharomyces cerevisiae has shown that the F₁ α-subunit aggregates in mitochondria of yeast lacking the β-subunit; likewise, the β-subunit aggregates in α-subunit null strains (5). In contrast, the α- and β-subunits can be recovered in soluble fractions when mitochondria are prepared from strains harboring a disrupted gene for the γ-subunit (6), δ-subunit (7), or ε-subunit (8), despite the fact that F₁ does not assemble in the absence of any of these three subunits. Moreover, in a strain disrupted for the γ-subunit, the α- and β-subunits show evidence of forming hetero-oligomers (6). Hence, it would appear that if αβ complexes can form, the proteins remain soluble. If, on the other hand, only one of the “partner” subunits is present (e.g. in an α- or β-subunit null strain), the natural tendency of free α- or free β-protein to aggregate becomes apparent. Notably, the α- and β-subunits of F₁ also aggregate in mitochondria of yeast carrying a nonfunctional allele of the ATP11 or ATP12 gene (5). The fact that the biochemical properties of these mutant strains are similar to those of α- or β-subunit null strains suggests that the products of the ATP11 (9) and ATP12 (10) genes are required to maintain the unassembled α- and β-subunits as soluble proteins during the early phase of enzyme assembly. As might be expected for proteins that bind free F₁ subunits, which constitute a very small fraction of the total pool of F₁ protein (11), Atp11p and Atp12p are present at a very low level in yeast mitochondria (9, 10). Based on the facts that Atp11p and Atp12p appear to function exclusively in F₁ assembly (5) and do not share sequence homology with other proteins of known function, they can be considered as “F₁-specific” assembly factors.

Atp11p is a 31-kDa monomeric protein of the mitochondrial matrix with an overall basic charge (12). Previous work has localized the functional domain of Atp11p to a region in the middle of the protein that is characterized by two stretches of hydrophobic sequence (13). Here we report results from coprecipitation experiments and yeast two-hybrid screens that probe for direct interactions between Atp11p and the F₁ α- and β-subunits and conclude that Atp11p binds to the β-subunit only. The binding site for Atp11p was mapped within a sequence of 205 amino acids in the nucleotide-binding domain of the β-subunit.

**EXPERIMENTAL PROCEDURES**

**Strains and Growth Media—**Coprecipitation experiments utilized yeast strain W303ΔG13 (MATα ade2-1 his3-1,15 leu2-3,112 ura3-1 trp1-1 his3-112 HIS3; 9). Yeast two-hybrid experiments employed strain Y190 (MATα ade2-1 his3-2Δ lys2-801 ade2-101 trp1-901 leu2-3,112 gal4Δ gal80Δ cyh2 lys2::GAL1(UAS)HIS31XAT(AES) HIS3 URA3::GAL1(UAS) GAL1(TATA)lacZ) (CLONTECH). Escherichia coli RR1 (proA leuB lacY galK xyl-5 mtl-1 ara-14 rpsL supE hsdS λ−) was the host bacterial strain for the recombinant plasmid constructions. Yeast cells were grown in the following media:YPD (2% glucose, 2% peptone, and 1% yeast extract), YPGal (2% galactose, 2% peptone, and 1% yeast extract), EG (2% ethanol, 2% glycerol, 2% peptone, and 1% yeast extract), and SD/−Leu,Trp,His (2% glucose and 0.67% yeast nitrogen base without amino acids), and SD/−Leu,Trp,His (2% glucose and 0.67% yeast nitrogen base without amino acids, supplemented with all essential amino acids and nucleotides except leucine, tryptophan, and histidine). Amino acids and other growth requirements were added at a final concentration of 20–150 µg/ml. The solid media contained 2% agar in addition to the components described above.

**Plasmid Constructions—**The plasmids used in this study are described in Table I. Atp11p is numbered from residues 1 to 318 (9); the F₁ β-subunit is numbered from residues 1 to 511 (15); and the F₁ α-subunit is numbered from residues 1 to 544 (16). In all cases, residue 1 is the initiator methionine in the primary translation products. The

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Atp11p binds to the F₁ β-Subunit

Table I

| Plasmid | Encoded product | Source |
|---------|----------------|--------|
| pG13/St5 | Atp11p, including leader peptide | Ref. 9 |
| pG13/ETEYP | Br-Atp11p, including leader peptide | Ref. 12 |
| pAS2–1/ATP1-(40–318) | Mature Atp11p (Gly₂⁶–Asn₃₁₅) | This study |
| pACT2/ATP1-(35–544) | Mature F₁ α-subunit (Leu₃₅–Phe₃₄⁴) | This study |
| pACT2/ATP2-(36–511) | Mature F₁ β-subunit (Ala₅₆–Asn₃₁₁) | Ref. 14 |
| pACT2/ATP2-(101–511) | F₁ β-subunit fragment (Gln₁₀¹–Asn₃₁₁) | This study |
| pACT2/ATP2-(36–511) | F₁ β-subunit fragment (Gln₁₆₆–Asp₃₈₂) | This study |
| pACT2/ATP2-(114–210) | F₁ β-subunit fragment (Gln₃₈–Ile₂₇₂) | This study |
| pACT2/ATP2-(319–511) | F₁ β-subunit fragment (Ala₁₁₂–Asn₅₁₁) | This study |
| pACT2/ATP2-(289–511) | F₁ β-subunit fragment (Asp₂₈₀–Asn₅₁₁) | This study |
| pACT2/ATP2-(36–318) | F₁ β-subunit fragment (Ala₅₆–Leu₃₁₆) | This study |
| pACT2/ATP2-(36–255) | F₁ β-subunit fragment (Gln₃₈–Ile₂₅₅) | This study |
| pACT2/ATP2-(36–210) | F₁ β-subunit fragment (Gln₃₈–Ala₂₁₀) | This study |
| pACT2/ATP2-(114–288) | F₁ β-subunit fragment (Gly₁₁₄–Ile₂₈₈) | This study |
| pACT2/ATP2-(211–288) | F₁ β-subunit fragment (Gly₁₁₄–Ile₂₇₈) | This study |
| pACT2/ATP2-(273–318) | F₁ β-subunit fragment (Gly₁₁₄–Ile₃₁₀) | This study |
| pACT2/ATP2-(273–288) | F₁ β-subunit fragment (Gly₁₁₄–Ile₂₇₈) | This study |
| pACT2/ATP2-(289–318) | F₁ β-subunit fragment (Asp₂₈₀–Leu₃₁₆) | This study |
| pACT2/ATP2-(153–288) | F₁ β-subunit fragment (Asp₁₅₃–Ile₂₈₀) | This study |

* Fusion protein with the DNA-binding domain of Ga14p.
* Fusion protein with the activation domain of Ga14p.
* Nine amino acids coded by vector sequence are fused to the carboxyl terminus of this β-subunit fragment.
* Fifteen amino acids coded by vector sequence are fused to the carboxyl terminus of this β-subunit fragment.

Plasmids carrying partial segments of a gene and the encoded products are named according to the codons/amino acids that are retained in the constructs. Plasmid pAS2-1/ATP1-(40–318) carries a 860-bp NcoI-BamHI fragment prepared from pTRCAT11-12 (in pAS1-1 [CLONTECH]. To make pACT2/ATP1-(35–544), a 1.6-kilobase BstXI (blunted)-DraI fragment carrying ATP1 DNA was first subcloned in the Smal site of YEp352 (17) and then prepared as an EcoRI (blunted)-BamHI fragment and ligated at the NcoI (blunted) and BamHI sites of pACT2 (CLONTECH). Plasmid pG13/St5, which carries the entire ATP2 gene in the vector pRS316 (18), was used for construction of most of the ATP2 two-hybrid plasmids. Plasmid pACT2/ATP2-(101–511) carries a 1.25-kilobase KpnI (blunted)-XhoI fragment prepared from pG13/St5 in the Smal and XhoI sites of pACT2. Plasmid pACT2/ATP2-(211–511) carries a 920-bp NcoI (blunted)-XhoI fragment prepared from pG13/St5 in the BamHI (blunted) and XhoI sites of pACT2. Plasmid pACT2/ATP2-(273–511) carries a 730-bp PvuI (blunted)-XhoI fragment prepared from pG13/St5 in the Smal and XhoI sites of pACT2. For construction of pACT2/ATP2-(35–511), plasmid pACT2/ATP2-(273–511) was digested with NcoI and BamHI and treated with Klenow enzyme, and the resultant 9.1-kilobase fragment was recircularized. Plasmid pACT2/ATP2-(329–511) carries a 600-bp MscI-XhoI fragment prepared from pG13/St5 in the XmaI (blunted) and XhoI sites of pACT2. To construct pACT2/ATP2-(36–511), plasmid pACT2/ATP2-(273–511) was digested with NcoI and BamHI and treated with Klenow enzyme, and the resultant 9.1-kilobase fragment was recircularized. The terminus code for the insert in pACT2/ATP2-(36–532) is provided by vector sequence fused to the carboxyl-terminal sequence of this β-subunit fragment. Plasmid pACT2/ATP2-(36–518) carries a 850-bp SfiI-MscI fragment prepared from pACT2/ATP2-(36–511) in the SfiI and Smal sites of pACT2. The terminus code for the insert in pACT2/ATP2-(36–318) is provided in the vector 45 nucleotides downstream from the ATP2 DNA; there are 9 amino acids (NGGSEFELEMNRNR) encoded by vector sequence fused to the carboxyl terminus of this β-subunit fragment. The ATP2 inserts in the remaining plasmids were synthesized by PCR using plasmid pG13/St5 as the template (unless indicated otherwise) with the primers listed in Table II. The forward and reverse primer pairs used to construct each plasmid are as follows: pACT2/ATP2-(289–511), β289–511-f and M13 #1323, pACT2/ATP2-(38–288), β38–288-f and β38–288-r (a 8.4-kilobase NcoI fragment prepared from this plasmid was filled in with Klenow enzyme and religated to make plasmid pACT2/ATP2-(211–288); pACT2/ATP2-(38–272), β38–288-f and β38–272-r; pACT2/ATP2-(38–253), β38–288-f and β38–253-r; pACT2/ATP2-(38–210), β38–288-f and β38–210-r; pACT2/ATP2-(38–116), β38–288-f and β38–116-r; pACT2/ATP2-(273–114), β114–288 and β114–288-r; pACT2/ATP2-(273–114), β114–288-f and β114–288-r; pACT2/ATP2-(273–88), β88–288-f and β88–288-r. PCR for construction of pACT2/ATP2-(273–318) employed a plasmid template bearing a 140-bp NcoI-MscI fragment that was subcloned from pACT2/ATP2-(273–511) in the NcoI and Smal sites of pACT2 with primers β273–318-f and β273–318-r. PCR for pACT2/ATP2-(289–318) used primers β289–511-f and β289–318-r with template pACT2/ATP2-(289–511). PCR for pACT2/ATP2-(273–288) used primers β273–318-f and β288–288-r with template pACT2/ATP2-(273–318). All resultant PCR products were flanked with restriction sites appropriate for subcloning in pACT2 (Table II).

Coprecipitation Experiments—Mitochondria were prepared as described (13) from yeast strains w303-1A/pG13/St5 and w303-1A/pG13/ETEYP, which produce native and biotin-tagged forms of Atp11p, respectively, from the 2μ vector Yep352. The mitochondria were suspended at 7 mg/ml (300-μl final volume) in TEA buffer (10 mM Tris-HCl, pH 8.0, 2 mM EDTA, and 4 mM ATP) supplemented with protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, 1 μM Leupeptin, and 1 μM pepstatin) and subjected to four 10-s bursts of sonic irradiation at full power (Branson Model 450 sonifier). The sonicated material was centrifuged at 50,000 rpm in a Beckman Ti-70.1 rotor for 30 min at 4 °C to sediment the membranes. The soluble extracts (130 μl) were mixed with 50 μl of avidin-Sepharose beads and brought to a 200-μl final volume in either buffer A (TEA buffer supplemented with 1% Triton X-100 and 0.14 mM NaCl) or buffer B (TEA buffer supplemented with 1% Triton X-100, 0.14 mM NaCl, and 2 mM biotin). The samples were rotated end-over-end at 4 °C for 30 min and subsequently centrifuged for 5 min (Beckman Microfuge) to sediment the avidin-Sepharose beads. The beads were washed three times by centrifugation with 400 μl of buffer A or buffer B (as appropriate), followed by an additional three washes with 400 μl of plain TEA buffer. The washed beads were suspended in 40 μl of Laemmli sample buffer in preparation for Western analysis. The proteins present in the initial post-bead

# The abbreviations used are: bp, base pair; PCR, polymerase chain reaction; X-gal, 5-bromo-4-chloro-3-indoyl β-D-galactopyranoside; Bt-Atp11p, biotinylated Atp11p.
Assays for Atp11p and the F1 β-subunit from CLONTECH. Yeast were grown in SD-medium as supplied in the MATCHMAKER Two-Hybrid System manual. Qualitative assessment of expression from the β-galactosidase reporter gene was by chemiluminescence using the ECL system from Amersham Pharmacia Biotech. Protein concentrations were estimated by coupling native avidin (Sigma) to CNBr-activated Sepharose from Amersham Pharmacia Biotech. Avidin-Sepharose resin was pre-equilibrated with biotin, there was only a background level of Bt-Atp11p, and no significant amounts of both Bt-Atp11p and the F1 β-subunit in the bead precipitates (*), a 4-fold excess of biotin was loaded on the gel versus the amount used to visualize the same proteins in the supernatant fractions.

RESULTS

Affinity Precipitation of Biotinylated Atp11p (Bt-Atp11p) with Avidin-Sepharose Beads—Yeast transformed with plasmid pG13/BTYEP produce a form of Atp11p that carries a sequence for in vivo biotinylation at the C terminus of the protein (12). The tagged protein (Bt-Atp11p) is fully functional in F1 assembly (9, 12) and can be used in combination with avidin-Sepharose beads in coprecipitation experiments aimed at identifying proteins that form complexes with Atp11p. For this purpose, soluble mitochondrial extracts, prepared from yeast cells that produce either native or biotinylated Atp11p, were incubated with avidin-Sepharose beads in the absence or presence of free biotin in the buffer (see “Experimental Procedures”). Western blots of the post-bead supernatants (Fig. 1) showed that the F1 α- and β-subunits were present in approximately equal amounts and that native Atp11p was more abundant than biotinylated Atp11p. In the bead precipitate from mitochondrial extracts containing native Atp11p, only background levels of Atp11p were detected compared with the strong signal for this protein in the supernatant. Moreover, there was no evidence that F1 α- or β-subunits were precipitated by the beads. In contrast, the bead precipitate from mitochondrial extracts containing biotinylated Atp11p showed significant amounts of both Bt-Atp11p and the F1 β-subunit. The specificity of this coprecipitation is attested by the fact that when precipitation was carried out in the presence of excess biotin, there was only a background level of Bt-Atp11p, and no F1 β-protein was detected in the bead precipitate. The absence of the α-subunit in the Bt-Atp11p precipitate suggests that this F1 protein either does not interact directly with Atp11p or forms only a very weak complex.

Identification of the β-Subunit Amino Acid Sequence That Binds Atp11p—The yeast two-hybrid assay was used to probe for binding interactions between Atp11p and the F1 β-subunit. The plasmids employed in this work (Table I) encode only the mature sequences (i.e. without the mitochondrial leader sequences) of the Atp11p and F1 proteins fused to either the transcriptional activation domain of Gal4p or avidin bead-precipitated samples (Bead ppt + biotin) were loaded on a 12% SDS gel, transferred to nitrocellulose, and probed with either a mixture of antibodies against the α- and β-subunits or with Atp11p antisera. To allow detection of even small quantities of F1 α- and β-subunits in the bead precipitates (*), a 4-fold excess of protein was loaded on the gel versus the amount used to visualize the same proteins in the supernatant fractions.

| Primer | Primer sequence (5’ → 3’)a | ATP2 gene location | Restriction sites for cloning |
|--------|----------------------------|--------------------|-----------------------------|
| 5′-GGAAGCAGTCATACCATATGAGTACGACATGACGTC-3′ | Neol | NcoI |
| 5′-CGGAGGTCACGTCACGACGTC-3′ | BamHI | BamHI |
| 5′-GGGTTCGCATGGAAGTCGTCAGTGCAGTCGTC-3′ | XhoI | XhoI |
| 5′-GGGTCACGTCACGACGTC-3′ | NcoI | NcoI |
| 5′-GGGTCACGTCACGACGTC-3′ | NcoI | NcoI |

a ATP2 nucleotides are shown in boldface.
Atp11p binds to the F₁ β-subunit.

FIG. 2. Yeast two-hybrid screen of the binding between Atp11p and the F₁ β-subunit. A protein map of the mature yeast F₁ β-subunit (Ala²⁸⁶–Asn³¹⁵) that shows the positions of three primary domains is given in the upper part of the figure. The designation of the yeast β-barrel domain (Ile⁴²–Ile¹¹⁶), the nucleotide-binding domain (Ser¹¹⁷–Val³⁹⁶), and carboxyl-terminal helix bundle domain (Gly³⁹⁷–Asn⁵¹¹) is based on the homologous β-subunit from beef heart mitochondria (3). The position and length of the β-subunit fragments that scored positively for binding Atp11p are shown by open rectangles; negatively scoring fragments are indicated by thick lines. The boundaries of the fragments tested are shown on the left. The amount of β-galactosidase activity measured in blue colonies is given on the right and is expressed in Miller units (20). The shaded box highlights the segment of β-subunit sequence that contains binding determinants for Atp11p. n.d., not determined.

The two-hybrid system was used further to locate the binding site for Atp11p on the β-subunit. There are three domains in the mature β-subunit: a β-barrel at the N terminus, a central domain that contains the adenine nucleotide-binding site, and a helix bundle at the C-terminal end (3, 4) (see Fig. 2). The fragment Glu¹⁰¹–Asn⁵¹¹, which lacks almost the entire β-barrel, and the fragment Ala³⁶–Asp³⁸², which lacks the helix bundle, both scored positively for binding Atp11p in this assay. There was no evidence of Atp11p binding to the sequence for the β-barrel domain (Ala³⁸–Ile¹¹⁶) or the helix bundle domain (Asp³⁸²–Asn⁵¹¹). Western analysis with antibodies against the Gal4p activation domain confirmed the presence of the non-interacting β-subunit fusion proteins in the yeast host (data not shown).

The results from initial mapping studies indicated that the binding determinants for Atp11p are contained entirely within the nucleotide-binding domain of the β-subunit. To define the boundaries of the Atp11p-binding site, fragments of the β-subunit, deleted for sequence from the amino- or carboxyl-terminal end of the adenine nucleotide-binding domain, were tested for binding Atp11p in the two-hybrid assay. This analysis disclosed positive results for the fragments His²¹¹–Asn⁵¹¹, Ala²⁷³–Asn⁵¹¹, Asp²⁸⁹–Asn⁵¹¹, Ala³⁶–Leu³¹⁸, Gln³⁸–Ile²⁸⁸, Gln³⁸–Ile²⁷², Gln³⁸–Gly²⁵³, Gly¹¹⁴–Ile²⁸⁸, Gly¹¹⁴–Ile²⁷², Gly¹¹⁴–Gly²⁵³ and Ala²⁷³–Leu³¹⁸. The largest segment of β-subunit residues that can be removed from the N or C terminus of the protein without disrupting binding interactions with Atp11p occurs proximal to Gly¹¹⁴ and distal to Leu³¹⁸, which suggests that the binding site for Atp11p is located between these 2 amino acids (Fig. 2, shaded box). The data also indicate that the structural elements recognized by Atp11p are distributed throughout this region since not all of the fragments that scored positive for binding overlap in sequence. Notably, there are a number of fragments that encompass portions of the sequence between Gly¹¹⁴ and Leu³¹⁸ that did not show evidence for binding overlap in sequence. On this basis, we suggest that the fragments Gln³⁸–Ala²¹⁰, Gly¹¹⁴–Ala²¹⁰, His³¹¹–Ile²⁸⁸, Ala²⁷³–Ile²⁸⁸, Asp²⁸⁹–Leu³¹¹, and Asp¹⁵³–Ile²⁸⁶ may not fold correctly, which could prevent their recognition by Atp11p.

DISCUSSION

We have used two different methods to detect binding interactions between Atp11p and the α- and β-subunits of F₁. First, avidin-Sepharose beads, which selectively bind biotinylated Atp11p in mitochondrial extracts, were shown to coprecipitate the F₁ β-subunit. Second, direct binding between Atp11p and the F₁ β-subunit was demonstrated by means of a yeast two-hybrid screen. Neither the affinity precipitation assay nor the two-hybrid screen provided indications that Atp11p binds also to the F₁ α-subunit. Additional work with the yeast two-hybrid screen has mapped the Atp11p-binding site to a sequence of 205 amino acids (Gly¹¹⁴–Leu³¹⁸) located within the nucleotide-binding domain of the β-subunit (Fig. 2). It is of note that only certain fragments of this sequence bind the assembly factor. For example, binding was observed for the β-subunit sequence Gly¹¹⁴–Ile²⁸⁸, but not for its smaller fragments, Gly¹¹⁴–Ala²¹⁰ and His²¹¹–Ile²⁸⁸. Similarly, the sequence Ala²⁷³–Ile²⁸⁸ scored positive for Atp11p binding, whereas the fragments Ala²⁷³–Ile²⁸⁸ and Asp²⁸⁹–Leu³¹⁸ did not. The β-subunit fragments that did not show evidence of binding Atp11p were detected in the cell by Western analysis in amounts comparable to the levels of the α-subunit.
to those of the other fragments that did show evidence for binding the assembly factor. Thus, it would appear that other factors such as a correct folding are required for recognition and binding by Atp11p. This observation, together with the fact that *atp11* mutants accumulate only the mature *β*-subunit (5), whereas strains defective in mitochondrial protein folding (i.e. *hsp80* (*mif4*) mutants) accumulate both the precursor and mature forms of the *β*-subunit (26), supports the view that Atp11p acts at a step downstream from Hsp60, and binds the folded form of the *β*-subunit.

It is conceivable that Atp11p prevents the aggregation of unassembled *β*-subunits by shielding sequence elements that would cause nonproductive *β/β* interactions. The availability of structural information for bovine F1 from x-ray studies (3) allows us to identify by homology modeling candidates for the sequence elements in the free *β*-subunit that are most likely to be protected by the assembly factor. The sequence Gly114-Leu318 of the yeast *β*-subunit, which we have shown to harbor binding determinants for Atp11p, is homologous to Ala80–Leu285 of the bovine F1 *β*-subunit (3). This region, which extends from β-strand 1 through the beginning of α-helix F in the nucleotide-binding domain of the bovine *β*-subunit, is colored in red in the ribbon diagram shown in Fig. 3A. Within this segment of the *β*-subunit, several amino acid side chains are involved in intersubunit contacts with the adjacent α-subunits. This feature is illustrated in Fig. 3B, which shows the secondary structural elements of the *β*20*-subunit sequence between Ala80 and Leu285 rendered as a schematic in red, the Cα traces of the adjacent αDP- and αCF-*subunits in cyan, and the amino acids at the interfaces between these subunits as stick models colored in yellow (contribution from the segment Ala80–Leu285 of the *β*-subunit) or blue (contribution from the adjacent α*-subunits). The participation of the Atp11p-binding region in the formation of the contact surfaces between α- and *β*-subunits suggests that during assembly of the oligomer, the α-*subunits may bind to the *β*-subunit in exchange for bound Atp11p. In consideration of the high tendency of *β*-subunit monomers to aggregate (5), this type of action would ensure that the *β*-subunit is never present as a free protein in solution.

Recent findings provide evidence that a similar mechanism (binding of Atp12p to the *α*-subunit) is adopted to prevent aggregation of the *α*-subunit during normal assembly of the enzyme. In this context, of particular interest is the observation that in the absence of Atp11p, also the *α*-subunit aggregates inside mitochondria (5). One possible explanation of this phenomenon is that whereas under normal conditions, the very small pool of unassembled *α*-subunit is maintained in solution via binding to Atp12p, under conditions in which the *β*-subunit aggregates (i.e. in *atp11* mutants), a large amount of unassembled *α*-subunit is likely to accumulate in excess over the Atp12p protein. Thus, under these circumstances, also the *α*-subunit is expected to aggregate in the mitochondrial matrix.

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