The cleaved presequence is not required for import of subunit 6 of the cytochrome bc_1 complex into yeast mitochondria or assembly into the complex*

Marie Laure DeLabre, Jürgen H. Nett, Bernard L. Trumpower*

Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755, USA

Received 8 March 1999

Abstract Subunit 6 of the yeast cytochrome bc_1 complex contains a 25 amino acid presequence that is not present in the mature form of the protein in the bc_1 complex. The presequence of subunit 6 is atypical of presequences responsible for targeting proteins to mitochondria. Whereas mitochondrial targeting sequences rarely contain acidic residues and typically contain basic residues that can potentially form an amphiphilic structure, the presequence of subunit 6 contains only one basic amino acid and is enriched in acidic amino acids. If the 25 amino acid presequence is deleted, subunit 6 is imported into mitochondria and assembled into the cytochrome bc_1 complex and the activity of the bc_1 complex is identical to that from a wild-type yeast strain. However, if the C-terminal 45 amino acids are truncated from the protein, subunit 6 is not present in the mitochondria and the activity of the bc_1 complex is diminished by half, identical to that of the bc_1 complex from a yeast strain in which the *QCR6* gene is deleted. These results indicate that the presequence of subunit 6 is not required for targeting to mitochondria or assembly of the subunit into the bc_1 complex and that information necessary for targeting and import into mitochondria may be present in the C-terminus of the protein.

Key words: Mitochondria import; Presequence; Cytochrome bc_1; *Saccharomyces cerevisiae*

1. Introduction

The cytochrome bc_1 complex is located in the inner mitochondrial membrane and contains 10 or 11 subunits that range in size from 7.2 to 44 kDa [1]. Subunit 6 of the yeast bc_1 complex is highly acidic and is located on the intermembrane space surface of the complex [2–4]. Like many nuclear encoded mitochondrial proteins, subunit 6 has an amino-terminal presequence which is absent from the mature subunit in the bc_1 complex [5]. The presequence of subunit 6 is atypical of proteins targeted to mitochondria. It contains only one basic amino acid (lysine) and is enriched in acidic amino acids. Mitochondrial targeting sequences rarely contain acidic residues and contain basic residues spaced so that they can form an amphiphilic structure that is thought to be essential for the targeting function of the signal sequence [6,7].

We have analyzed the role of the cleavable presequence of Qcr6p and of the C-terminus of the protein for targeting and import into the mitochondria. Surprisingly, the presequence of Qcr6p is not required for targeting to mitochondria or for assembly of this protein into the bc_1 complex. However, the C-terminus of Qcr6p apparently contains information necessary for the targeting and import of this protein into mitochondria.

2. Materials and methods

2.1. Yeast strains and plasmids

Yeast strains and plasmids are listed in Tables 1 and 2, respectively. The plasmid pGEM-3Zf(+) was purchased from Promega. The expression vector pRS200 [8] was obtained from ATTC.

2.2. Subcloning of the QCR6 gene

The *QCR6* reading frame and its 5′ and 3′ untranslated regions were amplified by a polymerase chain reaction (PCR) with yeast genomic DNA as template. The presequence deletion (Qcr6-Δ26p) was introduced by PCR amplification. The amplicons were subcloned into pGEM3Zf(+). pMLD01 harbors the DNA encoding the *QCR6* reading frame, amplified by PCR using pMES32 as a template [11]. pMES32 has an insertion of a G at position 295 of the *QCR6* reading frame, resulting in a frameshift that introduces a stop codon at position 307. The C-terminal sequence of the encoded truncated protein (Qcr6-102Δp) is changed to GGVC instead of EECA. The *QCR6* promoter was amplified by PCR and the DNA's encoding the full length Qcr6p, Qcr6-Δ26p and Qcr6-102Δp were subcloned in the CEN-TRP containing expression vector pRS200. All constructs were verified by sequencing using the Dye Deoxy Terminator Cycle sequencing kit from Applied Biosystems.

2.3. Western analysis and enzyme assays of mitochondrial membranes

Mitochondria were isolated as described elsewhere [12]. Qcr6p and cytochrome c_1 were detected by Western blotting using polyclonal antibodies affinity purified to the GST-Qcr6p [15] and monoclonal antibodies to the cytochrome c_1 [13]. Antibodies were detected by chemiluminescence (Amersham). Ubiquinol-cytochrome *c* oxidoreductase activities of mitochondrial membranes were assayed as described previously [14].

2.4. In vitro transcription and translation and import into mitochondria

In vitro transcription using SP6 RNA polymerase and in vitro translation using rabbit reticulocyte lysate were performed according to supplier recommendations (Promega). The in vitro translation products were labelled using [^{35}S]cysteine from Amersham. Import into mitochondria was performed as described previously [12].

3. Results

3.1. Qcr6p lacking the presequence complements the Qcr6 deletion strain

MMY18, a yeast strain in which *QCR6* has been deleted, exhibits a temperature-sensitive petite phenotype at 37°C [15]. To determine whether truncated forms of Qcr6p are imported into mitochondria and assembled correctly into the bc_1 complex, we first tested whether these would rescue the temperature-sensitive petite phenotype of MMY18. We transformed the *Δqcr6* deletion strain with the single copy expression vec-

*Corresponding author. Fax: (1) (603) 650-1389.
E-mail: trumpower@dartmouth.edu

PII: S0014-5793(99)00415-9

Qcr6p

MGMLELVGEYWEQLKITVVPVVAAA↓EDDDNEQHEEKAAEGEEKEEENG
DEDEDEDEDEDDDDDDDEDEEEEEEVTDQLEDLREHFKNTEEGKALVHHY
EECAERVKIQQQQPGYAELEHKEDCVEEFFHLQHYLDTATALPRLFDKLK

Qcr6-102Δp

MGMLELVGEYWEQLKITVVPVVAAA↓EDDDNEQHEEKAAEGEEKEEENG
DEDEDEDEDEDDDDDDDEDEEEEEEVTDQLEDLREHFKNTEEGKALVHHY
GGVC

Fig. 1. The deduced amino acid sequence of Qcr6p showing the site at which the presequence is cleaved and the C-terminal truncation introduced by site-directed mutagenesis. The arrow indicates the site at which the 25 amino acid presequence is cleaved to form mature sized Qcr6p. The amino terminus of mature Qcr6p, lacking the cleaved presequence, was determined by sequencing the protein isolated from the cytochrome bc_1 complex [5]. The amino acid sequence of Qcr6p was deduced from the *QCR6* genomic locus in yeast strain W303 and the sequence of the *QCR6* locus in the yeast genome data base and that of Qcr6-102Δp was deduced from the sequence of *QCR6-102Δ*. Qcr6-Δ26p is Qcr6p in which the 25 amino acid presequence has been deleted and replaced by an N-terminal methionine.

tor pRS200 encoding Qcr6p, Qcr6-Δ26p and Qcr6-102Δp (Table 2). Qcr6-Δ26p is subunit 6 in which the 25 amino acid presequence has been deleted and replaced by an N-terminal methionine. Qcr6-102Δp is subunit 6 in which the presequence is retained but which lacks the C-terminal 45 amino acids due to a stop mutation in *QCR6*. The deduced amino acid sequences of Qcr6p, Qcr6-Δ26p and Qcr6-102Δp are shown in Fig. 1.

When the transformants were first grown on medium containing dextrose as a carbon source and then replicated on ethanol/glycerol medium and incubated at 37°C, the temperature-sensitive petite phenotype of MMY18 was complemented by both the full length Qcr6p and by Qcr6-Δ26p (results not shown). These results suggest that the 25 amino acid presequence is not required for the import of subunit 6 into mitochondria and assembly of the cytochrome bc_1 complex. When the *Δqcr6* deletion strain was transformed with the plasmid encoding Qcr6-102Δp, in which the C-terminus of the protein is deleted (MLD09), the transformants did not grow on ethanol/glycerol medium at 37°C (results not shown). The protein in which the C-terminal 45 amino acids have been truncated is apparently unable to rescue the temperature-sensitive petite phenotype of the *Δqcr6* deletion strain, even though this protein contains the presequence.

3.2. Qcr6p lacking the presequence restores cytochrome c reductase activity to the qcr6 deletion strain

To confirm that complementation of the temperature-sensitive petite phenotype resulted from restoration of activity to the cytochrome bc_1 complex, we isolated mitochondria from the *Δqcr6* deletion strain and from the strains containing the truncated forms of Qcr6p and measured cytochrome *c* reductase activities of the mitochondrial membranes. When the *Δqcr6* deletion strain (MMY18) is grown at the permissive temperature, the ubiquinol-cytochrome *c* reductase activity of the mitochondrial membranes is reduced to ∼50% of that of the wild-type activity [5]. As can be seen in Fig. 2, the plasmids encoding Qcr6p (MLD05) and Qcr6-Δ26p (MLD06) restore the cytochrome *c* reductase activity of the mitochondrial membranes from the deletion strain to 100% of the wild-type activity. In contrast, the ubiquinol-cytochrome *c* reductase activity of mitochondria from the Δ*qcr6* deletion strain expressing Qcr6-102Δp (MLD09) is essentially the same as the activity of the mitochondria from MMY18.

3.3. Qcr6p lacking the presequence is imported into mitochondria in vivo

The failure of the plasmid encoding Qcr6-102Δp to rescue the temperature-sensitive petite phenotype could be due to a lack of import of the truncated protein into the mitochondria or a lack of assembly of the protein into the bc_1 complex. To confirm that Qcr6-Δ26p is imported into mitochondria and to determine whether the Qcr6-102Δp is imported into mitochondria in vivo, we performed a Western blot analysis on the mitochondrial membranes.

As expected, Qcr6p is absent from the mitochondrial membranes of the Δ*qcr6* deletion strain, MMY18 (Fig. 2). Mature sized Qcr6p is detected in membranes from the wild-type strain, YPH500, and in membranes from the deletion strain expressing either the full length Qcr6p (MLD05) or the truncated Qcr6-Δ26p (MLD06). Treatment of the mitochondria with proteinase K confirmed that the mature sized Qcr6p was present within a protease-protected space.

On close examination of the Western blots of the resolved mitochondrial membranes, it appears that there is somewhat less mature sized Qcr6p in the membranes of the yeast strain

Table 1

Yeast strain	Genotype	Source
YPH 500	Matα, *ura* 3-52, *lys*2-801(amber), *ade* 2-101 (ochre), *trp*1Δ63, *his*3Δ200, *leu* 2Δ1	[8]
MMY18	*ura* 3-52, *lys* 2-801 (amber), *ade* 2-101 (ochre), *trp*1Δ63, *his*3Δ200, *qcr6* Δ1::LEU2	[15]
MLD05	*ura* 3-52, *lys* 2-801 (amber), *ade* 2-101 (ochre), *trp*1Δ63, *his*3Δ200, *qcr6* Δ1::LEU2, pMLD13 (TRP1, CEN, QCR6)	This study
MLD06	*ura* 3-52, *lys* 2-801 (amber), *ade* 2-101 (ochre), *trp*1Δ63, *his*3Δ200, *qcr6* Δ1::LEU2, pMLD14 (TRP1, CEN, QCR6-Δ26)	This study
MLD09	*ura* 3-52, *lys* 2-801 (amber), *ade* 2-101 (ochre), *trp*1Δ63, *his*3Δ200, *qcr6* Δ1::LEU2, pMLD12 (TRP1, CEN, QCR6-102Δ)	This study
MLD01	*ura* 3-52, *lys* 2-801 (amber), *ade* 2-101 (ochre), *trp*1Δ63, *his*3Δ200, *qcr6* Δ1::LEU2, pRS200 (TRP1, CEN)	This study
W303-1A	*Mat* a, *ade* 2-1, *his* 3-11,15, *trp* 1-1, *leu* 2-3,112, *ura* 3-1, can 1-100	[9]
JPJ1	*Mat* a, *ade* 2-1, *his* 3-11,15, *trp* 1-1, *leu* 2-3,112, *ura* 3-1, can 1-100, *rip1*::LEU2	[10]
JN24	*Mat* a, *ade* 2-1, *his* 3-11,15, *trp* 1-1, *leu* 2-3,112, *ura* 3-1, can 1-100, *rip1*::LEU2, pFL39-RIP1 (TRP1, CEN, RIP1)	This study
JN26	*Mat* a, *ade* 2-1, *his* 3-11,15, *trp* 1-1, *leu* 2-3,112, *ura* 3-1, can 1-100, *rip1*::LEU2, pJN72 (TRP1, CEN, RIP1-Δ30)	This study
JN25	*Mat* a, *ade* 2-1, *his* 3-11,15, *trp* 1-1, *leu* 2-3,112, *ura* 3-1, can 1-100, *rip1*::LEU2, pFL39 (TRP1, CEN)	This study

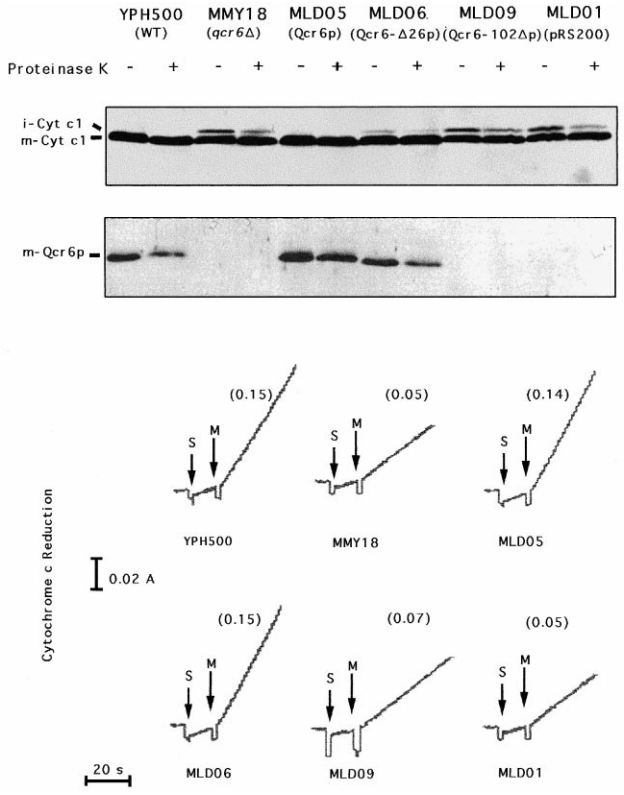

Fig. 2. Immunoblot analysis of Qcr6p and cytochrome c_1 and cytochrome c reductase activities of isolated mitochondria. Yeast strains, described in Fig. 1 and Table 1, were grown on selective media at 30°C with dextrose as a carbon source. Half of the mitochondria sample isolated from each strain was treated with proteinase K, after which 90 μg of mitochondrial proteins were used per lane for the immunoblot analysis. The top panel shows an immunoblot probed with the monoclonal antibody raised against cytochrome c_1. The migration positions of intermediate (i-Cyt c_1) and mature (m-Cyt c_1) cytochrome c_1 are indicated. The second panel shows the same immunoblot as the top panel, except stripped and probed with the polyclonal antibody raised against the GST-Qcr6 fusion protein. The migration position of mature Qcr6p (m-Qcr6p) is indicated. The tracings in the bottom panel show the ubiquinol-cytochrome c reductase activities of the same mitochondria samples as used for the immunoblots. The non-enzymatic rate of cytochrome c reduction was measured by the addition of 40 μM ubiquinol substrate (S), after which the enzymatic rate was initiated by addition of mitochondrial membranes (M). Numbers in parentheses alongside the cytochrome c reduction traces are rates of cytochrome c reduction, in μmoles/min/mg, which have been corrected for the non-enzymatic rate.

expressing the truncated Qcr6-Δ26p (Fig. 2). We then examined the amounts of mature sized Qcr6p and truncated forms of the protein in total cell lysates of yeast expressing the various forms of the protein. These Western blots (results not shown) confirmed that there is approximately one-half as much mature sized Qcr6p present in the cells of the strain expressing Qcr6-Δ26p, consistent with the lower levels of mature sized Qcr6p in the mitochondrial membranes.

The antibodies did not detect Qcr6-102Δp, either within or on the surface of the mitochondria (Fig. 2). However, the Western blots performed on the total cell lysate of this strain showed that a protein migrating at the expected position for the C-terminal truncated Qcr6-102Δp was present and the N-terminal presequence was not cleaved (results not shown). Since the affinity-purified antibodies do not cross-react with any other proteins in the total cell lysate, we conclude that the C-terminal truncated protein is expressed and present in the cytoplasm, but not imported into the mitochondria. From the intensity of staining in the Western blot and the appearance of diffuse protease degradation products, it appears that the C-terminal truncated Qcr6-102Δp is not as stable as the full length Qcr6p (results not shown).

Cytochrome c_1 is synthesized as a precursor that carries a 61 amino acid presequence that is cleaved in two steps during its import into mitochondria and assembly into the bc_1 complex [16]. Previously, we have shown that the second cleavage step, that processes cytochrome c_1 from an intermediate to mature size, is blocked coincident with the loss of Qcr6p [15]. The retardation of c_1 maturation and accumulation of intermediate sized cytochrome c_1 is evident in the mitochondrial membranes from the Δ*qcr6* deletion strain in Fig. 2.

The plasmid encoding Qcr6p corrects the defect in cytochrome c_1 processing, as evidenced by the absence of intermediate sized c_1. The plasmid encoding Qcr6-Δ26p partially corrects the defect in c_1 processing, since a small amount of intermediate sized c_1 is detectable in the mitochondrial membranes from that yeast strain (Fig. 2). The partial correction of the c_1 processing defect is consistent with the lower amounts of Qcr6p present in the mitochondria of the yeast strain expressing the protein without the N-terminus. Evidently, this extent of c_1 maturation is sufficient to fully restore the bc_1 activity, since the cytochrome c reductase activity of the mitochondrial membranes from MLD06 is identical to that of the membranes from YPH500 and MLD05. The expression of Qcr6-102Δp did not correct the defect in the maturation of cytochrome c_1. The amount of intermediate sized c_1 in mitochondrial membranes from MLD09 was identical to that in the deletion strain, MMY18 (Fig. 2).

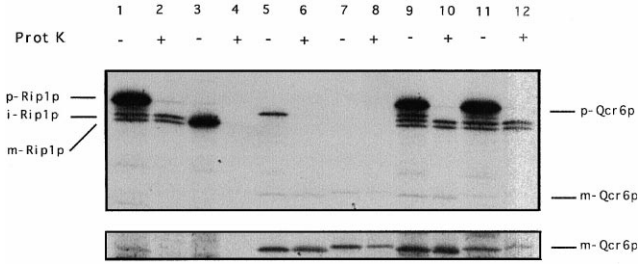

Fig. 3. Import of Qcr6p and Rip1p into mitochondria in vitro. The plasmids encoding Qcr6p, Rip1p and N-terminal truncated derivatives thereof (see Table 2) were transcribed and the RNA's were translated in vitro, after which the [^{35}S]cysteine-labeled translation products were incubated with mitochondria [12]. After import, half of each sample was treated with proteinase K. The migration positions of precursor, intermediate and mature Rip1p are indicated on the left and the migration positions of precursor and mature Qcr6p are indicated on the right. The samples are as follows: lanes 1 and 2, Rip1p; lanes 3 and 4, Rip1-Δ30p; lanes 5 and 6, Qcr6p; lanes 7 and 8, Qcr6-Δ26p; lanes 9 and 10, Qcr6p+Rip1p; lanes 11 and 12, Qcr6-Δ26p+Rip1p. In the last four lanes, equal radiochemically-labelled amounts of Qcr6p or Qcr6-Δ26p and Rip1p were mixed in the same tube, after which the samples were incubated with mitochondria under the conditions for in vitro protein import. In the top panel, the autoradiograph was exposed overnight and in the bottom panel, it was re-exposed for 6 days so that the radioactive bands corresponding to m-Qcr6p are visible.

Table 2

Transcription plasmid	In vivo expression plasmid	Encoding protein
pMLD05	pMLD13	Qcr6p
pMLD11	pMLD14	Qcr6-Δ26p
pMLD01	pMLD12	Qcr6-102Δp
pGEM3-RIP1	pFL39-RIP1	Rip1p
pJN73	pJN72	Rip1-Δ30p

3.4. *Qcr6p, with or without the presequence, is inefficiently imported into mitochondria in vitro*

Because the presequence of subunit 6 is atypical of mitochondrial targeting sequences, we were interested to know whether this protein was imported into mitochondria via the same pathway as other inner membrane proteins [17]. To study the import pathway of Qcr6p into mitochondria, we performed in vitro import experiments. The iron-sulfur protein of the bc_1 complex contains a presequence that is required for import of that protein into mitochondria. We used truncated forms of the iron-sulfur protein as controls to demonstrate the essential role of the presequence in the targeting and assembly of this protein into the bc_1 complex.

In spite of extensive attempts and numerous variations on the usual import protocols [12], we were unable to obtain efficient import of Qcr6p with or without the presequence into yeast mitochondria in vitro. Under conditions where the iron-sulfur protein is imported into mitochondria and becomes inaccessible to proteinase K (Fig. 3, lanes 1 and 2), it appears that the full length Qcr6p was not imported, as judged by the lack of mature sized protein (Fig. 3, lanes 5 and 6, top panel). However, if the autoradiograph is exposed for longer periods of time, it can be seen that a small amount of the mature sized fragment was formed when Qcr6p was incubated with mitochondria and this fragment was protected from proteinase K (Fig. 3, lanes 5 and 6, bottom panel). In contrast, the p-Qcr6p was completely digested with proteinase K. These results suggest that Qcr6p is imported into the intermembrane space and processed to mature size with a very low efficiency in vitro. To test whether import of Qcr6p requires additional factors that are absent from the reticulocyte lysate, we added yeast $100\,000 \times g$ supernatant to the reticulocyte lysate, but this did not result in Qcr6p import. We also tried co-translational import of Qcr6p from a homologous system containing yeast polysomes but were unable to improve the import of Qcr6p into yeast mitochondria. In all of these systems, iron-sulfur protein of the bc_1 complex was imported efficiently.

It appears that Qcr6-Δ26p lacking the presequence also did not bind to the mitochondria and therefore was not imported (Fig. 3, lanes 7 and 8, top panel). Again, however, if the autoradiograph is exposed for longer periods of time, it can be seen that a small amount of Qcr6-Δ26p bound to the mitochondria and a significant amount of this fragment was protected from proteinase K (Fig. 3, lanes 7 and 8, bottom panel). Qcr6-Δ26p contains an N-terminal methionine and for that reason migrates somewhat more slowly than mature sized Qcr6p (lanes 7 and 8 versus 5 and 6, bottom panel of Fig. 3).

From these results, we conclude that the Qcr6 protein, either with or without the presequence, is imported into mitochondria in vitro. However, the efficacy of import is too poor to use the in vitro import system to test the role of the C-terminus of the protein in import. From the amounts of labelled proteins that sediment with the mitochondria (Fig. 3, lanes 5 and 7, top panel), it is clear that Qcr6p with the presequence binds better to the outer mitochondrial membrane than does Qcr6-Δ26p. Most of the latter was stably retained and recovered in the supernatant after in vitro import and separation of the mitochondria.

Iron-sulfur protein in which the presequence was removed (lane 3) bound to the mitochondria but was not imported into a protease-protected space (lane 4). These results confirm that the presequence of the iron-sulfur protein is absolutely essential for import of this protein into mitochondria in vivo. When Qcr6p was incubated together with Rip1p, both proteins bound to the mitochondria (lane 9) but only the iron-sulfur protein was efficiently imported (lane 10). This result demonstrates that the mitochondria are import competent and that Qcr6p is not inhibitory to the import of iron-sulfur protein.

4. Discussion

From the results of our experiments, it is clear that the cleavable N-terminal extension is not essential for the targeting and import of subunit 6 (Qcr6p) of the bc_1 complex into mitochondria or for assembly of this subunit into the bc_1 complex. The presequence improves the efficacy of import of Qcr6p into mitochondria in vivo, but it is not essential for either of these processes. Our results also establish that information necessary for import of Qcr6p is located in the C-terminal region of the protein. Although we cannot rule out the possibility that the absence of Qcr6 protein in mitochondria might result from proteolysis after import, the C-terminal truncated protein is detectable in the cytoplasm.

We have not been able to establish whether the C-terminus itself is sufficient for import by the usual means of attaching this region to a passenger protein and examining import of the hybrid protein, since Qcr6p is imported very inefficiently into mitochondria in vitro. One possible explanation for the inefficient import is that there is no mechanism to concentrate the imported Qcr6p or fold it into its native conformation in an in vitro import system, since there is little, if any, ongoing assembly of the bc_1 complex. If the subunit cannot be assembled into the enzyme, the relatively small volume of the intermembrane space would limit the amount that can be taken up by the mitochondria.

Although our results establish that the presequence of Qcr6p is not required for the import of this protein into mitochondria, they leave the question unanswered why Qcr6p is translated with a presequence. The atypical acidic presequence that is present in this protein in yeast and mammals is absent in the homologous protein of potato mitochondria [18]. There are some mitochondrial and chloroplast proteins for which

the presequence is not necessary for their targeting but which plays a role in the specificity and efficiency of import, as well as in the correct localization or assembly of the protein into complexes. The yeast Qcr6 protein resembles Toc86p [19] and the mammalian phosphate carrier [20] in this regard, in that the presequence enhances import, but is not essential, and information essential for import and targeting is internal or at the C-terminus of the protein.

We also do not know how Qcr6p is imported into mitochondria, for example, whether it uses the Tim54p-dependent pathway for inserting membrane proteins, the Tim23p-dependent pathway for translocating precursors across the inner membrane into the matrix or another, yet uncharacterized, pathway [21,22]. These questions can only be addressed when it becomes possible to efficiently import Qcr6p into mitochondria in vitro.

Acknowledgements: This research was supported by National Institutes of Health Grant GM 20379.

References

[1] Brandt, U., Uribe, S., Schägger, H. and Trumpower, B.L. (1994) J. Biol. Chem. 269, 12947–12953.
[2] Zhang, Z., Huang, L., Shulmeister, V.M., Chi, Y.I., Kim, K.K., Hung, L.W., Crofts, A.R., Berry, E.A. and Kim, S.H. (1998) Nature 392, 677–684.
[3] Iwata, S., Lee, J.W., Okada, K., Lee, J.K., Iwata, M., Rasmussen, B., Link, T.A., Ramaswamy, S. and Jap, B.K. (1998) Science 281, 64–71.
[4] Ohta, S., Goto, K., Arai, H. and Kagawa, Y. (1987) FEBS Lett. 226, 171–175.
[5] Schmitt, M.E. and Trumpower, B.L. (1990) J. Biol. Chem. 265, 17005–17011.
[6] Hurt, E.C., Pesold-Hurt, B., Suda, K., Oppliger, W. and Shatz, G. (1985) EMBO J. 4, 2061–2068.
[7] Hartl, F.U., Pfanner, N., Nicholson, D.W. and Neupert, W. (1989) Biochim. Biophys. Acta 988, 1–45.
[8] Sikorski, R.S. and Hieter, P. (1989) Genetics 122, 19–27.
[9] Rothstein, R.J. (1983) Methods Enzymol. 101, 202–211.
[10] Beckmann, J.D., Ljungdahl, P. and Trumpower, B.L. (1989) J. Biol. Chem. 264, 3713–3722.
[11] Tron, T., Yang, M., Dick, F.A., Schmitt, M.E. and Trumpower, B.L. (1995) J. Biol.
[12] Nett, J.H. and Trumpower, B.L. (1996) J. Biol. Chem. 271, 26713–26716.
[13] Graham, L.A. and Trumpower, B.L. (1991) J. Biol. Chem. 266, 22485–22492.
[14] Trumpower, B.L. and Edwards, C.A. (1979) J. Biol. Chem. 254, 8697–8706.
[15] Yang, M. and Trumpower, B.L. (1994) J. Biol. Chem. 269, 1270–1275.
[16] Ohashi, A., Gibson, J., Gregor, I. and Schatz, G. (1982) J. Biol. Chem. 257, 13042–13047.
[17] Neupert, W. (1997) Annu. Rev. Biochem. 66, 863–917.
[18] Braun, H.P., Jansch, L., Kruft, V. and Schmitz, U.K. (1994) FEBS Lett. 347, 90–94.
[19] Muckel, E. and Soll, J. (1996) J. Biol. Chem. 266, 23846–23852.
[20] Zara, V., Palmieri, F., Mahlke, K. and Pfanner, N. (1992) J. Biol. Chem. 267, 12077–12081.
[21] Kerscher, O., Holder, J., Srinivasan, M., Leung, R.S. and Jensen, R.E. (1997) J. Cell. Biol. 139, 1663–1675.
[22] Sirrenberg, C., Bauer, M.F., Guiard, B., Neupert, W. and Brunner, M. (1996) Nature 384, 582–585.