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Minireview

Bifunctional role of the bc_1 complex in plants

Mitochondrial bc_1 complex catalyses both electron transport and protein processing

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Abstract

Nuclear encoded mitochondrial precursor proteins are cleaved to mature size products by the general mitochondrial processing peptidase (MPP). In contrast to non-plant sources where MPP is a matrix enzyme, the plant mitochondrial MPP is localised in the inner membrane and constitutes an integral part of the bc_1 complex of the respiratory chain. Core proteins of the complex are immunologically related and show high sequence similarity to the MPP subunits from non-plant sources. The bc_1 complex in plants is thus bifunctional, being involved both in respiration and in protein processing.

Key words: Mitochondrial processing peptidase; Protein processing; Protein import; bc_1 complex; Plant mitochondria; Organelle biogenesis

1. Introduction

Mitochondrial biogenesis requires the cooperation between the mitochondrial and nuclear genetic systems. Only a limited number of the mitochondrial proteins are encoded by the mitochondrial DNA and synthesised inside the organelle. Most of the proteins are nuclear encoded and synthesised in the cytosol as precursors with N-terminal extensions called presequences. The presequences are required for targeting and are proteolytically cleaved off inside mitochondria by a highly specific organellar processing system. This results in production of mature proteins, which are assembled in a process requiring organellar chaperones [1,2].

Most mitochondrial precursor proteins are cleaved to mature form in a single step by the mitochondrial general processing peptidase (MPP) [3], which is believed to be a metalloprotease. MPP has been purified from *Neurospora crassa* [4], *Saccharomyces cerevisiae* [5] and rat liver [6] and has been shown to consist of two structurally related proteins that cooperate in processing, α-MPP and β-MPP. Both subunits were shown to be matrix proteins, except for β-MPP from *N. crassa*, which is partially attached to the mitochondrial inner membrane. In *N. crassa*, β-MPP is identical to the Core 1 subunit of the bc_1 complex of the respiratory chain, indicating that this protein has a bifunctional role and is involved both in precursor processing and in electron transport [7]. In yeast and rat liver, β-MPP shows sequence similarity but not identity to the corresponding Core 1 proteins [7].

Recent studies of the plant mitochondrial processing system have demonstrated that in contrast to non-plant sources, the general processing activity is membrane-bound [8,9] and is totally integrated into the bc_1 complex of the respiratory chain [10–13]. This finding implies that the bc_1 complex in plants is bifunctional and is involved both in respiration and in protein processing. This finding opens several intriguing questions concerning interdependence of the oxidoreductase activity, protein processing and transport of proteins through the mitochondrial membrane. The aim of the present minireview is to give a brief summary of the present knowledge on the plant mitochondrial processing system.

2. Plant mitochondrial protein processing system

The first reports of in vitro plant mitochondrial protein import date from the late eighties. General characteristics of the system were shown to be analogous to fungi [14–16]. However, in contrast to sorting experiments using fungi mitochondria [18,19], studies of the homologous plant organelle system using isolated spin-

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ach leaf mitochondria and chloroplasts showed strict organellar specificity [15], in agreement with in vivo investigations using transgenic tobacco [17].

In vitro protein processing studies in plants were originally performed with total mitochondrial processing extract derived by extraction of mitochondria with Triton X-100 in the presence of salts [8,20,21]. The processing systems of *Vicia faba*, cauliflower [20], spinach leaf and potato mitochondria [8,21] were investigated using in vitro transcribed and translated precursors. Processing resulted in cleavage of the precursors to mature size products in a reaction which was inhibited by EDTA and orthophenanthroline. One of the components of the *V. faba* processing system was immunologically related to β-MPP from *N. crassa* [20]. Furthermore, the processing system was shown to be highly specific for mitochondrial precursor proteins and completely inert with chloroplast precursors [21].

2.1. MPP in plants is a membrane-bound enzyme

Fractionation of mitochondria from spinach leaves and potato tubers by sonication revealed that in contrast to fungi and mammals, the general processing peptidase of plant mitochondria was a membrane-bound enzyme [8,9]. The processing peptidase could not be dissociated from the membrane by high pH, high ionic strength, chaotropic reagents or weak detergent treatment, indicating that the enzyme is an integral membrane protein. The membrane-bound processing activity could not be stimulated by addition of matrix. In potato mitochondria fractionated with octylpolyoxyethylene [22] processing activity was also found only in the membrane fraction, however upon sonication in the presence of salts, part of the activity was found in the matrix [9]. Recent studies show that in spinach root mitochondria, the processing activity can be disassociated from the membrane by salt treatment [23].

2.2. MPP in plants constitutes an integral part of the bc_1 complex of the respiratory chain

Most interestingly, it has been found that the purified bc_1 complex of the respiratory chain of potato tuber mitochondria contained processing activity and that MPP constituted an integral part of the complex [10,12]. The potato bc_1 complex was purified from mitochondrial membranes monodispersed with Triton X-100 by affinity chromatography followed by gel filtration and ultrafiltration [10]. Another approach was used to isolate the general processing activity from spinach leaf mitochondria. Total processing activity was extracted from membranes with dodecyl-β-maltoside followed by FPLC anion-exchange and gel filtration chromatography. Here also, in photosynthetic tissue, total MPP was found in the bc_1 complex and was shown to be an integral part of the complex. No processing activity has been found in any other fractions [11,13].

2.3. Polypeptide composition of the integrated bc_1 / MPP complex

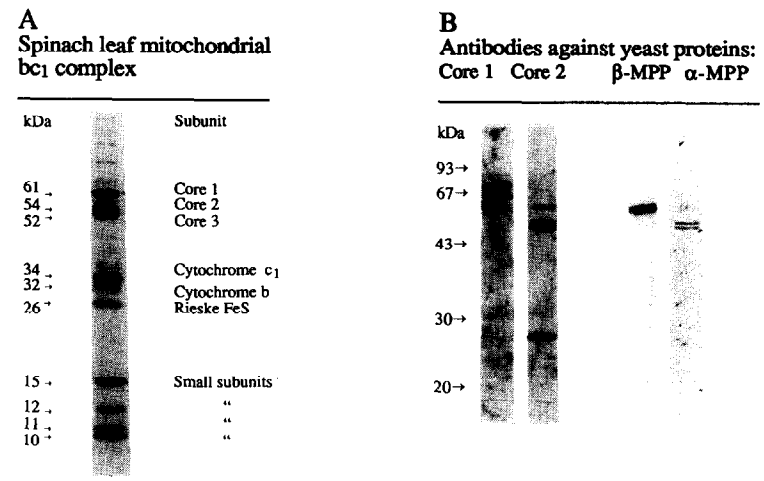
The bc_1 complex purified from both potato tubers and spinach leaves consists of ten protein bands [13,24,25], identified by immunological means [13,24] or sequence analysis [11, 12] as cytochromes b and c_1, FeS protein, Core proteins and low molecular mass subunits. Polypeptide composition and identification of bands with indication of the molecular mass of the spinach bc_1 complex is shown in Fig. 1A. In contrast to the bc_1 complex in fungi [26] and mammals [27], where only two Core proteins were found, the plant mitochondrial complex of spinach and potato appears to contain three Core proteins. Recent studies of wheat bc_1 complex report occurrence of four Core proteins [28]. The term Core protein has been traditionally used to describe high molecular mass components of the bc_1 complex. Suffixes 1, 2, 3 and 4 refer to decreasing molecular mass of these components. In spinach, Core 1 protein is immunologically related to yeast Core 1 protein and Core 2 and 3 proteins to yeast Core 2 protein [13], see Fig. 1B. In potato and wheat, Core 1 and 2 proteins show 70–81% sequence identity [12, 28] as also do Core 3 and 4 proteins in wheat. Generally there is 12–25% sequence similarity between plant Core proteins and Core proteins from other sources. An exception is the 49% sequence similarity of potato Core 1 and 2 proteins to *N. crassa* Core 1 protein, identical to β-MPP.

Taken together, these data suggest that in plant mitochondria components corresponding to Core proteins show moderate sequence similarity to non-plant Core proteins, they occur in different, multiple forms and the occurrence is organism and tissue specific.

2.4. Identification of the components of plant MPP

Subunits identified as Core proteins of the bc_1 complex in plants were shown to be immunologically related to MPP subunits from other sources [10,13,22]. Potato and wheat Core 1 and 2 proteins, and spinach Core 1 protein, corresponded to β-MPP, whereas spinach Core 2 and 3 proteins, potato Core 3 protein and wheat Core 3 and 4 proteins corresponded to α-MPP. Comparison of amino acid sequences of the potato and wheat Core proteins with sequences of MPP subunits from non-plant sources by Schmitz and coworkers [10,12] showed high homology between these proteins. Potato Core proteins 1 and 2 share 40–50% sequence identity with β-MPP of fungi and mammals [12], whereas Core 3 protein shows 30–35% identity with α-MPP [10]. Furthermore, a bipartite structure with a hydrophilic N-terminus and a hydrophobic C-terminus and hydrophobicity profiles of the potato Core proteins 1 and 2, as well as a highly conserved internal hydrophobic region of the Core 3 protein, supports identity of the Core proteins with MPP subunits.

Notably, plant Core proteins seem to be more closely



C

Processing with bc_1 complex	Intramitochondrial location of the precursor
pre- $F_1\beta$ → $F_1\beta$ →	extrinsic membrane protein
pre- FeS → FeS →	membrane protein facing intermembrane space
pre- MDH → MDH →	matrix protein

Fig. 1. Polypeptide composition (A) and immunological analysis (B) of the isolated spinach leaf mitochondrial bc_1 complex of the respiratory chain, using antibodies directed against *S. cerevisiae* Core 1 and Core 2 proteins of the bc_1 complex and against α- and β-MPP. C. Processing of precursor proteins of different intramitochondrial location with the isolated spinach leaf bc_1 complex. Left lanes show translation product alone; right lanes show processing of the translation product with the isolated spinach leaf bc_1 complex.

related to subunits of MPP than to Core proteins from non-plant sources. Moreover, comparison of the results in different plant species suggest species and/or tissue dependent differences in the occurence of these proteins within the bc_1 complex. However, both in spinach and in potato [12,13], attempts to separate MPP components from the complex have failed so far and therefore it still remains to be clarified if these subunits alone can support processing activity and if they have functions as Core proteins do in the bc_1 complex in other organisms.

2.5. *Properties of MPP in plants*

MPP in plants, although homologous to the soluble, matrix MPP from non-plant sources is completely integrated into an oligomeric protein complex with an additional function. What are the consequences of this integration?

As in non-plant sources, the enzyme has been shown to catalyse processing of precursor proteins of different intramitochondrial location [13,29], the $F_1\beta$ subunit of ATP synthase (extrinsic membrane protein), the Rieske FeS protein (intermembrane protein), adenine nucleotide translocator (integral membrane protein), and the malate dehydrogenase or Hsp-68 (matrix proteins), see Fig. 1C. The processing activity was not dependent on addition, but was stimulated by divalent cations Mn^{2+}, Zn^{2+} and Co^{2+}, Ca^{2+} and inhibited by the metal chelators, orthophenanthroline and EDTA, indicating that the enzyme is a metalloprotease. No effect on processing was seen with a variety of protease inhibitors such as PMSF, bestatin, leupeptin or pepstatin that are specific for Cys, Ser, or Asp proteases [9,29]. Consensus for processing is not characterised, but single mutation of −2 Arg to Gly in the presequence of soybean alternative oxidase (Whelan and Glaser, unpublished results) inhibits processing.

The plant enzyme also has several unique features. The enzyme is fully active at high pH (pH 9) [8,9,29], at high temperatures (50°C) [29] and at high salt concentrations (1.2 M NaCl) [12, 29]. Metal involved in catalysis is probably tightly bound. However, there is no classical zinc-binding motif (HEXXH), which led to classification of the enzyme to a new group of metalloendoproteases [29].

3. Interdependence of respiration and protein processing in plants

Integration of MPP into the bc_1 complex in plants

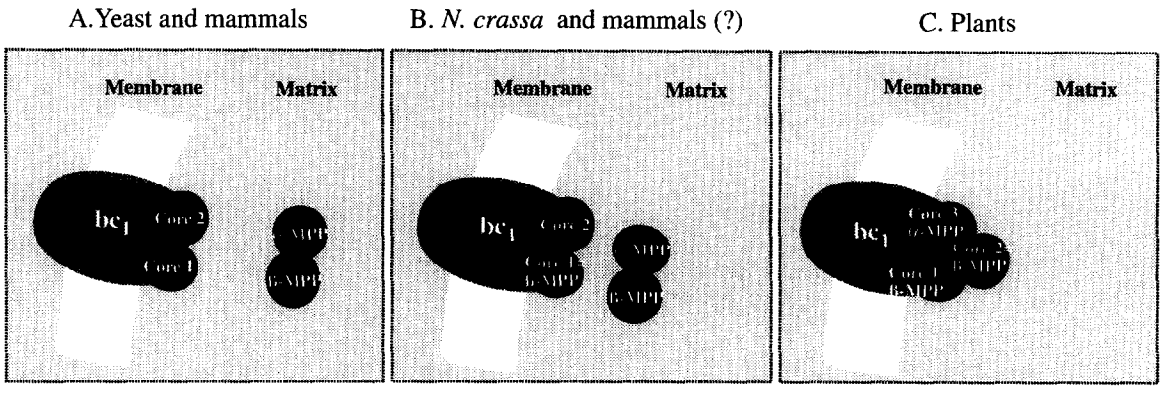


Fig. 2. Intramitochondrial occurrence of the Core proteins of the bc_1 complex and components of MPP in different sources. A illustrates studies with *S. cerevisiae* and rat; B, studies with *N. crassa* and bovine and C, studies with *S. tuberosum* but also refers to studies with *S. oleracea* and *T. aestivum*.

raises the question concerning interdependence of processing and respiration. We have investigated the interdependence using three approaches. We have studied inhibition of processing in the presence of inhibitors of the respiratory chain in the isolated bc_1 complex from spinach leaf mitochondria, in spinach submitochondrial particles under the control of membrane potential and in soybean and spinach leaf mitochondria upon in vitro import driven either by the alternative oxidase or by the ATPase, respectively [13, unpublished results]. Our studies point to the conclusion that the processing was not dependent on the redox state of the bc_1 complex or on respiration [13]. However, the processing activity of the isolated spinach leaf bc_1 complex and import driven by the alternative oxidase or by the ATPase was inhibited by antimycin A and myxothiazol [13]. Lack of sensitivity of processing to antimycin A and myxothiazol in the presence of Triton X-100 has been reported for the potato tuber bc_1 complex [29]. Although the titration curves of processing and respiration showed different profiles, the fact that an inhibitory effect on processing was seen in the isolated bc_1 complex indicates high affinity binding sites for the inhibitors within the complex [13].

4. Protein family of the Core proteins and MPPs

There is strong evidence that high molecular mass components of the bc_1 complex, traditionally called Core proteins, constitute subunits of MPP in plants [10–13]. In *N. crassa* [7] and possibly also in bovine [30] Core 1 protein of the bc_1 complex and β-MPP are identical. In *S. cerevisiae* and rat liver, β-MPP shows sequence similarity but not identity to the corresponding Core 1 proteins. Based on sequence similarity studies, the two proteins of the general processing enzyme, α-MPP and β-MPP and the Core proteins of the bc_1 complex have been grouped as a new family of proteins involved in both bioenergetics and biogenesis of the mitochondrion [7].

Core proteins belong to the group of subunits of the bc_1 complex without redox groups, they are not required for the electron transfer or proton translocation per se, but are required for activity, stability and assembly of the complex in eucaryots [31]. In *N. crassa*, bovine and potato it seems that at least one of the Core proteins may be substituted by a subunit of MPP. Results from sequence analysis suggest a two domain structure of Core 1 protein identical to β-MPP. The N-terminal domain is preferentially involved in the processing function whereas the C-terminal domain is involved in the Core function [32], which is consistent with the proposed bifunctional role of this protein.

5. Future perspectives

Present studies relate to an interesting evolutionary development of the mitochondrial processing peptidase. The occurrence of Core proteins and subunits of MPP in different organisms is illustrated in Fig. 2. For anaerobic growth of yeast it may be advantageous to express processing and respiration independently. On the other hand, in obligate aerobic organisms, integration of the biogenetic and respiratory events may be important for the regulatory processes.

Integration of the processing activity into the bc_1 complex opens intriguing questions concerning regulation of the biogenetic events in mitochondria. What is the topology of the complex in comparison to import sites? Is import of the precursors dependent on processing? Does the respiratory function of the complex influence import and processing? The physiological and biogenetic significance of the association of the respiratory function and the processing activity, as well as the regulation and mutual interdependence of the translocation of the precursor, processing and electron transfer in plants, are subjects of further research.

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