TALKING POINT

Puzzling subunits in mitochondrial cytochrome reductase

The ubiquinol–cytochrome *c* reductase complex, like the other proton-pumping respiratory complexes of mitochondria, is an assembly of many different subunits. However, only a few of these subunits participate directly in the electron transfer and proton translocation. The roles of the other subunits are largely unknown. We discuss here some intriguing features of two of these subunits.

CYTOCHROME REDUCTASE (also known as cytochrome bc_1 complex or complex III) is the second of three energy-transducing respiratory chain complexes of mitochondria. The function of the complex is to link the electron transfer from ubiquinol to cytochrome *c* with proton translocation across the inner membrane. In doing so a proton-motive force is generated which can subsequently drive ATP synthesis[1]. The actual mechanism of cytochrome reductase, which is best described by the ubiquinone cycle[2], involves only the three subunits of the enzyme which contain redox centres: the two heme-containing cytochromes, *b* and c_1, and the *Rieske*-iron–sulfur protein. However, in all organisms studied so far this mitochondrial enzyme is composed of many more subunits. Cytochrome reductase from fungi contains nine subunits, while the mammalian enzyme was found to have 11 subunits[3]. Since no mechanistic differences between the much simpler prokaryotic and the eukaryotic enzyme have yet been found, the role of the additional subunits in the mitochondrial enzyme remains mysterious. We discuss here the largest of these subunits, I and II (traditionally called core proteins) which contribute almost half of the total protein of the mitochondrial cytochrome reductase[4].

Structural properties

Electron microscopic studies of the three-dimensional structure of cytochrome reductase from *Neurospora crassa* suggest that subunits I and II are peripheral membrane proteins. When the enzyme was isolated using the detergent Triton and cleaved by salt treatment[5], two subcomplexes were obtained; a detergent-bound bc_1 subcomplex comprising the cytochromes *b* and c_1 and the four smallest subunits without redox centres, and a water-soluble subcomplex of the subunits I and II. Membrane crystals of the whole enzyme[6] and the bc_1 subcomplex[5] were prepared by incorporating the preparations into artificial phospholipid bilayers. Low resolution three-dimensional structures of the two proteins were determined from tilted electron-microscope views of the negatively stained membrane crystals (Fig. 1). Comparison of the structures clearly showed that the subcomplex of subunits I and II is located peripherally at the matrix side of the mitochondrial inner membrane[3,7]. The sequences of subunits I and II of

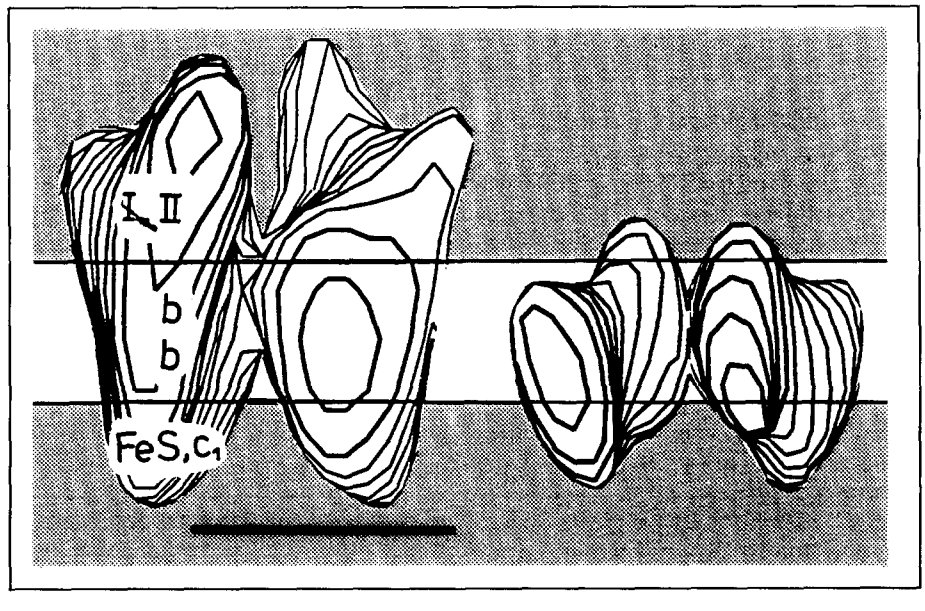

Figure 1

Side view parallel to the plane of the membrane of whole cytochrome reductase (left) and bc_1 subcomplex (right). Both preparations were obtained as dimers. The white section represents the membrane, and the upper and lower shaded areas the matrix space and the intermembrane space, respectively, of mitochondria. In the holoenzyme, cytochrome *b* with its two heme goups (b, M_r ~43 000) lies mainly in the membrane; cytochrome c_1 (c_1, M_r ~28 000) and the iron-sulfur protein (FeS, M_r ~22 000) protude into the intermembrane space, and the subcomplex of subunits I and II (M_r ~48 000 and ~44 000) is located peripherally on the matrix side of the membrane. The cytochrome bc_1 subcomplex comprises the cytochrome *b* and c_1 and the four smallest subunits without redox groups (M_r 16 000–9 000; not shown in the figure) and lacks the subcomplex of subunits I and II and the iron–sulfur protein. The scale bar represents 10 nm.

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the *S. cerevisiae* cytochrome reductase[8,9] and subunit I of the *N. crassa* enzyme[4] (see below) contain no hydrophobic stretches long enough to span the membrane and thus would also indicate that the two subunits could be peripheral proteins.

Speculation on the role of subunits I and II in electron transfer

For a long time no function could be attributed to subunits I and II. Their equivalents are not found in isolated cytochrome b_6f complex from chloroplasts, nor in bacterial *bc* complexes[10,11] although these complexes are closely related to the mitochondrial cytochrome reductase both functionally and structurally. This suggested that subunits I and II are not required for electron transfer and proton translocation *per se*. On the other hand, reconstitution experiments with isolated *N. crassa* cytochrome reductase indicated that the enzyme is inactive when it lacks subunits I and II[12]. Yeast mutants deficient in subunit II exhibit strongly reduced levels of enzymatically active cytochrome reductase[9,13] and mutants deficient in subunit I do not have any activity[8]. Furthermore, these mutants contain much less cytochrome *b* relative to cytochrome c_1. This led to the suggestion that the subunits are required for assembly of cytochrome reductase or maturation of cytochrome *b*. Alternatively, they might function as control subunits regulating the activity of the enzyme. All these ideas remain speculative, however, and await further experiments.

A new protein family

We have cloned and sequenced cDNA encoding subunit I of the *N. crassa* cytochrome reductase[4]. The deduced amino acid sequence (which was verified by partial protein sequences), is most surprisingly identical to the sequence of processing enhancing protein (PEP) which stimulates the activity of the matrix processing peptidase (MPP) in *N. crassa*[4,14]. In yeast, MPP and PEP are the products of the *Mas2* (or *Mif2*) and *Mas1* (or *Mif1*) genes[15–17]; however, yeast PEP is homologous but not identical to subunit I[16,17].

Alignment of the sequences of the yeast PEP, MPP, subunits I and II, and subunit I/PEP of *N. crassa* reveals significant similarities. Whenever one member of this family is compared to the other four members, *N. crassa* subunit I/PEP shows the highest degree of sequence identity (namely 51%) to yeast PEP, 26% to *N. crassa* MPP and 23% to yeast MPP[4].

Functional identity of subunit I and PEP in *N. crassa*

The ability of whole cytochrome reductase and isolated subunits I and II has been compared with that of PEP itself to stimulate the processing activity[4,14]. For the assay, a PEP-free MPP preparation was used to process the precursor of the β-subunit of ATP-synthase. MPP alone showed very low activity which was stimulated by either isolated PEP, isolated subunit I or whole cytochrome reductase, all having similar specific enhancing activity. Isolated subunit II was inactive[4] (Fig. 2).

The PEP function of the *N. crassa* subunit I may be located on a hydrophilic N-terminal domain since this domain shows a much higher similarity to the N-terminal half of yeast PEP (65%) than to the N-terminal half of yeast subunit I (23%). A more hydrophobic C-terminal domain might contact subunit II and contribute to the respiratory function. Compared to the N-terminal domain this domain has lower similarity to the C-terminal half of yeast PEP (43%) and a higher one to the C-terminal half of yeast subunit I (40%)[4].

New questions arise

Why should proteins with such different functions belong to a single family? So far the roles of the various members of this protein family are poorly understood. It seems that subunits I and II function by interacting with other subunits of the cytochrome reductase and thereby contribute to the assembly and stabilization of the complex. MPP has a proteolytic activity, and PEP is required for this reaction by interacting with MPP and/or the precursor proteins. The common denominator with all these functions might be that protein–protein interaction must occur. Thus, one essential function of all the members of the family may be to make contact to certain active epitopes on other proteins and thereby facilitate an ordered sequence of reactions.

However, these proteins may have other functions. For instance, subunits I and II might function as a control unit, sensing what is going on inside the mitochondria. Differential affects of the membrane potential on redox activity and/or H^+/e^- stoichiometry of cytochrome reductase might imply that the reaction pathway in the enzyme could

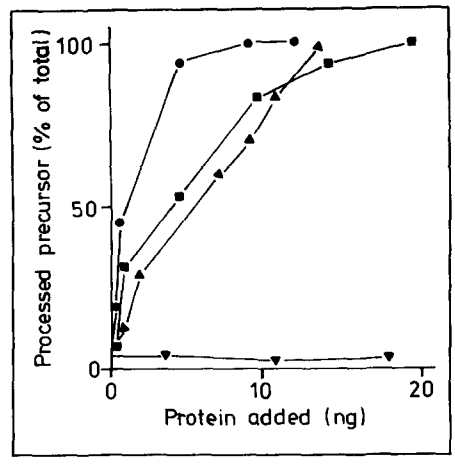

Figure 2
Stimulation of the activity of mitochondrial processing peptidase (MPP) by the processing enhancing protein (PEP), subunit I, subunit II, and whole cytochrome reductase. (▲) PEP, (●) cytochrome reductase, (■) subunit I, (▼) subunit II.

be affected[19]. PEP in yeast may have a role in translocation, in addition to enhancing the processing of precursor proteins. In PEP-deficient mutants the rate of the protein import was found to be reduced (F-U. Hartl, unpublished). It will be interesting to learn whether there are other organisms in which subunit I and PEP are identical. Recently the MPP from rat liver has been purified and described as a tight complex of two components[20]. Sequencing of this protein family in mammals might provide clues to their function(s). The existence of two different genes in yeast for subunit I and PEP may be related to the exceptional ability of this fungus to repress respiration under aerobic conditions. The promitochondria of fermenting cells lack the respiratory chain complexes, but still perform protein import and processing. Thus, requirement of PEP in repressed yeast might have led to divergence of PEP and subunit I.

More questions than answers

Could there be any function for PEP in *N. crassa* in association with cytochrome reductase? Membrane translocation of precursor proteins happens via contact sites between the outer and inner membranes, involves ATP-dependent unfolding of the preproteins and requires an electrical potential across the inner membrane. During or after translocation the presequences are cleaved off by the processing enzyme[18]. Does this imply that in *N. crassa* processing can only occur in vicinity of cytochrome reductase?

Although the puzzling findings on the structure and function of the subunits I and II of cytochrome reductase raise more questions than we can answer, they may contribute to an understanding of why the mitochondrial respiratory chain complexes contain so many additional subunits, which are not directly involved in energy transduction.

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LETTERS

Evolution of transport ATPases

In the recent discussion about the origins of transport ATPases[1–3], an essential physiological requirement has been ignored, i.e. cell-volume regulation[4]. Any cell surrounded by a semipermeable membrane and containing both permeable ions (e.g. H^+, Na^+) and impermeable ions (e.g. proteins) will swell infinitely unless: (1) there is a rigid cell wall that can sustain the elevated osmotic pressure resulting from the Donnan equilibrium distribution of ions; or (2) the membrane is impermeable to water; or (3) ion pumps (such as active transport ATPases) prevent the Donnan distribution of ions. Obvious functional problems for primitive cells result from solutions (1) and (2), which are, moreover, difficult to attain absolutely. For solution (3) an efficient approach to maintaining a non-equilibrium steady-state asymmetry of permeable ions is for the pump to match the leak[5]. This is most easily achieved if the major ion leaking in is the major ion pumped out[4]. Consequently, if the primitive cells arose in acidic environments (caused either by inorganic[2] or biological[3] processes), then a primordial H^+-ATPase could solve the problem of cell-volume maintenance; alternatively, if the primitive cells arose in saline environments, then a primordial Na^+-ATPase would be expected[4,6].

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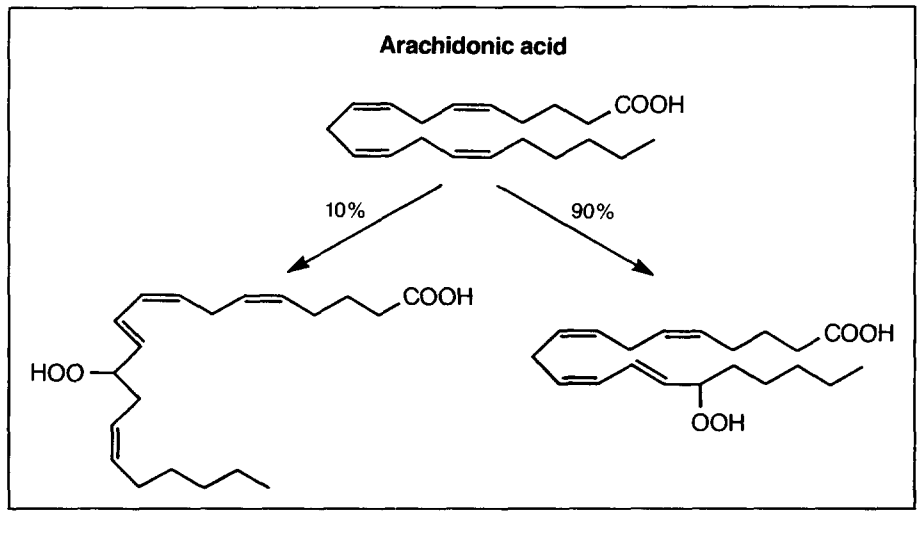
Lipoxgenase specificity

We read with great interest Tim Hunt's recent article on lipoxygenase and the maturation of reticulocytes (*TIBS* 14, 393–394). However, Fig. 1 of the article was misleading, since it showed only the formation of 12-hydroxyeicosatetraenoic acid (12-HETE) from arachidonic acid. In fact, lipoxygenase exhibits dual positional specificity; the major product formed from arachidonic acid is 15-HETE (see Figure), with 12-HETE formed in a side reaction.



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