Research News

A new dawn for plant mitochondrial NAD(P)H dehydrogenases

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The expression of complex I and two homologues of bacterial and yeast NADH dehydrogenases, NDA and NDB, have been studied in potato leaf mitochondria. The mRNA level of NDA is completely light dependent and shows a diurnal rhythm with a sharp maximum just after dawn. NDA protein quantity and internal rotenone-insensitive NADH dehydrogenase activity are also light dependent. These findings suggest that NDA has a role in photorespiration and might be identical to the previously unidentified internal rotenone-insensitive NADH dehydrogenase.

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Interactions between mitochondrial respiration and photosynthesis, notably in photorespiration, have long been recognized [1,2]. Recently, Staffan Svensson and Allan Rasmusson [3] reported a new and exciting development in this area – that the expression of respiratory chain components, not found in mammalian mitochondria, is light regulated.

The respiratory chain of plant mitochondria contains the standard four enzyme complexes (I–IV), which are present in virtually all mitochondria, and five additional enzymes. None of these additional enzymes pumps protons, making them energy wasteful. One of them is the alternative oxidase, the function of which has become much clearer in the past few years [4,5]. The other four are NADH and NADPH dehydrogenases, which are all insensitive to rotenone, the diagnostic inhibitor of the standard mitochondrial NADH dehydrogenase (also called complex I). Two of these additional NAD(P)H dehydrogenases are found on the outer surface of the inner mitochondrial membrane facing the intermembrane space (and thus the cytosol) and two on the inner surface facing the matrix like the standard mitochondrial NADH dehydrogenase (Fig. 1). The two external enzymes can oxidize cytosolic NAD(P)H, possibly mainly under stress conditions where they are activated by the increased cytosolic concentration of free Ca^{2+} [6–8]. The function of the two internal enzymes is not known, although it has been hypothesized that internal, rotenone-insensitive NADH dehydrogenase [NDin(NADH)] acts as an overflow mechanism when complex I is overburdened [9].

We have only recently begun to understand the molecular biology of the plant-specific NAD(P)H dehydrogenases. Two years ago, Rasmusson and co-workers [10] identified two genes encoding proteins homologous to bacterial NADH dehydrogenases. One, NDA, resides inside the inner membrane, but there is still no direct evidence that it is an NADH dehydrogenase. The other protein, NDB, contains a Ca^{2+}-binding motif and is located on the outer surface of the inner membrane. NDB is similar to a *Neurospora crassa* enzyme. The *N. crassa* enzyme was recently shown to be an external, rotenone-insensitive NADH dehydrogenase [NDex(NADPH)] [11,12], indicating that NDB might be an NADPH-specific enzyme.

A new dawn

Svensson and Rasmusson [3] reported that expression of NDA in potato leaves is completely light dependent in that the amount of mRNA decreases to ~0.1% (close to the level of detection) during incubation of intact plants in constant darkness for four days. When the dark-incubated plants are exposed to light, the mRNA levels in the leaves increases 600-fold in 12 h. Under normal day–night (16–8 h) conditions, the amount of mRNA has a diurnal cycle with a sharp peak of mRNA content just after

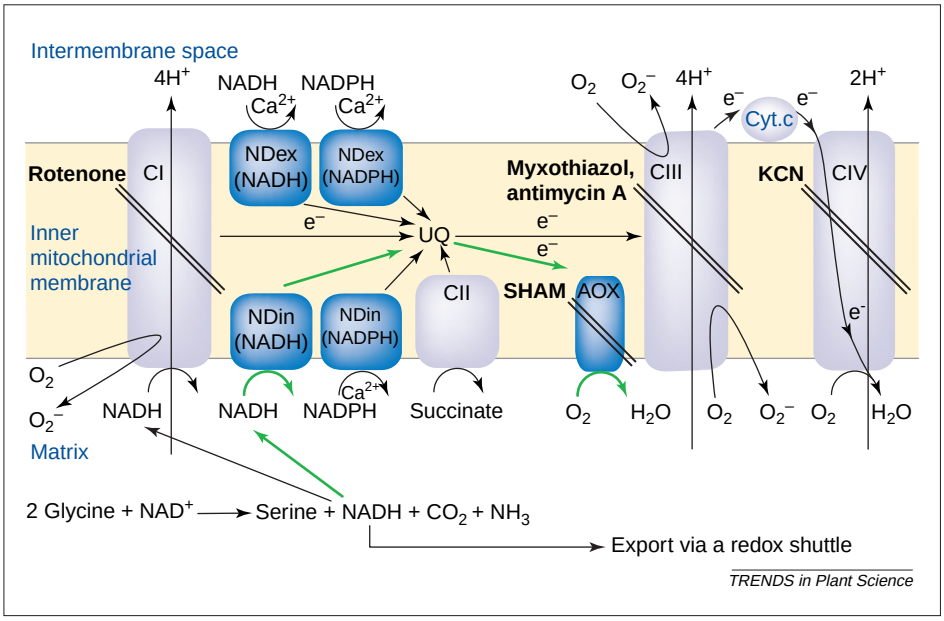

Fig. 1. Involvement of the electron transport chain of plant mitochondria in photorespiration. Plant-specific enzymes are shown in bright blue. H^+-pumping of complexes I, III and IV (per $2e^-$), inhibitors of complexes I, III and IV and reactive oxygen species production at the two main sites, complexes I and III, are also indicated. Because UQ is bound to two sites in complex III, one close to the inner surface of the inner mitochondrial membrane, the other close to the outer surface [19], reactive oxygen species might be formed on either side of the membrane. The non-phosphorylating pathway involving NDin(NADH) and the alternative pathway is indicated with green arrows. Abbreviations: AOX, alternative oxidase; CI–CIV, respiratory complexes; CI, complex I (NADH dehydrogenase); NDex(NADH), external, rotenone-insensitive NADH dehydrogenase; NDin(NADH), internal, rotenone-insensitive NADH dehydrogenase; CII, succinate dehydrogenase; CIII, cytochrome bc_1 complex; CIV, cytochrome c oxidase; cyt., cytochrome; KCN, potassium cyanide; SHAM, salicylhydroxamic acid; UQ, ubiquinone. (Modified from Ref. [7])

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dawn and the lowest level late in the day. This is similar to the expression pattern of photorespiratory enzymes, such as serine hydroxymethyltransferase (e.g. [13]) and catalase [14] in *Arabidopsis*. By comparison, the expression of NDB and two complex I genes, one mitochondrially encoded and the other nuclear-encoded, showed little or no change in potato leaves. Furthermore, the amount of NDA protein, as detected by antibodies raised against NDA, decreased by a factor of five when the plants were incubated in constant darkness for four days. Finally, the activity of NDin(NADH) decreased by 40% in constant darkness [3]. The results are consistent with an involvement of NDA in photorespiration and suggest that NDA is identical to NDin(NADH).

Identifying the affected mitochondrial NAD(P)H dehydrogenase activity is not easy

An important point made by Svensson and Rasmusson [3] that is often missed in other studies is the way activity of the NAD(P)H dehydrogenases is measured in intact plant mitochondria. Detergents should not be used to solubilize membranes because activity can then only be measured with ubiquinone or ubiquinone analogues as electron acceptors. This should be avoided because then the Ca^{2+} dependence and the rotenone-sensitivity of the enzymes disappear [15,16], making it impossible to distinguish between different dehydrogenases. In spite of these limitations, the external NADH and NADPH dehydrogenases are straightforward to measure in intact mitochondria with NADH and NADPH as electron donors, respectively, and oxygen as the electron acceptor.

The problem lies with the internal dehydrogenases. Measuring NDin(NADH) activity as rotenone-insensitive malate oxidation, which is commonly used, will not measure the full capacity unless NAD^+ is included in the assay medium, and even then the results are not simple to interpret because of the complexity of the reactions monitored; and NDin(NADPH) cannot be estimated in this way. A better method is to isolate inside-out submitochondrial particles [17], but this is difficult to do quantitatively from a small amount of leaf material. In addition, enzyme activity can be lost because of either inactivation or detachment of the enzyme(s) from the membrane.

Svensson and Rasmusson [3] solved the problem by rupturing the inner membrane in a hypo-osmolar medium in the presence of a Ca^{2+} chelator to inhibit NDex(NADH), which is Ca^{2+}-dependent [6], whereas neither complex I nor NDin(NADH) are affected by it [9,17]. This treatment made the inner membrane leaky to NADH, as judged by the partial rotenone inhibition of NADH oxidation (indicative that NADH reaches the active site of complex I on the inner surface of the inner membrane) and by the greatly increased malate dehydrogenase activity in the absence of detergents (indicative that NADH reaches the enzyme in the matrix inside the inner membrane). Under these conditions, NDin(NADH) activity (rotenone-insensitive NADH oxidation) and complex I activity (total minus rotenone-insensitive NADH oxidation) could be quantified reliably in mitochondria from the variously treated leaves.

These measurements showed that a decrease in NDin(NADH) activity in total darkness correlated with a decreased NDA mRNA level and a decreased amount of immunodetectable NDA, suggesting that NDin(NADH) is the NDA gene product. However, more direct proof is required.

Perspectives

During photorespiration, a massive flow of carbon passes through the glycine decarboxylase complex in the mitochondrial matrix, producing large amounts of NADH. Although as much as half is thought to be exported via redox shuttles [1], the other half needs to be reoxidized under conditions where ATP production is not at a premium. Under these conditions the upregulation of the non-proton-pumping NDin(NADH) would make excellent sense.

Ribas-Carbo and co-workers [18] reported that alternative oxidase activity was induced in soybean cotyledons during greening and that extended dark treatment of the green cotyledons led to a marked decrease in activity. This activity decrease seemed to be the result of a post-translational modification because the amount of alternative oxidase protein was constant. In this connection, it is interesting that Svensson and Rasmusson [3] report that immunodetectable alternative oxidase disappeared completely from potato leaf samples after four days in constant darkness. It is therefore possible that expression of the alternative oxidase in potato leaves follows a pattern similar to NDA and the photorespiratory enzymes. This would provide the mitochondria with a pathway for reoxidation of matrix NADH – from NDin(NADH) via ubiquinone to the alternative oxidase and oxygen (Fig. 1) – where no protons are pumped because all three sites of proton pumping are bypassed (Fig. 1). In this way, over-reduction, and the accompanying production of reactive oxygen species at complexes I and III, is minimized.

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