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Different respiratory-defective phenotypes of *Neurospora crassa* and *Saccharomyces cerevisiae* after inactivation of the gene encoding the mitochondrial acyl carrier protein

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Abstract The nuclear genes (*acp-1*, *ACP1*) encoding the mitochondrial acyl carrier protein were disrupted in *Neurospora crassa* and *Saccharomyces cerevisiae*. In *N. crassa* acp-1 is a peripheral subunit of the respiratory NADH: ubiquinone oxidoreductase (complex I). *S. cerevisiae* lacks complex I and its ACP1 appears to be located in the mitochondrial matrix. The loss of acp-1 in *N. crassa* causes two biochemical lesions. Firstly, the peripheral part of complex I is not assembled, and the membrane part is not properly assembled. The respiratory ubiquinol: cytochrome *c* oxidoreductase (complex III) and cytochrome *c* oxidase (complex IV) are made in normal amounts. Secondly, the lysophospholipid content of mitochondrial membranes is increased four-fold. In *S. cerevisiae*, the loss of ACP1 leads to a pleiotropic respiratory deficient phenotype.

Key words Acyl carrier protein · NADH: ubiquinone oxidoreductase · PET gene · Fatty acid synthesis

Introduction

The mechanism of fatty acid synthesis is the same in all organisms, whereas the enzymes involved are organized in different structural forms. In the cytoplasm of vertebrates a type-I fatty acid synthase (FAS) is present, which consists of one large multifunctional polypeptide in which different domains carry the catalytic activities (Smith 1994). The type-I FAS of fungi consists of two polypeptides carrying five and three functional domains, respectively (Köttig et al. 1991). In contrast, in bacteria (and in chloroplasts of plants) fatty acids are synthesized by distinct enzymes which together are called FAS type II. The growing acyl chain is attached via a phosphopantetheine group to an acyl carrier protein (ACP), which is a central domain in the FAS type I, whereas the ACP of the FAS type II is a distinct small polypeptide (Slabas and Fawcett 1992; Magnuson et al. 1993). Bacterial ACPs are involved not only in fatty acid synthesis but also in the synthesis of polyketides (Hopwood and Sherman 1990) and peptide antibiotics (Lipmann 1980), in the acylation of toxins (Issartel et al. 1991), and in the signalling for the nodulation of legumes by rhizobia (Göttfert 1993).

Recently, an ACP which is closely related to the ACP of the bacterial FAS type II has been found in the mitochondria of *Neurospora crassa* (Brody and Mikolajczyk 1988; Sackmann et al. 1991), bovine heart (Runswick et al. 1991) and *Arabidopsis thaliana* (Shintani and Ohlrogge 1994). The gene for a mitochondrial ACP (*ACP1*) has also been located on chromosome XI of *Saccharomyces cerevisiae* (Chéret et al. 1993). In *N. crassa* and bovine heart the mitochondrial ACP is tightly associated with the peripheral part of complex I, while *S. cerevisiae* does not possess a complex I (de Vries and Grivell 1988). In yeast a nuclear gene, *CEM1*, has been identified which encodes a protein homologous to the β-ketoacyl ACP synthases (condensing enzymes of the FAS type II). Inactivation of this gene leads to a respiratory deficient mutant (Harington et al. 1993). An extragenic repressor able to restore respiration in the mutant was found. The suppressor is predicted to encode a cytoplasmic long-chain fatty acid CoA ligase that has aquired a mitochondrial import sequence by a point mutation. The relocation of some of the enzyme from the cytosol to the mitochondrion results in its suppressor activity (Harington et al. 1994).

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There is experimental evidence that the mitochondrial ACP might be involved in a *de novo* fatty acid synthesis. Mikolajczyk and Brody (1990) reported that, after incorporation of [^{14}C]-malonate in *N. crassa* mitochondria, labeled hexanoic, caprylic and myristic acid can be released from the mitochondrial ACP by alkaline hydrolysis. Zensen et al. (1992) analyzed the acyl groups bound to the *N. crassa* mitochondrial ACP by gas-liquid chromatography and found the saturated C_6 to C_{18} fatty acids, as well as oleic acid, to be present. Runswick et al. (1991) recorded a mass difference of 302 Da between the acylated and deacylated mitochondrial ACP of bovine heart by means of mass spectrometry. According to their data the predominant proportion of the ACP is esterified with only one definite substance.

Considering the well-established transport of long-chain fatty acids by the carnitine-translocase system from the cytosol into the mitochondria, the question arises why mitochondria synthesize their own fatty acids. The phospholipids of the mitochondrial inner membrane are imported from the endoplasmic reticulum and the outer membrane. Cardiolipin is synthesized at the mitochondrial inner membrane, but the acylated precursors are also imported (Daum 1985; Voelker 1991).

In order to understand the function of the mitochondrial ACP and the significance of its association with the respiratory complex I, we disrupted the *acp-1* and *ACP1* genes in *N. crassa* and *S. cerevisiae*, respectively. The *N. crassa acp-1* mutant is defective in the assembly of complex I while the other respiratory complexes are made in normal amounts. The mitochondria of the mutant contain an unusually high amount of lysophospholipids. The *S. cerevisiae Δacp1* mutant shows a pleiotropic respiratory defect lacking the respiratory complexes while having a normal pattern of mitochondrial phospholipids. Two possible roles of the mitochondrial ACP will be discussed.

Materials and methods

Isolation and disruption of the acp-1 gene in N. crassa. [α-^{32}P] labelled cDNA of acp-1 (formerly called ncnuo12) was used as a probe for secreening the genomic λJ1 library of *N. crassa* (Orbach et al. 1986; Sackman et al. 1991). A 7-kb *Eco*RI fragment containing the entire acp-1 gene and flanking sequences of 4.3 kb in a 5′-direction and 1.5 kb in a 3′-direction was isolated from a recombinant clone and inserted into pT7T3 19U (Pharmacia). A 1.6-kb fragment including the N-terminal and most of the coding region of the *acp-1* gene was deleted with *Apa*I and *Nco*I. A 2.0-kb *Apa*I/*Nco*I fragment from pCSN43 (Staben et al. 1989) containing the bacterial gene for hygromycin B phosphotransferase flanked by the *A. nidulans TrpC* promotor and terminator was inserted as a selection marker. A linear 7.5-kb *Sph*I/*Bgl*II fragment was used for transformation of *N. crassa*. Heterocaryotic transformants were selected for hygromycin B resistance and then crossed with the parental strain to obtain homocaryotic transformants. For Southern-blot analysis genomic DNA of transformants was isolated, digested with *Pst*I and probed with a 800-bp *Cla*I/*Xba*I fragment (see Fig. 1). Standard recombinant techniques were performed according to Sambrook et al. (1989).

Disruption of the ACP1 gene in S. cerevisiae. *S. cerevisiae* strain JRY-675 [*MAT***a**, *his4-519*, *Δleu2*, *ura3-52*] (Lisowsky 1993) was used for transformation. A 1889-bp DNA fragment with the *ACP1* gene and flanking DNA was amplified from genomic DNA by the polymerase chain reaction (PCR). The primers scacpn (5′-GCATGTACGTGTTCAGCAGCGACCTCG-3′) and scacpc (5′-GATGTGCTTGATCTTGTTAAACGACAG-3′) were used (see Fig. 2). For the construction of the deletion-insertion vector the PCR product was digested with *Spe*I and *Pst*I and inserted in *Xba*I/*Pst*I-cut pT7T3 18U to give pSCACP. A 448-bp *Sty*I fragment was deleted and the protruding ends were blunted. The *Sma*I/*Hpa*I fragment of the plasmid YEp351, containing the *LEU2* gene, was inserted (Hill et al. 1986) (Fig. 2). Transformation of *E. coli* (Hanahan 1983) and *S. cerevisiae* (Soni et al. 1993) was performed as described. The disruptant obtained was transformed with the *ACP1* gene as an *Eco*RI/*Bam*HI fragment of pSCACP inserted in *Eco*RI/*Bam*HI-cut YCp50 (a centromeric plasmid with a *URA3* marker, Rose et al. 1987). Complete or minimal media with the appropriate supplements containing glucose (2%), glycerol (3%) or galactose (3%) were as described (Sherman et al. 1986).

Labelling of N. crassa with [^{14}C] panthothenate. The double-mutant *N. crassa nuo49/pan-1* was constructed from strain *pan-1A* (Fungal Genetics Stock Center 4063) and strain *nuo49* (unpublished, see below) according to Davis and de Serres (1970). Labelling with [^{14}C]-pantothenate was performed as described (Zensen et al. 1992).

Determination of mitochondrial respiratory activities, cytochrome contents, subunit composition of complex I and iron-sulphur clusters. The following procedures are described elsewhere: large and small scale preparation of *N. crassa* mitochondria (Weiss et al. 1970; Nehls et al. 1992), isolation of *S. cerevisiae* mitochondria (Rickwood et al. 1990), determination of mitochondrial respiratory activity and cytochrome content (Weiss et al. 1970), analysis of complex-I subunits by sucrose gradient centrifugation and immunoprecipitation (Nehls et al. 1992; Fecke et al. 1994), determination of iron-sulphur clusters of complex I by means of electron paramagnetic resonance spectroscopy (Wang et al. 1991).

Analysis of mitochondrial phospholipids. Mitochondrial lipids were extracted by the method of Folch et al. (1957) using chloroform/methanol (2:1, v/v). Phospholipids were separated by thin-layer chromatography on silica-gel 60 plates (Merck), which were first developed in chloroform/methanol/25% ammonia (75:35:5, v/v) and then in chloroform/acetone/methanol/acetic acid/water (60:20:10:10:4, v/v). The lipids were visualized by iodine vapour and scraped out for phosphorous analysis (Rouser et al. 1966; McClare 1971). Phospholipids were also separated by high-performance liquid chromatography (Nissen and Kreysel 1983) using a prepacked Nucleosil 100 column (diameter of particles 7 μm, 250.4 mm) with a Nucleosil 50 pre-column (5 μm, 30.4 mm; Macherey and Nagel). Phospholipids were detected by their absorption at 203 nm. Samples of 20–40 μl of the lipid extract containing 2–4 mg/ml lipid were injected. A gradient from 12 to 75% acetonitrile/water (80:20, v/v) was applied for separation. The flow-rate was 1 ml/min. Quantitative analysis of phosphorous was performed as described above using samples dried under nitrogen. Phospholipid standards were purchased from Sigma.

Results

Isolation and disruption of the *acp-1* gene in *N. crassa*

A 7-kb fragment containing the *acp-1* gene was isolated from a genomic *N. crassa* library using the cDNA of acp-1 as a probe (Sackmann et al. 1991). To determine

the position of the coding regions, we sequenced 3.1 kb of this fragment. The *acp-1* gene contains three introns of 589, 219, and 156 bp. The length of the first intron is highly uncommon for filamentous fungi (Bruchez et al. 1993). The transformation vector designed to replace the *acp-1* gene in *N. crassa* is shown in Fig. 1. A 1.6-kb fragment, including the N-terminal and most of the coding region of the gene, was replaced by the bacterial gene for hygromycin B phosphotransferase flanked by the *A. nidulans TrpC* promoter and *TrpC* terminator. A linear 7.5-kb fragment was used for transformation. Heterocaryotic transformants were selected for hygromycin B resistance and then crossed with the parental strain to obtain homocaryotic mutants. The result of the gene disruption was investigated by Southern-blot analysis (data not shown). Genomic DNA was isolated, digested with *Pst*I and probed with a 800-bp *Cla*I/*Xba*I fragment. In mutants in which the *acp-1* gene was replaced by the defective copy, the 3.6-kb *Pst*I fragment of the wild-type is missing while a fragment of 3.0 kb length was found due to an additional *Pst*I site. For Western-blot analysis mitochondrial protein of the mutant and the wild-type was separated by SDS/PAGE, blotted and probed with an antiserum against the acp-1. No band corresponding to, acp-1 was found with the mutant, whereas the band was detected with the wild-type (data not shown).

Disruption of the *ACP1* gene in *S. cerevisiae*

The one-step procedure described by Rothstein (1991) was applied. The *ACP1* gene and flanking DNA regions were amplified by PCR and the product was used for vector construction (Fig. 2). The *ACP1* presequence and the N-terminal half of the mature protein were deleted. Leucine prototrophs were selected, genomic DNA was isolated, digested, and probed for replacement of the gene (data not shown). For reintroduction of the *ACP1* gene into in the mutant *Δacp1*, we inserted a *Bam*HI/*Eco*RI fragment from pSCACP (see Fig. 2) into *Bam*HI/*Eco*RI-cut YCp50. No respiratory competent transformants were produced because the mutant has become rho$^-$ or rho^0. The transformant was outcrossed to the wild-type and sporulated, the presence of the deletion of the *ACP1* gene was confirmed by PCR. As expected, the introduction of the wild-type *ACP1* gene present on a centromeric plasmid restores the wild-type phenotype.

Growth impediments, mitochondrial cytochrome contents and respiratory activity of the *ACP* mutants of *N. crassa* and *S. cerevisiae*

The *N. crassa acp-1* mutant grows at one-half the rate of the wild-type in minimal medium with 2% sucrose at 25 °C when inoculated with the same number of conidia. The mitochondrial cytochrome content of the mutant and its respiratory activity with pyruvate/malate and succinate were comparable to those of the wild-type (see Weiss et al. 1970). Pyruvate/malate respiration, however, was insensitive to piericidin A, a specific inhibitor of complex I, but

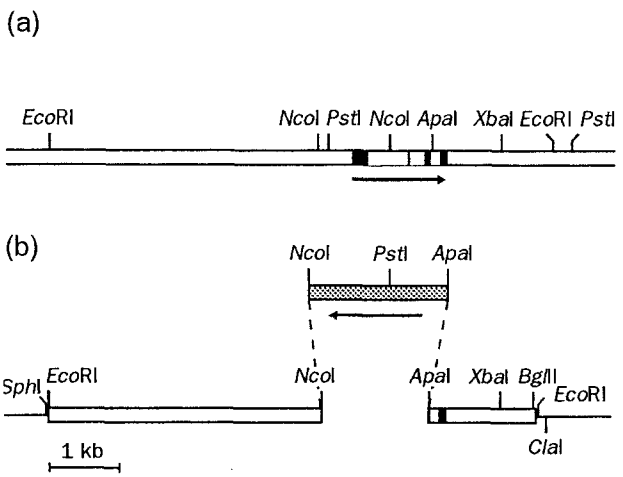

Fig. 1a, b Construction of the vector used for disruption of the *acp-1* gene in *N. crassa*. **a** a genomic 7-kb *Eco*RI fragment containing the *acp-1* gene with exons (*black*) and introns (*white*) and flanking sequences was isolated. **b** this fragment was inserted in pT7T3 19U. A 1.6-kb stretch of the gene was deleted by using *Apa*I and *Nco*I. A 2.0-kb *Apa*I/*Nco*I fragment of pCSN43 (*grey*) was inserted into the deletion gap. A linear *Sph*I/*Bgl*II fragment was used for transformation. The direction of transcription is indicated by *arrows*

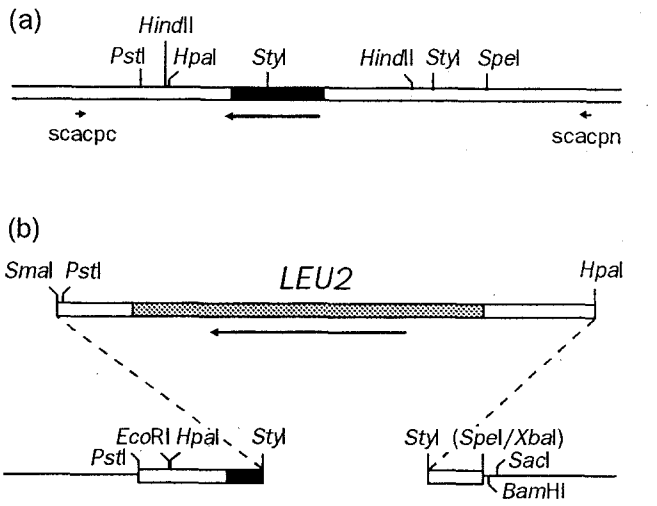

Fig. 2 Construction of the vector used for disruption of the *ACP1* gene in *S. cerevisiae*. **a** a 1.9-kb yeast DNA wild-type fragment (*white*) with the *ACP1* gene (*black*) was amplified by PCR. **b** a *Pst*I/*Spe*I fragment was cloned into pT7T3 18U to give pSCACP. A 448-bp *Sty*I fragment was deleted, and the protruding ends of the remaining plasmid were blunted. The *Sma*I/*Hpa*I fragment of plasmid YEp351 containing the *LEU2* gene was inserted. Transformation was carried out with a *Hpa*I/*Sac*I fragment. The direction of transcription is indicated by *arrows*

sensitive to antimycin A and KCN, inhibitors of the complexes III and IV. In the mutant, electrons are transferred from mitochondrial NADH by means of the alternative NADH:ubiquinone oxidoreductase, the ubiquinol:cytochrome *c* oxidoreductase (complex III) and the cytochrome *c* oxidase (complex IV). Thus the alteration in the respiratory chain of the *acp-1* mutant corresponds to the alteration found in other *N. crassa* complex I-mutants (Nehls et al. 1992; Fecke et al. 1994).

Disruption of the *ACP1* gene in *S. cerevisiae* resulted in the mutant *Δacp1* which is unable to grow on non-fermentable substrates. The mitochondrial cytochrome content of the mutant was decreased to undetectably low amounts (Fig. 3). *S. cerevisiae* thus responds to the loss of ACP1 with a pleiotropic respiratory deficient phenotype.

Disturbed assembly of complex I in the *acp-1* mutant of *N. crassa*

To investigate the assembly of complex I in the *acp-1* mutant of *N. crassa*, hyphae were radioactively labelled with [^{35}S]-methionine. Mitochondria were isolated, solubilized with Triton X-100 and subjected to sucrose-gradient centrifugation in the presence of Triton X-100. Radioactivity immunoprecipitated by means of an antiserum raised against the total complex-I protein, was measured across the gradient, as well as the NADH/ferricyanide reductase activity, an artificial electron transfer activity of the NADH dehydrogenase part of complex I (Fig. 4a). In the control experiment, performed with the *N. crassa* wild-type, the radioactive complex I protein and the NADH/ferricyanide reductase activity co-sedimented as a single peak between the third and the fourth quarter of the gradient (Fig. 4b; see also Nehls et al. 1992). With the *acp-1* mutant neither such a distinct radioactivity peak nor a peak of the NADH/ferricyanide reductase was found (Fig. 4a, c). SDS/PAGE of the immunoprecipitated protein followed by autoradiography did not show any of the peripheral subunits of complex I to be present. The membrane intrinsic subunits were found to sediment faster through the gradient than expected for a properly assembled membrane part of the complex (Fig. 4; see also Tuschen et al. 1990; Schmidt et al. 1991; Nehls et al. 1992; Schulte and Weiss 1995). We also searched for an iron-sulphur cluster in the *acp-1*

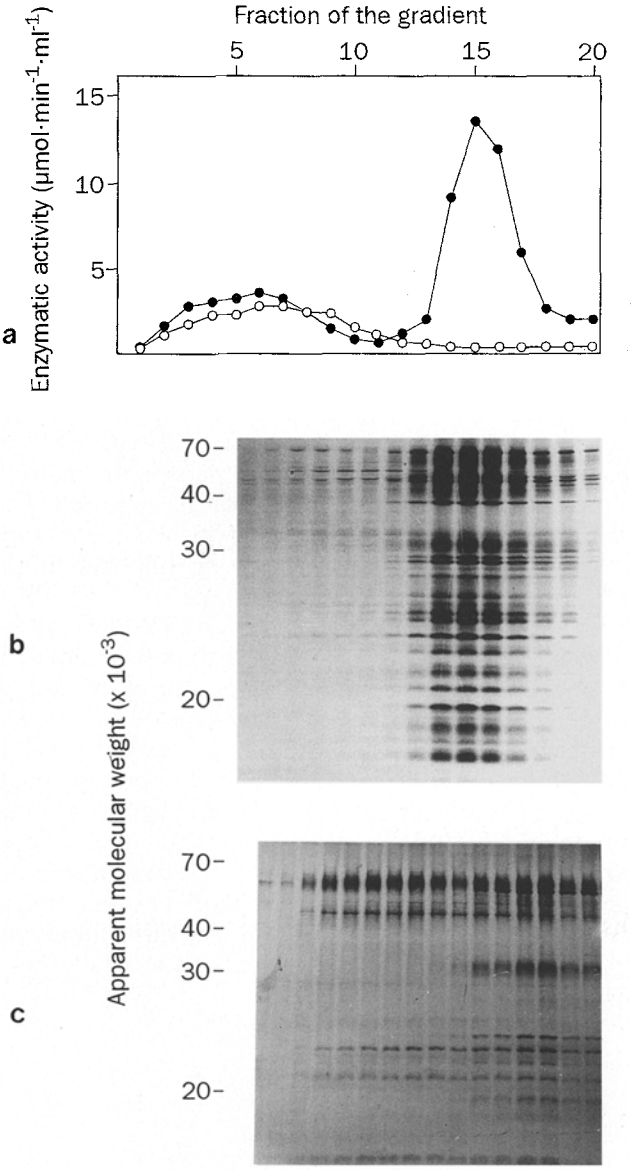

Fig. 4a–c Sucrose-gradient centrifugation of triton-solubilized mitochondria from the *N. crassa* wild-type and the *acp-1* mutant labelled with [^{35}S] methionine. **a** distribution of NADH/ferricyanide reductase activity across the gradient, (●) wild-type; (○) mutant *acp-1*; **b, c** radioactively labeled polypeptides immuoprecipitated by means of an antiserum raised against the entire complex I: **b** the wild-type and **c** the mutant *acp-1*

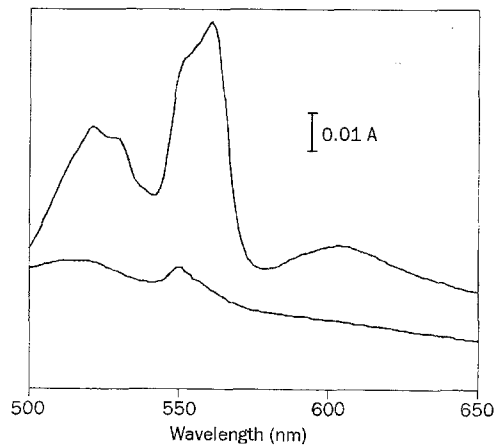

Fig. 3 Difference spectra of dithionite-reduced minus air-oxidized mitochondria of *S. cerevisiae* wild-type (upper curve) and mutant *Δacp1* (lower curve). The spectra were performed with a mitochondrial suspension containing 5 mg/ml of protein using a Perkin Elmer Lambda 2 spectrophotometer

mutant by electron paramagnetic resonance-spectroscopy of mitochondrial membranes. No signal of complex-I clusters could be found (data not shown; see also Wang et al. 1991; Nehls et al. 1992; Fecke et al. 1994).

Increased mitochondrial lysophospholipid contents in the *acp-1* mutant of *N. crassa* but normal content in the mutant *Δacp1* of *S. cerevisiae*

The phospholipid composition of mitochondrial membranes was analyzed by high-performance liquid chromatography and thin-layer chromatography (Fig. 5; Table 1). In the *N. crassa* wild-type and the *acp-1* mutant, all major classes of phospholipids were found to be present in nearly comparable amounts. The main difference between the wild-type and the mutant is the lysophospholipid content, being four-times higher in the mutant than in the wild-type.

To ensure that this high lysophospholipid content is caused by the loss of acp-1 rather than by complex I, we analyzed other complex-I mutants of *N. crassa* (Table 1). In the mutant *nuo51*, the gene of the 51-kDa peripheral subunit carrying binding sites for NADH, FMN and iron-sulphur cluster N-3 was disrupted. Complex I is almost completely assembled in this mutant but lacks the 51-kDa subunit and therefore is enzymatically inactive (Fecke et al. 1994). In mutant *nuo49*, the gene of the 49-kDa peripheral subunit is disrupted (for the primary structure of this subunit see Preis et al. 1990). The mutant lacks the whole peripheral part of complex I while the membrane part is assembled correctly together with iron-sulphur cluster N2 (unpublished data). In the mutant *nuo21.3*, the gene of the membrane-bound 21.3-kDa subunit is disrupted. The mutant is still capable of making the complete peripheral part of complex I together with acp-1 but cannot assemble the membrane part of the complex (Nehls et al. 1992). All these mutants have normal contents of lysophospholipids (Table 1). We also analysed the mitochondrial phospholipids in the *S. cerevisiae* wild-type and the mutant *Δacp1*. In the mutant the mitochondrial contents of cardiolipin and phosphatidylethanolamine are decreased and the contents of phosphatidylserine and phosphatidylinositol are increased. These alterations can also be observed in other respiratory deficient *pet* mutants (Jakovcic et al. 1971; Daum 1985). No increase in the mitochondrial lysophospholipid content was found (Table 1). The mutant *nuo49* cannot assemble the peripheral part of complex I, but still contains acp-1.

We generated the pantothenate auxotrophic mutant *nuo49/pan-1* which was then grown in the presence of [^{14}C]-pantothenate. Mitochondria were isolated, solubilized with Triton X-100 and centrifuged on a sucrose gradient as above. SDS/PAGE followed by autoradiography and immunoprecipitation of fractions of the gradient with an antiserum against the total complex I showed two radioactive protein bands corresponding to the acylated and de-acylated forms migrating with apparent molecular weights of 12 000 and 18 000, respectively (data not shown).

Table 1 Mitochondrial phospolipid composition of the *N. crassa* wild-type, the *acp-1* mutant and *N. crassa* mutants lacking other complex-I subunits, the *S. cerevisiae* wild-type and the *Δacp1* mutant. *N. c. N. crassa*; *S. c. S. cerevisiae*; *PC* phosphatidylcholine; *PE* phosphatidylethanolamine; *CL* cardiolipin; *PI* phosphatidylinositol; *PS* phosphatidylserine; *LPC* lysophosphatidylcholine; *LPE* lysophosphatidylethanolamine. The nomenclature of the mutants is explained in the text. The data are the average of six determinations both with thin-layer chromatography and high-performance liquid chromatography followed by phosphate determination

Strain	(Mol %)						
	PC	PE	PI	CL	PS	LPC	LPE
N. c. wild-type	43	30	8	7	3	2	1
N. c. nuo51 mutant	45	32	8	8	4	2	1
N. c. nuo21.3 mutant	46	31	8	8	4	2	1
N. c. nuo49 mutant	45	30	7	7	2	3	1
N. c. acp-1 mutant	45	28	8	5	3	8	4
S. c. wild-type	47	29	17	5	2	1	1
S. c. Δacp1 mutant	48	19	20	2	7	1	1

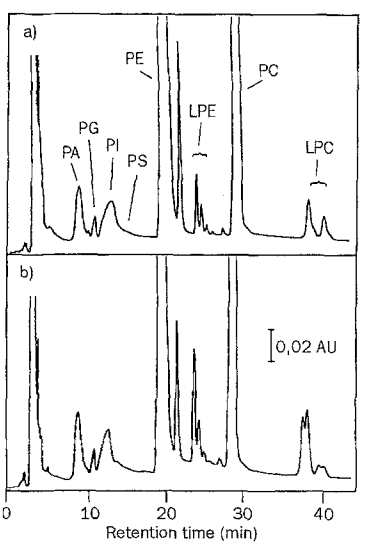

Fig. 5a, b Analysis of mitochondrial phospholipids from the *N. crassa* wild-type and the *acp-1* mutant by high-performance liquid chromatography. **a** wild-type and **b** *N. crassa* mutant *acp-1*. *PC* phosphatidylcholine; *PE* phosphatidylethanolamine; *PG* phosphatidylglycerol; *CL* cardiolipin; *PI* phosphatidylinositol; *PS* phosphatidylserine; *LPC* lysophosphatidylcholine; *LPE* lysophosphatidylethanolamine

Discussion

In this study we describe biochemical lesions caused by the loss of mitochondrial ACP in the obligate aerobic

fungus *N. crassa* and in the facultative anaerobic yeast *S. cerevisiae*. In *N. crassa* (as in bovine) acp-1 is a subunit of the respiratory complex I whereas *S. cerevisiae* does not possess complex I.

Complex I is a most complicated assembly of some 40 different subunits. They can be categorized into 14 "minimal" subunits, of which the homologues are also found in the simpler bacterial complex I, and the remaining "accessory" subunits, which are a characteristic feature of the mitochondrial complex I (Walker 1992; Friedrich et al. 1993; Weidner et al. 1993; Xu et al. 1993; Leif et al. 1995); acp-1 belongs to the "accessory" subunits. Complex-I subunits can also be subgrouped according to their topology and assembly. The mitochondrial complex has an L-shaped overall structure consisting of a peripheral and a membrane part (Hofhaus et al. 1991). The peripheral part contains the NADH dehydrogenase segment and the membrane part the ubiquinone reduction segment. These two parts are pre-assembled separately before they join to form the complex (Friedrich et al. 1989; Tuschen et al. 1990; Weiss et al. 1991; Nehls et al. 1992); acp-1 is located in the peripheral part (Sackmann et al. 1991).

So far we have disrupted the genes of four peripheral subunits in *N. crassa*: the 51-kDa (NADH-binding) subunit (Fecke et al. 1994), the 49-kDa subunit (unpublished result, for sequence see Preis et al. 1990), the 40-kDa subunit (unpublished result, for sequence see Röhlen et al. 1991) and the acp-1 (9.6-kDa) subunit. Duarte et al. (1995) have inactivated the gene of the 29.9-kDa peripheral-part subunit. Only the loss of the acp-1 subunit appears to affect the assembly of the membrane part of complex I. The loss of peripheral subunits other than acp-1 affects the formation of the peripheral part of complex I but leaves the membrane part intact. These observations suggest that acp-1 may participate in a synthetic pathway which delivers a product that is necessary for the assembly of the membrane part of complex I. This product must be a specific requirement for complex I because the other respiratory complexes are made in normal amounts in the *acp-1* mutant of *N. crassa*.

The second specific lesion caused in *N. crassa* by the loss of acp-1 is the four-fold increase in mitochondrial lysophospholipid content. Reactive oxygen species generated by the respiratory chain are known to lead to peroxidation of polyunsaturated fatty acids in membrane phospholipids. Fatty acyl hydroperoxides are then removed by a phospholipase yielding lysophospholipids (van Knijk et al. 1987; Sevanian 1991; van Ginkel and Sevanian 1994). Repair of the phospholipids by reacylation could occur in mitochondria, as in bacteria, by reacylation using ACP-bound fatty acids (Magnuson et al. 1993). If they are not available, as in the *acp-1* mutant, the lysophospholipids are further degraded and replaced by newly imported phospholipids. Such a mechanism could result in a higher level of lysophospholipids in the *acp-1* mutant of *N. crassa*.

In contrast to *N. crassa*, *S. cerevisiae* responds to the loss of the ACP1 with a pleiotropic respiratory deficient phenotype. *S. cerevisiae* is capable of satisfying its energy requirements by fermentation alone. Respiration is not essential for its viability. A large collection of *S. cerevisiae* strains have been described which lose their ability to utilize non-fermentable substrates as a result of mutations in nuclear DNA. These mutations concern genes that code for proteins which are either directly involved in oxidative phosphorylation or else are necessary for the expression of this activity (Tzagoloff and Dieckmann 1990). A direct involvement of ACP1 in oxidative phosphorylation or in the expression of this system, however, can be ruled out by the finding that the *N. crassa acp-1* mutant still contains functional respiratory complexes (and most likely also the ATPase-complex) since *N. crassa* depends on oxidative phosphorylation. A proton-impermeable phospholipid bilayer is essential for energy transduction via an electrochemical proton gradient across the mitochondrial inner membrane. Lipid peroxidation strongly reduces the molecular orientation of membrane phospholipids (van Ginkel and Sevanian 1994) and thus disturbs the respiratory energy conservation. If one function of ACP1 is to repair phospholipids damaged by oxidation, the *S. cerevisiae* mutant *Δacp1* might repress expression of the respiratory activity to prevent the oxidative injury of the membrane.

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