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Altered Mitochondrial Respiration in a Chromosomal Mutant of *Neurospora crassa*

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A mutant of *Neurospora crassa* (*cni*-1) has been isolated that has two pathways of mitochondrial respiration. One pathway is sensitive to cyanide and antimycin A, the other is sensitive only to salicyl hydroxamic acid. Respiration can proceed through either pathway and both pathways together in this mutant account for greater than 90% of all mitochondrial respiration. The *cni*-1 mutation segregates as a nuclear gene in crosses to other strains of *Neurospora*. Absorption spectra of isolated mitochondria from *cni*-1 show typical *b*- and *c*-type cytochromes but the absorption peaks corresponding to cytochrome aa_3 are not detectable. Extraction of soluble cytochrome *c*-546 from these mitochondria followed by reduction with ascorbate reveals a new absorption peak at 426 nm that is not present in wild-type mitochondria. This peak may be due to an altered cytochrome oxidase with abnormal spectral properties. Mitochondria from *cni*-1 have elevated levels of succinate-cytochrome *c* reductase but reduced levels of nicotinamide adenine dinucleotide reduced form cytochrome *c* reductase and of cyanide- and azide-sensitive cytochrome *c* oxidase. These studies suggest that the *cni*-1 mutation results in the abnormal assembly of cytochrome *c* oxidase so that the typical cytochrome aa_3 spectrum is lost and the enzyme activity is reduced. As a consequence of this alteration, a cyanide-insensitive respiratory pathway is elaborated by these mitochondria which may serve to stimulate adenosine 5'-triphosphate production via substrate level phosphorylation by glycolysis and the Krebs cycle.

We have recently described a method for selecting respiratory mutants of *Neurospora crassa* (4). Some of the mutants selected by this procedure have high levels of respiration that are insensitive to cyanide or antimycin A. In this regard, the respiration of these mutants is similar to that recently described in *poky*, an extrachromosomal mutant of *Neurospora* (10, 11). The cyanide-insensitive respiration of *poky* is inhibited by salicyl hydroxamic acid (SHAM), an inhibitor of cyanide-insensitive respiration in some species of higher plants (4, 10, 16). Cyanide-insensitive (SHAM sensitive) mitochondrial respiration can also be induced in wild-type strains of *Neurospora* by growth on medium containing chloramphenicol (10) or antimycin A.

One mutant that we isolated (*cni*-1) differed in its respiratory properties from *poky* or wild type grown on chloramphenicol in that it was insensitive to the addition of SHAM alone and was also insensitive to cyanide and antimycin A (4). In this paper we present further data on the respiratory properties of *cni*-1.

MATERIALS AND METHODS

Strains. An inositol-requiring strain (*inos*-89601) and a lysine auxotroph (*lys*-1) were obtained from the Fungal Genetics Stock Center, Arcata, Calif. Fungal Genetics Stock Center numbers of these strains are: *inos*-89601: FGSC no. 497, 498 *lys*-1: FGSC no. 74, 94. *inos*-89601 is referred to throughout the text as *inos*.

Growth of cells and respiration measurements. Cultures of *Neurospora* for these studies were grown at 30 C in a New Brunswick gyratory shaker on Vogel minimal medium containing 2% sucrose plus 50 μg of inositol per ml as described previously (4). Respiration measurements of both mycelium and isolated mitochondria were carried out at 30 C in a YSI biological oxygen monitor as described previously (4). Mitochondria for both respiratory and spectral measurements were isolated by the glusulase method of Greenawalt et al., and purified through two rounds of differential centrifugation (7). Mitochondria for respiration measurements were suspended in the respiration medium described by Hall and Greenawalt (8).

Mitochondria for spectral measurements were suspended in 0.5 M sucrose, 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride mM ethylenediaminetetraacetic acid, pH 7.5.

Cytochrome spectra. Difference spectra of mitochondrial preparations were determined at the temperature of liquid nitrogen in a Cary model 14 spectrophotometer equipped with a scattered transmission accessory. All spectra were measured in cells of 2-mm path length.

Enzyme assays. Cytochrome *c* oxidase was measured by the method of Wharton and Tzagoloff (19) by using 20 μM ferrocytochrome *c*.

Nicotinamide adenine dinucleotide reduced form (NADH)-cytochrome *c* reductase was measured by the method of Hatefi and Rieske (9) without addition of phospholipid. Succinate-cytochrome *c* reductase was measured by the method of Tisdale (17). All assays were carried out at room temperature.

Genetic crosses. Genetic crosses were carried out on slants of synthetic crossing medium as described by Davis and De Serres (2). Ascospores were germinated on 4% agar plates by heat shocking at 60 C for 45 min. Germinated spores were then transferred to slants of appropriate medium.

Miscellaneous. Salicyl hydroxamic acid was obtained from the Aldrich Chemical Co. Antimycin A was obtained from Sigma Chemical Co. Both inhibitors were dissolved in either absolute ethanol or dimethylformamide (16). Potassium cyanide (Baker) was prepared fresh for each experiment in aqueous solution at pH 6.9. Concentrations of all inhibitors were determined gravimetrically. Protein was measured by the method of Lowry et al. (14) by using bovine serum albumin as a standard.

RESULTS

Respiration of whole mycelium. Oxygen electrode traces of the respiration of mycelium from 24-h cultures of *cni*-1 are shown in Fig. 1. The addition of either KCN or SHAM alone has little effect on the respiration rate of the culture. When both inhibitors are present, however, the respiration of the culture is markedly inhibited.

Antimycin A also has little effect on the respiration of *cni*-1 unless SHAM is also present (Fig. 2). This is in marked contrast to the effects of cyanide and antimycin A on cultures of *inos*, the strain that *cni*-1 was derived from (4), or on wild-type strains of *Neurospora* (11). The addition of either millimolar cyanide or antimycin A to these strains inhibits respiration by more than 90%.

These observations indicate that *cni*-1 mitochondria have a branched election transfer chain similar to that recently described for *poky* (10, 11). The difference between the respiration of *cni*-1 and *poky* appears to be that in *cni*-1 either branch of the pathway is sufficient to support nearly all of the electron flux through the system, whereas in *poky* the SHAM-sensi-

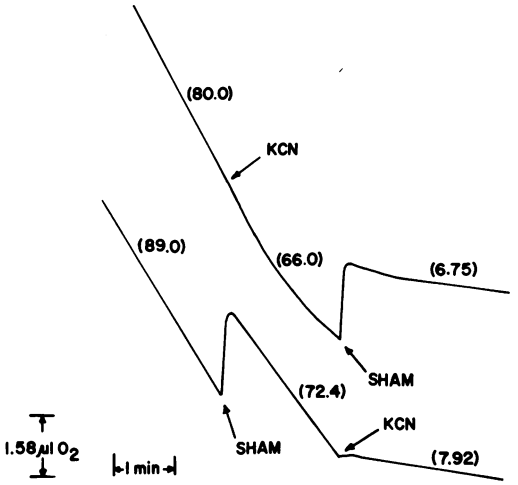

FIG. 1. *Oxygen electrode traces of 24-h cultures of cni-1. At the points indicated by arrows either potassium cyanide or SHAM was added to give final concentrations of mM or 120 µg/ml, respectively. The suspending medium is Vogel minimal medium plus 2% sucrose plus 50 µg of inositol per ml. Temperature is 30 C. Numbers in parenthesis are the rates of oxygen uptake per hour per milligram dry weight.*

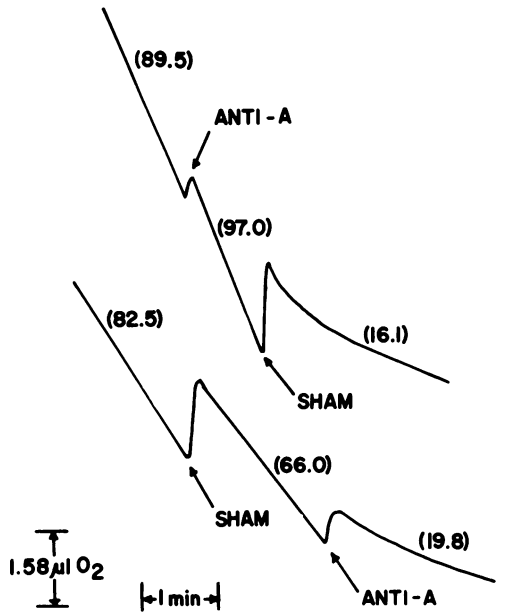

FIG. 2. *Effects of SHAM and antimycin A on the respiration of cultures of cni-1. The final concentration of antimycin A is 1 mM. Conditions are the same as in Fig. 1.*

tive pathway is sufficient to support the entire electron flux but the cytochrome chain is not (11). Thus, inhibitors of both pathways must be present before respiration of *cni*-1 mitochondria is inhibited, whereas SHAM alone will inhibit the respiration of *poky*.

It has been previously reported that the SHAM-sensitive pathway of mitochondrial respiration can be induced in wild-type strains of *Neurospora* by growth on medium containing chloramphenicol (10, 13). These conditions result in a reduction of cyanide-sensitivity and an increased sensitivity to SHAM. We have also observed this result with *inos* and found that growth of *cni*-1 in medium containing chloramphenicol results in a marked decrease in the cyanide-sensitive pathway in this mutant. Figure 3 shows that the respiration of chloramphenicol-treated *cni*-1 is now inhibited by 82% by SHAM alone, where previously SHAM alone inhibited respiration by only 19% (Fig. 1). The respiration of chloramphenicol-treated *inos* is now insensitive to KCN and is inhibited by 41% by SHAM where previously the respiration was inhibited by greater than 90% by KCN and was not affected by SHAM. These experiments also indicate that those components required for the SHAM-sensitive pathway are not the products of mitochondrial ribosomes.

Respiration of isolated mitochondria. Experiments carried out with isolated mitochondria indicate that the SHAM- and cyanide-sensitive pathways combined account for greater than 90% of all mitochondrial respiration in this

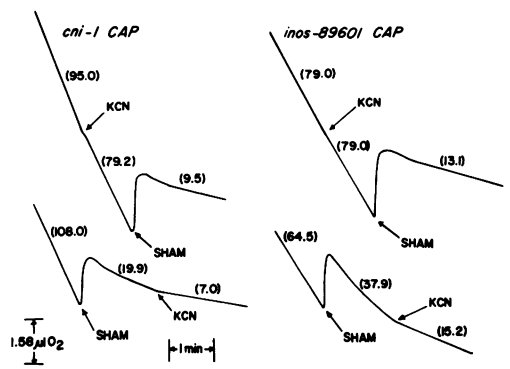

FIG. 3. *Effects of cyanide and SHAM on cultures of chloramphenicol-treated cni-1 and inos. Cells were grown for 8 h at 30 C on Vogel minimal medium plus 2% sucrose plus 50 µg of inositol per ml. Chloramphenicol was then added to give a final concentration of 2 mg per ml. The cultures were harvested after 16 h of growth with chloramphenicol and respiration measurements were made. Final concentrations of KCN and SHAM are 1 mM and 120 µg per ml, respectively.*

mutant. Titration of *cni*-1 mitochondria with KCN results in a maximal inhibition of respiration of 65% of the control value as compared with 93% inhibition of *inos* mitochondria (Fig. 4). If the titration is carried out in the presence of SHAM, respiration is inhibited by greater than 90%. Figure 5 shows the titration of *cni*-1 mitochondria with SHAM. In the absence of cyanide, SHAM inhibits respiration of these mitochondria by 51% of control values, whereas in the presence of cyanide SHAM inhibits respiration by greater than 90%. SHAM also has a slight inhibitory effect on *inos*-89601 mitochondria giving a maximal inhibition of 28% of control values.

The experiments presented here with isolated mitochondria are in marked contrast to the experiments carried out with whole mycelium. KCN or SHAM alone has a large inhibitory effect on the respiration of isolated *cni*-1 mitochondria, whereas they have little or no effect on the respiration of whole mycelium. One possible explanation for this anomaly is that both the alternate oxidase and the cytochrome chain have become partially inactivated during the isolation procedure. Under these conditions, neither chain would be sufficient to support the entire flux of electron in isolated mitochondria and SHAM or KCN alone would inhibit respiration. In this regard, however, control respiration rates of isolated *cni*-1 mitochondria are higher than those of *inos* mitochondria when NADH is used as a substrate (see legend of Fig. 4).

Cytochrome spectra. Difference spectra at the temperature of liquid nitrogen of *inos* and *cni*-1 mitochondria are shown in Fig. 6. *inos* mitochondria show typical *b*-, *c*-, and *a*-type cytochromes and the absorption maxima seen agree well with those previously reported by Weiss et al. (18) for a wild-type strain of *Neurospora* with the exception of a peak at 552 nm. Since this peak is also reducible with ascorbate plus KCN (Fig. 7), it appears to be a *c*-type cytochrome. Mitochondria from *cni*-1 have similar absorption maxima to those of *inos* in the *b*- and *c*-regions of the spectrum. The major difference in the spectral properties of these mitochondria is that cytochrome aa_3 is not detectable in the *cni*-1 spectrum. Absorption maxima for both the alpha (605 nm) and gamma (444 nm) absorption bands of cytochrome aa_3 are not observed in *cni*-1 mitochondria at this level of analysis.

With our present instrumentation which allows only qualitative or semiquantitative estimates of cytochrome content, we estimate that there is an approximate threefold increase in the concentration of cytochrome *c* 546 in *cni*-1

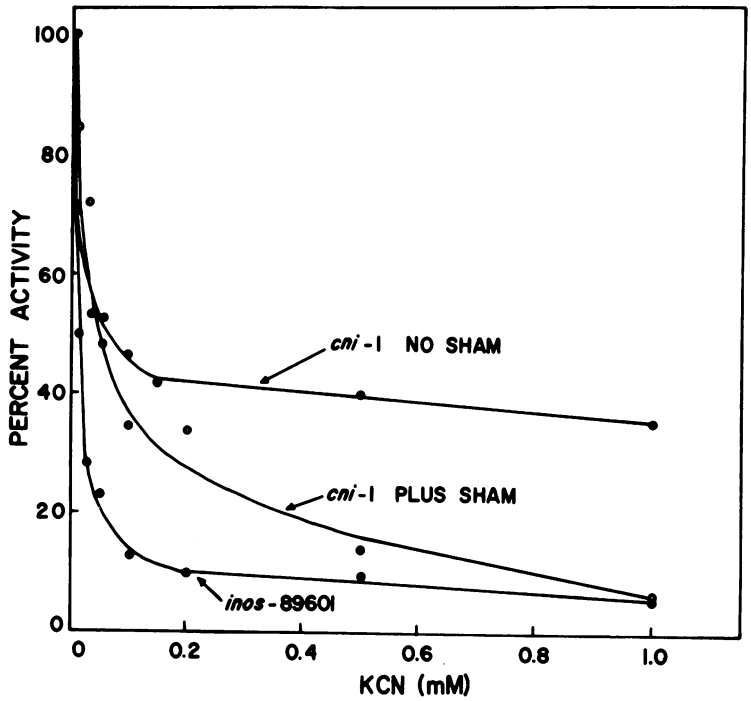

FIG. 4. *Effects of KCN on the respiration of isolated cni-1 mitochondria with NADH as a substrate. The initial concentration of NADH was 0.3 M. In one experiment SHAM (120 μg/ml) was added to the mitochondrial suspension before addition of KCN. Respiration rates are presented as percentages of initial rates measured in the absence of cyanide. Temperature is 30 C. Control respiration rates in μatoms O per min per mg are: cni-1, 0.61 ± 0.03; inos, 0.35 ± 0.03.*

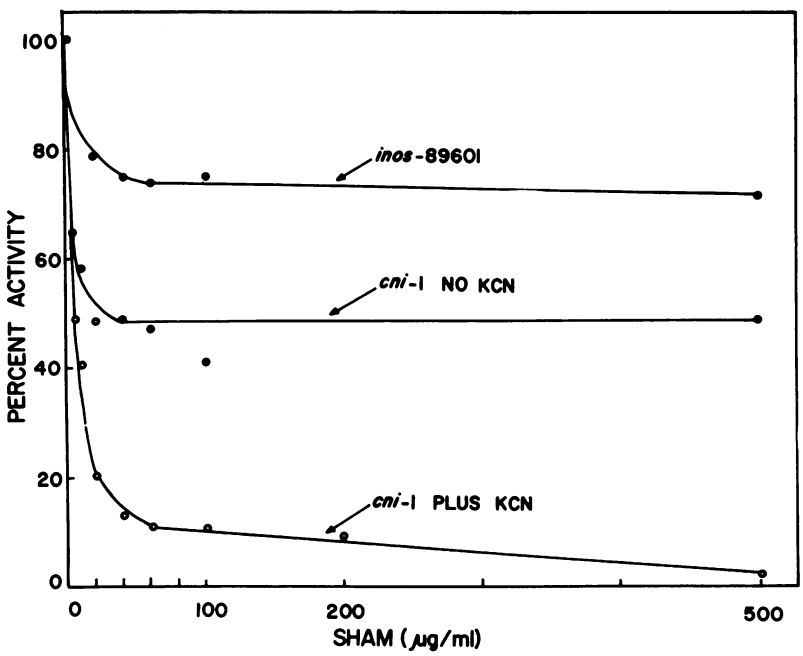

FIG. 5. *Effects of SHAM on the respiration of isolated cni-1 mitochondria with NADH as a substrate. The initial concentration of NADH was 0.3 M. In one experiment KCN (1.0 mM) was added to the mitochondrial suspension before addition of SHAM. Respiration rates are presented as percentages of initial rates measured in the absence of SHAM. Control respiration rates are the same as in Fig. 4.*

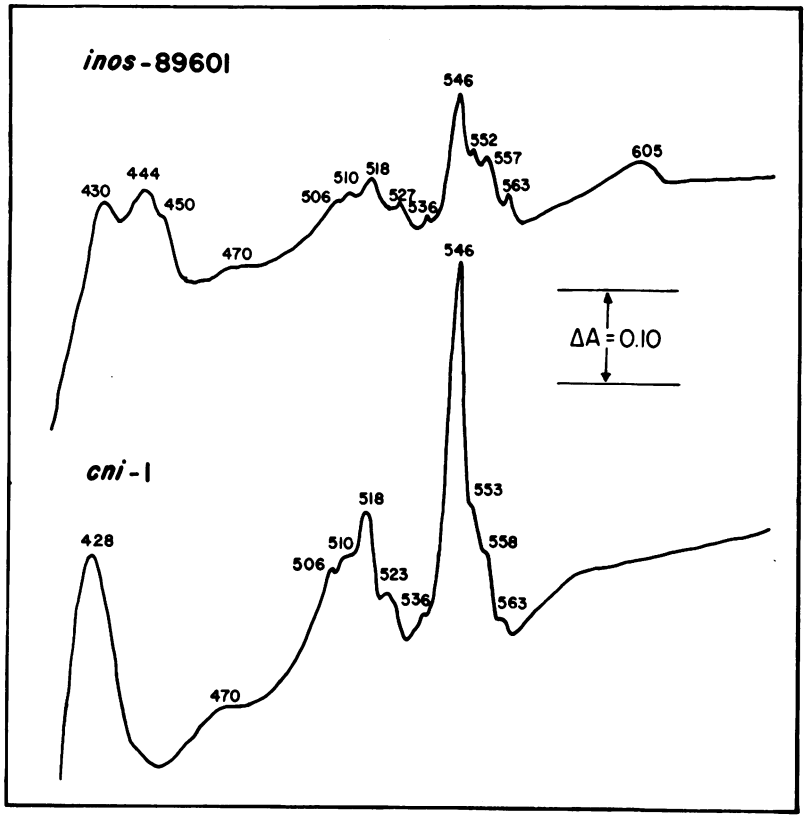

FIG. 6. *Difference spectra of dithionite-reduced minus oxidized mitochondria from inos and cni-1 mitochondria. Measurements were made at the temperature of liquid nitrogen. Path length is 2 mm. The protein concentrations of both mitochondrial suspensions was 6.6 mg/ml.*

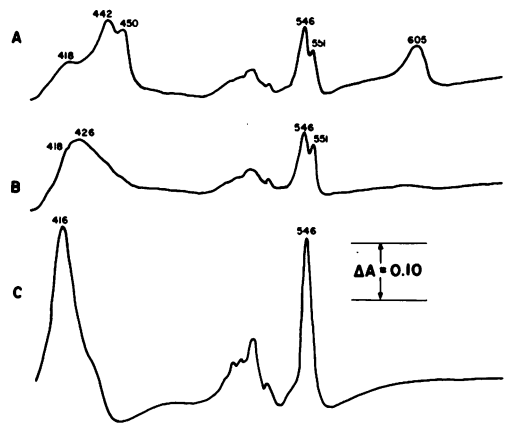

FIG. 7. *Extraction of soluble c 546 from inos and cni-1 mitochondria. A, Residue of inos mitochondria after extraction four times with 0.1 M phosphate buffer, pH 7.2. B, Residue of cni-1 mitochondria after extraction four times with phosphate buffer. C, First phosphate buffer extract of cni-1 mitochondria. Difference spectra of ascorbate plus KCN-reduced minus oxidized were measured at the temperature of liquid nitrogen. The protein concentration in trace A was 10.0 mg/ml, in trace B it was 9.3 mg/ml.*

over that of *inos*, whereas cytochrome aa_3 is not detectable in *cni*-1 at all. The concentrations of other cytochrome components remain relatively constant between the two strains.

In order to study the membrane-bound *c*- and *a*-type cytochromes of mitochondria from these two strains further, soluble cytochrome *c* 546 was extracted from them with phosphate buffer (12) and the mitochondrial residue remaining after extraction was reduced with ascorbate plus KCN. Low-temperature difference spectra of these ascorbate-reduced mitochondrial residues are shown in Fig. 7. The *inos* residue again has typical cytochrome aa_3 absorption maxima (605 and 442 nm), although these maxima are again not detectable in the *cni*-1 residue. A new broad absorption band is present in *cni*-1, however, with a maximum at 426 nm. This band may correspond to the absorption maximum of an altered cytochrome oxidase that has been previously described by Edwards and

Woodward (3) and by Woodward et al. (20) in a crude preparation of cytochrome oxidase from *poky*. A new absorption peak at 429 nm has also been recently reported in a detailed spectral study of *poky* (12).

Both mitochondrial residues contain *c*-type cytochromes with alpha absorption maxima at 546 and 551 nm, beta peaks at 505 to 526 nm, and at a Soret band at 418 nm. It has been suggested that cytochrome *c*-551 is analagous to cytochrome c_1 found in animal mitochondria (12). It is not possible to tell whether the cytochrome *c*-546 remaining in the mitochondrial residue is due to soluble cytochrome *c*-546 that is not completely extracted from the membrane or to a third *c*-type cytochrome. One observation that suggests that *c*-546 is a third *c*-type cytochrome is that after extensive extraction of *inos* and *cni*-1 mitochondria, which initially have widely varying concentrations of soluble *c*-546, the concentration of *c*-546 that remains in the mitochondrial membrane after extraction is the same in each of these strains.

Enzyme assays. The high respiration rate of *cni*-1 mycelium in the presence of SHAM (Fig. 1) and of *cni*-1 mitochondria in the presence of SHAM (Fig. 5), but the lack of cytochrome aa_3 in absorption spectra of *cni*-1 (Fig. 6 and 7) have prompted us to attempt to determine by enzyme assays what the nature of the terminal respiratory enzyme is in the cyanide-sensitive portion of the *cni*-1 respiratory chain. The results of these assays are shown in Table 1. Cytochrome *c* oxidase activity is present in *cni*-1 mitochondria but is only about 10% of the activity of *inos* mitochondria. This activity is sensitive to both cyanide and azide. The succinate-cytochrome *c* reductase activity of *cni*-1, however, is increased approximately fourfold over the activity of *inos*. In contrast to this, the activity of NADH-cytochrome *c* reductase in *cni*-1 is only half that of *inos*. Elevated levels of succinate-cytochrome *c* reductase activity have been previously reported in cytoplasmic mutants of *Neurospora* (1). The reduced level of NADH-cytochrome *c* reductase in *cni*-1 may well be due to the flow of electrons through the SHAM-sensitive respiratory pathway in this mutant. In this regard, we have tested the effects of SHAM and antimycin A on this system. SHAM (120 μg/ml) has no effect on succinate-cytochrome *c* reductase, whereas antimycin A inhibits the activity by greater than 95%. We have not been able to test the effects of these inhibitors on NADH-cytochrome *c* reductase directly as they are added as either ethanol solutions or dissolved in dimethylformamide (4). The addition of either of these reagents to the assay mixture completely abolishes any enzyme activity.

TABLE 1. *Measurement of enzyme activities in inos and cni-1 mitochondria*

Enzyme act	Spc act[a]	
	inos-89601	*cni*-1
Cytochrome oxidase[b]	2.38 ± 0.28	0.27 ± 0.01
Cytochrome oxidase + mM KCN	0.00	0.00
Cytochrome oxidase + mM NaN_3	0.14 ± 0.01	0.02 ± 0.002
Succinate-cytochrome *c* reductase[c]	0.21 ± 0.01	0.86 ± 0.08
NADH-cytochrome *c* reductase[d]	0.37 ± 0.02	0.18 ± 0.01

[a] All values are reported as micromoles of substrate oxidized per minute per milligram of protein. Values are presented as the mean ± standard deviation of the mean for at least five determinations. Temperature was 23 C.

[b] Measured by the method of Wharton and Tzagoloff (19) by using 20 μM ferrocytochrome *c*.

[c] Measured by the method of Tisdale (17).

[d] Measured by the method of Hatefi and Rieske (9) without added phospholipid.

Genetic studies. In order to determine the genetic nature of the *cni*-1 mutation, the mutant was crossed to various strains of *Neurospora*. The results of a random spore analysis of a reciprocal cross between *cni*-1 and a lysine auxotroph (*lys*-1) are shown in Table 2. When *cni*-1 as the conidial parent was crossed to a *lys*-1 (cyanide-sensitive respiration), it segregated as a normal mendelian gene. An analysis of 46 random ascorpores from the cross showed that half of the spores had cyanide-sensitive respiration and the other half were cyanide-insensitive. The lysine and inositol mark-

TABLE 2. *Random spore data from a reciprocal cross between cni-1 and lys-1*[a]

Mutant cross	Germination (%)	No. cyanide sensitive	No. cyanide insensitive	Cyanide insensitive (%)
lys-1 × *cni*-1	75	22	24	52.5
cni-1 × *lys*-1	20	32	7	17.9

[a] Ascospores were germinated by heat shock at 65 C for 45 min on 4% agar plates. Germinated spores were transferred to individual slants of complete medium. Respiration measurements were carried out on mycelium of 24-h liquid cultures grown from these slants as described previously (4).

ers in these strains also segregated normally. When individual asci from this cross were isolated, cyanide-sensitive and cyanide-insensitive respiration segregated 2:2 among the spore pairs.

When *cni*-1 as the protoperithecial parent was crossed to *lys*-1, the results were not as clearcut. A random spore analysis showed that only about 18% of the spores had cyanide-insensitive respiration. The germination of spores from this cross was also very poor. Asci isolated from this cross generally showed incomplete germination.

These studies indicate that the *cni*-1 mutation does segregate as a single mendelian gene. When *cni*-1 is the protoperithecial parent, however, the viability of spores from the cross is very poor. One possible explanation for this poor viability could be a loss in oxidative phosphorylation capacity of *cni*-1 mitochondria similar to that recently described for *poky* mitochondria (13). Preliminary measurements of oxidative phosphorylation in *cni*-1 support this hypothesis (4).

DISCUSSION

The studies presented here both with whole mycelium and with isolated mitochondria indicate that *cni*-1 mitochondria have a branched electron transfer chain similar to that previously reported for an extrachromosomal mutant of *Neurospora* and for wild-type strains of *Neurospora* grown on chloramphenicol. An altered oxidase is present in these mitochondria that is sensitive to SHAM.

The genetic studies presented here indicate that *cni*-1 segregates as a single mendelian gene. Since this branched electron transfer pathway has now been demonstrated to occur due to mutation in mitochondrial deoxyribonucleic acid (DNA), inhibition of mitochondrial protein synthesis, or mutation in a nuclear gene, it must represent a general pathway that is elaborated by the organism in response to alteration of the traditional mitochondrial electron transfer chain. This alteration could be due to either mutation or to the lack of the gene products required for the assembly of the system.

The function of the alternate oxidase system in *Neurospora* is not known. However, Lambowitz et al. (13) have speculated that it might be beneficial to the organism by preventing high levels of reduced pyridine nucleotides from accumulating which would permit glycolysis and the Krebs cycle to operate at an increased rate and thereby stimulate adenosine 5'-triphosphate (ATP) production. Our observations on the NADH and succinate-cytochrome *c* reductase systems of *cni*-1 (Table 1) suggest that the alternate oxidase may be associated with the NADH portion of the electron transfer chain. The rate of cytochrome *c* reduction by NADH is half that of *inos* mitochondria, whereas the rate of cytochrome *c* reduction by succinate is four times that of *inos*. SHAM, furthermore, has no effect on the activity of succinate-cytochrome *c* reductase, indicating that there is no electron flux through the alternate oxidase under these conditions of assay. These observations all suggest that some electrons from the oxidation of NADH pass through the alternate oxidase in *cni*-1. This suggestion is supported by the results with intact mitochondria shown in Fig. 5. In this experiment 51% of the mitochondrial respiration of *cni*-1 was sensitive to SHAM when NADH was a substrate. The lability of the deoxycholate preparation used in the NADH-cytochrome *c* reductase assay to ethanol and dimethylformamide precludes a direct measurement of this activity. We have also observed that the alternate oxidase of *cni*-1 does provide this mutant with a physiological advantage. The growth rate of cultures of *cni*-1 grown in the presence of SHAM is lowered as compared with cultures grown without SHAM (unpublished observations).

The non-detectability of cytochrome aa_3 in *cni*-1 mitochondria is of interest since there is considerable electron flux through the traditional electron transfer chain in this mutant (Fig. 1, 4). Cytochrome *c* oxidase activity which is sensitive to cyanide and azide is, however, present in these mitochondria (Table 1). We have also assayed *cni*-1 mitochondria for the presence of heme *a* by the pyridine hemochromogen method of Morrison and Horie (15). A typical *a*-type heme with absorption maximum at 583 and 430 nm was found to be present. In addition, we have determined that the cytochrome *c* oxidase activity of *cni*-1 is inhibited by antibodies prepared against subunits of wild-type cytochrome oxidase (unpublished observations). The cyanide and azide sensitivity of the enzyme, the presence of heme *a* and the inhibition of enzyme activity by antibodies against wild-type cytochrome oxidase indicate that the cytochrome *c* oxidase activity of *cni*-1 is due to the same enzyme as in wild-type rather than to a new enzyme that is produced due to the *cni*-1 mutation. The absorption peak at 426 nm found in *cni*-1 mitochondria may be due to an altered cytochrome oxidase similar to that previously described in

poky mitochondria (3, 20). The altered spectral properties of the enzyme in *cni*-1 could be due to improper assembly of enzyme subunits as has been suggested previously. We cannot, on the basis of our present data, rule out entirely the possibility of a small amount of wild-type cytochrome *c* oxidase being present in *cni*-1 and being responsible for the activity observed. A reduction of cytochrome aa_3 in wild-type mitochondria to 10% of its initial level would place the concentration of this cytochrome at the limit of detectability by our present instrumentation. Thus we cannot exclude the possibility that the cytochrome *c* oxidase activity of *cni*-1 mitochondria (10% of the wild-type level) may be due entirely to spectrally normal cytochrome aa_3 and that the peak at 426 nm may be due to an enzyme that is enzymatically inactive or, in fact, not associated with cytochrome *c* oxidase at all. In view of the previous studies on the cytochrome *c* oxidase from *poky* (3, 20), however, we feel that this possibility is unlikely. We feel that a more likely hypothesis is that the *cni*-1 mutation results in abnormal assembly of cytochrome oxidase with the resultant loss of the typical cytochrome aa_3 spectrum and a shift to a spectrum with an absorption peak at 426 nm.

It has now been demonstrated that the alternate oxidase system appears in *Neurospora* mitochondria due to mutation in mitochondrial DNA, mutation in chromosomal DNA, or to growth on chloramphenicol. All of these events serve to severely reduce mitochondrial oxidative phosphorylation capacity. Flavell and Woodward have discussed the regulation of Krebs cycle and glyoxylate shunt enzymes of *Neurospora* with respect to catabolite repression by ATP or a similar "high energy" intermediate (5, 6). When wild-type *Neurospora* is grown on sucrose as a carbon source, Krebs cycle enzymes are repressed. When mitochondrial energy metabolism is altered, however, due to either a chromosomal (cyt-1) or mitochondrial (*poky*) mutation, Krebs cycle enzymes are derepressed two- to threefold when grown on the same sucrose carbon source. We suggest that such a system may well regulate the alternate oxidase of *Neurospora* mitochondria as well. Growth under normal conditions of mitochondrial energy metabolism would result in repression of the alternate oxidase. Reduction of mitochondrial energy metabolism due to mutation or chloramphenicol treatment would result in derepression of the alternate oxidase which would serve to prevent a buildup of reduced pyridine nucleotides and thus stimulate ATP production via substrate-level phosphorylation by glycolysis and the Krebs cycle. In this regard, we have noted that the level of fumarate hydratase in sucrose-grown *cni*-1 is derepressed twofold over the level of that enzyme in sucrose-grown *inos* (unpublished observations).

Studies as to the nature of the cytochrome oxidase of *cni*-1 and the regulation of the alternate oxidase are continuing in our laboratory.

ACKNOWLEDGMENTS

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