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Altered Mitochondrial Membrane Activities Associated with Cytoplasmically-inherited Disease Sensitivity in Maize

P. A. PETERSON*, R. B. FLAVELL and D. H. P. BARRATT

Cytogenetics Dept., Plant Breeding Institute, Cambridge (England)

Summary. The effect of *Helminthosporium maydis*, race T, pathotoxin on mitochondria isolated from etiolated maize seedlings with different cytoplasmic genomes was investigated. Mitochondria isolated from plants with the Texas cytoplasm, which confers male sterility and field susceptibility to *Helminthosporium maydis* race T, were sensitive to the pathotoxin while mitochondria from male fertile plants with normal cytoplasm, which are resistant to race T of the fungus, were resistant to the pathotoxin. The pathotoxin induced uncoupling of oxidative phosphorylation, activation of cytochrome oxidase and succinate cytochrome c reductase, inhibited electron transport at an early site in the electron transport chain and overcame the malate and succinate inhibition of ATPase in sensitive mitochondria. All of the pathotoxin-induced abnormalities are consistent with the hypothesis that the pathotoxin has a binding site on the inner membrane of sensitive but not resistant mitochondria and this site is controlled by cytoplasmic DNA. It is concluded that a component of susceptibility of maize lines to *Helminthosporium maydis*, race T, is the uncoupling and inhibition of mitochondrial electron transport by race T pathotoxin.

Introduction

The so-called 'Texas' (T) cytoplasm of maize carries a genetic modification(s) of some kind which confers male sterility (Duvick, 1965). Plants with normal (N) cytoplasm are male fertile. The production of hybrid maize seed over the past three decades in the United States and Europe has largely been dependent upon the male sterility introduced by the 'Texas' cytoplasm. However in 1969, 1970 and 1971, a Southern Corn Leaf Blight epidemic occurred in the USA which selectively attacked inbreds and hybrids of maize with the 'Texas' cytoplasm (Tatum, 1971; Hooker *et al.*, 1970a, b; Hilty and Josephson, 1971). Plants with normal cytoplasm were resistant. The outbreak of blight was caused by the dispersal of a new pathogenic race — race T — of *Helminthosporium maydis* Nisikado and Miyake (Hooker *et al.*, 1970b). A pathotoxin has since been shown to be present in crude filtrates of cultures of *H. maydis* race T and also in extracts from leaves infected with the fungus which, when injected into non-diseased leaves, causes a sensitive reaction in plants carrying the 'Texas' cytoplasm but does not significantly affect plants with other cytoplasms (Hooker *et al.*, 1970a, Lim and Hooker, 1972; Turner and Martinson, 1972; Gracen, Grogan and Forster, 1972; Gracen, Forster and Grogan, 1971). This pathotoxin has not been found in other strains of *H. maydis* in significant quantities. The effects of the pathotoxin on a number of physiological processes have been investigated in an attempt to locate the sensitive subcellular site(s) altered by the cytoplasmic modification (Gracen, Grogan and Forster, 1972; Garroway, 1973; Halloin *et al.*, 1973; Arntzen *et al.* 1973; Arntzen, Haugh and Bobick, 1973; Tipton, Mondal and Uhlig, 1973). Miller and Koeppe (1971) first investigated the mitochondria from plants with N and T cytoplasms and found that in T mitochondria the pathotoxin caused uncoupling of oxidative phosphorylation, inhibition of oxoglutarate oxidation and irreversible swelling in KCl medium but had no effect on N mitochondria. This paper is concerned with a more detailed analysis of the effects of the pathotoxin on maize mitochondrial activities, correlated with the presence of the cytoplasmically-inherited modification conferring disease susceptibility.

Materials and Methods

Maize genotypes and growth of seedlings

The genotypes used were the single cross hybrids $F7^T \times F2$ with T cytoplasm and $F7^N \times F2$ with N cytoplasm. The seeds were well-washed and then germinated in the dark on water-saturated paper towelling at approximately 27 °C for 3 days.

Isolation of mitochondria

Etiolated shoots were ground at 1–5 °C for 40 secs in a mortar in a medium (grinding buffer) consisting of 0.5 M mannitol, 10 mM TES, 0.2% bovine serum albumin (BSA), 1 mM EGTA, 10 mM potassium phosphate at pH 7.2. The homogenate was filtered through 4 layers of close-weave terylene fabric and the filtrate centrifuged at 30,000 × g for 1 min and the white starch component of the pellet removed. The pellet was overlayered with grinding buffer without BSA and centrifuged at 17,000 × g for 1 min. The mitochondrial pellet was resuspended in 'assay buffer' which consisted of 0.3 M mannitol, 4 mM

* PAP on Faculty Leave from Dept. of Agronomy, Iowa State University, Ames, Iowa 50010, USA during 1972–73.

EGTA, 10 mM potassium phosphate, 10 mM TES, 5 mM $MgCl_2$, 0.01 M KCl and 0.2% BSA at pH 7.2 unless stated otherwise. This procedure consistently yielded mitochondria which had oxidative phosphorylation ratios of approximately 3.0 with oxoglutarate as substrate (unpublished results).

Mitochondrial assays

Oxidative phosphorylation was measured at 23 °C with an oxygen electrode (Rank Bros. Bottisham, Cambridge) in a 1.5 or 2 ml reaction volume of the mitochondrial pellet resuspended in 'assay buffer'. Additions, where appropriate, were succinate and malate 40 μmoles, oxoglutarate (α KG) 50 μmoles, NADH 2 μmoles and ADP 2.6 μmoles (approximately). For NADH oxidation approximately 0.26 mg mitochondrial protein was used, with the other substrates approximately 0.40 mg was used.

For ATP hydrolysis assays, mitochondria were resuspended in 0.4 M sucrose, 0.1% BSA, 50 mM Tris-HCl pH 7.2 containing 3 mM $MgCl_2$. The reaction volume of 1 ml contained 0.28 M sucrose, 35 mM Tris-HCl pH 7.2, 3 mM $MgCl_2$ and 4 mM ATP. Succinate, malate and dinitrophenol were included as shown in *Results*. Assays were carried out for 20 min at 27 °C, terminated by addition of trichloracetic acid to a concentration of 8% and the inorganic phosphate formed measured by a modification of the Fiske-Subbarow method (Taussky and Shorr, 1953).

Malate oxidation was determined in mitochondria resuspended in 'assay buffer', at 27 °C using 2'6, dichlorophenolindophenol (DCIP) as an electron acceptor in a 1.5 ml reaction mixture of 'assay buffer' at 0.74 × strength, 0.6 × 10^{-5} M DCIP, 1.5 mM sodium azide and 0.15 M L-malate. Malate dependent oxidation of DCIP was determined from the rate of optical density decrease at 600 nm (Flavell, 1974).

NADH oxidation was measured by the rate of decrease in optical density at 340 nm. Mitochondria were resuspended in 'assay buffer' without phosphate or bovine serum albumin but containing 22 μg NADH, and incubated at 27 °C.

Cytochrome oxidase was assayed in 'intact' mitochondria in 'assay buffer' by measuring the oxidation of 6 m μmoles of dithionite-reduced cytochrome c. The rate of oxidation was determined from the initial rate of change of optical density at 550 nm (Wharton and Tzagoloff, 1967).

Succinate cytochrome c reductase was assayed in intact mitochondria in 'assay buffer' containing 0.28 mM EDTA, 1.5 mM sodium azide and approximately .03 mM oxidised cytochrome c. The rate of cytochrome c reduction dependent on 0.019 M succinate was determined at 30 °C from the initial rate of change of optimal density at 550 nm (King, 1967).

In all spectrophotometric assays, a concentration of mitochondria was chosen which gave a rate of enzyme activity that could be followed optically for at least two minutes before a component became limiting. Shaking, to mix the contents of the cuvette, was strictly controlled as vigorous shaking was found to enhance oxidation of reduced cytochrome c, probably due to mitochondrial breakage.

Pathotoxin preparation

The pathotoxin was a gift from Dr. Carl Tipton. Concentrations of pathotoxin were chosen for this work which had significant effects on T mitochondria but no effect on N mitochondria.

Results

The effect of the pathotoxin on respiration and oxidative phosphorylation in mitochondria isolated from etiolated seedlings with male-fertile N and male-sterile T cytoplasms was investigated first. The two types of cytoplasm were in single cross hybrids with essentially genetically identical nuclei but different cytoplasmic genomes. With N mitochondria, the pathotoxin had no effect on respiration or oxidative phosphorylation irrespective of the substrate utilized (NADH, succinate, oxoglutarate or malate). However, as illustrated in Fig. 1 (see also Barratt and Flavell, 1974; Miller and Koeppe, 1971), 5 μl/ml of the pathotoxin preparation caused an immediate uncoupling of oxidative phosphorylation and stimulation of respiration in T mitochondria, when NADH or succinate served as substrates. When oxoglutarate (or malate) served as substrate, the pathotoxin caused severe inhibition of respiration. In a study of the differential effects of varied low doses of pathotoxin on inhibition of oxoglutarate respiration in T mitochondria (Fig. 2) inhibition of State 3 respiration in the presence of ADP occurred prior to inhibition of State 4 respiration and at a lower pathotoxin concentration. Also, the time taken for inhibition of respiration was inversely related to the pathotoxin concentration.

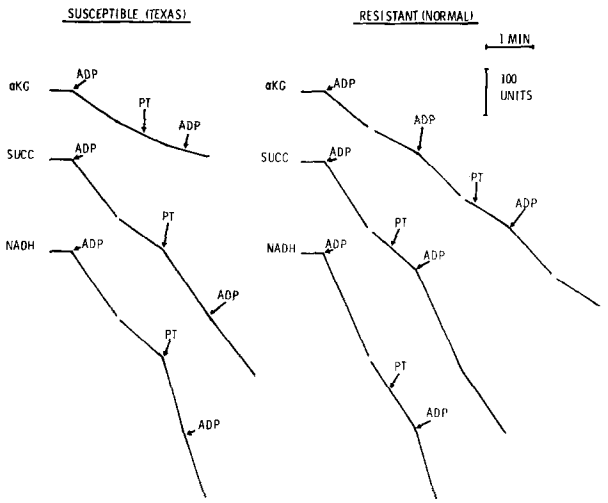

Fig. 1. Effect of pathotoxin on mitochondrial respiration and oxidative phosphorylation
The traces are redrawn from the original O_2 uptake traces. 100 units of O_2 = approximately 45 n moles O_2. ADP and pathotoxin (PT) were added where indicated

The effects of the pathotoxin on electron transport in the three oxidative phosphorylation enzyme complexes of the mitochondrial electron transport chain, i. e. cytochrome oxidase, succinate cytochrome c reductase and endogenous NADH dehydrogenase, were then investigated.

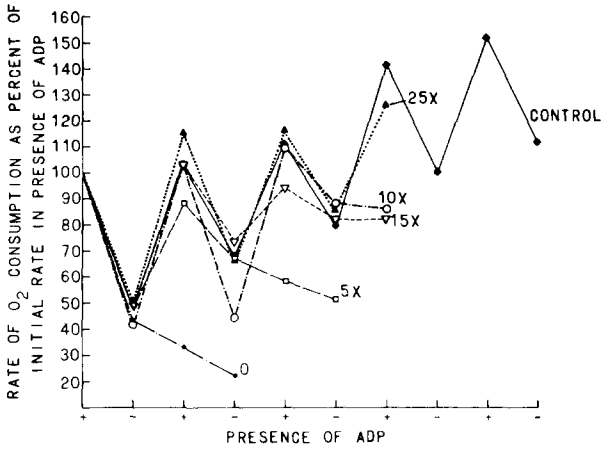

Fig. 2. Effects of different concentrations of pathotoxin on respiration and oxidative phosphorylation
Mitochondria were assayed using oxoglutarate (α KG) as substrate. 10 µl of different dilutions of pathotoxin were added at the same time as the first 2.6 µmoles ADP. Dilutions of pathotoxin used are shown alongside the appropriate lines. The points represent the rate of oxygen uptake following the addition of approximately 2.6 µmoles ADP (+) (State 3) and after utilisation of the ADP (−) (State 4) relative to the rate of oxygen uptake during the first period of State 3 respiration

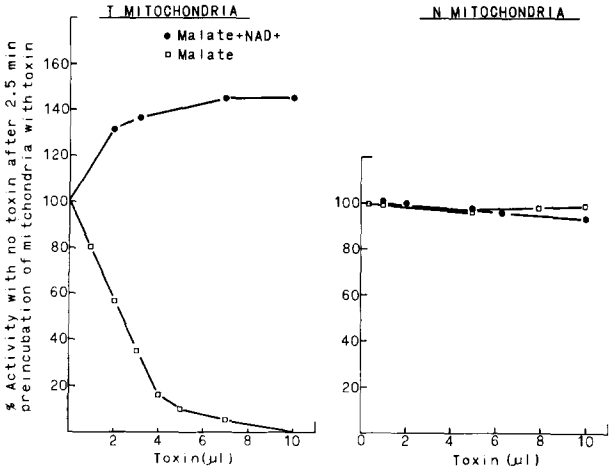

Fig. 3. Effect of pathotoxin on mitochondrial oxidation of malate with and without exogenous NAD^+
—□—□— = initial rate of malate oxidation relative to rate in absence of pathotoxin —●—●— = initial rate of malate oxidation dependent on 1 mM NAD^+ relative to rate in absence of pathotoxin. All rates are corrected for any changes occurring on addition of water instead of pathotoxin or NAD^+. The N and T mitochondrial preparations were of similar protein content

Cytochrome oxidase, assayed in 'intact' T mitochondria, using reduced cytochrome c as substrate (Wharton and Tzagoloff, 1967), was significantly activated 10.5 ± 3.5% by low concentrations (8 µl/ml) of pathotoxin. No significant effect of pathotoxin was observed on cytochrome oxidase activity in N mitochondria. Succinate cytochrome c reductase was assayed in 'intact' mitochondria using oxidised cytochrome c as electron acceptor (King, 1967). In T mitochondria, 8 µl/ml promoted 16 ± 4% activation of this enzyme while in N mitochondria no significant activation by pathotoxin was observed. The activities of cytochrome oxidase and succinate cytochrome c reductase greatly increased following disruption of mitochondrial membranes by freezing or detergent treatment (unpublished results). This was principally but not entirely, due to the increased accessibility of exogenous cytochrome c to the electron transport chain. It is therefore possible that the increase in activity of cytochrome oxidase and succinate cytochrome c reductase in intact T mitochondria promoted by the pathotoxin was due to disturbance of the mitochondrial membranes which allowed increased substrate accessibility. However, it is also known that partially purified cytochrome oxidase (Hinkle, Kim and Racker, 1972) and succinate cytochrome c reductase (Lichtman and Howland, 1973) enzymes are activated by uncouplers of oxidative phosphorylation.

The effect of pathotoxin on the first ATP-coupled site of the electron transport chain, which includes the endogenous NADH dehydrogenase, was studied using malate as a substrate in the absence of exogenous NAD^+ and using 2, 6 dichlorophenolindophenol as an electron acceptor. This dye accepts electrons before the site between cytochromes b and c which is sensitive to antimycin (Flavell, 1974). In T mitochondria, the pathotoxin strongly inhibited the oxidation of malate by 'intact' mitochondria (Fig. 3). Because the extent of inhibition is time-dependent for a given pathotoxin concentration (Flavell, 1974) the percentage inhibition of malate oxidation at each pathotoxin concentration was determined after preincubation of the mitochondria for a fixed time (2.5 min). Malate oxidation via endogenous NADH dehydrogenase in N mitochondria was unaffected by similar concentrations of pathotoxin. Upon the addition of NAD^+, however, there was a marked stimulation of malate oxidation in 'intact' T mitochondria (Fig. 3). This indicated the presence of an intermembrane malate dehydrogenase activity coupled to NAD^+ reduction in maize, similar to that in Jerusalem artichoke (Coleman and Palmer, 1972). This additional activity in T mitochondria was not inhibited by pathotoxin, as expected, if the NADH formed in the intermembrane space was oxidised by an exogenous NADH dehydrogenase which passes electrons to cytochrome b (Coleman and Palmer, 1972) bypassing the first ATP-coupling site of the electron transport chain. In T, but not N mitochondria, low concentrations of pathotoxin caused an initial and immediate stimulation of malate oxidation via this exogenous NADH dehydrogenase. The oxidation of exogenous NADH by intact T mitochondria, measured by following the decrease in optical density at 340 nm was similarly stimulated by low concentrations of pathotoxin (Table 1).

Table 1. *Effects of pathotoxin on oxidation of NADH by intact mitochondria*

Source of mitochondria (Cytoplasmic genotype)	Pathotoxin −	Pathotoxin +
N	0.29	0.29
T	0.49	1.88

Toxin was added to give a final concentration of 8 µl/ml. Results are $\triangle$ OD_{340}/min/0.05 ml mitochondrial suspension

The effect of the pathotoxin on mitochondrial ATP hydrolysis was also investigated. In isolated intact plant mitochondria, the rate of hydrolysis of exogenously supplied ATP is relatively low (Passam and Palmer, 1973). In mung bean mitochondria, ATP hydrolysis is enhanced by the presence of uncouplers (Blackmon and Moreland, 1971), while in castor bean endosperm mitochondria (Takeuchi, Yoshida and Sato, 1969) and Jerusalem artichoke mitochondria (Passam and Palmer, 1973) ATP hydrolysis is enhanced by uncouplers only in the presence of succinate. Table 2 shows that succinate and malate cause an inhibition of ATP hydrolysis in N and T maize mitochondria, as succinate does in artichoke mitochondria, but this inhibition is more than overcome by dinitrophenol (DNP), an uncoupler. With T, but not with N mitochondria, the pathotoxin at low concentrations caused an increase in ATP hydrolysis similar to that by DNP. This further illustrates the action of the pathotoxin on T mitochondria as an uncoupler of oxidative phosphorylation.

Table 2. *Effect of pathotoxin on ATP hydrolysis in 'intact' mitochondria*

Addition	N mitochondria nmole Pi formed/min	T mitochondria nmole Pi formed/min
None	9.2	8.2
Pathotoxin	9.2	7.8
Succinate	0.0	0.0
Malate	0.0	0.0
Succinate + Pathotoxin	0.0	6.6
Malate + Pathotoxin	0.0	7.2
None	—	5.8
Dinitrophenol	—	5.2
Succinate	—	0.0
Succinate + Dinitrophenol	—	8.0
None	—	3.6
Succinate	—	0.0
Succinate + pathotoxin 2 µl	—	2.0
Succinate + pathotoxin 10 µl	—	3.3
Succinate + pathotoxin 20 µl	—	3.6

ATP hydrolysis was assayed in isolated 'intact' mitochondria by measuring the amount of inorganic phosphate formed in 1 ml after incubation for 20 mins. at 27 °C. Where indicated, succinate, malate and dinitrophenol were included to give final concentrations of 0.1 M, 0.1 M and 80 µM respectively.

— denotes not measured

Discussion

The results reported in this paper illustrate that mitochondria of male sterile plants with 'Texas' cytoplasm can be distinguished from mitochondria of male fertile plants with normal cytoplasm in several ways using *Helminthosporium maydis*, race T pathotoxin. The uncoupling of oxidative phosphorylation, (Figures 1 and 2), the activation of ATPase activity in the presence of succinate and malate (Table 2) and the activation of succinate cytochrome c reductase and cytochrome oxidase activities in T mitochondria all occur very rapidly at low concentrations of our pathotoxin preparation. Whether all these phenomena result from the same pathotoxin-induced biochemical lesion cannot be concluded for certain but it is quite possible. All could be the consequence of an alteration in the mitochondrial inner membrane function induced by the pathotoxin.

The lack of inhibition by pathotoxin of (1) oxygen uptake when NADH and succinate are used as substrates, (2) succinate cytochrome c reductase and cytochrome oxidase activities, and (3) exogenous NADH oxidation (Table 1, Figs. 1 and 3), indicates that the inhibition by pathotoxin of (1) oxygen uptake when oxoglutarate or malate are used as substrates (Fig. 1 and 2) and (2) malate oxidation, (Fig. 3) is due to the action of the pathotoxin on an early step of the electron transport chain prior to the entry of electrons from succinate dehydrogenase, i. e. at the first ATP coupled site associated with the endogenous NADH dehydrogenase.

It is possible that inhibition of electron tranport using malate or oxoglutarate as substrate results from an inability to pass electrons from the associated Krebs Cycle dehydrogenases to the endogenous NADH dehydrogenase of the mitochondrial inner membrane. However, it seems more likely that the pathotoxin causes disruption within the first ATP-coupled site of the electron transport system. Gengenbach *et al.* (1973) have recently suggested from electron microscopical observations that the pathotoxin causes uncoupling by general disruption of the inner mitochondrial membrane conformation rather than by altering the properties at specific sites. From the observations reported here the consequences for electron transport of such disruption, if it occurs, are relatively site-specific.

Whether the uncoupling of oxidative phosphorylation and inhibition of electron transport result from the same pathotoxin-induced lesion cannot be decided, especially, since the pathotoxin preparation may contain multiple components (Karr, Karr and Strobel, 1974). However, we have found (Barratt and Flavell, 1974) that uncoupling of oxidative phosphorylation and inhibition of electron transport by pathotoxin are quantitatively correlated in different nuclear genetic backgrounds.

These findings strongly support the hypothesis that the pathotoxin has a specific binding site on the inner membrane of T but not of N mitochondria and that the differences between N and T mitochondria are due to the cytoplasmically-inherited genetic modification. However, the binding of the pathotoxin to the inner membrane of T but not N mitochondria has yet to be formally demonstrated. It is attractive to consider that this genetic modification is in the mitochondrial DNA of the 'Texas' cytoplasm but this must remain a speculation for the time being. It has been known for some time that some of the proteins synthesized in the mitochondria form part of the inner membrane (Coote and Work, 1971; Yang and Criddle, 1970) but until recently, evidence for an inner membrane protein being specified by cytoplasmic DNA has not been clearly established. Shannon *et al.* (1973) have reported alteration of an inner membrane-associated component in yeast which is part of the oligomycin sensitive ATPase and which is inherited through the cytoplasm. If the race-T pathotoxin binds to an inner membrane protein or if binding is at least dependent on inner membrane protein conformation, then this would constitute additional evidence that cytoplasmic DNA of maize specifies components of the inner mitochondrial membrane.

Although the sensitivity of T mitochondria to the disease-inducing pathotoxin is completely correlated with sensitivity of the plants to the disease, other studies on the effect of the pathotoxin on inhibition of root growth (Arntzen *et al.*, 1973) ion leakage from roots and leaves (Arntzen *et al.*, 1973; Halloin *et al.*, 1973; Gracen, Grogan and Forster, 1972), stomata function (Arntzen, Haugh and Bobick, 1973) and root plasma membrane ATPase (Tipton, Mondal and Uhlig, 1973) in plants with *T* cytoplasm, have suggested that the plasma membrane and not the mitochondria may be the primary site of action of the pathotoxin upon infection. Whatever cell function is inactivated or altered first when the pathotoxin pervades the cells, all sensitive sites including mitochondria will eventually be affected, and cell death would thus result from a combination of causes. From the results we report in this paper, it must be assumed that the sensitivity to T mitochondria plays a role in the susceptibility of plants carrying Texas cytoplasm to race-T pathotoxin. The results, therefore, contribute significantly to understanding disease susceptibility/resistance at the molecular level.

Consideration of sites sensitive to pathotoxin which are not mitochondrial (or chloroplast) in plants with T cytoplasm (but not N cytoplasm) raises the fundamental question of how cytoplasmic DNA might specify alterations in non-organelle components, for example in the plasma membrane. As far as we are aware no conclusive evidence exists for non-organelle proteins being specified by cytoplasmic DNA although evidence for the transport of messenger-like RNAs from animal mitochondria, presumably for translation elsewhere, has been reported (Attardi and Attardi, 1968).

The assays we have used here to demonstrate the specific effects of race-T pathotoxin on mitochondria from plants containing the 'Texas' cytoplasm conferring male sterility and sensitivity to race-T *Helminthosporium maydis* are very sensitive, require little toxin and mitochondrial substrate, are easy and quick to perform and do not require fertile pollen as does another recently reported assay method (Laughnan and Gabay, 1973). Therefore they should be of considerable use as assays of 'Texas' cytoplasm in the extensive world-wide maize breeding programmes concerned with T cytoplasm (Peterson, Flavell and Barratt, 1974). As assays for race-T *Helminthosporium maydis* pathotoxin, they should facilitate identification, purification and characterisation of the pathotoxin.

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Literature

Arntzen, C. J., Haugh, M. F., Bobick, S.: Induction of stomatal closure by *Helminthosporium maydis* pathotoxin. Plant Physiol. **52**, 569–574 (1973).

Arntzen, C. J., Koeppe, D. E., Miller, R. J., Peverly, J. H.: The effect of pathotoxin from *Helminthosporium maydis* (race T) on energy-linked processes of corn seedlings. Physiol. Plant Pathology **3**, 79–89 (1973).

Attardi, G., Attardi, B.: Mitochondrial origin of a membrane – associated heterogeneous RNA in Hela cells. Proc. Nat. Acad. Sci. US **61**, 261–268 (1968).

Barratt, D. H. P., Flavell, R. B.: Alterations in mitochondria associated with cytoplasmic and nuclear genes concerned with male sterility in maize. Theor. Appl. Genetics **45**, 315–321 (1975).

Blackmon, W. J., Moreland, D. E.: Adenosine triphosphatase activity associated with mung bean mitochondria. Plant Physiol. **47**, 532–536 (1971).

Coleman, J. O. D., Palmer, J. M.: The Oxidation of Malate by isolated Plant Mitochondria. Europ. J. Biochem. **26**, 499–509 (1972).

Coote, J. L., Work T. S.: Proteins coded by mitochondrial DNA of mammalian cells. Europ. J. Biochem. **23**, 564–574 (1971).

Duvick, D. N.: Cytoplasmic pollen sterility in corn. Adv. Genetics **13**, 1–56 (1965).

Flavell, R. B.: The inhibition of electron transport by *Helminthosporium maydis*, race T pathotoxin, in maize mitochondria altered by a cytoplasmically-inherited mutation. Physiological Plant Pathology, in press (1974).

Garroway, M. O.: Electrolyte and Peroxidase leakage as indicators of susceptibility of various maize inbreds to *Helminthosporium maydis* races 0 and T. Plant Disease Reporter **57**, 518–522 (1973).

Gengenbach, B. G., Miller, R. J., Koeppe, D. E., Arntzen, C. J.: The effect of toxin from *Helminthosporium maydis* (race T) on isolated corn mitochondria: swelling. Canad. J. Botany **51**, 2119–2125 (1973).

Gracen, V. E., Forster, M. J., Grogan, C. O.: Reactions of corn (*Zea mays*) genotypes and cytoplasms to *Helminthosporium maydis* toxin. Plant Disease Reporter **55**, 938–941 (1971).

Gracen, V. E., Grogan, C. O., Forster, M. J.: Permeability changes induced by *Helminthosporium maydis*, race T toxin. Canad. J. Botany **50**, 2167–2170 (1972).

Halloin, J. M., Comstock, J. C., Martinson, C. A., Tipton, C. L.: Leakage from corn tissues induced by *Helminthosporium maydis* Race T toxin. Phytopathology **63**, 640–642 (1973).

Hilty, J. W., Josephson, L. M.: Reaction of corn inbreds with different cytoplasms to *Helminthosporium maydis*. Plant Disease Reporter **55**, 195–198 (1971).

Hinkle, P. C., Kim, J. J., Racker, E.: Ion transport and respiratory control in vesicles formed from cytochrome oxidase and phospholipids. J. Biol. Chem. **247**, 1338–1339 (1972).

Hooker, A. L., Smith, D. R., Lim, S. M., Beckett, J. B.: Reaction of corn seedlings with male-sterile cytoplasm to *Helminthosporium maydis*. Plant Disease Reporter **54**, 708–712 (1970a).

Hooker, A. L., Smith, D. R., Lim, S. M., Musson, M. D.: Physiological races of *Helminthosporium maydis* and disease resistance. Plant Disease Reporter **54**, 1109–1110 (1970b).

Karr, A. L., Karr, D. B., Strobel, G. A.: Isolation and partial characterisation of four host-specific toxins of *Helminthosporium maydis* (Race T). Plant Physiol. **53**, 250–257 (1974).

King, T. E.: Preparations of succinate-cytochrome c reductase and the cytochrome b–c, particle and reconstitution of succinatecytochrome c reductase. Methods in Enzymology, Academic Press, X, 216–225 (1967).

Laughnan, J. R., Gabay, S. J.: Reaction of germinating maize pollen to *Helminthosporium maydis* pathotoxin. Crop Science **13**, 681–684 (1973).

Lichtman, A. H., Howland, J. L.: Influence of uncouplers on succinate-cytochrome c reductase. FEBS Letters **34**, 256–258 (1973).

Lim, S. M., Hooker, A. L.: Disease determinant of *Helminthosporium maydis* race T. Phytopathology **62**, 968–971 (1972).

Miller, R. J., Koeppe, D. E.: Southern corn leaf blight: susceptible and resistant mitochondria. Science **173**, 67–69 (1971).

Passam, H. C., Palmer, J. M.: The ATPase activity of Jerusalem artichoke mitochondria and submitochondrial particles. Biochim. Biophys. Acta **305**, 80–87 (1973).

Peterson, P. A., Flavell, R. B., Barratt, D. H. P.: A simple biochemical assay for "Texas" cytoplasm in corn by use of *Helminthosporium maydis* race T pathotoxin. Plant Disease Reporter, **58**, 777–780 (1974).

Shannon, C., Enns, R., Wheelis, L., Burchiel, K., Criddle, R. S.: Alterations in mitochondrial adenosine triphosphatase activity resulting from mutation of mitochondrial deoxyribonucleic acid. J. Biol. Chem. **248**, 3004–3011 (1973).

Tatum, L. A.: The Southern Corn Leaf Blight Epidemic. Science **171**, 1113–1116 (1971).

Takeuchi, Y., Yoshida, K., Sato, S.: Electron transport dependent adenosine triphosphatase activity in castor bean endosperm mitochondria. Plant Cell Physiol. **10**, 733–741 (1969).

Taussky, H. H., Shorr, E.: A microcolorimetric method for the determination of inorganic phosphorus. J. Biol. Chem. **202**, 675–685 (1953).

Tipton, C. L., Mondal, M. H., Uhlig, J.: Inhibition of the K^+ stimulated ATPase of maize root microsomes by *Helminthosporium maydis* race T pathotoxin. Biochem. Biophys. Res. Comm. **51**, 725–728 (1973).

Turner, M. T., Martinson, C. A.: Susceptibility of corn lines to *Helminthosporium maydis* toxin. Plant Disease Reporter **56**, 29–32 (1972).

Wharton, D. C., Tzagoloff, A.: Cytochrome Oxidase from Beet Heart Mitochondria. Methods in Enzymol., Academic Press, X, 245–250 (1967).

Yang, S., Criddle, R. S.: *In vitro* biosynthesis of membrane proteins in isolated mitochondria from *Saccharomyces carlsbergensis*. Biochemistry **9**, 3063–3071 (1970).

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P. A. Peterson
Dept. of Agronomy
Iowa State University
Ames, Iowa 50010 (USA)

R. B. Flavell
D. H. P. Barratt
Cytogenetics Dept.
Plant Breeding Institute
Cambridge CB2 2LQ (England)