Striated inclusions and defective mitochondria in the restricted form of the 'amycelial' mutant of *Neurospora crassa*[1]

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Summary. The cytoplasm of the restrictive form of the 'amycelial' mutant of *Neurospora crassa,* growing on sucrose-medium, contains paracrystalline, microfilamentous inclusions and mitochondria with internal membranous whorls lacking in acetate-grown cultures.

The morphological mutant 'amycelial' (amyc, linkage group IL[3]) grows semi-colonially on sugar-synthetic media. Its short, vesiculous hyphae form thickened walls and are characteristically poor in sparsely cristated mitochondria[4].

In another slow growing morphological mutant, 'snow-flake', striated bodies have been found and described in detail[5]. Paracrystalline inclusions had previously been found in the respiratory mutant 'abnormal'[6], and even in the wild type grown on high sugar media[7]. Recently, Mishra[8] has also described striated inclusions in a morphological mutant.

It has been observed by one of us[9] that 'amyc' (strain 305 A from the Fungal Genetic Stock Center, Humboldt University, Arcata, California, USA) is a dual, dimorphic mutant, and that when it is maintained for more than 10 days on sugar-synthetic medium, its initially dot-like, slow mycelial growth of the colonial type escapes into spreading hyphae, conferring to the whole colony a semi-colonial aspect. However, such dual growth can be dissociated into its components and the restricted (tight) form isolated from the more general spreading (escape) form when dot-like fragments are subcultured at the latest every 6–8 days. From such premises, we thought it interesting to reinvestigate the ultrastructural features of this maximally colonial and slow-growing, new form of 'amycelial'.

The then so-called 'restricted amyc' was grown from small inocula for 3 days on the surface of solid sucrose (2%)-nitrate (0.1%) synthetic Westergaard and Mitchell medium[10] in Petri plates incubated at 25 °C. Fragments from one of the dense colonies obtained which consisted of entangled short vesiculous hyphae, were fixed and embedded as following: 2% glutaraldehyde buffered in 0.1 M phosphate buffer (pH 6.3–6.5) for 1 h at room temperature (5 min under vacuum); several washings with the same buffer followed by postfixation in 2% osmic acid in phosphate buffer for 2 h at 25 °C; several washings in distilled water before staining with 2% aqueous uranyl acetate for 30 min at 25 °C. After dehydratation in a graded

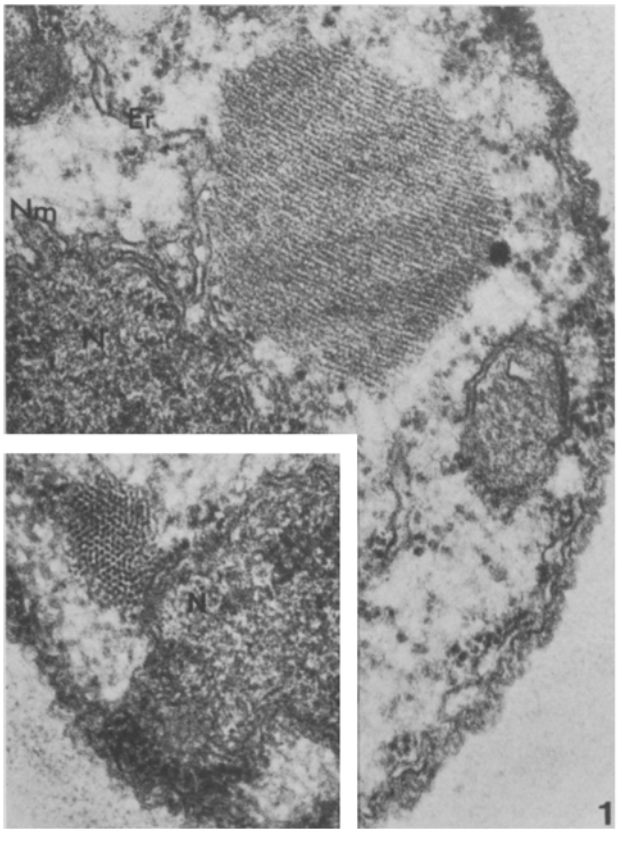

Fig. 1. Striated inclusion sectioned longitudinally in the cytoplasm of the 'restricted amycelial' strain of *Neurospora crassa* grown on sucrose-medium. × 31,000. Insert: transverse section of the same type of inclusion. × 31,000. N, nucleus; Nm, nuclear membrane; Er, endoplasmic reticulum.

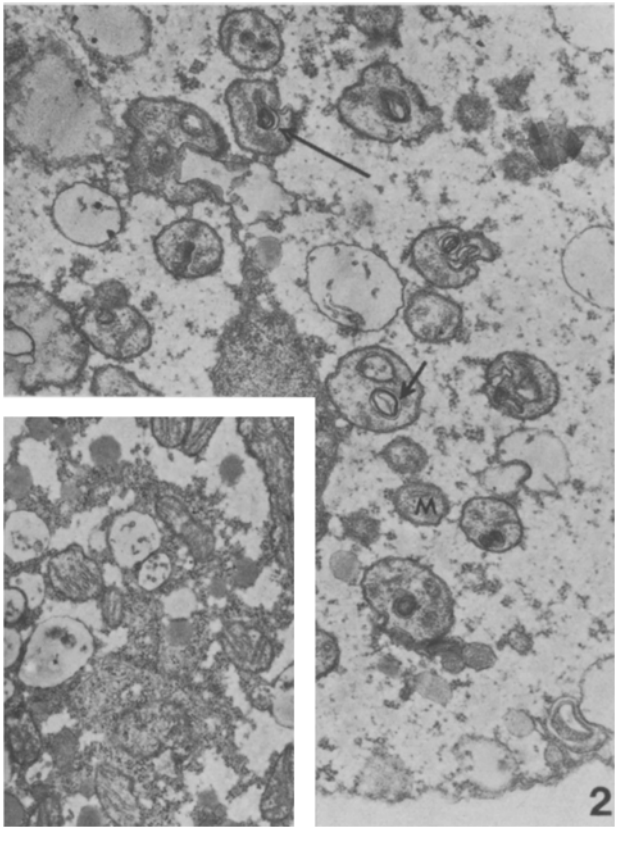

Fig. 2. Mitochondria (M) with internal membranous whorls (arrows) of the 'restricted amycelial' strain of *N. crassa* grown on sucrose-medium. × 11,000. Insert: normalized mitochondria on acetate-medium. × 11,000.

series of ethanol, the material was embedded in Spurr's medium[11]. Thin sections were cut with a diamond knife on a Reichert automatic microtome. They were stained for 20 min at room temperature with 2% aqueous uranyl acetate solution and poststained for 10 min with Reynold's lead citrate[12]. The sections were examined with an AEI EM6B electron microscope.

Finely striated inclusions have been observed (figure 1) in the cytoplasm of most of the vesiculous, repeatedly budding hyphae of 'restricted amyc'. These inclusions are not enclosed in a membrane and have often been seen proximal to the nuclear membrane. In longitudinal sections, the striae appear to correspond to microfilaments (figure 1) which show a regular, hexagonal organization on transverse sections (figure 1, insert). Selective isolation of these inclusions should permit us to check whether those of 'amyc' are polypeptidic, as shown in 'snow-flake'[5].

Another ultrastructural feature worth mentioning on the sections of 'restricted amyc' concerns the mitochondria, which are more underdeveloped than those of the original strain as they show not only a few cristae but, in addition, internal membranes (figure 2). Such membranous structures have previously been described in the mitochondria of especially high sugar-grown cultures of wild type *N. crassa*[13], in which case they were connected with the exaggerated synthesis of phospholipids induced in such abnormal growth conditions[14]. However, when the restricted form of 'amyc' is grown on acetate (2%) medium for 3 days, its short, densely septated hyphae contain greatly restandardized mitochondria practically deprived of internal membranous whorls and normally cristated (figure 2, insert).

A very low QO_2 had already been measured in cultures from the complete, dimorphic (restricted + spreading) strain of 'amycelial'[15]. Now it appears that respiration is practically nullified when measured in the restricted form containing internally whorled mitochondria[9]. If enough growth can be obtained with the restricted form, it will be of interest to check its low respiratory ability in isolated abnormal mitochondria, and to compare it with that of restandardized mitochondria from acetate-grown cultures.

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Effects of anaerobiosis on auxin- and fusicoccin-induced growth and ion transport

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Summary. In maize coleoptile segments, anaerobiosis completely inhibits IAA-induced cell enlargement, H^+ extrusion and K^+ uptake, while it only partially inhibits the stimulating effect of FC on the same processes. Very similar results are obtained by blocking protein synthesis with cycloheximide. As anaerobiosis blocks protein synthesis within 15 min, we conclude that the block of protein synthesis, and not the drop of ATP per se is the cause of the different response of IAA and FC to anaerobiosis.

Indole-3-acetic acid (IAA) and fusicoccin (FC) stimulate K^+ uptake, H^+ extrusion and hyperpolarization of the transmembrane potential difference as well as cell enlargement in many plant materials[2-8]. IAA-stimulated growth is completely inhibited by either anaerobiosis or inhibitors of cytochrome oxidase mediated respiration such as CO[9-12], while FC-induced cell enlargement in pea stem sections is only partially inhibited by CO[12]. If total inhibition of the effect of IAA by nitrogen or by CO is due to the drop of ATP level, we have to explain how a large effect of FC is still present under the same experimental conditions of inhibition of ATP synthesis.

In the present investigation we have studied the effect of anaerobiosis on IAA- and FC-induced cell enlargement, H^+ extrusion and K^+ uptake in maize coleoptile segments. Evidence is reported suggesting that the effect of anaerobiosis in suppressing the promoting action of IAA on growth and ion transport depends rather on the block of protein synthesis than on the decrease of ATP level per se.

Material and methods. Maize (*Zea mays* L. cv. Dekalb XL 640) seeds were germinated for about 80-90 h on poplar sawdust in the dark at 28 °C. Coleoptile segments, 3 mm long, were cut from the region between 5 and 15 mm from the tip. The segments were washed for 2 h in 5×10^{-4} M $CaCl_2$ and 2.5×10^{-4} M $MgCl_2$ (the solution was freshly changed after 30 min) and then transferred to the various media as described in the single experiments; 5×10^{-4} M $CaCl_2$, 2.5×10^{-4} M $MgCl_2$ and 10^{-3} M KCl were present in every treatment; pH of all solutions was adjusted to 5.7. The experiments were run in the dark in a thermoregulated water-bath with shaking (50 spm) at 28 °C. Anaerobic conditions were obtained by continuous bubbling in the incubation medium of 0_2-free N_2.

Growth was measured as increase in length. Titrations of H^+ released in the medium at the end of incubation were performed as already described[13]. In the experiments of K^+ uptake $^{86}Rb^+$ was used as the tracer for K^+. After incubation in the labelled solution, the samples were treated as described[2]. In the experiments of leucine uptake and incorporation the segments, after incubation in 10^{-2} M L-[1-^{14}C]leucine, were rapidly washed and then incubated for 10 min in 5 ml of ice-cold unlabelled solution of 10^{-2} M leucine. TCA soluble and TCA insoluble fractions were prepared as described[14]. All the data reported in this paper