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

are the average of 2 or more experiments performed in triplicate.

Results and discussion. The data of table 1 show that anaerobiosis completely suppresses IAA-induced cell enlargement, H^+ extrusion and K^+ uptake in maize coleoptiles; on the contrary, the similar effects of FC are reduced but still significantly detectable even under anaerobic conditions. This different behaviour of the FC- as compared with the IAA-treated samples under anaerobiosis does not seem to relate to different energy supply. In fact ATP level drops by about 40–50% under nitrogen, but the new level is the same for the controls (90 nmoles/g fresh wt) as for the IAA- and FC-treated samples (respectively 88 and 85 nmoles/g fresh wt).

The lack of correlation between the ATP decrease and the different responses of the FC- and of the IAA-treated segments to anaerobiosis suggests that anaerobiosis-induced inhibition does not depend directly, or only, on the decrease of ATP level.

Table 1. Effect of anaerobiosis on IAA- and FC-induced cell enlargement, H^+ extrusion and K^+ uptake

	Elongation (%)		$-\Delta H^{+}$*		$+\Delta K^{+}$**	
	Air	Nitrogen	Air	Nitrogen	Air	Nitrogen
			nEq./g fresh weight × h			
Control	1.6	0	−0.08	−0.11	0.55	0.28
IAA	5.9	0	+0.10	−0.10	0.90	0.29
FC	11.2	3.6	+0.70	+0.14	2.30	0.64

IAA or FC (2×10^{-5} M) were added after 30 min of preincubation under air or nitrogen. All measurements were performed after 1 h of treatment. * $-\Delta H^+$ = nEq. H^+ extruded from the tissue. ** $+\Delta K^+$ = nEq. K^+ taken up by the tissue.

Table 2. Effects of anaerobiosis and of cycloheximide on the rate of protein synthesis

	TCA soluble fraction		TCA insoluble fraction	
	15 min	30 min	15 min	30 min
	nmoles leucine/g fresh weight			
Control	859	1800	63.1	114.9
Nitrogen	311	707	3.4	6.1
Cycloheximide	786	1579	3.3	4.2

The segments were preincubated for 15 min in air or nitrogen or cycloheximide (50 µg/ml); then treated for 15 or 30 min with 10^{-2} M L-[1-^{14}C]leucine.

Table 3. Effects of cycloheximide on cell enlargement and K^+ uptake in maize coleoptile segments

	Elongation (%)		K^+ uptake (nmoles/g fresh weight)	
	−CH	+CH	−CH	+CH
Control	1.4	1.2	0.52	0.23
IAA	6.1	1.3	0.81	0.22
FC	12.1	7.0	2.18	0.81

IAA or FC (2×10^{-5} M) were added after 30 min of preincubation ± CH (50 µg/ml). Increase in length and K^+ uptake were measured after 1 h of treatment.

An energy-dependent process clearly involved in IAA and – to a lesser extent – in FC action is protein synthesis. In fact in pea stem segments the block of protein synthesis completely suppresses IAA- but not FC-induced cell enlargement[14]; also in maize and oat coleoptiles, the requirement of a normal RNA and protein synthesis is more stringent for IAA- than for FC-stimulated processes[15–17].

Oxygen deficiency might inhibit protein synthesis either by lowering the energy supply or the energy charge ratio[18–20] or by some other mechanism[21] also in our material.

The data of table 2 show that in maize coleoptiles anaerobiosis completely blocks protein synthesis within 15 min; a similar inhibition of protein synthesis is obtained with cycloheximide (CH) (50 µg/ml). CH, at the concentration used, does not affect ATP level and respiration for at least 1.30 h in our material (data not reported).

The block of protein synthesis induced by CH (table 3) prevents any stimulating effect of IAA on cell enlargement and K^+ uptake, while it reduces by only about 60% the effects of FC on the same processes. The larger inhibition induced by anaerobiosis (table 1) than by CH (table 3) on the effects of FC is probably due to the concurrence of the block of protein synthesis and of the decrease of ATP level under anaerobic conditions.

These data suggest that the block of protein synthesis probably related to the drop of ATP level and/or energy charge ratio, rather than the lowering of ATP level per se, causes the lack of effect of IAA under anaerobiosis. Therefore the different response to anaerobiosis of the IAA- as compared with the FC-induced effects seems to correlate to their different degree of dependence on protein synthesis already described[14,15].

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