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Promotion of Respiration by Auxin in the Induction of Cell Division in Suspension Culture

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Abstract. Auxins (IAA, NAA, p-CPA) caused 20–30% promotion of respiration of auxin-dependent cell cultures of *Nicotiana tabacum, Glycine max, Taraxacum mongolica,* and *Atriplex* sp. and had small if any effect on respiration of auxin-independent cell cultures of *Rubus* sp. and *Scorzonera hispanica*. Antiauxin (p-CPIBA) did not affect the respiration. The auxin effect on the respiration of tobacco cells was revealed 10 min after its addition to the suspension and reached a maximum value in 60 min. This stimulation preceded the induction of cell division by auxin. Mitochondria isolated from auxin-treated tobacco cells had greater oxidative and phosphorylative activity than mitochondria from untreated cells. However, isolated mitochondria did not respond to auxin. The inhibitors of respiration (cyanide, monoiodoacetate, malonate, and 2,4-dinitrophenol) eliminated auxin effect on the respiration and cell division. It is concluded that the promotion of respiration is a common event for the auxin effects both on cell extension and on cell division. This promotion is necessary for the induction of cell division and is exerted via direct activation of mitochondria *in situ*.

Auxins not only stimulate cell extension but also induce cell division, especially in isolated culture. The stimulation of cell extension is usually accompanied by the promotion of respiration (Audus 1960, Bonner and Bandurski 1952). However, it is uncertain if respiration is promoted when auxins induce cell division.

Normal (nontumorous) plant cells cultured in suspension are the most suitable objects to elucidate this problem, because their division has an obligatory dependence on exogenous auxin. Many authors have reported that initiation and growth of callus and cell cultures in the media with auxins was accom-

panied by an enhancement of respiration (Auerswald and Günter 1972, Komamine and Shimizu 1975, Le Tran Binh et al. 1977, Romani et al. 1981) and by an increased activity of mitochondria (Van der Plas and Wagner 1980). However, in these papers the rate of respiration was estimated after the induction of growth had taken place, and it may be considered a post effect.

It was shown in our previous study (Gamburg and Osharova 1973, Gamburg 1982) that the addition of auxin to tobacco cell suspensions that had been incubated previously without auxin caused an initiation of DNA replication and an increase of mitotic activity after a 10- to 12-h lag period. The aim of the present work is to study the early effect of auxin on the respiration of plant cells induced to divide and to relate this effect to the changes in mitochondrial activity.

Materials and Methods

Auxin-dependent tobacco cell culture (*Nicotiana tabacum* L., cv. Trapesond) was used in most of the experiments. The origin and maintenance of the culture have been described previously (Gamburg and Osharova, 1973). Some experiments were performed with auxin-dependent cell cultures of *Glycine max, Taraxacum mongolica, Atriplex* sp., and auxin-independent cell cultures of *Rubus* sp. and *Scorzonera hispanica.*

All cultures were maintained in Murashige and Skoog (1962) salt medium with the addition (mg $\times$ l^{-1}) of sucrose 20,000, thiamine 0.4, pyridoxine 0.1, inositol 80, NAA of 2,4-D (for auxin-dependent cultures) 1–2. The cultures were incubated in Ehrlenmeyer flasks (250 ml) on a horizontal shaker at 26°C in darkness, and aliquots of the grown suspension were transferred weekly to the fresh medium. Before the experiments all cultures were grown for 3–4 days without auxin. Auxin was then added, and portions of suspension (1.5 ml) were transferred to Warburg flasks or a polarographic cell for respiration measurements.

Mitochondria were isolated from tobacco cells by the method already described (Vojnikov 1979). The cells (5–7 g fresh weight) were separated from the medium on a glass filter, resuspended in the isolation medium (0.25 M sucrose + 0.05 M TRIS-HCl buffer + 0.005 M Na-EDTA + 0.075% BSA + 0.05% cysteine, pH 8.3), and homogenized with a homogenizer LE-402 (Medimpex, Hungary) and a Teflon pestle at 5600 rpm for 1 min. The brei was filtered through a nylon sieve (pore size 0.2 mm), and the resulted suspension was centrifuged at 2500*g* (K-24, Heintz Janetzki, GDR) for 3 min. The supernatant was centrifuged at 18,000*g* for 3 min. The pellet was resuspended in the isolation medium (pH 7.4, without cysteine), and centrifugation at 18,000*g* was repeated. Isolated mitochondria were resuspended in 0.2 ml 0.25 M sucrose + 0.05 M TRIS-HCl + 0.075% BSA, pH 7.4. Portions of the suspension (50 µgl) were injected into a polarographic cell prefilled with the incubation medium 1.5 ml (125 mM KCl + 18 mM KH_2PO_4 + 0.1% BSA, pH 7.4). Malate 10 mM or succinate 10 mM was used as substrate.

The rate of oxygen consumption was measured with a platinum electrode and a polarograph LP-60 (Czechoslovakia) in the presence and absence of ADP.

The protein content was determined by the method of Lowry et al. (1951). All experiments were repeated three or more times.

Results

As shown in Table 1, the stimulation of respiration during the first 3 h after the NAA addition was observed in all auxin-dependent cells and was small or absent in auxin-independent cells. The stimulation was caused by auxins (NAA, IAA, p-CPA); antiauxin (p-CPIBA) had no effect (Table 2). These data show that the promotion of respiration of cultured plant cells is an auxin-specific effect.

For time course study of the auxin effect on respiration, the portions of tobacco cell suspension (1.5 ml) were taken off at several time points after NAA addition and transferred to a polarographic cell. The oxygen absorption was measured for 5 min. It is seen in Fig. 1 that an increase in the respiration rate was detectable 2 min after the addition of auxin. Under prolonged incubation the stimulation increased and reached a maximum in 60 min. A similar time course of the auxin effect on respiration of maize coleoptile sections (registered by the method of microcalorimetry) was observed by Anderson et al. (1981). Our experiments on auxin uptake by cultured cells revealed that maximum content of auxins (IAA, 2,4-D) was registered in 40–60 min after auxin administration to suspension (Shvetsov and Gamburg 1981, 1982). It may be assumed that the rate of respiration is enhanced concomitantly with the increase of the cellular auxin content.

The auxin is believed to promote the respiration indirectly by increasing ATP consumption in enhanced cell growth, thus providing more intensive supply of ADP for mitochondria (Audus 1960, Anderson et al. 1981). In this case the activity of mitochondria isolated from auxin-treated and untreated cells should not differ. However, mitochondria isolated from auxin-treated tobacco cells showed a greater rate of oxygen absorption both in the presence (state 3) and in the absence of ADP (state 4) than mitochondria from untreated cells (Table 3, Fig. 2). Auxins failed to change the respiratory control and ADP/O ratio. The extent of stimulation of the mitochondrial activity (~28%) corresponded to an increase of tobacco cell respiration (see Table 1). The promotion of cell respiration may be concluded to be a consequence of direct effect of auxin on mitochondria, causing an increase in their ability to oxidize Krebs cycle acids and to phosphorylate ADP.

Our attempts to stimulate isolated mitochondria by auxin were unsuccessful. The addition of NAA at any concentration from 10^{-12} M to 10^{-5} M did not cause a reproducible increase in oxygen consumption (Table 4). Some authors (Bonner and Bandurski 1952, Poljakoff-Mayber 1955; Switzer 1957) reported that auxin had no effect on mitochondria isolated from elongating cells either. Therefore, auxins can probably increase the respiratory activity of mitochondria only when they are inside the cells.

The next question is whether the promotion of respiration is needed for the induction of cell division by auxin. To answer this question, we performed experiments with some inhibitors of respiration. Several concentrations of each

Table 1. The effect of NAA 1 mg × l^{-1} on the respiration of suspension-cultured plant cells

Cultures	O_2 absorption, μl × h^{-1} × g^{-1} fresh weight		Stimulation (%)
	Control	NAA	
Nicotiana tabacum	433 ± 17[a]	553 ± 23	27
Glycine max	1047 ± 23	1357 ± 17	30
Taraxacum mongolica	450 ± 10	560 ± 7	24
Atriplex sp.	500 ± 10	593 ± 10	19
Rubus sp.[b]	1083 ± 7	1163 ± 10	7
Scorzonera hispanica[b]	1187 ± 13	1297 ± 10	9

[a] Data are means ± SE of three tests.
[b] Auxin-independent cultures.
The cells were precultivated for 3–4 days in fresh media without auxin. Then the aliquots of the suspensions and NAA were added to flasks of Warburg's respirometer, and O_2 absorption was registered for 3 h.

Table 2. The effects of 4-chlorophenoxyacetic acid (p-CPA) and 4-chlorophenoxy-*iso*-butyric acid (p-CPIBA) on the respiration of suspension-cultured plant cells

Cultures	Concentration (μM)	O_2 absorption, μl × h^{-1} × g^{-1} fresh weight		
		Control	p-CPA	p-CPIBA
Nicotiana tabacum	3	545 ± 7[a]	622 ± 10	536 ± 7
Glycine max	0.3	885 ± 6	1108 ± 8	884 ± 1
Rubus sp.[b]	0.3	537 ± 9	544 ± 1	481 ± 7

[a] Data are means ± SE of three tests.
[b] Auxin-independent culture.
The cells were precultivated for 3–4 days in fresh media without auxin. Then the aliquots of the suspensions and regulators were added to flasks of Warburg's respirometer, and O_2 absorption was registered for 3 h.

inhibitor were tested, and the results obtained with the concentrations providing full elimination of auxin-induced cell division are presented in Table 5. One can see that the inhibitors used eliminated auxin effects both on cell division and on cell respiration. An experiment with monoiodoacetate was particularly significant, because this inhibitor did not affect the respiration in the absence of auxin but removed auxin-induced increase in the respiration rate and in cell number. The oxygen consumption by tobacco cells increased in the presence of 2,4-dinitrophenol, but the difference between auxin-treated and control cells disappeared. Therefore auxin did not stimulate uncoupled respiration. Accordingly, auxin-induced cell division was also removed by 2,4-dinitrophenol. It may be presumed that the promotion of respiration by auxin is necessary for the induction of cell division in order to meet an enhanced demand for energy and substrates.

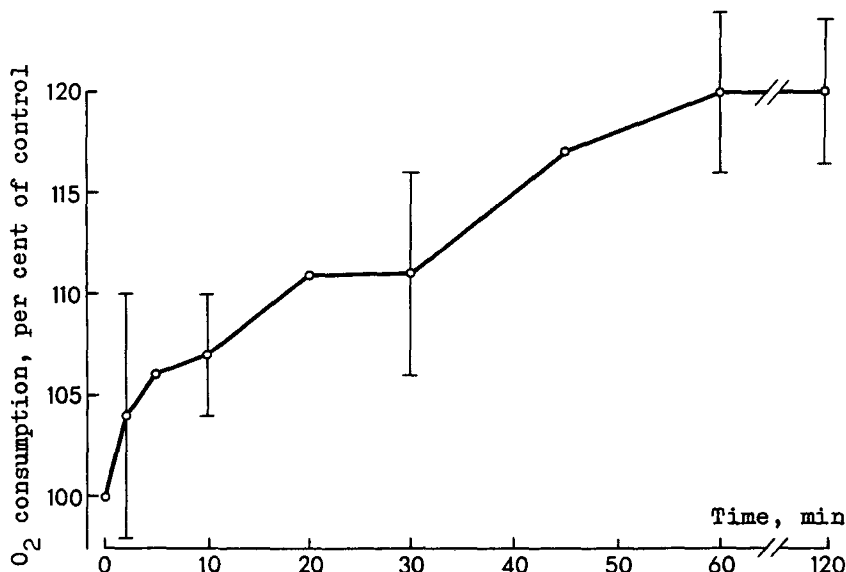

Fig. 1. Time course of NAA 1 mg × l^{-1} effect on oxygen consumption by tobacco cells grown previously in the medium without auxin for 3 days. Statistical errors are indicated by vertical bars.

Table 3. Oxidative and phosphorylative activity of mitochondria isolated from auxin-treated and untreated tobacco cells

Exp.	Auxin (μM)	O uptake, natom × min^{-1} × mg^{-1} protein		RC[a]	ADP/O
		State 3	State 4		
1	—	63 ± 1[b]	23 ± 1	2.78	2.60[c]
	—	151 ± 2	76 ± 1	1.99	1.58[d]
	NAA, 5.38	81 ± 3	24 ± 1	3.22	2.85[c]
	NAA, 5.38	178 ± 2	83 ± 2	2.15	1.78[d]
2	—	161 ± 0	61 ± 0	2.65 ± 0	1.25 ± 0.01[d]
	IAA, 10	214 ± 22	77 ± 13	2.78 ± 0.22	1.21 ± 0.13[d]
3	—	221 ± 8	130 ± 7	1.70 ± 0.09	1.32 ± 0.06[d]
	IAA, 10	294 ± 14	179 ± 8	1.64 ± 0.02	1.24 ± 0.02[d]

[a] Respiratory control.
[b] Data are means ± SE of three tests.
[c] Malate was used as substrate.
[d] Succinate was used as substrate.

Tobacco cells were grown for 3 days in fresh medium without auxin. Then NAA or IAA was added, and mitochondria were isolated after incubation for 2 h. Mitochondria were incubated in a polarographic cell with the addition of 150–200 nmol ADP 4–5 times. RC and ADP/O were estimated in the third cycle of phosphorylation.

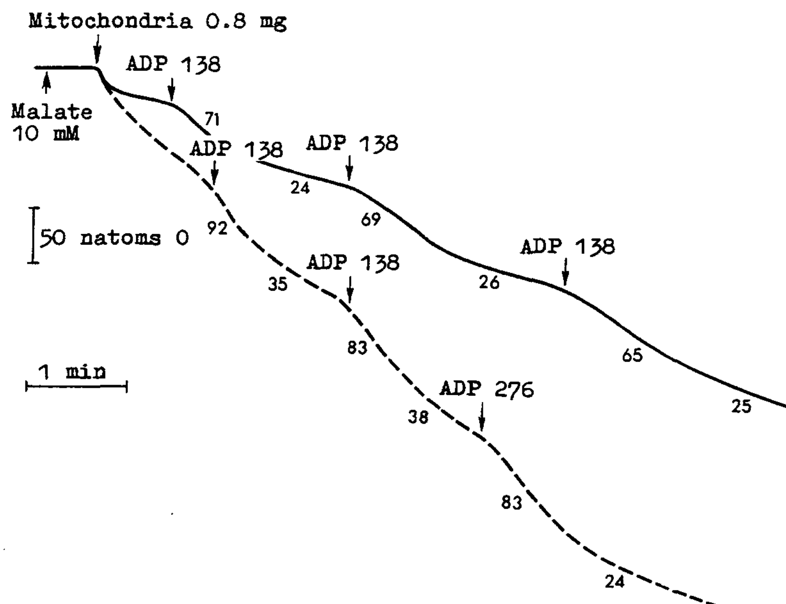

Fig. 2. A polarographic record of oxygen absorption by mitochondria isolated from control tobacco cells (solid line) and auxin-treated cells (dashed line). Tobacco cells were grown previously in fresh medium without auxin. Then NAA 1 mg × 1^{-1} was added, and incubation proceeded for 2 h.

Table 4. The effect of NAA on the oxidative and phosphorylative activity of mitochondria isolated from suspension-cultured tobacco cells (0 natoms × min^{-1} × mg^{-1} protein).

		NAA concentrations (M)						
Exp.	ADP	0	10^{-12}	10^{-11}	3×10^{-11}	10^{-10}	3×10^{-10}	10^{-9}
1	+	79.2	77.4	66.6	64.1	95.4	83.4	64.9
	–	37.1	37.8	30.6	35.7	36.5	38.6	32.1
2	+	63.3	63.9	47.8	58.6	60.6	61.5	54.2
	–	21.2	21.0	15.2	22.1	20.8	21.5	19.8

		0	10^{-8}	5×10^{-8}	10^{-7}	2×10^{-7}	5×10^{-7}	2×10^{-6}	10^{-5}
3	+	52.1	51.0	49.2	43.8	45.0	53.2	54.4	49.4
	–	22.4	27.9	26.0	21.2	23.9	26.9	19.4	34.6
4	+	59.3	—	—	43.3	56.8	68.6	48.1	49.8
	–	27.3	—	—	21.0	22.6	28.6	20.4	25.1

Tobacco cells were cultured for 3 days in fresh medium without auxin, then mitochondria were isolated. Polarographic cell contained glutamate 20 mM, malate 10 mM, $MgCl_2$ 1 mM, EDTA 5 mM, polyvinylpyrrolidone (MW 10,000 daltons) instead of BSA 0.1%, NAA in concentrations indicated, and mitochondrial suspension. ADP (200 nmol) was added repeatedly during the incubation. Protein content of this mixture was determined after the estimation of oxygen absorption rate.

Table 5. The effects of NAA 1 mg × l^{-1}, KCN 2 × 10^{-4} M, monoiodacetate (MIA) 5 × 10^{-5} M, malonate (Mal) 10^{-2} M, and 2,4-dinitrophenol (DNP) 10^{-4} M on the division and respiration of tobacco cells

		O_2 absorption, $\mu l \times h^{-1}$				Cell number per 1 tube, $\times 10^6$			
Inhibitor	NAA	KCN	MIA	Mal	DNP	KCN	MIA	Mal	DNP
–	–	74	62	56	58	1.33	1.40	1.64	1.40
–	+	95	74	72	70	2.41	2.50	3.10	2.52
+	–	43	61	38	95	1.28	—	1.50	—
+	+	46	61	27	102	1.28	1.40	1.60	1.61

Tobacco cells were grown for 3 days in fresh medium without auxin. The aliquots (1.5 ml of suspension) were then transferred to flasks of Warburg's respirometer together with NAA and inhibitors. The absorption of oxygen was measured for 3 h. The portions of the same suspension (4 ml) were transferred to the tubes together with NAA and inhibitors. The cell number was determined after incubation for 24 h.

Discussion

The results reported here show that the stimulation of respiration is one of the most earliest metabolic events in the induction of cell division by auxins. The extent of the stimulation and its time course were very similar to those observed in the action of auxin on cell elongation (Bonner and Bandurski 1952, Audus 1960, Anderson et al. 1981). These data indicate that the promotion of respiration appears to be a common event for the action of auxin on cell extension and cell division.

The enhancement of respiration rate seems to be necessary for the additional supply of energy and precursors in such processes as protein and RNA syntheses, DNA replication, and cell division, which are promoted or induced by auxin (Gamburg 1982). This supposition is confirmed by experiments on the interaction of respiratory inhibitors and auxin in respiration and cell division (Table 5).

Auxin-induced increase of cell respiration is the result of the promotion of the activity of mitochondria in cells. This change may be a consequence of some modification of preexistent mitochondria or may have resulted from the stimulation of their growth, differentiation, and propagation, as shown by Key et al. (1960) in experiments concerning 2,4-D influence on soybean hypocotyls. The second possibility is unlikely to be realized in our experiments, because the time needed for the full expression of auxin effect on respiration was too short (1 h). It seems more probable that auxin causes some changes of preexistent mitochondria (perhaps their membranes), which results in an increase of cell respiration. The nature of such changes remains unknown.

Our data, in accordance with those of most other experimenters (Bonner and Bandurski 1952, Poljakoff-Mayber 1955, Switzer 1957), showed that the increase of mitochondrial activity under auxin influence occurred only when they were within the cells, not when they were isolated. This contradiction may be accounted for by the supposition that certain cofactors required for

auxin action on mitochondria (perhaps receptor) are lost during the isolation procedure. Another possibility may involve auxin-induced formation of some cytoplasmic constituent that incorporates into the mitochondria, causing an increase of their activity.

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