Reducing Power of Hyphal Tips and Vegetative Apical Dominance in Fungi[1]

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Summary. The activity of reducing glycolytic enzymes is significantly higher in extracts from vegetative compared with differentiated cultures of *Neurospora* and *Allomyces*. The reducing power, which is cytochemically shown to be localized in the vegetative hyphal tips, fades at differentiation.

Hyphal tips are deprived of mitochondria[2] but filled with small vesicles associated with wall extension[3]. In 1957, we had mentioned that the apices of hyphae from *Allomyces* can reduce thionine and methylene blue[4]. In *Neurospora*, the hyphal apices were shown to be negative to cytochemical tests for succinate dehydrogenase and cytochrome oxidase while being especially positive for -SH groups[5].

Specific activities[a] of D-lactate dehydrogenase (D-LDH) and ethanol dehydrogenase (ADH) compared in homogenates from vegetative and differentiated stages of *Allomyces arbuscula* and *Neurospora crassa*.

Developmental stage	D-LDH	ADH
A. arbuscula		
Vegetative	1.66	
Differentiated (gametangia)	0.52	
N. crassa		
Vegetative		6.75
Differentiated (conidiated)		1.52

[a] △ O.D. 340 nm (NADH) / min / μg protein[13]; average of 3 experiments.

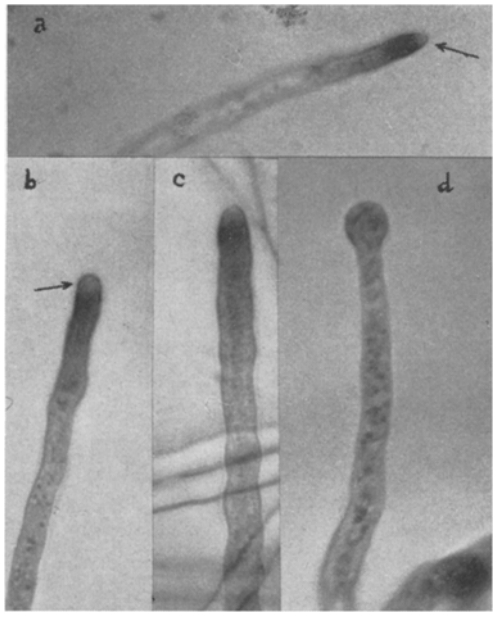

Fig. 1. Neutral red staining (10^{-4} in phosphate buffer pH 7.0) of vegetative hyphae (a–c) compared with that of an aerial hypha induced to the first conidiogenous apical enlargement (d). The vegetative tips (arrows) are yellow. ×500 (a) and ×700 (b–d).

In a search for a functional explanation of the mechanism of control of hyphal elongating growth versus apical differentiation to gametangia or to conidia[6,7], we have further compared the reducing power of vegetative hyphal tips with that of differentiating apices, both by enzymatic and cytochemical techniques.

For the enzymatic studies, we have chosen two terminal glycolytic enzymes, namely D-lactate-dehydrogenase (LDH) for *Allomyces*[8] and ethanol-dehydrogenase (ADH) for *Neurospora*[9]. In *A. arbuscula*, soluble fractions extracted (100,000 *g*[10]) from mycelial balls bearing only vegetative hyphal tips (3 days growth in agitated GCY medium at 25°C[11]) were compared for their LDH activity with homogenates with balls bearing differentiated gametangia (3 days, in agitated G_2Y medium at 25°C[11]). In *N. crassa*, homogenates (10,000 *g*) from vegetative mycelial mats obtained after 60 h of stationary growth in an ammonium-sucrose medium[12] were compared with those from the same mats but 8 h after their transfer into a phosphate solution (0.1 *M*, pH 7.2), on glass beads, to induce apical differentiation of macroconidia (with Kenny, unpublished). Our results show that both vegetative *Allomyces* and *Neurospora* have significantly higher glycolytic activities (Table) in agreement with the previously recorded abundant liberation into the filtrates of lactic acid[10] and ethanol[9], respectively. They also reinforce our previous view[10] that the negative reactions obtained with the cytochemical tetrazolium tests for both LDH and ADH, which are necessarily made in the oxidizing direction for substrates (with NAD reduction), were due to the reducing power localized in the hyphal tips.

From the enzymatic results, we have assumed that the required reduced coenzyme NADH should be more highly concentrated in what can be considered the differential zone when vegetative hyphae are compared to sporogenic ones, namely in the amitochondrial tips in which active glycolysis should therefore occur. Such localized high production of NADH generating either lactic acid or ethanol, superimposed on the presumptively high con-

[1] The support of the Fonds national suisse de la Recherche Scientifique is gratefully acknowledged.

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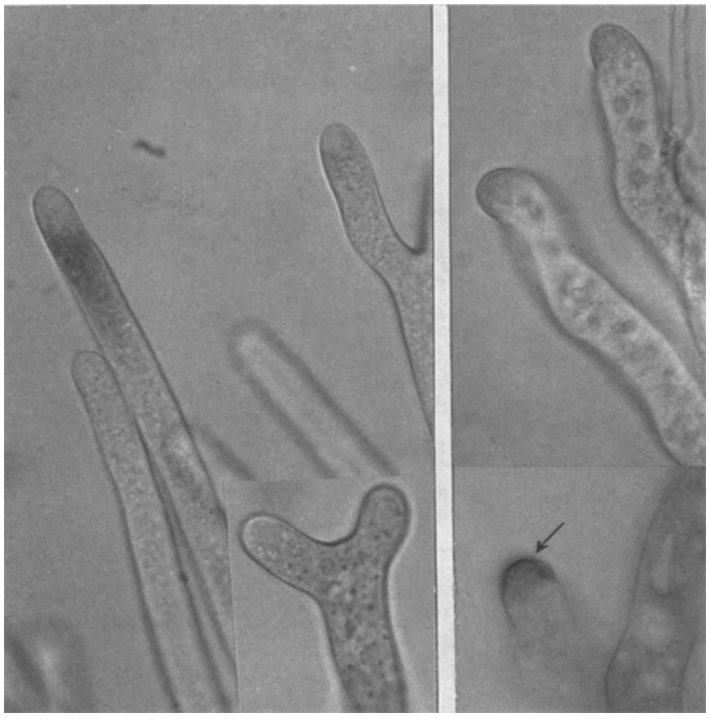

Fig. 2. Right side: vegetative hyphae of *Allomyces arbuscula* bathed in an aqueous solution (10^{-4}) of phenazine methosulfate (PMS) and presenting an apical blue hue after 2 min (top) rarely intensified (arrow), with brownish transition to yellowish, after around 5 min (below). Left side: vegetative hyphae of *A. arbuscula* progressively reoxidizing methylene blue (see text). ×1000 and 1200.

centration of reducing, SH-rich glycolytic enzymes in that zone, strongly suggested the occurrence of a cytochemically demonstrable high reducing power in the hyphal tips of both species studied.

We first tested a vital staining reagent with low redox potential, neutral red (E'_o around -0_V30 at pH 7.0, i.e. only slightly above the -0_V32 value of $NAD^+/NADH$[14]). After short-time bathing in a 10^{-4} phosphate buffered (pH 7.0) solution of this reagent, the vegetative hyphae of *Neurospora crassa* showed under the optical microscope a distinctive yellow staining of their tips contrasting with the red coloration developing from their subapical zones (Figure 1, a–c). Interestingly, the yellow reaction corresponding to the shift to the reduced form of neutral red did not occur in enlarged hyphal tips triggered by preliminary transfer into phosphate buffer to initiate conidial differentiation (Figure 1, d). In other experiments, we could see a decreasing yellow to red gradient from the tip of germinative tubes to their basis on the conidium.

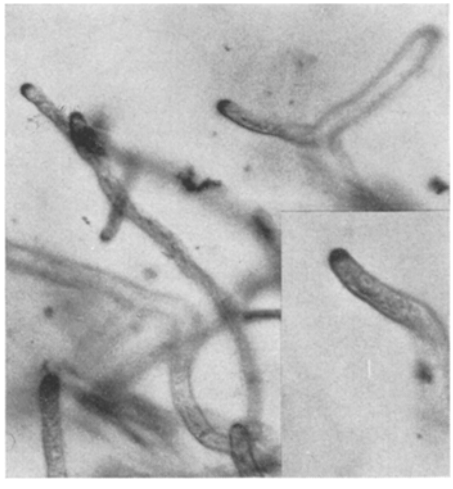

Fig. 3. Dark sites of Fe^{+3} to Fe^{+2} reduction as revealed by the positive Turnbull blue reaction in the tips of vegetative hyphae of *Neurospora crassa*. ×350 and 500.

Phenazine methosulfate (PMS, $E'_o = +0_V080$[14]) was next tested, because of its direct interaction with pyridino-proteins without intervention of flavoproteins[14]. Vegetative hyphae of both *Neurospora* and *Allomyces* transferred under coverslip in a drop of a dilute aqueous solution of PMS rapidly developed a somewhat transient, subvital blue hue in their apices, optically more visible in the wider hyphae of *Allomyces* (Figure 2, right). The unreduced form of the reagent then stained lethally, in pale yellow, the subapical and distal zones of the hyphae. In a dilute solution of methylene blue ($E'_o = +0_V010$[14]), only the hyphal tips remained colourless after a brief lifting of the coverslips, leading to reoxidation of the leucobase in the subapical mitochondrial-rich zone (Figure 2, left).

The strong reducing power of the hyphal tips has been further demonstrated by a new technique involving the initial bathing of a bunch of vegetative hyphae in a dilute aqueous solution of $FeCl_3$ (10^{-3}, average 10 min) followed by several washings in distilled water before exposure to a dilute aqueous solution of K-ferricyanide (10^{-3}, average 5 min); after final replacement of the ferricyanide solution by distilled water, microscopical examination of the hyphal apices showed a clear blue staining of Fe^{+2}-ferricyanide concentrated in their tips. The blue colour obtained was denser in the hyphal tips of *Neurospora* (Figure 3) than in those of *Allomyces*. Significantly, the Turnbull blue reaction faded in the hyphal tips induced to differentiate (1 h into phosphate buffer). The iron reduction in the tips of growing hyphae is interesting from the eco-physiological point of view; it leads

[14] H. R. MAHLER and E. H. CORDES, *Biological Chemistry* (Harper Intern. Edit., New York 1969), p. 872.

us to think that the often described iron oxidation by fungi[15] should intervene secondarily, in the subapical, mitochondrial zone of the hyphae. The known close redox interaction of Fe^{+3} and cysteine suggests that the strong Fe^{+3} to Fe^{+2} reducing power of the hyphal tips is reflecting less the NADH production (revealed more specifically above with the PMS test) than the apical concentration of the sulfhydryl-rich enzymes (glyceraldehyde-phosphate dehydrogenase, hexokinase, ADH).

The cylindrical, polarized extension of the hyphal tips is maintained as long as pro-glycolytic conditions such as semi-anaerobiosis, high glucose in ammonium medium, excess of antioxidants (cysteine, diphenylamine, etc.) are maintained. Contrary effects such as oxidative starvation, inactivation of the –SH compounds (iodoacetate, Hg-compounds, quinones, etc.) lead to a premature disappearance of the apical reducing zone which is 'invaded' by mitochondria. Thus, in *Neurospora*, the conidiogenic transition is marked by a progressive enlargement of the hyphal tip accompanied by a loss of the PMS or Fe^{+3} reducing power and a generalization of the oxidative vital staining with neutral red (Figure 1, d) or Janus green[16]. In *Allomyces*, the club-like stage shows a generalized reoxidation of the methylene blue and no reduction of phenazine methosulfate.

We can admit that glycolytic, apical dominance over the oxidative activity of the subapical mitochondrial population enforces and maintains an acropetally reductive gradient (tip redox value negative, averaging those of NADH and –SH). Such a redox gradient could provide the electrochemical power postulated by BARTNICKI-GARCIA[17], to insure continuous polarized transportation to the growing apex of the wall precursors-containing vesicles. Conversely, the progressive (by ageing) or prematurely induced (see above) loss of the apical reducing power (through lifting of the glycolytic dominance), would then lead to a uniform distribution of the mitochondrial oxidative activity in the enlarging hyphal tip. The uniform 'oxidative climate' thus created would restrict vesicles transport and delay setting of the wall (plasticized for apical enlargement) while triggering transcription of the sporogenic genes in the apical nuclei.

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Pigeons Homing: Some Experiments for Testing the Olfactory Hypothesis[1]

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Summary. Homing experiments on Swiss pigeons show that the birds use olfactory cues for navigational purposes and that outward journey detours influence their initial orientation.

A few years ago, Italian researchers presented a new hypothesis of pigeon homing, according to which olfaction plays an important and specific role in the navigation mechanism of these birds[2]. This hypothesis has been supported by the results of a long series of experiments performed in Tuscany and in the surrounding areas[3]. In addition to the olfactory cues detected while aloft, pigeons may also use those detected during the outward journey, while they are carried to the release site. Indeed, when 2 groups of pigeons were carried by different routes, with the first part of the 2 routes being very divergent, each group showed in most cases the tendency to fly in a direction which was roughly between the home direction and the direction opposite that of the first segment of its outward journey. Moreover, the orientation of one group was often at random, whereas that of the other was not (detour effect)[4].

American authors confirmed that experimental pigeons whose olfactory nerves had been cut were much poorer at homing than the control birds, both in terms of speeds and of the number of birds lost[5]. However, their attempt to repeat two other experiments produced results which did not agree with those of the Italian workers[6,7]. This fact gave rise to the idea that pigeons may use different navigational cues according to their strain, the region in which they live, or the way in which they are reared or trained[8]. It is commonly believed that birds may utilize cues some of them repetitive, for navigational purpose[9]. Therefore, the possibility that different strains may use different navigational cues, should be considered. However, other explanations for the discrepancies between the results of the two research teams are possible.

In this situation, it seemed appropriate to repeat 3 of the experiments of the Italian workers, using Swiss pigeons. The first 2 experiments had already been repeated in the States, but with different results.

Materials and methods. The pigeons came from the loft of one of us (G. W.) at Grächwill and from the Swiss Army loft at Sand. Both localities are near Berne. The birds were different in age and experience. The pigeons for the 2 groups used in the 1st and 2nd experiments were chosen by lot, and for the 3rd experiment so that age differences were minimized. α-pinene was used in a mixture of 10 ml with 50 g of pure vasoline. This mixture was spread just before the release onto the beak of each experimental bird; pure vasoline was applied to the

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