Isolation and morphological studies of a variant of *Ceratodon purpureus*

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Summary. Morphological variants from protonema of *Ceratodon purpureus* (Hedw.) were obtained using nitrosoguanidine (NTG). In one of the variants, reduced growth was accompagnied by hyperbranching and inhibition of caulogenesis. The deposition of branches in this strain can be studied by enzymatic analysis.

Light[1,2], biochemical[3] and cellular[4] factors, among others act on the branching of the protonema of Bryales. Few results have been published concerning genetic studies on the morphogenesis of this system[5-8]. These organisms merit further study with the same techniques as are used for fungi. The essential contribution of genetics to the better understanding of mycelium growth and branching does not need to be emphasized[9,10].

Bryales mutants are often auxotroph strains. Results obtained with fungi[11] suggest that only morphological variants can lead us to understand the morphogenesis of filamentous systems. In auxotroph mutants, the block in development is a function of an organic molecule, which restores morphogenesis of the wild type. On the contrary, morphological mutants do not have any new metabolic needs but are characterized by changes in the regulation of metabolic processes in the later stage of development.

The attainment of hyperbranching mutants in fungi permitted the partial elucidation of the mechanisms leading to the production of lateral buds. In the case of Bryales protonema, we have not yet obtained morphological mutants which are easy to characterize even though we used parameters which permitted us to define morphogenesis (growth speed, mitotic rate, cell length, branching capacity). We tried therefore to isolate morphological mutants of *Ceratodon purpureus* and determine the branching mechanism of this filamentous system.

Material and methods. Mutagenesis was carried out on aqueous suspensions of spores of *Ceratodon purpureus* at a concentration of 2×10^3 cells/ml. Nitrosoguanidine (NTG) was added at concentrations varying from 10 to 750 µg · ml^{-1} during 30 min, in agitated medium, at room temperature. The spores were collected by centrifugation, then washed and spread in Petri dishes on Kofler A medium[3]. The culture development was continued under multilateral light (fluorescent tubes 'Lumière du jour de luxe' type) at 14,000 erg cm^{-2} sec^{-1}, at 23 °C during 8 days.

NTG concentrations greater than 500 µg · ml^{-1} gave the percentage of survival (2%) necessary to obtain the most probable variants. At 750 µg · ml^{-1} thalli were picked and maintained on medium A. Implants were then taken from the edges of cultures and inoculated in van Tieghem cells according to the usual technique[3] under light of 1500 erg cm^{-2} sec^{-1}. Kofler A medium[3] was used in some cases supplemented with α-D(+) glucose (Ag).

Results. Figures 1 and 2 summarize the results obtained with the wild strain and one of the most interesting morphological variants (133,750). In culture chambers the growth rate of this strain was very inferior to that of the wild type (fig.1, a), and the elongation of filaments was 29% of that in the wild strain. This growth rate inhibition was a consequence of a 50% decrease in the rate of mitosis, perpendicular along the axes. The cell lengths were decreased only slightly (20%) (figs 1 and 2, b).

Branching capacity measured by the ratio R/N (total No. of branches over total No. of cells along the main axes) was multiplied by 1.5 in the variant on glucose medium and by 5 on medium A, in contrast to the wild type (figs 1 and 2, c). The protonema mutants were hyperbranched, which was a result we obtained for the first time in illuminated cultures. The main axes frequently aborted (figs 1 and 2, e). This is in agreement with the observations made of the mycelium of *Neurospora* and *Podospora* variants[11]. After 75 days culture, the strain 133750 never produced a gametophyte with leaves. Differentiation remained blocked at an early stage of spherical cells. This contrasted with the wild type, in which the gametophyte plants were well developed. Media supplemented with β-indolyl acetic acid (10^{-4}M) or 6-furfurylaminopurine (10^{-5}M) did not restore the budding of caulonema. Additions of purine bases (adenine 500 µg · l^{-1}), growth factors (Ca panthotenate 4 µM, pyridoxine 1.2 µM, thiamine 1.5 µM, biotin 40 µM, choline Cl 20 µM, inositol 22 µM, nicotinic acid 8 µM, p-amino benzoic acid 1.8 µM), casamino acids (200 mg/ml) or yeast extract (100 mg/l) did not restore morphogenesis of the wild type. This

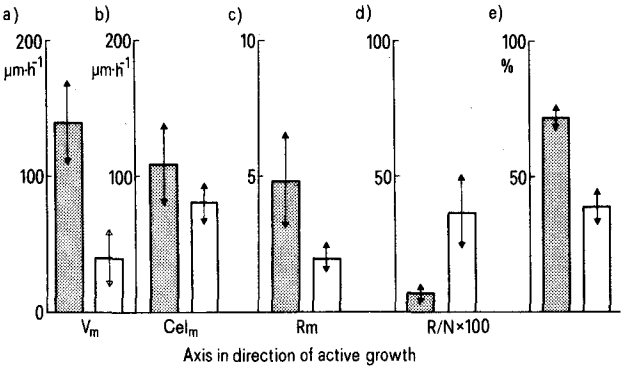

Figure 1. Comparative study of growth parameters of a wild type of *Ceratodon purpureus* (Hedw.) and its mutant 133750 on medium A. (M. Larpent-Gourgaud 1969). Mean values ± SE from 83 cultures are presented. ▩ Wild type; □ mutant 133750.

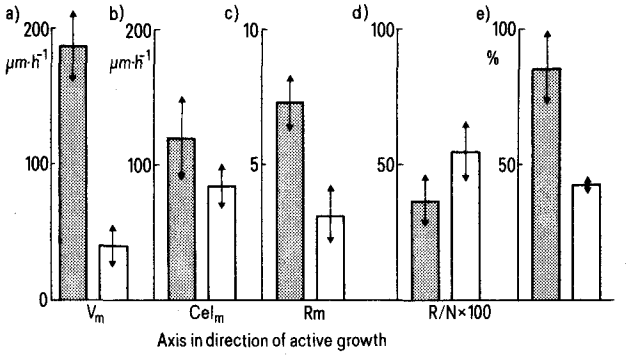

Figure 2. Comparative study of growth parameters of a wild type of *Ceratodon purpureus* (Hedw.) and its mutants 133750 on medium Ag (M. Larpent-Gourgaud 1969). Mean values ± SE from 85 cultures are presented. V_m, mean growth rate µm h^{-1}; Cel_m, mean length of cell (µm); R_m, mitotic rate (cell divisions · 24 h^{-1} $filament^{-1}$); R/N, number of ramifications × 100/number of cells.

hyperbranched variant is probably not an auxotrophic mutant.

Discussion and conclusions. The isolation of morphological mutants, easy to characterize by classical parameters, allows genetic and biochemical analysis of the branching processes. What has been possible in the case of hyperbranched mutants of *Neurospora* and *Podospora* is now possible for the protonema of *Ceratodon*. Even though it is too early to analyze these morphological processes at the level of regulation it is now possible to characterize them at the enzymatic level. This would elucidate the mechanisms controlling the deposition of lateral buds.

Cell walls have a primordial role during protonema morphogenesis and cell walls of Bryales possess glucane polymers. To ensure normal growth, cell wall component synthesis and deposition must be highly coordinated and the enzymes involved in cell wall polysaccharide synthesis must be regulated. Activities of glucane hydrolases and glucane synthetases should be studied to understand the regulation and the possible implication of their morphogenetic roles, in apical growth an protonema branching.

The mutant obtained allows analysis of the processes of caulogenesis. Is the blockage of this morphological stage in the mutant due to a need for certain growth substances, as in Bryophytes (β-indolylacetic acid, cytokinin, unknown growth factor) rather than to a deficient sequential enzymatic regulation as in certain mutants of *Penicillium baarnense*[12]? The preliminary examination does not give an explanation for the lack of budding in our mutant strain.

The only limitation to the genetic analysis of the morphogenesis of Bryales protonema lies in the difficulty of obtaining sexual reproduction in vitro. However the techniques used with protoplasts can overcome this obstacle. It is not clear, at this time, why the somatic hybrids have a different morphology from haploid strains[6-8].

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Synchronization of deposition of daily growth layers in the cuticle of the cockroach *Blaberus fuscus* by gating of moult

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Summary. In *Blaberus fuscus*, combined light/dark-warm/cold cycles induce a rhythm of moulting. Most of the moults take place in the middle of the dark cold phases. Because of a stable phase-relation between moulting time and endocuticle growth rhythm, the latter is synchronized with the environmental cycles, too; lamellated layers are deposited during the light warm phases, which correspond to the time of resting of the cockroaches. Therefore, the cuticle growth is internally synchronized with rhythmic locomotory activity and metabolic rhythms.

In many pterygote insects the endocuticle growth after moulting occurs in a daily pattern: on each day a lamellated and a non-lamellated layer are deposited[1]. In all probability this daily growth rhythm is controlled by an oscillator which is localized in the hypodermis[2-4]. In locusts[4,5] as well as in *Blaberus fuscus*[2,3], the cuticle growth rhythm satisfies 2 properties of circadian rhythms: free-running in constant conditions, and temperature compensation. In locusts, the deposition is light-sensitive[5,6]. In cockroaches, the growth rhythm cannot be influenced by constant light (*Periplaneta*[5], *Blaberus*[3]); it cannot be synchronized by LD cycles (*Blaberus*[3]) or shifted by light pulses of high intensity (*Leucphaea*[7]). Once started, the clock which controls cuticle growth cannot be influenced to change frequency and phase.

This point raises the question of the biological significance of the circadian rhythm of cuticle growth. Circadian rhythms allow organisms to entrain to environmental cycles and, more important, they allow an internal synchronization of rhythms of different physiological processes. Internal synchronization of these rhythms can be achieved by internal coupling meachnisms, or by external 'Zeitgebers'.

If the daily growth pattern cannot be synchronized by external 'Zeitgeber' or by internal synchronization, it must be assumed that the regular sequence of layers is of significance on its own, regardless of the time of day at which the layers are deposited (frequency control only).

We examined whether there is any evidence for a phase control of cuticle growth. There exists a stable phase

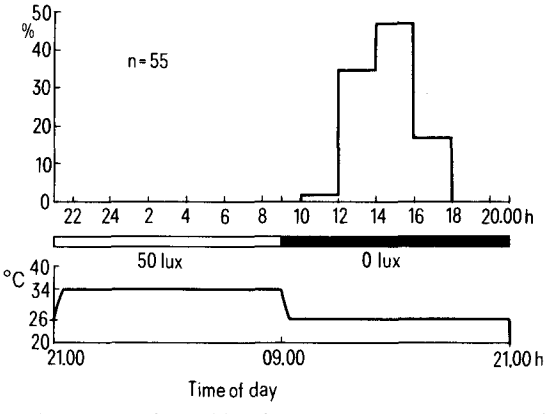

Figure 1. Pattern of moulting in a population synchronized by combined light/dark-warm/cold cycles. The animals prefer the middle of the dark/cold phase.