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Disruption of the bipolar gametangial segregation by anticytoskeletal chemicals in *Allomyces*

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Abstract

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The genetically-controlled inverse bipolar sexualizing gradient of the *Allomyces* can be dissipated by adequate concentrations of both the antiactin cytochalasin E and anti-tubulins DMSO and benlate.

In cell morphogenesis, the process of establishment and fixation of the polar axis precedes the unequal distribution of cytoplasmic components along that axis (Quatrano 1978). Such a sequence is well exemplified in the fucoid algae and possibly *Acetabularia*, in which certain macromolecules are synthesized throughout the cell and then redistributed to some predetermined site specified by the polar axis. Two mechanisms could control this type of segregation: a cytoplasmic, electrical potential gradient or field which could electrophoretically segregate macromolecules on the basis of their net charge and/or a mechanical contractile mechanism involving actin microfilaments.

Sex differentiation and its segregation into inversely superposed gametangia in the epigynous *Allomyces macrogynus* and hypogynous *A. arbuscula* is genetically-controlled (Emerson & Wilson 1954), as confirmed by DNA-mediated cross-inversion of sexual bipolarity (Ojha & Turian 1971).

The control of differentiation of gametangial territories could be effected through the gradiental segregation of a respiration inhibitory factor as suggested by the masculinizing effect of acridines (Turian et al. 1969; Rønne & Olson 1976). However, the chemo-mechanical mediator of this genetical polarity is still unknown. Consequently, and in view of the recent biochemical trend to attribute such a role to the cytoskeleton (Quatrano 1978, Fulton 1984, McKerracher & Heath 1987), it became tempting to ascribe the vectorial cytoplasmic movement involved in such bipolar sexual morphogenesis to microfibrillar-microtubular elements of the cytoskeleton.

The antiactin chemical cytochalasin B can selectively interfere with the process of axis establishment and fixation at the germination of fucoid eggs (Quatrano et al. 1979). However, microtubules have also been implicated in the processes of maintenance of polar axiation at hyphal growth in fungi (Howard & Aist 1977). In fact, both microfilamentous and microtubular components of the cytoskeleton might coordinately

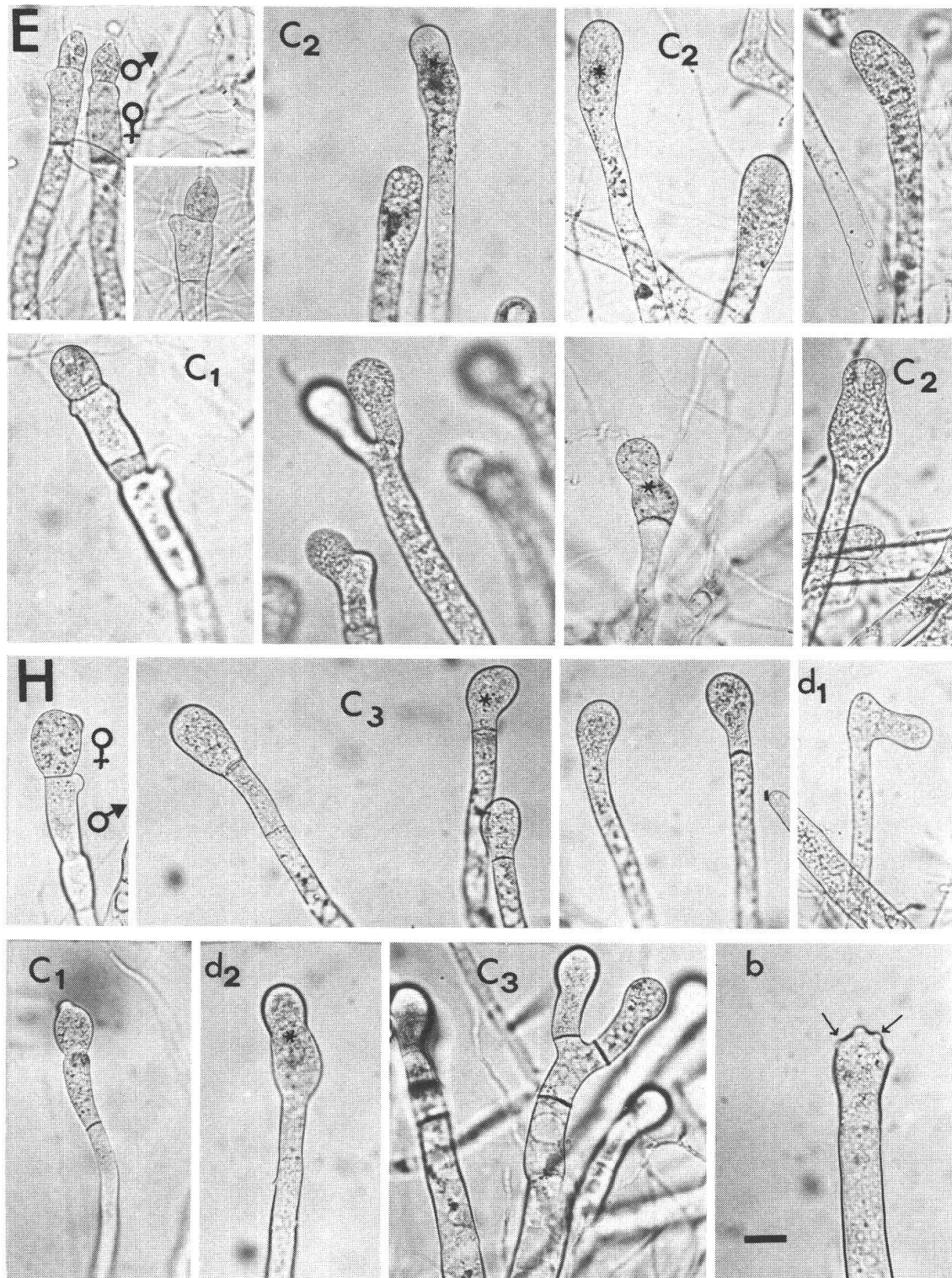


Plate I. Progressive disruption (from left to right) of the bipolar segregation of male-female epigynous (E) gametangia of *Allomyces macrogynus* and female-male hypogynous (H) gametangia of *A. arbuscula*. Dissipation of the inversely bipolar sexual gradients leads to uniformization of the cytoplasmic content, male dominance being visualized by the spreading of yellow, carotene-containing lipid granules (*) over the non-delimited, sexual "territories". Small vegetative colonies produced in semi synthetic medium (PYG) and transferred into wells of 1 ml dilute saline solution

establish and sustain polar axiation (Lloyd 1982). In this context, we therefore have explored the effect of inhibitors of both actin (cytochalasin E, Tannenbaum 1978) and tubulin (dimethylsulfoxide or DMSO, Capková 1986, and benlate, Howard & Aist 1977) on the process of axial, sexual segregation in the inversely bipolar species of *Allomyces*.

At relatively moderate concentration of the anticytoskeletal drugs used, the first morphological criteria of polar axiation, as shown by the development of club-like primers of gametangial couples, could generally still be reached (Plate I, c₁, c₂, d₂). Along such normally axiated structures, it was mainly the inverse bipolar segregation of sexual biochemical properties (male γ -carotene synthesizing enzymes and elicitors of nuclear multiplication *versus* female high ribonucleic acid synthesis and respiratory-competent mitochondria, see Turian 1975) which were selectively prevented in parallel to the presumed disruption of the polarizing microfibrillar and microtubular tracks. Only in a few extreme cases was the polar axis itself deviated to produce cross-like structures (Plate I, d₁) or simply shortened to an inflated apex (Plate I, e₂).

The effects described suggest that the presumed disruption of bipolarizing tracks would permit the overflow by the dominant male tendency of the presumptive female "territories" on the sexually differentiating hyphal apices of *both* epi- and hypogynous species. The suggestive conclusion of such effect is that DNA-controlled inverse bipolarity is mediated by the inversely-directed function of filamentous actin and/or tubulin microstructures vectorially conveying the sex-determining elicitors.

Résumé

Le gradient de sexualisation bipolaire, génétiquement inversé selon l'espèce d'*Allomyces*, peut être dissipé par des concentrations adéquates soit de l'agent antiactine cytochalasin E soit d'agents antitubuline DMSO ou benlate.

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(DS, Machlis & Ossia 1953) for the differentiation of their hyphal apices into couples of gametangia (3 h) or the various regressive structures observed after 6–7 h in the presence of: cytochalasin E, added to DS solution at the beginning of induction to final concentrations of 57 $\mu\text{g/ml}$ (c₁), 76 $\mu\text{g/ml}$ (c₂) and 95 $\mu\text{g/ml}$ (c₃); DMSO, 40 $\mu\text{g/ml}$, with cross-like effect (d₁) or doubly inflated structures (d₂) as also obtained with colchicine (20 $\mu\text{g/ml}$); benlate, 2 $\mu\text{g/ml}$ (b), in which papillae (arrows) could appear on the club-like structures induced on widened hyphae. Bar = 10 μm .

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