Actin stringently accumulated in the specifically positioned, differentiating female gametangia of *Allomyces*

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**Abstract**

Female gametangia of the normal bisexual Allomyces species are richer in fluorescently probed (FITC) actin, independent of their apical or subapical positioning during differentiation on the fertile hyphae. The anti-actin, cytochalasin D, can selectively suppress female differentiation in both species.

**Reference**


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Short communication

Actin stringently accumulated in the specifically positioned, differentiating female gametangia of Allomyces

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Summary. Female gametangia of the normal bisexual Allomyces species are richer in fluorescently probed (FITC) actin, independent of their apical or subapical positioning during differentiation on the fertile hyphae. The anti-actin, cytochalasin D, can selectively suppress male differentiation in both species.

Key words: Actin – Female Gametangia – Differentiation – Allomyces

Introduction

The aquatic molds of the genus Allomyces (Phycomycetes) present an experimentally attractive system of superposed gametangia specifically differentiated in an inverse position, namely epigynous (male-female) in A. macrogynus versus hypogynous (female-male) in A. arbuscula (Emerson and Wilson 1954). Their value in sex-ratio studies is enhanced by the easy recognition of the male gametangia by their yellowish-orange, $\gamma$-carotene pigmentation (Turian 1963; Ojha 1985).

By means of fluorescent probing we have recently shown the predominant localization of actin in female gametangia apically differentiated on the fertile hyphae of hypogynous A. arbuscula (Turian et al. 1992). However, this selective accumulation of actin may only have been the normal consequence of apical localization, which has been observed in all fungal hyphae studied so far (see references in Heath 1990). To establish a link between actin and female gametangial differentiation in the Allomyces, it was therefore deemed necessary to further probe actin localization in the epigynous A. macrogynus. The positive cytotopological link that was established led to the question of its stringency, which also could be positively answered by the selective prevention of female gametangial differentiation by the most effective anti-actin drug available, cytochalasin D (Tanenbaum 1978; Bereiter-Hahn and Strohmeier 1987; Betina 1989).

Materials and methods

The gametophytic phases of Allomyces arbuscula Butl. (strain Stumm) and A. macrogynus Em. and W. (strain Burma) were grown on standard Difco YpsS agar medium (Turian and Ojha 1987), and meiospores were liberated into sterile water. They were then germinated, grown to small colonies in semi-synthetic medium PYG (peptone, yeast extract, glucose) (Turian 1963), and differentiated into gametangia following their transfer to DS solution (Machlis and Ossia 1953).

For single-labeling fluorescence microscopy, the preparations were incubated in fluorescein isothiocyanate (FITC)-phalloidin (Sigma Biochemicals, St. Louis, Mo.) at a concentration of 10 $\mu$g/ml for 1 h at room temperature (Turian et al. 1992). After a final rinse the cells were mounted in p-phenylenediamine-glycerol (10% w/v) (Johnson and De Nogueira-Araujo 1981). A Leitz Orthoplan epiillumination microscope (Ernst Leitz, Wetzlar, FRG) equipped with fluoroptics and a selective filter combination was used for viewing the FITC-phalloidin fluorescence patterns. Photographs were taken on HP 5 Ilford black and white film (Ilford, Basel, Switzerland).

For the inhibition experiments, Cytochalasin D or CD (Serva Feinbiochemica, Heidelberg, FRG) was used. Meiospores and vegetative hyphae were grown on dialysis membranes (1.5 x 1.5 cm) placed on YpsSs medium. After 15 h of culture individual membrane-bound colonies were transferred either onto the same medium supplemented with the cytochalasin or into DS solution containing different concentrations of CD, dissolved in dimethyl sulfoxide (DMSO) to a final stock concentration of 1 mg/100 ml. The concentrations tested varied from 10 $\mu$g to 100 $\mu$g/ml. Controls consisted of mycelia treated with DMSO at the working concentrations as well as untreated mycelia. An Olympus microscope coupled with an Olympus camera C-35 AD-4 was used, and micrographs were taken on Kodak Ektachrome 64T.

Results and discussion

Actin has now been found to be predominantly localized not only in the apical female gametangium of the hypogynous A. arbuscula (Fig. 1b) but also in the female gametangium of the epigynous A. macrogynus (Fig. 1a).
Fig. 1a, b. Predominant localization of actin fluorescently probed with fluorescein-isothiocyanate (FITC)-phalloidin in the female gametangia of Allomyces; localization is independent of the specific positioning of the female gametangial with respect to that of the males. a Epigynous in A. macrogynus, b hypogynous in A. arbuscula. Note that fluorescent actin dots are restricted to the female gametangia. Bar: 20 μm

In both of the inversely positioned female gametangia, actin is visualized as fluorescent dots and as more diffuse fluorescent areas over the ribosomal nuclear caps of the differentiating gametes. In contrast, males, whether apically or subapically positioned, show a weak fluorescence during gametic differentiation (Fig. 1). These observations indicate that a close relationship exists between actin and female differentiation that is independent of their positioning on the fertile hyphae.

To further ascertain the stringency of the topo-cytological symmetry of actin-female differentiation, we attempted to prevent the synthesis/accumulation of actin in order to see which type of differentiation would be more detrimentally affected – the female type or the male type. We then tested the effects of the anti-actin cytochalasin D (CD), which is known to efficiently disrupt the fine, fibrillary actin meshwork (Bereiter-Hahn and Strohmeier 1987; Schliwa 1986).

Colonies of both species of Allomyces grown in the presence of 20–40 μg/ml of CD could only differentiate a few bisexual couples of gametangia. On 40 μg/ml, there were predominantly abnormal couples of incompletely separated male gametangia – an effect previously observed with CE (Turian and Ojha 1987; Turian et al. 1992) – as well as a few single males. It is only with 60 μg/ml CD that female differentiation was fully prevented, leaving only single yellowish-orange male gametangia on the tips of the fertile hyphae, and this occurred independently of their specific positioning in the control cultures (Fig. 2a–d).

Our observation of the differential accumulation of actin in the female gametangia presents the problem of whether the differential control is expressed by the actin gene(s), as suggested (Nguyen Thi et al. 1991); this gene is still uncharacterized in the Allomyces. Preliminary results obtained with a practically fully feminized (98 females/2 males) hybrid strain (A. arbuscula × A. macrogynus, Emerson and Wilson 1954) have shown that its sex-ratio drops to an average of 45/55 (females/males) after having been grown in the presence of 60 μg/ml CD. Inversely, the genetic male (“mas”) mutant of A. arbuscula (Stumm 1958) is undisturbed by the same concentration of CD (unpublished observation). Conversely to the situation in the female strain, male differentiation on the short hyphae of the “mas” mutant thus supports a relaxed expression of the actin gene(s).

The sex-linked difference in CD sensitivity confirms that the major localization of actin in the female gametangia is of functional significance for that type of sexual orientation. Presumably, it could be related to its cytoplasmic status – oxidatively competent mitochondria (Turian 1975; Olson 1984) – as regards the monocious type of Allomyces sexuality. There might therefore be a close interrelationship between the selective enrichment in ribosomal RNA that leads to the larger basophilic nuclear caps of the female gametes (Turian 1963) – as opposed to a deficiency in ribosomal genes in the male gametes (Ojha and Turian 1978) – and actin accumulation in the female zones. A subtle relationship could then occur between the high actin content of female gametangia and their high ribosomal RNA content, as has also been suggested for mRNA in the yeast system (Singer 1992).
The observation of a gradient of synthesis/accumulation of actin with its top either in the apical or the subapical female gametangium is relevant to the fundamental problem of the direction of the bipolar axis of sexual differentiation previously found to be controlled by an interspecifically transferable, positioning DNA (Ojha and Turian 1971).

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