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Inositol-phosphate signaling as mediator for growth and sexual reproduction in *Podospora anserina*

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ABSTRACT

The molecular pathways involved in the development of multicellular fruiting bodies in fungi are still not well known. Especially, the interplay between the mycelium, the female tissues and the zygotic tissues of the fruiting bodies is poorly documented. Here, we describe *PM154*, a new strain of the model ascomycetes *Podospora anserina* able to mate with itself and that enabled the easy recovery of new mutants affected in fruiting body development. By complete genome sequencing of *spod1*, one of the new mutants, we identified an inositol phosphate polykinase gene as essential, especially for fruiting body development. A factor present in the wild type and diffusible in mutant hyphae was able to induce the development of the maternal tissues of the fruiting body in *spod1*, but failed to promote complete development of the zygotic ones. Addition of *myo*-inositol in the growth medium was able to increase the number of developing fruiting bodies in the wild type, but not in *spod1*. Overall, the data indicated that inositol and inositol polyphosphates were involved in promoting fruiting body maturation, but also in regulating the number of fruiting bodies that developed after fertilization. The same effect of inositol was seen in two other fungi, *Sordaria macrospora* and *Chaetomium globosum*. Key role of the inositol polyphosphate pathway during fruiting body maturation appears thus conserved during the evolution of *Sordariales* fungi.

1. Introduction

Genetic analysis has been invaluable in deciphering the molecular events shaping development in animals and plants (Wolpert et al., 2010). The recovery of mutants blocked at various stages of morphogenesis has enabled to define the complex regulatory networks and cell-to-cell communications involved in the formation of organs in both these domains of life. As yet, no comparable regulatory scheme is available for fungi, although the fruiting bodies differentiated during sexual reproduction by many species are truly multicellular structures adopting defined shapes produced by precise developmental programs involving differentiated cells. Filamentous Ascomycota (*Pezizomycotina*) are well suited to study fruiting body development as most are readily cultivated in the lab and production of fruiting bodies can often be obtained rapidly. In ascomycetes, fruiting body formation is studied mostly in *Podospora anserina* (Silar, 2013, 2014), *Sordaria macrospora* (Teichert et al., 2014), *Neurospora crassa* (Chinnici et al., 2014) and *Aspergillus nidulans* (Dyer and O'Gorman, 2012). Fruiting bodies are composed of two tissues with different origins, zygotic and maternal. The binucleated zygotic tissue results from the fertilization of a female gametangium (ascogonium) by a male gamete (antheridium or spermatium) and produces the progeny (ascospores). Nonetheless, development starts without an obvious fertilization event in homothallics, although a binucleated zygotic tissue is present. The zygotic tissue is mixed with hyphae of maternal origin in the centrum, the mass of cells that is constituted of both maternal hyphae, such as paraphyses and related hyphae, and zygotic tissues (Fig. 1). The maternal hyphae of centrum are called the hamathecium. The centrum is protected by an outer layer of maternal tissues called the peridium. In addition, proper fruiting body development requires appropriate growth conditions of the mycelium and is affected by genetic defects unrelated to development *per se*, since many auxotrophic mycelia are unable to nurture fruiting bodies to maturation (Debuchy et al., 2010). Indeed, fruiting bodies likely rely only on the nutrient reserves present in the mycelium to mature and these must be acquired and stored properly. How the three tissues of

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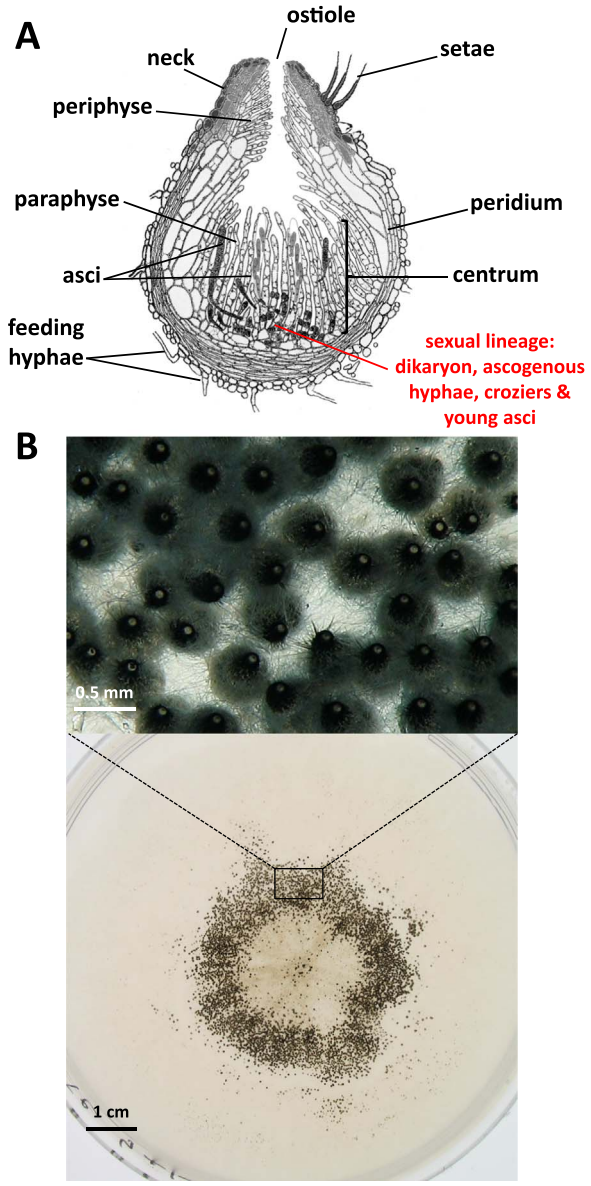

Fig. 1. *P. anserina* fruiting bodies. (A) Schematic representation of a nearly mature fruiting body. The peridium is constituted exclusively from maternal tissues, while the centrum contains the zygotic lineage embedded with maternal hyphae that structure the perithecial content (*i.e.*, the paraphyses), enabling proper orientation of the asci, whose ascospores are ejected through the ostiole. (B) Actual fruiting bodies four days after fertilization and about to expel ascospores. As seen on the bottom Petri plate, their repartition is not random on the thallus.

different origins interact to produce fruiting bodies is at the present time unknown. It is known that in the four model ascomycetes, ascogonia, and spermatia when present, are differentiated first (ascohymenial development), in opposition to some other species in which the maternal tissues protecting the fruiting bodies are differentiated before the ascogonium and the male gametes (ascolocular development). The ascogonia often recruit maternal hyphae to form a resting structure that awaits fertilization in heterothallic species, the fungi that require two genetically distinct partners for sexual reproduction (*e.g.*, the protoperithecium). Once fertilized, there is a coordinated growth of the maternal and zygotic tissues, as to obtain fruiting bodies, which will accommodate the production of ascospores. Uncontrolled proliferation of the zygotic tissues may for example results in the bursting of the fruiting body, which could impair further development. In ascohymenial development, it is thus likely that the zygotic tissues orchestrate development of the whole fruiting body. The molecular signaling pathway(s) coordinating development is(are) presently unknown. However, important roles of MAP kinases, the STRIPAK complex and NADPH oxidases have been evidenced in the studied species (Debuchy et al., 2010; Peraza-Reyes and Malagnac, 2016; Teichert et al., 2014).

The filamentous ascomycete *P. anserina* is a good model to study development of fruiting bodies (called perithecia in this species; Fig. 1) through a direct genetic approach (Debuchy et al., 2010; Lalucque et al., 2017; Silar, 2013, 2014). Indeed, its one-week life cycle enables the rapid screening of sterile mutants and many mutants lacking sexual reproduction are available. Moreover, fruiting bodies localization (Fig. 1) is not random on the thallus (Coppin et al., 2012; Malagnac et al., 2008), suggesting that complex interactions between the mycelium and the fruiting bodies exist, so that only a subset of fertilized protoperithecia located in the appropriate zone will continue their maturation. Perithecia are indeed mostly located in a ring-shape region whose internal diameter is about 1 cm and whose width is about 1 cm. Mutants affecting the pattern of repartition of perithecia are available (Ait Benkhali et al., 2013; Coppin et al., 2012; Malagnac et al., 2013), as well as mutants affecting fruiting development, especially mutants that are sterile. Most of the available sterile mutants in *P. anserina* are affected in the maternal tissues of the perithecia or in the mycelium (Coppin et al., 2012; Debuchy et al., 2010; Silar, 2013, 2014), which is required to properly nurture the fruiting bodies. Indeed, fertilization in *P. anserina* necessitates two genetically different partners, rendering selection of recessive mutants affected in the zygotic tissue a work-consuming process (Simonet and Zickler, 1972), unlike in the homothallic *S. macrospora*, for which many developmental mutants are available (Teichert et al., 2014). To facilitate the screening of such mutations, we describe here the construction of a strain carrying in a stable manner the two mating type idiomorphs of *P. anserina*, and the use of this self-fertile strain in the generation of mutants affected at various stages of development and in the two different tissues of the perithecium. This strain renders the screening recessive mutation as easy as in *S. macrospora*. We then go on by identifying the gene responsible for the early blockage of *spod1*, one such mutant, as encoding an inositol polyphosphate kinase (IPK) orthologous to ARG82p of *S. cerevisiae*. Our analyses of the mutant point towards inositol and its phosphate derivatives as likely key players of sexual reproduction in *P. anserina* by promoting differentiation of both the maternal and zygotic tissues of the fruiting bodies and regulating the position of the fruiting bodies on the mycelium.

2. Materials and methods

2.1. Strains and growth conditions

All strains of *P. anserina* used in this study derived from the "S" (big S) wild-type strain that was used for sequencing of the *P. anserina* genome (Espagne et al., 2008; Grognet et al., 2014), except when indicated. The *S. macrospora* strain was 000 (Gagny et al., 1997) and the *C. globosum* one was DSM62.110. Both were grown on the M2 minimal medium used for *P. anserina*. The most recent protocols for standard culture conditions, media and genetic methods for *P. anserina* can be accessed at http://podospora.igmors.u-psud.fr/more.html. The *S Δmus51::phleoR* mutant strain differed from the S wild-type reference strain by a single deletion of the *mus51* gene, which increased frequency of targeted gene replacement (Lambou et al., 2008). Strains are available upon request.

The *PaIPK2*$^{\Delta}$ *mat-*/*PaIPK2*$^{+}$ *mat+* strains was maintained on medium containing nourseothricin (40 μg/mL), as to avoid the loss of nuclei carrying *PaIPK2*$^{\Delta}$. For the recovery of homokaryotic thalli, the mycelium of this strain was fragmented using a Fastprep instrument (MPBiomedicals, Santa Ana, CA., U.S.A.) at speed 4.0 for 20 s.