Workshop III

Mating and sexual development
Chair:Gillian Turgeon

IIIo-1

HOMOTHALLISM VS HETEROTHALLISM: DETERMINANTS

*B. Gillian Turgeon, Shun-Wen Lu, Shinichi Oide, and Sung-Hwan Yun**
Dept. of Plant Pathology, Cornell University, Ithaca, NY, 14853
Ph: 607 254 7458, fax 607 255 8835
**Division of Life Sciences, Soonchunhyang University, Asan 336-745, Korea*

Fungi capable of sexual reproduction use heterothallic (self-sterile) or homothallic (self-fertile) mating strategies. In most ascomycetes, a single mating type locus, MAT, with two alternate forms (MAT1-1 and MAT1-2) called idiomorphs, controls mating ability. In heterothallic ascomycetes, these alternate idiomorphs reside in different nuclei. In contrast, most homothallic ascomycetes carry both MAT1-1 and MAT1-2 in a single nucleus, usually closely linked. Using a pair of closely related loculoascomycete species, heterothallic Cochliobolus heterostrophus and homothallic C. luttrellii as models, we undertook a series of MAT manipulations to determine whether deletion of the MAT loci from either species and replacement with the MAT genes from the alternate species which differs in reproductive strategy, could confer heterothallism on a MAT-deleted strain of the homothallic species or, conversely, homothallism on a MAT-deleted strain of the heterothallic species. In both cases we effected a change in reproductive life style, indicating that the determinants of reproductive life style reside at MAT. In addition, we have experimented with the single MAT locus in the pyrenomycete, Gibberella zeae, a homothallic species that is capable of both selfing and outcrossing. We asked if G. zeae could be made strictly heterothallic by manipulation of MAT. Targeted gene replacement was used to differentially delete MAT1-1 or MAT1-2 from a wild type haploid MAT1-1;MAT1-2 strain, resulting in MAT1-1;mat1-2, mat1-1;MAT1-2 strains that were self-sterile, yet able to cross to wild type testers and more importantly, to each other. These results indicated that differential deletion of MAT idiomorphs eliminates selfing ability of G. zeae, but the ability to outcross is retained. They also indicated that both MAT idiomorphs are required for self fertility.

IIIo-2

THE PHEROMONE RESPONSE PATHWAY OF THE HOMOTHALLIC ASCOMYCETE SORDARIA MACROSPORA

Nicole Nolting, Severine Mayrhofer, Cathrin Wörmann, Christian Würtz and Stefanie Pöggeler,
Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, 44780 Bochum, Germany,
Phone: 0049-234-3224264, Fax: 0049-234-3214184,
e-mail; nicole.nolting@ruhr-uni-bochum.de
Session: 3. Mating and Sexual Development

In order to analyze the involvement of pheromones in cell recognition and mating in homothallic and heterothallic ascomycetes, two putative pheromone precursor genes, named ppg1 and ppg2, were isolated from the homothallic fungus Sordaria macrospora. The ppg 1 gene is predicted to encode a precursor pheromone that is structurally similar to the alpha-factor of the yeast Saccharomyces cerevisiae. The ppg2 gene encodes a 24-amino-acid polypeptide that contains a putative farnesylated and carboxy-methylated C-terminal cysteine residue (1). The expression of both pheromone genes was analysed in vivo by means of the egfp reporter gene. Disruption of both pheromone precursor genes revealed that pheromones are involved in the fruiting body development. In addition to the pheromone genes, we have identified two pheromone receptor genes, named pre1 and pre2. The deduced gene products are putative seven-transmembrane proteins (2). We also have identified the coding capacity for a set of proteins that could be involved in the pheromone response pathway. We were able to shown that all of these genes were transcriptionally expressed in S. macrospora. Among the genes we analysed was the S. macrospora mcm1 gene encoding a putative MADS-box transcription factor. In yeast the MCM1 protein has been shown to serve as a transcriptional regulator for mating-type specific genes. Little is known about its role in fruiting-body development in a homothallic species. A yeast two hybrid-analyses revealed that the S. macrospora MCM1 protein has the capability to interact with the mating-type protein SMTA-1. This result suggests an involvement of the MCM1 protein in the transcriptional regulation of mating-type specific genes in S. macrospora.

(1) Pöggeler S (2000) Two pheromone precursor genes are transcriptionally expressed in the homothallic ascomycete *Sordaria macrospora*. Curr Genet 37: 403-411
(2) Pöggeler S, Kück U (2001) Identification of transcriptionally expressed pheromone receptor genes in filamentous ascomycetes. Gene 280: 9-17

SC15 OF SCHIZOPHYLLUM COMMUNE MEDIATES FORMATION OF AERIAL HYPHAE AND ATTACHMENT TO HYDROPHOBIC SURFACES IN THE ABSENCE OF SC3

J.F. de Jong [2], O.M.H. de Vries [1], R. Jalving [1], J. Dijksterhuis [3], H.A.B. Wösten [2], J.G.H. Wessels [1] & L.G. Lugones [1,2]

[1]Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Department of Molecular Plant Biology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands, [2]Microbiology, Institute of Biomembranes, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands, Tel: 31 30 2533854, Fax: 31 30 2513655, [3]Centraal Bureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. E-mail: j.f.dejong@bio.uu.nl; l.g.lugones@bio.uu.nl

Formation of aerial hyphae in S. commune is used as a model to understand the initial stages of fruiting body formation in basidiomycetes. The SC3 hydrophobin plays a pivotal role in this process. It lowers the surface tension of the medium, enabling aerial growth, and it coats the aerial hyphae rendering them hydrophobic. SC3 also allows hyphae to attach to hydrophobic surfaces. A 15 kDa protein (SC15) co-purifies with self-assembling SC3. SC15 is secreted into the medium but is also found in the cell walls of aerial hyphae. Both SC3 and SC15 are regulated by the B mating type genes and the thn gene.

To investigate whether SC15 has a function in aerial hyphae formation and attachment of hyphae to hydrophobic substrates, the SC15 gene was deleted. The ΔSC15 strain formed hydrophobic aerial hyphae like the wild-type strain and attachment to hydrophobic surfaces was not affected. In contrast, formation of aerial hyphae and attachment was reduced in the ΔSC3 strain and almost completely abolished in the ΔSC3ΔSC15 strain. The absence of aerial hyphae in static liquid cultures of the latter strain is due to the inability to lower the water surface tension of the medium. These data show that SC15 mediates formation of aerial hyphae and attachment in the the absence of SC3. A natural isolate obtained from a forest near Wageningen in The Netherlands produced both SC3 and SC15 on minimal medium but only SC15 when grown on wood. From this it is concluded that in nature SC15 may function to enable growth in a non-aqueous environment.

REGULATING NETWORKS RELATING FILAMENTOUS GROWTH AND PATHOGENICITY IN USTILAGO MAYDIS

Miroslav Vranes, Mario Scherer, Jörg Kämper
Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., 35043 Marburg, Germany; Phone:+496421-178632; FAX: +496421-178609; vranes@staff.uni-marburg.de

The phytopathogenic fungus Ustilago maydis is the causal agent of smut disease on corn. For successful infection of the host plant two compatible haploid sporidia have to fuse and form a filamentous, infectious dikaryon. Sexual development is controlled by two loci, a and b. The a locus encodes a pheromone/receptor system that is required for cell recognition and cell fusion. The transition of the resulting dikaryon to a true filament and all subsequent steps in sexual and pathogenic development are controlled by the multiallelic b-mating type locus. It encodes the two distinct homeodomain proteins bE and bW. The heterodimeric complex formed by the bE and bW proteins is thought to achieve its function as a transcriptional regulator of pathogenicity genes either directly by binding to cis regulatory sequences or indirectly via b-dependent regulatory cascades.
The formation of a functional bE/bW-heterodimer is accompanied by morphological changes from yeast-like to filamentous growth. Independently from b, filamentous growth can be induced by decreasing the cellular cAMP level. Deletion mutants lacking the adenylyl cyclase (uac1) exhibit filamentous growth, but are not pathogenic.

Based on the U. maydis genome sequence, an Affymetrix GeneChip® was designed that allows parallel expression analysis of more than 6200 U. maydis genes. We have employed the DNA array technology to compare the expression profiles of U. maydis genes during cAMP-dependent and b-dependent morphological changes. Our aim is to use expression kinetics to dissect the distinct roles of the b-mating type during morphogenesis and pathogenicity.

IIIo-5

FRUITING BODY DEVELOPMENT OF SORDARIA MACROSPORA: MUTANT COMPLEMENTATION AND FUNCTIONAL GENOMICS ANALYSES IDENTIFY REGULATORY NETWORKS

Minou Nowrousian[1], Christine Rech[1], Ines Wißmann[1], Carol Ringelberg[2], Jennifer J. Loros[2], Jay C. Dunlap[2], Stefanie Pöggeler[1], Ulrich Kück[1]

[1]Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Universitätstr. 150, 44780 Bochum, Germany, phone +49 (0)234 3224588, fax +49 (0)234 3214184, email: minou.nowrousian@ruhr-uni-bochum.de, [2]Dartmouth Medical School, Departments of Genetics and Biochemistry, Hanover, NH 03755, USA

The filamentous fungus Sordaria macrospora forms complex fruiting bodies (perithecia) which protect the developing ascospores and ensure their proper discharge. To gain insights into the molecular mechanisms that control this developmental process, we are analyzing sterile mutants that are blocked at the stage of protoperithecia formation. Mutants pro1, pro11, and pro22 have already been complemented by transformation with an indexed cosmid library. pro1 encodes a transcription factor whereas pro11 is a membrane-associated protein that has been implied as a scaffold protein of several signal transduction pathways (Masloff et al. (2002) Fung Genet Biol 36:107-116; Pöggeler and Kück (2004) Eukaryot Cell, in press). pro22 is a homolog of the N. crassa ham-2 gene, a putative membrane protein mediating hyphal fusion. In addition to the identification of genes essential for fruiting body formation, we are currently trying to establish relationships between these genes. To this end, we have conducted microarray hybridizations of N. crassa cDNA arrays with S. macrospora targets. We were able to identify a number of genes that are more than twofold up- or downregulated in mutant pro1 compared to the wild type. Among the genes that are up- or downregulated in the mutant strain are the pheromone precursor genes, several genes involved in cell wall biosynthesis and structure, and putative regulatory genes. One of the genes that is downregulated in the pro1 mutant encodes a novel protein which as a GFP fusion protein locates to the cell wall/membrane region of the hyphae. Transcript levels of some of the genes regulated in mutant pro1 were analyzed in mutants pro11, pro22, and all combinations of double mutants using real time PCR. These molecular epistasis analyses show that the pro-mutants can be grouped in at least two different classes according to their expression patterns. They also indicate that in some of the mutants, residual mutant gene products have an effect on gene expression.

IIIo-6

SEX AND MATING IN ASPERGILLUS; ASEXUAL OR NOT?

Mathieu.Paoletti, Elke Schwier[§], David Archer and Paul Dyer.
School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, UK.
Phone: 44 (0) 115 951 3203, Fax 44 (0) 115 951 3251,
e-mail: Paul.Dyer@Nottingham.ac.uk
[§]Institute of Microbiology and Genetics, Georg-August-Universität, Grisebachctrasse 8, D-37077 Göttingen, Germany.

The aspergilli provide an ideal opportunity to investigate sex and mating as the genus includes both (supposedly) asexual species, and also sexual species - with the latter exhibiting either homothallic or heterothallic (requires compatible MAT-1 and MAT-2 isolates) breeding systems. The genus includes the model organism Aspergillus nidulans, as well as a number of species important to the food industry, and species pathogenic to humans such as A. fumigatus. Only three heterothallic (obligate outcrossing) species are known.
We first investigated reproduction in the homothallic species A. nidulans. A combination of experimental molecular biology and genomic approaches lead to the identification of MAT-1 and MAT-2 idiomorphs, as well as a number of genes involved in the pheromone response pathway of heterothallic species. Semi-quantitative RT-PCR analysis revealed that these genes are expressed at a low level under conditions favouring asexual conidiation, but are highly stimulated under conditions promoting sexual reproduction. Thus, this homothallic species has the functional 'sexual machinery' characteristic of heterothallism. In addition, microsynteny around MAT loci indicates that A. nidulans probably originated from an ancestral heterothallic species by chromosome translocation event(s).
Using known sequences of MAT-1 and MAT-2 genes from Aspergillus we designed sets of mating-type specific degenerate primers and analysed isolates of a range of Aspergillus species which exhibit different reproductive strategies. Isolates of the heterothallic species A. heterothallicus contained either a MAT-1 or MAT-2 gene. Surprisingly, all 6 asexual species analysed (A. fumigatus, A. flavus, A. sojae, A. parasiticus, A. niger, A. oryzae) also contained mating-type genes, with a 'heterothallic' distribution evident, with different isolates having either a MAT-1 or a MAT-2 gene. In addition, preliminary results indicate that a number of genes involved in the pheromone response pathway are present and expressed in asexual aspergilli, indicating that asexual species might have the potential for sexual reproduction.