Available online at www.sciencedirect.com

SCIENCE DIRECT

Gene 348 (2005) 33–44

GENE
SECTION
FUNCTIONAL GENOMICS

www.elsevier.com/locate/gene

Sexuality and parasitism share common regulatory pathways in the fungus *Parasitella parasitica*

Kornelia Schultze, Christine Schimek, Johannes Wöstemeyer, Anke Burmester*

Institute for Microbiology, Friedrich-Schiller-Universität Jena, Chair of General Microbiology and Microbe Genetics, Neugasse 24, D-07743 Jena, Germany

Received 27 November 2003; received in revised form 27 November 2004; accepted 6 January 2005

Received by W. Martin

Abstract

Parasitella parasitica, a facultative mycoparasite of zygomycetous fungi, forms cytoplasmic fusions with its hosts during infection. Thus, the organism is an efficient donor of genetic material in parasexual host–parasite interactions. Recognition between parasite and host is mediated by trisporoids, which are also responsible for sexual communication. The *TDH* gene for one of the key enzymes of trisporic acid biosynthesis, 4-dihydromethyl-trisporate dehydrogenase, was cloned and its transcription analysed. *TDH* was cloned on a 6175-bp insert and was found to map in a complex cluster of genes that suggest post-transcriptional antisense regulation. Histochemical TDH analysis in developing parasitic or sexual structures shows high enzymatic activity in *Parasitella*. *TDH* is linked to a gene for a putative acyl-CoA thioesterase (*ACT*). Two *ORF*s were identified in the 5′-region of the *TDH* gene, a third one, coding for 176 amino acids overlaps the *ACT* gene in antisense direction completely. Expression levels of *ACT* and *ORF1* depend on parasitic and sexual interactions.

Keywords: Acyl-CoA-thioesterase; Antisense RNA; Retinoid; Trisporic acid; Zygomycetes

1. Introduction

Parasitella parasitica is a biotrophic fusion parasite establishing a limited cytoplasmatic continuum with its host during infection. At the morphological level, fusion followed by interspecific nuclear migration was seen as early as 1924 (Burgeff, 1924). The interaction between *P. parasitica* and its hosts was anticipated to be a parasexual system by Lederberg (1952), but genetic and molecular evidence for efficient horizontal gene transfer of essentially any gene in this system was supplied only half a century later (Kellner et al., 1993; Wöstemeyer et al., 2002).

P. parasitica infects many different mucoralean species from nearly all major genera (Burgeff, 1924; Wöstemeyer et al., 1997). Several host species, especially from the genera *Absidia*, *Blakeslea*, *Mucor*, *Rhizopus*, and *Thamnidium* are used for a variety of biotechnical purposes like regio- and stereospecific biotransformation of steroids, isolation of rennin-like acidic proteases for cheese fermentation or, with increasing importance, the production of β-carotene by mated *Blakeslea trispora* cultures. Especially, this latter process has stimulated the interest in sexual differentiation of zygomycetes, as the yield of β-carotene production is strongly increased in sexually stimulated cultures. The parasexual *Parasitella* system has been proposed as a tool for genetic manipulation within its host

Abbreviations: ACT, acyl-CoA thioesterase; ATCC, American Type Culture Collection, USA; bp, base pairs; *carB*, carotene biosynthesis gene; CBS, Centraalbureau voor Schimmelcultures, The Netherlands; cDNA, DNA complementary to RNA; EMBL, European Molecular Biology Laboratory; kb, kilobase; mRNA, messenger RNA; nt, nucleotide; ORF, open reading frame; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; rRNA, ribosomal RNA; RT-PCR, reverse transcriptase polymerase chain reaction; TDH, 4-dihydromethyl-trisporate dehydrogenase; U, unit.

* Corresponding author. Tel.: +49 3641 949 310; fax: +49 3641 949 312.
E-mail address: b5buan@rz.uni-jena.de (A. Burmester).

0378-1119/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2005.01.007

range, an extremely appealing option, as zygomycetous fungi in general are not amenable to strain improvement by conventional genetics due to intrinsic difficulties with the germination of sexual spores (Wöstemeyer et al., 2003).

Interactions between *P. parasitica* and *Absidia glauca* depend on mating type compatibility. Exclusively the plus mating type of *P. parasitica* infects minus type *A. glauca* strains, whereas minus mating type *P. parasitica* strains parasitise on plus type *A. glauca*. This observation led to the assumption that the sexual hormone system, comprising the retinoid compound trisporic acid (Austin et al., 1969) and its precursors, is also involved in host recognition and may thus be regarded as pathogenicity factor. Although the metabolic diversity between trisporoids from different species is intriguingly pronounced at the level of isoforms, the pheromone communication system is not strictly species specific. Physiologically complementary mating types of different species tend to undergo early steps of the sexual differentiation pathway (Blakeslee and Cartledge, 1927). The molecular basis of interspecific recognition relies on trisporoid compounds. Recently, Schimek et al. (2003) have provided physiological evidence that trisporoids represent the general principle of mating recognition in Mucorales and, surprisingly, in the only distantly related Mortierellales.

Mating type specific pheromones, derived via the breakdown of β-carotene, stimulate the development of sexually committed hyphae, which later develop into gametangia as soon as contact between mating partners is established. The mating type specific pheromones are converted to trisporic acid by the cooperative enzymatic action of complementary partners (Nieuwenhuis and van den Ende, 1975). One of the key enzymes of that biosynthetic pathway, the 4-dihydromethyl-trisporate dehydrogenase, was purified from *Mucor mucedo,* and the corresponding gene was isolated and sequenced (Czempinski et al., 1996).

Analysis of genomic organisation and transcriptional regulation of the 4-dihydromethyl-trisporate dehydrogenase (*TDH*) gene and adjacent regions in *P. parasitica* will help to elucidate the relationship between sexual and parasitic lifestyle.

2. Material and methods

2.1. Microbial strains

Genomic libraries were maintained in *Escherichia coli* strain XL1blue (Stratagene). *P. parasitica* and *A. glauca* strains listed in Table 1 are available from Dr. K. Voigt at Fungal Reference Centre, University of Jena (b5kevo@uni-jena.de). Expression analysis of host–parasite combinations were performed with the *A. glauca* strain pair FSU 659/660 and the *P. parasitica* pair FSU 387/388.

Table 1
Strain list

Species	Mating type	Strain No.	Origin
Absidia glauca	plus	FSU 659	ATCC 6776a
	minus	FSU 660	ATCC 6776b
Parasitella parasitica	plus	FSU 387	CBS 412.66
	minus	FSU 388	ATCC 11077
	plus	FSU 327	CBS 207.28
	minus	FSU 328	CBS 208.28

2.2. Isolation of genomic regions and DNA sequencing

Genomic DNAs of *P. parasitica* strains were prepared as described previously (Schilde et al., 2001). Southern hybridizations using the heterologous *Mucor TDH* probe allowed the choice of suitable restriction enzymes for creating partial genomic libraries. *P. parasitica* FSU 388 genomic DNA was restricted with *Eco*RI and *Pst*I, and random fragments with lengths between 9 and 12 kb were ligated into pTZ19R, followed by transformation of *E. coli* by electroporation (Dower et al., 1988). Approximately 3000 clones were used for colony filter hybridization. They were probed with the heterologous *TDH*-PCR fragment of *M. mucedo* (Czempinski et al., 1996). Four independent clones with a 10-kb insert were selected. Subclones containing the *TDH* genes were identified by PCR using primers derived from *Mucor* DNA sequences. A region of 6175 bp was sequenced completely following methods described by Voigt and Wöstemeyer (2001) using a combined subclone and primer walking strategy (EMBL accession no. AJ509873). PCR fragments containing the corresponding DNA region of the plus type strain FSU 387 were also cloned and sequenced completely (EMBL accession no. AJ604529).

2.3. Hybridization analyses and RT-PCR

Isolation of total RNA and hybridization conditions for Northern (Vetter et al., 1994) and Southern hybridization analyses (Burmester and Czempinski, 1994) were as described previously. PCR products obtained with primers 3 and 5 were radioactively labelled using the 'hexalabel' DNA label kit of Fermentas, Germany. The labelled probe detects *ORF3* transcripts as well as *ACT* transcripts. PCR with primers 5 and 19 amplify a 380-bp fragment of *ACT,* which was used for strand-specific labelling. Reaction mix for *ACT* amplification with a final volume of 25 μl contained 200 ng PCR fragment, 50 mM Tris–Cl pH 8.8, 16 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 1.7 mM $MgCl_2$, 26.4 μM of dATP, dGTP, and dTTP each, 0.4 μM α^{32}P-dCTP (111 TBq/mmol), 0.4 μM primer 19, and 1 U Taq polymerase. Amplification conditions were 5 min 94 °C, 0.3 min 55 °C, 1 min 72 °C for cycle 1, 0.3 min 94 °C, 0.3 min 55 °C, 1 min 72 °C for cycles 2–30. The reverse transcription mix contained 0.7 μg total RNA, 0.32 μM specific primer, 0.31 mM dNTP mix, 31 mM Tris–Cl pH