

RESEARCH ARTICLE

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M. E. Schweingruber**Cordycepin in *Schizosaccharomyces pombe*: effects on the wild type and phenotypes of mutants resistant to the drug**Received: 28 March 2003 / Revised: 13 May 2003 / Accepted: 18 May 2003 / Published online: 25 June 2003
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Abstract The adenosine analogue cordycepin (3'-deoxyadenosine) inhibits growth and causes aberrant cell morphology in the fission yeast, *Schizosaccharomyces pombe*. Exogenously added thiamine, the pyrimidine moiety of the thiamine molecule, and adenine alleviate its growth-disturbing effect. At concentrations that do not inhibit growth, the drug reduces mating and sporulation and causes a decrease in the mRNA level of gene *ste11* and the *ste11*-dependent gene, *mei2*. The mating- and sporulation-inhibiting effect of cordycepin is overcome by adenine. A mutant disrupted for the *ado1* gene encoding adenosine kinase exhibits a cordycepin-resistant and methionine-sensitive phenotype, excretes adenosine into the medium and mates and sporulates poorly in the presence of adenine. A *S. pombe* mutant containing a frameshift mutation at the beginning of the carboxy-terminal half of gene *ufd1* (the *Saccharomyces cerevisiae* *UFD1* homologue) is cordycepin-resistant and sterile. Strains disrupted for the *ufd1* gene only form microcolonies.

Keywords Cordycepin · *Schizosaccharomyces pombe* · Adenosine kinase · *ado1* · *UFD1* homologue

Introduction

The antibiotic cordycepin (3'-deoxyadenosine) is an adenosine analogue which in eukaryotic cells, including yeast, was shown to inhibit polyadenylation by preventing the addition of the poly(A) tail to the 3'-cleaved mRNA (Zeevi et al. 1981; Butler et al. 1990). Cordyce-

pin 5'-triphosphate is added by poly(A) polymerase to RNA ends and blocks further extension because of the lack of a 3'-OH.

Early studies suggested that *Saccharomyces cerevisiae* is resistant to cordycepin (Anderson and Roth 1974). Later it was found that strains cultivated in thiamine-free media are more sensitive to cordycepin than those in thiamine-containing media and that thiamine increases growth resistance for the drug (Iwashima et al. 1992). Being interested in the thiamine metabolism of the fission yeast *Schizosaccharomyces pombe* and drugs that interfere with this metabolism (Schweingruber et al. 1992; Fankhauser et al. 1995; Hilti et al. 1999), we investigated the effects of cordycepin in fission yeast. In this report, we show that cordycepin inhibits growth, mating and sporulation in this organism and describe the cordycepin-resistant phenotype of mutants defective in adenosine kinase and the *S. cerevisiae* *UFD1* homologue.

Material and methods

Strains, media and chemicals

All strains used in this study (972 *h*⁻, 975 *h*⁺, 968 *h*⁹⁰, *ura4*-D18 in the different mating-type configurations) were from our collection in Bern, Switzerland. The diploid strain *h*⁻*ade6*-M210*ura4*-D18/*h*⁺*ade6*-M216*ura4*-D18 used for disruption of the *ufd1* gene was constructed by standard genetic methods (Gutz et al. 1974). Liquid Difco yeast nitrogen base without amino acids (YNB) or the synthetic liquid (MM) and solidified (MMA) minimal media described by Schweingruber and Edenharter (1990) were used. Added supplements were as indicated in the text. Cordycepin was from Sigma.

crs strains (cordycepin-resistant, sterile) mentioned in this communication were selected by plating 10⁷ cells of strain *ura4*-D18 *h*⁹⁰ on a MMA plate containing 80 mg uracil/l and 200 μM cordycepin and by isolating those strains that formed colonies which did not stain with the iodine vapour assay on MMA plates containing no cordycepin.

Mating and sporulation assay

The protocol for quantifying mating and sporulation in liquid MM was described in detail by Schweingruber and Edenharter (1990). A

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total of 500–1,000 units of vegetative cells, zygotes and asci were counted.

Disruption of genes *ado1* and *ufd1*

ado1

The disruptant strain $h^{90-}ado1::kanMX\ ura4-D18$ was constructed using the PCR-based gene-targeting strategy described by Bähler et al. (1998). Strain $h^{90}ura4-D18$ was transformed with a PCR fragment containing the *kanMX* cassette flanked by sequences homologous to the 5'- and 3'-flanking sites of the adenosine kinase encoding gene SPCC338.14. As template, 50 ng of plasmid pFA6-kanMX6 (a gift from J. Bähler) were used. The PCR fragment was generated using the primers DelAKforw (5'-CATAATATCGCTCTTTGAACATTAAGATACTTTACCTGTTGGTGATTTTC TCGTGGTTTTCTTTAACTTTTCCCTTCCCTTACTACAA TCATTTATCCGGATCCCCGGGTTAATTAA-3') and DelAKrev (5'-TTGCAATCATAGTGTGCACAATTTTAAAGTTAA ACATCTAATTAACGAAGTGCCTTATCTACGTTGACA GTTTAAAAAATCACTAAAATGCAATAGTGAATTCGAGC TCGTTTAAAC-3'). The product of five PCR reactions was pooled, precipitated and directly used for transformation. G418-resistant cells were selected and tested by PCR for correct integration of the *kanMX* cassette.

ufd1

This gene was disrupted in the diploid strain $h^{-}ade6-M210ura4-D18/h^{+}ade6-M216ura4-D18$, using a cassette containing the *ura4* gene as described for the disruption of *sam1* gene (Hilti et al. 2000). Plasmid pUR19 *ufd1A1*, a genomic clone from the gene library pURSP1 containing the *ufd1* gene, was digested with *Clal*. The generated fragment containing the complete *ufd1* ORF was cloned into pGEM-7Zf(+) (Promega) and the resulting construct, pGEM-7Zf*ufd1*, was digested with *HindII* and *BstEII* to remove about 1,100 bp of the *ufd1* ORF, including the start codon. Primers containing the restriction sites *BstEII* and *HindII* (5'-GCGCGC GGTNACCAAGCTTCCACTGGCTATATGTATGC-3', 5'-GC CGCCGYRACCCAACACCAATGTTTATAACC-3') were used to generate a PCR fragment containing the ORF of the *ura4* gene, using plasmid pB4-2[+ SphI] as template. This fragment was digested with *HindII* and *BstEII* and ligated into pGEM-7Zf*ufd1* to create plasmid pGEM-7Zf *ufd1::ura4*. The *ufd1::ura4* insert of this plasmid was cut-out by a *Clal* digestion and used to transform the diploid strain $ade6-M210\ ade6-M216\ ura4-D18$. Correct integrants (which had the *ufd1* gene replaced by the *ufd1::ura4* fragment) were verified by PCR and Southern blot analyses. Diploid strains having one *ufd1* copy disrupted were sporulated and tetrads were analysed by standard methods.

Cloning of the genes *ado1* and *ufd1*

ado1

Cosmid c338 (obtained from the Sanger Center) was digested with the restriction enzyme *PstI*. The largest fragment (about 17 kb) was extracted from the gel, using the QIAquick gel extraction kit

(Qiagen), and was digested with *AvaI* and *HindIII* to obtain a 4,037-bp fragment containing the complete ORF of the *ado1* gene. The fragment was ligated into the shuttle vector pUR19 (Barbet et al. 1992) which was digested with the same enzymes and dephosphorylated with shrimp alkaline phosphatase (Roche). Ligation was achieved using the Rapid DNA ligation kit (Roche). Plasmids were checked by restriction enzyme digestions for correct integration.

ufd1

The non-sporulating strains *crs3*, *crs5* and *crs6* were transformed with the pURSP genomic library (Barbet et al. 1992). Sporulating transformants (selected by the iodine vapour assay) exhibiting a wild-type phenotype in terms of cordycepin resistance and morphology were selected. Methods for transformation, amplification and subcloning of plasmids were described by Hilti et al. (2000). For unknown reasons, transforming cells with the empty vector pUR19 alone yields transformants that are slightly more resistant to cordycepin than the wild type.

RNA extraction and Northern blot analysis

Cells were cultivated at 30 °C in MM or MM containing 5 g NH₄Cl/l to an optical density at 530 nm (OD₅₃₀) of about 1 unit, centrifuged, washed in H₂O and shifted into the different media. Samples were taken as described in the legend of Fig. 4. Total RNA was extracted, denatured by glyoxal, separated on 1% agarose gels and blotted on Gene Screen Nylon membranes as described by Schweingruber et al. (1992). Twenty micrograms of RNA were loaded on each lane. Radioactively labelled restriction fragments from genes *ste11* and *mei2* (as described in detail by Hilti et al. 1999) were used as hybridisation probes. A 413-bp fragment from gene *act1* was used as control.

Sequencing

The sequence of the *ufd1* gene was amplified by PCR, using the following two primers corresponding to the 5' and 3' untranslated regions, respectively, of the gene: 5'-CAACGA ATGCTGTACA TAGC-3' and 5'-TCGGAAGCACTTGGCTTAC-3'. The generated DNA was purified on an agarose gel and used for custom-made sequencing.

Determination of adenosine by HPLC

Cells of the wild type and the *ado1* mutant were cultivated in YNB containing different amounts of adenine to an OD₅₃₀ of 2.7–3.9 units (as indicated in Table 1) and were centrifuged for 5 min at 10,000 g. The supernatant was filtered through a filter (pore size 0.2 µm; Schleicher and Schuell) and 90 µl of the filtrate were analysed by HPLC using an ODS 5 µm column (4.6×250 mm; Beckmann Ultrasphere) according to a modified method of Lecoq et al. (2001). Buffer A (10 mM KH₂PO₄) and buffer B (80% methanol, 20% 10 mM KH₂PO₄) were used to set up gradients. The following gradient elution protocol (at a flow rate of 1 ml/min)

Table 1 Adenosine concentrations of the *ado1* disruption strain and its parent strain. Strains were grown on the stated media (optical density at 530 nm given in parentheses), cells were centrifuged and

the concentration of the adenosine in the supernatant was determined as described in Materials and methods. *bdl* Below detection limit (0.2 µM), *ura* uracil, *YNB* yeast nutrient broth

Strain	YNB + ura	YNB + ura + 10 mg adenine/l	YNB + ura + 200 g adenine/l
<i>ura4-D18 h⁻</i>	bdl (3.5)	bdl (3.7)	0.2 µM (3.7)
<i>ado1::kanMX h⁹⁰ ura4-D18 h⁻</i>	2.0 µM (3.9)	4.6 µM (3.3)	10.6 µM (2.7)

was used: 10 min with 97% A and 3% B, 10 min with 89% A and 11% B, 20 min with 85% A and 15% B and then 30 min with 75% A and 25% B. The retention times of the markers inosine and adenosine were approximately 12.7 min and 22.5 min, respectively, under the given elution conditions. The material identified as adenosine in the supernatant had the same retention time as adenosine. Upon treatment with adenosine deaminase, the adenosine peak disappeared and a new peak with the retention time of inosine appeared. Deamination was achieved by adding 25 μ l 1 M Tris to 500 μ l supernatant following incubation for 30 min at 25 °C in the presence of 0.62 units (1 μ l) adenosine deaminase (type VI from calf intestinal mucosa; Sigma). Detection occurred at 260 nm, and Borwin 1.20 software was used for quantification of peaks.

Results

Cordycepin disturbs growth, mating and sporulation

Cordycepin causes aberrant cell morphology (cells become elongated, multiseptated, branched) and growth inhibition in *S. pombe*. The effect on growth is shown in Fig. 1. At a concentration of 25 μ M, growth is clearly inhibited and, at 100 μ M, cells cannot divide more than three times in liquid medium. Growth inhibition and aberrant cell morphology can be reversed by exogenously added adenine and thiamine (Fig. 2). Thiamine consists of a pyrimidine moiety (4-amino-5-hydroxymethyl-2-methyl-pyrimidine) and a thiazole moiety [5-(2-hydroxyethyl)-4-methylthiazole] and it is specifically the pyrimidine moiety of the thiamine molecule that is responsible for the relief of growth inhibition (Fig. 2). The effects of thiamine can be observed at concentrations as low as 0.1 μ M. Adenosine at concentrations ten times higher than that of cordycepin only weakly relieves growth inhibition by cordycepin, indicating that adenosine does not efficiently compete with its toxic analogue under our experimental conditions. Except for guanine, which causes only a very slight effect, we find no growth-inhibition relief for any other tested substances, including cytosine, uracil, thymine, inosine, all 20 natural amino acids, *p*-aminobenzoic acid, vitamins B₂, B₆, B₁₂, pantothenic acid and biotin (data not shown).

When nutritional conditions are not favourable, in particular when cells are starved for nitrogen, haploid *S. pombe* cells of opposite mating type mate, fuse to a diploid zygote and sporulate. One of the genes involved in the regulation of mating and sporulation is *ste11*. Its expression is induced upon nitrogen starvation and other stress conditions; and it encodes a transcription factor which activates a number of genes necessary for the mating and sporulation process, including *mei2* and many others (Yamamoto et al. 1997).

As shown in Fig. 3, cordycepin (at concentrations that do not or only marginally affect growth) severely inhibits mating and sporulation. This effect can be reversed by adenine. The effect of thiamine cannot be tested since, as shown previously, thiamine drastically reduces mating and sporulation under the given experimental conditions (Schweingruber and Edenharter

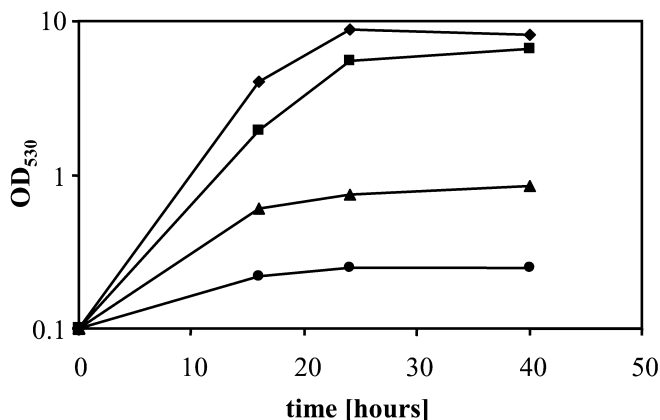


Fig. 1 Growth inhibition by cordycepin. Aliquots of 10 ml of liquid minimal medium (MM) containing different concentrations of cordycepin in 50-ml Erlenmeyer flasks were incubated with precultures of *S. pombe* wild-type 972 *h*⁻ cells at 30 °C in a shaker, to an optical density at 530 nm (OD_{530}) of 0.1 unit. Growth (OD_{530}) was then followed for 40 h. Concentration of cordycepin: diamonds none, squares 25 μ M, triangles 100 μ M, circles 250 μ M

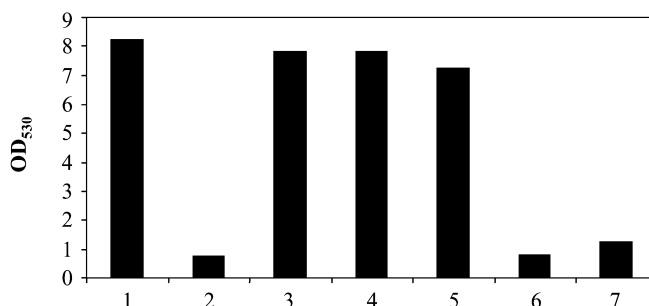


Fig. 2 Relief of the growth-inhibitory effect of cordycepin. Samples of liquid MM containing cordycepin and the substances indicated were inoculated and incubated with *S. pombe* 972 *h*⁻ cells, as described in the legend of Fig. 1. Growth was measured after 40 h. Column 1 MM alone, column 2 MM containing 100 μ M cordycepin, column 3 MM containing 100 μ M cordycepin and 0.74 mM adenine, column 4 MM containing 100 μ M cordycepin and 10 μ M thiamine, column 5 MM containing 100 μ M cordycepin and 10 μ M 4-amino-5-hydroxymethyl-2-methyl-pyrimidine, column 6 MM containing 100 μ M cordycepin and 10 μ M 5-(2-hydroxyethyl)-4-methylthiazole, column 7 MM containing 100 μ M cordycepin and 1 mM adenosine

1990). As revealed by Northern blots, the levels of *ste11* and *mei2* mRNA are drastically reduced by cordycepin (Fig. 4), indicating that inhibition of mating and sporulation is mediated at least partially by a *ste11*-dependent pathway.

Phenotypes of a mutant disrupted for the *ado1* gene encoding a putative adenosine kinase

Knowing that cordycepin is an adenosine analogue and that adenosine kinase (adenosine 5'-phosphotransferase, EC 2.7.1.20) catalyses phosphorylation of adenosine to AMP, we wanted to examine the phenotype of adenosine kinase disruption in *S. pombe*. In mammals,

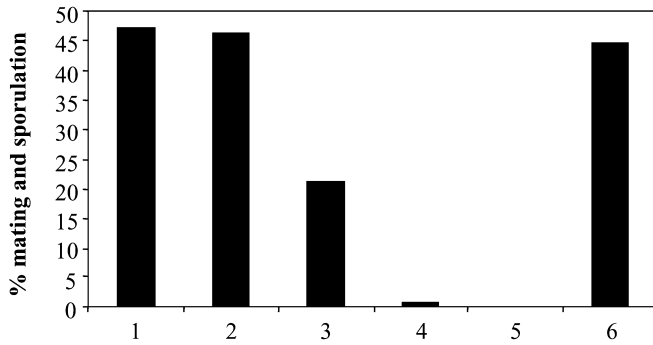


Fig. 3 Inhibition of mating and sporulation by cordycepin and its relief by adenine. Cells of opposite mating type ($975 h^+$, $972 h^-$) were pre-cultivated separately in MM; and cells of the two mating types were then mixed in MM containing cordycepin and adenine and were incubated to allow mating and sporulation. After 18 h, zygotes, asci and free spores were counted. The protocol for the quantitative determination of mating and sporulation was described in detail by Schweingruber and Edenharter (1990). Column 1 MM alone, columns 2–5 MM containing cordycepin at concentrations of 1 μM (column 2), 5 μM (column 3), 10 μM (column 4) and 25 μM (column 5), column 6 MM containing 25 μM cordycepin and 0.74 mM adenine

the enzyme plays an important role in the regulation of intra- and extracellular adenosine levels (Kowaluk et al. 1998); and in *S. cerevisiae* it is thought to be primarily involved in the recycling of adenosine produced by the methyl cycle (Lecoq et al. 2001). Sequence comparisons reveal that *S. pombe* contains a gene which codes for a protein exhibiting very significant sequence identity to the adenosine kinase of other eukaryotes (38% to human, 41% to *Caenorhabditis elegans*, 40.7% to *S. cerevisiae*). We assume this gene encodes adenosine kinase and name it *ado1*. The gene is disrupted by the *kanMX* cassette (see Materials and methods). Growth of the mutant on MMA is retarded (Fig. 5a) and, in contrast to the wild type, the disruption strain grows on MMA containing 200 μM cordycepin (Fig. 5b). On MMA containing methionine (100 mg/l), growth is almost completely inhibited but growth inhibition can be overcome by extracellularly added adenine (Fig. 5a). Thiamine has no obvious effect on the mutant phenotype. On MMA, the mutant strain stains slightly darker by iodine vapour and on media where the wild type sporulates poorly, e.g. YEA containing 10 mg inositol/l (Niederberger et al. 1998), the percentage of zygotes, asci and free spores is about two-fold increased in areas on the plate where the colony density is high (data not shown). When adenine (100 mg/ml) is added to the MMA, we observe, in contrast to the wild type, a drastic reduction (about 80%) in mating and sporulation. However, no corresponding reduction in *ste11* and *mei2* mRNA is observed (data not shown).

As measured by HPLC, adenosine is detected in the growth medium of the *ado1*-disrupted mutant. If adenine is added to the growth medium, the concentration of adenosine increases (Table 1). At a concentration of 200 mg adenine/l very small amounts of adenosine can

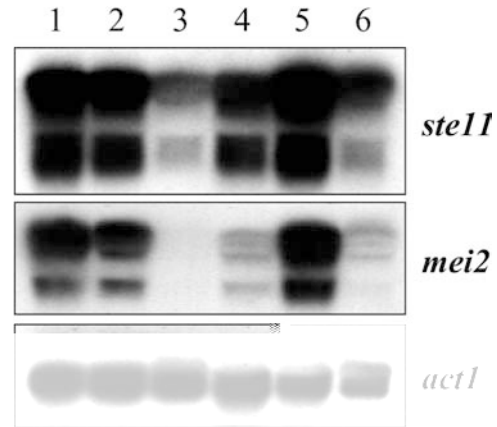


Fig. 4 Levels of *ste11* and *mei2* mRNA from cells shifted to a medium containing cordycepin. Lanes 1–3: 968 h^{90} cells were cultivated in MM and then shifted to MM alone or MM containing 25 μM cordycepin, as described in Materials and methods. One sample was taken at the time of the shift (lane 1) and another sample at 4 h after the shift to MM (lane 2) or to MM containing cordycepin (lane 3). Lanes 4–6: 968 h^{90} cells were cultivated in MM containing 5 g $\text{NH}_4\text{Cl/l}$ and then shifted to nitrogen-depleted MM alone or nitrogen-depleted MM containing cordycepin. One sample was taken at the time of the shift (lane 4) and another sample at 4 h after the shift to nitrogen-depleted MM (lane 5) or to nitrogen-depleted MM containing cordycepin, respectively (lane 6). RNA from the samples was extracted and blotted as indicated in Materials and methods. The *act1* probe was used as a control

also be detected in the wild type. Thiamine has no detectable effect on adenosine excretion, neither in the mutant nor in the wild type.

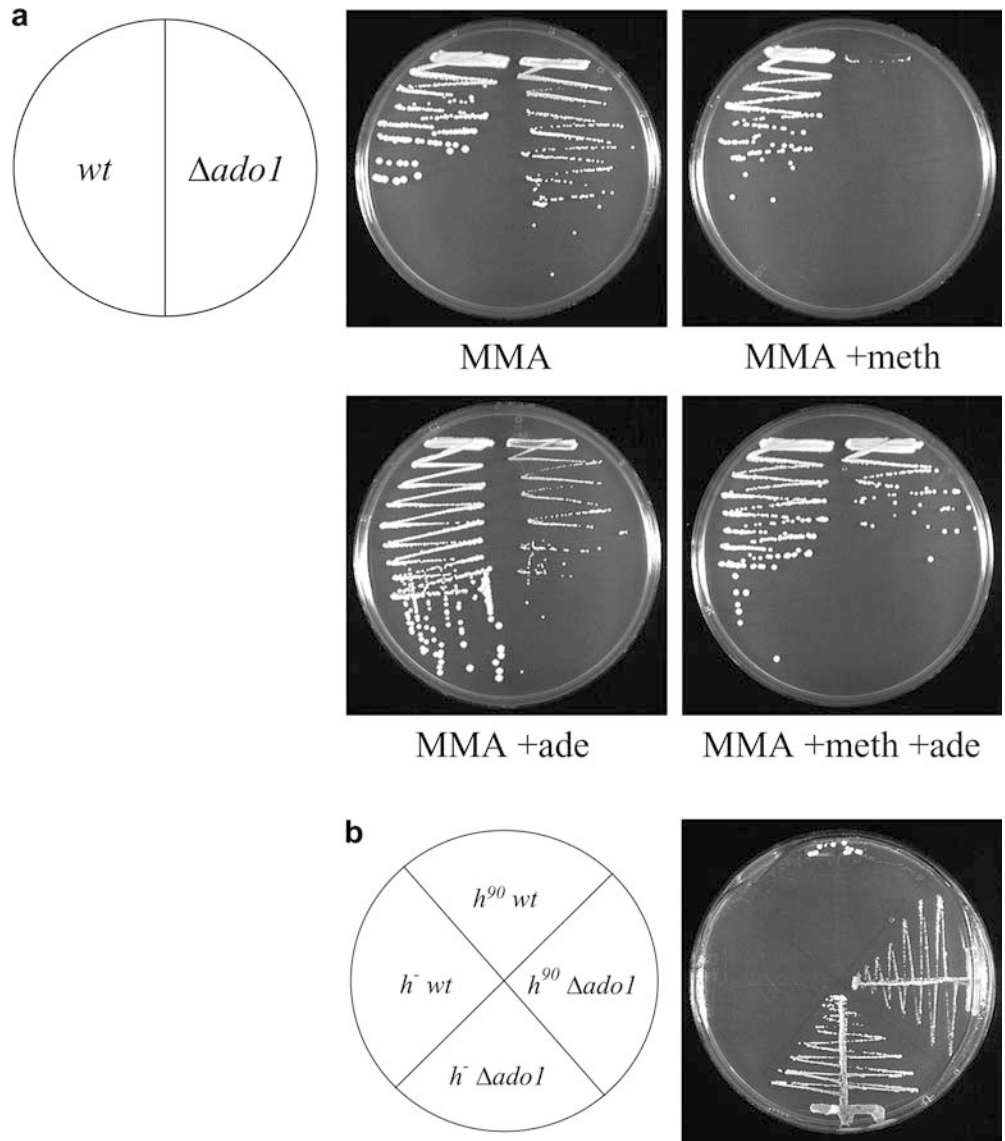
We cloned the *ado1* gene on a multicopy vector (pUR19) and transformed wild-type and mutant cells. As expected, the gene on the vector complements the mutant phenotypes, but neither in the mutant nor in the wild-type strain could overexpression phenotypes (as for example cordycepin hypersensitivity) be observed on MMA plates (data not shown).

As in the case for *s*-adenosylmethionine (SAM) synthase (Hilti et al. 2000), we find for the wild type a significant decrease in the adenosine kinase mRNA in stationary-phase cells, indicating that synthesis and recycling of SAM operates mainly in the exponential phase (data not shown).

Phenotypes of mutants defective in the gene encoding the *S. cerevisiae* *UFD1* homologue *ufd1*

To identify additional genes responsible for cordycepin sensitivity, we selected on a thiamine-free medium spontaneous mutants that grow at a cordycepin concentration of 200 μM . Knowing that cordycepin interferes with mating and sporulation, we were mainly interested in genes that are responsible not only for cordycepin sensitivity but also for fertility and sporulation; and thus we selected from the cordycepin-resistant mutants only those that exhibit no sporulation at 30 °C on MMA plates, as judged by the iodine vapour method.

Fig. 5a, b Growth of the *ado1* disruption strain. **a** Growth of strain *ura4-D18 ado1::kanMX h⁹⁰* on solid MM (MMA), MMA containing methionine (*meth*), or adenine (*ade*) and MMA containing both methionine and adenine. All supplements were added at a concentration of 100 mg/l and all media contained in addition uracil (80 mg/l). As a comparison, growth of the parent strain *ura4-D18 h⁹⁰* is shown (*wt*) on the same media. **b** Growth of the *ado1* disruption strain and its parent strain on MMA containing 200 mg cordycepin/l



Five strains were selected, all exhibiting reduced growth, slightly aberrant cell morphology (somewhat rounder, more irregular in shape than wild-type cells) and all unable to mate and sporulate. We called them *crs* strains (cordycepin resistant, sterile). Using a partial *Sau3A* library derived from the wild type, three of the mutants, *crs3*, *crs5* and *crs6*, could be transformed to phenotypic wild-type strains. From this library, we isolated 22 plasmids (ten from strain *crs3*, nine from *crs5*, three from *crs6*). The plasmids varied in size but all contained the complete sequence of a gene exhibiting 40% similarity to the *UFD1* gene of *S. cerevisiae* (Johnson et al. 1995). We called this *S. pombe* gene *ufd1*. One of the plasmids was subcloned so that it contained essentially only the *ufd1* gene. This plasmid efficiently transformed the three *crs* strains mentioned above to strains exhibiting phenotypes like the wild type, indicating that the gene product of the *ufd1* homologue is probably responsible for the observed *crs* mutant phenotypes (data not shown). To verify that the plasmid borne *ufd1*

gene does not represent a multicopy suppressor of an unknown gene, we sequenced the *ufd1* gene of strains *crs3* and *crs5*. The wild-type gene contains four introns, codes for 342 amino acids and, in the last exon, there is a row of six cytosine nucleotides encoding two prolines at positions 208 and 209. In both mutant strains, this cytosine row is extended by one more cytosine, leading to a chain-termination triplet at amino acid position 213. The cordycepin-resistant phenotype of the two strains is shown in Fig. 6. We cannot totally exclude the possibility that the mutation alone is lethal but that it is suppressed by a hidden mutation in the *crs* strains. The fact, however, that the mutants arose spontaneously and that they originated from independent experiments makes this possibility unlikely.

Cells of the three *crs* strains and the wild type were cultivated in MM containing high amounts of nitrogen to repress mating and were shifted to a medium containing no nitrogen. The same increase in *ste11* and *mei2* mRNA as in the wild type was observed for the *ufd1*

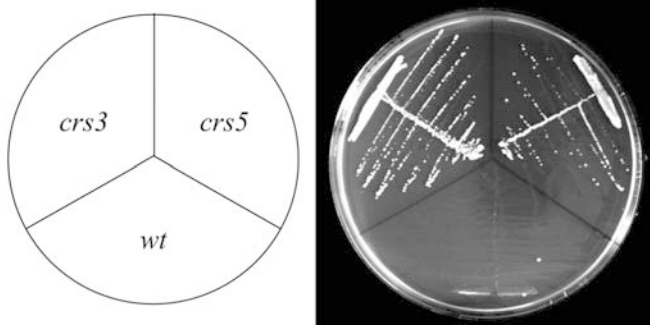


Fig. 6 Growth of strains *crs3 ura4-D18 h⁹⁰* and *crs5 ura4-D18 h⁹⁰* and the parent strain on MMA containing 200 mg cordycepin/l and 80 mg uracil/l. Strains *crs3* and *crs5* do not stain by the iodine vapour assay, although the wild type does

mutant strains, indicating that the sterile phenotype of the mutants is not due to a repression of gene *ste11* expression (data not shown).

Attempts to disrupt the *ufdl* gene in haploid cells failed. One copy of the *ufdl* gene was therefore disrupted in four diploid strains heterozygous at the mating type locus and the strains were allowed to sporulate. A total of 32 asci were dissected (the results for one of the strains are shown in Fig. 7). For all of them, only two spores in every tetrad gave rise to normal colonies and two developed to hardly visible microcolonies after 7 days incubation, indicating that disruption of the gene leads to a very drastic reduction in growth.

Discussion

Our studies show that cordycepin inhibits growth, mating and sporulation in *S. pombe*. The molecular mechanisms for these effects are unknown, except that cordycepin interferes with the *ste11* signalling cascade, which is involved in the regulation of mating and sporulation. In analogy to the situation in higher eukaryotes, we assume that cordycepin is phosphorylated in *S. pombe*. The fact that inhibition of growth, mating and sporulation can be reversed by exogenously adding adenine could indicate that the concentration of one or several components of the adenine nucleotide pool for which cordycepin can compete is increased by feeding cells with adenine. Knowing that the *ste11*-dependent meiotic pathway is cAMP-dependent (Yamamoto et al. 1997), we speculate that cordycepin or one or several of its phosphorylated derivatives interferes with this cAMP signalling cascade. In agreement with this view, we find that cAMP-dependent induction of a mating-independent enzyme, fructose-biphosphate phosphatase, is also inhibited by cordycepin (our unpublished data).

Interestingly, thiamine can reverse growth inhibition by cordycepin. We showed previously that thiamine also relieves growth inhibition caused by amiloride (Niederberger et al. 1996). It is possible that thiamine, amiloride and cordycepin have a common target. We find that a

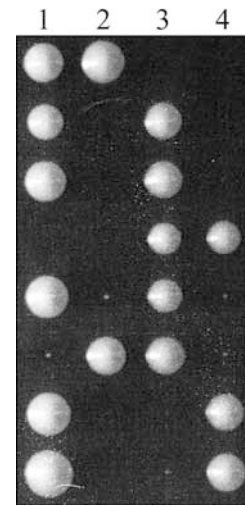


Fig. 7 Growth of spores on YEA (Niederberger et al. 1998) from eight tetrads resulting from the diploid *h⁻ade6-M210ura4-D18/h⁺ade6-M216ura4-D18 ufd1::ura4*. In some tetrads, microcolonies are visible

mutant with a disrupted adenosine kinase gene is cordycepin-resistant and accumulates adenosine in the growth medium. Similar observations have also been reported for *S. cerevisiae* (Iwashima et al. 1995; Lecoq et al. 2001). Apparently, adenosine kinase is involved in the control of adenosine accumulation and excretion. It is conceivable that intracellularly accumulated adenosine successfully competes with the toxic analogue and thereby renders cells resistant and/or that cordycepin is not phosphorylated to 3' deoxy-AMP in the *ado1* mutant and therefore is less harmful to cells. Growth retardation observed in the mutant might be due to harmful effects caused by adenosine accumulation.

Exogenously added methionine drastically increases the SAM pool in fission yeast (Hilti et al. 2000). The inability of the *ado1* mutant to grow on a methionine-containing medium could be due to the fact that adenosine generation in the recycling process of SAM is increased due to the elevated SAM pool and, since adenosine can no longer be reused (phosphorylated), it is possible that the mutant therefore becomes adenine-auxotrophic. The observation that the inhibitory effect of methionine is partially released by exogenously added adenine favours this interpretation.

Mating and sporulation are slightly more efficient in the mutant and, in contrast to the wild type, they are clearly reduced by exogenously added adenine. The reason for this is unknown; and the observation just implies that adenosine kinase is involved in the control of mating and sporulation by adenine or one of its derivatives.

The two *S. pombe* mutants with a frameshift mutation in the carboxy-terminal half of the *ufdl* gene are cordycepin-resistant and sterile; and the mutant disrupted for the *ufdl* gene is almost completely blocked in growth. In *S. cerevisiae*, a *UFD1* deletion is lethal in a haploid strain and a diploid strain homozygous for the

mutated gene (Val-to-Asp substitution at position 94) leads to a non-sporulating phenotype (Johnson et al. 1995), indicating that loss of the *ufd1* gene leads to similar phenotypes in the two yeasts. The precise function of the *UFD1* gene in *S. cerevisiae* is unknown. It seems to be involved in several processes. Results from Johnson et al. (1995) suggest that it is involved in ubiquitin-mediated proteolysis. Other results indicate that it interacts with poly(A) polymerase and stimulates polyadenylation (del Olmo et al. 1997). In particular, the gene product of *UFD1* can form complexes with *cdc48p* and *npl4p*, which on the one hand can mediate in ubiquitin-dependent processing of intra-membrane proteins in the ER and nuclear envelope (Bays and Hampton 2002) and on the other hand are involved in nuclear transport, especially of poly(A) RNA (Dehoratius and Silver 1996). The fact that the two *S. pombe* mutants with a frameshift mutation in the carboxy-terminal half of the *ufd1* gene can grow suggests this half of the gene is essential for mating and sporulation but not for viability. It is compatible with the view that this frameshift-associated truncation leaves the most highly conserved part of the *ufd1* protein intact, which thereby is sufficient to provide the essential activity for growth. How the less stringently conserved carboxy-terminal part selectively affects mating efficiency remains to be determined. The cordycepin-resistant phenotype of the *ufd1* frameshift mutants could possibly be indicative for a lowered poly(A) polymerase activity. A decreased activity could lead to an accumulation of ATP, which could compete with the cordycepin 5'-triphosphate and lead to a lower incorporation of the toxic triphosphate in the mRNA ends. It is also possible that the mutations lowers the affinity of poly(A) polymerase for cordycepin 5'-triphosphate.

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