Allelism of methionine-sensitive mutants of *Schizosaccharomyces pombe* to loci involved in adenine biosynthesis

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SUMMARY

Methionine-sensitive mutants of *Schizosaccharomyces pombe* were found to map at 16 loci, *min1–min16*, seven of which are allelic to known *ade* loci involved in adenine biosynthesis. The mutant *min1–106* was shown to be located within the *ade6* locus by intragenic recombination analysis. Methionine sensitivity of these mutants is reversible by adenine supplementation and their pigmentation is similar to that of the corresponding *ade* mutants. We conclude, therefore, that these *min* mutants are incompletely blocked in adenine biosynthesis. These observations further indicate that adenine biosynthesis in *S. pombe* is inhibited by methionine or a metabolite derived from it. For some other *min* mutants showing reversibility of methionine sensitivity by leucine or proline, no allelism to known *leu* or *pro* loci was detected.

1. INTRODUCTION

Like auxotrophic mutants, metabolite-sensitive mutants represent a useful tool for discovering and investigating biochemical pathways and their regulation. Among the few reports existing on amino acid sensitive mutants in fungi, two deal with methionine sensitivity. In *Aspergillus nidulans*, a methionine-sensitive mutant was found to be defective in an enzyme of methionine catabolism (Pieniazek, Stepien & Paszewski, 1973). One of three methionine-sensitive mutants isolated in *Saccharomyces cerevisiae*, *met-i₃*, has two outstanding properties: (1) Its methionine sensitivity is reversed by adenine supplementation; (2) it was found to be allelic to the *ade2* locus, which codes for an enzyme in adenine biosynthesis (Meuris, Lacroute & Slonimski, 1967; Meuris, 1969). This points to a relationship between methionine and adenine biosynthesis. Further evidence for this comes from the observation of Cutts & Rainbow (1950) that wild-type yeasts form a red pigment when grown in the presence of high methionine and suboptimal biotin concentrations. This red pigment also accumulates in mutant strains blocked in two steps of adenine biosynthesis (Smirnov *et al.* 1967; Dorfman, 1969; Gutz, 1963; Strauss, 1972). In *Schizosaccharomyces pombe* this relationship between

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methionine and adenine biosynthesis is also indicated by the finding of Clarke (1965) that a leaky *ade7* mutant has an increased adenine requirement when grown on medium containing methionine.

In the present study we investigated by genetical means the possibility of a relationship between methionine-sensitivity and adenine biosynthesis in *S. pombe*.

2. MATERIALS AND METHODS

(i) *Strains*

The following strains of *S. pombe* were received from the stock collection of Prof. U. Leupold, Institute of General Microbiology, University of Bern, Switzerland: Wild-type strains 972 h^- and 975 h^+; auxotrophic strains *ade1–40* h^-/h^+, *ade2–17* h^-/h^+, *ade3–58* h^-/h^+, *ade4–31* h^-/h^+, *ade5–36* h^-/h^+, *ade6–406* h^-/h^+, *ade6–250* h^-/h^+, *ade6–706* h^-/h^+, *ade6–473* h^-/h^+, *ade6–704* h^-/h^+, *ade6–525* h^-/h^+, *ade6–430* h^-/h^+, *ade6–432* h^-/h^+, *ade6–M210* h^-/h^+, *ade7–50* h^-/h^+, *ade8–106* h^-/h^+, *ade9–8H* h^-/h^+, *ade10–3H* h^-, *pro1–1* h^-/h^+, *pro2–1* h^-/h^+, *leu1–32* h^-/h^+, *leu2–120* h^-/h^+, *leu3–155* h^-/h^+.

(ii) *Media*

Culture media for *S. pombe* (YEA, MMA, MEA) were previously described by Gutz *et al.* (1974). Strains were usually cultured on tubes or plates containing MEA + adenine (50 mg/l). Media for proline- or leucine-requiring strains were additionally supplemented with L-proline (50 mg/l) or L-leucine (50 mg/l), respectively. Amino acids and nucleic acid bases of the highest purity available were used for supplementation.

(iii) *Mutant isolation*

Mutagenization by N-methyl-N'-nitro-N-nitrosoguanidine of wild type cells of *S. pombe*, 972 h^- or 975 h^+, was performed as described elsewhere (Strauss, 1976). Treated cells were plated on unsupplemented MEA and incubated for 6 days at 30 °C. Colonies were then replicated on MMA and MMA + methionine (100 mg/l). After 3 days incubation at 30 °C all clones showing any degree of growth inhibition on MMA + methionine compared to MMA were picked up, reisolated and back-crossed to the wild type for genetical purification and introduction of the opposite mating type.

(iv) *Growth and pigmentation*

For preculturing, cell material of different strains was inoculated as dots on plates with MEA + adenine (50 mg/l) and incubated for 3 days at 30 °C. The resulting cell material was then transferred to various media by replica plating. Growth and pigment formation were examined qualitatively on these media after 2, 3 and 6 days incubation at 30 °C.

(v) *Genetical procedures*

In general, the genetical techniques for *S. pombe* described by Gutz *et al.* (1974) were followed. In contrast to studies with auxotrophic strains, wild type recombinants from crosses involving *min* mutants were selected on MMA + methionine (100 mg/l). Isolated *min* mutants were genetically classified into loci by the criss-cross technique (Leupold, 1955). The same test was applied for demonstrating allelism between *min* mutants and *ade* mutants. Quantitative methods for determination of recombination frequencies (tetrad analysis, free spore analysis) were performed after Gutz *et al.* (1974). The complementation test was carried out (again on MMA + methionine) according to Leupold (1970).

3. RESULTS

(i) *Genetic classification of* min *mutants*

Mutants of *S. pombe* with wild-type like growth on MMA but with only slight or no growth on MMA + methionine (100 mg/l) were isolated and further analysed. Out of 44 such methionine-sensitive mutants, 34 could be allocated to 16 different loci by means of the criss-cross technique. These newly described loci, called *min1–min16*, contained one to four alleles (Table 1).

Table 1. *Genetical classification of methionine-sensitive mutants of* S. pombe *and the reversion of their sensitive phenotypes by supplements*

Locus	Standard allele	Number of alleles	Supplements reversing methionine sensitivity
min1	106	4	ade*
min2	65	4	ade
min3	73	4	pro†
min4	83	4	ade
min5	51	3	ade
min6	139	2	ade
min7	128	2	
min8	109	2	
min9	142	2	ade
min10	15	1	ade
min11	22	1	ade
min12	148	1	leu‡
min13	32	1	ade
min14	85	1	ade
min15	113	1	leu
min16	52	1	

* ade = adenine (20 mg/l).   † pro = proline (50 mg/l).   ‡ leu = leucine (50 mg/l).

(ii) *Further characterization of mutant phenotypes*

Standard alleles at all *min* loci were tested to see whether amino acids other than methionine were capable of inhibiting growth on minimal medium. Some amino acids occasionally had a slight inhibitory effect, but in all cases methionine was clearly the most effective inhibitor.

Methionine sensitivity of *min* mutants disappeared partly or fully on YEA + methionine (100 mg/l), indicating that some component of yeast extract alleviates the growth inhibition caused by methionine. We therefore examined the possibility that methionine sensitivity on minimal medium could be reversed by additional supplementation with adenine, guanine, uracil or any of the remaining amino acids. As shows Table 1, methionine sensitivity of the standard alleles was in fact reversible by adenine in ten cases (*min1*, *min2*, *min4*, *min5*, *min6*, *min9*, *min10*, *min11*, *min13* and *min14*), by proline in one case (*min3*) and by leucine in two cases (*min12* and *min15*).

(iii) *Allelism of* min *mutants to auxotrophic mutants*

In order to check for allelism, the standard alleles of all *min* loci showing a reversion effect by adenine were crossed to representative alleles of the ten *ade* loci known in *S. pombe*. The outcome of these crosses, performed by the criss-cross technique, suggests allelism in the following seven cases: *min1–ade6*, *min4–ade1*, *min6–ade9*, *min10–ade2*, *min11–ade3*, *min13–ade4*, *min14–ade10*. In tetrad analyses of all these crosses only parental ditypes occurred (Table 2), thus confirming the

Table 2. *Tetrad analysis of crosses between* min *and* ade *mutants*

	Number of tetrads		
	PD*	NPD†	T‡
min1–106 h⁻ × *ade6-M210* h⁺	105	0	0
min4-83 h⁻ × *ade1-40* h⁺	23	0	0
min6–139 h⁻ × *ade9–8H* h⁺	51	0	0
min10–15 h⁻ × *ade2–17* h⁺	29	0	0
min11–22 h⁻ × *ade3–58* h⁺	17	0	0
min13–32 h⁻ × *ade4–31* h⁺	29	0	0
min14–85 h⁺ × *ade10-3H* h⁻	25	0	0

* PD = parental ditype.   † NPD = nonparental ditype.   ‡ T = tetratype.

allelism found by the criss-cross technique. The remaining four *min* standard alleles showing a reversion effect by adenine were not allelic to any of the *ade* loci tested. Two of them, namely *min3–73* and *min5–51*, were found to map closely to *ade2* and *ade6*, respectively (Kohli *et al.* 1977).

In the case of the allelism *min1–ade6*, the approximate map position of a *min* allele was determined at the intragenic level. From crosses of *min1–106* with nine *ade6* alleles which are dispersed all over the *ade6* recombination map as described by Gutz (1963), recombination frequencies were obtained showing that *min1–106* is located within the *ade6* locus close to *ade6–525* (Table 3).

In a similar way, the standard alleles of the *min* loci showing a reversion effect by proline or leucine were checked for allelism with proline auxotrophic mutants (*pro1–1* and *pro2–1*) and leucine auxotrophic mutants (*leu1-32*, *leu2-120* and *leu3–155*). No allelism could be detected.

Table 3. *Intragenic recombination frequencies and complementation reactions in crosses of* min1–106 *with some* ade6 *alleles*

Cross	Recombination frequency*	Complementation reaction†
min1–106 × ade6–406	472	—
min1–106 × ade6–250	464	—
min1–106 × ade6–706	330	—
min1–106 × ade6–473	78	—
min1–106 × ade6–704	72	—
min1–106 × ade6–525	10	—
min1–106 × ade6–430	73	—
min1–106 × ade6–432	52	—
min1–106 × ade6–M210	260	+

* Recombination frequencies are expressed in prototrophic recombinant spores/10^6 plated spores and represent mean values from six crosses.

† + stands for complementation, — stands for no complementation.

(iv) *Pigmentation of* min *and* ade *mutants*

Ade6 and *ade7* mutants of *S. pombe*, grown under limiting concentration of adenine, accumulate a red pigment (Gutz, 1963). The pigment probably consists largely of polymerized AIR (5-aminoimidazole ribonucleotide), an intermediate of purine biosynthesis (Smirnov *et al.* 1967; Strauss, 1972). Red pigmentation occurred also in *min1* mutants grown on MMA, but disappeared when adenine was supplied. If conditions were used where wild type cell material was also pigmented, namely under biotin starvation together with high methionine concentrations (Cutts & Rainbow, 1950), three different responses of pigmentation could be observed among *ade* and *min* mutants (Table 4): dark red, light red and white. Considering the gene–enzyme relationships in purine biosynthesis of *S. pombe* (Fluri, Coddington & Flury, 1976), we found that *ade* mutants blocked directly after AIR were dark red, while those blocked before AIR remained white on this medium. All other strains tested, including wild type, showed a light red pigmentation. Pigmentation reactions for *min* mutants were the same as those observed for the corresponding allelic *ade* mutants.

4. DISCUSSION

Three lines of evidence make it likely that part of the isolated methionine-sensitive mutants are impaired in adenine biosynthesis: (1) Methionine sensitivity of many *min* mutants is reversible by adenine supplementation; (2) *min* mutants at seven loci are allelic to adenine auxotrophic mutants; it was shown for *min1–106* that it maps clearly within the *ade6* gene; (3) pigmentation reactions under conditions of biotin starvation and high methionine concentration are the same in *min* mutants and in the corresponding allelic *ade* mutants. These results, together with the observation by Clarke (1965) that the growth of an incompletely blocked *ade7* mutant is additionally inhibited by high methionine concentrations in the medium and that it thus behaves like a *min* mutant, suggest that *min*

mutants mapping at *ade* loci in fact represent leaky *ade* mutants showing their auxotrophic character only under methionine supply. Wild-type like growth on MMA of these *min* mutants could be explained by a mutant enzyme activity which is reduced but still sufficient to support apparently full growth. Apart from this divergent growth behaviour on MMA, these *min* mutants resemble

Table 4. *Pigmentation reactions of residually grown* ade *and* min *mutants on minimal medium with a high methionine concentration and without biotin*

Pigmentation on MMA + methionine (500 mg/l) + adenine (10 mg/l) − biotin	Wild type	*ade* mutants	*min* mutants
Dark red		*ade6–M210* *ade7–50*	*min1–106*
Light red	972, 975		
		ade2–17 *ade8–106*	*min10–15*
		ade10–3H	*min14–85* *min3–73* *min5–51* *min7–128* *min8–109* *min9–142* *min12–148* *min15–113* *min16–52*
White		*ade1–40* *ade3–58* *ade4–31* *ade5–36* *ade9–8H*	*min4–83* *min11–22* *min13–32* *min6–139* *min2–65*

Allelic *ade* and *min* mutants are noted on the same line.

leaky *ade* mutants in every respect tested, i.e. in their adenine requirement under high methionine supply, in their chromosomal location and in their pigmentation. We assume that *min* alleles exist at all *ade* loci but that because of the limited number of mutants isolated, *min* mutants were discovered at only seven out of ten possible *ade* loci. The alleviating effect of adenine on the methionine sensitivity of other *min* mutants which do not map at *ade* loci remains unexplained by this hypothesis.

The increase in the adenine requirement caused by methionine supply in *min* mutants and leaky *ade* mutants indicates that methionine or a metabolite derived from it inhibits adenine biosynthesis in *S. pombe*. Increase in pigment formation due to high methionine doses in all tested strains which are not blocked before AIR in adenine biosynthesis is consistent with this hypothesis. It further shows that the postulated inhibition of adenine biosynthesis takes place after the intermediate AIR, which is known to be accumulated and converted into the red pigment in *ade6* and *ade7* mutants (Strauss, 1972).

Although the physiological basis of the inhibition of adenine biosynthesis by methionine or its derivative is not obvious, it is likely that it is related to the biosynthesis of S-adenosylmethionine. This compound accumulates in yeast cells which are cultivated in medium containing methionine (Schlenk & DePalma, 1957). Enzymatic analysis will be required for confirmation and further characterization of the postulated inhibition.

Adenine biosynthesis appears also to be inhibited by methionine in *Saccharomyces cerevisiae*, as wild-type cells also form a red pigment when grown at high methionine concentrations and at low biotin concentrations (Cutts & Rainbow, 1950). Furthermore, the methionine-sensitive mutant *met-i$_3$* behaves like the *min1* mutants of *Schizosaccharomyces pombe* in all ways tested (Meuris, 1969). This applies to the pigmentation under conditions of adenine limitation and to the reversion of methionine sensitivity by adenine as well as to the allelism with the structural gene for AIR-carboxylase (EC 4.1.1.21). The enzyme AIR-carboxylase in *S. cerevisiae* was shown to be coded by the *ade2* locus (Yanulaitis, Nikolaeva & Smirnov, 1975) whilst indirect data suggest that in *S. pombe* it is coded by the *ade6* locus (Strauss, 1972). In analogy to the situation for the *min1* mutants of *S. pombe*, the *met-i$_3$* mutant of *S. cerevisiae* could thus be a leaky *ade2* mutant which expresses its auxotrophic character only at high methionine supply.

The physiological basis for methionine sensitivity of *min* mutants of *S. pombe* not mapping at *ade* loci is unknown. Some of these mutants are perhaps defective in methionine catabolism, as it was shown for one methionine-sensitive mutant of *A. nidulans* (Pieniazek *et al.* 1973).

This work formed part of a Ph.D. thesis submitted to the University of Bern.

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