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4 Serine hydroxymethyltransferase: a key player
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9 connecting purine, folate and methionine metabolism
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Serine hydroxymethyltransferase: a key player

connecting purine, folate and methionine metabolism

in *Saccharomyces cerevisiae*

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Abstract

Previous genetic analyses showed phenotypic interactions between 5-Amino-4-Imidazole CARboxamide Ribonucleotide 5'-phosphate (AICAR) produced from the purine and histidine pathways with methionine biosynthesis. Here, we revisited the effect of AICAR on methionine requirement due to AICAR accumulation in the presence of the *fau1* mutation invalidating folinic acid remobilization. We found that this methionine auxotrophy could be suppressed by overexpression of the methionine synthase Met6 or by deletion of the serine hydroxymethyl transferase gene *SHM2*. We propose that in a *fau1* background, AICAR, by stimulating the transcriptional expression of *SHM2*, leads to a folinic acid accumulation inhibiting methionine synthesis by Met6. In addition, we uncovered a new methionine auxotrophy for the *ade3 bas1* double mutant that can be rescued by overexpressing the *SHM2* gene. We propose that methionine auxotrophy in this mutant is the result of a competition for 5,10-methylene-tetrahydrofolate (THF) between methionine and deoxythymidine monophosphate (dTMP) synthesis. Altogether, our data show intricate genetic interactions between one-carbon units, purine and methionine metabolism through fine-tuning of serine hydroxymethyl transferase by AICAR and the transcription factor Bas1.

Introduction

A growing number of metabolites are found to play important regulatory roles thereby directly connecting metabolic status and cellular functions. This is the case for 5-amino-4-imidazole carboxamide ribonucleotide 5'-phosphate (AICAR) which is an intermediate in the purine *de novo* synthesis pathway (Fig. 1) (Daignan-Fornier and Pinson 2012). In yeast, AICAR co-regulates purine synthesis and phosphate utilization by promoting interaction of the transcription factor Pho2 with either Bas1 or Pho4 (Pinson et al. 2009). In mammals, AICAR is an agonist of the AMP-activated kinase (AMPK) (Corton et al. 1995, Sullivan et al. 1994) and feeding sedentary mice with AICA-riboside, an AICAR precursor, mimics muscular exercise (Narkar et al. 2008). Over-accumulation of AICAR is probably detrimental, as suggested by the multiple deficiencies associated with AICAR

1 transformylase IMP-cyclohydrolase (ATIC) defect in human (Marie et al. 2004). Importantly, its
2 antiproliferative effects (Rattan et al. 2005) are largely AMPK-independent (Ceschin et al. 2014, Liu
3 et al. 2014). In yeast, AICAR accumulation is found in an *ade16 ade17* double mutant lacking ATIC
4 (Pinson et al. 2009). Massive AICAR accumulation associated to constitutive activation of the first
5 step of the purine pathway combined to *ade16 ade17* mutations abolishes yeast proliferation (Rebora
6 et al. 2005). However, when the purine pathway is not hyper-activated, the *ade16 ade17* mutant is
7 viable but displays a yet unexplained auxotrophy for histidine (Tibbetts and Appling 2000). This
8 auxotrophy is not dependent on the Pho2 or Bas1 transcription factors that regulate the purine and
9 histidine pathways and can be suppressed by overexpression of the putative phosphomutase Pmu1
10 (Rebora et al. 2005) or by mutations resulting in decreased AICAR concentration (Hurlimann et al.
11 2011). Additionally, when combined with the *fau1* mutation affecting folinic acid utilization (Fig. 1),
12 the *ade16 ade17* mutant is unable to grow in the absence of methionine (Holmes and Appling 2002).
13 This phenotype can be suppressed by upstream mutations in the purine and histidine pathways
14 blocking AICAR synthesis (Holmes and Appling 2002). Thus, while a triple *ade16 ade17 fau1* mutant
15 is auxotrophic for methionine, a quintuple *ade16 ade17 fau1 ade2 his4* mutant is not. Appling and
16 coworkers proposed that the methionine auxotrophy of *ade16 ade17 fau1* could be due to inhibition of
17 methionine metabolism by combined elevated levels of AICAR and folinic acid (Holmes and Appling
18 2002). It is noteworthy that similarly to the *ade16 ade17* double mutant, an *ade3* mutant unable to
19 synthesize 10-formyl-THF, a co-substrate for ATIC (Fig. 1), accumulates AICAR (Hurlimann et al.
20 2011) and displays methionine auxotrophy when associated with a *fau1* knock-out mutation (Rebora et
21 al. 2005). In this work, we used genetic approaches to explore how AICAR connects purine and
22 methionine metabolism in yeast.
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53 **Material and methods**

54 **Yeast media**

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60 SD is a synthetic minimal medium containing 0.5% ammonium sulfate, 0.17% yeast nitrogen base
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1 without amino acids and ammonium sulfate (Difco), 2% glucose and supplemented or not with
2 adenine (0.30 mM; + A), histidine (0.06 mM; + H), leucine (0.40 mM; + L), methionine (0.13 mM; +
3 M) and/or uracil (0.30 mM; + U). S-Adenosyl methionine, folic acid, glycine, homocysteine, or serine
4 were added in the medium when indicated at 0.10 mM, 2.20 mM, 0.25 mM, 0.20 mM, and 3.5 mM
5 respectively.
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10 Strains and plasmids

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13 All yeast strains are listed in Table 1 and belong to, or are derived from, a set of disrupted strains
14 isogenic to BY4741 or BY4742 purchased from Euroscarf. Multi-mutant strains were obtained by
15 crossing, sporulation and micromanipulation of meiosis progeny. The plasmid allowing expression of
16 *SHM2* gene under the control of a tetracycline repressible promoter (tet-*SHM2*; p3487) was obtained
17 by PCR amplification of the *SHM2* open reading frame using yeast genomic DNA as template and
18 oligonucleotides 5'-CGCGGATCCACATGCCTTACTCTAT-3' and 5'-
19 CGCCTGCAGTTACACACAGCCAATGGGTATTC-3'. The PCR product was digested with *Bam*HI
20 and *Pst*I and cloned in PCM189 plasmid (Gari et al. 1997) opened with the same restriction enzymes.
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38 Growth test

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42 Yeast cells from an overnight pre-culture were re-suspended in sterile water at 3.10^7 cells/ml and
43 submitted to 1/10 serial dilutions. Drops (5 μ l) of each dilution were spotted on freshly prepared
44 medium plates and were incubated at 30°C or 37°C for 48-72 h before imaging.
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51 Northern blot analysis

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55 The transcript levels of *SHM2* and *ACT1* were determined by northern blot analysis as described
56 (Pinson et al. 2004). The *SHM2* radiolabeled probe was obtained by PCR using oligonucleotides 5'-
57 ATGCCTTACTCTATCCGAC-3' and 5'-CATAGCACCACGTGGACCTCTC-3' on yeast
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genomic DNA as template. The *ACT1* probe was already described (Denis et al. 1998).

Isolation of multicopy suppressors of the methionine auxotrophy phenotypes

Multicopy suppressors of the *ade3 bas1* methionine auxotrophy phenotype were obtained by transforming the Y2844 strain with a plasmid multicopy library (PFL46LII backbone, 2 μ *LEU2*; generous gift from F. Lacroute). Positive clones were obtained by replica-plating of the transformants on SD +AHU medium containing or not methionine. Thus, the multicopy plasmid (p3147) was extracted from the unique positive clone. Sequencing revealed a DNA fragment from chromosome XII (coordinates from 255850 bp to 261890 bp) containing *SHM2* and *REX2* genes. Similarly, multicopy suppressors of the *ade3 fau1* methionine auxotrophy phenotype were obtained by transforming the Y2031 strain with the same plasmid multicopy library (PFL46LII backbone). Positive clones were then identified by replica-plating of the transformants on SD +AHU medium containing or not methionine. Three positive clones were so identified, all containing the *MET6* encoding region. The plasmid (p3157) containing the chromosome V fragment with coordinates from 339444 bp to 342871 bp was further studied.

Results

The methionine auxotrophy due to AICAR accumulation in the *fau1* background is dependent on Bas1 but not Pho2 or Pho4

As mentioned in the introduction, a triple *ade16 ade17 fau1* mutant is auxotrophic for methionine due to AICAR accumulation (Holmes and Appling 2002) (Fig. 2a) and similarly, an *ade3 fau1* double mutant is auxotrophic for methionine (Rebora et al. 2005) (Fig. 2b). Of note, as reported before, we noticed that the methionine auxotrophy was enhanced at high temperature (Rebora et al. 2005) and therefore several experiments were ran at 37°C. As for *ade2 his4 ade16 ade17 fau1* (Holmes and

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Appling 2002), we found that the *ade8 his1* upstream mutations abolishing AICAR synthesis (Fig. 1) reversed the methionine auxotrophy of an *ade3 fau1* mutant (Fig. 2b). AICAR accumulation was thus critical for the methionine auxotrophy of these mutants. How does AICAR result in this methionine requirement phenotype? A well-known physiological effect of AICAR is to promote interaction between two pairs of transcription factors (Bas1/Pho2 and Pho4/Pho2) and thereby activate transcription of a set of about 60 genes (Pinson et al. 2009). We asked whether this documented mechanism could be responsible for the methionine auxotrophy observed in a *fau1* mutant accumulating AICAR and thus whether the transcription factors Bas1, Pho2 and/or Pho4 were involved in the methionine requirement phenotype. This was done by combining *ade3 fau1* with *bas1*, *pho2* or *pho4* mutations. The growth defect of the *ade3 fau1* double mutants in the absence of methionine was clearly not rescued by the *pho2* or *pho4* mutations (Fig. 2c), thus suggesting that the methionine auxotrophy is not strictly dependent on the activation of these transcription factors by AICAR. For the Bas1 transcription factor, we could not reach a conclusion in this genetic background, since we made the unexpected observation that a **double *ade3 bas1* mutant (Y2043)** was itself auxotrophic for methionine even in the absence of the *fau1* mutation (Fig. 2c, see section below). To circumvent the methionine auxotrophy of *ade3 bas1*, we constructed a triple *ade16 ade17 bas1* mutant. Growth of this AICAR-accumulating mutant was clearly not affected in the absence of methionine (Fig. 2d), indicating that for this specific phenotype, *ade3* did not mimic *ade16 ade17*. We thus took advantage of the fact that *ade16 ade17 bas1* did not require methionine for growth, to construct an *ade16 ade17 bas1 fau1* mutant that allowed us to evaluate the role of Bas1p in the *ade16 ade17 fau1* methionine requirement. Our results establish that, by opposition to Pho2 and Pho4 (Fig. 2c), Bas1p is strictly required for methionine auxotrophy of the *fau1* mutant under AICAR-accumulating conditions (Fig. 2e). It is therefore likely that AICAR accumulation in the *ade16 ade17* mutant activates a Bas1p-specific target that makes the *ade16 ade17 fau1* triple mutant methionine-requiring. Of note, while most of the genes requiring Bas1 for their expression also require Pho2, expression of several of these genes is much more affected by the absence of Bas1 than that of Pho2 (Denis and Daignan-Fornier 1998, Denis et al. 1998).

1 The methionine auxotrophy due to AICAR accumulation in the *fau1* mutant is suppressed by over
2 expression of *MET6* and by deletion of *SHM2*
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6 In order to find clues for the methionine auxotrophy of *fau1* mutants accumulating AICAR, the *ade3*
7 *fau1* double mutant was transformed with a genomic library carried on a multicopy plasmid to identify
8 genes suppressing this methionine auxotrophy when overexpressed. As a result, three positive clones
9 were isolated, all containing a plasmid allowing overexpression of the *MET6* gene. One of them

10 containing only the *MET6* gene fully bypassed the methionine auxotrophy of the *ade3 fau1* strain (Fig.
11 3a). This result suggested that there is insufficient Met6p activity in *fau1* mutants accumulating

12 AICAR and that overexpression of *MET6* can allow sufficient methionine synthesis for prototrophy.

13 *MET6* encodes methionine synthase which catalyzes the conversion of homocysteine into methionine
14 (Fig. 1, orange box). Met6 activity requires the one-carbon unit donor, 5-methyl-THF and is thereby
15 directly connected metabolically to Fau1 which is required for mobilization of folinic acid (5-formyl-
16 THF), a major source of one-carbon units (Fig. 1, yellow box). We reasoned that in the absence of

17 *fau1*, specific folate derivatives could be limiting and/or inhibitory. We first asked whether folate
18 limitation could be responsible for methionine auxotrophy of the *ade3 fau1* mutant. The methionine

19 auxotrophy of *ade3 fau1* was clearly not rescued by addition of folic acid (Fig. 3b), as an external
20 source of folate derivatives (Fig. 1, blue box) (Holmes and Appling 2002). This result suggested that

21 folate limitation due to folinic acid sequestration in the *fau1* mutant is probably not the major reason
22 for methionine auxotrophy. To address the second hypothesis, i.e. inhibition of Met6 by a one-carbon

23 unit compound, the *ade3 fau1* mutant was grown on various organic sulphur sources. The methionine
24 auxotrophy of the *ade3 fau1* mutant was clearly rescued by AdoMet, which decreases the requirement

25 for methionine (Fig. 1, green box), while it was not rescued by homocysteine, the methionine
26 precursor (Fig. 3c, orange box). This result indicates that methionine auxotrophy of the *ade3 fau1*

27 mutant is due to insufficient synthesis of methionine from homocysteine by Met6 (Fig. 1). Since the
28 *fau1* mutant accumulates folinic acid, and that this accumulation is further enhanced ~4 times in the

29 *ade16 ade17 fau1* mutant (Holmes and Appling 2002), we explored the possibility that folinic acid
30 could have an inhibitory effect on methionine synthesis. We thus combined the *ade16 ade17 fau1*

1 mutations with a *shm2* knock-out, because the serine hydroxymethyltransferase Shm2 was proposed to
2 be responsible for synthesis of folinic acid (Holmes and Appling 2002, Stover and Schirch 1990)
3 which cannot be remobilized in the *fau1* mutant (Fig. 1, yellow box). Importantly, the *ade16 ade17*
4 *fau1* methionine auxotrophy was fully suppressed by *shm2* knock-out (Fig. 3d), strongly suggesting
5 that folinic acid synthesis via Shm2p is responsible for methionine auxotrophy, possibly through
6 Met6p dysfunction as suggested by Holmes and Appling (Holmes and Appling 2002) and as indicated
7 by isolation of *MET6* as a multicopy suppressor gene (Fig. 3a). All together, these results suggested
8 that the methionine auxotrophy of *fau1* mutants could be the result of Met6 inhibition by folinic acid
9 that is increased in cells accumulating AICAR (Holmes and Appling 2002) in which transcriptional
10 expression of *SHM2* is drastically increased (Hurlimann et al. 2011, Pinson et al. 2009).

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26 The methionine auxotrophy of *ade3 bas1* is suppressed by overexpression of *SHM2*

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31 The newly uncovered methionine *ade3 bas1* auxotrophy (Fig. 2c) was further examined. As expected,
32 it was complemented by introduction of either *ADE3* or *BAS1* wild-type genes carried on centromeric
33 plasmids (Fig. 4a). We then searched for multicopy suppressors that would restore methionine
34 prototrophy. This approach allowed us to identify a unique multicopy suppressor plasmid which
35 turned out to contain the *SHM2* and *REX2* genes. As *SHM2* appears intimately linked to methionine
36 auxotrophy (see previous section), we constructed a plasmid allowing expression of *SHM2* alone (tet-
37 *SHM2*) and observed that this overexpression was sufficient to restore growth of the *ade3 bas1* mutant
38 in the absence of methionine (Fig. 4b). This result suggests that low expression of *SHM2* due to the
39 *bas1* mutation (Denis and Daignan-Fornier 1998) could lead to methionine auxotrophy when
40 combined with *ade3*. It is noteworthy that the *shm2* knock-out is synthetic lethal with *ade3* (Rebora et
41 al. 2005) and expression of *SHM2* is activated by Bas1p at the transcriptional level (Denis and
42 Daignan-Fornier 1998). Hence, in the *ade3 bas1* double mutant, low expression of *SHM2* due to the
43 *bas1* mutation (Denis and Daignan-Fornier 1998) could lead to a synthetic auxotrophy to methionine
44 when combined to the *ade3* mutation. Importantly, while the *ade3* and *ade16 ade17* mutants affect a

1 common step in the purine *de novo* pathway (Fig. 1), the *ade16 ade17 bas1* mutant is not auxotrophic
2 for methionine (Fig. 2d). Together these results indicate that the methionine auxotrophy of the *ade3*
3 *bas1* mutant is due to the combined low expression of *SHM2* in the *bas1* mutant and to a specific
4 effect of the *ade3* mutation. Shm2 and Ade3 enzymes both contribute to the synthesis of 5,10-
5 methylene THF, a precursor of methyl-THF which is required for methionine synthesis (Fig. 1). The
6 methionine auxotrophy of the *ade3 bas1* double mutant could therefore result from a limitation for
7 5,10-methylene THF. In addition, 5,10-Methylene THF, is utilized for dTMP synthesis and therefore
8 methylenetetrahydrofolate reductase (Met12, Met13) and thymidilate synthase (Cdc21) compete for
9 5,10-methylene THF as a substrate (Fig. 1). In a double *ade3 shm2* mutant, there would be no
10 **cytoplasmic** route left for 5,10-methylene-THF synthesis and thus the lethality of this double mutant is
11 probably due to the lack of dTMP synthesis which is essential for life. A partial limitation for 5,10-
12 methylene-THF in the *ade3 bas1* mutant would affect methionine synthesis but allow sufficient dTMP
13 synthesis to maintain viability. Accordingly, methionine auxotrophy of the *ade3 bas1* mutant was
14 rescued by AdoMet, which diminishes the requirement for methionine (Fig. 1), but not by
15 homocysteine which requires methylene THF to be metabolized to methionine (Fig. 4c). Thus, by
16 supplying AdoMet to the cell, the residual methionine synthesis would become sufficient to allow
17 growth in the absence of exogenous methionine (Fig. 1). By contrast, medium supplementation with
18 folic acid, **an external precursor of folate derivatives** which requires Shm2 to be metabolized to 5,10-
19 methylene-THF (Fig. 1), could not rescue the methionine auxotrophy (Fig. 4d).

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47 Shm2 a key enzyme at the crossing point between purine, methionine and folate metabolisms

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51 In this work, we have found that the *shm2* knock-out suppressed the methionine auxotrophy of the
52 *ade16 ade17 fau1* mutant (Fig. 3d), while on the opposite, *SHM2* overexpression suppressed the
53 methionine auxotrophy of the *ade3 bas1* mutant (Fig. 4a). Consistently with the suppression of the
54 methionine auxotrophy by *bas1* but not *pho2* knock-out (Fig. 2c), we found that *SHM2* transcriptional
55 expression is much more dependent on Bas1 than on Pho2 (Fig. 5a). This result establishes that the
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ade3 fau1 methionine auxotrophy is the result of the transcriptional activation of *SHM2* by Bas1 in strains accumulating AICAR. Hence, both too low and too high expression of *SHM2* can result in a methionine requirement in the *ade3* background. Accordingly serine and glycine, which can be substrates or products of Shm2 (Fig. 1), had very different effects on the *ade3 fau1* and *ade3 bas1* mutants (Fig. 5b). Indeed glycine rescued the methionine auxotrophy of *ade3 fau1*. By contrast, serine rescued the methionine auxotrophy of *ade3 bas1* (Fig. 5b). These results suggest that the serine/glycine balance favoring either the forward or the reverse reaction catalyzed by Shm2 can result in very different phenotypical outcomes. In conclusion, all these results point to the central role of the serine hydroxymethyl transferase Shm2 in the crosstalk between purine, methionine and folate pathways.

Discussion

In this paper we show that the methionine auxotrophy of *ade3 bas1* and *ade3 fau1* double mutants is due to inadequate expression of *SHM2* which is too low in the *ade3 bas1* mutant and too high in the *ade3 fau1* mutant. Our results point to regulation of *SHM2* expression as a critical controlling step connecting purine, methionine and one-carbon metabolism in yeast and potentially in other organisms. The main role for Shm2 in this process appears to be folate inter-conversion required to maintain proper amount of folate intermediates for methionine synthesis.

The newly uncovered methionine auxotrophy of the *ade3 bas1* double mutant can be rescued by overexpression of Shm2. We propose that methionine auxotrophy observed for this mutant results from a competition for 5,10-methylene-THF between two branches of the pathway, methionine and dTMP synthesis (Fig. 1). On the opposite, the knock-out of *SHM2* can efficiently suppress the methionine requirement of a *fau1* mutant accumulating AICAR. Importantly both *ade3 fau1* and *ade3 bas1* mutants were rescued by methionine or by S-adenosylmethionine but not by homocysteine (Fig. 3c and 4c) strongly suggesting that these mutants affect the methionine synthetase step either by inhibiting Met6 activity, as proposed for *ade3 fau1*, or by limiting its supplying with 5,10-methylene-THF co-substrate, as proposed for *ade3 bas1*. All together our data show intricate genetic interactions

1 between purine, one-carbon units and methionine metabolism through fine-tuning of serine
2 hydroxymethyl transferase.
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4 The Bas1 transcription factor is a critical regulator of *SHM2* expression (Denis and Daignan-
5 Fournier 1998, Subramanian et al. 2005) and responds to both AICAR (Pinson et al. 2009) and glycine
6 (Subramanian et al. 2005), thus integrating these signals to properly modulate one-carbon units inter-
7 conversion. Importantly, in wild-type yeast cells, physiological AICAR variations in response to
8 adenine availability significantly impact on expression of *SHM2* (3.8 fold up-regulation when adenine
9 is lacking) (Servant et al. 2012), an effect similar to what is found for all the purine *de novo* pathway
10 genes (except *ADE16*) (Daignan-Fournier and Fink 1992, Denis et al. 1998, Servant et al. 2012).
11 Physiological AICAR variations thereby modulate expression of the key *SHM2* gene which connects
12 purine, methionine and folate metabolic pathways. Importantly, AICAR (also named ZMP) was
13 recently shown to co-regulate purine and one-carbon metabolism in bacteria *via* a riboswitch
14 mechanism (Kim et al. 2015). This evolutionary conservation further confirms the highly strategic role
15 of this metabolite in cross-pathway regulation.
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35 **References**

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50 Ceschin J, Saint-Marc C, Laporte J, Labriet A, Philippe C, Moenner M, Daignan-Fournier B, Pinson B.
51 2014. Identification of yeast and human 5-aminoimidazole-4-carboxamide-1-beta-d-
52 ribofuranoside (AICAr) transporters. J Biol Chem 289: 16844-16854.
53
54
55
56
57
58 Corton JM, Gillespie JG, Hawley SA, Hardie DG. 1995. 5-aminoimidazole-4-carboxamide
59 ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells?
60
61
62
63
64
65

Eur J Biochem 229: 558-565.

1
2
3 Daignan-Fornier B, Fink GR. 1992. Coregulation of purine and histidine biosynthesis by the
4
5 transcriptional activators BAS1 and BAS2. Proc Natl Acad Sci U S A 89: 6746-6750.
6

7
8 Daignan-Fornier B, Pinson B. 2012. 5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranosyl 5'-
9
10 Monophosphate (AICAR), a Highly Conserved Purine Intermediate with Multiple Effects.
11
12 Metabolites 2: 292-302.
13
14

15
16 Denis V, Daignan-Fornier B. 1998. Synthesis of glutamine, glycine and 10-formyl tetrahydrofolate is
17
18 coregulated with purine biosynthesis in *Saccharomyces cerevisiae*. Mol Gen Genet 259: 246-
19
20 255.
21
22

23
24 Denis V, Boucherie H, Monribot C, Daignan-Fornier B. 1998. Role of the myb-like protein bas1p in
25
26 *Saccharomyces cerevisiae*: a proteome analysis. Mol Microbiol 30: 557-566.
27
28

29
30 Gari E, Piedrafita L, Aldea M, Herrero E. 1997. A set of vectors with a tetracycline-regulatable
31
32 promoter system for modulated gene expression in *Saccharomyces cerevisiae*. Yeast 13: 837-
33
34 848.
35
36

37
38 Holmes WB, Appling DR. 2002. Cloning and characterization of methenyltetrahydrofolate synthetase
39
40 from *Saccharomyces cerevisiae*. J Biol Chem 277: 20205-20213.
41
42

43
44 Hurlimann HC, Laloo B, Simon-Kayser B, Saint-Marc C, Couplier F, Lemoine S, Daignan-Fornier B,
45
46 Pinson B. 2011. Physiological and toxic effects of purine intermediate 5-amino-4-
47
48 imidazolecarboxamide ribonucleotide (AICAR) in yeast. J Biol Chem 286: 30994-31002.
49

50
51 Kim PB, Nelson JW, Breaker RR. 2015. An ancient riboswitch class in bacteria regulates purine
52
53 biosynthesis and one-carbon metabolism. Mol Cell 57: 317-328.
54
55

56
57 Liu X, et al. 2014. Discrete mechanisms of mTOR and cell cycle regulation by AMPK agonists
58
59 independent of AMPK. Proc Natl Acad Sci U S A 111: E435-444.
60
61
62
63
64
65

- 1 Marie S, Heron B, Bitoun P, Timmerman T, Van Den Berghe G, Vincent MF. 2004. AICA-
2 ribosiduria: a novel, neurologically devastating inborn error of purine biosynthesis caused by
3 mutation of ATIC. *Am J Hum Genet* 74: 1276-1281.
4
5
6
7 Narkar VA, et al. 2008. AMPK and PPARdelta agonists are exercise mimetics. *Cell* 134: 405-415.
8
9
10 Pinson B, Merle M, Franconi JM, Daignan-Fornier B. 2004. Low affinity orthophosphate carriers
11 regulate PHO gene expression independently of internal orthophosphate concentration in
12 *Saccharomyces cerevisiae*. *J Biol Chem* 279: 35273-35280.
13
14
15
16
17
18 Pinson B, Vaur S, Sagot I, Couplier F, Lemoine S, Daignan-Fornier B. 2009. Metabolic intermediates
19 selectively stimulate transcription factor interaction and modulate phosphate and purine
20 pathways. *Genes Dev* 23: 1399-1407.
21
22
23
24
25
26 Rattan R, Giri S, Singh AK, Singh I. 2005. 5-Aminoimidazole-4-carboxamide-1-beta-D-
27 ribofuranoside inhibits cancer cell proliferation in vitro and in vivo via AMP-activated protein
28 kinase. *J Biol Chem* 280: 39582-39593.
29
30
31
32
33
34 Rebora K, Laloo B, Daignan-Fornier B. 2005. Revisiting purine-histidine cross-pathway regulation in
35 *Saccharomyces cerevisiae*: a central role for a small molecule. *Genetics* 170: 61-70.
36
37
38
39
40 Servant G, et al. 2012. Tye7 regulates yeast Ty1 retrotransposon sense and antisense transcription in
41 response to adenylic nucleotides stress. *Nucleic Acids Res* 40: 5271-5282.
42
43
44
45 Stover P, Schirch V. 1990. Serine hydroxymethyltransferase catalyzes the hydrolysis of 5,10-
46 methenyltetrahydrofolate to 5-formyltetrahydrofolate. *J Biol Chem* 265: 14227-14233.
47
48
49
50
51 Subramanian M, Qiao WB, Khanam N, Wilkins O, Der SD, Lalich JD, Bognar AL. 2005.
52 Transcriptional regulation of the one-carbon metabolism regulon in *Saccharomyces cerevisiae*
53 by Bas1p. *Mol Microbiol* 57: 53-69.
54
55
56
57
58 Sullivan JE, Carey F, Carling D, Beri RK. 1994. Characterisation of 5'-AMP-activated protein kinase
59
60
61
62
63
64
65

1 in human liver using specific peptide substrates and the effects of 5'-AMP analogues on enzyme
2 activity. *Biochem Biophys Res Commun* 200; 1551-1556.
3

4
5 Tibbetts AS, Appling DR. 2000. Characterization of two 5-aminoimidazole-4-carboxamide
6
7 ribonucleotide transformylase/inosine monophosphate cyclohydrolase isozymes from
8
9 *Saccharomyces cerevisiae*. *J Biol Chem* 275: 20920-20927.
10
11
12
13
14
15
16
17
18
19
20
21
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Table 1 Yeast strains used in this study

Strain	Genotype	Reference
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Euroscarf
BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Euroscarf
Y1162	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade16::KanMX4 ade17::KanMX4</i>	This study
Y2031	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 fau1::KanMX4</i>	This study
Y2043	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 bas1::KanMX4</i>	This study
Y2816	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 fau1::KanMX4</i>	This study
Y2829	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 fau1::KanMX4</i>	This study
Y2831	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 fau1::KanMX4 pho2::KanMX4</i>	This study
Y2839	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 bas1::KanMX4</i>	This study
Y2840	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 bas1::KanMX4 fau1::KanMX4</i>	This study
Y2844	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 bas1::KanMX4</i>	This study
Y2845	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4</i>	This study
Y2946	<i>MATα his3Δ1 leu2Δ0 ura3Δ0</i>	Pinson et al 2009
Y2954	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade16::KanMX4 ade17::KanMX4 bas1::KanMX4</i>	Pinson et al 2009
Y4252	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 pho2::KanMX4</i>	This study
Y4276	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 ade16::KanMX4 ade17::KanMX4</i>	This study
Y4277	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade16::KanMX4 ade17::KanMX4 fau1::KanMX4</i>	This study
Y4278	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 ade16::KanMX4 ade17::KanMX4 fau1::KanMX4 shm2::kanMX4</i>	This study
Y4279	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade16::KanMX4 ade17::KanMX4 shm2::KanMX4</i>	This study
Y4924	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 fau1::KanMX4 pho4::KanMX4</i>	This study
Y4925	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 pho4::KanMX4</i>	This study
Y4985	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 fau1::KanMX4 ade8::KanMX4 his1::KanMX4</i>	This study
Y5613	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 ade16::KanMX4 ade17::KanMX4 fau1::KanMX4 bas1::kanMX4</i>	This study
Y7210	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 ade3::KanMX4 fau1::KanMX4</i>	This study

Legends of figures

Fig. 1 Schematic representation of the *de novo* purine and histidine pathways in yeast and their connection to the one-carbon unit metabolism and the methyl cycle. Only the enzymes mentioned in the text are listed in green or dark blue (Fau1). Ado: adenosine; AdoMet: S-adenosyl-methionine; AICAR: 5-amino-4-imidazole carboxamide ribonucleotide 5'-phosphate (in red); DHF: dihydrofolic acid; dTMP: deoxythymidine 5'-monophosphate; dUMP: deoxyuridine 5'-monophosphate; IMP: inosine 5'-monophosphate; PRPP: 5-phosphoribosyl-1-pyrophosphate; SAH: S-adenosyl homocysteine; SAICAR: succinyl-AICAR; S-AMP: Succinyl adenosine 5'-monophosphate. THF: tetrahydrofolic acid.

Fig. 2 AICAR accumulation leads to a Bas1-dependent methionine auxotrophy of *fau1* mutants. **a)** The triple *ade16 ade17 fau1* mutant is auxotroph for methionine. **Strains: Y1162 and Y4277** **b)** Methionine auxotrophy of *ade3 fau1* double mutant is abolished in the absence of AICAR. **Strains: Y2816, Y2845 and Y4985** **c)** Deletion of *PHO2* and *PHO4* genes does not restore growth of the *ade3 fau1* mutant on methionine free medium. **Strains: Y2816, Y2831, Y2043, Y2840, Y4252 and Y4925** **d)** The *ade16 ade17 bas1* triple mutant is not methionine auxotroph. **Strains: Y1162, Y2839, Y2845, Y2946 and Y2954** **e)** The Bas1 transcription factor is required for methionine auxotrophy of an *ade16 ade17 fau1* mutant. **Strains: Y4276, Y5613 and Y7210** **(a-e)** Yeast strains were serial diluted and grown for 48 h at 37°C on SD +HLUA medium supplemented (+ Met) or not (- Met) with methionine.

Fig. 3 Overexpression of *MET6* gene, S-adenosyl methionine supplementation and *SHM2* gene deletion restore methionine prototrophy of *fau1* mutants accumulating AICAR. **a)** Growth in the absence of methionine of the *ade3 fau1* mutant is restored by overexpressing the methionine synthase gene *MET6*. Cells were serial diluted and grown on SD +AHU medium for 48 h at 37°C before imaging. **b-c)** Growth of the *ade3 fau1* mutant on methionine-free medium is restored by AdoMet **(c)** but not by Folic acid supplementation **(b)**. **d)** Methionine auxotrophy of the *ade16 ade17 fau1* mutant

1 is fully suppressed by deletion of the serine hydroxymethyl transferase gene *SHM2*. Yeast strains were
2 serial diluted and grown on SD +AHLU medium containing (+ Met) or not (- Met) methionine and
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4 supplemented with S-adenosyl methionine (AdoMet), Homocystein (HomoCys) or folic acid (Folic
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6 Ac.) as indicated. Plates were imaged after incubation for 48 h at 37°C.
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11 **Fig. 4** The methionine auxotrophy of *ade3 bas1* is suppressed by overexpression of the *SHM2* gene or
12 S-Adenosyl methionine supplementation. **a)** Complementation of the *ade3 bas1* methionine
13 auxotrophy by either *BAS1* or *ADE3* wild-type gene expressed from a centromeric plasmid. The
14 Y2844 strain was transformed with a plasmid allowing expression of either *BAS1* or *ADE3* or by the
15 cognate empty vector (YCp50). Transformants were spotted on a SD +AHL medium containing (+
16 Met) or not (- Met) methionine at 30°C. **b)** Overexpression of the *SHM2* gene restores growth of an
17 *ade3 bas1* mutant on medium lacking methionine. The Y2043 strain was transformed with either a
18 plasmid allowing expression of *SHM2* under the control of a tetracycline repressible promoter (Tet-
19 *SHM2*; p3487) or the cognate empty vector (pCM189). Transformants were spotted on a SD +AHL
20 medium containing (+ Met) or not (- Met) methionine at 30°C. **c)** S-adenosyl methionine
21 supplementation is sufficient to restore growth of an *ade3 bas1* mutant (Y2043) on methionine free
22 medium. **d)** Methionine auxotrophy of the *ade3 bas1* (Y2844) mutant is not rescued by folic acid
23 supplementation. **c-d)** Cells were serial diluted and spotted on SD +AHLU medium containing (+
24 Met) or not (- Met) methionine and supplemented with S-adenosyl methionine (AdoMet),
25 Homocystein (HomoCys) or folic acid (Folic Ac.) when indicated. Plates were imaged after incubation
26 for 48 h at 30°C.
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49 **Fig. 5** The serine hydroxymethyl transferase Shm2 is central for connecting purine, methionine and
50 folate metabolism. **a)** Transcriptional expression of the *SHM2* gene monitored by northern blotting.
51 Total RNAs were extracted from cells grown in SD +AHLU medium containing Methionine.
52 *SHM2/ACT1* ratios were determined using a phosphorimager and were set at 1 for the wild-type cells.
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54 **b)** Glycine and serine supplementation allows growth on methionine-free medium of *ade3 fau1* and
55 *ade3 bas1* mutants, respectively. Yeast strains were serial diluted and grown on SD +AHLU medium
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containing (+ Met) or not (- Met) methionine and supplemented or not with glycine (+ Gly) or serine (+ Ser). Plates were imaged after incubation for 48 h at 30°C.

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Figure 1

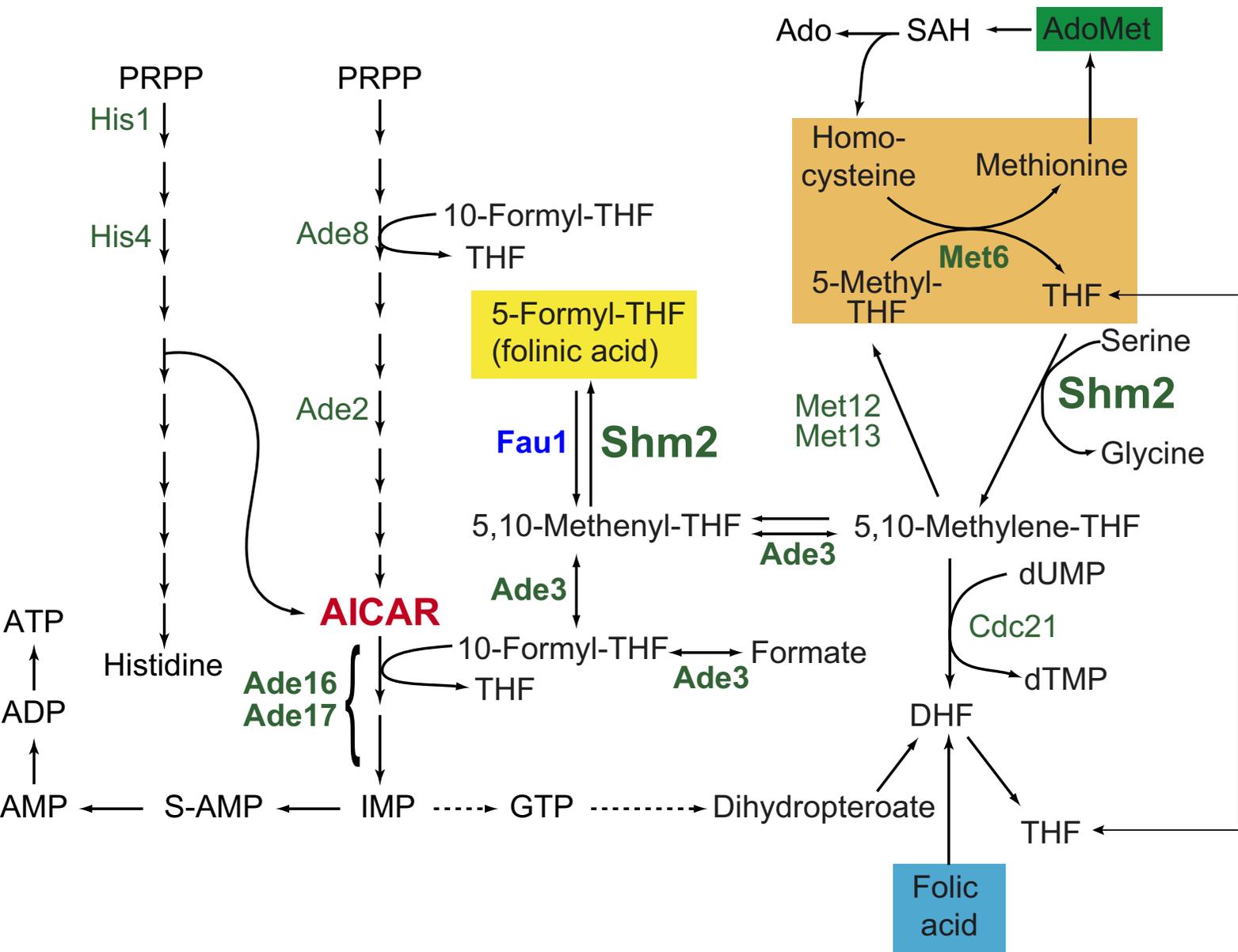


Figure 2

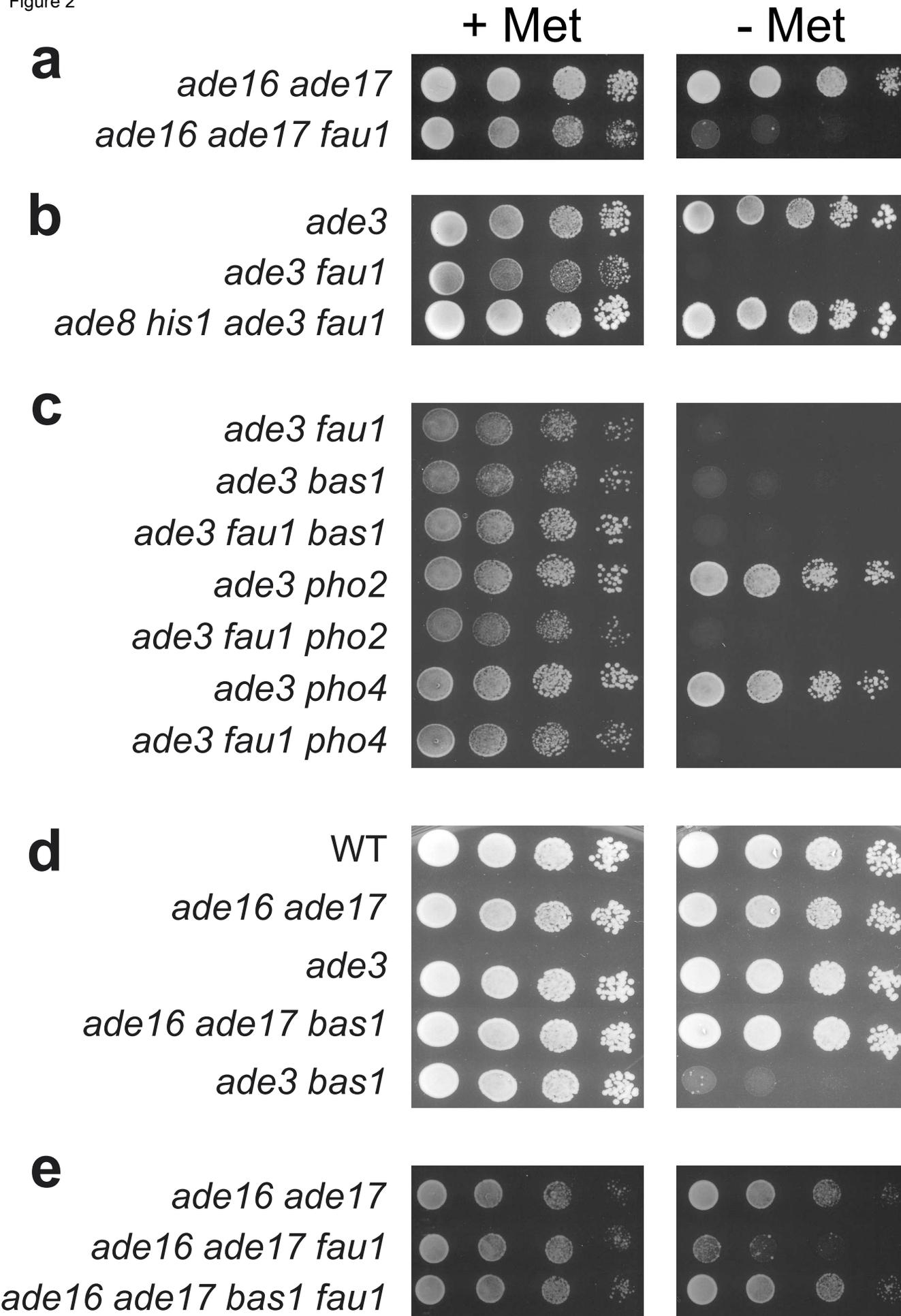


Figure 3

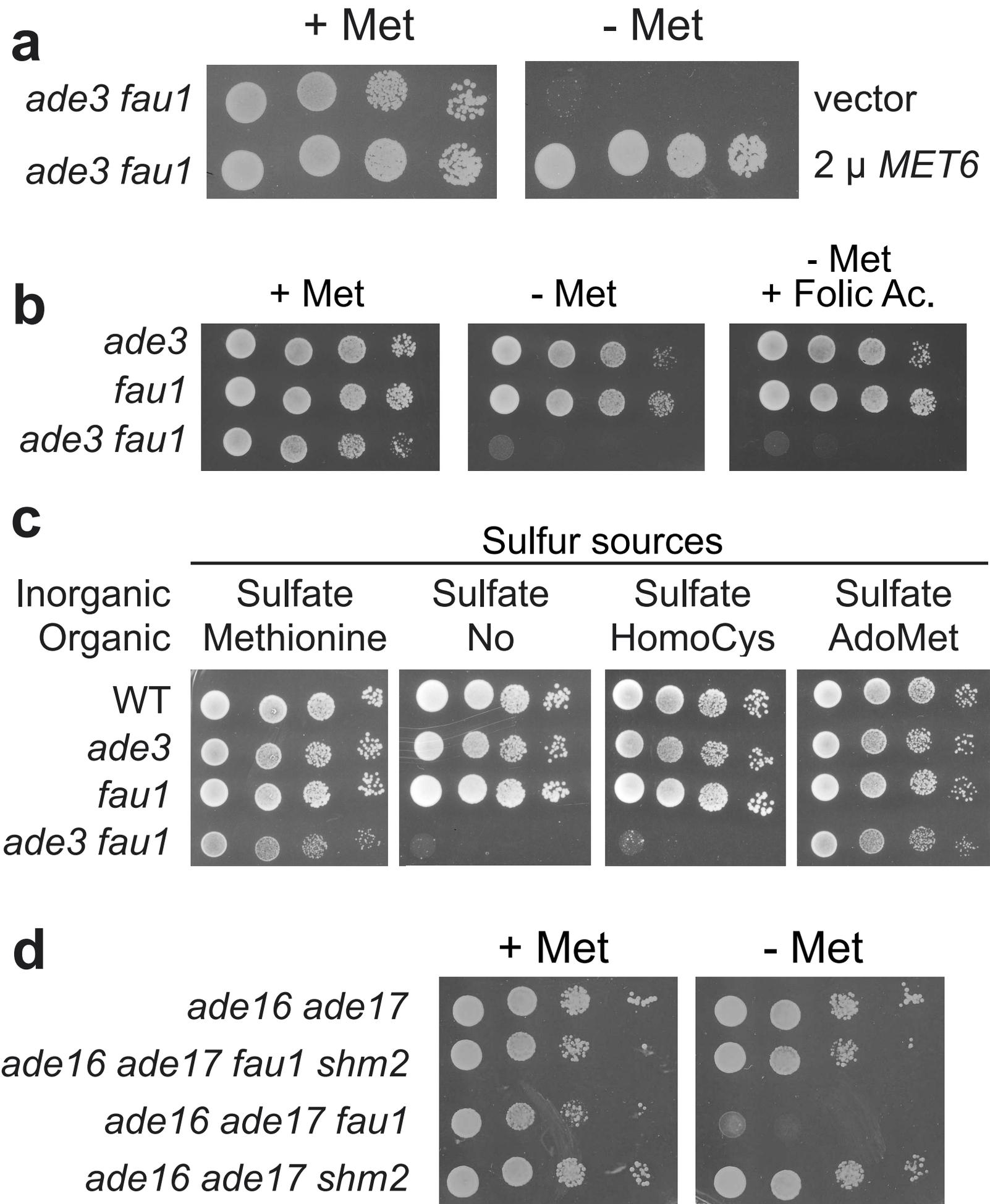


Figure 4

