S-Adenosylmethionine Synthetase Is Required for Cell Growth, Maintenance of G0 Phase, and Termination of Quiescence in Fission Yeast
Article

S-Adenosylmethionine Synthetase Is Required for Cell Growth, Maintenance of G0 Phase, and Termination of Quiescence in Fission Yeast

Takeshi Hayashi,1 Takayuki Teruya,1 Romanas Chaleckis,1,2 Susumu Morigasaki,1 and Mitsuhiro Yanagida1,3,*

SUMMARY

S-adenosylmethionine is an important compound, because it serves as the methyl donor in most methyl transfer reactions, including methylation of proteins, nucleic acids, and lipids. However, cellular defects in the genetic disruption of S-adenosylmethionine synthesis are not well understood. Here, we report the isolation and characterization of temperature-sensitive mutants of fission yeast S-adenosylmethionine synthetase (Sam1). Levels of S-adenosylmethionine and methylated histone H3 were greatly diminished in sam1 mutants. sam1 mutants stopped proliferating in vegetative culture and arrested specifically in G2 phase without cell elongation. Furthermore, sam1 mutants lost viability during nitrogen starvation-induced G0 phase quiescence. After release from the G0 state, sam1 mutants could neither increase in cell size nor re-initiate DNA replication in the rich medium. Sam1 is thus required for cell growth and proliferation, and maintenance of and exit from quiescence. sam1 mutants lead to broad cellular and drug response defects, as expected, since S. pombe contains more than 90 S-adenosylmethionine-dependent methyltransferases.

INTRODUCTION

Methylation, the transfer of a methyl group from one molecule to another, is one of the most important and universal biochemical reactions in cells, where it is involved in numerous cellular processes, such as transcriptional control, lipid metabolism, and signal transduction. The diversity of functions involving methylation is sustained by numerous methyltransferases that are present in all living organisms. Although a broad range of molecules including proteins, nucleic acids, lipids, and cellular metabolites can be substrates for methyltransferases, almost all methyltransferases use S-adenosylmethionine (SAM, also known as SAMe or AdoMet) as the common methyl donor (Chiang et al., 1996). SAM is also required in other important processes, including synthesis of polyamines (Fontecave et al., 2004), which are essential for various cellular functions affecting growth and development (Pegg and Casero, 2011). Thus, SAM is the most versatile biomolecule, second to ATP, in biochemical processes (Loenen, 2006).

SAM is synthesized from methionine and ATP by SAM synthetase (also known as methionine adenosyltransferase [MAT]), and converted to S-adenosylhomocysteine (SAH) via methyl transfer reactions. Then it is regenerated via the methionine cycle (Figure 1A). SAM synthetases are highly conserved among diverse organisms and have been extensively studied in bacteria (Wei and Newman, 2002), fungi (Gerke et al., 2012; Hilti et al., 2000; Thomas and Surdin-Kerjan, 1991), plants (Li et al., 2011; Shen et al., 2002), and animals, including humans (Ding et al., 2015; Obata and Miura, 2015; Ramani and Lu, 2017). However, genetic analyses of SAM synthesis disruption are scarce, because multiple genes encode SAM synthetases in the majority of eukaryotic organisms, including the budding yeast, Saccharomyces cerevisiae.

The fission yeast, Schizosaccharomyces pombe, is an excellent model organism to study eukaryotic molecular and cellular biology (Hoffman et al., 2015; Yanagida, 2002). S. pombe has more than 90 genes predicted to encode SAM-dependent methyltransferases, according to PomBase (Wood et al., 2012). The physiological roles of methylation have been investigated by inactivating specific methyltransferases involved in a wide range of cellular processes, such as biomolecule synthesis (Hayashi et al., 2014a; Iwaki et al., 2008; Kanipes et al., 1998; Pluskal et al., 2014), ribosome function (Bachand and Silver, 2004; Shirai et al., 2010), transcriptional control (Ekwall and Ruusala, 1994; Morris et al., 2005; Thon et al., 1994), and DNA damage response (Sanders et al., 2004). However, cellular defects in the genetic control of SAM synthesis are not well understood. S. pombe possesses a single gene for SAM synthetase, sam1+,” which is
essential for cell viability, and low expression of \textit{sam1}\textsuperscript{+} affects growth, mating, and sporulation (Hilti et al., 2000).

In this study, we report isolation by PCR random mutagenesis and characterization of temperature-sensitive (ts) mutant strains of fission yeast SAM synthetase and demonstrate that \textit{sam1}\textsuperscript{+} is a super-housekeeping (SHK) gene, essential for both proliferation and quiescence (Sajiki et al., 2009). Mutations in the \textit{sam1} gene block cell growth and cell cycle progression in vegetative culture and also cause failure to exit from nitrogen starvation-induced G0 quiescence. Furthermore, \textit{sam1} mutants lose cell viability during G0 quiescence.
RESULTS
Isolation of Temperature-Sensitive Mutants of the sam1 Gene
Because the sam1" gene is essential for cell viability (Hilti et al., 2000; Kim et al., 2010), we examined the effects of SAM limitation on cellular functions by isolating S. pombe ts mutants of SAM synthetase Sam1. To obtain ts mutants of the sam1" gene, we employed a PCR-based random mutagenesis screen (Hayashi et al., 2014b) (Figure S1). The sam1 DNA fragment, in which the hygromycin-resistance-encoding marker gene, hph, was inserted just downstream of the stop codon of the sam1" gene open reading frame, was amplified by PCR under error-prone conditions, containing increased MgCl2 (Eckert and Kunkel, 1990). Mutagenized DNA fragments were introduced into wild-type (WT) cells for replacement of the chromosomal sam1" gene with the mutated gene by homologous recombination. Hygromycin-resistant transformants were selected at 26°C and then tested for colony formation at 36°C on rich YPD medium plates. After confirmation of linkage of the ts phenotype to the hygromycin-resistant phenotype, five ts mutant strains of the sam1 gene were obtained and designated sam1-1 to sam1-5.

We then identified mutation sites in the sam1 gene of the sam1 ts mutants. sam1-1 and sam1-2 contained single amino acid substitutions (F367L and D36N, respectively), whereas sam1-3, sam1-4, and sam1-5 contained two amino acid substitutions (L292S R299H, I24M E115G, and T90A Q370R, respectively) in the sam1 gene (Figure 1B). All mutation sites except for Q370 are conserved among humans, rats, and fission yeast. Based on the three-dimensional structure of the rat ortholog of Sam1 (González et al., 2003), no mutations were found to locate near the binding site of the substrates, ATP and methionine (Figure S2). To identify the mutations responsible for the ts phenotype, we introduced one of the five mutant sequences (sam1-1–sam1-5) or one of two amino acid substitutions in sam1-3–sam1-5 mutants into the WT genome using linearized plasmids carrying the hygromycin resistance marker. The resulting transformants, containing chromosomal gene replacements with the sam1-1–sam1-5 mutant genes, showed the ts phenotype on both rich YPD and synthetic minimal EMM2 plates, whereas the transformants containing one of two amino acid substitutions in sam1-3–sam1-5 mutants did not show the ts phenotype (Figure 1C). In conclusion, sam1 gene mutations in the mutants caused the ts phenotype and both the amino acid substitutions in sam1-3–sam1-5 were necessary for the ts phenotype. Since sam1-3 showed the most severe growth defects and sam1-1 showed a moderate ts phenotype at 36°C on YPD plates (Figure 1C), sam1-1 and sam1-3 were used for further investigation. It was confirmed that the colony formation defects of sam1-1 and sam1-3 at 36°C were rescued by plasmid carrying the sam1" gene (Figure 1D).

Defective SAM Synthetase in sam1 Mutant Cells
To detect Sam1 protein, polyclonal antibodies against a mixture of two peptides (I77GYDDSEKFDYKTC91 and N230TYGSSKTAELV343) of S. pombe Sam1 were raised and used for immunoblots after affinity purification. We validated the specificity of the α-Sam1 antibodies by detecting a single band at the expected molecular weight of ~42 kD in immunoblots of WT cell extracts (no tag, Figure 2A). The assignment of the band was confirmed using extracts of Sam1-FLAG cells in which the chromosomal sam1" gene was replaced with the sam1"-FLAG gene. The band detected by immunoblot using α-Sam1 antibodies was shifted to ~45 kD in extracts of Sam1-FLAG cells (Figure 2A). Untagged Sam1 was not detected by α-FLAG antibody. Thus, polyclonal antibodies against Sam1 specifically detected Sam1 protein in cell extracts. We then compared protein levels of Sam1 in WT and sam1 mutant cells cultured at 36°C for 0–6 hr in rich YPD medium. Sam1 mutant proteins of sam1-1 and sam1-3 were diminished, compared with WT, even at the permissive temperature of 26°C (0 hr, Figure 2B), and further decreased at 36°C. These results suggest that sam1-1 and sam1-3 mutants are loss-of-function mutants of SAM synthetase Sam1, partly due to the decreased protein level.

If sam1-1 and sam1-3 mutants are indeed defective in SAM synthetic activity, SAM should be decreased in the mutant cells. We employed metabolomic analysis using liquid chromatography-mass spectrometry to assay the levels of SAM and related metabolites in extracts of WT and sam1 mutant cells grown in rich YPD medium. WT and sam1 mutant cells were grown at 26°C and then shifted to 36°C for 0–24 hr. Levels of SAM detected in sam1-1 and sam1-3 cell extracts were rather low, even at the permissive temperature of 26°C (0 hr, Figure 2C), and further decreased at 36°C, whereas the high level of SAM in WT was maintained at 36°C for 0–24 hr (Figure 2C). Similar results were obtained for SAH, a by-product of SAM-dependent methyl transfer reactions (Figure 2D). Levels of methionine, a substrate of Sam1 enzyme, were decreased in WT cells, but not in sam1 mutants at 36°C (Figure 2E). These results strongly support the notion that SAM synthesis was defective in sam1-1 and sam1-3 mutant cells.
Figure 2. sam1 Mutants Are Defective in SAM Synthesis and Histone H3K4 Methylation, and Are Rescued by Choline

(A) Immunoblot of extracts of WT (no tag) and Sam1-FLAG cells using affinity-purified anti-Sam1 and anti-FLAG antibodies.

(B) Levels of mutant Sam1 proteins were diminished. Immunoblotting was done using antibodies against Sam1 to detect Sam1 protein in the extracts of WT, sam1-1, and sam1-3 cells cultured at 36°C for 0–6 hr in rich YPD medium. Antibody against α-tubulin (Tub) was used as the loading control.

(C–E) The time course change of the normalized peak areas of SAM and related metabolites. Metabolites were extracted from WT and sam1 mutant cells cultured at 36°C for 0–24 hr in rich YPD medium and analyzed. Normalized peak areas of the following metabolites are shown: (C) S-adenosylmethionine (SAM); (D) S-adenosylhomocysteine (SAH); (E) methionine (Met).

(F) Levels of methylated histone H3 K4 in WT, sam1-1, and sam1-3 cells. WT, sam1-1, and sam1-3 cells were cultured at 36°C for 0–8 hr in rich YPD medium. Antibodies against histone H3 and mono-, di-, and trimethylated H3K4 (H3K4me1, H3K4me2, and H3K4me3, respectively) were used to detect the total H3 and methylated H3K4.

(G) Cells were diluted serially 10-fold, spotted onto EMM2 or EMM2 containing 2 mM choline chloride, and incubated at 30°C, 33°C, or 36°C for 3 days.
**Impairment of Methylation Reactions in sam1 Mutants**

Because the levels of SAM, a universal methyl group donor, were greatly decreased in sam1 mutant cells, we next investigated histone methylation in sam1 mutant cells. WT and sam1 mutant cells were shifted from 26°C to 36°C for 0–8 hr, and immunoblot analysis was carried out using three antibodies specific to mono-, di-, and trimethylated lysine 4 of histone H3 (H3K4me1, H3K4me2, and H3K4me3, respectively). In sam1-1 and sam1-3 mutant cells, the levels of H3K4me2 and H3K4me3 were greatly decreased after the shift to 36°C, whereas only a slight decrease was observed in H3K4me1 (Figure 2F). These results suggested that a global change of histone methylation was caused by mutations of SAM synthetase and that levels of di- and trimethylated Lys4 of histone H3 were more susceptible to SAM deficiency.

Many eukaryotes, including S. pombe, produce phosphatidylcholine (PC) via two pathways. One is methylation of phosphatidylethanolamine, a major methylation target in mammals and budding yeast (Noga et al., 2003; Stead et al., 2006; Ye et al., 2017). Another PC biosynthetic pathway is the CDP-choline pathway, which is independent of methylation and requires choline (Kanipes et al., 1998; Kent, 1995). We presumed that exogenous choline relieves the ts phenotype of sam1 mutants by rescuing the PC synthesis defect. As expected, colony formation of WT and mutants was rescued by adding choline (Figure 2G). These results strongly suggested that the sam1 mutations impaired cellular methylation reactions.

**Diminished Cell Growth and Cell-Cycle Arrest in G2 Phase Occur in sam1 Mutants**

We examined the cellular phenotypes of sam1-1 and sam1-3 mutant cells at the restrictive temperature (36°C). WT and sam1 mutant cells were grown at 26°C and then shifted to 36°C in rich YPD medium. The cell number increase of sam1 mutants stopped after one or two cell divisions at 36°C (Figure 3A), although the high viability of mutant cells was still maintained after 8 hr (Figure 3B). Since fluorescence microscopic observation of DNA-specific probe DAPI-stained cells demonstrated that most sam1-1 and sam1-3 cells were mononucleate 8 hr after the shift to 36°C (Figure 3C), the sam1 mutants probably arrest the cell cycle at the higher temperature. The length of sam1 mutant cells did not increase significantly compared with WT after the shift to 36°C. Average cell lengths of WT, sam1-1, and sam1-3 were 11.6 ± 2.3, 11.5 ± 2.4, and 11.8 ± 2.4 μm, respectively, after 8 hr at 36°C (Figure 3D), suggesting that cell growth (i.e., the extension of cell length) halted at 36°C in sam1 mutant cells, unlike cdc mutants that develop extremely elongate cells because of growth without cell division (Nurse et al., 1976).

To monitor cell cycle progression in WT and sam1 mutant cells at 36°C, we measured the septation index (percentage of cells with a septum), the cellular DNA content, and the spindle index (percentage of cells with mitotic spindle). The septation index was maintained at ~12% in WT cells, whereas it dropped to 0.8% 4 hr after the shift to 36°C in sam1-3 mutant cells and to 1.2% at 8 hr in sam1-1 mutant cells (Figure 3E). These results indicate that sam1 mutant cells were arrested before the onset of cytokinesis. In cellular DNA content analysis by fluorescence-activated cell sorting (FACS), a single peak appeared before the shift to 36°C (0 hr) in WT and sam1 mutants, meaning 2C DNA content, because most vegetatively growing S. pombe cells are mononucleate in post-replicative (G2) phase and pre-replicative nuclei exist in binucleate cells (Figure 3F). After the shift to 36°C (2–8 hr), only one peak at the 2C DNA content was still present in sam1-1 and sam1-3 mutants, like WT cells, indicating that cell-cycle arrest in sam1 mutants at the restrictive temperature occurred after completion of DNA replication. The spindle index was measured using WT and sam1 mutant strains expressing α-tubulin-GFP and Cut11-mCherry, which visualized spindle microtubules and the nuclear envelope, respectively (Figures 3G and 3H). The spindle index was maintained around 8%–10% in WT cells at 36°C for 0–6 hr, whereas it dropped to 0% at 4 hr after the shift to 36°C in sam1-3 mutant cells and to 0.4% at 6 hr in sam1-1 mutant cells (Figure 3G), indicating that sam1 mutant cells arrested before the onset of M phase. Consequently, sam1 mutant cells arrest specifically in G2 phase at the restrictive temperature in rich YPD medium.

**sam1 Mutant Cells Lose Cell Viability in the Maintenance of G0 Phase Induced by Nitrogen Starvation**

To determine the capacity of sam1 mutants to maintain viability in the nitrogen starvation-induced quiescent G0 phase, we monitored cell viability under nitrogen starvation (Sajiki et al., 2009; Shimamura et al., 2007). WT and sam1 mutant cells first grown in EMM2 medium were transferred to the nitrogen-deficient EMM2–N at 26°C for 24 hr to enter a quiescent G0 state. After 24 hr, the cell number increase in the sam1-1 mutant was only 2.4-fold, whereas that in WT and sam1-3 mutants was around 3.6-fold (Figure 4A). FACS
Figure 3. Diminished Cell Growth and Cell-Cycle Arrest in G2 Phase Occur in sam1 Mutants

(A–F) WT, sam1-1, and sam1-3 cells were grown at 26°C in rich YPD medium and then shifted to 36°C. The cell number increase (A), the viability change (B), fluorescence microscopic images of DAPI-stained cells (C), the average cell length with standard deviations (D), the proportion of cells with a septum (septation index) (E), and the distributions of cellular DNA content (F) are shown. Cell viability was measured by plating cells onto YPD agar and counting the number of colonies formed after incubation at 26°C.

(G and H) Time courses of changes in spindle index (G) and fluorescence microscopic images (H) of WT, sam1-1, and sam1-3 cells expressing GFP-fused α-tubulin and mCherry-fused Cut11 after the shift from 26°C to 36°C in YPD medium. GFP and mCherry fluorescence is shown in green and magenta, respectively.
analysis revealed that ~80% of WT and sam1-3 cells contained 1C DNA, whereas 1C DNA-containing sam7-1 cells accounted for only ~40% (Figure 4B). These results indicated that the sam1-1 mutant was somewhat defective in pre-quiescence division under nitrogen starvation at 26°C. Nitrogen starvation-induced quiescent G0 cells were further cultivated at either 26°C or 36°C for 12 days. G0-arrested WT and sam1-3 cells maintained high viability at 26°C, whereas G0-arrested sam1-1 cells gradually lost viability even at 26°C (Figure 4C). At 36°C, the viability of sam1-1 and sam1-3 decreased to <25% after 4 days and to <0.3% after 8 days, whereas the viability of WT was ~40% after 12 days at 36°C (Figure 4D). Together, functional Sam1 is required for the maintenance of G0 phase under nitrogen starvation.

Figure 4. sam1 Mutant Cells Lose Cell Viability in the Maintenance of Nitrogen Starvation-Induced G0 Phase
(A and B) WT, sam1-1, and sam1-3 cells were brought into the G0 quiescent state at 26°C under nitrogen-deficient medium, EMM2–N for 24 hr. Increase in cell number as the fold increase (A) and the distribution of cellular DNA content (B) after 24 hr in EMM2–N medium at 26°C are shown.
(C and D) Resulting G0 quiescent cultures were kept at 26°C (C) or shifted to 36°C (D) for 12 days. The cell viability percentages were scored at different time points (days). sam1-1 mutant cells lost cell viability both at 26°C and 36°C, whereas sam1-3 mutant cells lost cell viability only at 36°C.
(E) Levels of Sam1 and methylated histone H3 K4 in WT, sam1-1, and sam1-3 cells in nitrogen starvation-induced G0 phase at 26°C. WT, sam1-1, and sam1-3 cells were brought into the G0 quiescent state under nitrogen-deficient medium, EMM2–N for 24 hr (day 0), and further incubated at 26°C for 8 days. Immunoblotting was done using antibodies against Sam1, α-tubulin (Tub), histone H3, and mono-, di- and trimethylated H3K4 (H3K4me1, H3K4me2, and H3K4me3, respectively).
Since sam1-1 lost viability at 26°C during nitrogen starvation, whereas sam1-3 did not, we compared the mutant Sam1 protein abundance in sam1-1 with that in sam1-3 mutants in G0 phase 0–8 days after culture for 24-hr quiescence entry in the EMM2–N medium. Although the Sam1 proteins of both sam1-1 and sam1-3 were diminished compared with that of WT under nitrogen starvation at 26°C, the mutant Sam1 protein level was lower in sam1-1 than in sam1-3 (Figure 4E). In addition, levels of H3K4me2 and H3K4me3 were already greatly decreased in sam1-1 at day 0 in G0 (24 hr after nitrogen removal), whereas only a slight decrease of H3K4me3 was observed in sam1-3 (Figure 4E). These results are consistent with the data in Figure 4C.

sam1 Mutant Cells Fail to Exit from Nitrogen-Starved Arrest

We next addressed the question of whether sam1 mutant cells could exit from a nitrogen starvation-induced G0 state in which cells became small and contained pre-replicative 1C DNA (Su et al., 1996) (see also Figures 5C and 5D). WT and sam1 mutants were first cultured in nitrogen-deficient medium, EMM2–N, at 26°C for 24 hr to induce G0 arrest, and then shifted to rich YPD medium for release from G0 arrest at 36°C. The cell number increase was completely blocked in sam1-1 and sam1-3 mutant cells after transfer to rich YPD medium at 36°C, whereas the cell number of WT started increasing after 6 hr (Figure 5A). However, the high viability of sam1-1 and sam1-3 mutant cells was maintained for 8 and 24 hr, respectively, in rich YPD medium at 36°C (Figure 5B). sam1-1 and sam1-3 mutant cells remained small and round even after 24 hr in rich YPD medium, whereas WT cells were a normal rod shape after 8 hr (Figure 5C), demonstrating that sam1 mutants were unable to initiate cell growth at the restrictive temperature. FACS analysis showed that sam1-1 and sam1-3 mutant cells did not initiate DNA replication even after 24 hr, whereas WT cells completed DNA replication after 4 hr (Figure 5D). These results imply that Sam1 is necessary to initiate cell growth and DNA replication after the release from nitrogen starvation-induced G0 state.

Rapamycin Rescues sam1 Mutants

The target of rapamycin (TOR) complex is the main regulator of cell growth and controls nutrient signaling pathways in eukaryotes (Kennedy and Lamming, 2016). We investigated the effect of rapamycin (an inhibitor of TOR kinase) on the ts phenotype of sam1 mutants on rich YPD medium, as sam1 mutants were defective in cell growth, as described above (Figures 3C and 5C). Rapamycin (0.1 μg/mL) rescued the growth defect of sam1-1 mutant cells at 36°C and slightly suppressed the ts phenotype of sam1-3 mutants at 33°C (Figure 6A), suggesting that reducing TOR activity relieved the growth defects of sam1 mutants. Rapamycin may rescue sam1 mutants in a way different from protein level restoration because the mutant Sam1 protein amount was not affected in sam1-1 and sam1-3 mutants by the addition of rapamycin at 26°C and 36°C (Figures 6B and 6C).

sam1 mutants were also defective in the maintenance of G0 phase induced by nitrogen starvation (Figures 4C and 4D), so we next tested whether rapamycin could rescue the viability loss of sam1 mutants in G0 phase. Viability loss of sam1-1 in G0 at 26°C was significantly suppressed by the addition of rapamycin, and the viability was >40% after 12 days, whereas the viability in the absence of rapamycin was ~3% after 8 days (Figure 6D). At 36°C, viability loss of sam1-1 and sam1-3 in G0 was delayed in the presence of rapamycin (Figure 6E). Thus, rapamycin rescued sam1 mutants in both proliferative and quiescent phases.

sam1 Mutants Show Pleiotropic Drug Sensitivities

To search further the physiological roles of Sam1, we examined the drug sensitivity of sam1 mutants. The response of sam1-1 and sam1-3 mutants to various stress agents is summarized in Table 1 (data are shown in Figure S3). Both mutants were sensitive to 25 mM nicotinamide, which inhibits class III histone deacetylases (HDACs) (Sauve et al., 2006), but not to 12.5 μg/mL trichostatin A (TSA), an inhibitor of class I and class II HDACs (Yoshida et al., 1995), at 30°C. These mutants were also sensitive to 4 μg/mL actinomycin D, an inhibitor of transcription (Bensaude, 2011), at 30°C. At 33°C, sam1-1 was moderately sensitive to 4 mM hydroxyurea (HU, an inhibitor of DNA synthesis) (Singh and Xu, 2016), 8 μg/mL phleomycin (inducing double-strand DNA breaks) (Moore, 1988), and 4 μM camptothecin (CPT, an inhibitor of topoisomerase I that induces single-strand DNA breaks) (Pommier, 2006) and showed similar sensitivity to 100 J/m² UV irradiation (inducing the formation of thymine dimers) compared with the WT strain. No significant difference in the sensitivity of sam1-3 to HU, phleomycin, CPT, and UV irradiation was observed, partly due to no growth at 33°C even without the treatments. Thus, sam1 mutants showed hypersensitivity to a variety of drugs related to DNA damage and chromatin remodeling.
Extensive Changes Occur in the Metabolic Profiles of sam1 Mutants

We performed metabolomic analysis of WT and sam1 mutant cells grown at 36°C for 8 hr in rich YPD medium to understand the impact of SAM limitation on metabolic homeostasis beyond the methionine cycle (Table S1). A comparison of WT with sam1-1 is shown in a scatterplot (Figure 5A). The normalized peak area showed more than a 2-fold difference for 54 compounds (61%) out of 88 identified metabolites in comparison of WT.
with \textit{sam1-1}, whereas only \sim 10\% of the identified metabolites usually change more than 2-fold in comparisons of two independent experiments under identical conditions (Pluskal et al., 2010), indicating that the profile of metabolites was significantly altered in \textit{sam1-1}. Similar results were obtained from comparisons of \textit{sam1-3} with WT: the normalized peak areas of approximately 64\% of identified metabolites had greater than a 2-fold change (Figure 7B). Beside the remarkable decrease of SAM and SAH described above (Figures 2C and 2D), eight compounds (phosphoribosyl pyrophosphate [PRPP], hercynylcysteine sulfoxide, CDP, UMP, adenine, UDP, sedoheptulose-7-phosphate, and pentose-phosphate) decreased over 10-fold in \textit{sam1-1} and/or \textit{sam1-3} mutant cells (Figure 7C). Hercynylcysteine sulfoxide is a precursor of ergothioneine, an antioxidant compound (Pluskal et al., 2014), and the others are involved in nucleotide metabolism. These extensive changes in the metabolic profiles of \textit{sam1} mutants may reflect the importance of SAM in diverse cellular functions implicated in anti-oxidation, nucleotide metabolism, and pentose pathways.

**DISCUSSION**

SAM-dependent methylation occurs in a wide variety of cellular compounds, and in the fission yeast, \textit{S. pombe}, one enzyme, the product of the \textit{sam1} gene, is solely responsible for the synthesis of SAM.
from methionine and ATP. There are ~90 methyltransferase genes in the genome of S. pombe, so these enzymes are the sources of diversity of methylated substrates. In this study, we examined the cellular defects produced by the genetic impairment of SAM synthesis in the fission yeast, S. pombe, using five sam1 mutants that produced the ts phenotype. Two of them, moderate sam1-1 and severe sam1-3, were further investigated. Both mutants showed Sam1 protein instability, and a great decline of SAM and SAH, whereas increased methionine was observed at the restrictive temperature. As expected, the degree of histone H3 K4 methylation was diminished at the restrictive temperature. The ts phenotype was suppressed by the presence of choline at the semi-restrictive and restrictive temperatures. Exogenous choline, containing three methyl groups, may relieve the ts phenotype by suppressing the defect of PC synthesis and/or reducing the required amount of SAM for cell proliferation. The findings, indeed, indicated that sam1 mutations cause deterioration of a wide spectrum of methylation reactions.

sam1 mutants showed diverse phenotypes. Two sam1 mutants (sam1-1, sam1-3) are hypersensitive to drugs such as nicotinamide, actinomycin D, HU, phleomycin, and CPT. Considering these drug sensitivities, broad cellular functions implicating methylation seem to necessitate Sam1 enzyme. In addition, rapamycin relieved the defects of sam1 mutants in both proliferative and quiescence phases. A possible explanation is that the TOR signaling regulates Sam1 via direct or indirect phosphorylation as rapamycin attenuates TOR kinase. Another possibility is that rapamycin, perhaps through TOR kinase, alters methyltransferase activity to give priority to methylation reactions essential to growth. We also identified 12 other groups of ts mutants that were rescued by rapamycin (Sajiki et al., 2018); thus, perhaps Sam1 with some methyltransferases, composes an additional group. According to the report proposing that rapamycin re-balances TOR activity with the activities of identified gene products (Sajiki et al., 2018), the balance between TOR kinase and Sam1 appears important for cell proliferation, and severe impairment of the balance blocks cell growth. The detailed mechanism of this rapamycin-induced rescue should be the subject of further investigation.

Mutations in the S. pombe sam1 gene affected cell growth (cell size increase) and cell cycle progression at two transition points: the G2/M transition in vegetative culture and the G1/S transition after release from nitrogen-starvation-induced G0 phase. Since fission yeast cells need to increase their size to a critical threshold to pass these two regulatory points (Nurse and Thuriaux, 1977; Nurse, 1975; Shiozaki, 2009), we interpreted our results to mean that a primary defect of mutants resided in cell growth, rather than in the transition of specific cell cycle stages. Our metabolomic analysis of sam1 mutants suggested that the sam1 mutation disturbed many metabolic pathways, especially attenuating nucleotide biosynthesis. These metabolic changes appear to cause defects of both cell growth and cell cycle progression, although the precise roles of Sam1 in cell growth remain to be determined.

Sam1 is also required in G0 phase quiescence induced by nitrogen starvation, so Sam1 belongs to a family of SHK gene products, involved in the control of G0 phase, as well as cell growth (Sajiki et al., 2009). sam1-1

|        | 30°C | 33°C |
|--------|------|------|
|        | WT   | sam1-1 | sam1-3 | WT   | sam1-1 | sam1-3 |
| Control| ++++ | ++++ | ++++ | ++++ | +++ | – |
| 25 mM nicotinamide | +++ | – | – | +++ | – | – |
| 12.5 μg/mL TSA | +++ | +++ | +++ | +++ | ++ | – |
| 4 μg/mL actinomycin D | ++ | – | – | +++ | – | – |
| 4 mM HU | ++ | ++ | ++ | +++ | + | – |
| 8 μg/mL phleomycin | +++ | ++++ | +++ | +++ | ± | – |
| 4 μM CPT | +++ | +++ | +++ | +++ | + | – |
| 100 J/m² UV | ++++ | ++++ | ++++ | ++++ | +++ | – |

Table 1. Summary of Colony Formation Abilities of WT and sam1 Mutants in the Presence of Various Stress Agents

++++, normal growth; +, slow growth; ±, diminished colony formation; –, no colony formation.
See also Figure S3.
lost viability at 26°C in G0 phase, whereas sam1-3 did not. Why is sam1-1 more sensitive to this condition than sam1-3 although the ts phenotype of sam1-1 was less severe in vegetative culture? One reason is that sam1-1 seems more defective at 26°C as the SAM content of sam1-1 is about half of sam1-3 at 26°C (Figure 2C, 0 hr) and the doubling time of sam1-1 (3.4 hr) is longer than that of sam1-3 (3.2 hr) at 26°C. It is also possible that Sam1 protein is differently regulated in G0 phase, as the mutant Sam1 protein level in G0 phase was lower in sam1-1 than in sam1-3 (Figure 4E), contrary to the case in vegetative culture. In G0 phase, the level of SAM increases (Shimanuki et al., 2013), and nitrogen sources are recycled for the degradation and synthesis of nucleic acids, proteins, and other nitrogen-containing substrates. Hence, methylated compounds may be central to recycling of nitrogen-containing metabolites.

We isolated and characterized fission yeast ts mutants for the SAM synthetase gene. As the yeast is an excellent model organism for genetic analyses, further study by using these sam1 mutant strains is expected. However, this single-cell eukaryote does not reveal higher order phenotypes at the level of tissues or organs. In humans, mutations in MAT I/III have been identified as clinical blood variations with hypermethionine and various brain symptoms (Chamberlin et al., 2000; Nagao et al., 2013). Accumulation of methionine seems to be characteristic to all SAM synthetase mutants isolated in different species. In C. elegans, S-adenosylmethionine synthetase 1 (SAMS-1) mutants produced enlarged lipid droplets throughout their life cycle and impaired PC synthesis (Ehmke et al., 2014). In both C. elegans and S. pombe, methyl group-enriched PC is a key metabolite, perhaps because choline is the basic compound for phospholipid

Figure 7. Metabolomic Analysis of sam1 Mutants

(A and B) A scatterplot comparing the normalized peak areas of all identified metabolites in extracts of cells cultured at 36°C for 8 hr in rich YPD medium. WT versus sam1-1 (A) and WT versus sam1-3 (B) are shown. Red diagonal lines indicate a 2-fold difference. Most changed compounds are annotated according to the list in (C).

(C) Normalized peak areas of compounds that decreased more than 10-fold in sam1-1 or sam1-3 compared with WT. See also Table S1.
methionine. In *Arabidopsis thaliana*, a mutation in the S-adenosylmethionine synthetase 3 gene caused high free methionine and decreased lignin (Goto et al., 2002; Shen et al., 2002). In *Neurospora crassa*, mutations of the S-adenosylmethionine synthetase-encoding gene has been identified as an allele resistant to the toxic methionine analog, ethionine (Barra et al., 1996). Taken together, although the phenotypes of methylation-related biological events, which may be quite complex.

**METHODS**

All methods can be found in the accompanying Transparent Methods supplemental file.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Transparent Methods, three figures, and one table and can be found with this article online at https://doi.org/10.1016/j.isci.2018.06.011.

**ACKNOWLEDGMENTS**

We thank Dr. Steven Aird for editing the manuscript. We acknowledge the generous support and funding provided by the Okinawa Institute of Science and Technology Graduate University.

**AUTHOR CONTRIBUTIONS**

T.H. designed the experiments. T.H. and S.M. performed the experiments. T.T. and R.C. performed met abolomic analyses. T.H., S.M., and M.Y. analyzed data and wrote the manuscript.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

Received: March 21, 2018
Revised: June 4, 2018
Accepted: June 27, 2018
Published: July 27, 2018

**REFERENCES**

Bachand, F., and Silver, P.A. (2004). PRMT3 is a ribosomal protein methyltransferase that affects the cellular levels of ribosomal subunits. EMBO J. 23, 2641–2650.

Barra, J.L., Mautino, M.R., and Rosa, A.L. (1996). A dominant negative effect of eth-1r, a mutant allele of the *Neurospora crassa* S-adenosylmethionine synthetase-encoding gene conferring resistance to the methionine toxic analogue ethionine. Genetics 144, 1455–1462.

Bensaude, O. (2011). Inhibiting eukaryotic transcription: which compound to choose? how to evaluate its activity? Transcription 2, 103–108.

Chamberlain, M.E., Ubagha, T., Mudd, S.H., Thomas, J., Pao, V.Y., Nguyen, T.K., Levy, H.L., Greene, C., Freehauf, C., and Chou, J.Y. (2000). Methionine adenosyltransferase I/III deficiency: novel mutations and clinical variations. Am. J. Hum. Genet. 66, 347–355.

Chiang, P.K., Gordon, R.K., Tal, J., Zeng, G.C., Doctor, B.P., Pandhasaradhi, K., and McCann, P.P. (1996). S-Adenosylmethionine and methylation. FASEB J. 10, 471–480.

Ding, W., Smulan, L.J., Hou, N.S., Taubert, S., Watts, J.L., and Walker, A.K. (2015). s-Adenosylmethionine levels govern innate immunity through distinct methylation-dependent pathways. Cell Metab. 22, 633–645.

Eckert, K.A., and Kurtz, T.A. (1990). High fidelity DNA synthesis by the *Thermus aquaticus* DNA polymerase. Nucleic Acids Res. 18, 3739–3744.

Ehnke, M., Luthe, K., Schnabel, R., and Döring, F. (2014). S-adenosyl methionine synthetase 1 limits fat storage in *Caenorhabditis elegans*. Genes Nutr. 9, 386.

Elwall, K., and Ruusala, T. (1994). Mutations in rik1, clr2, clr3 and clr4 genes asymmetrically derepress the silent mating-type loci in fission yeast. Genetics 136, 53–64.

Fontecave, M., Atta, M., and Mulliez, E. (2004). S-adenosylmethionine: nothing goes to waste. Trends Biochem. Sci. 29, 243–249.

Gerke, J., Bayram, Ö., and Braus, G.H. (2012). Fungal S-adenosylmethionine synthetase and the control of development and secondary metabolism in *Aspergillus nidulans*. Fungal Genet. Biol. 49, 443–454.

González, B., Pajares, M.A., Hermoso, J.A., Guillern, D., Guillern, G., and Sanz-Aparicio, J. (2003). Crystal structures of methionine adenosyltransferase complexes with substrates and products reveal the methionine-ATP recognition and give insights into the catalytic mechanism. J. Mol. Biol. 331, 407–416.

Goto, D.B., Ogi, M., Kijima, F., Kumagai, T., van Werven, F., Onouchi, H., and Naito, S. (2002). A single-nucleotide mutation in a gene encoding S-adenosylmethionine synthetase is associated with methionine over-accumulation phenotype in *Arabidopsis thaliana*. Genes Genet. Syst. 77, 89–95.

Hayashi, K., Ogiyama, Y., Yokomi, K., Nakagawa, T., Kaino, T., and Kawamukai, M. (2014a). Functional conservation of coenzyme Q biosynthetic genes among yeasts, plants, and humans. PLoS One 9, e99038.

Hayashi, T., Ebe, M., Nagao, K., Kokubu, A., Sajiki, K., and Yanagida, M. (2014b). Schizosaccharomyces pombe centromere protein Mis19 links Mis16 and Mis18 to recruit CENP-A through interacting with NMD factors and the SWI/SNF complex. Genes Cells 19, 541–554.

Hayashi, T., Hatanaka, M., Nagao, K., Nakaseko, Y., Kanoh, J., Kokubu, A., Ebe, M., and Yanagida, M. (2007). Rapamycin sensitivity of the *Schizosaccharomyces pombe* tor2 mutant and...
organization of two highly phosphorylated TOR complexes by specific and common subunits. Genes Cells 12, 1357–1370.

Hilti, N., Graub, R., Jörg, M., Arnold, P., Schweinruber, A.M., and Schweinruber, M.E. (2000). Gene sam1 encoding adenosylmethionine synthetase: effects of its expression in the fission yeast Schizosaccharomyces pombe. Yeast 16, 1–10.

Hoffman, C.S., Wood, V., and Fantes, P.A. (2015). An ancient yeast for young geneticists: a primer on the Schizosaccharomyces pombe model system. Genetics 201, 403–423.

Iwaki, T., Iefuji, H., Hiraga, Y., Hosomi, A., Morita, T., Giga-Hama, Y., and Takegawa, K. (2008). Multiple functions of ergosteryl in the fission yeast Schizosaccharomyces pombe. Microbiology 154, 830–841.

Kanipes, M.I., Hill, J.E., and Henry, S.A. (1998). An ancient yeast for young geneticists: a primer on the Schizosaccharomyces pombe model system. Genetics 150, 553–562.

Kennedy, B.K., and Laming, D.W. (2016). The mechanistic target of rapamycin: the grand ConductTor of metabolism and aging. Cell Metab. 23, 990–1003.

Kent, C. (1995). Eukaryotic phospholipid biosynthesis. Annu. Rev. Biochem. 64, 315–343.

Kim, D.-U., Hayles, J., Kim, D., Wood, V., Park, H.-O., Won, M., Yoo, H.-S., Duhi, T., Nam, M., Palmer, G., et al. (2010). Analysis of a genome-wide set of gene deletions in the fission yeast Schizosaccharomyces pombe. Nat. Biotechnol. 28, 617–623.

Li, W., Han, Y., Tao, F., and Chong, K. (2011). Knockdown of SAM5 genes encoding S-adenosyl-l-methionine synthetases causes methylation alterations of DNAs and histones and leads to late flowering in rice. J. Plant Physiol. 168, 1837–1843.

Linnen, W.A.M. (2006). S-adenosylmethionine: Jack of all trades and master of everything? Biochem. Soc. Trans. 34, 330–333.

Moore, C.W. (1988). Internucleosomal cleavage and chromosomal degradation by bleomycin and phleomycin in yeast. Cancer Res. 48, 6837–6843.

Morris, S.A., Shibata, Y., Noma, K.-I., Tsukamoto, Y., Warren, E., Tempand, B., Grewal, S.I.S., and Strahl, B.D. (2005). Histone H3K36 methylation is associated with transcription elongation in Schizosaccharomyces pombe. Eukaryot. Cell 4, 1446–1454.

Nagao, M., Tanaka, T., and Furuo, M. (2013). Spectrum of mutations associated with methionine adenosyltransferase III deficiency among individuals identified during newborn screening in Japan. Mol. Genet. Metab. 110, 460–464.

Noga, A.A., Stead, L.M., Zhao, Y., Brosnan, M.E., Brosnan, J.T., and Vance, D.E. (2003). Plasma homocysteine is regulated by phospholipid methylation. J. Biol. Chem. 278, 5952–5955.

Nurse, P., and Thuriaux, P. (1997). Controls over the timing of DNA replication during the cell cycle of fission yeast. Exp. Cell Res. 107, 365–375.

Nurse, P. (1975). Genetic control of cell size at cell division in yeast. Nature 256, 547–551.

Nurse, P., Thuriaux, P., and Nasmyth, K. (1976). Genetic control of the cell division cycle in the fission yeast Schizosaccharomyces pombe. Mol. Gen. Genet. 146, 167–178.

Obata, F., and Miura, M. (2015). Enhancing S-adenosyl-l-methionine catabolism extends Drosophila lifespan. Nat. Commun. 6, 8332.

Pegg, A.E., and Casero, R.A. (2011). Current status of the polyamine research field. Methods Mol. Biol. 720, 3–35.

Plasulz, T., Nakamura, T., Villar-Brimnes, A., and Yanagida, M. (2010). The metabolic profiling of the fission yeast S. pombe: quantification of compounds under different temperatures and genetic perturbation. Mol. Biosyst. 6, 182–198.

Plasulz, T., Ueno, M., and Yanagida, M. (2014). Genetic and metabolic dissection of the ergothionine and selenonene biosynthetic pathway in the fission yeast, S. pombe, and construction of an overproduction strain. PLoS One 9, e97774.

Pommier, Y. (2006). Topoisomerase I inhibitors: camptothecins and beyond. Nat. Rev. Cancer 6, 789–802.

Ramani, K., and Lu, S.C. (2017). Methionine adenosyltransferases in liver health and diseases. Liver Res. 1, 103–111.

Sajiki, K., Hatanaka, M., Nakamura, T., Takeda, K., Shimanuki, M., Yoshida, T., Hanu, Y., Hayashit, T., Nakaseko, Y., and Yanagida, M. (2009). Genetic control of cellular quiescence in S. pombe. J. Cell Sci. 122, 1418–1429.

Sajiki, K., Tahara, Y., Villar-Brimnes, A., Plasulz, T., Teruya, M., Mori, A., Hatanaka, M., Ebe, M., Nakamura, T., Aoki, K., et al. (2018). Genetic defects in SAKP, a serine–threonine kinase, and CoA-related lipid metabolism are rescued by rapamycin in fission yeast. J. Cell Sci. 121, 603–614.

Saue, A.A., Wollberger, C., Schramm, V.L., and Boeke, J.D. (2006). The biochemistry of sirtuins. Annu. Rev. Biochem. 75, 435–465.

Shen, B., Li, C., and Tarczynski, M.C. (2002). High free-methionine and decreased lignin content result from a mutation in the Arabidopsis S-adenosyl-l-methionine synthetase 3 gene. Plant J. 29, 371–380.

Shimanuki, M., Chung, S.-Y., Chikashige, Y., Kawasaki, Y., Uehara, L., Tsutsui, C., Hatanaka, M., Hiraoka, Y., Nagao, K., and Yanagida, M. (2007). Two-step, extensive alterations in the transcriptome from G0 arrest to cell division in Schizosaccharomyces pombe. Genes Cells 12, 677–692.

Shimanuki, M., Uehara, L., Plasulz, T., Yoshida, T., Kolubu, A., Kawasaki, Y., and Yanagida, M. (2013). Klf1, a C2H2 zinc-finger transcription factor, is required for cell wall maintenance during long-term quiescence in differentiated G0 phase. PLoS One 8, e78545.

Shizoka, K. (2009). Nutrition-minded cell cycle. Sci. Signal. 2, pe74.

Shirai, A., Sadaie, M., Shinmyozu, K., and Nakayama, J. (2010). Methylation of ribosomal protein L42 regulates ribosomal function and stress-adapted cell growth. J. Biol. Chem. 285, 22448–22460.

Singh, A., and Xu, Y.J. (2016). The cell killing mechanisms of hydroxyurea. Genes (Basel) 7, 99.

Stead, L.M., Brosnan, J.T., Brosnan, M.E., Vance, D.E., and Jacobs, R.L. (2006). Is it time to reevaluate methyl balance in humans? Am. J. Clin. Nutr. 83, 5–10.

Su, S.S., Tanaka, Y., Samejima, I., Tanaka, K., and Yanagida, M. (1996). A nitrogen starvation-induced dormant G0 state in fission yeast: the establishment from uncommitted G1 state and its delay for return to proliferation. J. Cell Sci. 109, 1347–1357.

Thomas, D., and Sardin-Kerjan, Y. (1991). The synthesis of the two S-adenosyl-methionine synthetases is differently regulated in Schizosaccharomyces cerevisiae. Mol. Gen. Genet. 226, 224–232.

Thon, G., Cohen, A., and Klar, A.J. (1994). Three additional linkage groups that repress transcription and meiotic recombination in the mating-type region of Schizosaccharomyces pombe. Genetics 138, 29–38.

Wei, Y., and Newman, E.B. (2002). Studies on the role of the metK gene product of Escherichia coli K-12. Mol. Microbiol. 43, 1651–1656.

Wood, V., Harris, M.A., McDowall, M.D., Rutherford, K., Vaughan, B.W., Staines, D.M., Aslett, M., Lock, A., Bähler, J., Kersey, P.J., et al. (2012). PomBase: a comprehensive online resource for fission yeast. Nucleic Acids Res. 40, D695–D699.

Yanagida, M. (2002). The model unicellular eukaryote, Schizosaccharomyces pombe. Genome Biol. 3, COMMENT2003.

Ye, C., Sutter, B.M., Wang, Y., Kuang, Z., and Tu, B.P. (2017). A metabolic function for phospholipid and histone methylation. Mol. Cell 66, 180–193.e8.

Yoshida, M., Horinouchi, S., and Beppu, T. (1995). Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. Bioessays 17, 423–430.
Supplemental Information

S-Adenosylmethionine Synthetase Is Required for Cell Growth, Maintenance of G0 Phase, and Termination of Quiescence in Fission Yeast

Takeshi Hayashi, Takayuki Teruya, Romanas Chaleckis, Susumu Morigasaki, and Mitsuhiro Yanagida
Five ts mutants of *sam1* were identified after screening more than 3,000 hygromycin-resistant transformants.
Figure S2

Figure S2. Locations of mutations of sam1 mutants in the three-dimensional structure, Related to Figure 1

The three-dimensional structure of a homodimer of rat liver MAT, the orthologue of Sam1, is depicted with the positions of amino acids mutated in sam1 mutants in three orientations. Subunits in the dimer appear as cartoon (monomer A) and molecular surface (monomer B), and N-terminal, central, and C-terminal domains of each subunit are colored light blue, light pink, and light green, respectively. The substrates, ATP and methionine, are represented as orange-colored sticks. Mutated amino acids in sam1 mutants are colored red (sam1-1), green (sam1-2), blue (sam1-3), yellow (sam1-4), and
magenta (*sam1*-5), and labeled with amino acid residue numbers adapted for *S. pombe* Sam1. Amino acid residue numbers of monomer A are underlined. D36, T90, E115, R299, F367 and Q370 are at solvent-exposed surfaces of the dimer, whereas I24 and L292 are not.
Figure S3. *sam1* mutants are sensitive to nicotinamide, actinomycin D, hydroxyurea, phleomycin and camptothecin, Related to Table 1

*sam1-1* and *sam1-3* were spotted on YPD plates in the presence or absence of nicotinamide (A), trichostatin A (B), actinomycin D (C), hydroxyurea (D), phleomycin (E), camptothecin (F), and ultraviolet irradiation (G), and then incubated at the indicated temperatures for 2–3 days. Wild-type, *ppc1-537* and *Δrad3* were used as controls.
## Transparent Methods

### Strains

The *S. pombe* strains used in this study are listed below.

| Strain | Genotype |
|--------|----------|
| 972    | $h^-$    |
| THO80  | $h^-\text{sam1-1}[hph]$ |
| THO48  | $h^-\text{sam1-2}[hph]$ |
| THO116 | $h^-\text{sam1-3}[hph]$ |
| THO50  | $h^-\text{sam1-4}[hph]$ |
| THO51  | $h^-\text{sam1-5}[hph]$ |
| THO55  | $h^-\text{sam1-L292S}[hph]$ |
| THO56  | $h^-\text{sam1-R299H}[hph]$ |
| THO52  | $h^-\text{sam1-I24M}[hph]$ |
| THO54  | $h^-\text{sam1-E115G}[hph]$ |
| THO53  | $h^-\text{sam1-T90A}[hph]$ |
| THO57  | $h^-\text{sam1-Q370R}[hph]$ |
| THO43  | $h^-\text{leu1 sam1-1}[hph]$ |
| THO415 | $h^-\text{leu1 sam1-3}[hph]$ |
| THO3   | $h^-\text{sam1-FLAG}[hph]$ |
| THO289 | $h^+\text{lys1}^+:\text{nda2p-atb2-GFP cut11-mCherry[nat]}$ |
| THO367 | $h^-\text{sam1-3}[hph] \text{ lys1}^+:\text{nda2p-atb2-GFP cut11-mCherry[nat]}$ |
| TH2F81 | $h^-\text{tor2-287}$ |
| THO144 | $h^-\text{ppc1-537}$ |
| THO100 | $\text{ura4 Δrad3::ura4}^-$ |

### Media and general techniques

Growth media and basic methods for *S. pombe* were described (Forsburg and Rhind, 2006; Sherman, 2002). YPD (rich medium) and EMM2 (synthetic minimal medium) were used for cultivation of fission yeast. Nitrogen-starved G0 cells were prepared according to procedures previously described (Su et al., 1996). Briefly, cells exponentially grown in EMM2 to a density of $2 \times 10^6$ cells/mL at 26 °C were harvested by vacuum filtration using a nitrocellulose membrane (0.45 µm pore size), washed in...
EMM2-N (EMM2 lacking a nitrogen source) once on the membrane, and then re-suspended in EMM2-N at a concentration of $2 \times 10^6$ cells/mL and incubated at 26°C for 24 hr. Cells were counted using a Sysmex CDA-500 particle analyzer.

**Construction of yeast strains**

To tag endogenous *sam1*+ with FLAG, the termination codon of the *sam1*+ gene was changed to the NotI site to incorporate 3FLAG, and was cloned into the pFA6a-hphMX6 derivative plasmid (Hentges et al., 2005), followed by integration into the endogenous *sam1* locus. Correct integration was confirmed by PCR. A similar strategy was used to tag endogenous *cut11*+ with mCherry using the natMX6 marker (Hentges et al., 2005). The resulting strain expressing Cut11-mCherry was crossed with the strain expressing α-tubulin-GFP (Nakazawa et al., 2016) to obtain strains expressing both α-tubulin-GFP and Cut11-mCherry.

**Isolation of ts alleles of sam1**

Error-prone PCR was performed as described previously (Hayashi et al., 2014). Briefly, the *sam1*+ gene was cloned into the pFA6a-hphMX6 derivative plasmid, and the fused DNA fragment containing the *sam1*+ ORF, the *hph* marker gene and the 0.5-kb downstream sequence of *sam1*+ ORF was amplified by PCR in the presence of increased MgCl$_2$ (8 mM), which significantly reduced the fidelity of polymerization and produced mutated gene fragments (Eckert and Kunkel, 1990). Amplified fragments were used for transformation of wild-type 972 cells. Hygromycin-resistant clones were selected at 26°C and then tested for growth at 36°C on YPD. Mutation sites in isolated strains were determined by sequencing the genomic *sam1* region after PCR amplification. Subsequently, mutations were introduced into the wild-type strain by site-directed mutagenesis, and temperature sensitivity of the resulting integrant strains was examined for reproducibility. The image of mutation sites in the three-dimensional structure was made using the molecular visualization system, PyMOL (Schrödinger, LLC). The structure of a homodimer of rat liver MAT was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/structure/1O9T).

**Metabolite analysis**

Metabolomic analysis was performed as previously described (Pluskal and
Yanagida, 2016; Pluskal et al., 2010b). Cells from liquid cultures (40 mL/sample, 5.0 × 10^6 cells/mL) were collected and immediately quenched in 25 mL of -40°C methanol. Cells were harvested by centrifugation at -20°C and internal standards (10 nmol of HEPES and PIPES) were added to each sample. Cells were disrupted using a Multi-Beads Shocker (Yasui Kikai) in 500 µL of 50% methanol. Proteins were removed by filtering on an Amicon Ultra 10-kDa cut-off filter (Millipore) and samples were concentrated by vacuum evaporation. Finally, each sample was re-suspended in 40 µL of 50% acetonitrile and 1 µL was used for LS-MS analysis using a Paradigm MS4 HPLC system (Michrom Bioresources) coupled to an LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific). Peak areas of metabolites of interest were measured using MZmine 2 software (Pluskal et al., 2010a) and normalized by the peak areas of spiked internal standards. Among detected peaks, 86 metabolites were identified by comparing their m/z values and retention times with pure standards. Glycerophosphoethanolamine and hercynylecysteine sulfoxide were identified by their theoretical m/z values and MS/MS fragmentation data.

**Immunoblotting**

Total protein extracts were prepared using the trichloroacetic acid precipitation method (Nagao et al., 2004). Cell were disrupted using glass beads in 10% trichloroacetic acid. Cell extracts were boiled in LDS sample buffer (Invitrogen) and loaded onto a NuPAGE 4-12% Bis-Tris Protein Gel (Invitrogen). Antibodies used were: anti-FLAG (M2, Sigma-Aldrich), anti-α-tubulin (TAT1, a gift from K. Gull), anti-Histone H3 (Abcam, ab1791), anti-monomethyl histone H3 (Lys4) (MABI0302, Takara), anti-dimethyl histone H3 (Lys4) (MABI0303, Takara), and anti-trimethyl histone H3 (Lys4) (MABI0304, Takara). Polyclonal antibodies against *S. pombe* Sam1 were prepared by immunizing rabbits using two synthetic peptides (IGYDDSEKGFDYKTC and NTYGTSSKTSVEY) as antigens (SCRUM Inc., Japan).

**Microscopy**

DAPI staining was performed as described previously (Adachi and Yanagida, 1989). Cells were fixed with 2% glutaraldehyde for 30 min on ice, washed three times with phosphate-buffered saline (PBS), and observed with a fluorescence microscope (BZ9000 or BZ-X710, Keyence, Japan). Cell length was measured using the “Between
2-points” tool of the “Measurement module” in BZ-II Analyzer software (Keyence, Japan). Methanol-fixed cells were used to observe GFP- and mCherry-tagged proteins, as previously described (Hayashi et al., 2004). Cells were harvested by vacuum filtration and fixed by immersion in 100% methanol at -80°C. After 30 min, PBS was added and cells were washed three times with PBS, and observed with a fluorescence microscope. Ten z-axis sections at 0.2 µm intervals were scanned using a DeltaVision system (GE Healthcare). Obtained images were deconvolved and projected on a 2D plane using SoftWoRx software.

FACS analysis

To measure DNA content of cells, flow cytometry was performed as described previously (Su et al., 1996) with minor modifications. 1×10⁷ cells were collected, washed with sterile distilled water, and resuspended in ice-cold 70% ethanol, and then kept at 4ºC. Cells were subsequently washed with 0.5 ml 50 mM sodium citrate (pH 7.0) and digested with RNase A (1 mg/ml) for 3 hr at 37ºC. Cells were stained with propidium iodide (12.5 µg/ml) and analyzed by FACSCalibur (Becton Dickinson).
Supplemental References

Adachi, Y., and Yanagida, M. (1989). Higher order chromosome structure is affected by cold-sensitive mutations in a Schizosaccharomyces pombe gene crm1+ which encodes a 115-kD protein preferentially localized in the nucleus and its periphery. J. Cell Biol. 108, 1195–1207.

Eckert, K.A., and Kunkel, T.A. (1990). High fidelity DNA synthesis by the Thermus aquaticus DNA polymerase. Nucleic Acids Research 18, 3739–3744.

Forsburg, S.L., and Rhind, N. (2006). Basic methods for fission yeast. Yeast 23, 173–183.

Hayashi, T., Ebe, M., Nagao, K., Kokubu, A., Sajiki, K., and Yanagida, M. (2014). Schizosaccharomyces pombe centromere protein Mis19 links Mis16 and Mis18 to recruit CENP-A through interacting with NMD factors and the SWI/SNF complex. Genes Cells 19, 541–554.

Hayashi, T., Fujita, Y., Iwasaki, O., Adachi, Y., Takahashi, K., and Yanagida, M. (2004). Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres. Cell 118, 715–729.

Hentges, P., Van Driessche, B., Tafforeau, L., Vandenhaute, J., and Carr, A.M. (2005). Three novel antibiotic marker cassettes for gene disruption and marker switching in Schizosaccharomyces pombe. Yeast 22, 1013–1019.

Nagao, K., Adachi, Y., and Yanagida, M. (2004). Separase-mediated cleavage of cohesin at interphase is required for DNA repair. Nature 430, 1044–1048.

Nakazawa, N., Mehrotra, R., Arakawa, O., and Yanagida, M. (2016). ICRF-193, an anticancer topoisomerase II inhibitor, induces arched telophase spindles that snap, leading to a ploidy increase in fission yeast. Genes Cells 21, 978–993.

Pluskal, T., and Yanagida, M. (2016). Metabolomic Analysis of Schizosaccharomyces pombe: Sample Preparation, Detection, and Data Interpretation. Cold Spring Harb Protoc 2016, pdb.top079921.
Pluskal, T., Castillo, S., Villar-Briones, A., and Orešić, M. (2010a). MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics 11, 395.

Pluskal, T., Nakamura, T., Villar-Briones, A., and Yanagida, M. (2010b). Metabolic profiling of the fission yeast S. pombe: quantification of compounds under different temperatures and genetic perturbation. Mol Biosyst 6, 182–198.

Sherman, F. (2002). Getting started with yeast. Meth. Enzymol. 350, 3–41.

Su, S.S., Tanaka, Y., Samejima, I., Tanaka, K., and Yanagida, M. (1996). A nitrogen starvation-induced dormant G0 state in fission yeast: the establishment from uncommitted G1 state and its delay for return to proliferation. J. Cell. Sci. 109, 1347–1357.