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Synthetic Gene Circuit-Mediated Monitoring of Endogenous Metabolites: Identification of *GAL11* as a Novel Multicopy Enhancer of *S*-Adenosylmethionine Level in Yeast

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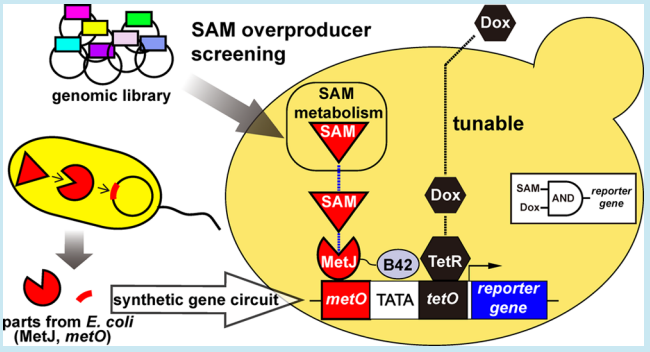
Supporting Information

ABSTRACT: Monitoring levels of key metabolites in living cells comprises a critical step in various investigations. The simplest approach to this goal is a fluorescent reporter gene using an endogenous promoter responsive to the metabolite. However, such a promoter is often not identified or even present in the species of interest. An alternative can be a synthetic gene circuit based on a heterologous pair consisting of a promoter and a transcription factor known to respond to the metabolite. We exploited the *met* operator and MetJ repressor of *Escherichia coli*, the interaction between which depends on *S*-adenosylmethionine (SAM), to construct synthetic gene circuits that report SAM levels in *Saccharomyces cerevisiae*. Using a dual-input circuit that outputs selection marker genes in a doxycycline-tunable manner, we screened a genomic library to identify *GAL11* as a novel multicopy enhancer of SAM levels. These results demonstrate the potential and utility of synthetic gene circuit-mediated metabolite monitoring.



KEYWORDS: *AND gate, SAM, metO, MetJ, doxycycline, tunability*

Monitoring the intracellular levels of a given metabolite in living cells often comprises a critical step in both basic and applied research: it can lead to better understanding of metabolic responses as well as improved production of the metabolite for industrial and pharmaceutical use. The simplest approach to this goal would be a fluorescent reporter gene based on an endogenous promoter that responds to the metabolite. However, it is often not known how such a promoter responds to the metabolite: it may be directly controlled by a metabolite-responsive transcription factor (TF) that binds to the metabolite or indirectly affected by altered metabolic state. While the former scenario is desirable for developing a reporter gene system, such a TF has often not yet been identified or may not even be present in the species of interest. These limitations may be overcome by using a synthetic gene circuit based on a promoter and metabolite-responsive TF pair that are known to directly respond to the metabolite in species other than the host cell or a heterologous promoter and TF pair. While interdomain transplantation of TFs from prokaryotes to eukaryotes is potentially useful, only a limited number of practical applications have been reported so far.[1−3]

Here we intended to construct a synthetic gene circuit that specifically responds to a key metabolite, *S*-adenosylmethionine (SAM), in the budding yeast *Saccharomyces cerevisiae*, in which no SAM-responsive TF has been identified to date. SAM serves as the primary methyl donor for the modification of a plethora of biomolecules, ranging from DNA, RNA, and proteins to various small molecules. Accordingly, a simple method for monitoring the intracellular SAM levels would be useful in many aspects of basic biology. It should be also noted that SAM is an important industrial product used as an essential research reagent, pharmaceutical, and supplement. As SAM is produced in *S. cerevisiae*,[4] strains that overproduce SAM have been pursued through a variety of approaches,[5−7] even including a spaceflight culture.[8] However, these screenings have focused on alteration of known SAM metabolic pathways or were conducted using a brute force approach based on measurement of SAM by high-performance liquid chromatography (HPLC) in every randomly picked clone from a mutagenized yeast population.

We hypothesized that a synthetic gene circuit that can fluorescently report the SAM level would provide a much simpler assay than those currently used, enabling high throughput (HTP) screenings of genomic and chemical libraries to identify genes and chemicals, respectively, that affect the level. The circuit can also utilize selection marker genes as its outputs, in order to enrich for cells with elevated SAM levels to facilitate the screening.

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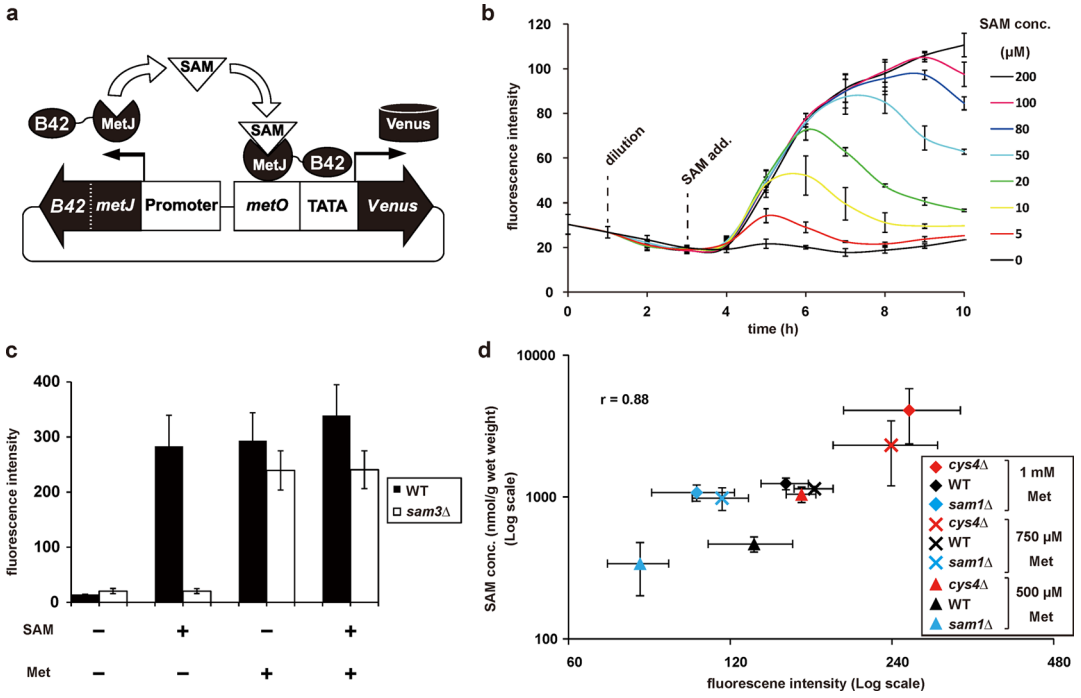

Figure 1. A single-input synthetic gene circuit for reporting SAM levels in budding yeast. (a) Scheme of the SAM-responsive synthetic gene circuit. (b) Response of the circuit. SAM was added to the medium at 2 h after a 16-fold dilution of overnight culture, and cellular fluorescence was measured by FCM at every 1-h interval. (c) Response of wild-type and *sam3*Δ cells to SAM and Met. Cellular fluorescence was measured at 8 h after the addition of SAM and/or Met to a concentration of 100 μM and 1 mM, respectively. (d) Comparison of cell fluorescence and SAM concentration. Each strain was cultured for 24 h under the indicated condition and harvested for FCM and HPLC analyses. The correlation coefficient (r) between the results of FCM and HPLC analyses was 0.88. The means and standard deviations are shown (n = 3 for panel b; n = 4−8 for panels c and d).

Here we used the methionine operator *metO* and repressor MetJ from *Escherichia coli* to construct single- and dual-input synthetic gene circuits that are responsive to SAM in the yeast. The DNA-binding activity of MetJ depends solely on complex formation with SAM and does not require any other covalent modifications or binding to regulatory subunits,[9−13] thereby ensuring its orthogonality to the host system. We used the dual-input circuit developed here to screen a genomic library and successfully identified *GAL11* as a novel multicopy enhancer of SAM levels.

A Single-Input Synthetic Gene Circuit for Reporting SAM Levels in Budding Yeast. To construct a SAM-responsive reporter gene system, we designed the synthetic transcriptional activator MetJ-B42 by fusing the MetJ repressor to the transcriptional activation domain B42. The MetJ-B42 should bind SAM to form a holocomplex, SAM−MetJ-B42, which can bind *metO* to activate the expression of genes placed downstream of *metO* (Figure 1a). We used the fluorescent protein Venus as the reporter for quantification by flow cytometry (FCM). We cloned *metO-CYC1pr-Venus* and *MetJ-B42* modules into a single plasmid for "one-step installation" of the synthetic gene circuit to the cell (Figure 1a). The yeast cells installed with the circuit commenced fluorescing 1 h after addition of SAM to the medium (Figure 1b). The maximum fluorescence intensity was dependent on the dose of SAM added to the medium (Figure 1b). The dynamics of this gene circuit was consistent with the result of a simulation model (Supplementary Figure 1), suggesting that it operates as we intended.

To validate the proper operation of the circuit genetically, we tested the effect of deleting *SAM3*, which encodes the high affinity SAM permease on the plasma membrane.[14] If the circuit operates according to the scenario described above, then *sam3*Δ cells should be incapable of responding to SAM added to the medium. Indeed, we found that *sam3*Δ cells, in contrast to the wild-type cells, failed to fluoresce in the presence of SAM (Figure 1c). We also examined the effect of methionine (Met), which serves as a precursor for SAM and has been known to increase the intracellular SAM concentration.[15,16] Addition of Met to the medium induced the expression of the *Venus* reporter gene not only in the wild-type but also in the *sam3*Δ cells (Figure 1c), because both cells can take up Met to synthesize SAM.

To confirm the SAM-driven operation of the circuit biochemically, we quantified the intracellular SAM levels by an HPLC method and compared the value with the cell fluorescence. We used different Met concentrations in the medium as well as 2 mutant strains, namely, *sam1*Δ and *cys4*Δ strains, to prepare cells with differential SAM levels. *SAM1* encodes SAM synthetase and its deletion leads to a decrease in SAM production, whereas *CYS4* encodes cystathionine-β-synthase and its deletion disturbs sulfur metabolism to increase the intracellular SAM level.[17] As expected, the HPLC method showed that the intracellular SAM level was the highest in the *cys4*Δ cells, followed by the wild-type cells, and then by the *sam1*Δ cells regardless of the Met concentration (Figure 1d). The fluorescence signals were largely consistent with the results obtained by the HPLC method (Figure 1d).

Taken together, we concluded that the single-input synthetic gene circuit using MetJ-B42 reflects the intracellular SAM level. Importantly, monitoring of SAM levels by this system is much simpler and less time-consuming than by the conventional

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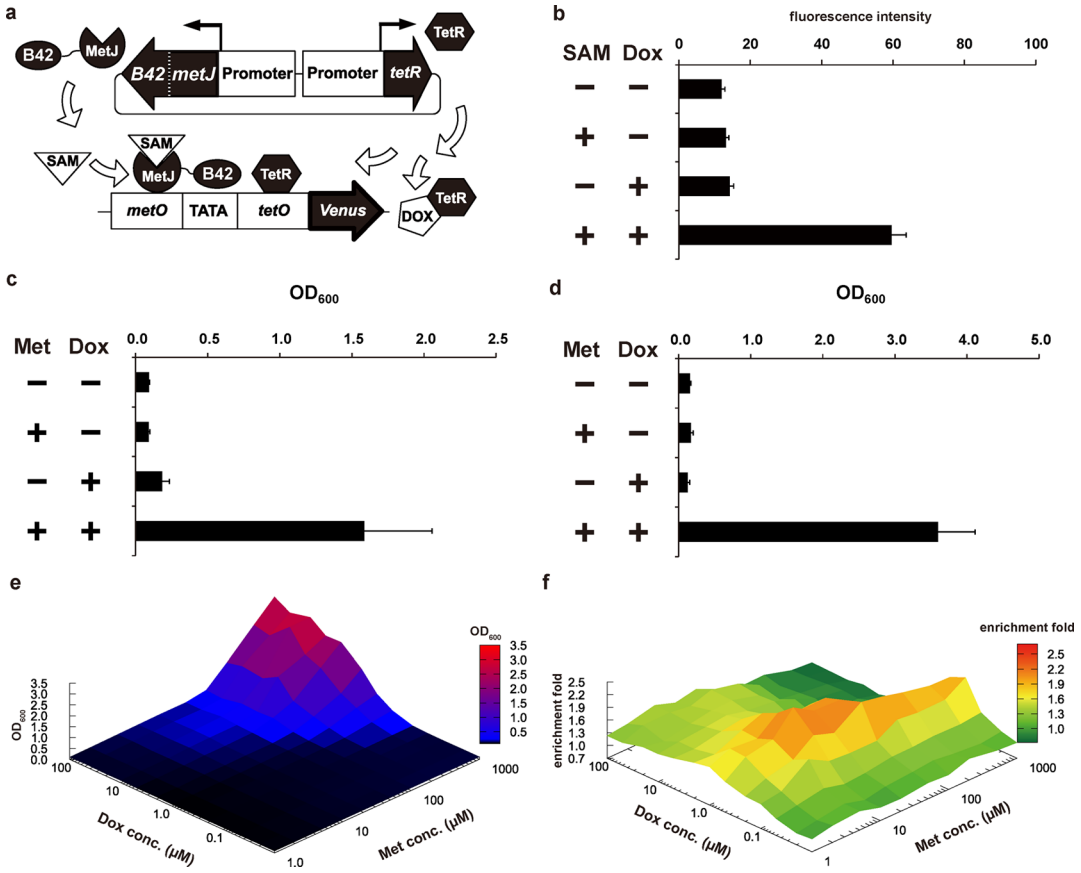

Figure 2. A dual-input tunable synthetic gene circuit to enrich SAM overproducers. (a) Design of the SAM- and Dox-responsive AND gate. (b) Fluorescence of the cells installed with the AND gate. (c) His-independent growth of the cells installed with the AND gate using *HIS3* reporter. (d) Leu-independent growth of the cells installed with the AND gate using *LEU2* reporter. (e) Fitness landscape of the screening host strain YTU225. (f) Competitive growth assay between the strains with and without an additional copy of *SAM1*. The former and the latter were labeled with RFP and GFP, respectively. The means (n = 3) are shown for panels b–f, and the standard deviations are shown for panels b–d.

HPLC method. Furthermore, it enables live cell analysis. These features make this system ideal for various applications. For instance, it can be used for HTP screening of chemical libraries for small molecules that affect SAM level, some of which can provide leads for drug development.[18−20] It is also applicable to the screening of genomic and mutant libraries for DNA clones and mutants, respectively, that improve SAM production. While HTP fluorometric screening is possible, an enrichment step would also be useful. We thus modified the circuit to use auxotrophic reporter genes as its output.

A Dual-Input Tunable Synthetic Gene Circuit tTo Enrich SAM Overproducers. We replaced the *Venus* reporter gene with the auxotrophic marker gene *HIS3*, induction of which enables the cell to grow in the absence of histidine (His), and examined the strain for His-independent growth in the presence and absence of Met. While we had expected that cells would grow better in the presence than in the absence of Met, we failed to observe any effect of Met on growth (Supplementary Figure 2). It is conceivable that the basal expression level of *HIS3* (i.e., the expression level of *HIS3* in the absence of Met) was sufficient to confer His-independent growth to the cells. While we may make this system work either by competitively inhibiting the activity of His3 protein (i.e., imidazoleglycerol-phosphate dehydratase) with 3-aminotriazole or by weakening the reporter gene promoter in an *ad hoc* manner, we preferred to develop a more *generic* solution to tune the output of the reporter system.

As an approach to tunable modulation of the reporter gene expression, we attempted to integrate the Dox-responsive repressor TetR into the system by inserting the *tet* operator (*tetO*) downstream of *metO*. Such a dual-input system was expected to serve as an "AND gate" to output only when both SAM and Dox are present in the medium (Figure 2a). We also expected that it would be possible to modulate the intensity of the output by changing the concentration of Dox.

To test this idea, we first validated the dual-input system using the *Venus* reporter gene. As intended, the cells fluoresced only when both SAM and Dox were added to the medium (Figure 2b). We next used the *HIS3* reporter and quantified cell growth in the absence of His. While the cells equipped with the dual-input system failed to grow in media lacking either Met or Dox, they showed robust growth in the presence of both Met and Dox (Figure 2c). Notably, the basal growth rate was controlled by adjusting the concentration of Dox (see below). This tunable characteristic would enable screening under different selection pressures, thereby enhancing the utility of the system.

Tuning of the Dual-Input Gene Circuit for Efficient Screening. We improved the Met-dependence in the fitness landscape of the cells installed with the dual-input system as follows. First, we adjusted the level of TetR by truncating the *TDH1* promoter that controls its expression. Second, we shifted the position of *tetO* from a site immediately downstream of the TATA box (*metO-CYC1pr-tetO*(TATA)) to a site around the

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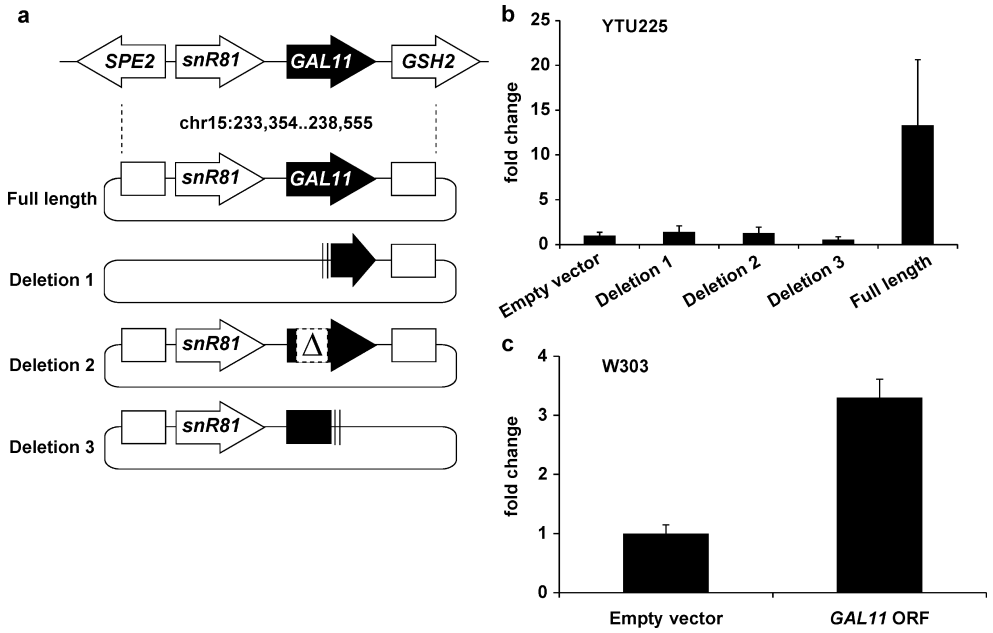

Figure 3. *GAL11* as a multicopy enhancer of SAM level. (a) Genomic structure around the region cloned in the 5 library plasmids that elevated the SAM levels. Plasmid structures are also shown for the full-length and partially deleted clones. (b) Comparison of the SAM levels among YTU225 transformed with the 3 subclones and the full-length clone. The means and standard deviations are shown (n = 3). (c) Elevation of SAM level in W303 transformed with a low copy plasmid that overexpresses *GAL11*. The means and standard deviations are shown (n = 5).

transcription start site (*metO-CYC1pr-tetO*(TSS)). Third, to reduce the effect of the intrinsic noise of reporter gene expression,[21] we employed *LEU2* as a second auxotrophic reporter gene, as we had confirmed that the *LEU2* system alone confers a Met- and Dox-dependent growth phenotype, as did the *HIS3* system (Figure 2d).

We generated the screening host strain YTU225 by integrating all of these modules, namely, 2 auxotrophic and 1 fluorescent reporters (i.e., *HIS3*, *LEU2*, and *Venus*), a *MetJ-B42* module, and a *TetR* module, into the host genome (Supplementary Figure 3). We then measured the growth of YTU225 under various concentrations of Met and Dox to reveal its fitness landscape (Figure 2e, Supplementary Figure 4). The landscape suggests that due to the flexible nature of this system differently inclined planes can be used for screening; cells with high SAM levels can be searched for in the presence of a low Dox concentration, whereas those with intermediate levels can be searched for in the presence of a high Dox concentration.

We next intended to identify appropriate concentrations of Dox and Met for the screening. For this purpose, we took advantage of the fact that a single additional copy of *SAM1* can increase the intracellular SAM level by approximately 1.3-fold (Supplementary Figure 5) and designed a competitive growth assay between the *HIS3*-reporter strains, one bearing a low copy *SAM1* plasmid and the other bearing a control empty vector. The former and the latter cells were labeled with red and green fluorescent proteins, respectively, and counted by FCM. The enrichment was evaluated by the ratio between the numbers of red and green cells. A series of competitive growth assays under various concentrations of Met and Dox were conducted to reveal the conditions for maximum enrichment (Figure 2f). Similar results were obtained when the fluorescent labels were swapped (Supplementary Figure 6). These conditions should enrich even for mutants with a marginal increase in SAM levels, similar to that conferred by an additional copy of *SAM1*. The cells with a higher increase in SAM levels would be enriched more efficiently.

Identification of *GAL11* as a Multicopy Enhancer of SAM Level. To screen for multicopy enhancers of SAM level, we constructed a genomic library from *sam1*Δ cells using a 2μ-type vector and transformed YTU225 with the library plasmids. We performed a screening under weak selection pressure by cultivating the transformants for 3 days in the presence of 200 μM Met and 2 μM Dox. The plasmids were then extracted *en masse* from the culture and used for transformation of freshly prepared YTU225 cells, which were subsequently cultivated under the same conditions. Following single colony isolation on agar plates, we measured the fluorescence and SAM levels from each clone by FCM and HPLC, respectively. We selected 5 clones with the highest SAM levels for further analysis.

We sequenced the plasmids in these clones and found that they shared an identical plasmid derived from Chr XV: 233,354−238,555. Intriguingly, an independent screening of the same library revealed that a plasmid carrying almost the same genomic fragment (ChrXV: 233,454−238,555) in the opposite direction also elevated SAM levels. The genomic locus shared by these plasmids includes intact *GAL11* (a component of the tail module of Mediator complex), *snR81* (snoRNA), and the 5′-half of both *SPE2* (SAM decarboxylase involved in spermine synthesis) and *GSH2* (glutathione synthetase) (Figure 3a). To delineate the critical region of the insert, we used appropriate restriction enzymes to generate 3 subclones and examined these for the ability to elevate SAM levels. All of these subclones, in each of which *GAL11* was partially deleted, failed to confer any increment of SAM (Figure 3b). These results indicated that intact *GAL11* is required for the increase of SAM. To examine whether *GAL11* is sufficient to elevate SAM levels, we overexpressed this ORF from the *TDH1* promoter on a low copy centromeric plasmid and quantified SAM levels by HPLC. Cells transformed with the *GAL11* plasmid showed an approximately 3.3-fold increase in the level

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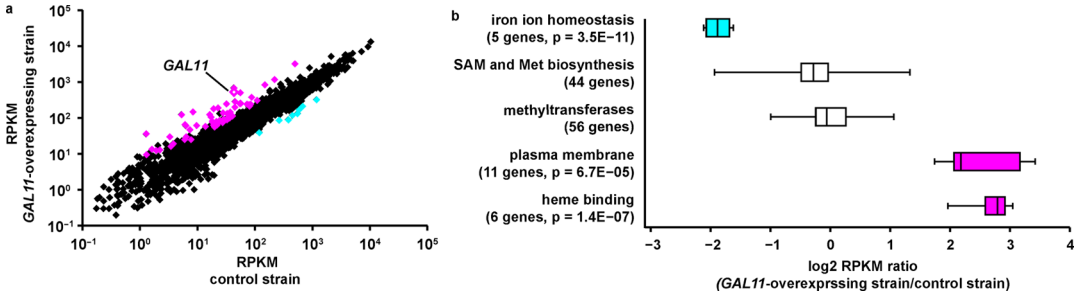

Figure 4. Transcriptome changes upon *GAL11* overexpression. (a) Comparison of RNA-Seq data between cells with and without overexpression of *GAL11*. Genes called differentially expressed at the false discovery rate of 0.08 are colored: magenta and cyan spots indicate 52 up-regulated and 8 down-regulated genes, respectively. (b) Box plots for the expression ratio between cells with and without overexpression of *GAL11*. Data are shown for genes associated with each of the 3 GO terms significantly enriched in the differentially expressed genes. Number of genes in each GO category and p-value for the enrichment are indicated in the parentheses. Data were also shown for 44 genes involved in SAM and Met biosynthesis and 56 genes encoding methyltransferases that consume SAM. All of the genes used for this analysis are listed in Supporting Information. RPKM; reads per kilobase of exon per million reads.

of SAM (Figure 3c). From these results, we concluded that *GAL11* serves as a novel multicopy enhancer of SAM level in *S. cerevisiae*.

Elusive Mechanisms for *GAL11*-Induced SAM Elevation. Since *GAL11* encodes a component of the Mediator complex, its overexpression would primarily affect the transcriptome. We thus performed RNA-Seq of cells with and without overexpression of *GAL11*.

Of the 7,126 genes in the yeast, 60 were called differentially expressed at the false discovery rate of 0.08 (Figure 4a). Gene Ontology (GO) analysis indicated that the 52 up-regulated genes were enriched for those associated with "heme binding" and "plasma membrane proteins", whereas the 8 down-regulated ones were enriched for those associated with "iron ion homeostasis" (Figure 4b and Supporting Information). However, it is currently not clear whether and how these changes contribute to the observed SAM elevation. Counter-intuitively, we found that genes involved in SAM and Met biosynthesis were unaffected or even down-regulated (Figure 4b and Supporting Information). We also failed to find any change suggestive of reduced SAM consumption: no significant changes in expression level were found for genes encoding methyltransferases (Figure 4b and Supporting Information). Taken together, the mechanism underlying *GAL11*-induced elevation of SAM is not immediately obvious based on current knowledge (also see Supporting Information).

Summary and Conclusion. We used *metO* and MetJ to construct SAM-responsive synthetic gene circuits in yeast. A Dox-tunable circuit allowed us to screen a genomic library, leading to the identification of *GAL11* as a novel multicopy enhancer of SAM level. A potential advantage of this system would be its tunable nature, since it enables screenings under different selection pressures. Indeed, we screened the same library under a different selection pressure to identify another multicopy enhancer that encodes a previously uncharacterized protein (to be published elsewhere). A SAM-responsive riboswitch, which reversibly modulates prokaryotic translation initiation through masking/unmasking of ribosome-binding sites, could be used as the sensor module.[22] This study avoided the riboswitch, because the cap-dependent ribosome scanning mechanism in eukaryotes prevents its straightforward use in 5′-untranslated regions, thereby necessitating its further engineering, and because its high affinity to SAM is disfavored for screening of SAM overproducers.[23] Nevertheless, we note that riboswitches can serve as another rich source of sensor modules for synthetic gene circuit-mediated metabolite monitoring. We expect that the ongoing explosion of genomic sequence data, together with the progress of HTP *cis*-element identification, protein engineering, and bioinformatics,[24,25] will expand the repertoire of metabolites that can be monitored in this approach, enabling far broader applications in the near future.

METHODS

Plasmid Construction. The open reading frame of *metJ* was PCR-amplified from *E. coli* genomic DNA so that MetJ bears a classical nuclear localization signal (NLS) (PKKKRKV) from SV40 T-antigen at its N terminus. The linker to connect MetJ and B42 transcriptional activation domain was composed of λ cI repressor linker (92−132) followed by a synthetic linker GENLFQSGG. The *MetJ-B42* was expressed from a truncated *ADH1* promoter. The reporter gene was constructed by inserting chemically synthesized *metO* upstream of *CYC1* promoter (*CYC1pr*) followed by *Venus*. For one-step installation of the gene circuit, the *MetJ-B42* module and the *metO-CYC1pr-Venus* module were cloned in a head-to-head configuration at the multiple cloning site of pRS415.

To construct a SAM- and Dox-responsive AND gate, *tetR* was PCR-amplified so that TetR bears the SV40 NLS at its the N terminus. The *TetR* was expressed from *TDH1* promoter or its truncated variant. The *MetJ-B42* and *TetR* modules were inserted into the multiple cloning site of pRS415 in a head-to-head configuration. For the integration of *MetJ-B42* and *TetR* modules to *TRP1* locus, these modules were cloned in a head-to-head configuration in pRS404

The *metO-CYC1pr* was mutated to design *metO-CYC1pr-tetO*(TSS) and *metO-CYC1pr-tetO*(TATA) as shown in Supplementary Table 1. For the integration of reporter genes, *metO-CYCpr-tetO*(TATA)-*Venus* was cloned in pRS406, a URA3-marked YIp integration vector. Similarly, *metO-CYC1pr-tetO*(TSS)-*LEU2* was cloned in pRS402, an ADE2-marked YIp. *metO-CYC1pr-tetO*(TATA)-*HIS3* was cloned in a LYS2-marked YIp vector.

Strains. The screening host strain YTU225 was constructed by integrating the *MetJ-B42* and *TetR* modules, the *metO-CYC1pr-tetO*(TATA)-*LEU2* module, the *metO-CYC1pr-tetO*(TSS)-*HIS3* module, and the *metO-CYC1pr-tetO*(TATA)-*Venus* module in the YIp vectors described above into *TRP1*, *ADE2*, *LYS2*, and *URA3* loci, respectively, followed by disrupting the functional *URA3* copy by *KanMX*. To construct the strains for the competitive growth assay, either *ACT1pr-gfp*

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or *ACT1pr-rfp* was integrated into *URA3* locus, whereas the *MetJ-B42* and *TetR* modules and the *metO-CYC1pr-tetO*(TSS)-*HIS3* were integrated as described above.

■ ASSOCIATED CONTENT

Supporting Information

Supporting text, methods, figures, tables, and data sheets. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

All authors contributed to design of the research; T.U. and S.O. performed experiments; T.U. and T.I. wrote the paper.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Bacchus, W., Lang, M., El-Baba, M. D., Weber, W., Stelling, J., and Fussenegger, M. (2012) Synthetic two-way communication between mammalian cells. *Nat. Biotechnol. 30*, 991−996.

(2) Weber, W., and Fussenegger, M. (2007) Inducible product gene expression technology tailored to bioprocess engineering. *Curr. Opin. Biotechnol. 18*, 399−410.

(3) Weber, W., and Fussenegger, M. (2011) Molecular diversity–the toolbox for synthetic gene switches and networks. *Curr. Opin. Chem. Biol. 15*, 414−420.

(4) Shiozaki, S., Shimizu, S., and Yamada, H. (1984) Unusual intracellular accumulation of *S*-adenosyl-L-methionine by microorganisms. *Agric. Biol. Chem. 48*, 2293−2300.

(5) Shobayashi, M., Mukai, N., Iwashita, K., Hiraga, Y., and Iefuji, H. (2006) A new method for isolation of *S*-adenosylmethionine (SAM)-accumulating yeast. *Appl. Microbiol. Biotechnol. 69*, 704−710.

(6) Shiomi, N., Fukuda, H., Murata, K., and Kimura, A. (1995) Improvement of *S*-adenosylmethionine production by integration of the ethionine-resistance gene into chromosomes of the yeast *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol. 42*, 730−733.

(7) Chan, S. Y., and Appling, D. R. (2003) Regulation of *S*-adenosylmethionine levels in *Saccharomyces cerevisiae*. *J. Biol. Chem. 278*, 43051−43059.

(8) Huang, Y., Gou, X., Hu, H., Xu, Q., Lu, Y., and Cheng, J. (2012) Enhanced *S*-adenosyl-L-methionine production in *Saccharomyces cerevisiae* by spaceflight culture, overexpressing methionine adenosyltransferase and optimizing cultivation. *J. Appl. Microbiol. 112*, 683−694.

(9) Parsons, I. D., Persson, B., Mekhalfia, A., Blackburn, G. M., and Stockley, P. G. (1995) Probing the molecular mechanism of action of co-repressor in the *E. coli* methionine repressor-operator complex using surface plasmon resonance (SPR). *Nucleic Acids Res. 23*, 211−216.

(10) Lawrenson, I. D., and Stockley, P. G. (2004) Kinetic analysis of operator binding by the *E. coli* methionine repressor highlights the role(s) of electrostatic interactions. *FEBS Lett. 564*, 136−142.

(11) Marincs, F., Manfield, I. W., Stead, J. A., McDowall, K. J., and Stockley, P. G. (2006) Transcript analysis reveals an extended regulon and the importance of protein-protein co-operativity for the *Escherichia coli* methionine repressor. *Biochem. J. 396*, 227−234.

(12) Augustus, A. M., Reardon, P. N., Heller, W. T., and Spicer, L. D. (2006) Structural basis for the differential regulation of DNA by the methionine repressor MetJ. *J. Biol. Chem. 281*, 34269−34276.

(13) Augustus, A. M., Sage, H., and Spicer, L. D. (2010) Binding of MetJ repressor to specific and nonspecific DNA and effect of *S*-adenosylmethionine on these interactions. *Biochemistry 49*, 3289−3295.

(14) Rouillon, A., Surdin-Kerjan, Y., and Thomas, D. (1999) Transport of sulfonium compounds. Characterization of the *S*-adenosylmethionine and *S*-methylmethionine permeases from the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem. 274*, 28096−28105.

(15) Ferro, A. J., and Spence, K. D. (1973) Induction and repression in the *S*-adenosylmethionine and methionine biosynthetic systems of *Saccharomyces cerevisiae*. *J. Bacteriol. 116*, 812−817.

(16) Thomas, D., Rothstein, R., Rosenberg, N., and Surdin-Kerjan, Y. (1988) *SAM2* encodes the second methionine *S*-adenosyl transferase in *Saccharomyces cerevisiae*: physiology and regulation of both enzymes. *Mol. Cell. Biol. 8*, 5132−5139.

(17) Christopher, S. A., Melnyk, S., James, S. J., and Kruger, W. D. (2002) *S*-adenosylhomocysteine, but not homocysteine, is toxic to yeast lacking cystathionine beta-synthase. *Mol. Genet. Metab. 75*, 335−343.

(18) Mischoulon, D., and Fava, M. (2002) Role of *S*-adenosyl-L-methionine in the treatment of depression: a review of the evidence. *Am. J. Clin. Nutr. 76*, 1158S−1161S.

(19) Martínez-Chantar, M. L., García-Trevijano, E. R., Latasa, M. U., Pérez-Mato, I., Sánchez del Pino, M. M., Corrales, F. J., Avila, M. A., and Mato, J. M. (2002) Importance of a deficiency in *S*-adenosyl-L-methionine synthesis in the pathogenesis of liver injury. *Am. J. Clin. Nutr. 76*, 1177S−1182S.

(20) Purohit, V., Abdelmalek, M. F., Barve, S., Benevenga, N. J., Halsted, C. H., Kaplowitz, N., Kharbanda, K. K., Liu, Q. Y., Lu, S. C., McClain, C. J., Swanson, C., and Zakhari, S. (2007) Role of *S*-adenosylmethionine, folate, and betaine in the treatment of alcoholic liver disease: summary of a symposium. *Am. J. Clin. Nutr. 86*, 14−24.

(21) Maheshri, N., and O'Shea, E. K. (2007) Living with noisy genes: how cells function reliably with inherent variability in gene expression. *Annu. Rev. Biophys. Biomol. Struct. 36*, 413−434.

(22) Smith, A. M., Fuchs, R. T., Grundy, F. J., and Henkin, T. M. (2010) The SAM-responsive SMK box is a reversible riboswitch. *Mol. Microbiol. 78*, 1393−1402.

(23) Tomsic, J., McDaniel, B. A., Grundy, F. J., and Henkin, T. M. (2008) Natural variability in *S*-adenosylmethionine (SAM)-dependent riboswitches: S-box elements in *Bacillus subtilis* exhibit differential sensitivity to SAM *in vivo* and *in vitro*. *J. Bacteriol. 190*, 823−833.

(24) Sharon, E., Kalma, Y., Sharp, A., Raveh-Sadka, T., Levo, M., Zeevi, D., Keren, L., Yakhini, Z., Weinberger, A., and Segal, E. (2012) Inferring gene regulatory logic from high-throughput measurements of thousands of systematically designed promoters. *Nat. Biotechnol. 30*, 521−530.

(25) Collins, C. H., Leadbetter, J. R., and Arnold, F. H. (2006) Dual selection enhances the signaling specificity of a variant of the quorum-sensing transcriptional activator LuxR. *Nat. Biotechnol. 24*, 708−712.