Complete conversion of sugars synthesized by photosynthesis into commodity products

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Research objective

MazF, a protein toxin of the bacterial toxin-antitoxin system, is a sequence-specific endoribonuclease that recognizes “ACA”. Given that ACA sequences are present in almost all mRNAs, most mRNAs are degraded in MazF-expressing cells. In contrast, mRNAs lacking the ACA sequence do not undergo cleavage by MazF and are translated in large quantities by occupying the ribosome. Accordingly, by removing ACA sequences from all genes encoding enzymes in a particular metabolic pathway, we could create cells in which a particular metabolic pathway functions preferentially. In the present study, we developed a system to inhibit cellular functions using MazF to create cells in which specific metabolic pathways preferentially function.

Methods

First, we examined whether the expression of MazF improves the translation efficiency of mRNAs lacking the ACA sequence in *Escherichia coli* using GFP as a model. There are 26 ACA sequences in the *gfp* gene. All ACA sequences in the *gfp* gene were replaced with other sequences without changing the amino acid sequence of GFP to create the *gfpP* gene. Plasmids pKMgP and pKMg, which express *gfpP* and *gfp*, respectively, from the arabinose-inducible promoter P_{BAD}, were created based on pBR322. In addition, we created the plasmid pKMmR, which expresses MazF from the tetracycline-inducible promoter P_{R0040}, based on pACYC177. A control plasmid, pKM, was created by removing P_{R0040} and *mazF* from the pKMmR vector. *E. coli* strains KMmR+g and KMmR+gP were generated by transforming pKMg or pKMgP, respectively, into *E. coli* HST08 carrying pKMmR. Strains KM+g and KM+gP, harboring pKM instead of pKMmR, were used as controls. In 96-well plates, each strain was cultured in 200 μL of LB medium, supplemented with 100 μg/ml ampicillin and 30 μg/ml kanamycin, for 2 h at 37°C with shaking. Subsequently, arabinose (final concentration 0.1%) and anhydrotetracycline (aTc) were added to induce the expression of GFP and MazF. GFP fluorescence intensity was normalized to an optical density at 600 nm.

Results

To determine whether the translation efficiency of the *gfpP* gene lacking the ACA sequence was similar to that of the original *gfp* gene, GFP expression was induced by adding arabinose (range, 0.001–1%) in KMmR+g and KMmR+gP, followed by the measurement of GFP fluorescence intensity. In both strains, GFP fluorescence intensity increased with increasing arabinose concentrations, and there was no significant difference in fluorescence intensity at each concentration. Accordingly, there was no difference in translation efficiency between *gfpP* and *gfp*.

To examine the effect of MazF expression on GFP expression, aTc and arabinose were added simultaneously to induce MazF expression (Figure 1). GFP fluorescence increased immediately after induction in KM+g but did not increase in KMmR+g until 60 min after induction. These results suggested that MazF could suppress protein synthesis from *gfp*. However, GFP fluorescence increased after 60 min, and there was no significant difference in the fluorescence intensity between KM+g and KMmR+g at 120 min. Therefore, the MazF function was considered to be suppressed after 60 min. Moreover, the MazF-mediated

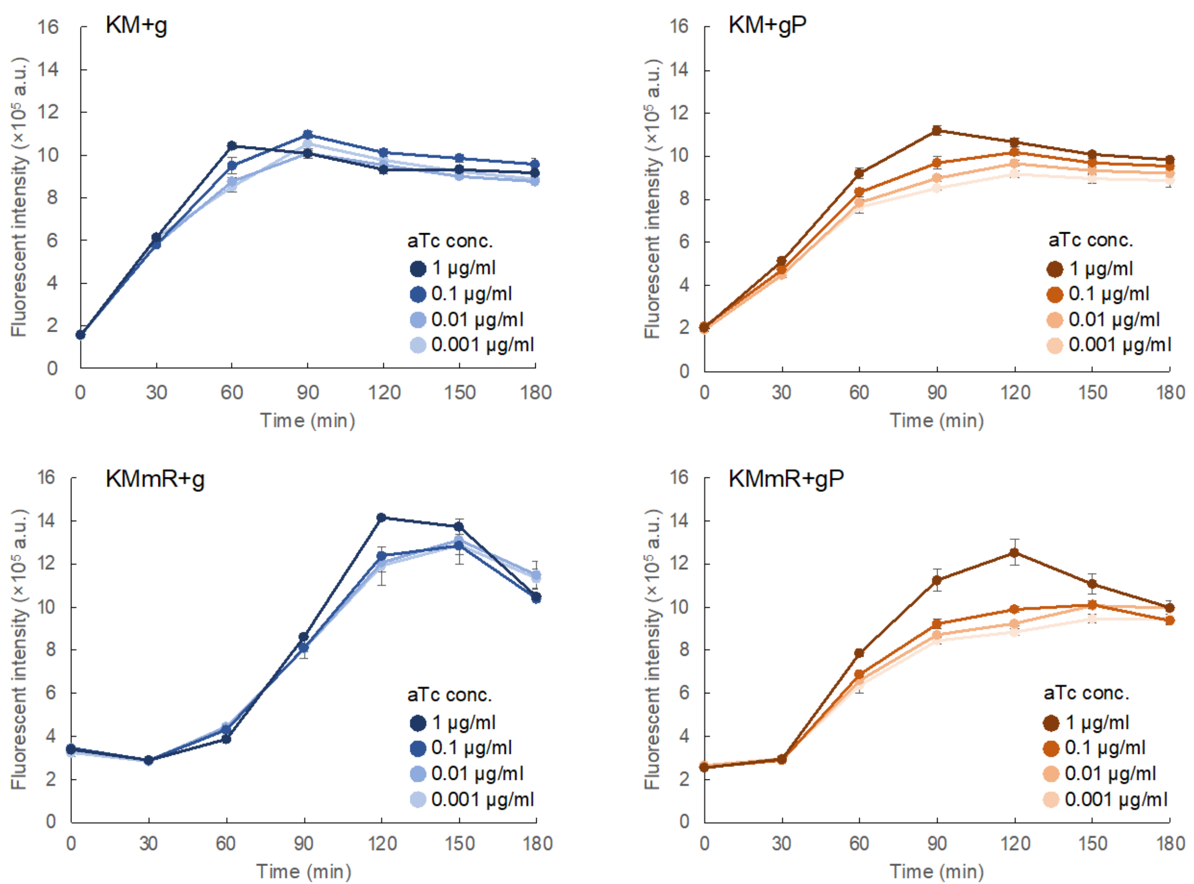

Figure 1. Effects of MazF on expression of GFP

0.1% arabinose and aTc were added to cultures at 0 min, and then GFP fluorescence intensity was measured. KMmR indicates MazF-expressing strains and KM were non-expressing strains. gP strains harbor *gfpP* lacking the ACA sequence, and g strains have the original *gfp*.

suppression of GFP fluorescence occurred independent of the aTc concentration, suggesting that differences in MazF expression levels had no effect on the suppression of GFP expression and that markedly low levels of MazF expression could suppress GFP synthesis even in the absence of aTc.

Next, we compared the effects of MazF on *gfp* and *gfpP* expression. GFP fluorescence increased at 60 min in KMmR+gP, indicating that deleting the ACA sequence reduced the MazF-mediated suppression of GFP synthesis. Moreover, in KMmR+gP, GFP fluorescence intensity was significantly increased at an aTc concentration of 1 μg/ml than at lower aTc concentrations. Thus, higher MazF expression was associated with enhanced *gfpP* translation, thereby suggesting that degradation of endogenous mRNA by MazF increased the number of ribosomes binding to *gfpP* mRNA.

Conclusion

The present study revealed that MazF expression could suppress protein synthesis. However, the inhibitory effect was abolished after 60 min, suggesting that *mazF* mRNA was also degraded because there were eight ACA sequences in the *mazF* mRNA. Sustained suppression of protein synthesis would be achieved by removing ACA sequences from *mazF*. In addition, MazF could suppress protein synthesis even in the absence of aTc, thereby implying that MazF functions well, even at markedly low expression levels. At high MazF expression levels, protein synthesis from *gfpP* was preferred over that from other proteins. These results indicate that protein synthesis from genes lacking the ACA sequence occurs preferentially in MazF-expressing cells. However, given that the effect of MazF is significant even at low expression levels, it is necessary to develop a strict gene expression control system in cyanobacteria to apply the results of this experiment to cyanobacteria.

References

1) Zhang, Y., Zhang, J., Hoeflich, K.P., Ikura, M., Qing, G., and Inouye, M. (2003) MazF cleaves cellular mRNAs specifically at ACA to block protein synthesis in *Escherichia coli*. *Mol. Cell* **12:** 913-923.