Harnessing the intracellular triacylglycerols for titer improvement of polyketides in *Streptomyces*

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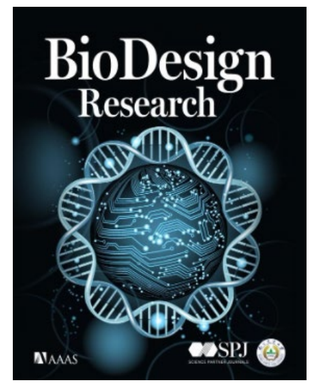


Introduction

Pharmaceutically important polyketides are mainly produced as secondary metabolites during the stationary phase of growth of *Streptomyces* species. The source of intracellular metabolites that are funneled into polyketide biosynthesis has proven elusive.

We applied multi-omics to reveal that intracellular triacylglycerols (TAGs), which accumulates in primary metabolism, are degraded during stationary phase. This process could channel carbon flux from intracellular TAGs and extracellular substrates into polyketide biosynthesis.

We devised a strategy named 'dynamic degradation of TAG' (ddTAG) to mobilize the TAG pool and increase polyketide biosynthesis. Our strategy could improve polyketide titers for pharmaceutical production.

Identification of intracellular metabolites

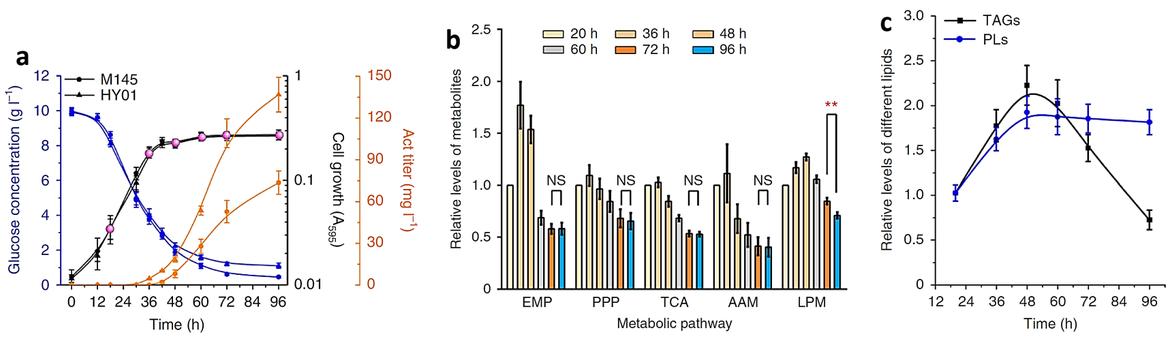
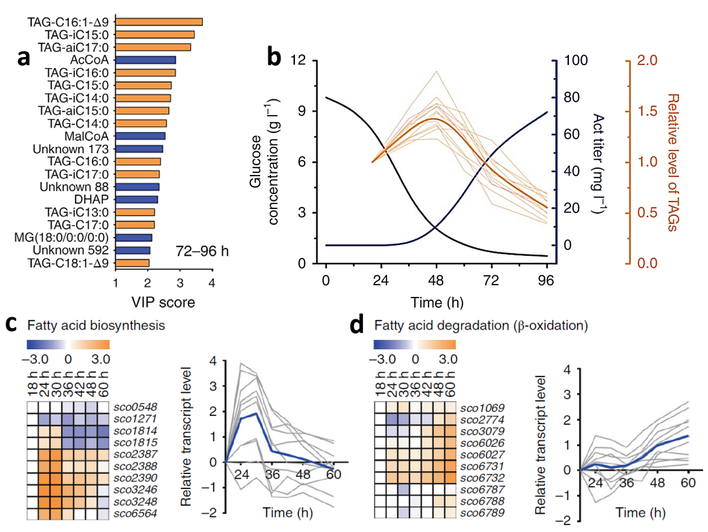


Fig.1 a, Glucose consumption, cell growth and Act production of the fermentation run. M145, a model strain that produces the well-known polyketide Act, and HY01, a mutant strain derived from M145 that overproduces Act. **b**, Trends of different metabolic pathways in M145. **c**, Temporal profile of phospholipids (PLs) and TAGs by thin-layer chromatography (TLC).

By comparative metabolomic analyses, we noted a difference between patterns of metabolites in the lipid metabolic pathway with that of the EMP, PPP, TCA and AAM pathways. By TLC, we inferred that the TAG pool, which is catabolized into FFAs and MAGs, might represent one group of our proposed key intracellular metabolites for polyketide biosynthesis.

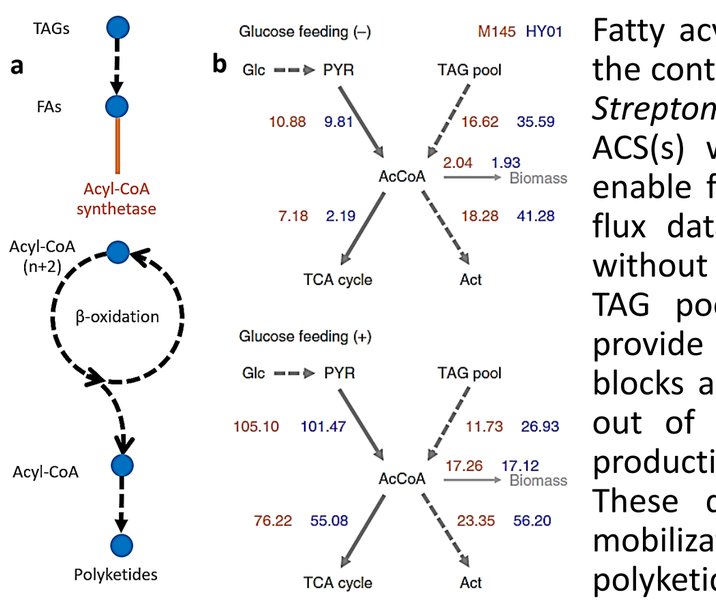
Cellular TAG pool contributes to Act yield



More intracellular TAGs were degraded in HY01 during late stationary phase compared with M145. These results indicate that the cellular TAG pool contributes to the higher Act yield in HY01. The TAG pool accumulated during primary metabolism and degraded during Act biosynthesis. The time-course transcriptome consistent with cellular TAG profiles.

Fig.2 a, Top 20 metabolites with significant change between M145 and HY01 during late stationary phase. **b**, Profiles of glucose consumption (black), TAG metabolism (orange) and Act production (blue). Light and thick orange curves indicate the profiles of fatty acid moieties of cellular TAG pool and their overall trend, respectively. c, d, Transcriptional profiles of genes involved in fatty acid biosynthesis and β-oxidation pathways. Blue curves indicate the overall transcriptional trend of the selected genes.

High-yield mechanism of cellular TAG pool



Fatty acyl-CoA synthesis is more likely the control point of TAG degradation in *Streptomyces* and that the appropriate ACS(s) will need to be identified to enable further engineering. Metabolic flux data of fermentations with and without feeding showed that cellular TAG pool degradation in HY01 can provide more acetyl-CoA building blocks and redirects more carbon flux out of this node toward polyketide production by depressing the TCA cycle. These data suggested that rational mobilization of TAG might improve polyketide titers.

Fig.3 a, A brief illustration of metabolic pathway from cellular TAG pool to polyketides. **b**, Mass balance of the acetyl-CoA (AcCoA) node. Fluxes were expressed in unit of the acetyl unit. Data for MFA were collected during stationary phase (72–120 h).

Genes involved in cellular TAG mobilization

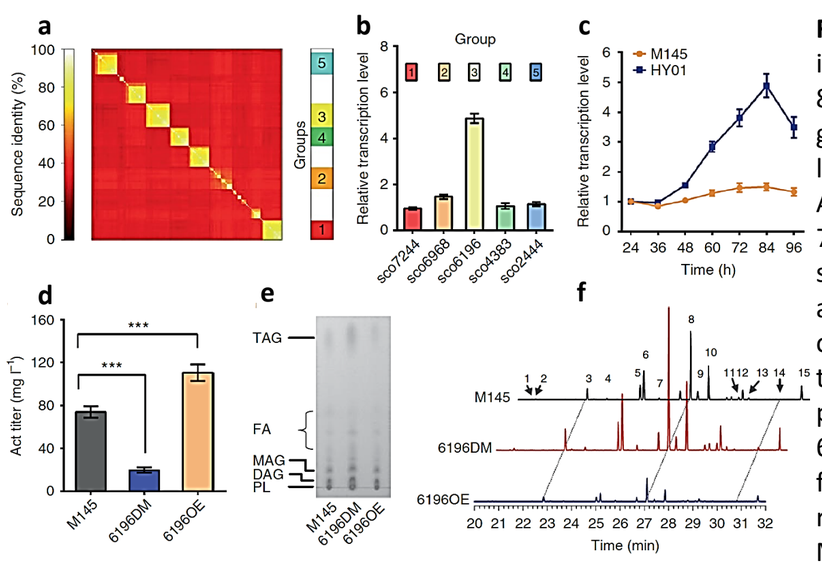
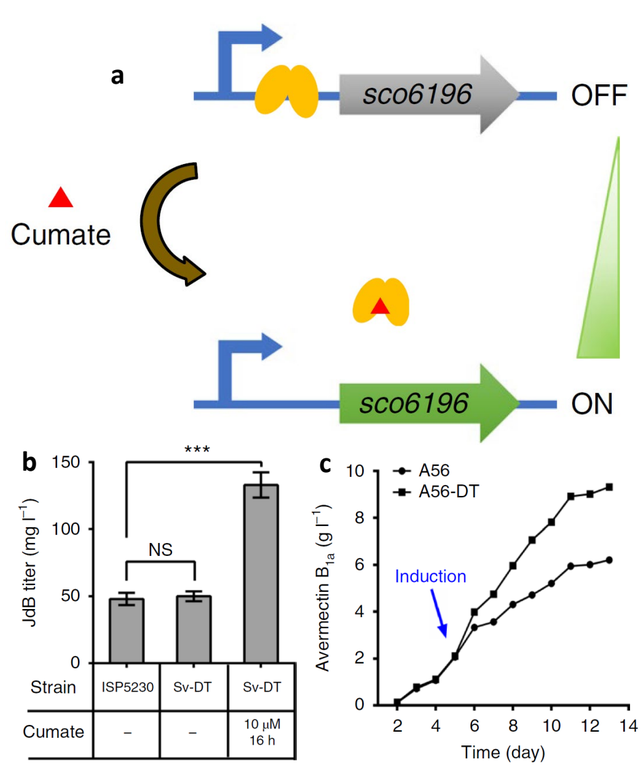


Fig.4 a, Pairwise sequence identity analysis among the 888 ACSs in 125 *Streptomyces* genomes. **b**, Relative transcript levels of the five conserved ACSs in *S. coelicolor* M145 at 72 h. **c**, Temporal profiles of sco6196 transcripts in M145 and HY01. **d**, Effect of SCO6196 on Act production. **e**, TLC assay the remaining cellular TAG pools in M145, 6196DM and 6196OE at 96 h. **f**, Levels of fatty acid moieties from remaining cellular TAG pools of M145, 6196DM and 6196OE.

We implemented a pairwise sequence identity assay among the 888 ACSs present in 125 *Streptomyces* genomes in NCBI, and found five groups of ACSs that are conserved in *S. coelicolor* and other *Streptomyces*. The expression of sco6196 was significantly higher in HY01 compared with M145. To analyze the role of SCO6196 further, we created a sco6196 deletion strain (6196DM) and a sco6196 overexpression strain (6196OE) in M145. Compared with parental strain M145, 6196DM produced substantially less Act, and harbored more cellular TAG pool in late stationary phase. that deletion of sco6196 blocked the degradation of cellular TAGs with a broad range of fatty acid moieties.

ddTAG strategy for titer improvement



We placed the *sco6196* gene under the control of a cumate-inducible promoter to generate a ddTAG module, to enable selective control of the timing and strength of TAG degradation. Under optimized induction conditions, engineered strain Sv-DT produced 133.0 ± 9.4 mg l^{-1} of jadomycin B in 48 h, which is 1.7-fold higher than the parent strain ISP5230. To ascertain whether our ddTAG strategy could be scaled up, we carried out experiments in a stirred-tank bioreactor. In a 180 m^3 fermenter, the engineered strain A56-DT produced 50% higher titers of avermectin B_{1a}, with production increased from 6.20 to 9.31 g l^{-1}

Fig.5 a, Schematic of temporal control of TAG mobilization. **b**, Jadomycin B (JdB) titer improvement using the ddTAG strategy. S. venezuelae ISP5230 and Sv-DT are the parent and the engineered strain, respectively. **c**, Avermectin B_{1a} titer improvement using the ddTAG in a 180-m_3 fermenter. A56 and A56-DT are the start strain and the engineered *S. avermitilis* strain, respectively.

References

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