Analysis of molecular mechanisms by which the ribosomal *rpsL* mutation activates secondary metabolism in *Streptomyces coelicolor* A3(2)

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【Introduction】

The K88E mutation in the *rpsL* gene, which encodes the ribosomal protein S12, enhances the protein synthetic activity of *Streptomyces coelicolor* A3(2) during the late growth phase, resulting in the deep blue-pigment polyketide actinorhodin (ACT) overproduction (Fig. 2)[1]. Moreover, recent studies with comparative expression analysis by RT-PCR and Western blotting analyses and subsequent genetic verification revealed that increased expression of several translation factors could be responsible for the enhanced protein synthesis during the late growth phase in an ACT-overproducing *S. coelicolor* A3(2) (Fig. 3)[2]. However, it has been mystery why the ribosomal mutation leads to the increased expression of the translation factors at transcription levels.

【Results and Discussion】

In the present study, to clarify the mechanisms that the increased levels of translation factors caused by the ribosomal mutation is responsible for antibiotic overproduction, we conducted a detailed investigation of the effects of the *rpsL*K88E mutation on transcriptional apparatus in *S. coelicolor* A3(2) by using DNA microarray analysis. Intriguingly, the results demonstrated that the expression profiles of almost all of 65 sigma factors were very different between the wild-type strain and the ACT-overproducing *rpsL*K88E mutant (Fig. 4). It is therefore highly probable that the altered expression of sigma factors holds the key to increasing the expression of the translation factors at transcriptional levels. Although, the mechanisms in the altered expression profiles of sigma factors in the ACT-overproducing *rpsL*K88E mutant may be much more complicated than it appears, our results presented here provided a big clue to uncover the question "why a specific ribosomal mutation enhances antibiotic production in *Streptomyces* strains."

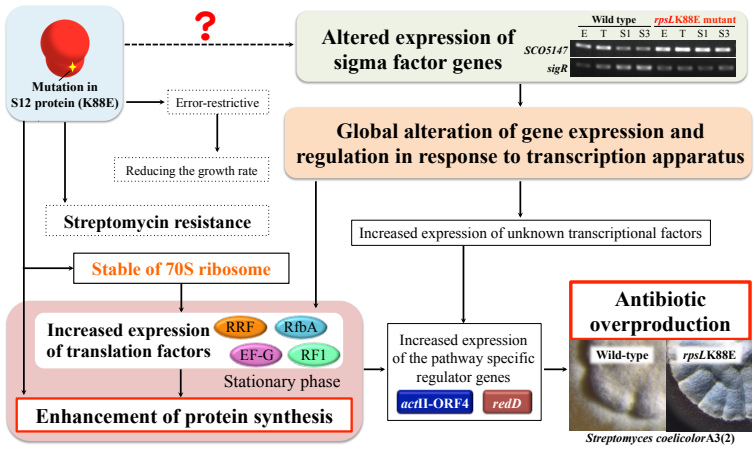


Figure 1 Schematic diagram showing the mechanism by which the *rpsL*K88E mutation in ribosomal protein S12 enhances antibiotic production in *S. coelicolor* A3(2)

Why the *rpsL*K88E mutation alters the transcription of sigma factor genes ?

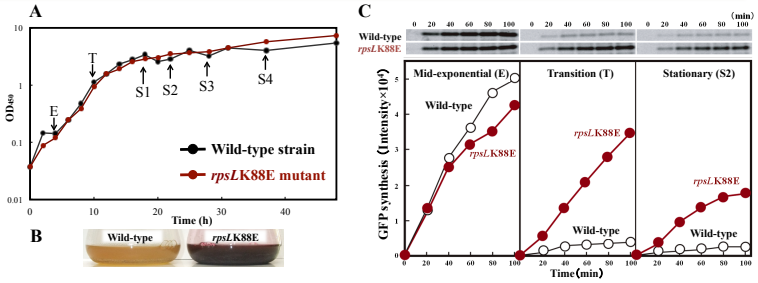


Figure 2 Profiles of growth, antibiotic production and protein synthesis in wild-type strain and *rpsL*K88E mutant of *S. coelicolor* A3(2)

(A) Strains wild-type and *rpsL*K88E mutant were grown in YEME medium at 30˚C. Growth was monitored by measuring OD_{450}. The zero time point represents 20–23 h after inoculation of fresh spores ; at that time OD_{450} was 0.03. At the times designated by arrows E (mid-exponential phase), T (transition phase), and S (S1-S4, stationary phase), cells were harvested for preparation of washed ribosomes, the S-150 fraction and total RNA. **(B)** Production of the actinorhodin by wild-type strain and *rpsL*K88E mutant. Cultivation was continued for 7 days at 30˚C. **(C)** *In vitro* synthesis of GFP using wild-type and *rpsL*K88E mutant ribosomes. Cell-free translation of GFP mRNA was carried out with washed ribosomes and the S-150 fraction prepared from wild-type and *rpsL*K88E mutant strains grown to the indicated growth phase (and see arrows E, T and S2 in A). The upper panel shows fluorographs of the GFP synthesized *in vitro*. The intensity of the GFP bands was determined by scanning the fluorographs.

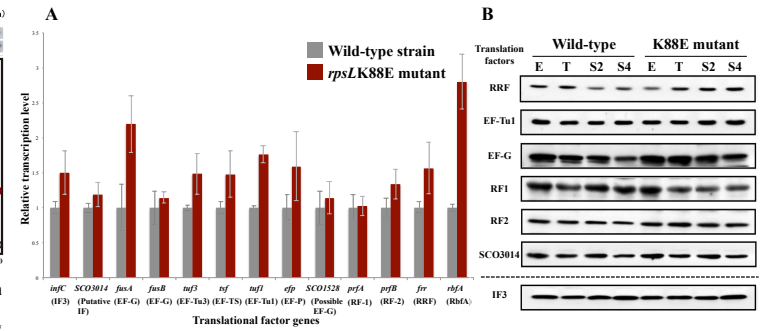
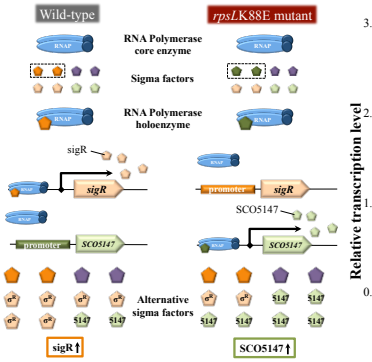


Figure 3 Profiles of expression of the translational factor genes in the wild-type strain and *rpsL*K88E mutant

(A) Transcriptional analysis using DNA microarrays. The total RNA were extracted from cells grown to stationary phase (S3). Data were normalized to *hrdB* expression from the same samples. The maximum expression levels were compared, taking the maximum expression levels of the wild-type strain as unity.

(B) Western blotting analysis. Protein samples (S-150 fraction and ribosome fraction) were prepared from cells grown to the indicated growth phase. Only IF3 was analysis in the ribosome fraction.



Global alteration of gene expression and regulation in response to transcriptional apparatus.

The Big Question : How the *rpsL*K88E mutation alters the expression and regulation of sigma factors ?

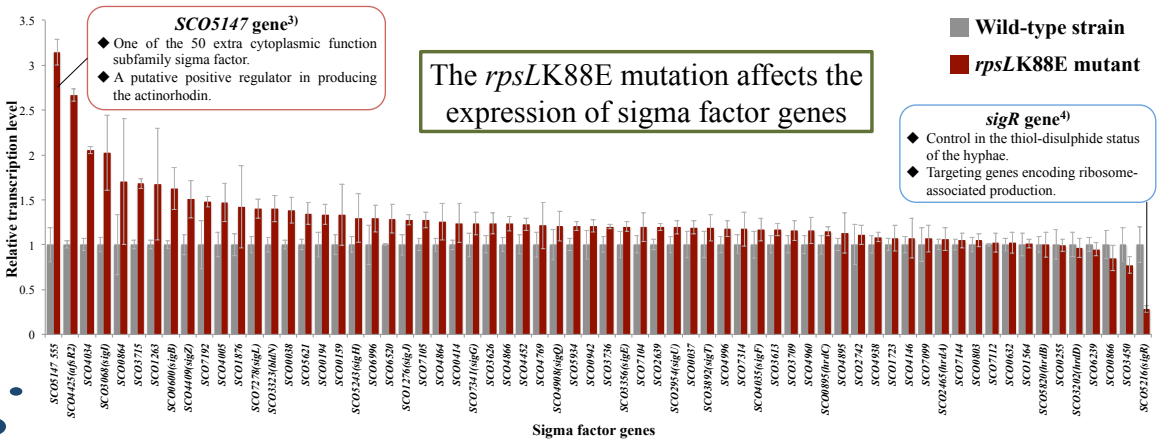


Figure 4 Transcriptional analysis of sigma factor genes in the wild-type strain and *rpsL*K88E mutant

The total RNA were extracted from cells grown to stationary phase (S3). Data were normalized to *hrdB* expression from the same sample. The maximum expression levels were compared, taking the maximum expression levels of the wild-type strain as unity.

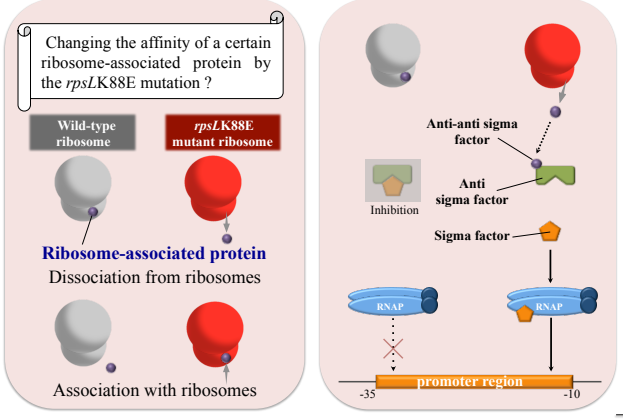


Figure 5 The hypothesis of mechanism by which the *rpsL*K88E mutation alters the expression and regulation of sigma factors

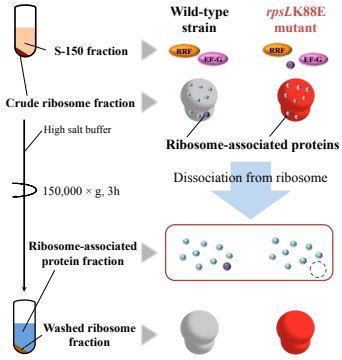


Figure 6 Preparation of ribosome-associated protein fractions

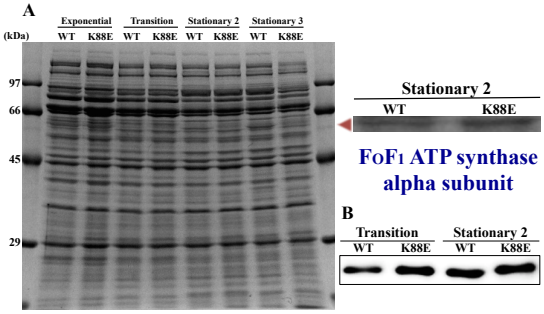


Figure 7 Comparative analysis of ribosome-associated proteins prepared from the wild-type strain and *rpsL*K88E mutant

(A) SDS-PAGE. (B) Western blotting analysis. Sample were prepared from cells shown to the indicated growth phases. Each lane contained 6 μg (A) and 1 μg (B), respectively, of protein samples.

References

1) Okamoto-Hosoya Y. *et al. Microbiol.* 149 : 3299-3309 (2003). **2)** Hosaka T. *et al. Mol. Microbiol.* 61(4) : 883-879 (2006). **3)** Kong S. *et al. J. Bacteriol.* 189 : 4135 (2007). **4)** Paget M. *et al. Mol. Microbiol.* 42 : 1007-1020 (2001).