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The Biosynthesis of Kasugamycin, an Antibiotic against Rice Blast Disease, with Particular Reference to the Involvement of *rpoZ*, a Gene Encoding RNA Polymerase Omega Subunit

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Biosynthetic genes of kasugamycin (KSM), an aminoglycoside antibiotic produced by *Streptomyces kasugaensis*, forms a cluster within the chromosome. A cloned 22.4-kb cluster region contains almost all the enzyme-coding genes required for KSM biosynthesis together with *kac*^{338}, a gene for KSM acetyltransferase, and *kasKLM*, a set of genes encoding an ABC transporter, both of which participate in KSM self-resistance as well as *kasT* encoding a KSM-synthesis-specific transcriptional activator of the biosynthetic genes. Furthermore, *rpoZ*, encoding a 90-amino acid omega (ω) subunit of RNA polymerase (RNAP), is required for the simultaneous production of KSM and aerial mycelium, and *rpoZ*-disrupted wild-type *S. kasugaensis* produces neither KSM nor aerial mycelia. Transcriptional analysis of the biosynthetic genes and forcible expression of *kasT* in the mutant revealed that the presence of *rpoZ*, which results in formation of RNAP carrying the ω subunit, facilitates initiation of *kasT* transcription and is thus crucial for KSM production.

Key Words : *Streptomyces kasugaensis*, *rpoZ*, RNA polymerase ω subunit, kasugamycin biosynthesis

INTRODUCTION

Members of the genus *Streptomyces* produce an enormous variety of biologically active antibiotics and morphologically differentiate in a manner similar to eukaryotic fungi. Mature colonies of these Gram-positive prokaryotic bacteria possess spore-bearing aerial mycelia that cover a lower layer of substrate mycelia growing on the surface of agar medium (Figure. 1). The production of antibiotics and the formation of aerial hyphae, which appear to be independent events, have been considered genetically correlated based on the occurrence of pleiotropic mutants that neither produce antibiotics nor form aerial mycelia. This pleiotropic regulation is thus an area of considerable interests in *Streptomyces* genetics [1].

The translational regulation of this pleiotropy has, for instance, been demonstrated by extensive studies on *bldA* mutants obtained from two representative strains of streptomycetes : *Streptomyces coelicolor* A3(2) that produces the blue and red pigments, actinorhodin and undecylprodigiosin, and *Streptomyces griseus* that accumulates streptomycin (SM) [2, 3]. The *bldA* gene encodes the leucyl tRNA for a rarely used UUA codon in streptomycetes, and the TTA codon specifying leucine is present in the regulatory genes for synthesis of the pigments in *S. coelicolor* A3(2) and for the production of SM and aerial mycelium in *S. griseus*. In these streptomycetes, therefore, *bldA* defects that prevent leucyl tRNA synthesis lead to the pleiotropic phenotype.

The antibiotic biosynthetic genes are normally located on the streptomycete chromosome as a cluster of the structural, regulatory and self-resistance genes [4]. In *S. griseus*, the

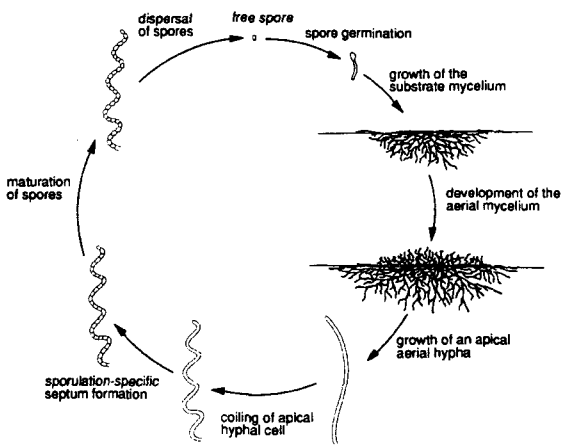

Figure 1 Life cycle of *Streptomyces* strains from a free spore.

regulatory cascade of SM biosynthesis and self-resistance occurs as follows [5]. At a specific point of growth, A-factor, a γ-butyrolactone hormone-like autoregulator, is produced and binds to its receptor protein ArpA. This binding relieves the AdpA repression of *adpA* whose product, AdpA, regulates *strR*, the SM-biosynthesis-specific transcriptional activator gene located in the SM biosynthetic gene cluster. Subsequently, the product, StrR, activates the transcription of the biosynthetic and self-resistance genes by binding to their promoter regions, thereby inducing *S. griseus* to concomitant SM production and self-resistance.

Streptomyces kasugaensis produces kasugamycin (KSM, Figure. 2), an aminoglycoside antibiotic effective against rice blast disease caused by the fungus, *Piricularia oryzae*. KSM is composed of two sugars, D-*chiro*-inositol (Figure. 2(A)) and a derivative of kasugamine (2,4-diamino-2,3,4,6-tetradeoxy-D-arabino-hexose, Figure. 2(B)), together with a carboxy-imino-methyl group (Figure. 2(C)) attached to the amino group

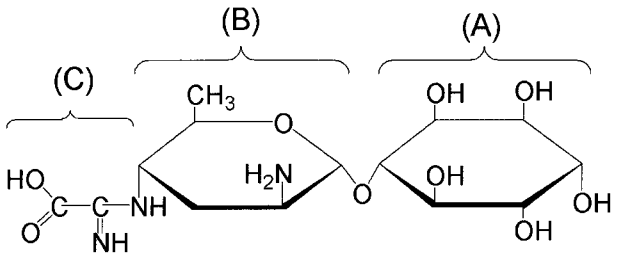

Figure 2 Chemical structure of KSM. (A), (B) and (C) represent D-*chiro*-inositol moiety, kasugamine moiety, and a carboxy-imino-methyl group.

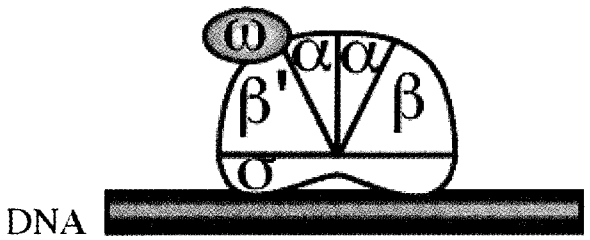

Figure 3 Schematic view of the holo RNA polymerase. The subunit ω binding to the β' subunit facilitates the assembly of $\alpha_2\beta$ and the β' subunit. The holoenzyme binds to the promoter region of genes via the σ subunit.

at C4 of the kasugamine moiety. We previously cloned a 22.4-kb chromosomal DNA region (*kas* cluster) containing the biosynthetic genes for KSM together with *kac*[338], the KSM acetyltransferase gene, *kasKLM*, ABC transporter genes, both of which are responsible for KSM self-resistance, and *kasT*, the KSM-biosynthesis-specific transcriptional activator gene [6-9].

Meanwhile, we found that disruption of *rpoZ*, the gene encoding the RNA polymerase (RNAP) subunit omega (ω), turned the wild-type *S. kasugaensis* into pleiotropic mutants, which were phenotypically similar to the *bldA* mutation, and clarified that the gene is required for KSM production and aerial mycelium formation in the streptomycete [10] by use of a host-vector system established for gene cloning and analysis [11].

In both prokaryotes and eukaryotes, RNAP participates in the initial step for transfer of information stored in DNA to protein synthesis by synthesizing messenger RNAs from a DNA template. The core RNAP of prokaryotic bacteria is composed of four subunits with a stoichiometry of $\alpha_2\beta\beta'\omega$ and the ω subunit is considered to bind to the β' subunit to facilitate association of the $\alpha_2\beta$ assembly and β'. The core enzyme requires an additional σ subunit to constitute the holoenzyme for initiation of transcription (Figure. 3). Despite the structural contribution of the ω subunit to RNAP, however, little has been reported on its physiological role.

This paper summarizes our recent molecular genetic studies especially on KSM biosynthesis in *S. kasugaensis* and its regulation with particular reference to *kasT* and *rpoZ*.

RESULTS AND DISCUSSION

kas cluster, a gene cluster of kasugamycin biosynthesis

Antibiotic-producing streptomycetes generally harbor the self-resistance gene within the biosynthetic gene cluster, and a variety of such clusters have been cloned using the self-resistance genes as probe [4]. In a similar attempt, we initially cloned a 7.6-kb *Pst*I-*Kpn*I DNA fragment, including the KSM acetyltransferase gene (*kac*[338]) and eight genes (*kasA-kasI*) as illustrated in Figure. 4, from the chromosomal DNA of wild-type *S. kasugaensis* [7]. In fact, when the *kac*[338] gene was introduced by plasmid-mediated transformation into KSM-susceptible *Escherichia coli*, the transformed cells became highly resistant to KSM (>800 μg/mL KSM). Furthermore, by repeated gene walking experiments around the 7.6-kb region, we obtained the *kas* cluster that contains 20 genes thought to be involved in KSM biosynthesis (Figure. 4) [8, 9]. Of these 20 genes, the nine genes deduced or identified to date are summarized in Table 1.

Previous biosynthetic analyses have demonstrated that the

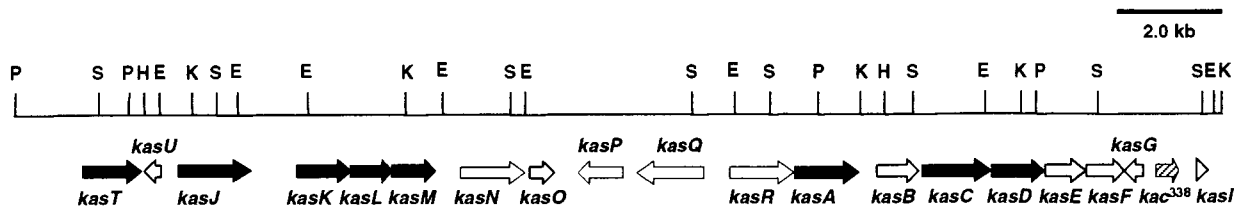

Figure 4 Restriction map of the KSM biosynthetic gene cluster (*kas* cluster) in a 22.4-kb DNA fragment cloned from the chromosome of *S. kasugaensis*. Arrows indicate putative genes related to KSM biosynthesis. In particular, the hatched arrow represents the KSM acetyltransferase gene, *kac*[338], and solid arrows indicate deduced/identified genes (see Table 1). Abbreviations : E, *Eco* RI ; H, *Hin*dIII ; K, *Kpn*I ; P, *Pst*I ; S, *Sac*I.

Table 1 Identified and deduced genes in *kas* cluster.

Gene	Identified/deduced function	Amino acid residues
kac^{338}	KSM acetyltransferase	141
kasA	Transferase (deduced)	391
kasC	Transaminase (deduced)	436
kasD	Dehydratase (deduced)	329
kasJ	Oxidoreductase (deduced)	445
kasK	ABC transporter, ATP-binding protein	329
kasL	ABC transporter, membrane protein	257
kasM	ABC transporter, membrane protein	240
kasT	KSM-specific transcriptional activator	346

kasugamine and D-*chiro*-inositol moieties originate from UDP-*N*-acetylglucosamine (UDP-GlcNAc) and *myo*-inositol, respectively, and that the carboxy-imino-methyl group attached to the C4 amino group of kasugamine is derived from glycine [12-17]. Based on these biosynthetic findings and the genes identified or deduced, we have proposed a pathway for KSM biosynthesis starting from UDP-GlcNAc as illustrated in Figure. 5 [18]. Initially, UDP-GlcNAc is dehydrated and then hydrated for formation of the keto functionality at C4. An amino group is then added to C4 by KasC, and glycine is subsequently attached to the amino group as a carboxy-imino-methyl group. After the acetyl group is removed from the acetamido group at C2, the resulting UDP-kasugamine moiety is glycosylated with *myo*-inositol by KasA. Finally, KSM is synthesized via epimerization of the incorporated *myo*-inositol in a hypothesized intermediate into D-*chiro*-inositol by KasJ. KSM accumulating within the cells is thought to be exported outside the cell by KasKLM, which constitute an ABC transporter that is a member of a membrane-associated, ATP-dependent transport system widely present in prokaryotic and eukaryotic cells [8]. The three consecutive genes, *kasK*, *L* and *M*, show a characteristic feature in that they overlap at the sequence, ATGA, which includes *kasL* and *M* transcriptional initiators (ATG) and *kasK* and *L* terminators (TGA). This implies that *kasKLM* is transcribed into a polycistronic messenger RNA that is then most probably co-translated into the KasK, L and M proteins [8]. Furthermore, the fact that KSM-susceptible *E. coli* cells were converted into KSM-resistant cells (200 μg/mL KSM) when *kasKLM* was expressed via a plasmid strongly suggests that the KasK, L and M proteins form a complex which, together with KSM acetyltransferase encoded by *kac*^{338} [7], participates in the KSM self-resistance of *S. kasugaensis*.

The *kasT* gene was found upstream of *kasKLM*. The product KasT possesses a helix-turn-helix motif as with many DNA-binding proteins and shows a 50% identity in its deduced amino acid sequence of *strR*, the transcriptional activator gene in the SM biosynthetic gene cluster [9]. Moreover, the fact that purified KasT protein was found to bind to the intergenic DNA region of *kasU* and *kasJ* implied that KasT is a pathway-specific activator of the KSM biosynthetic gene cluster [9].

Involvement of *rpoZ* in kasugamycin biosynthesis and morphological differentiation

In a mutagenesis study of *S. kasugaensis*, we isolated a pleiotropic mutant KSB that produced neither KSM nor aerial mycelia (Figure. 6A(b) and 7A(b)). Using the mutant as host, we cloned a 9.3-kb fragment of wild-type *S. kasugaensis* that restored the pleiotrophy. Deletion analysis of the cloned fragment revealed that *rpoZ*, the gene encoding the RNAP ω subunit of 90 amino acid residues, was responsible for the mutation. In fact, as shown in Figure. 8, sequence analysis of the mutated *rpoZ* gene of strain KSB demonstrated a 2-bp (GC) frameshift deletion, which probably resulted in a truncated, incomplete ω subunit of 47 amino acid residues [10].

To elucidate the specific functions of *rpoZ*, we disrupted the gene of wild-type *S. kasugaensis* by insertion of *aphII*, a neomycin resistance gene. The resulting mutant R6D4 exhibited a pleiotropic phenotype similar to the mutant KSB (Figure. 6B(e) and 7B(e)). Furthermore, when intact *rpoZ* genes derived from *S. kasugaensis* and *S. coelicolor* A3(2), were introduced into the *rpoZ*-disrupted mutant by plasmid-mediated transformation, each of the transformed mutants demonstrated the restored production of KSM and aerial mycelia (Figure. 6B(f) and (g) and 7B(f)

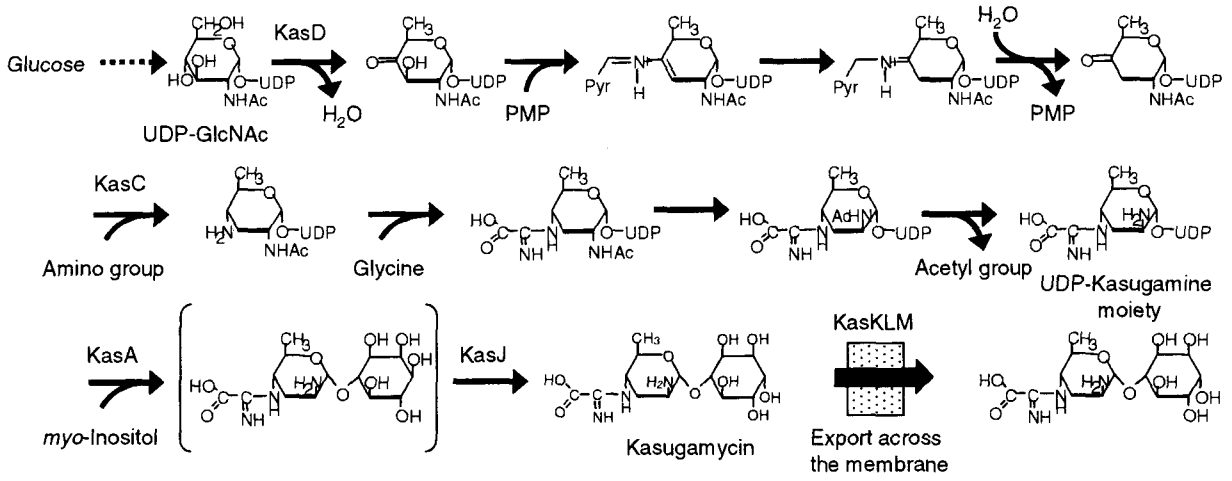

Figure 5 Biosynthetic pathway of KSM starting from UDP-GlcNAc derived from glucose. The enzymatic steps involved in KSM biosynthesis are indicated by arrows. The genes encoding KasD, KasC, KasA, KasJ and KasKLM are found in the *kas* cluster. Abbreviation : PMP, pyridoxamine 5'-phosphate.

and (g)). It is therefore evident that *rpoZ* plays a crucial role in both KSM biosynthesis and morphological differentiation [10].

Little has been reported on physiological functions of the subunit ω in bacteria except that the *rpoZ*-defective (or ω-less) *E. coli* mutant shows only a slow-growth phenotype [19]. In contrast, inactivation of *rpoZ* results in failure to produce KSM and aerial mycelium without affecting growth rate in *S. kasugaensis*. Our findings on *rpoZ*, therefore, provide an important clue to the yet undiscovered physiological function of *rpoZ* (or the subunit ω of RNAP) in prokaryotic bacteria.

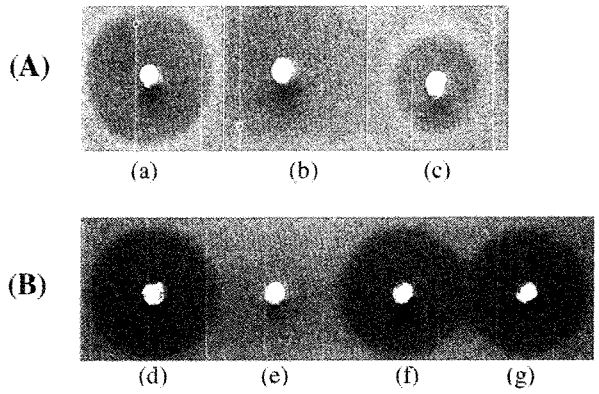

Figure 6 KSM production by transformation of KSB (A) and R6D4 (B) mutants. KSM production was estimated by an agar diffusion bioassay using KSM-susceptible *Pseudomonas fluorescens* IFO15334 as the test strain. Agar plugs, inoculated with transformants to be tested on the top, were placed on a bioassay agar medium inoculated with *P. fluorescens*. The halos formed around the plugs are indicative of the accumulation of KSM produced by the transformants. (a) and (d), transformation of wild-type strain A1R6 with a control plasmid as positive control; (b) and (e), transformation of mutants KSB and R6D4 with a control plasmid as negative control; (c) and (f), transformation of mutants KSB and R6D4 with a control plasmid carrying the *rpoZ* gene of *S. kasugaensis*; and (g), transformation of mutant R6D4 with a control plasmid carrying the *rpoZ* gene of *S. coelicolor* A3(2).

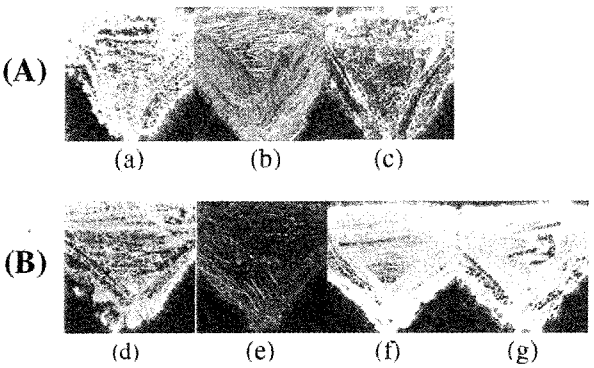

Figure 7 Aerial mycelium formation by transformation of mutants KSB (A) and R6D4 (B). Aerial hypha formation was examined by inoculation of the transformants onto GMY agar medium. (a) and (d), transformation of wild-type strain A1R6 with a control plasmid as positive control; (b) and (e), transformation of mutants KSB and R6D4 with a control plasmid as negative control; (c) and (f), transformation of mutants KSB and R6D4 with a control plasmid carrying the *rpoZ* gene of *S. kasugaensis*; and (g), transformation of mutant R6D4 with a control plasmid carrying the *rpoZ* gene of *S. coelicolor* A3(2).

Transcriptional dependence of *kasT* on *rpoZ*

As described in the preceding section, *S. kasugaensis* fails to synthesize KSM when *rpoZ* alone is specifically disrupted. To establish the underlying reason for this, we examined the transcriptional activity of the *kas* cluster biosynthetic genes by RT-PCR using the total RNA extracted from mutant R6D4 cells. As a result, no transcriptional activity was detected for *kasT* or the biosynthetic genes responsible for KSM synthesis. In contrast, however, we observed distinct transcription of *kasKLM* and *kac*[338], genes for KSM self-resistance [18]. Subsequently, for forcible expression of *kasT* in mutant R6D4, we constructed a hybrid *kasT* gene in which *kasT*p, the original promoter of *kasT* was replaced by *ermE*p, the *ermE* (an erythromycin-resistance gene) promoter recognized by the ω-less RNAP. Introduction of the *ermE*p-*kasT* construct into mutant R6D4 by plasmid-mediated transformation stimulated transcription of the biosynthetic genes in the *kas* cluster and in consequence induced the transformed *rpoZ*-disruptant to produce KSM [18].

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          M  S  S  S  I  T  A  P  E  G  I  I  N  P  P  I  D  E  L  L
A1R6  GTGTCCTCTTCCATCACCGCACCCGAGGGCATCATCAATCCGCCAATTGATGAGCTTCTC
KSB   GTGTCCTCTTCCATCACCGCACCCGAGGGCATCATCAATCCGCCAATTGATGAGCTTCTC
          M  S  S  I  T  A  P  E  G  I  I  N  P  P  I  D  E  L  L

          E  A  T  D  S  K  Y  S  L  V  I  Y  A  A  K  R  A  R  Q  I
A1R6  GAGGCCACCGACTCGAAGTACAGCCTCGTGATCTACGCCGCCAAGCGCGCGCGCCAGATC
KSB   GAGGCCACCGACTCGAAGTACAGCCTCGTGATCTACGCCGCCAAGCGCGCGC--CAGATC
          E  A  T  D  S  K  Y  S  L  V  I  Y  A  A  K  R  A     P  D

          N  A  Y  Y  S  Q  L  G  E  G  L  L  E  Y  V  G  P  L  V  D
A1R6  AACGCGTACTACTCGCAGCTCGGTGAGGGCCTGCTCGAGTACGTCGGCCCCCTCGTCGAC
KSB   AACGCGTACTACTCGCAGCTCGGTGAGGGCCTGCTCGAGTACGTCGGCCCCCTCGTCGAC
          Q  R  V  L  L  A  A  R  *

          T  H  V  H  E  K  P  L  S  I  A  L  R  E  I  N  A  G  L  L
A1R6  ACGCACGTCCACGAGAAGCCGCTGTCGATCGCGCTCCGCGAGATCAACGCGGGCCTGCTG
KSB   ACGCACGTCCACGAGAAGCCGCTGTCGATCGCGCTCCGCGAGATCAACGCGGGCCTGCTG

          T  S  E  A  I  E  G  P  A  Q  *
A1R6  ACCTCCGAGGCCATCGAGGGCCCGGCCCAGTAA
KSB   ACCTCCGAGGCCATCGAGGGCCCGGCCCAGTAA
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Figure 8 Comparison of the nucleotide sequence of the *rpoZ* genes from wild-type strain A1R6 and mutant KSB. The two hyphens in the *rpoZ*-encoding region of mutant KSB indicate gaps in the sequence. Asterisks denote stop codons.

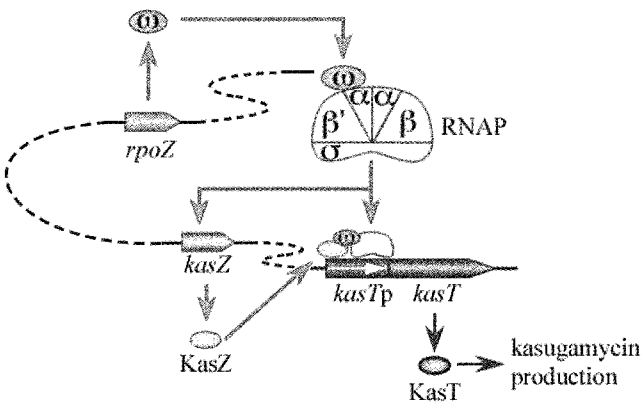

Figure 9 Involvement of RNAP with the ω subunit for *kasZ* transcription and *kasT* transcription with KasZ.

This result demonstrated that KSM production is controlled by transcriptional activation of the KSM biosynthetic genes by *kasT*. In addition, the recent study revealed that the *kasT* disruption turned the wild-type strains of *S. kasugaensis* into KSM non-producing mutants which still, however, formed aerial mycelia (data not shown here).

Overall, these results confirm that *kasT* is a pathway-specific transcriptional activator gene on the KSM biosynthesis and that the *kasT*p is not recognized by the ω-less RNAP, whereas the rest of the *kas* cluster biosynthetic genes together with *kasKLM* and *kac*[338] are transcribed by the ω-less RNAP. We have thus clarified that the KSM non-producing phenotype of the *rpoZ*-disrupted *S. kasugaensis* is due to the lack of *kasT* transcription. In other words, the transcription of *kasT* needs the presence of *rpoZ* and in consequence RNAP with the ω subunit.

Transcriptional cascades are observed for biosyntheses of antibiotics such as SM and tylosin in these antibiotic-producing streptomycetes [5, 20]. As described in the introduction, transcription of *strR*, the SM-biosynthesis-specific transcription activator gene and a structural and functional homolog of *kasT* in KSM biosynthesis, is demonstrated to require AdpA, the DNA binding transcriptional activator [5]. It seems, therefore, most likely that *kasZ*, a transcriptional activator gene such as *adpA*, also exists for the *kasT* transcription. We assume that RNAP containing the ω subunit might be required for the *kasZ* transcription and that the resulting KasZ might interact with the RNAP for the initiation of *kasT* transcription as shown in Figure 9. We are in the process of cloning of the *kasZ* gene to elucidate the transcriptional mechanism of the *kasT* gene.

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References

[1] Chater, K. F. and Bibb, M. J., "Regulation of bacterial antibiotic production," In Kleinkauf, H. and von Dohren, H. (ed), Biotechnology, VCH, Weiheim, Germany, vol.7, pp. 59-105, (1997).

[2] Fernandez-Moreno, M. A., Caballero, J. L., Hopwood, D. A. and Malpartida, F., "The *act* cluster contains regulatory and antibiotic export genes, direct targets for translational control by the *bldA* tRNA gene of *Streptomyces*," Cell 66(4), 769-780, (1991).

[3] Kwak, J., McCue, L. A. and Kendrick, K. E., "Identification of *bldA* mutants of *Streptomyces griseus*," Gene 171(1), 75-78, (1996).

[4] Meurer, G. and Hutchinson, C. R., "Genes for the biosynthesis of microbial secondary metabolites," In Demain, A. L. and Davis, J. E. (ed), Manual of industrial microbiology and biotechnology, second edition, ASM Press, Washington, D.C., pp. 717-724, (1999).

[5] Ohnishi, Y., Kameyama, H., Onaka, H. and Horinouchi, S., "The A-factor regulatory cascade leading to streptomycin biosynthesis in *Streptomyces griseus* : identification of a target gene of the A-factor receptor," Mol. Microbiol., 34(1), 102-111, (1999).

[6] Ikeno, S., Higashide K., Kinoshita, N., Hamada, M. and Hori, M., "Correlation between the presence of *kac*, kanamycin acetyltransferase gene, and the productivity of kasugamaycin in *Streptomyces*," Actinomycetol., 11(1), 6-10, (1997).

[7] Ikeno, S., Tsuji T., Higashide K., K., Kinoshita, N., Hamada, M. and Hori, M., "A 7.6 kb DNA region from *Streptomyces kasugaensis* includes some genes responsible for kasugamycin biosynthesis," J. Antibiot., 51(3), 341-352, (1998).

[8] Ikeno, S., Yamane, Y., Ohishi, Y., Kinoshita, N., Hamada, M., Tsuchiya, K. S. and Hori, M., "ABC transporter genes, *kasKLM*, responsible for self-resistance of a kasugamycin producer strain," J. Antibiot., 53(4), 373-384, (2000).

[9] Ikeno, S., Aoki, D., Sato, K., Hamada, M., Hori, M. and Tsuchiya, K. S., "*kasT* gene of *Streptomyces kasugaensis* M338-M1 encodes a DNA-binding protein which binds to intergenic region of *kasU-kasJ* in the kasugamycin biosynthesis gene cluster," J. Antibiot., 55(12), 1053-1062, (2002).

[10] Kojima I., Kasuga, K., Kobayashi, M., Fukasawa, A., Misuno, S., Arisawa, A. and Akagawa, H., "The *rpoZ* gene, encoding the RNA polymerase omega subunit, is required for antibiotic production and morphological differentiation in *Streptomyces kasugaensis*," J. Bacteriol., 184(23), 6417-6423, (2002).

[11] Kojima I., Kasuga, K., Kobayashi, M., Matsuo, M., Akagawa, H. and Mizuno, S., "Cloning system for *Streptomyces kasugaensis* using the hybrid melanin-synthesizing gene *melE* as a chromogenic marker," Actinomycetol., 17(2), 29-32, (2003).

[12] Fukagawa, Y., Sawa, T., Takeuchi, T. and Umezawa, H., "Studies on biosynthesis of kasugamycin. I : Biosynthesis of kasugamycin and the kasugamine moiety," J. Antibiot. 21(1), 50-54, (1968).

[13] Fukagawa, Y., Sawa, T., Takeuchi, T. and Umezawa, H., "Studies on biosynthesis of kasugamycin. II : Biosynthesis of the two-carbon-side chain of kasugamycin," J. Antibiot. 21(3), 182-184, (1968).

[14] Fukagawa, Y., Sawa, T., Takeuchi, T. and Umezawa, H., "Studies on biosynthesis of kasugamycin. III : Biosynthesis of the D-inositol moiety," J. Antibiot. 21(3), 185-188, (1968).

[15] Fukagawa, Y., Sawa, T., Homma, I., Takeuchi, T. and Umezawa, H., "Studies on biosynthesis of kasugamycin. IV : Biosynthesis of the kasugamycin moiety from [1-^{14}C]-glucosamine and [1,2 or 6-^{14}C]-glucose," J. Antibiot. 21(5), 358-360, (1968).

[16] Fukagawa, Y., Sawa, T., Homma, I., Takeuchi, T. and Umezawa, H., "Studies on biosynthesis of kasugamycin. V : Biosynthesis of the amidine group," J. Antibiot. 21(6), 410-412, (1968).

[17] Fukagawa, Y., Sawa, T., Homma, I., Takeuchi, T. and Umezawa, H., "Studies on biosynthesis of kasugamycin. VI : Some relationships between the incorporation of ^{14}C-compounds and the production of kasugamycin," J. Antibiot. 21(6), 410-420, (1968).

[18] Ikeno, S., "Molecular genetic studies on biosynthesis and export of kasugamycin, an aminoglycoside antibiotic produced by *Streptomyces kasugaensis* (in Japanese)," Ph.D Thesis, The University of Tokyo, (2004).

[19] Gentry, D. R. and Burgess, R. R., "*rpoZ*, encoding the omega subunit of *Escherichia coli* RNA polymerase, is in the same operon as *spoT*," J. Bacteriol. 171(3), 1271-1277, (1989).

[20] Bibb, M. J., "Regulation of secondary metabolism in streptomycetes," Curr. Opin. Microbiol. 8, 208-215 (2005).