Linear Plasmids from Actinomycetes

Haruyasu Kinashi

Department of Fermentation Technology, Faculty of Engineering, Hiroshima University
1-4-1 Kagamiyama, Higashihiroshima-shi, Hiroshima 724, Japan

(Received Sep. 27, 1994)

INTRODUCTION

Since Hayakawa *et al.*¹⁾ discovered pSLA2 in *Streptomyces rochei*, many linear double-stranded DNA plasmids of broad size ranges (12~1700 kb) have been isolated from actinomycetes including SCP1 from *Streptomyces coelicolor* A3(2)²⁾. Although linear plasmids have been found in a wide variety of eukaryotic organisms, they are rather rare in prokaryotes³⁾. The question why actinomycetes frequently possess linear plasmids has not been answered until recently. Lin *et al.*⁴⁾ reported a linear structure of the *Streptomyces lividans* 66 chromosome by comparing its ends with that of a linear plasmid SLP2. *S. coelicolor* A3(2), the genetically best studied *Streptomyces* strain, was also indicated to have a linear chromosome⁴⁾. If the linear chromosome is general in actinomycetes, the detection of many linear plasmids in this group of microorganisms seems rational. Namely, linear plasmids might be formed by deleting the central large part of the chromosome and by still retaining the terminal inverted repeats. In this paper, I summarize the accumulated data of linear plasmids from actinomycetes and discuss their origin, terminal structure, replication, maintenance and function in stressing our data on the SCP1 and pSLA2 plasmids.

SCP1

The plasmid SCP1, which carries the methylenomycin biosynthetic (*mmy*) gene cluster⁵⁾ in *S. coelicolor* A3(2), was shown to be a giant linear plasmid of 350 kb by pulsed field gel

electrophoresis (PFGE)⁶⁾. Physical characterization of SCP1 revealed that it has 80-kb terminal inverted repeats (TIRs) on both ends, that it contains an insertion sequence, IS466, at the inside end of the right TIR (TIR-R), and that the 5'-ends are attached to a protein⁶⁾. The restriction map of the 15 *EcoRV* fragments of SCP1 is shown in a racket frame structure (Fig. 1) according to Sakaguchi⁷⁾. Hybridization experiments indicated that the *mmy* gene cluster is present on the *EcoRV*-C fragment. In addition, SCP1 codes for several spore-associated proteins which might be involved in spore formation. While the *sapA* gene⁸⁾, which codes for the major SapA protein, is located on the *AseI*-F fragment of the chromosome⁹⁾, the *sapC*, *D*, *E* genes are present on the *EcoRV*-B fragment in the TIR arms of SCP1¹⁰⁾.

Extensive PFGE analysis of *S. coelicolor* A3(2) strains confirmed the existence of various forms of SCP1¹¹⁾ which had been indicated by genetic studies of Hopwood and his colleagues¹²⁾. Namely, (i) a free form of SCP1 in the wild type strain 1147, (ii) SCP1' plasmids containing a chromosomal fragment: SCP1'-*cysB* (550 kb) in strain 1984, a 1700-kb plasmid (possibly SCP1'-*cysD*) in strain 2106 and a series of plasmids (390~630 kb) in *Streptomyces violaceoruber* JCM4979 which form a plasmid ladder with a size difference of about 40 kb, and (iii) integrated states into the chromosome in NF strains 2612, A317 and A332, and in stable donor strains A608 and A634.

Hybridization experiments using the terminal probe indicated that all SCP1' plasmids have

Fig. 1. A physical map of the 15 *EcoRV* fragments of SCP1 drawn in a racket frame structure. The positions of insertion sequence IS466 and the sequence homologous to SCP2 are indicated by the open and filled boxes, respectively. The *mmy* genes are present on the *EcoRV*-C fragment. The *cysB* locus is integrated in the same fragment to make *EcoRV*-C' in the plasmid SCP1'-*cysB*.

the intact termini of SCP1. Digestion of SCP1'-*cysB* with *EcoRV* gave identical fragments with SCP1 except the 140-kb *EcoRV*-C' fragment compared to the 40-kb *EcoRV*-C fragment. The *cysB* locus may be present on *EcoRV*-C', although this cannot explain the gross size difference. A series of SCP1-related plasmids in strain JCM4979 were also revealed to have the identical restriction map with SCP1 except the size of the *EcoRV*-A' fragment which were 150, 190, 230 kb and so on compared with 80 kb for *EcoRV*-A. A 3.5-kb sequence homologous to the 30-kb circular plasmid SCP2 was found on the *EcoRV*-A fragment of SCP1, and on the *EcoRV*-A' fragments of the plasmid ladder a truncated SCP2 molecule was detected. Based on these results integration of one molecule of SCP2 into the *EcoRV*-A locus of SCP1 followed by deletion and amplification was speculated to form the plasmid ladder in JCM4979.

A unique feature of the NF strains of *S. coelicolor* A3(2) is that they donate chromosomal markers bidirectionally at high frequency during conjugal mating¹³, which is dissimilar to

Hfr strains of *Escherichia coli* that donate unidirectionally. In NF strain 2612, SCP1 is integrated in the chromosome at the 9 o'clock position¹⁴. Analysis of the two junctions between the SCP1 DNA and the chromosomal DNA revealed that the left junction had almost intact left terminus of SCP1, while the right junction was composed of the insertion sequence IS466, completely deleting TIR-R¹⁵. The integrated structure of SCP1 in NF strains A317 and A332 were proved to be identical with that in strain 2612. Based on these results, a model was proposed for generation of the NF strains, which is in accordance with the racket frame structure of SCP1^{15,16}.

In contrast to the NF strains, SCP1 integrated in the chromosome of strains A608 and A634 lost at least the 4.1-kb terminal DNA from both ends¹¹. The A608 and A634 strains were reported to show unidirectional and bidirectional gene transfers, respectively¹⁷. These results suggest that the terminus of SCP1 itself is not important for determining the direction of gene transfer. The molecular reason for uni- and bidirectional gene transfers might reflect on the

direction of insertion and the extent of deletion of SCP1 in various strains.

S. violaceoruber SANK95570, another methylenomycin producer, contains a 70-kb linear plasmid pSV2, which however was shown not to carry the *mmv* genes by hybridization¹¹. A large circular plasmid pSV1 (170 kb) was detected in this strain and suggested to be involved in methylenomycin production^{18,19}. The circularity of pSV1 was indicated by electron microscopy¹⁸ and conventional agarose gel electrophoresis¹⁹. If the *mmv* gene cluster is located on three different genomes, namely, the giant linear plasmid SCP1, the circular plasmid pSV1 and the chromosome, it is an extraordinary phenomenon which has never been reported.

pSLA2 plasmids

The 17-kb linear plasmid pSLA2 was isolated from the lankacidin producer *S. rochei* 7434 AN4. The linearity of pSLA2 was confirmed by restriction analysis and electron microscopy¹. Hirochika *et al.*^{20,21} revealed that it has 614-bp TIRs on both ends and the 5' ends are blocked by a protein. In the TIR regions of pSLA2, several short inverted repeat sequences are present, which could form hairpin loops and might serve as binding sites for specific proteins. The hypothetical DNA binding proteins were postulated to hold the two double-stranded DNA ends together and to form a four-stranded DNA structure. This hypothetical racket frame structure was proposed to play a key role in linear plasmid replication, as well as integration into and excision from host chromosomes⁷.

PFGE analysis of strain 7434AN4 revealed that it contains two additional linear plasmids of 200 and 100 kb beside pSLA2²². Three plasmids were named pSLA2-S (17 kb), M (100 kb) and L (200 kb), respectively. Strain 7434AN4 produces two structurally unrelated antibiotics, lankacidin and lankamycin. The involvement of pSLA2-L rather than pSLA2-S in the produc-

tion of both antibiotics was indicated by the following data²². (1) Analysis of various mutants of 7434AN4 showed a parallel relationship between the presence of pSLA2-L and the production of both lankacidin and lankamycin. (2) pSLA2-L was transferred by protoplast fusion from a wild type strain to a non-producer which gained the ability to produce both antibiotics. (3) pSLA2-L1 isolated from an antibiotic non-producing mutant was determined to be a symmetrical linear plasmid containing only the right 80-kb part of pSLA2-L. (4) pSLA2-L showed a weak homology to pSLA2-M and no homology to pSLA2-S.

The structural analysis of pSLA2-L revealed that it has 2-kb TIRs on both ends and the ends are blocked by a protein. As described above, a symmetrical deletion plasmid pSLA2-L1 was isolated from a non-producer. Kikuchi *et al.*²³ reported a similar symmetrical linear plasmid F1 formed during transformation of *Saccharomyces cerevisiae* by a linear plasmid pGKL1. pSLA2-L1 might be formed by an incomplete replication of pSLA2-L as proposed for F1. Comparison of the restriction maps of pSLA2-L and pSLA2-L1 suggested that the antibiotic biosynthetic genes are located on the deleted left part of pSLA2-L (Fig. 2).

Large linear plasmids were found in four additional lankacidin producing *Streptomyces* strains²². Although the sizes of plasmids were different each other, the pSLA2-L probe hybridized to the plasmids from all the producers. Solenberg *et al.*²⁴ studied the transposition of Tn5096 in *Streptomyces griseofuscus* C581, another lankacidin producer. Insertion of Tn5096 into the 200-kb linear plasmid eliminated the capacity of the strain to produce lankacidin, indicating its involvement in lankacidin production.

Recently, we have cloned two pSLA2-L fragments homologous to an *eryA* probe which contains the polyketide synthase gene of *Saccharopolyspora erythraea*. The partial nucleo-

tide sequence analysis of one clone indicated a high homology to the characteristic domains of the *eryA* genes such as acyl carrier protein, β -ketoacyl synthase and acetyl transferase. The identical carbon skeleton of erythromycin and lankamycin suggests that this clone may code for the biosynthetic genes of lankamycin rather than lankacidin. In addition, an *actI* homologous fragment has been cloned from pSLA2-L whose location on the plasmid is distinct from those of the *eryAI* homologs (Fig. 2). This result suggests a possibility that the *actI* homolog is related to lankacidin production (unpublished results).

SLP2

SLP2 is a 50-kb linear plasmid isolated from *S. lividans* 66²⁵⁾. The sequence analysis of the terminal fragments indicated that it has 44-bp imperfect TIRs. The SLP2 termini were resistant to both λ -exonuclease and exonuclease III, suggesting that the protein bound to the 5' ends also protects the 3' ends. A truncated copy of Tn4811 was found to be present near the right end of SLP2. While SLP2 exists as a free form, a 16-kb sequence corresponding to the segment of SLP2 from Tn4811 to the right end is also present elsewhere in the genome of *S. lividans*.

A careful study of the location of this sequence led Lin *et al.*⁴⁾ to the finding of the linear structure of the *S. lividans* chromosome. The *S. lividans* chromosome was also detected as a 8-Mb linear molecule by PFGE using the condition for megabase-sized linear DNA, which will be described by Chen *et al.* in this issue.

pSCL and other linear plasmids in β -lactam producers

pSCL is a 12-kb linear plasmid²⁶⁾ isolated from *Streptomyces clavuligerus* NRRL3585, a producer of several β -lactam antibiotics. The complete nucleotide sequences of pSCL indicated that it has TIRs about 900 bp long, of which the first 690 bp are absolutely identical²⁷⁾. Seven or eight open reading frames were suggested to be present on the plasmid, none of which, however codes for the B family or other known DNA polymerases.

Screening for linear plasmids in β -lactam producing actinomycete strains indicated the presence of plasmids of 12~450 kb for the following strains²⁸⁾; *Streptomyces argenteolus* JCM4229 (pSAR1, 300 kb), *S. argenteolus* JCM4623 (pSAR2, 200 kb), *Streptomyces griseus* subsp. *griseus* IFO14059 (pSGR1, 160 kb) and *S. clavuligerus* IFO13307 (pSCL1, 12

Fig. 2. Physical maps of pSLA2-L and pSLA2-L1. pSLA2-L1 is a symmetrical deletion plasmid containing only the 80-kb right part of pSLA2-L. The *eryAI* and *actI* probes, hybridized to the positions indicated by arrows. D, *DraI*; E, *EcoRV*; H, *HindIII*.

kb; pSCL2, 150 kb; pSCL3, 450 kb). pSCL1 may be identical to pSCL from *S. clavuligerus* NRRL3585, because both originated from the same strain. Hybridization experiments using the biosynthetic probes indicated that all plasmids do not contain the *pcbC* gene for isopenicillin synthase nor the *cefD* and *cefE* genes for isopenicillin N epimerase and deacetoxycephalosporin C synthase (expandase), respectively. However, pSAR1 from *S. argenteolus* JCM 4229 hybridized to the total DNA digest of several β -lactam producers, whether they contain a linear plasmid or not. This suggests an original relationship between pSAR1 and the chromosomal DNA of these strains.

Linear plasmids in *Streptomyces rimosus*

A 43-kb linear plasmid, pSRM was isolated from an oxytetracycline (OTC) producer, *Streptomyces rimosus* RCC by 0.4% agarose gel electrophoresis²⁹. Acridine orange treatment gave aerial mycelium-deficient mutants in which pSRM was not detected but their OTC production and resistance were not modified. *S. rimosus* R6, another OTC producer, was found to carry a giant linear plasmid of about 390 kb³⁰. During a strain improvement program, derivatives of R6 were obtained with increased oxytetracycline production and resistance. Most of them had DNA rearrangement in the chromosome, except one (MV17) which possessed a larger linear plasmid of about 1 Mb. Hybridization experiments indicated that the *otrB* resistance gene is present both on the chromosome and on the larger plasmid.

pHG plasmids

Several giant linear plasmids were detected in the functionally lithoautotrophic Gram-positive bacterium *Rhodococcus*³¹. By means of DNA probes for genes of soluble hydrogenase and ribulose-bisphosphate carboxylase, these plasmids were shown to be the carriers of the genetic information for these enzymes. Analysis

of plasmid pHG207, a possible recombination product of the wild type plasmids pHG204 and pHG205, revealed that it carries a protein attached to the 5' ends and contains highly homologous sequences within the terminal 583 bp (left end part) and 560 bp (right end part)³². The non-identity of the TIR sequences of pHG207 could be explained by the fact that it is a recombinant plasmid and the two ends originated from two different native plasmids.

pBL1

A 43-kb linear plasmid pBL1 was isolated from *S. lividans* TK64 after interspecific mating with *Streptomyces bambergiensis* S712 harboring a giant linear plasmid pSB1 of 640 kb³³. Hybridization experiments showed that two of the three *EcoRI* fragments of pBL1 was identical to those of pSB1, suggesting that pBL1 originated from plasmid pSB1. Cloning and gene disruption experiments using the non-conjugative circular vector pIJ702 suggest that at least five previously unknown genes of pBL1 are required for an efficient plasmid transfer³⁴.

Other linear plasmids

Application of PFGE to *Streptomyces* DNA led to the detection of linear plasmids in addition to the SCP1 and pSLA2 plasmids for the following antibiotic producing *Streptomyces* strains³⁵; *Streptomyces lasaliensis* NRRL 3382R (lasalocid and echinomycin, 520 kb), *Streptomyces fradiae* C373.1 (tylosin, 420 kb), *Streptomyces parvulus* JCM4068 (actinomycin, 520, 560, 580 kb) and *Streptomyces venezuelae* JCM4526 (chloramphenicol, 130 kb). The structure and function of these linear plasmids have not been studied except the 520-kb linear plasmid pKSL from *S. lasaliensis* NRRL3382R. Derivatives of strain NRRL3382R showed the correlation between the presence of pKSL and the production of both lasalocid and echinomycin. pKSL was transferred to a non-producer not containing the plasmid which gained the capaci-

Streptomyces avermitilis, an avermectin producer shows a site-specific DNA modification like *S. lividans*, which causes DNA degradation and makes the detection of large DNAs difficult. Evans *et al.*³⁷⁾ succeeded to isolate two novel giant linear plasmids of 100 and 250 kb from this strain by using a modified electrophoresis buffer.

Linear DNA genomes have a special terminal structure to prevent the progressive loss of terminal nucleotides and to prime DNA replication³⁸). (i) Circular intermediates: bacteriophage λ ; (ii) self-complementary terminal hairpins: vaccinia viruses, *Paramecium* mitochondria and *Borrelia* plasmids; (iii) terminal proteins bound to the 5'-ends: adenovirus, bacteriophage $\phi 29$ and yeast killer plasmids; and (iv) eukaryotic nuclear telomeres.

mycetes possess terminal proteins bound to the 5'-ends, none of which has been characterized. All of them also have terminal inverted repeats on both ends. The sizes of TIRs are quite different from strain to strain and range from 44 bp to 80 kb. The terminal 60 bp of pSLA2-S²¹⁾, SLP2²⁵⁾, SCP1³⁹⁾ and pHG207³²⁾ are compared in Fig. 3 with that of pSCL1²⁷⁾ which indicated the highest homology to other plasmids. In the extreme terminal region, a conserved homologous sequence was detected in all plasmids. The conserved sequence was found even in the *Rhodococcus* plasmid pHG207. In the case of pSLA2-S and pSCL1, the homologous sequences extend up to 350 bp, suggesting the same origin for these TIRs.

One of the characteristics of the terminal sequences is the presence of many short palindromic inverted repeats which could make hairpin loops. Both the loop structures and the conserved terminal sequence are supposed to be the binding sites for specific proteins which might have an important role in maintenance or replication of linear plasmids. Another characteristic of the terminal structure is the presence

Fig. 3. The terminal nucleotide sequences of *Streptomyces* linear plasmids. The terminal 60 bp are compared with that of pSCL1. The nucleotides identical to those of pSCL1 are indicated by asterisks above the sequence. The gaps are indicated by dashes. In the cases of SLP2 and pHG207, both the right and left terminal sequences are shown and mismatched nucleotides are underlined.

of an insertion-like sequence. SCP1 contains insertion sequence IS466 at the inside end of TIR-R³⁹), while SLP2 has transposon Tn4811 at the inside end of TIR-R²⁵). These examples seem not to be coincidences. I would rather consider the possibility that these elements are involved in the origin of TIRs of linear plasmids.

The plasmid SLP2 and the *S. lividans* chromosome have the identical 16-kb terminal sequence. However, this was not the case in other *Streptomyces* linear plasmids. When the cloned termini of pSLA2-S and SCP1 were probed to the total DNA digests of *S. rochei* and *S. coelicolor* mutants which lost the linear plasmid, no hybridizing band was observed (unpublished results). This indicates that the ends of both plasmids do not have a homology to the chromosomal ends of these strains, although the presence of a very short homology cannot be ruled out.

Replication and maintenance of linear plasmids

Extensive studies of DNA replication in bacteriophage ϕ 29 and adenovirus indicated that the protein-primed synthesis of a full-length DNA strand proceeds continuously and processively from the telomere by a mechanism that displaces the corresponding strand of the DNA duplex⁴⁰). Based on the similar protein binding to the 5' ends, it has been thought that actinomycete linear plasmids also replicate by the same mechanism, which however has not been proved experimentally.

Chang and Cohen⁴¹) studied the replication of pSLA2-S. The central 7.5-kb *Bcl*I fragment, when ligated to the thiostrepton-resistant gene, can replicate at high copies as a circular molecule in *S. lividans*. Analysis of the replication forks of pSLA2-S itself by two-dimensional agarose gel electrophoresis showed that the replication proceeds bidirectionally from the replication origin present on the center of the

linear plasmid. They further detected double-stranded replication intermediates that have recessed (about 280 nucleotides) 5' ends. The 3' overhang may serve as template for the non-displacing synthesis of the lagging strand terminus primed by the covalently attached 5' DNA binding protein.

The replication origins (*oriC*) have been cloned from *S. lividans*⁴²) and *S. coelicolor*⁴³), respectively, as an autonomously replicating circular molecule or by hybridization to a conserved *dnaA* probe. Quantitative DNA hybridization analysis suggested that the replication proceeds bidirectionally from the origin in the center of the linear *S. lividans* chromosome⁴⁴). Taken together, these data suggest that the replication mechanism found for pSLA2-S may be more general for DNA synthesis in *Streptomyces*⁴¹).

The sequence analysis of pSCL1 suggests that it does not code for DNA polymerase. Since additional two large linear plasmids are present in this strain, it might be possible to speculate that the replication of pSCL1 depends on a machinery present on the larger plasmids. Mutagenic treatments of *S. rochei* 7434AN4 gave mutants with all combinations of the three plasmids except the one containing only pSLA2-S²²). The inability to isolate this mutant could have happened by chance or may suggest that the pSLA2-L or M plasmids are indispensable for the maintenance of pSLA2-S. Further, we successfully transferred the pSLA2 plasmids from a wild type *S. rochei* strain to a non-producer, but not to *S. lividans* TK64. This result could also suggest that the *S. rochei* chromosome contains a region necessary to maintain the pSLA2 plasmids which is missing in the *S. lividans* chromosome.

Origin and transfer of linear plasmids

Most of prokaryotic linear plasmids were isolated from actinomycetes specially from *Streptomyces*. This might be related to the linear

structure of *Streptomyces* chromosomes. Several linear plasmids were detected after interspecific mating of actinomycete strains. The recombination of two linear chromosomes during mating could afford a hybrid linear plasmid containing both chromosomal fragments. This speculation can be proved by analysis of the newly formed plasmid if are known the precise physical maps of the chromosomes of two strains used for mating. Possible hybrid plasmids pHG207 in *Rhodococcus* and pBL1 in *S. lividans* may be examples of recombination of linear DNAs.

Recent molecular analysis revealed that organizations of antibiotic biosynthetic gene clusters in different *Streptomyces* species were similar for some antibiotics and individual biosynthetic enzymes were also similar on both nucleotide and amino acid sequence levels⁴⁵⁻⁴⁷. The similarity is seen even beyond the barrier between prokaryote and eukaryote. For example, β -lactam antibiotics are produced by fungi and bacteria including *Streptomyces*. Individual genes⁴⁸ involved in the biosynthetic steps and their organization⁴⁹ are both conserved in the producers. From these results, horizontal transfer of the β -lactam biosynthetic gene cluster was proposed⁴⁸. Although plasmids or transposable elements were suggested to be involved, no experimental evidence has been obtained.

SCP1 contains the methylenomycin biosynthetic gene cluster and is integrated into the chromosomes of *S. coelicolor* A3(2) strains. Therefore, SCP1 is a candidate vehicle for horizontal transfer. It is thus possible to speculate that transfer of large linear plasmids and their integration into the chromosome have been involved in the spreading of the antibiotic biosynthetic gene clusters in microorganisms^{15,16}.

REFERENCES

1) Hayakawa, T.; T. Tanaka, K. Sakaguchi, N.

- Otake & H. Yonehara: A linear plasmid-like DNA in *Streptomyces* sp. producing lankacidin group antibiotics. *J. Gen. Appl. Microbiol.* 25: 255-260, 1979.
- 2) Kinashi, H.; M. Shimaji & A. Sakai: Giant linear plasmids in *Streptomyces* which code for antibiotic biosynthesis genes. *Nature* 328: 454-456, 1987.
- 3) Hinnebusch, J. & K. Tilly: Linear plasmids and chromosomes in bacteria. *Mol. Microbiol.* 10: 917-922, 1993.
- 4) Lin, Y.-S.; H.M. Kieser, D.A. Hopwood & C. Chen: The chromosomal DNA of *Streptomyces lividans* 66 is linear. *Mol. Microbiol.* 10: 923-933, 1993.
- 5) Chater, K. F. & C. J. Bruton: Resistance, regulatory and production genes for the antibiotic methylenomycin are clustered. *EMBO J.* 4: 1893-1897, 1985.
- 6) Kinashi, H. & M. Shimaji-Murayama: Physical characterization of SCP1, a giant linear plasmid from *Streptomyces coelicolor*. *J. Bacteriol.* 173: 1523-1529, 1991.
- 7) Sakaguchi, K.: Invertrons, a class of structurally and functionally related genetic elements that includes linear DNA plasmids, transposable elements, and genomes of Adeno-type viruses. *Microbiol. Rev.* 54: 66-74, 1990.
- 8) Guijarro, J.; R. Santamaria, A. Schauer & R. Losick: Promoter determining the timing and spatial localization of transcription of a cloned *Streptomyces coelicolor* gene encoding a spore-associated polypeptide. *J. Bacteriol.* 170: 1895-1901, 1988.
- 9) Kieser, H. M.; T. Kieser & D. A. Hopwood: A combined genetic and physical map of the *Streptomyces coelicolor* A3(2) chromosome. *J. Bacteriol.* 174: 5496-5507, 1992.
- 10) McCormick, J. R.; R. Santamaria & R. Losick: The genes for three spore-associated proteins are encoded on linear plasmid SCP1 in *Streptomyces coelicolor*, P1-125. Abstracts of the 8th International Symposium on Biology of Actinomycetes, Madison, WI, 1991.
- 11) Kinashi, H.; M. Murayama, H. Matsushita & O. Nimi: Structural analysis of the giant linear plasmid SCP1 in various *Streptomyces coelicolor* strains. *J. Gen. Microbiol.* 139: 1261-1269, 1993.
- 12) Hopwood, D.A.; D.J. Lydiate, F. Malpartida & H.M. Wright: Conjugative sex plasmids of *Streptomyces*. In "Plasmids in Bacteria" (eds. D.R.

- Helinski, S.N. Cohen, D.B. Clewell, D.A. Jackson and A. Hollaenders), pp.615-633, Plenum Press, New York and London, 1985.
- 13) Hopwood, D. A.; R. J. Harold, A. Vivian & H. M. Ferguson: A new kind of fertility variant in *Streptomyces coelicolor*. *Genetics* 62: 461-477, 1969.
 - 14) Hodgson, D. A. & K. F. Chater: A chromosomal locus controlling extracellular agarase production by *Streptomyces coelicolor* A3(2), and its inactivation by chromosomal integration of plasmid SCP1. *J. Gen. Microbiol.* 124: 339-348, 1981.
 - 15) Hanafusa, T. & H. Kinashi: The structure of an integrated copy of the giant linear plasmid SCP1 in the chromosome of *Streptomyces coelicolor* 2612. *Mol. Gen. Genet.* 231: 363-368, 1992.
 - 16) Kinashi, H.; M. Murayama & T. Hanafusa: Integration of SCP1, a giant linear plasmid, into the *Streptomyces coelicolor* chromosome. *Gene* 115: 35-41, 1992.
 - 17) Vivian, A. & D. A. Hopwood: Genetic control of fertility in *Streptomyces coelicolor* A3(2): new kinds of donor strains. *J. Gen. Microbiol.* 76: 147-162, 1973.
 - 18) Okanishi, M.; T. Manome, & H. Umezawa: Isolation and characterization of plasmid DNAs in actinomycetes. *J. Antibiot.* 33: 88-91, 1980.
 - 19) Aguilar, A. & D. A. Hopwood: Determination of methylenomycin A synthesis by the pSV1 plasmid from *Streptomyces violaceus-ruber* SANK95570. *J. Gen. Microbiol.* 128: 1893-1901, 1982.
 - 20) Hirochika, H. & K. Sakaguchi: Analysis of linear plasmids isolated from *Streptomyces*: Association of protein with the ends of the plasmid DNA. *Plasmid* 7: 59-65, 1982.
 - 21) Hirochika, H.; K. Nakamura & K. Sakaguchi: A linear DNA plasmid from *Streptomyces rochei* with an inverted terminal repetition of 614 base pairs. *EMBO J.* 3: 761-766, 1985.
 - 22) Kinashi, H.; E. Mori, A. Hatani & O. Nimi: Isolation and characterization of linear plasmids in lankacidin-producing *Streptomyces* species. *J. Antibiot.* 47: No.12, in press.
 - 23) Kikuchi, Y.; K. Hirai, N. Gunge & F. Hishinuma: Hairpin plasmid - a novel linear DNA of perfect hairpin structure. *EMBO J.* 4: 1881-1886, 1985.
 - 24) Solenberg, P. J. & R. H. Baltz: Transposition of Tn5096 and other IS493 derivatives in *Streptomyces griseofuscus*. *J. Bacteriol.* 173: 1096-1104, 1991.
 - 25) Chen, C. W.; T-W. Yu, Y-S. Lin, H. M. Kieser & D. A. Hopwood: The conjugative plasmid SLP2 of *Streptomyces lividans* is a 50 kb linear molecule. *Mol. Microbiol.* 7: 925-932, 1993.
 - 26) Keen, C.L.; S. Mendelovitz, G. Cohen, Y. Aharonowitz & K.L. Roy: Isolation and characterization of a linear DNA plasmid from *Streptomyces clavuligerus*. *Mol. Gen. Genet.* 212: 172-176, 1988.
 - 27) Wu, X. & K.L. Roy: Complete nucleotide sequence of a linear plasmid from *Streptomyces clavuligerus* and characterization of its RNA transcripts. *J. Bacteriol.* 175: 37-52, 1993.
 - 28) Kinashi, H.; M. Doi & O. Nimi: Isolation of large linear plasmids from β -lactam producing actinomycete strains. Submitted for publication.
 - 29) Chardon-Loriaux, I.; M. Charpentier & F. Percheron: Isolation of a linear plasmid from *Streptomyces rimosus*. *FEMS Microbiol. Lett.* 35: 151-155, 1986.
 - 30) Hranueli, D.; B. Gravius, G. Biukovic, M. Redenbach & J. Cullum: Characterization of a giant linear plasmid prime carrying the oxytetracycline genes in *Streptomyces rimosus*. In "Proceedings of the 5th European Congress on Biotechnology" pp.749-752, Florence, Italy, June 13-17, 1993.
 - 31) Kalkus, J.; M. Reh & H.G. Schlegel: Hydrogen autotrophy of *Nocardia opaca* strains is encoded by linear megaplasmids. *J. Gen. Microbiol.* 136: 1145-1151, 1990.
 - 32) Kalkus, J.; C. Dorrie, D. Fischer, M. Reh & H.G. Schlegel: The giant linear plasmid pHG207 from *Rhodococcus* sp. encoding hydrogen autotrophy: characterization of the plasmid and its termini. *J. Gen. Microbiol.* 139: 2055-2065, 1993.
 - 33) Zotchev, S.B.; L.I. Soldatova, A.V. Orekhov & H. Schrempf: Characterization of a linear extrachromosomal DNA element (pBL1) isolated after interspecific mating between *Streptomyces bambergensis* and *S. lividans*. *Res. Microbiol.* 143: 839-845, 1992.
 - 34) Zotchev, S. B. & H. Schrempf: The linear *Streptomyces* plasmid pBL1: analyses of transfer functions. *Mol. Gen. Genet.* 242: 374-382, 1994.
 - 35) Kinashi, H. & M. Shimaji: Detection of giant linear plasmids in antibiotic producing strains of *Streptomyces* by the OFAGE technique. *J. Anti-*

- biot. 40: 913-916, 1987.
- 36) Kinashi, H.; S. L. Otten, J. S. Duncan & C. R. Hutchinson: Frequent loss and restoration of antibiotic production by *Streptomyces lasaliensis*. J. Antibiot. 41: 624-637, 1988.
 - 37) Evans, M.; F.S. Kaczmarek, K. Stutzman-Engwall & P. Dyson: Characterization of a *Streptomyces-lividans*-type site-specific DNA modification system in the avermectin-producer *Streptomyces avermitilis* permits investigation of two novel giant linear plasmids, pSA1 and pSA2. Microbiol. 140: 1367-1371, 1994.
 - 38) Blackburn, E. H.: Structure and function of telomeres. Nature 350: 569-573, 1991.
 - 39) Kinashi, H.; M. Shimaji-Murayama & T. Hanafusa: Nucleotide sequence analysis of the unusually long terminal inverted repeats of a giant linear plasmid, SCP1. Plasmid 26: 123-130, 1991.
 - 40) Salas, M.: Protein-priming of DNA replication. Annu. Rev. Biochem. 60: 39-71, 1991.
 - 41) Chang, P.-C. & S.N. Cohen: Bidirectional replication from an internal origin in a linear *Streptomyces* plasmid. Science 265: 952-954, 1994.
 - 42) Zakrzewska-Czerwinska, J. & H. Schrepf: Characterization of an autonomously replicating region from the *Streptomyces lividans* chromosome. J. Bacteriol. 174: 2688-2693, 1992.
 - 43) Calcutt, M. & F.J. Schmidt: Conserved gene arrangement in the origin region of the *Streptomyces coelicolor* chromosome. J. Bacteriol. 174: 3220-3225, 1992.
 - 44) Musialowski, M.S.; F. Flett, G.B. Scott, G. Hobbs, C.P. Smith & S.G. Oliver: Functional evidence that the principal DNA replication origin of the *Streptomyces coelicolor* chromosome is close to the *dnaA-gyrB* region. J. Bacteriol. 176: 5123-5125, 1994.
 - 45) Distler, J.; K. Mansouri, G. Mayer, M. Stockmann & W. Piepersberg: Streptomycin biosynthesis and its regulation in *Streptomyces*. Gene 115: 105-111, 1992.
 - 46) Hara, O.; T. Murakami, S. Imai, H. Anzai, R. Itoh, Y. Kumada, E. Takano, E. Satoh, A. Satoh, K. Nagaoka & C. Thompson: The bialaphos biosynthetic genes of *Streptomyces viridochromogenes*: cloning, heterospecific expression, and comparison with the genes of *Streptomyces hygroscopicus*. J. Gen. Microbiol. 137: 351-359, 1991.
 - 47) Katz, L. & S. Donadio: Polyketide synthesis: prospects for hybrid antibiotics. Ann. Rev. Microbiol. 47: 875-912, 1993.
 - 48) Smith, D.J.; M.K.R. Burnham, J.H. Bull, J.E. Hodgson, J.M. Ward, P. Browne, J. Brown, B. Barton, A.J. Earl & G. Turner: β -Lactam antibiotic biosynthetic genes have been conserved in clusters in prokaryotes and eukaryotes. EMBO J. 9: 741-747, 1990.
 - 49) Weigel, B.J.; S.G. Burgett, V.J. Chen, P.L. Skatrud, C.A. Frolik, S.W. Queener & T.D. Ingolia: Cloning and expression in *Escherichia coli* of isopenicillin N synthetase genes from *Streptomyces lipmanii* and *Aspergillus nidulans*. J. Bacteriol. 170: 3817-3826, 1988.