Linear plasmids mobilize linear but not circular chromosomes in *Streptomyces*: support for the ‘end first’ model of conjugal transfer

Hsuan-Hsuan Lee, Chin-Chen Hsu, Yen-Ling Lin and Carton W. Chen

Correspondence
Carton W. Chen
cwchen@ym.edu.tw

Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei 112, Taiwan

Gram-positive bacteria of the genus *Streptomyces* possess linear chromosomes and linear plasmids capped by terminal proteins covalently bound to the 5' ends of the DNA. The linearity of *Streptomyces* chromosomes raises the question of how they are transferred during conjugation, particularly when the mobilizing plasmids are also linear. The classical rolling circle replication model for transfer of circular plasmids and chromosomes from an internal origin cannot be applied to this situation. Instead it has been proposed that linear *Streptomyces* plasmids mobilize themselves and the linear chromosomes from their telomeres using terminal-protein-primed DNA synthesis. In support of this ‘end first’ model, we found that artificially circularized *Streptomyces* chromosomes could not be mobilized by linear plasmids (SLP2 and SCP1), while linear chromosomes could. In comparison, a circular plasmid (pIJ303) could mobilize both circular and linear chromosomes at the same efficiencies. Interestingly, artificially circularized SLP2 exhibited partial self-transfer capability, indicating that, being a composite replicon, it may have acquired the additional internal origin of transfer from an ancestral circular plasmid during evolution.

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INTRODUCTION

Conjugation is a major process leading to genetic exchange among bacteria. It is commonly mediated by conjugative plasmids (reviewed by Clewell, 1993). Besides mediating self-transfer at relatively high frequencies, conjugative plasmids also mobilize the chromosomes from donor to recipient cells at lower frequencies. In the classical model for conjugal transfer of circular plasmids in Gram-negative bacteria and many low-G+C Gram-positive bacteria, transfer is initiated by nicking at a specific site on supercoiled plasmid DNA (*oriT*) by a relaxase (or ‘nickase’), followed by rolling-circle replication (RCR; Fig. 1a). The relaxase remains covalently bound to the 5' end of the nicked DNA strand, and leads the displaced strand into the recipient cell, where the DNA is circularized and converted into double-stranded form (reviewed by Byrd & Matson, 1997). Generally about 10–20 transfer genes (*tra*) are clustered on these circular conjugative plasmids, and encode components of various parts of the conjugation process, including the mating pair formation complexes and relaxosomes (reviewed by Grohmann *et al.*, 2003). Mobilization of the bacterial chromosomes is mediated by an integrated plasmid (‘Hfr’ chromosome) using the same RCR initiated at *oriT*, except that the transfer continues beyond the plasmid sequence (Fig. 1a).

The classical model is not applicable to the linear chromosomes and linear plasmids in the Gram-positive filamentous bacteria of the genus *Streptomyces*. These linear replicons are capped by terminal proteins (TPs) covalently bound to the 5' ends of the DNA (reviewed by Chen, 2007; Kirby & Chen, 2011). The telomere DNA sequences contain complex palindromic sequences that are important for the replication of the telomeres (Chen, 1996; Huang *et al.*, 1998; Qin & Cohen, 1998). Despite the linearity of the DNA, there is evidence that these *Streptomyces* linear replicons form supercoiled circular structures *in vivo* through interactions between the telomeres (Tsai *et al.*, 2011). Interactions between the chromosomal telomere and the plasmid telomere have also been observed (Tsai *et al.*, 2011). Moreover, *Streptomyces* chromosomes circularize spontaneously through fusion of the chromosomal arms at high frequencies (10^{-3} – 10^{-2} per sporulation cycle) (reviewed by Chen *et al.*, 2002).

Circular and linear plasmids are abundant in *Streptomyces*, and most of them are conjugative. Unfortunately, the efficient and synchronous conjugal transfer of plasmids seen in some bacteria such as *Escherichia coli* is not available for biochemical investigations in *Streptomyces*. In a typical laboratory setting, conjugation in *Streptomyces* occurs between multinucleoid hyphae during mixed growth of the donor and recipient cultures on solid medium over a period of several days. After that, the haploid spores are selected and scored for plasmid transfer

Abbreviations: RCR, rolling-circle replication; TP, terminal protein.

Fig. 1. Models of conjugal transfer for circular and linear plasmids in *Streptomyces* and other bacteria. (a) Paradigmatic model for circular plasmids and chromosomes in most bacteria (using *E. coli* as example). Plasmid DNA (free or integrated) is coloured in blue and the chromosomal DNA segment containing the integrated plasmid in yellow. The relaxase is represented by the white circles. The origin of transfer, *oriT*, is indicated by the white lines. The direction of transfer is indicated by the broad arrow. The type IV secretion system (T4SS) through which the DNA is transferred is represented by the orange circle. (b) Internal initiation model for transfer of naturally circular or artificially circularized plasmids. The leading and lagging strand synthesis in the bidirectionally progressing replication forks are represented by the single and double arrows, respectively. The TraB ring through which the DNA is transferred is represented as a green circle. (c) End first model for transfer of linear plasmids and chromosomes in *Streptomyces*. The linear plasmid (blue) and one end of the chromosome (yellow) are shown with covalently bound TP (black circles) at the 5' end of the transferred strand and the newly synthesized strand (blue and yellow arrows). TtrA helicase engaging in the unwinding of the telomere DNA is represented by white rings wrapping around the DNA strands. (d) Internal initiation model for transfer of linear plasmids in *Streptomyces*. The symbols used are the same as in (b) except for the circularity of the plasmid.

and chromosome recombination. Transfer of *Streptomyces* plasmids is generally very efficient (up to 100 %), and often causes a temporary inhibition of the development of the recipient mycelium, forming inhibition zones termed 'pocks', which are useful for tracking plasmid transfer (Bibb *et al.*, 1978). In addition, progeny exhibiting recombinant parent phenotypes, reflecting recombination between donor and recipient chromosomes, emerge at frequencies of 10^{-3} – 10^{-6} (Hopwood & Sermonti, 1963).

Unlike the classical model, conjugal transfer of circular plasmids in *Streptomyces* usually requires only a single plasmid-borne *traB* gene (reviewed by Hopwood & Kieser, 1993), which encodes an FtsK/SpoIIIE-type septal DNA translocase with an ATPase domain (with Walker A and B boxes), a transmembrane domain and a winged helix–turn–helix (wHTH) motif (Vogelmann *et al.*, 2011a).

The FtsK/SpoIIIE-type translocases play an important role in bacterial chromosome segregation during cell division and sporulation (reviewed by Bigot *et al.*, 2007; Burton &

Dubnau, 2010). They have been demonstrated *in vitro* to form oligomeric rings to allow DNA to pass through (Massey *et al.*, 2006) and to translocate DNA in an ATP-dependent manner (Bath *et al.*, 2000; Pease *et al.*, 2005). Recent studies by Burton *et al.* (2007) have shown that SpoIIIE forms DNA-conducting channels in the lipid bilayer and transports DNA across two bilayers during sporulation in *Bacillus subtilis*.

TraB of circular *Streptomyces* plasmid pSG5 has been shown to form foci at the hyphal tips (Reuther *et al.*, 2006a). TraB of circular *Streptomyces* plasmid pSVH1 possesses no nicking activity (Reuther *et al.*, 2006a) and forms hexamer-like rings on the membrane (Vogelmann *et al.*, 2011b). Based on these observations, it has been proposed that *Streptomyces* circular plasmids are transferred as double-stranded DNA from *oriT* through the membrane-anchored TraB ring at the hyphal tip (Grohmann *et al.*, 2003; Vogelmann *et al.*, 2011a, b) (Fig. 1b). This is consistent with the demonstration by Possoz *et al.* (2001) that the transfer of circular *Streptomyces*

plasmid pSAM2 was sensitive to the *Sall* restriction system present in the recipient, which digests incoming double-stranded, but not single-stranded, DNA.

Upon entry into the recipient, the plasmid requires the spread (*spd*) genes for spreading along the substrate and aerial mycelia and to spores. Inactivation of *spd* genes causes a reduction in pock sizes (Kataoka *et al.*, 1994; Kieser *et al.*, 1982). The *spd* gene products usually are small hydrophobic proteins. One *spd* gene product, SpdB2 of pSVH1, forms oligomers and interacts with TraB *in vitro* (Tiffert *et al.*, 2007), leading to the proposal that TraB, SpdB2 and other spread proteins form a channel at the septal crosswalls for intrahyphal spread of the plasmid DNA. Recently, the *traB* and *spd1* genes on linear plasmid SLP2 have been found to suppress the partitioning defect caused by deletion of the *parAB* operon of SLP2 (Hsu & Chen, 2010). Apparently, plasmidless hyphae resulting from the partitioning defect may regain plasmids from plasmid-containing hyphae through intramycelial transfer and spread during colony growth.

Little is known about initiation of transfer in circular *Streptomyces* plasmids. Pettis & Cohen (1994) identified a *cis*-acting non-coding element, *clt*, on pIJ101 that is required for conjugal transfer. Interestingly, *clt* is not required for mobilization of the host chromosome. Similar *clt* loci have since been found in several other circular plasmids in *Streptomyces* (Grohmann *et al.*, 2003; Pettis & Prakash, 1999; Servín-Gonzalez, 1996). Although *clt* may represent the origin of transfer, binding of TraB of pIJ101 to its *clt* was not observed (Ducote *et al.*, 2000). On the other hand, TraB of pSVH1 binds (albeit at low affinity) to imperfect GACCCGGA octameric repeats (*clt*) downstream of *traB* (Reuther *et al.*, 2006b; Vogelmann *et al.*, 2011a, b).

Much less is known about the transfer mechanism of linear *Streptomyces* plasmids and chromosomes. The topology of the linear replicons imposes specific constraints on the site of initiation and the directionality of transfer. The classical model is not applicable for transfer of linear plasmids, because the transfer of single-stranded DNA by RCR from an internal origin cannot transport a linear plasmid DNA completely. It also cannot transfer a linear chromosome completely, and Wang *et al.* (1999) have previously shown that donor chromosomes are mobilized in total by linear plasmids.

An 'end first' model was proposed by Chen (1996), in which the conjugal transfer of linear *Streptomyces* replicons is initiated at the telomere(s) using TP-primed DNA synthesis (Fig. 1c). The model resembles the classical RCR model of transfer (Fig. 1a) in that the leading 5' strand is capped by a covalently bound leader protein (relaxase in the classical model and TP in the end first model) and is displaced by a newly synthesized DNA strand. In the classical model, replication is primed by the 3' free end at the nick created by the relaxase, whereas, in the end first model, replication is primed by a new TP.

TP-primed DNA synthesis is known to participate in the fill-in of the 3' single-stranded gaps (of about 300 nt) at the telomeres ('end patching') during the replication of the linear *Streptomyces* replicons (reviewed by Chaconas & Chen, 2005). In the end first model, the TP-primed DNA synthesis must extend all the way to the other end in order to transfer the complete plasmid and chromosomal DNA as single entities. Such end-to-end replication using TPs as primers is commonly used for the TP-capped linear viral genomes, typified by the *Bacillus* phage ϕ 29 (reviewed by Meijer *et al.*, 2001) and adenoviruses (reviewed by Liu *et al.*, 2003).

The end first model is supported by the finding of a single conserved helicase-like gene, *ttrA*, located in the subtelomere region of all the wholly sequenced *Streptomyces* chromosomes that display the typical telomere sequences. Many linear plasmids also contain one or two *ttrA* homologues, albeit not always terminally located. The *ttrA* gene has been found to be involved in the conjugal transfer of the linear plasmid SLP2 and of the linear host chromosome (Huang *et al.*, 2003). Inactivation of *ttrA* on SLP2 blocked the conjugal transfer of the plasmid and the linear host chromosome. Inactivation of *ttrA* on the host chromosome, however, blocked the transfer of the chromosome but not SLP2. These results suggest that, during SLP2-mediated conjugation, SLP2 transfer is necessary for chromosome transfer, and each *ttrA* gene may act *in cis* – very probably on the proximally located telomere.

Another plausible model for complete transfer of the linear replicons is bidirectional transfer from an internal origin (Fig. 1d), which is basically identical to the proposed transfer model for circular *Streptomyces* plasmids (Fig. 1b) except for the linearity of the substrate. We designated this the 'internal initiation' model. Because transfer is driven by bidirectional replication, the DNA enters the recipient in double-stranded form, perhaps also through the TraB ring.

In this study, we tested these models of linear replicon transfer in *Streptomyces* by investigating whether the telomeres of the linear plasmids and chromosomes of *Streptomyces* were necessary for mobilization by linear plasmids. If the end first model were correct, circularization of the linear replicons would result in a defect in transfer. Indeed, this proved to be true for the chromosomes; i.e. artificially circularized chromosomes could be mobilized by a circular plasmid, but not by a linear plasmid. On the other hand, circularization of SLP2 greatly reduced its self-transfer, but did not eliminate it completely. The remaining transfer activity was attributed to an internal initiation mechanism. The existence of the dual transfer mechanisms in SLP2 presumably reflects the acquisition of an additional transfer function during the replicon fusion that shaped this composite plasmid (Huang *et al.*, 2003). Moreover, circularized SLP2 was segregationally unstable, which would be expected from the reduced self-transfer that would normally reinstate plasmids from

plasmid-containing hyphae into plasmidless hyphae during growth (Hsu & Chen, 2010).

METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. *Streptomyces* cultures were maintained on YM medium with the appropriate antibiotics (ATCC culture medium 196, containing, per litre: 4 g Difco yeast extract; 10 g Difco malt extract, 20 g Difco agar, pH 7.2) at 30 °C.

Recombinant DNA techniques. General recombinant DNA techniques were according to Sambrook *et al.* (2001). Genetic manipulations of *Streptomyces* were according to Kieser *et al.* (2000). Separation of DNA by PFGE was performed as previously described (Chen *et al.*, 1993) in 1 % agarose gel in 0.5 × TBE buffer containing 25 µM thiourea (Ray *et al.*, 1995) with a 120° reorientation angle and 200 V voltage gradient at 13 °C. DNA for hybridization was labelled using DIG-Nick Translation Mix (Roche) according to the specifications supplied.

Conjugation analysis. Conjugation was performed according to Kieser *et al.* (2000). Approximately 10⁶–10⁷ spores of each parental strain were mixed and plated on R5Cu (R2YE supplemented with 0.02 mM CuCl₂) or YM agar (Kieser *et al.*, 2000). Spores were collected after incubation at 30 °C for 7–14 days, and recombinant cultures were isolated and counted on minimal medium containing appropriate supplements and/or antibiotics. In each mating, 10⁷–10¹⁰ spores were analysed, and 200 to over 1000 recombinants scored. The parental cultures were individually plated on the same selection medium to determine the frequencies of spontaneous mutation. The presence of conjugative plasmids was determined by (i) gel electrophoresis of total extracted DNA, (ii) thiostrepton resistance

(for plasmids containing *tsr*), or (iii) replicating colonies onto a sensitive (plasmid-free) lawn and observing the formation of ‘pocks’ around the donor colonies on a recipient lawn (Kieser *et al.*, 2000). The plasmid transfer efficiencies were calculated as the percentage of recipient spores that had received the plasmid.

Construction and isolation of circular SLP2 plasmid. Two terminal sequences of SLP2 (293–3200 bp from the left end and 12–1540 bp from the right end) were placed on two sides of the *tsr* gene on *E. coli* plasmid pCRII-TOPO (Invitrogen; containing kanamycin resistance and ampicillin resistance genes) to form a suicide plasmid, pLUS530 (see Results). pLUS530 was introduced into *S. lividans* 1326 by transformation. Thiostrepton-resistant and kanamycin-sensitive segregants were isolated, in which SLP2 had presumably been circularized through homologous recombination. Plasmid DNA was isolated from the candidate cultures using the procedure of Zhong *et al.* (2010) and analysed by PFGE, restriction and Southern hybridization. Cultures containing free circular SLP2 DNA of the correct size were used in further genetic studies.

RESULTS

SLP2 replicates in *Streptomyces* mutants possessing artificially circularized chromosomes

The end first transfer model for linear *Streptomyces* replicons (Chen, 1996) predicts that mobilization of circular donor chromosomes by linear conjugative plasmids would be defective. Unfortunately, *Streptomyces* strains possessing a circular chromosome and a linear plasmid were not readily available. Circularization of the *Streptomyces* chromosomes occurs frequently, and the resulting variants may be readily

Table 1. Bacterial strains and plasmids used in this study

Strain/plasmid	Genotype/description	Source/reference
<i>S. lividans</i>		
1326	SLP2 ⁺ SLP3 ⁺	Hopwood <i>et al.</i> (1983)
TK64	<i>pro-2 str-6</i>	Hopwood <i>et al.</i> (1983)
TK64/SLP2tsr	TK64 containing SLP2tsr	Hsu & Chen (2010)
TK64/cSLP2tsr	TK64 containing cSLP2tsr	This study
TK64/SCP1	<i>pro-2 str-6</i> SCP1 ⁺ (=strain 3200)	Hopwood <i>et al.</i> (1983)
ZX7	<i>pro-2 str-6 rec-46 ΔdndA</i>	Zhou <i>et al.</i> (1988)
ZX7/SLP2tsr	<i>pro-2 str-6 rec-46 ΔdndA</i> SLP2tsr ⁺	Hsu & Chen (2010)
TK54	<i>his-2 leu-2 spc-2</i>	Hopwood <i>et al.</i> (1983)
TK54C3	<i>his-2 leu-2 spc-2</i> Km ^s ; spontaneously circularized chromosome	This study
YSC26	<i>pro-2 str-6</i> Km ^r ; artificially circularized chromosome	Lin & Chen (1997)
YSC26/SLP2tsr	YSC26 harbouring SLP2tsr	Lin & Chen (1997)
YLR5~YLR8	Km ^s derivatives of YSC26; SLP2tsr ⁺	Lin (1998)
YLR5S	SLP2 [−] segregant (Ts ^s) of YLR5	This study
YLR5S/pIJ303	YLR5S containing pIJ303	This study
MR04	<i>pro-2 str-6 Δcmr</i> SLP2 [−] SLP3 [−] ; spontaneously circularized chromosome	Redenbach <i>et al.</i> (1993)
<i>S. coelicolor</i>		
M145	SCP1 [−] SCP2 [−]	Hopwood <i>et al.</i> (1983)
Plasmids		
pIJ303	pIJ101:: <i>tsr</i>	Kieser <i>et al.</i> (1982)
pLUS530	<i>E. coli</i> pCRII-TOPO plasmid containing terminal fragment of SLP2 flanking <i>tsr</i> gene	This study (Fig. 4a)
SLP2tsr	SLP2 with an integrated <i>tsr</i> gene in Tn4811	Hsu & Chen (2010)
cSLP2tsr	Artificially circularized SLP2 containing <i>tsr</i>	This study (Fig. 4)

isolated among spontaneous chloramphenicol-sensitive (Cm^s) mutants (Lin *et al.*, 1993), in which the chloramphenicol resistance gene (*cmr*) near the right terminus of the chromosome has been deleted (Redenbach *et al.*, 1993). However, the *cmr* deletions generally include the more distal *tap*-*tpg* operon, which encodes a telomere-associating protein and the TP (Bao & Cohen, 2001; Yang *et al.*, 2002). Thus, these Cm^s mutants cannot support replication of linear plasmids that lack an intact *tap*-*tpg* set, such as SLP2 (Denapaite & Cullum, 1998; Lin, 1998). SLP2 contains a functional *tpg* homologue and a *tpg* pseudogene but no *tap* homologue (Fig. 2), and requires an extrinsically supplied *tap* gene for replication (C.-C. Yang, unpublished results).

Instead, we chose to use *S. lividans* strains with an artificially circularized chromosome, which was constructed previously by Lin *et al.* (1993), in which the two chromosomal arms were joined by a kanamycin (Km) resistance gene (*aphII*) using a targeted recombination procedure. The artificially circularized chromosomes in these strains were highly unstable. Under kanamycin selection, *aphII* existed in tandem repeats, and, when the cultures were released from the kanamycin selection, the *aphII* repeats were deleted together with neighbouring sequences at frequencies approaching 100% (Lin & Chen, 1997).

One of these strains, YSC26, under Km selection, could be transformed by SLP2tsr, an SLP2 derivative containing a thiostrepton resistance gene *tsr* (Hsu & Chen, 2010). In the

Fig. 2. Genetic maps of *S. lividans*. The *S. lividans* chromosome is represented by the horizontal line, with the TP represented by the filled circles. The designated 'left' and 'right' ends are marked by 'L' and 'R'. The genetic locations of the alleles used in this study are marked. The right end of the *S. lividans* chromosome is enlarged below to show the 15.4 kb sequence (open box) that is homologous to the right end of SLP2 (Huang *et al.*, 2003). A 3.5 kb stretch of the homologous region to the left of the inserted *tsr* was removed during the construction of SLP2tsr (shown further below) such that relocation of *tsr* to the chromosome through homologous recombination is impossible. The terminal inverted repeat of the *S. lividans* chromosome is indicated by the double arrow. The *tpg* pseudogene on SLP2 is not functional; the *tpg* homologue, *tpg*_{SLP2} is functional, but it is not accompanied by a *tap* gene that is required for replication of SLP2 (C.-C. Yang, personal communication).

absence of Km, YSC26/SLP2tsr segregated approximately 25% Km^s spores in a sporulation cycle (Lin, 1998), and among these Km^s derivatives, approximately 38% had become thiostrepton sensitive (Ts^s) and had lost SLP2tsr, while the others remained thiostrepton resistant (Ts^r) and Cm^r and had retained SLP2tsr.

Nine such $\text{Km}^s \text{Ts}^r \text{Cm}^r$ strains, YLR1–YLR9, were characterized with respect to the extents of deletions by hybridization of two probes, 436 and 427L, located at 35.7 kb from the right end and 34.3 kb from the left end of the chromosome, respectively, and adjacent to the boundaries of the 31 kb terminal inverted repeats (Fig. 3a). Of the nine strains, YLR5 appeared to have suffered the smallest deletions. It retained both 436 and 427L, indicating less than 70 kb of deletions. YLR8 had lost both 436 and 427L, and the rest had retained one of the two. Four strains, YLR5, YLR 6, YLR7 and YLR8, which maintained SLP2tsr stably (Fig. 3b), were chosen for subsequent mating experiments. They inherited the proline auxotrophic (*pro-2*) and streptomycin resistance (*str-6*) markers from their ancestral strain TK64. These genetic markers were as stable in these strains as in TK64 (reversion frequencies $<10^{-7}$ to $<10^{-9}$).

Circular *Streptomyces* chromosomes are not mobilized by SLP2

YLR5/SLP2tsr–YLR8/SLP2tsr were individually mated with a plasmidless spectinomycin-resistant (*spc-1*) strain of *Streptomyces lividans*, TK54 (Table 2, matings Ia–Id). Transfer of SLP2tsr was very efficient (approx. 50% per recipient), as previously reported. However, all attempts to select for streptomycin-resistant (Sm^r) and spectinomycin-resistant (Sp^r) recombinants (for genetic map see Fig. 2) failed among 10^8 – 10^{10} spores screened in 11 independent matings. In addition, YLR5/SLP2tsr–YLR8/SLP2tsr were individually mated with TK54C3, a Cm^s derivative of TK54 containing a circular chromosome isolated in this study (matings Ie–Ih). Likewise, no $\text{Sm}^r \text{Sp}^r$ recombinants were detected in any of the cases ($<10^{-8}$).

To test whether the lack of recombination in these matings was specific for the particular markers chosen, another set of markers, Sm^r and Pro^+ (proline prototrophy), which were more distantly separated (Fig. 2), was chosen for recombination selection. In these matings (Ia, Ib, Ie, If), YLR5 and YLR7 were the donor strains and TK54 and TK54C3 were the recipient strains. Again, no recombinant was detected ($<2.0 \times 10^{-7}$ to $<4 \times 10^{-8}$). These results indicated that the absence of recombination was general, and reflected a lack of chromosome mobilization in these matings.

Linear chromosomes are mobilized by linear plasmids

The situation was different when the donor chromosomes were linear. Mating between ZX7/SLP2tsr and TK54

Fig. 3. *S. lividans* strains containing artificially circularized chromosomes. (a) Extents of deletions in the circularized chromosome. The fusion region of the circular chromosome of YSC26 is shown above. 'L' and 'R' indicate the left and right arms of the linear chromosome before circularization. The tandem amplifications containing the *aphII* gene are indicated by the open triangles. Based on the Km^s Cm^r Ts^r phenotype, the chromosomes of YLR1–YLR9 were inferred to have lost (–) *aphII* (Km^s), but retained (+) the *tap*-*tpg* operon (Ts^s) and *cmr* (Cm^r). The extents of deletions were further refined by hybridization to the 427L and 436 probes (+/–). The locations of 436 and 427L (flanking the 31 kb terminal inverted repeats), the *tap*-*tpg* operon and *cmr* are marked and their distances (in kb) from the ends are indicated above. (b) Presence of SLP2tsr in YLR5–YLR8. The physical presence of SLP2tsr in YLR5–YLR8 and the absence of SLP2tsr in YLR5S were confirmed by PFGE (top panel: 2–20 s ramp; run time 15 h; 200 V) and hybridization with the probe of the 3.2 kb *Hind*III left terminal fragment of SLP2 (bottom panel). (c) Comparison of YLR5 and YLR5S genomic DNA. The genomic DNA of YLR5 and YLR5S was digested with *Asel* and separated by PFGE (5–10 s ramp for 160 min, 40–60 s ramp for 560 min, 80–100 s ramp for 560 min, 120–140 s ramp for 160 min; 200 V). The *Asel* fragments of *S. lividans* chromosomes (A–L) and the 36 kb *Asel* fragment of SLP2tsr (marked SLP2') in YLR5 (absent in YLR5S) are indicated. The *Asel* site on SLP2tsr was present in the inserted *tsr* gene cassette. An unknown approximately 85 kb fragment marked '?', probably generated during the artificial circularization and rearrangements, was also found in YLR6–YLR8, which exhibited essentially the same *Asel* digestion patterns as YLR5.

produced Sm^r Sp^r recombinants at frequencies of 4.2×10^{-5} (Table 2, mating IIa). This was comparable with the frequencies of recombination (2×10^{-4} – 3×10^{-4}) between more distant marker pairs (*his*-*spc* or *leu*-*spc*; Fig. 2) in SPL2-mediated conjugation in *S. lividans* reported previously (Hopwood *et al.*, 1983). Similar results were obtained when the recipient chromosome (TK54C3) was circular (mating IIb). In these matings, SLP2tsr was transferred at 91–100% efficiency. ZX7 was a descendant of TK64 containing a *rec-46* (intraplasmid recombination deficiency; Tsai & Chen, 1987) and a $\Delta dndA$ (DNA sulfur modification deficiency; Zhou *et al.*, 2005) mutation. To remove the possible effect of these mutations, TK64/SLP2tsr was mated with TK54C3 (mating IIc). Similar recombination frequencies were observed.

Another linear plasmid SCP1 (Kinashi *et al.*, 1987) originally found in *Streptomyces coelicolor* was tested. Mating of *S. lividans* TK64 harbouring SCP1 (Hopwood *et al.*, 1983) with TK54 (mating IId) or its circular derivative TK54C3 (mating IIe) also produced recombinants, but at frequencies approximately one order of magnitude lower. These frequencies were in accord with those obtained by Hopwood *et al.* (1983).

Circular chromosomes are mobilized by circular plasmids

To test whether the failure of the circular chromosomes to be mobilized during conjugation was specific to linear plasmids, circular plasmid pIJ303, a derivative of pIJ101 (Kieser *et al.*, 1982), was used to mediate conjugation. Firstly, YLR5 was cured of SLP2tsr by protoplasting and cold storage (Hopwood *et al.*, 1983) and screening for Ts^s derivatives. A representative, YLR5S, the genome of which was indistinguishable from that of YLR5 in *Asel* restriction patterns (Fig. 3c) except for the loss of SLP2 (Fig. 3b), was chosen, and pIJ303 was introduced into it by transformation. When YLR5S/pIJ303 was mated with TK54, Sm^r Sp^r recombinants were recovered at frequencies of approximately 3.7×10^{-6} (Table 2, mating IIIa).

Similarly, pIJ303 was introduced into MR04, a spontaneous Cm^s mutant containing a circular chromosome with a large (approx. 700 kb) deletion (Lin *et al.*, 1993; Redenbach *et al.*, 1993). Matings between MR04/pIJ303 and TK54 gave recombination frequencies of approximately 1.5×10^{-4} (mating IIIb). Matings between TK64/pIJ303 and TK54C3 (mating IIIc) gave similar recombination frequencies. These

Table 2. Mobilization of linear and circular chromosomes by naturally occurring linear and circular plasmids

Spontaneous mutation giving rise to the recombinant phenotype occurred at frequencies of $<10^{-9}$ to $<10^{-8}$ in the donor strains and $<10^{-9}$ to 10^{-7} in the recipient strains.

Mating*	Topology† of:			Transfer frequency‡	Selection§	Recombination frequency
	Plasmid	Donor chr.	Recip. chr.			
Circular chromosomes are not mobilized by linear plasmid						
Ia. YLR5/SLP2tsr (<i>pro-2 str-6</i>) × TK54 (<i>spc-1</i>)	L	C	L	52 ± 29 (4)	Sm ^r Sp ^r Pro ⁺ Sm ^r	<1.6 × 10 ⁻⁸ (4) <2.0 × 10 ⁻⁷ (1)
Ib. YLR7/SLP2tsr (<i>pro-2 str-6</i>) × K54 (<i>spc-1</i>)	L	C	L	48 ± 32 (3)	Sm ^r Sp ^r Pro ⁺ Sm ^r	<2.5 × 10 ⁻⁸ (5) <4.7 × 10 ⁻⁸ (1)
Ic. YLR6/SLP2tsr (<i>pro-2 str-6</i>) × TK54 (<i>spc-1</i>)	L	C	L	ND	Sm ^r Sp ^r	<4.4 × 10 ⁻⁹ (1)
Id. YLR8/SLP2tsr (<i>pro-2 str-6</i>) × TK54 (<i>spc-1</i>)	L	C	L	ND	Sm ^r Sp ^r	<9.9 × 10 ⁻¹⁰ (1)
Ie. YLR5/SLP2tsr (<i>pro-2 str-6</i>) × TK54C3 (<i>spc-1</i>)	L	C	C	80 ± 7 (1)**	Sm ^r Sp ^r Pro ⁺ Sm ^r	<2.4 × 10 ⁻⁸ (4) <4.1 × 10 ⁻⁸ (1)
If. YLR7/SLP2tsr (<i>pro-2 str-6</i>) × TK54C3 (<i>spc-1</i>)	L	C	C	ND	Sm ^r Sp ^r Pro ⁺ Sm ^r	<2.3 × 10 ⁻⁸ (5) <3.7 × 10 ⁻⁸ (1)
Ig. YLR6/SLP2tsr (<i>pro-2 str-6</i>) × TK54C3 (<i>spc-1</i>)	L	C	C	ND	Sm ^r Sp ^r	<7.1 × 10 ⁻⁹ (1)
Ih. YLR8/SLP2tsr (<i>pro-2 str-6</i>) × TK54C3 (<i>spc-1</i>)	L	C	C	ND	Sm ^r Sp ^r	<3.1 × 10 ⁻⁹ (1)
Linear chromosomes are mobilized by linear plasmid						
IIa. ZX7/SLP2tsr (<i>str-6</i>) × TK54 (<i>spc-1</i>)	L	L	L	91 ± 10 (3)	Sm ^r Sp ^r	4.2 ± 1.2 × 10 ⁻⁵ (2)
IIb. ZX7/SLP2tsr (<i>str-6</i>) × TK54C3 (<i>spc-1</i>)	L	L	C	100 ± 11 (1)**	Sm ^r Sp ^r	4.4 ± 3.2 × 10 ⁻⁵ (3)
IIc. TK64/SLP2tsr (<i>str-6</i>) × TK54C3 (<i>spc-1</i>)	L	L	C	ND	Sm ^r Sp ^r	1.7 ± 1.9 × 10 ⁻⁵ (4)
IId. TK64/SCP1 (<i>str-6</i>) × TK54 (<i>spc-1</i>)	L	L	L	ND	Sm ^r Sp ^r	3.4 ± 3.1 × 10 ⁻⁶ (2)
IIe. TK64/SCP1 (<i>str-6</i>) × TK54C3 (<i>spc-1</i>)	L	L	C	ND	Sm ^r Sp ^r	1.6 ± 1.1 × 10 ⁻⁶ (3)
Circular and linear chromosomes are mobilized by circular plasmid						
IIIa. YLR5S/pIJ303 (<i>str-6</i>) × TK54 (<i>spc-1</i>)	C	C	L	ND	Sm ^r Sp ^r	3.7 ± 0.4 × 10 ⁻⁶ (1)**
IIIb. MR04/pIJ303 (<i>str-6</i>) × TK54 (<i>spc-1</i>)	C	C	L	ND	Sm ^r Sp ^r	1.5 ± 0.4 × 10 ⁻⁴ (3)
IIIc. TK64/pIJ303 (<i>str-6</i>) × TK54C3 (<i>spc-1</i>)	C	L	C	ND	Sm ^r Sp ^r	9.6 ± 3.5 × 10 ⁻⁵ (3)

*Donor and recipient strains listed with only the relevant genetic markers.

†C, circular; L, linear.

‡Frequency of plasmid transfer (%), calculated by dividing the number of Ts^r recipients by the number of total recipients scored. The mean is followed by standard deviation and the number of repeat experiments (in parentheses). In mating experiments without repeat (**), the standard deviations were estimated by Poisson distribution based on the numbers of c.f.u. scored. ND, Not determined.

§The selection used for the calculations of recombination frequencies. Sm^r, streptomycin resistance; Sp^r, spectinomycin resistance; Pro⁺, proline prototrophy.

||Recombination frequencies: mean followed by standard deviation and the number of repeat experiments (in parentheses).

results were comparable with the recombination frequencies of 10^{-3} between more distantly separated marker pairs (*his-spc* and *leu-spc*) in similar matings (Kieser *et al.*, 1982), and indicated that circular chromosomes could be mobilized by circular plasmids as efficiently as linear chromosomes could be.

SLP2 is circularized by targeted recombination

The above tests dealt with the effect of topology of the chromosomes on mobilization by plasmids. The next question was whether circularization of linear plasmids would affect their self-transfer and chromosome mobilization. To test this, we chose SLP2 for circularization using

the same targeted recombination procedure as used by Lin *et al.* (1993). A suicide vector, pLUS530, was constructed that contains the *tsr* gene flanked by the left and right terminal sequences of SLP2 (Fig. 4a). *S. lividans* 1326 (SLP2⁺) was transformed by this plasmid, and Ts^r transformants were selected. After one round of sporulation in the absence of Km, Ts^r Km^s segregants were isolated and examined for circularization of SLP2.

The procedure of Zhong *et al.* (2010) was used to isolate the 51 kb circular plasmid DNA, followed by examination by PFGE, restriction and Southern hybridization. In some of the Ts^r Km^s strains, SLP2 DNA appeared to have undergone rearrangements, and these strains were not

Fig. 4. Circularization of SLP2. (a) Circularization scheme. The two terminal segments at the left (red bar) and right (blue bar) arms of SLP2 (top) were placed on the suicide vector pLUS530 flanking *tsr* (yellow arrow), which also contained *aphII* (kanamycin resistance). $\Delta 292$ and $\Delta 11$ indicate the distances (in bp) between these segments and the ends of SLP2 DNA, which would be deleted during circularization. Recombination between these homologous sequences circularized SLP2 DNA (cSLP2tsr). Selected genes relevant to conjugation, partitioning, transfer and spread, as well as the *Clal* restriction sites (C), on cSLP2tsr are shown. The expected sizes of the five larger *Clal* fragments [detected in (b)] are indicated. The 3.2 kb *HindIII* (H) left terminal fragment of SLP2 used as the probe in Southern hybridization

(below) is indicated by the open bars. (b) Physical characterization of cSLP2tsr. Left panel. cSLP2tsr DNA (C) was isolated from TK64/cSLP2tsr, digested with *HindIII* (H), subjected to PFGE (pulse time 3–5 s ramp; run time 24 h) and hybridized with the left-terminal DNA probe. The lambda DNA size markers are indicated on the left. L, SLP2tsr DNA. U, uncut DNA. OC, open circle form of cSLP2tsr. SC, supercoiled form of cSLP2tsr. The expected sizes (in kb) of the restriction fragments are shown in white numbers in the black boxes. Right panel. cSLP2tsr DNA preparation treated with *Clal*, separated by agarose gel electrophoresis and photographed. Southern hybridization using SLP2 DNA as probe would lead to hybridization to contaminating homologous chromosomal DNA as well as SLP2 fragments, and therefore was not used.

used. In the others, circularized SLP2 DNA could be identified in the gel as a DNA species migrating at an anomalously lower mobility than the linear counterpart (Levene & Zimm, 1987; Sobral & Atherly, 1989) (Fig. 4b). The size and circularity of the SLP2 DNA was confirmed by restriction digestion and Southern hybridization. This circularized SLP2 plasmid was designated cSLP2tsr. It contained the complete SLP2 sequence except for the 292 bp and 11 bp of the left and right ends, respectively (Fig. 4a). cSLP2tsr was unstably maintained in TK64, suffering a loss of approximately 30 % in one round of a sporulation cycle in the absence of thiostrepton selection. Its linear counterpart, SLP2, was highly stable in TK64 (Hopwood *et al.*, 1983; Hsu & Chen, 2010).

Circularized SLP2 may be transferred by a telomere-independent mechanism

S. lividans 1326 contains another conjugative element, SLP3, which is competent for self-transfer and chromosome mobilization (Hopwood *et al.*, 1983). To eliminate the interference of SLP3, cSLP2tsr was transferred to TK64 by transformation for subsequent conjugation experiments. TK64/cSLP2tsr and TK64/SLP2tsr were mated with TK54 and *S. coelicolor* M145 (Table 3, matings IVa–IVd) to examine the effect of SLP2 circularization on self-transfer and chromosome mobilization. Unlike the circularized chromosomes (Table 2, matings Ia–Ih), cSLP2tsr remained competent for transfer. However, the transfer frequency was reduced 50-fold (2 % versus 100 % and 1.5 % versus 75 %, respectively) in both matings. The transfer of cSLP2tsr and SLP2tsr plasmids in these matings was confirmed in selected samples by PFGE and Southern hybridization (data not shown). The decrease in cSLP2tsr self-transfer supports the involvement of the end first mechanism in SLP2 transfer; yet the residual transfer also indicated the presence of a telomere-independent mechanism for SLP2 transfer, which accounted for about 2 % of the transfer events.

Recombination frequencies in these matings were also similarly affected by the circularization of SLP2: they were

Table 3. Effect of circularization of SLP2 on self-transfer and chromosome mobilization

All the footnotes are as the same as in Table 2. Spontaneous mutation giving rise to the recombinant phenotype occurred at frequencies of 10^{-9} to 10^{-8} in the donor strains and $<10^{-9}$ to 10^{-7} in the recipient strains.

Mating*	Topology† of:			Transfer frequency‡	Selection§	Recombination frequency
	Plasmid	Donor chr.	Recip. chr.			
IVa. TK64/SLP2tsr (<i>pro-2 str-6</i>) × TK54 (<i>spc-1</i>)	L	L	L	107.6 ± 23.5 (5)	Sm ^r Sp ^r	$3.4 \pm 4.8 \times 10^{-6}$ (5)
IVb. TK64/cSLP2tsr (<i>pro-2 str-6</i>) × TK54 (<i>spc-1</i>)	C	L	L	1.9 ± 1.2 (5)	Sm ^r Sp ^r	$3.1 \pm 4.0 \times 10^{-7}$ (5)
IVc. TK64/SLP2tsr (<i>pro-2 str-6</i>) × M145	L	L	L	$74.8 \pm 7.5^{**}$ (1)	Pro ⁺ Sm ^r	$1.3 \pm 0.2 \times 10^{-6}$ (2)
IVd. TK64/cSLP2tsr (<i>pro-2 str-6</i>) × M145	C	L	L	1.5 ± 0.6 (6)	Pro ⁺ Sm ^r	$<6 \times 10^{-8} - <2 \times 10^{-7}$ (6)

reduced from 3.4×10^{-6} to 3.1×10^{-7} in the TK64 to TK54 transfer, and from 1.3×10^{-6} to non-detectable ($<6 \times 10^{-8}$ to $<2 \times 10^{-7}$) in the TK64 to M145 transfer. This also supported the end first mechanism for the transfer of linear chromosomes.

DISCUSSION

Retro-transfer is unlikely

Previous genetic studies indicate that recombination during *Streptomyces* mating occurs via merozygotic intermediates, which is similar to other bacterial systems (Hopwood *et al.*, 1984). In their comparative analysis, Wang *et al.* (1999) noticed that the composition of the merozygotes depended on the topology of the conjugative plasmids. In circular-plasmid-mediated conjugation, the merozygotes contain a partial chromosome from the donor and a complete chromosome from the recipient (as in other bacteria), whereas in linear-plasmid-mediated conjugation, the situation is reversed – the merozygotes consist of a complete chromosome from the donor and an incomplete chromosome from the recipient. Two alternative possibilities were raised to account for the latter: (i) the linear-plasmid-driven transfer exerted an unknown destructive effect on the integrity of the recipient chromosomes; and (ii) there was efficient retro-transfer of a partial chromosome from the recipient to the donor where recombination takes place.

This uncertainty may be addressed by considering the results of the matings between a donor containing a circular chromosome plus a linear plasmid (SLP2tsr) with a recipient containing a linear chromosome (Table 2, matings Ia and Ib). In these matings, the plasmid was transferred at about 50 % efficiency, but no recombination was detected. Apparently, acquisition of the plasmids in the recipients had not promoted retro-transfer at a significant level. Thus, the possibility of retro-transfer may be discounted in our assessment of (at least) linear-plasmid-driven conjugal transfer.

Chromosome mobilization by integrated linear plasmids is unlikely

We do not know how *Streptomyces* chromosomes are mobilized by linear plasmids, and it might be argued that they are mobilized via integration of the plasmid DNA and that the failure of the circular chromosomes to be mobilized is due to some terminal sequences, which were removed during circularization, containing homologous sequences necessary for recombination with the linear plasmids.

Indeed the deletions in the artificially circularized chromosomes in YLR5–YLR8 do include the 15.4 kb terminal sequence of the *S. lividans* chromosomes shared by the right end of SLP2 (Fig. 2) (Lin, 1998). One may argue that the 15.4 kb homologous sequence is required for integration of SLP2. However, homologous recombination between two linear molecules cannot form a single cointegrate. In the case of SLP2 and the *S. lividans* chromosomes, homologous recombination between the terminal homologous sequences would be futile, giving rise to two products with unchanged sequences.

The main obstacle for the integration model is how a linear plasmid may integrate into (or excise from) a linear chromosome without any sequence loss. In the NF (Hopwood *et al.*, 1969) and NF-like (Vivian & Hopwood, 1973) strains of *S. coelicolor*, the integrated SCP1 mobilizes the chromosome efficiently, but they are truncated at both ends (Hanafusa & Kinashi, 1992; Kinashi *et al.*, 1992; Yamasaki *et al.*, 2001). A circular-chromosome-mobilizing genetic element, pSAM2 of *Streptomyces ambifaciens*, is normally integrated at a specific site of the chromosome (Boccard *et al.*, 1989), but it is excised and replicates as a circular molecule before transfer to the recipient, where it is again integrated into the chromosome (Possoz *et al.*, 2001). Other than these, no integration of conjugative plasmids (linear or circular) has ever been detected in the donor or recipient chromosomes in matings driven by free plasmids in *Streptomyces*.

Moreover, no specialized enzymic system for integration and excision (such as those employed by integrative linear replicons like lambdoid phages, retroviruses and

retrotransposons) is found in SLP2, making it unlikely that it may mobilize the chromosomes by integration. We conclude that the inability of these linear plasmids to mobilize a circular chromosome is not due to a defect in plasmid integration but due to the absence of functional telomeres on the chromosomes.

Linear plasmids mobilize *Streptomyces* chromosomes from the ends

In these circumstances, the end first mechanism remains the favoured model to account for the deficiencies in mobilization of circularized *Streptomyces* chromosomes. In this study, this has been demonstrated exhaustively in four different donor strains. No mobilization of circular donor chromosomes was ever detected ($<10^{-7}$ to $<10^{-9}$), while SLP2_{tsr} transfer was normal and efficient (at approximately 50%). The topology of the donor chromosomes appears to be the crucial element, as all the three other topological combinations of the donor genome – (i) linear plasmid + linear chromosome, (ii) circular plasmid + linear chromosome and (iii) circular plasmid + circular chromosome – allowed detectable transfer of the donor chromosome. The last combination is most noteworthy in that the same circular chromosomes that were not mobilized by linear plasmids could be readily mobilized by a circular plasmid (Table 2, matings IIIa and Ia). This implies a basic mechanistic difference in chromosome mobilization mediated by a linear plasmid and by a circular plasmid in *Streptomyces*. Presumably the linear plasmid mobilizes the chromosome from its telomeres by the same end first mechanism for self-transfer and the circular plasmid mobilizes the chromosome by the internal initiation mechanism.

The end first model is supported by the involvement of the terminally located putative helicase gene *ttrA* in linear-plasmid-mediated conjugal transfer. The defects in plasmid and chromosome transfer caused by *ttrA* mutations were initially observed in *S. lividans*-to-*S. coelicolor* interspecific transfers (Huang *et al.*, 2003). Later, two reports (Wang & Pettis, 2010; Xu *et al.*, 2006) failed to observe the same effect of *ttrA* deletions on plasmid transfer from *S. lividans* to *S. lividans*. The discrepancy could be attributed to the difference in the choices of mating partners: while the donor strains were all *S. lividans*, the recipient was *S. coelicolor* in the first case and *S. lividans* in the other two cases. This suspicion was supported by the recent observations that *ttrA* deletions in SCP1 also blocked plasmid transfer from *S. lividans* to *S. coelicolor*, but not from *S. lividans* to *S. lividans* (C.-H. Huang, personal communication). At present, the possible underlying mechanism for the discrepancy may only be speculated upon.

Linear plasmids are transferred by two mechanisms

Circularization of SLP2 reduced plasmid transfer 50-fold from TK64 to TK54 and to M145. A smaller reduction in

plasmid transfer was observed in a circularized partial SLP2 plasmid pQC588 (spanning ORFs SLP2.4–SLP2.23; Xu *et al.*, 2006). While these observations support the end first mechanism (Fig. 1c), the remaining transfer must represent a telomere-independent transfer mechanism, i.e. the internal initiation mechanism (Fig. 1d). As discussed in the Introduction, such an internal initiation mechanism must replicate bidirectionally in order to transfer the complete plasmid and for the DNA to enter the recipient in a double-stranded form.

In these matings, the end first mechanism appeared to contribute about 98 % of the observed transfer, whereas the internal initiation mechanism contributed 2 %. It is not clear how these two mechanisms interact. However, it is clear that both depend on *traB*, because no self-transfer of SLP2 can be detected ($<10^{-7}$) if *traB* is deleted (Hsu & Chen, 2010). Presumably, DNA mobilized by either mechanism is pumped through the TraB ring (Fig. 1c, d). If the TraB ring only transports double-stranded DNA, the TP-led DNA strand must be converted into duplex form in the donor prior to transfer.

Is the existence of such a dual transfer mechanism in SLP2 common among linear plasmids in *Streptomyces*? The answer to this question must await further investigation. It is possible that this is a special case for SLP2 and possibly some other large linear plasmids. The two transfer activities may represent independent functions inherited from two ancestral plasmids (a circular and a linear one) that now make up SLP2. Previous analysis of the SLP2 sequence has revealed: (i) apparent exchange with the host chromosomal telomeres; (ii) two dissimilar telomere sequences; and (iii) a *tpg* pseudogene (Fig. 2) and pseudo-telomere sequences. All these indicate that SLP2 is a composite plasmid that has evolved from several rounds of recombination involving at least three replicons (Huang *et al.*, 2003). It is likely that one of the ancestral replicons was a circular plasmid, which brought in the internal initiation transfer function. This additional function would complement the end first mechanism and provide a selective advantage in helping the spread of SLP2 in nature, particularly when it has suffered accidental circularization. Recently, a 2 kb internal *cis*-acting element for conjugal transfer was located in SLP2 (Hsueh, 2009), which may be involved in the internal initiation mechanism. This sequence spans from the 3' terminus of *traB* to the 5' terminus of the downstream putative transglycosylase gene and contains many short repeats.

Similarly, an internal *cis*-acting element involved in conjugal transfer has also been identified in SCP1 (C.-H. Huang, personal communication). That SCP1 may also contain an internal initiation mechanism can be inferred from the conjugal behaviour of the chromosome in the NF strain of *S. coelicolor*. Transfer of the NF chromosome exhibits a distinct bidirectional polarity from the integrated SCP1 (100 % transfer) to more distant markers on both

sides (Hopwood *et al.*, 1969). This suggests a bidirectional transfer initiated internally from the integrated SCP1. The 350 kb sequence of SCP1 also reveals a mosaic nature (Bentley *et al.*, 2004).

The instability of cSLP2_{tsr} may reflect its defect in conjugal transfer

Despite its relatively low copy number (about two per chromosome; Chen *et al.*, 1993), SLP2 is stably maintained (Hopwood *et al.*, 1983; Hsu & Chen, 2010). The results in this study show that cSLP2_{tsr} was lost at high frequencies during a sporulation cycle. Likewise, plasmid pQC588, a circularized partial SLP2 (spanning ORF SLP2.4 to approximately SLP2.23) with a reduction in self-transfer efficiency (Xu *et al.*, 2006) also exhibits similar segregational instability (Z. Qin, personal communication). The segregational instability in these circularized SLP2 plasmids may be (at least partially) accounted for by the deficiency in self-transfer. It has been shown previously that *traB*-mediated transfer was involved in reintroduction of SLP2 from plasmid-containing hyphal segments to other segments that had suffered plasmid loss during colony growth (Hsu & Chen, 2010). Another non-exclusive possibility is that the telomeres of the plasmids may play a role in post-replicative segregation – perhaps through interaction between the plasmid telomeres and the chromosomal telomeres as previously proposed by Tsai *et al.* (2011).

Terminal proteins are crucial to end first transfer

In the end first model, TP plays the crucial dual roles of primer for replication initiation and leader for the covalently attached DNA to enter the recipient (Fig. 1c). In the classical model, transfer of the relaxase-capped DNA employs a type IV protein secretion system (T4SS; reviewed by Cascales & Christie, 2003) and it is possible that relaxase protein itself is transferred and the attached DNA is transferred passively. In the end first model, TP might also be transported by a protein secretion system, with the DNA following passively.

Moreover, *in vitro* TPs (both archetypal Tpg on most *Streptomyces* chromosomes and plasmids and atypical Tpc on SCP1) can also lead the attached DNA to the nuclei of human (Tsai *et al.*, 2008) and plant cells (Lai, 2010) using their nuclear localization signals (NLSs). In Ti-plasmid-mediated conjugation between *Agrobacterium tumefaciens* and plant cells, the Ti-plasmid-encoded relaxase VirD2 also contains an NLS, and doubles as a protein that leads Ti-DNA into the plant cell as well as a protein that targets T-DNA into the plant nucleus (Tinland *et al.*, 1992). This intriguing comparison hints at possible TP-mediated inter-kingdom conjugal transfers from *Streptomyces* to certain eukaryotes in the soil.

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REFERENCES

- Bao, K. & Cohen, S. N. (2001). Terminal proteins essential for the replication of linear plasmids and chromosomes in *Streptomyces*. *Genes Dev* **15**, 1518–1527.
- Bath, J., Wu, L. J., Errington, J. & Wang, J. C. (2000). Role of *Bacillus subtilis* SpoIIIE in DNA transport across the mother cell–prespore division septum. *Science* **290**, 995–997.
- Bentley, S. D., Brown, S., Murphy, L. D., Harris, D. E., Quail, M. A., Parkhill, J., Barrell, B. G., McCormick, J. R., Santamaria, R. I. & other authors (2004). SCP1, a 356,023 bp linear plasmid adapted to the ecology and developmental biology of its host, *Streptomyces coelicolor* A3(2). *Mol Microbiol* **51**, 1615–1628.
- Bibb, M. J., Ward, J. M. & Hopwood, D. A. (1978). Transformation of plasmid DNA into *Streptomyces* at high frequency. *Nature* **274**, 398–400.
- Bigot, S., Sivanathan, V., Possoz, C., Barre, F. X. & Cornet, F. (2007). FtsK, a literate chromosome segregation machine. *Mol Microbiol* **64**, 1434–1441.
- Boccard, F., Smokvina, T., Pernodet, J. L., Friedmann, A. & Guérineau, M. (1989). The integrated conjugative plasmid pSAM2 of *Streptomyces ambofaciens* is related to temperate bacteriophages. *EMBO J* **8**, 973–980.
- Burton, B. & Dubnau, D. (2010). Membrane-associated DNA transport machines. *Cold Spring Harb Perspect Biol* **2**, a000406.
- Burton, B. M., Marquis, K. A., Sullivan, N. L., Rapoport, T. A. & Rudner, D. Z. (2007). The ATPase SpoIIIE transports DNA across fused septal membranes during sporulation in *Bacillus subtilis*. *Cell* **131**, 1301–1312.
- Byrd, D. R. & Matson, S. W. (1997). Nicking by transesterification: the reaction catalysed by a relaxase. *Mol Microbiol* **25**, 1011–1022.
- Cascales, E. & Christie, P. J. (2003). The versatile bacterial type IV secretion systems. *Nat Rev Microbiol* **1**, 137–149.
- Chaconas, G. & Chen, C. W. (2005). Linear chromosomes in bacteria: no longer going around in circles. In *The Bacterial Chromosome*, pp. 525–539. Edited by N. P. Higgins. Washington, DC: American Society for Microbiology.
- Chen, C. W. (1996). Complications and implications of linear bacterial chromosomes. *Trends Genet* **12**, 192–196.
- Chen, C. W. (2007). *Streptomyces* linear plasmids: replication and telomeres. In *Microbial Linear Plasmids*, pp. 33–61. Edited by F. Meinhardt & R. Klassen. Berlin & Heidelberg: Springer-Verlag.
- Chen, C. W., Yu, T.-W., Lin, Y. S., Kieser, H. M. & Hopwood, D. A. (1993). The conjugative plasmid SLP2 of *Streptomyces lividans* is a 50 kb linear molecule. *Mol Microbiol* **7**, 925–932.
- Chen, C. W., Huang, C.-H., Lee, H.-H., Tsai, H.-H. & Kirby, R. (2002). Once the circle has been broken: dynamics and evolution of *Streptomyces* chromosomes. *Trends Genet* **18**, 522–529.
- Clewell, D. B. (1993). *Bacterial Conjugation*. New York: Plenum Press.
- Denapate, D. & Cullum, J. (1998). Chromosome end of *Streptomyces lividans* 66 is necessary for the maintenance of the linear plasmid SLP2. In *Society for General Microbiology 141st Ordinary Meeting*, p. 47. University of East Anglia, Norwich, UK.

- Ducote, M. J., Prakash, S. & Pettis, G. S. (2000). Minimal and contributing sequence determinants of the *cis*-acting locus of transfer (*clt*) of streptomycete plasmid pIJ101 occur within an intrinsically curved plasmid region. *J Bacteriol* **182**, 6834–6841.
- Grohmann, E., Muth, G. & Espinosa, M. (2003). Conjugative plasmid transfer in Gram-positive bacteria. *Microbiol Mol Biol Rev* **67**, 277–301.
- Hanafusa, T. & Kinashi, H. (1992). 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.
- Hopwood, D. A. & Kieser, T. (1993). Conjugative plasmids of *Streptomyces*. In *Bacterial Conjugation*, pp. 293–311. Edited by D. B. Clewell. New York: Plenum Press.
- Hopwood, D. A. & Sermonti, G. (1963). The genetics of *Streptomyces coelicolor*. *Adv Genet* **11**, 273–342.
- Hopwood, D. A., Harold, R. J., Vivian, A. & Ferguson, H. M. (1969). A new kind of fertility variant in *Streptomyces coelicolor*. *Genetics* **62**, 461–477.
- Hopwood, D. A., Kieser, T., Wright, H. M. & Bibb, M. J. (1983). Plasmids, recombination and chromosome mapping in *Streptomyces lividans* 66. *J Gen Microbiol* **129**, 2257–2269.
- Hopwood, D. A., Lydiate, D. J., Malpartida, F. & Wright, H. M. (1984). Conjugative sex plasmids of *Streptomyces*. In *Plasmids in Bacteria*, pp. 615–634. Edited by D. Helinski, S. Cohen & D. Clewell. New York: Plenum.
- Hsu, C. C. & Chen, C. W. (2010). Linear plasmid SLP2 is maintained by partitioning, intrahyphal spread, and conjugal transfer in *Streptomyces*. *J Bacteriol* **192**, 307–315.
- Hsueh, K.-W. (2009). Identification of a *cis*-acting element on *Streptomyces linear plasmid SLP2 for conjugal transfer*. MSc thesis, Department of Biotechnology, National Taipei University of Technology.
- Huang, C.-H., Lin, Y.-S., Yang, Y.-L., Huang, S. W. & Chen, C. W. (1998). The telomeres of *Streptomyces* chromosomes contain conserved palindromic sequences with potential to form complex secondary structures. *Mol Microbiol* **28**, 905–916.
- Huang, C. H., Chen, C. Y., Tsai, H. H., Chen, C., Lin, Y. S. & Chen, C. W. (2003). Linear plasmid SLP2 of *Streptomyces lividans* is a composite replicon. *Mol Microbiol* **47**, 1563–1576.
- Kataoka, M., Kiyose, Y. M., Michisui, Y., Horiguchi, T., Seki, T. & Yoshida, T. (1994). Complete nucleotide sequence of the *Streptomyces nigrificans* plasmid, pSN22: genetic organization and correlation with genetic properties. *Plasmid* **32**, 55–69.
- Kieser, T., Hopwood, D. A., Wright, H. M. & Thompson, C. J. (1982). pIJ101, a multi-copy broad host-range *Streptomyces* plasmid: functional analysis and development of DNA cloning vectors. *Mol Gen Genet* **185**, 223–238.
- Kieser, T., Bibb, M., Buttner, M. J., Chater, K. F. & Hopwood, D. A. (2000). *Practical Streptomyces Genetics*. Norwich: The John Innes Foundation.
- Kinashi, H., Shimaji, M. & Sakai, A. (1987). Giant linear plasmids in *Streptomyces* which code for antibiotic biosynthesis genes. *Nature* **328**, 454–456.
- Kinashi, H., Shimaji-Murayama, M. & Hanafusa, T. (1992). Integration of SCP1, a giant linear plasmid, into the *Streptomyces coelicolor* chromosome. *Gene* **115**, 35–41.
- Kirby, R. & Chen, C. W. (2011). Genome architecture. In *Streptomyces: Molecular Biology and Biotechnology*, pp. 5–26. Edited by P. Dyson. Norfolk, UK: Caister Academic Press.
- Lai, C.-H. (2010). *Function of the nuclear localization signals of Streptomyces terminal proteins in plants*. MSc thesis, Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University.
- Levene, S. D. & Zimm, B. H. (1987). Separations of open-circular DNA using pulsed-field electrophoresis. *Proc Natl Acad Sci U S A* **84**, 4054–4057.
- Lin, Y.-L. (1998). *Involvement of the terminal sequence of the Streptomyces chromosome in the maintenance of linear plasmids*. MSc thesis, Institute of Genetics, National Yang-Ming University.
- Lin, Y.-S. & Chen, C. W. (1997). Instability of artificially circularized chromosomes of *Streptomyces lividans*. *Mol Microbiol* **26**, 709–719.
- Lin, Y.-S., Kieser, H. M., Hopwood, D. A. & Chen, C. W. (1993). The chromosomal DNA of *Streptomyces lividans* 66 is linear. *Mol Microbiol* **10**, 923–933.
- Liu, H., Naismith, J. H. & Hay, R. T. (2003). Adenovirus DNA replication. *Curr Top Microbiol Immunol* **272**, 131–164.
- Massey, T. H., Mercogliano, C. P., Yates, J., Sherratt, D. J. & Löwe, J. (2006). Double-stranded DNA translocation: structure and mechanism of hexameric FtsK. *Mol Cell* **23**, 457–469.
- Meijer, W. J., Horcajadas, J. A. & Salas, M. (2001). ϕ 29 family of phages. *Microbiol Mol Biol Rev* **65**, 261–287.
- Pease, P. J., Levy, O., Cost, G. J., Gore, J., Ptacin, J. L., Sherratt, D., Bustamante, C. & Cozzarelli, N. R. (2005). Sequence-directed DNA translocation by purified FtsK. *Science* **307**, 586–590.
- Pettis, G. S. & Cohen, S. N. (1994). Transfer of the pIJ101 plasmid in *Streptomyces lividans* requires a *cis*-acting function dispensable for chromosomal gene transfer. *Mol Microbiol* **13**, 955–964.
- Pettis, G. S. & Prakash, S. (1999). Complementation of conjugation functions of *Streptomyces lividans* plasmid pIJ101 by the related *Streptomyces* plasmid pSB24.2. *J Bacteriol* **181**, 4680–4685.
- Possoz, C., Ribard, C., Gagnat, J., Pernodet, J. L. & Guérineau, M. (2001). The integrative element pSAM2 from *Streptomyces*: kinetics and mode of conjugal transfer. *Mol Microbiol* **42**, 159–166.
- Qin, Z. & Cohen, S. N. (1998). Replication at the telomeres of the *Streptomyces* linear plasmid pSLA2. *Mol Microbiol* **28**, 893–903.
- Ray, T., Mills, A. & Dyson, P. (1995). Tris-dependent oxidative DNA strand scission during electrophoresis. *Electrophoresis* **16**, 888–894.
- Redenbach, M., Flett, F., Piendl, W., Glocker, I., Rauland, U., Wafzig, O., Kliem, R., Leblond, P. & Cullum, J. (1993). The *Streptomyces lividans* 66 chromosome contains a 1 Mb deletogenic region flanked by two amplifiable regions. *Mol Gen Genet* **241**, 255–262.
- Reuther, J., Gekeler, C., Tiffert, Y., Wohlleben, W. & Muth, G. (2006a). Unique conjugation mechanism in mycelial streptomycetes: a DNA-binding ATPase translocates unprocessed plasmid DNA at the hyphal tip. *Mol Microbiol* **61**, 436–446.
- Reuther, J., Wohlleben, W. & Muth, G. (2006b). Modular architecture of the conjugative plasmid pSVH1 from *Streptomyces venezuelae*. *Plasmid* **55**, 201–209.
- Sambrook, J., MacCallum, P. & Russell, D. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Servín-González, L. (1996). Identification and properties of a novel *clt* locus in the *Streptomyces phaeochromogenes* plasmid pJV1. *J Bacteriol* **178**, 4323–4326.
- Sobral, B. W. & Atherly, A. G. (1989). Pulse time and agarose concentration affect the electrophoretic mobility of cccDNA during PFGE and FIGE. *Nucleic Acids Res* **17**, 7359–7369.
- Tiffert, Y., Götz, B., Reuther, J., Wohlleben, W. & Muth, G. (2007). Conjugative DNA transfer in *Streptomyces*: SpdB2 involved in the intramycelial spreading of plasmid pSVH1 is an oligomeric integral membrane protein that binds to dsDNA. *Microbiology* **153**, 2976–2983.

- Tinland, B., Koukolíková-Nicola, Z., Hall, M. N. & Hohn, B. (1992). The T-DNA-linked VirD2 protein contains two distinct functional nuclear localization signals. *Proc Natl Acad Sci U S A* **89**, 7442–7446.
- Tsai, J. F.-Y. & Chen, C. W. (1987). Isolation and characterization of *Streptomyces lividans* mutants deficient in intraplasmid recombination. *Mol Gen Genet* **208**, 211–218.
- Tsai, H.-H., Huang, C.-H., Lin, A. M. & Chen, C. W. (2008). Terminal proteins of *Streptomyces* chromosome can target DNA into eukaryotic nuclei. *Nucleic Acids Res* **36**, e62.
- Tsai, H.-H., Huang, C.-H., Tessmer, I., Erie, D. A. & Chen, C. W. (2011). Linear *Streptomyces* plasmids form superhelical circles through interactions between their terminal proteins. *Nucleic Acids Res* **39**, 2165–2174.
- Vivian, A. & Hopwood, D. A. (1973). Genetic control of fertility in *Streptomyces coelicolor* A3(2): new kinds of donor strains. *J Gen Microbiol* **76**, 147–162.
- Vogelmann, J., Ammelburg, M., Finger, C., Guezguez, J., Linke, D., Flötenmeyer, M., Stierhof, Y.-D., Wohlleben, W. & Muth, G. (2011a). Conjugal plasmid transfer in *Streptomyces* resembles bacterial chromosome segregation by FtsK/SpoIIIE. *EMBO J* **30**, 2246–2254.
- Vogelmann, J., Wohlleben, W. & Muth, G. (2011b). *Streptomyces* conjugative genetic elements. In *Streptomyces: Molecular Biology and Biotechnology*, pp. 27–42. Edited by P. Dyson. Norfolk, UK: Caister Academic Press.
- Wang, J. & Pettis, G. S. (2010). The *tra* locus of streptomycete plasmid pIJ101 mediates efficient transfer of a circular but not a linear version of the same replicon. *Microbiology* **156**, 2723–2733.
- Wang, S.-J., Chang, H.-M., Lin, Y.-S., Huang, C.-H. & Chen, C. W. (1999). *Streptomyces* genomes: circular genetic maps from the linear chromosomes. *Microbiology* **145**, 2209–2220.
- Xu, M.-X., Zhu, Y.-M., Shen, M.-J., Jiang, W.-H., Zhao, G.-P. & Qin, Z.-J. (2006). Characterization of the essential gene components for conjugal transfer of *Streptomyces lividans* linear plasmid SLP2. *Progress in Biochemistry and Biophysics* **33**, 986–993.
- Yamasaki, M., Redenbach, M. & Kinashi, H. (2001). Integrated structures of the linear plasmid SCP1 in two bidirectional donor strains of *Streptomyces coelicolor* A3(2). *Mol Gen Genet* **264**, 634–642.
- Yang, C.-C., Huang, C.-H., Li, C.-Y., Tsay, Y.-G., Lee, S.-C. & Chen, C. W. (2002). The terminal proteins of linear *Streptomyces* chromosomes and plasmids: a novel class of replication priming proteins. *Mol Microbiol* **43**, 297–305.
- Zhong, L., Cheng, Q., Tian, X., Zhao, L. & Qin, Z. (2010). Characterization of the replication, transfer, and plasmid/lytic phage cycle of the *Streptomyces* plasmid-phage pZL12. *J Bacteriol* **192**, 3747–3754.
- Zhou, X., Deng, Z., Firmin, J. L., Hopwood, D. A. & Kieser, T. (1988). Site-specific degradation of *Streptomyces lividans* DNA during electrophoresis in buffers contaminated with ferrous iron. *Nucleic Acids Res* **16**, 4341–4352.
- Zhou, X., He, X., Liang, J., Li, A., Xu, T., Kieser, T., Helmann, J. D. & Deng, Z. (2005). A novel DNA modification by sulphur. *Mol Microbiol* **57**, 1428–1438.

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