

## MINI-REVIEW

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**Microbial linear plasmids**

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**Abstract** While plasmids were originally considered to be generally *circular* until almost two decades ago, *linear* elements were reported to exist as well. They are now known to be common genetic elements in both, pro- and eukaryotes. Two types of linear plasmids exist, the so-called hairpin plasmids with covalently closed ends and those with proteins bound to their 5' termini. Hairpin plasmids are common in human-pathogenic *Borrelia* spirochetes, in which they are instrumental in escape from the immunological response; cryptic hairpin elements are present in mitochondria of the plant pathogenic fungus *Rhizoctonia solani*. Plasmids with 5' attached proteins constitute the largest group. In actinomycetous bacteria they are conjugative and usually confer advantageous phenotypes, e.g. formation of antibiotics, degradation of xenobiotics, heavy-metal resistance and growth on hydrogen as the sole energy source. In contrast, the majority of linear plasmids from eukaryotes are cryptic, with only a few exceptions. In some yeasts a killer phenotype may be associated, the most thoroughly investigated elements being those from *Kluyveromyces lactis* killer strains. In *Neurospora* spp. and in *Podospira anserina*, senescence and longevity respectively are correlated with linear plasmids. This review focuses on the biology of linear plasmids, their environmental significance and their use as tools in molecular and applied microbiology.

**Introduction**

During replication, any linear DNA molecule faces a severe problem: in contrast to covalently closed circular DNA it requires specific mechanisms to circumvent progressive shortening during each replication round. In eukaryotes, chromosome shortening is prevented by the ribonucleoprotein enzyme telomerase, which compensates for reduction of the termini by repeatedly adding short sequence motifs (telomeric repeats) to the ends of the chromosomes. It is regulated such that telomeric DNA lengths are kept within defined bounds (McEachern and Blackburn 1995). However, the termini of both pro- and eukaryotic extrachromosomal linear DNA are different from those of telomeres. Microbial extrachromosomal linear DNA elements either carry covalently closed ends (hairpins) or proteins covalently attached to both 5' ends, similar to viral and phage genomes.

Linear plasmids were detected only some 20 years ago, the relatively late discovery being partly due to the fact that they are not regularly present but are associated with specific strains, such as *kalilo* in *Neurospora intermedia* (Bertrand et al. 1985) or pAL2-1 in *Podospira anserina* (Hermanns and Osiewacz 1996). In eukaryotes, most of them are associated with mitochondria and, in addition, the presence of 5' covalently attached proteins resulted in removal of the plasmids along with deproteinization steps routinely applied in isolation procedures. Many giant linear plasmids could only be detected when nucleic acid samples were submitted to pulsed-field gel electrophoresis, which was developed and frequently applied only a decade ago.

**Hairpin plasmids**

*Borrelia* spirochetes – among them the agents causing relapsing fever, *B. hermsii* and Lyme disease, *B. burgdorferi* – have linear chromosomes approximately 1000 kbp in size. In addition, they harbour numerous

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circular and linear plasmids, which can comprise one-third of the total genome. Their linear plasmids as well as their chromosomes have short terminal inverted repeats (TIR) and covalently closed hairpin loops at both termini (Barbour and Garon 1987; Hinnebusch and Barbour 1991). For further details see a review by Girons et al. (1994).

The genes for major surface proteins, namely *ospA* and *ospB* of *B. burgdorferi* and the *vmp* gene family of *B. hermsii* are located on linear plasmids. Two of the linear plasmids of *B. hermsii* each carry a nonexpressed *vmp* gene coding for a variable major surface protein. On an additional plasmid, one copy of these genes is governed by an expression signal. Recombination between the *vmp* genes located on the different plasmids causes antigenic variation, allowing the bacterium to escape the immunological response of its host (Plastkerk et al. 1985). The mitochondria of an isolate of the phytopathogenic fungus *Rhizoctonia solani* were reported to contain short linear plasmids (2.7 kbp) that have hairpin loops and TIR at both ends (Hashiba et al. 1984; Miyashita et al. 1990). It has been postulated that these plasmids might be instrumental in determining avirulence or virulence (Hashiba et al. 1984; Hashiba 1987; Hongo et al. 1994). Meanwhile, the occurrence of small linear extrachromosomal elements, ranging in size from 2.2 kbp to 7.0 kbp, seems common in *R. solani*, because plasmids were found in a great number of randomly chosen isolates (Miyasaka et al. 1990; Jabaji-Hare et al. 1994). The fact that plasmid-containing strains were as virulent as plasmid-less isolates indicated that plasmids do not play a role in pathogenicity (Jabaji-Hare et al. 1994). These findings are supported by extensive investigations, conducted in various laboratories in Canada, Spain and Japan (for details see a review by Rubio et al. 1996). A replication model was proposed for *Rhizoctonia* plasmids by analogy to viral systems, and it involves nicking the DNA at the beginning of the loops (Miyashita et al. 1990). Recent investigations in *Borrelia* led to the suggestion that circular intermediates are involved in replication of linear hairpin plasmids. Circularization occurs presumably by the cutting of opposite strands at identical positions close to the TIR, followed by unfolding and hybridization of the terminal inverted

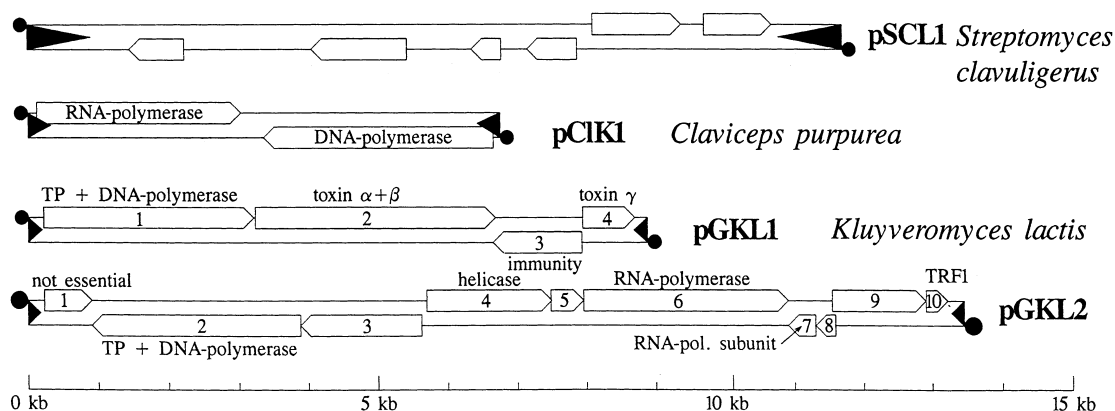
repeats. The same mechanism, acting in reverse, converts the circular molecules back to linear elements (Ferdows et al. 1996).

### Linear plasmids with 5' attached polypeptides

DNA plasmids with proteins covalently linked to their 5' ends constitute the largest group of extrachromosomal linear elements. Along with viruses having structurally similar linear genomes and with transposons they are also known as invertrons (Sakaguchi 1990). Linear plasmids of this kind exist in bacteria, particularly in Actinomycetes; in plants and fungi they are located in organelles, usually in mitochondria (reviewed by Meinhardt et al. 1990; Meinhardt and Rohe 1993; Griffiths 1995). Yeast linear plasmids, however, are located in the cytoplasm (reviewed by Stark et al. 1990; Gunge et al. 1995). Figure 1 shows examples of linear plasmids representing the different types.

Besides linearity and 5' attached proteins, all elements have terminal inverted repeats, varying in size from 44 bp in SLP2 of *Streptomyces lividans* (Chen et al. 1993), 95 kbp in pPZG 101 in *Streptomyces rimosus* (Gravius et al. 1994). Since the structure of linear plasmids is reminiscent of adenoviruses and linear phages, a viral mode of replication via protein priming has repeatedly been suggested (Paillard et al. 1985; Meinhardt et al. 1986; Sakaguchi 1990). Replication is initiated by covalent attachment of a deoxynucleotide monophosphate moiety to a free molecule of terminal protein, thereby providing the -OH group necessary to start polymerization. By forming a complex with the viral DNA polymerase the charged terminal protein binds to the TIR, allowing DNA synthesis to start. Unidirectional 5' to 3' DNA chain elongation results in

**Fig. 1** Schematic representation of a selection of linear plasmids with 5' attached proteins. Arrows open-reading frames; directions correspond to the orientation of the respective gene. Black triangles the terminal inverted repeats; black circles terminal proteins. (References: pSCL1: Wu and Roy 1993, pCIK1: Oeser and Tudzynski 1989, pGKL1: Hishinuma et al. 1984, Stark et al. 1984, pGKL2: Tommasino et al. 1988)



displacement of the parental strand. The displaced strand forms a panhandle-shaped structure due to hybridization of the self-complementary TIR, which then acts as the start site of a new replication round, such as occurs in adenoviruses (Desiderio and Kelly 1981), phage  $\Phi$ 29 (Hermoso and Salas 1980) or phage PRD1 (Bamford et al. 1983). Experimental evidence that replication of linear plasmids is due to a similar mechanism exists for pAI2 of the ascomycetous fungus *Ascobolus immersus*. It could be shown that a radioactive label was preferentially incorporated from the 5' termini (Kempken et al. 1989).

### Prokaryotic elements

For the only linear plasmid so far reported to exist in a gram-negative bacterium (pKO2 of *Klebsiella oxytoca*, Stoppel et al. 1995) it is not known whether proteins are attached. No function is known either, as is also true for elements from the gram-positive *Lactobacillus gasseri* (Roussel et al. 1993) and *Bacillus polymyxa* (Rosado and Seldin 1993).

However, while in eukaryotes most linear plasmids are also cryptic (see below), a phenotype can be attributed to many bacterial elements, particularly to those from Actinomycetes (Table 1). In the nocardioform

genus *Rhodococcus* several large linear plasmids were found that confer advantageous abilities on their hosts. The genus comprises nutritionally versatile soil bacteria, many of which are known to degrade a wide array of xenobiotics, such as phenol, insecticides, acrylamines, anilines, and also halogenated alkanes. *Rhodococcus* sp. (MR11 and MR22), formerly designated *Nocardia opaca*, have the ability to grow autotrophically on hydrogen as the sole energy source. This character, denoted Aut<sup>+</sup>, can be transferred to non-autotrophic strains and a number of other heterotrophic *Rhodococcus* species (Reh and Schlegel 1981). The ability to grow as an aerobic hydrogen bacterium is encoded on a giant linear plasmid, about 270 kbp in size. Recombination between different giant linear conjugative plasmids led to the formation of a new linear genetic element with tremendously enhanced transfer frequency (up to 1000-fold compared to the original situation; Kalkus et al. 1990, 1993).

Other members of the genus, e.g. *Rhodococcus erythropolis* BD2 have the ability to utilize isopropylbenzene as the sole carbon and energy source. The latter strain was shown to contain a giant linear plasmid (approx. 210 kbp); loss of the plasmid was accompanied by the loss of isopropylbenzene and trichlorethene degradation. In addition, resistance to arsenite and also mercury was shown to be plasmid-encoded in this

**Table 1** Selection of microbial linear plasmids with 5' attached proteins

Organism	Plasmid	Size (kbp)	Phenotype attributed	Reference
<b>Bacteria</b>				
<i>Rhodococcus</i> sp. (formerly <i>Nocardia opaca</i> )	pHG201	270	Autotrophy	Kalkus et al. 1990, 1993
	pHG204	180	Thallium resistance	
	pHG205	280	Autotrophy	
<i>Rhodococcus erythropolis</i>	pBD2	210	Isopropylbenzene and trichlorethene catabolism, arsenite and mercury resistance	Dabrock et al. 1994; Kessler et al. 1996
	pFiD188	200	Induction of fasciation	Crespi et al. 1992; 1994
<i>Streptomyces clavuligerus</i>	pSCL1	11.7	Cryptic	Wu and Roy 1993
<i>Streptomyces coelicolor</i>	SCP1	350	Methylenomycin synthesis	Kinashi et al. 1987, 1993
<i>Streptomyces fradiae</i>		420	Tylosin synthesis	Kinashi and Shimaji 1987
<i>Streptomyces lasaliensis</i>	pKSL	520	Lasalocid A synthesis	Kinashi et al. 1987
<i>Streptomyces parvulus</i>		520	Actinomycin D synthesis	Kinashi and Shimaji 1987
<i>Streptomyces rimosus</i>	pPZG101	387	Cryptic	Gravius et al. 1994
<i>Streptomyces venezuelae</i>		130	Chloramphenicol synthesis	Kinashi and Shimaji 1987
<b>Yeasts</b>				
<i>Debaromyces hansenii</i>	pDHL1	8.4	Associated with osmotolerance	Gunge et al. 1993
	pDHL2	9.2		
	pDHL3	15.0		
<i>Kluyveromyces lactis</i>	pGKL1	8.9	Killer	Gunge et al. 1981
	pGKL2	13.5		
<i>Pichia acaciae</i>	pPac1-1	13.6	Killer	Worsham and Bolen 1990
	pPac1-2	7.3		
<i>Pichia inositovora</i>	pPin11	18	Killer	Ligon et al. 1989
	pPin12	13		
	pPin13	10		
<i>Saccharomyces kluyveri</i>	pSKL	14.2	Cryptic	Hishinuma and Hirai 1991
<b>Filamentous fungi</b>				
<i>Claviceps purpurea</i>	pCLK1	6.8	Cryptic	Oeser and Tudzynski 1989
<i>Neurospora crassa</i>	Maranhar	7.1	Senescence	Court et al. 1991
<i>Neurospora intermedia</i>	Kalilo	9.0	Senescence	Bertrand et al. 1985, 1986
<i>Podospora anserina</i>	pAL2-1	8.4	Longevity	Hermanns and Osiewicz 1996

bacterium (Dabrock et al. 1994). Conjugational transfer to a cured strain restored the resistance as well as the degradation abilities.

*R. fascians* is a phytopathogenic representative of the genus, which induces fasciation, i.e. leafy galls, on dicots and several monocots. Strain D188 was reported to contain a conjugative, 200-kbp linear plasmid. Cured strains were avirulent, but virulence was restored by reintroduction of the linear 200-kbp plasmid by conjugation. It was proven that at least three virulence-requiring regions (*fas*, fasciation; *att*, attenuation; *hyp*, hypovirulence) are located on the plasmid (Crespi et al. 1992, 1994). The fact that all virulent strains of *R. fascians*, so far investigated, contained similar linear genetic elements may indicate that the conjugative ability of linear plasmids facilitates their dissemination amongst the genus and other related actinomycetes.

Linear plasmids of the gram-positive genus *Streptomyces* range in size from approximately 12 kbp to several hundred kbp (see Table 1). The replication mode of *Streptomyces* linear replicons was shown to be different from the strand-displacing mechanism outlined above for the adenoviral and phage systems. DNA synthesis of pSLA2, a 17-kbp linear plasmid of *Streptomyces rochei* occurs by bidirectional replication extending outward from a centrally located origin toward the ends of the plasmids (Chang and Cohen 1994). Since the chromosomes of several *Streptomyces* spp. are also linear with proteins and highly conserved inverted repeats at their termini (Lin et al. 1993), a similar replication mode was suggested. Support for this assumption came from experiments in which the linear *S. lividans* chromosome was circularized by joining both ends either by artificial targeted recombination or by spontaneous deletion spanning both inverted repeats (Lin et al. 1993).

Large plasmids, commonly over 50 kbp in size, are often referred to as giant linear plasmids; some of these have known phenotypes, and they particularly carry antibiotic biosynthesis genes (see Table 1). Irrespective of whether actinomycete linear plasmids contribute to the formation of antibiotics or not, all linear plasmids detected so far are conjugative (Hopwood and Kieser 1993). In addition, *Streptomyces* conjugative plasmids (linear and circular) are associated with pocks, macroscopically visible, circular areas of retarded growth that develop around colonies growing from individual plasmid-carrying spores seeded in a lawn of plasmid-free spores.

Conjugation provides bacteria with the ability for horizontal gene transfer, which is in most cases limited to one species or at least to closely related organisms. Among Actinomycetes, i.e. *Rhodococcus* and *Streptomyces*, there are several lines of evidence that conjugative transfer of DNA might cross the species barrier. Plasmid pBD2 of *Rhodococcus erythropolis* carries genes for isopropylbenzene and 3-isopropylcatechol dioxygenases, which exhibit a 55%–78% identity to analogous enzymes from gram-negative bacteria (Dabrock et al. 1994; Kessler et al. 1996). The corresponding

enzymes of the gram-negative bacteria all contain the same type of redox components and are functionally and evolutionarily related (Mason and Cammack 1992). Mating is possible between different *Streptomyces* species. Transfer of DNA was observed in combinations of *S. bambergiensis* and *S. lividans*, the latter contained a 42-kbp linear conjugative plasmid that is derived from a giant linear 640 kbp plasmid (pSB1) of the former (Zotchev et al. 1992). The linear plasmid SLP2 could be transferred from *S. lividans* to *S. coelicolor* and *S. parvulus* and vice versa. Consistent with these results is the fact that the inverted terminal repeats of many *Streptomyces* linear plasmids are almost identical (Chen et al. 1993). It is not yet clear whether plasmid-mediated conjugation is as promiscuous as that brought about by conjugative transposons of the gram-positive bacteria (Scott and Churchward 1995); however, actinomycetous soil bacteria may have the ability to share – via conjugation – useful genetic material, such as heavy-metal resistances, production of and resistance to antibiotics, degradation of xenobiotics, and possibly the ability to exist as a plant pathogen.

#### Linear plasmids of filamentous fungi

The number of eukaryotic linear plasmids with 5' attached proteins identified in various species is continuously growing, and they have been the subject of many reviews (e.g. Meinhardt et al. 1990; Meinhardt and Rohe 1993; Schründer and Meinhardt 1995a; Griffiths 1995; Kempken 1995a), to which we refer for further information. Table 1 lists a selection of elements, known to have associated phenotypes; upon request a complete list of linear plasmids reported so far is available.

In spite of the DNA sequence data available for many of them, very little is known about specific gene functions. In particular, most mitochondrial plasmids of filamentous fungi appear to be cryptic in function, thus representing selfish DNA. In general, linear plasmids of filamentous fungi and also plants appear to be simple and uniform in structure. A typical mitochondrial linear plasmid (pCIK1 of *Claviceps purpurea*) is shown in Fig. 1. It only encodes viral-like DNA and RNA polymerases. Mitochondrial linear plasmids shown to affect their hosts, i.e. *kalilo*, *maranhar* and pAL2-1, are the exceptions of this rule. *Kalilo* and *maranhar* are involved in the control of senescence in certain strains of the genus *Neurospora*. It has been shown that senescence was induced by integration of a copy of the plasmids into the mitochondrial DNA and by subsequent accumulation of the defective mitochondrial genome (for details see Griffiths 1995). In contrast to *Neurospora*, longevity is induced by the integration of plasmid pAL2-1 into the mitochondrial genome of *Podospora anserina* (Hermanns and Osiewacz 1992; Hermanns et al. 1994). Transformation and other genetic experiments using such elements are largely hampered by their mitochondrial localization, and that is why data are rather scarce.

Nevertheless, experimental evidence exists, that mitochondrial linear plasmids can be transferred between *Claviceps* strains by protoplast fusion (Gessner-Ulrich and Tudzynski 1994) and even the horizontal transfer of a linear plasmid from *Ascobolus immersus* to *Podospora anserina* has recently been reported (Kempken 1995b). However, stable propagation in the new host was not possible, leading to gradual plasmid loss. In compatible pairings of *Neurospora* strains, plasmids can readily spread out (Debets et al. 1994). Since there is a plasmid that is closely related to the *kalilo* DNA in *Gelasinospora*, this might be indicative that even intergeneric distribution of such elements is possible (Wei et al. 1996). Thus, although evolutionary calculations suggest that these elements are descended from a common ancestor and coevolution with their hosts (Rohe et al. 1992), horizontal transfer of mitochondrially inherited linear plasmids cannot be excluded a priori (Kempken et al. 1992).

#### Yeast linear plasmids

A recent survey of plasmids among 1800 yeast strains covering about 600 species revealed that linear DNA plasmids can be found at a frequency of about 1%–2% (Fukuhara 1995). Linear plasmids of yeasts differ from all other eukaryotic plasmids in some aspects. They are located in the cytoplasm and encode additional proteins other than viral-like DNA and RNA polymerases; furthermore, some confer an advantageous phenotype on their hosts (see Table 1).

The best-characterized yeast system is the killer plasmid pair pGKL1 and -2 of the dairy yeast *Kluyveromyces lactis* (see Fig. 1 for the structure). Originally detected by Gunge et al. (1981), they were also described by Wesolowski et al. (1982a,b,c) and termed k1 and k2 respectively. The killer system has been the subject of a number of reviews to which we refer for further citations of the original literature (Stark et al. 1990; Schründer and Meinhardt 1995a; Gunge 1995).

Killer plasmid pGKL1 gene functions are mainly understood. Open-reading frame ORF1 codes for the plasmid-specific DNA polymerase, ORF2 and ORF4 specify subunits  $\alpha$ ,  $\beta$ ,  $\gamma$  of the heterotrimeric killer toxin and ORF3 is essential for toxin immunity. The toxin causes an irreversible arrest of sensitive yeast cells in the unbudded (G1) phase of the cell cycle (Sugisaki et al. 1983). Toxicity exclusively resides within the  $\gamma$  subunit since intracellular ORF4 expression mimicked the effect of exogenously applied native toxin (Tokunaga et al. 1990; Butler et al. 1991a). The  $\alpha$  subunit shows chitinase activity, inhibition of which by allosamidin in vivo abolishes activity of the holotoxin (Butler et al. 1991b). Since the  $\beta$  subunit is remarkably hydrophobic, both  $\alpha$  and  $\beta$  subunits are considered to be instrumental in binding and/or uptake of the  $\gamma$  subunit.

pGKL2 appears to play a fundamental role in the killer system by providing essential functions for gene

expression and maintenance of both plasmids within the same cell. pGKL1 is strictly dependent on pGKL2, which carries ten genes: ORF1 is not essential for killer plasmid replication and maintenance (Schaffrath et al. 1992); ORF2 encodes the plasmid-specific DNA polymerase as well as the terminal protein of the plasmid (Schaffrath et al. 1995b; Takeda et al. 1996); no function is known for ORF3; ORF4 exhibits similarities to a viral helicase (Stark et al. 1990); ORF5 has been shown to be essential and functionally interchangeable between both plasmids and is presumably involved in plasmid stability (Schaffrath and Meacock 1995, 1996); ORF6 encodes the plasmid-specific RNA polymerase, considered to be instrumental in transcription of both plasmids (Schaffrath et al. 1995a,b); ORF7 might encode a subunit of the latter (Schaffrath et al. 1997); for ORF8 and ORF9 functions are not known; ORF10 encodes TRF1, a terminal recognition factor, likely to be involved in plasmid replication initiation (Tommasino 1991; McNeel and Tamanoi 1991).

When artificially transferred to different yeast species, such as *Saccharomyces cerevisiae*, *Kluyveromyces fragilis* and *Candida pseudotropicalis*, the killer plasmids are stably maintained and confer the killer phenotype on their new hosts (Gunge and Sakaguchi 1981; Gunge et al. 1982; Sugisaki et al. 1985; Tokunaga et al. 1990). However, in *S. cerevisiae* the plasmids are only stable in haploid  $\rho^-$  strains (Gunge and Yamane 1984; Gunge et al. 1990).

The killer plasmids have all the parameters needed for yeast vectors: broad host range, a high copy number of 50–100/cell, ensuring a high gene dosis, extreme mitotic stability without selective pressure (Kämper et al. 1991), and cytoplasmic localization making them independent from nuclear control in terms of replication and transcription.

First attempts to manipulate linear plasmids pGKL1 and pGKL2 genetically in vitro involved nuclear *Saccharomyces cerevisiae* genes as selectable traits and resulted exclusively in the formation of circular vectors replicating in the nucleus (de Louvencourt et al. 1983; Fujimura et al. 1987). In vivo experiments, aimed at integration of the nuclear *LEU2* gene into the killer toxin gene, also resulted in nucleus associated hybrid plasmids (Kämper et al. 1989a,b, 1991). Cytoplasmic linear hybrid plasmids could, however, be obtained by fusing the 5' non-coding region of ORF2 (killer toxin gene) to selectable marker genes [*LEU2*, Kämper et al. 1989b; *HIS3*, Gallo and Galeotti 1990; G418 resistance (*aph*), Tanguy-Rougeau et al. 1990]. There is at least one non-essential locus on each plasmid that is dispensable and not necessary for plasmid maintenance, i.e. the killer toxin ORF2 of the smaller plasmid pGKL1 and ORF1 of the larger plasmid pGKL2 (Schaffrath et al. 1992). Thus, these loci represent potential target sites for integration of foreign DNA.

DNA sequence and transcript analysis revealed that all 14 plasmid genes are transcribed independently (Romanos and Boyd 1988; Tommasino et al. 1988) and

preceded by a cytoplasmic promoter, also known as the upstream conserved sequence (Stark et al. 1990). Transcription in general appears to be rather weak, as judged from an assay based on the bacterial glucose dehydrogenase reporter gene (Schründer and Meinhardt 1995b). Applying this reporter system made it evident that the level of expression can vary upto 12-fold depending on the upstream conserved sequence motif used. Sequences further upstream do not influence expression, whereas deletion of the upstream conserved sequence motifs lead to complete loss of expression (Schaffrath et al. 1996; Schickel et al. 1996). Since linear plasmids presumably have evolved from a postulated viral ancestor (Meinhardt et al. 1990; Rohe et al. 1992; Kempken et al. 1992) this balanced low level of expression evidently ensures both stable plasmid propagation and viability of the host cell.

Several heterologous genes, including biotechnologically relevant enzymes (Schründer et al. 1996), have been expressed using killer plasmids as vectors (Kämper et al. 1991; Gallo and Galeotti 1990; Tanguy-Rougeau et al. 1990; Meinhardt et al. 1994; Schaffrath et al. 1995b). Expression, in general, occurred at a low level, thus the main application of linear yeast plasmids remains, for the present, in the area of basic research. For biotechnological exploitation it is necessary to increase efficiency of expression, e.g. by site-directed mutagenesis and promoter optimization. As exemplified by the *K. lactis* killer plasmids (pGKL1, pGKL2) and plasmid pSKL of *Saccharomyces kluyveri* (Hishinuma and Hirai 1991) these elements constitute a closely related group (Rohe et al. 1992). Further investigations on the *K. lactis* killer plasmids should thus be highly encouraged, as this will contribute to our understanding of these widely distributed genetic traits.

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## References

- Bamford DH, McGraw T, Mackenzie G, Mindich L (1983) Identification of a protein covalently bound to the termini of bacteriophage PRD1 DNA. *J Gen Virol* 47: 311–316
- Barbour AG, Garon CF (1987) Linear plasmids of the bacterium *Borrelia burgdorferi* have covalently closed ends. *Science* 237: 409–411
- Bertrand H, Chan BSS, Griffiths AJF (1985) Insertion of a foreign nucleotide sequence into mitochondrial DNA causes senescence in *Neurospora intermedia*. *Cell* 41: 877–884
- Bertrand H, Griffiths AJF, Court DA, Cheng CK (1986) An extrachromosomal plasmid is the etiological precursor of kal-DNA insertion sequences in the mitochondrial chromosome of senescent *Neurospora*. *Cell* 47: 829–837
- Butler AR, Porter M, Stark MJR (1991a) Intracellular expression of *Kluyveromyces lactis* toxin  $\gamma$ -subunit mimics treatment with exogenous toxin and distinguishes two classes of toxin-resistant mutants. *Yeast* 7: 617–625
- Butler AR, O'Donnell RW, Martin VJ, Gooday GW, Stark MJR (1991b) *Kluyveromyces lactis* toxin has an essential chitinase activity. *Eur J Biochem* 199: 483–488
- Chang PC, Cohen SN (1994) Bidirectional replication from an internal origin in a linear *Streptomyces* plasmid. *Science* 265: 952–954
- Chen CW, Yu TW, Lin YS, Kieser HM, Hopwood DA (1993) The conjugative plasmid SLP2 of *Streptomyces lividans* is a 50 Kbp linear molecule. *Mol Microbiol* 7: 925–932
- Court DA, Griffiths AJ, Kraus SR, Russell PJ, Bertrand H (1991) A new senescence-inducing mitochondrial linear plasmid in field-isolated *Neurospora crassa* strains from India. *Curr Genet* 19: 129–137
- Crespi M, Messens E, Caplan AB, van-Montagu M, Desomer J (1992) Fasciation induction by the phytopathogen *Rhodococcus fascians* depends upon a linear plasmid encoding a cytokinin synthase gene. *EMBO-J* 11: 795–804
- Crespi M, Vereecke D, Temmerman W, van-Montagu M, Desomer J (1992) The *fas* operon of *Rhodococcus fascians* encodes new genes required for efficient fasciation of host plants. *J Bacteriol* 176: 2492–2501
- Dabrock B, Kessler M, Averhoff B, Gottschalk G (1994) Identification and characterization of a transmissible linear plasmid from *Rhodococcus erythropolis* BD2 that encodes isopropylbenzene and trichloroethene catabolism. *Appl Environ Microbiol* 60: 853–860
- Debets F, Yang X, Griffiths AJF (1994) Vegetative incompatibility in *Neurospora*: its effect on horizontal transfer of mitochondrial plasmids and senescence in natural populations. *Curr Genet* 26: 113–119
- Desiderio SV, Kelly TJ Jr (1981) Structure of the linkage between adenovirus DNA and the 55,000 molecular weight protein. *J Mol Biol* 145: 319–337
- Ferdows MS, Serwer P, Griess GA, Norris SJ, Barbour AG (1996) Conversion of a linear to a circular plasmid in the relapsing fever agent *Borrelia hermsii*. *J Bacteriol* 178: 793–800
- Fujimura H, Hishinuma F, Gunge N (1987) Terminal segment of *Kluyveromyces lactis* linear DNA plasmid pGKL2 supports autonomous replication of hybrid plasmids in *Saccharomyces cerevisiae*. *Curr Genet* 12: 99–104
- Fukuhara H (1995) Linear DNA plasmids of yeasts. *FEMS Microbiol Lett* 131: 1–9
- Gallo E, Galeotti CL (1990) Transplacement of a nuclear gene into the cytoplasmic killer plasmids of *Kluyveromyces lactis*. In: Abstracts of the 15th International Conference on Yeast Genetics and Molecular Biology. Wiley, London New York, p 547
- Gessner-Ulrich K, Tudzynski P (1994) Studies on function and mobility of mitochondrial plasmids from *Claviceps purpurea*. *Mycol Res* 98: 511–515
- Girons IS, Old IG, Davidson BE (1994) Molecular biology of the *Borrelia*, bacteria with linear replicons. *Microbiology (Reading)* 140: 1803–1816
- Gravius B, Glocker D, Pigac J, Pandza K, Hranueli D, Cullum J (1994) The 387 kbp linear plasmid pPZG101 of *Streptomyces rimosus* and its interactions with the chromosome. *Microbiology (Reading)* 140: 2271–2277
- Griffiths AJF (1995) Natural plasmids of filamentous fungi. *Microbiol Rev* 59: 673–685
- Gunge N (1995) Plasmid DNA and the killer phenomenon in *Kluyveromyces*. In: Kück U (ed) *The mycota. II. Genetics and biotechnology*. Springer, Berlin Heidelberg New York, pp 189–209
- Gunge N, Sakaguchi K (1981) Intergeneric transfer of deoxyribonucleic acid killer plasmids, pGKL1 and pGKL2, from *Kluyveromyces lactis* into *Saccharomyces cerevisiae* by cell fusion. *J Bacteriol* 147: 155–160
- Gunge N, Yamane C (1984) Incompatibility of linear DNA killer plasmids pGKL1 and pGKL2 from *Kluyveromyces lactis* with mitochondrial DNA from *Saccharomyces cerevisiae*. *J Bacteriol* 159: 533–539
- Gunge N, Tamaru A, Ozawa F, Sakaguchi K (1981) Isolation and characterization of linear deoxyribonucleic acid plasmids from

- Kluyveromyces lactis* and the plasmid-associated killer character. *J Bacteriol* 145: 382–390
- Gunge N, Murata K, Sakaguchi K (1982) Transformation of *Saccharomyces cerevisiae* with linear DNA killer plasmids from *Kluyveromyces lactis*. *J Bacteriol* 151: 462–464
- Gunge N, Murakami K, Takesako T, Moriyama H (1990) Mating type locus-dependent stability of the *Kluyveromyces* linear pGKL plasmids in *Saccharomyces cerevisiae*. *Yeast* 6: 417–427
- Gunge N, Fukuda K, Morikawa S, Murakami K, Takeda M, Miwa A (1993) Osmophilic linear plasmids from the salt-tolerant yeast *Debaryomyces hansenii*. *Curr Genet* 23: 443–449
- Gunge N, Fukuda K, Takahashi S, Meinhardt F (1995) Migration of the yeast linear DNA plasmid from the cytoplasm into the nucleus in *Saccharomyces cerevisiae*. *Curr Genet* 28: 280–288
- Hashiba T (1987) An improved system for biological control of damping-off by using plasmids in fungi. In: Chet I (ed) *Innovative approaches to plant disease control*. Wiley-Interscience, New York, pp 337–351
- Hashiba T, Homma Y, Hyakumachi M, Matsuda I (1984) Isolation of a DNA plasmid in the fungus *Rhizoctonia solani*. *J Gen Microbiol* 130: 2067–2070
- Hermanns J, Osiewacz HD (1992) The linear mitochondrial plasmid pAL2-1 of a longlived *Podospora anserina* mutant is an invertron encoding a DNA and RNA polymerase. *Curr Genet* 22: 491–500
- Hermanns J, Osiewacz HD (1996) Induction of longevity by cytoplasmic transfer of a linear plasmid in *Podospora anserina*. *Curr Genet* 29: 250–256
- Hermanns J, Asseburg A, Osiewacz HD (1994) Evidence for a life span-prolonging effect of a linear plasmid in a longevity mutant of *Podospora anserina*. *Mol Gen Genet* 243: 297–307
- Hermoso JM, Salas M (1980) Protein p3 is linked to the DNA of phage phi 29 through a phosphoester bond between serine and 5'-dAMP. *Proc Natl Acad Sci USA* 77: 6425–6428
- Hinnebusch J, Barbour AG (1991) Linear plasmids of *Borrelia burgdorferi* have a telomeric structure similar to those of a eukaryotic virus. *J Bacteriol* 173: 7233–7239
- Hishinuma F, Hirai K (1991) Genome organization of the linear plasmid, pSKL, isolated from *Saccharomyces kluyveri*. *Mol Gen Genet* 226: 97–106
- Hishinuma F, Nakamura K, Hirai K, Nishizawa R, Gunge N, Maeda T (1984) Cloning and nucleotide sequences of the linear DNA killer plasmids from yeast. *Nucleic Acids Res* 12: 7581–7597
- Hongo M, Miyasaka A, Suzuki F, Hashiba T (1994) Expression of the linear DNA plasmid pRS64 in the plant pathogenic fungus *Rhizoctonia solani*. *Mol Gen Genet* 245: 265–271
- Hopwood DA, Kieser T (1993) Conjugative plasmids in *Streptomyces*. In: Clewell DB (ed) *Bacterial conjugation*. Plenum, New York, pp 293–311
- Jabaji-Hare SH, Burger G, Forget L, Lang BF (1994) Extrachromosomal plasmids in the plant pathogenic fungus *Rhizoctonia solani*. *Curr Genet* 25: 423–431
- Kalkus J, Reh M, Schlegel HG (1990) Hydrogen autotrophy of *Nocardia opaca* strains is encoded by linear megaplasmids. *J Gen Microbiol* 136: 1145–1151
- Kalkus J, Dorrie C, Fischer D, Reh M, Schlegel HG (1993) The giant linear plasmid pHG207 from *Rhodococcus* sp. encoding hydrogen autotrophy: characterization of the plasmid and its termini. *J Gen Microbiol* 139: 2055–2065
- Kämper J, Meinhardt F, Gunge N, Esser K (1989a) New recombinant linear DNA-elements derived from *Kluyveromyces lactis* killer plasmids. *Nucleic Acids Res* 17: 1781
- Kämper J, Meinhardt F, Gunge N, Esser K (1989b) *In vivo* construction of linear vectors based on killer plasmids from *Kluyveromyces lactis*: selection of a nuclear gene results in attachment of telomeres. *Mol Cell Biol* 9: 3931–3937
- Kämper J, Esser K, Gunge N, Meinhardt F (1991) Heterologous gene expression on the linear DNA killer plasmid from *Kluyveromyces lactis*. *Curr Genet* 19: 109–118
- Keen CL, Mendelovitz S, Cohen G, Aharonowitz Y, Roy KL (1988) Isolation and characterization of a linear DNA plasmid from *Streptomyces clavuligerus*. *Mol Gen Genet* 212: 172–176
- Kempken F (1995a) Plasmids in mycelial fungi. In: Kück U (ed) *The mycota. II. Genetics and biotechnology*. Springer, Berlin Heidelberg New York, pp 169–187
- Kempken F (1995b) Horizontal transfer of a mitochondrial plasmid. *Mol Gen Genet* 248: 89–94
- Kempken F, Meinhardt F, Esser K (1989) *In organello* replication and viral affinity of linear, extrachromosomal DNA of the ascomycete *Ascobolus immersus*. *Mol Gen Genet* 218: 523–530
- Kempken F, Hermanns J, Osiewacz HD (1992) Evolution of linear plasmids. *J Mol Evol* 35: 502–513
- Kessler M, Dabbs ER, Averhoff B, Gottschalk G (1996) Studies on the isopropylbenzene 2,3-dioxygenase and the 3-isopropylcatechol 2,3-dioxygenase genes encoded by the linear plasmid of *Rhodococcus erythropolis* BD2. *Microbiology* 142: 3241–3251
- Kinashi H, Shimaji M (1987) Detection of giant linear plasmids in antibiotic producing strains of *Streptomyces* by the OFAGE technique. *J Antibiot (Tokyo)* 40: 913–916
- Kinashi H, Shimaji M, Sakai A (1987) Giant linear plasmids in *Streptomyces* which code for antibiotic biosynthesis genes. *Nature* 328: 454–456
- Kinashi H, Murayama M, Matsushita H, Nimi O (1993) Structural analysis of the giant linear plasmid SCP1 in various *Streptomyces coelicolor* strains. *J Gen Microbiol* 139: 1261–1269
- Ligon JM, Bolen PL, Hill DS, Bothast RJ, Kurtzman CP (1989) Physical and biological characterization of linear DNA plasmids of the yeast *Pichia inositovora*. *Plasmid* 21: 185–194
- Lin YS, Kieser HM, Hopwood DA, Chen CW (1993) The chromosomal DNA of *Streptomyces lividans* 66 is linear. *Mol Microbiol* 10: 923–933
- Louvencourt L de, Fukuhara H, Heslot H, Wesolowski M (1983) Transformation of *Kluyveromyces lactis* by killer plasmid DNA. *J Bacteriol* 154: 737–742
- Mason JR, Cammack R (1992) The electron-transport proteins of hydroxylating bacterial dioxygenases. *Annu Rev Microbiol* 46: 277–305
- McEachern MJ, Blackburn EH (1995) Runaway telomere elongation caused by telomerase RNA gene mutations. *Nature* 376: 403–408
- McNeel DG, Tamanoi F (1991) Terminal region recognition factor 1, a DNA-binding protein recognizing the inverted terminal repeats of the pGK1 linear DNA plasmids. *Proc Natl Acad Sci USA* 88: 11398–11402
- Meinhardt F, Rohe M (1993) Extranuclear inheritance: Linear protein-primed replicating genomes in plants and microorganisms. *Prog Bot* 54: 334–357
- Meinhardt F, Kempken F, Esser K (1986) Proteins are attached to the ends of a linear plasmid in the filamentous fungus *Ascobolus immersus*. *Curr Genet* 11: 243–246
- Meinhardt F, Kempken F, Kämper J, Esser K (1990) Linear plasmids among eukaryotes: fundamentals and applications. *Curr Genet* 17: 89–95
- Meinhardt F, Wodara C, Larsen M, Schickel J (1994) A novel approach to express a heterologous gene on *Kluyveromyces lactis* linear killer plasmids: expression of the bacterial *aph* gene from a cytoplasmic promoter fragment without in-phase fusion to the plasmid open reading frame. *Plasmid* 32: 318–327
- Miyasaka A, Chen CL, Hashiba T (1990) Detection and properties of plasmid-like DNA in isolates from nine anastomosis and intraspecific groups of *Rhizoctonia solani*. *J Gen Microbiol* 136: 1791–1798
- Miyashita S, Hirochika H, Ikeda JE, Hashiba T (1990) Linear plasmid DNAs of the plant pathogenic fungus *Rhizoctonia solani* with unique terminal structures. *Mol Gen Genet* 220: 165–171
- Oeser B, K, Tudzynski P (1989) The Zhe linear mitochondrial plasmid pCLK1 of the phytopathogenic fungus *Claviceps purpurea* may code for a DNA polymerase and an RNA polymerase. *Mol Gen Genet* 217: 132–140

- Paillard M, Sederoff RR, Levings III CS (1985) Nucleotide sequence of the S1 mitochondrial DNA from the S-cytoplasm of maize. *EMBO-J* 4: 1125–1128
- Plasterk RH, Simon MI, Barbour AG (1985) Transposition of structural genes to an expression sequence on a linear plasmid causes antigenic variation in the bacterium *Borrelia hermsii*. *Nature* 318: 257–263
- Reh M, Schlegel HG (1981) Hydrogen autotrophy as a transferable genetic character of *Nocardia opaca* 1b. *J Gen Microbiol* 126: 327–336
- Rohe M, Schründer J, Tudzynski P, Meinhardt F (1992) Phylogenetic relationships of linear, protein-primed replicating genomes. *Curr Genet* 21: 173–176
- Romanos MA, Boyd A (1988) A transcriptional barrier to expression of cloned toxin genes of the linear plasmid k1 of *Kluyveromyces lactis*: evidence that native k1 has novel promoters. *Nucleic Acids Res* 16: 7333–7350
- Rosado A, Seldin L (1993) Isolation and partial characterization of a new linear DNA plasmid isolated from *Bacillus polymyxa* SCE2. *J Gen Microbiol* 139: 1277–1282
- Roussel Y, Colmin C, Simonet JM, Decaris B (1993) Strain characterization, genome size and plasmid content in the *Lactobacillus acidophilus* group (Hansen and Mocquot). *J Appl Bacteriol* 74: 549–556
- Rubio V, Tavantzis SM, Lakshman DK (1996) Extrachromosomal elements and degree of pathogenicity in *Rhizoctonia solani*. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds) *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and control. Kluwer, Dordrecht, pp 127–138
- Sakaguchi K (1990) 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
- Schaffrath R, Meacock PA (1995) *Kluyveromyces lactis* killer plasmid pGKL2: Molecular analysis of an essential gene, ORF5. *Yeast* 11: 615–628
- Schaffrath R, Meacock PA (1996) A cytoplasmic gene shuffle system in *Kluyveromyces lactis*: use of epitope-tagging to detect a killer plasmid encoded gene product. *Mol Microbiol* 19: 545–554
- Schaffrath R, Stark MJR, Gunge N, Meinhardt F (1992) *Kluyveromyces lactis* killer system: ORF1 of pGKL2 has no function in immunity expression and is dispensable for killer plasmid replication and maintenance. *Curr Genet* 21: 357–363
- Schaffrath R, Soond SM, Meacock PA (1995a) Cytoplasmic gene expression in yeast: A plasmid-encoded transcription system in *Kluyveromyces lactis*. *Biochem Soc Trans* 23: 128
- Schaffrath R, Soond SM, Meacock PA (1995b) The DNA and RNA polymerase genes of yeast plasmid pGKL2 are essential loci for plasmid integrity and maintenance. *Microbiology (Reading)* 141: 2591–2599
- Schaffrath R, Meinhardt F, Meacock PA (1996) Yeast killer plasmid pGKL2: molecular analysis of UCS5, a cytoplasmic promoter element essential for ORF5 gene function. *Mol Gen Genet* 250: 286–294
- Schaffrath R, Meinhardt F, Meacock PA (1997) ORF7 of yeast plasmid pGKL2: Analysis of gene expression *in vivo*. *Curr Genet* (in press)
- Schickel J, Helmig C, Meinhardt F (1996) *Kluyveromyces lactis* killer system: analysis of cytoplasmic promoters of the linear plasmids. *Nucleic Acids Res* 24: 1879–1886
- Schründer J, Meinhardt F (1995a) Yeast linear killer plasmids as a tool in genetic engineering. *Prog Bot* 56: 332–353
- Schründer J, Meinhardt F (1995b) An extranuclear expression system for analysis of cytoplasmic promoters of yeast linear killer plasmids. *Plasmid* 33: 139–151
- Schründer J, Gunge N, Meinhardt F (1996) Extranuclear expression of the bacterial xylose-isomerase (*xyIA*) and the UDP-glucose-dehydrogenase (*hasB*) genes in yeast using *Kluyveromyces lactis* linear killer plasmids as vectors. *Curr Microbiol* 33: 323–330
- Scott JR, Churchward GC (1995) Conjugative transposition. *Annu Rev Microbiol* 49: 367–397
- Stark MJR, Mileham AJ, Romanos MA, Boyd A (1984) Nucleotide sequence and transcription analysis of a linear DNA plasmid associated with the killer character of the yeast *Kluyveromyces lactis*. *Nucleic Acids Res* 12: 6011–6030
- Stark MJR, Boyd A, Mileham AJ, Romanos MA (1990) The plasmid-encoded killer system of *Kluyveromyces lactis*. *Yeast* 6: 1–29
- Stoppel RD, Meyer M, Schlegel HG (1995) The nickel resistance determinant cloned from the enterobacterium *Klebsiella oxytoca*: conjugational transfer, expression, regulation and DNA homologies to various nickel-resistant bacteria. *Biometals* 8: 70–79
- Sugisaki Y, Gunge N, Sakaguchi K, Yamasaki M, Tamura G (1983) *Kluyveromyces lactis* killer toxin inhibits adenylate cyclase of sensitive yeast cells. *Nature* 304: 464–466
- Sugisaki Y, Gunge N, Sakaguchi K, Yamasaki M, Tamura G (1985) Transfer of DNA killer plasmids from *Kluyveromyces lactis* to *Kluyveromyces fragilis* and *Candida pseudotropicalis*. *J Bacteriol* 164: 1373–1375
- Takeda M, Hiraishi H, Takesako T, Tanase S, Gunge N (1996) The terminal protein of the linear plasmid pGKL2 shares an N-terminal domain of the plasmid-encoded DNA polymerase. *Yeast* 12: 241–246
- Tanguy-Rougeau C, Chen XJ, Wesolowski-Louvel M, Fukuhara H (1990) Expression of a foreign Km<sup>R</sup> gene in linear killer DNA plasmids in yeast. *Gene* 91: 43–50
- Tokunaga M, Kawamura A, Hishinuma F (1989) Expression of pGKL killer 28K subunit in *Saccharomyces cerevisiae*: identification of 28K subunit as a killer protein. *Nucleic Acids Res* 17: 3435–3446
- Tokunaga M, Kawamura A, Kitada K, Hishinuma F (1990) Secretion of killer toxin encoded on the linear DNA plasmid pGKL1 from *Saccharomyces cerevisiae*. *J Biol Chem* 265: 17274–17280
- Tommasino M (1991) Killer system of *Kluyveromyces lactis*: the open reading frame 10 of the pGKL2 plasmid encodes a putative DNA binding protein. *Yeast* 7: 245–252
- Tommasino M, Ricci S, Galeotti CL (1988) Genome organization of the killer plasmid pGKL2 from *Kluyveromyces lactis*. *Nucleic Acids Res* 16: 5863–5878
- Wesolowski M, Algeri A, Fukuhara H (1982a) Killer DNA plasmids of the yeast *Kluyveromyces lactis*. III. Plasmid replication. *Curr Genet* 5: 205–208
- Wesolowski M, Algeri A, Goffrini P, Fukuhara H (1982b) Killer DNA plasmids of the yeast *Kluyveromyces lactis*. I. Mutations affecting the killer phenotype. *Curr Genet* 5: 191–197
- Wesolowski M, Dumazert P, Fukuhara H (1982c) Killer DNA plasmids of the yeast *Kluyveromyces lactis*. II. Restriction endonuclease maps. *Curr Genet* 5: 199–203
- Worsham PL, Bolen PL (1990) Killer toxin production in *Pichia acaciae* is associated with linear DNA plasmids. *Curr Genet* 18: 77–80
- Wei YW, Yang X, Griffiths AJF (1996) Structure of a *Gelasinospora* linear plasmid closely related to the *kalilo* plasmid of *Neurospora intermedia*. *Curr Genet* 29: 150–158
- Wu X, Roy KL (1993) Complete nucleotide sequence of a linear plasmid from *Streptomyces clavuligerus* and characterization of its RNA transcripts. *J Bacteriol* 175: 37–52
- Zotchev SB, Soldatova LI, Orekhov AV, Schrepf H (1992): Characterization of a linear extrachromosomal DNA element (pBL1) isolated after interspecific mating between *Streptomyces bambergiensis* and *S. lividans*. *Res Microbiol* 143: 839–845