**REVIEW
ARTICLE****Conditionally lethal genes associated with bacterial plasmids**

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Overview

Bacterial cells commonly serve as hosts for plasmids and bacteriophages. Since both plasmids and bacteriophages are not capable of proliferation without the machinery of the bacterial host, their stable coexistence with the host would be expected to be symbiotic. However, this is not always the case. Bacteriophages are good examples of parasites since often the killing and the lysis of their host is part of their life cycle. In contrast, bacterial plasmids are generally thought of as stable extra-chromosomal elements (Nordström & Austin, 1989). During the course of co-evolution of bacteria and plasmids, many non-essential genes were concentrated on plasmids from which they could be utilized by the host bacterium. Thus, bacterial plasmids could be considered as temporary endosymbionts (Sonea, 1991). However, new observations indicate that several bacterial plasmids carry genes capable of killing their host in a seemingly spiteful manner. In addition, recent experiments have identified plasmid genes that are conditionally lethal in a host-specific manner. What all these systems have in common is that they kill their host from within (the killer plasmid is initially present in the target cell), unlike colicins, microcins or antibiotics. Whilst most killing genes were identified as such and only operationally at the time of their discovery, their study is revealing a variety of mechanisms of interaction between plasmids and their hosts. The focus of previous reviews has been on post-segregational plasmid stabilization systems (Nordström & Austin, 1989; Jensen & Gerdes, 1995). The intention here is to provide a broader description of reasonably well-studied killer mechanisms employed by various plasmids. The killing of susceptible cells by bacteriocins and type II restriction-modification systems has been described elsewhere

(Naito *et al.*, 1995; Konisky, 1982) and is beyond the scope of this article.

Post-segregational killing of plasmid-free cells

Plasmids are normally inherited by both daughter cells when their host bacterium divides. If, however, the plasmid DNA has not replicated prior to cell division or the plasmid copies are physically joined to each other at the time of cell division, a plasmid-free daughter cell can be produced. To prevent this segregational loss, several independent mechanisms that ensure the stable maintenance of plasmids in the population have evolved. Some of these systems increase the likelihood of both daughter cells receiving a copy of the plasmid by selectively positioning plasmid DNA molecules (Fig. 1a) and are often called 'true' partitioning systems (reviewed in Williams & Thomas, 1992; Hiraga, 1992). Other stabilization systems are involved in the resolution of plasmid multimers into monomers, thus increasing the probability of equal distribution of plasmid molecules into daughter cells (Fig. 1b) (Summers & Sherratt, 1984; Austin *et al.*, 1981). Unlike these partitioning systems, the post-segregational killing systems found on some plasmids do not increase the likelihood of a daughter cell receiving the plasmid. Rather, plasmids carrying such genes are capable of selectively killing plasmid-free segregants after they are produced (Fig. 1c). This post-segregational killing was observed and described in the IncFII plasmids R1 and F (Loh *et al.*, 1988; Gerdes *et al.*, 1986b) and there is also evidence that plasmids of the incompatibility groups P [RK2; (Young *et al.*, 1985)] and bacteriophage P1 (Lehnher *et al.*, 1993) encode gene products that have an analogous role. Post-segregational killing systems always contain two components: a stable killer toxin and an unstable factor that prevents expression of the toxin or functions as an antidote to it. While the killer toxin is always a protein, the antitoxin is either antisense

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Fig. 1. Diagrammatic representation of three distinct plasmid stabilization mechanisms. (a) Some plasmids are selectively positioned inside the bacterial cell, thus ensuring that each of the daughter cells will receive a copy of a plasmid. (b) A multimer-resolution mechanism resolves plasmid multimers (when they are formed) into monomers, thus increasing the likelihood that both daughter cells will receive at least one copy of a plasmid. (c) Post-segregational killing systems selectively kill daughter cells that fail to receive a copy of a plasmid, thus enriching the subpopulation of plasmid-harboring cells.

RNA or a protein. The latter has also been named the proteic killer gene system (Jensen & Gerdes, 1995).

Systems in which both the killer and its antidote are proteins

The *ccd* locus [coupled cell division (Jaffé *et al.*, 1985); or control of cell death (Van Melder *et al.*, 1994)] of the F plasmid is probably the best characterized post-segregational killer system in which both components are proteins. The *ccd* region has two components: *ccdA* (also called H or *letA*), which is a suppressor of inhibition, and *ccdB* (also called G, *letB* or *letD*), which codes for a lethal protein (Fig. 2). Although it was originally thought that the function of *ccd* is to couple cell division to the proliferation of the plasmid (Ogura & Hiraga, 1983), it was later shown that the presumptive coupling is achieved by the killing of the plasmid-free segregants (Jaffé *et al.*, 1985). Jaffé *et al.* (1985) and later Hiraga *et al.* (1986) showed that if the replication of the plasmid was prevented, the plasmid-free segregants became non-viable and formed long filaments. This phenomenon was explained by the non-viable segregant model. When the replication of a thermosensitive-replication derivative of the plasmid was inhibited by a temperature shift, plasmid molecules were diluted by cell division and eventually a plasmid-free subpopulation of cells arose. These were, however, inhibited in their growth, formed filaments and became non-viable. It was speculated (Jaffé *et al.*, 1985) that this was due to the rapid decay of the CcdA protein, which enabled the toxic *ccdB* gene product to kill the cells. Hiraga *et al.* (1986) showed in a separate experiment that this must indeed be the case since the viability of the coexisting plasmid-free cells, which never carried the plasmid, was not affected. These experiments suggested that the killing action does not work by cell-to-cell contact and therefore the killing must be occurring from within.

Several studies have focused on the control and regulation of the *ccd* operon. It has been shown that expression of *ccdA* and *ccdB* is negatively controlled at the level of transcription (Tam & Kline, 1989a). The repression of the *ccd* operon is achieved only if both the CcdA and CcdB proteins are present. It was also shown

(Tam & Kline, 1989b) that the CcdA and CcdB proteins form a complex even in the absence of the *ccd* operator. A recent study by Salmon *et al.* (1994) indicated that the domain of the CcdA protein responsible for autoregulation is different from the domain involved in the antidote effect; several missense CcdA polypeptides that lost their autoregulatory activities retained the ability to antagonize the lethal activity of CcdB. The same was shown for the CcdB toxin; mutants that inactivated CcdB killer activity retained their autoregulatory properties (Bahassi *et al.*, 1995).

The CcdB protein (11700 Da) acts by poisoning DNA-gyrase complexes (Maki *et al.*, 1992; Bernard & Couturier, 1992). The tetrameric A₂B₂ DNA-gyrase is an essential cellular enzyme that catalyses negative supercoiling of DNA. DNA-gyrase introduces a transient double-strand nick in its substrate DNA and passes a double helix through the break in the direction that will decrease supercoiling of DNA. During the breaking and rejoining reaction, the 5'-ends of the substrate DNA are covalently linked to the A subunit of gyrase (Bernard & Couturier, 1992). CcdB stabilizes the cleaved DNA-gyrase complex and does not allow resealing of the nicked DNA (Bernard *et al.*, 1993). These induced complexes then interfere with replication and transcription, causing DNA lesions which in turn induce the SOS response and bacterial death (Salmon *et al.*, 1994). In this respect, its action resembles that of the quinolone antibiotics and other antitumour drugs although the CcdB protein is less efficient (Jaffé *et al.*, 1985). It was shown by *in vitro* and *in vivo* studies (Bernard & Couturier, 1992) that the CcdB protein is responsible for plasmid DNA linearization, which suggests that it can promote gyrase-mediated double-stranded DNA breakage. As expected, an *Escherichia coli* strain resistant to CcdB (*ccdB^r*) was found to have a mutation in the A subunit of the DNA-gyrase (Miki *et al.*, 1992). In this strain, the ability of CcdB to induce linearization of the plasmid DNA as well as SOS induction was suppressed. Interestingly, the UV-light-triggered SOS induction was unaffected. Bernard & Couturier (1992) proposed a model in which CcdB-promoted SOS induction is a consequence of DNA damage produced by a CcdB-gyrase-DNA complex rather than inhibition of gyrase

Fig. 2. Genetic organization of the *ccd*-like operons found on different plasmids, bacteriophage P1 and the *E. coli* chromosome (indicated on the left). Cross-hatched boxes represent lethal genes; open boxes, antitoxin-encoding open reading frames; bent arrows, transcriptional start sites; and black boxes, negative autoregulatory elements where they have been identified.

activity. However, Maki *et al.* (1992) reported that an increased dosage of the wild-type DNA-gyrase overcame the growth inhibition of the CcdB product. This observation suggested that the target of CcdB protein is the DNA-gyrase, particularly the A subunit. It was found that in the CcdB-overproducing strain the DNA supercoiling activity was decreased to a nondetectable level (Maki *et al.*, 1992). This was due to the inactivation of DNA-gyrase activity since the free DNA-gyrase isolated from these strains was also completely inactivated. Addition of the CcdA protein (8700 Da) to the extract of the CcdB-overproducing strain led to full restoration of gyrase activity (Maki *et al.*, 1992). These results indicated that the CcdA and CcdB proteins form an opposing pair in modulating the activity of DNA-gyrase. It is believed that CcdA interacts directly with CcdB, forming a complex of about 69000 Da (Tam & Kline, 1989b). DNA-gyrase plays a fundamental role in many cellular processes such as DNA replication, transcription, recombination and the resolution of

daughter chromosomes. An interesting possibility is that the CcdB protein also causes the inability of the bacterial chromosome to decatenate or to position the daughter chromosome, which could lead to the observed filamentation and formation of anucleated cells (Miki *et al.*, 1992; Bernard & Couturier, 1992). Certain conditional mutations in the *gyrA* gene have essentially the same phenotype (Kato *et al.*, 1989; Hussain *et al.*, 1987). It is possible that the CcdB protein preferentially inhibits the decatenation activity of the DNA-gyrase and the resulting higher topological order of the chromosomal DNA might interfere with proper chromosome segregation.

Differential stability of toxin and antidote is critical for post-segregational killing

For proper functioning as a post-segregational killing system, the stabilities of the CcdA and CcdB proteins must be different. It was proposed by Jaffé *et al.* (1985)

that in the plasmid-free cells, *de novo* synthesis of the Ccd proteins ceases and the CcdA protein is diluted, enabling the CcdB protein to kill the cell. In more recent work, Van Melderer *et al.* (1994) obtained evidence that the half-life of the CcdA protein is shorter than that of the CcdB protein. Using pulse-chase experiments, the half-life of the CcdA protein was estimated to be about 1 h whilst the CcdB protein remained stable for over 2 h. A number of proteases were suggested to play critical roles in fast turnover of proteins in the prokaryotic cell. The two best-characterized ATP-dependent serine proteases in *E. coli* are Lon and Clp (Gottesman & Maurizi, 1992). Interestingly, the stability of the CcdA protein improved in a Lon⁻ strain, suggesting that Lon is the major protease responsible for the degradation of CcdA. It was found that under conditions in which the CcdB protein is also produced, the stability of CcdA improved (Van Melderer *et al.*, 1994). This partial resistance to proteolysis was shown for other unstable proteins degraded by the Lon protease and may be due to the fact that the motifs recognized by the protease are also involved in protein-protein interactions (Van Melderer *et al.*, 1994). Another interesting observation is that the half-life of the CcdA protein is long when compared with other Lon targets such as the SulA, RcsA or N protein (Van Melderer *et al.*, 1994). This, however, might be necessary considering the essential function of the antidote. Since Lon is a heat-shock protein, rapid changes in the surrounding environment could lead to the degradation of the antidote even in the plasmid-harboring cells and eventually to the killing of the cells. Degradation of the antidote that is too slow would, on the other hand, lead to dilution of the toxin protein and thus the system would not be efficient.

ccd-like operons are found on many unrelated plasmids

The *ccd* operon of the F plasmid is not the only plasmid-borne post-segregational system with both the toxin and antidote occurring as proteins. The *parD* stability system of the R1 plasmid also codes for two genes: cytotoxin Kid (12 kDa) and the antidote protein Kis (9.3 kDa) (Ruiz-Echevarría *et al.*, 1991b). The ParD system is exactly conserved in the closely related R100 plasmid where it has been described as the Pem system with the two genes *pemI* (identical to *kis*) and *pemK* (identical to *kid*) (Tsuchimoto *et al.*, 1988). It was shown in several studies that the mode of action of the ParD system is remarkably similar to that of the Ccd system. The *parD* operon is autoregulated at the level of transcription and both protein products are needed for this autoregulation in a manner similar to the autoregulation of the *ccd* operon of the F plasmid (Ruiz-Echevarría *et al.*, 1991a). *kis* and *kid* mRNAs have similar half-lives and are found in similar amounts. Differences in the dosage of Kis and Kid are due to a partial degradation of polycistronic *parD* mRNA, suggesting the importance of RNA processing and translational coupling for *parD* expression (Ruiz-Echevarría *et al.*, 1995a). Similarly to *ccd*, the half-life of the toxic

Kid (PemK) protein is longer than that of the antidote Kis (PemI) (Tsuchimoto *et al.*, 1992). The degradation of the PemI antidote was also shown to be due to Lon protease (Tsuchimoto *et al.*, 1992). In a comparative study of *ccd* and *parD*, Ruiz-Echevarría *et al.* (1991b) showed that the ParD system of R1 and the Ccd system of F share not only functional but also structural and sequence similarities. However, despite structural similarities, the CcdA and Kis proteins are not interchangeable. Also, the killer proteins of these two systems, the CcdB and Kid proteins, do not share any structural similarities. While the DNA-gyrase complexes are believed to be a molecular target of *ccd*, the killer protein of *parD* is proposed to be an inhibitor of DnaB-dependent DNA replication at the initiation stage (Ruiz-Echevarría *et al.*, 1995b).

A functionally similar system was also found in the bacteriophage P1. As in the case of the *ccd* locus, there are two proteins involved in the post-segregational killing system of P1. The action of the toxic protein Doc (126 amino acids) is prevented in cells that retain P1 by the antidote protein Phd (73 amino acids) (Lehnher *et al.*, 1993). These two genes are organized in an operon (called addiction module) and the synthesis of Doc is translationally linked to that of Phd. Unlike killing by CcdB, expression of *doc* does not trigger SOS induction. Also, whilst killing by *ccd* or *pem* (*parD*) leads to filamentation, cells that have lost P1 show a reduced size and a decreased capacity for SOS induction (Lehnher *et al.*, 1993). There is no evidence to suggest that DNA-gyrase is the target of Doc protein; moreover, since the expression of *doc* does not trigger the SOS response, it seems unlikely that DNA-gyrase is the target. As in the case of *ccd* and *pem* (*parD*), the shorter half-life of the antidote protein is due to its preferential degradation by protease. However, unlike the CcdA and PemI proteins, Doc protein is degraded by the ATP-dependent serine protease ClpXp and not Lon protease (Lehnher & Yarmolinsky, 1995). Despite these differences, the *doc/phd* addiction module shares the same organizational features as the *ccd* or *pem* systems (Fig. 2).

The *parDE* operon of the broad-host-range plasmid RK2 also contributes to stabilization through the post-segregational killing of plasmid-free cells (Roberts *et al.*, 1994). The *parDE* operon has the organization of a proteic killer system and encodes toxic ParE protein (83 amino acids) and an antidote protein ParD (103 amino acids) (Fig. 2). It has been shown that ParE exists as a dimer, which binds to dimeric ParD to form a tetramer (Johnson *et al.*, 1996). In addition to its antidote function, ParD protein also acts as a negative regulator of the *parDE* operon (Roberts *et al.*, 1993; Johnson *et al.*, 1996). Similarly to killing by *ccd*- or *pem*-specified proteins, killing by ParE leads to growth inhibition accompanied by filamentation (Roberts *et al.*, 1994). While the *parDE* operon is necessary for cell filamentation, a region outside *parDE* and called *psa* (post-segregational arrest) is required for cell-growth arrest in the absence of filamentation (Jovanovic *et al.*, 1994). The *parDE* operon has been shown to stabilize RK2 in a

Fig. 3. Genetic organization of the *hok/sok* and related systems associated with plasmids and the *E. coli* chromosome (indicated on the left). Cross-hatched boxes represent killer genes; open boxes, overlapping regulatory genes; black boxes, antisense RNAs; bent arrows, transcription start sites; asterisks, transcription termination points; and short black bars, inverted repeats which can form stem-loop structures. (Adapted from Nielsen *et al.*, 1991).

broad spectrum of hosts, suggesting the importance of this locus for broad-host-range or RK2 plasmids (Sia *et al.*, 1995).

Recently, a novel killer system has been suggested for plasmid Rts1 (Tian *et al.*, 1996). The *hig* locus (host inhibition of growth) consists of *higA* (encoding an 11.7 kDa antidote protein) and *higB* (encoding a 10.7 kDa killer protein). Contrary to other proteic killer systems, the toxic part of the *hig* locus is located upstream of the antidote (Fig. 2). The interaction between HigA and HigB, as well as the molecular target of HigB, is yet to be characterized.

Systems in which the killer is a protein, the synthesis of which is controlled by antisense RNA

In other post-segregational killing systems the role of the antidote is played by antisense RNA. The best understood and characterized system of this type is the *parB* locus of the plasmid R1 (Gerdes *et al.*, 1990a). When the *parB* region was inserted into a plasmid with a temperature-sensitive replicon, stabilization in the inheritance of such a construct at non-permissive temperatures was observed (Gerdes *et al.*, 1986b). Moreover, a large fraction of the population of plasmid-free cells was found to be non-viable. These non-viable cells, however,

differed significantly from those observed with the *ccd* locus. The presence of *parB* did not induce filamentation; on the contrary, ghost cells were formed. The *parB* region was mapped to within a 580 bp region and consists of two genes: *hok* (host killing) and *sok* (suppressor of killing) (Fig. 3) (Gerdes *et al.*, 1986a). The product of the *hok* gene is a small 52 amino acid hydrophobic polypeptide associated with the cellular membrane which, when overexpressed, causes cell death. It was observed that shortly after the synthesis of the Hok product, there is a rapid decrease in the rate of oxygen consumption and a decrease in the membrane electrochemical potential (Gerdes *et al.*, 1986a). However, short-circuiting of membrane potential did not lead to inhibition of respiration and, vice versa, inhibition of respiration did not lead to the collapse of the membrane potential. It was therefore proposed that the Hok polypeptide interacts with a component of the membrane, the inactivation of which simultaneously leads to the observed changes in respiration and membrane potential (Gerdes *et al.*, 1986a). The potential target for the Hok polypeptide was proposed to be some component of the respiratory chain since the inhibition of this function should lead to an inhibition of electron transport and proton pumping. The morphological changes associated with the expression of *hok* (ghost cell appearance) could be due to the permeabilization of the cell membrane (Gerdes *et al.*, 1986a). Due to the osmotic

pressure and loosening of the peptidoglycan layer, water accumulates in the cell, resulting in the expansion of the cell volume and dilution of the intracellular material.

hok RNA is regulated by antisense RNA

The expression of *hok* is regulated at the post-transcriptional level by the *sok*-encoded 67 nucleotide antisense RNA which is transcribed in the opposite direction to *hok* (Fig. 3) (Thisted *et al.*, 1994b). It was shown that the target for Sok RNA is in the Hok mRNA leader region (SokT) (Thisted *et al.*, 1994b). This region overlaps with the translation initiation region (TIR) of the third open reading frame *mok* (mediation of killing). It is likely that Sok RNA inhibits *mok* translation by obstructing the *mok* TIR and preventing the entry of ribosomes to this region. The *mok* gene starts upstream of the *hok* gene

and overlaps with *hok* out-of-frame. Translation of *mok* is very tightly regulated by Sok RNA and the translation of *mok* is also a prerequisite for *hok* translation (Thisted & Gerdes, 1992). This genetical arrangement allows for the regulation of *hok* expression indirectly via *mok*.

Gerdes *et al.* (1990b) found that *hok* mRNA is very stable but *sok* RNA decays rapidly. Also, a new truncated *hok* mRNA species was found and its formation was found to be negatively regulated by Sok RNA. The truncated *hok* mRNA was found when the *sok* promoter was inactivated by site-specific mutagenesis or if rifampicin was added to cells containing the *hok/sok* locus. Addition of rifampicin also led to efficient expression of the Hok protein. On the other hand, addition of streptolydigin, which also led to the rapid decay of Sok RNA, had no effect on the truncation of

Fig. 4. Schematic illustration of proposed model of post-transcriptional regulation of *hok/sok* genes. In plasmid-carrying cells, binding of either the *fbi* region or the Sok RNA to the *mok* TIR region prevents translation of the Hok protein by targeting Hok RNA for degradation by RNase III. The absence of Sok RNA in plasmid-free cells leads to eventual translation of the Hok protein and cell death. A similar mode of regulation was proposed for other members of the *hok/sok* family. See text for details. (Adapted from Thisted *et al.*, 1994a).

hok mRNA or the synthesis of the Hok protein. It was therefore inferred that for the efficient translation of *hok* mRNA, 3'-end processing is required (Gerdes *et al.*, 1990b). In agreement with these findings, the truncated *hok* mRNA was found only in plasmid-free cells.

The regulation of *hok* mRNA translation by the antisense RNA seems very efficient, but it poses one problem. In plasmid-carrying cells, no *hok* translation is observed, implying very efficient inhibition by Sok RNA, whereas in plasmid-free cells the *hok* mRNA should be reactivated, leading to synthesis of the Hok protein. However, there has been no mechanism shown to operate in prokaryotes that can remove an antisense RNA from its target (Thisted *et al.*, 1994a). It has been shown that the *hok* mRNA is specifically cleaved at its 3'-end and that this processing leads to the translational activation of *hok* mRNA (Thisted *et al.*, 1994a, b; 1995). Northern blot analysis of *in vivo*- and *in vitro*-produced Hok mRNA showed that there are two full-length mRNA species present: Hok mRNA-1 and Hok mRNA-2 (Thisted *et al.*, 1994a). While Hok mRNA-1 was very stable both *in vivo* and *in vitro*, Hok mRNA-2 decayed slowly. This decay was consistent with the appearance of the new, truncated Hok mRNA. A potential stem-loop structure was identified in the 3'-end of the full-length Hok mRNA-1 and was proposed to act as a barrier for 3'→5' exonuclease degradation which could protect Hok mRNA-1 (Thisted *et al.*, 1994a). Using a cell-free system, Thisted *et al.* (1994a) showed that the full-length Hok mRNA-1 is translationally inactive whereas the truncated mRNA is translationally active and the translation inhibition motif *fbi* was mapped to the 3'-end region. Mapping of the full-length Hok mRNA indicated that the *fbi* region can interact directly with the *mok* TIR. Based on these results, Thisted *et al.* (1994a) proposed the following model for the regulation of post-segregational killing of plasmid-free cells (Fig. 4). In a plasmid-carrying cell, the 3'-end *fbi* region binds to the 5'-end *mok* TIR region. This 'closed' Hok mRNA is slowly processed from its 3'-end and the *fbi* region is removed. Removal of the *fbi* region will cause Hok mRNA to open and to disclose the *mok* TIR region, which is rapidly recognized and bound by the Sok RNA. The binding of either *fbi* or Sok RNA to the *mok* TIR region will prevent translation of the Hok protein. On the full-length closed Hok mRNA, the binding of *fbi* to the *mok* TIR is slowly replaced by the binding of Sok RNA (Thisted *et al.*, 1994b). This competitive binding of antisense RNA is significantly slower and ensures the presence of the pool of the full-length Hok mRNA in the cell. Once the Sok-Hok RNA complex is formed, it is recognized by RNase III and is rapidly degraded. The binding of the *fbi* region to the *mok* TIR region is essential for the proper functioning of this system. Since the Sok-Hok RNA duplex is very rapidly degraded, a fast turnover of the *hok* mRNA in the cell might be expected. However, binding of *fbi* to *mok* TIR protects Hok mRNA from being degraded. In plasmid-free cells, the pool of Sok RNA decays rapidly since the Sok RNA is highly unstable. The full-length Hok mRNA is slowly

processed to its truncated active form. Since this truncated mRNA cannot be degraded in the absence of Sok RNA, it is efficiently translated. The synthesis of the Hok protein eventually leads to the killing of the cells. This system represents a unique 'molecular memory' (Thisted *et al.*, 1994b).

Analogues of *hok/sok* have also been found on other bacterial plasmids. The F plasmid carries the *srnB'* (Onishi, 1975) and *flm* (Loh *et al.*, 1988) loci, and the IncI plasmid R483 and the IncB plasmid R16 each carry the *pnd* locus (Akimoto & Ohnishi, 1982). These loci were originally identified from their ability to induce membrane damage, RNase I influx, degradation of stable RNA and eventually cell killing upon addition of rifampicin. It was shown later by Nielsen *et al.* (1991) that these genes in fact mediate post-segregational killing of plasmid-free cells, are regulated by antisense RNAs, and that they also share the same structural and functional organization as *hok/sok* (Fig. 3). It was shown recently (Thisted *et al.*, 1994a) that the 3'-end processing of the killer mRNAs is essential for the activation of translation of these genes in a fashion similar to that of the Hok mRNA. It seems clear that these genes constitute a family of *hok*-homologous killer genes.

Conditionally lethal genes associated with the *E. coli* chromosome

Surprisingly, homologues of the *hok* family killer genes have also been found on the chromosome of *E. coli*. The *relF* gene of the *relB* operon is related to the *hok* gene both structurally and functionally (Fig. 3) (Gerdes *et al.*, 1986a). It encodes a 51 amino acid residue polypeptide that has 40% homology to the Hok protein. Induction of the *relF* gene leads to the same physiological response as the expression of the *hok* gene: cessation of respiration, dissipation of the membrane potential, appearance of ghost cells and cell death (Gerdes *et al.*, 1986a). Another chromosomal gene, *gef*, was identified on the chromosome of *E. coli* K-12 (Poulsen *et al.*, 1989), the protein product of which was found to be structurally and functionally similar to the proteins encoded by *hok* and *relF*. The Gef protein is a toxin of 50 amino acid residues and was found associated with the bacterial membrane (Poulsen *et al.*, 1991b). It was found that this protein forms dimers. However, the mutation which prevented the dimer formation had only a small effect on the toxicity of Gef protein (Poulsen *et al.*, 1991b). It has been shown that even though the *gef* gene is transcribed constitutively, Gef protein cannot be detected in the cells (Poulsen *et al.*, 1991a). The regulatory model for the control of the expression of *gef* was proposed to be similar to that of the regulation of the expression of *hok* (Poulsen *et al.*, 1991a). According to this model, translation of *gef* is coupled to the translation of the upstream open reading frame called *orf69* which is negatively regulated by the antisense RNA *Sof*. This *Sof* RNA can bind to the first three codons and the ribosome-binding site of the *orf69*

mRNA and thus prevent translation of *gef* (Poulsen *et al.*, 1991a). Two chromosomal mutations in *E. coli* were identified that rendered cells resistant to the Gef protein (Poulsen *et al.*, 1992). The function of the proteins involved in the Gef^r phenotype is at present unknown. Even though *relF* and *gef* share structural and functional homology with the plasmid-borne killing systems of the *hok* family, the chromosomal killer genes, unlike their plasmid counterparts, were not shown to be able to stabilize plasmid inheritance (Poulsen *et al.*, 1989).

An entirely different killing system was also found to be associated with the chromosome of *E. coli*. Two genes, *kicA* and *kicB*, were identified upstream of *mukB*, a gene essential for chromosome partitioning (Fig. 2) (Feng *et al.*, 1994). KicA (antidote) and KicB (toxin) were found to be non-essential for the normal function of the cell. The *kicA* and *kicB* genes were cloned onto a cloning vector and their effect on the physiology of the host was investigated. Similarly to the *ccd* system of F, KicA can prevent killing by KicB, presumably by protein-protein interaction with KicB. Preliminary experiments suggested that KicB interferes with protein synthesis, resulting in the killing of the cell (Feng *et al.*, 1994). Unlike other chromosome-borne killing systems, when *kicA* and *kicB* were transferred from the chromosome to a plasmid they were able to act in a 'true' post-segregational killing manner by killing plasmid-free segregants (Feng *et al.*, 1994). Recently, another operon of the *E. coli* chromosome was identified to be homologous to the *ccd/pem* family. The functional and structural homologue of the *pem* locus was located at 60 min (*chpA*) and at 100 min (*chpB*) (Fig. 2) (Masuda *et al.*, 1993). *chpA* is located within the *relA* operon and *chpB* is located in close proximity to the *ppa* region. Both of these regions are believed to be involved in the stringent response, suggesting a possible role of *chpA* and *chpB* in this response (Masuda *et al.*, 1993).

***kil/kor* genes of plasmids of incompatibility groups P and N**

Bacterial plasmids of the incompatibility groups P and N have been found to carry an entirely different set of genes that are potentially lethal to their host. It was found that certain regions of the IncP plasmid RK2 (indistinguishable from RP4) could not be cloned unless there were other regions of the same plasmid present *in cis* or *in trans* (Figurski *et al.*, 1982). These genes were designated *kil* and genes that can prevent killing were called *kor* (killing override). Later experiments showed that the *kil/kor* genes constitute a network of coregulated genes that are also involved in the control of replication (Schreiner *et al.*, 1985; Motallebi-Veshareh *et al.*, 1992) and conjugation (Motallebi-Veshareh *et al.*, 1992). Four *kil* operons have been identified: *kilA*, consisting of the genes *klaA*, *klaB* and *klaC* (Saltman *et al.*, 1991; Goncharoff *et al.*, 1991); *kilB*, consisting of *trbB* and *trbC* (Thomson *et al.*, 1993; Larsen & Figurski, 1994); *kilC*, consisting of *klaA*, *klaB* and *korB* (Larsen & Figurski, 1994); and *kilE*, consisting of *klaA*-F

(Kornacki *et al.*, 1993). At least five *kor* genes (-A, -B, -C, -E and -F) have been identified to date (Larsen & Figurski, 1994). The *kil* and *kor* genes are organized into a *kil-kor* regulon which resides within a 26 kb region of RK2 (Larsen & Figurski, 1994). It is not known whether these genes are involved in the post-segregational killing of plasmid-free cells or whether they contribute to plasmid stability by some other mechanism. It was shown (Saltman *et al.*, 1991; Goncharoff *et al.*, 1991; Thomson *et al.*, 1993; Kornacki *et al.*, 1993; Larsen & Figurski, 1994) that unregulated expression of the *kil* genes leads to killing of the host cells. The cells overexpressing the Kil proteins became elongated, had distorted outer membranes and their electron-dense materials were visible in the periplasmic spaces (Saltman *et al.*, 1991). This cell killing was always prevented if the corresponding *kor* region was present.

Incompatibility group N plasmids pKM101 and pCU1 were also shown to carry genes that are potentially lethal to their host. Even though there is no sequence homology to the *kil/kor* genes of RK2 and *kor* genes of these plasmids cannot inhibit lethality of RK2 *kil* genes, Winans & Walker (1985) used the same terminology for pKM101 genes. Two *kil* genes (*kilA* and *kilB*) and two *kor* genes (*korA* and *korB*) were identified in the sequence of pKM101 (Winans & Walker, 1985) and a third *kil* gene (*kilC*) was identified in the sequence of pCU1 (M. Holčík, M. Rodríguez, A. Couse, G. Cherton-Horvat & V. N. Iyer, unpublished). Recently, *kilA* and *kilB* were found to be allelic with *traL* and *traE*, respectively (Moré *et al.*, 1996). Both *kil* genes are required for conjugation and are homologous to transfer genes of *Agrobacterium tumefaciens* (*vir* operon) and toxin export genes (*ptl*) of *Bordetella pertussis* (Pohlman *et al.*, 1994).

Several studies indicated that the function of *kor* genes is to regulate expression of *kil* genes at the level of transcription (Williams & Thomas, 1992; Motallebi-Veshareh *et al.*, 1992; Thomson *et al.*, 1993; Kornacki *et al.*, 1993; Moré *et al.*, 1996). Detailed study of *korA* and *korB* of pKM101 indicated that KorA and KorB together repress transcription from the *kil* promoter region as well as their own transcription. Interestingly, neither protein alone was capable of repression. DNase I footprinting and gel retardation assays identified 29 nucleotide *kor* boxes located upstream of *kil* or *kor* promoters where the KorA/KorB heterodimers bind (Moré *et al.*, 1996). This type of regulation, however, is not expected for a system which would preferentially kill plasmid-free segregants since it cannot function as a molecular memory. Interestingly, the *kilA* and *korA* sequences of RK2 are part of the cryptic tellurite resistance transposon Tn521 (Grewal, 1990), and the *korA* and *korB* sequences of RK2 are conserved throughout the incompatibility group P plasmids (Figurski *et al.*, 1982; Thomas *et al.*, 1995).

As mentioned above, the *kil/kor* regulatory system of pKM101 is superficially similar to that of plasmid RK2. Furthermore, both systems are located within the

conjugal transfer system regions of the respective plasmids. Tight regulation of transfer genes may be required to achieve a fine balance between appropriate expression of these genes for plasmid transfer and their potentially negative effects such as zygotic induction or susceptibility to bacteriophages (Moré *et al.*, 1996).

Plasmid genes lethal in *Klebsiella oxytoca* but not in *E. coli*

All known conjugative plasmids of the incompatibility groups N, P and W confer a phenotype called Kik⁺. This phenotype was characterized by marked reduction of the viability of *K. oxytoca* recipients, but not of *E. coli* recipients, following matings with the donor host (Rodríguez & Iyer, 1981). This phenotype is called Kik (killing of *Klebsiella*) to distinguish it from *kil* which is manifested in *E. coli* and has been described in detail in the IncN group plasmid pCU1. Transposon insertions defined a region that abolished this phenotype. This region was called *kikA* and subsequent studies showed that intracellular expression of *kikA* in *K. oxytoca* was lethal whilst expression in *E. coli* was not (Hengen *et al.*, 1992). Two other loci, *kikB* (Thatte *et al.*, 1985) and *kikC* (Rodríguez *et al.*, 1995), have also been implicated in reducing the viability of *K. oxytoca* and remain to be analysed. The *kikA* locus consists of two separate genes, *orf70* and *pep* (PRD1 entry protein), and their co-expression causes reversible growth inhibition without any apparent morphological changes (Holčík & Iyer, 1996b). Even though the *kikA* locus lies outside the N *tra* region as it was defined in *E. coli*, mutations in *pep* resulted in reduced frequency of transfer in *K. oxytoca* and abolished susceptibility to N-pilus-specific bacteriophage PRD1 in both *K. oxytoca* and *E. coli* (Holčík & Iyer, 1996a). *Pep* is an 8.9 kDa protein located in the periplasmic space, suggesting that *pep* (or *kikA*) is part of an extended *tra* region of pCU1. No plasmid region that overrides the lethality of *kikA* has been found. However, it was found that *K. oxytoca* cells that survived mating with pCU1 had acquired a chromosomal mutation (*kik*-immune) that protected them against killing by pCU1 in subsequent matings (Rodríguez *et al.*, 1995). Mating experiments using prototrophic *E. coli* donors carrying pCU1:*lacZ* with auxotrophic *K. oxytoca* recipients demonstrated that the plasmid pCU1 is deleterious to the *K. oxytoca* host when it is present in the cell (Rodríguez *et al.*, 1995). The observed lethality was therefore not a consequence of segregational plasmid loss followed by the killing of plasmid-free cells as has been observed in *E. coli* with a number of plasmids (see above). Analysis of *lacZ* expression from pCU1:*lacZ* in developing colonies following conjugation suggested that the mutation conferring immunity to the Kik⁺ phenotype occurs late during the development of a bacterial colony. These observations led to the proposal of a hypothesis whereby the expression of *K. oxytoca*-lethal genes arrests the growth of the recipient, thus allowing for a rare secondary chromosomal mutation which renders the cell immune to killing by *kik* genes (Rodríguez *et al.*,

1995). The mutation induced in *K. oxytoca* is likely to be specific as the screening of several Kik-immune strains did not indicate that they had acquired other identifiable mutations (Rotheim *et al.*, 1991). This suggests that the mutation induced by pCU1 is site-specific. The nature of this mutation, as well as the mechanism of its occurrence, is at present unknown.

Lethal genes in *Streptomyces* plasmids

While the majority of the killer genes described above were studied in *E. coli*, at least two distinct and species-specific killer systems have been described. Plasmid transfer in Gram-positive *Streptomyces* is associated with the formation of 'pocks' corresponding to areas of growth inhibition in the recipient strain (Bibb & Hopwood, 1981). Genetic studies with the plasmid pIJ101 identified four genes, *traA* (formerly *kilA*), *kilB*, *spdA* and *spdB*, which are necessary for conjugal transfer and pock formation in *Streptomyces lividans* (Kendall & Cohen, 1988). Similarly to the *kil/kor* genes from RK2 or pKM101, *kil* genes of pIJ101 cannot be cloned unless their corresponding *kor* genes are present *in cis* or *in trans*. The *spdA* and *spdB* genes are responsible for the spreading of the plasmid in the mycelium after transfer (Kendall & Cohen, 1988). The *traA* gene is negatively regulated by the divergently transcribed *korA* gene, the promoter of which overlaps the control region of *kilA* (Stein *et al.*, 1989). A second regulatory gene, *korB*, represses its own expression as well as the expression of *kilB* (Zaman *et al.*, 1992). *KorB* is synthesized as a 10 kDa protein which is subsequently processed to its active 6 kDa form. The footprint of *KorB* was found to overlap the *sti* locus, which is involved in plasmid incompatibility, copy number and pock formation during mating, suggesting that *KorB* is likely to be involved in plasmid DNA replication during or after mating (Tai & Cohen, 1993). Mutational analysis of *KorB* also suggested its central importance for plasmid transmission (Tai & Cohen, 1994). Analogous *kil/kor* genes were also identified in the plasmid pSAM2 from *Streptomyces ambofaciens* and the multicopy plasmid pSN22 from *Streptomyces nigrifaciens* (Kataoka *et al.*, 1991). Conservation of these genes in *Streptomyces* plasmids and their involvement in conjugal transfer strongly suggests that they are involved in the regulation of the transfer processes.

Other phenotypes associated with bacterial plasmids

Two further lethal phenomena are connected with conjugative plasmids: lethal zygosis (Gross, 1963) and zygotic induction (Hayes, 1965) are associated with plasmid transfer by conjugation. Lethal zygosis was first observed as reduced viability of the recipients under conditions where F⁻ bacteria were used in matings with an excess of Hfr donor cells (carrying F factor integrated into their chromosome). Recipients were inhibited in the synthesis of macromolecules and active transport across the membrane, and leakage of the cytoplasm was

observed (Skurray & Reeves, 1973). Lethal zygosis is attributed to the fact that during prolonged mating, membrane rearrangement, which occurs to facilitate plasmid transfer, can cause permeability of the recipient membrane followed by cell death (Skurray & Reeves, 1973; Ou, 1980). Bacterial cells that survive killing by lethal zygosis are immune. This immunity is, however, dependent on the presence of the F plasmid; curing of F from immune cells rendered them subsequently sensitive to lethal zygosis. Immunity to lethal zygosis is caused by entry (surface) exclusion, which is a mechanism by which a plasmid-carrying population of *E. coli* reduces the acceptance of a second, closely related plasmid of the same exclusion group (Ou, 1980). The surface exclusion genes of plasmid F were identified as *traT* and *traS*. TraS is a small inner-membrane protein that is believed to block DNA transfer after a mating pair has been established. In contrast, TraT is an outer-membrane lipoprotein that acts primarily to inhibit the initial steps in mating formation (for a review see Frost *et al.*, 1994). Zygotic induction is, on the other hand, caused by the transfer of a lysogenic bacteriophage inserted in the chromosome of the Hfr donor strain into a non-lysogenic recipient (Hayes, 1965). The transfer of the phage genome from the donor to a non-lysogenic recipient results in the induction of phage development in the recipient followed by cell lysis and the release of phage particles. The induction of lysogenic prophage is due to the lack of the repressor protein, which maintains the prophage in its lysogenic state in the donor. The production of phage particles has been shown to be inhibited by transfer of the *rac* locus from the *E. coli* chromosome into a *Rac*⁻ recipient (Feinstein & Low, 1982). However, evidence suggests that the *rac* locus is a cryptic, and possibly defective prophage that is excised during the induction process. Zygotic induction has also been implicated in the induction of expression of specific plasmid genes such as *psiB* and *ssb*, suggesting they have a role in plasmid conjugation (Jones *et al.*, 1992).

Concluding discussion

Bacterial plasmid genes often encode optional characteristics that are selectively advantageous to bacteria in some environments but not others. The best known example of such genes are probably the antibiotic resistance determinants carried on R plasmids. At the end of the twentieth century, when almost all infectious diseases are treated with natural or synthetic antibiotics, R plasmids still represent a serious threat due to their abilities to spread rapidly between different bacterial species, many of which are human or animal pathogens. Bacterial genes move between chromosomes and plasmids as well as from one plasmid to another. It is therefore not surprising that the same, or very similar sets of lethal genes, are found on different plasmids and that some of these genes are also shared with the chromosome (Table 1). Interestingly, some plasmids carry genes of more than one system on the same replicon (e.g. *ccd*, *srnB'* and *flmA* on F). This may seem superfluous since one stability system should be suffi-

cient to ensure inheritance of a given plasmid. In addition to the lethal genes, many, especially large, plasmids carry other, non-lethal stability determinants (for reviews see Williams & Thomas, 1992; Nordström & Austin, 1989). It is interesting then that the presence of multiple stability determinants on a given plasmid is often paralleled by the presence of multiple replicons on the same plasmid. While there is no direct evidence to support this, a plausible hypothesis could be that these plasmids arose by the recombination of multiple, initially separate plasmids, each with its own replication and maintenance systems. If true, a common origin of plasmid replicons and stability determinants could be inferred by their relative location on the new plasmid. A similar hypothesis has been proposed for the origin of the bacterial chromosome that would emerge as a gradual concentration of smaller replicons with all the essential genes for bioenergetic activity (Sonea, 1991). Interestingly, *gef* and *ref* analogues are conserved in several bacterial species including *E. coli* B and C, *Agrobacterium* and *Rhizobium*, suggesting that these genes play an important role in the physiology of the cell (Poulsen *et al.*, 1989). It has been shown recently that *hok/sok* genes are involved in phage exclusion of bacteriophage T4 and this mechanism could play an important role in the evolution of *hok/sok*-like genes found on bacterial chromosomes (Pecota & Wood, 1996).

The origin of lethal genes is open to speculation. An attractive hypothesis is that they emerged early during the evolution of prokaryotic cells, as a means to ensure co-segregation of self-replicating DNA molecules. These replicons carried different groups of essential genes and their stability and co-segregation was essential for cell survival. As these independent replicons fused together to form a bacterial chromosome the function of lethal genes became redundant. Lethal genes may, however, have been acquired by autonomously replicating DNA molecules and gave rise to a variety of stability determinants found on bacterial plasmids. Alternatively, they may have evolved on plasmids in the course of establishing opportunistic relationships with bacterial cells and this was followed by the evolution of a system to control the *kil* system, either as a cognate *kor* system of the same plasmid, or appropriate changes on the bacterial chromosome. Some of the plasmid lethal genes (*kil-kor* regulons) play a critical role in the regulation of plasmid replication and conjugal transfer. These genes may thus not be 'true' lethal genes. Rather, their lethality could be a consequence of unregulated gene expression. Since many of these *kil* genes are part of the plasmid transfer region it is possible to imagine that overexpression of membrane or pilus proteins would be detrimental to the cell. The function of the cognate *kor* loci is then to control expression of the *kil* genes. We have nevertheless included a discussion of these genes in this review to demonstrate the wide variety of conditionally lethal genes associated with bacterial plasmids, and to emphasize that other classes of such genes may remain to be uncovered. As suggested here, the presence

Table 1. Lethal genes associated with different plasmids, bacteriophage P1 and the *E. coli* chromosome, and genes that antagonize this lethality

Host	Plasmid, bacteriophage or chromosome	Lethal gene	Gene antagonizing the lethality and its functional product		Site/mode of action of the lethal gene product
<i>E. coli</i>	F	<i>ccdB</i>	<i>ccdA</i>	Protein	DNA-gyrase complex
<i>E. coli</i>	R1	<i>kid</i>	<i>kis</i>	Protein	DnaB-dependent DNA replication
<i>E. coli</i>	R100	<i>pemK</i>	<i>pemI</i>	Protein	DnaB-dependent DNA replication
<i>E. coli</i>	RK2	<i>parE</i>	<i>parD</i>	Protein	?
<i>E. coli</i>	P1	<i>doc</i>	<i>phd</i>	Protein	?
<i>E. coli</i>	Rts1	<i>higB</i>	<i>higA</i>	Protein	?
<i>E. coli</i>	Chromosome	<i>chpAK</i> , -BK	<i>chpAI</i> , -BI	Protein	?
<i>E. coli</i>	Chromosome	<i>kicB</i>	<i>kicA</i>	Protein	Protein synthesis inhibition
<i>E. coli</i>	R1	<i>hok</i>	<i>sok</i>	Antisense RNA	Membrane (respiration?)
<i>E. coli</i>	F	<i>srnB'</i>	<i>srnC</i>	Antisense RNA	Membrane (pore?)
<i>E. coli</i>	F	<i>flmA</i>	<i>flmB</i>	Antisense RNA	Membrane (pore?)
<i>E. coli</i>	R483, R16	<i>pndA</i>	<i>pndB</i>	Antisense RNA	Membrane (pore?)
<i>E. coli</i>	Chromosome	<i>relF</i>	?	Antisense RNA?	Membrane (pore?)
<i>E. coli</i>	Chromosome	<i>gef</i>	<i>sof</i>	Antisense RNA	Membrane (pore?)
<i>E. coli</i>	RK2	<i>kilA</i> , -B, -C, -E*	<i>korA</i> , -B, -C, -D, -E, -F	Protein	?
<i>E. coli</i>	pCU1	<i>kilA</i> , -B, -C	<i>korA</i> , -B	Protein	Membrane?
<i>E. coli</i>	pKM101	<i>traL</i> , -E	<i>korA</i> , -B	Protein	Membrane?
<i>S. lividans</i>	pIJ101	<i>traA</i> , <i>kilB</i>	<i>korA</i> , -B	Protein	?
<i>K. oxytoca</i>	pCU1	<i>kikA</i> , -C	?	?	Membrane (<i>kikA</i>)

* *kilA*, -B, -C and -E are operons consisting of several genes each (see text for details).

of these genes is important at several levels of plasmid biology: (i) they will ensure selective killing of plasmid-free cells and thus effectively ensure stable inheritance of plasmids (as in the case of post-segregational killing systems e.g. *hok/sok* or *ccd*); (ii) they are involved in controlling the expression and regulation of complicated networks of replication and transfer genes (such as *kil/kor* genes of RK2 and pKM101); (iii) the existence of *kik* (killing of *Klebsiella*) genes in some plasmids such as pCU1 and pKM101 suggests that they have a role in determining or modulating the host range of plasmids; the *kikA* gene was shown to be involved in modifying host range of conjugal transfer as well as susceptibility to bacteriophages (Holčák & Iyer, 1996a); (iv) a gene (*klaA*) of the *kilA* operon of RK2 has been reported to affect septum formation in *E. coli* (Saltman *et al.*, 1991); (v) the chromosomal *kicB* gene has been reported to interfere with protein synthesis (Feng *et al.*, 1994) and a novel killer system has been suggested for plasmid Rts1 (Tian *et al.*, 1996); and (vi) the indication that carriage of pCU1 by *K. oxytoca* results in a specific chromosomal mutation hints at the possible involvement of plasmids in directly modifying the genetic diversity of bacterial hosts (Rodríguez *et al.*, 1995). It is likely that future investigations will identify as yet unknown functions

associated with these systems. It seems clear that the discovery of families of such genes as operationally-defined *kil* genes followed by their characterization will continue to provide useful insights into the plasmid-bacterium relationship.

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References

- Akimoto, S. & Ohnishi, Y. (1982). R483 and F plasmid genes promoting RNA degradation: comparative restriction mapping. *Microbiol Immunol* **26**, 779–793.
- Austin, S., Ziese, M. & Sternberg, N. (1981). A novel role for site-specific recombination in maintenance of bacterial replicons. *Cell* **25**, 729–736.
- Bahassi, E. M., Salmon, M. A., Van Melder, L., Bernard, P. & Couturier, M. (1995). F plasmid CcdB killer protein: *ccdB* gene mutants coding for non-cytotoxic proteins which retain their regulatory functions. *Mol Microbiol* **15**, 1031–1037.

- Bernard, P. & Couturier, M. (1992). Cell killing by the F plasmid CcdB protein involves poisoning of DNA-topoisomerase II complexes. *J Mol Biol* **226**, 735–745.
- Bernard, P., Kezdy, K. E., Van Melder, L., Steyaert, J., Wyns, L., Pato, M. L., Higgins, P. N. & Couturier, M. (1993). The F plasmid CcdB protein induces efficient ATP-dependent DNA cleavage by gyrase. *J Mol Biol* **234**, 534–541.
- Bibb, M. J. & Hopwood, D. A. (1981). Genetic studies of the fertility plasmid SCP2 and its SCP2* variants in *Streptomyces coelicolor* A3(2). *J Gen Microbiol* **126**, 427–442.
- Feinstein, S. I. & Low, K. B. (1982). Zygotic induction of the *rac* locus can cause cell death in *E. coli*. *Mol Gen Genet* **187**, 231–235.
- Feng, J., Yamanaka, K., Niki, H., Ogura, T. & Hiraga, S. (1994). New killing system controlled by two genes located immediately upstream of the *mukB* gene in *Escherichia coli*. *Mol Gen Genet* **243**, 136–147.
- Figurski, D. H., Pohlman, R. F., Bechhofer, D. H., Prince, A. S. & Kelton, C. A. (1982). Broad host range plasmid RK2 encodes multiple *kil* genes potentially lethal to *Escherichia coli* host cells. *Proc Natl Acad Sci USA* **79**, 1935–1939.
- Frost, L. S., Ippen-Ihler, K. & Skurray, R. A. (1994). Analysis of the sequence and gene products of the transfer region of the F sex factor. *Microbiol Rev* **58**, 162–210.
- Gerdess, K., Bech, F. W., Jørgensen, S. T., Løbner-Olesen, A., Rasmussen, P. B., Atlung, T., Boe, L., Karlstrom, O., Molin, S. & von Meyenburg, K. (1986a). Mechanism of postsegregational killing by the *hok* gene product of the *parB* system of plasmid R1 and its homology with the *relF* gene product of the *E. coli* *relB* operon. *EMBO J* **5**, 2023–2029.
- Gerdess, K., Rasmussen, P. B. & Molin, S. (1986b). Unique type of plasmid maintenance function: postsegregational killing of plasmid-free cells. *Proc Natl Acad Sci USA* **83**, 3116–3120.
- Gerdess, K., Poulsen, L. K., Thisted, T., Nielsen, A. K., Martinussen, J. & Andreassen, P. H. (1990a). The *hok* killer gene family in gram-negative bacteria. *New Biol* **2**, 946–956.
- Gerdess, K., Thisted, T. & Martinussen, J. (1990b). Mechanism of post-segregational killing by the *hok/sok* system of plasmid R1: *sok* antisense RNA regulates formation of a *hok* mRNA species correlated with killing of plasmid-free cells. *Mol Microbiol* **4**, 1807–1818.
- Goncharoff, P., Saadi, S., Chang, C. H., Saltman, L. H. & Figurski, D. H. (1991). Structural, molecular, and genetic analysis of the *kilA* operon of broad-host-range plasmid RK2. *J Bacteriol* **173**, 3463–3477.
- Gottesman, S. & Maurizi, M. R. (1992). Regulation by proteolysis: energy-dependent proteases and their targets. *Microbiol Rev* **56**, 592–621.
- Grewal, K. K. (1990). Plasmid RP4 host-lethal function *kilA* and its repressor *korA* sequences are part of the cryptic tellurite resistance transposon Tn521. *FEMS Microbiol Lett* **57**, 97–99.
- Gross, J. D. (1963). Cellular damage associated with multiple mating in *Escherichia coli*. *Genet Res* **4**, 463–469.
- Hayes, W. (1965). *The Genetics of Bacteria and Their Viruses*, 2nd edn. Chichester: John Wiley.
- Hengen, P. N., Denicourt, D. & Iyer, V. N. (1992). Isolation and characterization of *kikA*, a region on IncN group plasmids that determines killing of *Klebsiella oxytoca*. *J Bacteriol* **174**, 3070–3077.
- Hiraga, S. (1992). Chromosome and plasmid partition in *Escherichia coli*. *Annu Rev Biochem* **61**, 283–306.
- Hiraga, S., Jaffé, A., Ogura, T., Mori, H. & Takahashi, H. (1986). F plasmid *ccd* mechanism in *Escherichia coli*. *J Bacteriol* **166**, 100–104.
- Holčík, M. & Iyer, V. N. (1996a). A novel plasmid gene involved in bacteriophage PRD1 infection and conjugative host-range. *Plasmid* **35**, 204–210.
- Holčík, M. & Iyer, V. N. (1996b). Structure and mode of action of *kikA*, a genetic region lethal to *Klebsiella oxytoca* and associated with conjugative antibiotic-resistance plasmids of the IncN group. *Plasmid* **35**, 189–203.
- Hussain, K., Elliott, E. J. & Salmond, G. P. (1987). The *parD*⁺ mutant of *Escherichia coli* also carries a *gyrA*^{am} mutation. The complete sequence of *gyrA*. *Mol Microbiol* **1**, 259–273.
- Jaffé, A., Ogura, T. & Hiraga, S. (1985). Effects of the *ccd* function of the F plasmid on bacterial growth. *J Bacteriol* **163**, 841–849.
- Jensen, R. B. & Gerdess, K. (1995). Programmed cell death in bacteria: proteic plasmid stabilization systems. *Mol Microbiol* **17**, 205–210.
- Johnson, E. P., Strom, A. R. & Helinski, D. R. (1996). Plasmid RK2 toxin protein ParE: purification and interaction with the ParD antitoxin protein. *J Bacteriol* **178**, 1420–1429.
- Jones, A. L., Barth, P. T. & Wilkins, B. M. (1992). Zygotic induction of plasmid *ssb* and *psiB* genes following conjugative transfer of Inc1 plasmid Col1b-P9. *Mol Microbiol* **6**, 605–613.
- Jovanovic, O. S., Ayres, E. K. & Figurski, D. H. (1994). Host-inhibitory functions encoded by promiscuous plasmids. Transient arrest of *Escherichia coli* segregants that fail to inherit plasmid RK2. *J Mol Biol* **237**, 52–64.
- Kataoka, M., Seki, T. & Yoshida, T. (1991). Regulation and function of the *Streptomyces* plasmid pSN22 genes involved in pock formation and inviability. *J Bacteriol* **173**, 7975–7981.
- Kato, J., Nishimura, Y. & Suzuki, H. (1989). *Escherichia coli* *parA* is an allele of the *gyrB* gene. *Mol Gen Genet* **217**, 178–181.
- Kendall, K. J. & Cohen, S. N. (1988). Complete nucleotide sequence of the *Streptomyces lividans* plasmid pIJ101 and correlation of the sequence with genetic properties. *J Bacteriol* **170**, 4634–4651.
- Konisky, J. (1982). Colicins and other bacteriocins with established modes of action. *Annu Rev Microbiol* **36**, 125–144.
- Kornacki, J. A., Chang, C. H. & Figurski, D. H. (1993). *kil-kor* regulon of promiscuous plasmid RK2: structure, products, and regulation of two operons that constitute the *kilE* locus. *J Bacteriol* **175**, 5078–5090.
- Larsen, M. H. & Figurski, D. H. (1994). Structure, expression, and regulation of the *kilC* operon of promiscuous IncPα plasmids. *J Bacteriol* **176**, 5022–5032.
- Lehnher, H. & Yarmolinsky, M. B. (1995). Addiction protein Phd of plasmid prophage P1 is a substrate of the ClpXP serine protease of *Escherichia coli*. *Proc Natl Acad Sci USA* **92**, 3274–3277.
- Lehnher, H., Maguin, E., Jafri, S. & Yarmolinsky, M. B. (1993). Plasmid addiction genes of bacteriophage P1: *doc*, which causes cell death on curing of prophage, and *phd*, which prevents host death when prophage is retained. *J Mol Biol* **233**, 414–428.
- Loh, S. M., Cram, D. S. & Skurray, R. A. (1988). Nucleotide sequence and transcriptional analysis of a third function (*Flm*) involved in F-plasmid maintenance. *Gene* **66**, 259–268.
- Maki, S., Takiguchi, S., Miki, T. & Horiuchi, T. (1992). Modulation of DNA supercoiling activity of *Escherichia coli* DNA gyrase by F plasmid proteins. Antagonistic actions of LetA (CcdA) and LetD (CcdB) proteins. *J Biol Chem* **267**, 12244–12251.
- Masuda, Y., Miyakawa, K., Nishimura, Y. & Ohtsubo, E. (1993). *chpA* and *chpB*, *Escherichia coli* chromosomal homologs of the

- pem* locus responsible for stable maintenance of plasmid R100. *J Bacteriol* 175, 6850–6856.
- Miki, T., Park, J. A., Nagao, K., Murayama, N. & Horiuchi, T. (1992). Control of segregation of chromosomal DNA by sex factor F in *Escherichia coli*. Mutants of DNA gyrase subunit A suppress *letD* (*ccdB*) product growth inhibition. *J Mol Biol* 225, 39–52.
- Moré, M. I., Pohlman, R. F. & Winans, S. C. (1996). Genes encoding the pKM101 conjugal mating pore are negatively regulated by the plasmid-encoded KorA and KorB proteins. *J Bacteriol* 178, 4392–4399.
- Motallebi-Veshareh, M., Balzer, D., Lanka, E., Jagura-Burdzy, G. & Thomas, C. M. (1992). Conjugal transfer functions of broad-host-range plasmid RK2 are coregulated with vegetative replication. *Mol Microbiol* 6, 907–920.
- Naito, T., Kusano, K. & Kobayashi, I. (1995). Selfish behavior of restriction-modification systems. *Science* 267, 897–899.
- Nielsen, A. K., Thorsted, P., Thisted, T., Wagner, E. G. & Gerdes, K. (1991). The rifampicin-inducible genes *srnB'* from F and *pnd* from R483 are regulated by antisense RNAs and mediate plasmid maintenance by killing of plasmid-free segregants. *Mol Microbiol* 5, 1961–1973.
- Nordström, K. & Austin, S. J. (1989). Mechanisms that contribute to the stable segregation of plasmids. *Annu Rev Genet* 23, 37–69.
- Ogura, T. & Hiraga, S. (1983). Mini-F plasmid genes that couple host cell division to plasmid proliferation. *Proc Natl Acad Sci USA* 80, 4784–4788.
- Onishi, Y. (1975). F factor promotes turnover of stable RNA in *Escherichia coli*. *Science* 187, 257–258.
- Ou, J. T. (1980). Role of surface exclusion genes in lethal zygosis in *Escherichia coli* K12 mating. *Mol Gen Genet* 178, 573–581.
- Pecota, D. C. & Wood, T. K. (1996). Exclusion of T4 phage by the *hok/sok* killer locus from plasmid R1. *J Bacteriol* 178, 2044–2050.
- Pohlman, R. F., Genetti, H. D. & Winans, S. C. (1994). Common ancestry between IncN conjugal transfer genes and macromolecular export systems of plant and animal pathogens. *Mol Microbiol* 14, 655–668.
- Poulsen, L. K., Larsen, N. W., Molin, S. & Andersson, P. (1989). A family of genes encoding a cell-killing function may be conserved in all gram-negative bacteria. *Mol Microbiol* 3, 1463–1472.
- Poulsen, L. K., Refn, A., Molin, S. & Andersson, P. (1991a). The *gef* gene from *Escherichia coli* is regulated at the level of translation. *Mol Microbiol* 5, 1639–1648.
- Poulsen, L. K., Refn, A., Molin, S. & Andersson, P. (1991b). Topographic analysis of the toxic Gef protein from *Escherichia coli*. *Mol Microbiol* 5, 1627–1637.
- Poulsen, L. K., Larsen, N. W., Molin, S. & Andersson, P. (1992). Analysis of an *Escherichia coli* mutant strain resistant to the cell-killing function encoded by the *gef* gene family. *Mol Microbiol* 6, 895–905.
- Roberts, R. C., Spangler, C. & Helinski, D. R. (1993). Characteristics and significance of DNA binding activity of plasmid stabilization protein ParD from the broad host-range plasmid RK2. *J Biol Chem* 268, 27109–27117.
- Roberts, R. C., Strom, A. R. & Helinski, D. R. (1994). The *parDE* operon of the broad-host-range plasmid RK2 specifies growth inhibition associated with plasmid loss. *J Mol Biol* 237, 35–51.
- Rodríguez, M. & Iyer, V. N. (1981). Killing of *Klebsiella pneumoniae* mediated by conjugation with bacteria carrying antibiotic-resistance plasmids of the group N. *Plasmid* 6, 141–147.
- Rodríguez, M., Holčík, M. & Iyer, V. N. (1995). Lethality and survival of *Klebsiella oxytoca* evoked by conjugative IncN group plasmids. *J Bacteriol* 177, 6352–6361.
- Rotheim, M. B., Love, B., Gill, S. & Iyer, V. N. (1991). Immunity: a plasmid-directed change in the *Klebsiella* genome. *Plasmid* 25, 155.
- Ruiz-Echevarría, M. J., Berzal-Herranz, A., Gerdes, K. & Díaz-Orejas, R. (1991a). The *kis* and *kid* genes of the *parD* maintenance system of plasmid R1 form an operon that is autoregulated at the level of transcription by the co-ordinated action of the Kis and Kid proteins. *Mol Microbiol* 5, 2685–2693.
- Ruiz-Echevarría, M. J., de Torrontegui, G., Giménez-Gallego, G. & Díaz-Orejas, R. (1991b). Structural and functional comparison between the stability systems ParD of plasmid R1 and Ccd of plasmid F. *Mol Gen Genet* 225, 355–362.
- Ruiz-Echevarría, M. J., de la Cueva, G. & Díaz-Orejas, R. (1995a). Translational coupling and limited degradation of a polycistronic messenger modulate differential gene expression in the *parD* stability system of plasmid R1. *Mol Gen Genet* 248, 599–609.
- Ruiz-Echevarría, M. J., Giménez-Gallego, G., Sabariego-Jareño, R. & Díaz-Orejas, R. (1995b). Kid, a small protein of the *parD* stability system of plasmid R1, is an inhibitor of DNA replication acting at the initiation of DNA synthesis. *J Mol Biol* 247, 568–577.
- Salmon, M. A., Van Melder, L., Bernard, P. & Couturier, M. (1994). The antidote and autoregulatory functions of the F plasmid CcdA protein: a genetic and biochemical survey. *Mol Gen Genet* 244, 530–538.
- Saltman, L. H., Kim, K. S. & Figurski, D. H. (1991). The *kilA* operon of promiscuous plasmid RK2: the use of a transducing phage (λ *pklA*-1) to determine the effects of the lethal *klaA* gene on *Escherichia coli* cells. *Mol Microbiol* 5, 2673–2683.
- Schreiner, H. C., Bechhofer, D. H., Pohlman, R. F., Young, C., Borden, P. A. & Figurski, D. H. (1985). Replication control in promiscuous plasmid RK2: *kil* and *kor* functions affect expression of the essential replication gene *trfA*. *J Bacteriol* 163, 228–237.
- Sia, E. A., Roberts, R. C., Easter, C., Helinski, D. R. & Figurski, D. H. (1995). Different relative importances of the *par* operons and the effect of conjugal transfer on the maintenance of intact promiscuous plasmid RK2. *J Bacteriol* 177, 2789–2797.
- Skurray, R. A. & Reeves, P. (1973). Physiology of *Escherichia coli* K-12 during conjugation: altered recipient cell functions associated with lethal zygosis. *J Bacteriol* 114, 11–17.
- Sonea, S. (1991). Bacterial evolution without speciation. In *Symbiosis as a Source of Evolutionary Innovation*, pp. 145–155. Edited by L. Margulis & R. Fester. Cambridge, MA & London: MIT Press.
- Stein, D. S., Kendall, K. J. & Cohen, S. N. (1989). Identification and analysis of transcriptional regulatory signals for the *kil* and *kor* loci of *Streptomyces* plasmid pIJ101. *J Bacteriol* 171, 5768–5775.
- Summers, D. K. & Sherratt, D. J. (1984). Multimerization of high copy number plasmids causes instability: ColE1 encodes a determinant essential for plasmid monomerization and stability. *Cell* 36, 1097–1103.
- Tai, J. T. & Cohen, S. N. (1993). The active form of the KorB protein encoded by the *Streptomyces* plasmid pIJ101 is a processed product that binds differentially to the two promoters it regulates. *J Bacteriol* 175, 6996–7005.
- Tai, J. T. & Cohen, S. N. (1994). Mutations that affect regulation of the *korB* gene of *Streptomyces lividans* plasmid pIJ101 alter plasmid transmission. *Mol Microbiol* 12, 31–39.
- Tam, J. E. & Kline, B. C. (1989a). Control of the *ccd* operon in plasmid F. *J Bacteriol* 171, 2353–2360.
- Tam, J. E. & Kline, B. C. (1989b). The F plasmid *ccd* autorepressor

is a complex of CcdA and CcdB proteins. *Mol Gen Genet* 219, 26–32.

Thatte, V., Gill, S. & Iyer, V. N. (1985). Regions on plasmid pCU1 required for the killing of *Klebsiella pneumoniae*. *J Bacteriol* 163, 1296–1299.

Thisted, T. & Gerdes, K. (1992). Mechanism of post-segregational killing by the *hok/sok* system of plasmid R1. Sok antisense RNA regulates *hok* gene expression indirectly through the overlapping *mok* gene. *J Mol Biol* 223, 41–54.

Thisted, T., Nielsen, A. K. & Gerdes, K. (1994a). Mechanism of post-segregational killing: translation of Hok, SrnB and Pnd mRNAs of plasmids R1, F and R483 is activated by 3'-end processing. *EMBO J* 13, 1950–1959.

Thisted, T., Sorensen, N. S., Wagner, E. G. & Gerdes, K. (1994b). Mechanism of post-segregational killing: Sok antisense RNA interacts with Hok mRNA via its 5'-end single-stranded leader and competes with the 3'-end of Hok mRNA for binding to the *mok* translational initiation region. *EMBO J* 13, 1960–1968.

Thisted, T., Sorensen, N. S. & Gerdes, K. (1995). Mechanism of post-segregational killing: secondary structure analysis of the entire Hok mRNA from plasmid R1 suggests a fold-back structure that prevents translation and antisense RNA binding. *J Mol Biol* 247, 859–873.

Thomas, C. M., Smith, C. A., Ibbotson, J. P., Johnston, L. & Wang, N. (1995). Evolution of the *korA-oriV* segment of promiscuous IncP plasmids. *Microbiology* 141, 1201–1210.

Thomson, V. J., Jovanovic, O. S., Pohlman, R. F., Chang, C. H. & Figurski, D. H. (1993). Structure, function, and regulation of the *kilB* locus of promiscuous plasmid RK2. *J Bacteriol* 175, 2423–2435.

Tian, Q. B., Ohnishi, M., Tabuchi, A. & Terawaki, Y. (1996). A new plasmid-encoded proteic killer gene system: cloning, sequencing, and analyzing *hig* locus of plasmid Rts1. *Biochem Biophys Res Commun* 220, 280–284.

Tsuchimoto, S., Ohtsubo, H. & Ohtsubo, E. (1988). Two genes, *pemK* and *pemI*, responsible for stable maintenance of resistance plasmid R100. *J Bacteriol* 170, 1461–1466.

Tsuchimoto, S., Nishimura, Y. & Ohtsubo, E. (1992). The stable maintenance system *pem* of plasmid R100: degradation of PemI protein may allow PemK protein to inhibit cell growth. *J Bacteriol* 174, 4205–4211.

Van Melderen, L., Bernard, P. & Couturier, M. (1994). Lon-dependent proteolysis of CcdA is the key control for activation of CcdB in plasmid-free segregant bacteria. *Mol Microbiol* 11, 1151–1157.

Williams, D. R. & Thomas, C. M. (1992). Active partitioning of bacterial plasmids. *J Gen Microbiol* 138, 1–16.

Winans, S. C. & Walker, G. C. (1985). Identification of pKM101-encoded loci specifying potentially lethal gene products. *J Bacteriol* 161, 417–424.

Young, C., Prince, A. S. & Figurski, D. H. (1985). *korA* function of promiscuous plasmid Rk2: an autorepressor that inhibits expression of host-lethal gene *kilA* and replication gene *trfA*. *Proc Natl Acad Sci USA* 82, 7374–7378.

Zaman, S., Richards, H. & Ward, J. (1992). Expression and characterisation of the *korB* gene product from the *Streptomyces lividans* plasmid pIJ101 in *Escherichia coli* and determination of its binding site on the *korB* and *kilB* promoters. *Nucleic Acids Res* 20, 3693–3700.