

Gene and cell survival: lessons from prokaryotic plasmid R1

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Plasmids are units of extrachromosomal genetic inheritance found in all kingdoms of life. They replicate autonomously and undergo stable propagation in their hosts. Despite their small size, plasmid replication and gene expression constitute a metabolic burden that compromises their stable maintenance in host cells. This pressure has driven the evolution of strategies to increase plasmid stability—a process accelerated by the ability of plasmids to transfer horizontally between cells and to exchange genetic material with their host and other resident episomal DNAs. These abilities drive the adaptability and diversity of plasmids and their host cells. Indeed, survival functions found in plasmids have chromosomal homologues that have an essential role in cellular responses to stress. An analysis of these functions in the prokaryotic plasmid R1, and of their intricate interrelationships, reveals remarkable overall similarities with other gene- and cell-survival strategies found within and beyond the prokaryotic world.

Keywords: plasmid R1; stability system; parD; toxin–antitoxin pair; RNA decay

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R1 architecture: a winning design for DNA survival

Plasmid R1 is a good example of how the need to be stably maintained in host cells shapes the architecture and building blocks of extrachromosomal elements. Four properties can be distinguished in R1, all of which contribute to plasmid survival: antibiotic-resistance genes, horizontal DNA transfer genes, stability systems and the basic replicon (Fig 1A; Womble & Rownd, 1988).

R1 carries ampicillin- (*bla*), chloramphenicol- (*cat*), kanamycin- (*aph*), streptomycin/spectinomycin- (*aad*) and sulfonamide- (*sul*) resistance genes. These are clustered in the R-determinant, which contains three insertion sequences and a transposon, Tn4. Insertion sequences are minimal transposable elements that encode functions involved in insertion events, and they are found in plasmids and in the chromosomes of prokaryotes, eukaryotes and archaea (Mahillon & Chandler, 1998). They mediate various DNA rearrangements,

including the integration of R1 into host chromosomes, which can also influence the transcription of neighbouring genes. Genes flanked by two identical insertion sequences become transposable elements; therefore, antibiotic-resistance genes in R1 can be mobilized as transposons in different combinations.

R1 can also move horizontally into plasmid-free cells by conjugation. To achieve this, R1 induces the synthesis of a pilus that attaches the donor to a recipient cell. One DNA strand of R1 is then cleaved at *oriT* (origin of transfer) and transferred through the pilus to the recipient cell, in which it is re-circularized and converted into double-stranded DNA. Most genes required for transfer are encoded in the *tra* operon. In R1, a regulatory network inhibits expression of this operon, limiting the frequency of conjugation to 1×10^{-3} per R1 copy. However, once transfer occurs, the absence of the limiting regulatory factors in recipient cells allows R1 to spread rapidly in dense bacterial populations (Pözlleitner *et al.*, 1997).

Resistance genes confer growth advantages to R1 hosts in niches in which antibiotics are present, and genetic mobility allows R1 to acquire and evolve these survival strategies (Frost *et al.*, 2005). However, few, if any, of these genes are under strong selection at all times and even if they were, their mobilization into the host chromosome would allow cell survival without the costs of maintaining the plasmid. Therefore, R1 has evolved additional functions to ensure that its survival does not depend on a single strategy, but rather on their additive effects. These functions lie outside the R-determinant. Therefore, loss of antibiotic resistance does not completely compromise plasmid survival.

Low copy number also contributes to R1 survival, minimizing the metabolic burden that the plasmid imposes on host cells. In *Escherichia coli*, the ratio between R1 and the *oriC* (origin of chromosomal replication) is approximately 1:1. Chromosomal replication requires the initiator protein DnaA; however, R1 encodes its own initiator factor, RepA. This protein is limiting for the replication of R1, and the plasmid keeps its copy number low by tightly regulating RepA synthesis. Furthermore, RepA uncouples R1 replication from the host cell cycle, a process largely regulated by DnaA (Nordström, 2006).

Apart from the *repA* gene, the basic replicon of R1 contains *oriR1* (origin of replication in plasmid R1) and two copy-number control genes, *copA* and *copB*. *RepA* can be transcribed from two promoters, *PrcopB* and *PrrrepA*. Weak transcription from *PrcopB* maintains the low R1 copy number. It generates a bicistronic transcript that

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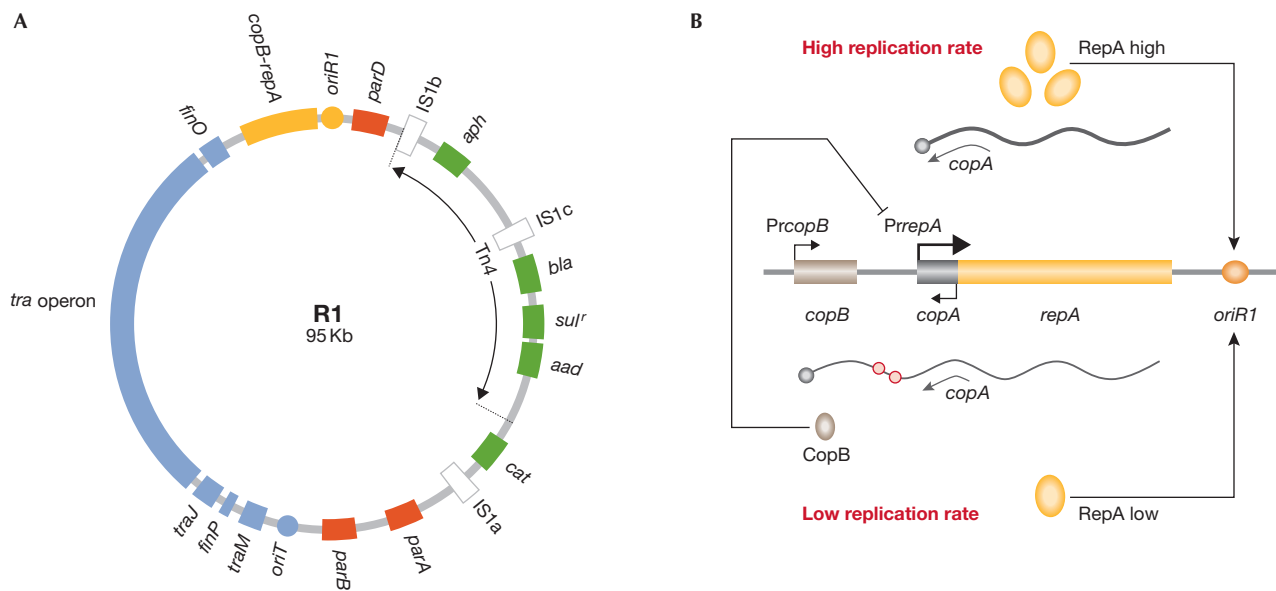


Fig 1 | R1 and copy-number control. (A) A map of R1 showing antibiotic resistance genes (green), insertion sequences (white), its basic replicon (yellow), conjugation genes (blue) and stability systems (red). (B) *PrcopB* produces some RepA as well as CopB, a repressor of *PrrepA*, which keeps R1 copy number low. In the absence of CopB, stronger *PrrepA* increases RepA and R1 copy number. Antisense RNA *copA* limits translation of RepA and is less effective when *PrrepA* is active. Red circles on RNA denote UUACU sites. *Cop*, copy-number control gene; *ori*, origin of replication; *Pr*, promoter; *Rep*, replication initiation factor.

encodes both CopB and RepA. CopB represses *PrrepA*, which then limits the production of RepA and the rate of R1 replication. The other gene, *copA*, encodes an antisense RNA that limits the translation of RepA, especially when *PrrepA* is fully repressed (Fig 1B; Nordström, 2006).

Copy number is a quantitative genetic trait of plasmids, but it fluctuates depending on the host and growth conditions. Indeed, the frequency of R1 replication is inversely proportional to its copy number. Control of RepA synthesis allows R1 to correct these fluctuations. This might be essential during conjugation, when a single copy of R1 is transferred into plasmid-free cells and needs to equal the number of *oriCs* rapidly in order to survive. This is possible because recipient cells lack CopB, allowing de-repression of *PrrepA* until R1 reaches its normal copy number.

Despite its benefits for plasmid survival, low copy number could reduce the chances of R1 being transmitted to daughter cells. To counteract this, the plasmid has evolved three stability systems: *parA*, *parB* and *parD*. *ParA* is a partition system that segregates copies of R1 to daughter cells. It is organized as a bicistronic operon that encodes proteins ParM (M for motor) and ParR (R for repressor). ParR binds to *parC*, an upstream *cis*-acting centromere-like region that also contains the promoter of *parA* (*PrparA*). Binding of ParR to *parC* generates a partition complex that represses transcription from *PrparA* *in vivo* and induces plasmid pairing *in vitro* (Fig 2A; Ebersbach & Gerdes, 2005).

ParM is an ATPase. When bound to ATP, it polymerizes into filaments that grow at an equal rate from each end. Hydrolysis of ATP within the filaments destabilizes the polymers, but polymerization and de-polymerization do not occur simultaneously. Filament growth occurs for some time before the process switches to unidirectional decay. This switch seems to be stochastic and, once

initiated, disintegration of ParM filaments proceeds to completion and new polymerization requires the rejuvenation of ParM-ADP by nucleotide exchange. Such dynamic instability is important for the search, capture and bipolar alignment of R1 copies. Capture occurs through (*parC*-*ParR*)-ParM interactions and requires paired R1 sister copies. It inhibits filament disintegration and stimulates polymer growth at the ParR-ParM interface, promoting constant growth of ParM filaments and pushing plasmids to opposite cell poles. Overall plasmid-filament contacts during partition are sustained by lateral interactions between parallel proto-filaments that grow asynchronously (Fig 2B). Although prolonged stability of ParM filaments requires pairing of sister copies of R1, segregation does not depend on plasmid replication *per se*, but on its outcome and location: it requires two paired R1 copies positioned at mid-cell. This regulates R1 segregation both temporally and spatially (Ebersbach & Gerdes, 2005, and references therein).

ParB is a post-segregational killing (PSK) system (Franch *et al*, 1997). It kills host cells that, despite all the efforts made by R1, still lose the plasmid during cell division. *ParB* encodes Hok (Host killing), a toxin that kills cells by damaging their membrane. Hok translation is coupled to the translation of upstream Mok (Mediator of killing) from a *mok-hok* messenger RNA. Folding of this mRNA hides the Shine-Dalgarno sequence of *mok* (*SDmok*) from ribosomes, which impedes the translation of both genes (Fig 3A). Partial 3'-processing of this transcript induces refolding, exposing *SDmok* to ribosomes, and enabling translation of Mok and Hok to trigger cell death. However, this is not possible in cells that contain R1 because of the action of *sok* (*suppressor of killing*), the third component of *parB*. *sok* is an antisense RNA complementary to *sokT*, a sequence downstream of *SDmok*. Different folding allows recognition of *sokT* by *sok* only

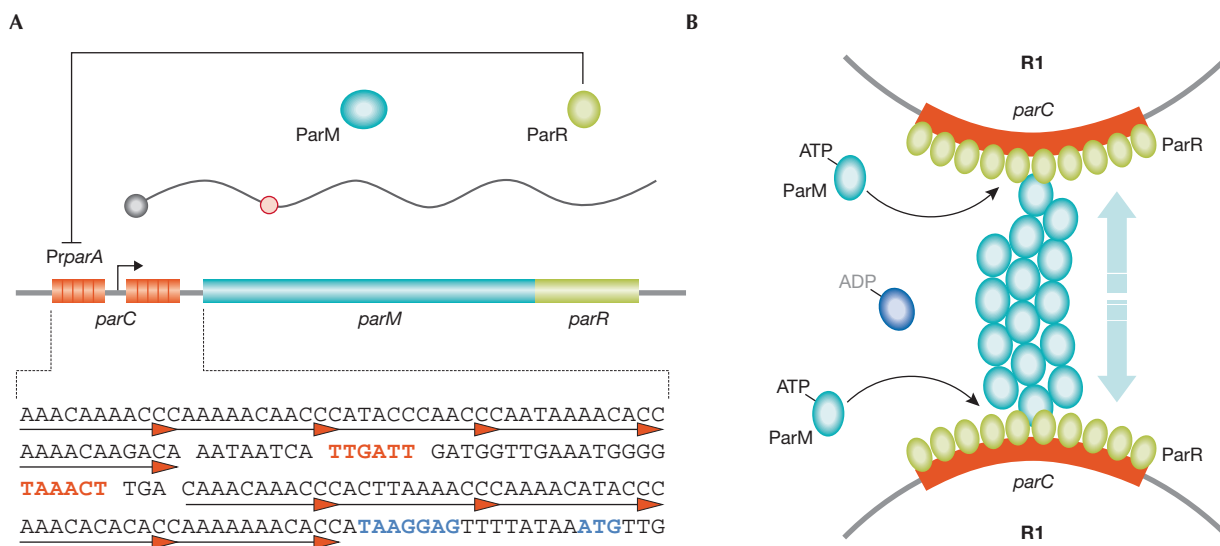


Fig 2 | *parA* and segregation of R1. (A) Transcription from *PrparA* generates ParM and ParR. Binding of ParR to direct repeats in *parC* (underlined) represses *PrparA*. Transcriptional (indicated in red text) and translational (indicated in blue text) signals of *parA* are embedded in *parC*. Red circles on RNA denote UUACU sites. (B) *parC*–ParR stimulates apical growth and increases the stability of ATPParM filaments, allowing segregation of paired copies of R1. Pr, promoter.

in the processed—that is, translatable—*mok-hok* transcript. This generates a dsRNA structure that is cleaved by RNase III, triggering rapid decay of the *mok-hok* mRNA and avoiding synthesis of Hok and cell killing. However, antisense *sok* RNA is unstable and only cells maintaining R1 are able to supply sufficient amounts to escape cell death (Fig 3B; Franch *et al*, 1997).

***parD*: 20 years of pre-segregational ‘kidding’**

parD is immediately downstream of *oriR1*. It encodes the toxin Kid (Killing determinant) and the antitoxin Kis (Killing suppressor; Bravo *et al*, 1988). Cells containing R1 synthesize both proteins, which form a complex that neutralizes Kid toxicity and represses transcription of *parD* (Fig 4A). Kis is less stable than Kid and, by analogy to *parB*, it was assumed that a constant supply of neutralizing amounts of Kis was only possible in plasmid-containing cells, suggesting that *parD* is also a PSK system. Despite this, experimental observations made since the discovery of *parD* 20 years ago do not fit well with its attributed biological role and mode of action. First, when compared with other PSK systems, *parD* seemed to be functionally inactive and contributed little to plasmid stability (Jensen *et al*, 1995). However, for reasons that were not understood at the time, *parD* seemed to ‘wake’ from its ‘dormant’ state when the replication of R1 was compromised (Ruiz-Echevarría *et al*, 1995a). Second, the replicative helicase in *E. coli*, DnaB, was proposed to be the cellular target of Kid (Ruiz-Echevarría *et al*, 1995b). Despite this, neither physical interaction between DnaB and Kid nor inhibition of DnaB activity by Kid could be shown. Indeed, this mode of action was difficult to reconcile with the activation of *parD* that is observed when R1 replication, which depends on DnaB, is compromised. Finally, attempts to eliminate *parD*⁺ plasmids from *E. coli* caused cell growth inhibition rather than cell killing. Intriguingly, arrested cells still contained the plasmid (Jensen *et al*, 1995), which is incompatible with a post-segregational activation of Kid.

Recently, we showed that Kid cleaves mRNA at 5′-UUACU-3′ sites, which shuts off host gene expression and stops cell growth (Pimentel *et al*, 2005). Activation of *parD* occurs when R1 copy number decreases but before it is lost from cells—that is, pre-segregationally—which allows the cleavage of plasmid-encoded *copB*–*repA* mRNA at two UUACU sites (Fig 1B). This decreases the levels of CopB, de-represses *PrrepA* and leads to a higher level of synthesis of RepA. Consequently, the replication rate of R1 increases and plasmid copy number recovers. Simultaneous cleavage of host-encoded mRNA arrests cell growth and avoids plasmid loss. These results explain how *parD* stabilizes plasmid R1 in bacteria. The stability of Kis, which is degraded by the host protease Lon, and the transcription of *parD*, which is not repressed by Kid alone, are functionally linked to sense R1 copy-number fluctuations. If a decrease in R1 copy number threatens plasmid stability, pre-segregational activation of Kid occurs. Cell-growth arrest prevents plasmid loss and greater amounts of RepA stimulate replication of R1 to restore its normal copy number. Furthermore, Kid alone cannot repress *PrparD* and, as one molecule of antitoxin neutralizes two molecules of toxin, synthesis of excess Kis from *parD* promotes re-neutralization of Kid while R1 recovers. Interestingly, *repA* and *kis* lack UUACU sites making them resistant to cleavage, and allowing copy-number rescue and Kid re-neutralization (Fig 4B; Pimentel *et al*, 2005).

Chromosomal homologues of *parD*: cell stress managers

ParD is a plasmid stress element. Copy-number fluctuations that threaten R1 stability activate Kid to eliminate the stress rather than killing host cells. Once this is achieved, Kid promotes its own re-neutralization and re-sets the stress element. Archaea and many bacteria encode stress elements that are functionally and structurally similar to *parD*. *E. coli* has five such elements, which enable cells to manage stressful growth conditions, such as starvation (Gerdes *et al*, 2005). Their similarities suggest that plasmid- and

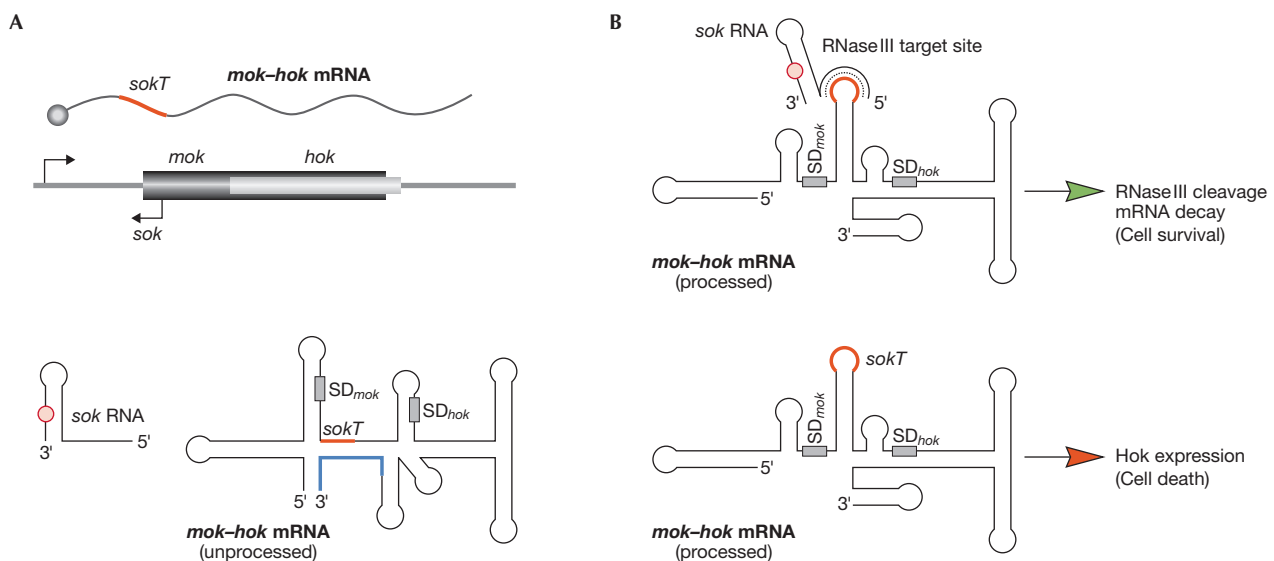


Fig 3 | *parB* and post-segregational killing. (A) *parB* encodes a messenger RNA-encoding toxin Hok (*mok-hok*) and a labile antisense RNA, *sok*. Folding of *mok-hok* mRNA impedes toxin production and hides the sequence *sokT* (red) in a double-stranded RNA structure. Partial processing of the 3' end (blue) of *mok-hok* mRNA induces refolding. (B) Refolded mRNA can produce Hok and exposes *sokT* in a single-stranded RNA loop. In cells containing R1, *sok-sokT* pairing induces rapid mRNA decay by RNase III. Instability of *sok* makes this impossible in cells that lose R1, which are then killed by Hok. Red circles on RNA denote UUACU sites. Hok, host killing; Mok, mediator of killing; SD, Shine–Dalgarno sequence; *sok*, suppressor of killing.

chromosomal-stress elements have co-evolved with the assistance of horizontal mobilization and DNA exchange, both of which occur in bacterial plasmids, such as R1, and in the archaeal world.

In *E. coli*, *relBE* reduces the global level of translation during amino-acid starvation. This stress activates RelE, which cleaves mRNA stop codons positioned at the ribosomal A site. Release factors (RF) cannot act on mRNA with disrupted stop codons; therefore, RelE traps translating ribosomes, cleaved mRNAs and unfinished polypeptides in inactive complexes. Instead of RFs, a specialized molecule—transfer-messenger RNA (tmRNA)—occupies the A site in these ribosomes. Through a process termed *trans-translation*, tmRNA promotes tagging of the unfinished proteins with a peptide that marks them for proteolysis. This frees and recycles stalled ribosomes and, by promoting degradation of trapped polypeptides, renews amino-acid pools, helping cells to relieve the stress and survive starvation (Pedersen *et al*, 2003).

Amino-acid starvation also induces *mazEF* in *E. coli*. MazF cleaves mRNA at ACA sites independently of ribosomes. The activities of RelE and MazF inhibit cell growth, and this can be reverted by their antitoxins, RelB and MazE. This suggests that, similar to *parD*, these systems are switched off after elimination of the stress (Gerdes *et al*, 2005).

Meta-regulating survival elements: a key to success

The presence of several stability functions in plasmid R1 raises interesting questions about how they coordinate their activities to maximize plasmid survival. The specificity of Kid explains how it interferes with the translation of *copB* but not of *repA* or *kis*, thereby allowing recovery of R1 copy number and re-neutralization of Kid. But *parD* also ensures that other cellular functions remain intact—for example, transcription, translation, replication and ATP regeneration, which

are essential for the correct functioning of the rescue system. Resistance or sensitivity of mRNAs to the action of Kid, as well as the stability of proteins produced before *parD* is activated, must have a role in this process. This is evident when the activity of Kid is examined in relation to the other stability functions in R1. *parA*, *parB* and *parD* act synergistically to reduce R1 loss to 1×10^{-7} per cell per generation, and to achieve this they must work in a coordinated and non-interfering manner.

For example, activation of *parD* must neither impede *parB*-mediated killing of cells that lose R1, nor induce *parB*-mediated killing in R1-containing cells. *mok-hok* mRNA lacks UUACU sites, so it is resistant to Kid and ensures that *parB* is ready to act if R1 is lost. Of equal importance, Kid does not degrade *sok* antisense-RNA, and so premature activation of *parB* is avoided. Interestingly, *sok* contains one UUACU site; however, it is positioned in a stable double-stranded RNA structure (Franch *et al*, 1997), and Kid only cleaves single-stranded RNA.

Similarly, *parA* and *parD* must function in a coordinated manner because conditions that activate *parD* are unfavourable for plasmid segregation. First, pairing of sister copies of R1 must be infrequent at low plasmid copy numbers, when Kid is active. Second, Kid inhibits host cell division and this impedes plasmid segregation before normal R1 copy number is restored. Therefore, *parA* cannot interfere with *parD* function. But can *parD* interfere with *parA*? *parA* contains one UUACU site and it is unknown whether Kid cleaves at this location. However, binding of ParR to *parC*, which is essential to generate the partition complex, inhibits transcription from *PrparA* (Ebersbach & Gerdes, 2005, and references therein). This suggests that ParR and ParM are relatively stable, as effective plasmid segregation does not require—indeed inhibits—their synthesis *de novo*. All this might help to synchronize *parD* and *parA*

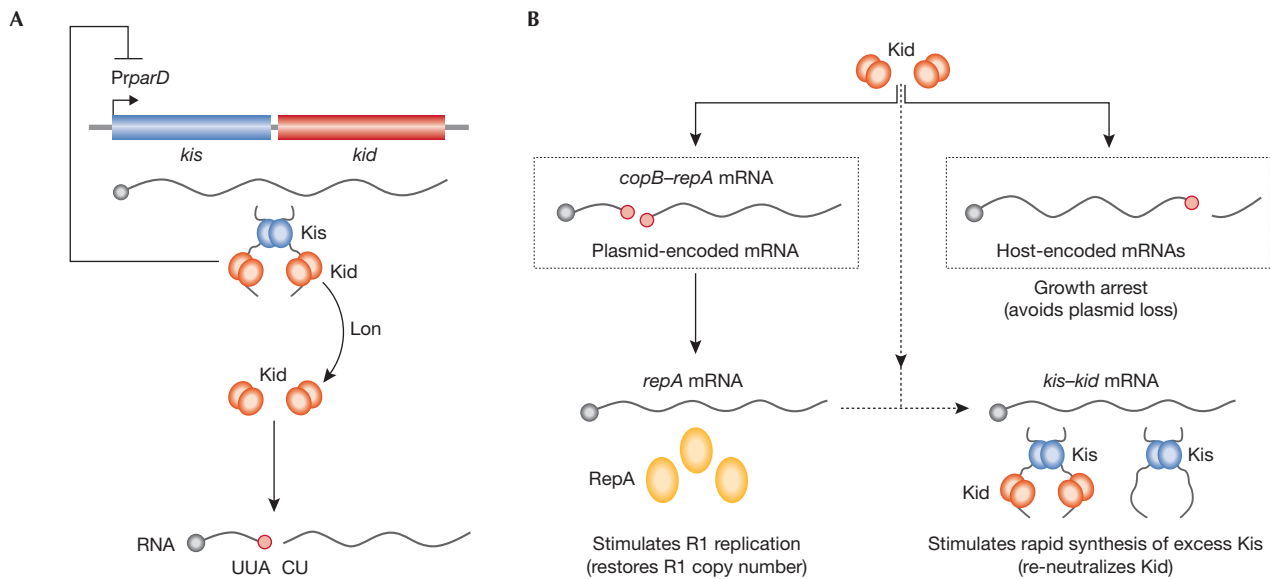


Fig 4 | *parD* and R1 rescue. (A) *parD* produces the toxin Kid and the antitoxin Kis, which assemble in a 2:1 complex that neutralizes Kid and represses *PrparD*. Lon degrades Kis, allowing Kid to cleave RNA at UUACU. (B) When R1 copy number decreases, Kid activates pre-segregationally and cleaves host- and plasmid-encoded messenger RNAs. This inhibits cell growth, avoiding plasmid loss, and restores R1 copy number. Kid alone cannot repress *PrparD*, enabling re-neutralization of Kid when R1 copy number recovers. Red circles on RNA denote UUACU sites. *Cop*, copy-number control gene; Kid, killing determinant; Kis, killing suppressor; Pr, promoter; Rep, replication initiation factor.

functions, allowing pairing of new R1 copies as they replicate after Kid activation but segregating them only when R1 copy number is restored and Kid is re-neutralized. It seems likely that a similar relationship contributing to plasmid survival must have evolved between *parD* and other functions in R1.

Meta-regulation is also important for the correct functioning of chromosomal stress elements. In *E. coli*, depletion of charged transfer RNAs during amino-acid starvation activates RelA to produce ppGpp (Guanosine-tetraphosphate). This bacterial ‘alarmone’ induces stringent control of RNA synthesis, which inhibits transcription of stable RNAs and stimulates that of amino-acid biosynthetic proteins and starvation regulatory factors. ppGpp also activates Lon, which degrades RelB, linking the stringent response to activation of RelE (Gerdes *et al.*, 2005). This protein competes with RFs to occupy the A site in the ribosome to cleave mRNA stop codons. Among these, RelE has a preference for UAG, the stop codon recognized by RF1. Conspicuously, RF1 ends at UAG. This might help to decrease the intracellular concentration of RF1 when RelE is active and favour RelE occupancy of the ribosomal A-site at UAG stop codons during starvation. Another protein, RF2, can terminate translation at UAA and UGA stop codons. Interestingly, the stop codon for RF2, RelB, RelE and Lon is UGA, which is the least sensitive to RelE. Furthermore, UAG is underrepresented in genes that are essential during the stringent response—for example, tmRNA and enzymes involved in amino-acid synthesis. As seen before for *kis* and *repA*, which lack UUACU, this feature might have been selected to facilitate the synthesis of essential proteins when RelE is active. This seems to be a general strategy, as MazF sustains transcription, translation—of mRNAs lacking ACA sites—and ATP regeneration *in vivo* over long periods of time (Suzuki *et al.*, 2005). Clearly, evolution

has shaped *E. coli* so that genes essential for surviving starvation are either resistant to the action of these endoribonucleases or encode stable proteins.

Functionally related survival strategies are physically linked on DNA, such as *parD* and *oriR1*, or *relA* and *mazEF* in some bacteria. Perhaps evolution has favoured these links, reflecting their meta-regulatory relevance. Interestingly, these types of stress elements are abundant in archaea—for example, 32 in *Sulfolobus tokodaii* (Gerdes *et al.*, 2005). Such density must have required the establishment of intricate regulatory networks, as well as functional and physical links, between these elements. If this is the case, it is worth noting that these relationships must have evolved in the eukaryotic-like molecular context that is inherent to archaea.

Concluding remarks

The metabolic burden that plasmids impose on host cells drives the evolution of strategies that work in a coordinated, non-interfering manner to maximize plasmid stability. The basic regulatory strategy used by plasmids to achieve this is autogenous control of gene expression. Operons encode at least an autorepressor, which ensures that gene expression is downregulated once a functional level of protein has been produced (Bingle & Thomas, 2001). This strategy has been adopted by R1 to control the expression of its survival functions—*copB-repA*, *parA*, *parD* and the *tra* operons. An additional level of control might be provided by coordinating the regulation of plasmid replication, transfer and stable maintenance genes, either by grouping them together in one operon or by physically associating their respective operons (Bingle & Thomas, 2001). This could facilitate co-regulation and contribute to the maintenance of physical links during plasmid evolution—for example, their co-acquisition by evolving

episomes. Such juxtaposition of functionally related survival roles is present in plasmid R1—that is, *parD* is located immediately downstream of the plasmid basic replicon. The relevance of this physical link was hidden by the traditional view of *parD* as a PSK system. Our understanding that Kid acts pre-segregationally and reversibly to rescue R1 copy number unveils the importance of this link and connects *parD* and the basic replicon of R1.

More sophisticated than simple juxtaposition is the coordinated regulation of survival functions found in some Gram-positive (pSM19035) and Gram-negative (RK2) plasmids (Bingle & Thomas, 2001, and references therein). In pSM19035, a small repressor (ω) is encoded in the same operon as antitoxin ϵ and toxin ζ . Interestingly, ω binds to specific sequences found in the promoters of distant genes involved in plasmid copy number control (*copS*) and plasmid partition (δ), and regulates their transcription. Furthermore, ω represses its own transcription and therefore that of antitoxin ϵ and toxin ζ , components of the PSK system in pSM19035 (Zielenkiewicz & Ceglowski, 2005). In RK2, the partition proteins IncC and KorB are encoded in a central control operon that also encodes KorA. KorB binds to 12 centromere-like DNA sequences scattered throughout the plasmid backbone. IncC is an ATPase that recognizes KorB–DNA complexes, interacts with them and directs active segregation of RK2 copies during cell division. Six of those centromeric-DNA sequences are located at, or close to, promoters for genes involved in plasmid replication (*trfA*), stable inheritance (*kle* and *krfA*) and conjugative transfer (*trbB* and *traG*). Binding of KorB to DNA represses transcription of these genes. To do so, KorB normally cooperates with two other repressors: KorA, also part of the central control operon, and TrbA. This ability to cooperate facilitates strong transcriptional repression without the need to increase repressor concentration and allows the entire system to be finely balanced (Bingle & Thomas, 2001, and references therein).

Plasmid R1 does not seem to follow global regulatory strategies such as those present in pSM19035 and RK2. However, the expression and activities of some of its survival genes are intimately coordinated to sense and correct negative copy-number fluctuations. Activation of *parD* switches on a programme that influences expression of survival genes both negatively (CopB) and positively (RepA and Kis). Several regulatory loops link the activities of Kid and Kis to plasmid copy number and its control. These involve the transcriptional regulation of survival genes *repA* by CopB and *parD* by Kid and Kis; the relative stability of survival proteins—for example, Kis compared with Kid—and its impact on the stoichiometry of the complexes they can form; differential sensitivity of mRNAs transcribed at survival operons to Kid, such as *copB–repA*, *repA*, *kis–kid*; and the consequences of the latter for *de novo* synthesis of survival proteins, RepA and Kis, and, ultimately, for the replication rate of R1, which is limited by RepA availability (Pimentel *et al*, 2005). Similar regulatory loops must have evolved between *parD* and other R1 and host genes, as Kid does not interfere with plasmid stability and cell viability despite its toxic activity.

Our understanding of *parD* function raises interesting questions about the roles attributed to other plasmid-encoded toxin–antitoxin (TA) protein pairs. The experimental set-up traditionally used to analyse the activities of TA pairs and their potential role in PSK might not be ideal. Promoters and/or replicons different from those naturally associated with the TAs are frequently used (Jensen *et al*, 1995; Deane & Rawlings, 2004). Clearly, in the case of *parD* and R1, such an approach would lead to inaccurate conclusions.

Therefore, it might be worth re-examining if other TA pairs exert similar roles to *parD*.

Members of all eight known families of TA pairs can be found in plasmids (Gerdes *et al*, 2005), and some plasmids encode more than one pair (Moritz & Hergenrother, 2007). Similar to *parD*, other TA pairs, such as *ccdAB* (controlled cell death) and *pasAB* (plasmid addiction system), are physically linked to—and even embedded into—the basic replicon of the plasmids that bear them (Ogura & Hiraga, 1983; Deane & Rawlings, 2004). There is evidence suggesting that the activation of some toxins, such as CcdB, can occur pre-segregationally, exerts a cytostatic effect, forces a large proportion of affected cells to retain plasmids and can be re-neutralized by their cognate antitoxin (Ogura & Hiraga, 1983; Jaffé *et al*, 1985; Maki *et al*, 1992; Bahassi *et al*, 1999). This indicates that these might be important features of the function of these plasmid-encoded TA pairs.

The modes of action of TA pairs are diverse and in many cases unknown (Gerdes *et al*, 2005). Acting as a plasmid rescue system might not be an exclusive attribute of *parD*, and might not even be limited to TA pairs encoding ribonucleolytic toxins. For example, CcdB of plasmid F and ParE of RK2 are gyrase inhibitors (Bahassi *et al*, 1999; Jiang *et al*, 2002). Interestingly, the activity of CcdB stimulates expression of chaperones DnaK and DnaJ and relaxes plasmid DNA (Maki *et al*, 1992; Kaneko *et al*, 1996). The limiting factor for replication of plasmid F—and RK2—is not the availability of the initiator protein but the ratio between its dimeric—inactive for replication—and monomeric—active for replication—forms. Notably, it has been shown that increasing negative superhelicity promotes initiation of this type of replication, and that DnaK and DnaJ are essential for the conversion of dimeric initiators into monomeric ones (Chattoraj, 2000, and references therein; Zzaman & Bastia, 2005). Therefore, it might be possible that the activity of CcdB (and ParE) helps to rescue the copy number of plasmid F (and RK2). Indeed, *ccd* activation seems to occur when plasmid F copy number decreases (Ogura & Hiraga, 1983). If this is the case, it would still be possible that pre- (rescuing) and post-segregational (killing) activities of plasmid-encoded TA pairs are not mutually exclusive.

R1 has evolved different survival functions, each of them operating at specific levels—for example, replication, partition and PSK. Despite their particular levels of action, all of them must function in a coordinated, non-interfering, manner to optimize plasmid, and host, survival. This need for coordination has an important role in the evolution of these systems, and might be even more important in the case of plasmids that contain more than one survival system of the same type (Moritz & Hergenrother, 2007). The same applies to plasmids that share host cells with other resident episomes. Therefore, it would be interesting to analyse whether the specific arsenal of survival functions in one plasmid influences the evolution and function of those found in co-resident plasmids. This might be the case, as at least one example has been reported in which the activity of a plasmid-encoded survival function depends on the presence of another—in this case host-encoded—system of the same type (Hazan *et al*, 2001).

Despite their prokaryotic origin, it is remarkable how R1 survival strategies resemble others found in eukaryotes. For example, the control of plasmid copy number and DNA mobilization into host chromosomes, and how these two events modulate episomal, and host cell, gene expression, are important for the stability of human papilloma virus, the survival of its infected cells and, of course, the aetiology of human cancer (Longworth & Laimins, 2004).

Similarly, it is remarkable how R1 has evolved systems that—similar to eukaryotic checkpoints—act before (*parD*), during (*parA*) and after (*parB*) cell division to guard DNA stability and integrity. It is even more striking how failure to achieve this activates a programme that triggers bacterial cell death, as with apoptosis in eukaryotes.

Bacterial and archaeal chromosomes encode survival systems that are similar in function and structure to those in R1. They have specialized in cell—rather than DNA—survival under nutritional starvation, and their activity is exquisitely regulated to avoid interference with other metabolic functions that are essential for this process. Their presence in bacteria and archaea suggests that this is possible in metabolic contexts that resemble both prokaryotes and eukaryotes. It is surprising how the overall function and consequences of these bacterial systems resemble those of autophagy—the eukaryotic strategy to survive cell starvation. This resemblance extends to the molecular arena. Some bacterial and archaeal stress elements contain PIN domains (Arcus *et al*, 2005), which are homologous to those found in eukaryotic ribonucleases that are linked to RNA interference and nonsense-mediated RNA decay—a quality-control system that degrades potentially toxic aberrant mRNAs.

We wonder whether these strategies evolved from common ancestors or whether it is simply that nature can only find a handful of similar solutions to a particular problem. Until these questions are answered, we can at least admire the precision with which evolution has sculpted R1 and its host cells to survive under stress.

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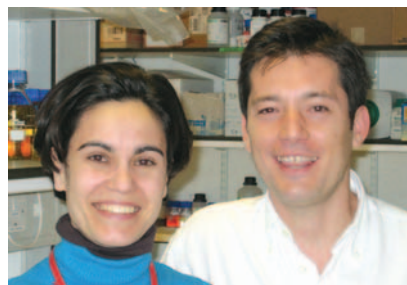
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