View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Apollo

Plasmid 78 (2015) 59–64

Contents lists available at ScienceDirect

Plasmid

journal homepage: www.elsevier.com/locate/yplas

Plasmids in the driving seat: The regulatory RNA Rcd gives plasmid ColE1 control over division and growth of its *E. coli* host

Hannah Gaimster [1,*], David Summers

Department of Genetics, Downing Site, University of Cambridge, Cambridge CB2 3EH, United Kingdom

ARTICLE INFO

Article history:
Available online 11 November 2014
Communicated by Manuel Espinosa

Keywords:
Plasmid maintenance
Regulatory RNA
Cell division checkpoint
Signalling
Indole

ABSTRACT

Regulation by non-coding RNAs was found to be widespread among plasmids and other mobile elements of bacteria well before its ubiquity in the eukaryotic world was suspected. As an increasing number of examples was characterised, a common mechanism began to emerge. Non-coding RNAs, such as CopA and Sok from plasmid R1, or RNAI from ColE1, exerted regulation by refolding the secondary structures of their target RNAs or modifying their translation. One regulatory RNA that seemed to swim against the tide was Rcd, encoded within the multimer resolution site of ColE1. Required for high fidelity maintenance of the plasmid in recombination-proficient hosts, Rcd was found to have a protein target, elevating indole production by stimulating tryptophanase. Rcd production is upregulated in dimer-containing cells and the consequent increase in indole is part of the response to the rapid accumulation of dimers by over-replication (known as the dimer catastrophe). It is proposed that indole simultaneously inhibits cell division and plasmid replication, stopping the catastrophe and allowing time for the resolution of dimers to monomers. The idea of a plasmid-mediated cell division checkpoint, proposed but then discarded in the 1980s, appears to be enjoying a revival.

© 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

If the ambition of every bacterium is to become two bacteria (Jacob, 1974), then the ambition of every plasmid is to make sure it gets into both of them. To persist in a cell population a plasmid must minimise the probability of loss at division and neutralise the growth advantage that any plasmid-free cells are likely to enjoy. This short review focuses on the role of one regulatory RNA, Rcd, in multicopy plasmid maintenance. However, to set the story in context, we should review briefly the mechanisms of action of the multiple small, non-coding RNAs involved in the control of plasmid replication, conjugation and host-killing. Their targets are typically *cis*-encoded complementary RNAs so these regulators are sometimes called anti-sense RNAs. By binding to their targets they alter function by changing higher-order RNA structure or regulating translation (reviewed by Brantl, 2007).

2. Creating the paradigm of plasmid regulatory RNAs

Keeping copy number high enough to ensure that plasmids are delivered to both daughters at cell division, but not so high that it imposes an unnecessary load on the host, is particularly important for multicopy plasmids. ColE1 provides a well characterised example of copy number control by a small RNA (Cesareni et al., 1991). An RNA pre-primer

* Corresponding author. School of Biology, University of East Anglia, Norwich, Norfolk NR4 7TJ, United Kingdom. Tel.: +01 223 333991.
E-mail address: h.gaimster@uea.ac.uk (H. Gaimster).
[1] Present address: School of Biology, University of East Anglia, Norwich, Norfolk, NR4 7TJ.

http://dx.doi.org/10.1016/j.plasmid.2014.11.002
0147-619X/© 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

(RNAII) transcribed from a constitutive promoter 555 bp upstream of the origin of replication folds into a complex secondary structure with multiple stem-loops (Masukata and Tomizawa, 1986). As an essential prelude to replication initiation, RNAII forms a stable complex with DNA at the origin of replication and the primer for leading strand replication is created by RNaseH cleavage of the RNA strand in the complex (Itoh and Tomizawa, 1980). Primer formation, and hence replication initiation, is regulated by small, non-coding counter-transcript (RNAI) that is expressed from a constitutive promoter 445 bp upstream of the origin of replication and on the opposite strand to RNAII (Tomizawa and Itoh, 1981). RNAI is therefore perfectly complementary to RNAII. When RNAI binds RNAII the secondary structure of the pre-primer is changed and it can no longer form the stable complex with DNA at the origin (Masukata and Tomizawa, 1986). In the absence of the complex, RNaseH cannot cleave RNAII to form a primer, so replication cannot initiate.

At the heart of ColE1 replication control is the ability of a small regulatory RNA to change the higher-order folding, and hence the function, of a relatively large RNA target. The mechanism is reiterated in pT181, a small multicopy Staphylococcal plasmid (Novick, 1989). In this case initiation is triggered by the RepC protein nicking the origin DNA and regulation of initiation is at the level of transcription of *repC* mRNA. Binding of an antisense regulatory transcript to the nascent mRNA alters its secondary structure, leading to the formation of a transcription terminator and attenuation of *rep* gene transcription. A third example of this common mechanism is provided by the low copy number plasmid RI where replication is triggered by the RepA protein. A small, unstable RNA (CopA) inhibits replication by binding to a complementary region in the *repA* mRNA, inhibiting the translation of RepA (Nordström et al., 1984). Here again functionality depends upon altered target folding although this time the system operates through the mechanism of translational coupling where CopA regulates the translation of an ORF (Tap) that is translationally linked to RepA (Blomberg et al., 1992, 1994).

Elsewhere on plasmid R1 we find a remarkably similar regulatory RNA mechanism controlling a host killing system The *parB* locus of R1 is comprised of three genes: *hok, mok* and *sok* (Gerdes et al., 1988). The *hok* (host killing) gene encodes a protein toxin whose expression is regulated at the level of translation by the anti-toxin Sok (suppression of killing), a small anti-sense RNA complementary to the 5 end of *hok–mok* mRNA. Finally, *mok* (modulation of killing) provides the regulatory link between the toxin and anti-toxin components.

The molecular mechanism behind the operation of Hok–Sok is surprisingly complex (Franch and Gerdes, 1996) but at its heart is the contrast between the stability of *hok* mRNA and the instability of Sok. In plasmid-containing cells, Sok is present at high concentrations and by complementary base pairing to *hok–mok* mRNA it prevents translation of the Mok ORF. Hok is translationally coupled to Mok so there is no expression of the Hok toxin either. Following plasmid loss the Sok RNA concentration falls rapidly, allowing translation of *hok* mRNA and killing of the plasmid-free cell by the Hok protein.

3. Challenging the paradigm

The discovery of these and many other regulatory systems during the heyday of plasmid biology in the 1980s and 1990s revealed the important role of regulatory RNAs long before their ubiquity was recognised in eukaryotes. They seemed to share a simple mechanism of action, exerting their effects through interactions with RNA targets with specificity provided by complementary base pairing. Proteins were not major players in these systems, although they were not entirely absent and in several cases they assisted the key RNA–RNA interactions. In ColE1, for example, the Rom (or Rop) protein was identified as a secondary repressor of replication (Cesareni et al., 1982; Twigg and Sherratt, 1980). Although thought initially to regulate transcription of the rom gene, it was shown subsequently to increase the rate of binding of the RNA I repressor to RNA II (Tomizawa and Som, 1984) by interacting with the double stranded stems of inhibitor and target (Helmer-Citterich et al., 1988). In a similar way, the FinO protein acts as a secondary repressor of F plasmid conjugative transfer. Again, its role is indirect and repression is exerted by stabilising the small regulatory transcript FinP and assisting its interaction with its target, *traJ* mRNA (Lee et al., 1992; van Biesen and Frost, 1994).

In the early 1990s, it was reported that a small regulatory RNA (Rcd) was encoded within the multimer resolution site of ColE1 (Patient and Summers, 1993) and that knocking out its promoter (P_{cer}) was detrimental to plasmid maintenance. Following the precedent established by the many plasmid regulatory RNAs described previously, it seemed likely that Rcd would exert its effect by interaction with a larger RNA target. However, Rcd was to challenge the antisense RNA paradigm as its primary target was shown eventually to be a protein rather than an RNA.

4. The role of Rcd in plasmid stability

The discovery of Rcd arose from studies of plasmid dimers and their effect on the stability of ColE1-like plasmids. In the absence of active partitioning, multicopy plasmids must maintain a high copy number in dividing cells to ensure distribution to both daughters. Anything that reduces the number of independent plasmids in a cell presents a threat to plasmid stability; for example non-random distribution or clustering of plasmids (Yao et al., 2007). Plasmid dimerisation also reduces copy number (Chiang and Bremer, 1988) and has long been known to correlate with instability (Summers and Sherratt, 1984).

Plasmid dimers form at low frequency by homologous recombination between monomers and even a relatively small proportion of dimers has a large detrimental effect on plasmid stability (Summers and Sherratt, 1984). The reason for this was unclear until a combination of computer modelling and experimental work led the formulation of the dimer catastrophe hypothesis (Summers et al., 1993) which explained that dimers were clustered in relatively few cells, rather than being distributed through the cell population. At the heart of hypothesis was the trivial observation that plasmid dimers have two replication origins in each molecule. Crucially these origins fire independently so a

dimer replicates twice as often as a monomer, causing dimers to accumulate rapidly and clonally in the descendants of the cell in which a single dimer has arisen by recombination. Dimer-only cells appear just a few generations after formation of the first dimer and these cells have a depressed copy number and a very much higher probability of producing plasmid-free segregants. This process seems to have the potential to run away; tetramers should out-replicate dimers, octamers should out-replicate tetramers etc. However this is balanced by a growth disadvantage for multimer-containing cells that eventually brings the system to equilibrium.

The dimer catastrophe is bad news for both plasmid ColE1 and its bacterial host. An increased loss rate is an obvious problem for the plasmid but it also represents a direct threat to fitness of its bacterial host since plasmid-free cells are killed by exogenous colicin (Cascales et al., 2007). Recent reanalysis of the dimer catastrophe hypothesis (Field and Summers, 2011) confirmed the detrimental effect of plasmid loss but highlighted the reduced growth rate of dimer-containing cells as an even more serious threat to host fitness.

In response to the threat posed by the dimer catastrophe, site-specific recombination is employed to resolve plasmid multimers to monomers. The Xer–*cer* multimer resolution system of plasmid ColE1 has been studied in detail (Blakely et al., 1993; Colloms et al., 1990; Stirling et al., 1988, 1989; Summers and Sherratt, 1984). The plasmid and host cooperate in this process because, although the *cer* recombination site is of necessity on the plasmid, four essential proteins (XerC, XerD, ArgR and PepA) are all encoded on the host chromosome. At first sight Xer–*cer* recombination appears to offer a complete solution to the problem of plasmid multimers but this was called into question when the discovery of Rcd showed that although dimer resolution is necessary, it is not sufficient to ensure plasmid high fidelity maintenance of ColE1 (Patient and Summers, 1993). The reason is that it is not sufficient to possess a mechanism of dimer eradication but that the timing of their eradication is also crucial (Summers, 1998). Specifically it must be complete before the cell divides as this is when the plasmid can potentially be lost by the cell. The role of the Rcd transcript appears to coordinate dimer resolution and cell division.

5. The control of Rcd synthesis

Rcd is transcribed from a promoter, P_{cer}, encoded within the ColE1 multimer resolution site (Summers and Sherratt, 1988) and mutations in the P_{cer} promoter or the *rcd* coding region (Balding et al., 2006) reduce plasmid stability without affecting dimer resolution by XerCD. P_{cer} is up-regulated in cells containing plasmid multimers (Patient and Summers, 1993) so the transcript is produced in cells undergoing a dimer catastrophe. It was observed that cells which over express Rcd arrest their cell cycle prior to division, leading to the hypothesis that Rcd (regulator of cell division) was a component of a checkpoint that prevents division of cells suffering a catastrophe (Patient and Summers, 1993).

It is not immediately obvious how a promoter can detect whether it is in a plasmid monomer or dimer. However, one possibility is that *cer* sites interact more often and more stably in a dimer than sites in two monomers (Summers, 1998). Thus persistent *cer*–*cer* interactions indicate the presence of dimers. Blaby and Summers (2009) proposed a model of P_{cer} control that suggested P_{cer} is inactive in the nucleoprotein complex that assembles at an individual *cer* site in a plasmid monomer because it is repressed by proximity of the XerCD recombinase. However, when the two sites in a plasmid dimer interact, the nucleoprotein complex isomerises removing XerCD from one of the P_{cer} promoters and inducing Rcd transcription. However, whilst plausible, this model still lacks experimental testing.

6. The target of Rcd: Tryptophanase and indole production

When the role of Rcd in plasmid stability was first described, the fact that its size and predicted secondary structure (Balding et al., 2006) were similar to many plasmid-encoded regulatory RNAs suggested that it would most likely follow their example and target a complementary RNA. However, extensive sequence analysis and hybridisation assays failed to identify a convincing antisense target and raised the possibility that Rcd might interact with a protein.

More than a decade after it was first described, RNA affinity chromatography identified tryptophanase as an Rcd binding protein (Chant and Summers, 2007). Tryptophanase converts tryptophan to indole, pyruvate and ammonia (Newton and Snell, 1964). In the presence of Rcd the affinity of tryptophanase for tryptophan is increased by approximately fivefold, which stimulates indole production. Over the range 3–5 mM indole was shown to exert significant inhibition of cell growth and division (Chant and Summers, 2007) suggesting that an increased indole concentration is the mechanism by which Rcd prevents the division of cells containing plasmid dimers.

The mechanism by which indole inhibits *E. coli* cell division has recently been elucidated. Indole acts as an ionophore to reduce the proton gradient across the cytoplasmic membrane. This prevents MinD oscillation, which in turn disrupts the proper localisation of FtsZ, inhibiting septum formation (Chimerel et al., 2012). This is the first example of a natural ionophore regulating a fundamental biological process.

7. The indole concentration paradox

A potentially serious criticism of the suggestion that Rcd inhibits cell division by stimulating indole production is that the experimental inhibition of *E. coli* cell division requires the addition of 3–5 mM indole to a culture supernatant. This is approximately 10 times the maximum concentration of indole that is detected in culture supernatants of *E. coli* grown in LB medium under standard laboratory conditions (approximately 0.5 mM (Li and Young, 2013)). Unsurprisingly the biological relevance of higher indole concentrations has been questioned.

A possible solution to the concentration paradox has come from recent measurements of indole production during

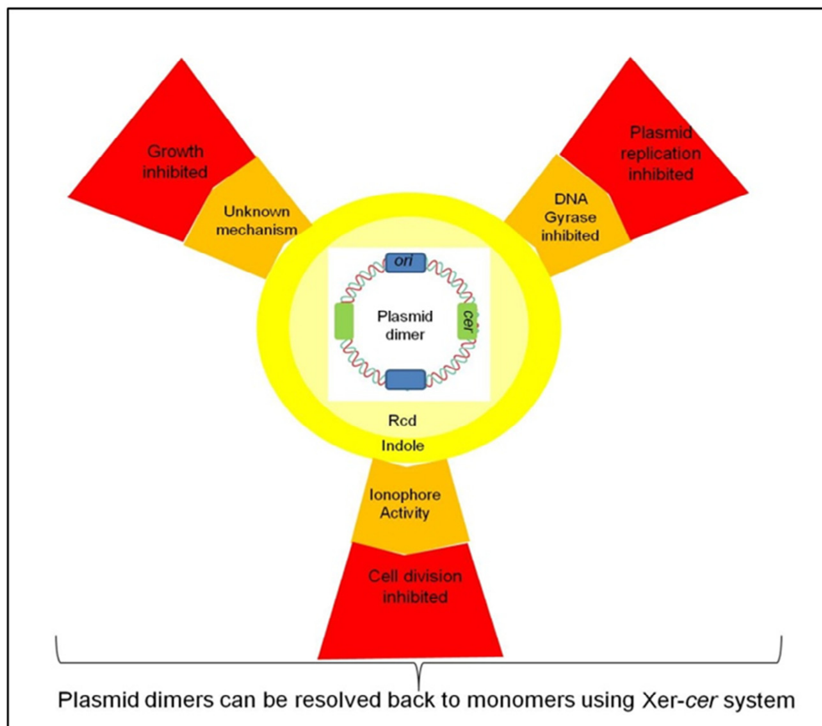

Fig. 1. Red-mediated response to the ColE1 dimer catastrophe. Plasmid dimers express the non-coding RNA, Red, from the cer site. Red binds to tryptophanase increasing the production of indole. High levels of indole inhibit plasmid replication, cell division and growth, allowing the Xer–cer recombination system to resolve plasmid dimers back to monomers.

stationary phase entry by plasmid-free cells (Gaimster et al., 2014). It was shown that indole production rate peaked rapidly as cells entered stationary phase and this led to a high but transient concentration of cell-associated indole similar to those resulting from the addition of 4–5 mM indole to the culture supernatant, which is sufficient to inhibit both cell division and growth. It is therefore possible that tryptophanase stimulation by Rcd during a dimer catastrophe raises cell-associated indole to a level sufficient to block cell division.

It should be emphasised that the elevation of cell-associated indole during stationary phase is achieved by a different mechanism from that proposed for Rcd. During stationary phase entry indole production is stimulated by the up-regulation of tryptophanase expression (Gaimster and Summers, in preparation) while Rcd achieves its effect by stimulating the action of existing enzyme. It remains to be shown experimentally that tryptophanase stimulation by Rcd can result in a pulse of cell-associated indole similar to that seen during stationary phase entry.

8. Beyond cell division: The effects of Rcd and indole on growth and plasmid replication

Reanalysis of the dimer catastrophe theory in 2011 suggested that simply preventing the division of dimer-containing cells would be insufficient to contain the dimer catastrophe, as dimers would continue to out-replicate monomers in a non-dividing cell (Field and Summers, 2011). This led to speculation that the Rcd checkpoint, and therefore indole, might inhibit plasmid replication as well as cell division. Subsequent investigation supported this idea and it was shown that 3–5 mM indole inhibits plasmid replication *in vivo*, probably through the inhibition of DNA gyrase (Field and Summers, 2012). Finally it is worth noting that indole inhibits the growth of cells as well as their division. Thus cells exposed to 5 mM indole for 2 hours appear frozen until the indole is removed and growth and division resumes (Chimerel et al., 2012).

It is striking that Rcd stimulation of indole production is responsible for the inhibition of three key processes: cell division, plasmid replication and cell growth. In the absence of evidence, Occam's razor might have encouraged the view that one common mechanism underpins all three. However, it is clear that the mechanisms of by which plasmid replication and cell division are inhibited are radically different; for one the target of indole is a protein but for the other it is the cytoplasmic membrane. This makes it difficult to speculate on the mechanism by which indole inhibits growth. One possibility is that making the membrane permeable to protons down-regulates oxidative phosphorylation and that the resulting ATP depletion inhibits growth. However preliminary experiments (Field and Summers, unpublished) have shown that the ATP concentration in cells treated with 5 mM indole declines only slowly while growth inhibition is immediate.

9. Back to the future: Plasmid control of cell division is rehabilitated

Shunning a conventional anti-sense target, the mechanism of action proposed for Rcd is strikingly different from that of most regulatory RNAs in plasmid biology (the hypothetical mechanism is summarised in Fig. 1). Interestingly, the idea that a plasmid might regulate division of its host is not new. In the 1980s it was proposed that the *ccd* (coupled cell division) system of plasmid F to blocked division of cells containing a single plasmid in order to prevent the formation of a plasmid-free daughter (Miki et al., 1984; Ogura and Hiraga, 1983). This idea was abandoned when it was found that a cell containing a single plasmid remained capable of division (Hiraga et al., 1986; Jaffe et al., 1985). The mechanism was reinterpreted as one of post-segregational killing, with the CcdB toxin becoming active in plasmid-free cells where it binds DNA gyrase and leads to the introduction of double strand breaks in the DNA. However in recent years plasmid-mediated cell division control has been making a come back. In addition to Rcd and the response of multicopy plasmid ColE1 to the dimer catastrophe, the idea has also been revisited in studies of the low copy number plasmid R1.

Besides a well-characterised toxin–antitoxin system (*hok–sok*), R1 encodes a plasmid rescue system *kis–kid* (Bravo et al., 1987). The toxin component, Kid, is activated in cells which are under threat of loosing the plasmid through copy number depression (Ruiz-Echevarría et al., 1995). It has been shown recently that Kid activation results in inhibition of cell division and stimulation of DNA replication, preventing plasmid loss and increasing its copy number (Pimentel et al., 2014). The mechanism of cell division inhibition differs from the Rcd-indole system and Kid directs cleavage of mRNA of two proteins, FtsZ and ZapA, involved in cell division.

With examples now emerging in both high and low copy number plasmids, it is clear that plasmids are no longer passive passengers but are once again back in the driving seat, controlling the most fundamental processes of their host cell.

Acknowledgments

HG was funded by a BBSRC DTG studentship, http://www.bbsrc.ac.uk/home/home.aspx, grant number PCAG-EJNF.

References

Balding, C., et al., 2006. A mutational analysis of the ColE1-encoded cell cycle regulator Rcd confirms its role in plasmid stability. Plasmid 56, 68–73.

Blaby, I.K., Summers, D.K., 2009. The role of FIS in the Rcd checkpoint and stable maintenance of plasmid ColE1. Microbiology 155, 2676–2682.

Blakely, G., et al., 1993. 2 related recombinases are required for site-specific recombination at *dif* and *cer* in *Escherichia coli* K12. Cell 75, 351–361.

Blomberg, P., et al., 1992. Replication control of plasmid R1: RepA synthesis is regulated by CopA RNA through inhibition of leader peptide translation. EMBO J. 11, 2675–2683.

Blomberg, P., et al., 1994. Replication control of plasmid R1: disruption of an inhibitory RNA structure that sequesters the *repA* ribosome-binding site permits *tap*-independent RepA synthesis. Mol. Microbiol. 12, 49–60.

Brantl, S., 2007. Regulatory mechanisms employed by cis-encoded antisense RNAs. Curr. Opin. Microbiol. 10, 102–109.

Bravo, A., et al., 1987. Identification of components of a new stability system of plasmid R1, ParD, that is close to the origin of replication of this plasmid. Mol. Gen. Genet. 210, 101–110.

Cascales, E., et al., 2007. Colicin biology. Microbiol. Mol. Biol. Rev. 71, 158–229.

Cesareni, G., et al., 1982. Control of ColE1 DNA replication: the rop gene product negatively affects transcription from the replication primer promoter. Proc. Natl. Acad. Sci. U.S.A. 79, 6313–6317.

Cesareni, G., et al., 1991. Control of ColE1 plasmid replication by antisense RNA. Trends Genet. 7, 230–235.

Chant, E.L., Summers, D.K., 2007. Indole signalling contributes to the stable maintenance of Escherichia coli multicopy plasmids. Mol. Microbiol. 63, 35–43.

Chiang, C.S., Bremer, H., 1988. Stability of pBR322-derived plasmids. Plasmid 20, 207–220.

Chimerel, C., et al., 2012. Indole prevents Escherichia coli cell division by modulating membrane potential. Biochim. Biophys. Acta 1818, 1590–1594.

Colloms, S.D., et al., 1990. Recombination at ColE1 *cer* requires the *Escherichia coli xerC* gene product, a member of the lambda integrase family. J. Bacteriol. 172, 6973–6980.

Field, C.M., Summers, D.K., 2011. Multicopy plasmid stability: revisiting the dimer catastrophe. J. Theor. Biol. 291, 119–127.

Field, C.M., Summers, D.K., 2012. Indole inhibition of ColE1 replication contributes to stable plasmid maintenance. Plasmid 67, 88–94.

Franch, T., Gerdes, K., 1996. Programmed cell death in bacteria: translational repression by mRNA end-pairing. Mol. Microbiol. 21, 1049–1060.

Gaimster, H., et al., 2014. The indole pulse: a new perspective on indole signalling in Escherichia coli. PLoS ONE 9, e93168.

Gerdes, K., et al., 1988. Translational control and differential RNA decay are key elements regulating postsegregational expression of the killer protein encoded by the *parB* locus of plasmid R1. J. Mol. Biol. 203, 119–129.

Helmer-Citterich, M., et al., 1988. Control of ColE1 replication: low affinity specific binding of Rop (Rom) to RNAI and RNAII. EMBO J. 7, 557–566.

Hiraga, S., et al., 1986. F plasmid *ccd* mechanism in *Escherichia coli*. J. Bacteriol. 166, 100–104.

Itoh, T., Tomizawa, J., 1980. Formation of an RNA primer for initiation of replication of ColE1 DNA by ribonuclease H. Proc. Natl. Acad. Sci. U.S.A. 77, 2450–2454.

Jacob, F., 1974. The Logic of Living Systems: A History of Heredity. Allen Lane, London, p. viii. 348 p.

Jaffe, A., et al., 1985. Effects of the *ccd* function of the F plasmid on bacterial growth. J. Bacteriol. 163, 841–849.

Lee, S.H., et al., 1992. FinOP repression of the F plasmid involves extension of the half-life of FinP antisense RNA by FinO. Mol. Gen. Genet. 235, 131–139.

Li, G., Young, K.D., 2013. Indole production by the tryptophanase TnaA in Escherichia coli is determined by the amount of exogenous tryptophan. Microbiology 159, 402–410.

Masukata, H., Tomizawa, J., 1986. Control of primer formation for ColE1 plasmid replication: conformational change of the primer transcript. Cell 44, 125–136.

Miki, T., et al., 1984. Control of cell division by sex factor F in Escherichia coli. I. The 42.84–43.6 F segment couples cell division of the host bacteria with replication of plasmid DNA. J. Mol. Biol. 174, 605–625.

Newton, W.A., Snell, E.E., 1964. Catalytic properties of tryptophanase, a multifunctional pyridoxal phosphate enzyme. Proc. Natl. Acad. Sci. U.S.A. 51, 382–389.

Nordström, K., et al., 1984. Control of replication of bacterial plasmids: genetics, molecular biology, and physiology of the plasmid R1 system. Plasmid 12, 71–90.

Novick, R.P., 1989. Staphylococcal plasmids and their replication. Annu. Rev. Microbiol. 43, 537–565.

Ogura, T., Hiraga, S., 1983. Mini-F plasmid genes that couple host cell division to plasmid proliferation. Proc. Natl. Acad. Sci. U.S.A. 80, 4784–4788.

Patient, M.E., Summers, D.K., 1993. ColE1 multimer formation triggers inhibition of E. coli cell division. Mol. Microbiol. 8, 1089–1095.

Pimentel, B., et al., 2014. Toxin kid uncouples DNA replication and cell division to enforce retention of plasmid R1 in Escherichia coli cells. PNAS 111, 2734–2739.

Ruiz-Echevarría, M.J., et al., 1995. A mutation that decreases the efficiency of plasmid R1 replication leads to the activation of parD, a killer stability system of the plasmid. FEMS Microbiol. Lett. 130, 129–135.

Stirling, C.J., et al., 1988. The arginine repressor is essential for plasmid stabilizing site-specific recombination at the ColE1 *cer* locus. EMBO J. 7, 4389–4395.
Stirling, C.J., et al., 1989. *xerB*, an *Escherichia coli* gene required for plasmid ColE1 site-specific recombination is identical to *pepA*, encoding aminopeptidase A, a protein with substantial similarity to bovine lens leucine aminopeptidase. EMBO J. 8, 1623–1627.
Summers, D., 1998. Timing, self-control and a sense of direction are the secrets of multicopy plasmid stability. Mol. Microbiol. 29, 1137–1145.
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.
Summers, D.K., Sherratt, D.J., 1988. Resolution of ColE1 dimers requires a DNA sequence implicated in the three-dimensional organization of the *cer* site. EMBO J. 7, 851–858.
Summers, D.K., et al., 1993. Multicopy plasmid instability: the dimer catastrophe hypothesis. Mol. Microbiol. 8, 1031–1038.
Tomizawa, J., Itoh, T., 1981. Plasmid ColE1 incompatibility determined by interaction of RNA I with primer transcript. Proc. Natl. Acad. Sci. U.S.A. 78, 6096–6100.
Tomizawa, J., Som, T., 1984. Control of ColE1 plasmid replication: enhancement of binding of RNAI to the primer transcript by the Rom protein. Cell 38, 871–878.
Twigg, A.J., Sherratt, D.J., 1980. Trans-complementable copy-number mutants of plasmid ColE1. Nature 238, 216–218.
van Biesen, T., Frost, L.S., 1994. The FinO protein of IncF plasmids binds FinP antisense RNA and its target, *traJ* mRNA, and promotes duplex formation. Mol. Microbiol. 14, 427–436.
Yao, S., et al., 2007. Localization of the naturally occurring plasmid ColE1 at the cell pole. J. Bacteriol. 189, 1946–1953.