The antisense leitmotif: A prelude

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Before the plethora of the many small non-coding RNAs (microRNAs, dsRNAs, siRNAs, sRNAs and riboswitches) encoded either in eukaryote or bacterial chromosomes became a hot topic for research, their regulatory role in different aspects of plasmid biology had been already established. As early as 1979, a transcriptional unit in the ColE1 plasmid encoding a small RNA of unknown function (*RNAI*) was found (Morita and Oka, 1979). In a series of seminal papers related to ColE1 replication published in the early 1980s, Jun-Ichi Tomizawa, Tateo Itoh, and colleagues found that such antisense RNA (asRNA) was responsible for the replication control of ColE1 (Tomizawa, Itoh, 1981 and Tomizawa, 1981), thus setting the bases for the discovery of the fundamental role of these molecules in gene expression. In the following years, an eclosion of findings demonstrated that the control of plasmid copy number by counter-transcribed RNAs was not exclusive to the ColE1-like plasmids that depend on a preprimer RNA to initiate replication, but also occurred in plasmids encoding a replication initiation protein. So, the list of asRNA-controlled plasmid replication examples increased exponentially. Members of either type of plasmid replication, named theta, rolling-circle and strand-displacement, swelled such list. asRNAs showed up as the sole replication control mechanism (as in the cases of plasmids pT181 (Carleton, 1984 and Kumar, Novick, 1985) and ColE2 (Sugiyama and Itoh, 1993)), as well as accompanied by other regulatory elements, such as transcriptional repressor proteins (e.g. R1 (Light, Molin, 1982 and Light, Molin, 1983), pMV158 (del Solar et al., 1995), RSF1010/R1162 (Kim, Meyer, 1986 and Maeser, 1990), and pIP501 (Brantl and Behnke, 1992)). Replication control by asRNAs is versatile in its mode of action (del Solar and Espinosa, 2000): (i) blocking translation of the replication initiator mRNA either by sequestering the Shine–Dalgarno sequence (exemplified by pMV158 (del Solar and Espinosa, 1992) and RSF1010/R1162 (Kim and Meyer, 1986)) or a leader mRNA region (as in R1 (Blomberg et al., 1992) and ColE2 (Takechi, 1994 and Yasueda, 1994)); (ii) transcription attenuation (as in plasmids pT181 (Novick et al., 1989), pIP501 (Brantl et al., 1993), and pAMβ1 (Le Chatelier et al., 1996)), and (iii) translation attenuation (pSK41 (Kwong et al., 2006)). It was soon noticed that post-transcriptional regulation by asRNA was not limited to plasmid replication, but other plasmid functions were also targeted.

In loci *parB* of R1 (Gerdes et al., 1988), *flm* (Loh et al., 1988) and *srn* (Nielsen et al., 1991) of F, *pnd* of R483 (Nielsen et al., 1991) and *par* of pAD1 (Weaver and Tritle,1994), sRNAs *sok*, *flmB*, *srnC*, *pndB* and*RNAII* were respectively found to prevent translation of the toxin transcripts involved in plasmid maintenance by post-segregational killing of plasmid-free cells. They were not exceptions; a bunch of type I toxin-antitoxin operons have been found in plasmids and chromosomes (reviewed in Fozo, 2008, Goeders, Van Melderen, 2014 and Wen, Fozo, 2014). Conjugative transfer of the IncF plasmids is another paradigm of regulation by asRNA. FinP is an antisense RNA (Dempsey, 1989 and Finlay, 1986) of the fertility inhibition FinOP system that hybridizes to the mRNA of the activator TraJ (Koraimann, 1991 and van Biesen, 1993), preventing the expression of the transfer operon. Finally, transcriptional attenuation by asRNA is one of the mechanisms involved in the regulation of conjugation genes in *Enterococcus faecalis* pheromone-responsive plasmids (Bae, 2004 and Tomita, Clewell, 2000). Under this light of regulatory expression of so many bacterial genes by asRNAs, it is interesting to recollect that a number of years ago a group of European scientists coordinated by Kenn Gerdes (then at the Odense University in Denmark), presented a grant application to the BIOTECH Programme of the European Union with the aim of using asRNAs to perform gene silencing and, therefore, control of the expression of genes involved in bacterial pathogenicity or virulence. The proposal was not granted because ‘it has little biotechnological interest’. This anecdotal reminder happened, of course, before asRNAs were ‘rediscovered’ by scientists working in the eukaryotic world, who demonstrated the value of small RNAs as the today's excellent gene silencing tools that they have become. Soon after the proposal rejection, scientists from Smith Kline Beecham reported that induction of a staphylococcal asRNA could reduce synthesis of the bacterium's alpha-toxin both *in vitro* and *in vivo* (Ji et al., 1999), setting the basis of the employment of asRNA technology in controlling expression of virulence-related genes. In the bacterial world, regulated expression by asRNA has proved to be a very useful tool in the study of genetic functions in several bacterial systems, Gram-positive (staphylococci, streptococci) as well as in *Escherichia coli* (Stach, Good, 2011 and Wagner, Flärdh, 2002).

In addition, asRNA used as antibacterials has developed enormously during the last decade because of the design of novel antisense oligonucleotides to silence essential bacterial genes or to target genes that can trigger bacterial cell death (Chan et al., 2015). Modifications of the phosphodiester backbone or the sugar of antisense oligonucleotides have allowed the development of new generation of chemically-modified 'asRNAs', some of them having passed preclinical and clinical trials (reviewed in Bai and Luo (2012)). In this Special Issue of *Plasmid*, we have gathered nine manuscripts: six reviews and three 'wet' research papers dealing with the state-of-the-art situation of asRNA molecules within the mobile genetic elements world. The first contribution summarizes the replication control of the pIP501 plasmid as a paradigm of the transcriptional attenuation by asRNA. It is followed by a research article that provides new insights on the importance of secondary structures of the asRNA in the regulation of plasmid pSK41 replication by translation attenuation. In turn, modulation of these regulatory elements by ribonucleases is reviewed. Two articles dissect the asRNA mutations that result in plasmid compatibility among rhizobial repABC replicons. The prevention of the dimer catastrophe by the ColE1-encoded regulatory RNA Rcd is reappraised, as well as the regulation of the toxin–antitoxin *par* locus of the pAD1 plasmid and the role of small regulatory RNAs in lambdoid bacteriophages and phage-derived plasmids. Closing this issue, the regulation of F-like plasmid conjugative transfer by the FinOP system is revisited, stressing the role of FinO-like RNA chaperones from a structural biology perspective. We are grateful to all the authors and reviewers who contributed to this Special Issue. We hope that it will provide valuable information for studying the function of related systems, but also for raising new questions since, far from being a closed subject, there is still much to learn from plasmid-encoded asRNAs. The articles gathered here are examples of how much we currently know about the biology of asRNA encoded by mobile genetic elements. We also hope these articles will provide the readers with a taste of how far we still have to go to grasp a complete understanding of the regulation of gene expression, of which the plasmids have constituted the first example. We would like to dedicate this Special Issue of Plasmid to Prof. Tomizawa and his former associates: they were the ones who started it all.