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# Structural analysis of the Anti-Q–Qs interaction: RNA-mediated regulation of *E. faecalis* plasmid pCF10 conjugation

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ABSTRACT

Conjugation of the *E. faecalis* plasmid pCF10 is triggered in response to peptide sex pheromone cCF10 produced by potential recipients. Regulation of this response is complex and multi-layered and includes a small regulatory RNA, Anti-Q that participates in a termination/antitermination decision controlling transcription of the conjugation structural genes. In this study, the secondary structure of the Anti-Q transcript and its sites of interaction with its target, Qs, were determined. The primary site of interaction occurred at a centrally-located loop whose sequence showed high variability in analogous molecules on other pheromone-responsive plasmids. This loop, designated the specificity loop, was demonstrated to be important but not sufficient for distinguishing between Qs molecules from pCF10 and another pheromone-responsive plasmid pAD1. A loop 5′ from the specificity loop which carries a U-turn motif played no demonstrable role in Anti-Q–Qs interaction or regulation of the termination/antitermination decision. These results provide direct evidence for a critical role of Anti-Q–Qs interactions in posttranscriptional regulation of pCF10 transfer functions.



## 1. Introduction

The pCF10 pheromone-inducible conjugative system (Bae et al., 2000, 2004; Bae and Dunny, 2001) is highly regulated with a number of checkpoints. Pheromone import by responsive donor cells triggers the cytoplasmic molecular switch, PrgX, increasing the initiation and processivity of transcription of the *prgQ* operon (refer to Fig. 1. for a genetic map of the pCF10 conjugation regulatory region). In general, the transcripts from the 5′ and 3′ end of the *prgQ* operon are involved in regulatory and conjugative functions, respectively (Nakayama et al., 1994). The major 5′ transcript, Qs, is constitutively expressed and encodes a functional peptide, the iCF10 inhibitor (Nakayama et al., 1994). The function of iCF10 is to suppress the expression of the conjugative machinery at low levels of the pheromone, cCF10, as a competitive inhibitor (Hedberg et al., 1996). Upon induction with cCF10, Qs levels increase and antitermination of Qs transcripts leads to formation of a longer mRNA species, $Q_L$ (Chung and Dunny, 1995). The downstream genes of the *prgQ* operon encode the conjugation proteins including aggregation substance (PrgB), surface exclusion protein (PrgA), etc. The small regulatory RNA Anti-Q is proposed to regulate the termination/antitermination decision at the Qs terminator IRS1 by suppressing readthrough into the conjugative structural genes.

Regulatory, or antisense, RNAs were first identified in studies of mobile genetic elements like plasmids, transposons, and bacteriophage, but have been more recently

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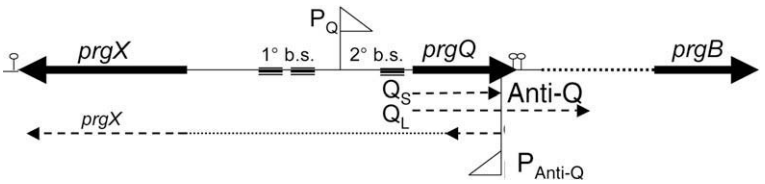


**Fig. 1.** Genetic map of the pCF10 conjugation regulatory region. The triangular arrows above and below the line represent the *prgQ* ($P_Q$) and Anti-Q ($P_{Anti\text{-}Q}$) promoters, respectively. $P_Q$ directs the transcription of the Qs transcript which is terminated at the IRS1 terminator, the first lollipop structure to the right of *prgQ*, in the absence of cCF10. cCF10 induction leads to readthrough of terminators IRS1 and IRS2, production of $Q_L$ and eventually the conjugation structural genes. Transcription from $P_{Anti\text{-}Q}$ generates both the regulatory Anti-Q RNA and the *prgX* mRNA which encodes the negative transcriptional regulator of the system. Anti-Q is processed from this larger transcript by an unknown mechanism. The hatched boxes labeled 1° b.s. and 2° b.s. indicate the primary and secondary binding sites for PrgX which control transcription from $P_Q$.

found to be ubiquitous on bacterial chromosomes as well (Brantl, 2002a, 2007; Fozo et al., 2008; Gerdes et al., 1997; Gottesman, 2005; Wagner et al., 2002; Weaver, 2007). In general, regulatory RNAs are not translated, although exceptions exist (Benito et al., 2000; Novick, 2003). These molecules regulate gene expression by several different mechanisms including RNA folding interference, translational repression or activation, premature transcriptional termination, and target RNA degradation. Regulation by the majority of antisense RNAs involves complex formation by complementary nucleotide pairing with their target RNAs. These RNAs have evolved several structural characteristics, which allow an accelerated pairing with the target RNA within a short time-frame. The rate-limiting step in antisense-target RNA complex formation is usually an initial base-pairing interaction between two loops, or a loop and a single-stranded RNA (Franch and Gerdes, 2000). The presence of a common structural motif, the U-turn motif, was revealed upon comparative sequence analysis of antisense RNAs. Consisting of a conserved 5′-YUNR-3′ sequence, the U-turn motif was first identified as a structural element found on the anticodon loops of tRNAs (Ashraf et al., 1999; Quigley and Rich, 1976), where they facilitate rapid codon–anticodon interaction. The U-turn motif is considered to be a general binding rate accelerator that promotes initial RNA–RNA pairing in some, but not all, RNA-regulated systems.

According to the current model of Anti-Q-mediated transcription attenuation (Bae et al., 2004), when Anti-Q is in excess it interacts with its complementary region in the nascent *prgQ* transcript and promotes the formation of a terminator structure producing Qs RNA and preventing expression of the downstream conjugative machinery. When Qs is in excess, unpaired *prgQ* RNA favors an anti-terminator structure allowing transcription of the downstream conjugation structural genes. Anti-Q is a *cis*-acting antisense RNA (Brantl, 2007), being transcribed from the opposite strand of its target. However, Anti-Q is unusual in that it is part of a longer transcript that includes the *prgX* gene encoding the transcriptional repressor of *prgQ*. Anti-Q RNA is processed from the full length Anti-Q–*prgX* mRNA and is positively regulated by PrgX by an unknown mechanism.

In this manuscript molecular details of the interaction between Anti-Q and Qs are reported. Structural analysis of Anti-Q revealed that it formed a cloverleaf like structure with three stem-loops. Loop I, the 5′ most loop, contains a 5′-YUNR-3′ sequence that could provide a U-turn motif for Anti-Q–Qs interaction, but both *in vitro* and *in vivo* results indicated that it is not essential for Anti-Q function. In contrast, structural analysis of Anti-Q–Qs complexes suggested that critical contacts existed at Anti-Q Loops II and III. Phylogenetic analysis suggested that, while the majority of the Anti-Q sequence was conserved among related pheromone-responsive conjugative plasmids, considerable variation was observed in Loop II, suggesting that it was important for interaction specificity. While mutational analysis confirmed that Loop II was important for determining specificity, it was not sufficient. The implications of these results for the overall mechanism of conjugation control in pheromone-responsive plasmids are discussed.

## 2. Materials and methods

### 2.1. Bacterial strains, media and culture conditions

*Escherichia coli* strain, DH5α (Invitrogen) was used for constructing the templates for generating *in vitro* transcripts. It was routinely cultured in Luria Bertani (LB) (Sambrook et al., 1989) broth at 37 °C. *E. faecalis* strain OG1X (pAD1) (Clewell et al., 1982) was used as a source of pAD1 DNA. *E. faecalis* strain OG1Sp (Fixen et al., 2007) was used for β-galactosidase assays. *E. faecalis* strains were cultured in Todd Hewitt broth (DIFCO) and M9-YE media (Dunny et al., 1985). Erythromycin (Sigma) was used at concentration of 300 μg/ml.

### 2.2. Production of in vitro transcripts

Plasmid pXPCAT (Bae et al., 2000) was used as a PCR template for generating the pCF10 Anti-Q and Qs *in vitro* transcription templates used in this study. Genomic DNA from OG1X (pAD1) was used as a PCR template for generating mD and Qs-pAD1 *in vitro* transcription templates. The likely starting points for these RNAs (for mD, −121 base pairs from the start codon of ORF-TTS1; for Qs-pAD1, −36 bp from the start codon of *iad*) were based on sequence provided by Pontius and Clewell (1992) and alignment with the pCF10 homologs. Genomic DNA was extracted using the Masterpure™ gram positive DNA purification kit from Epicentre Biotechnologies as per manufacturer's instructions. Primers used for all constructs are