The FinO family of bacterial RNA chaperones

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ABSTRACT
Antisense RNAs have long been known to regulate diverse aspects of plasmid biology. Here we review the FinOP system that modulates F plasmid gene expression through regulation of the F plasmid transcription factor, TraJ. FinOP is a two component system composed of an antisense RNA, FinP, which represses TraJ translation, and a protein, FinO, which is required to stabilize FinP and facilitate its interactions with its traJ mRNA target. We review the evidence that FinO acts as an RNA chaperone to bind and destabilize internal stem-loop structures within the individual RNAs that would otherwise block intermolecular RNA duplexing. Recent structural studies have provided mechanistic insights into how FinO may facilitate interactions between FinP and traJ mRNA. We also review recent findings that two other proteins, Escherichia coli ProQ and Neisseria meningitidis NMB1681, may represent FinO-like RNA chaperones.

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1. Introduction

The importance of small, non-coding RNAs (ncRNAs) that regulate gene expression in all kingdoms of life is becoming increasingly clear. ncRNA function involves base pairing with either RNA or DNA targets in an energetically favorable interaction that in theory should require no enzyme co-factors. However, the interaction of two nucleic acid polymers often involves the destabilization of internal structures (e.g., hairpins) within the individual nucleic acids. Although these internal structures are less energetically favorable than the final paired structure (e.g., duplex) between the two molecules, the bimolecular interaction may be kinetically unlikely in the absence of factors that can destabilize the internal structures as a first step in the process. This paradox lead to the proposal of the RNA chaperone hypothesis by Herschlag (1995) and it is now widely believed that RNA-based regulatory processes often require protein co-factors that may act as chaperones to regulate RNA-target interactions. In bacteria, the best characterized RNA chaperone is the Sm protein Hfq, which forms hexameric ring structures that provide multiple binding surfaces for RNAs (for recent reviews, see Weichenrieder, 2014; Sauer, 2013; Wagner, 2013; Wilusz and Wilusz, 2013). Here we review the evidence that the regulatory protein FinO of F-like plasmids represents another class of bacterial RNA chaperones. Whereas FinO is best characterized as a critical inhibitor of the F plasmid tra operon, recent findings indicate that FinO-like proteins with RNA chaperone activity may be widespread throughout bacterial species.

2. Discovery of an antisense RNA system that regulates F plasmid gene expression

Antisense RNA, defined as a transcript that is expressed in cis to its target RNA, has a long and important association with plasmid biology. It has been the subject of intense study on the regulation of replication initiation and copy number control, expression of antidotes to post-segregational
killing, control of the frequency of bacterial conjugation, and the expression of traits carried as cargo by plasmids such as Tn10 transposition and heavy metal resistance. One of the best-described systems is the fertility inhibition system of IncF (F-like) plasmids that downregulates conjugative gene expression.

Bacterial conjugation by transfer systems encoded on IncF plasmids requires the expression of transcripts for pilus synthesis, DNA processing, mating pair stabilization, surface and entry exclusion as well as regulation (reviewed in Gubbins et al., 2005; Arutunov and Frost, 2013). Positive activation of tra transcription requires the TraJ protein, which de-silences the tra promoters and positively regulates the main transfer promoter P_Y in conjunction with host-activation of finP whereasthe finP transcript encoding an antisense RNA is complementable (in cis) and short (4–6 bases) single-stranded regions at the 5′ end. Mutations in the spacer region between SLI and SLII were also found to destabilize the stem-loops of FinP RNA, leading to increased sensitivities to RNase E (Lee et al., 1992). Incidentally, overexpression of finP from foreign promoters can compensate for the absence of FinO leading to almost wild-type levels of repression (Lee et al., 1992; Koraimann et al., 1991, 1996).

In the 60s, Japanese researchers identified plasmids carrying antibiotic resistance (R factors) that, when introduced into F− donor cells, inhibited F conjugation (Egawa and Hirota, 1962; Watanabe and Fukasawa, 1962). This was termed i or fi by Hirota and Fukasawa, respectively. These R factors were transferred 50–100-fold less well than F but transfer-proficient or derepressed (HFT) levels of transfer. In the absence of new recipient cells, it took approximately seven generations to re-establish fertility inhibition (Stocker, 1963). This “epidemic spread” and (Willetts, 1974; Cullum et al., 1978) was attributed to relief from fertility inhibition by an unknown mechanism.

In vitro, FinO binds to FinP and the traJ UTR with similar kinetics (Jerome and Frost, 1999). However, in vivo, FinO does not affect the stability or translational activity of the traJ mRNA prior to its role in promoting FinP-traJ UTR duplex formation. The function of a third stem-loop (SLIII) in the traJ UTR, 25 bases from SLII, is not readily apparent. However, it is involved in Hfq-mediated degradation of the traJ mRNA (Jerome and Frost, 1999) possibly in conjunction with a small RNA (uprP), found in the “dark side” of the F plasmid, opposite the tra operon (Will, Majdalani and Frost, unpublished observations (Gubbins et al., 2005). A schematic
A diagram that summarizes the role of the FinOP system in the regulation of TraJ is shown in Fig. 2.

4. Early characterization of FinO

The finO gene is located at the distal end of the tra operon, approximately 33 kb away from finP. In F, it is interrupted by an IS3 element rendering F naturally derepressed. This is unusual since most IncF plasmids have wild-type finO genes. finO encodes a 22.2 kDa protein that was shown to be required for FinP repression of traJ translation (reviewed in Frost et al., 1994). The promoter for finO transcription has not been determined unequivocally. It might be transcribed from the main promoter Pγ, 33 kb away or it might also be expressed from secondary promoters at the distal end of the operon (Fowler and Thompson, 1986; Ham et al., 1989; Nuk et al., 2011). Willetts and Maule (1986) identified two variants of finO among F-like plasmids, types 1 and 2 that repressed transfer 100–1000-fold or 20–50-fold. This difference was traced to the presence of orf286 upstream of finO in plasmids capable of high levels of transfer repression rather than sequence differences in finO itself. Interestingly, orf286 extended the half-life of finO mRNA in cis but not in trans. The orf286-finO mRNA transcript is able to fold into a complex secondary structure that increases the stability of the finO transcript. The ribosome binding site for finO is exposed in a loop that allows access to ribosomes and translation of the mRNA (van Biesen and Frost, 1992).
5. Towards a structural mechanism of FinO function

Amino acid sequence analysis of the FinO protein indicated that it likely adopted a novel structure and was highly positively charged, consistent with its role in RNA processes. Proteolytic mapping of purified FinO both alone and in complex with its target FinP RNA indicated that N- and C-terminal regions of the protein are flexible in the absence of RNA but become resistant to proteolysis in the presence of RNA, suggesting that the terminal tails of FinO may contact RNA (Ghetu et al., 1999). The crystal structure of a fragment of FinO lacking the flexible N-terminal 25 amino acids revealed a largely helical structure resembling a right-handed fist with extended index finger and thumb, corresponding to N- and C-terminal helices (Ghetu et al., 2000) (Fig. 1). Positively charged surfaces near the N-terminus of α1, as well as a large positively charged patch on the body of the protein suggested two possible RNA contact surfaces. This idea was tested through RNA–protein crosslinking and FRET experiments utilizing a series of 12 single cysteine substituted FinO mutants (Ghetu et al., 2002). The crosslinking experiments demonstrated that cysteine residues positioned near either positively charged patch could efficiently crosslink to FinP SLII, however cysteine residues positioned near a negatively charged patch (bottom of FinO as illustrated in Fig. 1) did not crosslink to SLII RNA. The FRET experiments utilized a technique that enabled the analysis of FRET on distinct FinO-SLII complexes that had been separated by native gel electrophoresis, thereby reducing background from unbound protein or RNA. A two-stranded version of SLII RNA was used that could be 5′ tagged with fluorescein at either the 5′ single stranded tail or at the top of the duplex where the loop would be in a natural SLII hairpin. FRET was measured between the fluorescein linked to the RNA and specific fluorophores covalently linked to the single cysteine positions on FinO. The results indicated that efficient FRET occurred between the fluorescein linked to the 5′ single stranded tail of SLII and any position on FinO, however, lower levels of FRET were observed between RNA molecules labeled at the opposite end of the molecule at the position of the hairpin loop. This suggested that FinO specifically contacts the single stranded regions and perhaps the base of the SLII stem. The regions of the SLII RNA that contact FinO were probed in more detail by RNase digestion experiments (Arthur et al., 2011). These results showed that FinO strongly protected the 3′ single stranded tail from RNases, as well as the duplex stem extending ∼1/2 turn from the single stranded tails. Interestingly, the results suggested that, unlike the 3′ tail, the susceptibility of the 5′ single stranded tail to RNase degradation was actually enhanced by FinO binding. Not only was the 3′ tail highly protected against RNase degradation by FinO, but it was also found that modification of the 3′ nucleotide, either by phosphorylation or oxidation, markedly reduced FinO binding to SLII RNA, suggesting an intimate recognition of the 3′ nucleotide. Intriguingly, both Hfq (Wilusz and Wilusz, 2013) as well as RNA polymerase III regulatory factor La (Dong et al., 2004) specifically recognize 3′ nucleotides of their substrate RNAs.

To date, FinO-RNA complexes have eluded high-resolution structural analyses, however structural models for FinO and its interactions with SLII have been developed using an approach that integrates the crosslinking, FRET and footprinting results described above, with small angle X-ray scattering (SAXS) data obtained from purified FinO–SLII complexes (Arthur et al., 2011). SAXS can yield moderate resolution structural information for flexible macromolecular complexes in solution, and, together with constraints provided by other experimental methods as well as models of the individual molecular components, it can be a powerful tool to develop molecular models for such complexes (Rambo...
and Tainer, 2013). In this method, a molecular docking program, HADDOCK, was used that allows the definition of probable contact residues in both the RNA and protein to limit the number of possible docked complexes. Starting with 2500 docked FinO-SLII complexes, these were ranked based on their agreement with the experimental SAXS data and the top 10% were then clustered into five groups based on structural similarity. The top group in terms of agreement with the SAXS data has the RNA oriented against FinO such that the large, positively charged patch on the body of the protein contacts the 3′ single-stranded tail of SLII as well as the base of the duplex (Fig. 3). The duplex portion of SLII is directed toward α1 (the “index finger”). Interestingly, while models were produced that satisfied the biochemical restraints within the initial 2500 docked complexes in which the SLII duplex was directed away from α1, these models did not provide good fits to the SAXS data. Thus, SAXS was able to provide key information that likely has defined the overall orientation of FinO interaction with a minimal RNA substrate.

6. Mechanism of FinO RNA chaperone activity

Early work using full length FinP and traj mRNA suggested that FinO could enhance pairing between these RNAs ∼5-fold (van Biesen and Frost, 1994). Pairing between these highly structured RNAs is thought to initiate with interactions between complementary hairpin loops via “kissing” interactions (Gubbins et al., 2003) however the highly stable duplex regions within each RNA were predicted to block the attainment of the complete FinP-traj mRNA duplex. To test the idea that FinO might function to overcome energy barriers associated with the internal structures within FinP and the complementary region of traj mRNA, Arthur et al. used an assay which tested the ability of FinO to catalyze RNA strand exchange between a two-stranded version of SLII, and a complementary single strand (Arthur et al., 2003). The results indicated that FinO could dramatically enhance strand exchange activity, consistent with the notion that FinO functions to destabilize the internal hairpins within target RNAs. Deletions of the flexible N-terminal regions progressively reduced strand exchange activity so that FinO45-186, which lacksthe flexible N-terminus as well as the N-terminal portion of α1, exhibited no strand exchange activity. Point mutations in FinO suggested that the N-terminus of α1 is especially critical for strand exchange activity. This region is highly positively charged but also contains conserved hydrophobic residues that appear to be particularly important for strand exchange. The behavior of the same mutants in assays that measured the ability of FinO to catalyze duplexing between complementary RNAs derived from FinP and traj mRNA strongly suggested that the ability of FinO to catalyze RNA strand exchange is linked to its ability to facilitate sense-antisense RNA interactions. Interestingly, an inverse correlation was observed between either strand exchange or RNA duplexing activity and the SLII binding affinity of
the N-terminal mutants such that mutants with the lowest strand exchange activities actually exhibited significantly greater RNA binding. It was suggested that FinO might utilize the free energy of RNA binding to offset the unfavorable destabilization of hairpin base pairing that would be expected to be part of the RNA strand exchange activity. The finding that FinO RNA chaperone activity is dependent on a highly flexible region of polypeptide chain is a common theme in both RNA and protein chaperones (Tompa and Csermely, 2004). While this disordered region is required for chaperone activity, it appears to be dispensable for FinP stabilization in cells (Arthur et al., 2003). Interestingly however, N-terminal deletions strongly abrogate the ability of FinO to repress conjugation, such that FinO45–186 exhibits no inhibition of conjugation, even though this mutant is able to protect FinP against degradation similarly to the full-length protein (Arthur et al., 2003).

While more detailed structural studies are required to clearly elucidate the molecular mechanisms underlying FinO activity, the results to date provide a basis to begin to imagine how FinO acts to regulate tra expression (Fig. 4). Upon initial entry and replication of F plasmid in a newly infected cell, traJ is transcribed and translated, in turn activating the tra operon that leads to the accumulation of FinO. FinO, utilizing its RNA binding core (amino acids 45–186), binds FinP SLII, thereby protecting it from degradation and allowing FinP accumulation. We suggest FinP initially recognizes the complementary structure in traJ mRNA via kissing interactions between the complementary loops in the two RNAs, perhaps facilitated by the N-terminal region of FinO (Ghetu et al., 2000). The current model for FinO-SLII binding would direct the N-terminus toward the region of the loop, providing further support for this suggestion. The N-terminus of α1 appears to be most critical for chaperone activity, and we suggest that the highly positively charged nature of this region brings it into contact with the RNA near or at the loops. The conserved hydrophobic residues in this region, in particular Trp36, could interact with the bases, potentially via stacking interactions to destabilize the internal stem-loop base pairs and thereby allow propagation of intermolecular pairing between the two RNAs. The ability of FinO to not only bind FinP but also traJ mRNA presents the possibility that FinO could either act by binding only one RNA of the pair, or in a pseudo-symmetric arrangement in which both FinP and traJ mRNA are bound by FinO. Eventually, the progression of the reaction would unwind the stem-loop structures, leading to disengagement of FinO45–186, however it is possible that the N-terminal region of FinO might still be associated with the two RNAs as they complete the annealing process.

### 7. The FinO family of RNA chaperones – NMB1681 and ProQ

In a class of its own for two decades, recent work has established a FinO family of RNA chaperones consisting of at least three proteins to date based on amino acid sequence as well as structural similarity. The second member of this RNA chaperone family is the Neisseria meningitidis protein NMB1681. While mechanisms of gene regulation in N. meningitidis remain poorly understood, it is becoming clear that RNA chaperones, in particular the N. meningitidis Hfq ortholog, are associated with stress and virulence responses (Fantappie et al., 2009; Hey et al., 2013). The crystal structure of protein NMB1681 revealed a highly alpha-helical C-terminal core domain, highly similar to the folded core domain of FinO (Chaulk et al., 2010). NMB1681 also contains flexible N- and C-terminal tails although the length of these tails are shorter than the N-terminal flexible region of FinO. The RNA chaperone capacity of NMB1681 was demonstrated by RNA binding, RNA strand-exchange and RNA duplexing assays developed for FinO (Chaulk et al., 2010). Like FinO, NMB1681 binds RNA tightly with a KD in the nanomolar range and prefers double stranded over single-stranded RNA. Consistent with these in vitro results, NMB1681 was able to repress conjugation as well as stabilize FinP mRNA in a finO deficient Escherichia coli strain. While there is convincing evidence that NMB1681 can act as a RNA chaperone both in vitro and in vivo, it remains to be determined what role this RNA chaperone plays in N. meningitidis biology.

The third member of the FinO family of RNA chaperones is ProQ, best known as a regulator of the E. coli proline transporter ProP. ProQ not only regulates ProP as part of the osmotic response enabling E. coli growth in high osmolality media, but also has been shown to play a role in biofilm formation (Sheidy and Zielke, 2013). Though the genetic connection between ProP and ProQ has been established, the mechanism of regulation of ProP by ProQ remains elusive. ProQ is comprised of three domains: an N-terminal FinO-like domain (similar to FinO45–186), a linker region; and an Hfq-like C-terminal domain. Using a full-length construct along with N-terminal and C-terminal domain constructs Chaulk et. al. assessed the RNA chaperone activities of the full length protein as well as the individual domains (Chaulk et al., 2011). While the nanomolar scale RNA binding affinity of ProQ resides in the N-terminal FinO-like domain, both the N-terminal and C-terminal domains display RNA strand exchange and RNA duplexing activities on FinP/traJ based substrates. The combination of weaker RNA binding affinity with retention of strand exchange and duplexing activity in the Hfq domain of ProQ is reminiscent of the N-terminal residues 1–61 of FinO, which harbours the region necessary for FinO chaperone activity. In this manner, the FinO domain may serve as an RNA binding anchor for the chaperone activity within the Hfq domain (Chaulk et al., 2011). Although the effect of ProQ is thought to extend beyond regulation of ProP, identification of RNAs that bind ProQ has remained elusive. Recent work has shown that ProQ associates with ribosomes (Jiang et al., 2007) dependent on the presence of mRNA (Sheidy and Zielke, 2013). While ProQ can tightly bind mRNA, it has not been shown to exhibit specificity for ProP mRNA (Sheidy and Zielke, 2013). Identification of the direct targets of ProQ is necessary to truly determine the mechanism of action of this RNA chaperone.

### 8. Concluding remarks

The FinO RNA chaperone provides an elegant and simple mechanism that allows an initial burst of tra expression, coupled to a gradual, FinOP-dependent repression of the system. The subsequent repression is likely necessary to reduce the metabolic burden of the plasmid on the host, as
Fig. 4. A potential mechanism for FinO RNA chaperone function in the regulation of FinP-traM mRNA duplexing. (i) Plasmid transcription leads to production of traJ mRNA and antisense FinP. (ii) FinO binds to stem-loop structures in the RNAs utilizing the folded core of the protein which recognizes the base of the stem-loop duplex as well as the single stranded tails. (iii–iv) FinO facilitates interactions between the complementary RNAs, likely involving “kissing” interactions between the RNA loops in a manner that may be stimulated by the N-terminal regions of FinO (red). Particularly critical is the region at the N-terminus of α1 containing a cluster of lysine residues as well as Pro34 and Trp36 which are critical for RNA strand exchange and RNA duplexing. (v) The RNA chaperone activity of the N-terminal region of FinO facilitates the propagation of intermolecular base pairing spreading from the initial loop-loop interactions. (vi) Disengagement of the body of FinO as stem-loop structures are incorporated into the growing regions of intermolecular duplex. Final duplexing could be facilitated by the N-terminal regions of FinO that do not absolutely require the main body of FinO for chaperone function. (vii) Formation of the inhibitory FinP-traJ mRNA duplex. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
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