**In Vitro Analysis of the Interaction between the FinO Protein and FinP Antisense RNA of F-like Conjugative Plasmids**

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The FinO protein regulates the transfer potential of F-like conjugative plasmids through its interaction with FinP antisense RNA and its target, *traJ* mRNA. FinO binds to and protects FinP from degradation and promotes duplex formation between FinP and *traJ* mRNA *in vitro*. The FinP secondary structure consists of two stem-loop domains separated by a 4-base spacer and terminated by a 6-base tail. Previous studies suggested FinO bound to the smooth 14-base pair helix of stem-loop II. In this investigation, RNA mobility shift analysis was used to study the interaction between a glutathione *S*-transferase (GST)-FinO fusion protein and a series of synthetic FinP and *traJ* mRNA variants. Mutations in 16 of the 28 bases in stem II of FinP that are predicted to disrupt base pairing did not significantly alter the GST-FinO binding affinity. Removal of the single-stranded regions on either side of stem-loop II led to a dramatic decrease in GST-FinO binding to FinP and to the complementary region of the *traJ* mRNA leader. While no evidence for sequence-specific contacts was found, the results suggest that FinO recognizes the overall shape of the RNA and is influenced by the length of the single-stranded regions flanking the stem-loop.

RNA-protein interactions are important in the post-transcriptional regulation of RNA metabolism and expression. An excellent example is the FinO*P* fertility inhibition system, which controls the transfer frequency of F-like conjugative plasmids. Expression of the plasmid transfer genes is positively regulated by the TraJ protein, which is required for activation of transcription from the major transfer operon promoter, pY. Expression of *traJ* is negatively regulated by the combined actions of the *finO* and *finP* gene products. FinP is a plasmid-specific ~79-base antisense RNA molecule, complementary to part of the 5′-untranslated region (UTR) of *traJ* mRNA (1–3), which includes the ribosome binding site and first two codons of *traJ*. The FinP secondary structure (GenBank accession number U01159) consists of 2 stem-loop (SL) domains, separated by a 4-base spacer and terminated by a 6-base tail (Fig. 1). The 5′-UTR of *traJ* mRNA forms the mirror image of FinP, with the *traJ* RBS and start codon being localized to SLIc (complementary to FinP SLI). The *traJ* leader also contains an extensive single-stranded region and a third stem-loop, SLIII, at its 5′ end. Binding of FinP to the *traJ* UTR is believed to sequester the *traJ* RBS, preventing its translation and repressing plasmid transfer.

FinP requires a protein cofactor, FinO, to exert its negative effect on *traJ* expression. FinO is a 21.2-kDa basic protein (5), which has been shown to bind FinP SLII and *traJ* mRNA *in vitro*, increasing the rate of duplex formation (6). Binding of FinO to FinP also protects FinP from RNase E-mediated degradation (7, 8), increasing its steady-state concentration to a level that allows sequestration and reduced expression of the *traJ* mRNA (9). The F plasmid is derepressed for transfer (i.e. it transfers constitutively) due to the insertion of an IS3 element within its *finO* gene (10, 11). This effect can be overcome by supplying a functional FinO gene from a related plasmid such as R6–5 (5) or R100 (12) *in trans*, resulting in a 100–1000-fold reduction in F plasmid transfer.

The focus of this study is the specificity of the RNA-protein interaction between FinP antisense RNA and the FinO protein. Eight alleles of FinP have been described for F-like conjugative plasmids (Fig. 2), with sequence identity residing in the stem, spacer and tail regions and variability in the two loops (2, 13). Two alleles of FinO exist (13), which show very little sequence variation, but are classified on the basis of their levels of repression of F-like plasmids which is tied to their levels of expression (5). FinO is not plasmid-specific, which suggests that the loops are unimportant for RNA-protein recognition. The FinO protein does not share homology with any of the protein sequence motifs found in other RNA-binding proteins (14–16), providing no clues to the amino acid residues involved in RNA recognition.

This report presents a preliminary characterization of the RNA targets recognized by FinO. RNA mobility shift analysis was used to determine the binding affinity of a glutathione *S*-transferase (GST)-FinO fusion protein for a series of synthetic FinP and *traJ* mRNA variants. High affinity binding by GST-FinO was shown to be dependent on the presence of SLII, flanked on either side by single-stranded regions. Unexpectedly, mutations that disrupted base pairing in SLII were tolerated, as was the introduction of internal loops. Furthermore, decreasing the length of the 3′ tail reduced GST-FinO binding while altering the sequence had no effect. The same structural features were recognized in *traJ* mRNA, suggesting that FinO does not make sequence-specific contacts with the RNA, but recognizes its overall shape.

**Experimental Procedures**

Generation of RNA Templates for *in Vitro* Transcription of RNAs—Plasmid pLJ5–13 contains a 105-base pair PCR product with the *finP* gene fused to the T7 promoter sequence in pUC19 (8). During the construction of pLJ5–13, seven spontaneous mutant clones were obtained with single base mutations in stem II (G42:A, G45:A, C46:T, A47:G, G48:A, G49:A, A51:G) and one clone with a double mutation in stem II (C41:T C46:T). RNAs were generated by *in vitro* transcription.
from these BamHI-linearized pLI5–13 mutant plasmids, resulting in the addition of 7 bases (GGGGGAUC) to their 3’ ends derived from the BamHI site in the vector. All other RNAs were transcribed from gel-purified PCR products prepared with Vent DNA polymerase (New England Biolabs) using the appropriate primers and DNA templates, as detailed in Tables I and II. pOX38-Km has been described previously (17).

**Preparation of RNAs**—Uniformly labeled RNAs were prepared by in vitro transcription of DNA templates in reaction mixtures containing 1× T7 RNA polymerase buffer (Boehringer Mannheim), 1 unit/μl RNAGuard (Amersham Pharmacia Biotech), 10 μCi of [α-32P]UTP (Mandel Scientific), 0.5 mM GTP, ATP, and CTP, and 0.02 mM UTP at 37 °C for 1 h. DNA was removed with 10 units of RNase-free DNase I (Boehringer Mannheim) for 15 min at 37 °C, and the labeled RNA was gel-purified as described (8). RNA was stored at −20 °C for 1 week without noticeable degradation. A step of RNA denaturation-renaturation did not improve or modify the binding affinity of the protein; therefore, gel-purified RNA was used directly for gel-shift analysis.

**Gel-shift Analysis of RNA-Protein Interactions**—pGEX-FO2 (6) was used for expression of a protein fusion between GST and R6–5 FinO. GST-FinO was purified using glutathione-agarose affinity as described (6), except that the cells were lysed using a French press, rather than by sonication (18). Furthermore, GST-FinO and FinO obtained from thrombin cleavage of the fusion protein bind to FinP RNA with equal affinity.2 In the present study, the relative equilibrium association constants for GST-FinO binding to FinP variants were determined by performing gel-shift assays in which radiolabeled RNA was combined with increasing amounts of purified GST-FinO protein. Labeled RNA was synthesized by in vitro run-off transcription using PCR-generated templates or cloned fragments (see “Experimental Procedures”). Transcripts prepared from cloned fragments contained 7 additional bases (GGGGGAUC) at their 3’ ends derived from the BamHI site in the vector used to linearize the plasmids. To ensure that the 7 bases from the vector did not alter the binding constants, FinP affinity.2 In the present study, the relative equilibrium association constants for GST-FinO binding to FinP were calculated from the protein concentration that caused 50% of the labeled RNA to shift in the gel (6, 19). For RNA variants that gave more than one band shift, Kₐ values were calculated by considering all bound RNA as a single species. Except where noted, Kₛ values were calculated from three independent determinations. The percent binding relative to FinP was calculated as 100 × (Kₛ variant/Kₛ FinP).

**RESULTS**

We have previously shown that a fusion of FinO from the related plasmid, R6–5, to GST is active in vivo and in vitro (6, 18). Furthermore, GST-FinO and FinO obtained from thrombin cleavage of the fusion protein bind to FinP RNA with equal affinity. In the present study, the relative equilibrium association constants for GST-FinO binding to FinP variants were determined by performing gel-shift assays in which radiolabeled RNA was combined with increasing amounts of purified GST-FinO protein. Labeled RNA was synthesized by in vitro run-off transcription using PCR-generated templates or cloned fragments (see “Experimental Procedures”). Transcripts prepared from cloned fragments contained 7 additional bases (GGGGGAUC) at their 3’ ends derived from the BamHI site in the vector used to linearize the plasmids. To ensure that the 7 bases from the vector did not alter the binding constants, FinP RNA and the quadruple mutant, FinP C41:U/C46:U/G66:U/G71:U, with or without the additional 3’ bases, were synthe-
The presence of the 7 extra bases at the 3' end had no effect on GST-FinO binding (data not shown).

A comparison of GST-FinO binding affinities to FinP, SLI (nucleotides 1–34, Fig. 1) and SLII (nucleotides 35–79), which were transcribed from PCR-generated templates, is shown in Fig. 3. The $K_a$ for GST-FinO binding to FinP was $2.0 \times 10^7$ M$^{-1}$, 50-fold higher than that for SLI and 2-fold higher than SLII (summarized in Table III). These values are higher than previously reported (6) and likely reflect an increase in the fraction of active GST-FinO in preparations using the French press method for cell lysis, rather than sonication. Since the fraction of GST-FinO that is active was not determined, these values may still be underestimated. As a negative control, incubation of either FinP, SLI or SLII with GST alone did not result in the formation of shifted species in the gel (data not shown). This indicated that the complexes formed in the presence of the fusion protein were the result of RNA interaction with FinO. In agreement with the results of van Biesen and Frost (6), these results suggest that the major determinants for FinO binding reside in SLII, which prompted us to look for differences between SLI and SLII. The most notable structural difference between SLI and SLII is an A-A mismatch within stem I (Fig. 1). SLI A12:U, which creates an A:U base pair (bp) at this site, results in a fully duplexed SLI. Its ability to bind GST-FinO was tested and when compared with SLI with the natural A-A mismatch, was not increased (Table III). Thus, this 11-bp helix was not sufficient for recognition. A second obvious difference between stem-loops I and II is the sequence of the loops. To determine if the loops contributed to the specificity of GST-FinO binding, FinP RNAs from ColB2 and R100–1 were

### Table I

| Primer | Sequence |
|--------|----------|
| LJE1   | ATACATAGGAACCTC |
| LJE5   | AAAATCCGCGAGTACAGG |
| LJE6   | TGCAATTCTAACCCGACAG |
| LJE7   | CGGAATTCATACATAGGAACCTCCTCACAAAGG |
| LJE8   | CATAGAAGCTTGTGAGGA |
| LJE9   | AAAATCCGCGAGTACAGG |
| LJE10  | AAAATCCGCGAGTACAGG |
| LJE11  | GCCGATGCAAGGGGAC |
| LJE12  | TGCAATTCTAACCCGACAG |
| LJE13  | TTITTCGCGATGCAAGGG |
| LJE14  | AAAATCCGCGAGTACAGG |
| LJE17  | TGCCATAGATCTTGTGAGGA |
| LJE19  | TCCGATGCAAGGGGAC |
| LJE20  | AATACATAGGAACCTCCTCACAAAGG |
| LJE21  | TGCCATAGATCTTGTGAGGA |
| LJE22  | TGCAATTCTAACCCGACAG |
| LJE24  | AAAATCCGCGAGTACAGG |
| LJE25  | GACAGTCGATGCAAGGGGAC |
| LJE27  | AAAATCCGCGAGTACAGG |
| LJE28  | CGATGCAAGGGGAC |
| TVB14  | CCTGAATCTGCGGAG |
| TVB15  | TGCAATTCTAACCCGACAG |
| TVB18  | CATAGAATCTTGTGAGGA |
| TVB24  | TGCAATTCTAACCCGACAG |

### Table II

| RNA | 5' primer | 3' primer | PCR template |
|-----|-----------|-----------|--------------|
| FinP | LJE7 | LJE5 | pLJ5-13 |
| FinP G66:U G71:U | LJE7 | LJE9 | pLJ5-13 |
| FinP C41:U G66:U G71:U | LJE7 | LJE9 | pLJ5-13 |
| FinP C62:A C63:A C64:A | LJE7 | LJE10 | pLJ5-13 |
| FinP C70:A G71:U G72:U C73:A | LJE7 | LJE21 | pLJ5-13 |
| FinP G75:C C76:U | LJE7 | LJE27 | pLJ5-13 |
| FinP A2:A3:A4 | LJE22 | LJE5 | pLJ5-13 |
| Spacer-SLII-GA | TVB24 | LJE5 | pLJ5-13 |
| Spacer-SLII-GA | TVB24 | LJE20 | pLJ5-13 |
| Spacer-SLII-GA | TVB24 | LJE21 | pLJ5-13 |
| Spacer-SLII-GA | TVB24 | LJE19 | pLJ5-13 |
| Spacer-SLII | TVB24 | LJE11 | pLJ5-13 |
| SLI-GAUUUU | LJE12 | LJE5 | pLJ5-13 |
| SLI-GAAAAA | LJE12 | LJE13 | pLJ5-13 |
| SLI | LJE12 | LJE11 | pLJ5-13 |
| SLI | LJE7 | TVB18 | pLJ5-13 |
| SLI A12:U | LJE7 | LJE8 | pLJ5-13 |
| SLI-spacer | LJE7 | LJE17 | pLJ5-13 |
| SLI-tail | LJE7 | LJE14 | pLJ5-13 |
| traJ184 | TVB15 | TVB14 | pOX38-Km |
| traJ100 | TVB15 | LJE1 | pOX38-Km |
| traJ77 | TVB15 | LJE25 | pOX38-Km |
| ColB2 | LJE6 | LJE5 | ColB2 |
| R100–1 | LJE6 | R100–1 | ColB2 |
**TABLE III**

*p* values for SLI-spacer and SLI-tail are 1.2 and 1.4, respectively.

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**FIG. 3. Comparison of GST-FinO binding to FinP, SLI, and SLII.** 7.5 fmol of uniformly labeled RNA was incubated with increasing amounts of GST-FinO (0, 0.1, 0.5 (0.5 for SLI only), 1.1, 2.1, 5.3, 10.6, and 31.9 pmol) in a total volume of 30 μl for 30 min at room temperature. Samples were resolved on a nondenaturing 8% polyacrylamide gel. The association equilibrium constants (Kₐ) were calculated as described under “Experimental Procedures”. a, percentage of binding relative to FinP (see “Experimental Procedures”).

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**TABLE III**

GST-FinO binding to FinP variants

| FinP variant | 10ᵪ Kₐ (μ⁻¹) | Binding (%) |
|--------------|---------------|-------------|
| FinP         | 2.00          | 100         |
| SLII         | 1.10          | 55          |
| SLI          | 0.04          | 2           |
| SLI A12:U    | 0.04          | 2           |
| Stem II mutations |
| G42:A        | 1.39          | 70          |
| G45:A        | 1.54          | 77          |
| C46:U        | 1.57          | 79          |
| A47:G        | 1.39          | 70          |
| G48:A        | 1.14          | 57          |
| G49:A        | 1.82          | 91          |
| A51:G        | 1.13          | 57          |
| C41:U/C46:U | 1.36          | 68          |
| G66:U/G71:U | 1.30          | 65          |
| C46:U/G66:U/G71:U | 1.50 | 75          |
| C41:U/C46:U/G66:U/G71:U | 1.80 | 90          |
| C62:A/C63:A/C64:A | 1.70 | 85          |
| C70:A/G71:U/G72:U/C73:A | 1.40 | 68          |
| Other        |               |             |
| ColB2        | 1.95          | 98          |
| R100-1       | 2.34          | 117         |

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* Variants are F unless otherwise stated.

*Ka* values are an average of two or more independent gel shifts.

* Percentage of binding relative to F FinP (i.e., 100 × (Ka variant/Ka FinP)).
TABLE IV

The binding affinity of GST-FinO for SLI with the attached spacer or tail sequence was determined using the gel shift conditions described in the legend to Fig. 4, with the following amounts of GST-FinO: 0, 0.1, 0.5, 1, 1.1, 2.1, 5.3, 10.6, and 31.9 pmol. b, percentage of binding relative to FinP (as described under "Experimental Procedures").

| F FinP variant | $10^7 K_a$ | Binding |
|---------------|------------|---------|
| FinP          | 2.00 ± 0.20 | 100     |
| FinP ΔA2, U3, A4 | 1.50       | 75      |
| FinP G74:C/A75:C | 1.40       | 68      |
| SLII Spacer-SLI-GAUUUU | 1.10 ± 0.02 | 55      |
| SLII-GAUUUU (no tail) | 0.20 ± 0.04 | 10      |
| SLII (no spacer, no tail) | 0.82 ± 0.05 | 41      |
| SLII (no spacer, tail) | 0.08 ± 0.01 | 4       |
| SLII-GAUU  | 0.38 ± 0.05 | 19      |
| SLII-GA     | 0.06 ± 0.01 | 3       |
| SLII-GAAAAA | 0.69       | 35      |
| SLII-GACA   | 0.36       | 18      |
| SLII-spacer | 0.13 ± 0.06 | 6       |
| SLII-tail   | 0.43 ± 0.05 | 21      |

a Unless otherwise stated, $K_a$ values are averages ± standard deviation of at least three independent gel shifts.

b Percentage binding relative to F FinP ($= 100 \times (K_a, variant/F FinP)$).

$K_a$ values are averages of two independent gel shifts.

Fig. 4. The spacer and 3′ tail contribute to high affinity binding by GST-FinO to SLII. Labeled RNA (7.5 fmol) was incubated with increasing amounts (0, 0.1, 1, 1.1, 2.1, 5.3, 10.6, and 31.9 pmol) of GST-FinO. Samples were resolved on a nondenaturing 8% polyacrylamide gel. b, percentage of binding relative to FinP (as described under "Experimental Procedures").

Fig. 5. The spacer and 3′ tail improve SLI binding by GST-FinO. The binding affinity of GST-FinO for SLI with the attached spacer or tail sequence was determined using the gel shift conditions described in the legend to Fig. 4, with the following amounts of GST-FinO: 0, 0.1, 0.5, 1, 1.1, 2.1, 5.3, 10.6, and 31.9 pmol. b, percentage of binding relative to FinP (as described under "Experimental Procedures").

examined since addition of a 7-base extension had no effect (see above). Shortening of the SLII 3′ tail from 6 bases (GAUUUU) to 4 bases (GAUUU) decreased the $K_a$ 3-fold to $0.38 \times 10^7\text{ M}^{-1}$ (Fig. 6; Table IV). A further reduction in tail length to 2 bases (GA) decreased the $K_a$ another 6-fold to $0.06 \times 10^7\text{ M}^{-1}$. These results suggest that a minimum 6-base 3′ tail is necessary for efficient binding by GST-FinO. To determine whether the presence of the 3′ tail reflected a sequence-specific or general requirement for additional bases flanking SLII, variants SLII-GAAAAA, spacer-SLII-GACA, and FinP G74:C/A75:C were constructed (Table IV). The sequence of each variant was chosen to avoid introducing any other obvious secondary structural features. Comparison of the variant pairs (SLII-GAAAAA with SLII-tail; spacer-SLII-GACA with spacer-SLII-GAUU and FinP G74:C/A75:C with wild-type FinP) in Table IV shows that these base transversions in the 3′ tail had minor effects on GST-FinO binding. These results indicate that the length, but not sequence, of the FinP 3′ tail is important for high affinity binding by GST-FinO.

GST-FinO Recognizes the Same Structural Features in traJ mRNA—The results of an earlier study (6) showed that GST-FinO binds to a truncated 184-base version of the sense mRNA, traJ, with a $K_a$ similar to that for FinP. The sequence and secondary structure of the first 117 bases of F traJ are shown in Fig. 1. The secondary structure of traJ RNA between nucleotides 33 and 111 is almost identical to FinP, with the following exceptions: SLIC of traJ mRNA has an additional mismatch (A-C), which is paired in FinP SLII; SLIC of traJ is 2 bases shorter than SLII of FinP, resulting in a 6-base traJ spacer, as compared with 4 bases for FinP.

To further characterize the interaction between GST-FinO and F plasmid-encoded traJ184 (previously called TraJ211, Ref. 6), a series of 3′ truncated traJ variants were created and their binding to GST-FinO was compared with that of FinP. As seen in Fig. 7, addition of increasing amounts of GST-FinO led to the conversion of low molecular weight bands to one or more higher molecular weight bands (see "Discussion" for further comments). GST-FinO bound traJ184 almost as well as FinP (95%), with a $K_a$ of $1.9 \times 10^7\text{ M}^{-1}$. Deletion of 74 bases from the 3′ end of traJ184, creating traJ110 (see Fig. 1), yielded a modest 25% reduction in GST-FinO binding to $1.4 \times 10^7\text{ M}^{-1}$. Removal of SLIC from traJ110, which results in a transcribed...
product of 77 bases (traJ77; similar to SLII of FinP, Fig. 1) reduced GST-FinO binding by 48% to 0.73 × 10^7 M^-1. This is similar to the 45% reduction in the binding constant observed for the removal of SLI from FinP (see Fig. 3; Table III). Further mutation of traJ77 to eliminate the spacer residues 3' to SLIIc (A72 to C77), creating traJ71, decreased the K_a for GST-FinO binding another 53% to 0.34 × 10^7 M^-1. As with FinP, this result suggests that SLIIc and its 3' flanking sequence are important determinants for high affinity GST-FinO binding.

**DISCUSSION**

This report describes the structural features of RNA recognized by the FinO protein. RNA-binding proteins have generally been shown to target single-stranded regions caused by loops, bulges, and mismatches or between helical stems, rather than the duplexed regions themselves (14, 20, 21). Duplexed regions are necessary, however, for spacing and presentation of the single-stranded nucleotides in the correct orientation (20). The hairpin loops represent an obvious single-stranded region of FinP available for protein binding. Three lines of evidence suggest that FinO does not make sequence-specific contacts with bases in the FinP loops. First, although the loop sequences vary between finP alleles (2), FinO is exchangeable among F-like plasmids (5, 22). Second, the traJ mRNA loops are complementary in sequence to FinP, and yet GST-FinO bound traJ and FinP with nearly equal affinity (Figs. 3 and 7). Third, GST-FinO bound F, ColB2, and R100–1 FinPs with the same relative affinity, even though the sequences of the loops vary considerably, especially within loop II.

The results reported by van Biesen and Frost (6) and in this study indicate that the sequence between nucleotides 35 and 79 (SLII) of FinP is sufficient and necessary for high affinity GST-FinO binding. Stem II is fully duplexed in FinP and presents a relatively poor sequence-specific target for FinO. Attempts to disrupt the helical nature of SLII by introduction of internal loops and base substitutions were freely tolerated, indicating that a continuous duplex is not necessary for FinO binding. In agreement with this, single mutations at three of the sites tested (C41, C46, G49) and at G50, had no effect on FinP repressor activity from the related conjugative plasmid R1, as measured by conjugation frequency (23). Thus, the mutant FinP RNAs are also fully stabilized in vivo by FinO (9). These results indirectly demonstrate that FinO binding is not affected by these base substitutions in vivo, in accord with the results obtained in this study, in vitro.

Earlier studies (6) showed that GST-FinO could bind to a FinP/traJ184 duplex or a duplex formed between SLI and the complementary sequence of traJ mRNA. However, the present results indicate that the 14-bp duplex, SLII alone, was not sufficient for high affinity binding by GST-FinO. These apparently conflicting results can be reconciled by the finding that single-stranded regions adjacent to the duplexed RNA were necessary for binding by GST-FinO. In this respect, FinO resembles the stem-loop binding protein (SLBP), which binds to the 3' end of histone mRNA in mammalian cells (24). Efficient binding by SLBP requires at least three nucleotides each 3' and 5' of a stem-loop. FinO requires at least 6 nucleotides 3' to SLII and as many as 4 nucleotides 5' to SLII, although the length of the 5' spacer was not examined in this study. Like FinO, SLBP does not have a strict loop size requirement, suggesting that specific contacts do not involve the loop. However, unlike the interaction between FinO and FinP, the sequence of the stem and flanking regions is important for SLBP binding.

Since no evidence was obtained for sequence-specific contacts between GST-FinO and FinP, our data suggest that FinO
recognizes the overall shape of the RNA conferred by a stem-loop structure, flanked on either side by single-stranded regions. Congruent with this, GST-FinO recognizes the same structural features in tral mRNA. The requirement for a 6-nucleotide flanking region 3′ to SLIIe is fulfilled by the tral spacer, which is 2 bases longer than the FinP spacer (Fig. 1). In addition the tral spacer, which differs from the FinP 3′ tail at 4 of the 6 bases, can serve as a functional 3′ flanking region, indicating that sequence is unimportant for binding by FinO. Addition of the FinP tail sequence (GAUUUU) to the 3′ side of SLI conferred moderate GST-FinO binding, although the binding constant was 2-fold lower than that for SLII with the equivalent 3′ tail. These results suggest that the RNA conformation recognized by GST-FinO can be adopted quite efficiently by three different sequences: tral mRNA with its 6-base single-stranded spacer, SLI with the FinP 3′ tail, and SLII with the 3′ tail.

The functionally related RNA-binding protein, Rom, has also been shown to recognize RNA in a structure-dependent, rather than sequence-dependent fashion. Rom binds to an unstable complex formed between the complementary hairpin loops of RNA I and RNA II of ColE1 plasmids (25). The results of three independent studies indicate that Rom is capable of binding and stabilizing any complex formed by pairs containing fully single-stranded spacer, SLI with the FinP 3′ tail, and SLII with the 3′ tail. The RNA conformation recognized by GST-FinO can be adopted quite efficiently by three different sequences: tral mRNA with its 6-base single-stranded spacer, SLI with the FinP 3′ tail, and SLII with the 3′ tail.

Interestingly, binding of GST-FinO to tral184 gave rise to four shifted bands of different mobility, with the largest complex being retained in the well (Fig. 7). The fastest migrating complex was converted to more slowly migrating complexes observed with the tral RNA variants may represent complexes formed due to GST-FinO aggregation. Cooperative binding has been reported for the human immunodeficiency virus Rev-RRE interaction (32, 33, 34, 35) and between p24 (a 220- amino acid truncated polypeptide of the protein kinase, PKR) and human immunodeficiency virus dsTAR RNA (36). A closer look at GST-FinO binding to its antisense RNA targets (Fig. 3) shows that only one complex was formed between GST-FinO and either SLI or SLII, whereas two complexes were formed with full-length FinP (more readily distinguishable by under-exposure of this gel; data not shown). Although the strongest GST-FinO binding was achieved with SLII, low affinity binding was observed between GST-FinO and SLI with its attached 4-base spacer. Thus, binding of GST-FinO to its primary binding site, SLII, may promote binding to the low affinity site, SLI, resulting in the more slowly migrating complex observed at high GST-FinO concentrations. Similarly, the higher molecular weight complexes observed with the tral RNA variants may represent successive GST-FinO binding to stem-loops IIc, Ic, and III.

Further experiments are needed to determine the specificity of FinO binding to these potential low affinity sites. In addition, since the physiological FinO concentration is not presently known but is thought to be low, based on mRNA levels (5), the relevance of the higher molecular weight complexes, which are formed at high FinO concentrations, remains to be established.

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