Figures and figure supplements

A crystal structure of a collaborative RNA regulatory complex reveals mechanisms to refine target specificity

Chen Qiu et al
**Figure 1.** Identification of a minimal fragment of LST-1 that interacts with FBF-2. (A) Yeast 2-hybrid analyses of interaction between the FBF-2 PUM domain fused to a GAL4 activation domain (A.D.) and LST-1 fragments fused to the LexA DNA-binding domain (D.B.D.). A negative control empty vector (EV) is used as the control. **Figure 1 continued on next page.**

Qiu et al. eLife 2019;8:e48968. DOI: https://doi.org/10.7554/eLife.48968
vector (EV) with no FBF-2 fused to the activation domain and a positive control with the FBF-2 PUM domain fused to the activation domain were assessed with LST-1 34–328 fused to the DNA-binding domain and are shown at the top of the graph. (B) LST-1 L83 is critical for interaction with FBF-2. Yeast 2-hybrid analyses were conducted with LST-1 residues 55–105 fused to a GAL4 activation domain and the PUM domain of FBF-2 fused to the LexA DNA-binding domain. Mutants in LST-1 that interfered with FBF-2 interaction are colored green and those that were competent for interaction are colored gray. Binding activity is shown as units of β-galactosidase (β-gal) activity normalized to cell count. Error bars indicate the standard deviation of three biological replicate measurements. A schematic representation of the yeast 2-hybrid assay is illustrated in Figure 1—figure supplement 1 and results of yeast 2-hybrid analyses of LST-1 and FBF homologs are shown in Figure 1—figure supplement 2.

DOI: https://doi.org/10.7554/eLife.48968.002
Figure 1—figure supplement 1. A schematic of the yeast two-hybrid assay. To quantify binding activity, fusion proteins are introduced into yeast with a ‘bait’ protein (purple) fused to the LexA DNA-binding domain. The LexA protein provides a tether to the promoter region of the β-gal reporter gene by virtue of its association with the LexA operator site. ‘Prey’ proteins (peach) are introduced as fusions to the GAL4 transcriptional activation domain. Transcription of the reporter gene is dependent on interaction between the bait and prey proteins, which recruits RNA polymerase to the reporter gene.
DOI: https://doi.org/10.7554/eLife.48968.003
Figure 1—figure supplement 2. LST-1 interacts with FBF but not homologous PUF proteins. Yeast-two hybrid assays were conducted with LST-1 residues 55–105 fused to the Lex-A DNA-binding domain (D.B.D.) and the GAL4 activation domain (A.D.) was fused to the PUM domain of the indicated PUF protein homologs: FBF-1 (residues 121–614), human PUM1 (residues 456–1064), human PUM2 (residues 456–1064), C. elegans PUF-8 (full length), and D. melanogaster dPUM (residues 1091–1426). Binding activity is shown as units of β-gal activity normalized to cell count. Error bars indicate the standard deviation of three biological replicate measurements. DOI: https://doi.org/10.7554/eLife.48968.004
Figure 2. Crystal structure of an FBF-2/LST-1/RNA ternary complex reveals hotspots for protein-protein interaction. (A) Crystal structure of an FBF-2/LST-1/RNA ternary complex. FBF-2 is shown as a ribbon diagram with cylindrical helices. PUM repeats are colored alternately red and blue. RNA recognition side chains from each PUM repeat are shown with dotted lines indicating interactions with the RNA bases. LST-1 (green) and the RNA Figure 2 continued on next page
Figure 2 continued

(beige) are shown as stick representations colored by atom type (red, oxygen; blue, nitrogen; orange, phosphorus). (B) LST-1 contacts FBF-2 at conserved interaction hotspots. Zoomed-in view of interaction between FBF-2 and LST-1. Three interaction hotspots are labeled, and LST-1 L83 and L76 at hotspots 1 and 3, respectively, are shown with space-filling atoms. LST-1 K80 and FBF-2 Q448 at hotspot 2 are shown as stick models. Interactions between LST-1 and FBF-2 are indicated by dotted lines. Electron density for the LST-1 peptide is shown in Figure 2—figure supplement 1. (C) Conservation of LST-1 interacting residues in CPB-1 and GLD-3. Amino acid sequence alignment of the LST-1 interacting peptide and conserved sequences in CPB-1 and GLD-3. Residues at the interaction hotspots in (B) are highlighted and conserved residues are in boldface. (D) LST-1 L83 and Y85 at interaction hotspot 1 are essential for tight binding to FBF-2. Yeast 2-hybrid analyses were conducted with LST-1 residues 55–105 fused to the LexA DNA-binding domain (D.B.D.) and the PUM domain of FBF-2 fused to the GAL4 activation domain (A.D.). Mutants in LST-1 that interfered with FBF-2 interaction are colored green and those that were competent for interaction are colored gray. (E) FBF-2 Q448G at hotspot 2 has a minor effect on interaction with LST-1. FBF-2 variants that interfered with LST-1 interaction are colored red and those that were competent for interaction are colored gray. Binding activity is shown as units of β-gal activity normalized to cell count. Error bars indicate the standard deviation of three biological replicate measurements.

DOI: https://doi.org/10.7554/eLife.48968.009
Figure 2—figure supplement 1.  
$F_o-F_c$ simulated annealing omit map for the LST-1 peptide, contoured at 3 $\sigma$.

DOI: https://doi.org/10.7554/eLife.48968.010
Figure 3. The FBF-2 R7-R8 loop is essential for interaction with LST-1. (A) The essential residue LST-1 L83 interacts with FBF-2 at the base of the FBF-2 R7-R8 loop. FBF-2 L444 and Y479 at the R7-R8 loop are shown with space-filling atoms. (B) Yeast 2-hybrid analyses were conducted with LST-1 residues 55–105 fused to the LexA DNA-binding domain (D.B.D.) and the PUM domain of FBF-2 fused to the GAL4 activation domain (A.D.). (C) Yeast 2-hybrid analyses of mutations in Y479. Mutants in FBF-2 that interfered with LST-1 interaction are colored red and those that were competent for interaction are colored gray. Binding activity is shown as units of β-gal activity normalized to cell count. Error bars indicate the standard deviation of three biological replicate measurements.

DOI: https://doi.org/10.7554/eLife.48968.013
Figure 4. FBF-2 in the ternary complex binds to RNA using a 1:1 recognition mode and its curvature is more pronounced. (A) FBF-2 recognizes the central nucleotides in a compact RNA using repeats 4 and 5. The crystal structure of the FBF-2/LST-1/RNA ternary complex is shown with FBF-2 displayed as a ribbon diagram with cylindrical helices. PUM repeats are colored alternately red and blue. RNA recognition side chains from each PUM repeat are shown with dotted lines indicating interactions with the RNA bases. Central nucleotides 4–6 (green) within a compact RNA element (beige) are shown as stick representations colored by atom type (red, oxygen; blue, nitrogen; orange, phosphorus). Electron density for the compact RNA nucleotides 4–6 is shown in Figure 4—figure supplement 1. (B) FBF-2 binds to directly stacked and flipped central nucleotides in the extended gld-1 RNA motif. The crystal structure of the FBF-2/gld-1 RNA binary complex (PDB ID 3V74) is shown as a ribbon diagram with cylindrical helices. Central nucleotides 4–6 (green) within the gld-1 RNA (mauve) are shown as stick models. (C) Superposition of FBF-2 within ternary and binary complexes reveals increased curvature in the FBF-2/LST-1/RNA ternary complex. RNA-binding helices and RNA cartoons are shown for FBF-2 in the binary (mauve) and ternary (red) complexes.

DOI: https://doi.org/10.7554/eLife.48968.016
Figure 4—figure supplement 1. F_o-F_c simulated annealing omit map for the cFBE RNA nucleotides 4–6, contoured at 3 σ.

DOI: https://doi.org/10.7554/eLife.48968.017
**Figure 5.** SEQRS analysis of FBF-2/LST-1 and FBF-2 reveals distinct specificities. (A) Diagram of the SEQRS procedure. (B) Motif from SEQRS analysis of the FBF-2/LST-1 complex. (C) Motif from SEQRS analysis of FBF-2. Inset, superposition of the upstream C pocket in structures of the FBF-2/LST-1/RNA complex.
ternary and FBF-2/RNA binary complexes demonstrates that LST-1 L76 occupies the upstream C pocket in the structure of the ternary complex. (D) Comparative analysis of biases at base +4 in compact vs extended motifs. Sequences that conform to either the compact 8-nt or extended 9-nt sites were quantified in SEQRS data for FBF-2 alone (pink), the LST-1/FBF-2 complex (cyan), or CLIP data for FBF-2 (gray). (E) GO term analysis of FBF-2 mRNA targets. P-values were corrected using the Benjamini-Hochberg method (Kuleshov et al., 2016). Enrichment for compact sequences or extended binding elements was determined using the grep command on FBF-2 CLIP targets (Prasad et al., 2016). The abbreviation N.S. indicates that enrichment failed to achieve significance (adjusted p<0.05).

DOI: https://doi.org/10.7554/eLife.48968.018
Figure 5—figure supplement 1. Representative EMSA gels and corresponding binding curves are shown for binding to gld-1 (A) and compact FBE (cFBE, (B) RNAs. Triangles above the gels indicate increasing
concentrations of FBF-2 from 0.49 to 4000 nM. The left lanes in each gel contained no protein. $K_d$ values for triplicate experiments are presented in Table 2.

DOI: https://doi.org/10.7554/eLife.48968.019