Supporting Information

Kershner and Kimble 10.1073/pnas.1000495107

SI Materials and Methods

Immunoprecipitations. Immunoprecipitations (IPs) were done as described (1), but with modifications to accommodate our reagents. Age-synchronous FBF-GFP and TUB-GFP adults were harvested 24 h after L4 stage and washed with M9 buffer until the supernatant was clear, and then washed once in buffer A (20 mM Tris [pH 8.0], 150 mM NaCl, 10 mM EDTA) and twice in lysis buffer [buffer A plus 1.5 mM DTT, 0.1% Nonidet P-40, 0.2 mg/mL heparin, 1× EDTA-free Complete Protease Inhibitor Mixture (Roche), 100 U/mL RNaseOUT (Promega)]. Worm pellets were frozen in liquid nitrogen and homogenized using a mortar and pestle, followed by 30 passes with a 2-mL glass dounce (Pyrex). To remove insoluble material, extracts were centrifuged two times at 10,000 × *g* for 10 min at 4 °C, and 25.5 mg of 15 mg/mL extracts was precleared with 50 μL Protein A Trisacryl beads (Pierce). Precleared extracts were then added to ~7.5 μg of mouse monoclonal anti-GFP antibody 3E6 (Qbiogene) prebound to 50 μL of protein A beads, and incubated for 2.5–3 h at 4 °C on a rotating platform. IP beads were subsequently washed once in lysis buffer and four times in wash buffer [20 mM Tris (pH 8.0), 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 10% glycerol, 0.1% Nonidet P-40, 10 U/mL RNaseOUT (Promega)]. To elute bound proteins, 15% of IP beads were boiled for 5 min in 2× SDS/PAGE sample buffer. RNA was eluted from remaining beads using 300 μL TRIzol reagent (Invitrogen) and then purified using RNeasy Minelute columns (Qiagen). Purified RNA samples were run on a BioAnalyzer 2100 (Agilent) to confirm RNA integrity.

Microarrays. Microarrays were probed by the University of Wisconsin–Madison Gene Expression Center. RNA from IPs (50% of total) was subjected to one round of linear amplification and labeling using a MessageAmp Biotin II-Enhanced kit (Ambion). For each of four biological replicates, 100% or up to 10 μg of FBF-GFP IP-amplified RNA (aRNA) and the same amount of TUB-GFP IP aRNA by volume were hybridized in the GeneChip 640 hybridization oven (Affymetrix) to *Caenorhabditis elegans* whole-genome GeneChip arrays (Affymetrix), which have probe sets against >22,150 unique *C. elegans* transcripts representing ~85% of protein-coding genes in *C. elegans* (WormBase release WS190). Arrays were postprocessed in GeneChip Fluidics Station 450 (Affymetrix) and scanned using a GC3000 G7 scanner (Affymetrix). Array data were extracted and background was subtracted using GeneChip Operating Software v.1.4 (GCOS; Affymetrix), and unscaled intensities were exported to Excel (Microsoft). Data were then converted to $\log_2$ ratios (FBF/TUB), normalized by median centering the control AFFX probe sets, and filtered for probe sets called "Present" by GCOS in at least three of four FBF-GFP replicates. Reproducibility of array data was analyzed using Spearman's rank correlation coefficient (ρ), which ranged from 0.45 to 0.82. Data were analyzed in R with the BioConductor package siggenes using one-class significance analysis of microarrays (SAM) (2).

Real-Time Quantitative PCR. For real-time quantitative PCR (qPCR) experiments, random-primed cDNA was prepared from input and IP RNA samples using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qPCR was carried out using TaqMan Gene Expression Assays (Applied Biosystems) and TaqMan Universal PCR Master Mix (Applied Biosystems) in a 7500 Fast Real-Time PCR System (Applied Biosystems) with the standard ramp speed. *eft-3* was used as the endogenous control for normalization, and data were analyzed using the $\Delta\Delta C_T$ method (3). Occasionally, C_T readings for *hmit-1.3* and F53A3.1 in the TUB-GFP IP sample were undetectable and were set to the maximum cycle number. TaqMan assays were as follows: *gld-1*, Ce02409901_g1; *eft-3*, Ce02448437_gH; *rps-25*, Ce02464216_g1; *ced-4*, Ce02446175_g1; F53A3.1, Ce02443519_m1; F54D10.5, Ce02430714_g1; *gld-2*, Ce02408169_g1; H20J04.6, Ce02431576_g1; *ima-3*, Ce02455692_g1; *lin-45*, Ce02407558_g1; *hmit-1.3*, Ce0244681_m1; *smg-6*, Ce02443932_g1.

Bioinformatics. For FBE analysis, all *C. elegans* 3′UTR sequences (WS190) were downloaded from BioMart (http://www.biomart.org). A gene was considered FBE-positive if it had at least one annotated 3′UTR with at least one FBE. Genes with no annotated 3′UTRs were excluded. SAGE data were obtained from the Genome Sciences Centre *C. elegans* Gene Expression Consortium (http://elegans.bcgsc.bc.ca), WS190 mappings. For analysis of conserved putative PUF targets, Ensembl orthologs were downloaded from BioMart with the following database releases: *C. elegans*, WS190; human, NCBI36; *Drosophila*, BDGP5.4; *Saccharomyces cerevisiae*, SGD1.01.

1. Gerber AP, Luschnig S, Krasnow MA, Brown PO, Herschlag D (2006) Genome-wide identification of mRNAs associated with the translational regulator PUMILIO in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 103:4487–4492.
2. Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 98:5116–5121.
3. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* 25:402–408.

Tissue/cell type	FBF target	binding assay §	FBF/mRNA CoIP	protein	misregulated in *fbf(-)* * mRNA	misregulated in *fbf(-)* * reporter	misregulated in ΔFBE† mRNA	misregulated in ΔFBE† reporter	references
Germ line	*fbf-1*	+		+					1
Germ line	*fbf-2*	+		+					1
Germ line	*fog-1*	+		+		+		+	2,3
Germ line	*fem-3*	+					+		4,5,6,7
Germ line	*gld-1*	+	+	+		+		+	3,5,8,9,10
Germ line	*gld-3S*	+		+					11
Germ line	*lip-1*	+		+	+				12
Germ line	*mpk-1*	+	+	+					8
Neuron	*egl-4*	+				+		+	13

[1] Lamont et al, 2004
[2] Thompson et al, 2005
[3] Merritt et al, 2008
[4] Zhang et al, 1997
[5] Bernstein et al, 2005
[6] Barton et al, 1987
[7] Ahringer and Kimble, 1991
[8] Lee et al, 2007
[9] Crittenden et al, 2002
[10] Suh et al, 2009
[11] Eckmann et al, 2004
[12] Lee et al, 2006
[13] Kaye et al, 2009

Fig. S1. Known FBF target mRNAs. Known FBF targets are listed with a summary of the evidence that supports each as a direct FBF target. FBF target mRNAs and the tissue where they are regulated by FBF are shown (left). "+" indicates a positive result in the experiment listed (top). §, includes gel-shift and yeast three-hybrid assays. *, includes both *fbf* mutants and *fbf* RNAi. Gene expression increases when FBF is depleted [*fbf(–)*], except for *egl-4* in neurons, which decreases. †, gene expression increases when the FBF binding element is removed (ΔFBE), except for *egl-4* in neurons, which decreases.

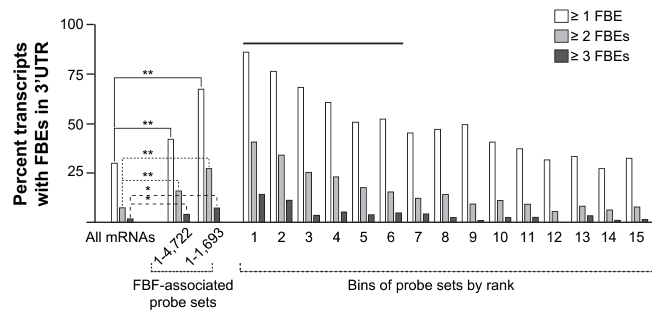

Fig. S2. FBF-associated probe sets are enriched for transcripts with multiple FBEs. Percentage of probe sets with ≥1 (white bars), ≥2 (light gray bars), or ≥3 FBEs (dark gray bars) in corresponding mRNA 3′UTRs is shown for all protein-coding genes (left), for two categories of FBF-associated probe sets (middle), and for 15 bins (4722 FBF-associated probe sets were placed in bins based on their ordered SAM rank, with each bin containing ∼314 probe sets). The black bar denotes bins containing the top 1693 probe sets. *$P < 10^{-30}$; **$P < 10^{-95}$. P values were calculated using the hypergeometric distribution.

Table S1. Shared PUF target mRNAs in humans, *C. elegans*, and *Drosophila*

Human	*C. elegans*	*Drosophila*	Protein description
ARAF*†	*lin-45*	*phl*‡	MAP kinase kinase kinase/RAF (MAPK pathway)
ECT2§	*ect-2*	*pbl*‡	RhoGEF
RKTG*§	Y67A10A.8	CG7530‡¶	Haemolysin-III-related protein/RAF inhibitor
PDK1§	*pdk-1*	*Pk61C*‡	3-Phosphoinositide-dependent protein kinase (PI3/Akt pathway)
FOXO3*§	*daf-16*	*foxo*‡	Forkhead/HNF3 transcription factor (PI3/Akt pathway)
FOXO1A*			
BCL3§	C33A11.1	*cact*‡	NF-κB transcription factor and NF-κB transcription factor inhibitor proteins (NF-κB pathway)
NFKBIA*†§			
NFKBIZ*†			
CSL*§	*lag-1*	*Su(H)*‡	CSL transcription factor (Notch pathway)
LMBR1*§	R05D3.2	CG5807‡	Lipocalin transmembrane receptor family
PARD3B*	*par-3*	*baz*‡	Atypical PKC-interacting protein
PARD3§			
CCNT2*	*cit-1.2*	*CycT*‡	Cyclin T
	cit-1.1		
CCNB1§	*cyb-1*	*CycB*‡	Cyclin B
CCNB2*§	*cyb-2.1*		
	cyb-2.2		
ITSN2*	*itsn-1*	*Dap160*‡	Intersectin
SMAP1*	W09D10.1	CG8243‡	ARF GTPase-activating protein
AP1S1*†	*aps-1*	*AP-1σ*‡	Clathrin adapter complex, σ1 subunit
RAB5B*†§	*rab-5*	*Rab5*‡	Rab GTPase
SLC25A40*†	C16C10.1	*Tyler*‡	Mitochondrial solute carrier
SLC37A3§	T10C6.6	CG10069‡	Major facilitator superfamily solute carrier
LARP5§	*larp-5*	CG11505‡	Metazoan-specific La protein
RBM7*	Y37D8A.21	CG11454‡	RRM-containing RNA-binding protein
HNRNPA3†	H28G03.1	*Hrb98DE*‡	hnRNA-binding protein
TIS11*§	*oma-1*	*Tis11*‡	CCCH zinc finger protein
	oma-2		
	mex-1		
	mex-5		
	mex-6		
MTPAP§	*pup-2*	CG11418‡	Nucleotidyltransferase protein
ZCCHC11§		*mkg-p*‡	
EIF4E*†§	*ife-5*	*eIF-4E*‡	Translation initiation factor 4E
RBM25*†§	W04D2.6	CG4119‡	snRNP complex protein
MBTD1*	*lin-61*	*Sfmbt*‡	Malignant brain tumor domain-containing Polycomb group protein
	mbtr-1		
HMG20A§	W02D9.3	CG9418¶	High-mobility group DNA-binding protein
EPC2†	*epc-1*	*E(Pc)*‡	Enhancer of Polycomb family
CYP3A4†	*cyp-13B1*	Cyp6a2‡	Cytochrome P450
		Cyp6a22¶	
		Cyp6d4¶	
		Cyp6a23‡	
		Cyp6a19‡¶	
OSBPL9*§	*obr-4*	CG1513‡	Oxysterol-binding protein
OSBPL10*§			
ZDHHC6*	M18.8	CG5196‡	DHHC zinc finger, putative palmitoyltransferase
MAPRE3*	*ebp-2*	Eb1‡	Microtubule-binding protein
CHPF*	*mig-22*	CG4351‡	Chondroitin *N*-acetylgalactosaminyltransferase
WIPI2*	*atg-18*	CG8678‡	Autophagy protein

*Human PUM1 (1).
†Human PUM2 (1).
‡*Drosophila* adult Pumilio (2).
§Human PUM1 (3).
¶*Drosophila* embryo Pumilio (2).

1. Galgano A, et al. (2008) Comparative analysis of mRNA targets for human PUF-family proteins suggests extensive interaction with the miRNA regulatory system. *PLoS One* 3:e3164.
2. Gerber AP, Luschnig S, Krasnow MA, Brown PO, Herschlag D (2006) Genome-wide identification of mRNAs associated with the translational regulator PUMILIO in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 103:4487–4492.
3. Morris AR, Mukherjee N, Keene JD (2008) Ribonomic analysis of human Pum1 reveals *cis-trans* conservation across species despite evolution of diverse mRNA target sets. *Mol Cell Biol* 28:4093–4103.

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)
Dataset S3 (XLS)
Dataset S4 (XLS)
Dataset S5 (XLS)
Dataset S6 (XLS)

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