

Table S1

Mass Spectrometry Data: Pull-down with Rny-3FL

Protein	# AA (peptides) ^a	Coverage (%) ^b	Abundance ^c (IS75-1)	Abundance ^c (IS75-2)	Abundance ^c (Rny-3FL-1)	Abundance ^c (Rny-3FL-2)	Ratio ^d	T test
Rny	520 (72))	85.77	2.1	3	173.5	221.4	77.2	0.01
RicT	275 (29)	80.0	6.5	8	191	194.5	26.6	0.00
RicF	149 (3)	27.52	0	0	1 peptide ^e	0	-	0.42
RicA	143 (2)	18.18	49.5	114.6	94.4	141.4	1.4	0.47
Eno	430 (28)	73.26	72.1	99.4	118	110.5	1.3	0.18
PfkA	319 (31)	79.62	81.5	109.9	121.7	86.9	1.1	0.74
Pnp	705 (42)	56.03	82.3	105.2	114.5	98	1.1	0.47
CshA	494 (39)	63.56	44.7	90.1	166.3	98.8	2.0	0.25
RnjB	555 (44)	82.34	6.4	97.1	184	112.6	2.9	0.24
RnjA	Not detected							

^aTotal number of amino acid residues (predicted number of peptides after trypsinization).

^bCombined identified amino acids/total amino acids in the protein.

^cReflects the signal strength in the raw data.

^d(Average abundance for Rny-3FL/average abundance for IS75).

^eOne peptide was detected in this sample.

Table S2

Strains

Strain number	Relevant genotype ^a	Source
IS75 ^a	<i>his leu met</i>	Lab strain
BD9000	<i>rny-3FL (spc)</i>	This work
BD9170	<i>P_{spac}-ricT-3FL::thr (erm) ricT::kan^b</i>	This work
BD9171	<i>P_{spac}-ricF-3FL::thr (erm) ricF::(kan^b</i>	This work
BD9172	<i>P_{spac}-ricA-3FL::thr (erm) ric::kan^b</i>	This work
BD9195	<i>rny-3FL (spc) ΔricA^c</i>	This work
BD9196	<i>rny-3FL (spc) ΔricF^c</i>	This work
BD9210	<i>rny-3FL (spc) ricT C161S</i>	This work
BD9211	<i>rny-3FL (spc) ricT C198S</i>	This work
BD9212	<i>rny-3FL (spc) ricT C167S</i>	This work
BD9217	<i>P_{spac}-ricT-3FL::thr (erm) rny::kan^b</i>	This work
B9218	<i>P_{spac}-ricT-3FL::thr (erm) rny::kan^b ricA::spc^d</i>	This work

^aAll strains were constructed in the IS75 background.

^bThese knockouts were obtained from the Bacillus Genetic Stock Center and were constructed by Koo et al (36).

^cThese deletions are markerless. Cassettes were removed using pDR244 (36).

^dA gift from the late A. A. Neyfakh.

Table S3

Oligonucleotides

Name	Sequence ^a
Rny-F	GTCAT CGAATT CTTCGTAAAACCATGCCC
Rny-R	GTCAT CGGATC CTTTTGCATACTCTACGGC
RicT-F1	CGAG GGTACC CTTGGTGTTAATGTTGCAGG
RicT-R1	GTATCCGCACAAACCACAGATAGCGCT ATCG ^b
RicT-F2	CCAAAAA ACTGCTGCCTTCGGATCC GATATCCTAACAGCACAAGAG
RicT-R2	GGGCTTTTTTCCATGCAAGCTAATTCGGATCC AAGCTT ATCGAATTCGATA
RicA-F	TGATCTAGAGTCGAG GGTACC GAAAGGAGAAAATATGACATG
RicA-R	CAAATAACAGCTGTTCTCT CATCGAT CGGCCGGATTATAAGGAT
RicF-F	TGATCTAGAGTCGAG GGTACC CTGGAGGTGCATGTTATG
RicF-F	CTGCGGATGTAAAGTGTCC ATCGAT CGGCCGGATTATAAGGAT

^aRelevant restriction sites are in bold face.

^bThis incomplete *Cla*I site is completed by the Gibson Assembly step.

Fig. S1. Verification of the Ric-3FL constructs. Panels A, B and C show that the RicT-3FL, RicF-3FL and RicA-3FL express the appropriate fusion proteins, detectable using both anti-Ric and anti-FLAG antisera and that the constructs are IPTG-inducible. These strains did not carry knockouts of the native *ric* genes and thus signals for the untagged proteins were detected.

Fig. S2. Ric-3FL and Rny-3FL proteins support processing of the *cggR gapA* transcript. Northern blotting was carried out using a probe complementary to the coding strand of *gapA*. Each of the four fusion strains were the only source of RicA, RicF, RicT and Rny in the cells. U and P show the positions of the unprocessed and processed RNA species (~2.2 and ~1.2 kb, respectively). The *ricT* deletion and wild-type (IS75) control lanes show the positions of these two species, respectively.

Fig. S3. Verification of anti-serum specificities and of the anti-FLAG magnetic beads. (A) The appropriate Ric proteins bands are missing from total lysates of each indicated deletion strain. (B) In a lysate of the Rny-3FL strain, the anti-FLAG and anti-Rny antisera reveal the fusion protein, but only the wild-type Rny signal is evident in a wild-type lysate. (C) An eluant fraction is shown from a pull-down experiment using an Rny-3FL lysate and anti-FLAG antiserum. The magnetic beads successfully pull down Rny-3FL. (D) Eluant fractions from pull-down experiment using wild-type and Rny-3FL lysates. The blots were developed with anti-Ric antiserum. RicT does not bind non-specifically to the beads. (E) No signal is detected in the eluant fraction from a mock pull-down experiment with a wild-type lysate, using anti-Rny antiserum.

Fig. S4. The three Ric-3FL constructs can pull one another down with anti-FLAG beads. In all three panels the blots were developed with anti-Ric antiserum. The empty arrow shows a cross-reacting band.

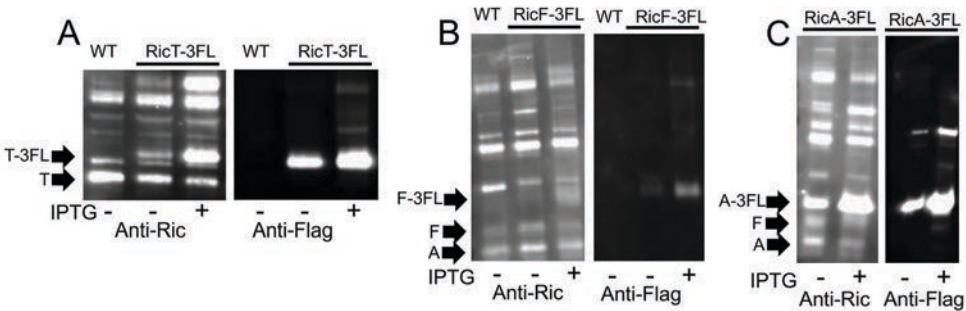


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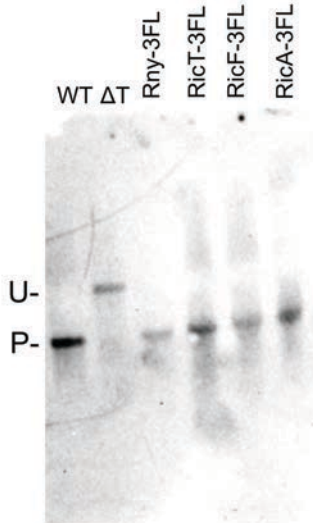


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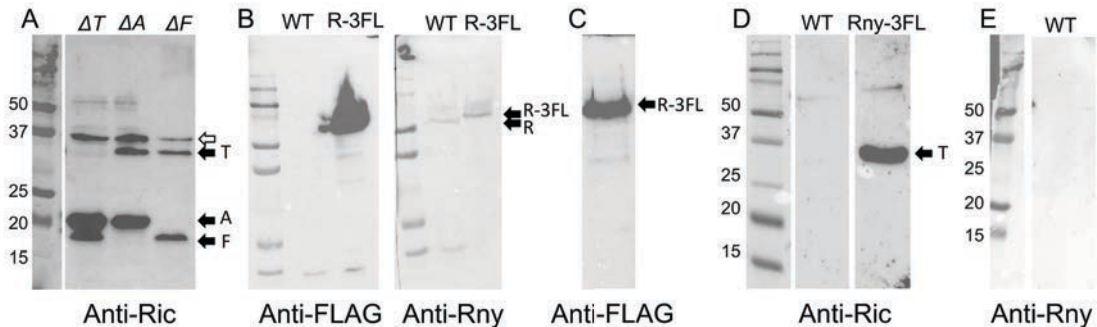


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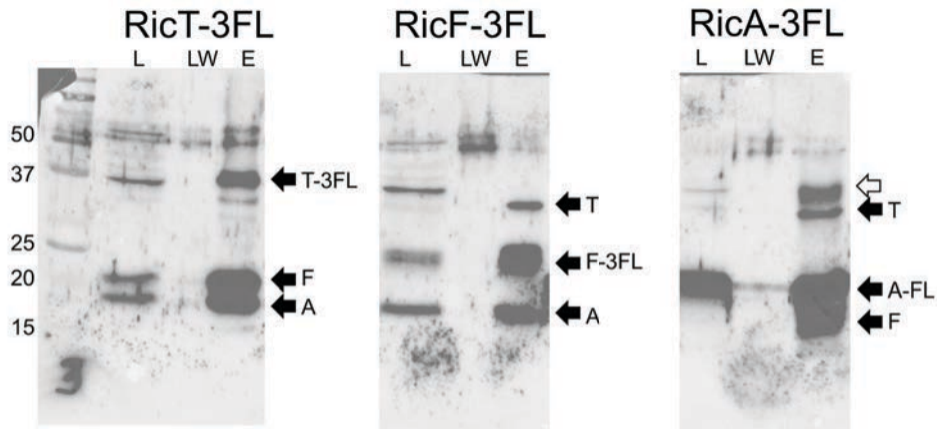


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