SI Text

Protein Preparation. The portion of *RNQ1* encoding amino acid residues 153 to 405 was amplified from pKanMXRNQ1–GFP (1) using primers 423 (CCCCATATGCAAGGTCAGGGACAAGGTCAA) and 424 (CCCAAGCTTTTCAGTGATGGTGATGATGGTGGTAGCGGTTCTGGTTGCCGTT), digested with NdeI and HindIII and ligated to pET-21a(+) (Novagen, Darmstadt, Germany) cut with the same enzymes to form p1178. *E. coli* strain BL21-CodonPlus (DE3)-RIPL carrying p1178 was grown overnight in rich medium containing 100 μg/ml ampicillin, 75 μg/ml streptomycin, and 34 μg/ml chloramphenicol.

Media for labeling were based on described methods (2, 3). Minimal medium contained, per liter, 200 ml of salts (65 g/liter KH_2PO_4, 50 g/liter K_2HPO_4, 45 g/liter Na_2PO_4, 10 g/liter NH_4Cl), 10 ml of trace elements (6 g/liter $FeSO_4$, 6 g/liter $CaCl_2$, 1.2 g/liter $MnCl_2$, 0.8 g/liter $CoCl_2$, 0.7 g/liter $ZnSO_4$, 0.3 g/liter $CuCl_2$, 0.25 g/liter $(NH_4)_6Mo_7O_{24}$, 5 g/liter EDTA), 10 ml of 1 M $MgSO_4$, 12 ml of 40% dextrose, 100 ml of TAU (0.3 g/liter thiamine, 2 g/liter adenine sulfate, 2 g/liter uracil), 100 ml of amino acid mix (1 g/liter of each amino acid except tyrosine), 100 ml of 0.1% tyrosine, and 50 μg/ml ampicillin. Cells are grown (500 ml per 2 l flask) at 37C to OD_{550} = 0.5, collected by centrifugation, and suspended in fresh medium with 200 mg/liter of labeled tyrosine or leucine in place of that unlabeled amino acid. To make Ala-3-^{13}C labeled protein, 1 g/liter of Ala-3-^{13}C replaced alanine in the medium. After 15 min growth, the culture was made 1 mM in IPTG and growth continued overnight. Uniformly ^{13}C, ^{15}N-labeled Rnq1$^{153\text{-}405}$ was prepared by the same method except that at OD_{550} = 0.5, cells were resuspended in media lacking amino acids and containing 2 g/liter $^{15}NH_4Cl$ and 0.5 g/liter U-^{13}C-glucose. After 15 min of shaking at 37C, 1 mM IPTG and 4.5 g/liter U-^{13}C-glucose were added.

Cells from two liters of culture are harvested, suspended in 25 ml of 8 M guanidine HCl, 100 mM Tris Cl pH 8.0, 150 mM NaCl, with one “complete, EDTA-free” protease inhibitor tablet (Roche, Mannheim, Germany) incubated for 2 h to complete lysis, spun in a 45Ti rotor for 60 min at 30,000 rpm. The supernatant was mixed with 4 ml packed of NiNTI volume per liter culture, mixed gently at room temperature for 20 min. The mixture was poured into a column,

washed extensively with 8 M urea, 100 mM Tris Cl pH 8.0, 150 mM NaCl, and the Rnq1$^{153-405}$ was eluted using the same buffer with 200 mM imidazole. Protein was precipitated by addition of four volumes of methanol, and dissolved for amyloid formation in 4 M urea, 150 mM NaCl, 5 mM KPO_4 pH 7.4. After 2-5 days incubation at room temperature with gentle agitation, amyloid formation was complete. Samples were washed with H_2O four times, and dried by lyophilization.

To examine the effect of dilution, Rnq1$^{153-405}$ fully labeled with Tyr-1-^{13} was dissolved in 8 M guanidine, 100 mM Tris Cl pH8.0, 150 mM NaCl, and mixed with four times the weight (as determined by OD_{280}) of unlabeled material, methanol-precipitated, and dissolved in 4 M urea, 150 mM NaCl, 5 mM KPO_4 pH 7.4 for amyloid formation.

1. Nakayashiki T, Kurtzman CP, Edskes HK, Wickner RB (2005) Yeast prions [URE3] and [*PSI*$^+$] are diseases. *Proc Natl Acad Sci USA* 102:10575-10580.

2. Blanco FJ, Hess S, Pannell LK, Rizzo NW, Tycko R (2001) Solid-state NMR data support a helix-loop-helix structural model for the N-terminal half of HIV-1 Rev in fibrillar form. *J Mol Biol* 313:845-859.

3. Cai ML, Huang Y, Sakaguchi K, Clore GM, Gronenborn AM, Craigie R (1998) An efficient and cost-effective isotope labeling protocol for proteins expressed in *Escherichia coli. J Biomol NMR* 11:97-102.

Sequence of $Rnq1^{153-405}$

(M) GQGQGQGQGQGQGQGQGQGSFTALASL
ASSFMNSNNNNQQGQNQSSGGSSFGALASM
ASSFMHSNNNQNSNNSQQGYNQSYQNGNQN
SQGYNNQQYQGGNGGYQQQQGQSGGAFSSL
ASMAQSYLGGGQTQSNQQQYNQQGQNNQQQ
YQQQGQNYQHQQQGQQQQQGHSSSFSALAS
MASSYLGNNSNSNSSYGGQQQANEYGRPQQ
NGQQQSNEYGRPQYGGNQNSNGQHESFNFS
GNFSQQNNNGNQNRY (H_6)

Amino acid composition of $Rnq1^{153-405}$.

Ala	A	13	Asn	N	41
Glu	E	3	Pro	P	2
Phe	F	9	Gln	Q	67
Gly	G	42	Arg	R	3
His	H	4	Ser	S	39
Leu	L	7	Thr	T	2
Met	M	5	Tyr	Y	15