Supplementary methods

Yeast Strains and Methods

Standard methods and media were used (Forsburg & Rhind, 2006). A complete list of strains is displayed in Table S1. C-terminal TAP-tagged versions of Rng3p, Swo1p and the four myosins were constructed using a PCR-based approach (Bähler et al, 1998; Tasto et al, 2001). All strains grew normally and displayed normal cell shape, showing that the tagged proteins were functional. RIp-chip experiments and polysome profiling were carried out with cells grown in yeast extract medium at 32°C. For the determination of myosin protein levels, cells were grown in yeast extract medium at 25°C and shifted to 36°C for 4 hours to inactivate Rng3p.

Preparation of denatured protein extracts for the determination of protein levels

$2x10^8$ cells were resuspended in 100 µl lysis buffer (10 mM Tris-HCl pH 7.6, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS), and incubated at 100°C for 7 minutes. The cells were lysed with glass beads using a FastPrep 24 machine (QBiogene, Montreal, Canada). Cell extracts were recovered from the beads after addition of 250 µl of lysis buffer. The extracts were clarified by a 5-minute centrifugation at 3,000g, and the concentration of protein in the supernatant was determined and adjusted to ensure that equal amounts of protein were loaded for all strains.

Detailed RIp-chip protocol

The method is based on published protocols (Gerber et al, 2004). 10^9 cells were resuspended in 100 µl lysis buffer (20 mM Tris-HCl pH 8.0, 140 mM KCl, 1.8 mM $MgCl_2$, 0.1% NP40) containing 0.2 mg/ml heparin, 200 units SuperaseIN (Ambion) and the following protease inhibitors: 20 µg/ml leupeptin, 20 µg/ml aprotinin, 1 mg/ml AEBSF, 40 µg/ml bestatin, 10 µg/ml E-64 and 1 mM PMSF. The cells were lysed with glass beads using a FastPrep 24

machine (QBiogene, Montreal, Canada). Cell extracts were recovered from the beads after addition of 500 µl of lysis buffer prepared as above except that it contained 100 units of SuperaseIN. The extracts were clarified by two sequential 5-minute centrifugations (at 9,000g and 15,000g). 50 µl of extract were used for the preparation of reference RNA and the rest for immunoprecipitation. Immunoprecipitation was carrying out using monoclonal antibodies against protein A (Sigma) coupled to magnetic beads (Invitrogen). Extracts were incubated with the beads for 2 hours, and the beads washed four times with 1 ml of lysis buffer containing 0.2 mg/ml heparin, twice with 1 ml of wash buffer (20 mM Tris-HCl pH 8.0, 140 mM KCl, 1.8 mM $MgCl_2$, 0.01% NP40, 10% glycerol) with 0.2 mg/ml heparin and twice in 1 ml wash buffer containing 20 units of SuperaseIN. The immunoprecipitated RNA was purified using an RNAqueous-Micro kit (Ambion). The beads were directly resuspended in lysis solution, the supernatant recovered and RNA purified following the manufacturer's instructions. Purification of immunoprecipitated RNA in this way resulted in a major improvement compared to the use of phenol chloroform extraction and ethanol precipitation. The amount of RNA in the immunoprecipitate was sufficient to allow direct labelling without RNA amplification. Total RNA was purified using RNeasy columns (Qiagen) following the manufacturer's protocol. For the sequential immunoprecipitation experiment, Rng3-TAP was purified as described above. To release Rng3-TAP from the beads, the beads were resuspended in 200 µl of wash buffer containing 1 mM DTT, 5 units of SuperaseIN and 60 units of AcTEV protease (Invitrogen) and incubated at 19°C for 1 hour. The Rng3p-containing supernatant was split into two: one half was used for immunoprecipitation with magnetic beads coupled to an antibody against 5.8S RNA (Y10b, Abcam) and the other with an antibody against myc (9E11, Abcam). The beads were washed and the RNA purified as described above for one-step immunoprecipitations.

SUPPLEMENTARY REFERENCES

Bähler J, Wu JQ, Longtine MS, Shah NG, McKenzie A, 3rd, Steever AB, Wach A, Philippsen P, Pringle JR (1998) Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* **14:** 943-951

Forsburg SL, Rhind N (2006) Basic methods for fission yeast. *Yeast* **23:** 173-183

Gerber AP, Herschlag D, Brown PO (2004) Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast. *PLoS Biol* **2:** E79

Tasto JJ, Carnahan RH, McDonald WH, Gould KL (2001) Vectors and gene targeting modules for tandem affinity purification in *Schizosaccharomyces pombe*. *Yeast* **18:** 657-662

Table S1: Strains used in this study.

Genotype	Source
rng3-TAP::kanMX6 h^{90}	This study
swo1-TAP::kanMX6 h^{90}	This study
myo1-TAP::kanMX6 h^{90}	This study
myo2-TAP::kanMX6 h^{90}	This study
myp2-TAP::kanMX6 h^{90}	This study
myo52-TAP::kanMX6 h^{90}	This study
rng3-65 ade6-M21? leu1-32 h^{-}	K. Gould
myo1-TAP::kanMX6 rng3-65 leu1-32	This study
myo2-TAP::kanMX6 rng3-65 leu1-32	This study
myp2-TAP::kanMX6 rng3-65	This study
myo52-TAP::kanMX6 rng3-65 leu1-32?	This study

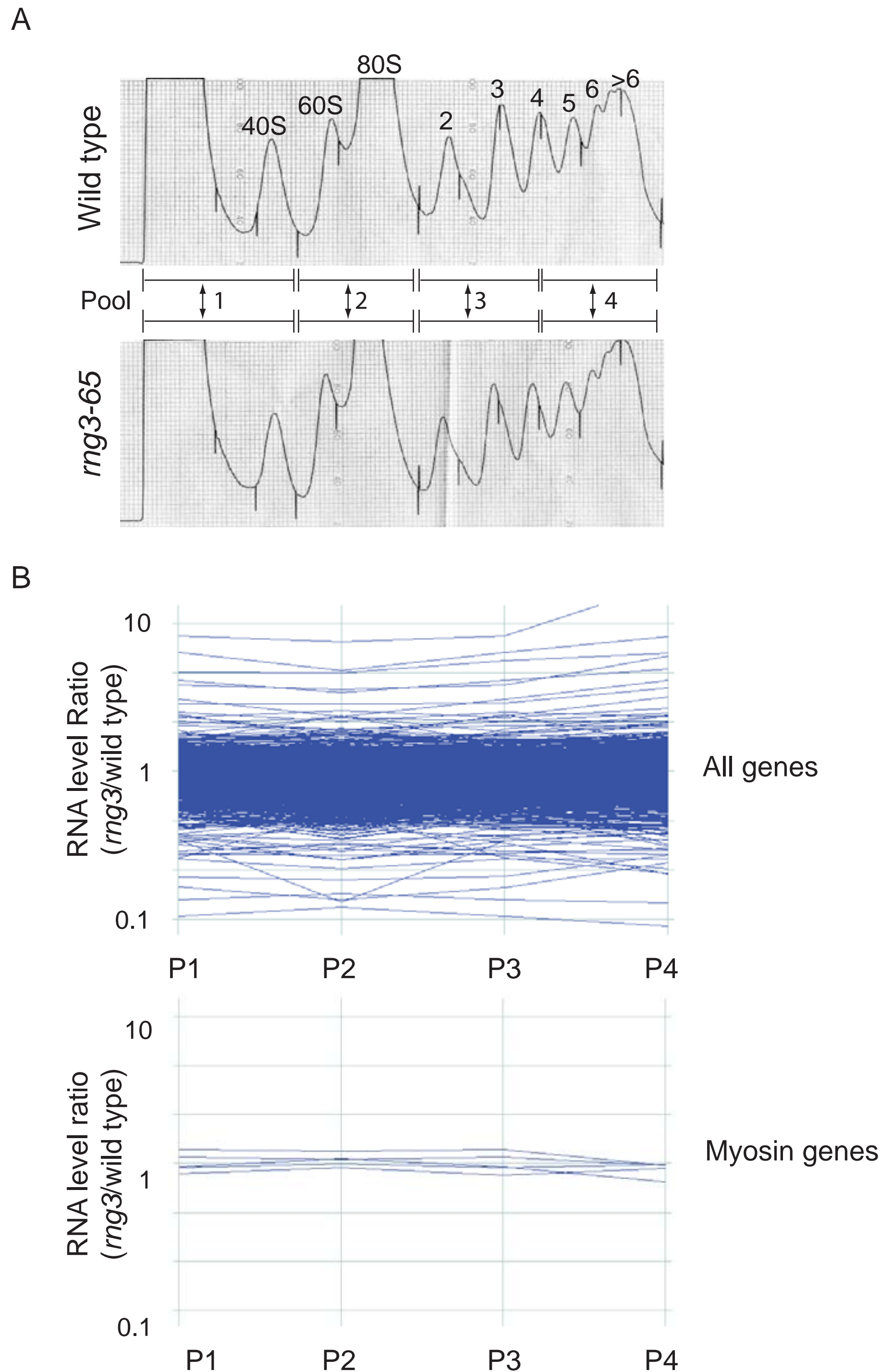

Figure S1. Genome-wide polysomal profiling of *rng3* mutants. (A) Polysomal profiles (absorbance at 254 nm) and experimental design. Extracts from wild type or *rng3-65* cells were fractionated using sucrose gradients. The fractions were pooled, RNA extracted, and RNAs from the corresponding fractions of both strains were directly compared using DNA microarrays. (B) Comparison of RNA levels between the fractions. Each line corresponds to a single RNA. Alterations in translation of specific RNAs would result in a change in the number of ribosomes associated with specific RNAs, and thus in an altered distribution of RNAs in specific fractions. Note that changes in RNA levels that do not affect translation cause identical changes in all fractions. The data are shown for all genes (left) and the five myosin-encoding genes (right).

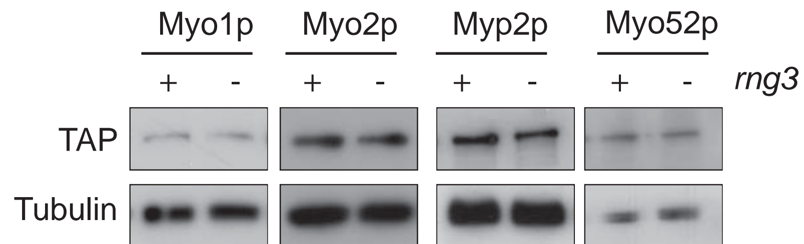

Figure S2. Comparison of myosin protein levels in wild type and *rng3* mutants. Levels of TAP-tagged myosin proteins were assessed by Western blot in wild type cells (+) or *rng3-65* mutants (-) after a 4 hour incubation at 36°C. Tubulin was used as a loading control.