Supplemental Materials

Molecular Biology of the Cell

Huang et al.

Separate responses of karyopherins to glucose and amino acid availability regulate nucleocytoplasmic transport

Huang and Hopper

Supplementary materials

Supplementary method

Protein purification

Yeast cultures were grown at 30˚C to OD_{600} of 0.4-0.6. Harvested cells were rapidly frozen in liquid nitrogen and broken by milling. Cryolysis was performed using a planetary ball mill. Pull down assays were performed as described (Alber et al, 2007) with some alterations for optimizations. In brief, 0.5 g of frozen ground cells were resuspended in 4.5 ml of the extraction buffer (20 mM Hepes, pH 6.1, 110 mM KOAc, 2 mM $MgCl_2$, 75 mM NaCl, 0.5% Triton, 0.1% Tween-20, 1 mM DTT) with 1: 100 dilutions of protease inhibitor cocktail set IV (Calbiochem). The suspension was clarified by centrifugation at 3000 xg, 4˚C for 10 min. The soluble extract was incubated with IgG-conjugated magnetic beads at 4˚C for 30 min. The magnetic beads were then collected with a magnet, washed six times with 1 ml of the ice-cold extraction buffer and once with 0.1 M NH_4OAc, 0.1 mM $MgCl_2$, 0.02% Tween-20. Proteins were eluted with 0.5 M NH_4OH, 0.5 mM EDTA by incubation for 20 min at room temperature. The eluates were lyophilized in a SpeedVac (Thermo Savant) overnight. The pellets were resuspended in NuPAGE LDS sample buffer (Life technologies), and separated on a 4-12% NuPAGE Novex Bis-Tris precast gel (Life technologies) according to the manufacturer's specifications.

Mass spectrometry analysis

Protein bands within gels were excised and the samples were analyzed by the Ohio State University Campus Chemical Instrument Center Mass Spectrometry and Proteomics Facility (http://www.ccic.ohio-state.edu/MSP).

Oligonucleotide sequences

The following primer sequences were used: IVY79/forward primer for C-terminal tagging of Msn5 (CATTGAAGACGGTGCTGTGGGTAATCTCTTTGATGACAACcggatccccgggttaattaa), IVY80/reverse primer for C-terminal tagging of Msn5 (CTTCAGAAATACCTTGTTCAGACCCAC ATTAAAACGCTTGgaattcgagctcgtttaaac), IVY163/forward primer for C-terminal tagging of Los1 (GTTTGTAGATAATTTCATTTCTGTTTTAAAGCAAGGTCAAcggatccccgggttaattaa), IVY164/reverse primer for C-terminal tagging of Los1 (GCTAAACCAAAGTGAATGATAAGT CTAATTACATATTACCgaattcgagctcgtttaaac), IVY169/forward primer for C-terminal tagging of Kap95 (AAGATGGGCTAGAGAGCAACAGAAGCGTCAATTATCCTTAcggatccccgggttaattaa), IVY170/reverse primer for C-terminal tagging of Kap95 (GGTAAAATAACAAATTAATTATGAT CAAAGAGAAAT GCCCgaattcgagctcgtttaaac). An upper case letter indicates the sequence for homologous recombination of target gene. A lower case letter indicates the sequence targeting to the tagging cassette plasmid (Longtine et al., 1998).

Table S1. Post-translational modifications of Msn5 and Los1

1. Msn5

K_{430}-Acetylation of Msn5						
Peptide Sequence	Theoretical m/z	Measured m/z	Mass error (PPM)	Relative Abundance (%)		
				Fed	-aa	-glu
$_{430}$KFAEIDFQSK$_{439}$[a]	606.8166 $^{2+}$	606.8231^{2+}	10.8	96.46	97.72	98.22
$_{430}$K$_{(Acetylation)}$FAEIDFQSK$_{439}$[b]	627.8219^{2+}	627.8206^{2+}	-2.07	3.54	2.28	1.78

K_{1013}-DiMethylation of Msn5						
Peptide Sequence	Theoretical m/z	Measured m/z	Mass error (PPM)	Relative Abundance (%)		
				Fed	-aa	-glu
$_{997}$IVID$_{(CAM)}$CVGQGNANPNSAK$_{1013}$[a]	878.9360^{2+}	878.9393^{2+}	3.8	99.98	99.98	99.95
$_{997}$IVID$_{(CAM)}$CVGQGNANPNSAK$_{(DiMethyl)1013}$[b]	892.9516^{2+}	892.9321^{2+}	-21.84	0.02	0.02	0.05

a. Relative Abundance of unmodified peptide =Peak Area$_{(unmodified)}$/(Peak Area$_{(modified)}$+Peak Area$_{(unmodified)}$)*100

b. Relative Abundance of modified peptide =Peak Area$_{(modified)}$/(Peak Area$_{(modified)}$+Peak Area$_{(unmodified)}$)*100

2. Los1

K_{426}-Methylation of Los1

Peptide Sequence	Theoretical m/z	Measured m/z	Mass error (PPM)	Relative Abundance (%)		
				Fed	-aa	-glu
$_{415}$DFLDNFQQIC$_{(CAM)}$FK$_{426}$[a]	787.8691^{2+}	787.8688^{2+}	-0.38	98	98	98
$_{415}$DFLDNFQQIC$_{(CAM)}$FK$_{(Me)426}$[b]	794.8769^{2+}	794.8788^{2+}	2.39	2	2	2

E_{1047} or E_{1049}--- Methylation or K_{1052}- Methylation of Los1

Peptide Sequence	Theoretical m/z	Measured m/z	Mass error (PPM)	Relative Abundance (%)		
				Fed	-aa	-glu
$_{1035}$ELFIVSSNPTTNENEC$_{(CAM)}$VK$_{1052}$[a]	1040.9964^{2+}	1041.0012^{2+}	-4.6	93	93	93
$_{1035}$ELFIVSSNPTTNE$_{(Me)}$NEC$_{(CAM)}$VK$_{1052}$[b] or $_{1035}$ELFIVSSNPTTNENE$_{(Me)}$C$_{(CAM)}$VK$_{1052}$[b]	1048.0042^{2+}	1048.0038^{2+}	-0.38	3	2	2
$_{1035}$ELFIVSSNPTTNENEC$_{(CAM)}$VK$_{(Me)1052}$[b]	1048.0042^{2+}	1048.0038^{2+}	-0.38	5	5	5

a. Relative Abundance of unmodified peptide =Peak Area$_{(unmodified)}$/(Peak Area$_{(modified)}$+Peak Area$_{(unmodified)}$)*100

b. Relative Abundance of modified peptide =Peak Area$_{(modified)}$/(Peak Area$_{(modified)}$+Peak Area$_{(unmodified)}$)*100

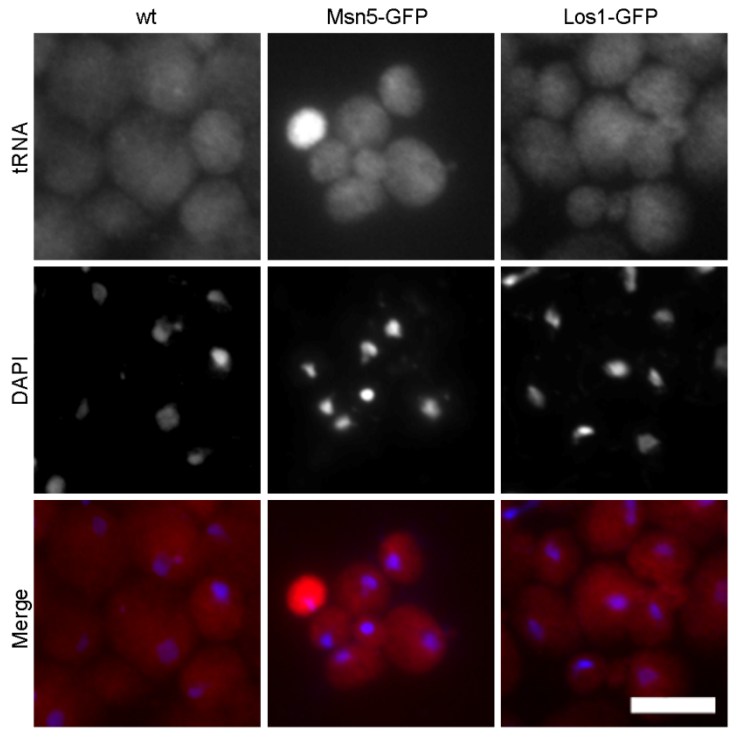

Figure S1. FISH analysis of the subcellular distribution of $tRNA^{Tyr}$ in wt, Msn5-GFP, and Los1-GFP cells. DAPI staining of DNA shows the location of the nucleus. Bar = 5 μm.

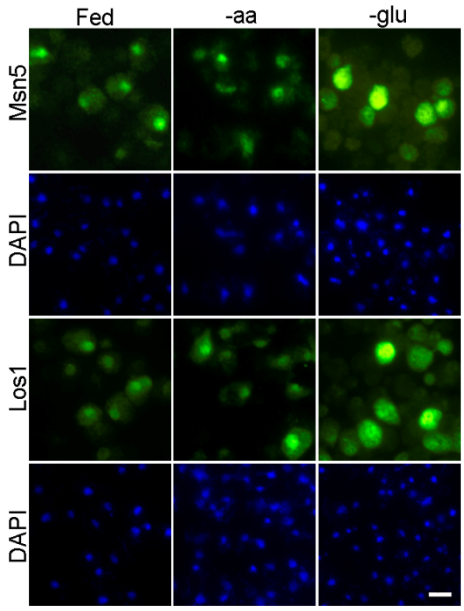

Figure S2. IF analysis of the subcellular distribution of multi-copy Msn5-Myc and Los1-Myc. Bar = 5 μm.

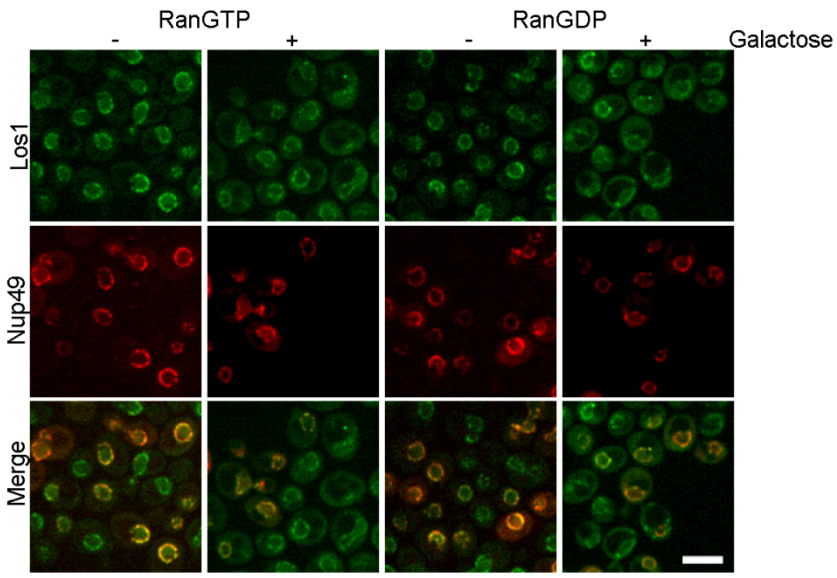

Figure S3. Confocal imaging of Los1-GFP in cells with galactose-inducible Ran locked in the GTP- or GDP-bound state."–", indicates the locations of proteins prior to addition of galactose."+", indicate the locations of proteins after addition of galactose for 2 hr. Bar = 5 μm.

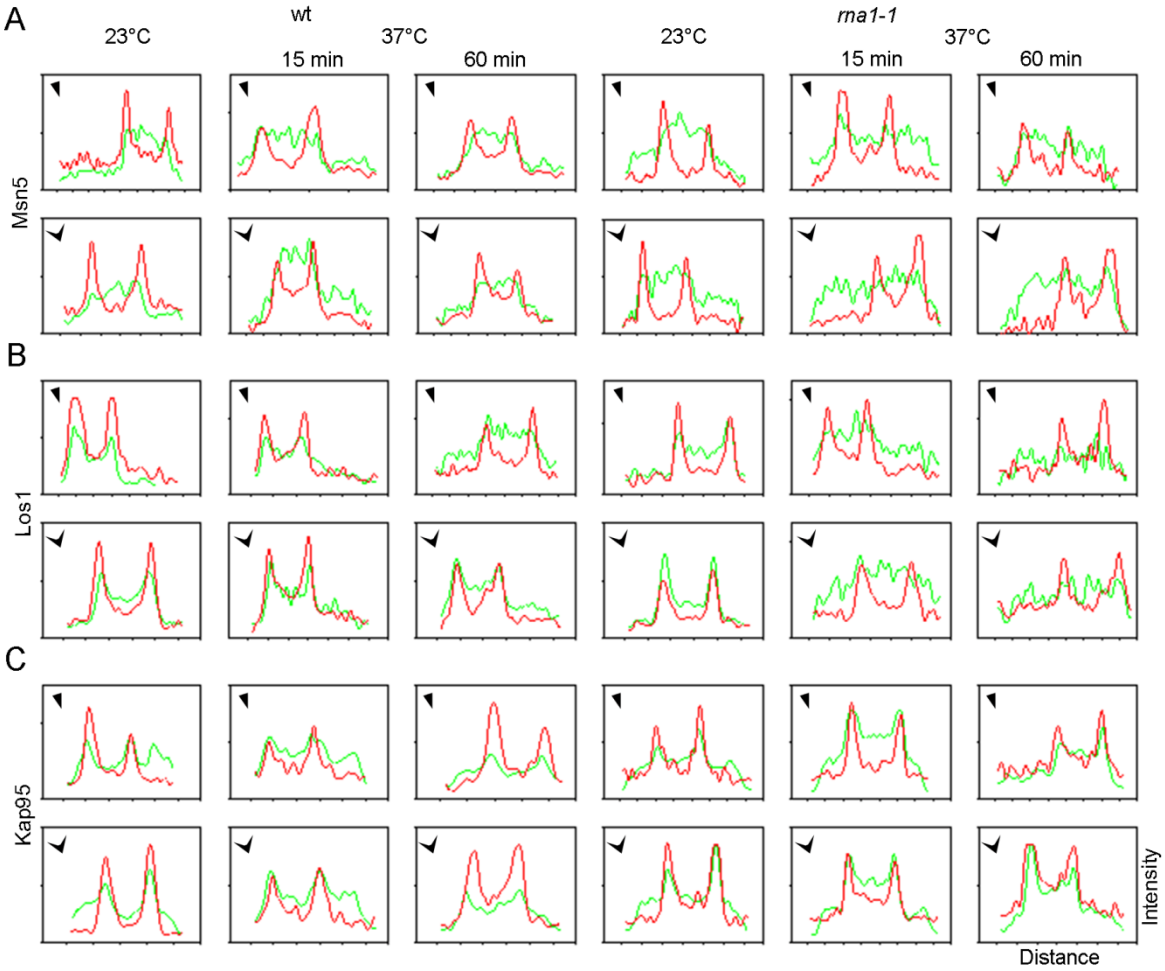

Figure S4. Intensity plot profiles of the subcelluar distribution of (A) Msn5-GFP, (B) Los1-GFP, and (C) Kap95-GFP in wt and *rna1-1* ts mutant. The cells in Fig. 6 were scanned and plotted in Fig. S3 and are indicated with the same shape arrowheads. Red lines: Nup49-mCherry intensities; green lines: karyopherins-GFP intensities.

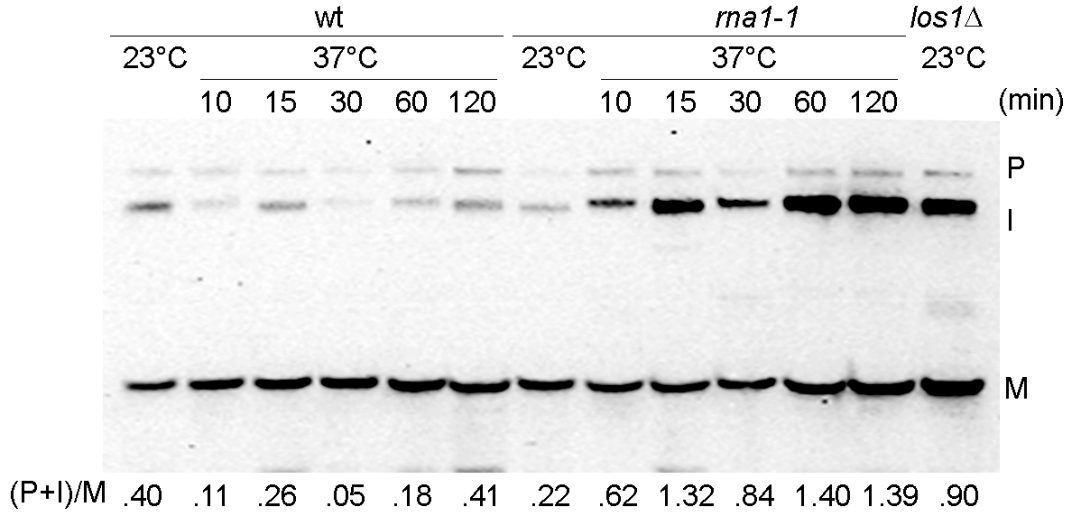

Figure S5. Pre-tRNAs accumulate in *rna1-1* cells. Northern analysis of pre-tRNA^{Ile} and mature tRNA^{Ile} in wt and mutant cells at permissive and non-permissive temperature for indicated times P, primary tRNA transcript; I, end-processed intron-containing pre-tRNA; M, mature tRNA. Ratio for (P+I)/M, both precursor tRNAs to mature tRNA.