Supplemental Materials for

Identification of a quality control mechanism for mRNA 5′-end capping

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Supplemental Figure Legends

Fig. S1. Rai1 decapping activity is restricted to a cap linked to an RNA moiety and does not occur on cap structure. ^{32}P-labeled m^{7}GpppG, GpppG or GpppGp were used as substrates and incubated with or without 50nM recombinant Rai1 and Rat1 at 37^{o}C for 15 minutes in decapping buffer as indicated. The asterisk denotes the labeled phosphate. Reaction products were resolved by PEI- TLC developed in 0.45 M $(NH_4)_2SO_4$. Migrations of standard markers were labeled on the right.

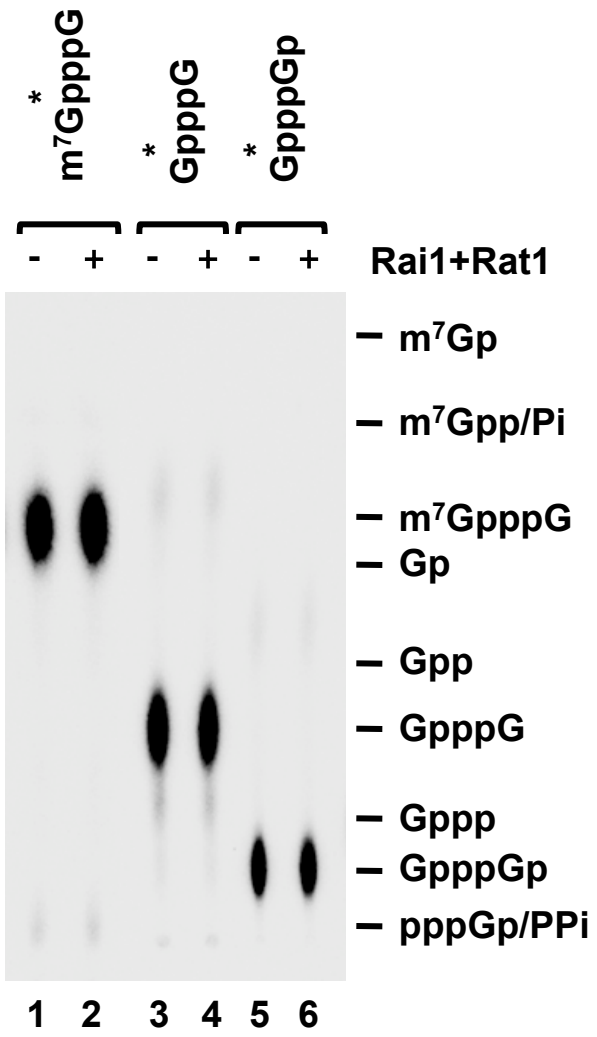
Fig. S2. Rai1 generates GpppN as a reaction product. The products from an unmethylated capped pcP RNA incubated with Rai1 are resistant to calf intestinal alkaline phosphatase (CIP) and nucleoside diphosphate kinase (NDPK). The reaction product of Rai1 hydrolyzed ^{32}P-labeled unmethylated capped RNA was treated with either 1 unit CIP or NDPK as indicated. Control treatments of GpppG$_{OH}$, GpppGp, Gpp and m^{7}Gpp with CIP or NDPK are as noted. The asterisk denotes the position of the ^{32}P. Reaction products were resolved on TLC as described in the legend to Fig. S1. Migrations of markers are indicated on the right.

Fig. S3. Rai1 has comparable pyrophosphohydrolase and decapping endonuclease phosphodiesterase activities. 50nM Rai1 and Rat1 recombinant proteins were incubated with 0.5 pmol ^{32}P-cap-labeled unmethylated pcP RNA or ^{32}P 5′ end labeled triphosphate uncapped RNA at 37^{o}C in decapping buffer for the indicated times. Hydrolysis products were resolved by PEI-TLC developed in 0.45 M $(NH_4)_2SO_4$ to resolve the GpppG cap analogue or in 0.75M

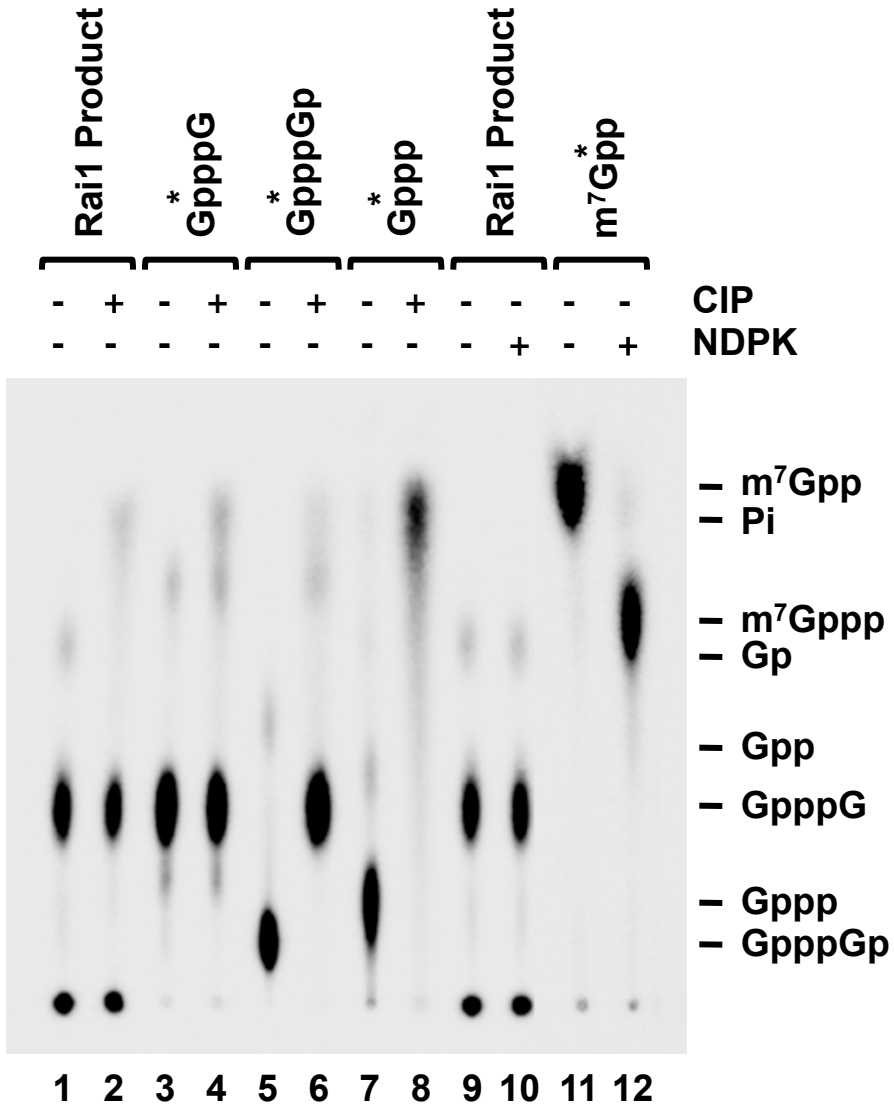
KH_2PO_4 (pH 3.4) to resolve pyrophosphate (PPi). Migrations of markers are shown on the right. Percent of product released are indicated at the bottom.

Fig. S4. Immunopurification of methyl-capped RNA. The monoclonal anti-trimethylguanosine antibody column can distinguish between methylated and unmethylated capped RNA. A mixture of 0.5 pmol ^{32}P -cap-labeled monomethylated or unmethylated pcP RNA (m^7G*ppp RNA or G*ppp RNA, respectively) with 20μg of total yeast RNA was immunoprecipitated with monoclonal anti-trimethylguanosine antibody (αTMG) Agarose beads. Immunoprecipitated RNA (IP) isolated from the αTMG agarose column and the corresponding supernatant containing the unbound RNA (Supernatant) were resolved by denaturing 5% PAGE and are shown.

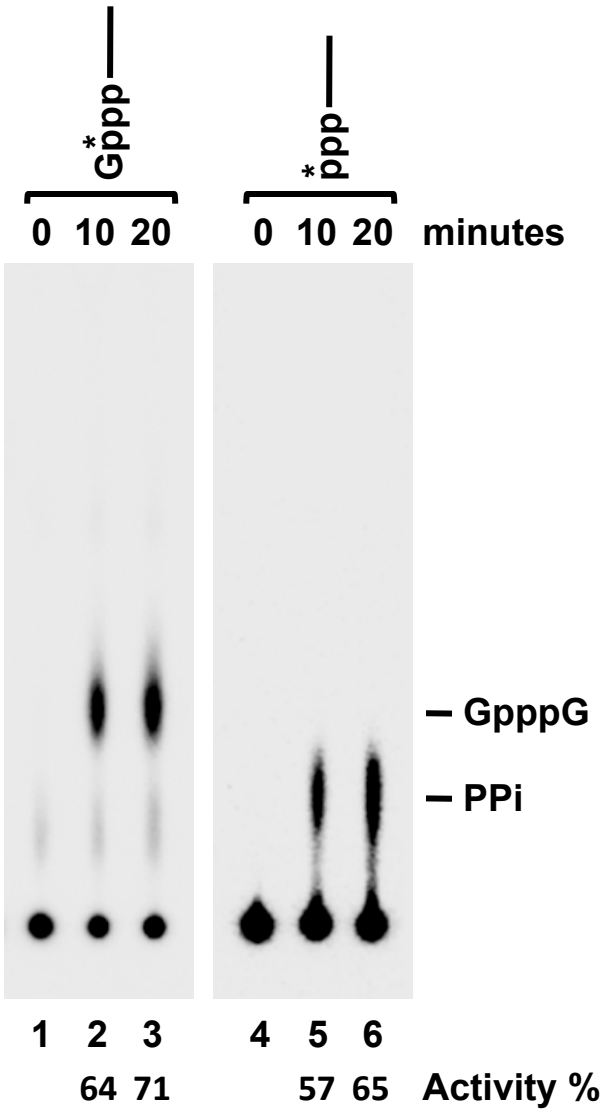
Fig. S5. Generation of an aberrant 5′ cap is an early event. The percentage of aberrantly capped intron containing pre-mRNA is not altered following nutrient starvation indicating the aberrant cap does not arise from a previously normally capped and methylated RNA. Methyl-capped and aberrant capped mRNAs were fractionated by immunopurification utilizing monoclonal anti-trimethylguanosine antibody column as in Fig 4c. Presented are the ratios of aberrantly capped intron containing CYH2 pre-mRNA relative to methyl-capped intron containing CYH2 pre-mRNAs from the rai1Δ strain grown in the indicated medium. The ratio obtained with cells grown in complete medium was arbitrarily set to 1. The ratios remain constant under the different growth conditions. The analyses were carried out as in Figure 4c.



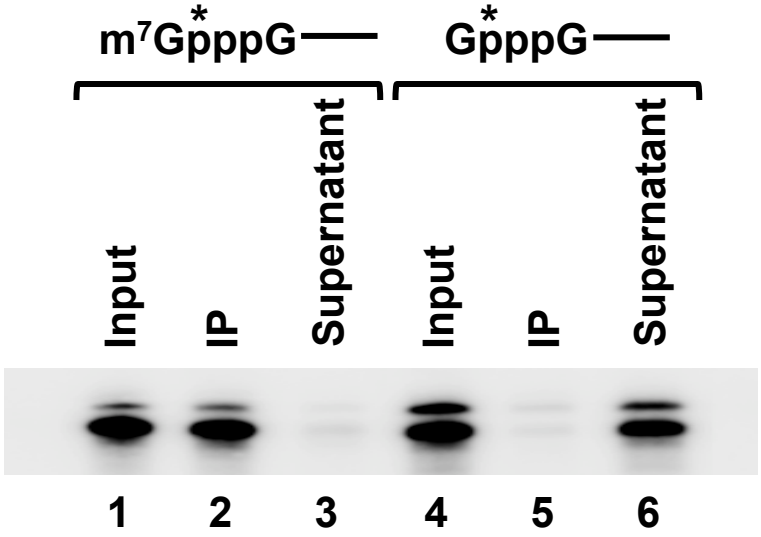
Jiao et al. Fig. S1



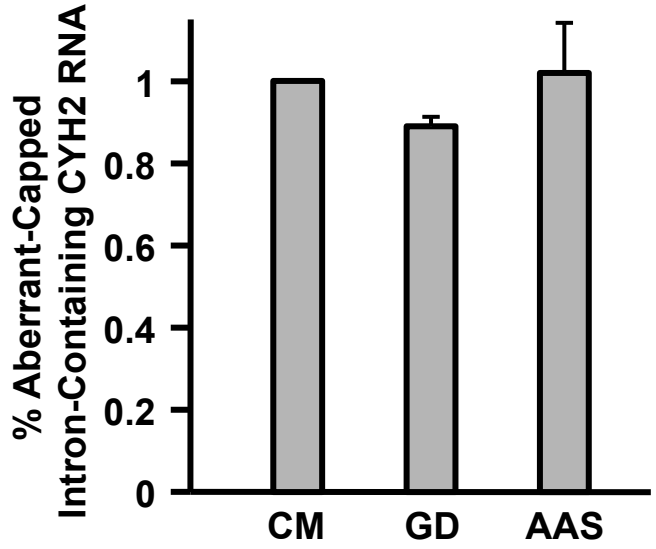
Jiao et al. Fig. S2



Jiao et al. Fig. S3



Jiao et al. Fig. S4



Jiao et al. Fig. S5

Table S1. Yeast Strains Used in This Study

Strain Name	Genotype	Reference
ABD1 WT	*MATα, leu2, ura3, lys2, trp1, his3, abd1::LEU2, p358-ABD1 (TRP1, CEN)*	Schwer et al., 2000
abd1-5	*MATα, leu2, ura3, lys2, trp1, his3, abd1::LEU2, p358-abd1-5 (TRP1, CEN)*	Schwer et al., 2000
ABD1; rai1Δ	*MATα, leu2, ura3, lys2, trp1 his3, abd1::LEU2, p358-ABD1 (TRP1, CEN), rail1Δ::kanMAX4*	This study
abd1-5; rai1Δ	*MATα, leu2, ura3, lys2, trp1, his3, abd1::LEU2, p358-abd1-5 (TRP1, CEN), rail1Δ::kanMAX4*	This study
abd1-5; dcp2Δ	*MATα, leu2, ura3, lys2, trp1, his3, abd1::LEU2, p358-abd1-5 (TRP1, CEN), dcp2Δ::kanMAX4*	*This study*
DTY-10A	*MATa, leu2-3, leu2-112, can1-100, ura-3-1, ade2-1, his3-11, his3-15 (TRP1^{+})*	This study
YGL246C	*Mat a, his3, leu2, met15, ura3, YGL246c::kanMX4*	Open Biosystems
YNL118C	BY4743, Mat a/a, his3/his3, leu2/leu2, lys2/LYS2, MET15/met15, ura3/ura3, YNL118c::kanMX4/YNL118c	Open Biosystems