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Cdk8 Kinase Module Modifies Expression of Specific Translation-Related Proteins Before and After Stress

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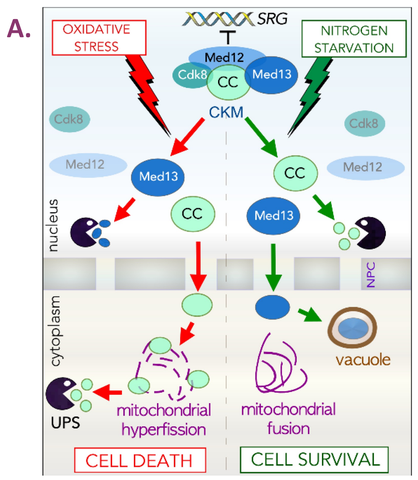
Abstract

Translation is tightly coupled to growth status. Efficient protein synthesis is necessary for cell growth in nutrient rich environments, while global translation inhibition combined with selective translation of stress-responsive mRNAs helps limit growth in times of stress. Environmental stress cues which inhibit the nutrient-sensing complex TORC1 are known to reduce general translation, but how does the cell alter protein synthesis machinery to adapt to these conditions? A few mechanisms to promote cell survival in nitrogen starvation include post-translational modification and selective degradation of specific mRNA-binding translation factors, as well as inhibition of activators of genes whose products are required for general translation. How and when these occur, however, have remained elusive. Here, we demonstrate in *Saccharomyces cerevisiae* that the highly conserved Cdk8 kinase module (CKM) of the mediator complex (cyclin C, Cdk8, Med13, and Med12) transcriptionally upregulates specific 60S ribosome proteins and translation initiation factors such as eIF4G1 to maintain steady state levels of translation-related proteins in physiological conditions. Yeast CKM is known to predominantly repress stress response genes (SRG), and our previous findings revealed that SRG suppression is relieved through the degradation of Med13 and cyclin C following both cell survival and death cues. Our recent data further suggest that degradation of the CKM following nitrogen starvation also plays a transcriptional role in fine-tuning the expression of translation-related proteins. The CKM is thus a multi-faceted hub that can provide insight to how the cell adapts to stress at the levels of transcription, translation, and degradation.

The Cdk8 Kinase Module (CKM) is a mediator of life and death decisions in times of stress.



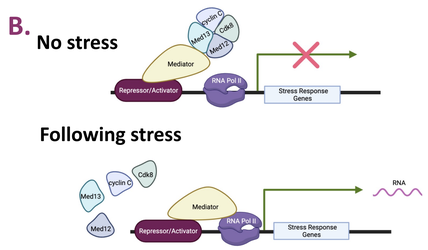


Figure 2. A) Model of the mechanisms used by the CKM to relieve repression on stress response genes (SRG's) following either cell death (oxidative stress) or cell survival (nitrogen starvation) cues in yeast. **B)** Model of mechanism used by CKM of the RNA polymerase II mediator complex to repress and de-repress SRG before and after stress, respectively.

CKM enhances gene expression of specific translation-related proteins in physiological conditions.

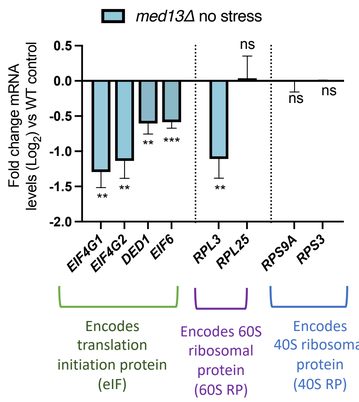


Figure 3. Quantitative RT-PCR (RT-qPCR) analysis of genes encoding translation-related machinery including translation initiation proteins (eIF), 60S ribosomal proteins (60S RP) and 40S ribosomal proteins (40S RP). Expression levels of eIF and RP mRNAs relative to *ACT1* were measured in wild-type (WT) and *med13*Δ strains. Bars indicate Log_2 fold change of gene expression in *med13*Δ relative to WT.

CKM assembly and kinase activity is required to maintain steady-state levels of translation-related proteins in physiological conditions.

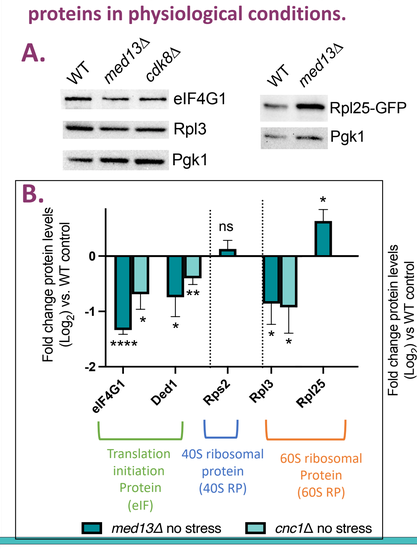


Figure 4. A) Western blot analyses demonstrate decreased eIF4G1 and Rpl3 protein levels (left) and increased Rpl25-GFP protein level (right) in CKM deletion mutants compared to WT. Pgk1 levels were used as a loading control. **B)** Fold change (Log2) protein levels of several eIF, 40S RP, and 60S RP in CKM mutants relative to WT. **C)** Western blot analysis demonstrates a decrease in eIF4G1 protein level in a Cdk8 kinase-dead mutant compared to WT. **D)** Fold change (Log_2) measurements of eIF4G1 and Rpl3 protein levels in kinase-dead mutants relative to WT.

CKM mutants demonstrate abnormal expression of specific 60S ribosomal proteins.

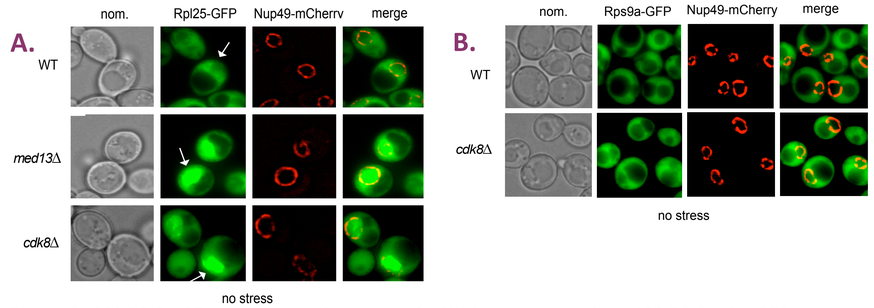


Figure 5. 60S RP Rpl25 accumulates in nucleus in CKM mutants, while a 40S RP Rps9a-GFP demonstrates normal localization in CKM mutants. Fluorescence microscopy to detect **(A)** Rpl25-GFP or **(B)** Rps9a-GFP localization in WT versus CKM mutants. Nup49 is a nuclear pore protein.

CKM mutants are more sensitive to translation inhibition.

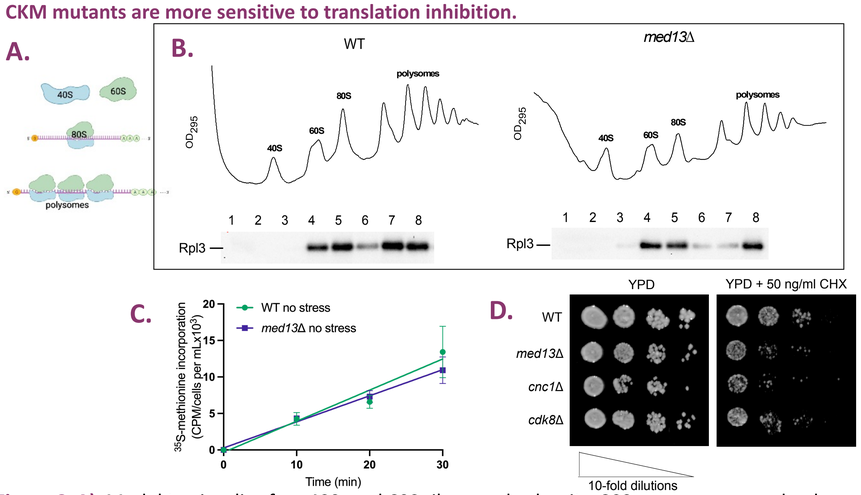


Figure 6. A) Model to visualize free 40S, and 60S ribosomal subunits, 80S monosomes, and polysomes. **B)** Sucrose density centrifugation analysis of polysomes in WT (left) and *med13*Δ (right) non-stressed cells. **C)** Protein synthesis rates in *med13*Δ are similar to WT in non-stressed cells, as determined by radiolabeled methionine incorporation. However, **D)** CKM mutant cultures demonstrate reduced growth relative to WT when spotted in ten-fold serial dilutions on YPD containing 50 ng/ml of cycloheximide (a translation inhibitor).

Altered expression of translation initiation factor eIF4G1 following Nitrogen Starvation (SD-N).

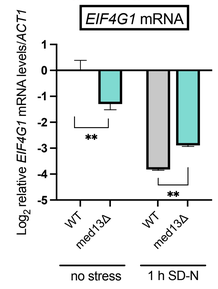
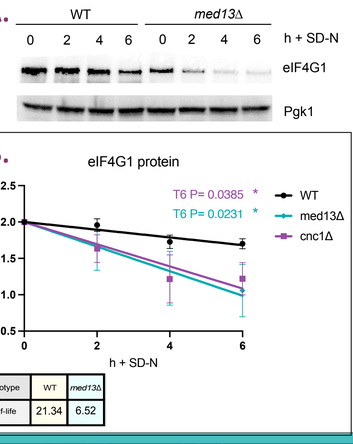


Figure 7. Expression levels of *EIF4G1* mRNAs relative to *ACT1* transcript levels were measured with RT-qPCR in wild-type and *med13*Δ strains, grown in complete media or nitrogen starvation for 1 h. Bars indicate Log_2 fold change of gene expression in experimental conditions relative to non-stressed WT.

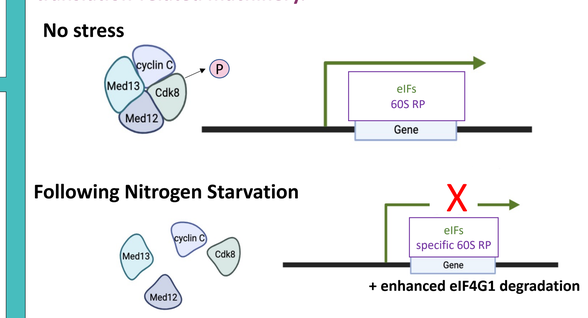
Enhanced degradation of eIF4G1 in CKM mutant following SD-N.



Genotype	WT	*med13Δ*
Half-life	21.34	6.52

Figure 8. A) Western blot analysis of eIF4G1 protein degradation following 0, 2,4, and 6 hours of SD-N in WT and *med13*Δ. Pgk1 levels were used as a loading control. **B)** Quantification of eIF4G1 degradation rates in biological replicates of WT and CKM mutants.

Figure 9. Working model of transcriptional role of CKM with translation-related machinery.



Conclusions

1. CKM transcriptionally upregulates several genes encoding specific translation initiation factors and 60S ribosomal proteins.
2. Proper assembly and kinase activity of the CKM is required to maintain steady-state levels of translation-related proteins in physiological conditions.
3. CKM disassembly following TORC1 inhibition plays a transcriptional role in stress-induced remodeling of specific translation-related proteins, including efficient down-regulation of *EIF4G1* expression and regulated eIF4G1 degradation.

References

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4. Kelly SP, Bedwell DM. Both the autophagy and proteasomal pathways facilitate the Ubp3p-dependent depletion of a subset of translation and RNA turnover factors during nitrogen starvation in Saccharomyces cerevisiae. RNA. 2015 May;21(5):898-910.