Circadian Clock Control of tRNA Synthetases in *Neurospora crassa*

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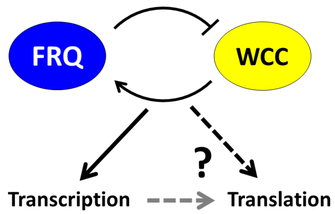
BACKGROUND

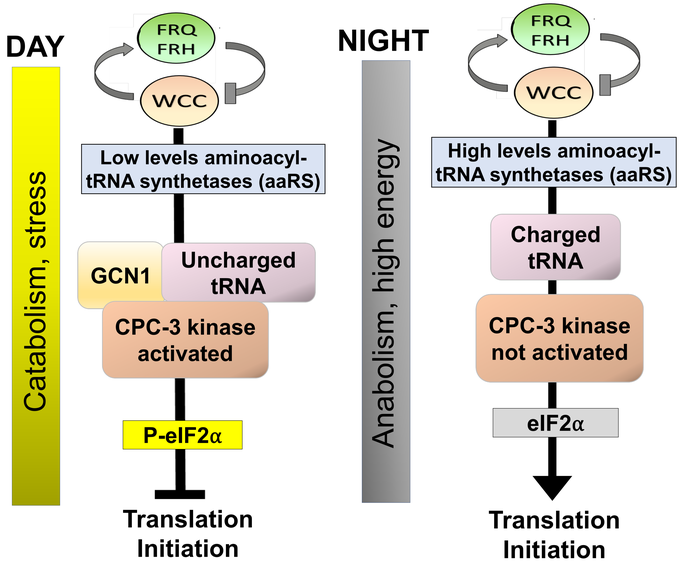
The Circadian Clock

The *N. crassa* core oscillator is known to control transcription, leading to rhythms in mRNA, but the clock's role in translation is understudied.



Rhythmic control of P-eIF2α

Our lab discovered that the *N. crassa* controls rhythmic mRNA translation through the regulation of the eIF2α kinase CPC-3, which phosphorylates and inactivates eIF2α. We showed that clock control of CPC-3 activity requires the rhythmic accumulation of valyl-tRNA synthetase (ValRS) (Karki et al., 2020).



HYPOTHESIS

We hypothesized that clock control of amino acid tRNA synthetases (aaRS's) drives rhythmic mRNA translation, as well as rhythmic growth and development in *N. crassa*.

METHODS

Select aaRS to test

Using RNA-seq and ribosome profiling (ribo-seq) datasets, identify aaRS's which are clock-controlled in both fungi and mammals.

Tag aaRS w/ reporter

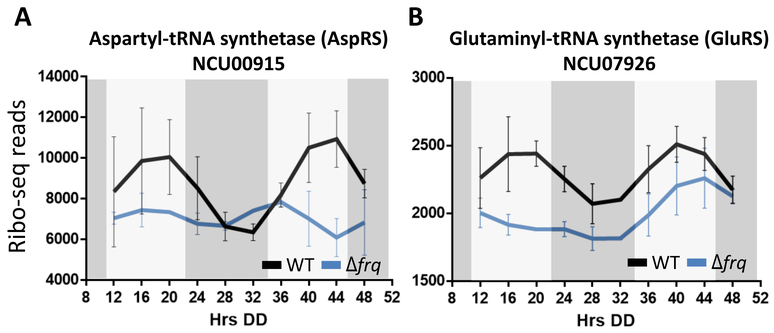
Create luciferase translational fusions to the aaRS's (aaRS::LUC) via 3-way fusion PCR. Transform construct into WT *N. crassa* cells.

Check rhythms

Examine rhythmic luciferase activity in WT and arrhythmic clock mutant Δ*frq* cells.

RESULTS

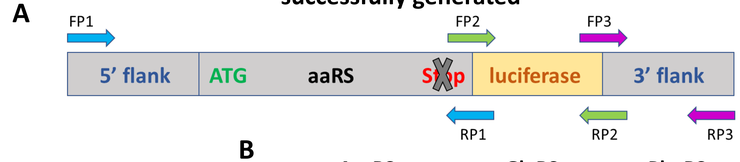
AspRS, GluRS, and PheRS, are clock-controlled based on circadian genomic datasets



Averaged ribo-seq reads for **(A)** AspRS and **(B)** GluRS, or FPKM for **(C)** PheRS in WT (black line) or clock mutant Δ*frq* (blue line) cells grown in DD and harvested at the indicated times (hrs).

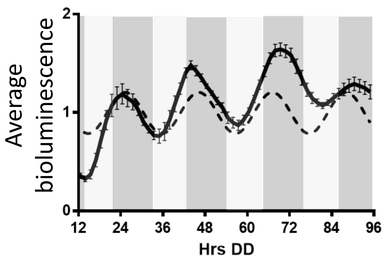
RESULTS

Luciferase translational fusions to AspRS, GluRS, and PheRS were successfully generated



(A) PCR-based strategy to generate luciferase translational fusions to aaRS's
(B) PCR products on a 1% agarose gel.

PheRS::LUC translational reporter fusion is rhythmic in DD, peaking during the subjective night



Luciferase activity from PheRS::LUC in WT cells, grown in DD over 4 d (Hrs DD). The average bioluminescence signal is plotted (mean ± SEM, n=12). PheRS::LUC in WT cells is rhythmic as indicated by a better fit to a wave ($p<0.05$, dotted black lines).

CONCLUSIONS

1. We have identified three clock-controlled aaRS from genomic datasets namely, AspRS, GluRS and PheRS.
2. We have successfully created luciferase translational fusions to AspRS, GluRS and PheRS to examine protein levels *in vivo* over several days.
3. PheRS protein rhythms are rhythmic in WT, validating the genomics data.

FUTURE DIRECTIONS

1. Examine rhythms of AspRS::LUC and GluRS::LUC in WT cells; and of AspRS::LUC, GluRS::LUC and PheRS::LUC in Δ*frq* cells.
2. If confirmed to be clock-controlled, the rhythmic expression of each will be abolished to determine how loss of rhythmicity alters rhythmic translation, growth, and development.

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