Effect of aromatic amino acid starvation-induced by glyphosate-based herbicides on splicing efficiency in Saccharomyces cerevisiae

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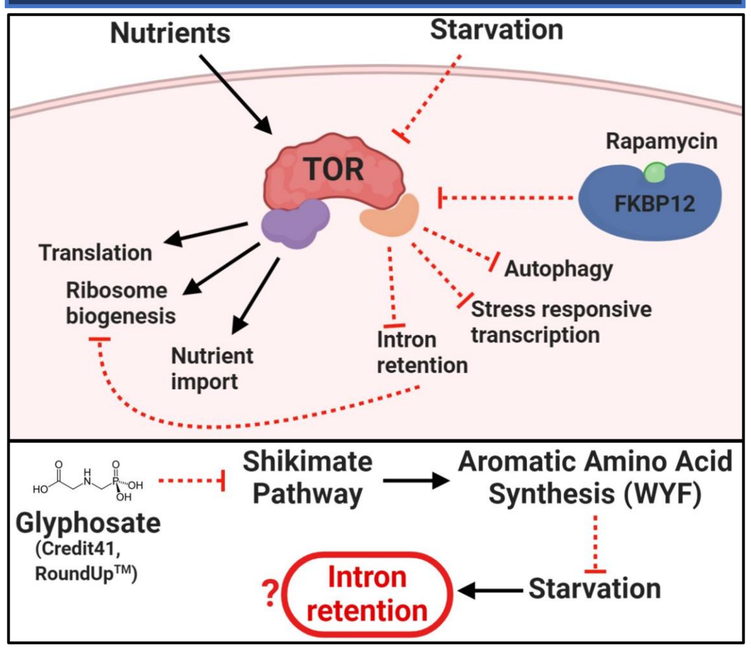
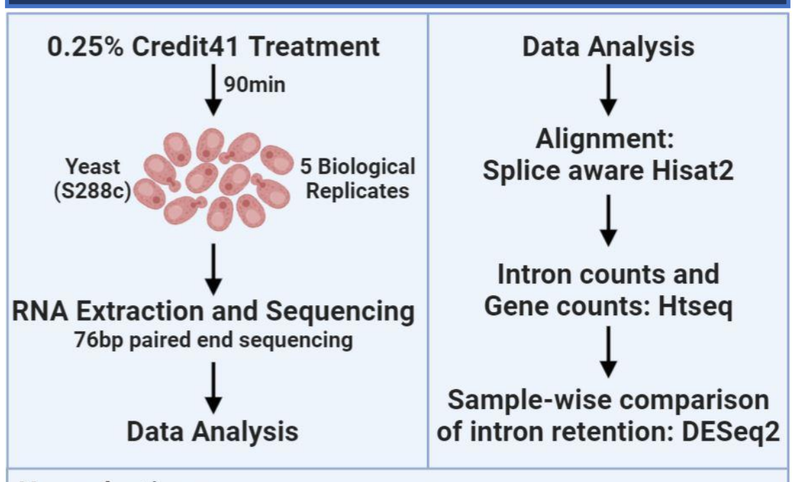
Abstract

While it is well understood that cells regulate growth during starvation, the molecular and genetic factors that contribute to this response are not clear. In yeasts, starvation leads to exit from the cell cycle into a quiescent state until the availability of nutrients returns. During short-term starvation, autophagy is induced to recycle building material until nutrients are replenished; however, long term starvation is detrimental for cells. The availability of nutrient activates the TOR pathway which is the central regulator of cell growth including transcription, ribosome biogenesis, translation, and cell cycle progression. One of the cellular responses to increase survivability during starvation is to modulate splicing which leads to the stabilization of a subset of diverse introns. Most of the introns in yeasts are in the ribosomal protein-encoding genes. One possible function for these stabilized introns during starvation is to sequester splicing factors which further reduces splicing. Production of ribosomes is energy-intensive and is rapidly downregulated during any stress including starvation. During growth, TOR inhibits these accumulation of stable introns. Yeasts exposed to glyphosate-based herbicides (GBH), such as RoundUp™ and Credit41, undergo starvation and inhibit the TOR pathway. GBH inhibits the shikimate pathway which is responsible for the production of aromatic amino acids i.e., phenylalanine, tryptophan, and tyrosine. Quantitative Trait analysis identified a splicing factor that when knockout out increased yeast tolerance to GBH, which led to our hypothesis that downregulation of splicing increases survival during nutrient limitation. Comparison of RNA-seq from the sensitive and resistant strains found hundreds of differentially express transcripts and we have quantified the changes in splicing efficiency. We expect to see modulation in splicing profile of various ribosomal protein genes. In the future, we will manually curate the list to look for candidate genes that are previously known to be associated with starvation and how modulation of splicing provides survival benefits to cells during starvation.

1 Objective



2 Method

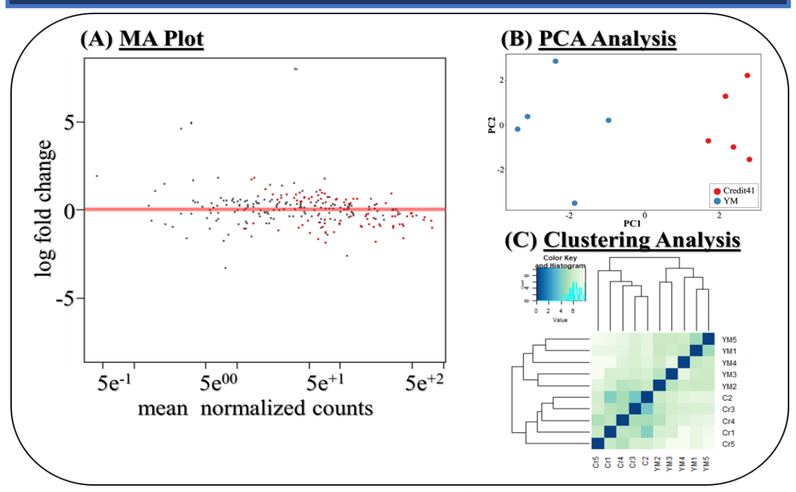


Normalzation:

$$\text{Normalized Intron Counts} = \text{Round-off}\left(\frac{IC}{GC} X1000\right)$$

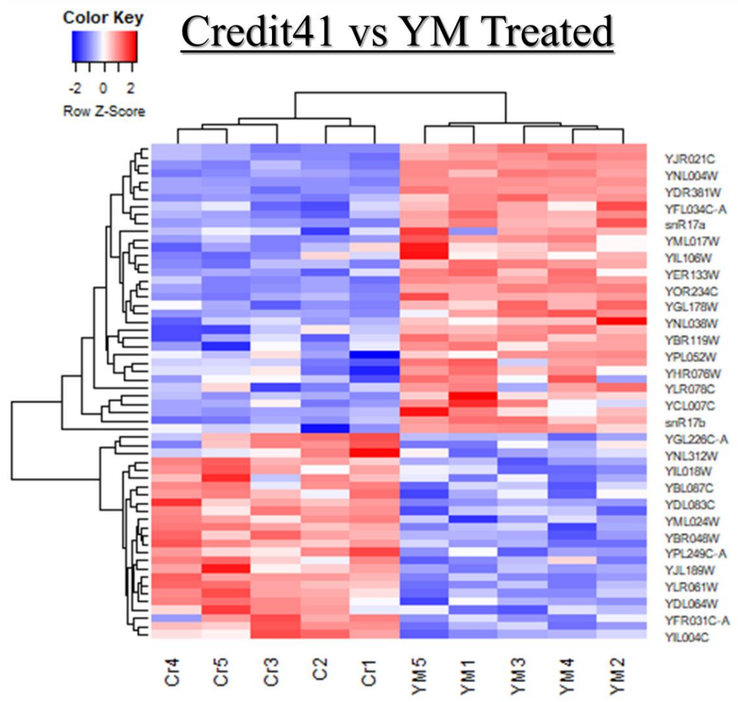
To remove expression level differences the intron counts (IC) were first normalized by gene counts (GC) for each repliicate. This value was then multiplied by 1000 and then rounded-off to neares integer.

3 Results: Normalization



(A) MA plot of Credit41 vs. YM treated samples indicate that samples have been correctly normalized as the mean red line passes through 0 on y-axis. Red dot indicate intron with significant differential retention. (B) Principal component analysis show that YM samples and Credit41 samples cluster separately along PC1 axis. Blue dots are YM samples and Red dots are Credit41 treated samples. (C) Clustering analysis also show that YM samples cluster together and Credit

4 Results: Heatmap of introns with differential retention



5 Upregulated Intron retention: Ribosomal Protein Genes (RPGs) have increased intron retention

Gene_Name	log2FoldChange	padj
UBC9	1.808821512	0.000825252
OST5	1.745527641	1.53E-05
RUB1	1.70388925	5.41E-05
RPL2A	1.153633645	0.019974167
PCC1	1.090658679	0.048407092
RPL22A	0.941757447	1.46E-05
RPL37B	0.935722554	1.28E-07
RFA2	0.915994082	0.049031479
YPR063C	0.878645905	0.007104055
RPL35B	0.878312308	0.000195895
RPL31B	0.861469666	0.017821113
RPL23A	0.787816211	0.000326324
RPS11A	0.754455424	0.031584503
RPL39	0.701991553	0.002585342
BET1	0.698830462	0.000196152
RPS17A	0.666655	0.014790388
RPS4A	0.653966977	0.003727131
RPL2B	0.632021164	0.000826995
RPS14B	0.624258307	8.20E-06
RPS16B	0.608989621	3.42E-06
RPL36B	0.606524398	0.00383512
RPL40A	0.598006257	0.013190753
RPL23B	0.563013639	0.002770297
RPL37A	0.546209445	3.95E-06
RPS11B	0.512876228	0.021689041

GO biological process complete	Fold Enrichment	FDR (p-value)
translational termination (GO:0006415)	31.6	7.40E-30
cellular protein complex disassembly (GO:0043624)	27.86	6.68E-29
cytoplasmic translation (GO:0002181)	27.53	5.84E-29
ribosomal small subunit assembly (GO:0000028)	26.9	3.15E-03
protein-containing complex disassembly (GO:0032984)	23.66	1.47E-27
cellular component disassembly (GO:0022411)	17.77	9.61E-25
translation (GO:0006412)	12.9	1.55E-21
peptide biosynthetic process (GO:0043043)	12.76	1.72E-21
ribosome assembly (GO:0042255)	11.92	9.79E-03
peptide metabolic process (GO:0006518)	11.71	1.15E-20
amide biosynthetic process (GO:0043604)	11.26	2.58E-20
cellular amide metabolic process (GO:0043603)	9.67	8.77E-19
ribosomal large subunit biogenesis (GO:0042273)	9.11	7.35E-03
ribosomal small subunit biogenesis (GO:0042274)	7.9	1.46E-02
protein-containing complex subunit organization (GO:0043933)	6.71	4.68E-15
ribosome biogenesis (GO:0042254)	5.74	8.16E-05
organonitrogen compound biosynthetic process (GO:1901566)	5.64	2.38E-14
rRNA processing (GO:0006364)	5.38	1.28E-02

6 Downregulated Intron retention: snRNA17A and snRNA 17B reduce intron retention

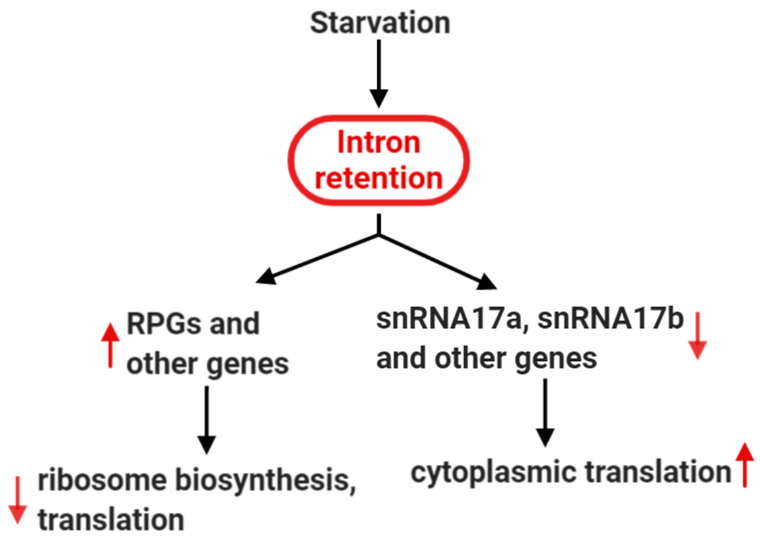
Gene_Name	log2FC	padj	Gene_Name	log2FC	padj
OM14	-1.87379	3.43E-06	IMD4	-0.92283	1.61E-31
SNR17B	-1.84418	1.18E-15	YCL007C	-0.91177	0.001871
YRA1	-1.67614	1.62E-25	PTC7	-0.90275	0.021829
YJR112W-A	-1.61523	0.000108	OAZ1	-0.85475	0.023359
HRB1	-1.34785	1.13E-14	YOR318C	-0.84547	8.51E-05
REC107	-1.31663	1.96E-17	TAD3	-0.83388	0.000156
SNR17A	-1.27996	1.19E-15	GCR1	-0.82294	3.41E-28
MUD1	-1.26368	1.05E-06	APE2	-0.80936	5.06E-10
YHR097C	-1.1546	0.015719	RPL22B	-0.80806	0.00012
MOB1	-1.09384	0.048407	GLC7	-0.80091	0.025258
REC102	-1.02022	1.96E-22	GPI15	-0.80068	0.002008
BET4	-1.01753	0.049031	DBP2	-0.78448	8.60E-08
RPS22B	-1.01142	2.43E-155	PSP2	-0.65134	1.26E-06
IWR1	-1.00007	0.000397	RPL33B	-0.64302	1.24E-50
BOS1	-1.00003	0.013865	RPL17B	-0.61699	0.000531
NCB2	-0.95721	4.55E-15	RPL43B	-0.58601	4.11E-26
YDR381C-A	-0.94239	0.000146	MPT5	-0.54018	3.42E-06
			CMC4	-0.51496	0.047401

GO biological process complete	Fold Enrichment	FDR (p-value)
translational termination (GO:0006415)	9.52	9.23E-03
cellular protein complex disassembly (GO:0043624)	8.39	1.14E-02
cytoplasmic translation (GO:0002181)	8.29	8.25E-03
protein-containing complex disassembly (GO:0032984)	7.13	1.81E-02

7 Conclusions

- Several RPGs increase intron retention under credit41 induced nutrient starvation
- snRNA17a and snRNA17b (U3 snoRNA) downregulate intron retention under nutrient starvation by Credit41
- Intron retention increase in transcripts responsible for ribosome biogenesis, translation etc.
- Intron retention decrease in transcripts responsible for translation termination, cytoplasmic translation etc.

8 Model



9 Funding

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