Gene expression analysis and temporal ablation of AMA-1/Pol II show that transcriptional regulation supports survival deep into starvation

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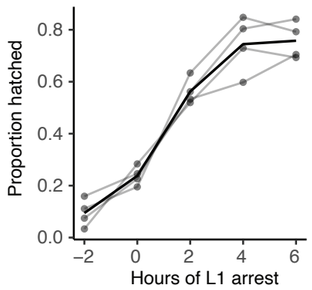
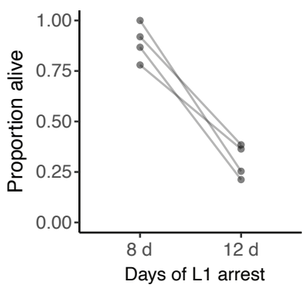
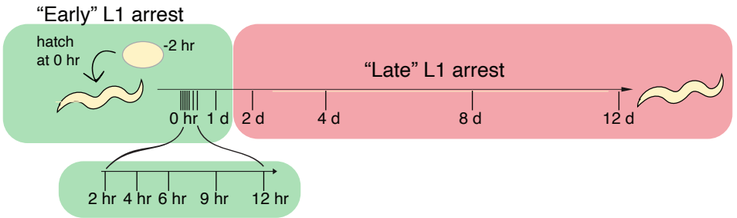
1. Introduction and RNA-seq design: *C. elegans* arrest development in the first larval stage in the absence of food (L1 arrest)



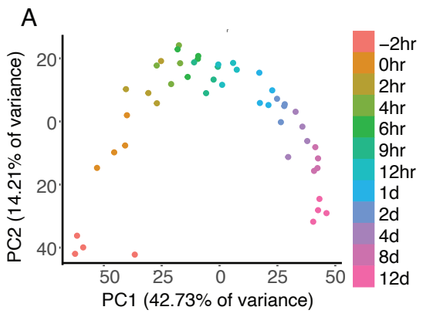


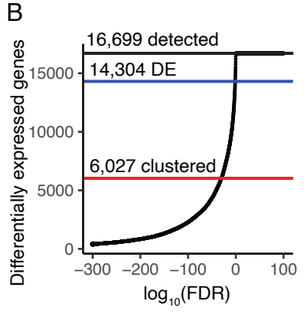
L1 larvae hatch during an approximately four hour window, and die within about two weeks of arrest in the absence of food.

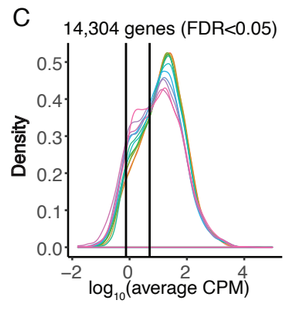


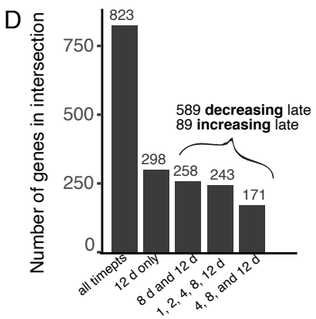
Previous work has assessed transcription up to 24 hours into L1 arrest [1], and transcription appeared to reach a steady state. However, late transcription that may be relevant to starvation resistance has not been assessed. We sequenced the mRNA of 4 biological replicates across the 12 time points shown, spanning early and late L1 arrest. We then assessed the importance of transcription late in arrest for survival.

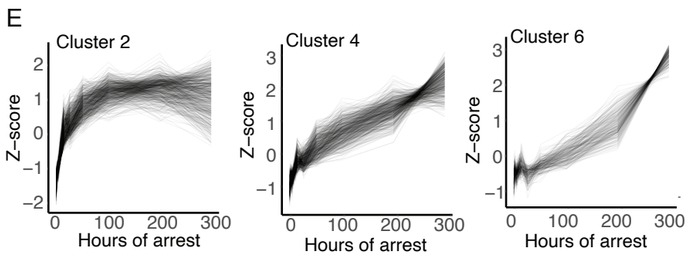
2. Results: Transcriptional changes occur rapidly early in L1 arrest but slow down later. Hundreds of genes are up-regulated or stably expressed late in starvation.



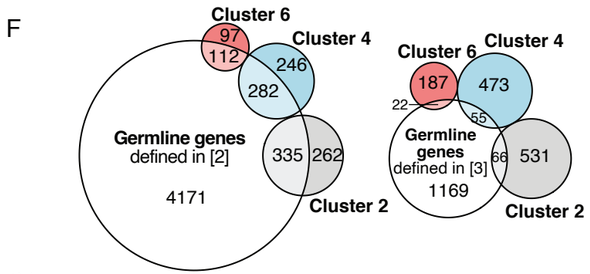




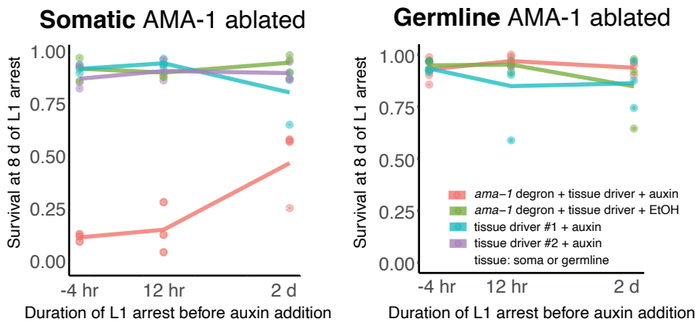




Principal component analysis separated time points across PCs 1 and 2 (A). We performed differential expression analysis on 16,699 detected genes across all 12 time points and found the vast majority (14,304) are differentially expressed (DE) at FDR < 0.05 (B). We found that the DE genes tended to be expressed at lower counts-per-million (CPM) in the later time points (C, between the two black lines). Of low-expressed genes from C found at multiple late time points, we found the majority of these genes are decreasing in expression, with some (~12%) increasing (D). Despite this transcriptome-wide pattern, among 6027 highly DE genes, 3 of the largest 6 clusters consist of genes that are stable or increasing late in arrest (E). These clusters are enriched for germline genes, as assessed by hypergeometric p-value (F).



3. Results: AMA-1/Pol II is required in the soma, but not the germline, for survival late in L1 arrest.



To assess the importance of mRNA transcription during arrest, we used the auxin-inducible degron (AID) system to ablate AMA-1 in the soma or germline at various time points. Survival was scored at 8 days of L1 arrest. Survival is dependent on AMA-1 in the soma, but the effect appears to weaken if AMA-1 is degraded later in L1 arrest (2 days). We do not see an effect if AMA-1 is ablated in the germline. This suggests that the gene expression changes occurring beyond 24 hr of arrest in the soma are essential for survival.

4. Conclusions and ongoing directions

Gene expression changes slow down throughout L1 arrest, but many genes still change late in arrest. The CPM distribution shifts consistently over the course of arrest, with an increase in low-abundance transcripts, primarily driven by genes decreasing in expression over time. However, among the most significantly differentially expressed genes, hundreds of genes are part of clusters that are stable or increasing in expression late in arrest, and these are, perhaps surprisingly, enriched for germline genes. To assess whether late transcription was required for survival, we ablated AMA-1/PolII in the soma and germline and found it is required in the soma but not the germline. These results raise questions that we are currently addressing using the AMA-1 AID system: Are the "germline genes" in Clusters 2, 4, and 6 actually increasing in the germline? What are the relative contributions of transcript stability and new transcription in shaping late gene expression patterns? Are germline transcripts preferentially stabilized late in L1 arrest?

References:
[1] Baugh *et al., RNA Pol II Accumulates as Promoters of Growth Genes During Developmental Arrest, Science*, 2009.
[2] Packer *et al., A lineage-resolved molecular atlas of C. elegans embryogenesis at single-cell resolution, Science*, 2019.
[3] Lee *et al., Nanos promotes epigenetic reprogramming of the germline by down-regulation of the THAP tranrscription factor LIN-15B, eLife*, 2017.