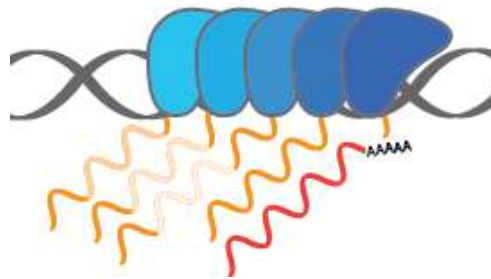


RNA as a dynamic molecule: how and when our cells regulate mRNA splicing



Athma A. Pai
November 8, 2021

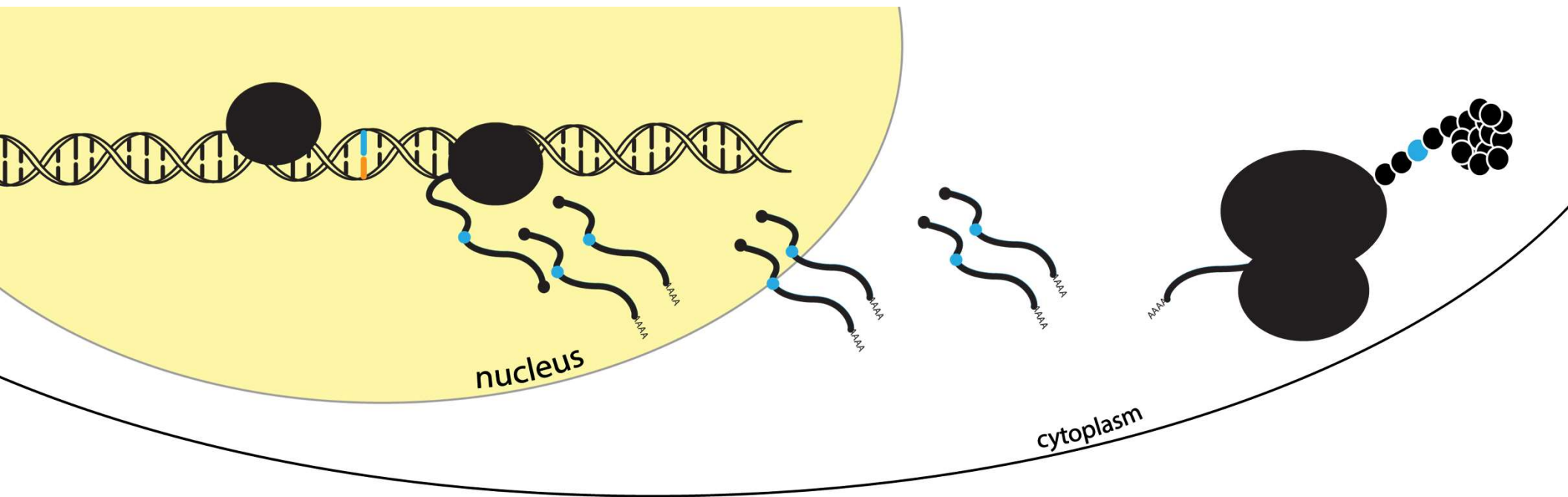


RNA
THERAPEUTICS
INSTITUTE



@athmapai

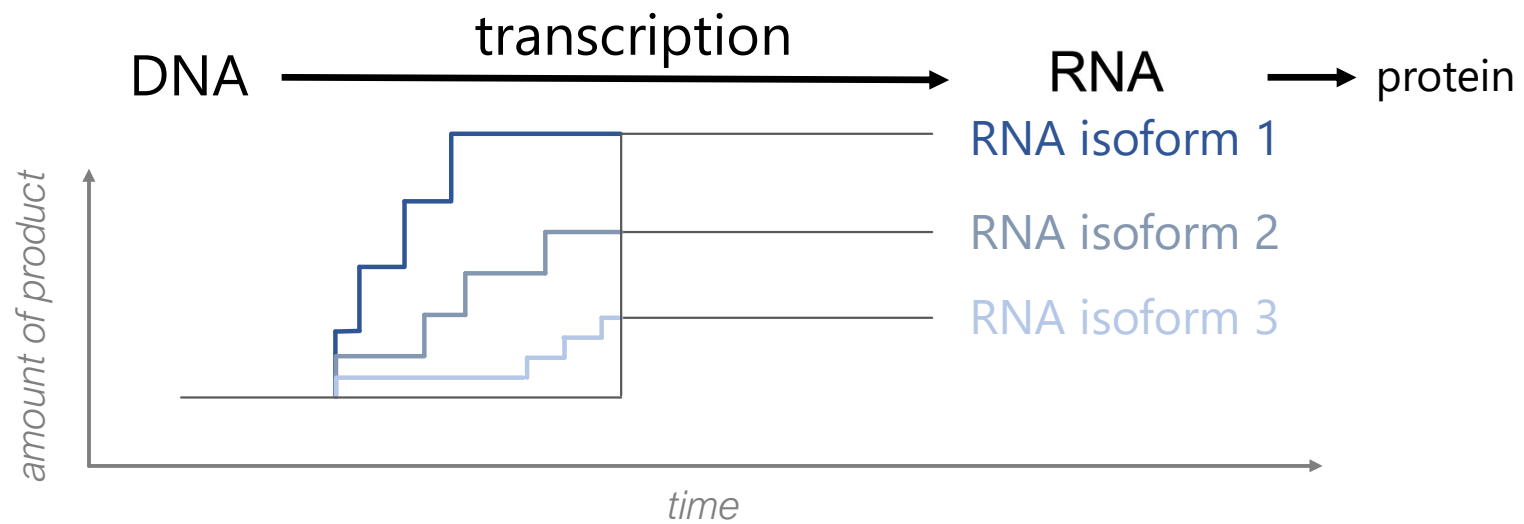
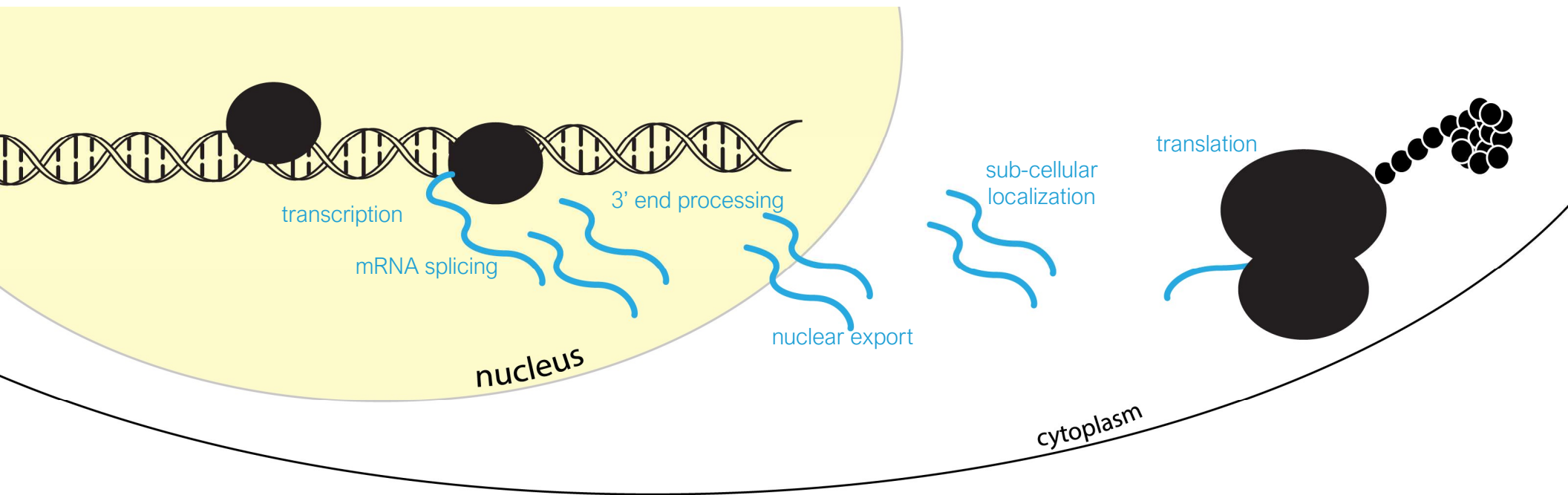


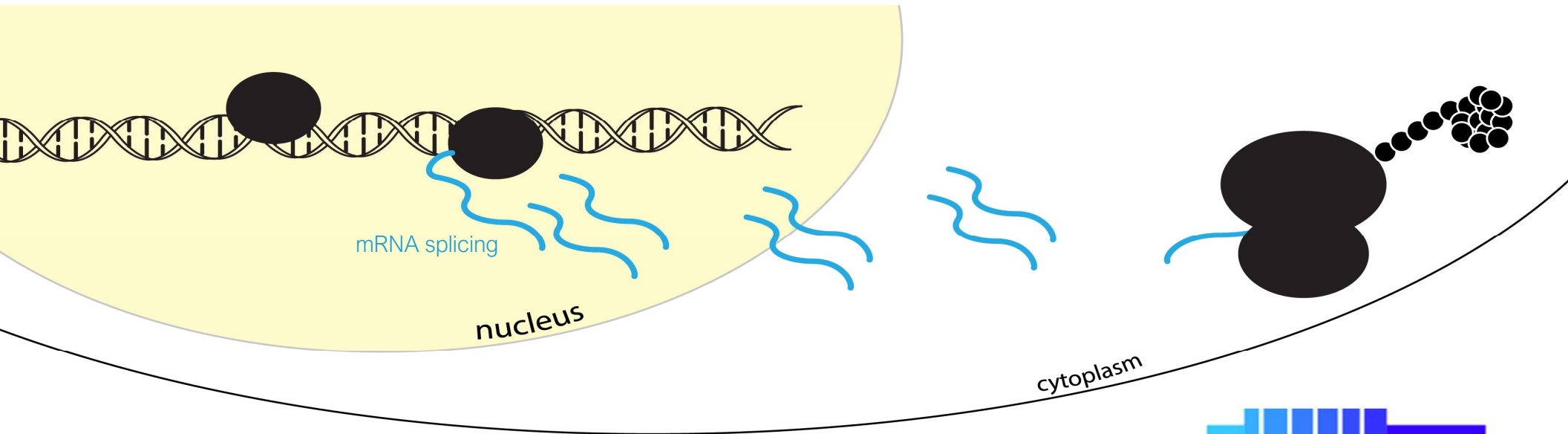


genetic information

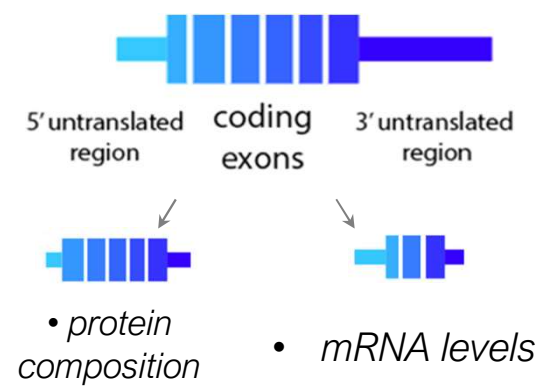
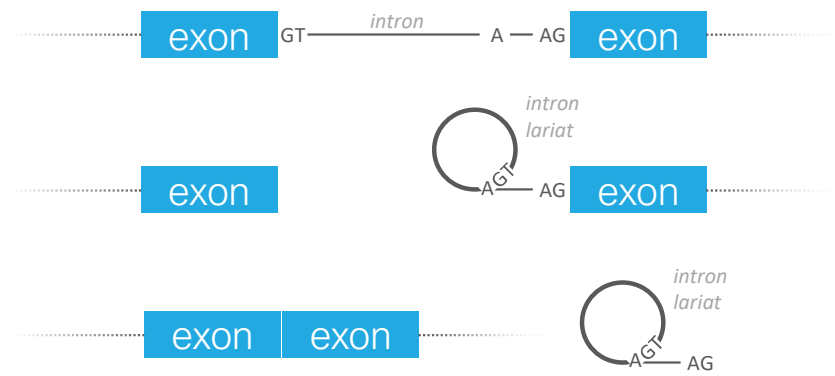
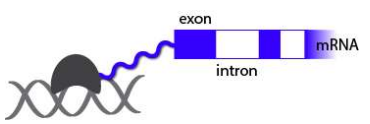
molecular mechanisms regulating transcriptome dynamics

diversity of cellular states and phenotypes



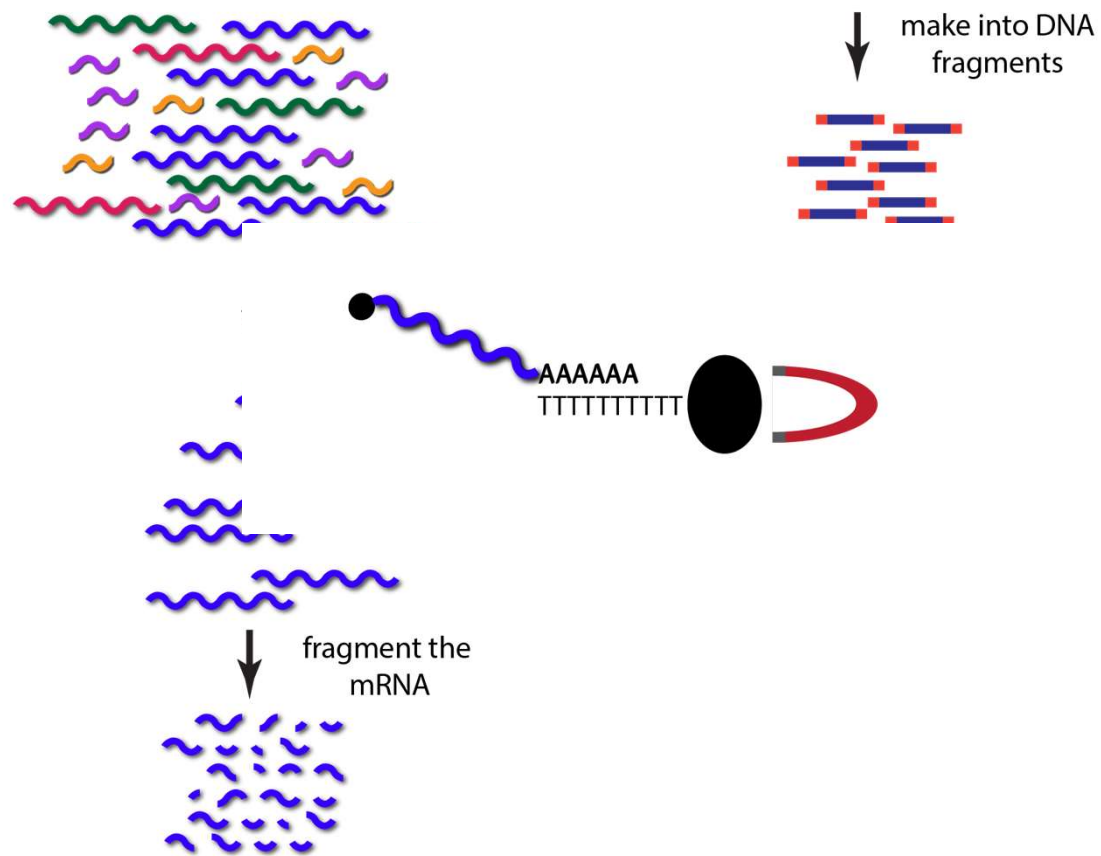


Splicing is an essential step in the mRNA lifecycle

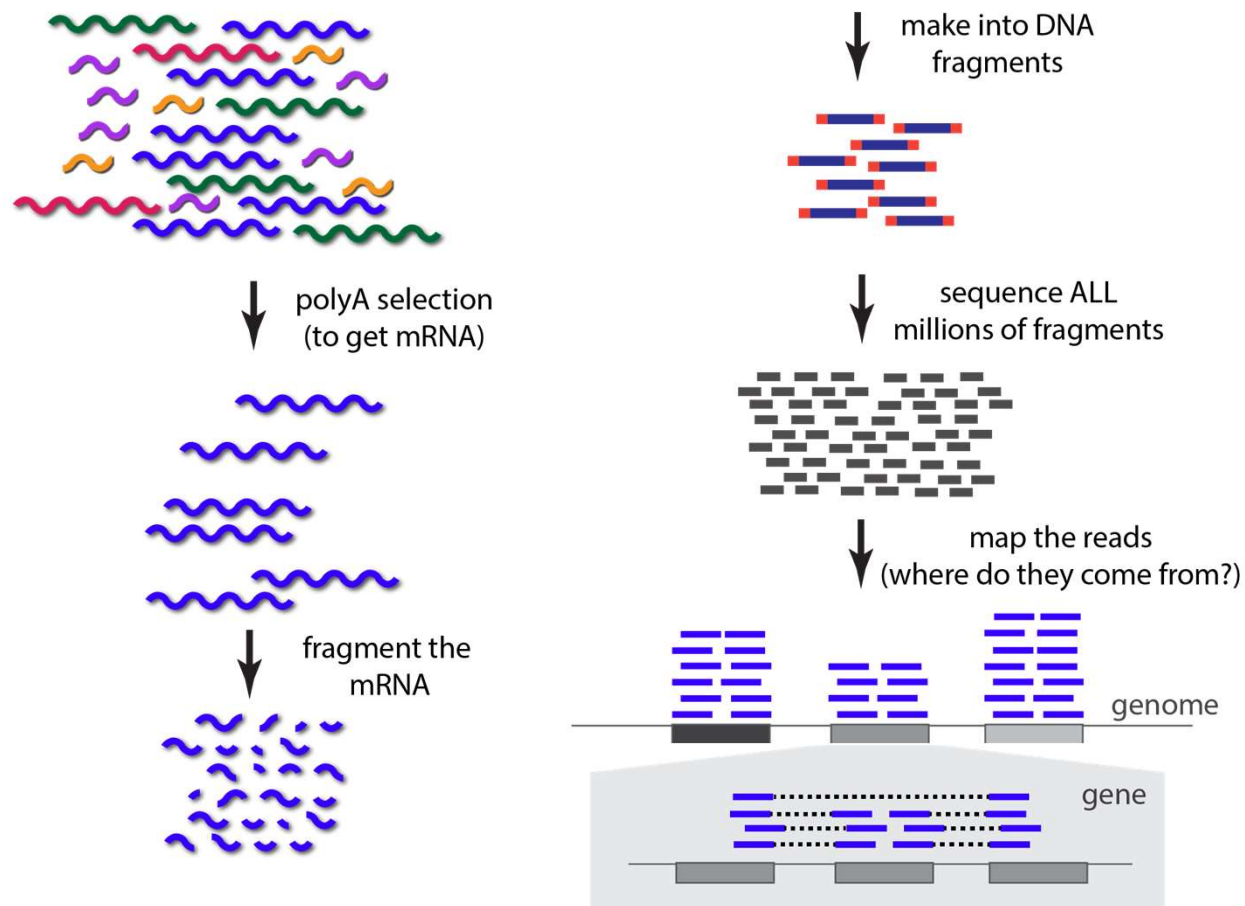


>90% of mammalian genes have alternative isoforms
cell-type and tissue-specific regulation

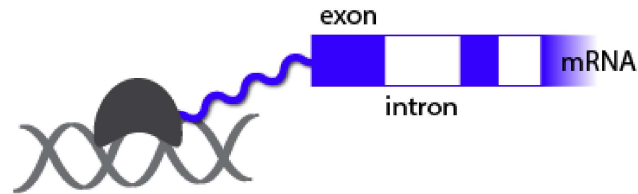
High-throughput RNA sequencing to measure RNA processing



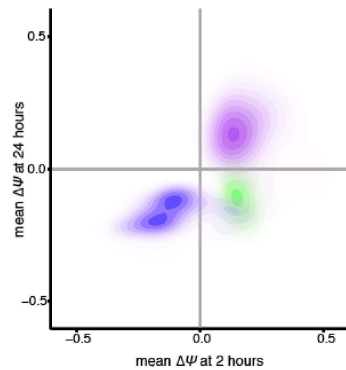
High-throughput RNA sequencing to measure RNA processing



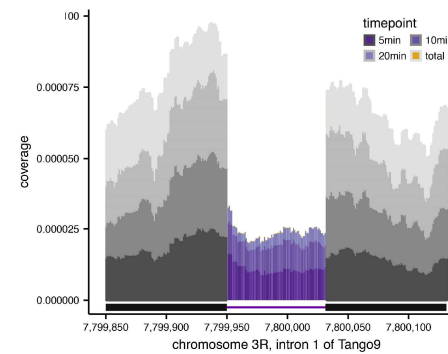
Exploring the dynamics of mRNA splicing



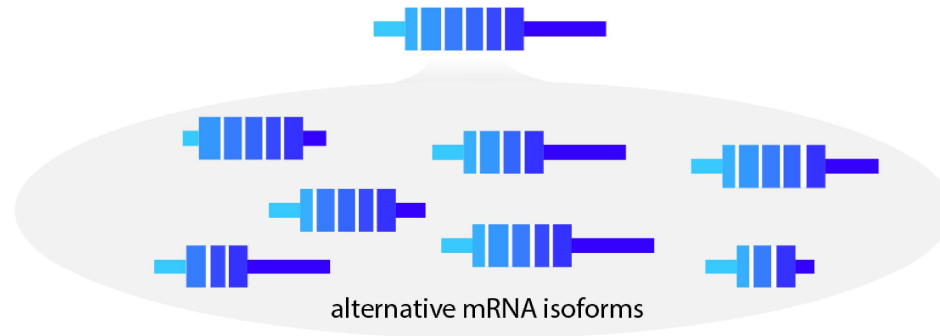
RNA processing dynamics in human immune response



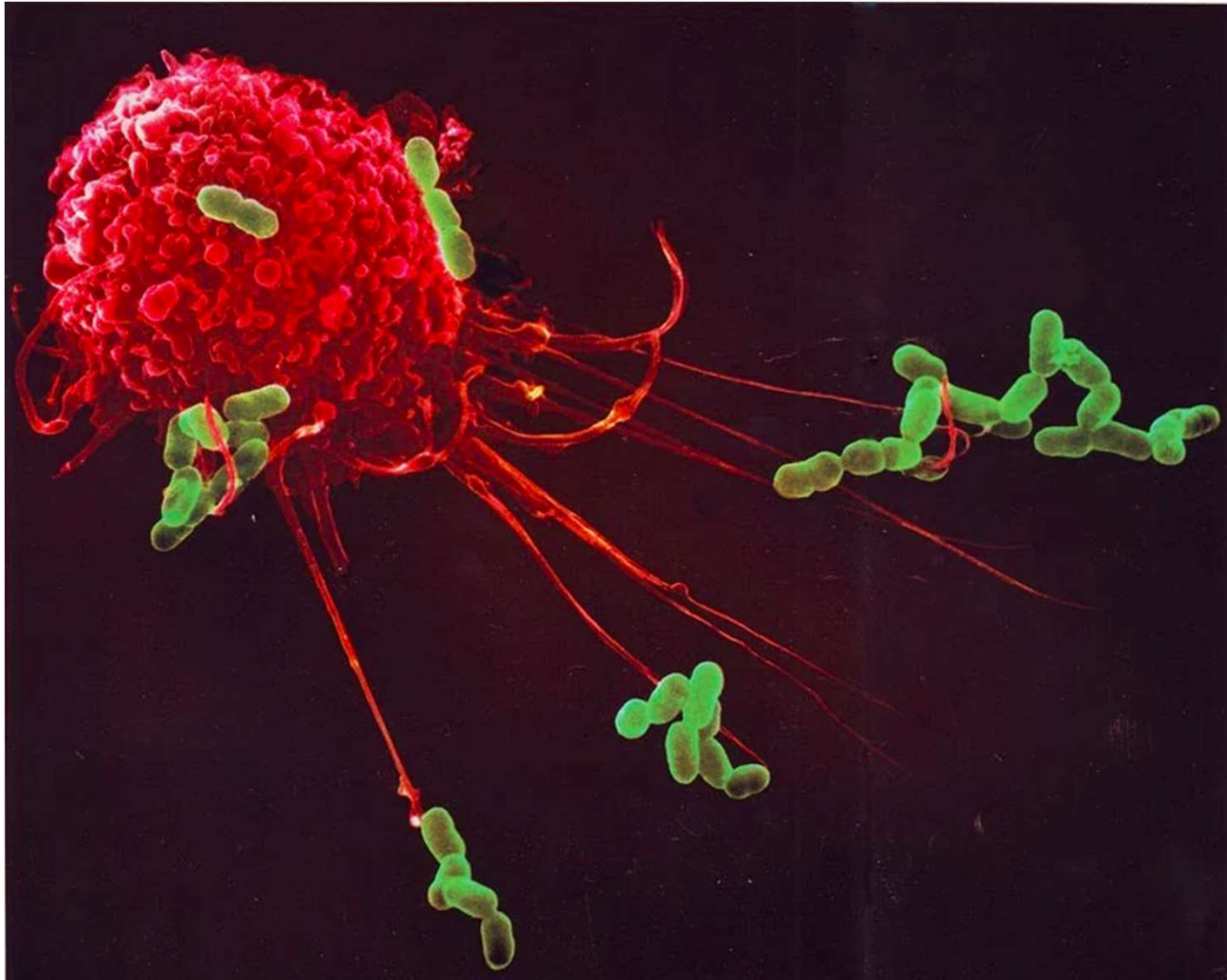
mRNA splicing kinetics in flies



How is mRNA processing dynamically regulated in response to an external stimulus?

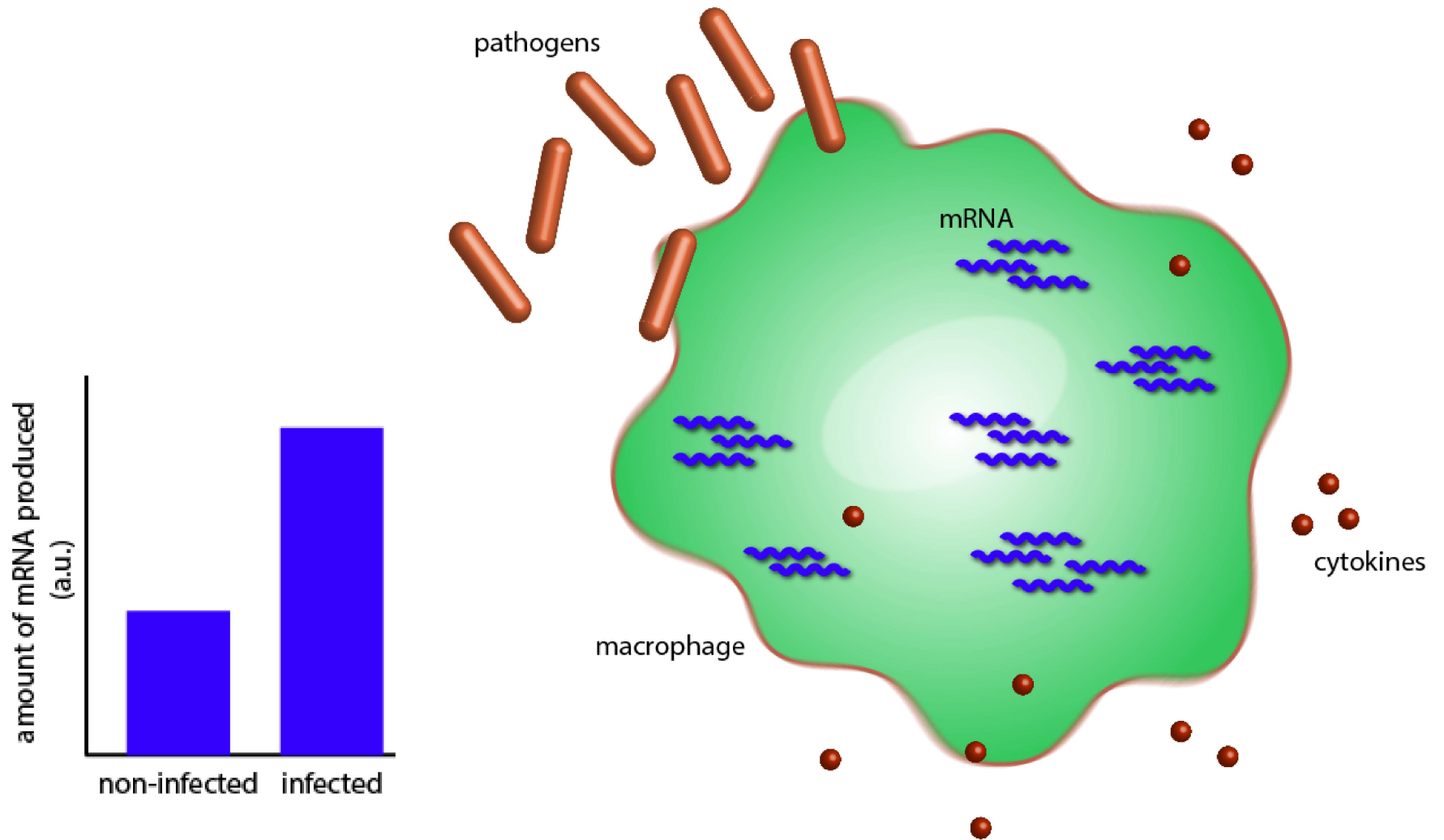


Bacterial infection induces the innate immune response

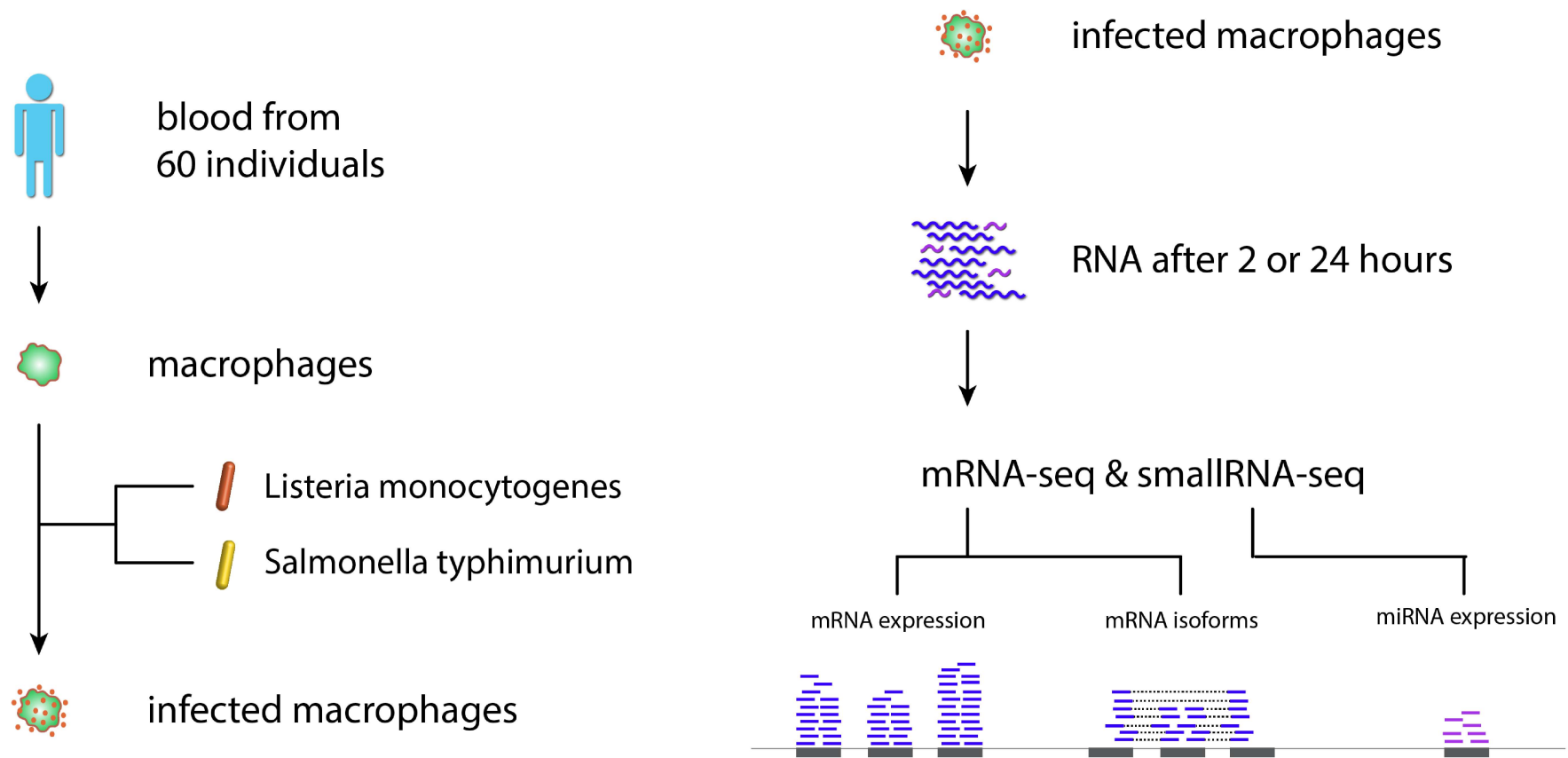


National Geographic

Transcriptional Activation upon Immune Response



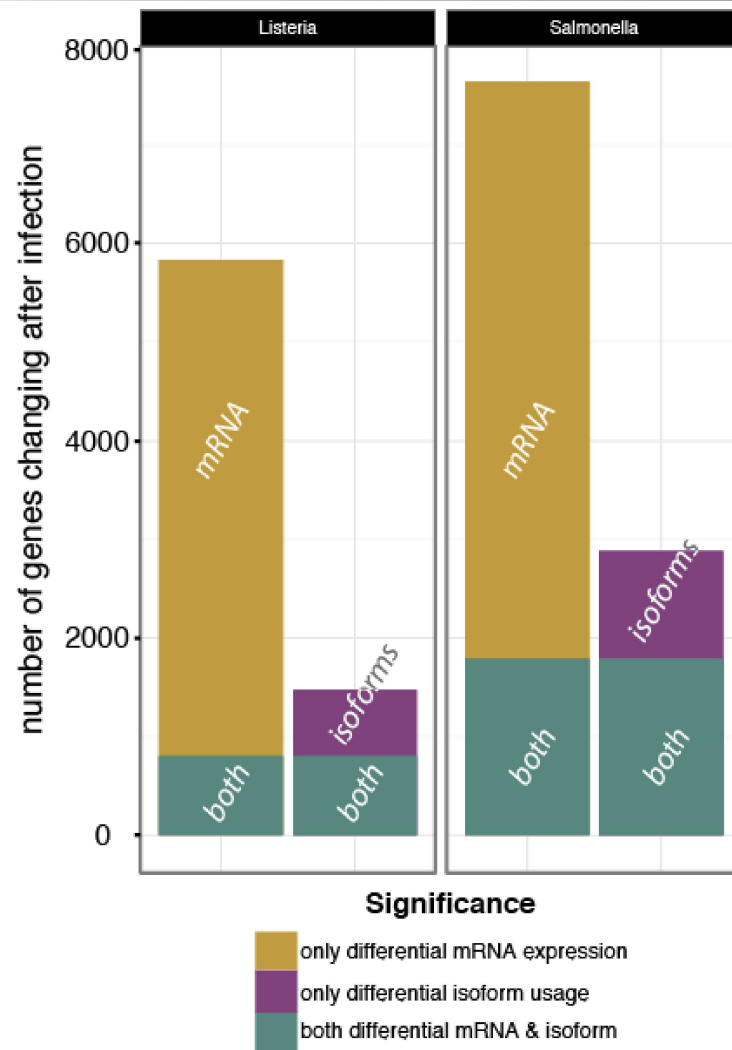
Studying immune response after bacterial infection



Pai, Baharian, *et al.* 2016
Nédélec, Sanz, Baharian, *et al.* 2016

In collaboration with Luis Barreiro (Université de Montréal/UChicago)

Many changes in gene expression & isoform usage



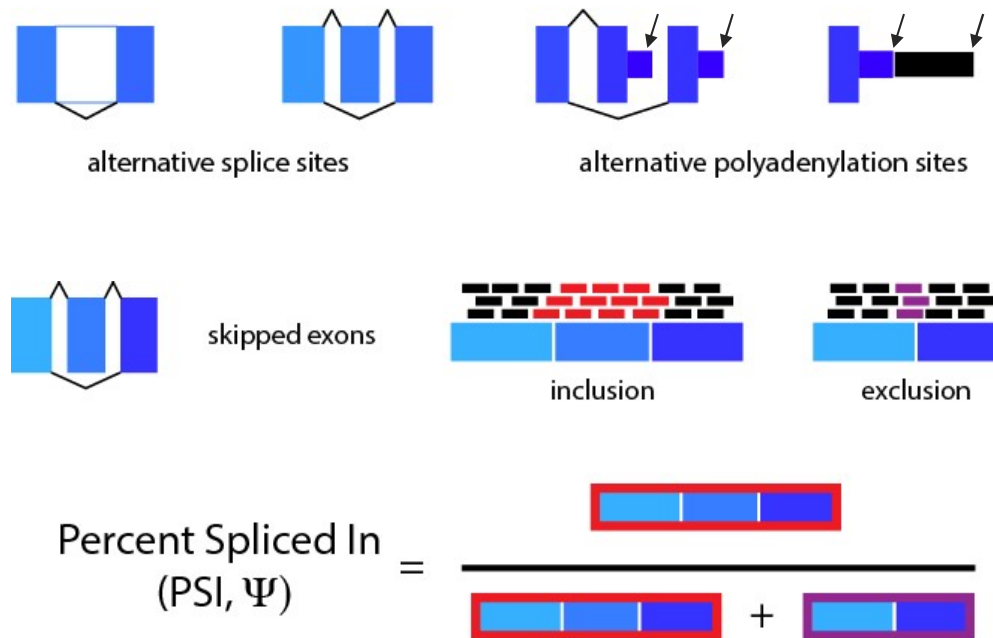
30-45% of genes
differentially expressed

13-25% of genes with
differential isoform usage

10% of genes ONLY
change isoform usage

both sets of genes:
enriched in immune-related genes

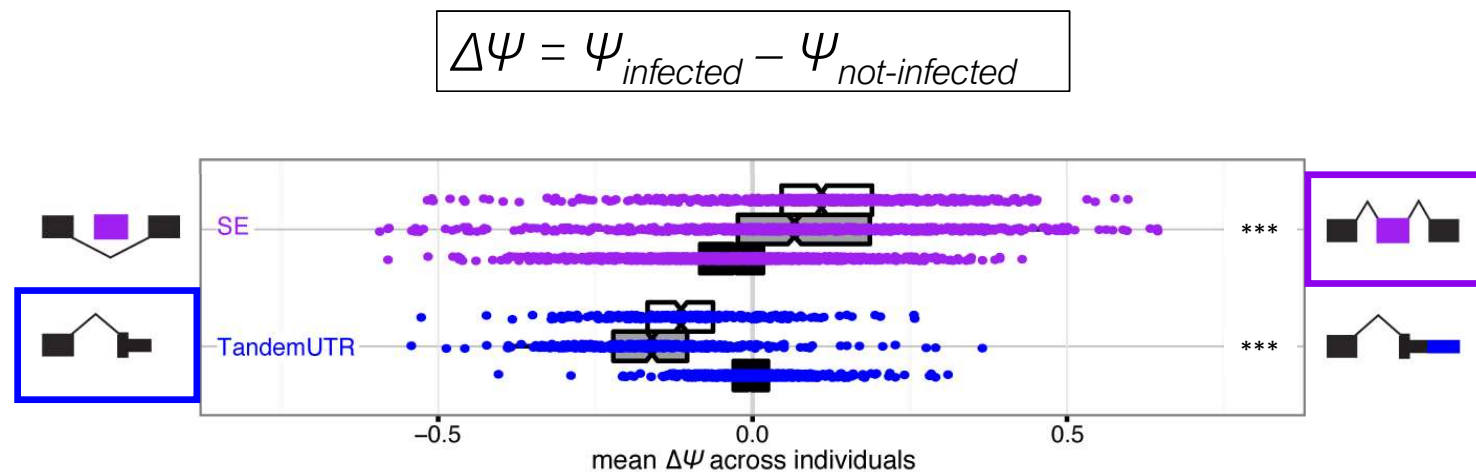
Exon-specific dynamics after infection



Calculations performed using
Mixture of Isoforms (MISO) software

Wang *et al.* 2008
Katz *et al.* 2010

Global shifts in specific RNA processing mechanisms

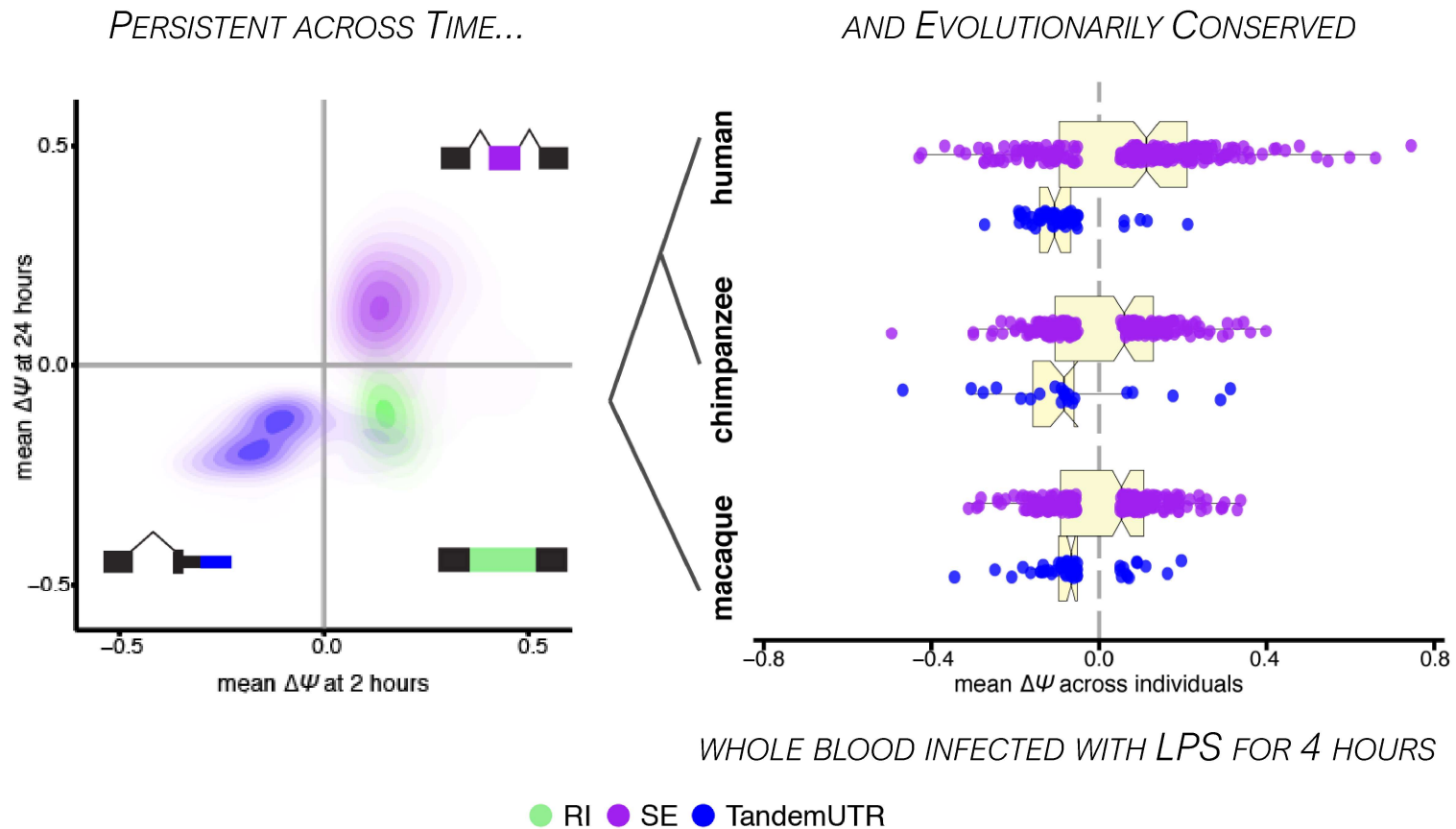


after infection:

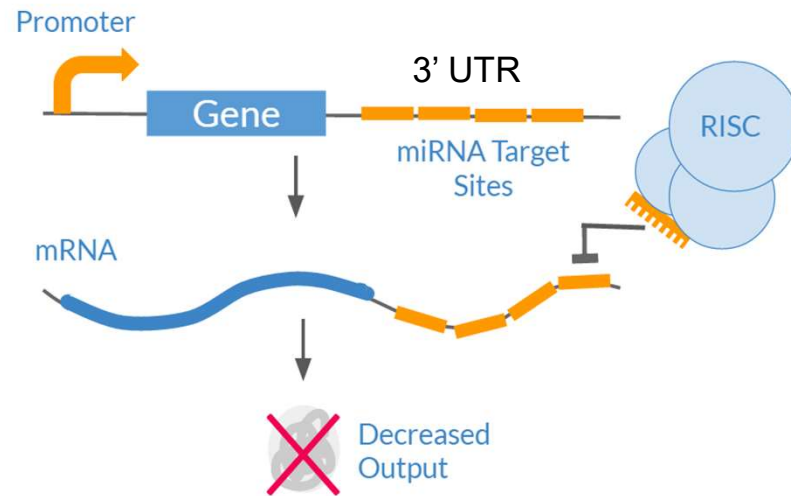
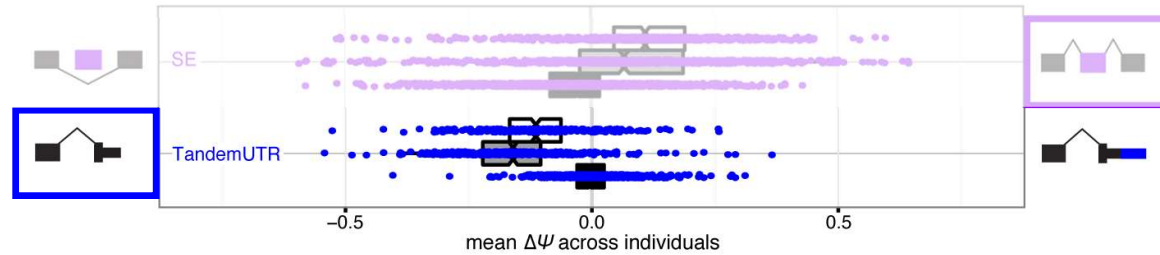
- inclusion of alternatively skipped exons
- usage of shorter 3' UTRs



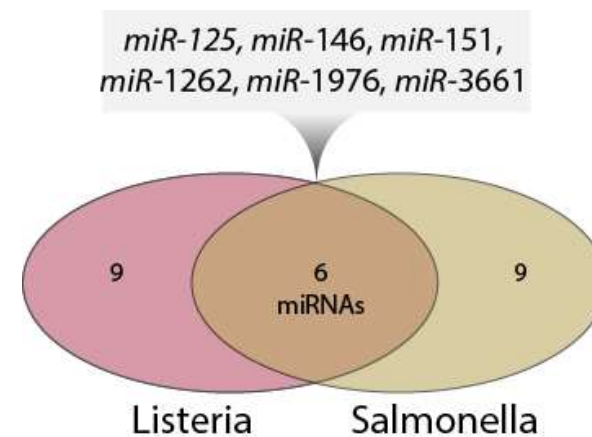
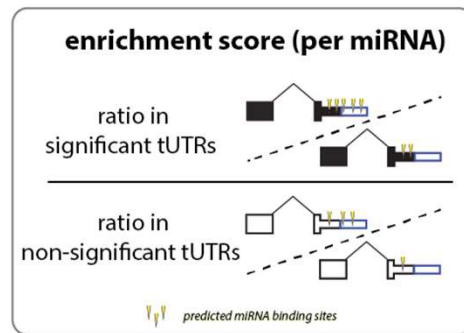
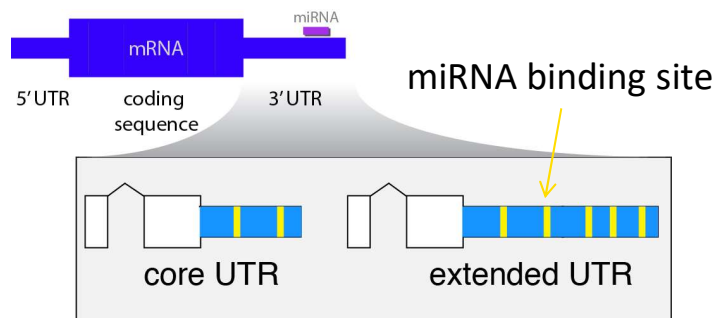
Exon & 3' UTR regulation are general properties of immune response



Why are 3' UTRs shortened upon infection?



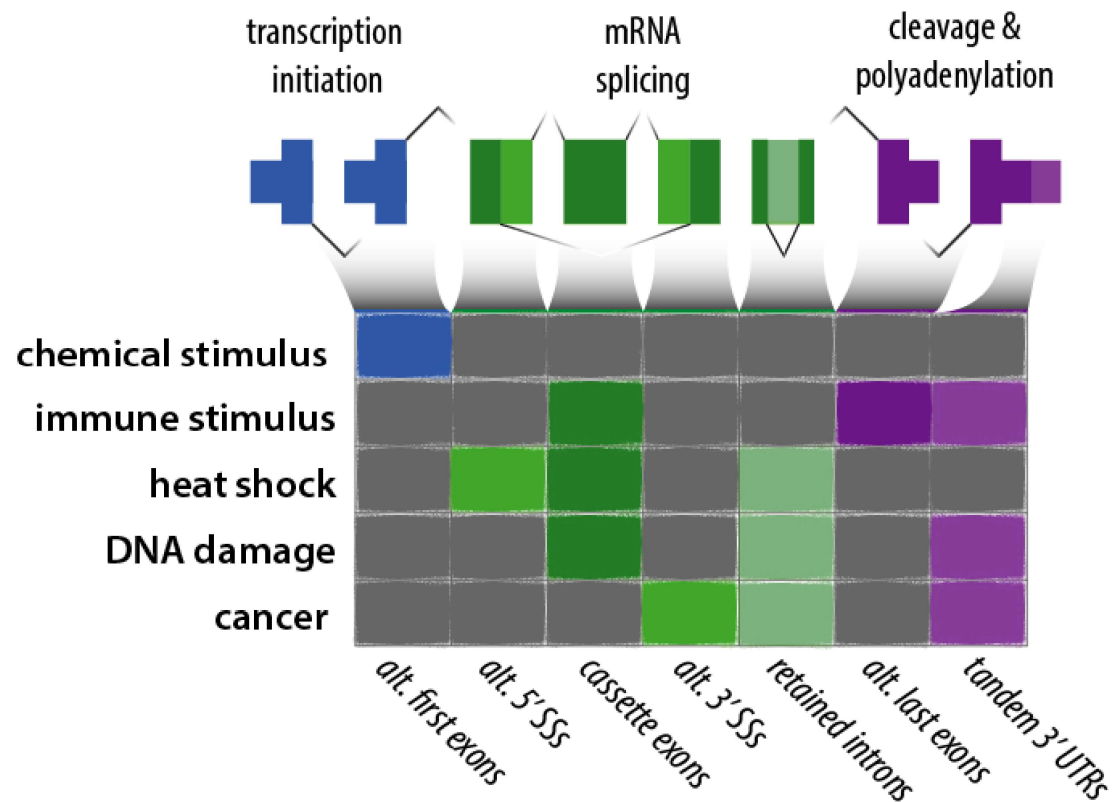
Shorter 3' UTRs evade regulation by specific miRNAs



miRNAs with enriched target sites are up-regulated following infection

miRNA target sites from TargetScan predictions in human

Global shifts in RNA processing upon cellular perturbation or stimulation



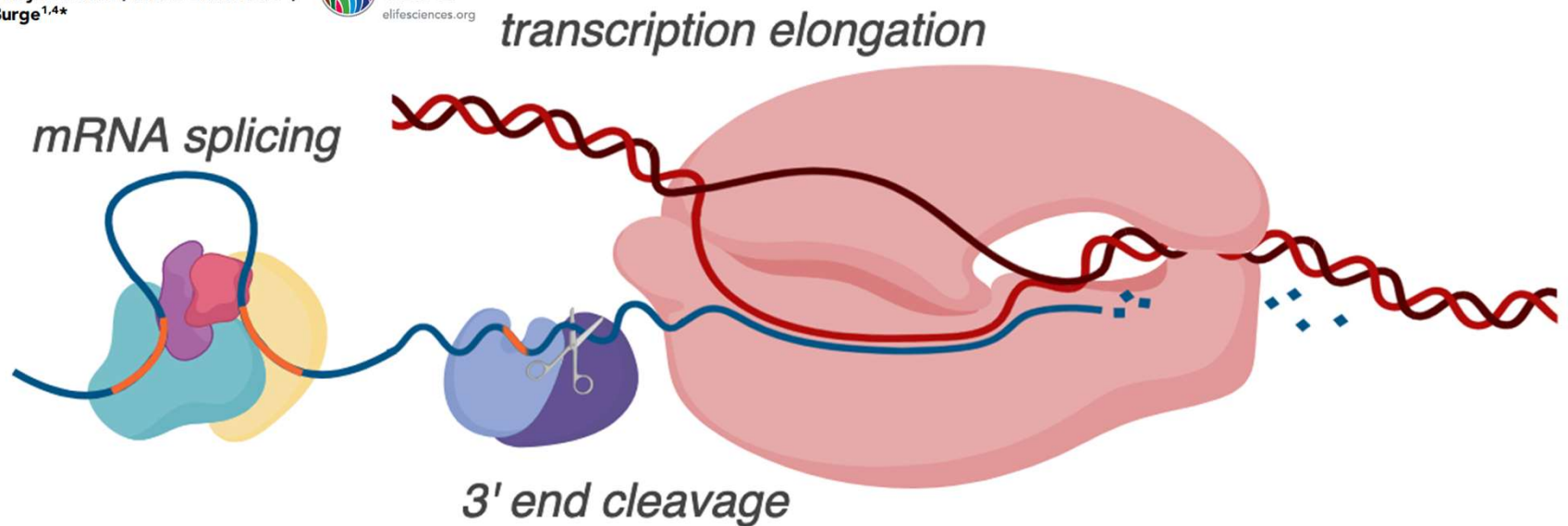
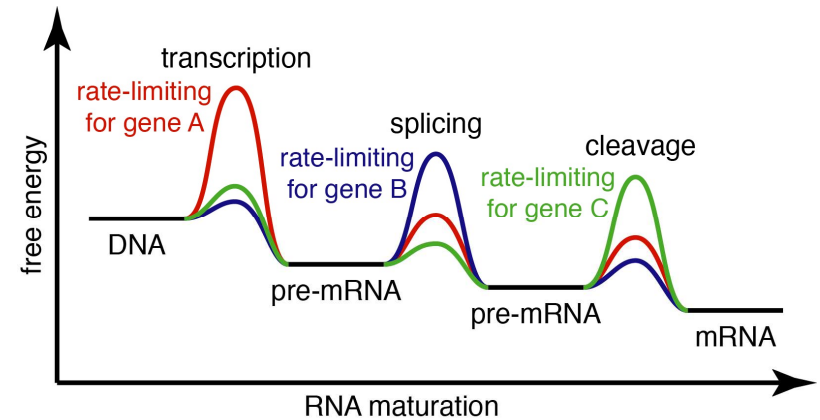
Estimating rates of mRNA biogenesis and maturation

The kinetics of pre-mRNA splicing in the *Drosophila* genome and the influence of gene architecture

Athma A Pai¹, Telmo Henriques^{2,3}, Kayla McCue⁴, Adam Burkholder⁵,
Karen Adelman^{2,3}, Christopher B Burge^{1,4*}

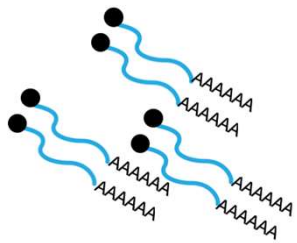


eLIFE
elifesciences.org

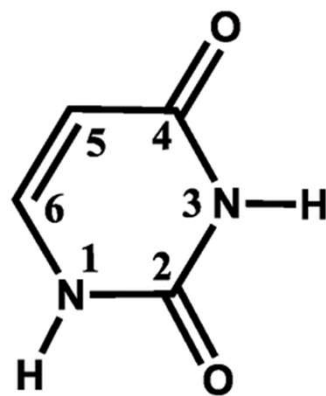
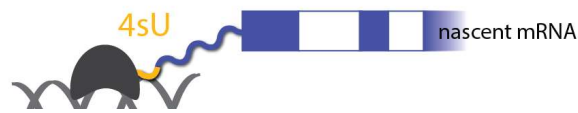
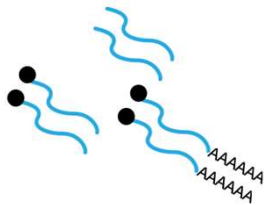


Experimentally identifying intermediate RNA products

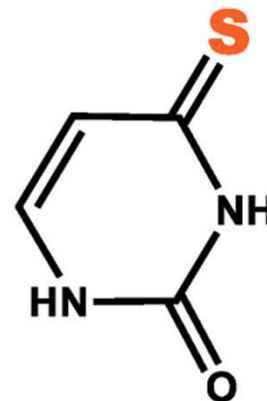
RNA-sequencing captures
mature, stable mRNA



Instead, capture **nascent**
RNA molecules (before
maturation & degradation)



Uracil (**Ura**)



4-thiouracil

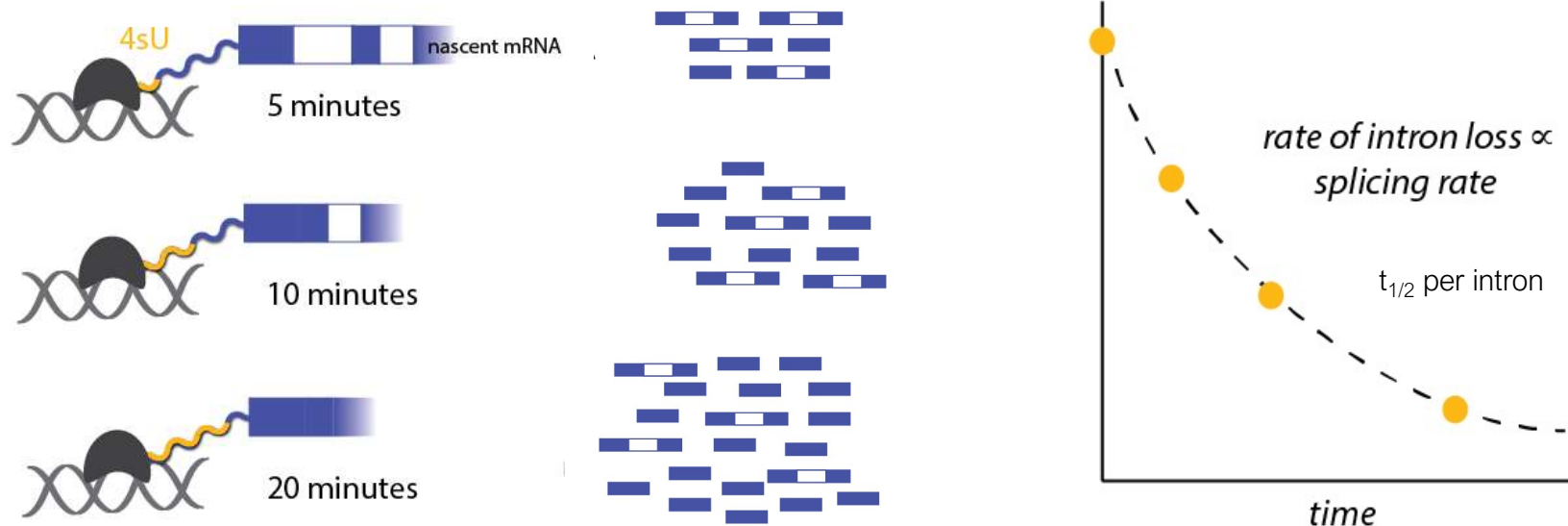
short 4sU labeling periods



Illumina HT sequencing
millions of short reads

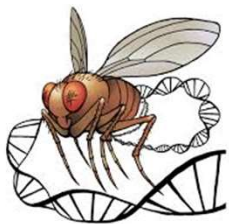


Estimating splicing rates with metabolic labeling of RNA



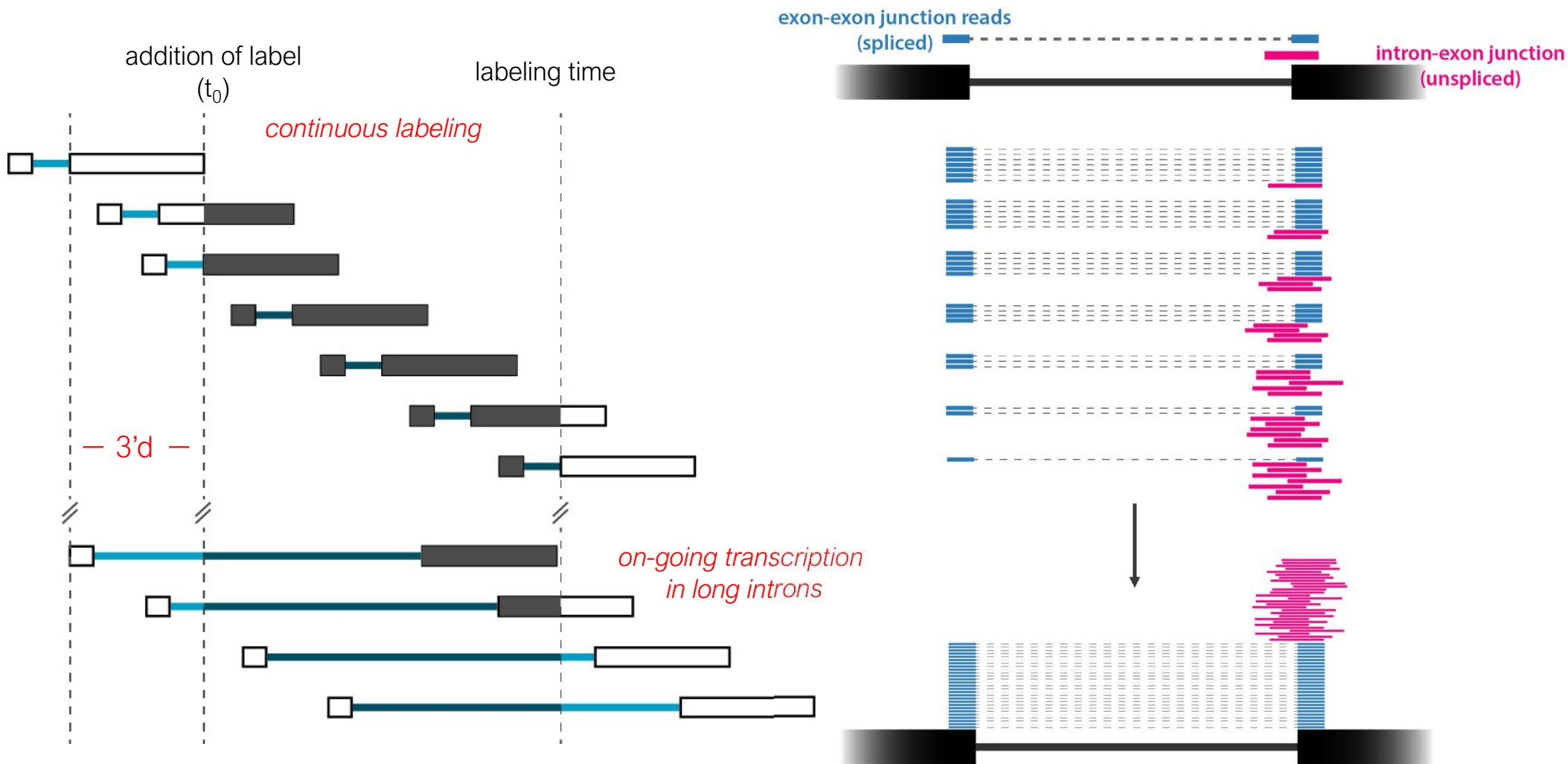
5m, 10m, and 20m incubations with 4sU in *Drosophila melanogaster* S2 cells
+ total RNA (~steady-state)
(3 replicates per timepoint)

protocol adapted from Windhager *et al.* 2012; **data from Karen Adelman (NIEHS/Harvard)**

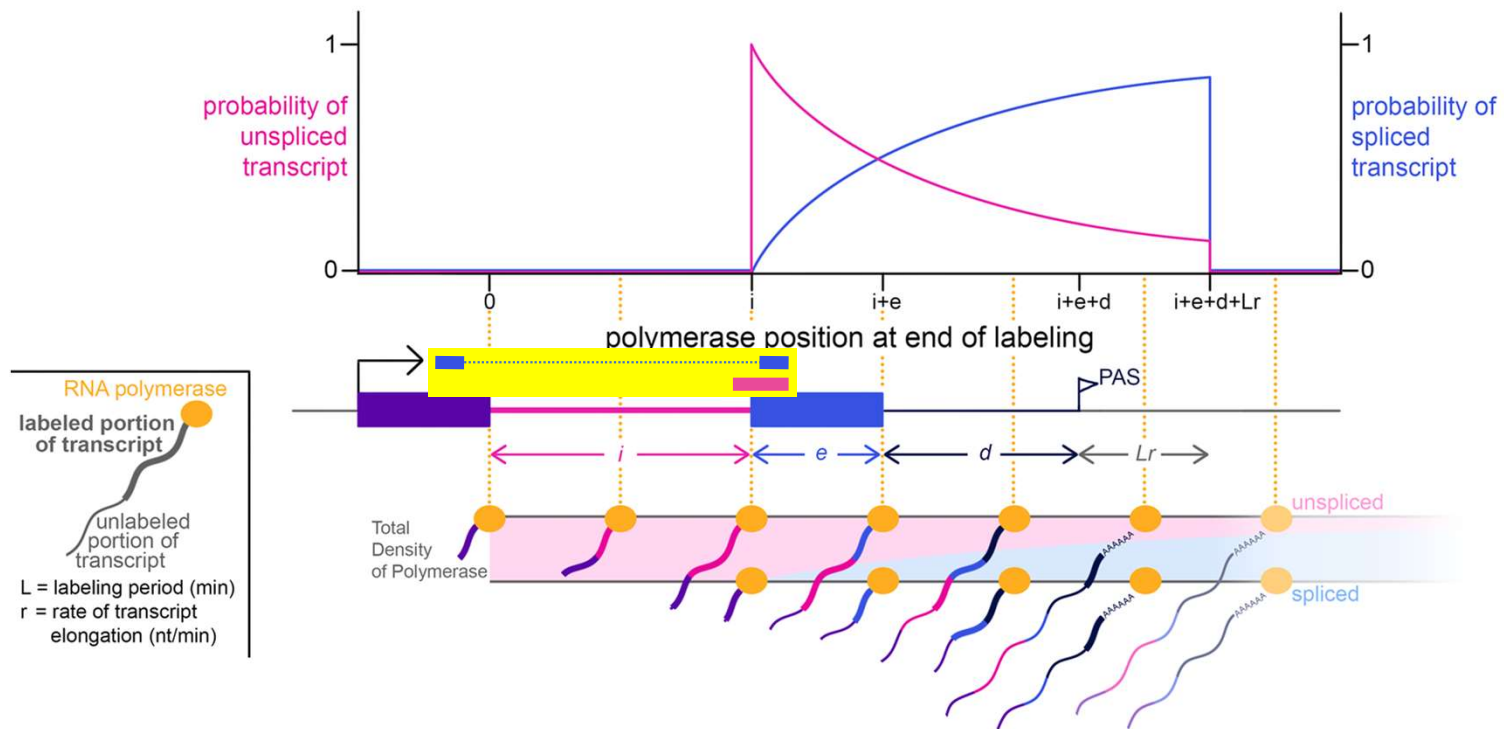


Pai *et al.* 2017 eLife

Inferring the lifetime of an intron with 4sU-seq

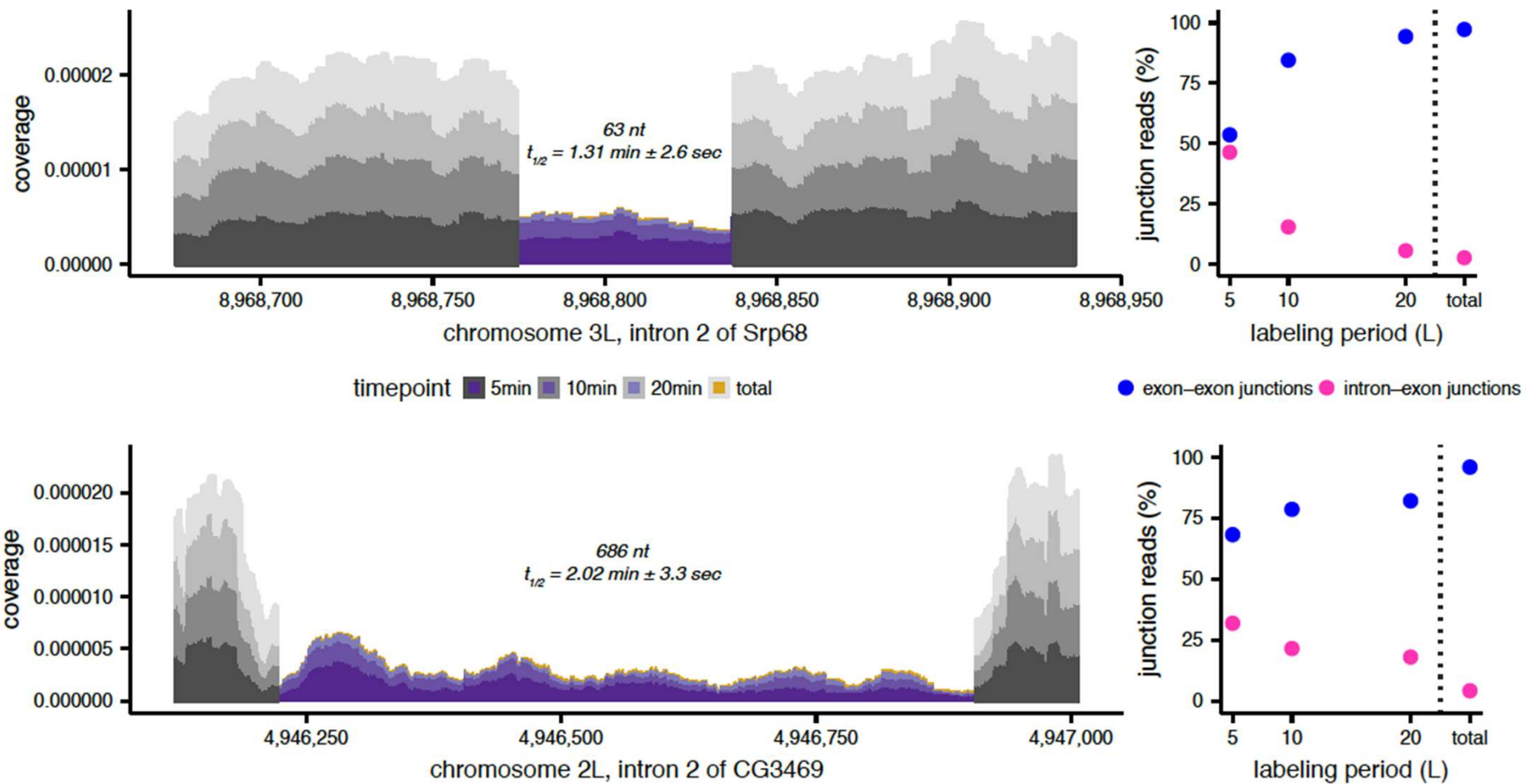


Contribution of reads from unspliced | spliced transcripts



$$\frac{\# \text{ of intron-exon junction reads}}{\# \text{ of exon-exon junction reads}} \approx \frac{\frac{hr}{\log(2)} \left(1 - 2^{-\frac{e+d+Lr}{hr}}\right)}{e + d + Lr - \frac{hr}{\log(2)} \left(1 - 2^{-\frac{e+d+Lr}{hr}}\right)}$$

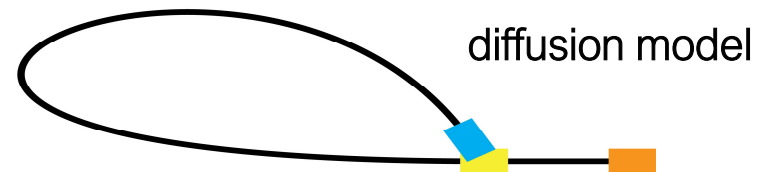
Observing the rate of splicing



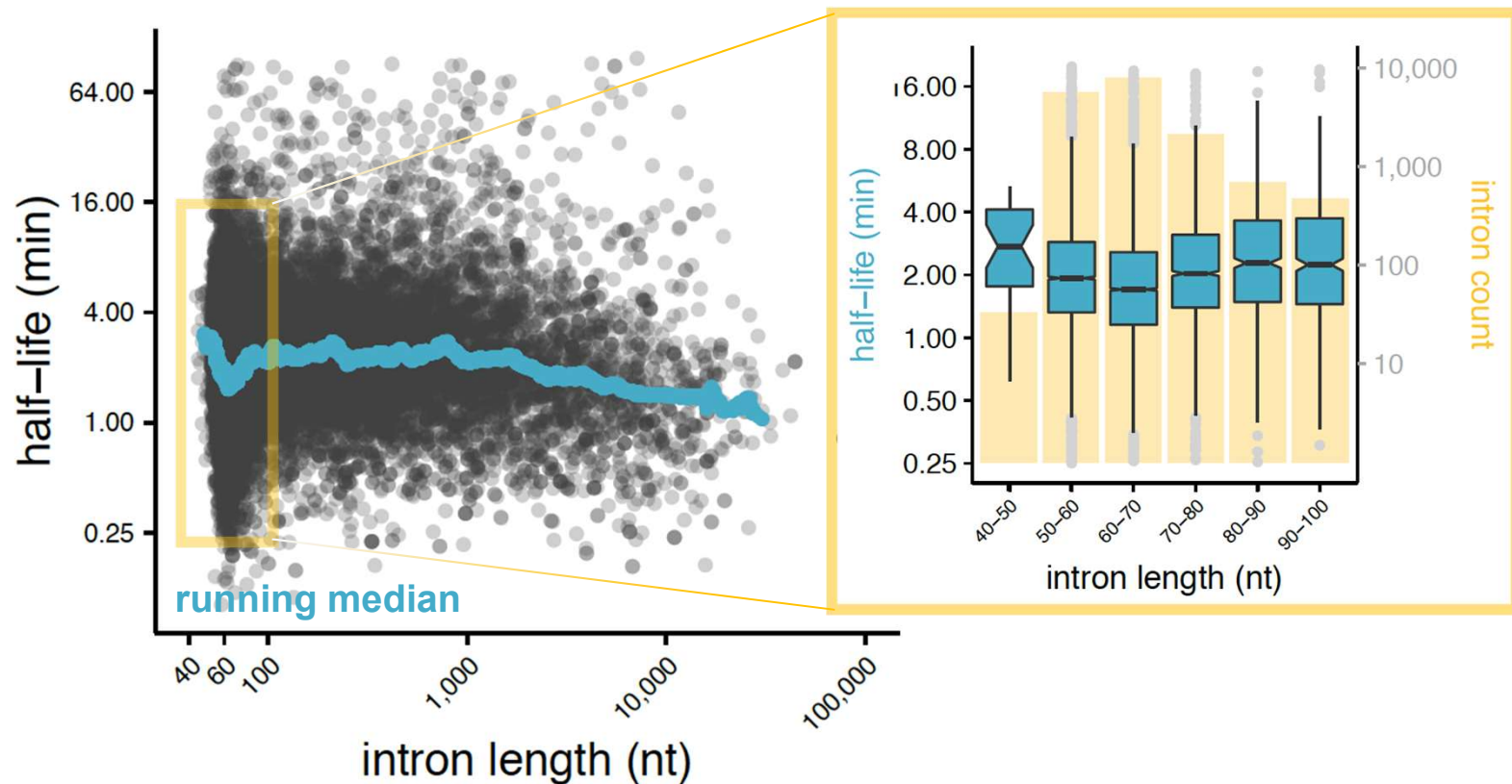
What underlies variability in splicing rates?

Calculated intron half-lives for 25,575 introns in 5,608 genes
median = 1.96 min

5' splice site
branchpoint
3' splice site



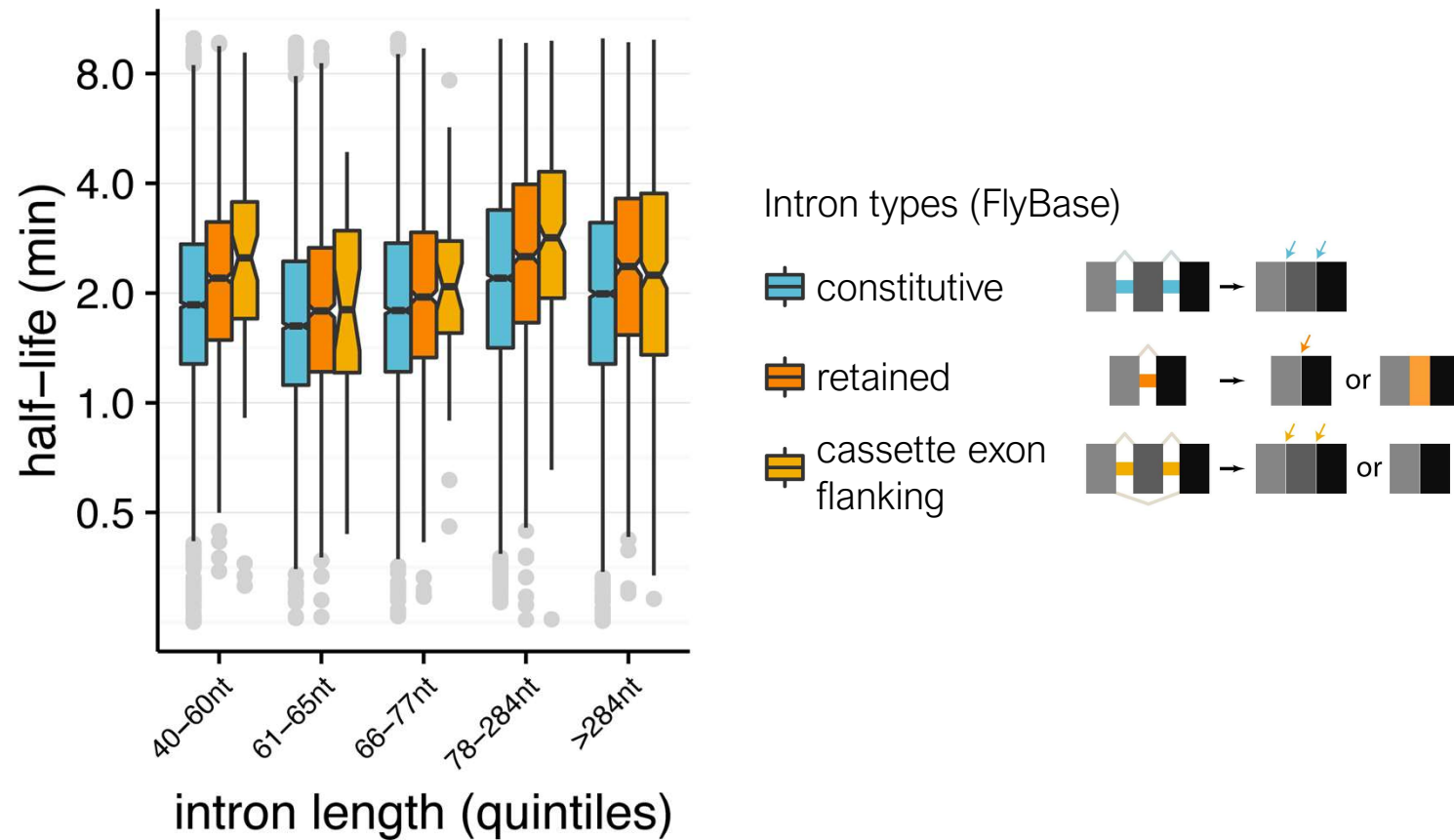
Optimal splicing for 60-70nt introns



mode: 63nt

>30% introns between 60-70nt
selective pressures favor short introns

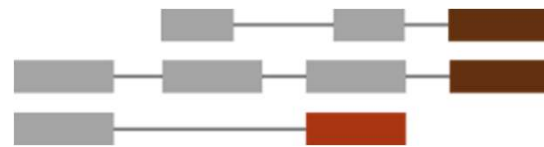
Introns involved in alternative splicing are spliced slowly



Cleavage at alternative PASs occurs more slowly



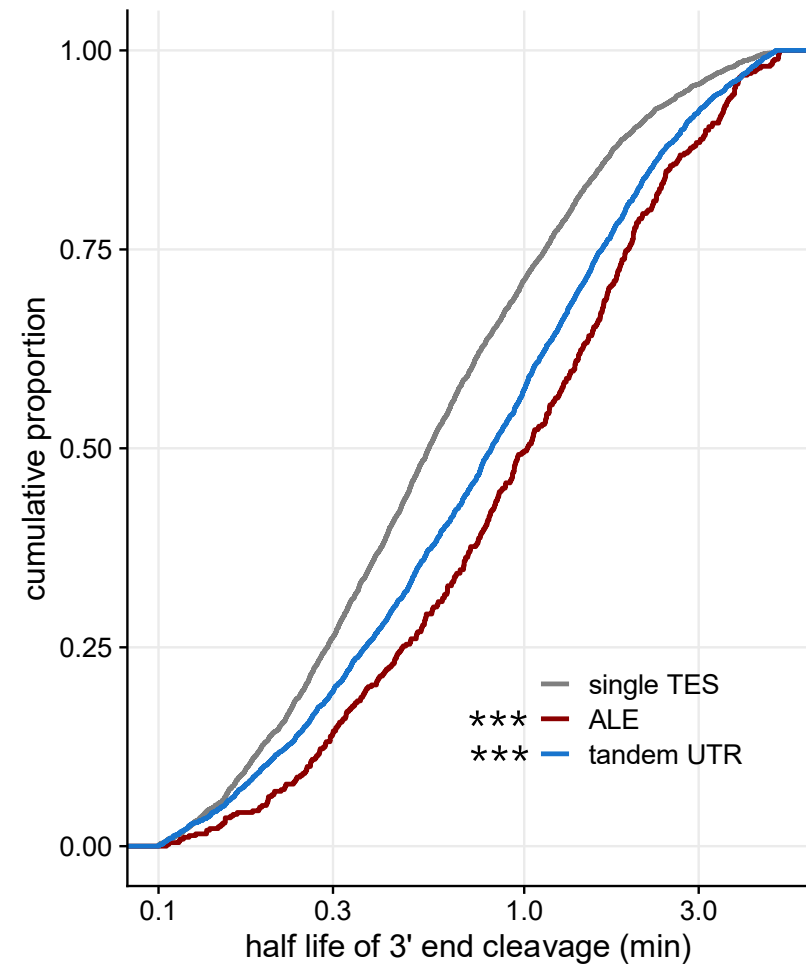
single TES (n=2,782)



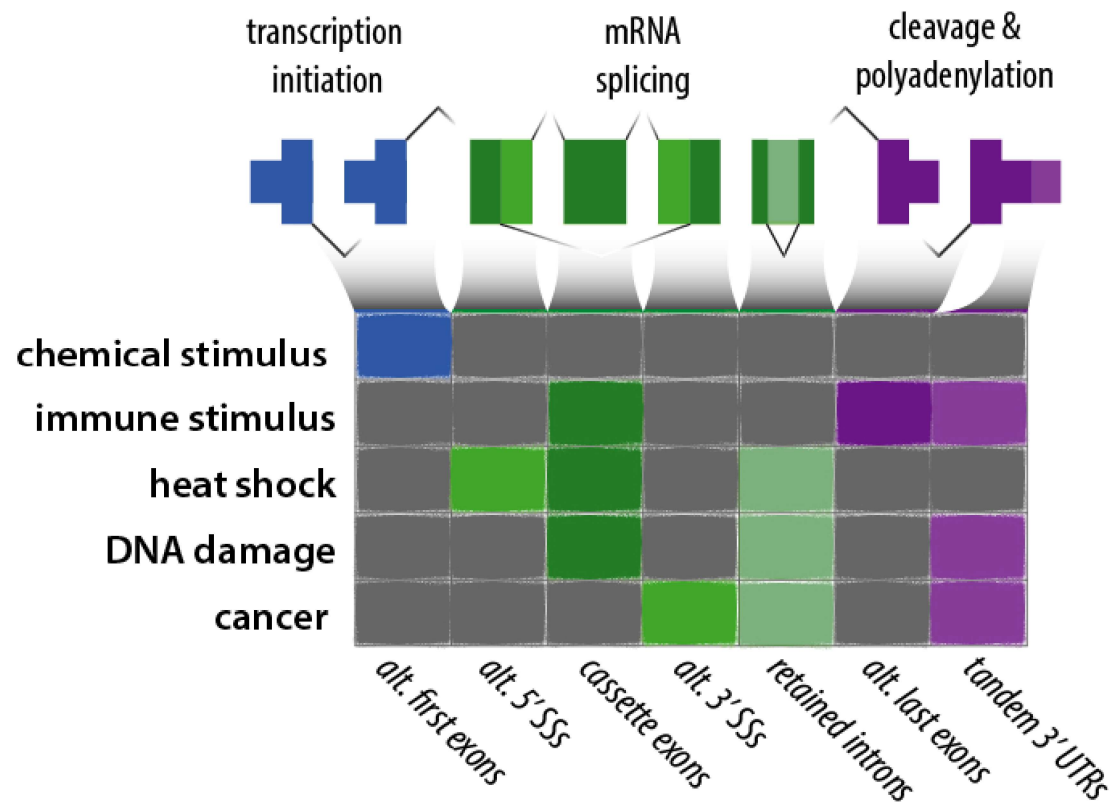
alternative last exon (n=261)



tandem 3' UTRs (n=4,253)



Global shifts in RNA processing upon cellular perturbation or stimulation



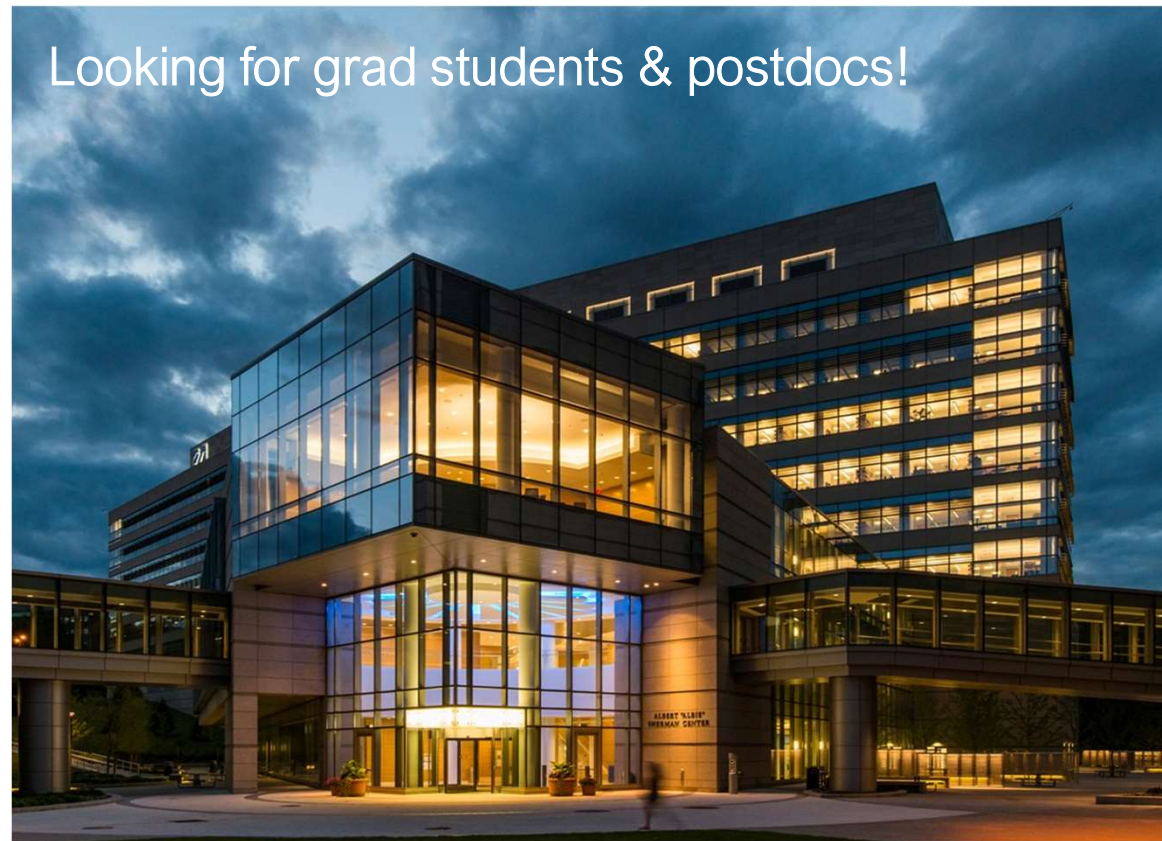
Acknowledgments



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Golshid Baharain

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Telmo Henriques

Chris Burge & Lab
Kayla McCue
Joe Paggi



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