RNA degradation and Alternative Poly-Adenylation

Goal of this session

The goal of this session is to introduce concepts of RNA degradation, methods to study it, and to discuss an analysis of alternative poly-adenylation as an example.

Concepts

Control of RNA degradation in the cell

Shyu et al. describe the mechanisms for determining mRNA degradation or translation in “Messenger RNA Regulation: To Translate or To Degrade” as nonsense-mediated mRNA decay (NMD) and microRNA-mediated mRNA decay. NMD identifies and eliminates mRNAs whose expression produces proteins with shortened termination codons, proving to be deleterious, and microRNA-mediated mRNA decay uses gene silencing by miRNAs which is achieved by mRNA decay triggered by deadenylation. Additional mechanisms involved in mRNA degradation include sequence-specific endonuclease cleavage and deadenylation-independent decapping; however, the primary goal of each of these degradation mechanisms is to essentially regulate which genes are expressed and when these genes are expressed.


What impacts mRNA half-lives?

mRNA half-lives reveal its lifetimes (stability), which can be affected by many factors, e.g. mRNA sequences, the mRNA’s primary and secondary structure, like poly(A) tail and steam loop, determined by its sequence, can alter mRNA’s accessibility to enzymes.

RNA stability can also be influenced by proteins which can either function as enzymes, like RNases, which degrade mRNA or RNA-binding proteins, like poly(A)-poly(A)-binding protein, which protect mRNA from degradation.

RNA stability can also be influenced by other factors, such as: hormones, like phorbol esters and lipopolysaccharide up-regulating mRNA, growth factors, ion, like calcium.


Methods to measure mRNA degradation rates

Methods based on transcription inhibitors and on metabolic labeling.

RATEseq to measure mRNA degradation rates

RNA approach to equilibrium sequencing (RATE-seq) is a method used to determine the genome-wide absolute RNA synthesis and degradation rates in vivo without disturbing cellular physiology. The rates of decay for different types of RNA are measured using strand-specific sequencing and ribosomal depletion. And multiple spike-in RNAs are used as normalization method to identify and correct technical artifacts.

What is Alternative Poly-Adenylation and its relevance to biology?

Alternative poly-adenylation is the mechanism whereby the same gene has multiple 3' ends as a result of multiple PAS (poly-adenylation signal) elements, thus allowing the creation of multiple messenger RNA transcripts. The length of the 3'UTR of the messenger RNA is changed due to alternative poly-adenylation. This influences the fate of the messenger RNA and ultimately, the dynamics of gene regulation, by affecting the availability of miRNA binding sites and RBP binding sites. Alternative poly-adenylation exhibits tissue-specificity and de-regulation has been linked
to several human diseases. This mechanism is important for cell proliferation and differentiation, and encourages proteomic and functional diversity.

OR: Alternative Poly-Adenylation is a mechanism of RNA-processing that results in mRNA isoforms with different 3' UTRs, which adds to the complexity of the transcriptome. It is a form of gene regulation and also plays a role in several cellular processes. APA sites located in 3' UTRs can have an effect on mRNA stability and translation by influencing microRNA targeting and motifs recognized by RNA-binding proteins (RBPs). It also affects nuclear export and cellular localization by varying 3' UTR sequence or length. APA sites upstream of the last exon (upstream region, UR) may cause alternative terminal exon expression, resulting in changes in coding sequence as well as the 3' UTR. These changes lead to protein diversification and can also function to inhibit gene expression by generating truncated transcripts. APA patterns also tend to be cell type specific, which could potentially aid in disease diagnosis and characterization.

What are basic principles of the LITE-Seq method that the Gunsalus lab developed?

The basic principles of the LITE-Seq method include the synthesis of cDNA from RNA, which is then amplified twice to allow for getting enough RNA to perform 3'-enriched sequencing. The primer is biotinylated in order to capture 3'-ends for paired end sequencing and library preparation. They applied LITE-Seq to C. elegans germ cell development and found that the 3' UTRs in the sperm producing region tend to be shorter than those in the oocyte producing region.

This section could benefit from some more explanation and perhaps a figure.