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 ploy(A) tail and steam loop, determined by its sequence, can alter mRNA’s accessiblility 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.

Degradation equation: Degradation= \( k_d \cdot RNA \) with \( K_d \) = degradation rate; RNA = RNA concentration

First order rate equation- amount mRNA present is proportional with amount RNA present

Change in RNA over time: \( dRNA/dt = ks \cdot DNA-kt\cdotRNA \)

Relationship between degradation and half life: \( t_{1/2} = \ln(2) / k_d \)

Exponential decay: \( RNA_{t} = RNA_{t=0} \cdot e^{-k_{d} \cdot t} \)

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

Inhibit transcription

i) This method is not effective because inhibiting transcription has side effects that confound observation of degradation.
Examples:

Pol II shut off induces some mRNA synthesis

Thiolutin stabilizes mRNA (less likely to degrade)

**Estimate degradation rates from concentration time series data**

i) This approach involves labeling mRNA and watching the labels (i.e. those mRNA molecules) disappear.

ii) Create mRNA with 4SU label (mRNA will use 4SU instead of uracil)

iii) We can covalently bind biotin to SH group of 4SU and separate it with column separation. This allows us to separate biotin-labeled mRNA from unlabeled mRNA.

iv) Transcriptomic analysis using the separated samples can be used to simultaneously calculate mRNA synthesis and decay rates

**Pulse-chase labeling techniques**

Pulse-chase is a method for examining a cellular process occurring over time by successively exposing the cells to labeled compound (pulse) and then to the same compound in an unlabeled form (chase). Then it is easy to observe changes in labeled composition. Radioactivity is a commonly used label.

1) Pulse
   a) Prime sample with 4TU label (same concept as described above).

2) Chase
   a) At this point, you stop synthesizing RNA with 4TU and introduce uracil.
   b) Include a spike in of 4TU- control RNA for reference.
   c) Biotinylate total RNA. Biotin should only bond to 4TU-containing molecules.
   d) Enrich for mRNA.
   e) Select for biotin-labeled mRNA.
   f) RNA-seq

ii) Results

1) mRNA degradation follows exponential decay curve.

2) The average half-life for an mRNA in yeast is 20 minutes.

3) Ribosomal mRNAs are more stable than other mRNAs

4) mRNAs degradation helps cells adjust to different conditions
   a) The study found that mRNAs were stabilized when cell environments shifted from glucose heavy environments to galactose heavy environments.
   b) On the other hand, ribosomal and translational mRNAs were de-stabilized when cell environments shifted from glucose heavy environments to galactose heavy environments.

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.

This section could benefit from some more explanation and perhaps a figure. Methods based on transcription inhibitors and on metabolic labeling.

**What is alternative splicing?**

**What is an example of alternative splicing?**

**Fig:** The exon/intron structure of the Nox1 gene and its alternative splicing pathways. Open boxes indicate exons. Numbers in the box denote the size of each exon (bp). The size of the intron is indicated under the broken lines (bp). Closed boxes show the open reading frames of the transcript.

Voltage-gated proton (H+) channels play an important role in cellular defense against acidic stress

Nox1 encodes 3 different mRNAs; two long (f and c types) and one short versions (a type). All forms are integral membrane proteins; but short one don’t have NADPH binding site. The three forms are produced by alternate splicing in tissue specific manner. The short form is able to transport H+ ions (produced in colon); whereas the long ones encode NADPH oxidase (produced in colon and uterus).

References:

[1]“Novel transcripts of Nox1 are regulated by alternative promoters and expressed under phenotypic modulation of vascular smooth muscle cells”

Noriaki Arakawa, Masato Katsuyama, Kuniharu Matsuno, Norifumi Urao, Yoshiaki Tabuchi, Mitsuhiro Okigaki, Hiroaki Matsubara, Chihiro Yabe-Nishimura

[2]“Analysis of mRNA Transcripts from the NAD(P)H Oxidase 1 (Nox1) Gene” Miklós Geiszt, Kristen Lekstrom And Thomas L. Leto
How do we measure alternative splicing?

RNA seq can be used to detect alternative splicing variants, but we will need longer reads to detect splice variants. First generation RNA seq produces short reads that are too short to monitor splice variants, but second and third generation RNA-seq produces long reads that can cover entire transcripts.

There are two approaches that accomplish this task.

Method 1: Pac Bio

1. Polymerase fixed to the bottom of a well with a template sequence and is flooded with phospholinked nucleotides.
2. A complementary sequence is made with fluorescent nucleotides. Changes in fluorescence indicate the addition of a nucleotide
3. Detect the fluorescence to obtain the nucleotide added.
4. Compile to get the sequence.
5. Note: This method looks at one molecule at a time and is prone to error.

Method 2: Nanopore

1. Begin with a motor protein and channel.
2. Thread molecule of interest through the channel. As each nucleotide passes through the channel, it creates a signature change in current.
3. Changes in current can be detected and used to generate a sequence

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.

Methods to investigate alternative poly-adenylation

Alternative polyadenylation is an RNA processing mechanism that generates distinct mRNA transcripts by adding a 3' terminal stretch of A residues. The polyA tail is added to the pre-RNA in the nucleus after transcription and plays several roles for the mRNA. Alternative polyadenylation affects mRNA stability, translation, nuclear export, and protein localization. There are different types of polyadenylation - CR-APA and UTR-APA.

CR-APA results in both distinct mRNA isoforms and distinct protein isoforms because it occurs in the coding region.

UTR-APA results in distinct mRNA isoforms but the same protein isoforms because it occurs in the untranslated region. A method used to identify alternative polyadenylation is PAL-seq. In this method, mRNA is isolated from total total RNA and ligated to a biotinylated splint primer. The mRNA is then partially digested with RNase T1. The mRNA then undergo Biotin-streptavidin based purification. Adaptors are ligated onto the mRNA containing biotylated primer and the mRNA are reverse transcribed to cDNA. Sequencing primers are annealed to the 3' end of the polyA sequences and are extended using dTTP and biotinylated dUTP. Regions near the polyA tail are sequenced, and fluorescently labeled streptavidin molecules are attached to the biotinylated dUTPs. Signal intensity is measured to determine polyA tail length. This method is useful because it avoids direct sequencing of the polyA tail. In recent experiments, PAL-seq has revealed that polyA tail lengths are relatively conserved across organisms, and that polyA tail lengths change during development, indicating an embryonic switch in translational control.

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.