Mapping and exploring the functions of $N^6$-methyladenosine in mRNA

Kayla Lee
Summer Research Program in Genomics 2012
RNA and Cell Regulation

- The roles of RNA within a cell include the regulation of genes and the synthesis of proteins

- Post transcriptional modifications:
  - 5' capping
  - 3' polyadenylation
  - RNA splicing
  - Base modifications

- Limited amounts of hypotheses and analytical methods leave many of these modifications uncharacterized
N⁶-methyladenosine (m⁶A)

- Most common, internal base modification on eukaryotic messenger RNA (mRNA)
- Occurs on almost 50% of expressed transcripts within the consensus motif RRACH
  - where $R$=purine, $A$=m⁶A, and $H$=A, C, or U
m\textsuperscript{6}A is highly conserved

Highly conserved between mouse and human genomes and strongly enriched in long exons and near stop codons

Phenotypic observations suggest regulatory role

- Catalyzed by a multi-component conserved enzyme
  - Only known subunit: methyltransferase like 3 (METTL3)

- Silencing of METTL3 leads to:
  - Apoptosis in *Homo sapiens*
  - Impaired gametogenesis in *S. cerevisiae* and *D. melanogaster*
How can we elucidate the functions of $m^6A$?
Objectives

Map m$^6$A in selected model systems

Pull down associated proteins

Understand m$^6$A in the life cycle of RNA

Understand genome wide trends

Identify unknown components recognizing this modification

How does methylation affect cellular processing?
Mapping of m$^6$A

### Intriguing $m^6A$ observations in model systems

<table>
<thead>
<tr>
<th>Selected organisms</th>
<th>Samples</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yeast</strong>&lt;br&gt;$S.\text{ cerevisiae}$&lt;br&gt;$ime4\Delta/ime4\Delta$ and wild type</td>
<td>IME4 required for induction of meiosis; increased methylation during sporulation</td>
<td></td>
</tr>
<tr>
<td><strong>Fruit fly</strong>&lt;br&gt;$D.\text{ melanogaster}$&lt;br&gt;Ovary and body tissues</td>
<td>IME4 homolog expressed in ovaries and testes; $ime4\Delta$ has fused-egg chambers</td>
<td></td>
</tr>
<tr>
<td><strong>Zebrafish</strong>&lt;br&gt;$D.\text{ rerio}$&lt;br&gt;Developmental time points</td>
<td>Decrease in METTL3 throughout embryonic development</td>
<td></td>
</tr>
</tbody>
</table>

Locate trends on genome-wide $m^6A$ maps
Zebrafish m^6A enrichment show similar conservation to human and mouse genomes
**D. melanogaster** and **S. cerevisiae** SK1 show signs of enrichment

- **Drosophila** and **S. cerevisiae** show distinctive enrichment peaks throughout the genome

- Does not follow the consensus motif

- Data is currently being replicated
Objectives

Map m^6A in selected model systems

Pull down associated proteins

Understand genome wide trends

Understand m^6A in the life cycle of RNA

Identify unknown components recognizing this modification

How does methylation affect cellular processing?
Pulling down associated proteins

I. Biotinylated RNA fragment preparation

Linearized Plasmid DNA (~100 nt)

In-vitro transcription
T7 RNA Polymerase

Biotinylated UTPs

Methylated RNA

Non-methylated RNA

II. Protein pull down

Add 2 µg RNA to pre-cleared cell lysate

RNA binds to protein

Streptavidin beads pull down proteins bound to RNA fragments

Visualize precipitated proteins on coomassie protein gel
Biotin-methylated RNA show unique protein bands

- Two distinct protein bands are observed in m\(^6\)A + biotin lanes
  - Estimated size: ~49 - 62 kDa
**Objectives**

1. **Map m⁶A in selected model systems**
   - Understand genome wide trends

2. **Pull down associated proteins**
   - Identify unknown components recognizing this modification

3. **Understand m⁶A in the life cycle of RNA**
   - How does methylation affect cellular processing?
Constructs designed to eliminate consensus sites followed by qPCR

Construct design with endogenous methylation site:

Test 1 (MCM2):

Control 1:  

Test 2 (ZFPM1):

Control 2:  

Test 3 (CYB561D2):

Control 3:  

Transfection

RNA extraction

m^6^-RIP

Construct-specific and Luciferase primer qPCR
Conclusions/Future Directions

• *S. cerevisiae* show distinct peaks of enrichment with a strong tendency to occur at the 3′ end of genes
  - Replicate m^6^A-RIP and continue computational analysis of mapped organisms

• Zebrafish data provides a developmental model of methylation enrichment that shows similar conservation to human and mouse genomes

• Protein mass spectrometry of potential candidates as identified by pull down

• Ensure the constructs and their mutant strains design a system that can selectively methylate
Acknowledgments

Regev and Lander Groups
  Aviv Regev
  Eric Lander
  Schragi Schwartz
  Ivo Wortman
  Dima Ter-Ovanesyan
  Dawn Thompson

Diversity Initiative
  Bruce Birren
  Ebony Smith
  Francie Latour
  Brandon Ogbunu

Summer Research Program in Genomics

Broad Institute