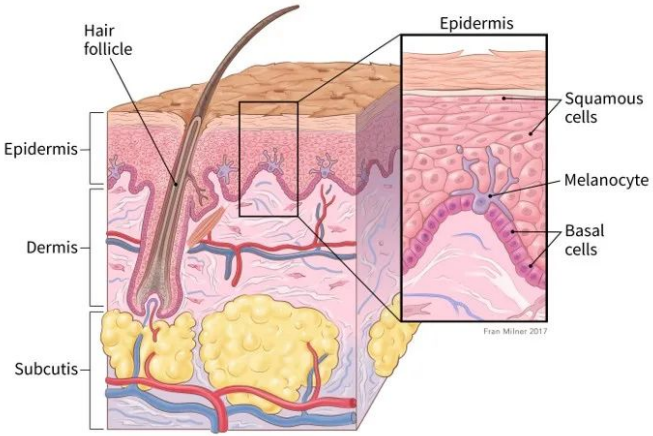
PAX3's Role in Nonsense-Mediated Decay: Implications for Melanoma



Lulu Jiang

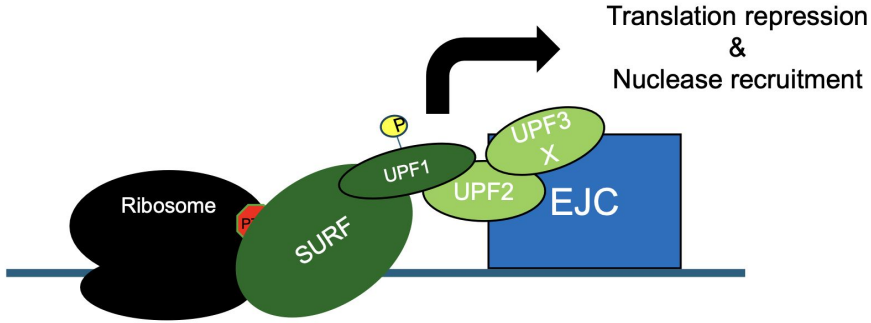
Background

PAX3 gene

- The PAX3 gene is a member of the paired box (PAX) family of transcription factors, which are essential in regulating cell growth and differentiation.
- Normal Conditions:
  - PAX3 regulate cell growth and differentiation genes and can act as both an activator and repressor of transcription, depending on the target gene and cellular context.
- PAX3 in Cancer:
  - PAX is often found to be overexpressed → downregulate genes that induce apoptosis

Nonsense mediated decay (NMD)

- NMD is an RNA quality control pathway that degrades improperly spliced transcripts containing premature termination codons (PTCs)
- It plays a crucial role in maintaining the quality of gene expression and protecting cells from the deleterious effects of mutated mRNAs.
- In cancers, NMD suppresses expression of tumor-suppressors and neoantigens
    - Increased NMD activity associated with reduced survival in some cancers



PTC = Premature Termination Codon
EJC = Exon Junction Complex

Source: Noah

Unmet Needs

Unclear Role of PAX3 in NMD:

- Current cancer therapies don't target NMD mechanisms
- PAX3's influence on NMD could be a novel therapeutic avenue
- Research Gaps: Need to understand if PAX3 affects NMD directly or indirectly
- Understanding how PAX3 modulates NMD could reveal new aspects of its function in cancer biology.

Hypothesis and Research questions

General Hypothesis: PAX3 is having an effect on nonsense-mediated decay (NMD).

Research Questions:

How is PAX3 affecting NMD?

Two main hypotheses:

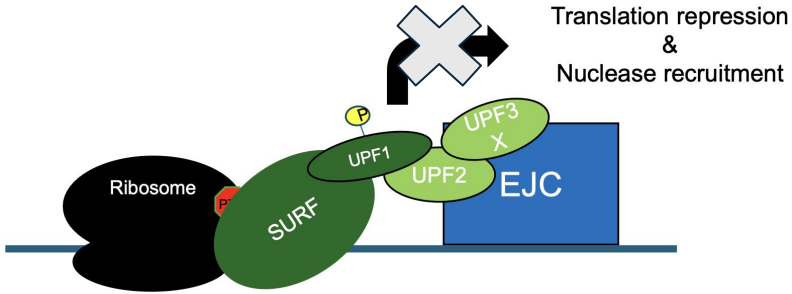
- By altering splicing of transcripts to increase the proportion of transcripts that will then be targeted by NMD
- Due to NMD activity being increased

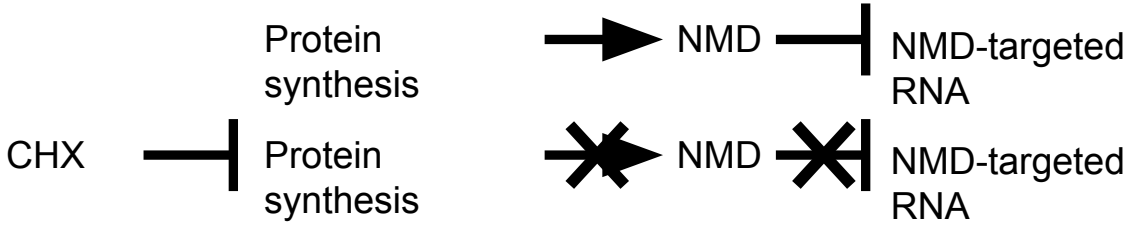
Is it possible that both mechanisms are occurring simultaneously?

Methods / Plan

PAX3 and NMD-targeted transcripts

- Method for separating transcriptional and NMD-based RNA regulation: **Cycloheximide (CHX) treatment**
- CHX inhibits ribosome function, inhibiting PTC NMD and increasing abundance of NMD-targeted transcripts
- Transcripts <u>downregulated via NMD</u> will **increase** after CHX, transcripts <u>downregulated via transcriptional regulation</u> will NOT.





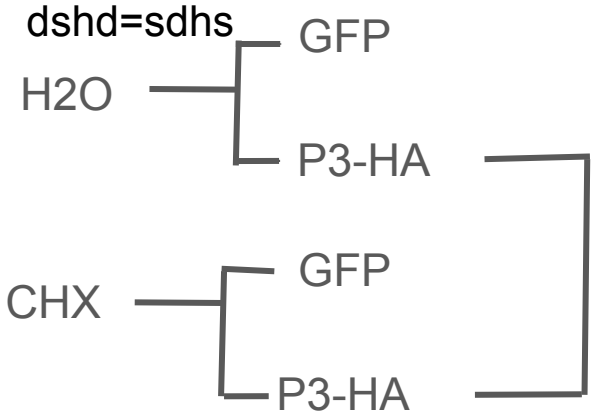
Huth, Michelle, et al., Genes Dev 36:1-20

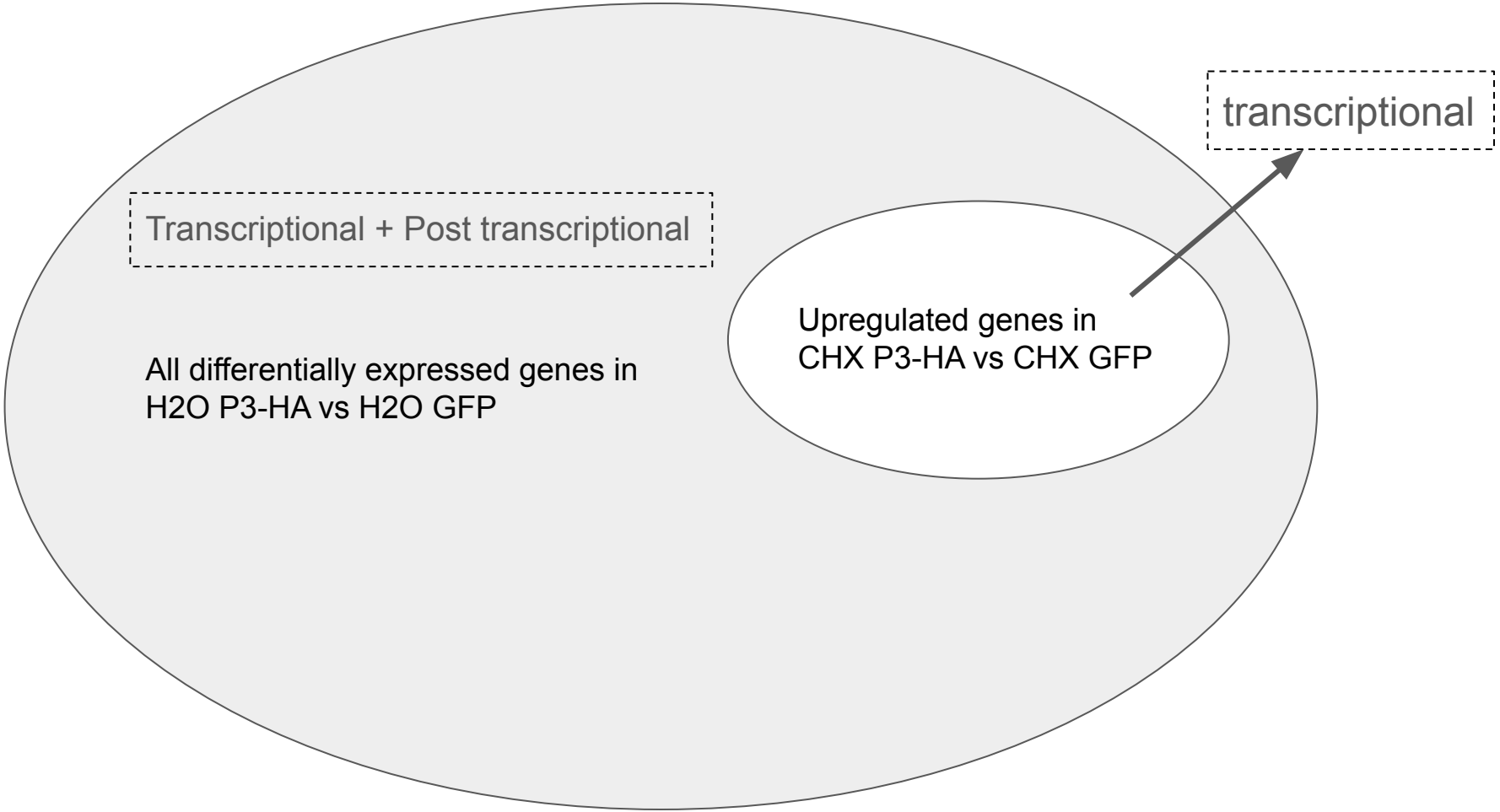
Study Design

- Using cell culture models with PAX3 manipulation
- Analyzing RNA sequencing data to study gene expression and splicing
- Employing cycloheximide treatment to isolate direct effects

Cycloheximide (CHX) Treatment

- Comparison groups: GFP vs PAX3 overexpression, with/without cycloheximide



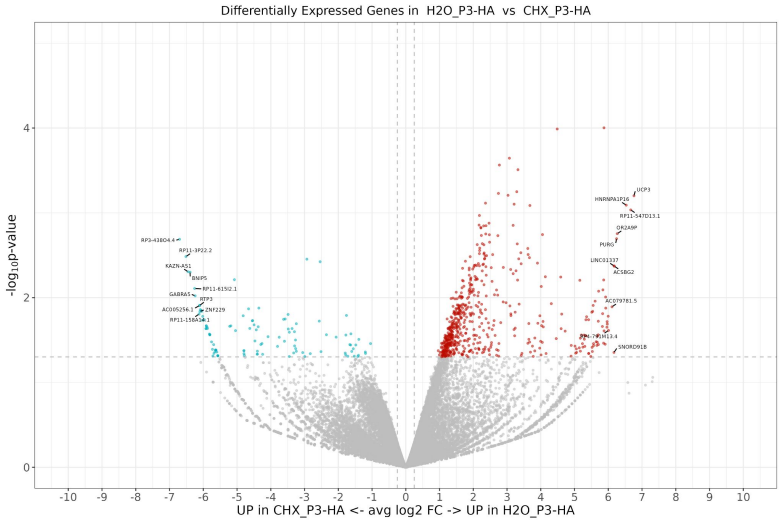

transcriptional
Transcriptional + Post transcriptional
Upregulated genes in
CHX P3-HA vs CHX GFP
All differentially expressed genes in
H2O P3-HA vs H2O GFP

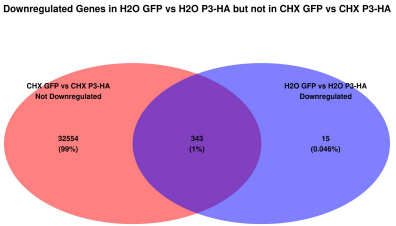
Data Processing Workflow:

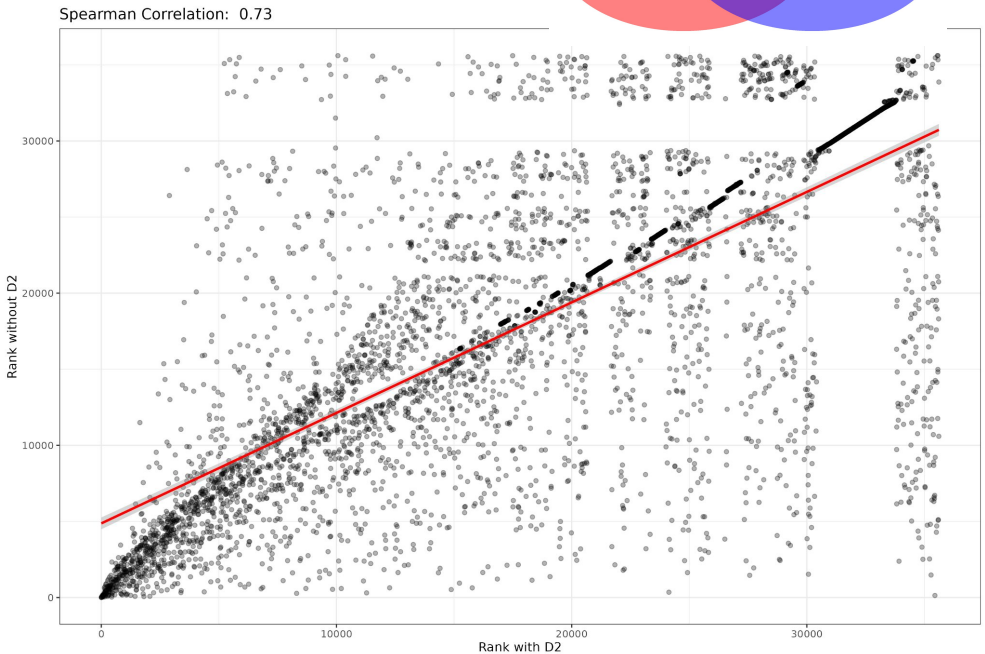
Paired end reads data preprocessing→ PCA plots
→ DESeq2

Visualization:

Volcano plots, Venn Diagram
Spearman Correlation Analysis







Challenges and Approaches in RNA-seq Analysis

PAX3 Function

- Traditional view: Transcriptional activator
- New perspective: Can also act as repressor
- Approach: Include all differentially expressed genes
    - Upregulated and downregulated
    - Captures full range of PAX3 effects

Analysis Strategy

1. Plot all significantly changed genes
2. Group genes based on:
    - Up/downregulation
    - Significance of change
3. Identify patterns across conditions
4. Confirm normalization of background noise

Challenges and Approaches in RNA-seq Analysis

Normalization

- Crucial for accurate analysis
- Accounts for:
  - Sequencing depth differences
  - RNA composition variations
  - Technical factors
- Improper normalization may lead to misleading results

Broader Context

- The project aims to elucidate the mechanism by which PAX3 influences NMD
- It explores both transcriptional and post-transcriptional effects of PAX3
- The research could reveal novel insights into gene regulation in the context of diseases like melanoma