Background
Better understanding of how the nucleosome affects transcription
  Important for understanding the nucleosome’s role in gene expression

Treats each component and region of the nucleosome distinctly
  Change from previous work in the field

Works toward enabling control of gene expression in vivo
General barrier to transcription and regulator of gene expression

**Three distinct regions:**
1. Entry - first H2A/H2B dimer encountered
2. Central - H3/H4 tetramer
3. Exit - second H2A/H2B dimer encountered

**Three main components:**
1. Histone Tails
2. Histone-DNA Contacts
3. DNA Sequence
**Histone Tails:**
Subjected to many post-translational modifications

**Histone-DNA Contacts:**
Strongest histone-DNA interactions are mediated by the core domains

**DNA Sequence:**
Influences RNA Polymerase arrest probability and pausing
**Relevant Histone Modifications**

- **Acetylation** - addition of an acetyl group to a lysine residue
- **Deacetylation** - removal of an acetyl group from a lysine residue
- **Tail Cleavage** - removal of the tail of a histone protein
- **Sin Mutations** - SWI/SNF independent mutations, can occur in H3 and H4
  
  SWI/SNF is a chromatin remodeling complex

<table>
<thead>
<tr>
<th>Modification</th>
<th>Barrier to Transcription</th>
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</thead>
<tbody>
<tr>
<td>Acetylation</td>
<td>Decreases</td>
</tr>
<tr>
<td>Deacetylation</td>
<td>Increases</td>
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<tr>
<td>Tail Cleavage</td>
<td>Decreases</td>
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Previous Work


Established **optical tweezers assay** to follow RNA Pol II complexes during transcription and showed initial results.

Determined nucleosomes **act as barriers** that increase the RNA pause density, slow pause recovery, and reduce transcription velocity.

Built a rudimentary **computational model** of the transcription through a nucleosome.
Current Paper

Uses methods developed in Hodges paper
Investigates how each of the nucleosomal components affect the strength of the nucleosomal barrier
Builds a kinetic model of transcription through a nucleosome
Determines effects of transcription barrier on polymerase dynamics
Specific Questions

➢ How is the **stability** of the nucleosomes affected by the modifications?

➢ How are the **wrapping and unwrapping rates** of the DNA around the histone core altered, and how do these affect polymerase dynamics?

➢ What is the role of the enzyme’s **pausing** in this modified behavior?

➢ What is the spatial **extent and distribution** of these effects?
Separate and quantify the roles played by the various nucleosomal elements in establishing the magnitude and spatial distribution of the barrier to transcription.
Methods
Purification and Assembly of Nucleosomes

Yeast histone proteins with deletions/substitutions → expressed in E. coli
Purified, assembled into octamers
Loaded octamers on a 574 bp DNA
**Transcription**

Complexed DNA with antidigoxigenin (AD) coated bead

Complexed RNA polymerase modified with biotin with streptavidin (SA) coated bead

Single-molecule transcription assays: used dual-trap optical tweezers to trap AD and SA coated beads

Measure force and length of the fixed DNA while assuming DNA behaved like a worm-like chain
Pause Analysis

Record changes in position of Pol II on DNA template
Identify pauses by dividing position vs time data into 3 bp bins and compute dwell time Pol II spends in each of these bins
Pauses = dwell times at least 1.5 times longer than average dwell time
Methods

Analysis of Nucleosome Wrapping/Unwrapping Events

Load histone octamers on DNA fragment using PCR from modified plasmid

Formation of DNA tether between AD and SA coated beads in optical trap

Increase force by stepping one of the traps by 2 nm every 60 ms → study inner and outer wraps unfolding under force

Two beads held at constant positions for 1 min and applied constant force → measure nucleosome wrapping and unwrapping rates
Results
Histone Modifications Alter Passage Probabilities and Crossing Times
Histone Tails Gate the Nucleosome Entry Region
Histone-DNA Contacts at the Dyad Control Nucleosomal Barrier Height
(Sin H₃ and Sin H₄ Mutants)
Direct Measurements of Nucleosomal Wrapping/Unwrapping Dynamics

(Both Histone Modifications)
Template Sequence Modulates Strength of the Nucleosomal Barrier (DNA Sequence)

DNA sequence around histone influences barrier in two ways:

1. Increasing affinity of DNA to histones
2. Directly modulating the tendency of Pol II to pause
A Kinetic Model that Integrates Histone-DNA Interactions and Sequence Effects
Discussion and Future Work
Discussion

Built a *kinetic model of the transcription* through a nucleosome using the experimental results
   Includes RNA pol pausing/backtracking, RNA pol recovery, and nucleosome wrapping and unwrapping

**Independent spatial domains** of nucleosome (entry, central, and exit regions) are affected differently by elements of the nucleosomal barrier

The main nucleosomal components *control transcription elongation*

Allows for alternative mechanisms for *control of gene expression* by chromatin remodeling and transcription factors
**Conclusion**

**Histone Tails:**
- Both acetylation and removal of tails *reduced* the barrier to transcription
- *Decreased* the pause density and pause duration in the entry region

**Histone-DNA Contacts:**
- Sin mutations *decreased* the barrier to transcription
- *Decreased* the pause density and duration dramatically in the center region
- *Decreased* the wrapping rate of the nucleosome

**DNA Sequence:**
- DNA sequence primarily affects the secondary structure of the nascent RNA
- A stronger secondary structure *decreases* the pausing of RNA Pol by preventing backtracking
Continued Work with Optical Tweezers Method

Fitz et al. “Nucleosomal arrangements affects single-molecule transcription dynamics.” (2016)

Used the same dual trap optical tweezers method but isolated two nucleosomes instead of one

Used different di-nucleosomal arrangements

Characterized a drift coefficient that describes ability of enzyme to recover from nucleosome backtracking

Predicts probability of RNA Pol passing the first nucleosome

Presence of second nucleosome changes the drift coefficient
Future Directions

➢ What is the molecular basis of how the second nucleosome influences the transcription dynamics through the first one?
➢ To what extent do histone modifications modulate nucleosome geometry, and what is the impact on the transcribing RNA Polymerase II enzyme?
➢ How do tightly organized nucleosomes affect chromatin transcription?
Thank you!

Questions?