A role for CENP-S,T,W,X at *Neurospora crassa* centromeres?

Mu Feng
Mentor: Dr. Jonathan Galazka
Sept 17th, 2012
Centromere function during mitosis

Each chromosome has a centromere.

The centromere is where the kinetochore assembles during mitosis.

Microtubules attached to the centromere via kinetochore and separated sister chromatids.

Therefore, centromeres are essential for eukaryotic life as we know it!
Kinetochore

Chromatin structure

Chromosome structure
What are centromeres?

• Centromeres are a specific region of the chromosome.
• They consist of a DNA sequence and associated proteins.
• Many proteins have been identified but we do not understand the details of their function.

CenH3( CENP-A)
Significance

- Sometimes, cells can acquire an improper number of chromosomes and this results in aneuploidy.
- Aneuploidy increases the risk of neoplastic transformation.
- Misfunctioning centromeres can lead to aneuploidy. Therefore, understanding centromeres is critical to understanding tumorigenesis.
Background
To establish a functional kinetochore structure, a subset of kinetochore proteins must make strong and specific contacts with centromeric DNA (Nishino, 2011).

In Dr. Nishino’s experiment, CENP-T-W complex and CENP-S-T complex have been found in chicken cells.
Our goal:

defining the molecular mechanism by which kinetochore proteins specify the position on the chromosome and generate stable contacts with DNA to drive kinetochore assembly in *Neurospora crassa* (model fungus).

Our interested kinetochore proteins:

- Centromere Protein S (CENP-S)
- Centromere Protein X (CENP-X)
- Centromere Protein T (CENP-T)
- Centromere Protein W (CENP-W)

Hypothesis: CENP-T-W complex and CENP-S-X complex are essential to the kinetochore assembly in *N. crassa*.

How to test our hypothesis?

Knock out encoding DNA to make certain protein deficient type and test their growth after mitosis.
Approach

Deletion of CENP-S, CENP-X, CENP-T, CENP-W

Eliminating target gene by using 2 split markers
Test the importance of centromere proteins

Wild type chromosome deletion

K.O.
TAG CENP-W, CENP-X, CENP-T, CENP-W
GFP—Green Fluorescent Protein tag
V5—epitope tag
FLAG—epitope tag
Help to define the binding point

Wild type chromosome

modified type
<table>
<thead>
<tr>
<th>Transformations</th>
<th>Strain</th>
<th>Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TJG10</strong></td>
<td>N3011</td>
<td>CENP-W (NCU03400) KO (replace ORF with hph)</td>
</tr>
<tr>
<td><strong>TJG11</strong></td>
<td>N3011</td>
<td>CENP-S (NCU03629) KO (replace ORF with hph)</td>
</tr>
<tr>
<td><strong>TJG12</strong></td>
<td>N3011</td>
<td>CENP-X (NCU09478) KO (replace ORF with hph)</td>
</tr>
<tr>
<td><strong>TJG13</strong></td>
<td>N3011</td>
<td>CENP-S V5 6xhis</td>
</tr>
<tr>
<td><strong>TJG14</strong></td>
<td>N3011</td>
<td>CENP-S FLAG</td>
</tr>
<tr>
<td><strong>TJG15</strong></td>
<td>N3011</td>
<td>CENP-S GFP</td>
</tr>
<tr>
<td><strong>TJG16</strong></td>
<td>N3011</td>
<td>CENP-X V5 6xhis</td>
</tr>
<tr>
<td><strong>TJG17</strong></td>
<td>N3011</td>
<td>CENP-X FLAG</td>
</tr>
<tr>
<td><strong>TJG18</strong></td>
<td>N3011</td>
<td>CENP-X GFP</td>
</tr>
<tr>
<td><strong>TJG19</strong></td>
<td>N3011</td>
<td>CENP-W V5 6xhis</td>
</tr>
<tr>
<td><strong>TJG20</strong></td>
<td>N3011</td>
<td>CENP-W FLAG</td>
</tr>
<tr>
<td><strong>TJG21</strong></td>
<td>N3011</td>
<td>CENP-W GFP</td>
</tr>
</tbody>
</table>
Methods

1. PCR for flanks of target genes and split markers

2. Electroporate spores

3. Inoculate strains onto hyg containing media
Methods

4. Select individual transformants

5. Observe **GFP-transformed** strains
GFP-tagged strains

CENP-W (GFP modified)

CENP-S (GFP modified)

CENP-X (GFP modified)
Methods

6. Grow more good GFP modified transformants

7. Make a cross of GFP-tagged strains with RFP-modified Cen-H3 strains (NMF426)

Co-localization
Co-localization

CENP-T tagged with GFP
CENP-A tagged with RFP

Green channel
Red channel
Merged channel
Acknowledgement

Dr. Jonathan Galazka
Dr. Michael Freitag & Entire Freitag Lab!!
Dr. Kevin Ahern
HHMI and all the funding sources