Molecular mechanism of the DNA replication checkpoint in fission yeast

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Perturbation factors of DNA replication

- Depletion of dNTPs
- Damage to DNA templates
- Inhibitors of polymerases
- Mutations in replisome
The replication checkpoint in fission yeast

Sensor

Mediator

Effector

Replisome stability
Inhibition of initiation
Delay of cell cycle
dNTP production
**Checkpoint proteins are highly conserved**

<table>
<thead>
<tr>
<th>Checkpoint Proteins</th>
<th>Budding yeast</th>
<th>Fission yeast</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PIKK</strong></td>
<td>Mec1</td>
<td>Rad3</td>
<td>ATR</td>
</tr>
<tr>
<td></td>
<td>Tel1</td>
<td>Tel1</td>
<td>ATM</td>
</tr>
<tr>
<td><strong>PIKK subunit</strong></td>
<td>Ddc2</td>
<td>Rad26</td>
<td>ATRIP</td>
</tr>
<tr>
<td><strong>RFC-like</strong></td>
<td>Rad24</td>
<td>Rad17</td>
<td>hRAD17</td>
</tr>
<tr>
<td><strong>“9-1-1”</strong></td>
<td>Ddc1</td>
<td>Rad9</td>
<td>hRAD9</td>
</tr>
<tr>
<td></td>
<td>Rad17</td>
<td>Rad1</td>
<td>hRAD1</td>
</tr>
<tr>
<td></td>
<td>Mec3</td>
<td>Hus1</td>
<td>hHUS1</td>
</tr>
<tr>
<td><strong>Mediator</strong></td>
<td>Mrc1</td>
<td>Mrc1</td>
<td>Claspin</td>
</tr>
<tr>
<td></td>
<td>Rad9</td>
<td>Crb2</td>
<td>BRCA1</td>
</tr>
<tr>
<td><strong>Effector kinases</strong></td>
<td>Rad53</td>
<td>Cds1</td>
<td>hCHK2</td>
</tr>
<tr>
<td></td>
<td>Chk1</td>
<td>Chk1</td>
<td>hCHK1</td>
</tr>
</tbody>
</table>
The replication checkpoint in fission yeast

I. Signal transduction from Rad3 to Cds1
II. Autoactivation of Cds1

Replisome stability
Inhibition of initiation
Delay of cell cycle
dNTP production
I. Signal transduction from Rad3 to Cds1

Mrc1 phosphorylation requires Rad3 and Tel1
Mrc1 phosphorylation has two nonredundant functions

I. Signaling from Rad3 to Cds1

Control 2.0mM HU 2.5mM HU 5.0mM HU

WT Δmrc1 mrc1 T645A+T653A T645A T653A S572A+S599A+S604A+S614A S599A+S604A+S614A S599A+S604A S604A

SQ Cluster TQ repeats
Three domains of Cds1 are required for its activation.
Phosphorylated Mrc1 TQ repeats bind Cds1 FHA domain

**Mrc1 T653:** biotin-TQLDSTIPTQTDSVD

**Mrc1 S604:** biotin-SLYVQNSQPSASQLTIVDAT

No binding

I. Signaling from Rad3 to Cds1
Cds1 FHA domain binds phosphorylated T11 of another Cds1 and promote autophosphorylation in trans

Cds1 T11: biotin-EEPEEATQATQEAPLHVSQ

I. Signaling from Rad3 to Cds1
Induced dimerization of Cds1 kinase domain activates the kinase in vitro
Dimerization of Cds1 bypasses Rad3 and Mrc1 in vivo

I. Signaling from Rad3 to Cds1
Two-stage mechanism for Cds1 activation

I. Signaling from Rad3 to Cds1
II. Autoactivation of Cds1

Three phosphorylatable sites are essential
Three possible mechanisms for Cds1 activation

1. Phosphorylation of T328, T332, or Y352
2. Direct activation by dimerization
3. Phosphorylation of multiple redundant sites
T328 and T324 in the activation loop are autophosphorylated \textit{in vitro}
T328 is autophosphorylated in Cds1 purified from *E. coli*

II. Activation of Cds1
Phosphorylation of T328 is the only covalent modification required for Cds1 activation

II. Activation of Cds1
**Autophosphorylation of T328 activates Cds1 in vivo**

<table>
<thead>
<tr>
<th></th>
<th>Cds1(WT)</th>
<th>Cds1(D312E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
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</tr>
</tbody>
</table>

**0.5 1 3 (h in HU)**

- T328-P
- T332-P
- Y352-P
- Cds1
- IgG

**Kinase**

- MyBP-32P
- MyBP

II. Activation of Cds1
Proposed mechanism for Cds1 autoactivation

Autoinhibition & Priming

Activation by *trans* autophosphorylation of T328
Properties of the replication checkpoint

- **Sensitivity**  autoamplification
- **Specificity**  protein-protein interactions, site specific phosphorylation
- **Noise immunity**  low concentration, unfavorable substrate, C-terminus
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F. Ishikawa
C-terminal tail inhibits Cds1 autoactivation *in vitro* and *in vivo*

II. Activation of Cds1

<table>
<thead>
<tr>
<th>Cds1(WT)</th>
<th>Cds1(ΔC)</th>
<th>Activated Cds1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ice</td>
<td>37°C</td>
<td>ice</td>
</tr>
</tbody>
</table>

ATP

Cds1

MyBP

MyBP

Wee1

Wee1

Cds1(WT)

Cds1(D3121E)

Cds1(ΔC)
C-terminal tail inhibits Cds1 autoactivation *in vitro* and *in vivo*.