Mouse Models to Investigate Cell Cycle and Cancer

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Crystal structure of Cdk2/ cyclin A

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Cyclin expression: periodicity

G1 S G2 M

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Cdk2
- Binds to Cyclin E, A, B
- Activity peaks in S phase
- Major target of p27
- Phosphorylates Rb and other targets
- Can replace yeast Cdk1
- Essential for DNA replication
- Inactivation arrest cells in G1

Cdk2 is important (essential?)
regulator of S phase

Cyril Berthet
Eiman Aleem

Cdk2^-/- knockout mice

Cdk2 knockout mice are viable!

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Atrophy in testis of Cdk2−/− mice

Absence of oocyte development in Cdk2−/− mice

Expression of cell cycle regulators in mouse tissues
Kinase activity and Cdk/cyclin complexes in spleen extract

Growth defect in Cdk2⁻/⁻ mouse embryo fibroblasts

Cdk complexes and activity in mouse embryonic fibroblasts

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Cdk2-/- MEF analysis after starvation

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Cdk activity in synchronized MEFs

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Delayed immortalization in Cdk2-/- MEFs
**Summary**

- Cdk2 is essential for meiosis but not mitosis
- Cdk2-/- MEFs display growth defects
- Which genes/ proteins compensate Cdk2 function?

**Cdk2 is not an essential gene in the mitotic cell cycle**

**The Retinoblastoma (Rb) protein pathway**

- Cdk1, cyclin A, Cdc25, Cyclin E, Cdk6, etc.
Cyril Berthet

\[ Cdk2^{-/-} Cdk4^{-/-} \]
double knockout mice

Developmental Cell 2006

Cdk2\(^{-/-}\)Cdk4\(^{-/-}\) mutants die around E15

WT DKO WT DKO WT DKO

Heart defects in Cdk2\(^{-/-}\)Cdk4\(^{-/-}\) embryos

Wild type

\[ Cdk2^{-/-} Cdk4^{-/-} \]
**In vivo proliferation in Cdk2\(^{-/-}\)Cdk4\(^{-/-}\) embryos**

- Wild type (WT)
- DKO (DKO)
- BrdU Liver

**Hypophosphorylated Rb leads to decreasing expression of Cdk1 and cyclin A**

- E13.5 E14.5 E16.5
- E13.5 E14.5 E16.5
- Cdk2
- Cdk4
- Cdk6
- p27
- pRb
- pRb\(^{378}\)
- E2F1

**Cdk2\(^{-/-}\)Cdk4\(^{-/-}\) MEFs display impaired proliferation**

- P2 MEFs
- P4 MEFs
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S phase entry defect in Cdk2<sup>-/-</sup>Cdk4<sup>-/-</sup> MEFs

Rb phosphorylation in Cdk2<sup>-/-</sup>Cdk4<sup>-/-</sup> MEFs

E2F target gene expression in Cdk2<sup>-/-</sup>Cdk4<sup>-/-</sup> MEFs

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Senescence in Cdk2\(^{-/-}\)Cdk4\(^{-/-}\) MEFs

SA-β-galactosidase staining

- P2: WT 14 ± 4%, DKO 29 ± 5%
- P4: WT 36 ± 7%, DKO 77 ± 10%

HPV-E7 neutralizes Rb and rescues Cdk2\(^{-/-}\)Cdk4\(^{-/-}\) phenotype

- HPV-E7 mutants cannot rescue Cdk2\(^{-/-}\)Cdk4\(^{-/-}\) phenotype

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### Summary: Cdk2−/−Cdk4−/−

- DKO die due to heart defects
- Rb is not phosphorylated and E2F mediated transcription is repressed
- Cdk2 and Cdk4 are essential genes, regulating the G1/S transition

Cdk2 and Cdk4 control the expression of Cdk1 through the Rb/E2F pathway
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The p27\textsuperscript{kip1} network

- TGF\textbeta, Cell cycle exit (GO)
- Ubiquitin-mediated degradation

Eiman Aleem

\textit{Cdk2}\textsuperscript{-/-}p27\textsuperscript{-/-} double knockout mice

- Increased body size
- Smaller body size
- Female sterility, disordered estrus, impaired luteal cell differentiation
- Pituitary tumors
- Retinal dysplasia
- Thymic hyperplasia, increased T cell proliferation, hematopoietic progenitors
- MEFs: lower proliferation, 4h delay in S phase entry
- MEFs: normal cell cycle kinetics
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**Cdk2⁻/-p27⁻/- mice**

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**Ovary tumors in Cdk2⁻/-p27⁻/- mice**

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**Pituitary tumors**

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**Cdk1 binds to p27 and cyclin E**

**Proliferation of mouse embryonic fibroblasts**

**Silencing of Cdk1 in Cdk2−/− MEFs**
Summary Cdk2⁻/⁻p27⁻/⁻
- P27 regulates Cdk2 and Cdk1
- Cdk1 interacts with cyclin E and forms active complexes
- Cdk1/cyclin E complexes most likely promote S phase and compensate for Cdk2

Cdk1 binds to p27 and cyclin E

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<thead>
<tr>
<th>Wild type</th>
<th>S phase</th>
<th>G2</th>
<th>M phase</th>
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<tbody>
<tr>
<td>Cdk2</td>
<td>Cdk2</td>
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<tr>
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Cdk1/cyclin E complexes can drive S phase

Cdk1 compensates for Cdk2 functions in S phase
Cdk1 translocates to the nucleus prematurely in the absence of Cdk2

Satya Ande

DNA damage response in Cdk2-/- knockout mice

Response to γ-irradiation in MEFs

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Response to γ-irradiation in MEFs

In vivo response to γ-irradiation: partial hepatectomy model

Cdk2⁺/⁺ and Cdk2⁻/⁻ mice subjected to 70% partial hepatectomy (surgery)

In vivo response to γ-irradiation: molecular outcome
Cdk1 and p21 can interact because they co-localize.

Proliferation of MEFs after irradiation

Cdk2<sup>-/-</sup> mice are sensitive to γ-irradiation.
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DNA damage in the absence of Cdk2
- Cdk2-/- mice are sensitive to $\gamma$-irradiation
- Cdk1 compensates for Cdk2 and is inhibited by p21
- DNA damage foci formation is disturbed
- DNA damage repair is delayed
- Cdk1 is less efficient in DNA damage repair

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