Investigating the role of CDK4/6 during the cell cycle by live-cell imaging

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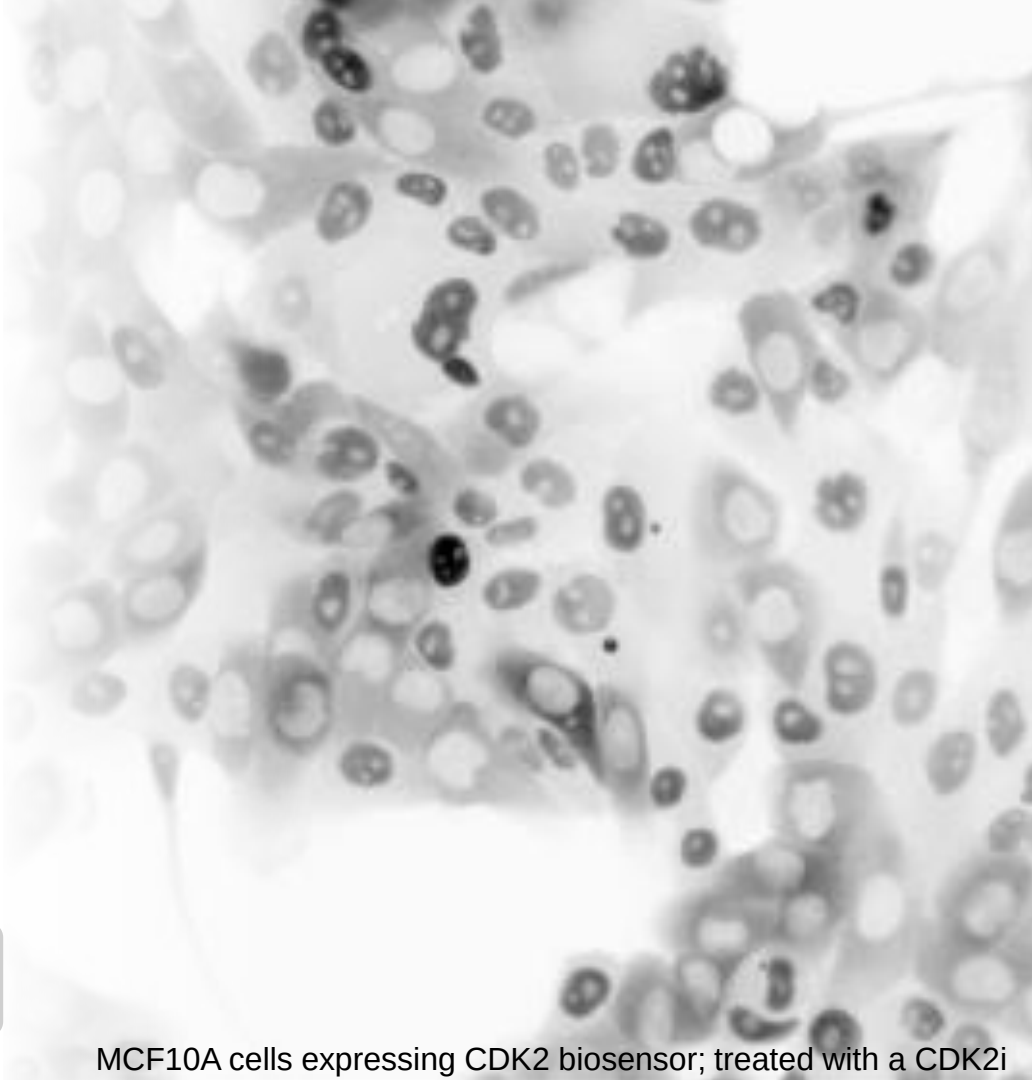
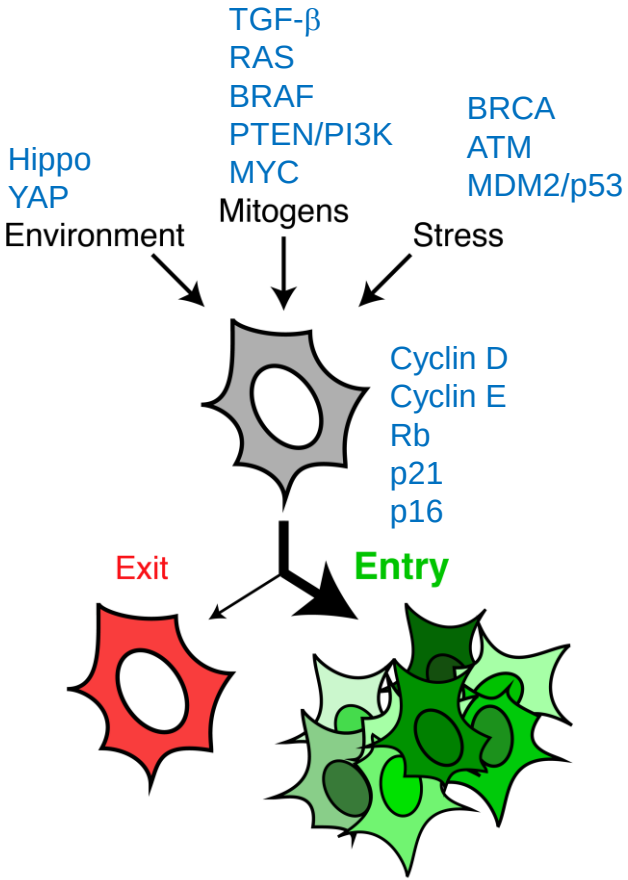
NIH NATIONAL CANCER INSTITUTE
Center for Cancer Research



MCF10A cells expressing CDK2 biosensor; treated with a CDK2i

Cancer is fundamentally a disease of uncontrolled proliferation



Regulation:

- Tissue and stem cell maintenance
- Tissue repair
- Immune responses

Misregulation:

- Cancer
- Degenerative diseases

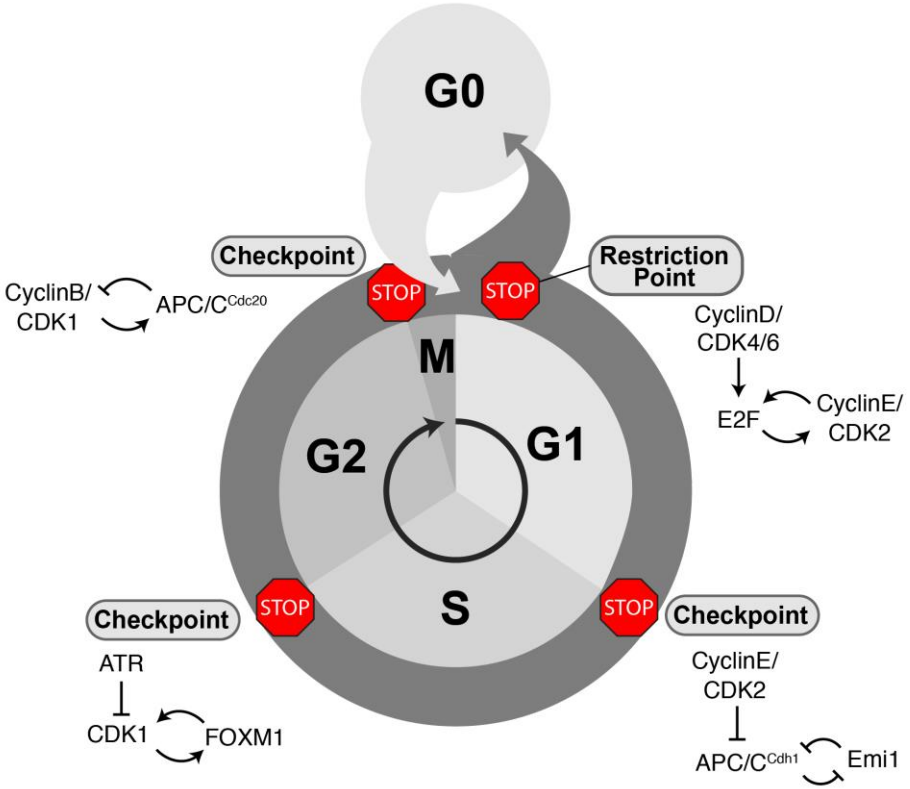
Goals of the lab:

- Investigate molecular mechanisms that regulate cell cycle **entry** and **exit**
- Elucidate how defects in these mechanisms contribute to human disease
- Exploit these mechanisms for new therapies

Cell cycle progression is regulated by a series of checkpoints

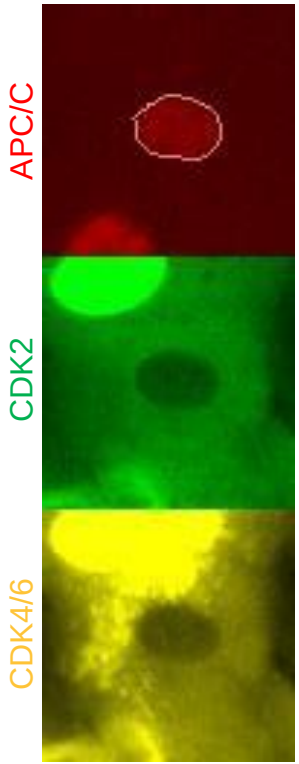
Problem:
Chemotherapies that impact the cell cycle don't always work as predicted by textbook models

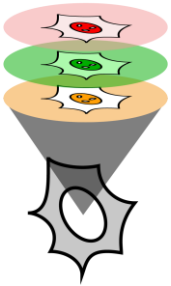
Challenge:
How to link cell cycle observations with molecular mechanisms?

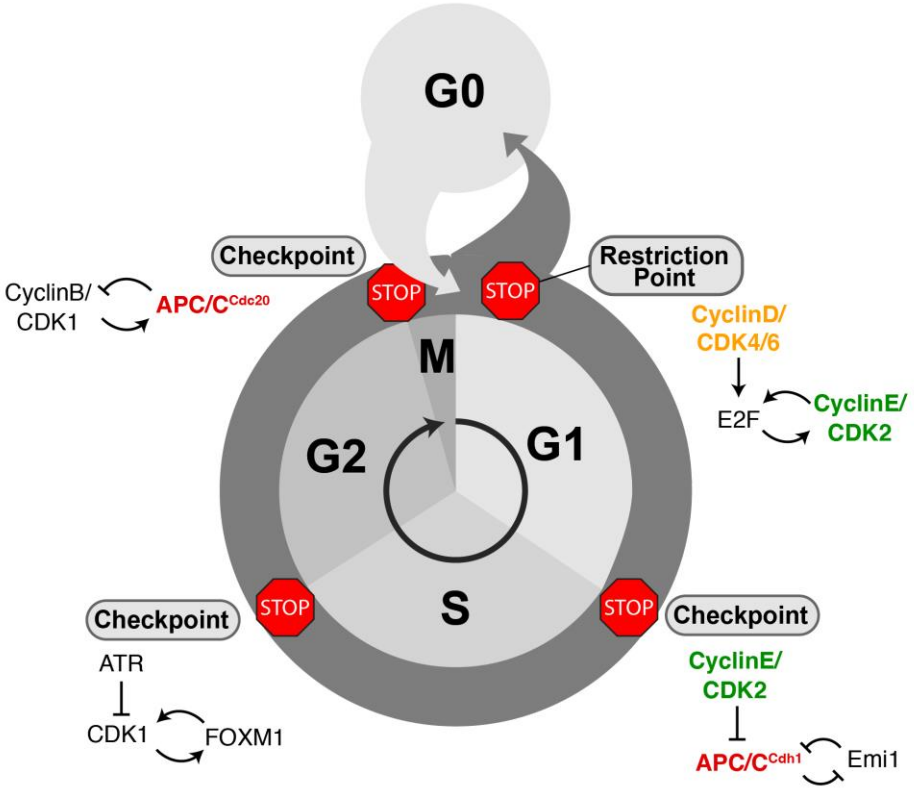


R point: Pardee, Zetterberg, Sherr, Weinberg, You, & others
G1/S: Lukas, Jackson, Cappell, & others
S/G2: Saldivar, et al. Science 2019
G2/M: Kirchner, Ferrell, Dunphy & others

Monitoring cell cycle progression in live cells using fluorescent biosensors



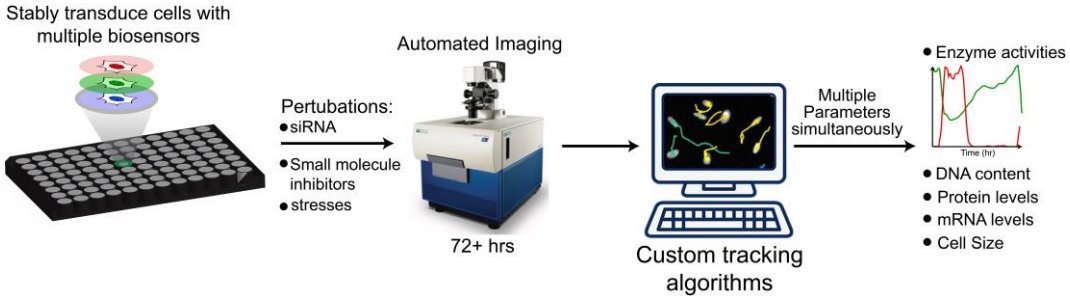




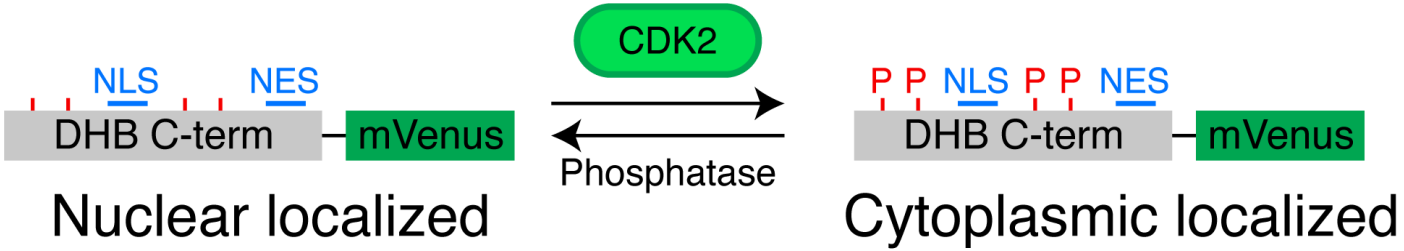
Spencer, Cappell et al., Cell 2013; Cappell et al., Nature 2018;
Cappell et al., Cell 2016; Yang*, Cappell*, Jaimovich*, et al., eLife 2020

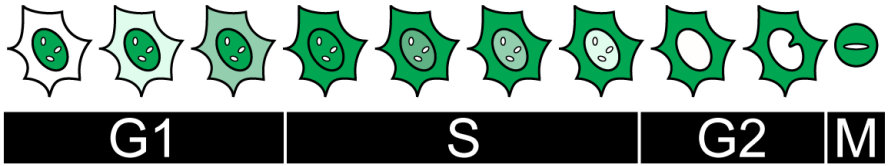
Automated live-cell imaging to capture cell-cycle dynamics in single cells

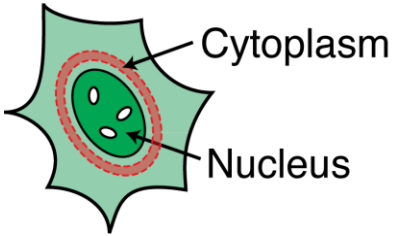
- Fluorescent biosensors for key cell cycle proteins
- Acute perturbations with small molecules
- Time-lapse microscopy and automated single-cell tracking



Monitoring Cyclin-dependent kinase (CDK) activity in live cells



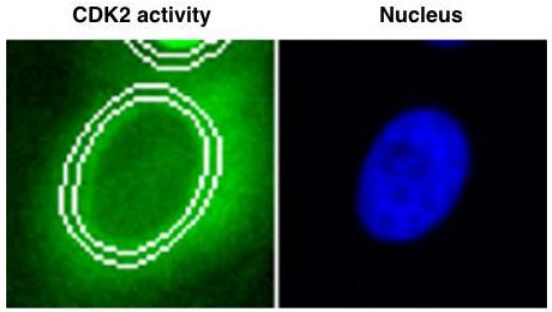


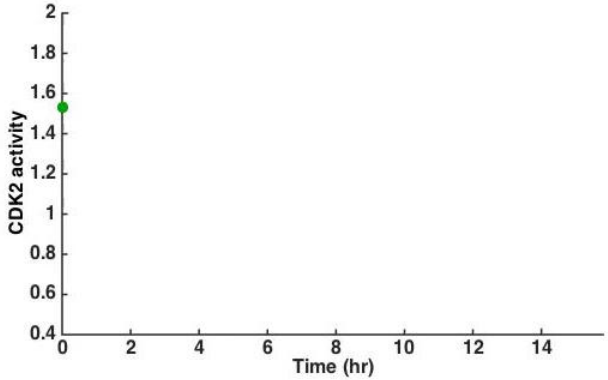


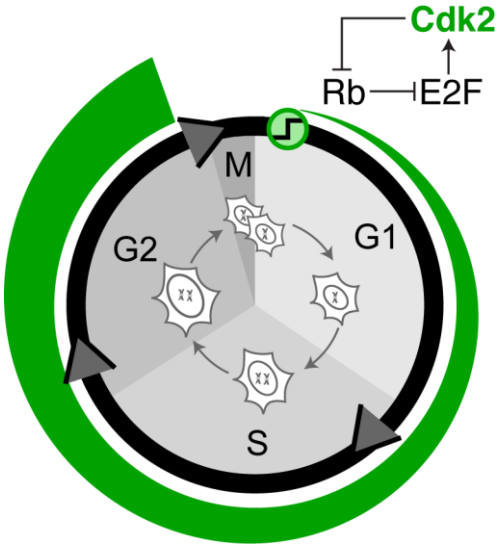
$$\text{CDK2 Activity} = \frac{\text{Cytoplasm}}{\text{Nucleus}}$$

Spencer, Cappell, et al., Cell 2013

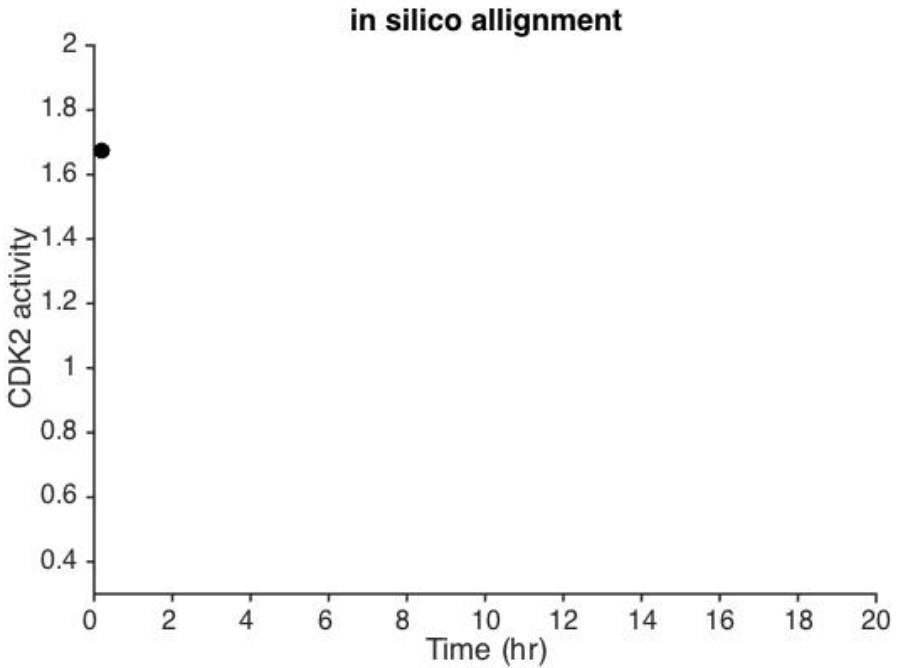
Monitoring CDK2 activity in live cells







in silico alignment of single-cell time courses



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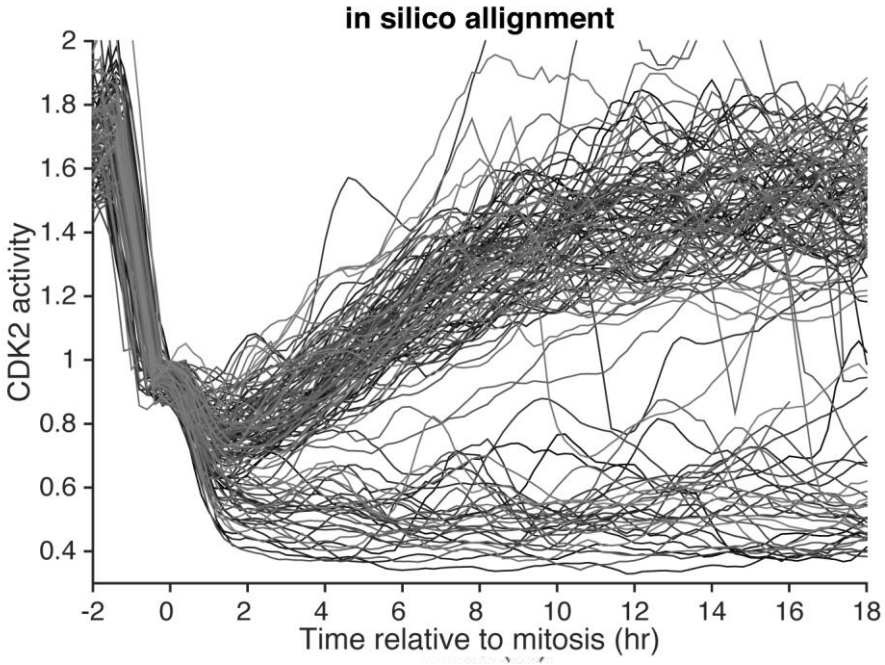

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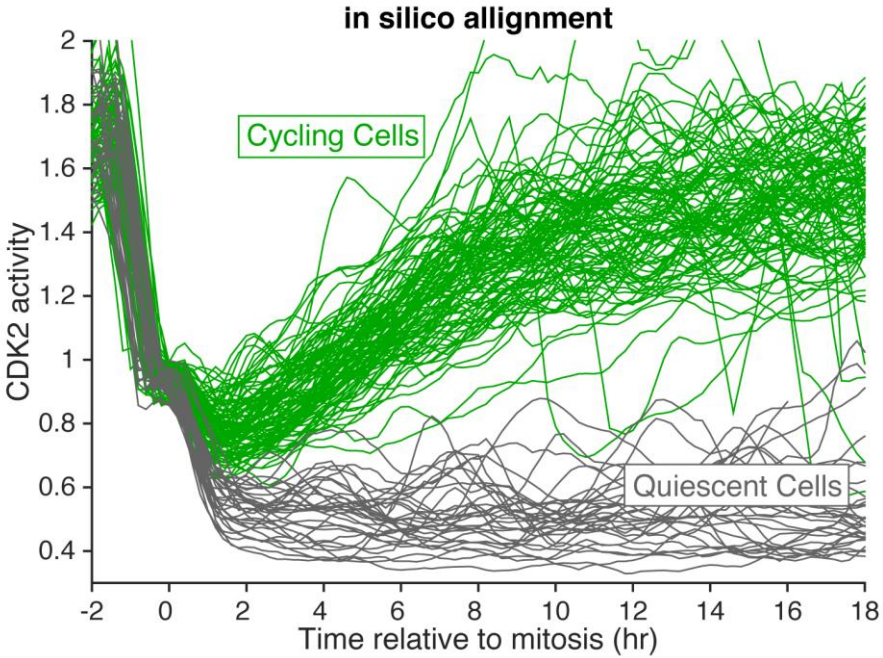
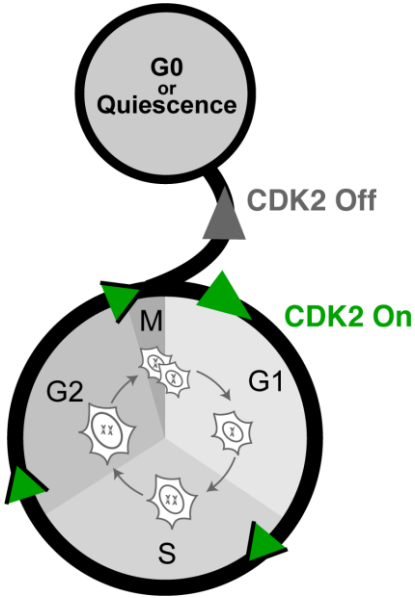
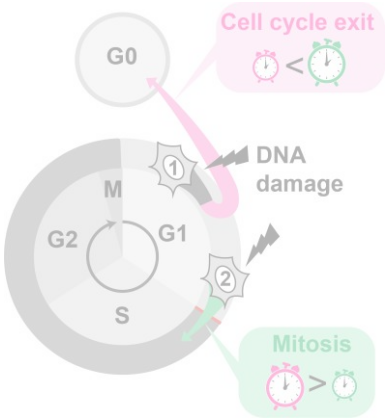




New insights into cell cycle regulation using live-cell imaging



Cells take divergent paths rather than pause at the G1/S checkpoint


Jenny Nathans

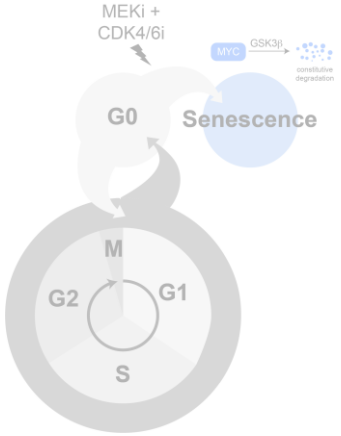

James Cornwell, PhD

Nathans, Cornwell…Cappell, Science Advances 2021

Senescence is irreversible because MYC is constantly degraded

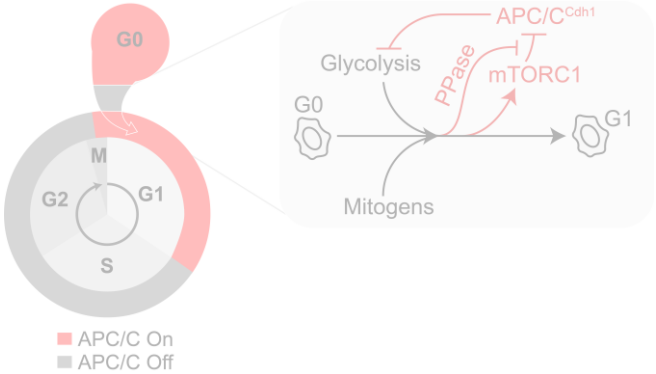

Marwa Afifi, BDS, PhD

Afifi…Cappell, Cell Reports 2023



Transient APC/C^{Cdh1} inactivation gives cells a metabolic boost during cell cycle entry

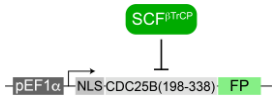
Paul…Cappell, In revision



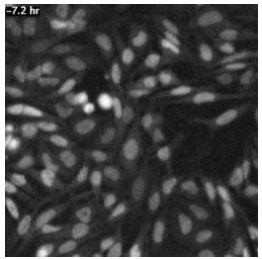
Paul…Cappell, Nature Comm 2022

Engineering a new biosensor for SCF$^{\beta TrCP}$

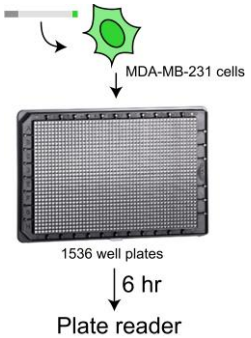
Design:



Validation:

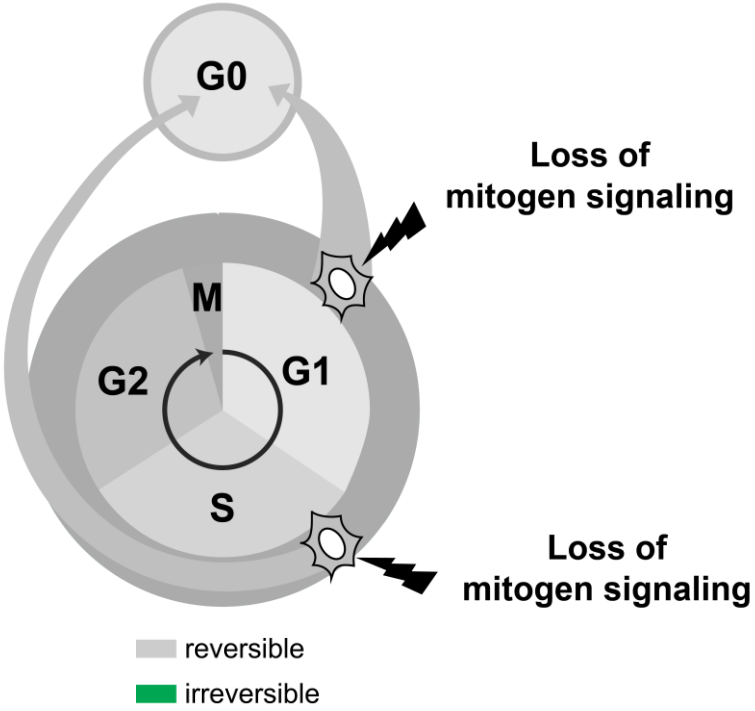


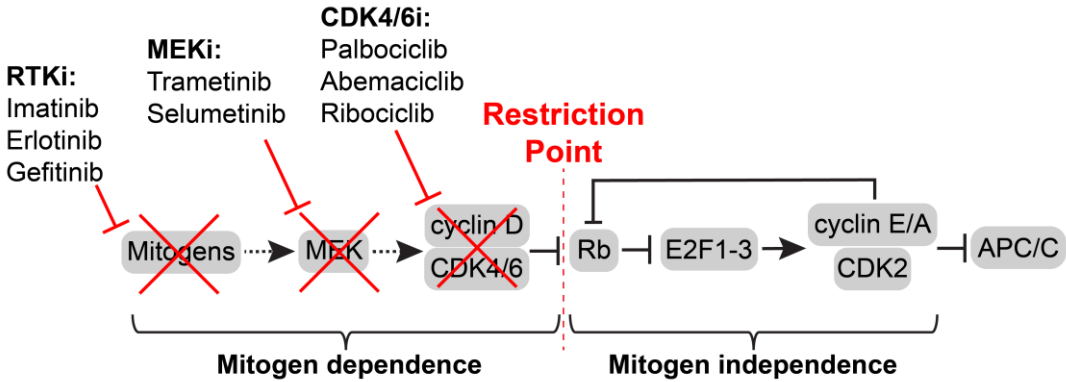
Assay development:




Debasish Paul, PhD

Irreversible Cell Cycle Commitment: **The Restriction Point Model**





James Cornwell, PhD

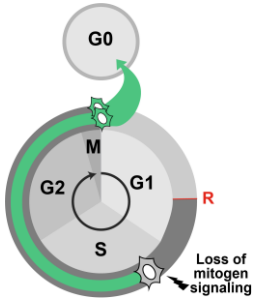
R point: Pardee, PNAS 1974; Zetterberg and Larsson, 1985

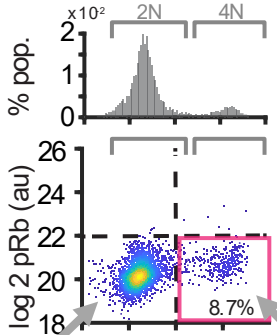
Feedback loop: Hunt, Nurse, Weinberg, Hershko, Sherr, Nasymth, Nevins, etc

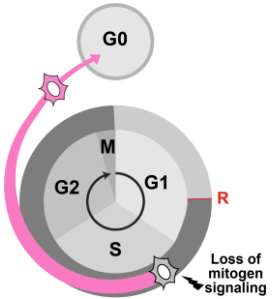
Cornwell…Cappell, Nature 2023

CDK4/6 inhibition can lead to post-R cells exiting the cell cycle

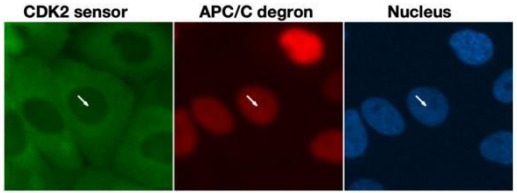
Mitosis then daughter cell exit:
CDK2 low
APC/C on
Rb hypophosphorylation
2N DNA content

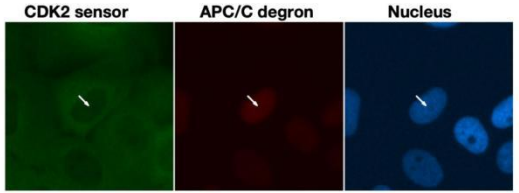


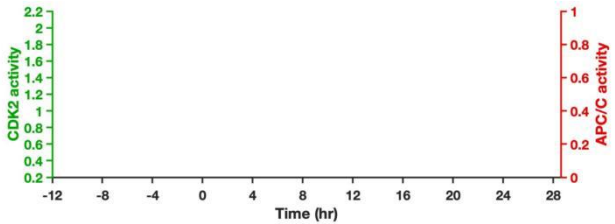


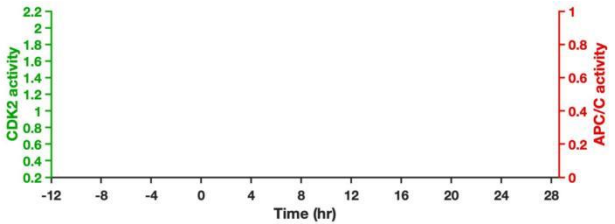


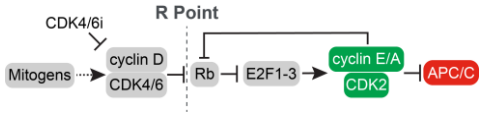
Cell cycle exit:
CDK2 low
APC/C on
Rb hypophosphorylation
4N DNA content





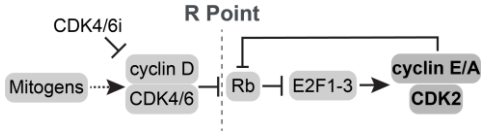


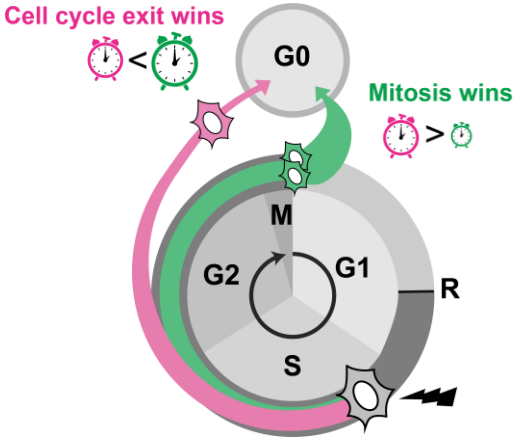




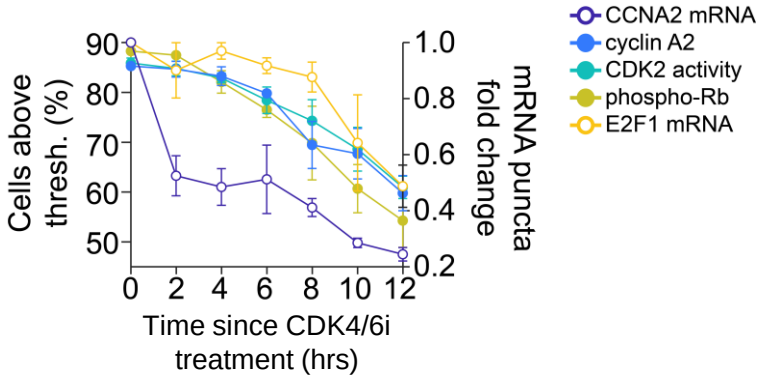
CDK2: Spencer, Cappell…Meyer 2013
APC/C: Sakaue-Sawano…Miyawaki 2008; Cappell…Meyer 2016

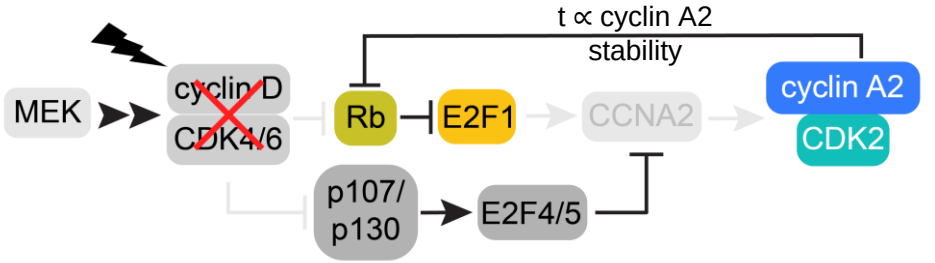
Loss of CDK4/6 leads to cell cycle reversal in all cells if mitosis is blocked or delayed

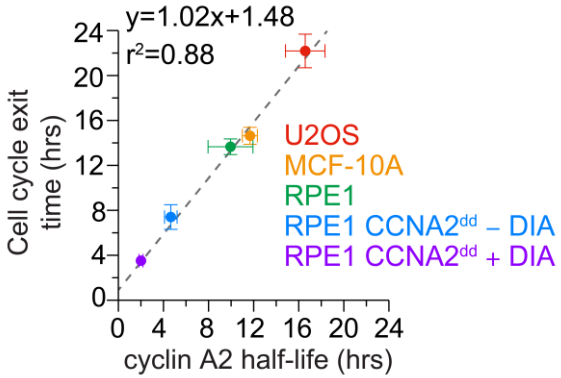




CDK4/6 inhibition in post-R cells leads to loss of CCNA2 mRNA

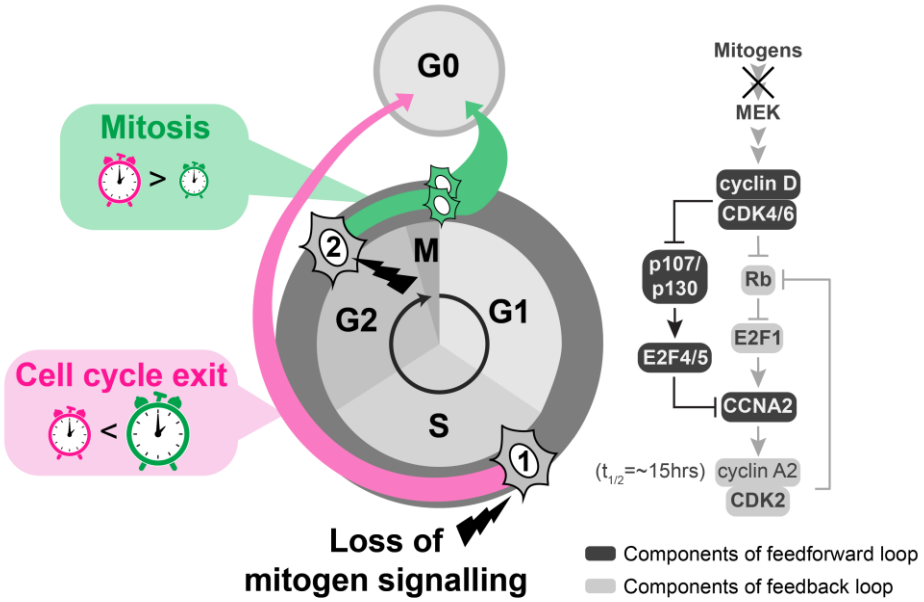






RPE1 CCNA2^{dd}: Hegarat*, Crncec*…Hochegger 2020

Summary: CDK4/6 activity is needed in S/G2 phase to sustain cyclin A/CDK2



Revising the textbook model of the cell cycle:

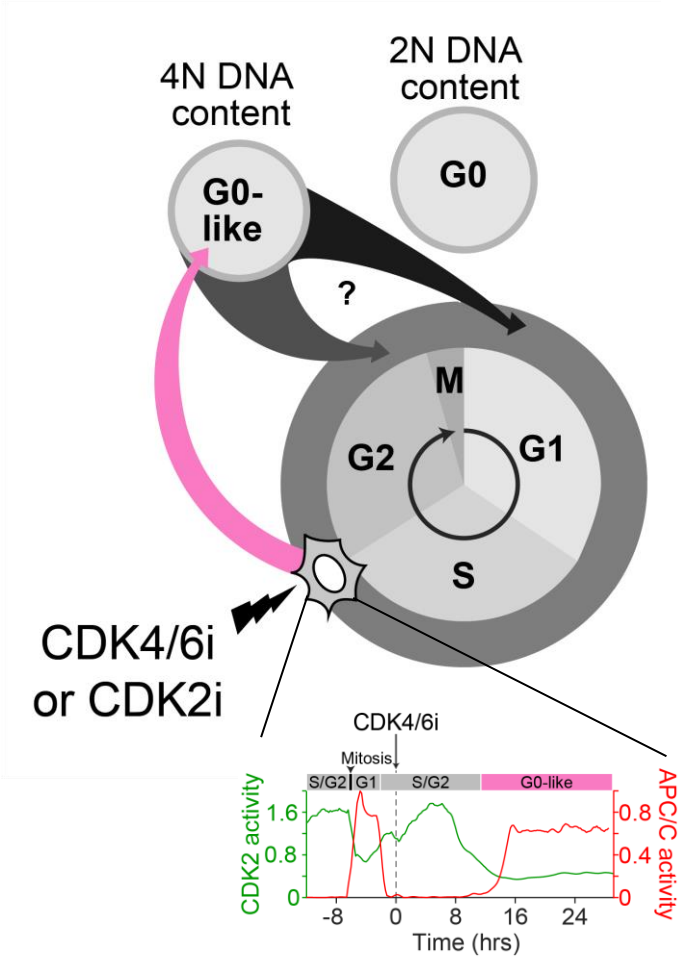
- The Restriction Point phenomenon is explained by temporal competition between mitosis and cell cycle exit
- CDK4/6 is active during entire cell cycle
- CDK inhibition can send cells to a G0-like state with 4N DNA content

Important implications for:

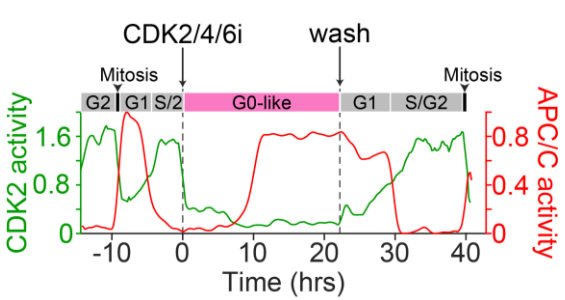
- Basic understanding of cell biology
- Clinical use of CDK inhibitors

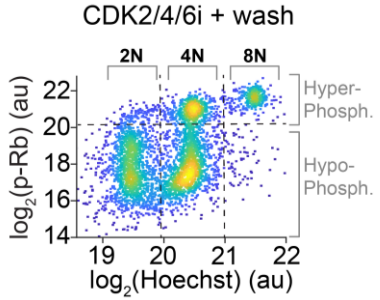
Cornwell…Cappell, Nature 2023

Next steps: What are the long-term consequences of this alternate cell cycle path?

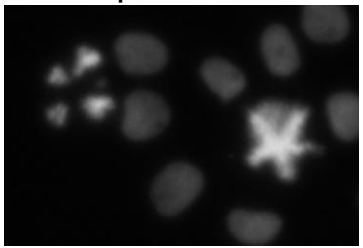


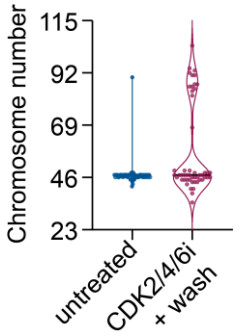
- CDK4/6 inhibitors are front-line therapy for HER+/HR+ breast cancer
- CDK2 inhibitors in development for treatment of CDK4/6i-resistant cancers





multi-polar mitoses





In collaboration Andre Nussenzweig (NCI)

Acknowledgments

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Helfrid Hochegger (Univ of Sussex)

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