Coping with stress:
Understanding how cells make the decision to enter or exit the cell cycle

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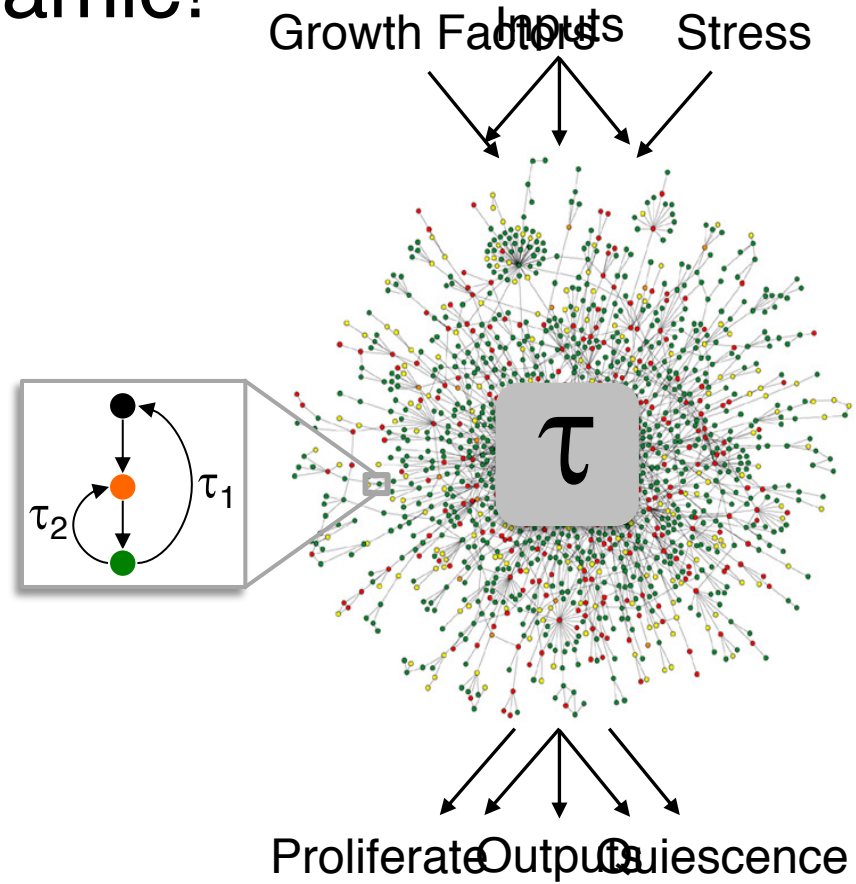
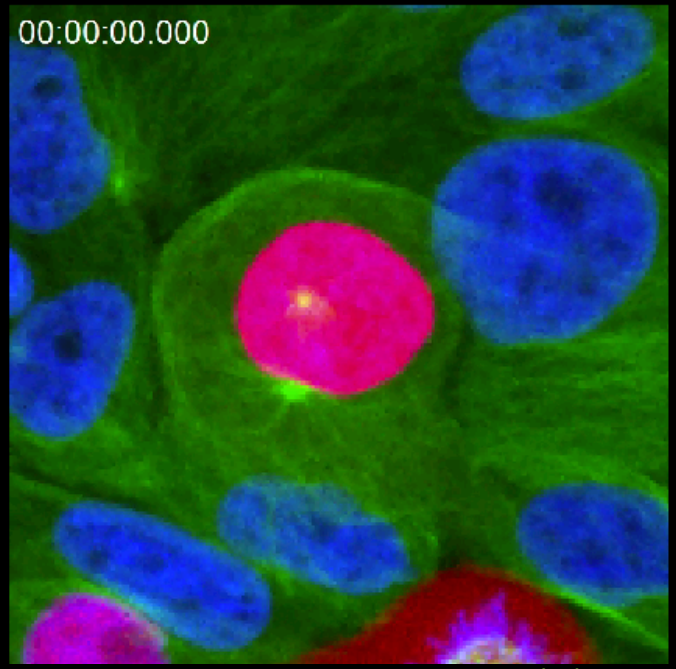
Center for Cancer Research

Signaling is Dynamic!

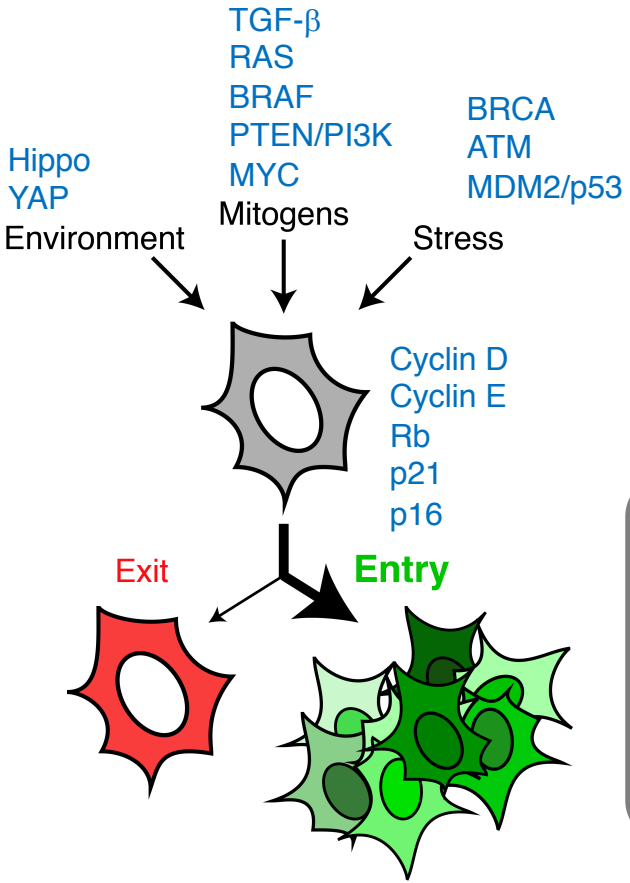


Visualizing The Cell Cycle

Mitosis



Cancer is fundamentally a disease of uncontrolled proliferation



Regulation:

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

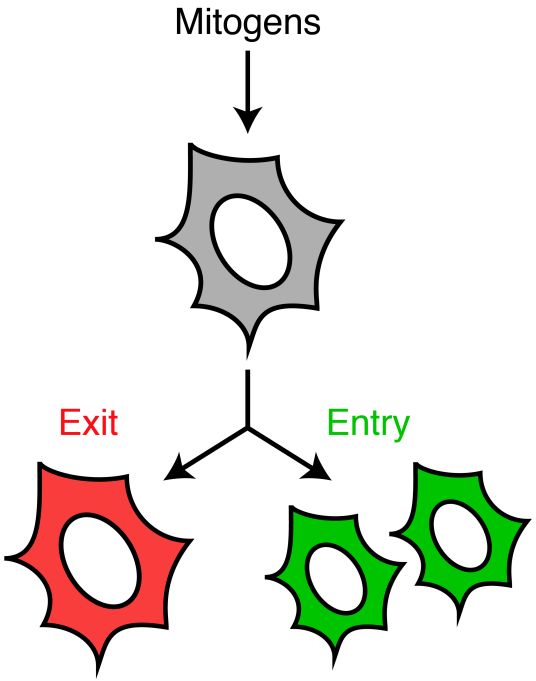
Misregulation:

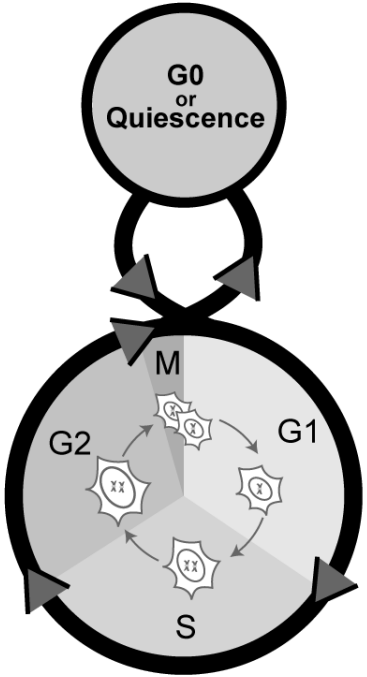
- Cancer
- Degenerative diseases

Goals of the lab:

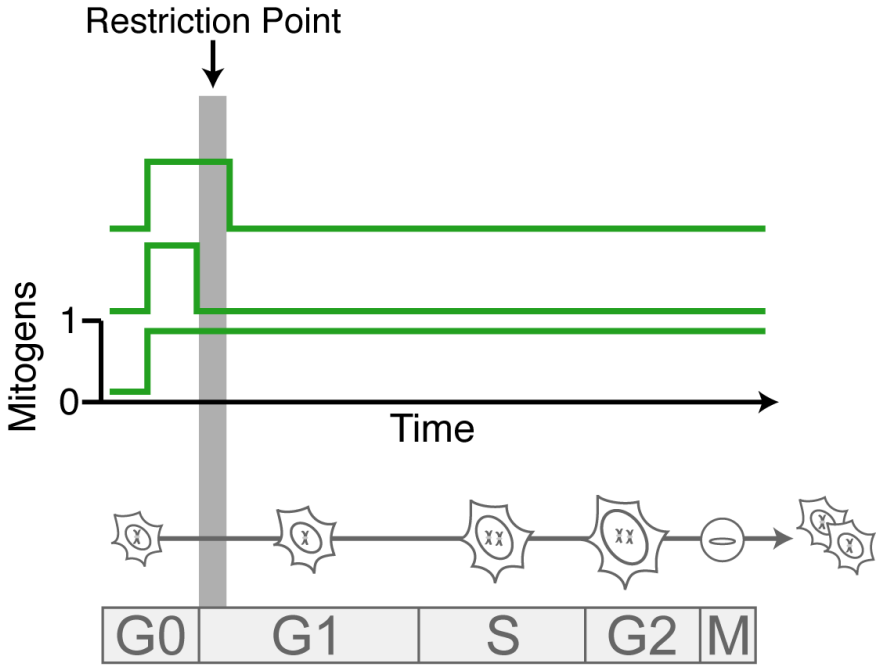
- Investigate molecular mechanisms that regulate cell cycle **entry** and **exit**
- Elucidate how defects in these mechanisms contribute to human disease
- Exploit these mechanisms for new therapies

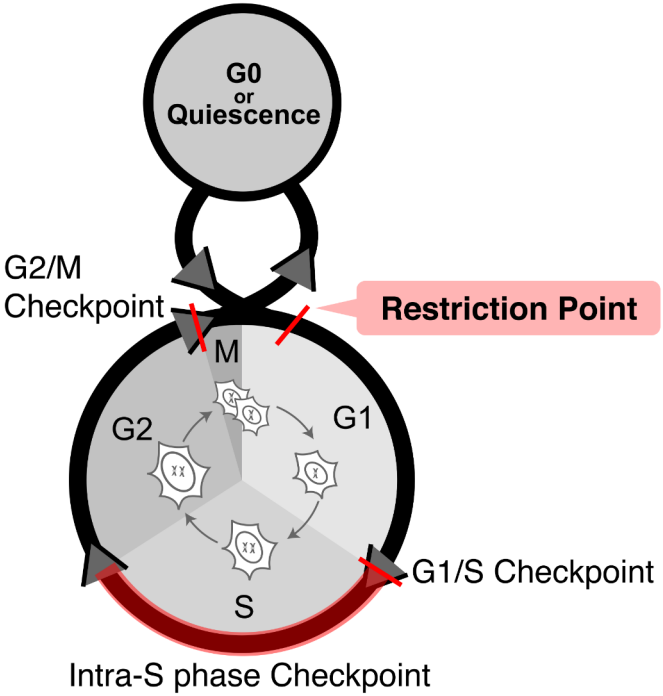
Mitogens trigger cell cycle entry





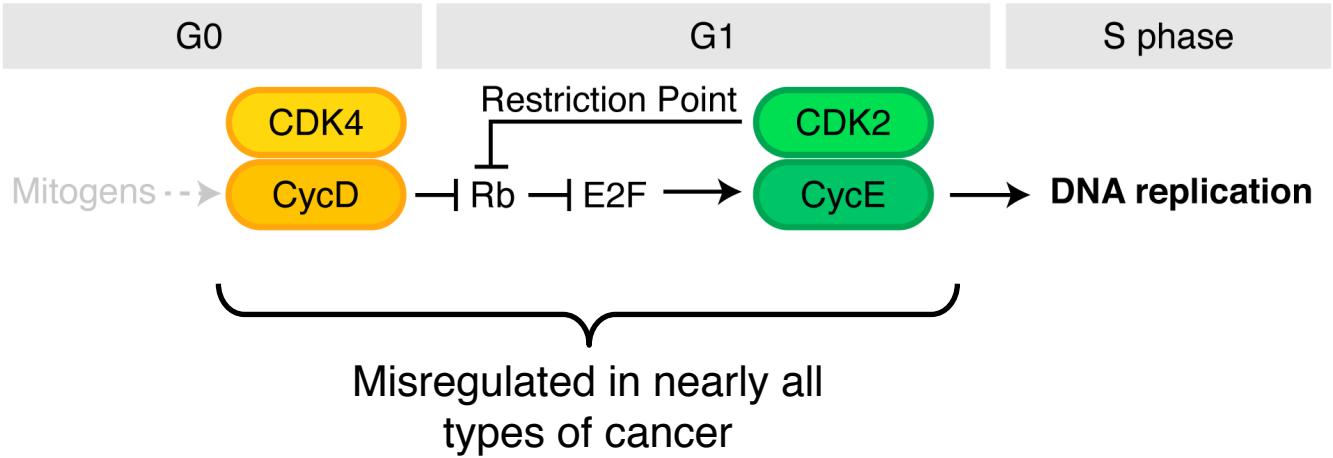
Cells become mitogen-independent after the Restriction Point





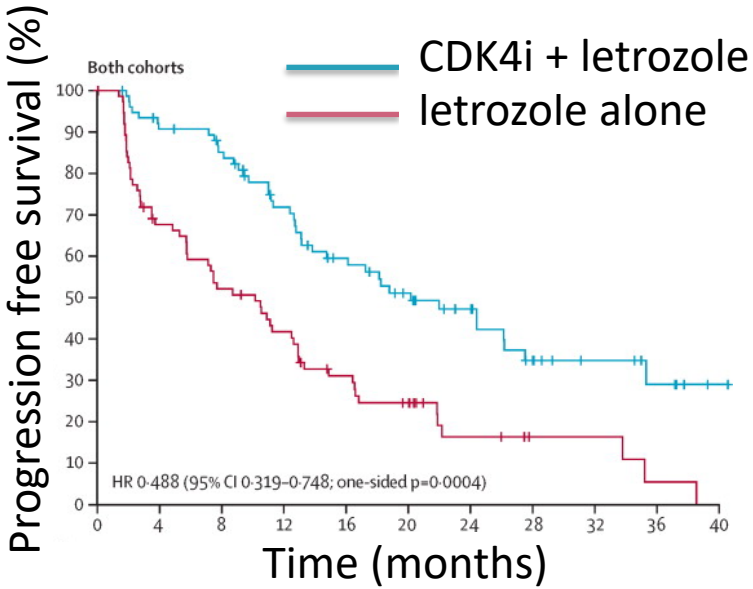
Pardee, PNAS 1974; Zetterberg and Larson, 1985

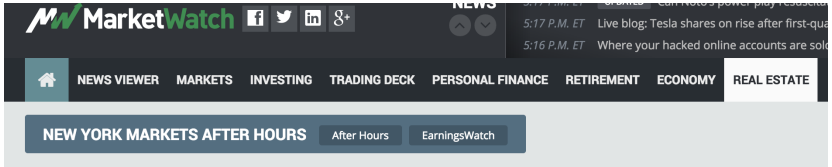
Molecular mechanism of the Restriction Point



1990's to 2000's: Hunt, Nurse, Weinberg, Hershko, Sherr, Nasmyth, Nevins, etc

Renewed interest in mechanisms of cell cycle entry

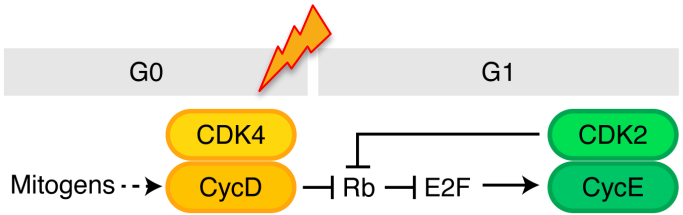




PRESS RELEASE

Pfizer Announces PALOMA-3 Trial For IBRANCE® (Palbociclib) Stopped Early Due To Efficacy Seen In Patients With HR+, HER2- Metastatic Breast Cancer Whose Disease Has Progressed Following Endocrine Therapy

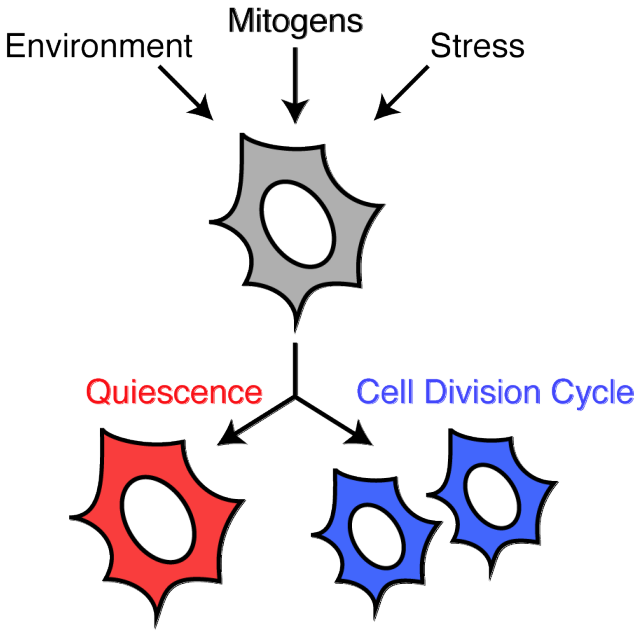
Published: Apr 15, 2015 8:00 a.m. ET

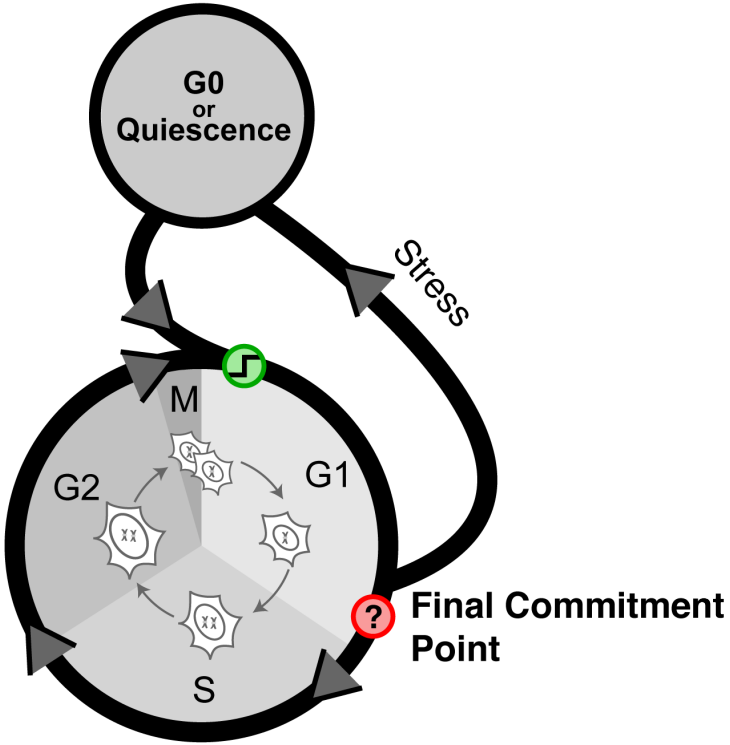


Palbociclib= CDK4i
Letrozole= anti-estrogen

Finn *et al.* The Lancet Oncology Jan 2015

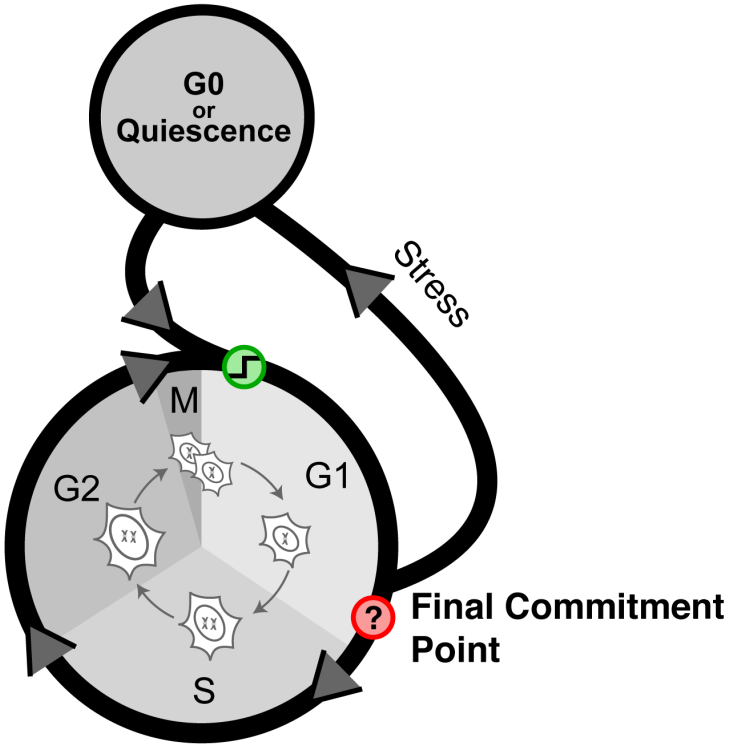
Q: How do cells integrate multiple signals to make the decision to divide?





When and how do cells execute the decision to divide?

1. When do cells execute the decision to divide?
2. What protein mediates the decision to divide?
3. What is the molecular mechanism underlying the decision to divide?

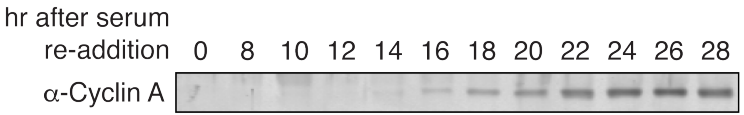


Cappell, et al. Cell 2016

Need to follow single-cells as they proceed through the cell cycle

Classic Approach:

- Synchronization (eg. Nocodazole, thymidine, serum removal)
- Bulk-Cell analysis
- End-point assays



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Classic Approach:
- Synchronization (eg. Nocodazole, thymidine, serum removal)
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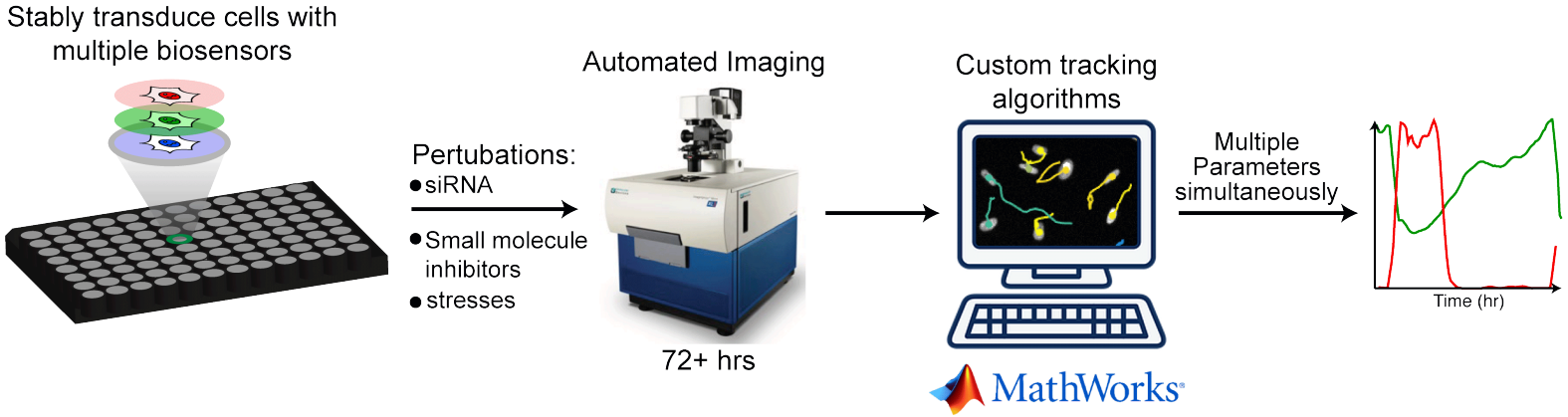
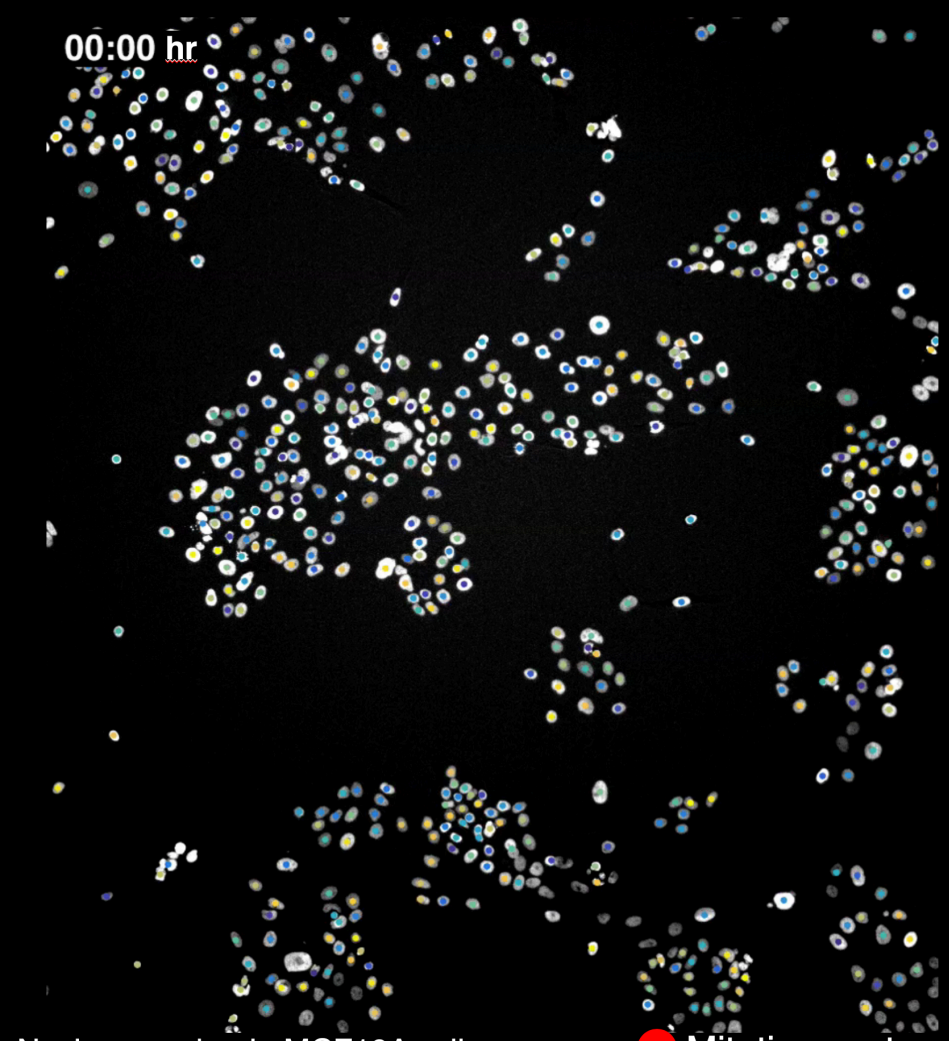
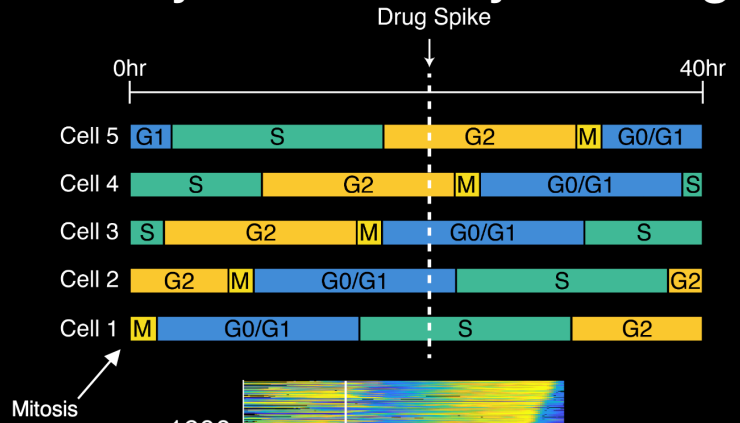
- End-point assays

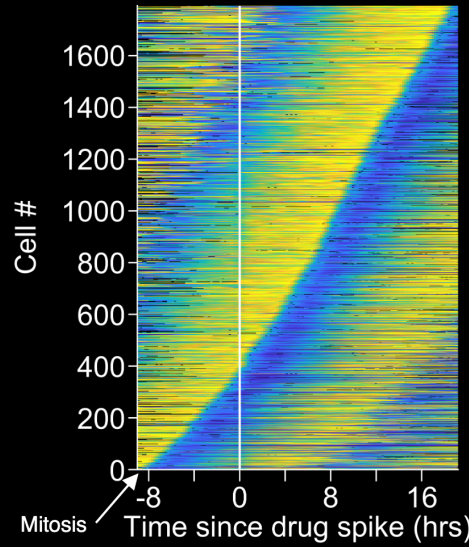
Our Approach:
- Asynchronously cycling cells
- Automated cell tracking
- Live-cell sensors
- Measure many parameters simultaneously



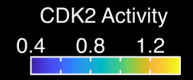
Automated tracking of thousands of asynchronously dividing cells



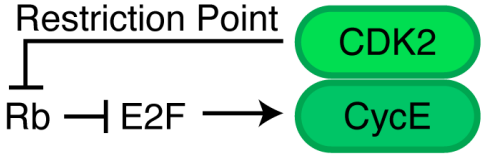




>3 million measurements per well



Monitoring CDK2 activity in live cells



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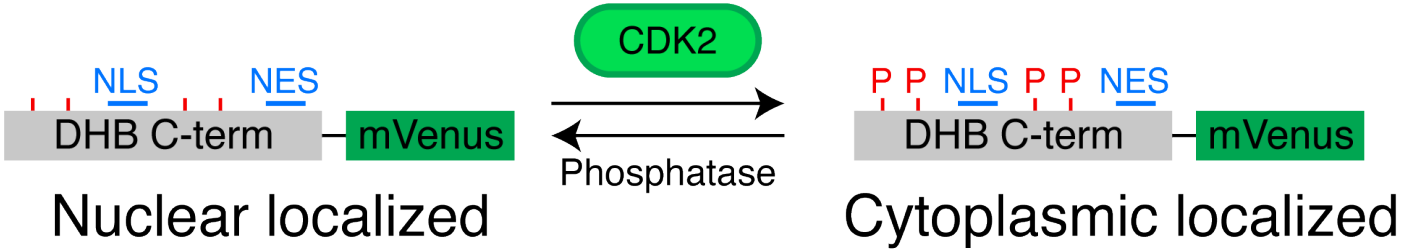
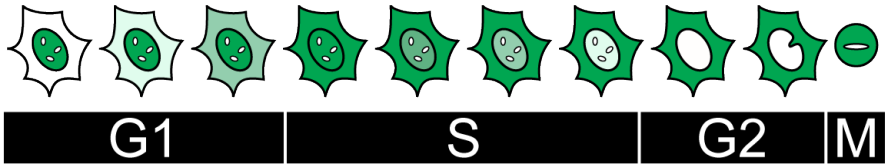
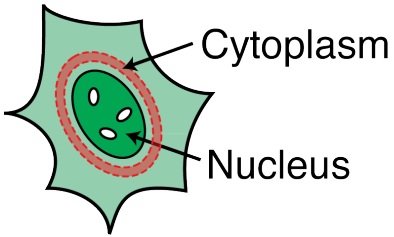


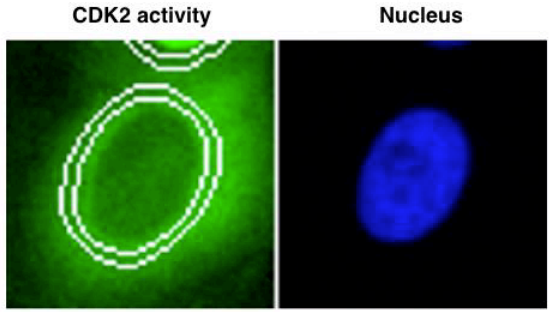


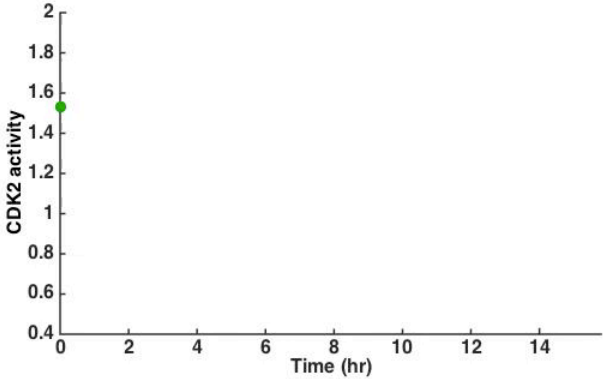


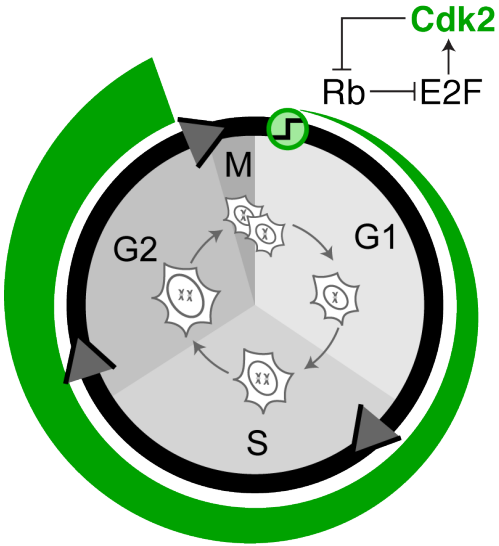
$$\text{CDK2 Activity} = \frac{\text{Cytoplasm}}{\text{Nucleus}}$$

Spencer, et al., Cell 2013

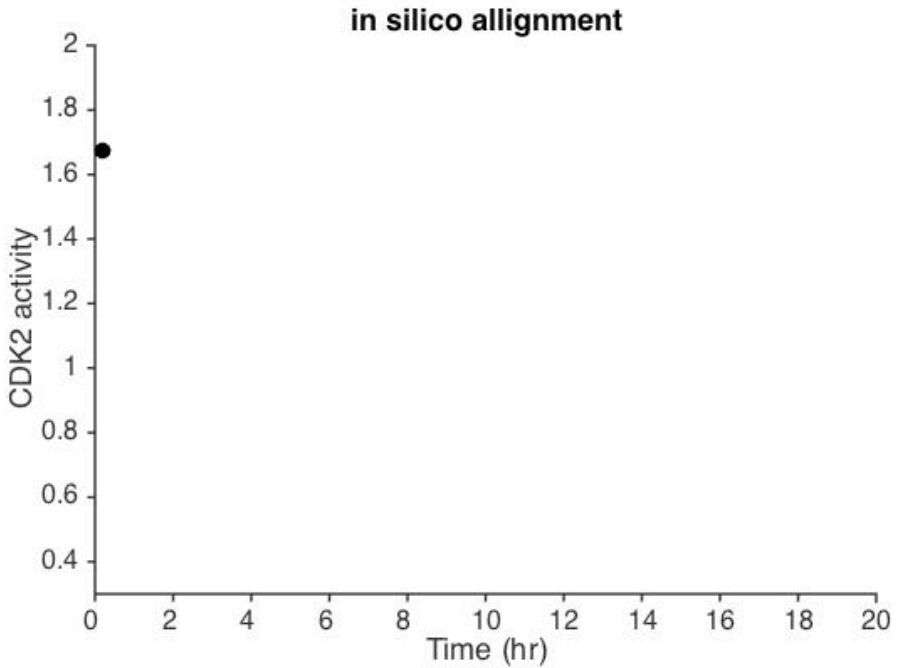
Monitoring CDK2 activity in live cells







in silico alignment of single-cell time courses



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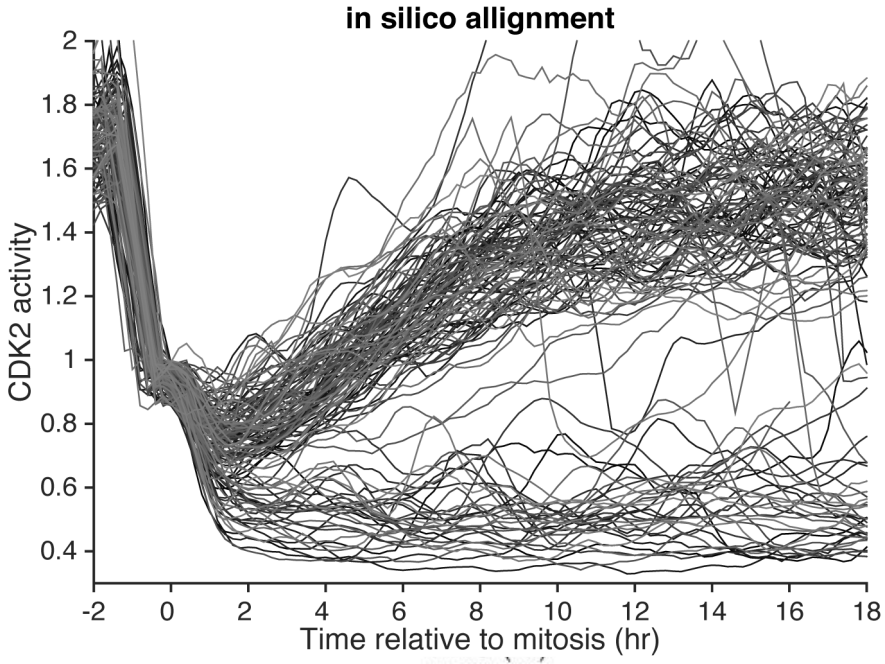

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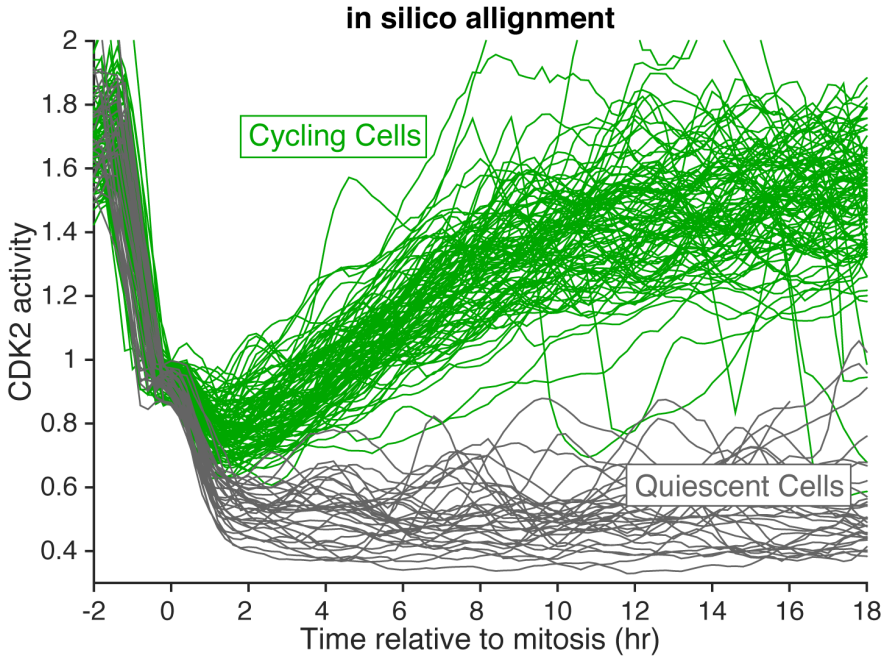
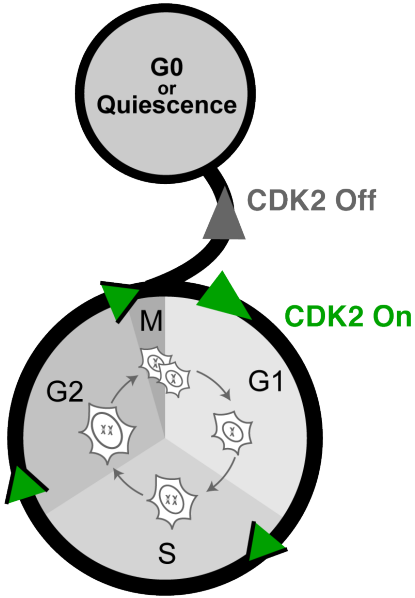
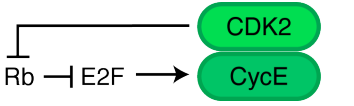
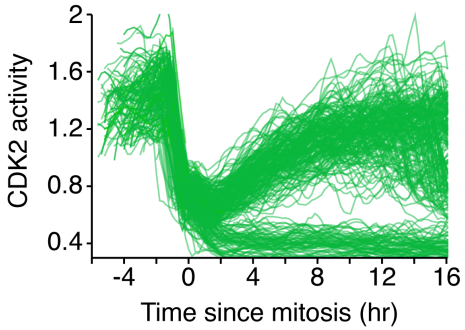
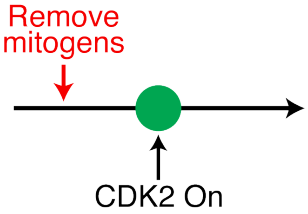


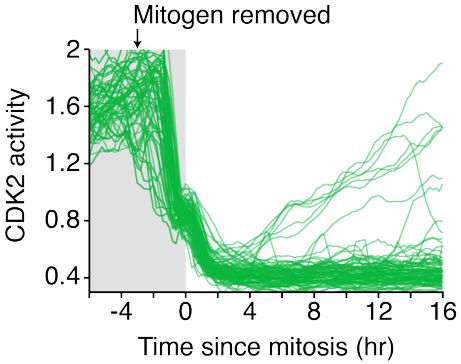


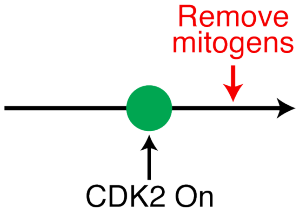
CDK2 sensor marks the Restriction Point

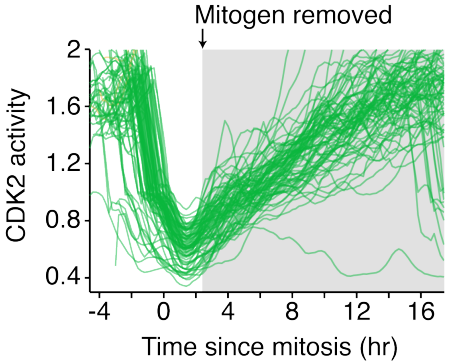












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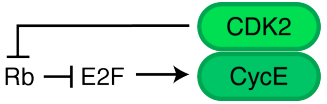
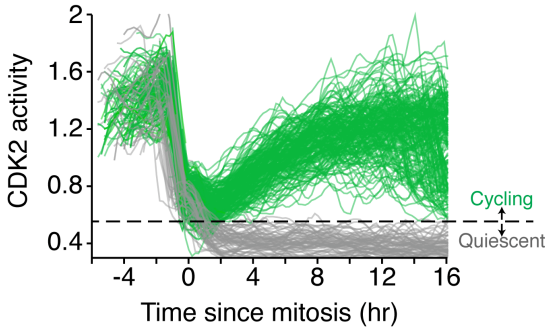
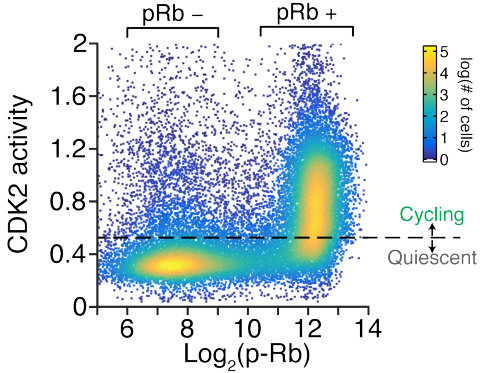
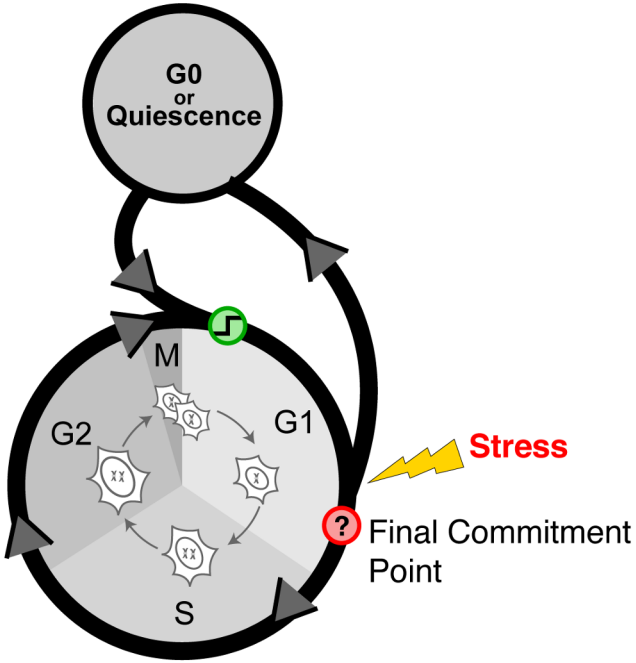
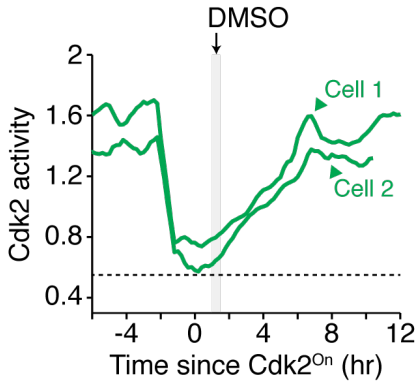


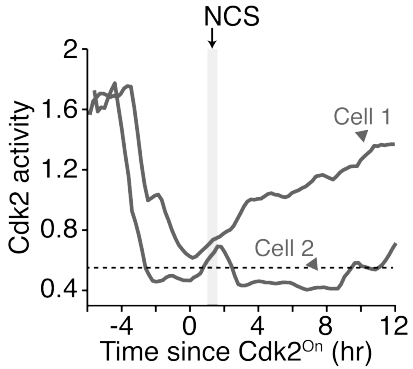


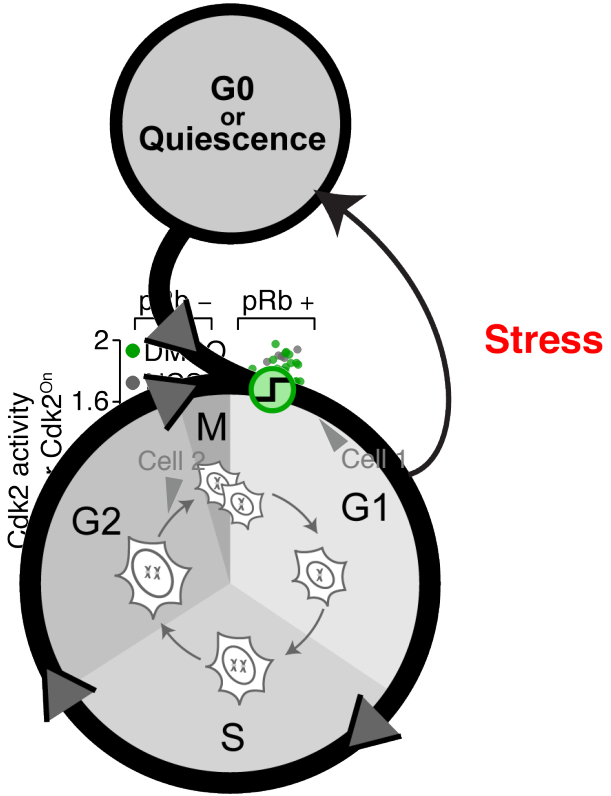




How does stress affect the decision to commit to the cell cycle?

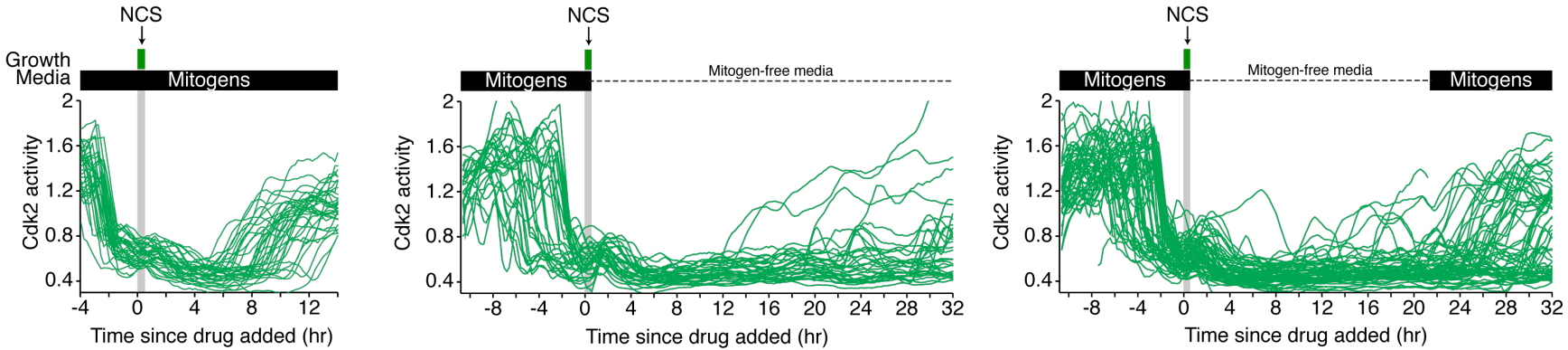




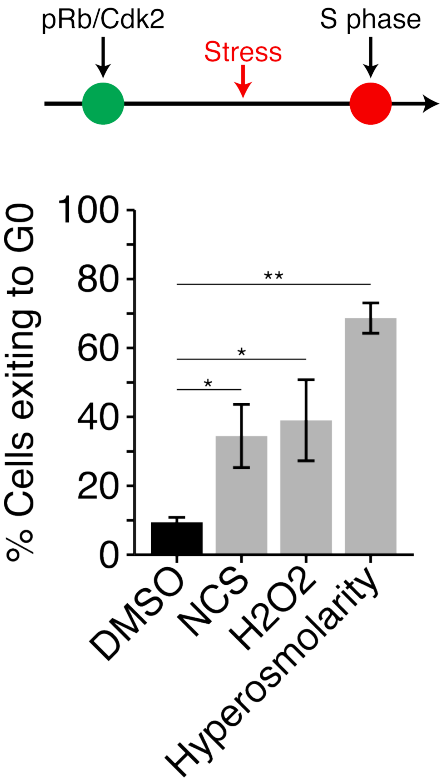


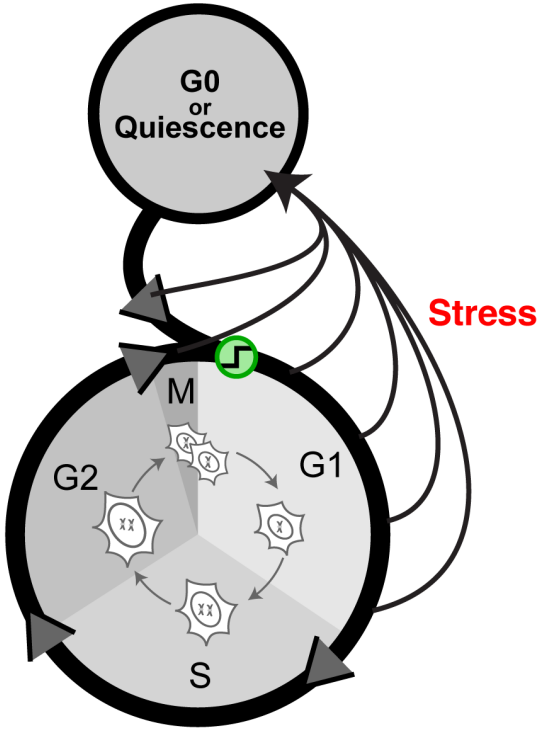
NCS: neocarzinostatin (DNA double-strand breaks)

Stressed cells exit to a mitogen-dependent quiescent state



Stress can send cells back to G0 **after** the Restriction Point

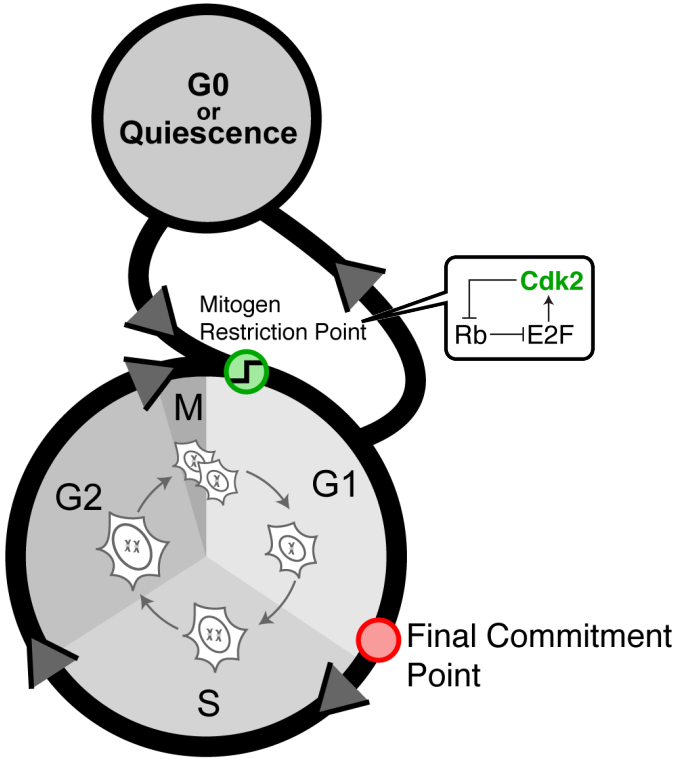




* p<0.05 **p<0.01

Cells become committed to the cell cycle in late G1 phase

1. When do cells execute the decision to divide?
2. What protein mediates the decision to divide?
3. What is the molecular mechanism underlying the decision to divide?

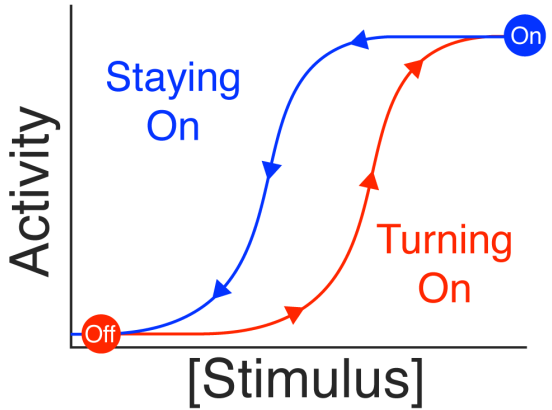


What features should a commitment point have?

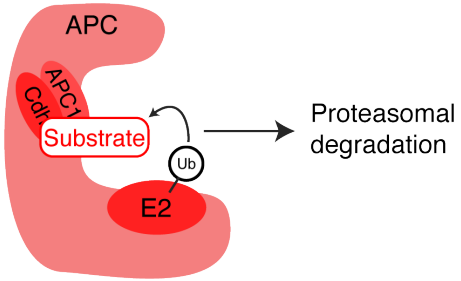
- Have global impact
- Bistable
- Irreversible
- Resistant to noise
- Should require a large stimulus to **turn** on

Hysteresis

Examples of hysteresis:
- Restriction Point
- SHH signaling: Posterior/Anterior
- Oct4-Sox2-Nanog: stem cell differentiation



Anaphase Promoting Complex/Cyclosome (APC/C) is a key regulator of G1 phase

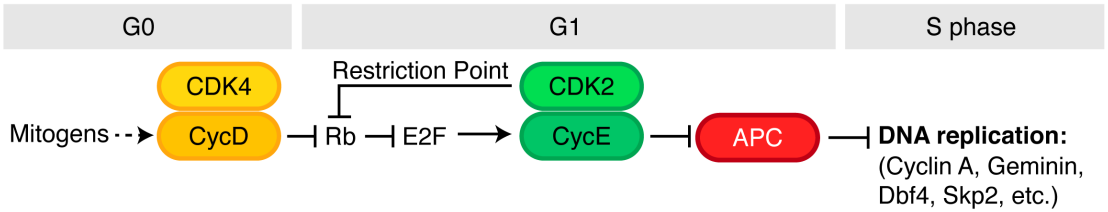


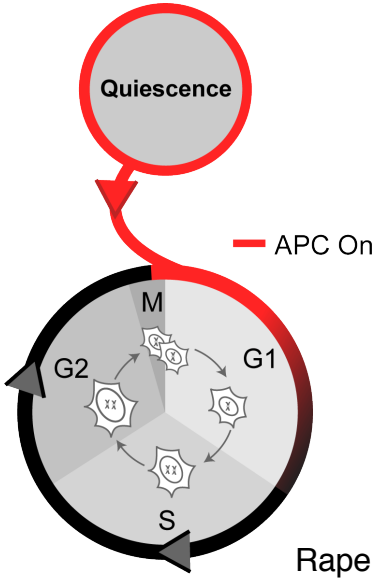
Mitosis
(Spindle Assembly Checkpoint)

S phase
Geminin
Cyclin A
Dbf4
Skp2
.....

Hallmarks of Commitment:

- ✓ Global impact
- X Bistable
- ○ Irreversible
- ○ Requires a strong stimulus to **turn it** on, but a small stimulus to **keep it** on

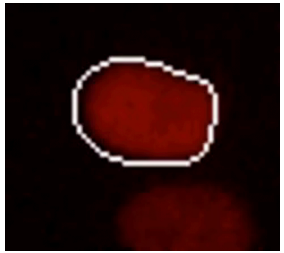




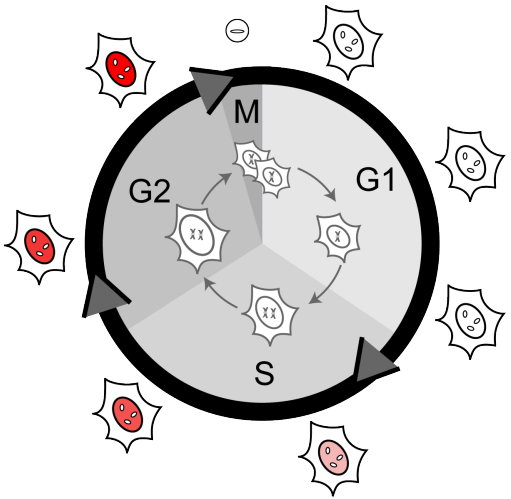
Rape and Kirschner, 2004

Searching for a live-cell sensor for APC/C activity

FUCCI: Miyawaki et al., Cell 2008



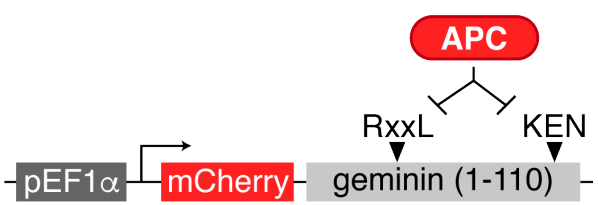
S/G2

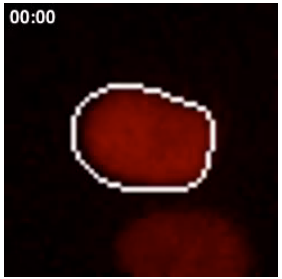


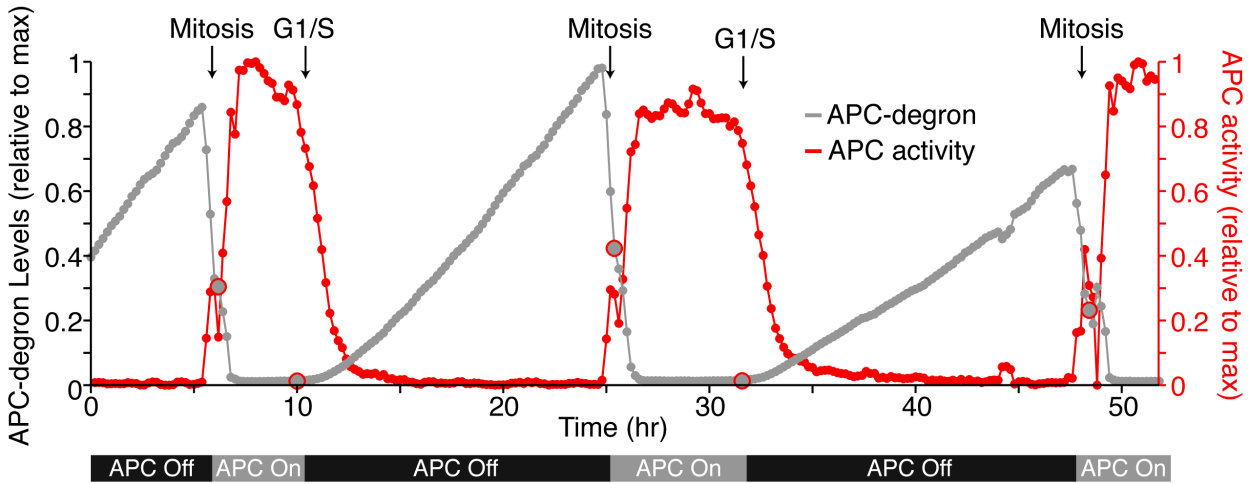
G0/G1

FUCCI: Fluorescent Ubiquitin Cell Cycle Indicators

Searching for a live-cell sensor for APC/C activity

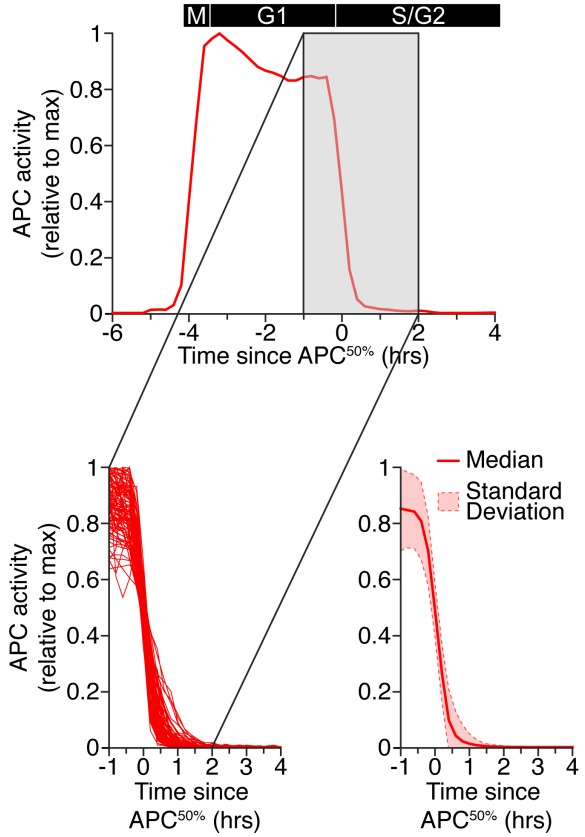






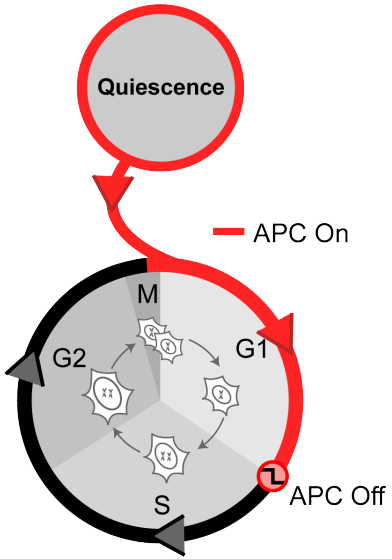
$$\frac{d[\text{APC degron}]}{dt} = K_{prom} - APC * [\text{APC degron}]$$

APC/C inactivates rapidly and with stereotypical kinetics

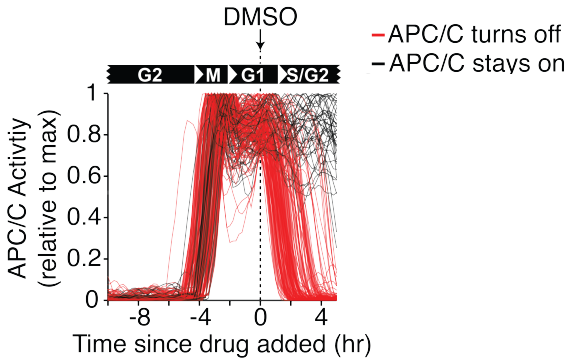


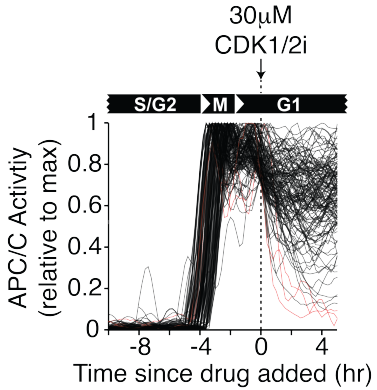
Hallmarks of Commitment:

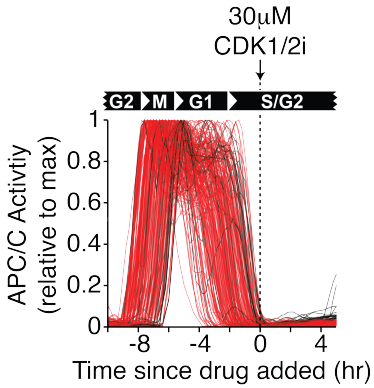
- ✓ Global impact
- ? Bistable
- ○ Irreversible
- ○ Requires a strong stimulus to **turn it** on, but a small stimulus to **keep it** on



APC/C inactivation is irreversible with respect to CDK2 activity

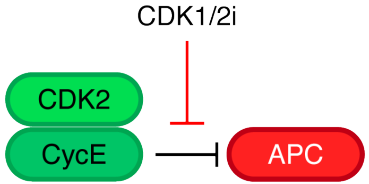


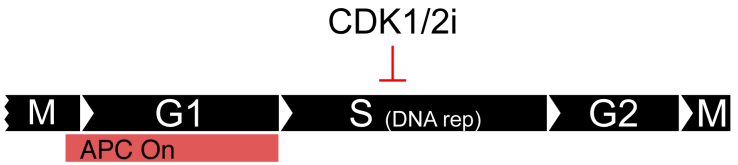




Hallmarks of Commitment:

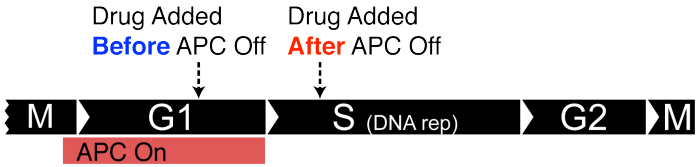
- ✓ Global impact
- ✓ Bistable
- ? ✓ Irreversible
- ? Requires a strong stimulus to **turn it** on, but a small stimulus to **keep it** on





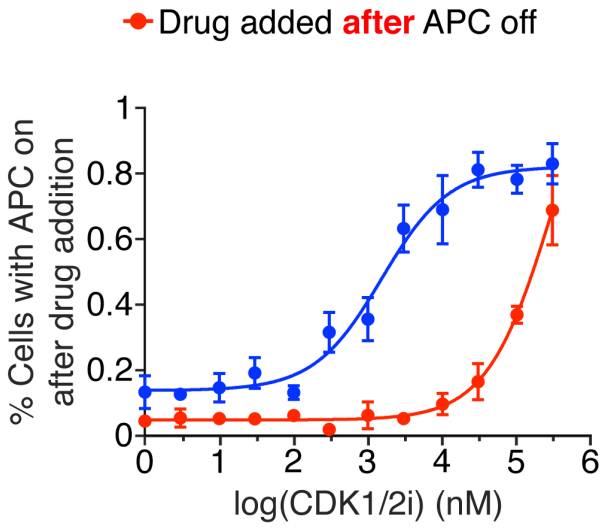
Lukas et al, Nature 1999

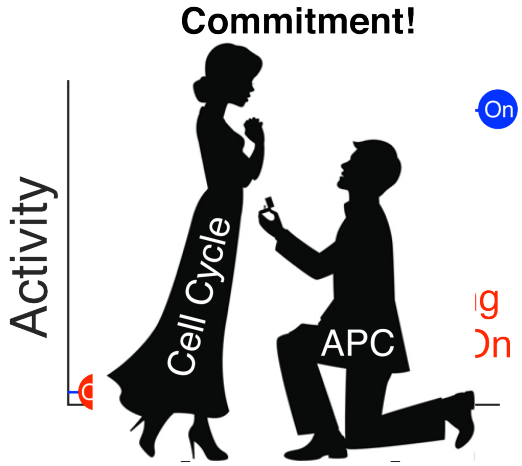
APC/C inactivation exhibits hysteresis



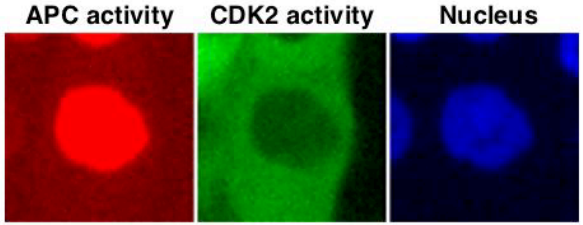
Hallmarks of Commitment:

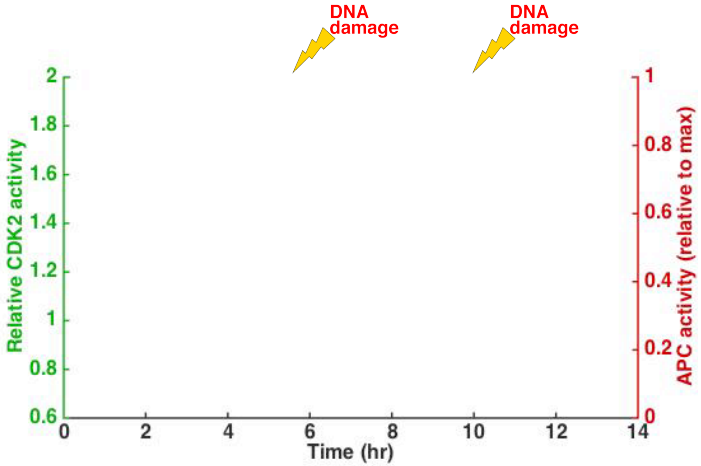
- ✓ Global Impact
- ✓ Bistable
- ✓ Irreversible
- ? ✓ Requires a strong stimulus to **turn it** on, but a small stimulus to **keep it** on

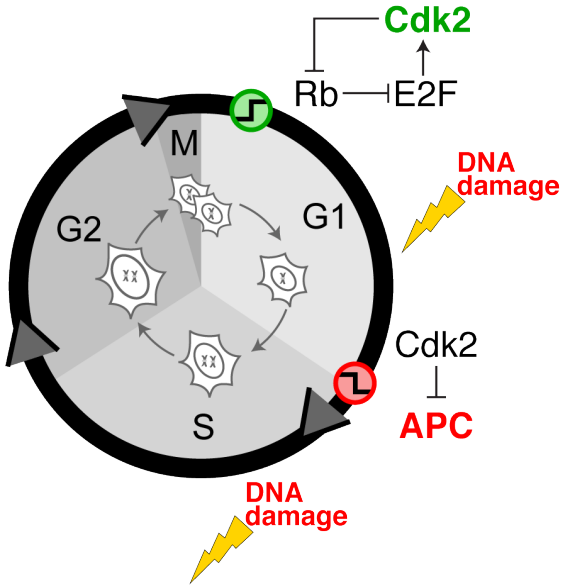




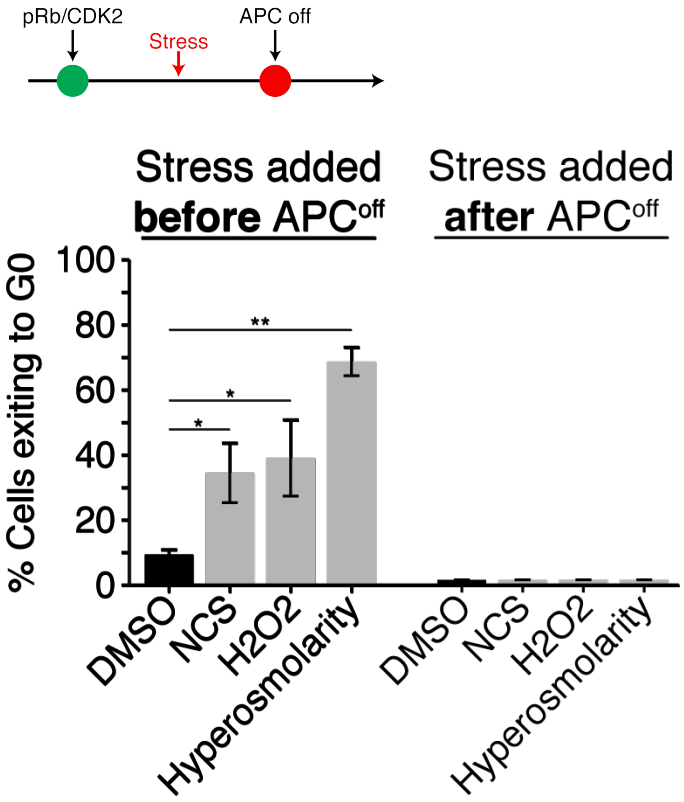
Is APC/C inactivation the Commitment Point for the cell cycle?

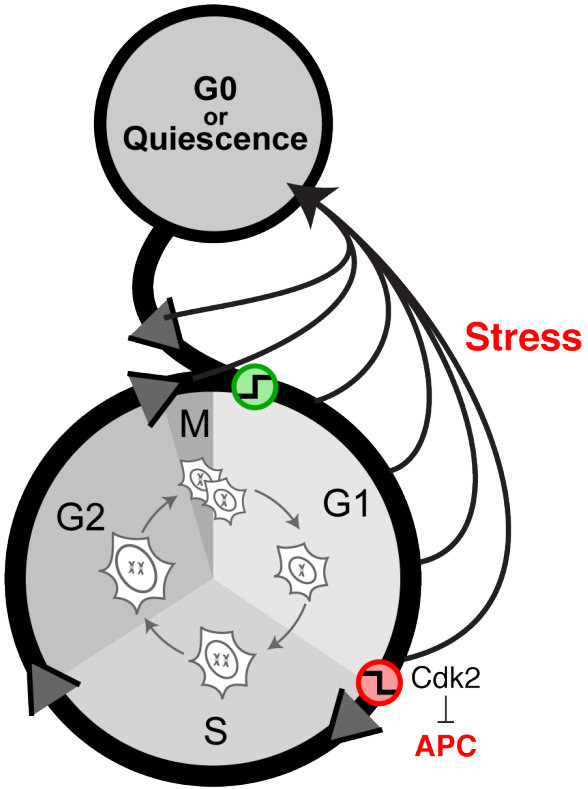






Stress sends cells back to G0 **before**, but not **after** APC/C inactivation

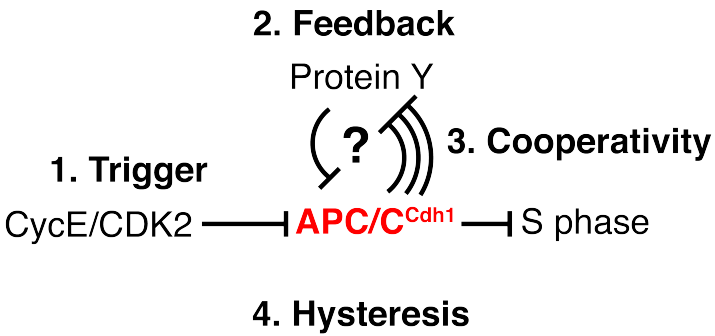


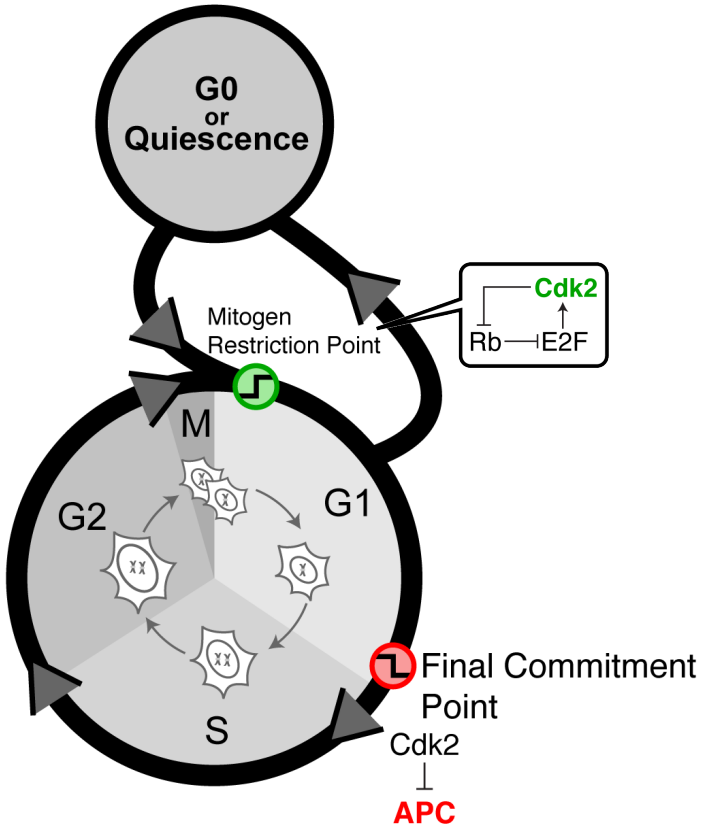


* p<0.05 **p<0.01

Cells commit to divide by inactivating the APC/C

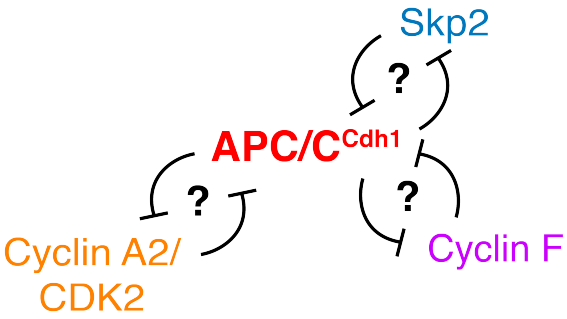
1. When do cells execute the decision to divide?
2. What protein mediates the decision to divide?
3. What is the molecular mechanism underlying the decision to divide?

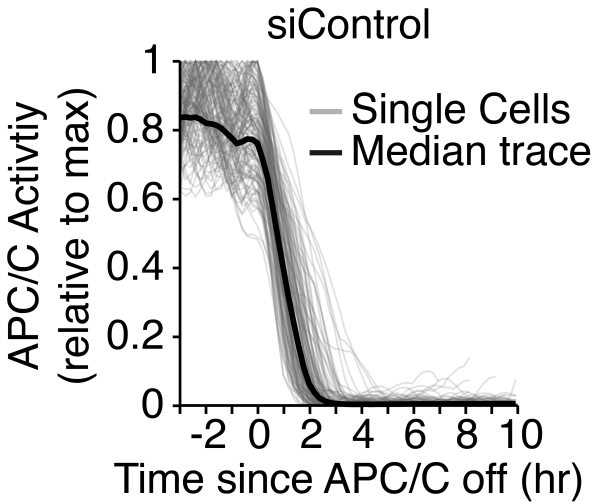


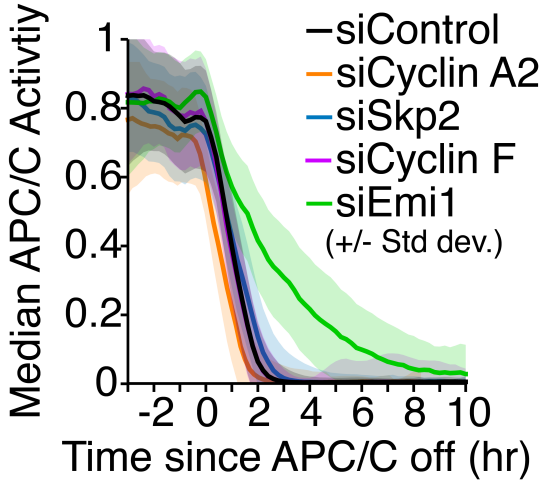


Cappell et al, Nature 2018

Bistable APC/C inactivation switch

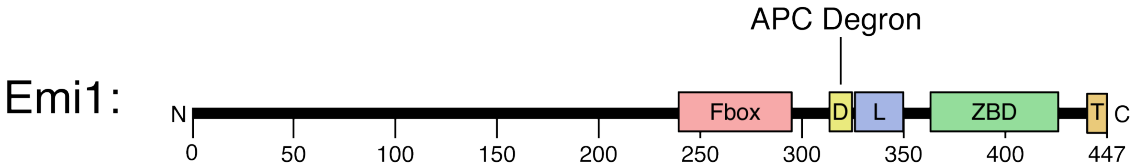




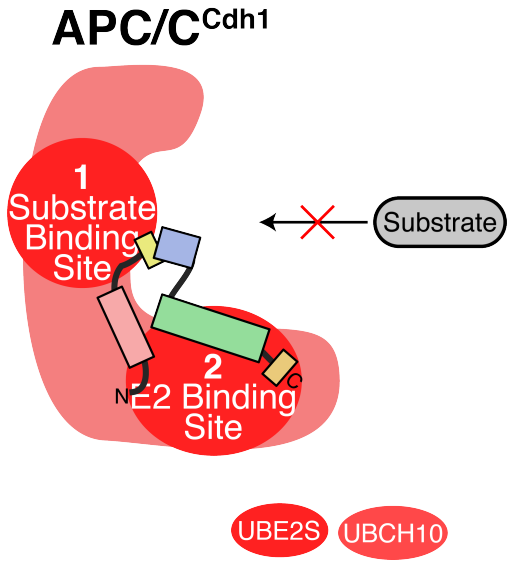


Choudhury et al., Cell Reports 2016; Fukushima et al., Cell Reports, 2013; Lukas et al, Nature 1999 Miller et al, Genes Dev 2006

Early Mitotic Inhibitor 1 (Emi1)

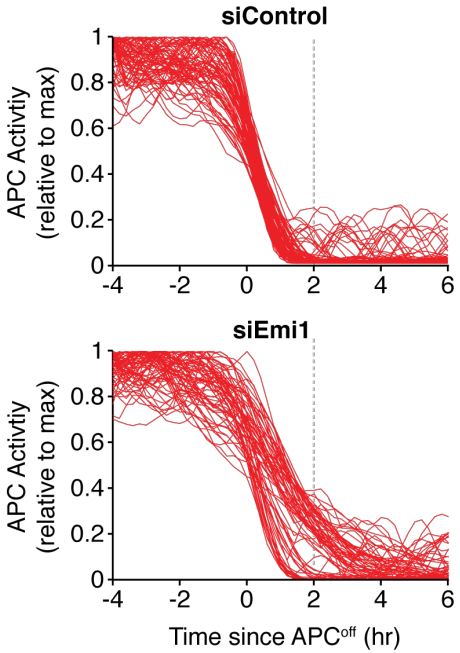


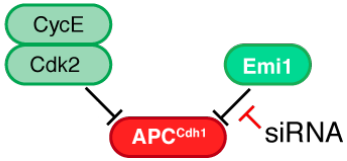
- Pseudosubstrate inhibitor of APC
- Inhibits E2 Ub conjugating enzyme
- Overexpressed in many cancers



Miller et al., 2006; Wang and Kirschner, 2013; Frye et al., 2013

Emi1 speeds up APC inactivation and makes it irreversible

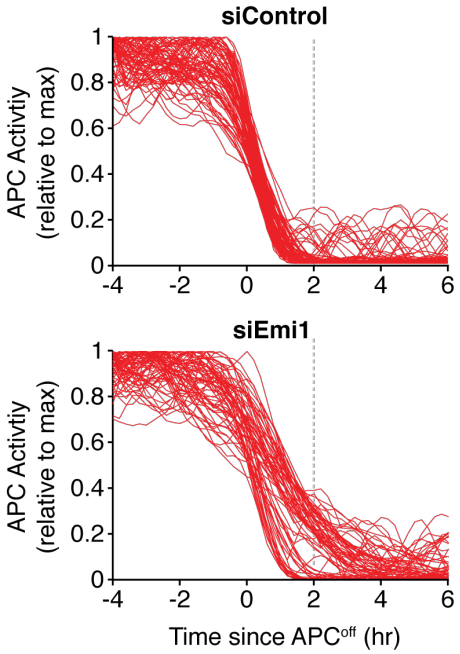


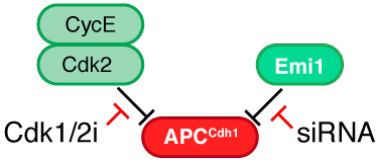


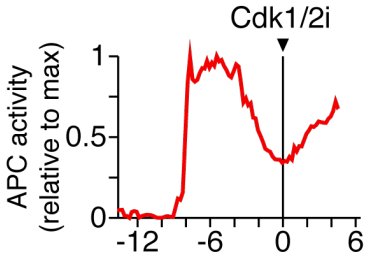
Hallmarks of Commitment:

- ✓ Global impact
- ✗ Bistable
- ✓ Irreversible
- ✓ Requires a strong stimulus to **turn it** on, but a small stimulus to **keep it** on

Emi1 speeds up APC inactivation and makes it irreversible



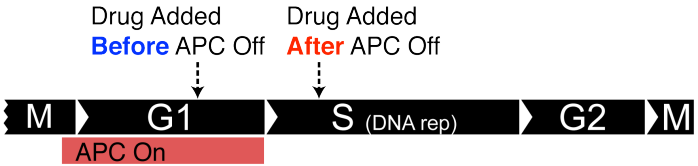




Hallmarks of Commitment:

- ✓ Global impact
- X Bistable
- X Irreversible
- ✓ Requires a strong stimulus to **turn it** on, but a small stimulus to **keep it** on

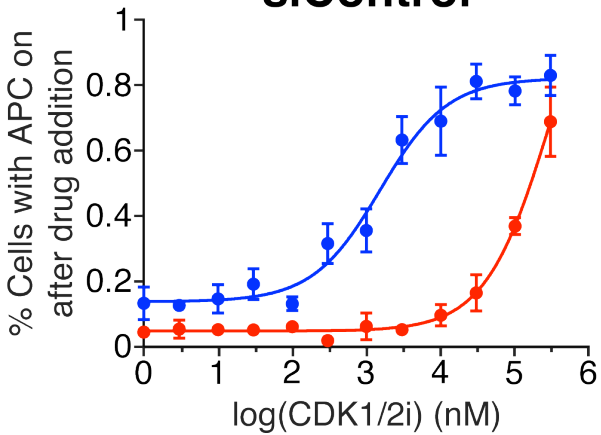
Emi1 conveys hysteresis to APC inactivation



Hallmarks of Commitment:

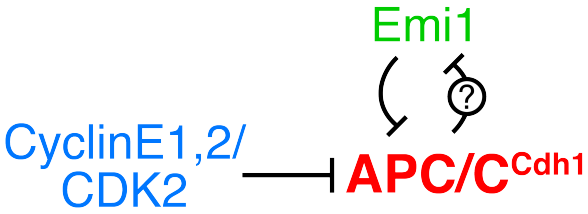
- ✓ Global impact
- X Bistable
- X Irreversible
- X Requires a strong stimulus to **turn it** on, but a small stimulus to **keep it** on

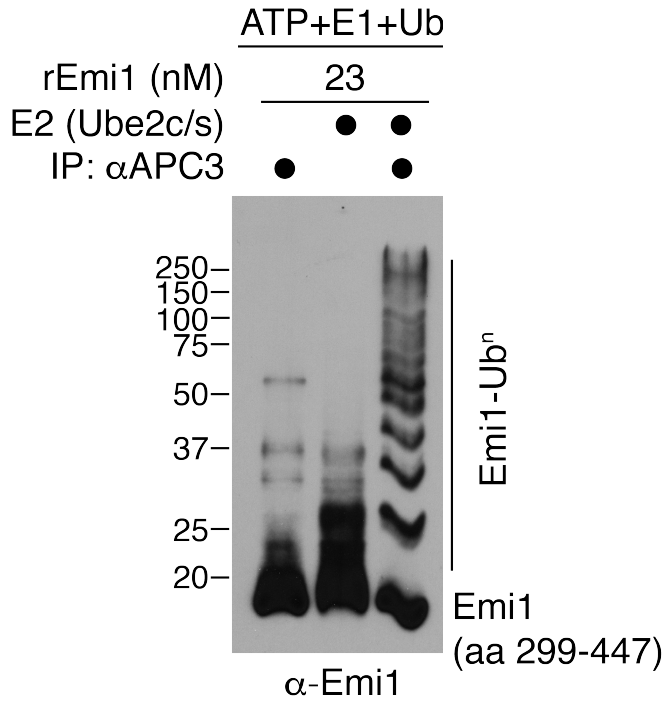
siControl



siEmi1

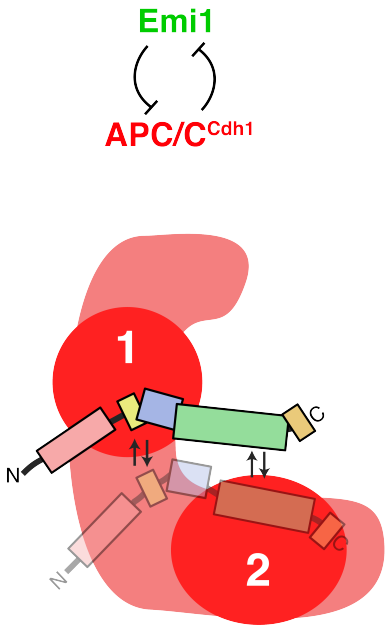
Is Emi1 a substrate of APC/C?

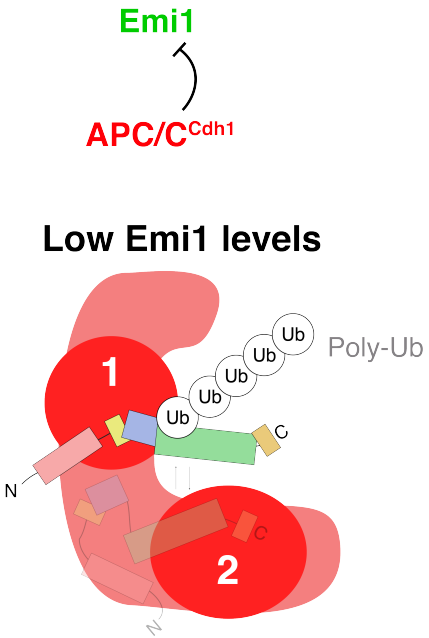


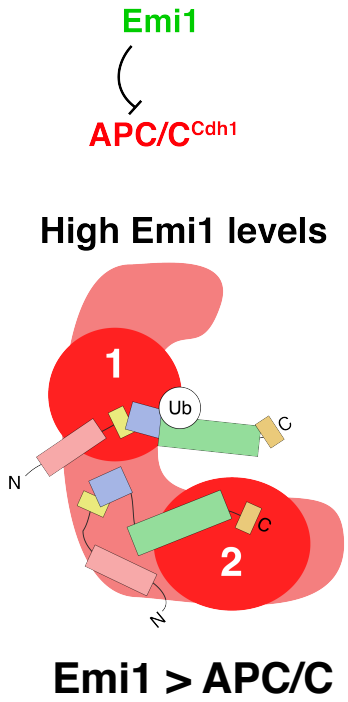


Collaboration with Kevin Mark and Michael Rape

How can Emi1 be both a substrate and an inhibitor of APC/C?



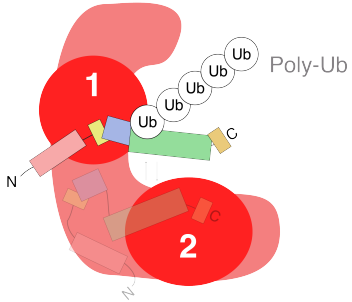




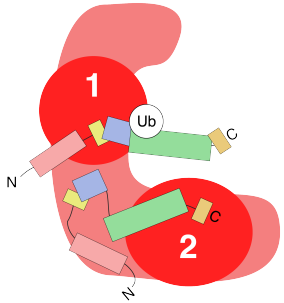
Miller et al., 2006; Wang and Kirschner, 2013; Frye et al., 2013

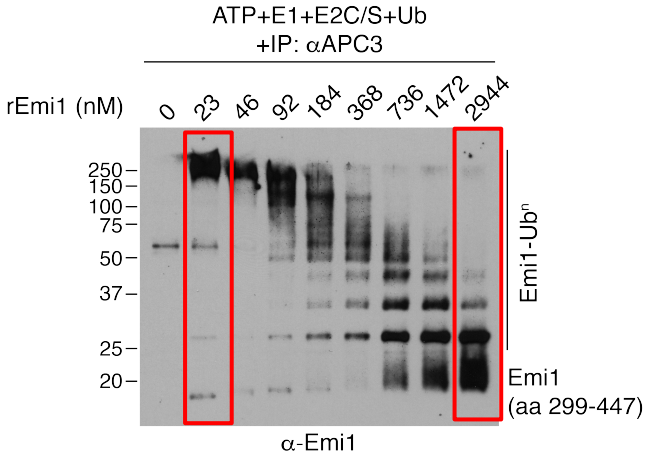
Emi1 switches from being a substrate to an inhibitor to switch APC/C off

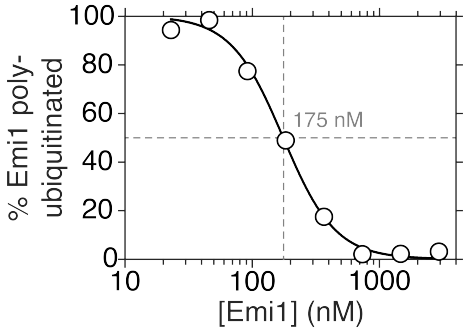
Low Emi1 levels

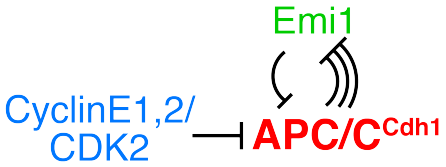


High Emi1 levels





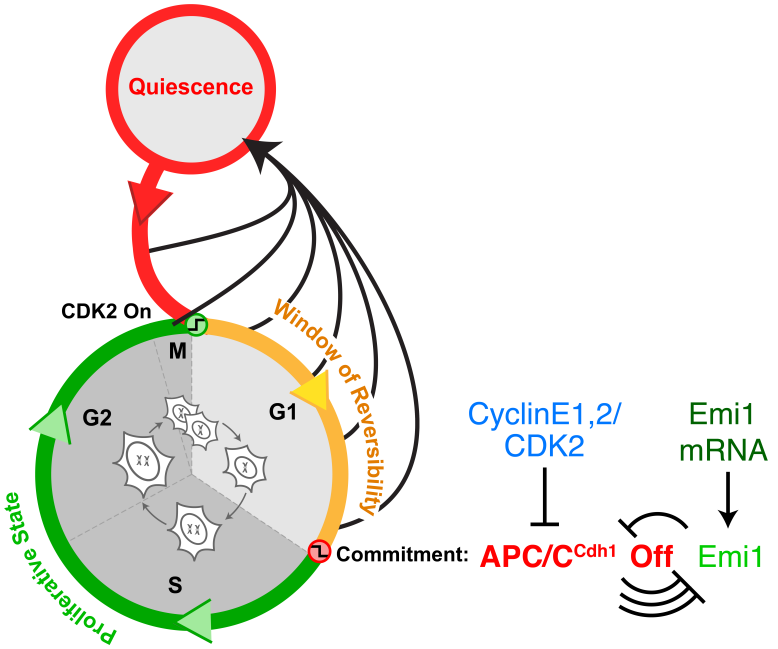




Collaboration with Kevin Mark and Michael Rape

Summary

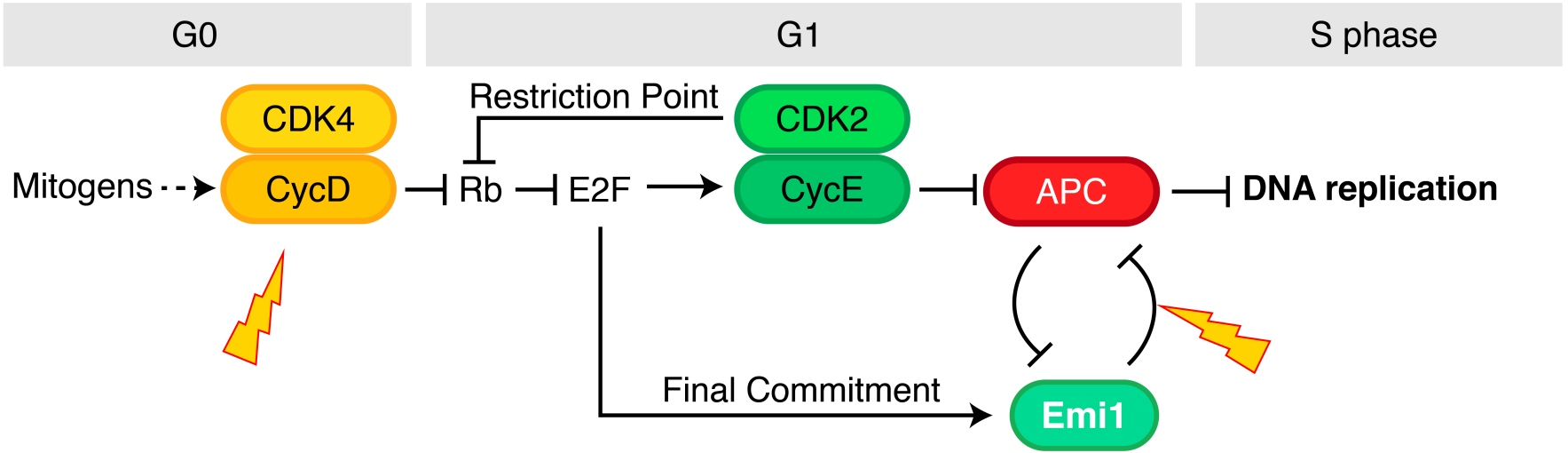
- Stress can trigger an exit back to quiescence any time during G1
- Developed a live-cell biosensor for APC/C
- Single-cell imaging shows APC/C inactivation is:
  - Bistable
  - Irreversible
  - Hysteresis
- APC/C inactivation mediates cell cycle commitment
- Cyclin E/CDK2 triggers APC/C inactivation
- Emi1 switches from being a substrate to an inhibitor of APC/C



Cappell, et al. Cell 2016

Cappell, et al. Nature 2018

Summary


G0
G1
S phase
CDK4
Restriction Point
CDK2
Mitogens
CycD
Rb
E2F
CycE
APC
DNA replication
Final Commitment
Emi1

Thank You

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James Ferrell (Stanford)
Marcus Covert (Stanford)
Sergi Regot (Johns Hopkins)
Sabrina Spencer (U of Colorado)
Sean Collins (UC Davis)

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Marwa Afifi, BDS, PhD
Jenny Nathans, Postbac

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