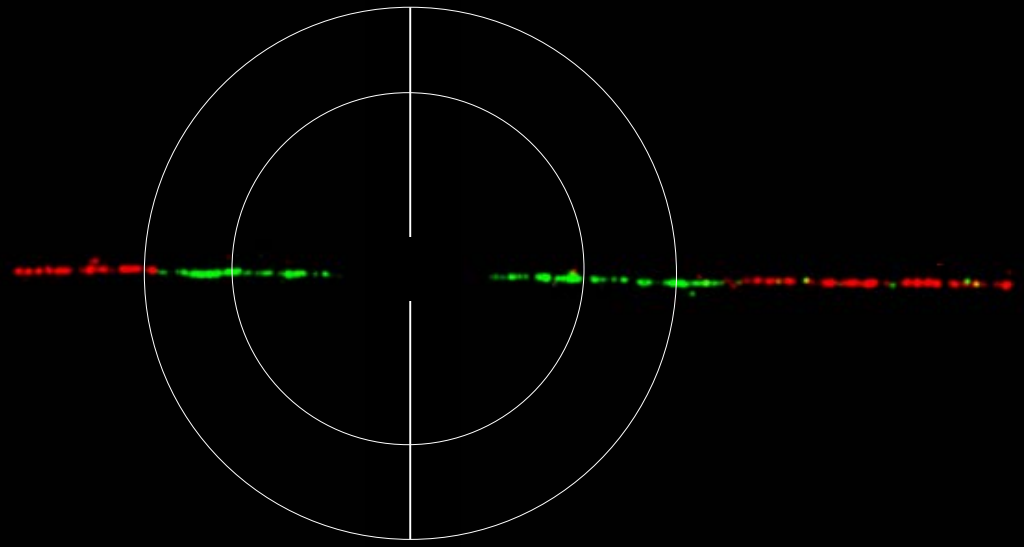
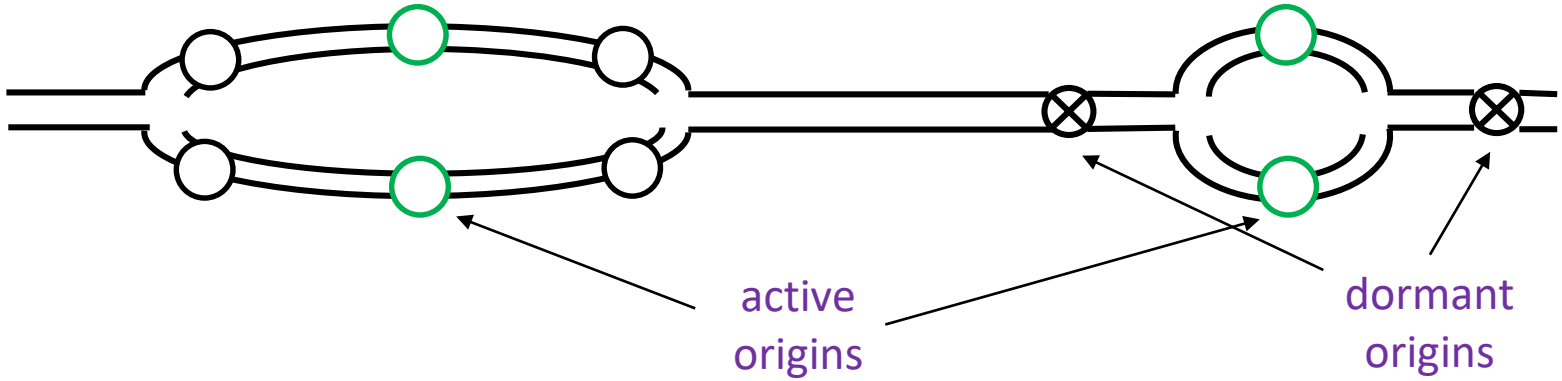
ATR/Chk1 axis controls replication origin firing



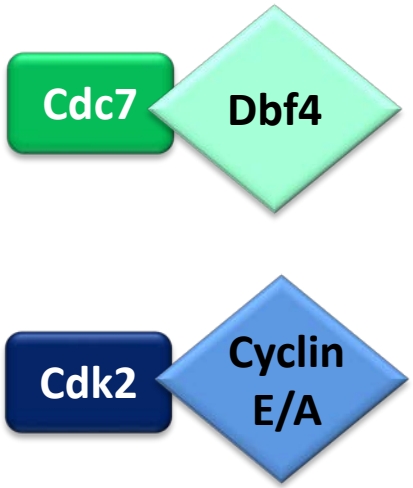
Tatiana Moiseeva
Postdoctoral associate
Chris Bakkenist lab @ University of Pittsburgh

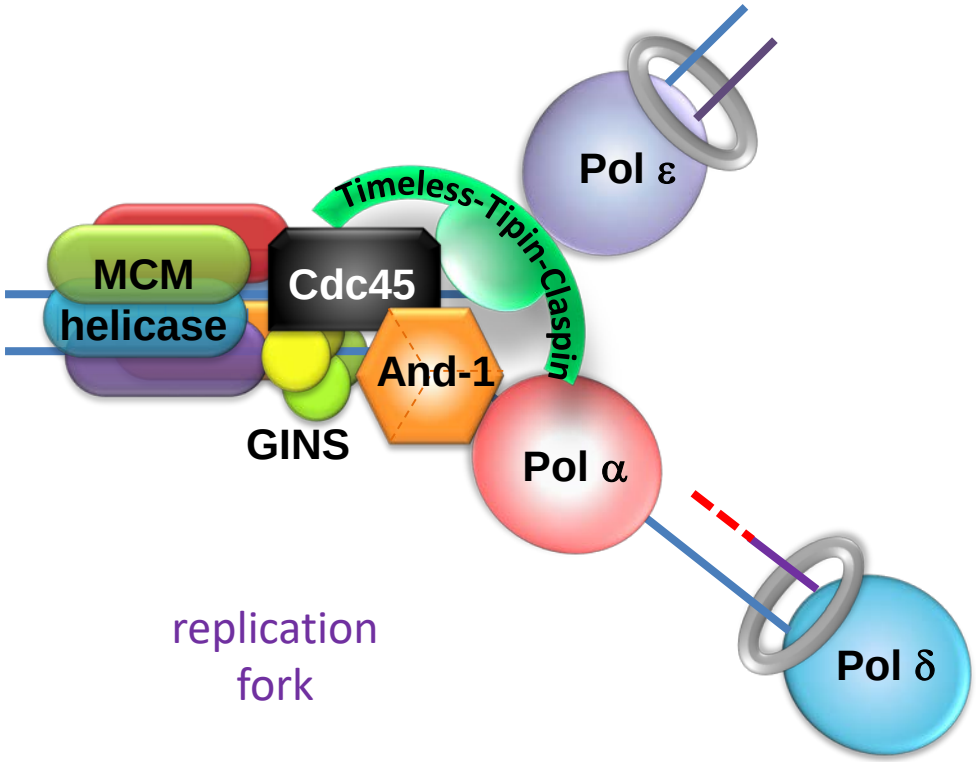
DNA repair interest group videoconference
April 24th 2018

DNA replication initiation



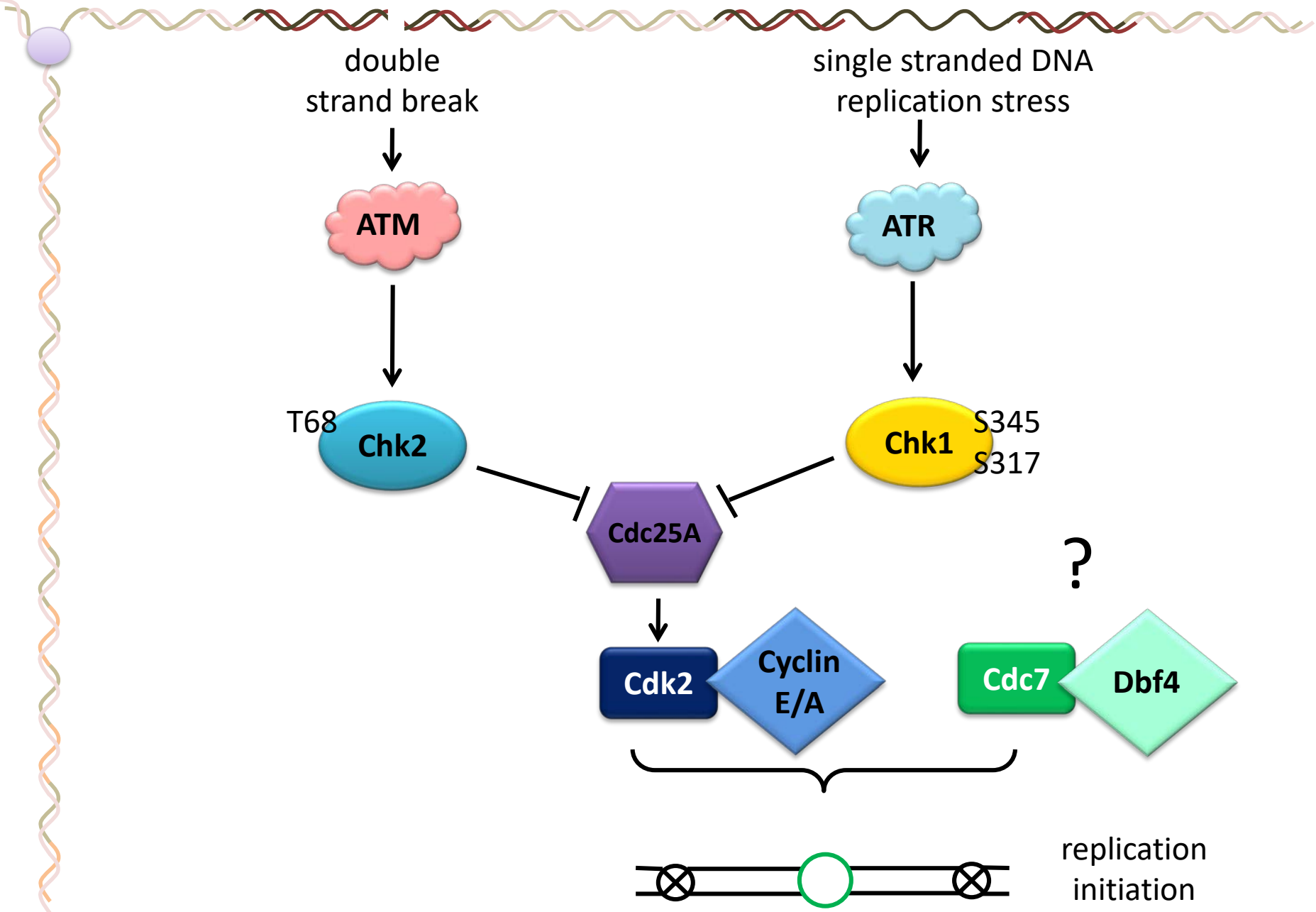
kinases responsible for replication initiation



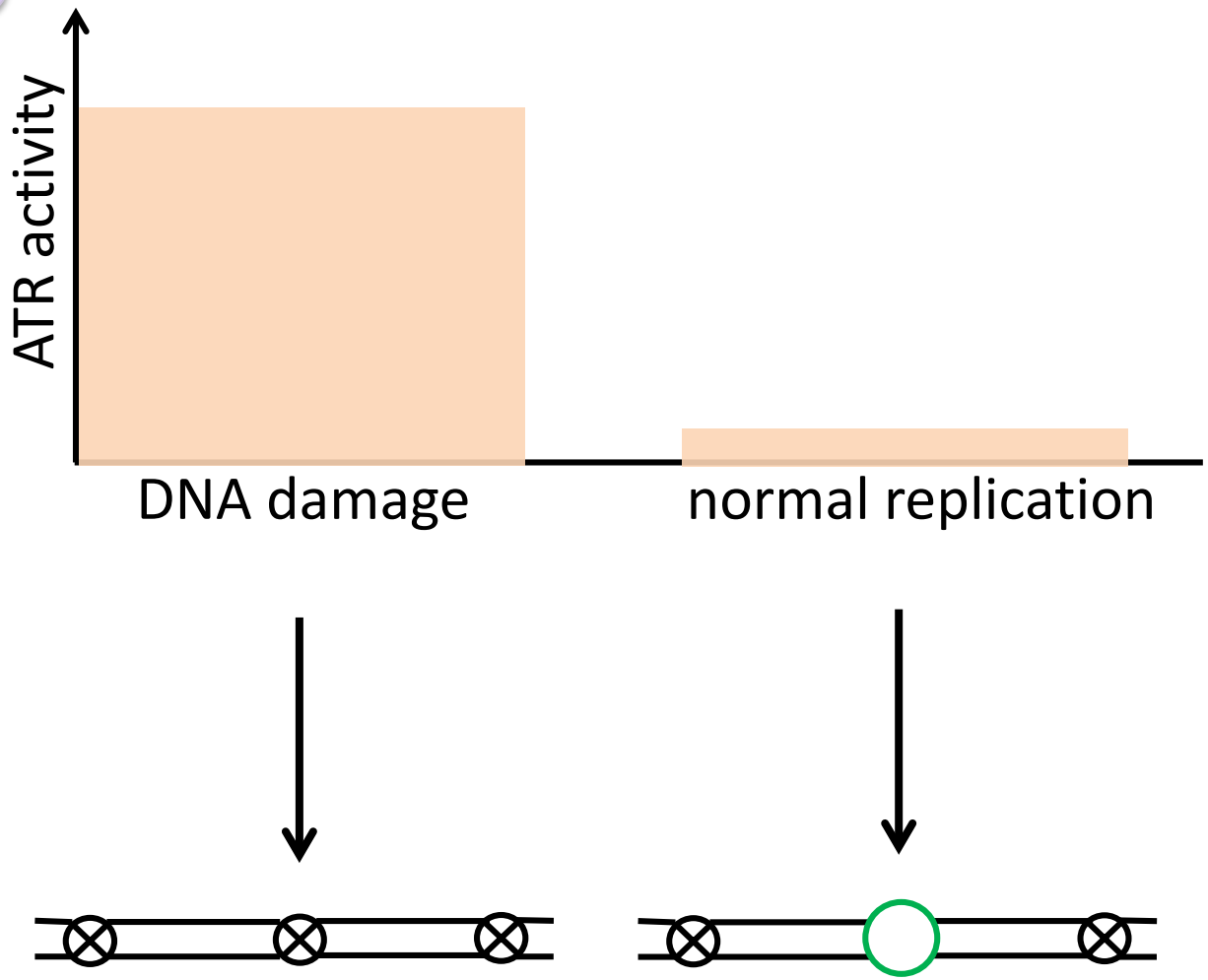


replication fork

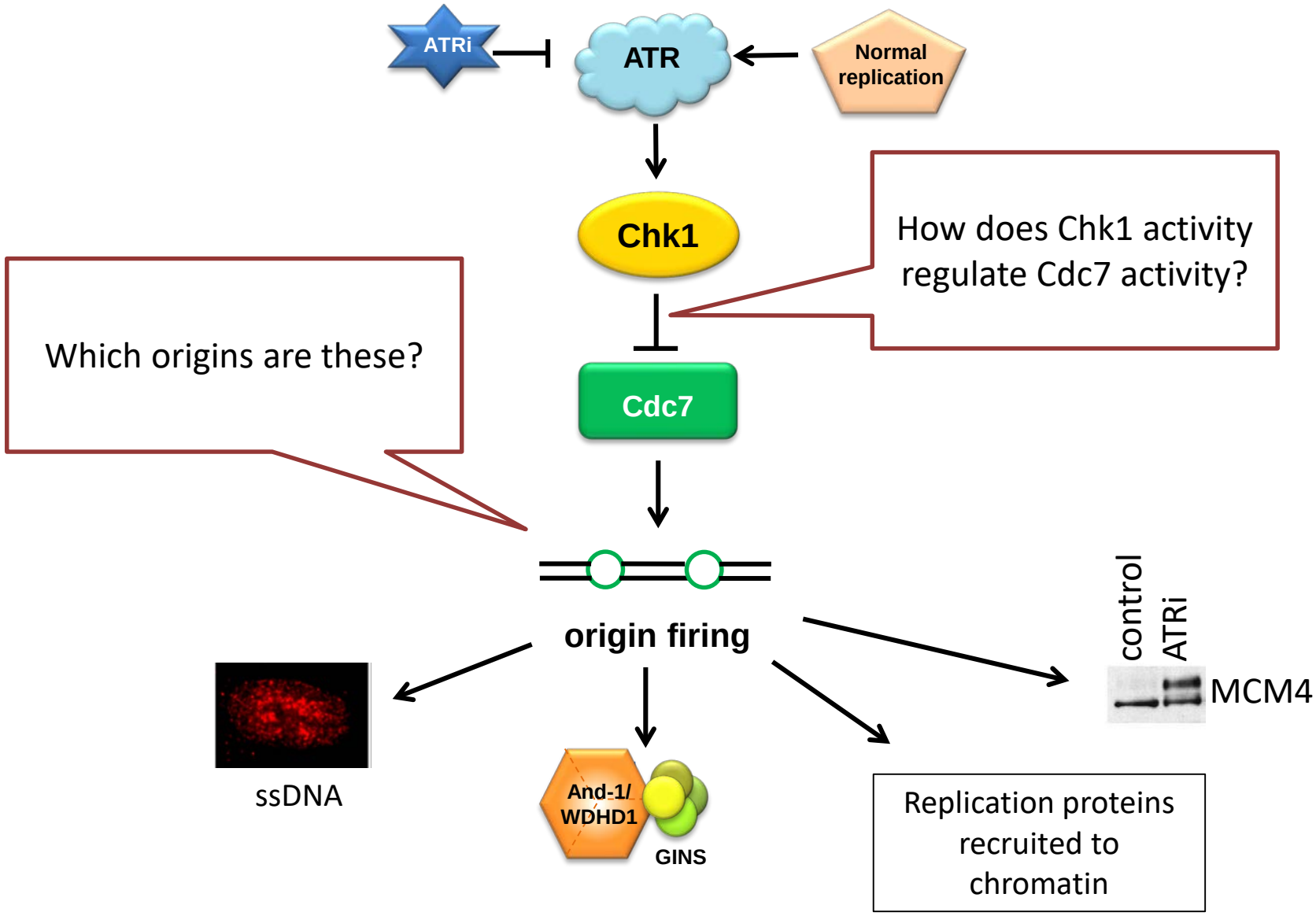
ATM/ATR-dependent replication checkpoint



ATR activity and replication



Model



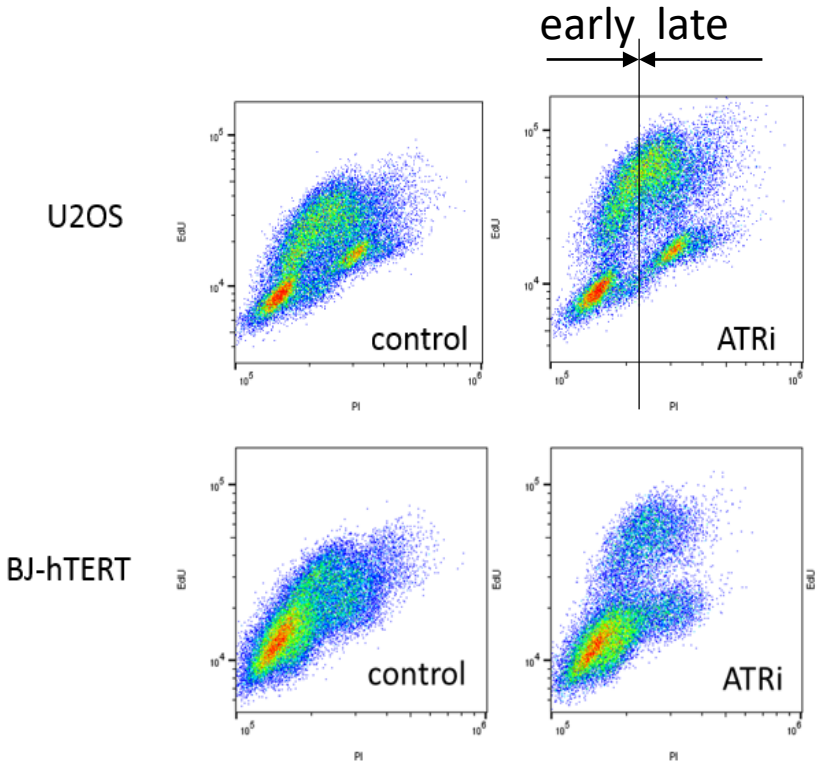
Moiseeva et al., Nature Communications, 2017

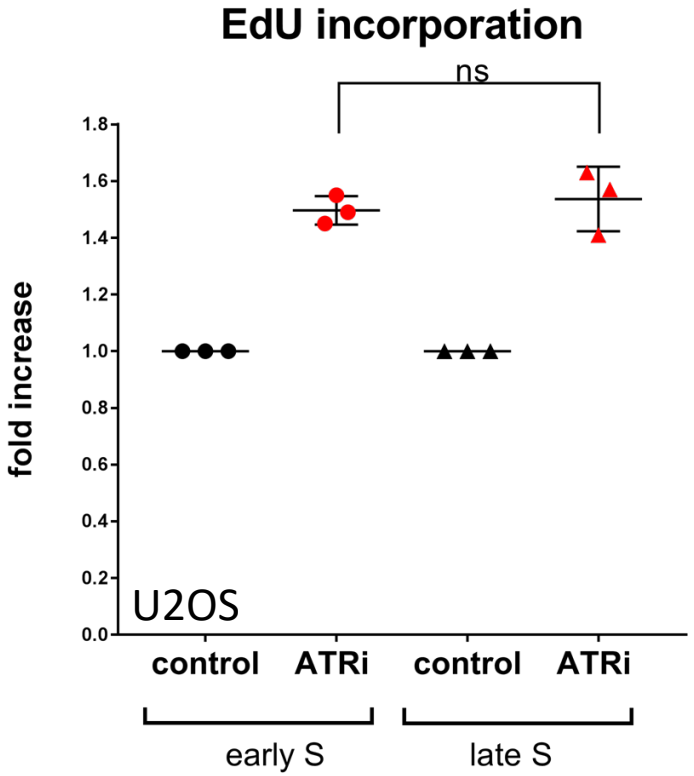
Which origins fire in response to ATR inhibition?

- Are ATRi effects specific to early or late origins?

- Is ATRi affecting the replication timing program (late origins would fire in early S-phase)?

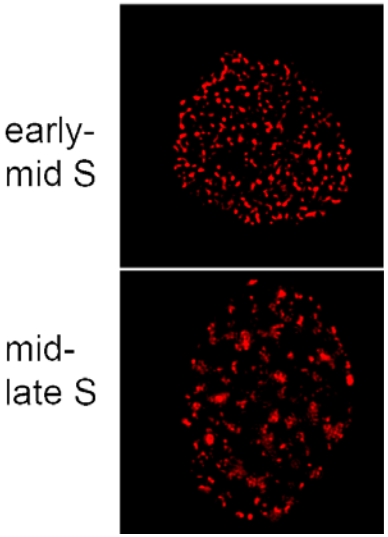
ATR inhibition → increased EdU incorporation throughout the S-phase



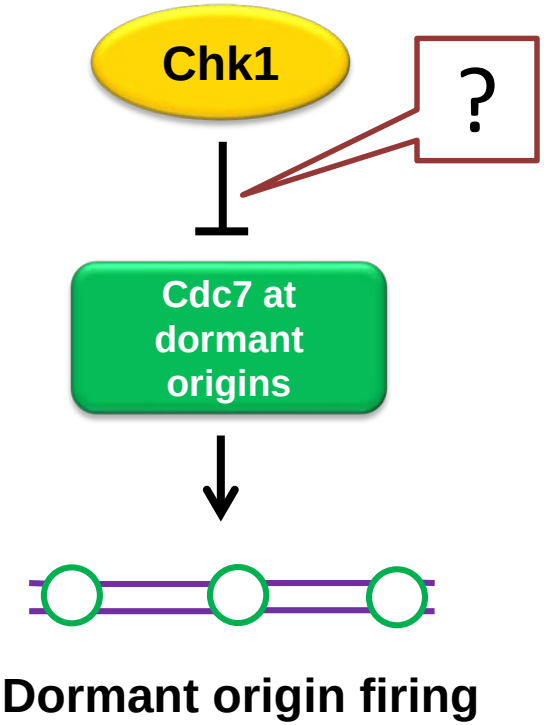


ATRi equally affects early and late S-phase cells

ATR inhibition → origins firing in the vicinity of ongoing replication

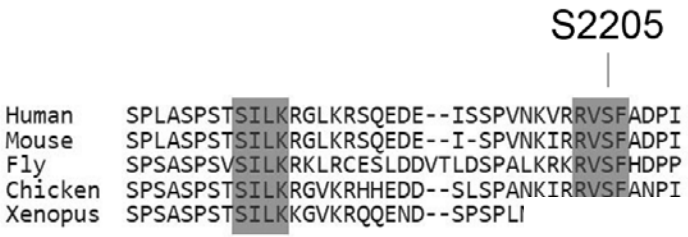


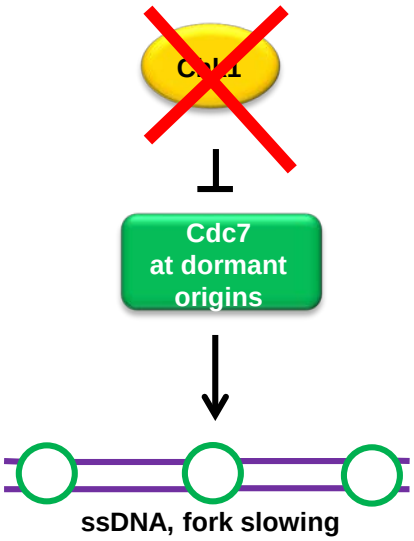
How does ATR/Chk1 activity prevent Cdc7-dependent origin firing?

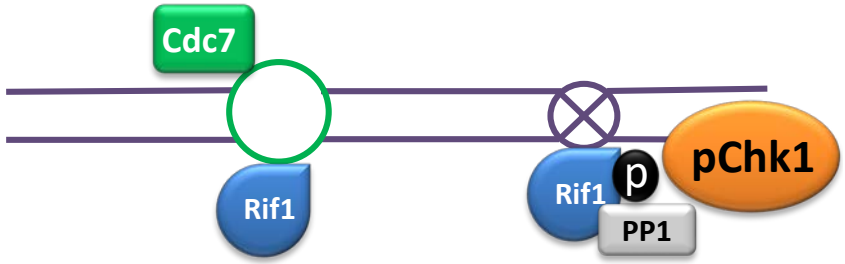


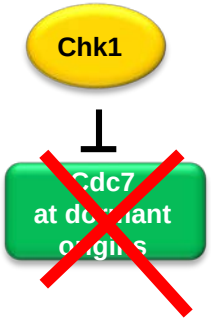
Model: Rif1-PP1 interaction is stabilized by phosphorylation

Rif1
(PP1-interacting motif)





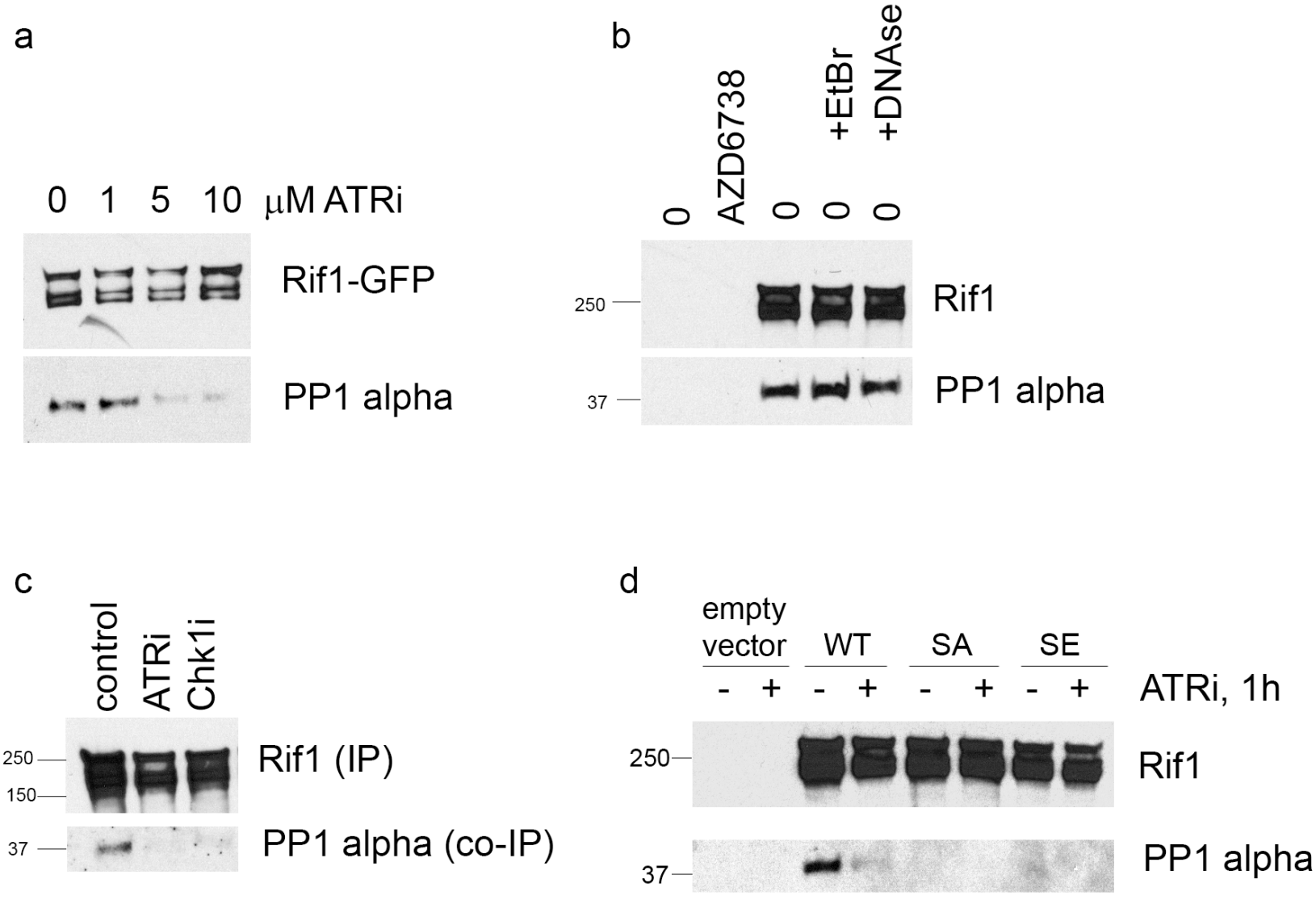




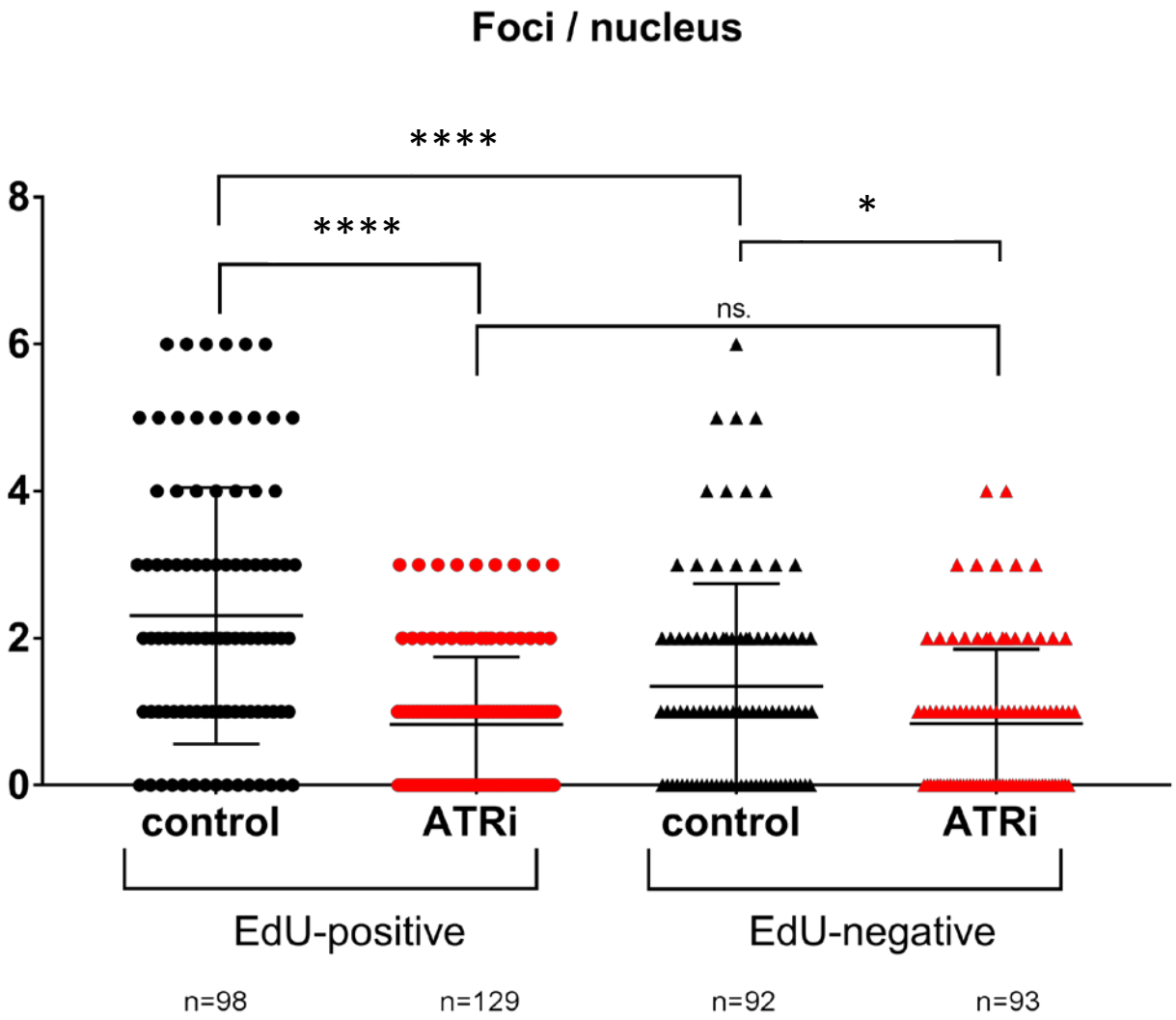
Hiraga et al., 2017:

Disruption of Rif1/PP1 interaction causes increased origin firing, fork stalling, MCM4 phosphorylation on chromatin.

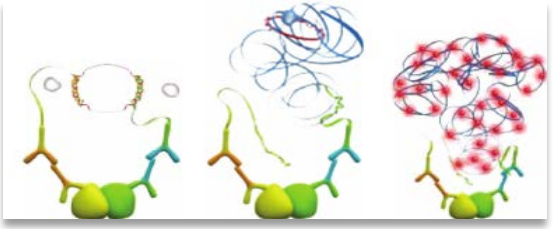
Rif1-PP1 interaction is blocked by ATR/Chk1 inhibition

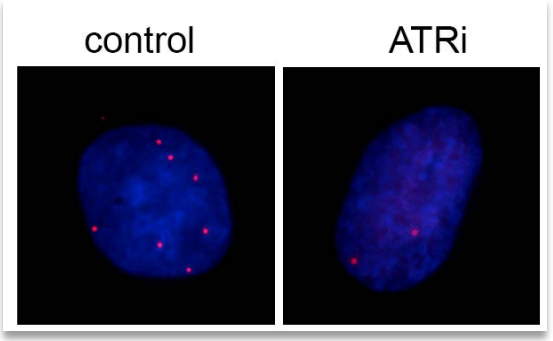


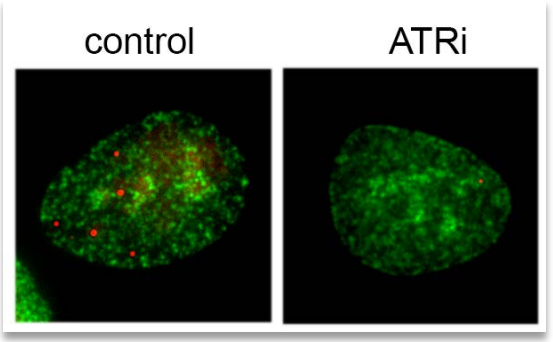
Rif1-PP1 interaction is blocked by ATR/Chk1 inhibition



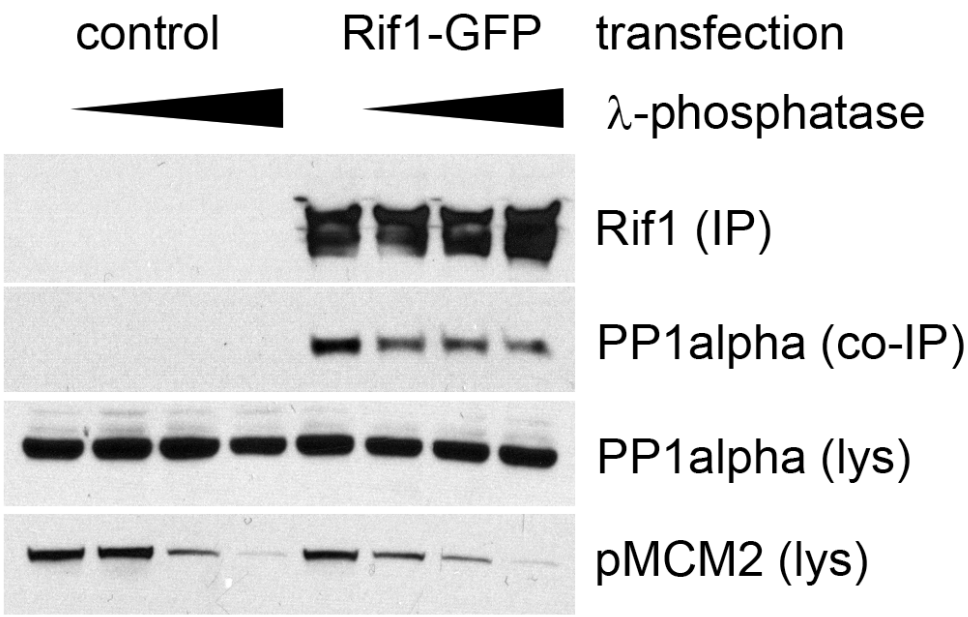
Proximity Ligation Assay



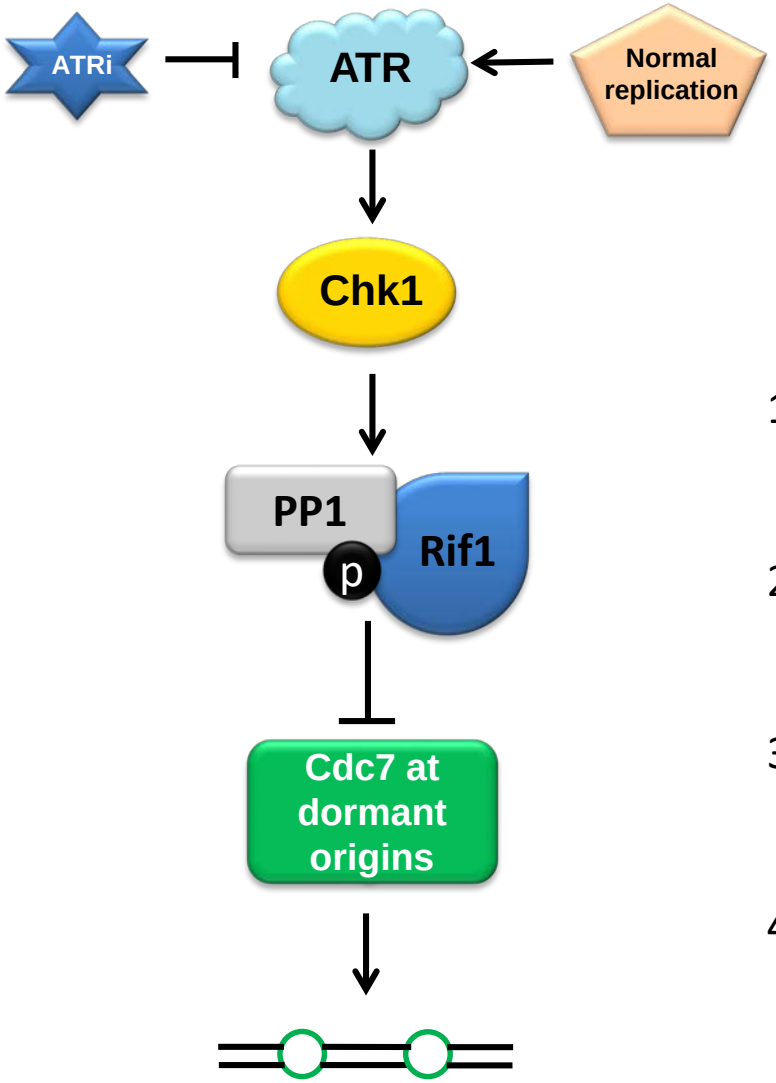




Rif1-PP1 interaction is inhibited by λ–phosphatase treatment



Conclusions:



local dormant origin firing

ATR/Chk1 activity stops dormant origins from firing by localizing PP1 phosphatase to Rif1 protein to reverse Cdc7-dependent phosphorylations

1. ATR/Chk1 activity blocks dormant origin firing throughout S-phase.
2. ATR inhibition does not affect replication timing program.
3. Rif1 interaction with PP1 phosphatase is blocked by ATRi or Chk1i.
4. Rif1 interaction with PP1 phosphatase is phosphorylation dependent.
5. Rif1 interaction with PP1 phosphatase is blocked by S2205 mutations.

Acknowledgements

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Chris Bakkenist, PhD
Sandy Schamus for all the mutations and constructs
all Bakkenist lab members and neighbors

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Brian Hood, PhD
} mass-spectrometry

THANK YOU!