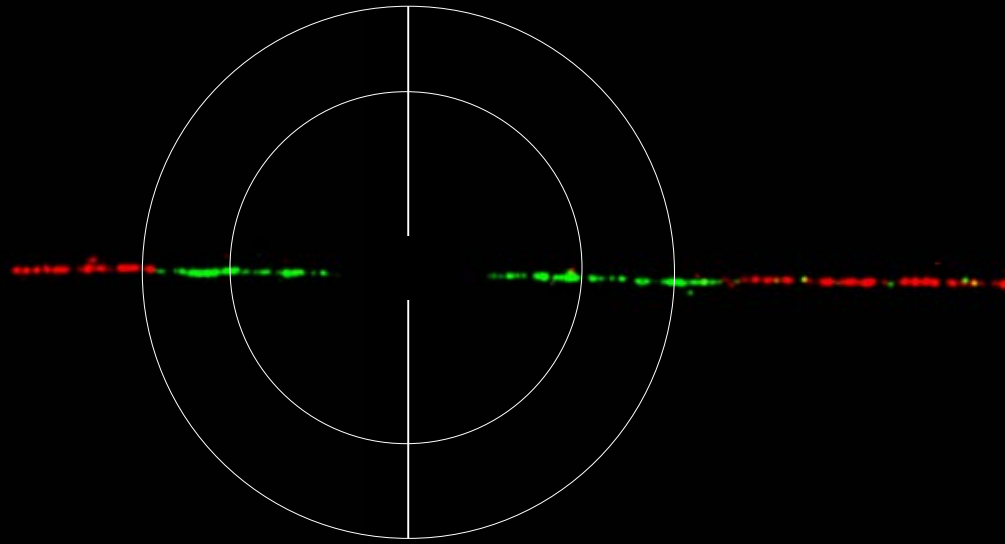


ATR/Chk1 axis controls replication origin firing



Tatiana Moiseeva

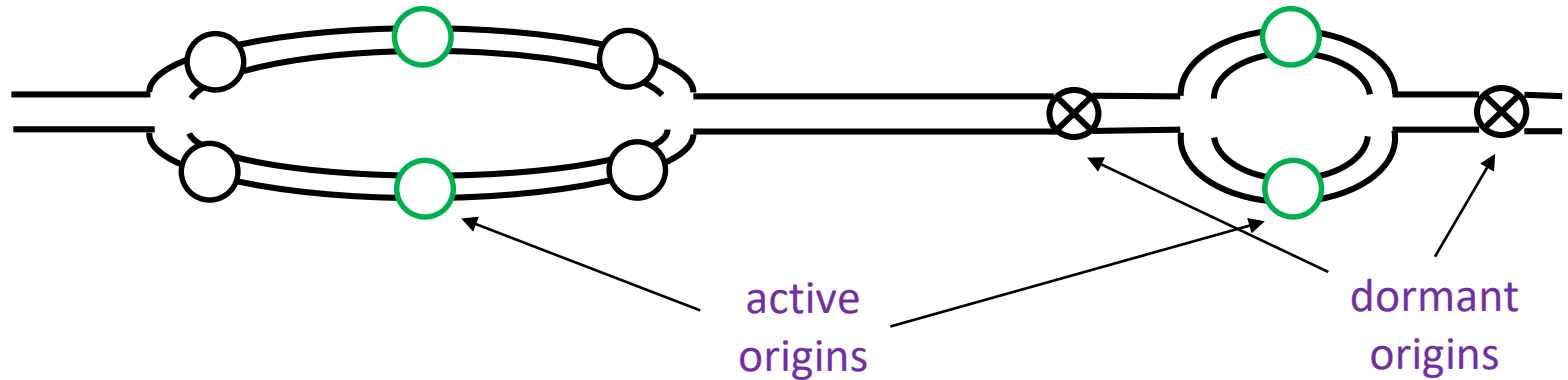
Postdoctoral associate

Chris Bakkenist lab @ University of Pittsburgh

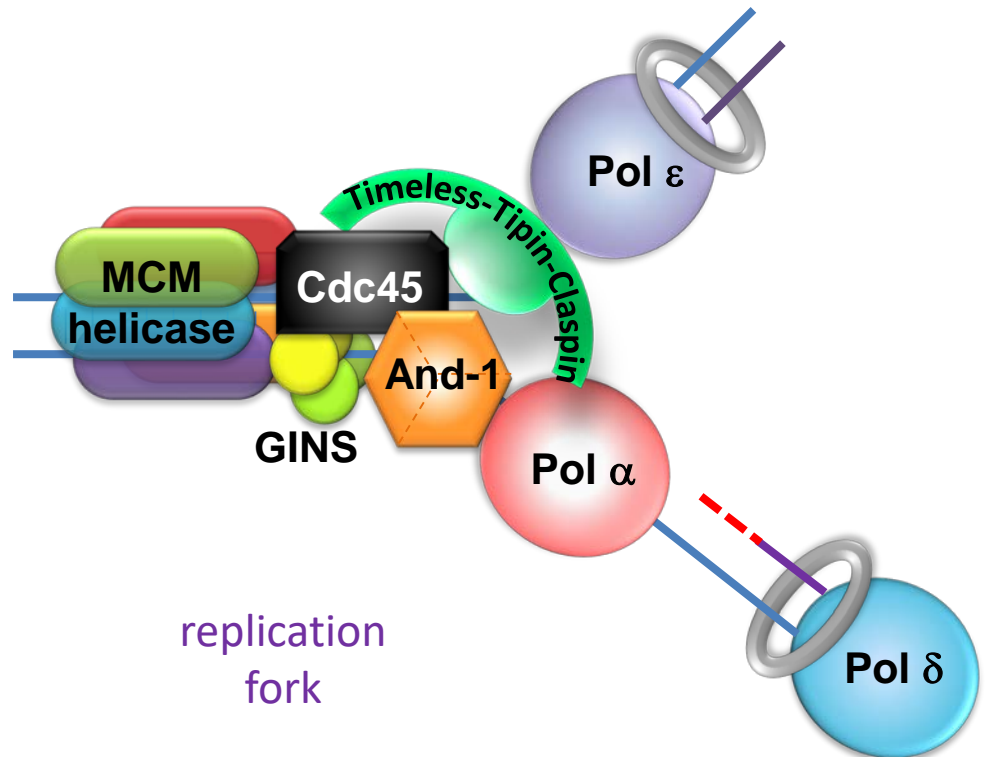
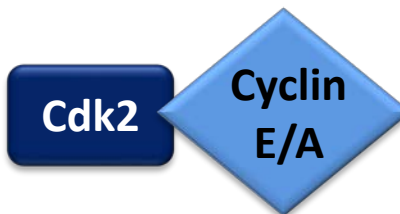
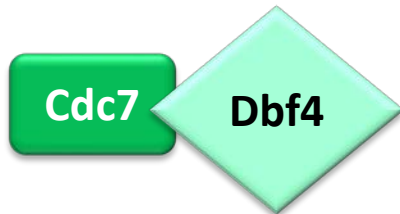
DNA repair interest group videoconference

April 24th 2018

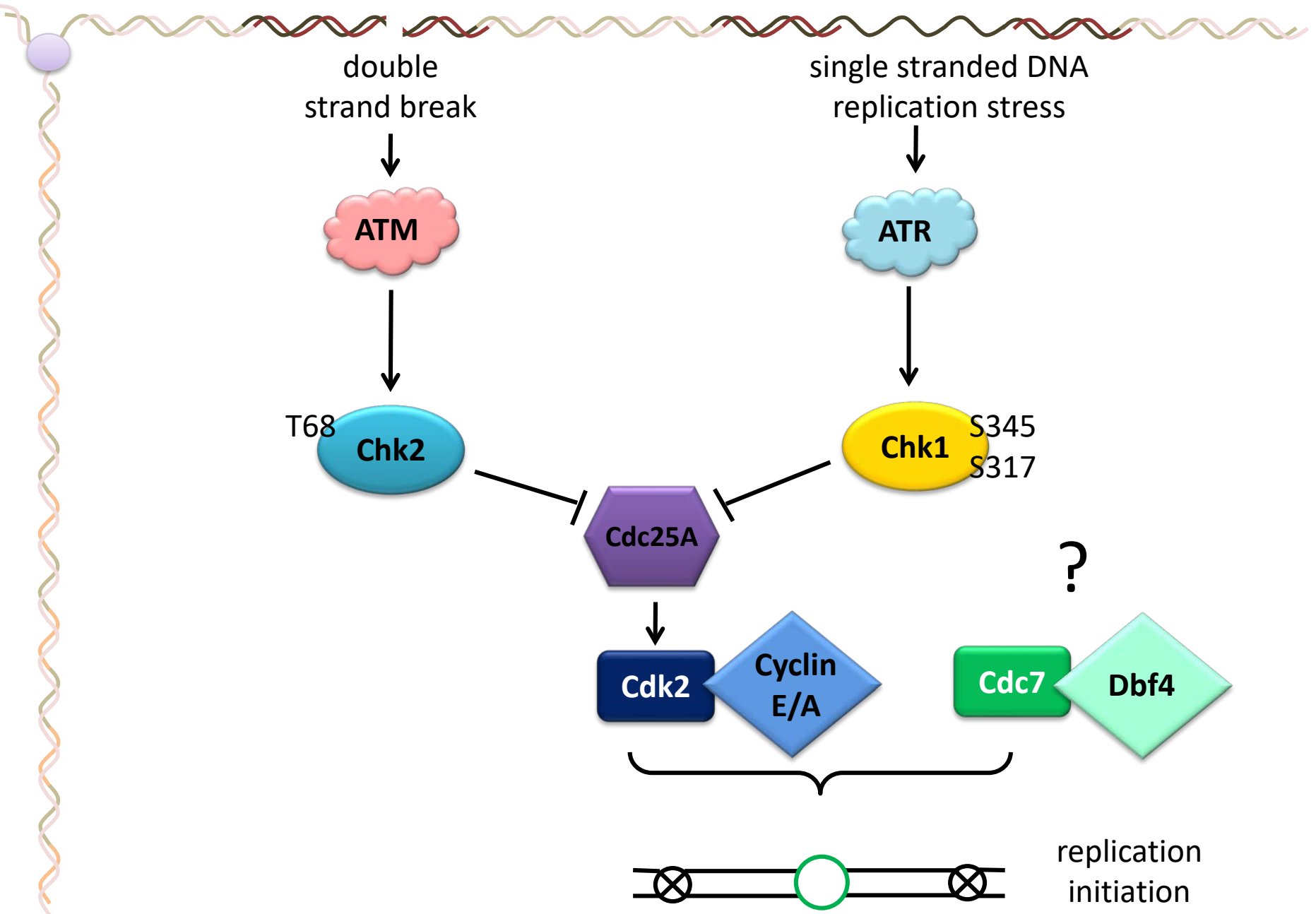
DNA replication initiation



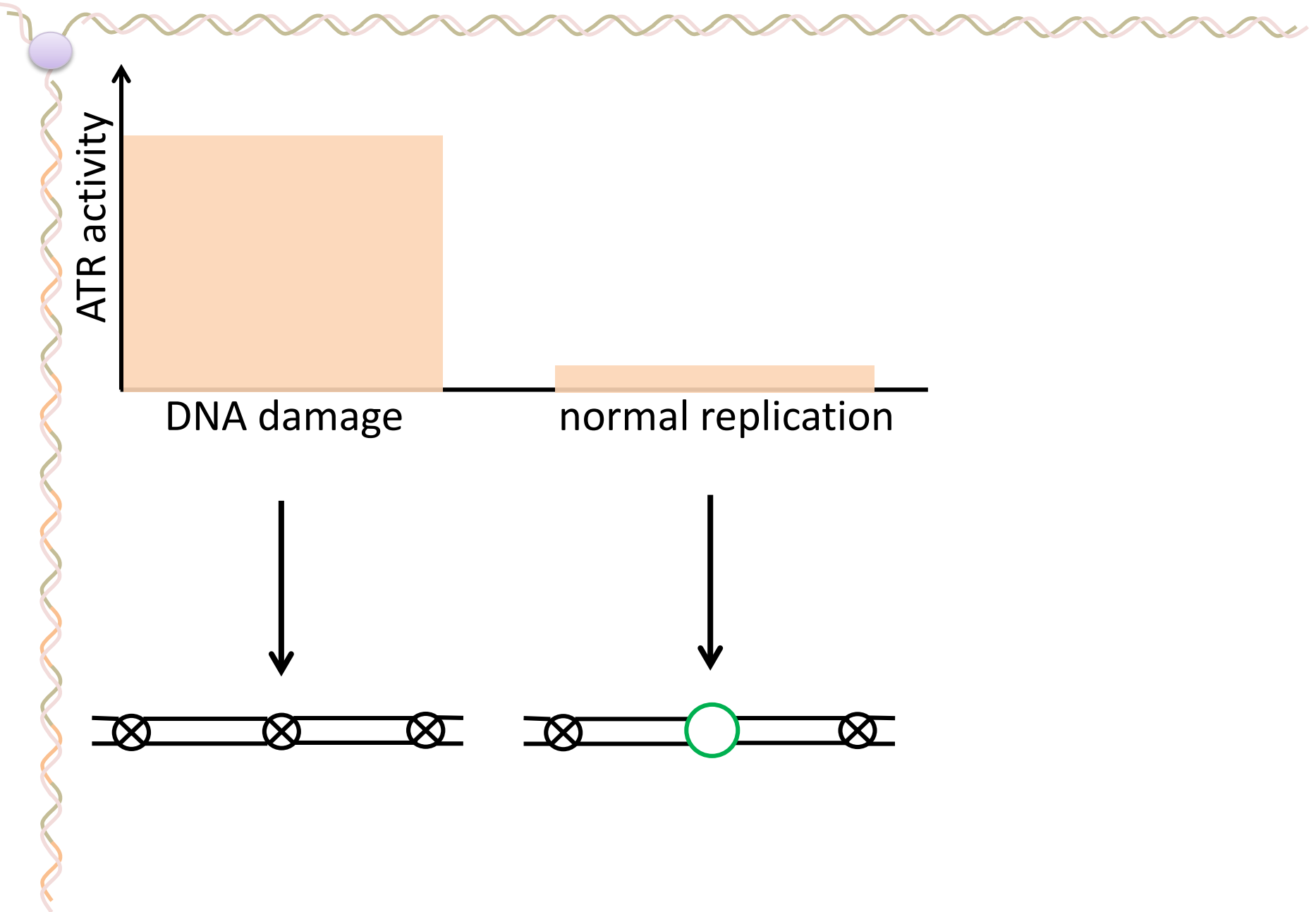
kinases responsible
for replication
initiation



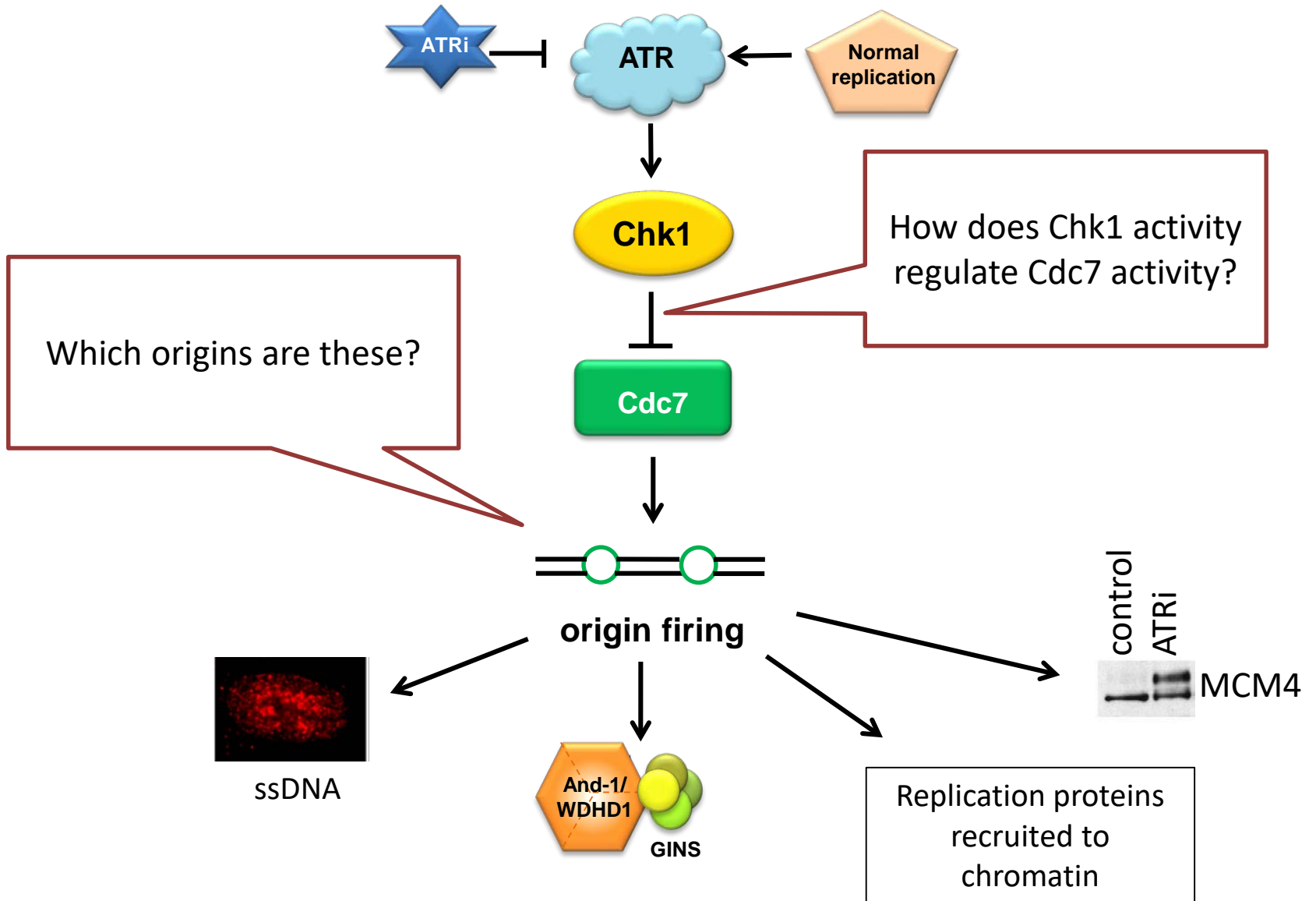
ATM/ATR-dependent replication checkpoint



ATR activity and replication



Model

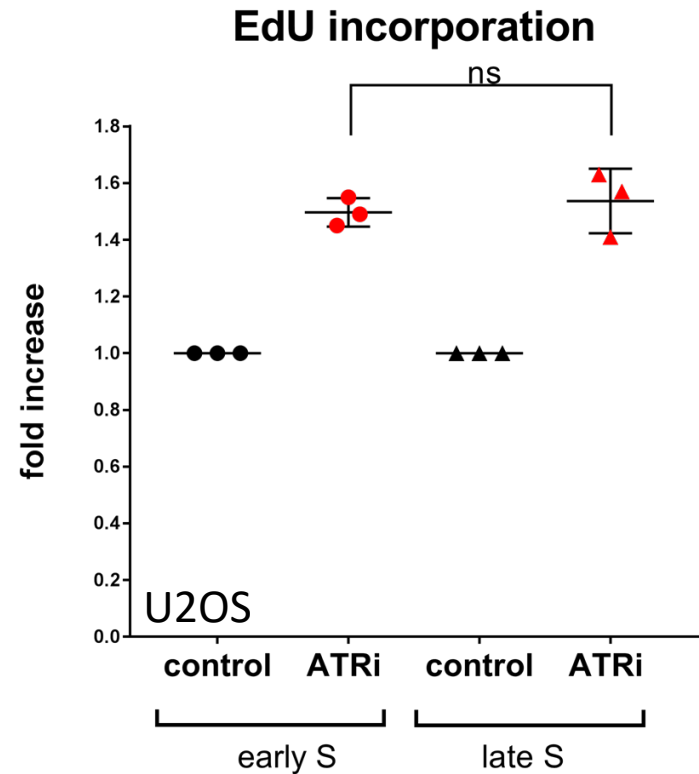
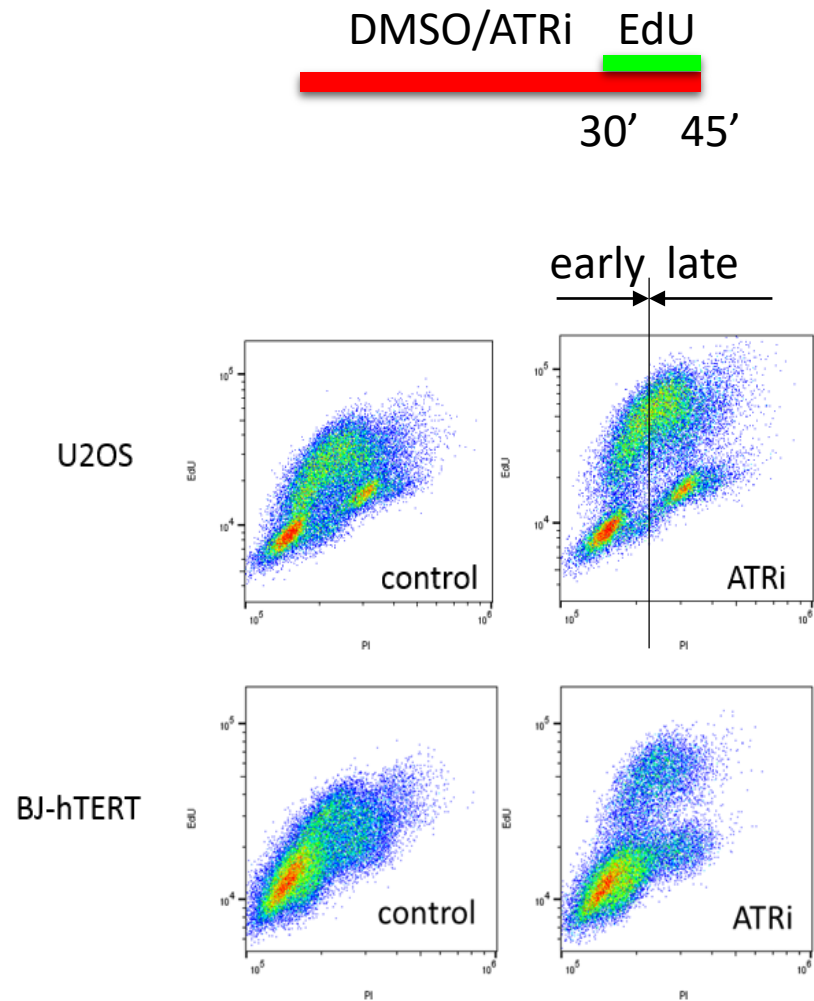


A decorative border consisting of a DNA double helix runs along the top and left edges of the slide. The helix is composed of two strands, one colored pink and the other green, intertwined. A purple sphere is attached to the left strand near the top left corner.

Which origins fire in response to ATR inhibition?

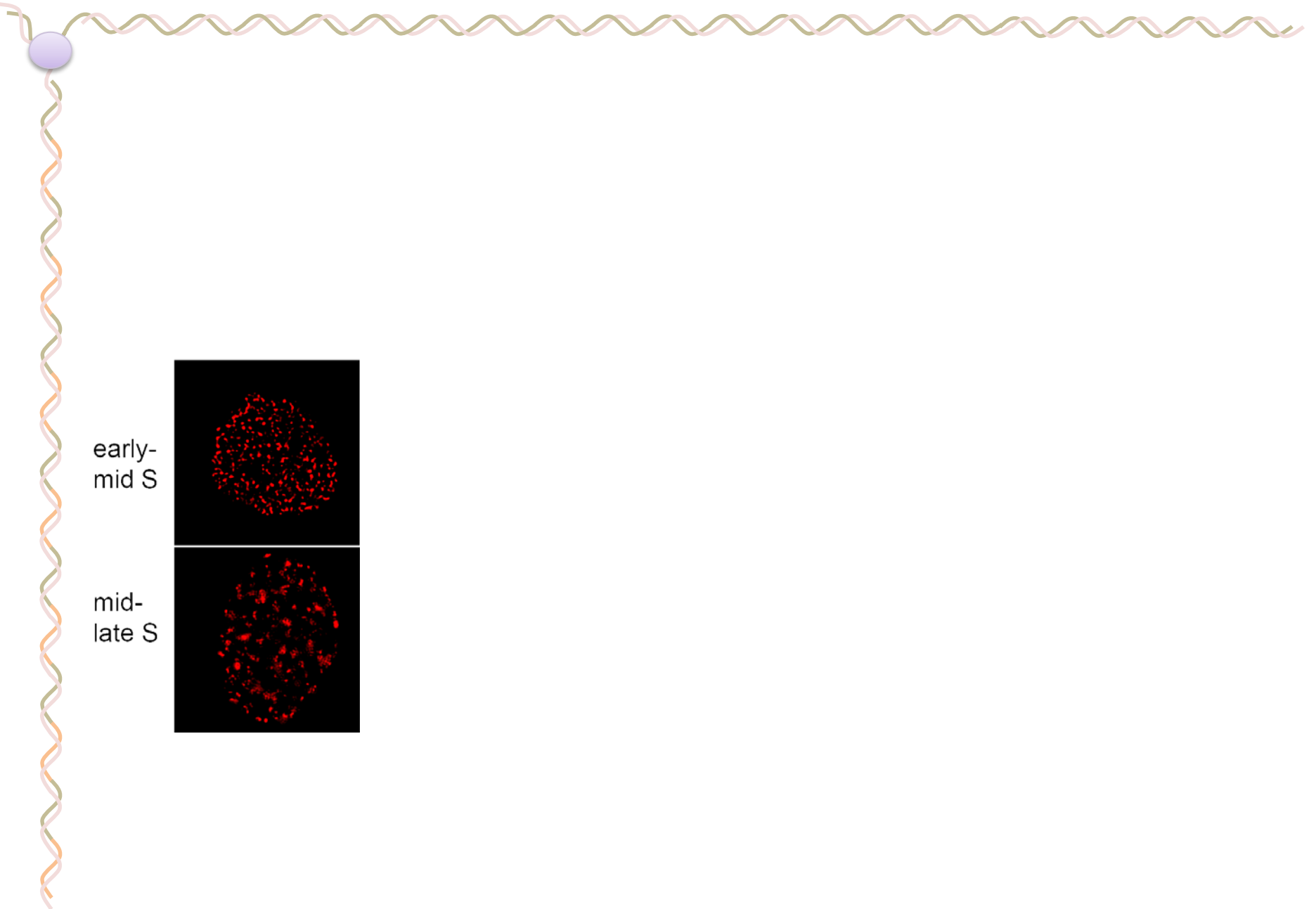
- Are ATRi effects specific to early or late origins?
- Is ATRi affecting the replication timing program (late origins would fire in early S-phase)?

ATR inhibition → increased EdU incorporation throughout the S-phase

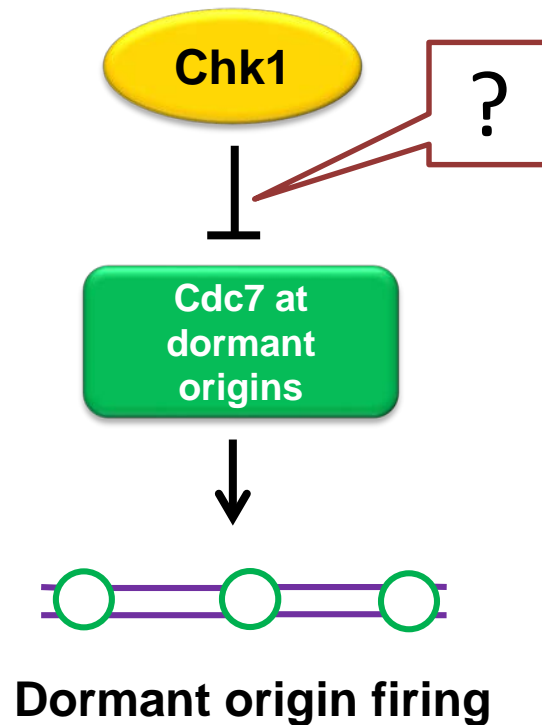


ATRi equally affects early and late S-phase cells

ATR inhibition → origins firing in the vicinity of ongoing replication



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

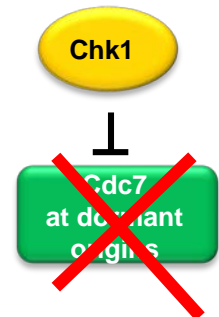
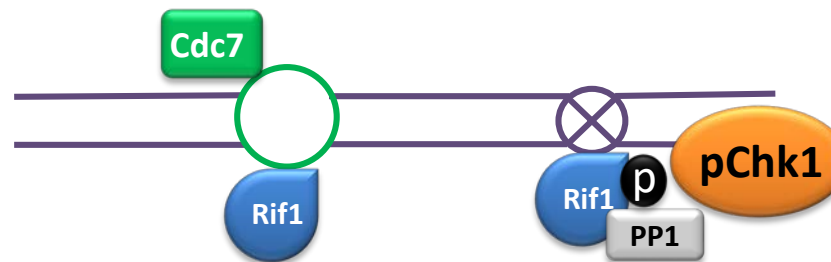
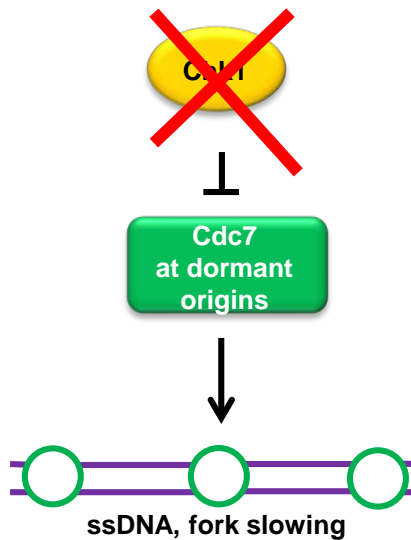


Model: Rif1-PP1 interaction is stabilized by phosphorylation

Rif1 (PP1-interacting motif)

| | | | |
|---------|----------|------|--------------------------------|
| Human | SPLASPST | SILK | RGLKRSQEDE--ISSPVNKVRRVVSFADPI |
| Mouse | SPLASPST | SILK | RGLKRSQEDE--I-SPVNKIRRVVSFADPI |
| Fly | SPSASPVS | SILK | RKLRCESLDDVTLDSPALKRKRVSFHDPP |
| Chicken | SPSASPST | SILK | RGVKRRHEDD--SLSPANKIRRVSEANPI |
| Xenopus | SPSASPST | SILK | KGVKRQQEND--SPSPLI |

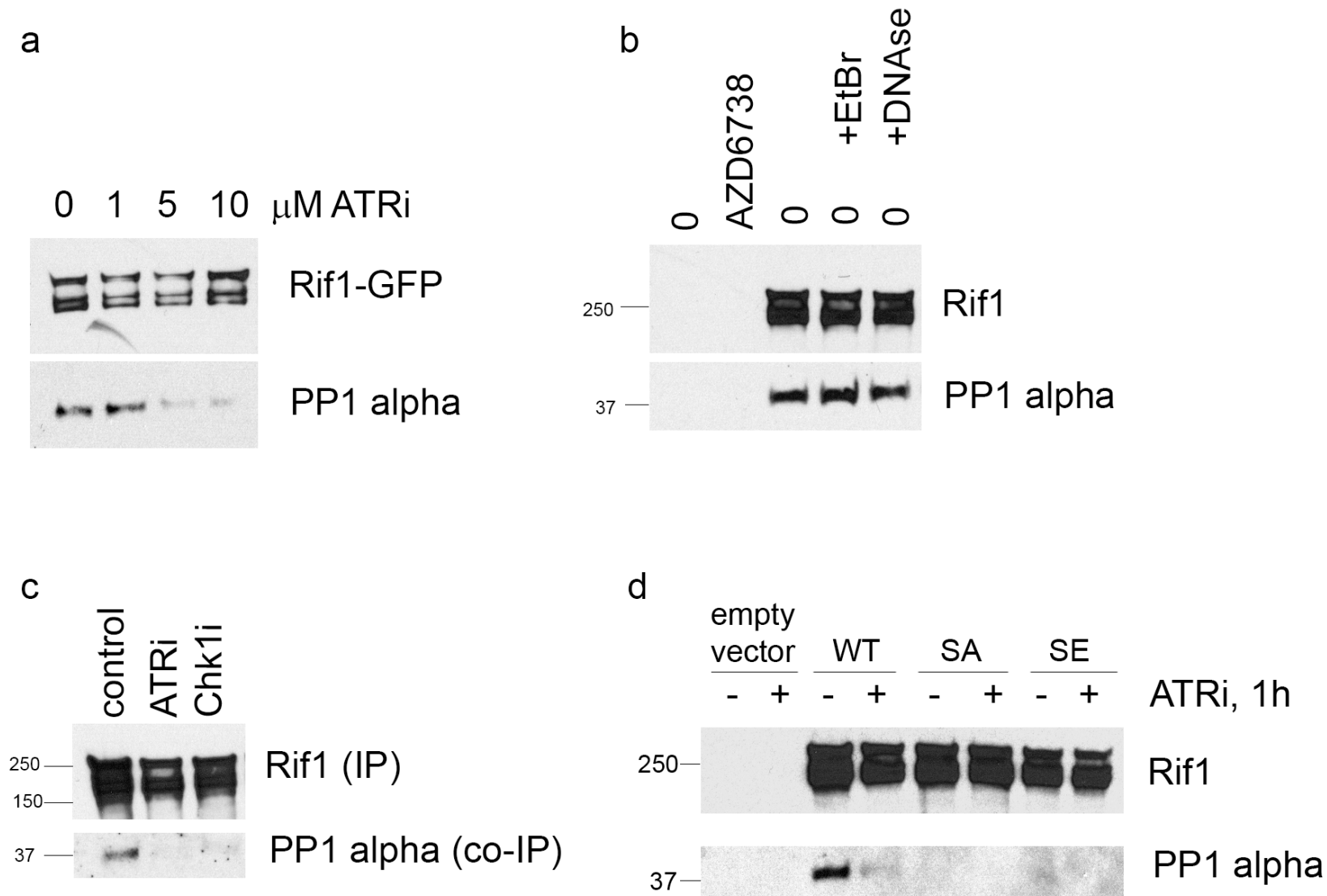
S2205



Hiraga et al., 2017:

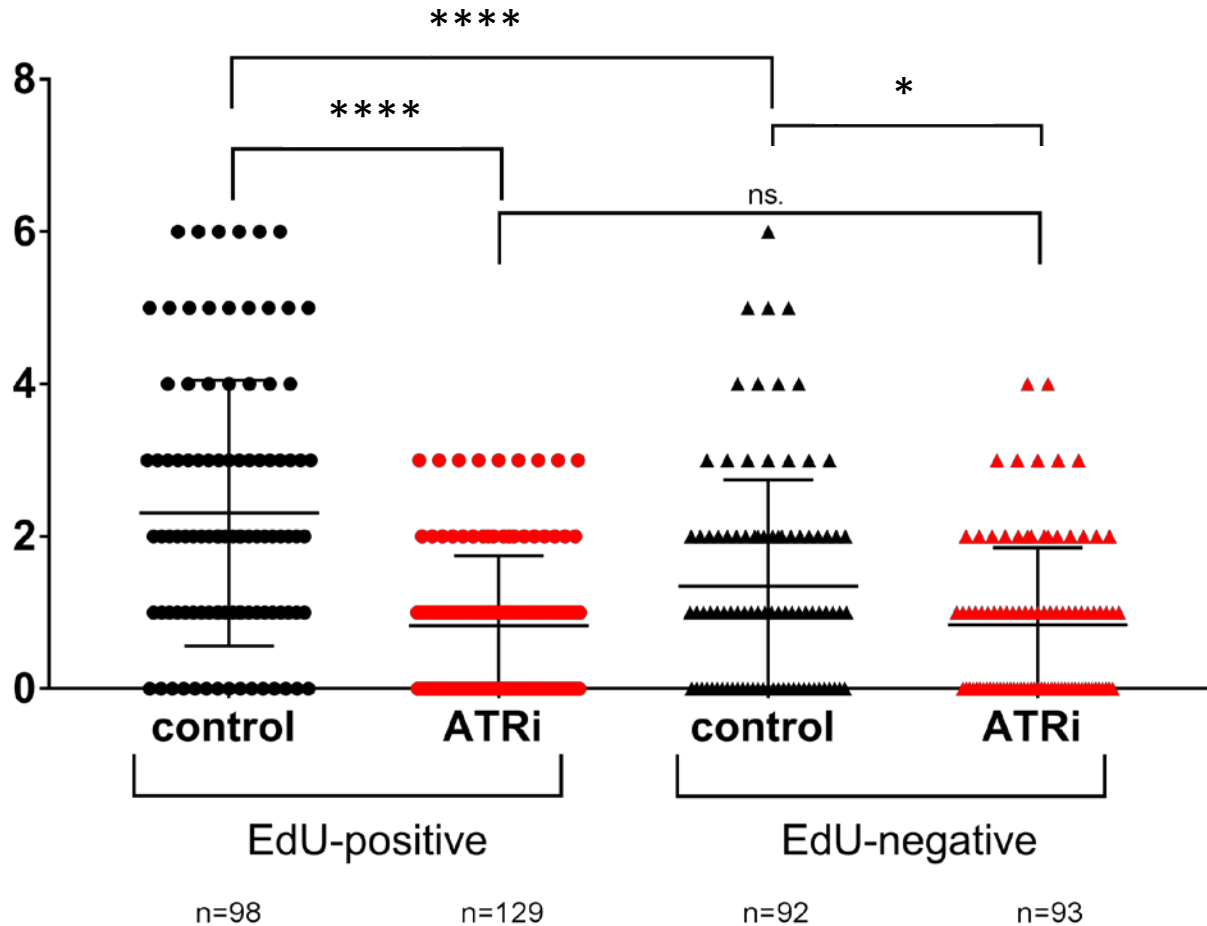
Disruption of Rif1/PP1 interaction causes increased origin firing, fork stalling, MCM4 phosphorylation on chromatin.

Rif1-PP1 interaction is blocked by ATR/Chk1 inhibition

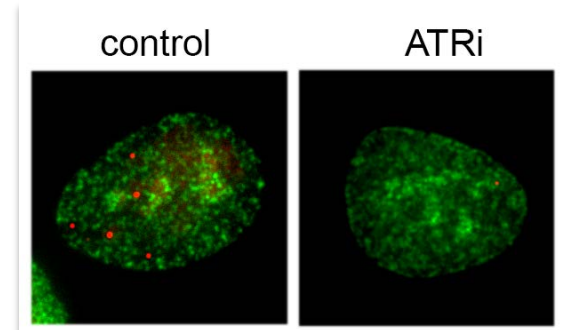
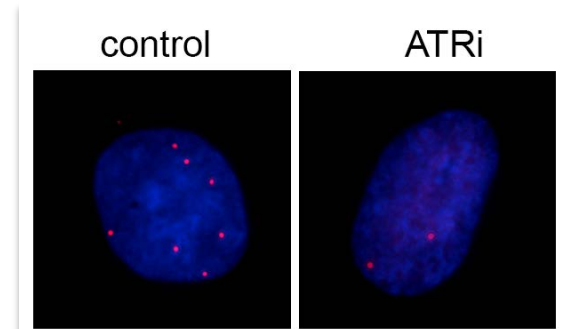
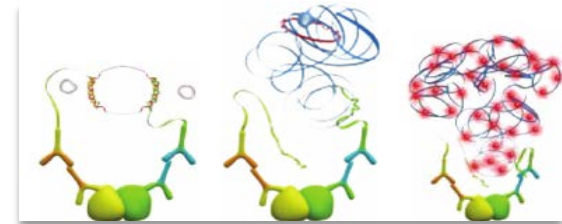


Rif1-PP1 interaction is blocked by ATR/Chk1 inhibition

Foci / nucleus

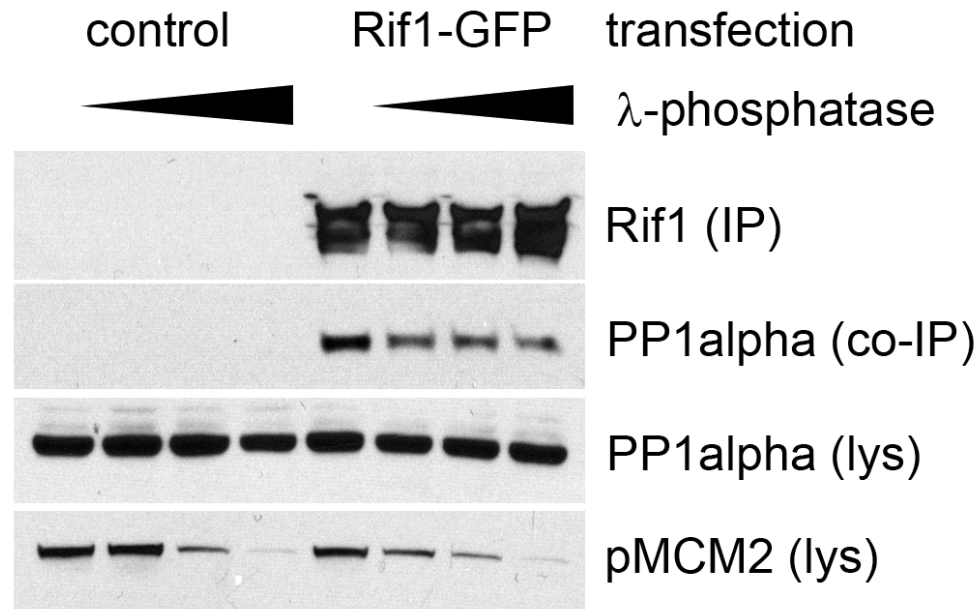


Proximity Ligation Assay

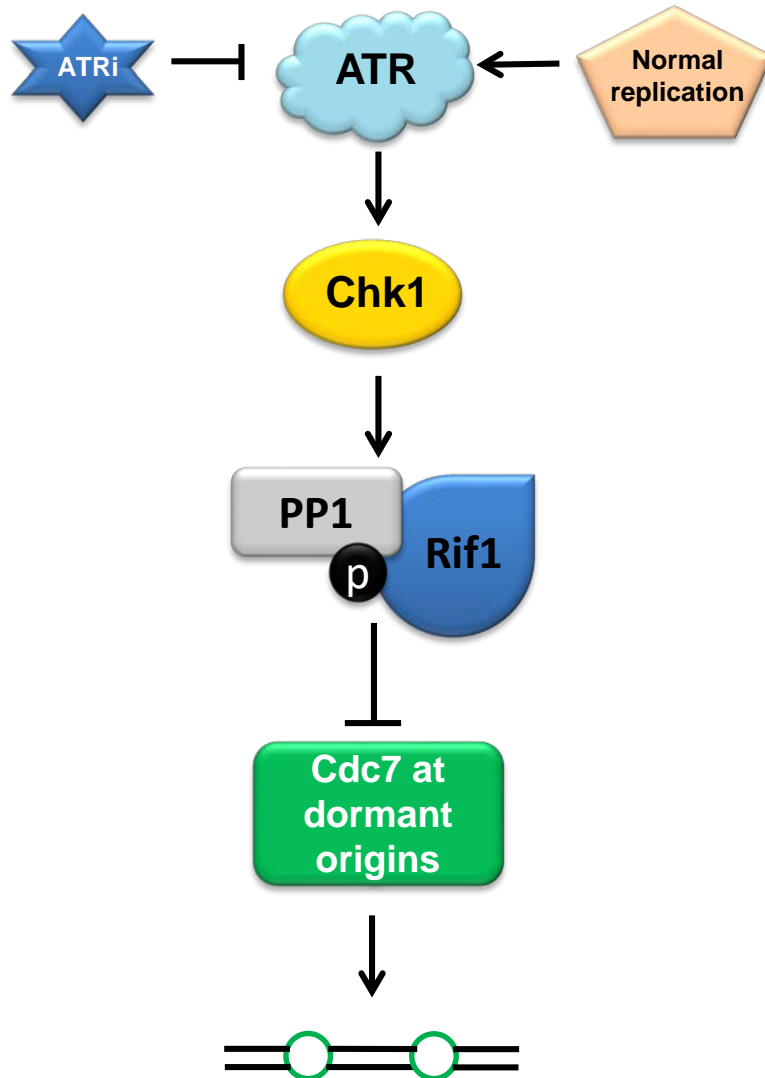


Western blot analysis showing the co-immunoprecipitation of Rif1-GFP with PP1alpha. The experiment includes a control and a Rif1-GFP transfection, each with a titration of lambda-phosphatase. The blots show four protein levels: Rif1 (IP), PP1alpha (co-IP), PP1alpha (lys), and pMCM2 (lys).

| control | Rif1-GFP | transfection |
|------------------------|----------|--------------|
| λ -phosphatase | | |
| Rif1 (IP) | | |
| PP1alpha (co-IP) | | |
| PP1alpha (lys) | | |
| pMCM2 (lys) | | |



Conclusions:



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

A decorative border on the left side of the slide, featuring a vertical DNA double helix. The helix is composed of two strands, one colored pink and the other green, with orange and yellow base pairs. A purple sphere is attached to the top of the pink strand.

University of Pittsburgh

Chris Bakkenist, PhD

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

Genome stability group

Inova Schar Cancer Institute, Annandale, VA

Thomas Conrads, PhD

Brian Hood, PhD

} mass-spectrometry

THANK YOU!