

Exploring the role of RAD51 interaction with G4s in replication



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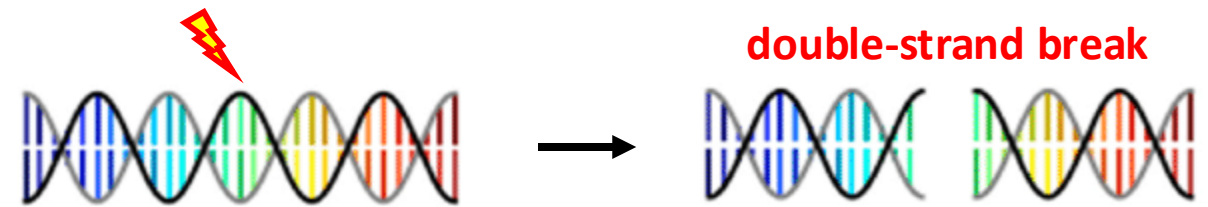
September 2024

RAD51 protein

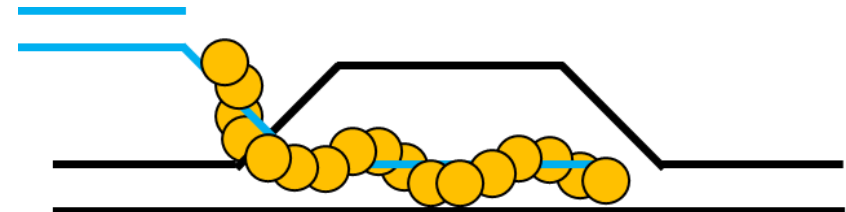
Homologous recombination

- DNA binding protein
- Essential protein
- Fanconi anemia
- Cancer

- Role in double strand break repair



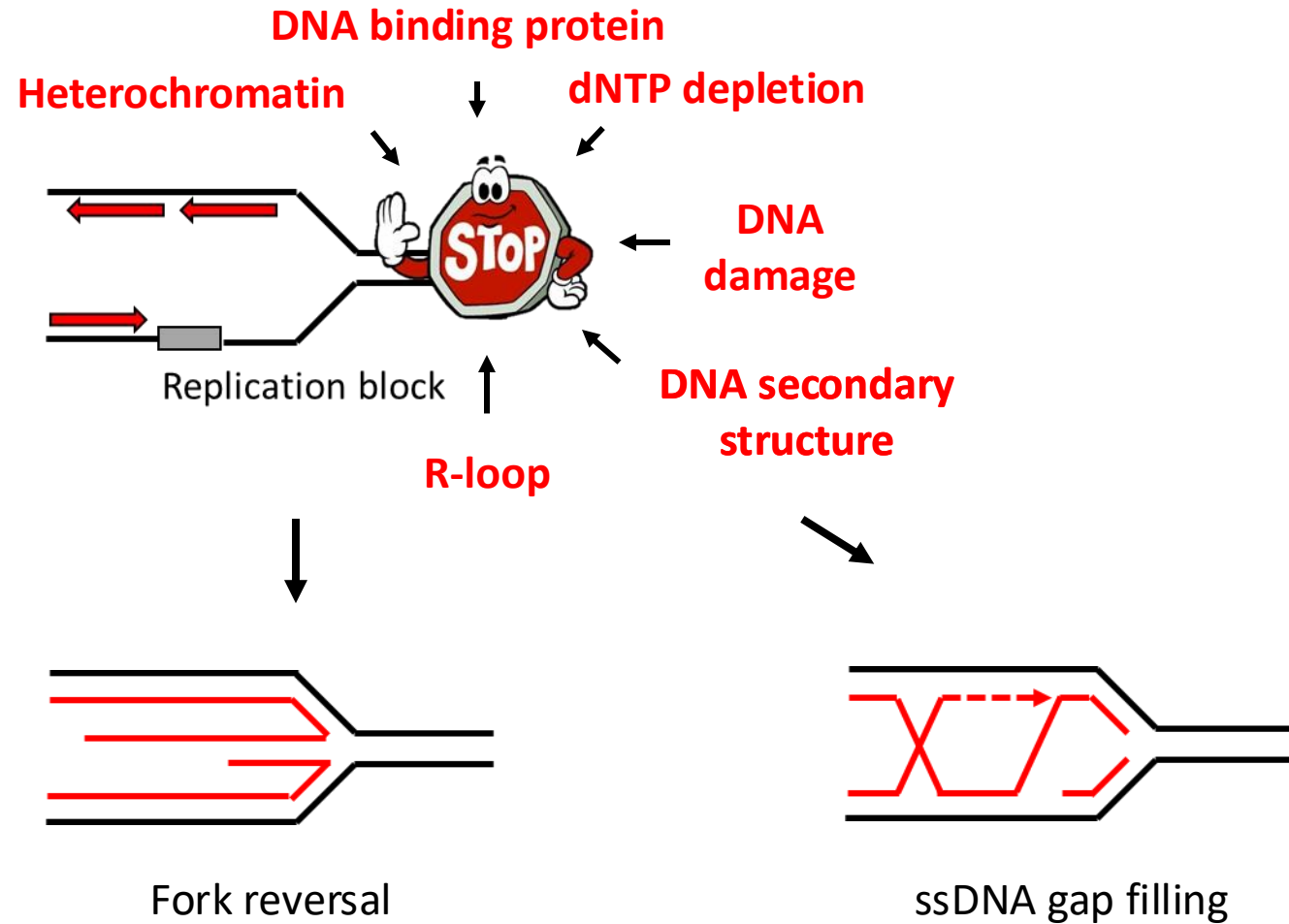
- RAD51 mediated searching for homologous sequence



RAD51 protein

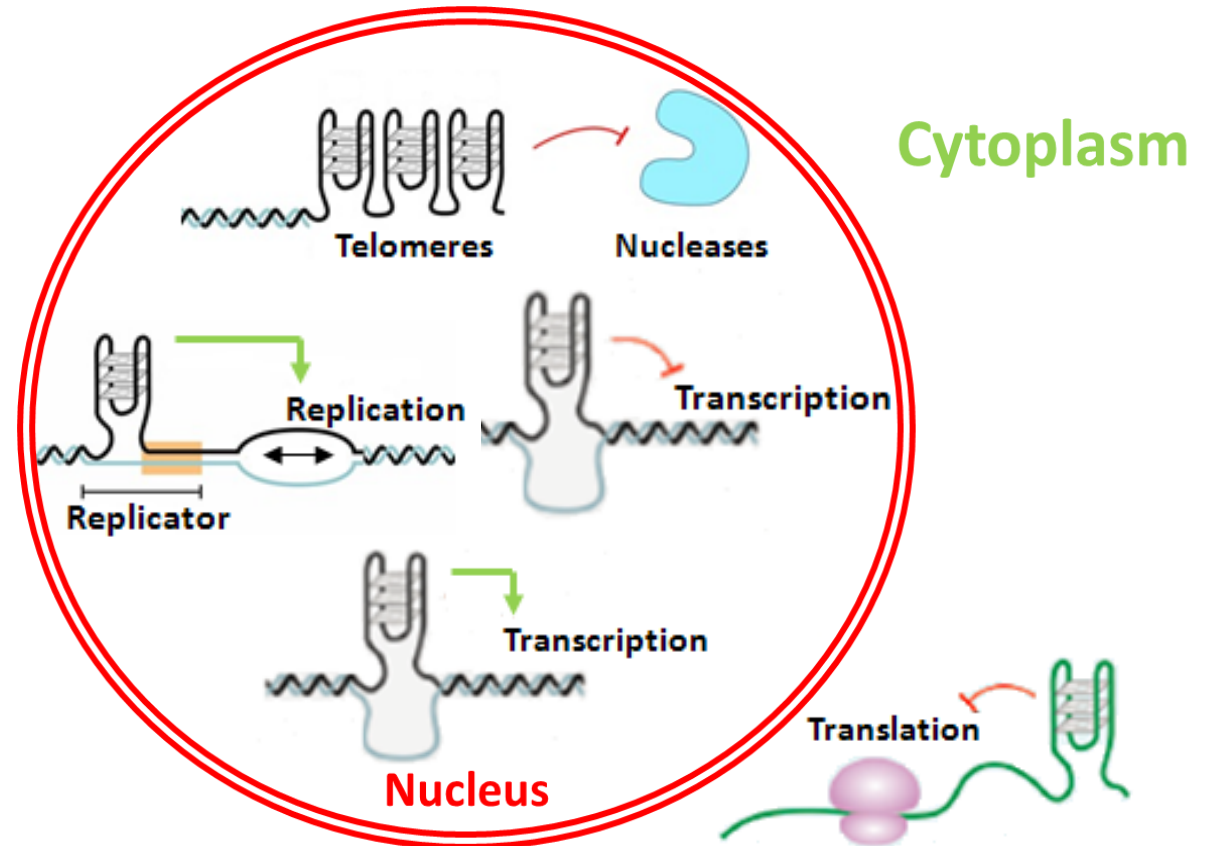
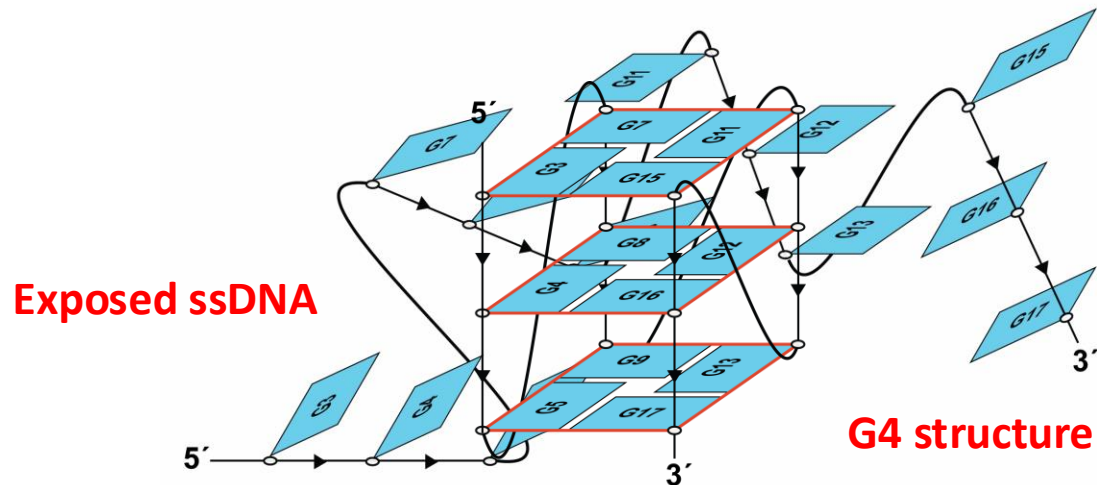
Role in DNA replication

- Stalled replication fork



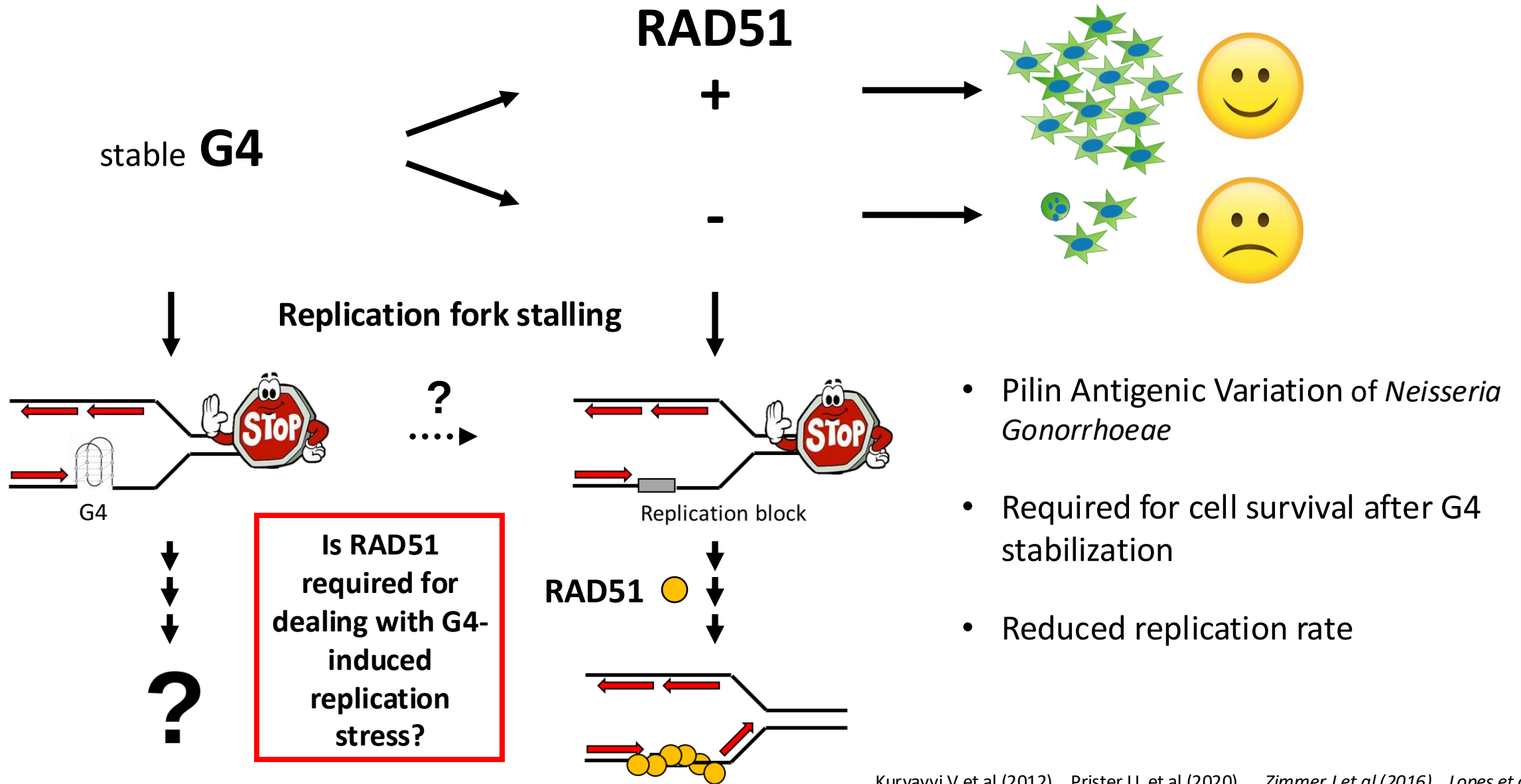
G4 structures

- Four stranded DNA secondary structures at guanine rich regions
- More than 700,000 potential G4 structures in human genome

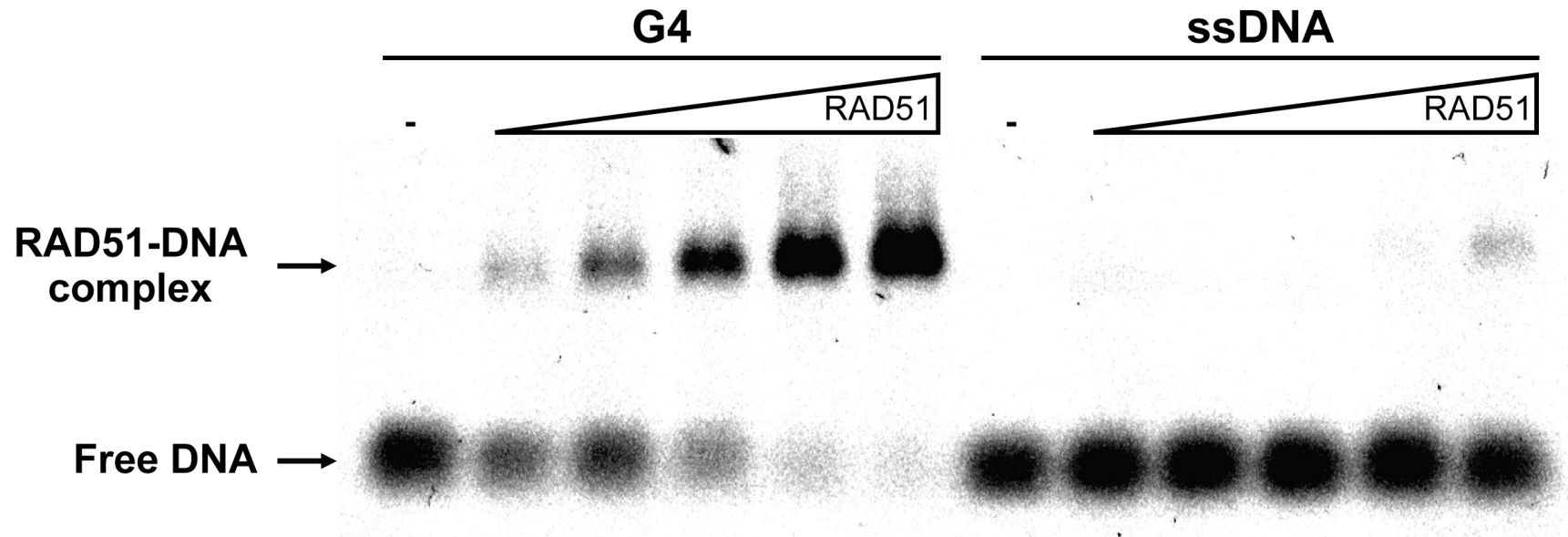
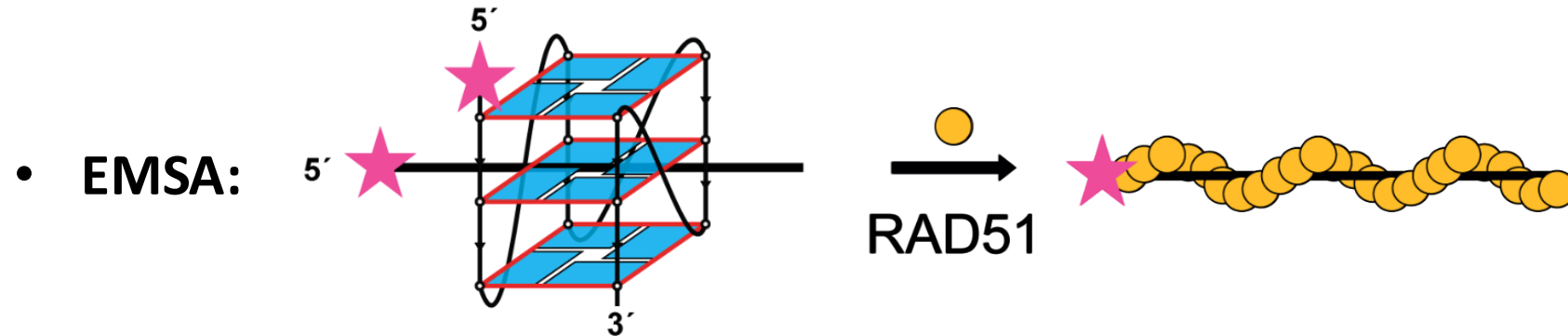


- Wide range of biological roles

Role of RAD51 protein at G4s



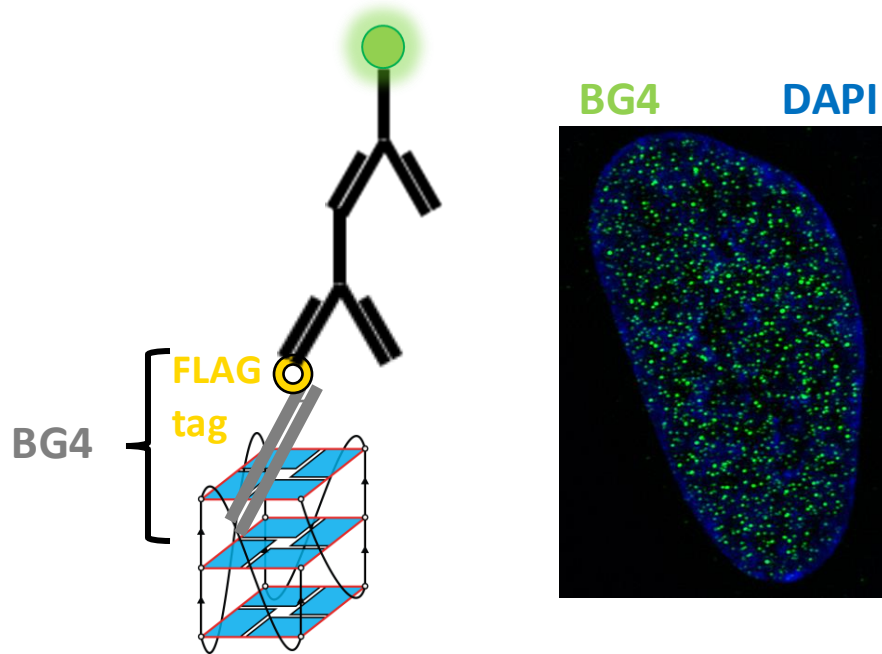
Does RAD51 bind G4 structures?



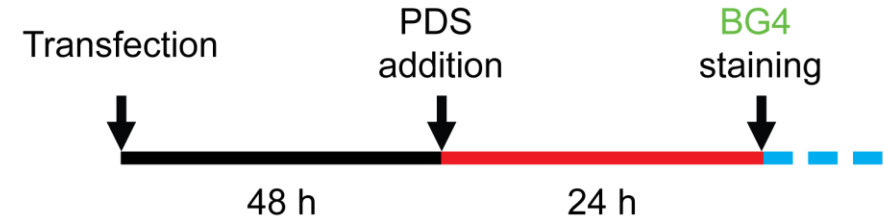
- RAD51 binds to G4 structures with higher affinity than equally long ssDNA *in vitro*

Does RAD51 interact with G4 in living cells?

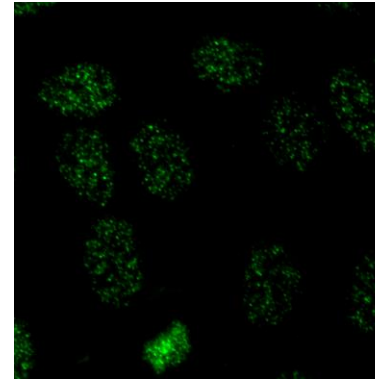
- BG4 staining



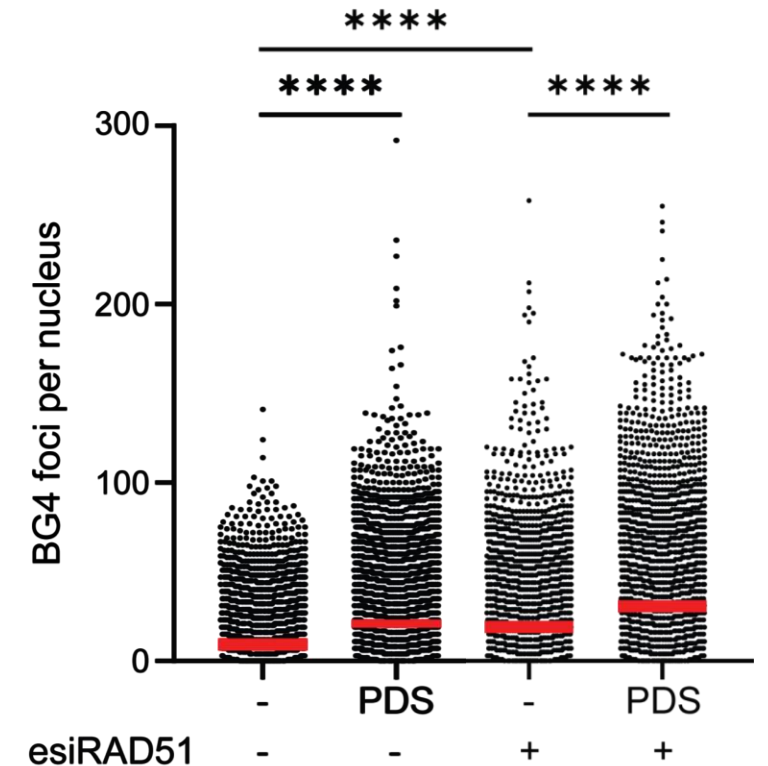
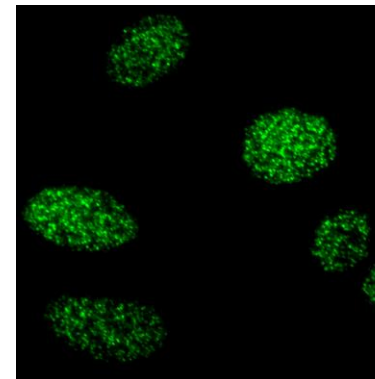
- RAD51 depletion results in increased level of BG4 foci



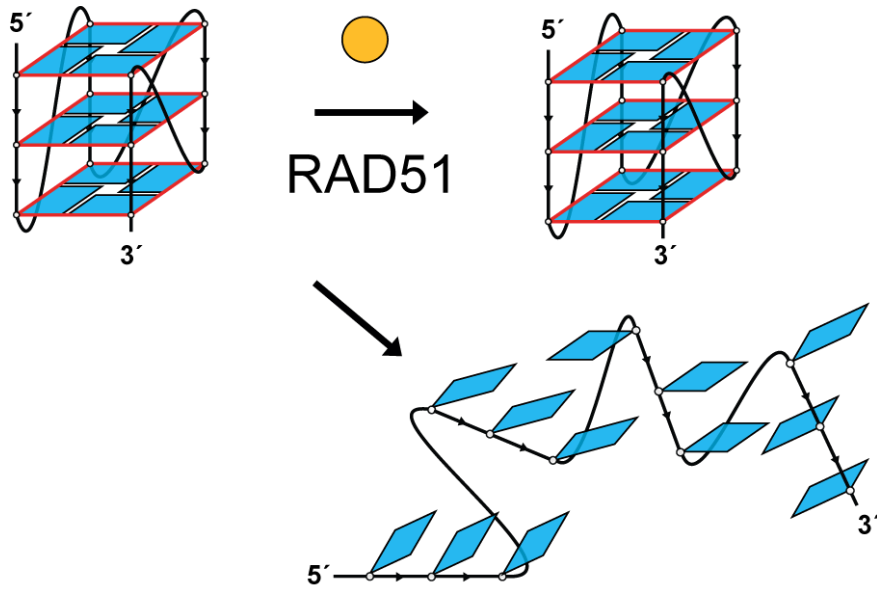
Control



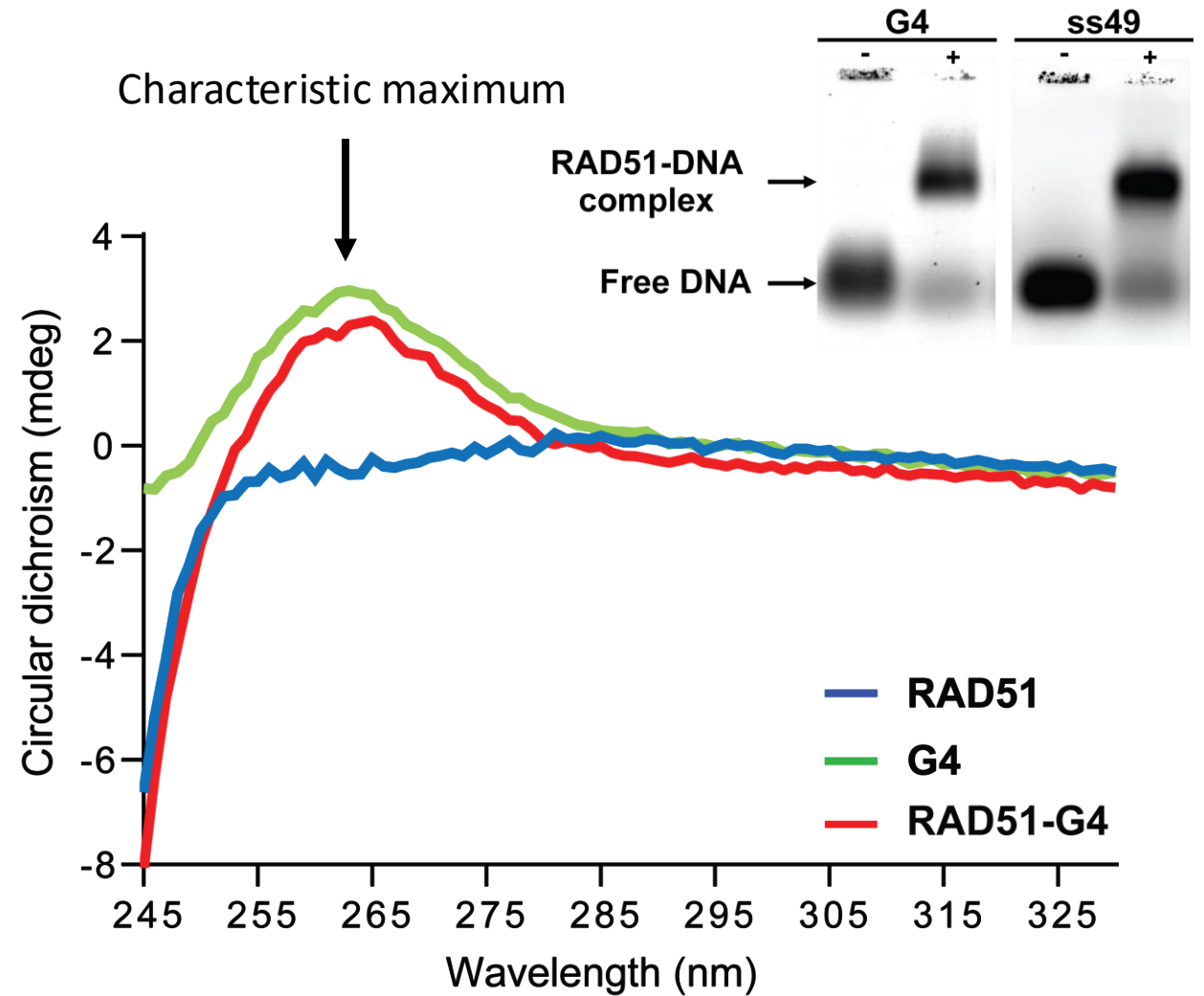
esiRAD51



Does RAD51 unfold G4 structures?



- Circular dichroism spectroscopy
- RAD51 does not unfold G4 structures



What is the RAD51-G4 structure?

- Cryo-EM

Sample preparation



Micrographs



2D classes

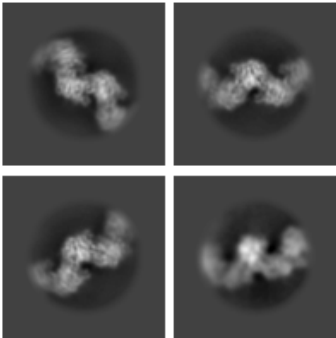


3D Map

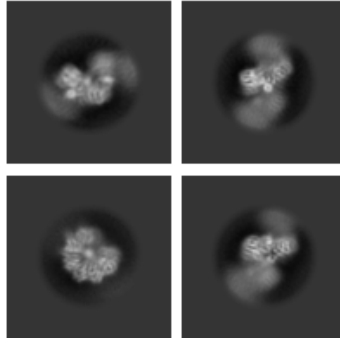


Structure

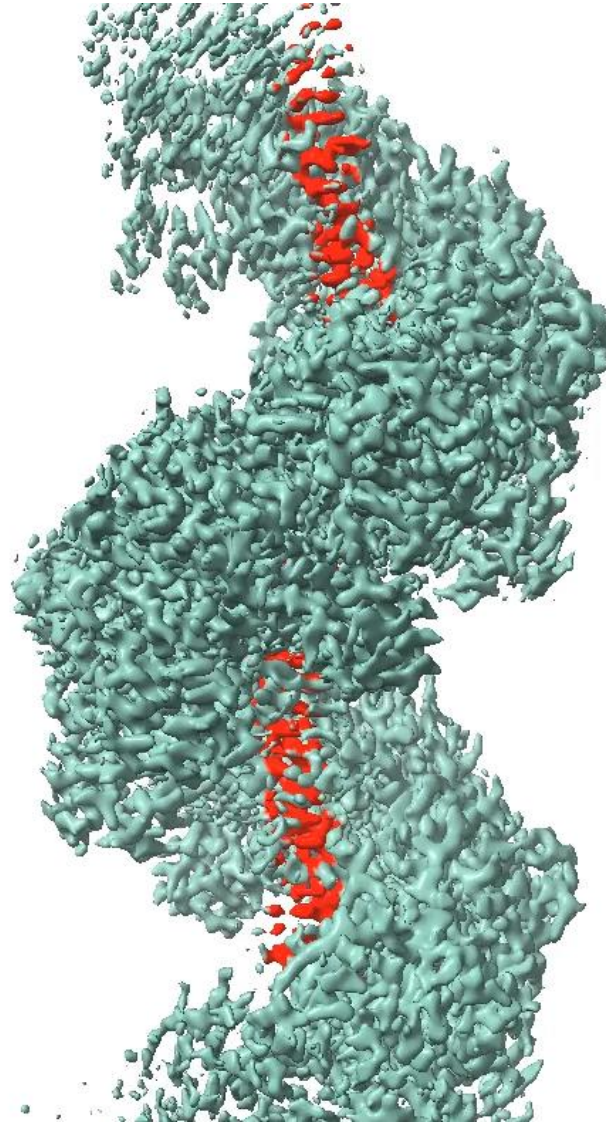
RAD51-ssDNA



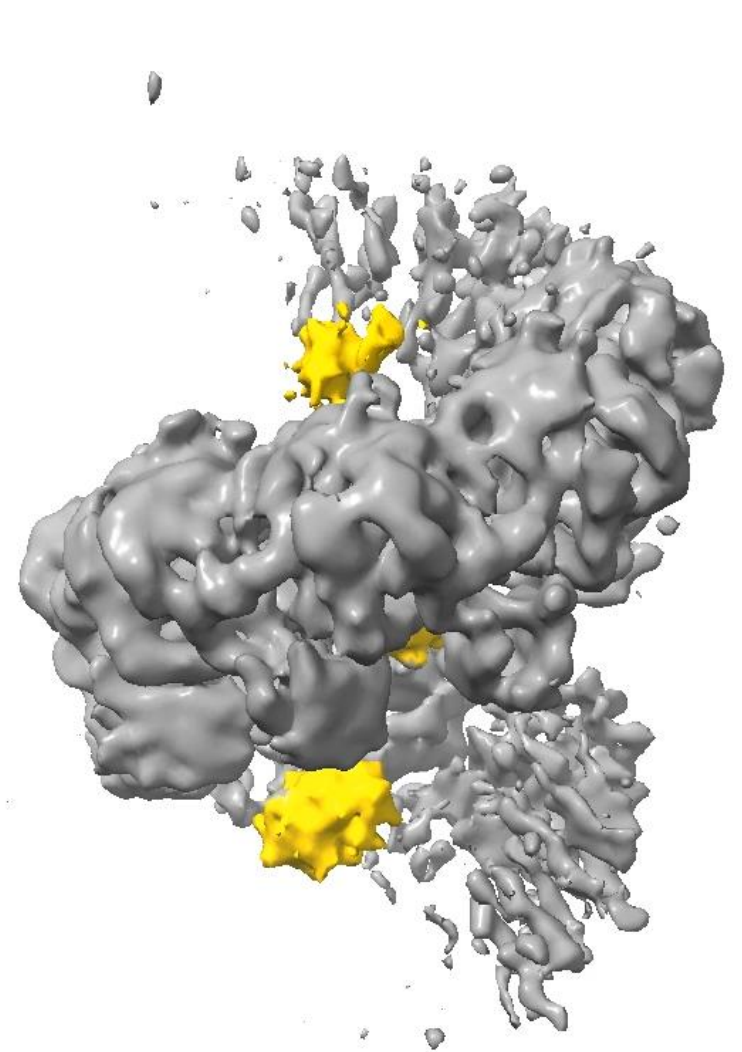
RAD51-G4



RAD51-ssDNA



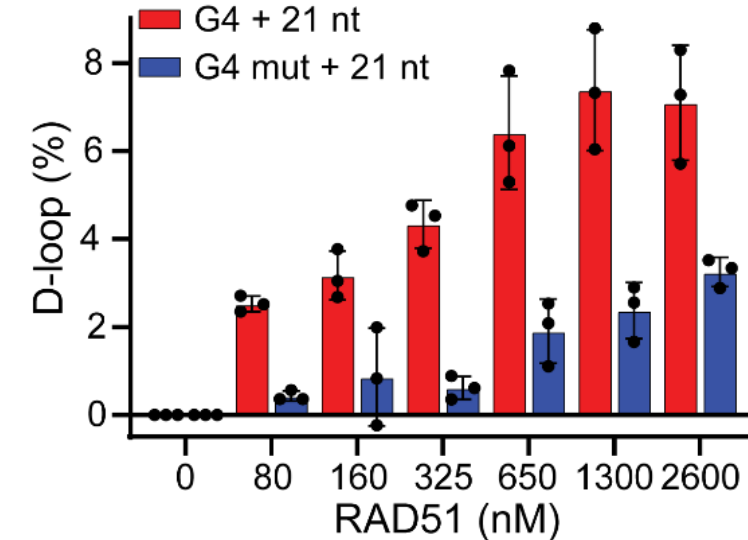
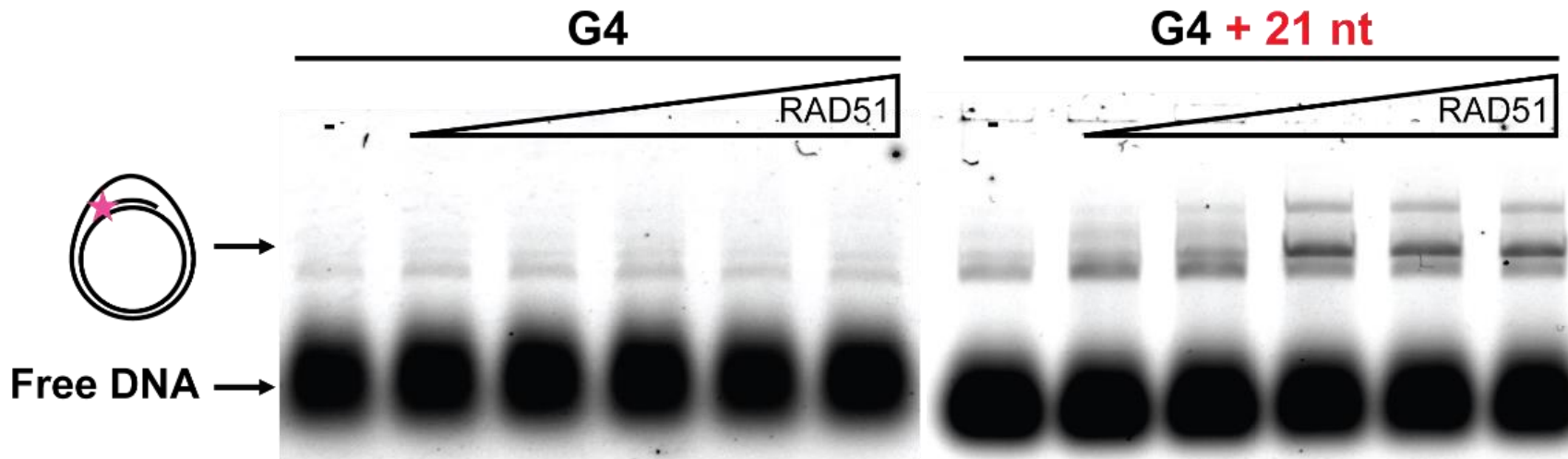
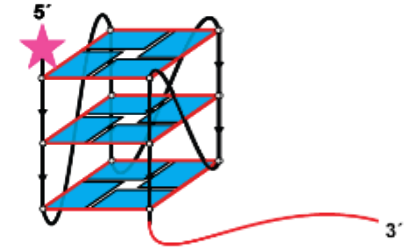
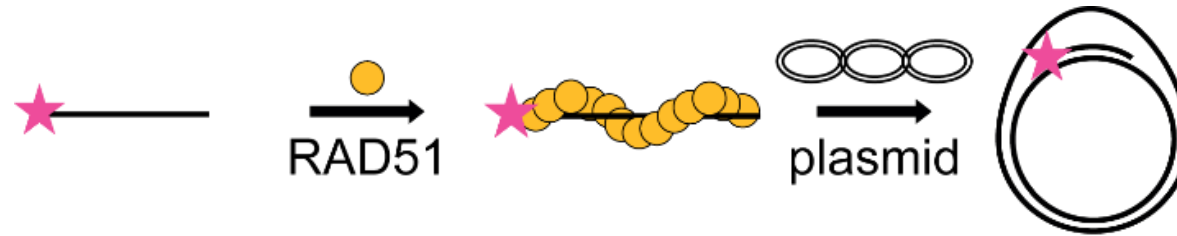
RAD51-G4



- RAD51-G4 complex form filament with altered helical parameters
- Role of G4 structure in regulation of RA

Does RAD51-G4 complex promote D-loop formation?

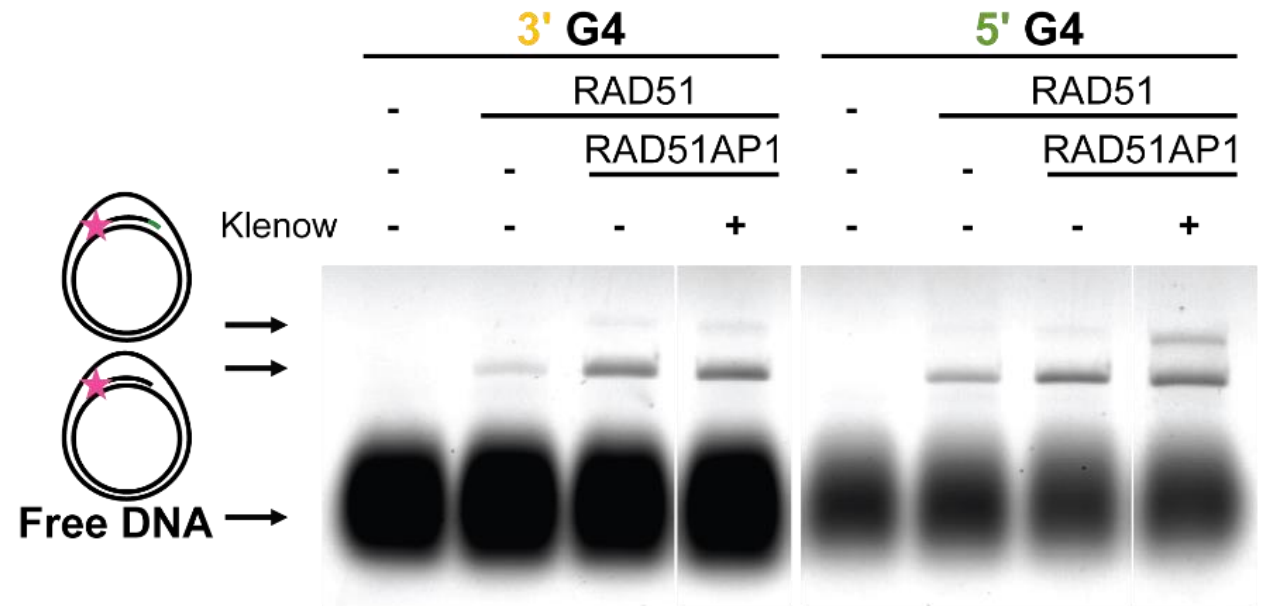
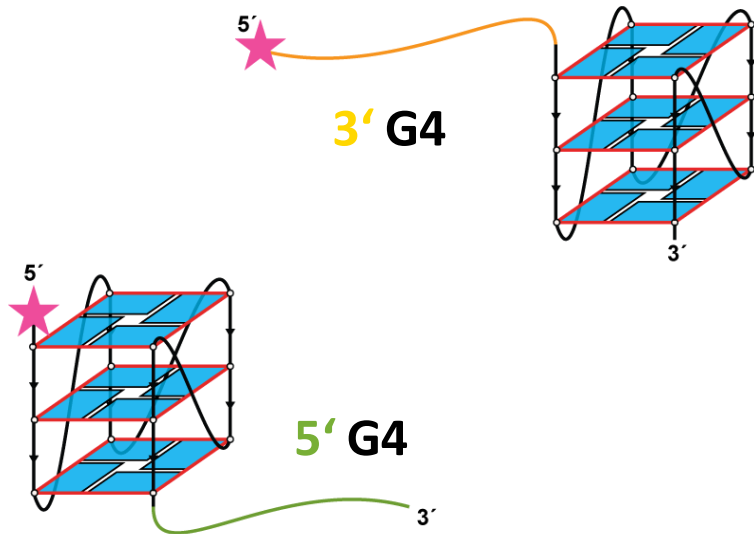
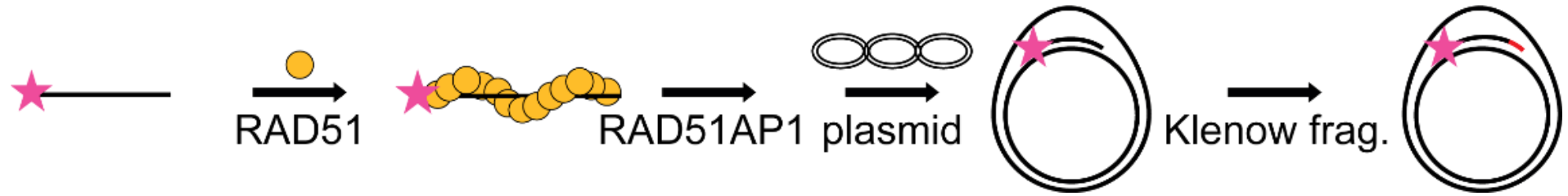
- D-loop assay



- G4 structure stimulates RAD51 mediated D-loop formation

What is the status of G4 within the D-loop?

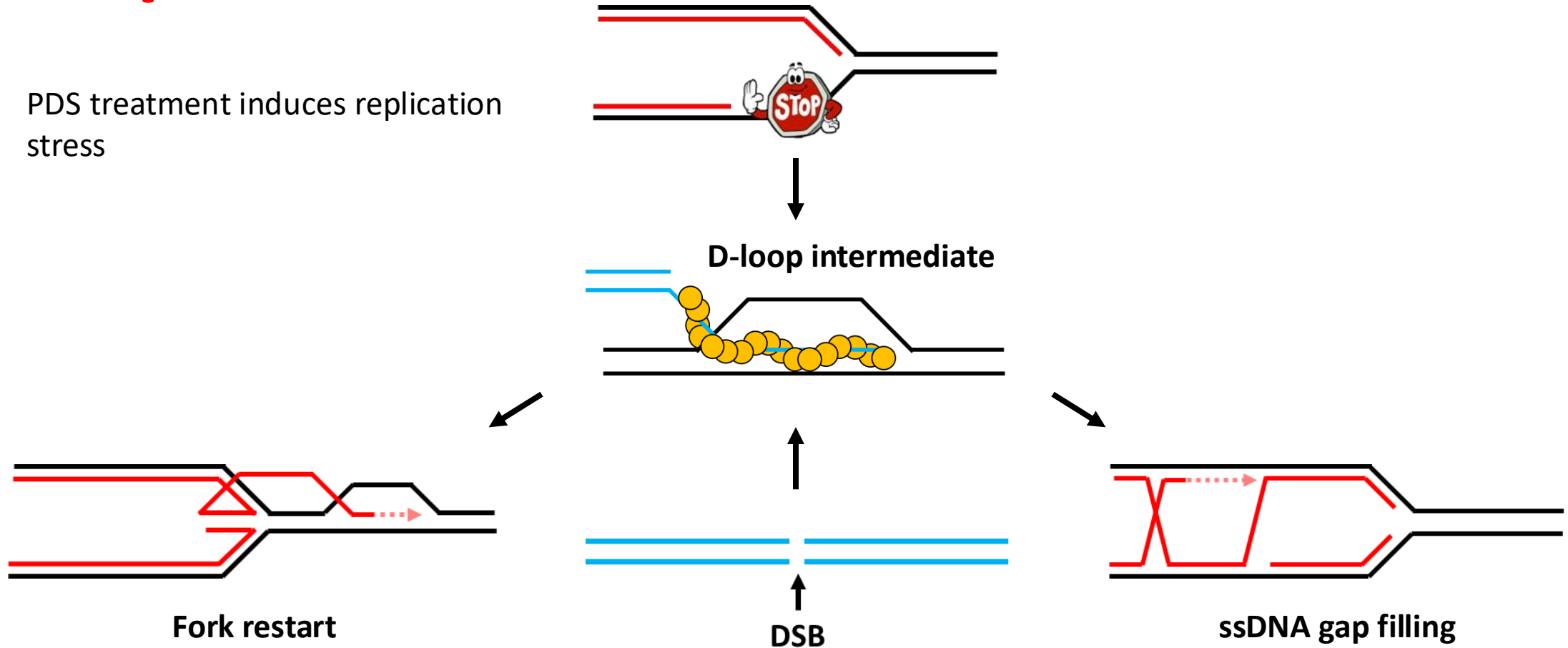
- D-loop assay



- Klenow polymerase cannot extend the D-loop product formed by G4 fused to ssDNA at 5'.

What is the biological role of RAD51-G4 complex?

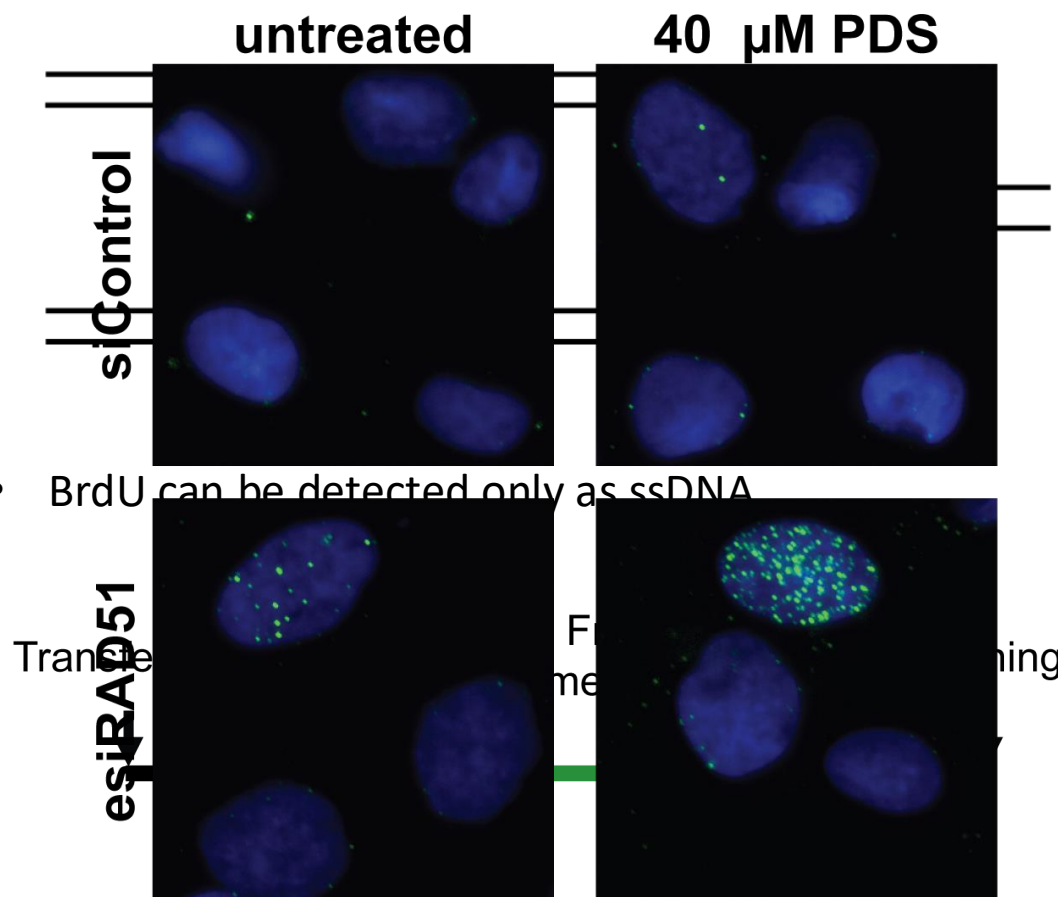
- PDS treatment induces replication stress



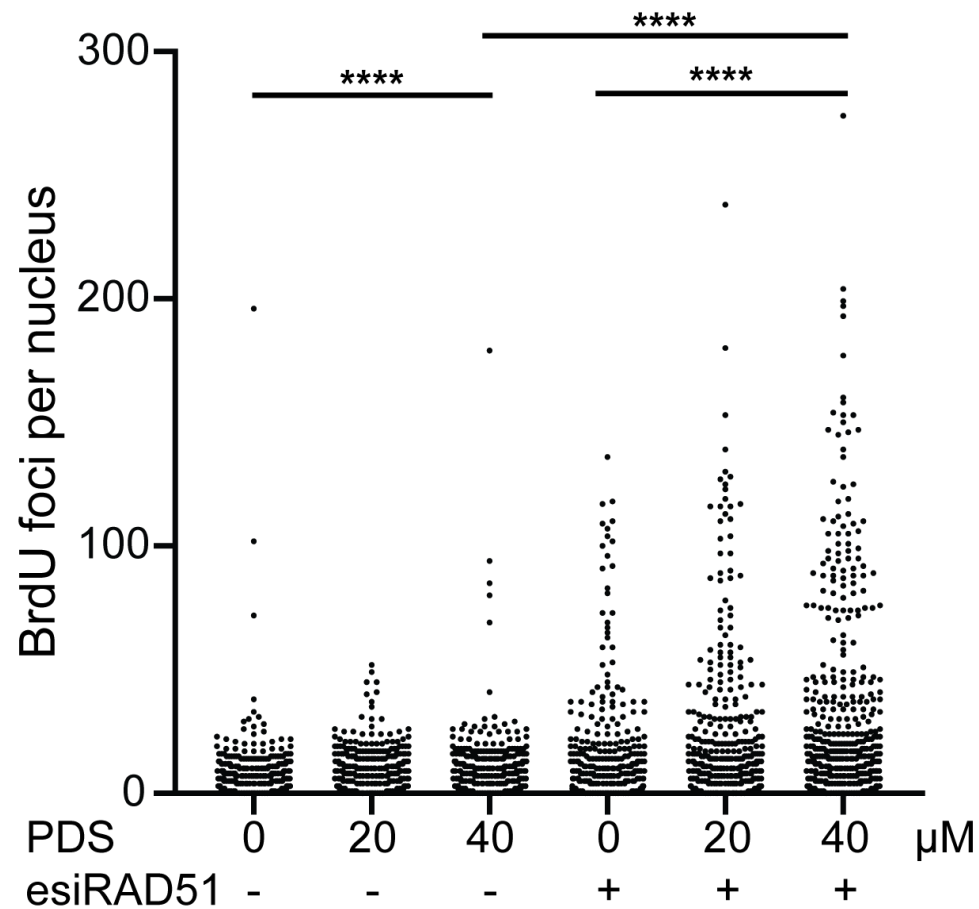
- Repriming of DNA synthesis behind G4 structures

Is RAD51-G4 complex involved in gap filling?

- ssDNA assay



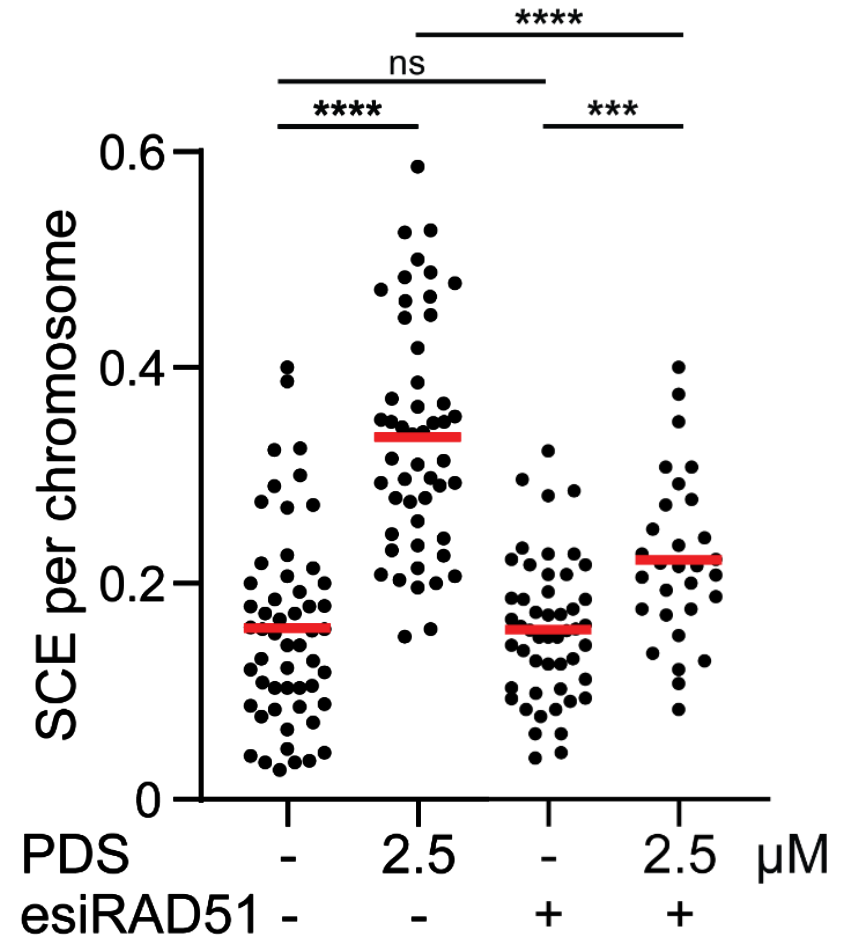
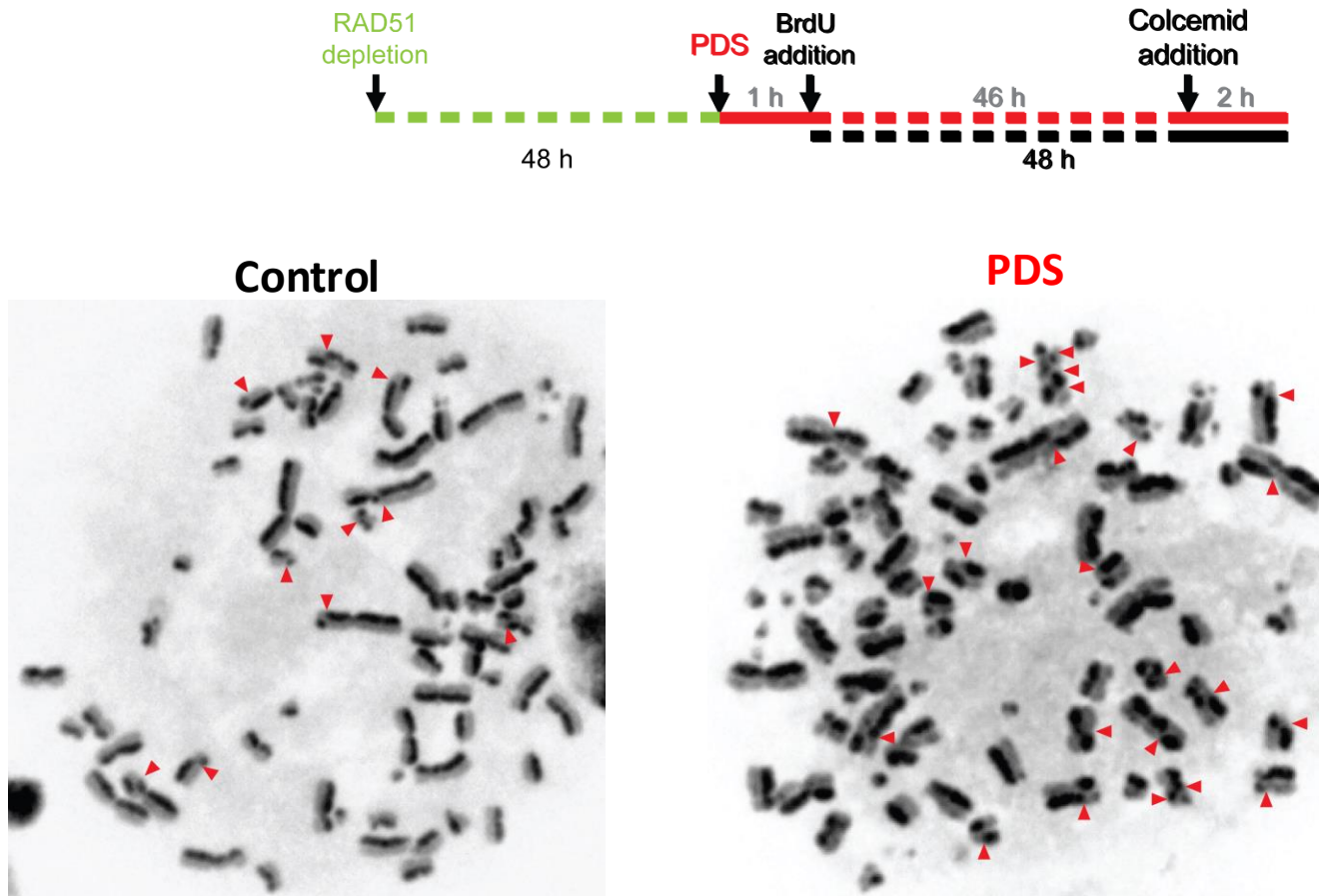
- BrdU can be detected only as ssDNA



- PDS treatment induces ssDNA gaps in RAD51 depleted cells

Are G4 gaps filled by template switch?

- Sister chromatid exchange

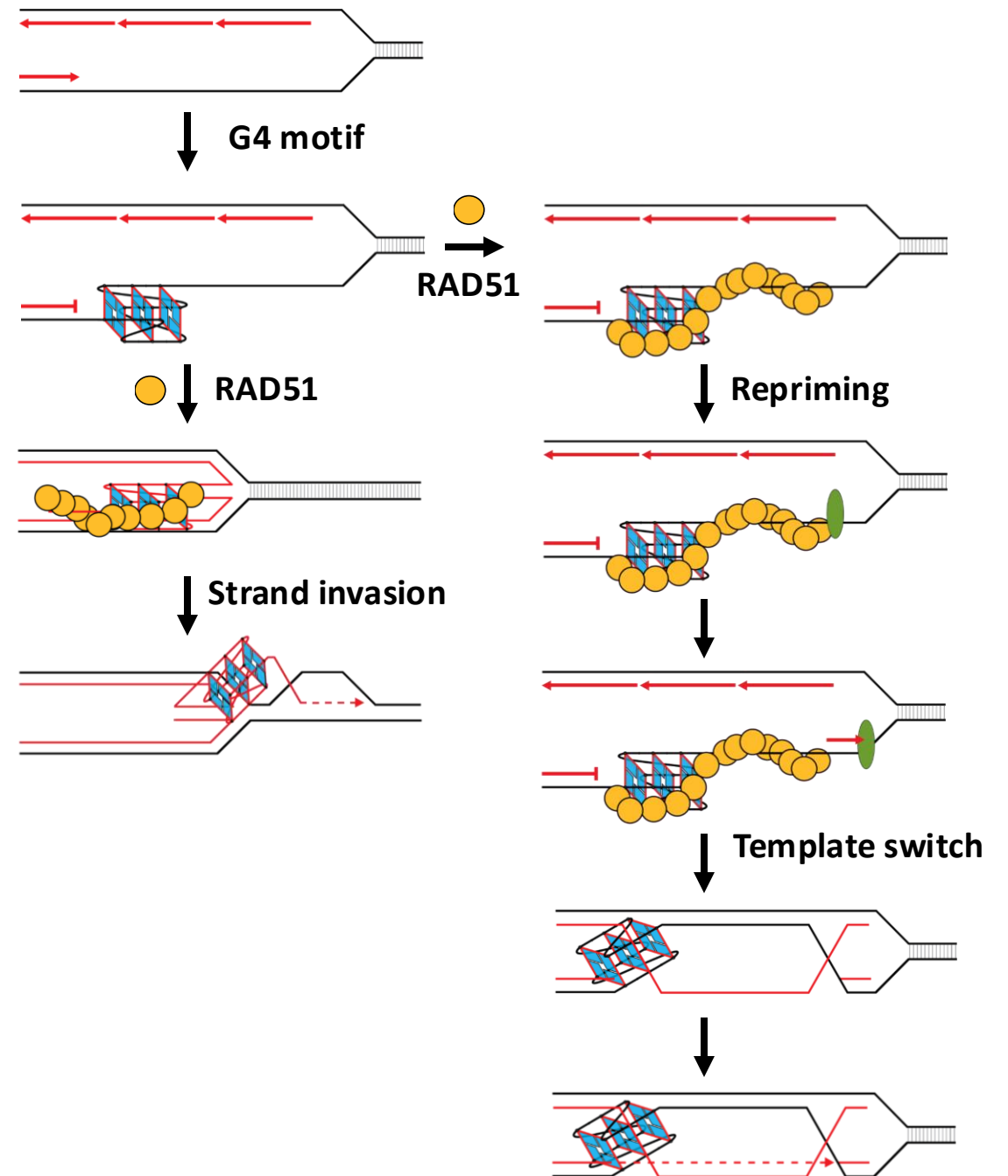


- SCEs induced by PDS treatment were significantly reduced in cells lacking RAD51.

with Lucia Sommerová

Summary

- RAD51 interacts with G4 structures *in vitro* and in living cells
- RAD51 does not unfold G4s
- RAD51-G4 complex form filamentous structure different from canonical filament
- RAD51-G4 complex seems to be involved in ssDNA gaps filling
- RAD51-G4 complex stimulates D-loop formation
- G4 stabilisation leads to increase of SCEs



Acknowledgement



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***Thank you for your
attention!***