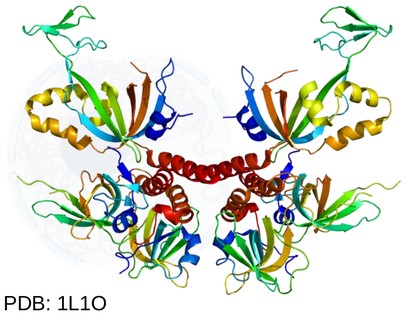

PDB: 1L1O

Characterizing the physical interaction between Replication Protein A (RPA), alt-RPA and the Human Shu Complex

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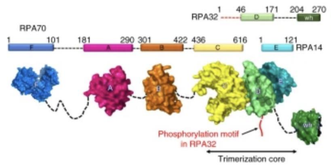
ABSTRACT

DNA damage is ubiquitous in cells and for this reason DNA repair is integral to the survival and proliferation of human cells. Throughout time, several different DNA repair processes have evolved to maintain genome integrity. While there are many DNA repair pathways, Replication Protein A (RPA), the main single-stranded binding protein, plays a role in almost every repair pathway. Some of these repair processes do not work at full efficiency in cancer cell lines as a consequence of the overexpression, underexpression, or mutation of specific DNA repair proteins. For this reason, understanding the interactions between RPA and the proteins involved in damage recognition and repair can inform cancer treatments and diagnostics. This summer I optimized the purification of human RPA and alt-RPA for the future study of its interaction with SWSAP1-SWS1. SWSAP1-SWS1 is a RAD51-paralog complex which functions in RAD51-dependent templated repair. It is both upregulated and mutated in several reproductive cancers and understanding its interaction with RPA will inform basic mechanistic understanding of this complex and enable future novel treatment strategies for these cancers. Understanding the specifics of this interaction will expand our general understanding of the mechanisms involved in RAD51-templated DNA repair and inform our ability to manipulate these processes for therapeutics.

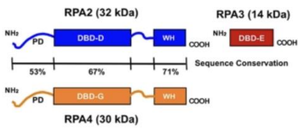
REPLICATION PROTEIN A



Pokhrel, et al., Nature Structural & Molecular Biology 2019

- RPA is the Eukaryotic single-stranded DNA binding protein
- RPA functions in DNA replication, DNA repair, recombination, cell cycle, and DNA damage checkpoints
- RPA is involved in over 40 protein-protein interactions
- RPA is a highly dynamic molecule with 3 subunits containing 7 DNA binding domains

ALTERNATIVE REPLICATION PROTEIN A (ALT-RPA)



Mason, et al., Journal of Biological Chemistry, 2008

- RPA2 (the 32kDa subunit) of RPA is replaced with a homolog known as RPA4
- Present only in primates and some mammals (excluding mice)
- DNA binding properties indistinguishable from the canonical RPA complex
- Alt-RPA is unable to support DNA replication and inhibits canonical RPA

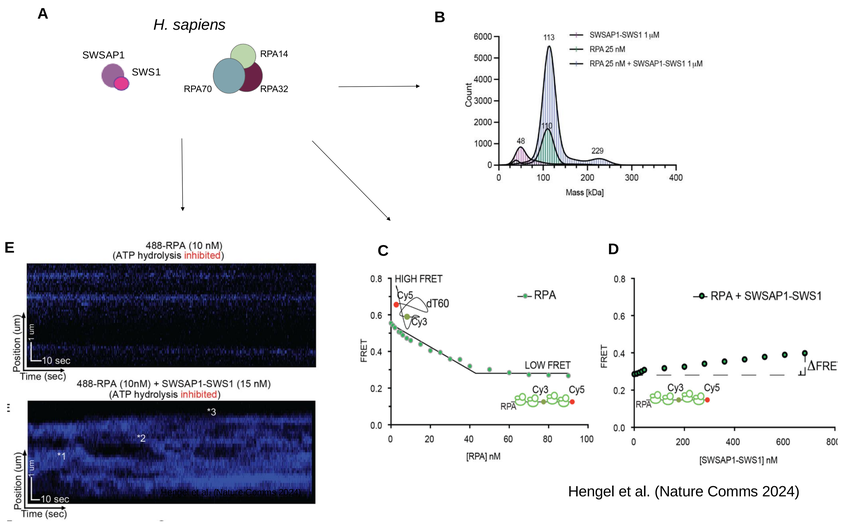
THE HUMAN SHU COMPLEX (SWSAP1-SWS1)

- SWSAP1-SWS1 is a RAD51 paralog containing complex
- SWSAP1 is amplified in Breast and Ovarian Cancers
- SWSAP1-SWS1 functions in RAD51-dependent repair
- SWSAP!-SWS1 has been shown to:
  - Stabilize RAD51 filament formation on ssDNA
  - Promote RPA diffusion and dynamics on ssDNA

Hengel et al., Nature Communications, 2024

RESULTS

RPA binds SWSAP1-SWS1 and enhances RPA diffusion on DNA.



Hengel et al. (Nature Comms 2024)

Figure 1

Hengel et al. 2024 demonstrated that RPA binds SWSAP1-SWS1 and enhances RPA diffusion on DNA. This was demonstrated via three methods: A) Schematic of human SWSAP1-SWS1 and human replication protein A complexes. B) Mass photometry which showed the formation of higher molecular weight complexes following the incubation of RPA and SWSAP1-SWS1, C) FRET (Förster resonance energy transfer) which shows human RPA binds to ssDNA (4 molecules of RPA on 1 ssDNA). D) FRET-based titration of SWSAP1-SWS1 onto RPA-coated ssDNA showing enhanced FRET. This means that the interaction of RPA to ssDNA is modulated in the presence of RPA. E) LUMICKS C-trap (combines single-molecule confocal fluorescence microscopy with optical tweezers) which showed increased rates of RPA diffusion and motion on ssDNA following the addition of SWSAP1-SWS1.

OVERVIEW OF THE PROJECT

INITIAL QUESTIONS

What subunit and binding domain(s) of RPA are involved in binding SWSAP1-SWS1?

How does the presence of various DNA substates impact how these proteins bind one another?

Does the human Shu complex also bind Alt-RPA?

Using either enzyme digest or PCR we will modify the RPA plasmid to express a protein that only includes the 32kDa and 14kDa subunits. Then pulldown results with this "RPA32+14" can be compared to pulldowns with normal RPA. This will help us determine if SWSAP1-SWS1 binds to RPA70 or RPA32.

After the subunit is determined, point mutations in different binding domains can be developed, purified, and tested to gain more specific information regarding the amino acids on RPA involved in binding to the Shu complex.

We will use Forester Resonance Energy Transfer (FRET) to observe the impact of various DNA substrates on RPA-Shu binding. FRET uses the configuration of ssDNA dually labeled with Cy3 and Cy5 fluorescent dyes to analyze protein-DNA interaction in equilibrium conditions.

We will conduct protein pulldown reactions to investigate whether Shu binds alt-RPA

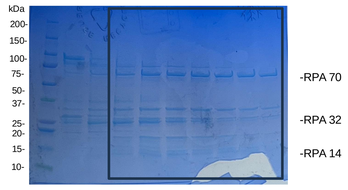
HOW DO WE EXPLORE THIS? METHODS

POSSIBLE FURTHER QUESTIONS AND DIRECTIONS

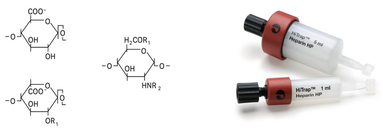
1) Are RPA diffusion rates impacted by SWSAP1-SWS1 mutants found in Breast and Ovarian cancers?
   A) Method: C-trap
2) We will screen libraries of chemicals to try and identify an inhibitor of RPA-Shu binding
   A) Method: ELISA
3) We could explore the interaction between alt-RPA and canonical RPA using the C-trap.
4) We could investigate the impact of RPA post-translational modifications on its ability to bind Shu.

PURIFICATION OF RPA AND ALT-RPA

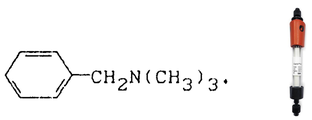
HiTrap Blue Column

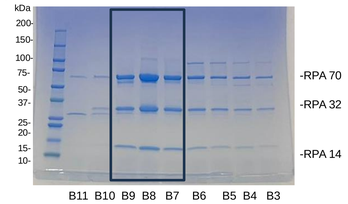


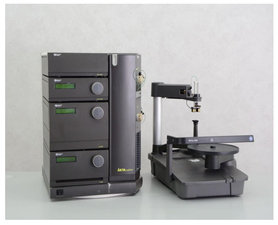
HiTrap Heparin HP

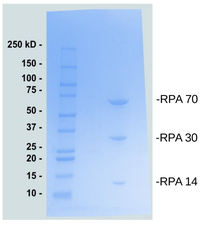


HiTrap Q HP anion exchange chromatography

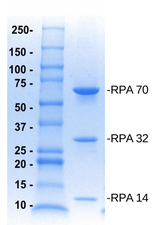








Purified Alt-RPA



Purified Canonical - RPA

CONCLUSIONS AND NEXT STEPS:

This summer, I expressed and optimized the purification of human RPA and Alt-RPA. Now that I have pure protein and the recombinant tools to test the functionality of the purified protein. This will be done using FRET experiments previously shown in Hengel et. al 2024 with RPA, SWSAP1-SWS1, and ssDNA.

Following this I will be able to conduct experiments that investigate RPA-Shu binding :

1) Protein pulldowns with RPA/alt-RPA and Shu
2) FRET experiments with RPA and Shu using different DNA substrates
(ssDNA, DNA fork, ssDNA with abasic site, fork with abasic site, d-loops and r-loops)

These experiments will provide information that could inform the development of future cancer therapeutics

Future steps will likely involve screening for inhibitors of RPA-Shu binding.

THANK YOU TO…

Thank you to everyone in the SRH lab for your help and support. Thank you to my mentor Dr. Sarah Hengel. Thank you to Tufts Biology for the amazing research community that I have gotten to be a part of. And thank you to the Laidlaw Foundation for the funding that made this summer possible

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