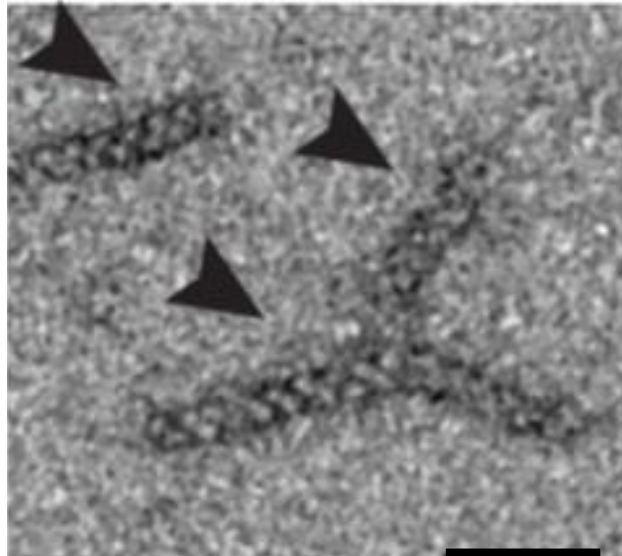


Promotion of Rad51 presynaptic filament assembly by the ensemble of *S. cerevisiae* Rad51 paralogs with Rad52



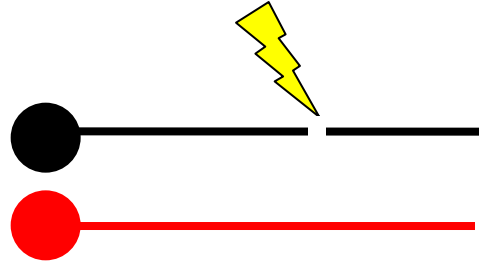
EM of Rad51 filaments

Stephen Godin

The Bernstein Lab

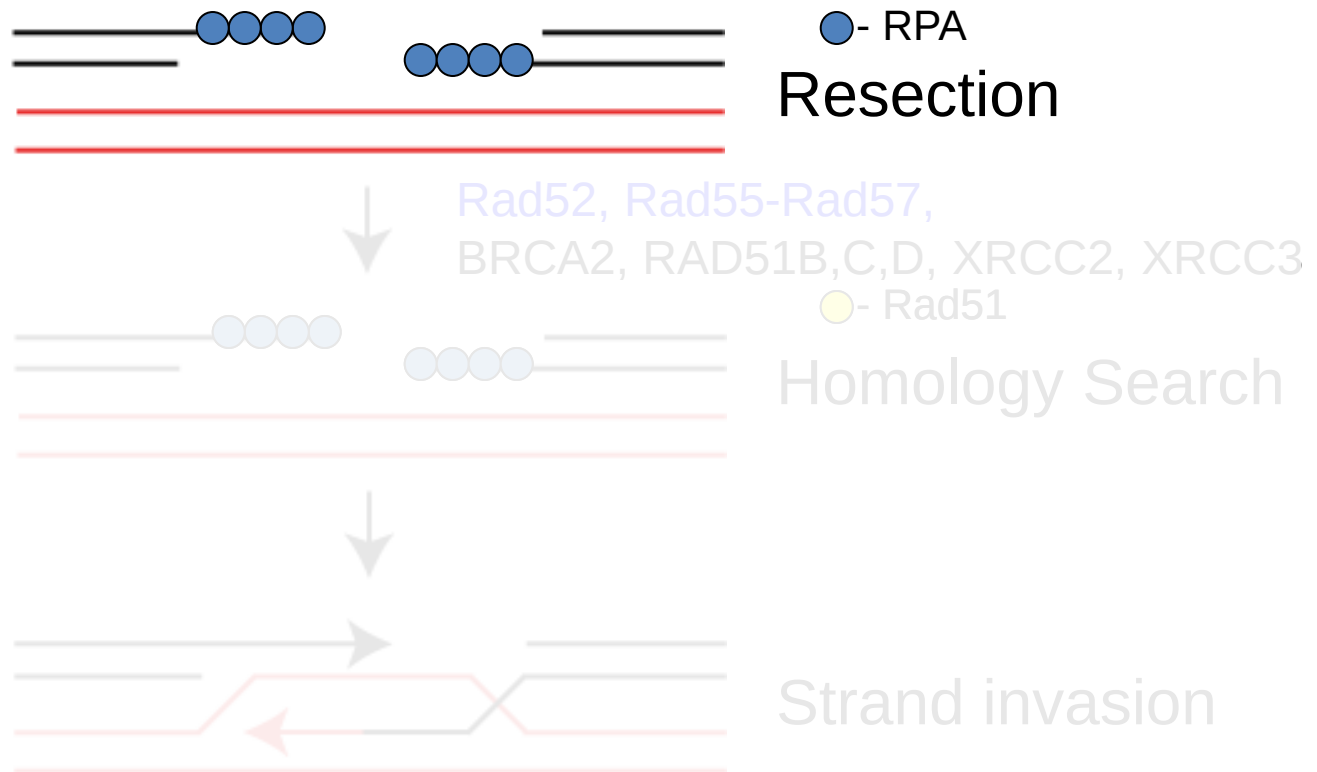
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Homologous Recombination



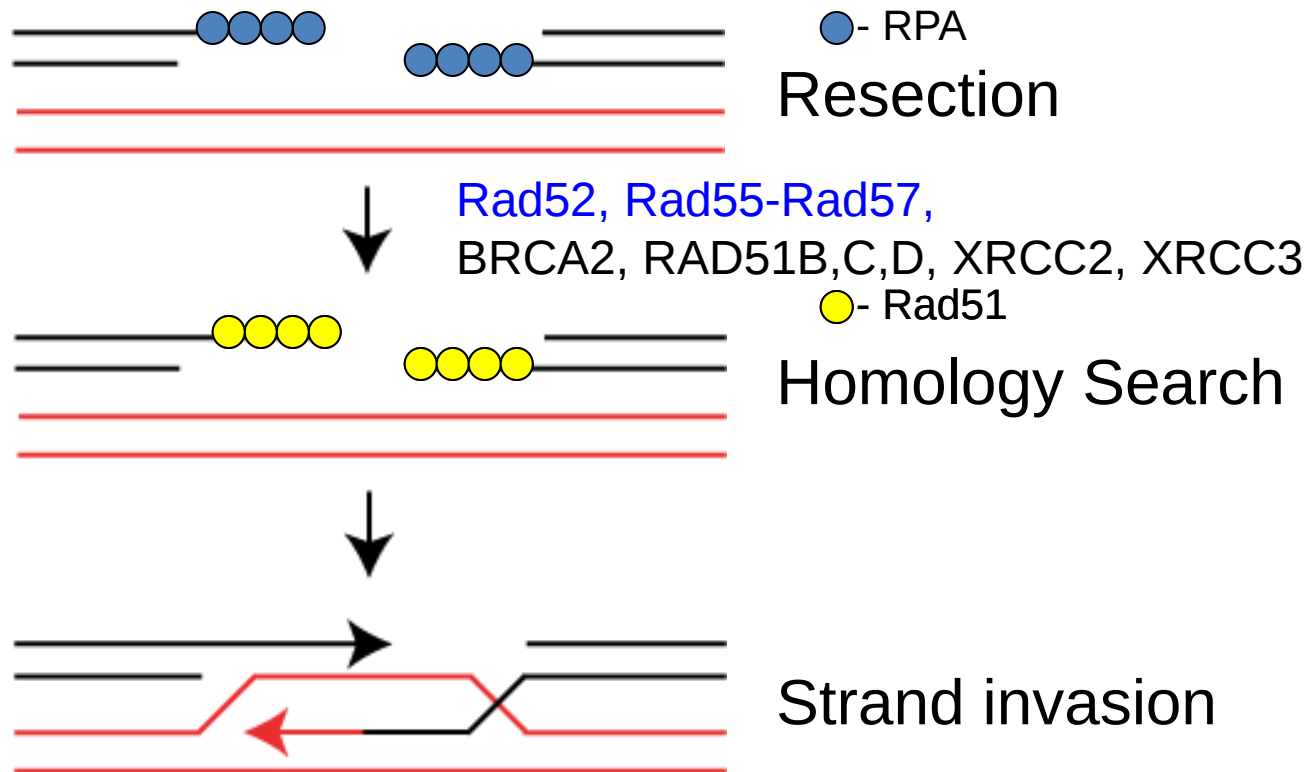
- Relies on availability of a homologous template for repair
- Considered an error-free repair mechanism
- Used during DNA replication, with endogenous DSBs, and in response to genotoxic chemicals (i.e. cisplatin) and physical agents (i.e. radon)

Homology search and strand invasion



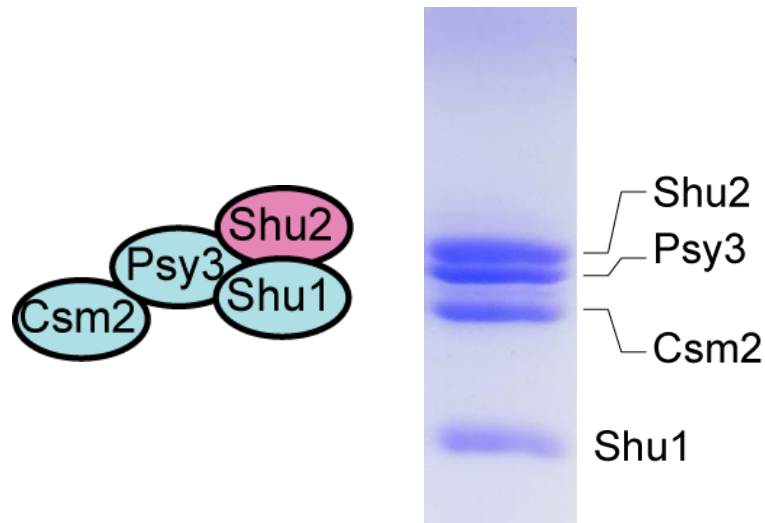
Yeast protein- blue
Human protein- black

Homology search and strand invasion



Yeast protein- blue
Human protein- black

The Shu complex proteins physically interact *in vivo* and *in vitro*



The Shu complex is important for;

- Resistance to DNA damaging agents such as MMS
- Suppressing mutations and gross chromosomal rearrangements

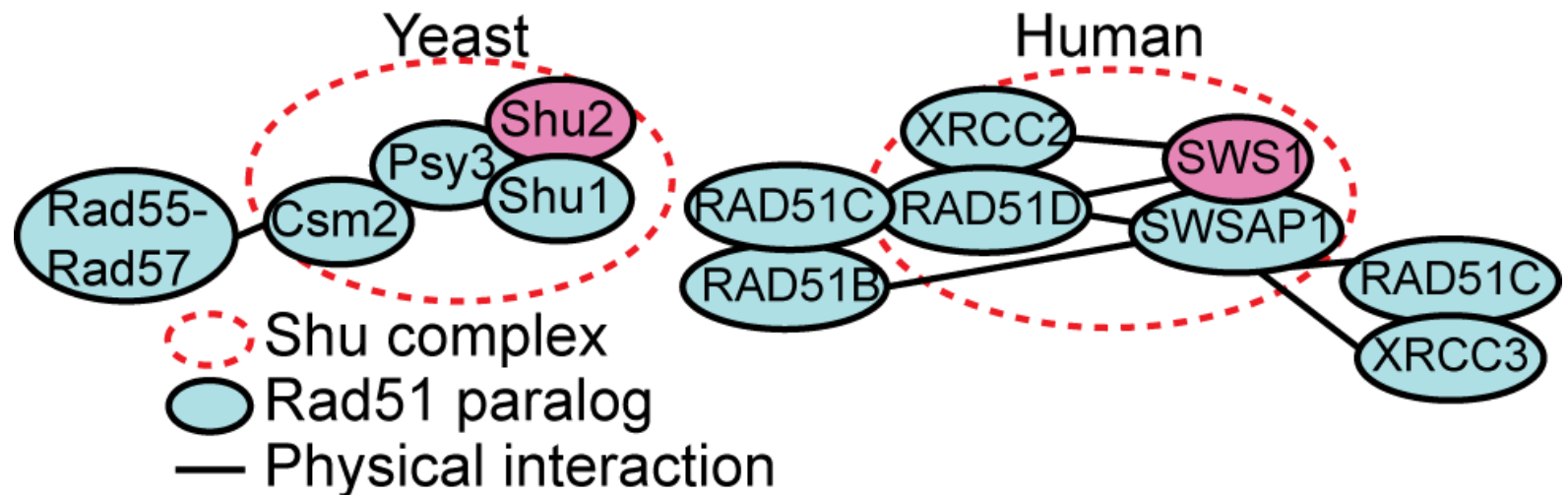
Shor et al (2005) Genetics 169: 1275

Mankouri et al (2007) MBC 18: 4062

Ball (2009) Mol. Microbiol. 73: 89

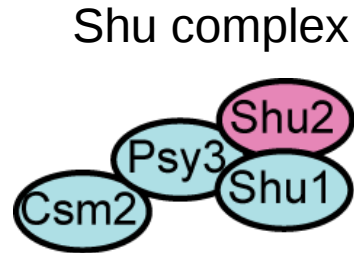
Huang et al (2003) PNAS 100: 11529

Potential human orthologs of the yeast Shu complex



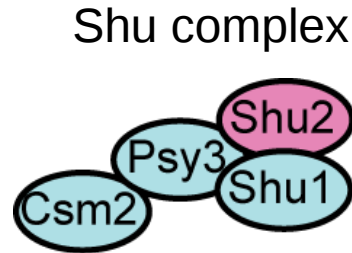
Martin et al (2006) EMBO J. 25: 2564
Liu et al (2011) JBC 286: 41758
Godin et al (2015) Genetics 199: 1023

How does the Shu complex promote recombination?



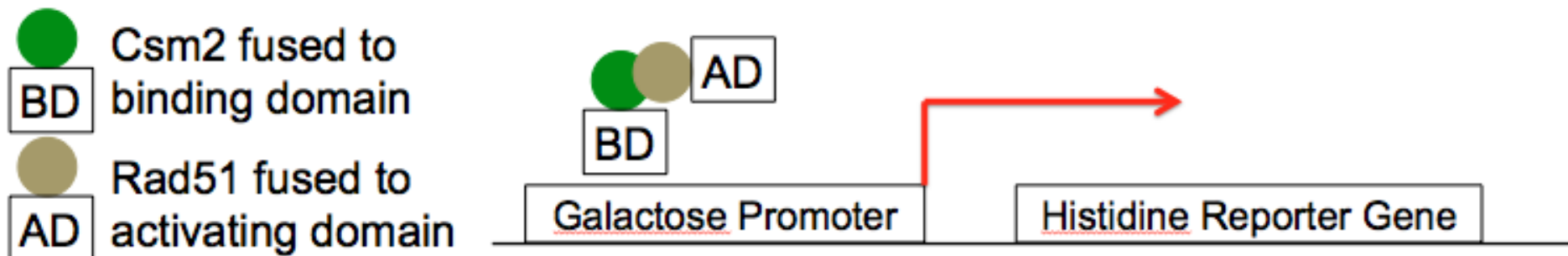
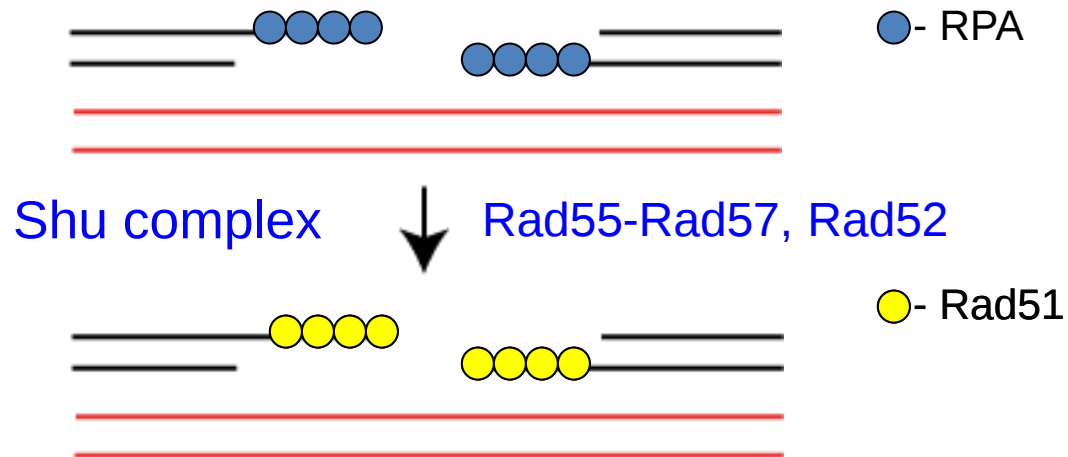
- Shu complex forms a higher order ensemble with Rad52 and Rad55-Rad57
- Rad52-Rad55-Rad57-Shu ensemble acts synergistically to promote Rad51 nucleation and presynaptic filament assembly
- The Shu complex interaction with Rad55 is essential for its role in Rad51 presynaptic filament assembly

How does the Shu complex promote recombination?

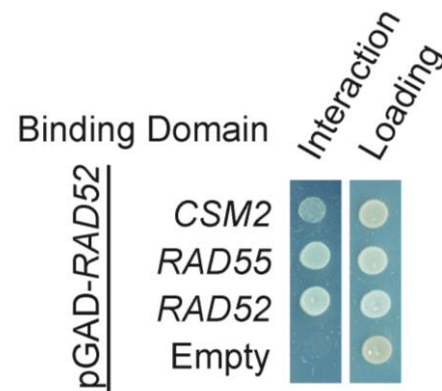
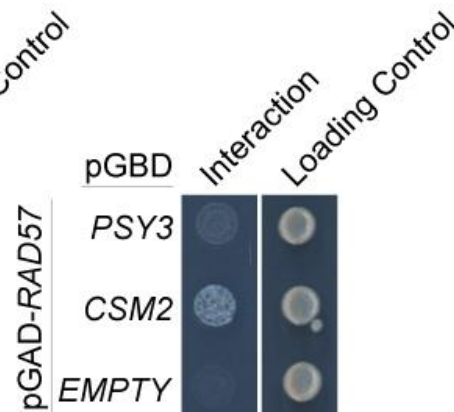
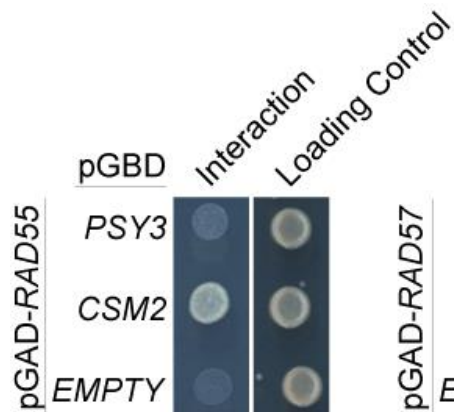
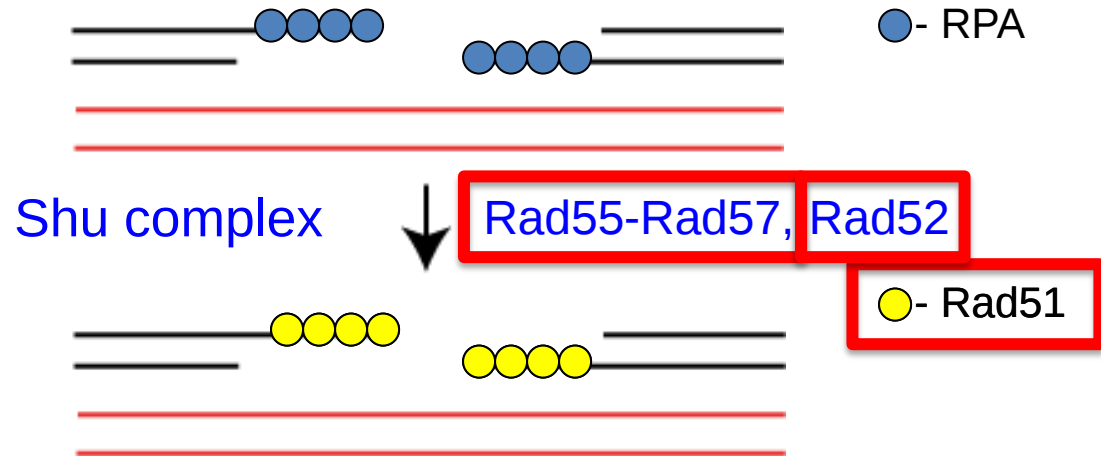
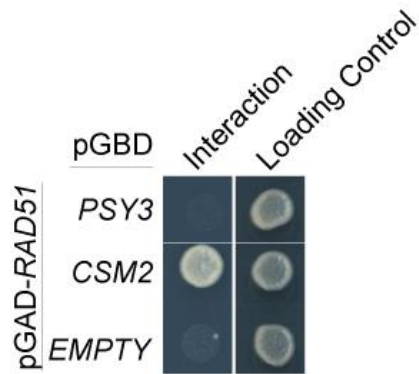


- Shu complex forms a higher order ensemble with Rad52 and Rad55-Rad57
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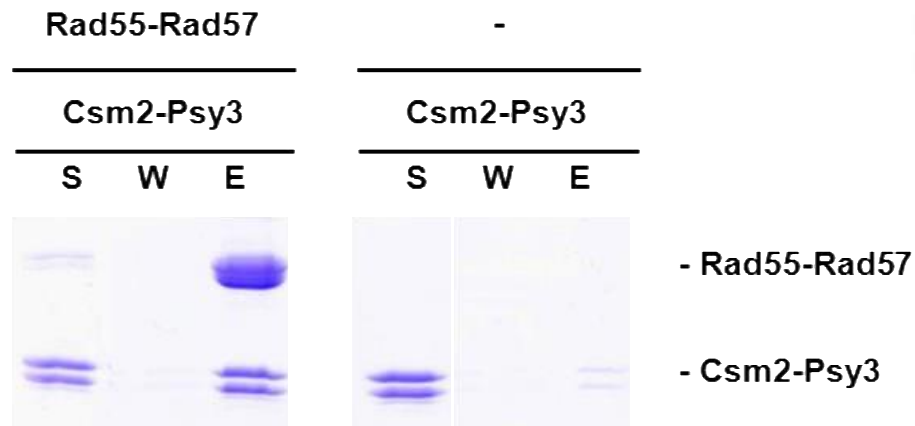
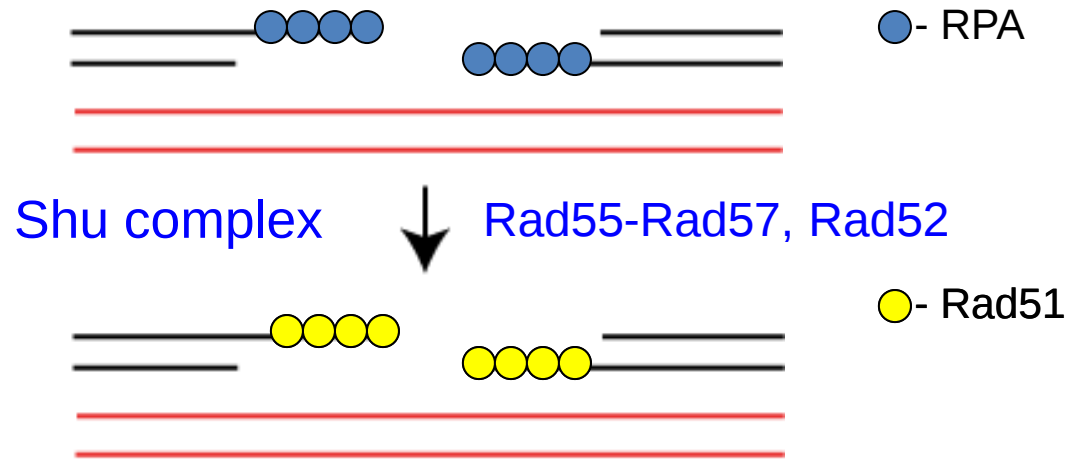
Does the Shu complex interact with other HR proteins?



Csm2 physically interacts with HR proteins (Rad51, Rad55-Rad57, Rad52) by Y2H

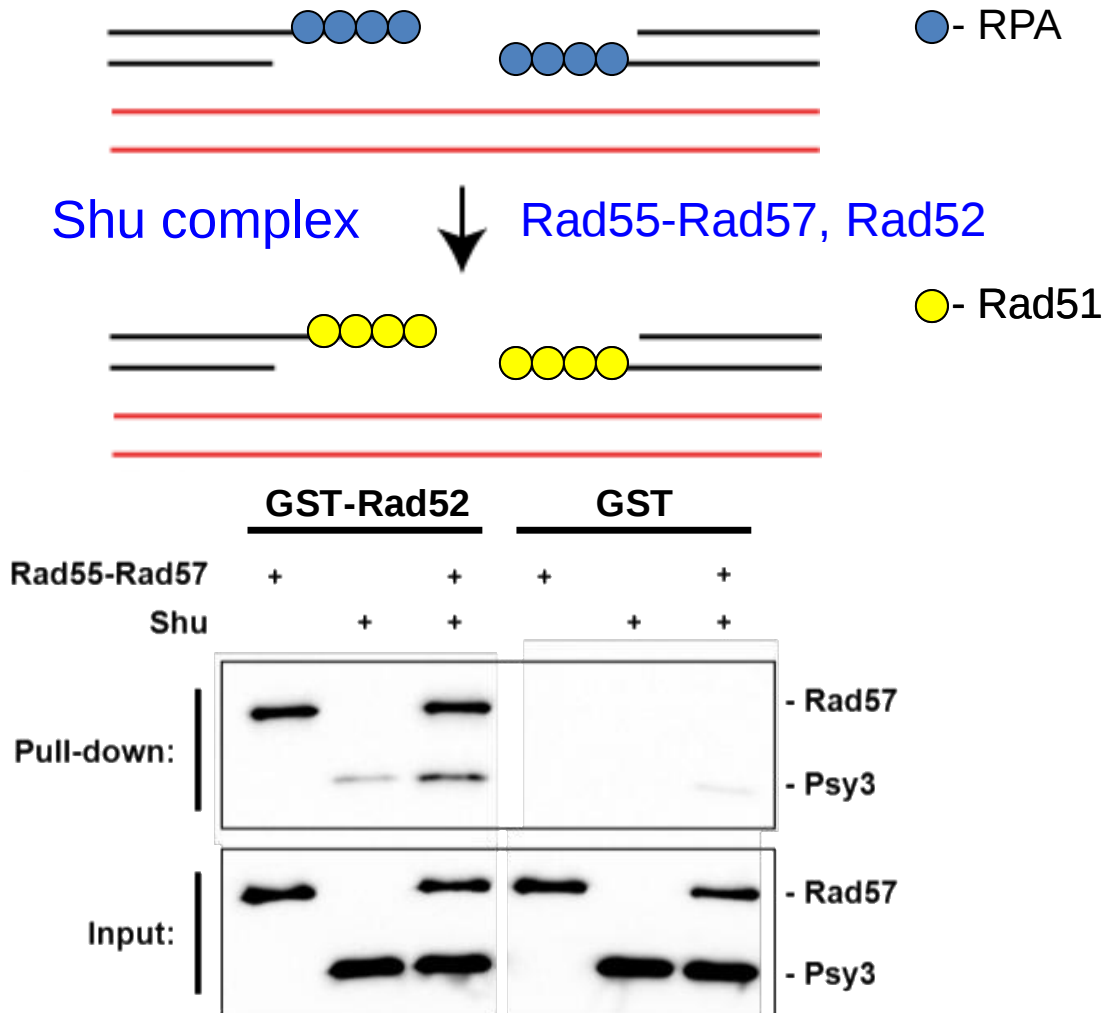


Csm2 directly interacts with Rad55

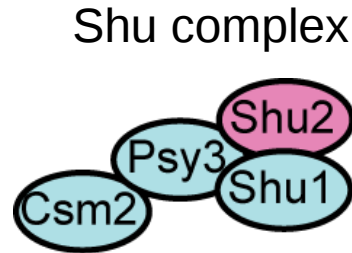


S = Supernatant
W = Wash
E = Eluate

Rad55 can directly interact with Rad52 and Csm2 simultaneously

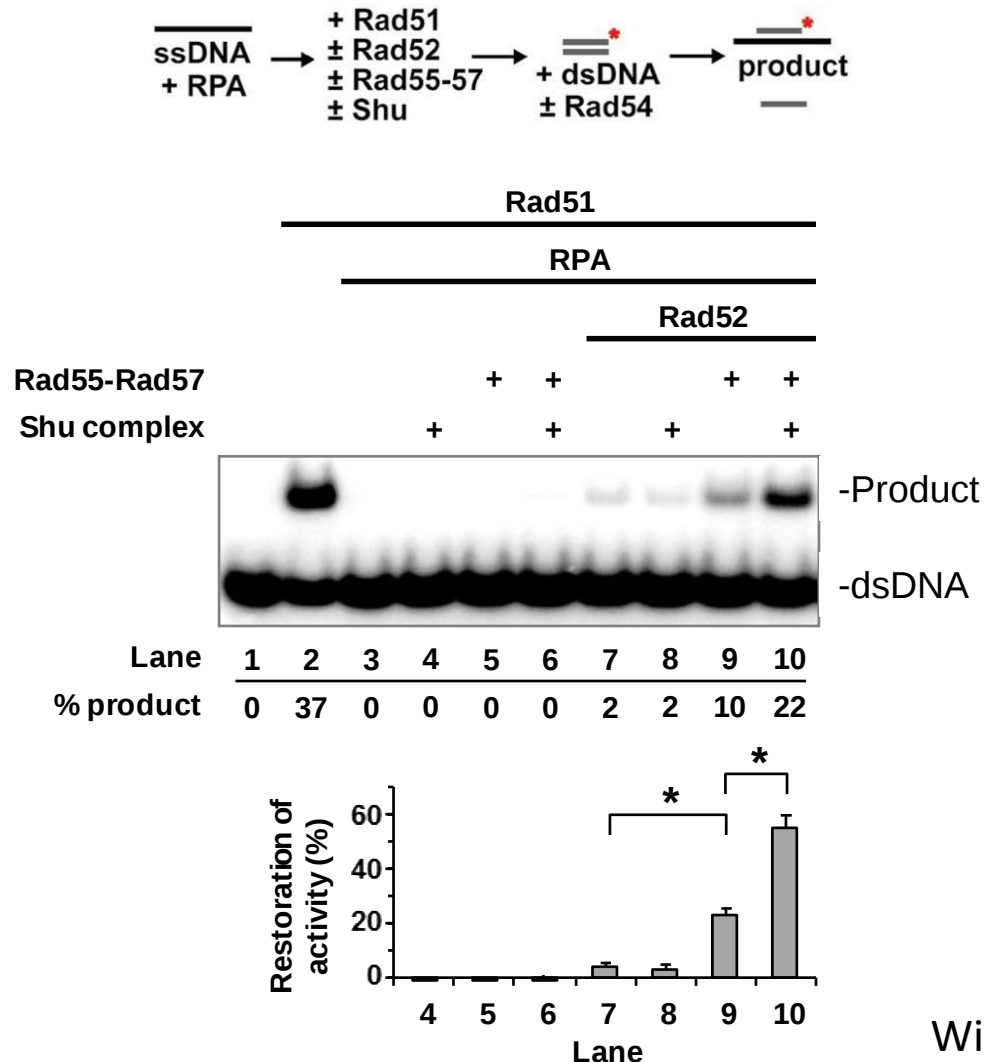


How does the Shu complex promote recombination?

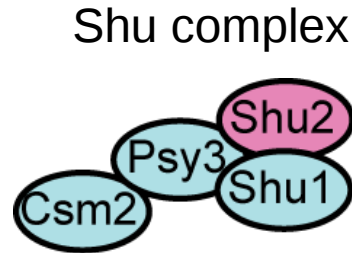


- Shu complex forms a higher order ensemble with Rad52 and Rad55-Rad57
- Rad52-Rad55-Rad57-Shu ensemble acts synergistically to promote Rad51 nucleation and presynaptic filament assembly
- The Shu complex interaction with Rad55 is essential for its role in Rad51 presynaptic filament assembly

Csm2-Psy3 synergizes with Rad55-57 and Rad52 to promote Rad51 filament formation to promote Rad51 filament formation

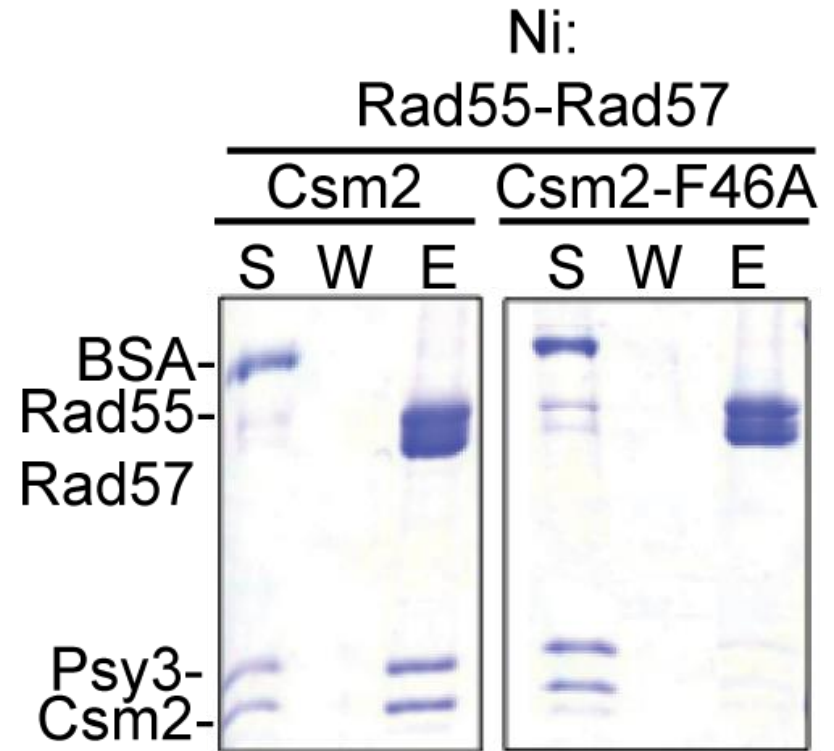
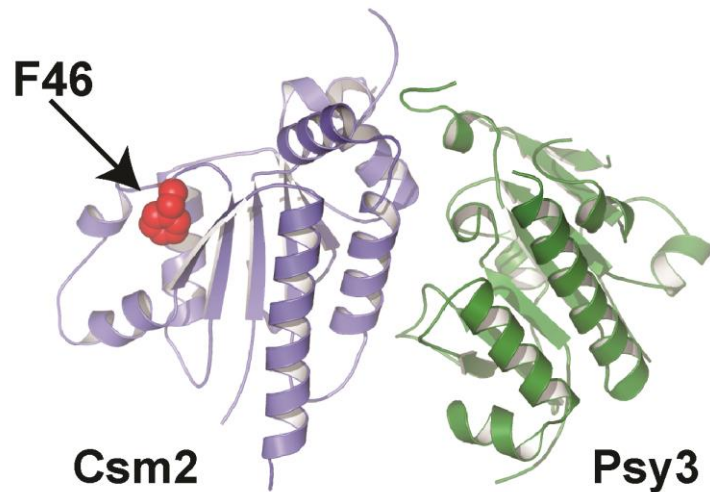


How does the Shu complex promote recombination?

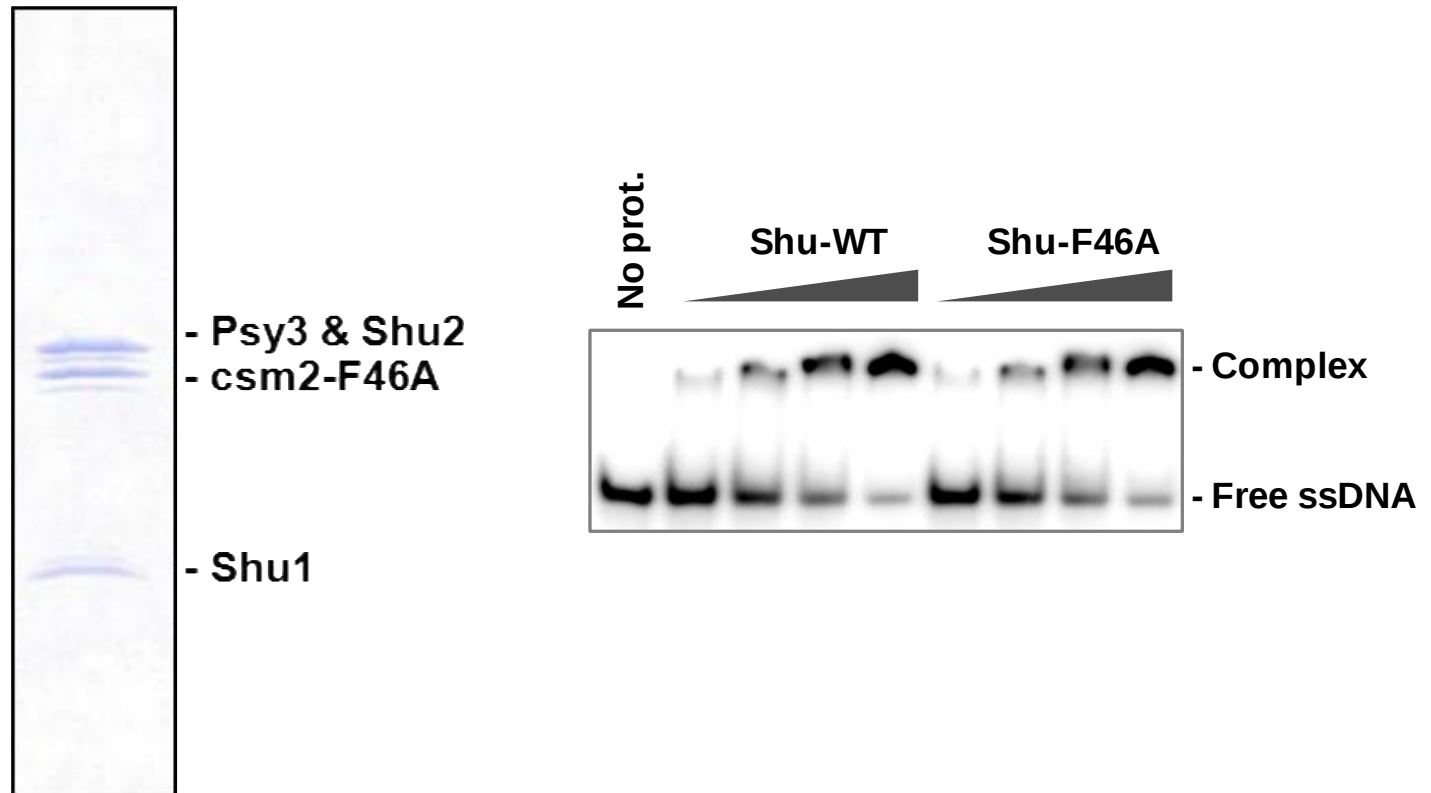


- Shu complex forms a higher order ensemble with Rad52 and Rad55-Rad57
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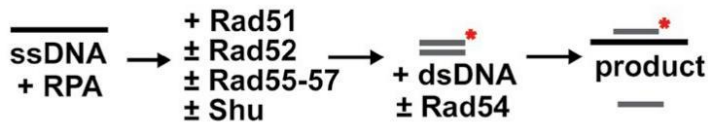
Csm2-F46A no longer interacts with Rad55



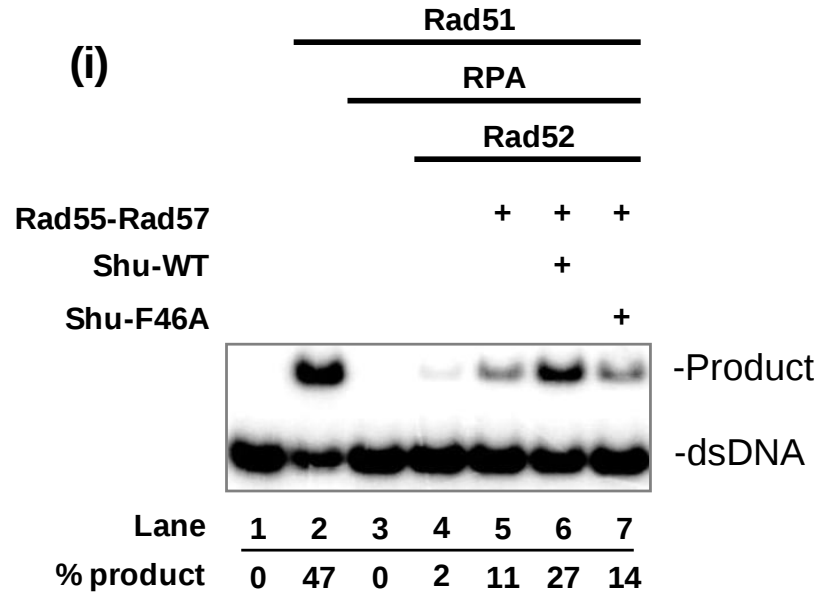
Csm2-F46A is incorporated into the Shu complex and maintains its DNA binding activity



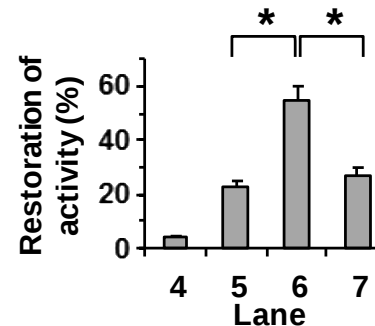
The Shu complex interaction with Rad55 is necessary to stimulate Rad51 filament formation



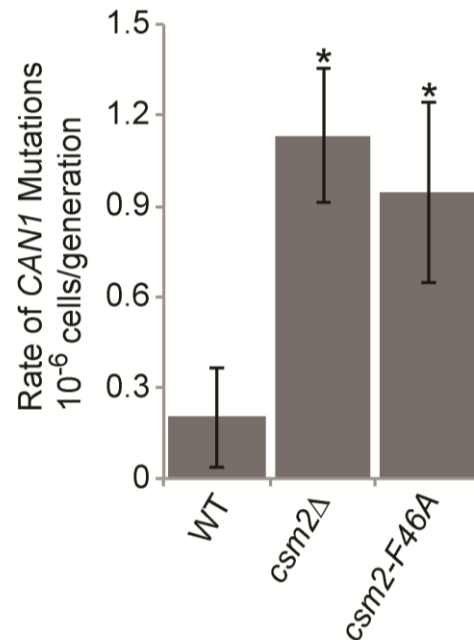
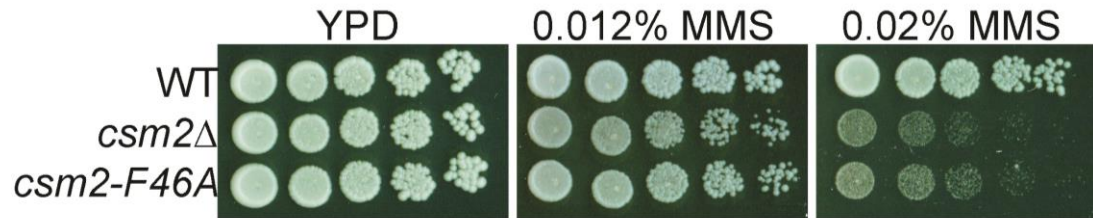
(i)



(ii)



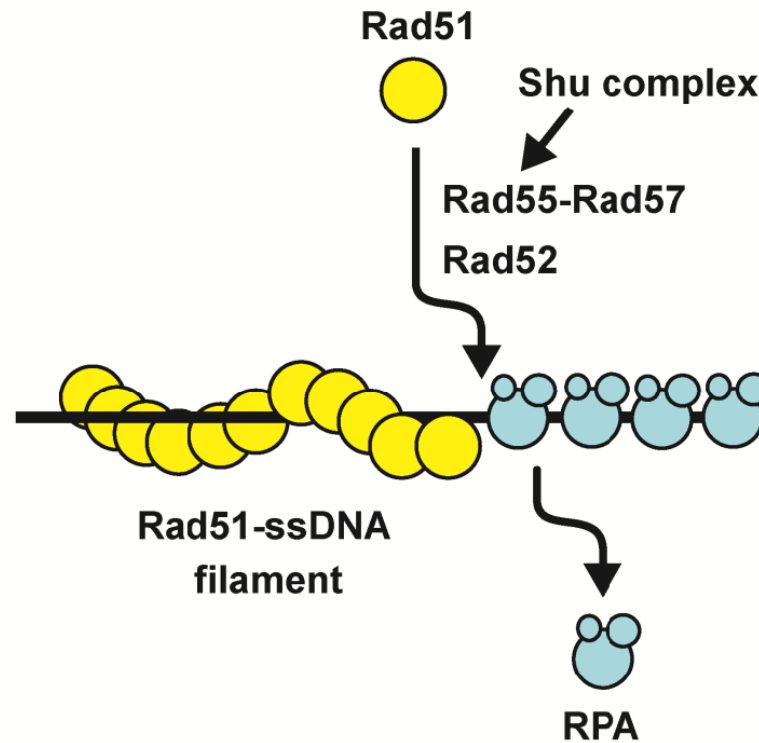
Loss of the Shu complex interaction with Rad55 leads to MMS sensitivity and increased mutagenesis



Outline of Today's Talk

- Overview of cancer and genetic instability
 - Yeast as a model to study human disease
- Novel complex that is an important regulator of DNA recombination
- Model and conclusions

How does the Shu complex promote recombination?



- Shu complex forms a higher order ensemble with Rad52 and Rad55-Rad57
- Rad52-Rad55-Rad57-Shu ensemble acts synergistically to promote Rad51 nucleation and presynaptic filament assembly
- The Shu complex interaction with Rad55 is essential for its role in Rad51 presynaptic filament assembly

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