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Role of MutSγ in Homologous Recombination: A biophysical and biochemical study

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Introduction

MutSγ or Msh4-Msh5 in yeast and humans plays a critical role in meiotic recombination by promoting crossovers, and facilitating proper assembly of the synaptonemal complex. Failure to form crossovers leads to improper segregation of chromosomes and aneuploidy.

MutSγ, a member of the MutS family of proteins, has high levels of sequence identity with MutS homologs involved in repair, but does not bind mismatches or insertion/deletion loops. MutSγ does exhibit strong affinity for Holliday Junctions and other intermediates involved in recombination. To better understand how Yeast Msh4-Msh5 recognizes and binds to these different intermediates, we have developed a structural model of MutSγ

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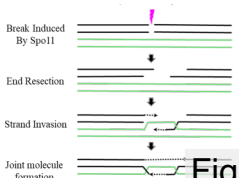

Fig. 1. Schematic model of the steps leading to crossover formation in homologous recombination (HR)..

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Msh4-Msh5 Binds to Model Recombination Intermediates

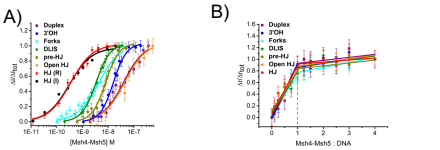
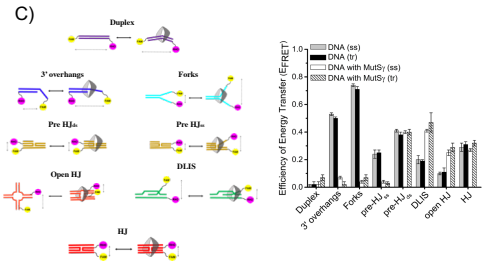


Table 1: *Sc* MutSγ-DNA Equilibrium Dissociation Constants and Stoichiometry

DNA substrates	Open HJ	Duplex	3'OH	Forks	DLIS	Pre-HJ	HJ
Kd (nM) (stoichiometry)	46 ± 7 (1:1)	40 ± 15 (1:1)	27 ± 8 (1:1)	5 ± 1 (1:1)	5 ± 2.5 (1:1)	6 ± 1 (1:1)	0.3 ± 0.1 (1:1)



MutSγ binds to DNA substrates resembling recombination intermediates. Fluorescence spectroscopy measurements of binding affinity demonstrate that Msh4-Msh5 exhibits some affinity for most recombination intermediates (A) and binds to them in a 1:1 stoichiometry (B). Subnanomolar affinity is observed for binding to the junction (Table 1). **C)** Protein binding induces structural changes into the DNA as measured by FRET. Generally, Msh4-Msh5 displaces the single strand in single-strand containing substrates and induces a stacked junction-like structure for D-loop with invading strand, pre-Holliday junction and the open junction.

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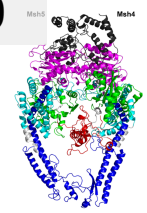
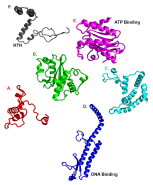
MD-Refined Homology-Modeled Structure of MutSγ

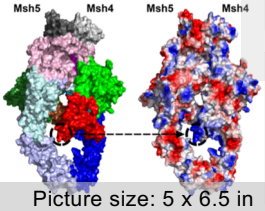
1. Structure-based Sequence Alignment



A. DNA Binding Region
B. Connector Domain
C. Lever Domain
D. Clamp Domain
E. ATPase Domain
F. C-terminal Dimerization Domain



- MD refined homology-modeled structure and assigned functional domains of MutSγ



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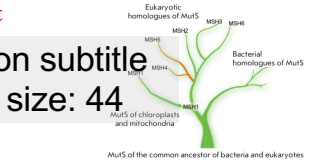

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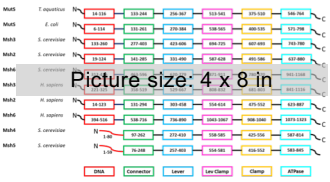

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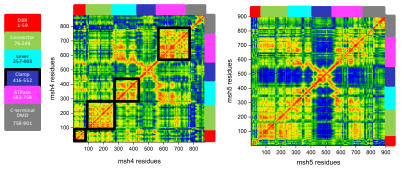
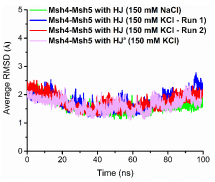
- Using the extensive homology of the MutS family, we generated a structural model of the Msh4-Msh5 protein with I-Tasser and refined it with MD simulation. As shown (right), there is little sequence and structural homology in the N-terminal DNA binding region
- *Sc* Msh4-Msh5 is structurally homologous to Msh2-Msh3 and Msh2-Msh6, but functionally distinct.

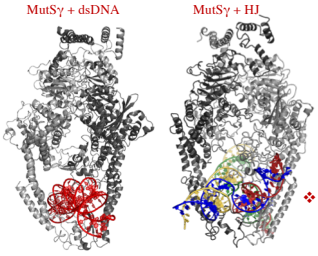


- Dynamic cross-correlation maps visualize correlated motions in the trajectory and identify protein domains and DBR
- Extensive interdomain (off-diagonal) interactions suggest possible allosteric interactions: ATPase, connector and lever domains for example

2. MD-Refined HJ and Duplex Docked Structures

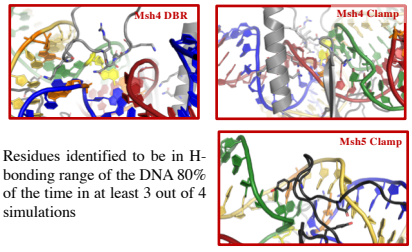
- Structures were generated by docking model HJ and duplex structures into the MD refined model of Msh4-Msh5.
- Docked structures were refined by MD simulation. We ran four parallel simulations of the Msh4-Msh5-HJ complex





- RMSD from four simulations of MutSγ-HJ complex shows an RMSD of 1.8 Å with respect to the average MD structure.

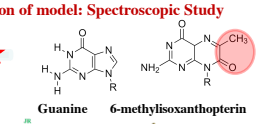
3 main points of contact with the junction: Msh5 clamp, Msh4 clamp and DBR

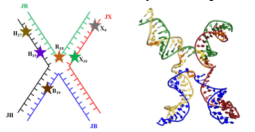


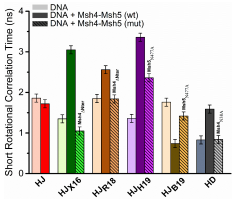
- Residues identified to be in H-bonding range of the DNA 80% of the time in at least 3 out of 4 simulations
- Msh4 DBR: Residues K33, R37, **N38**, **Q39**, **K40**
- Msh4 Clamp: N532, **R534**, K536, R489
- Msh5 Clamp: **N477**, Y480, Y517,

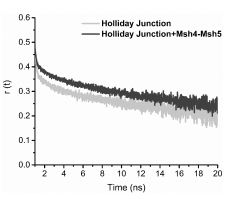
A. Experimental Validation of model: Spectroscopic Study

- Anisotropy measurements yield local and global dynamic information about the Msh4-Msh5-HJ complex
- 6-MI provides site-specific base information



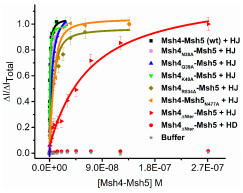






- Anisotropy measurements comparing WT and mutant Sc Msh4-Msh5 reveal that site specific dynamics with mutant protein resemble those of free junction. Global rotational information confirms that mutant and WT complexes are present.
- These results suggest that the protein residues identified in the MD simulations do play a role in interacting with the junction.

B. Experimental Validation of model: Mutational Study

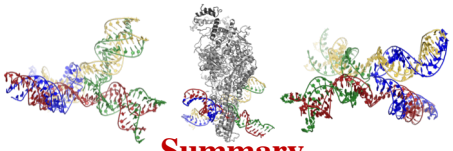


- Site-specific Msh4-Msh5 mutants bind to HJ with 10-fold lower affinity.
- Deletion of Msh4 N-terminal region reduces affinity for HJ 100-fold and abrogates binding to dsDNA.

Table 2

	Wild Type	Msh4N38A-Msh5	Msh4Q39A-Msh5	Msh4K40A-Msh5	Msh4R534A-Msh5	Msh4ΔN38,R534A-Msh5	Msh4-Msh5N477A
Intensity	0.4 ± 0.1	2 ± 0.4	3 ± 0.4	1 ± 0.3	4 ± 1	63 ± 10	5 ± 1

A tetrahedral intermediate to facilitate strand exchange?



Summary

- The MD-refined homology-modelled apo and bound structures reveal a putative DBR, in which MutSγ makes asymmetric contacts with the junction core and arms. Model is consistent with our mutational and spectroscopic studies.
- Domain-domain interactions support presence of DBR and potentially point to allosteric interactions
- In structure docked with HJ, the protein makes three points of contact with the DNA- through the Msh5 clamp and Msh4 DBR and clamp.