Elucidation of Msh4-Msh5's Binding and Hydrolysis of ATP via the Creation of ATPase mutants and Protein Purification using the Maltose Binding Protein

Daniel Mounier, Zane Lombardo and Ishita Mukerji

Department of Molecular Biology and Biochemistry
Wesleyan University, Middletown, CT, 06459

Introduction

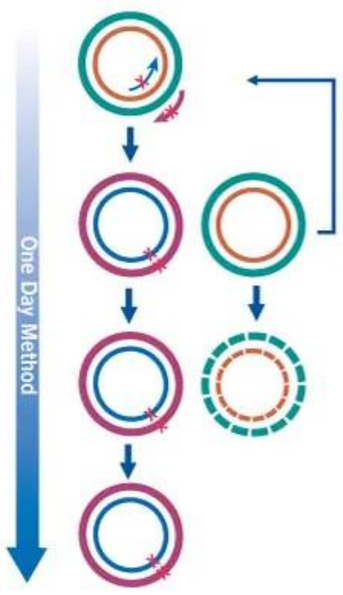
Msh4-Msh5 or MutSγ is a heterodimer that is critical for stabilizing joint molecule DNA recombination intermediates, for promoting crossover recombination, and for proper assembly of the synaptonemal complex.[1] The Cohen research group observed that a mutation in the ATPase domain of Msh5 resulted in disrupted homolog interactions and aberrant DNA repair, leading to a failure of forming any crossing-overs at the end of prophase.[2] Previous experiments have shown the existence of an asymmetry in ATP binding and hydrolysis and in binding DNA substrates in other MutS family proteins. In Msh2-Msh6, mutation of Lys in the Walker A motif disrupted ATP binding and consequent hydrolysis; while mutation of Glu in the Walker B motif disrupted ATP hydrolysis. Heterodimers prepared with Walker A and B mutations introduced into one subunit and one WT subunit revealed differential behavior in which Msh2 exhibited weaker ATP binding affinity and slower rates of hydrolysis relative to Msh6.[3] Introducing analogous mutations in Msh4-Msh5 by converting K640 and E713 to Alanine in Msh4 & K649 and E723 to Alanine in Msh5 will allow us to elucidate whether a similar binding/functional asymmetry exists in Msh4-Msh5.

Objectives

- Create ATPase mutants via site-directed mutagenesis
- Over-express mutated proteins in E. coli
- Purify Msh4-Msh5 mutated proteins using the MBP affinity tag, which increases the solubility of the protein in solution, using an amylose column
- Conduct ATPase assays with mutant proteins to elucidate potential binding/functional asymmetry

Methods

Site-directed mutagenesis:



1. PCR using mutagenic primers to synthesize plasmid containing the mutation of interest
2. DpnI digest of methylated template DNA (newly synthesized product is not methylated)
3. Transformation of synthesized DNA into competent cells for nick repair and selection + Grow overnight culture of chosen colonies

4. Miniprep plasmid to get plasmid with mutation
5. Restriction digest to verify correct sequence length of potential transformants
6. Sequencing of plasmid to make sure mutation was introduced successfully

Results

1. & 2. PCR + DpnI Digest:

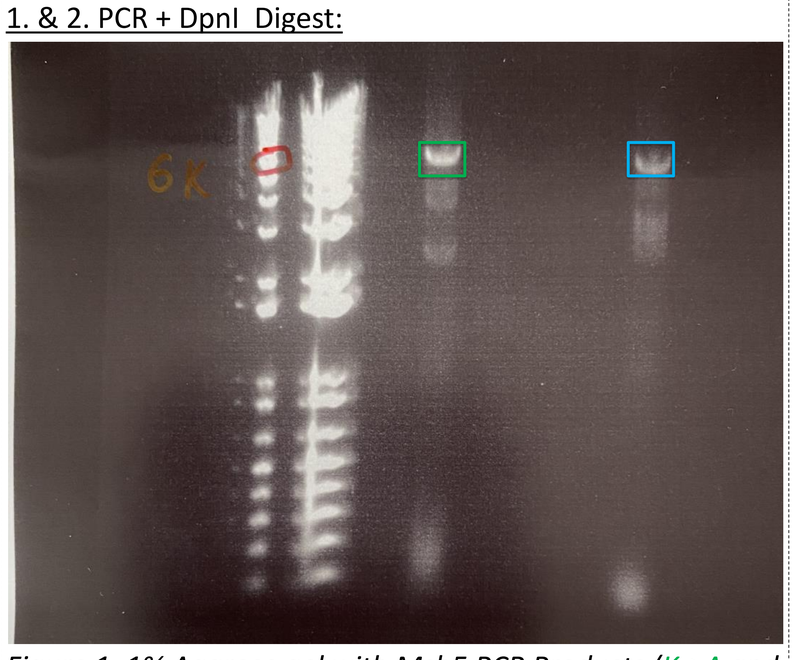


Figure 1. 1% Agarose gel with Msh5 PCR Products (K->A and E ->A)

Expected Product Size: ~6.5 kbp (whole plasmid)

*3. Transformation into NEB 5 alpha E. coli:

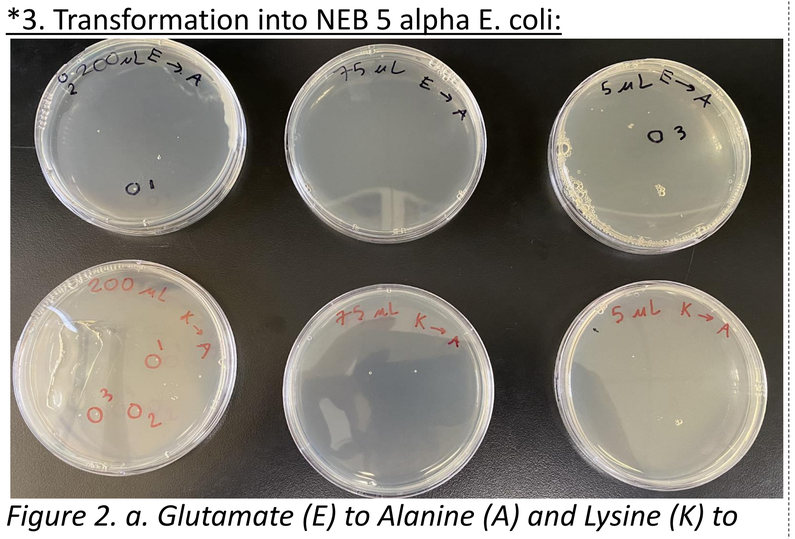


Figure 2. a. Glutamate (E) to Alanine (A) and Lysine (K) to Alanine (A) Msh5 Potential Transformants selected for on media containing Strep (plasmids contain Strep antibiotic resistance gene)

4. & 5. Miniprep + Restriction Digest (EcoRI):

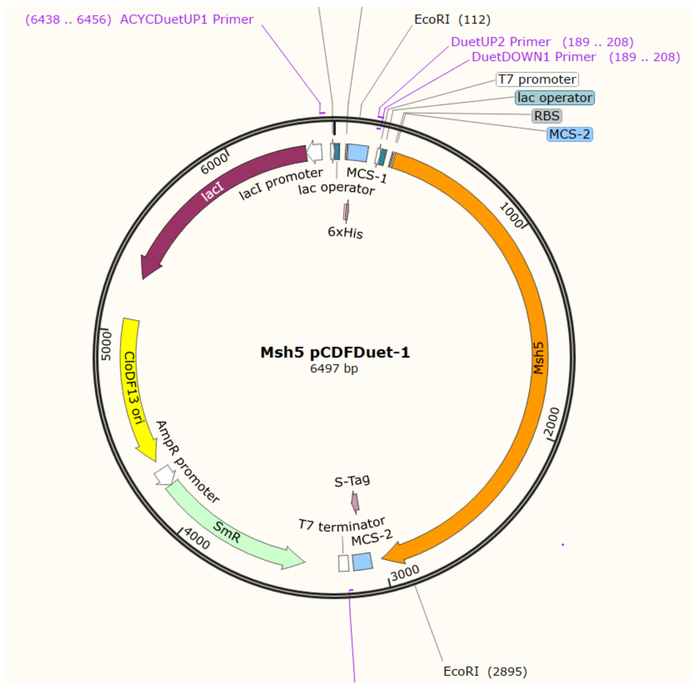


Figure 3. Msh5 pCDFDuet plasmid map with two EcoRI cut sites utilized for restriction digest with EcoRI

Two expected products (2 cut sites):

3714 bp

2783 bp

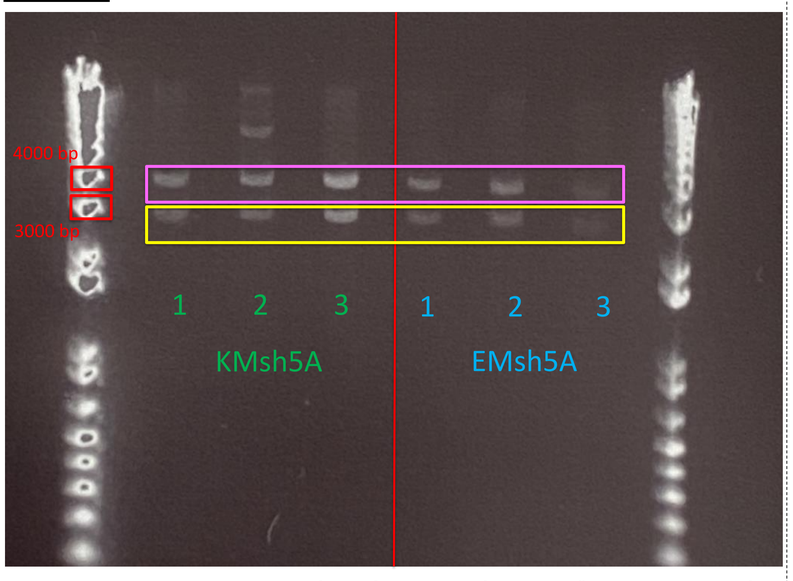


*Figure 4. 1% Agarose gel with EcoRI digest of miniprepped plasmids from different colonies on selection plates (*3.)*

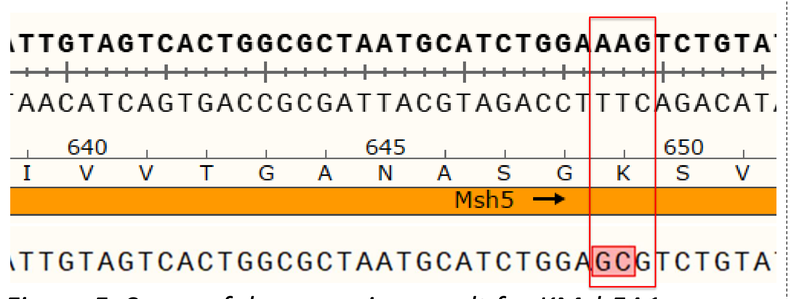
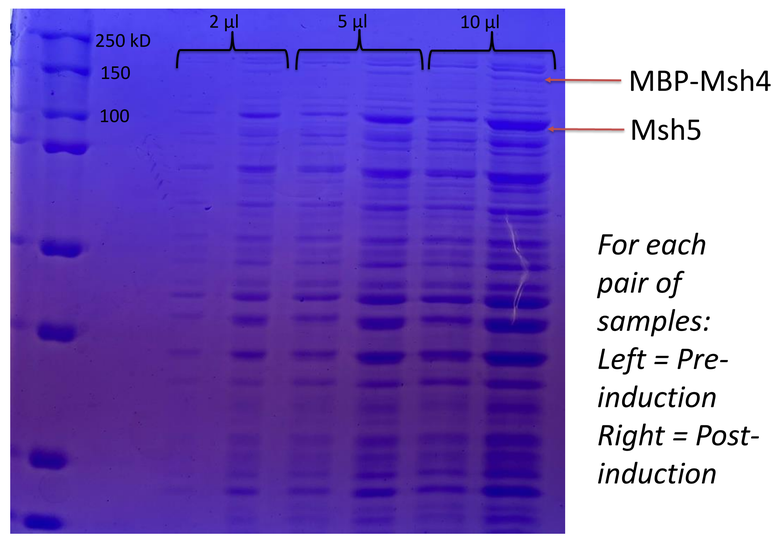


Figure 5. Successful sequencing result for KMsh5A1



For each pair of samples: Left = Pre-induction Right = Post-induction

Figure 6. Pre-induction and post-induction samples of cells containing MBP-Msh4-Msh5

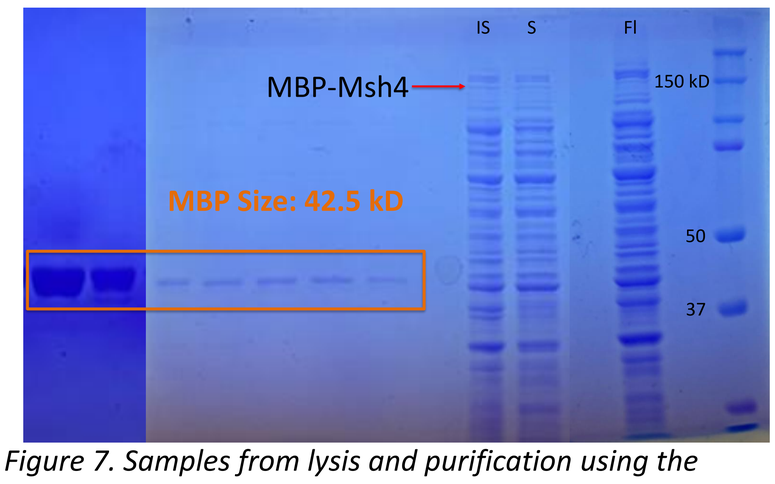


Figure 7. Samples from lysis and purification using the amylose column

References

1. Lahiri, S., Li, Y., Hingorani, M.M., Mukerji, I., 2018. MutSγ-Induced DNA Conformational Changes Provide Insights into Its Role in Meiotic Recombination. Biophysical Journal 115, 2087-2101.. doi:10.1016/j.bpj.2018.10.029
2. Milano, C.R., Holloway, J.K., Zhang, Y., Jin, B., Smith, C., Bergman, A., Edelmann, W., Cohen, P.E., 2019. Mutation of the ATPase Domain of MutS Homolog-5 (MSH5) Reveals a Requirement for a Functional MutSγ Complex for All Crossovers in Mammalian Meiosis. G3 Genes|Genomes|Genetics 9, 1839-1850.. doi:10.1534/g3.119.400074
3. Antony, E., Hingorani, M.M., 2003. Mismatch Recognition-Coupled Stabilization of Msh2-Msh6 in an ATP-Bound State at the Initiation of DNA Repair†. Biochemistry 42, 7682-7693.. doi:10.1021/bi034602h

Future Directions

- Send 6 miniprepped plasmids containing potential Msh5 E->A mutation out for sequencing to verify that the mutation was introduced successfully
- Co-transform all 4 different plasmids into E. coli expression strain with analogous WT subunit
- Grow large quantity of cells containing the various mutants
- Lyse cells and purify protein from cellular extract
- Perform ATPase assays with the various mutant versions of the protein to elucidate a potential functional asymmetry and/or DNA binding asymmetry between the different subunits