Exploring the Binding and ATPase Activity of Msh2-Msh6 with Four Way Junction DNA

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Introduction

Msh2-Msh6 is an ATP-dependent post-replicative mismatch repair protein that recognizes and initiates repair of single base pair mismatches and short insertion deletion loops. Msh2-Msh6 functions via a molecular switch mechanism, whereby hydrolysis and ADP/ATP exchange facilitates the binding and release of the protein from the mismatch. Msh2-Msh6 exhibits basal, DNA-independent ATPase activity. Prior experiments have demonstrated stimulated ATP hydrolysis when Msh2-Msh6 is bound to G:T mismatch and homoduplex DNA, with greater stimulation in G:T-bound protein compared to homoduplex-bound protein (Gradia et al. 1997, Li Yan 2018).

Electron microscopy and in-solution fluorescence assays show that Msh2-Msh6 binds specifically to Holliday Junction DNA with a high affinity similar to what is observed for G:T mismatch (Marsischky et al. 1999, Li Yan 2018). Based on the EM image, Msh2-Msh6 appears to bind the center of a junction in the open conformation. However, the structural and functional mechanisms of this binding interaction are not well characterized.

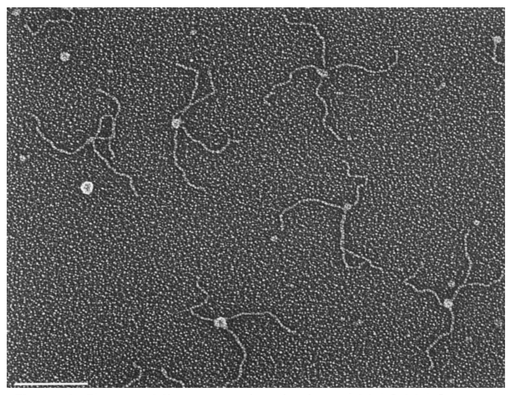


Figure 1. Electron Microscopy visualization of Msh2-Msh6 bound to the center of Holliday Junction DNA. (Marsischky et al. 1999)

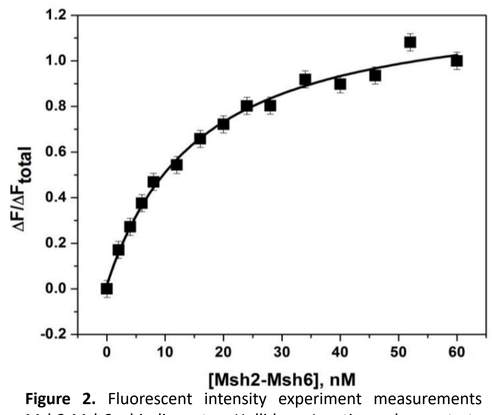
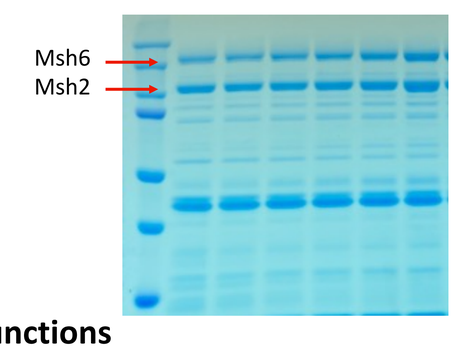


Figure 2. Fluorescent intensity experiment measurements of Msh2-Msh6 binding to Holliday Junction demonstrate a K_d=12.9±1.3 nM (Li Yan 2018)

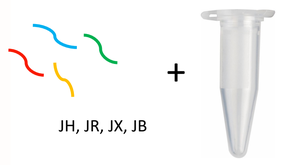
Materials and Methods

I. Protein Purification of Msh2-Msh 6

Co-Transformation into BL21-DE3
Growth; Over-expression; Lysis
Fast Protein Liquid Chromatography



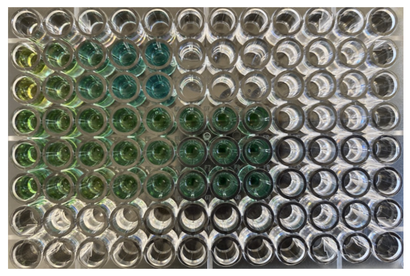
II. Annealing & Characterization of Mismatch Junctions



Combine C to T mutated J3 single strands arms in buffer
70ºC for 8 hours, cool to 25ºC for 16 hours, -20ºC for 24 hours
Followed by melting point experiments to determine T_m

III. ATPase Activity Assays

Colorimetric determination of free phosphate Release by Msh2-Msh6 incubated w/ DNA substrates per minute for 7 minutes (deeper blue green color represents higher [P_i] as shown to the right)



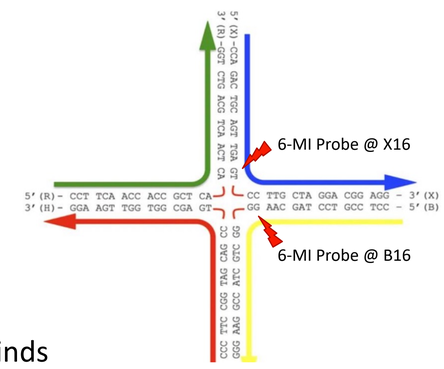
IV. Binding Assays

A. Gel Mobility Shift Assay

Fixed [DNA] + increasing [Msh2-Msh6]
Visualize via Sybr Green stain

B. Fluorescence In-Solution Assays

Fixed [6-MI labelled DNA] + increasing [Msh2-Msh6]
Measure change in fluorescence intensity as protein binds



Results

I. Melting Point Characterization of New Mismatch Junctions

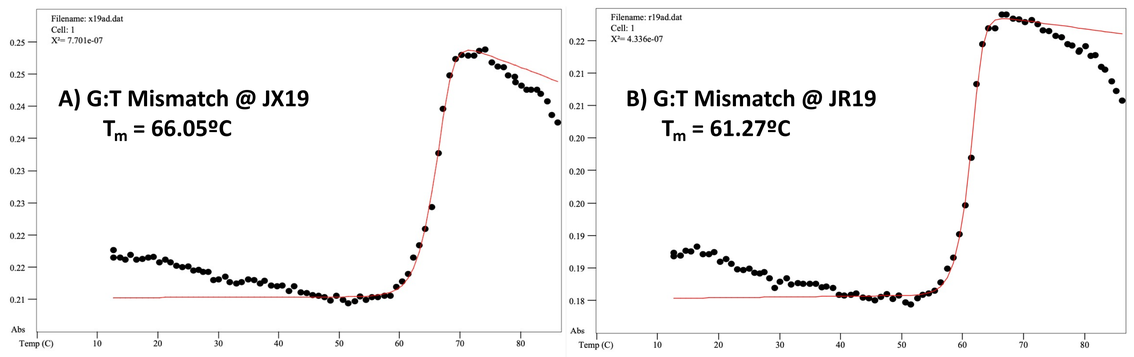


Figure 3. A) Melting data plotting absorbance vs temperature (Cº) of J3 Junction containing a G:T mismatch at position 19 of arm JX **B)** Melting data plotting temperature (Cº) vs absorbance of J3 Junction containing a G:T mismatch at position 19 of arm JR. T_m of normal J3 junction is around 64ºC, with measurement of errors of about 1-2ºC.

II. ATPase Malachite Green Assay

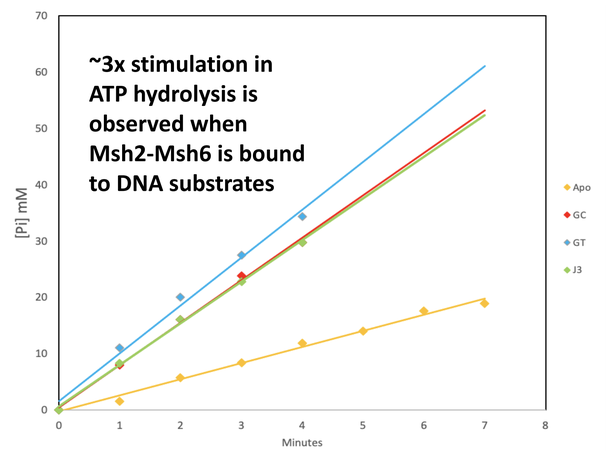
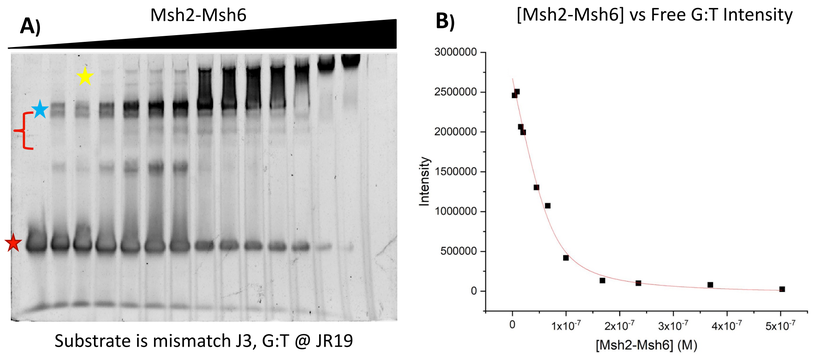


Figure 4. Plot of free phosphate concentration vs time when Msh2-Msh6 is incubated with no DNA (yellow), GC duplex (red), GT duplex (blue), and J3 junction (green). K_{cat} (s^{-1}) values are as follows: Apo = 0.62, GC = 0.63, GT = 0.66, J3 = 0.62.

III. Binding Assays

Gel Mobility Shift Assay Determination of K_d



C)

Substrate	K_d (nM)
G:T	10.1 ± 5.7
J3	9.1 ± 2.6
G:T @JX19	11.3 ± 7.6
G:T @ JR19	23.5 ± 11.8

Figure 5. A) 4.5% Native Gel illustrating increasing concentrations Msh2-Msh6 incubated with J3 junction containing a mismatch at JR position 19. The band labelled with a red star represents free DNA that disappears as Msh2-Msh6 binds. The larger band labelled with a blue star represents Msh2-Msh6 bound mismatch J3, and the highest band labelled with a yellow star represents non-specific binding activity of Msh2-Msh6. The bands in brackets represents bands seen uniquely in GMSAs with mismatch J3 that have yet to be characterized. B) Plot of Free DNA band intensity change vs concentration of Msh2-Msh6. C) K_d values of Msh2-Msh6 bound to G:T, J3, and two mismatch J3 junctions.

Fluorescence Intensity Determination of K_d

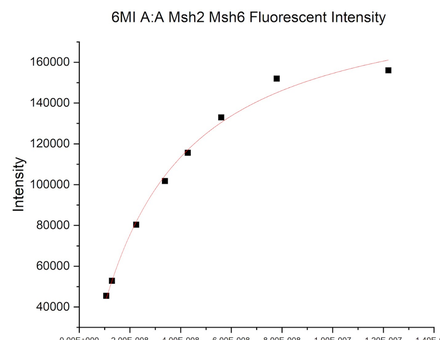


Figure 6. Msh2-Msh6 of increasing concentration incubated with 6MI A:A DNA plotted against fluorescence intensity change. Curve fit determined a K_d of 24 ± 6nM

Conclusions

1. Introducing a mismatch into Holliday Junction DNA does not significantly impede junction stability. New mismatch junctions will be annealed containing G:T mismatch in the center and G:T mismatch in the junction arm.
2. ATPase activity is weakly stimulated when Msh2-Msh6 binds to junction, but slightly less so than when Msh2-Msh6 is bound to G:T mismatch duplex DNA. Further ATPase and ATP affinity experimentation with J3/mismatch J3 are needed to understand Msh2-Msh6 ATPase behavior when bound to these various substrates.
3. Msh2-Msh6 binding affinity is not affected by the presence of a mismatch at the center of the junction, but binding may take on multiple conformations. More in-solution assays and GMSAs with 6-MI labelled J3/mismatch J3s are required to understand Msh2-Msh6 behavior in the presence of its well-characterized mismatch substrate vs. its behavior in the presence of junction DNA.

Acknowledgments

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References

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