Investigating the physical interaction between HMGB2 and the important players in genome organization, cohesin and CTCF

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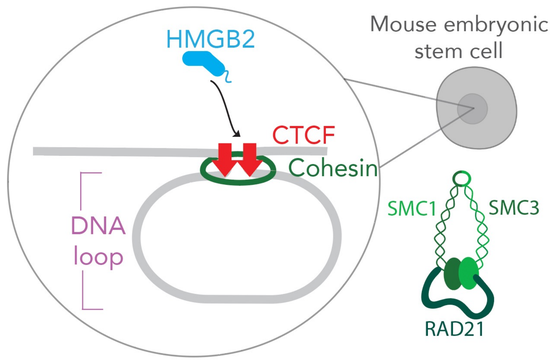
Introduction



Big Question: Does HMGB2 physically interact with the architectural proteins cohesin and/or CTCF?

Figure 1. Cohesin is a ring-like protein complex that extrudes DNA to organize into DNA loops, brining linearly distant segments of DNA into close physical proximity in the three-dimensional space. Cohesin interacts with a protein known as CTCF in order to form most DNA loops. Cohesin and CTCF are essential for genome organization at the DNA loop level as loss of either eliminates loop domains[1]. HMGB2 shares binding sites with CTCF across the genome. Loss of HMGB2 has also been shown to decrease the number of DNA loops[2].

Creation of HMGB2 Overexpression Plasmid via Gibson Cloning and Site Directed Mutagenesis

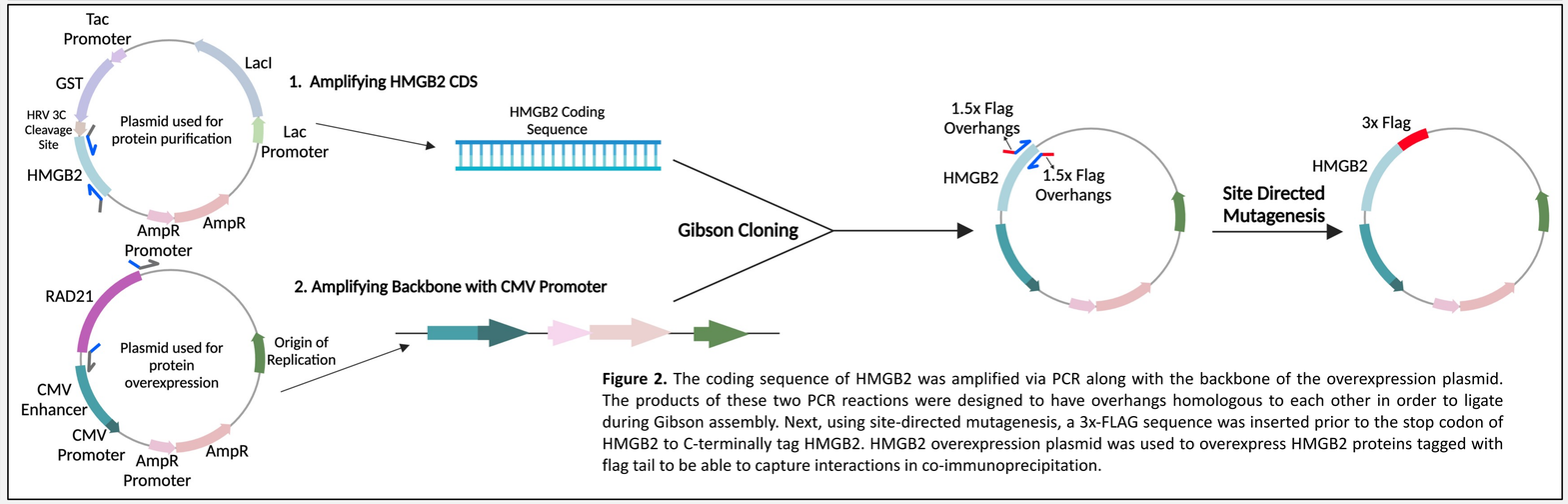


Figure 2. The coding sequence of HMGB2 was amplified via PCR along with the backbone of the overexpression plasmid. The products of these two PCR reactions were designed to have overhangs homologous to each other in order to ligate during Gibson assembly. Next, using site-directed mutagenesis, a 3x-FLAG sequence was inserted prior to the stop codon of HMGB2 to C-terminally tag HMGB2. HMGB2 overexpression plasmid was used to overexpress HMGB2 proteins tagged with flag tail to be able to capture interactions in co-immunoprecipitation.

Overexpression of HMGB2-3xFLAG in mouse embryonic stem cells

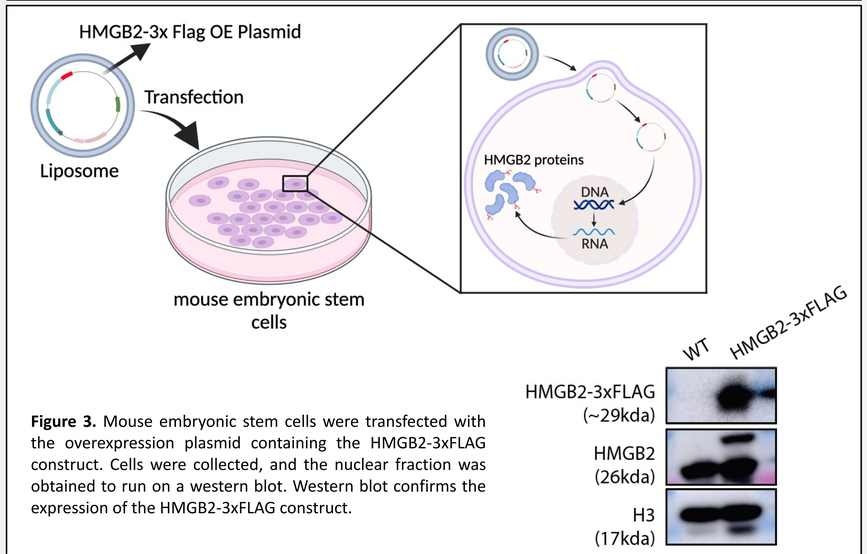


Figure 3. Mouse embryonic stem cells were transfected with the overexpression plasmid containing the HMGB2-3xFLAG construct. Cells were collected, and the nuclear fraction was obtained to run on a western blot. Western blot confirms the expression of the HMGB2-3xFLAG construct.

Identifying physical interactions between HMGB2, cohesin, and CTCF via co-immunoprecipitation

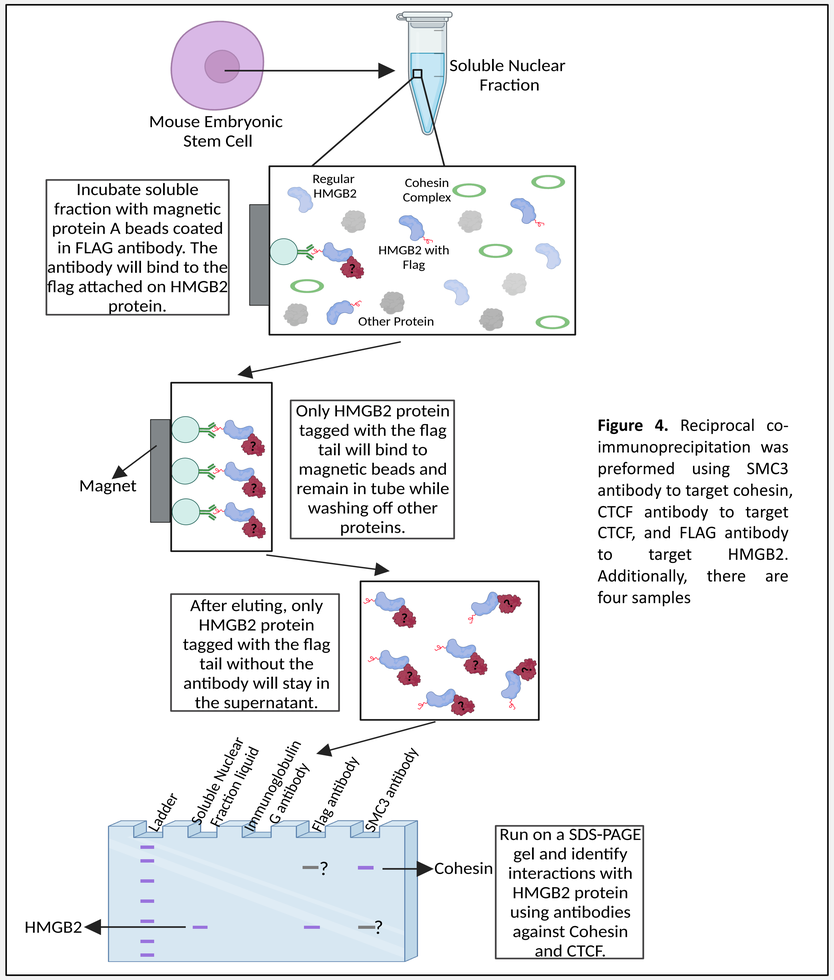


Figure 4. Reciprocal co-immunoprecipitation was preformed using SMC3 antibody to target cohesin, CTCF antibody to target CTCF, and FLAG antibody to target HMGB2. Additionally, there are four samples

HMGB2 does not interact with cohesin/CTCF under high stringency conditions

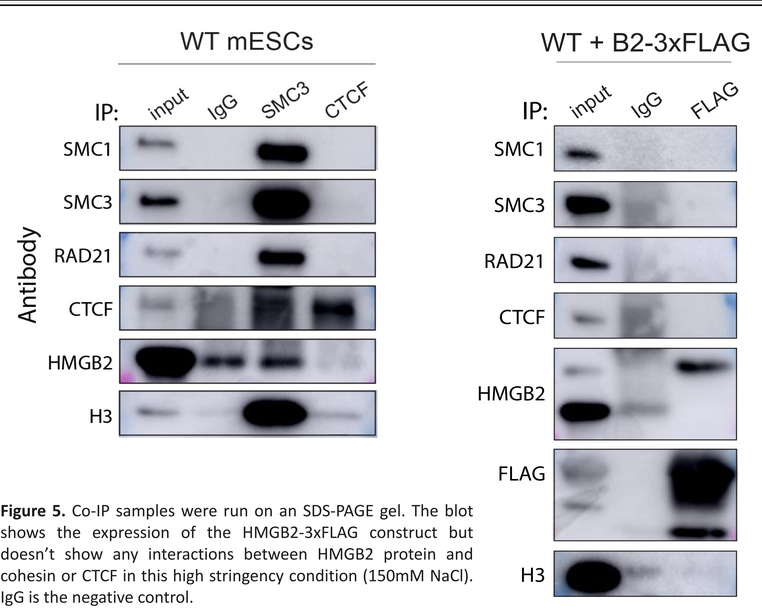


Figure 5. Co-IP samples were run on an SDS-PAGE gel. The blot shows the expression of the HMGB2-3xFLAG construct but doesn't show any interactions between HMGB2 protein and cohesin or CTCF in this high stringency condition (150mM NaCl). IgG is the negative control.

Future Direction

Our first designed co-immunoprecipitation was conducted under the high stringency condition (150mM NaCl). Under high stringency conditions, only strong interactions between proteins are captured. Therefore, if the interaction between HMGB2 and cohesin/CTCF is weak, it will not be captures under these conditions. However, under the lower stringency condition (75mM NaCl), weak interactions between proteins can be captured be detectable. Our future directions are to perform reciprocal co-immunoprecipitation with same samples under the low stringency condition. If there is a weak interaction between HMGB2 and cohesin/CTCF, we expect to see bands in the FLAG IP column when blotting for cohesin subunits (SMC1, SMC3, RAD21).

Acknowledgements

I want to express my special thanks of gratitude to my bench mentor Riya Gohil and Principal Investigator Dr. Jill Dowen. I appreciate the help from all members in the Jill Dowen Lab, including Natalie Rittenhouse, Benjamin Kornegay, Geoffrey Fox, Rebecca Lewis, and June Arricastres.

References

1. Rao SSP, Huang S-C, Glenn St Hilaire B, Engreitz JM, Perez EM, Kieffer-Kwon K-R, et al. Cohesin loss eliminates all loop domains. Cell. 2017 Oct 5;171(2):305-320.e24.
2. Zirkel A, Nikolic M, Sofiadis K, Mallm J-P, Brackley CA, Gothe H, et al. HMGB2 Loss upon Senescence Entry Disrupts Genomic Organization and Induces CTCF Clustering across Cell Types. Mol Cell. 2018 May 17;70(4):730-744.e6.