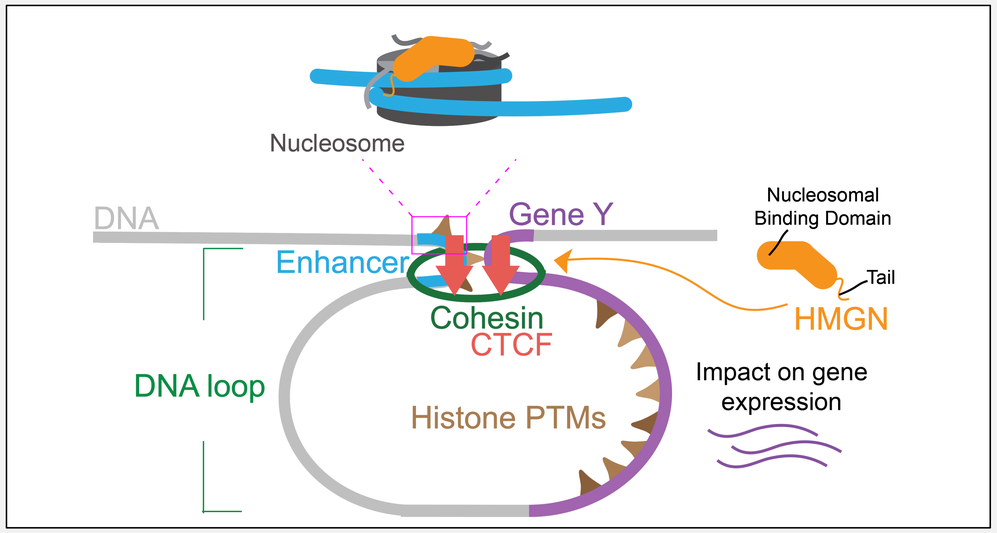
Investigating the role of HMGN proteins in genome organization

Becca Lewis[1], Riya Gohil[2] , Zhihan Gao[1], and Jill Dowen[2,3]
[1]Department of Biology, University of North Carolina, Chapel Hill, NC USA, [2]Curriculum in Genetics and Molecular Biology,
[3]Department of Biochemistry and Biophysics

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Role of HMGN proteins in chromatin organization and gene expression



HMGN1 and HMGN2 colocalize with marks of active chromatin

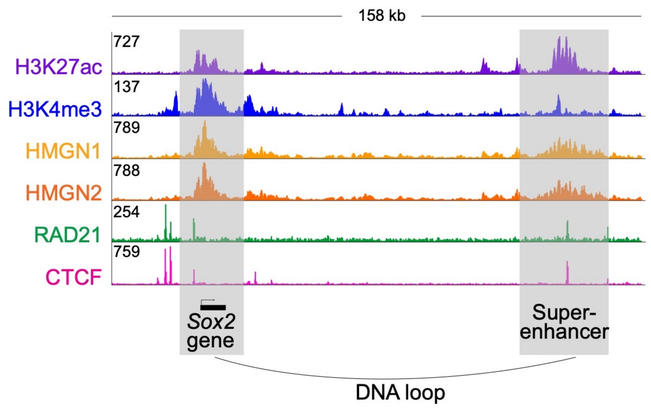


Figure 1: ChIP-seq data shows that HMGN1 and HMGN2 colocalize with active histone marks, Cohesin (RAD21), and CTCF.

Big Questions

1. How does loss of HMGNs affect gene expression?
2. What roles do HMGN proteins play in Cohesin/CTCF-mediated DNA looping?
    a) Do HMGNs interact with Cohesin and CTCF?
    b) How does loss of HMGNs affect Cohesin and CTCF localization?

Generation of *Hmgn1*$^{-/-}$ *Hmgn2*$^{-/-}$ mESC line

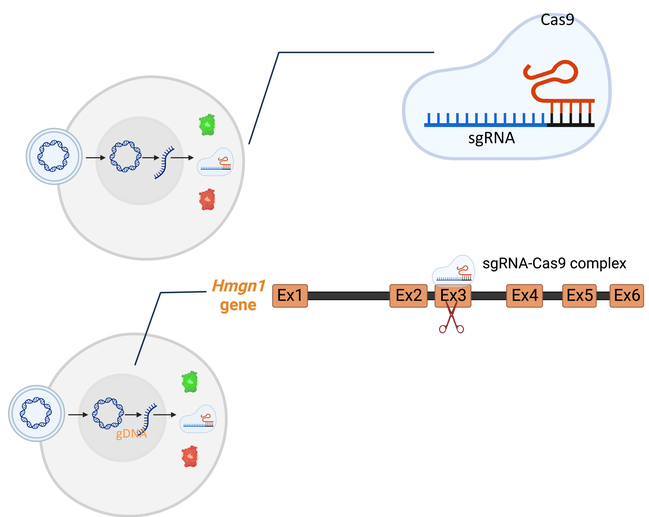


Figure 2: *Hmgn2*$^{-/-}$ cells were transfected with plasmids containing Cas9, GFP, mCherry, and an sgRNA targeting exon 3 of *Hmgn1*. Transfection efficiency was approximated to be about 25%. Individual cells were isolated, grown up, and screened via RT-qPCR and western blot.

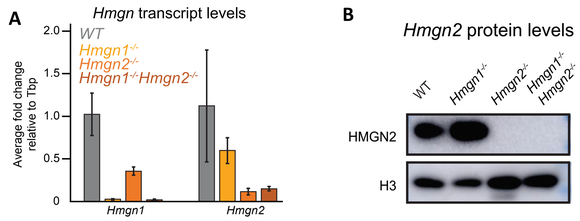


Figure 3: A) RT-qPCR confirmation of the loss of *Hmgn1* and *Hmgn2*. B) Western Blot confirmation of the loss of HMGN2.

RNA-seq reveals differential expression upon loss of *Hmgn1* and *Hmgn2*

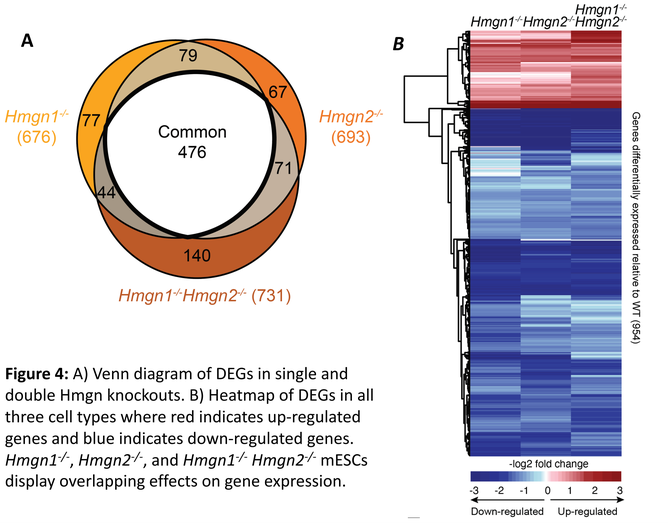


Figure 4: A) Venn diagram of DEGs in single and double Hmgn knockouts. B) Heatmap of DEGs in all three cell types where red indicates up-regulated genes and blue indicates down-regulated genes. *Hmgn1*$^{-/-}$, *Hmgn2*$^{-/-}$, and *Hmgn1*$^{-/-}$ *Hmgn2*$^{-/-}$ mESCs display overlapping effects on gene expression.

HMGN2 and Cohesin/CTCF do not co-purify

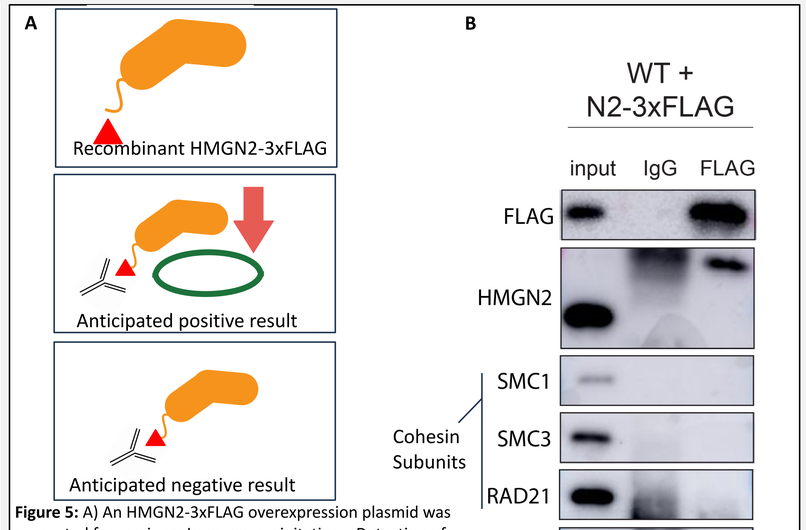


Figure 5: A) An HMGN2-3xFLAG overexpression plasmid was generated for use in co-Immunoprecipitations. Detection of Cohesin/CTCF in our FLAG-IP would be a positive result. No detection of Cohesin/CTCF is a negative result. B) Co-IPs were performed at 150mM NaCl (approximate physiological conditions) with nuclear extracts from wildtype cells transfected with HMGN2-3xFLAG. Co-IPs were run out on an SDS-PAGE gel and blotted for Cohesin, CTCF, H3, and FLAG.

Conclusions and Future Directions

Conclusions

- Loss of HMGN1 and HMGN2 leads to differential expression of approximately 700 genes.
- HMGN1 and HMGN2 colocalize on the genome with Cohesin and CTCF, yet do not copurify in a complex.

Future Directions

- Measure global changes in histone PTM levels in WT and HMGN knockout mESCs.
- Measure direct binding of HMGN proteins to nucleosomes with various histone PTMs and deduce preferential binding.
- Investigate how H3K27ac and H3K4me3 changes upon loss of HMGN proteins via ChIP-seq.

References

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