Investigating the role of HMGN proteins in genome structure

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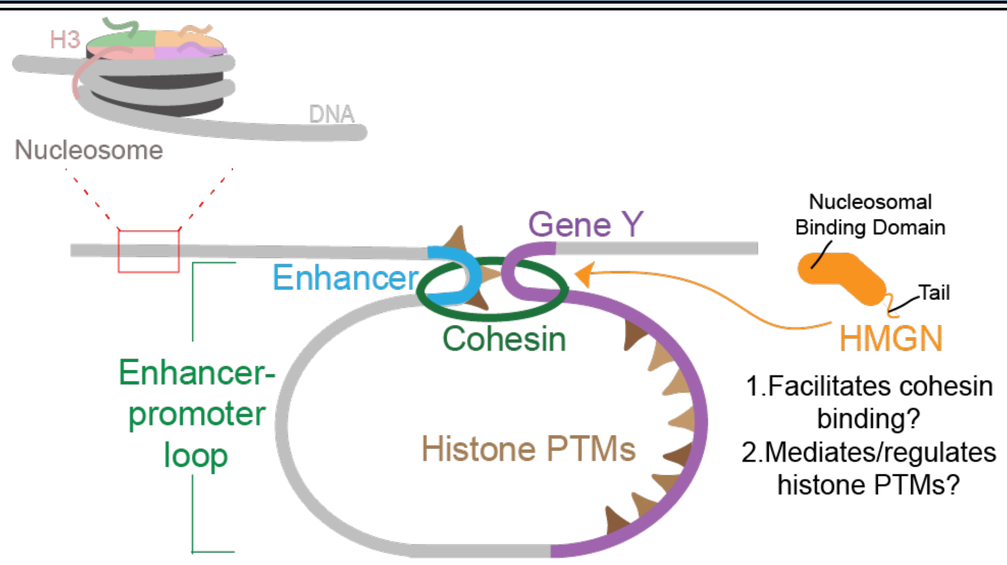
How do HMGN proteins shape the genome?



Introduction

Regulation of gene expression is governed by proper organization of the genome. The histone landscape regulates the accessibility of DNA for gene expression and DNA loops regulate the proximity of DNA regulatory elements to genes. This multi-level organization of the genome is essential for proper cell function, yet the precise details of this system are not well understood. The High Mobility Group Nucleosome-binding proteins (HMGNs) are found on the genome at active sites, such as enhancers and promoters, that are marked by specific histone post-translational modifications (PTMs) such as H32K27ac and H3K4me1[1]. Another important architectural complex found at enhancers and promoters is cohesin, which mediates DNA loops. HMGN1, HMGN2, cohesin, and histone PTMs are known to localize to enhancers and promoters, but it is not yet known how these elements may interact or influence each other at shared sites to regulate gene expression. HMGNs bind to the acidic patch on the nucleosome disc face and have a tail that is suggested to interacts with the histone tails[2]. We hypothesize that HMGNs directly bind to histone PTMs present at enhancers and promoters through their C-terminal tail, affecting the deposition of active histone marks and the recruitment of cohesin, which influences enhancer-promoter loops and gene expression. We will directly test the binding ability of HMGN proteins to various histone PTMs as well as determine the role of the C-terminal tail in these interactions via nucleosome binding assays. By elucidating the role of HMGNs in histone PTM regulation, we will better understand the mechanisms of genome organization that control gene expression, which is important to understand the mechanisms of cancer and developmental disorders.

Generating expression constructs for HMGN via Gibson cloning

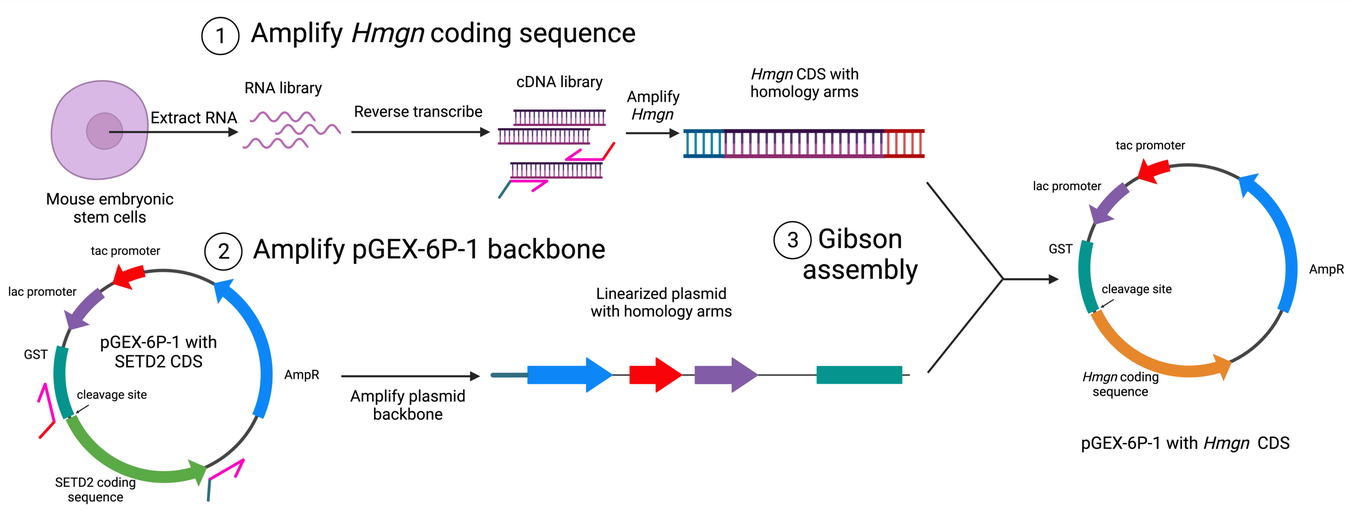


Figure 1: Cloning of *Hmgn* genes into an expression vector. The *Hmgn1* and *Hmgn2* coding sequences were cloned into a pGEX-6P-1 plasmid using Gibson cloning. The tac promoter allows for robust expression of the *Hmgn* coding sequence (CDS) with the addition of IPTG. The lac promoter and gene keep transcription turned off until the addition of IPTG. The *Hmgn* is tagged with an N-terminal GST tag to allow for downstream protein purification.

HMGN2 is highly expressed and soluble

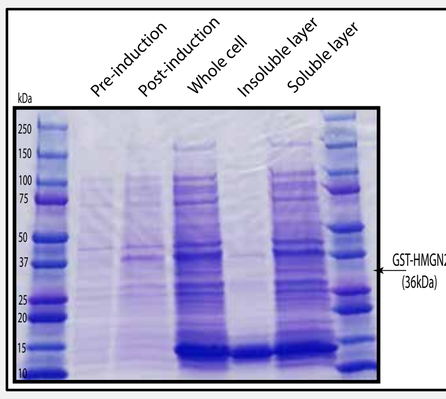


Figure 3: HMGN2 is highly expressed and soluble. Competent BL21 cells were transformed with the HMGN2 plasmid. Aliquots of culture were taken and pelleted pre- and post- IPTG induction. Cells were sonicated and separated into whole cell, insoluble, and soluble layers. Samples were run on a SDS-PAGE gel stained with Coomassie blue dye to visualize protein expression and solubility.

Protein Purification Strategy

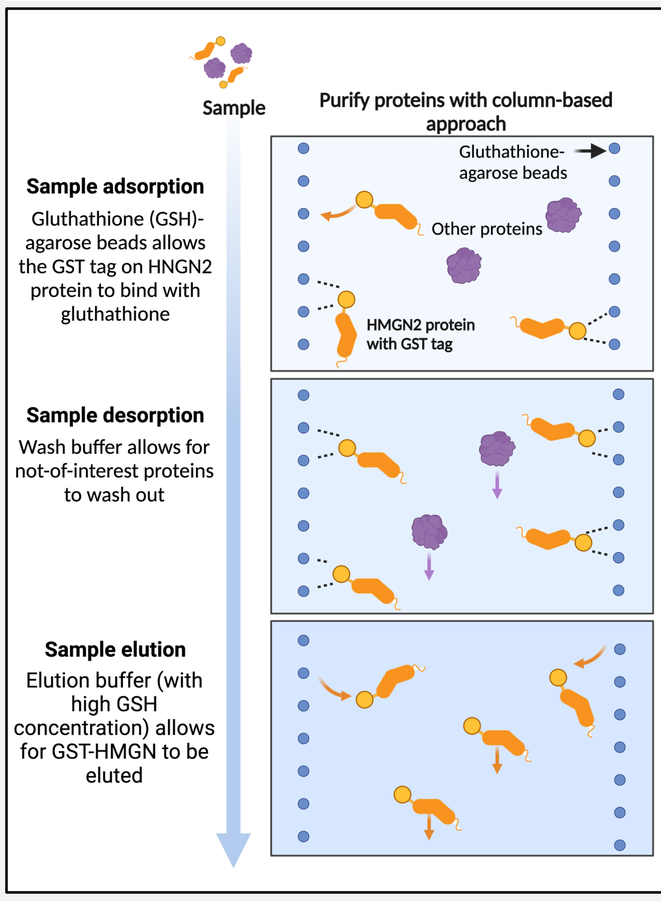


Figure 4: Protein Purification. HMGN2 was purified. First, the protein was expressed in bacterial cells and collected. The protein sample was purified using a glutathione agarose beads approach. The GST tag on HMGN2 binds to the beads. The beads were then washed, removing all proteins except those bound to the bead (HMGN). The GST-HMGN2 was then eluted from the bead mixture.

Generating expression constructs for tailless HMGN via site-directed mutagenesis

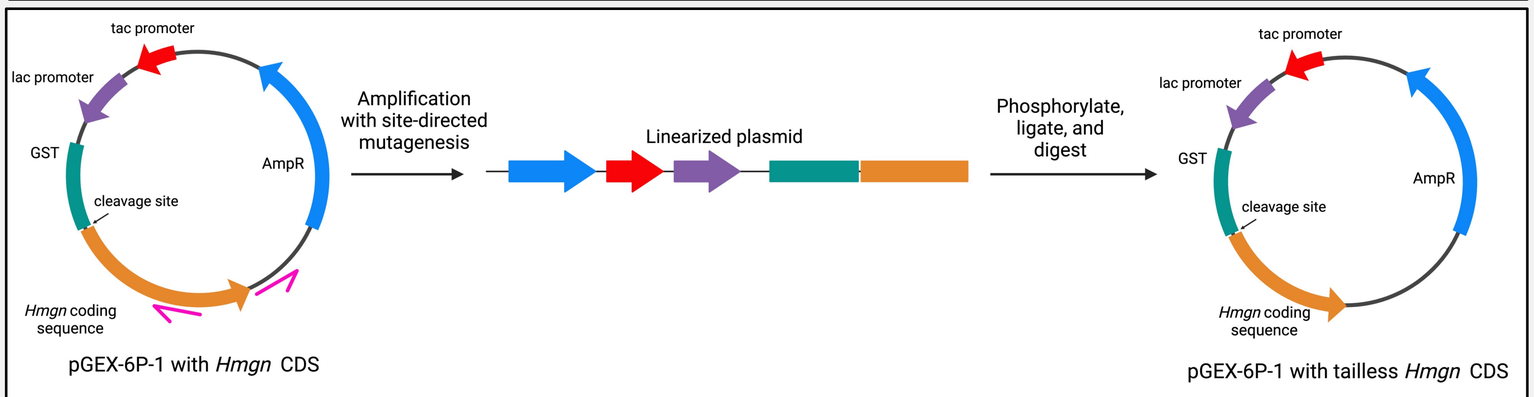


Figure 2: Cloning of *Hmgn* tailless mutants into an expression vector. Using the plasmids generated from Figure 3, mutant HMGN1 and HMGN2 proteins were made lacking the C-terminal tail (termed tailless mutants). We hypothesize that the C-terminal of HMGNs are thought to be the interface of interaction with histone PTMs. By generating tailless HMGN mutants, we can investigate the role of the tail in the proteins' functions.

Identifying HMGN binding preferences to specific histone PTMs

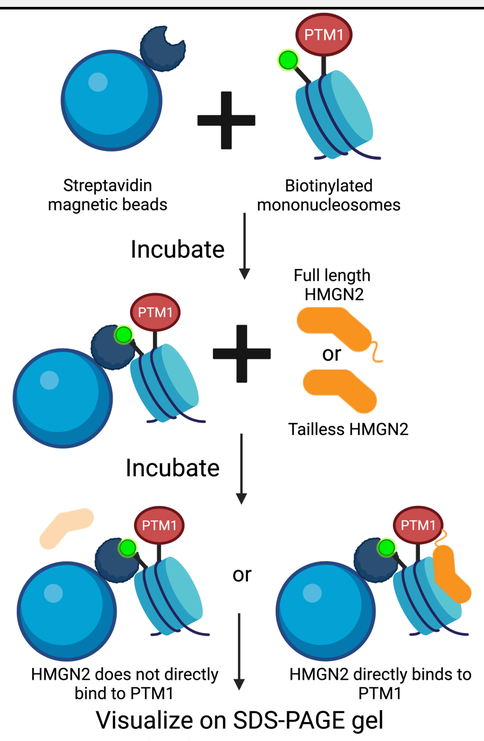


Figure 6: Nucleosome Binding Assay. Using purified HMGN full-length and tailless proteins, nucleosome binding assays will be used to determine direct binding of HMGNs to histone PTMs.

Purification of full length HMGN2

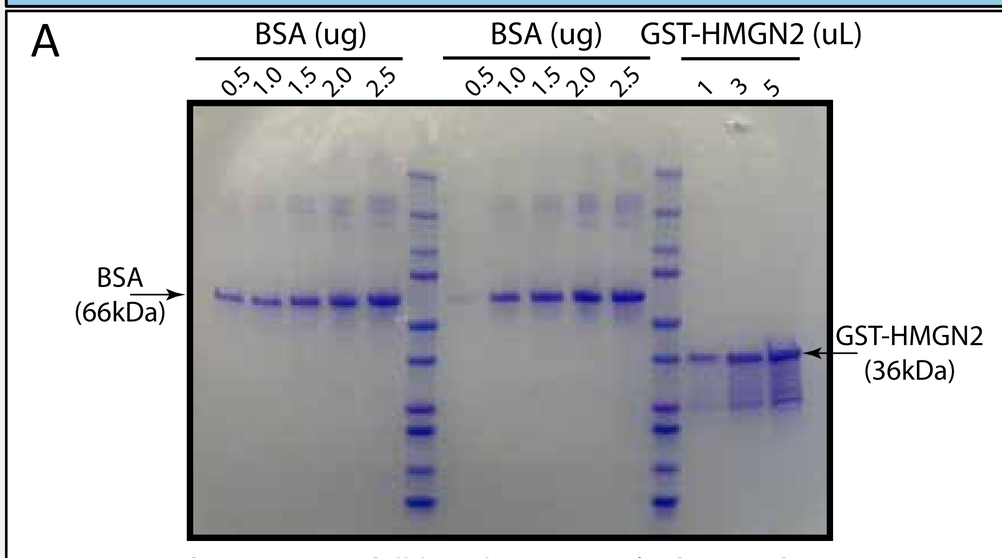


Figure 5: Purifying HMGN2 full-length protein. A) After purification, concentrated HMGN2 was run on an SDS-PAGE gel and stained with Coomassie blue dye. The concentration was quantified utilizing known amounts of BSA. B) The purified HMGN2 concentration was found to be 0.685 ug/uL utilizing the standard curve of known BSA concentrations.

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References

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2. Kato, H. *et al.* Architecture of the high mobility group nucleosomal protein 2-nucleosome complex as revealed by methyl-based NMR. *Proc Natl Acad Sci USA* **108**, 12283–12288 (2011).