Leveraging the HMGB1/protein VII interaction to interrogate HMGB1 function in the nucleus

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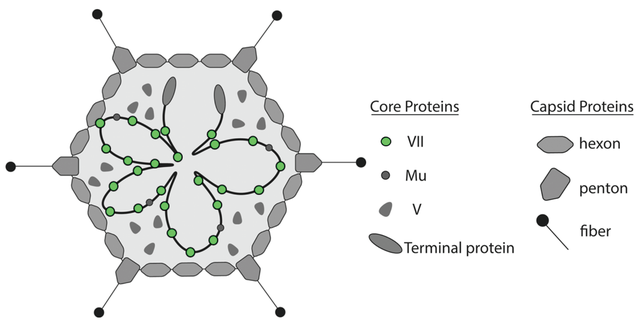
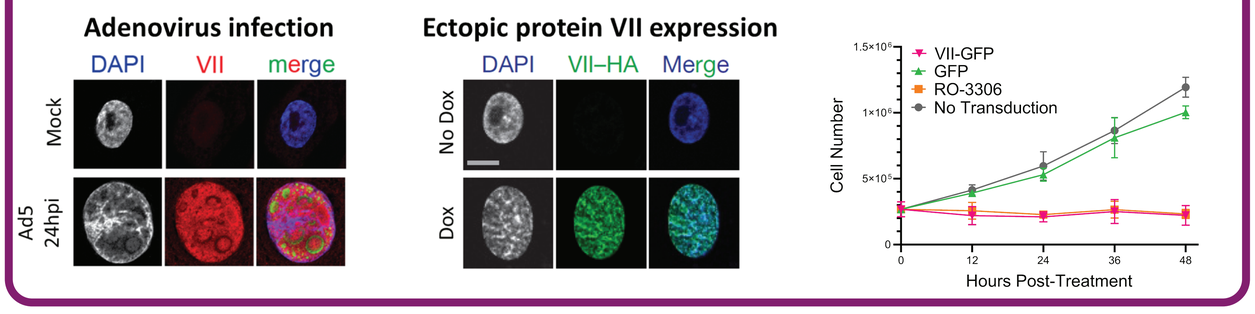
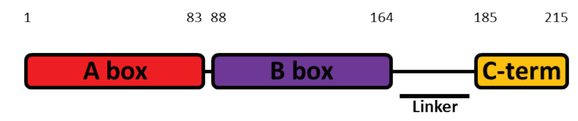
1. Adenovirus protein VII compacts the viral genome and localizes to host chromatin during infection



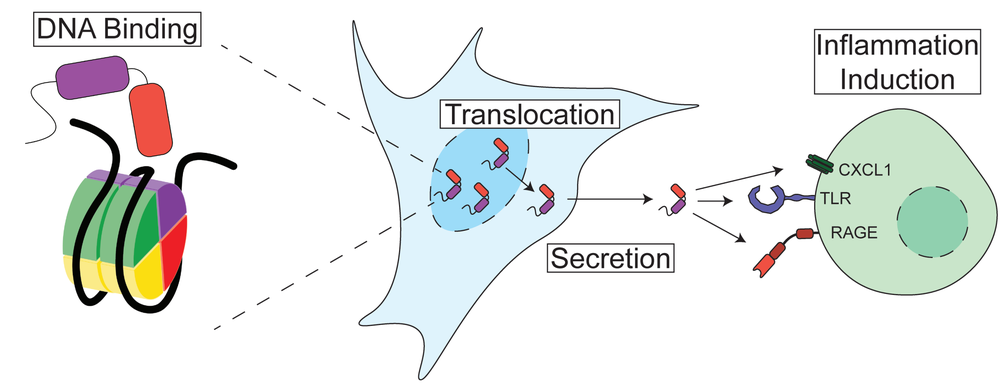
- **Small, basic, virally-encoded protein essential to viral infection**
- **Ectopic expression of VII is sufficient to cause chromatin distortion and impede cell cycle**



3. HMGB1 is a ubiquitous nuclear protein



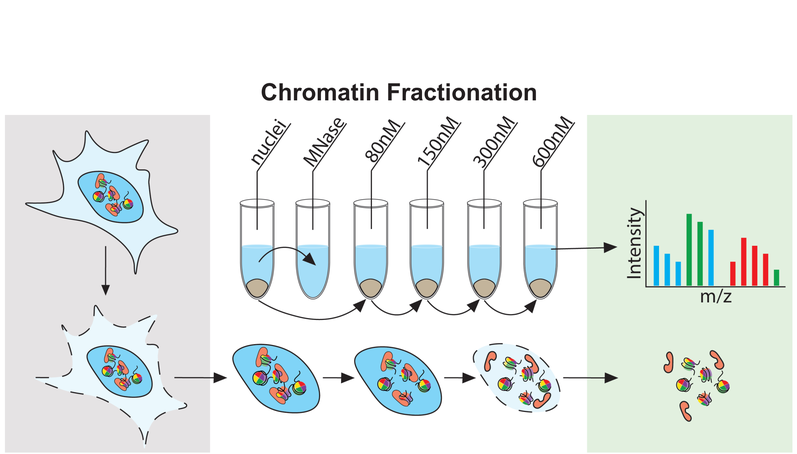
- **2 DNA binding Box domains: A and B**
- **Acidic C-terminus tail**

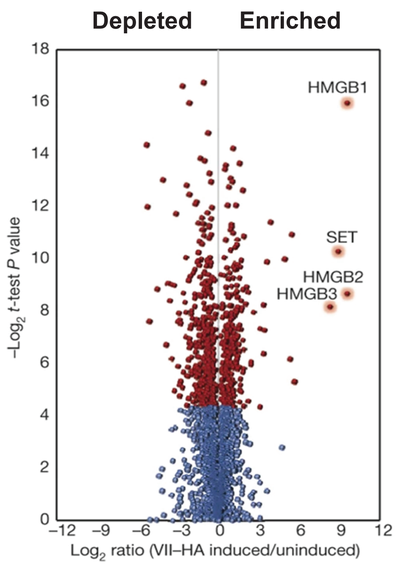


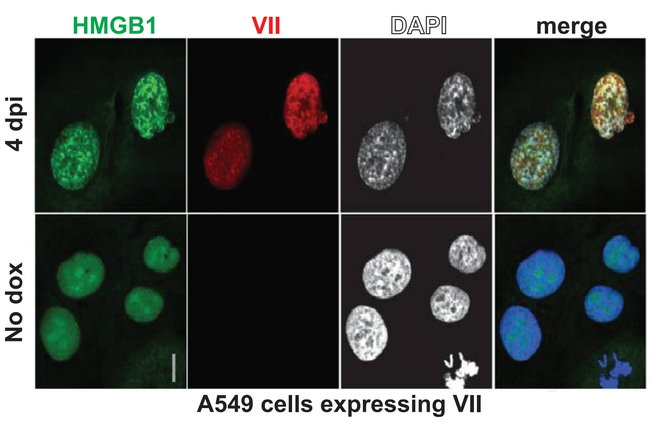
HMGB1 can recognize kinked forms of DNA or bend linear DNA at extreme angles to create a kinked confirmation in vitro

Following cell stress, HMGB1 can translocate out of the nucleus to be secreted into the extracellular space, acting as an alarmin and inducing inflammatory responses

2. Expression of protein VII leads to the mislocalization of HMGB1







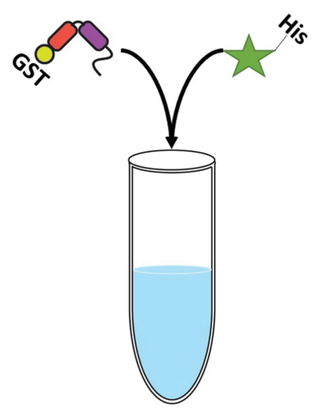
A549 cells expressing VII

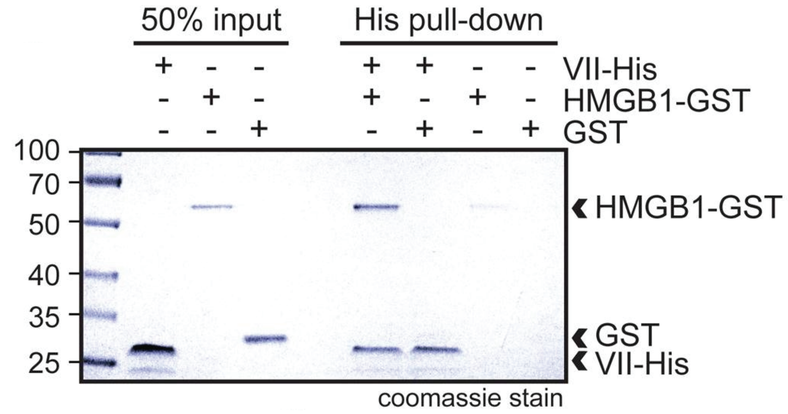
Chromatin fractionation shows significant enrichment of HMGB1 in the chromatin following ectopic VII expression

Hypothesis

The enrichment of HMGB1 in the chromatin fraction during protein VII expression is due to a direct interaction with protein VII

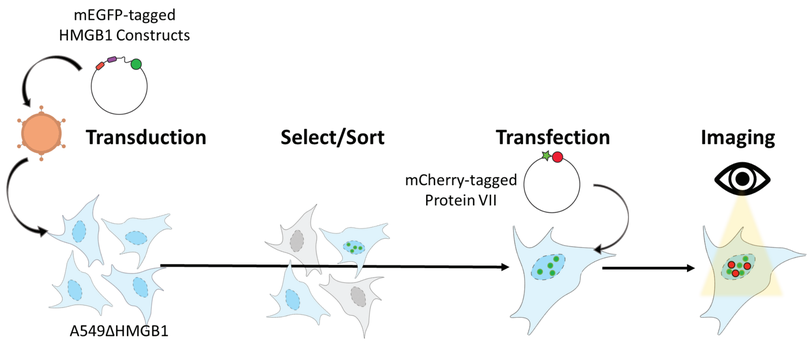
4. HMGB1 binds directly to adenovirus protein VII *in vitro*



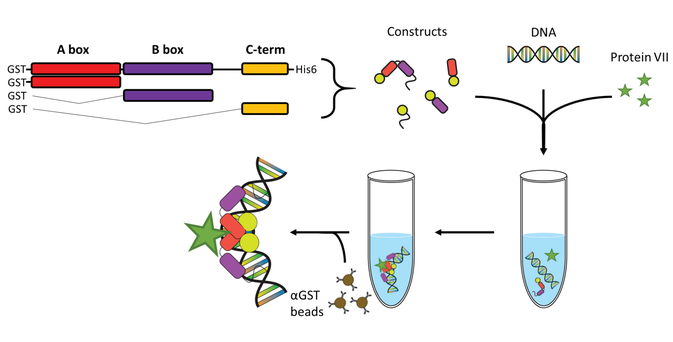


Preliminary pulldown experiments show that protein VII can directly bind HMGB1 in vitro

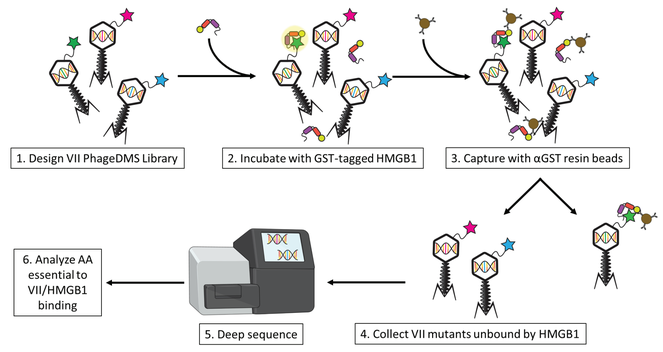
5. Defining the interaction between HMGB1 and adenovirus protein VII



Hypothesis: The mislocalization of HMGB1 during protein VII expression is domain-specific



Hypothesis: Binding of HMGB1 by protein VII is modulated in the presence of DNA



Hypothesis: Amino acids essential for the HMGB1/protein VII interaction can be mapped using DMS methods

6. Future Direction

Working model: Adenovirus protein VII immobilizes HMGB1 on the host chromatin during infection to halt its translocation from the nucleus

1
What other host factors are essential for this mislocalization?
CRISPR screen

2
Where does protein VII immobilize HMGB1 on the chromatin?
FRAPP/ChIP-seq

References/Acknowledgements

References: 1. Avgousti DC *et al,* Nature 2016
2. Lymch KL *et al,* BioRxiv 2020

Preliminary experiments conducted by C. Herrmann.
Funding: This research was funded in part by a Diverse Trainee Student Fellowship Award from the Office of Diversity, Equity, and Inclusion at Fred Hutchinson Cancer Research Center and NSF Grant DGE-1762114