Structure of Key Factors Bound to the Nucleosome at γ-Globin Promoter Help Reveal γ-Globin Control

Torry Li, Department of Chemistry; David C. Williams Jr., UNC School of Medicine Department of Pathology

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Background

β-hemoglobinopathies such as Sickle Cell Disease (SCD) and β-Thalassemia (β-Thal) are inherited diseases caused by irregular β-globin production. One promising therapy is the upregulation of fetal hemoglobin (HbF), the dominant oxygen transfer molecule in the fetus, which is made up of 2 units of γ-globin in place of β-globin. Treatments for these diseases could benefit from further research into HbF regulation.

The repression of γ-globin is a complex process involving the coordinated action of a multitude of proteins, one of them being BCL11a. Although it is known that BCL11a silence fetal hemoglobin around the γ-globin promoter on a nucleosome, the precise mechanism by which these proteins interact at the promoter is not well understood.

Objective

This project will determine the structure of BCL11A bound to a γ-globin promoter sequence wrapped around a nucleosome through Cryogenic Electron Microscopy, with implications for future β-hemoglobinopathy treatment.

Methods

Initial BCL11a and histones were purified from Rosetta2 DE3 competent cells. The 180 bp GG2 DNA oligomers were purified from Stellar competent cells. GG2 DNA nucleosomes were combined with BCL11a in a 2:1 ratio of BCL11a to nucleosome, purified using a sucrose gradient, and crosslinked using 0.1% glutathione. 3uM samples of the crosslinked BCL11a-nucleosome were buffer exchanged into BisTris buffer (25 mM BisTris pH 7.2, 100 mM NaCl, 1 mM DTT, 50 µM $ZnSO_4$) and then imaged using CryoEM.

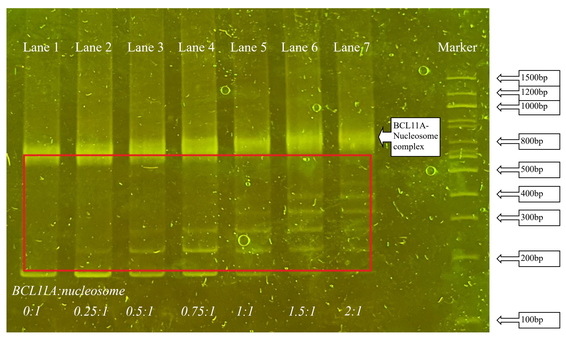

Figure 1. Gel shift of Bcl11a attached to a γ-globin locus native sequence nucleosome. The lanes represent titrations of BCL11A, from molar ratio of BCL11A:nucleosome of 0, 0.25, 0.5, 0.75, 1, 1.5, 2. The top band represents the BCL11A- nucleosome complex.

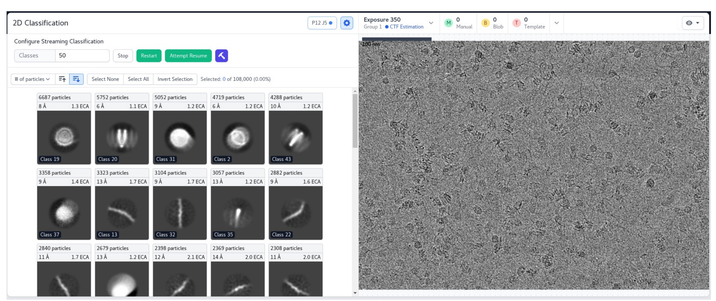

Figure 2. 2D classifications of the frozen sample. Though the samples gave clear images of the nucleosome, there was no indication of any additional bound protein. The first 3 images show images of the nucleosome. The second image shows a side view of stacking nucleosomes.

Results & Conclusion

The gel shift of BCL11a showed convincing evidence of binding to the nucleosome. Thus, only the BCL11a-nucleosome complex was imaged using CryoEM.

Imaging the cross-linked nucleosome complex revealed the structure of the nucleosome without the bound protein in the 2D classifications. However, the BCL11a protein was not apparent in the 2D classification, preventing determination of the 3D structure and mechanism of γ-globin repression.

Transcription factors are naturally weak binders of DNA, which itself is a weak binder to nucleosomes. The CryoEM samples had low concentrations of the BCL11a-nucleosome complex and were subjected to harsh conditions, which may have led to the dissociation of the complex, despite being crosslinked. Future experiments may aim at creating a more stable complex for imaging.

References

Vinjamur, D.S., Bauer, D.E. and Orkin, S.H. (2018), Recent progress in understanding and manipulating haemoglobin switching for the haemoglobinopathies. Br J Haematol, 180: 630-643. https://doi.org/10.1111/bjh.15038