

DNA and Chromatin Mechanics

Rohini Muthuswami¹

¹ School of Life Sciences, Jawaharlal Nehru University

Corresponding Author

Rohini Muthuswami

rohini_m@mail.jnu.ac.in

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Editorial

Chromatin is a dynamic, fluid structure maintained by protein-DNA as well as protein-protein interactions. Both histone and non-histone proteins are involved in maintaining the chromatin structure. How are these interactions mediated and how does the chromatin structure alter during processes that involve DNA as the substrate?

Many techniques have been developed to shed light on the dynamics of protein-DNA as well as protein-protein interactions. These include both biophysical techniques such as CD spectroscopy and biochemical methods such as gel filtration chromatography. This collection highlights few of these techniques that can be used to explore the dynamics of chromatin architecture.

DNA integrity can be analyzed by flow cytometery (FACS), and Ling et al.¹ adapt this method to detect human sperm functional defects. The sperm physiology can be assessed by using biomarkers such as DNA damage, mitochondrial membrane potential, and sperm apoptosis. Ling et al. show that FACS can be easily adapted to monitor each of these parameters to assess sperm physiology, thus demonstrating

that the technique is a practical and reliable toolkit for the diagnosis of male infertility.

CD spectroscopy is possibly one of the easiest methods to investigate the conformation of protein and DNA. Each conformer of DNA has a signature band pattern. The change in the band pattern can be correlated with the change in the conformation of the DNA and, thus, the shift in the DNA architecture can be monitored in the presence of interacting proteins by using a CD spectrophotometer. Arya et al.² use this technique to study change in the DNA induced by interaction with the ATP-dependent chromatin remodeling protein, SMARCAL1, in the absence and presence of ATP. They show that the alterations in the DNA structure can be correlated with the transcriptional activity of the protein.

Size exclusion chromatography and pull-down assays are routinely used for monitoring protein-protein interactions. Nucleosome assembly is facilitated by histone chaperones that bind to histones. Bobde et al.³ describe methods and assays that can be used to identify whether a given protein function is a histone chaperone or not.



How do we investigate the mechanisms of nucleosome assembly in real time? Single-molecule imaging techniques such as DNA curtain have been developed to visualize the assembly process in real time. DNA curtain is a hybrid technique that combines lipid fluidity, microfluidics, and total internal reflection microscopy to image the protein-DNA interaction in real-time. Kang et al.⁴ describe DNA curtain as a technique to study the mechanism underlying the assembly of the histone chaperone, Abo1, from *S. pombe*. The techniques described provide a way to examine chromatin dynamics, which play an important role both in genomic stability and the epigenetic regulation of processes involving the genetic material.

Traditional chromatin immunoprecipitation (ChIP) requires specific antibodies to the protein of interest. Many times, the antibodies are not specific enough, leading to false positive results, especially while probing the binding sites of the transcription factors. Antibodies are also not always available for proteins in all organisms. Candida albicans is one such organism for which antibodies are not available. The Cut and Run method circumvents these limitations by fusing MNase to the transcription factor of interest. Qasim et al. 5 describe the usage of this method to determine the binding sites of transcription factors in *C. albicans*.

The methods described in this collection provide a glimpse of the techniques available to researchers. All these methods are versatile and easily adaptable to address the questions that researcher are asking.

Disclosures

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References

- Ling, X. et al. Flow cytometric analysis of biomarkers for detecting human sperm functional defects. *Journal of Visualized Experiments*. (182), e63790 (2022).
- Arya, V., Dutta, A., Muthuswami, R. CD spectroscopy to study protein-DNA interactions. *Journal of Visualized Experiments*. (180), e63147 (2022).
- Bobde, C. R. et al. In vitro characterization of histone chaperones using analytical, pull-down and chaperoning assays. *Journal of Visualized Experiments*. (178), e63218 (2021).
- Kang, Y., Bae, S., An, S., Lee, J. Y. Deciphering molecular mechanism of histone assembly by DNA curtain technique. *Journal of Visualized Experiments*. (181), e63501 (2022).
- Qasim, M. N. et al. Genome-wide profiling of transcription factor-DNA binding interactions in Candida albicans: A comprehensive CUT&RUN method and data analysis workflow. *Journal of Visualized Experiments*. (181), e63501 (2022).