Single-molecule analysis of cytosolic DNA sensor sequestration by chromatin binding

Raquel Merino Urteaga[1], Alexander Strom[2], Xinyu A. Feng[1], Jungsan Sohn[2], Taekjip Ha[1,2,3,4]

1 Department of Biophysics, Johns Hopkins University, Baltimore, MD, USA
2 Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, USA
3 Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA
4 Howard Hughes Medical Institute, Chevy Chase, MD, USA

Cyclic-G/AMP synthase (cGAS) is responsible for initiating the innate immune response against cytosolic double-stranded DNA in eukaryotic cells. Although cGAS was thought to be confined to the cytoplasm, recent findings showed that cGAS is predominantly localized in the nucleus. It is believed that nuclear cGAS is tightly tethered to chromatin in an inactive state, concealing its key DNA-binding sites, and blocking the formation of active cGAS dimers, ultimately preventing autoreactivity to nuclear self-DNA. Cryo-EM reports suggest that a cGAS monomer directly binds to the acidic patch of the nucleosome core and nucleosomal DNA. These studies have also shown that two individual nucleosomes can be held together by two cGAS protomers. However, the underlying dynamics involved in the interaction between cGAS and nucleosomes remains poorly understood and most studies have only used the catalytic domain of cGAS ($cGAS^{CAT}$) to examine the basis of this association, excluding the unstructured N-terminus. Here, we utilized single-molecule fluorescence resonance energy transfer (FRET) to study the interaction and dynamics between cGAS and a nucleosome core particle (NCP). This assay revealed that $cGAS^{CAT}$ binds with high affinity to an NCP, with a dissociation constant in the subnanomolar range (Kd=0.72nM) and on average remains associated with the nucleosome for 13 seconds. By contrast, the full-length protein ($cGAS^{FL}$) displays a lower affinity to an NCP (Kd=1.35nM) and exhibits heterogeneous dissociation kinetics, indicating that two subpopulations with different stabilities may be formed. Notably, a subset of $cGAS^{FL}$ is able to remain stably tethered to nucleosomes for prolonged periods of time (up to an hour). We furthered this study by utilizing a dinucleosome construct with an internal FRET pair to monitor its conformational rearrangements upon cGAS binding. Two fluorophores were strategically placed on each nucleosomal DNA at a location in which the two NCPs become intimately associated when bridged by cGAS, forming a 2:2 cGAS-nucleosome complex. This assay revealed that cGAS draws the nucleosomes closer together and that this structural arrangement is stable over time. Additionally, $cGAS^{FL}$ displayed strong positive cooperativity when mediating dinucleosome compaction, substantially higher than $cGAS^{CAT}$. Real-time measurements shed light on the mechanism involved in nucleosome bridging by cGAS, revealing the existence of an intermediate state of very fast and dynamic FRET fluctuations that correspond to transiently compacted dinucleosomes. Moreover, direct observation of labeled $cGAS^{FL}$ bound to dinucleosomes indicates higher binding stoichiometry than structurally expected, which is not seen with $cGAS^{CAT}$. Collectively, these studies provide valuable insight into the dynamic picture of cGAS-nucleosome interaction, the generation of oligomeric assemblies between cGAS and chromatin, and provide detailed information on the mechanism ruling cGAS nuclear sequestration.