Title

An Integrative Approach Towards Understanding The Structure- Function Relationship Of The Epigenetic Regulator Methyl Cpg Binding Protein 2

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Abstract

Methyl CpG binding protein 2 (MeCP2) is a methylated DNA binding protein which, when mutated, leads to a severe neurodevelopmental disorder known as Rett syndrome (RTT). In the present work I have shown that MeCP2 is a highly unstructured protein with modular organization and harbors nine interspersed alpha-molecular recognition features (alpha-MoRFs). A detailed domain by domain analysis of the properties of human MeCP2 domains has revealed that MeCP2 binding to DNA is characterized by disorder-to-order transition and binding-dependent conformational selection. I have shown that in addition to the core Methyl-CpG Binding Domain (MBD), MeCp2 contains a chromatin (histone) binding domain, several autonomous DNA binding domains as well as domains which allosterically regulate DNA binding. Concerted binding through these domains lead to unprecedented chromatin compaction as well as nucleosome array oligomerization. Using a variety of biochemical, biophysical and imaging techniques, I have established the differences in physical principles driving both nonspecific and methyl-selective MeCP2 binding to DNA. Both binding modes are DNA length dependent. Even though MeCP2 exists as a monomer in solution, equilibrium exists between monomers and dimers on DNA. Consecutive interactions of MeCP2 monomers with DNA are cooperative, a property strongly correlated with methylation density and the presence of symmetric repeats of A/T stretches. Cooperativity in binding is abolished if the C-terminus of the protein is truncated and also in the severe RTT mutant F155S. I have also shown that Rett Syndrome-causing mutations in human MeCP2 result in diverse structural changes that impact folding, conformational coupling between domains and DNA interactions. The MeCP2 binding site on the nucleosome overlaps with that of linker histone H1 and is proximal to histone H3 at the nucleosomal dyad. MeCP2 mediated changes in nucleosome architecture result in compaction resembling the classical zigzag motif induced by histone H1, considered important for 30nm fiber formation. *In vivo*chromatin binding kinetics and *in vitro* steady state nucleosome binding of both MeCP2 and H1 provide strong evidence for competition between MeCP2 and H1 for common binding sites. This suggests that chromatin binding by MeCP2 and H1 *in vivo* should be viewed in the context of competitive multifactorial regulation.

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