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CpG island chromatin

A platform for gene regulation

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The majority of mammalian gene promoters are encompassed within regions of the genome called CpG islands that have an elevated level of non-methylated CpG dinucleotides. Despite over 20 years of study, the precise mechanisms by which CpG islands contribute to regulatory element function remain poorly understood. Recently it has been demonstrated that specific histone modifying enzymes are recruited directly to CpG islands through recognition of non-methylated CpG dinucleotide sequence. These enzymes then impose unique chromatin architecture on CpG islands that distinguish them from the surrounding genome. In the context of this work we discuss how CpG island elements may contribute to the function of gene regulatory elements through the utilization of chromatin and epigenetic processes.

Key words: CpG island, chromatin, histone, methylation, promoter, ZF-CxxC domain, transcription, histone lysine demethylase

Abbreviations: ZF-CxxC, zinc finger CxxC; HAT, histone acetyltransferase; HDAC, histone deacetylase; LPS, lipopolysaccharide; ES cell, embryonic stem cell; PRC, polycomb repressive complex; MBD, methyl CpG binding domain; ORI, origin of replication; PHD, plant homeodomain

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CpG Islands: Enigmatic Feature of the Mammalian Genome

In mammalian genomes, approximately 70–80% of CpG dinucleotides are post-replicatively marked by the addition of a methyl group to the 5 position of the cytosine ring.[1] This modification acts as a stable and heritable epigenetic mark that is generally associated with repressed chromatin states and inhibition of transcriptional initiation.[2] Despite the prevalence of CpG methylation, specific regions of mammalian genomes are refractory to this modification.[3,4] These regions, called CpG islands, correspond to contiguous non-methylated segments of the genome that have a higher than average level of CpG dinucleotides and GC content.[5] A large fraction of CpG islands encompass transcription start sites and approximately 70% of mammalian genes are associated with CpG islands. Interestingly, unlike classical TATA box containing promoters, CpG island promoters generally utilize dispersed transcription start sites, suggesting that the CpG island may act as a generalized element for transcriptional initiation.[6] Although CpG island promoters were originally considered a feature of housekeeping genes, it is now evident that many tissue-specific genes also utilize CpG island promoters.[7,8]

CpG islands often encompass mammalian gene promoters but their role in gene regulatory function is poorly understood. Certain transcription factors like Sp1, CREB and CTCF contain CpG in their binding site and DNA recognition is often blocked by CpG methylation.[9-13] This led to the hypothesis that non-methylated CpG islands act as preferential nucleation sites for these types of transcription factors. However, given that these DNA binding factors recognize CpG dinucleotides within the context of extended DNA motifs they are only targeted to a limited subset of CpG islands.[14-16] This suggests that additional, more broadly impinging mechanisms likely contribute to CpG island function.

ZF-CxxC Domain Proteins Bind Specifically to CpG Islands

In searching for additional DNA binding factors that might specifically recognize non-methylated CpG dinucleotides, Skalnik and colleagues identified a protein they called CpG Binding Protein

(CGBP).[17] CGBP encodes a ZF-CxxC DNA binding domain that specifically recognizes CpG dinucleotides and binding is blocked when the sequence is methylated. Therefore, CGBP could potentially recognize non-methylated CpG islands. CGBP was later renamed CxxC Finger Protein 1 (CFP1) based on the identification of an extended family of CpG binding proteins.[18] Despite their in vitro DNA binding activity, the relationship between ZF-CxxC proteins and CpG islands genome-wide has largely been overlooked.[17,19]

Based on the intriguing possibility that ZF-CxxC domain containing proteins may specifically recognize CpG islands, we recently analyzed the genome-wide localization of the ZF-CxxC domain containing KDM2A protein.[20,21] Chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-seq) revealed that KDM2A binds greater than 90% of CpG islands genome-wide and demonstrated that the ZF-CxxC domain is a CpG island targeting module. In a related study, Bird and colleagues demonstrated via a similar ChIP-seq based approach that the CFP1 protein also specifically nucleates at CpG islands.[21] Together this work demonstrates for the first time that CpG island elements are directly interpreted through recognition of non-methylated DNA. Interestingly, KDM2A and CFP1 are associated with histone modifying activities, suggesting that CpG islands may use chromatin based processes to elicit regulatory element function.

Translating the CpG Island Signal into Unique Chromatin Architecture

KDM2A is the founding member of a recently identified family of JmjC domain containing histone lysine demethylase enzymes. KDM2A specifically catalyzes removal of methylation from histone H3 lysine 36 with a preference for the di-methyl modification state (H3K36me2).[22] Mass-spectrometry analysis of histone modifications in a variety of cell types indicates that H3K36me2 is an abundant modification adorning 30–50% of histone H3.[23-25] Based on the observation that KDM2A nucleates at CpG islands, we hypothesized that this enzyme could function to remove H3K36me2 from CpG island promoters. Indeed, chromatin immunoprecipitation based analysis of H3K36me2 levels revealed that while inter- and intragenic regions show high and relatively constant levels of H3K36me2, KDM2A-bound CpG islands are depleted of this mark.[20] Importantly, knockdown of KDM2A by RNAi resulted in increased H3K36me2 at CpG islands indicating that KDM2A nucleation is responsible for this depletion. Like KDM2A, CFP1 modifies chromatin, but instead of removing histone methylation it associates with a histone H3 K4 methyltransferase complex (SET1 complex) to catalyze addition of the tri-methyl modification state (H3K4me3).[18] Histone H3K4me3 is not broadly distributed like H3K36me2, but is instead found associated with the 5' ends of genes.[26,27] When CFP1 was depleted in cells, a reduction of H3K4me3 was observed at CFP1 bound CpG island promoters.[21] Together, these observations reveal a simple yet elegant mechanism by which CpG island DNA is specifically interpreted by chromatin modifying enzymes encoding ZF-CxxC domains to create unique chromatin architecture characterized by depletion of H3K36me2 and nucleation of H3K4me3. Importantly, this architecture effectively differentiates CpG island elements from surrounding chromatin and suggests that chromatin environment may in part define CpG island function.

Transcriptionally Permissive Chromatin Architecture at CpG Islands

ZF-CxxC domain protein binding at CpG island promoters does not directly correlate with gene expression, fitting with the fact that ZF-CxxC proteins are recruited by the underlying non-methylated CpG content and not by the transcriptional state of the associated gene.[20,21] This is consistent with in vivo footprinting studies illustrating that active transcription has little or no effect on regions of protection at CpG islands.[28] By virtue of the fact that ZF-CxxC domains recognize DNA sequence it appears that they create CpG island chromatin architecture that is independent and therefore upstream of traditional transcription factor mediated gene activation or silencing. This prompts the question, what role does ZF-CxxC domain mediated CpG island chromatin architecture play in CpG island function?

To consider the implications of CpG island chromatin for gene function, it is useful to examine each of the unique CpG island chromatin features individually. Histone lysine methylation marks can act as binding sites for specific effector proteins that possess plant homeodomain (PHD) fingers or chromatin organization modifier (chromo) domains. A number of studies suggest that H3K4me3 acts as a binding site for the PHD finger of proteins that support transcription initiation, such as TFIID,[29] nucleosome remodeling factor (NURF)[30] and the ING4 containing histone acetyltransferase (HAT) complex.[31] In contrast, experiments in yeast suggest that H3K36me2 is inhibitory to transcriptional initiation by acting as a binding site for the chromodomain of the EAF3 component of the RPD3S histone deacetylase (HDAC) complex.[32-35] In addition to ZF-CxxC domain mediated chromatin features, early biochemical experiments examining CpG island chromatin revealed a distinct depletion of Histone H1 at these elements.[36] Histone H1 is considered repressive to transcription,[37-40] possibly due to stabilization of a higher order chromatin structure.[41] Together these features appear to create a CpG island chromatin environment that is an ideal substrate for transcriptional initiation.

How Do CpG Islands Contribute to Regulatory Element Function?

Up to 70% of mammalian genes are associated with CpG islands, almost all of which are free from DNA methylation. Their association with promoter and regulatory elements suggest they likely contribute to gene regulatory processes, but the presence of a CpG island alone does not define transcriptional output. Therefore, what do CpG islands and CpG island chromatin architecture contribute to gene regulation? Although the defined answers to these questions remain unclear, an emerging body of evidence support the possibility that CpG islands functionally

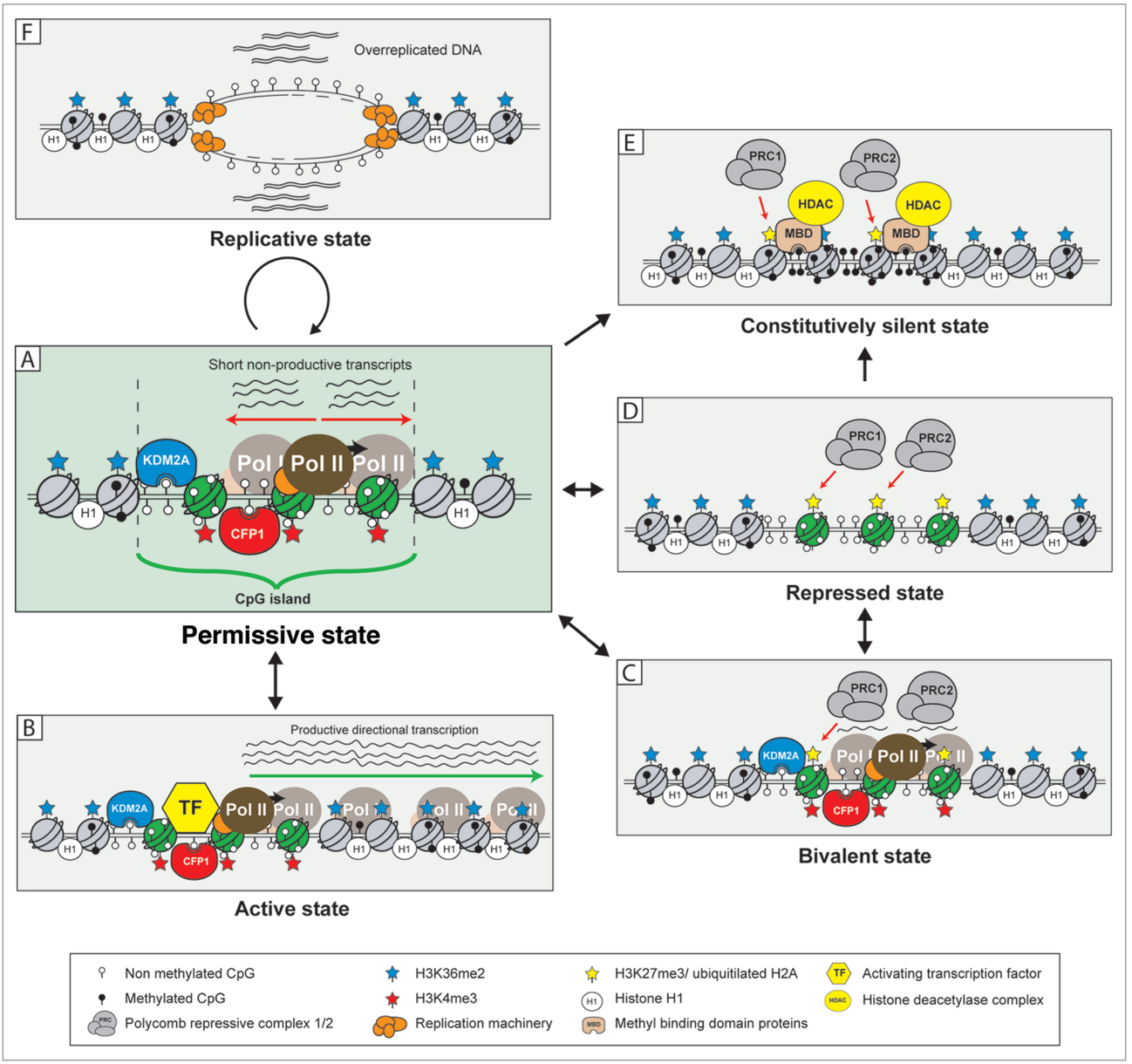

Figure 1. The various chromatin states of CpG islands. CpG islands have the capacity to adopt multiple chromatin states each with varying propensities for gene activation or repression (see main text for a description of these). Central to the capability of CpG islands to adopt and transition between these states is the default permissive state (A), partly imposed by the ZF-CxxC proteins KDM2A and CFP1. Nucleosomes within CpG islands are depicted in green and nucleosomes outside of CpG islands are grey. Straight arrows represent pathways of transition between individual CpG island states. Circular arrow represents a cell cycle involving initiation of DNA replication from CpG island ORIs.

impinge upon transcriptional potential through chromatin architecture and act as platforms for additional levels of transcriptional and epigenetic control. Below we discuss in more detail the nature of CpG island chromatin and how the function of additional signaling and chromatin modification pathways alter the default CpG island chromatin state leading to a series of more activated or repressed configurations (**Fig. 1**).

The permissive state. The default CpG island chromatin environment appears to have features that are permissive to transcriptional initiation, in part through the function of ZF-CxxC proteins.[20,21] We hypothesize that this system functions to highlight promoter regions within the vast expanse of the mammalian genome and focus nucleation of the transcriptional machinery at the 5' ends of genes. In support of this idea RNA pol II is enriched not only at the promoters of actively transcribed genes but many genes that are apparently silent.[27] Additionally, short bidirectional transcripts emanate from the 5' regions of genes, including some with no detectable full-length mRNA.[42-46] Interestingly, there is a strong correlation between RNA pol II enrichment, bidirectional transcripts and CpG island promoters (**Fig. 1A**).[27,42-44,46] One explanation for this relationship is that chromatin

structure at CpG islands creates an accessible environment that favors binding of RNA pol II and other components of the basal transcription machinery over surrounding non-regulatory chromatin. In the absence of additional regulatory factors that define activated directional transcriptional output, the CpG island may only permit futile and non-productive initiation/termination cycles of transcription that result in short bidirectional transcripts. In order to transition from this default state to a transcriptionally active state, additional regulatory signals outside of chromatin architecture alone are presumably required.

The active state. CpG islands appear to create a permissive chromatin environment but this does not define productive transcriptional output. This implies a specific signal must initiate the transition from this permissive state to the active state characterized by productive and directional transcription (**Fig. 1B**). This process likely relies on the function of sequence-specific DNA binding transcription regulators. For example, the immediate early genes, most of which have CpG island promoters and are usually silent or expressed at very low levels, can be rapidly activated in response to external stimuli such as lipopolysaccharide (LPS).[47] LPS stimulates the Toll-like receptor 4 pathway, resulting in activation of transcription factors such as NFκB and AP-1 that bind immediate early gene CpG island promoters and permit gene activation.[48,49] If the CpG island chromatin environment is permissive to transcription, one would envisage that activation of CpG island genes would be kinetically more favorable than genes that lack this environment. In support of this contention, induction of CpG island containing immediate early genes occurs rapidly and without the requirement of the chromatin remodeling machinery. In contrast, induction of non-CpG islands containing immediate early genes relies on the function of the ATP-dependent chromatin remodeling factor BRG-1 and suggests that chromatin environment at these genes is a barrier to activation.[27] These observations indicate that CpG island chromatin is permissive to transcription but that transcription factors ultimately define productive transcriptional output.

The bivalent state. In addition to permissive and active CpG islands, embryonic stem (ES) cells have an intriguing subset of CpG island genes that are characterized by nucleation of the polycomb mediated silencing machinery (**Fig. 1C**). These CpG islands retain the permissive H3K36me2 depleted/H3K4me3 enriched chromatin environment, but are also modified by histone H3 lysine 27 trimethylation (H3K27me3) through the function of the polycomb repressive complex 2 (PRC2).[26,50] This class of promoters are referred to as bivalent and are often associated with genes that must be actively silenced in ES cells but will often be activated later during development and lineage specific commitment. Interestingly, PRC2 nucleation and H3K27me3 profiles usually correspond to the underlying boundaries of the CpG island element suggesting that information within the CpG island itself may contribute to this profile.[26,51] The function of the polycomb system at CpG islands appears to constrain the permissive capacity of the CpG island chromatin environment and ensure the associated gene is not expressed. The mechanisms underlying this repression are not entirely clear, but there is experimental evidence that the polycomb repressive complex 1 (PRC1) restrains RNA pol II elongation at bivalent promoters.[52] Interestingly, a recent study demonstrated that short RNAs transcribed from bivalent CpG islands form hairpin structures that are directly recognized by polycomb components and aid in targeting PRC2 to these genes.[44] Therefore, PRC2 may have hijacked non-productive transcripts emanating from CpG islands as a gene-specific polycomb targeting mechanism. Clearly more work is required to understand how polycomb complexes recognize specific CpG islands to create bivalency.

The repressed state. In some tissues, expression of a given CpG island gene may very rarely or never be required. Therefore, maintenance of permissive or bivalent chromatin architecture may not be entirely beneficial. In mouse brain there is a population of CpG islands (<10%) that lacks DNA methylation yet does not display significant ZF-CxxC protein nucleation.[21] In general, it appears these genes are subject to polycomb-mediated repression and are modified by H3K27me3, but unlike bivalent genes in ES cells they lack H3K4me3 (**Fig. 1D**). This suggests in some instances ZF-CxxC protein binding and permissive chromatin architecture are excluded from CpG islands, perhaps through an as of yet undiscovered polycomb dependent silencing mechanism. Therefore, in some instances it appears that CpG islands transition from the default permissive state to a repressed state lacking ZF-CxxC dependent chromatin architecture as a means of actively repressing gene expression.

The constitutively silent state. A small number of CpG islands, such as those silenced during X chromosome inactivation or genomic imprinting, acquire DNA methylation during cellular differentiation. DNA methylation blocks recruitment of ZF-CxxC domain proteins and this abrogates the permissive chromatin environment usually defined by these factors.[20,21] At the same time methylated CpG acts as binding sites for methyl CpG-binding domain proteins (MBDs) that recruit HDAC complexes and are potent repressors of transcription (**Fig. 1E**).[2,53,54] This results in a very stable and epigenetically heritable form of silencing and makes reactivation of the affected gene extremely difficult. It still remains mechanistically unclear how this subset of CpG islands become susceptible to DNA methylation, but evidence from studies in cancer tissues indicate that a polycomb-silenced intermediate state may ultimately give rise to the constitutively repressed and DNA methylated state.[55,56] It remains poorly defined whether this is also true of CpG islands that acquire DNA methylation normally during development. Therefore, it will be important to understand if polycomb silencing is related to acquisition of DNA methylation.

The replicative state. The majority of work on CpG islands has focused on understanding their relationship with gene expression. Interestingly, another documented feature of CpG islands is that they often correspond to origins of DNA replication (ORIs).[57,58] The permissive nature of CpG island chromatin may

in analogy to acting a nucleation site for the transcriptional machinery, also preferentially act as assembly points for the DNA replication machinery. Intriguingly, it has been reported during S phase that CpG island ORIs undergo several rounds of short abortive replication, resulting in over-replication of these regions compared to other parts of the genome (**Fig. 1F**).[59] This phenomenon appears analogous to the short bidirectional transcripts initiating from CpG island promoters and could result from non-productive initiation of ORIs in the absence of factors needed for processive replication. More work is clearly required to understand what features of CpG islands make these preferential targets for initiating DNA replication.

Conclusion: The Many States of CpG Islands

The traditional view of gene promoters is one of defined transcription start sites that are either active or silent. However, genome-wide maps of RNA pol II binding and high resolution analysis of RNA transcripts reveal that CpG island promoters do not conform to this ideal. Rather than defining the active or repressed transcriptional state, CpG island elements utilize chromatin-based processes to create environments that contribute to the transcriptional potential of the associated gene (**Fig. 1**). From a default permissive state, CpG islands appear to act as a platform on which additional signaling and chromatin modification systems alter and modify this default state to help define the functional output of the associated gene (**Fig. 1**). Despite recent advances in our understanding of CpG islands, there are still significant gaps in our knowledge of these abundant yet poorly understood elements. For example, the mechanisms by which CpG islands are kept free of DNA methylation in an otherwise heavily methylated genome remain a mystery. A recent study suggests that TET1, an enzyme that hydroxylyates methylated DNA,[60,61] may be involved in the process of regulatory element demethylation.[62] It is interesting to note that Tet1 also possesses a ZF-CxxC domain[61] making it tempting to speculate that this domain targets DNA demethylase activity to CpG islands. With respect to CpG island chromatin architecture, more mechanistic investigation is required to directly define how chromatin impacts upon transcriptional regulation. Through defining these fundamental mechanisms, an understanding of how DNA methylation profiles and CpG islands provide a framework for gene regulatory processes will be achieved.

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