doi:10.1016/j.jmb.2011.07.032 J. Mol. Biol. (2011) 412, 634–646

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

Structural Constraints in Collaborative Competition of Transcription Factors against the Nucleosome

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Received 25 March 2011; received in revised form 3 July 2011; accepted 16 July 2011
Available online 29 July 2011

Edited by J. O. Thomas

Keywords:
chromatin; gene regulation; cooperativity; transcription factor; DNA

Cooperativity in transcription factor (TF) binding is essential in eukaryotic gene regulation and arises through diverse mechanisms. Here, we focus on one mechanism, collaborative competition, which is of interest because it arises both automatically (with no requirement for TF coevolution) and spontaneously (with no requirement for ATP-dependent nucleosome remodeling factors). Previous experimental studies of collaborative competition analyzed cases in which target sites for pairs of cooperating TFs were contained within the same side of the nucleosome. Here, we utilize new assays to measure cooperativity in protein binding to pairs of nucleosomal DNA target sites. We focus on the cases that are of greatest *in vivo* relevance, in which one binding site is located close to the end of a nucleosome and the other binding site is located at diverse positions throughout the nucleosome. Our results reveal energetically significant positive (favorable) cooperativity for pairs of sites on the same side of the nucleosome but, for the cases examined, energetically insignificant cooperativity between sites on opposite sides of the nucleosome. These findings imply a special significance for TF binding sites that are spaced within one-half nucleosome length (74 bp) or less along the genome and may prove useful for prediction of cooperatively acting TFs genome wide.
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Introduction

Cooperative action of multiple DNA binding transcription factors (TFs) is a hallmark of eukaryotic promoters and enhancers.[1–3] Cooperative binding is required for most eukaryotic TFs to identify functional DNA target sites because of the factors' short degenerate target sites (~15 bits of information) compared to eubacterial TF's (~25 bits).[4] Such information-poor target sites occur by happenstance every few thousand base pairs, raising the question of how the small fraction of *bona fide* functional sites are specified. Nucleosomal organization of eukaryotic DNA does not eliminate this specificity problem, as only 75–90% of the genomic DNA is wrapped in nucleosomes.[5] The smaller genomes of eubacteria are similarly occluded by DNA binding proteins,[6] yet eubacterial TFs have much greater specificity. Cooperative interactions between eukaryotic TFs allow TFs to collectively develop the sequence specificity that they lack individually.[4]

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Abbreviations used: TF, transcription factor; FRET, fluorescence resonance energy transfer; EDTA, ethylenediaminetetraacetic acid; NEB, New England Biolabs.

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Here, we extend our earlier analysis of a novel mechanism for cooperativity in TF binding, in which cooperativity arises spontaneously through synergistic action of two or more TFs against a nucleosome.[7,8]

Cooperativity between eukaryotic TFs can arise by conventional mechanisms. Two TFs can favorably bind each other while simultaneously binding their DNA targets, allowing the two TFs to effectively bind as a single unit with increased specificity. Alternatively, another protein can bridge two noninteracting TFs, again allowing the two TFs to bind DNA as a single unit. Conventional TF cooperativity can be augmented by a DNA bending protein that brings two separately bound TFs into proximity, facilitating their interaction.

The above mechanisms require coevolution of different proteins or of different binding surfaces of one protein because the cooperativity derives ultimately from favorable specific protein–protein interactions.

In contrast, the nucleosomal organization of DNA makes possible a novel cooperativity between eukaryotic TFs, which can occur with no requirement for coevolution. This cooperativity arises automatically when two or more arbitrary TFs each seek individually to bind to target sites contained within one nucleosome.[7–14] This cooperativity can arise with or without the assistance of an ATP-dependent nucleosome remodeling factor. In the former case, an initially bound TF can recruit a remodeler, which then disassembles or moves a nucleosome that covers a second TF's binding site, thereby facilitating binding by the second TF.[15,16] However, nucleosome-dependent cooperativity can also occur spontaneously without participation of remodelers. TFs can spontaneously bind to DNA target sites that, in the time average, are buried inside nucleosomes through frequent but transient "site exposure" conformational fluctuations that are inherent to nucleosomes;[17–20] binding of a first TF can enhance the accessibility of a second TF's binding site, facilitating spontaneous binding by the second TF.[7] The resulting "collaborative competition" of TFs against a nucleosome occurs both *in vitro*[7,9] and *in vivo*[8,10,21] without requirement for remodeler action[7,11,22] and may contribute to transcriptional regulation genome wide.[23,24] We seek to understand the structural constraints governing cooperative TF binding via this remodeler-independent collaborative competition.

Existing studies suggest that all that is required to evolve cooperatively acting pairs of TFs by remodeler-independent collaborative competition is for binding sites for the two TFs to be juxtaposed close together along the DNA within the same nucleosome.[7,12] However, previous *in vitro* analyses of collaborative competition only tested for cooperativity between sites on the same side of the nucleosome,[7,9] raising the question of whether comparable cooperativity also occurs between sites on opposite sides of the nucleosome. Indeed, binding to sites on opposite sides might occur with negative (unfavorable) cooperativity instead of positive (favorable) cooperativity.[25,26] Here, we use restriction enzyme digestion and fluorescence resonance energy transfer (FRET) assays to further characterize cooperative interactions in a nucleosome. As we discuss below, for reasons of both plausible biological relevance and technical feasibility, our analysis is restricted to cases in which at least one of the two potentially cooperating target sites is located not far from an end of the nucleosomal DNA. Our previous demonstration of reciprocity in cooperativity,[7] together with our earlier and new findings of cooperativity between many different pairs of sites, makes our findings more general. Our results again reveal energetically significant positive (favorable) cooperativity between pairs of sites on one side of the nucleosome, but, for the cases examined, cooperativity between sites on opposite sides of the nucleosome is energetically insignificant. Our results imply specific length scales at which clustering of TF binding sites may be functionally relevant and may prove helpful for improving genome-wide prediction of cooperatively acting TFs.

Results

Our earlier theoretical analysis of collaborative competition[7] was based on data obtained by others,[9] in which the binding sites investigated were located on the same side of the nucleosome. Those studies utilized a coupled equilibrium binding assay. Cooperative binding was manifested as an influence of the binding of one DNA binding protein on the binding of another DNA binding protein to the same nucleosome, with double-binding events detected using a gel mobility supershift assay.

If our understanding is correct, such nucleosome-dependent cooperative binding should be detectable using quite different assays as well. We therefore sought first to extend the previous work with two new assays for cooperative binding to nucleosomal target sites. One assay uses a restriction enzyme in place of one of the two DNA binding proteins that were used in the coupled binding assay described above. We then measure how a protein–DNA binding event at one location in the nucleosome influences the ease with which the restriction enzyme can cleave its target site, which is sterically occluded by the nucleosomal wrapping. This assay is attractive because it yields a positive signal (specific DNA cleavage) on a near-zero background while being carried out fully in solution, thereby

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