CTCF sites display cell cycle-dependent dynamics in factor binding and nucleosome positioning.

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Public Summary:
CCCTC-binding factor (CTCF) plays a key role in the formation of topologically associating domains (TADs) and loops in interphase. During mitosis TADs are absent, but how TAD formation is dynamically controlled during the cell cycle is not known. Several contradicting observations have been made regarding CTCF binding to mitotic chromatin using both genomics- and microscopy-based techniques. Here, we have used four different assays to address this debate. First, using 5C, we confirmed that TADs and CTCF loops are readily detected in interphase, but absent during prometaphase. Second, ATAC-seq analysis showed that CTCF sites display greatly reduced accessibility and lose the CTCF footprint in prometaphase, suggesting loss of CTCF binding and rearrangement of the nucleosomal array around the binding motif. In contrast, transcription start sites remain accessible in prometaphase, although adjacent nucleosomes can also become repositioned and occupy at least a subset of start sites during mitosis. Third, loss of site-specific CTCF binding was directly demonstrated using CUT&RUN. Histone modifications and histone variants are maintained in mitosis, suggesting a role in bookmarking of active CTCF sites. Finally, live-cell imaging, fluorescence recovery after photobleaching, and single molecule tracking showed that almost all CTCF chromatin binding is lost in prometaphase. Combined, our results demonstrate loss of CTCF binding to CTCF sites during prometaphase and rearrangement of the chromatin landscape around CTCF motifs. This, combined with loss of cohesin, would contribute to the observed loss of TADs and CTCF loops during mitosis and reveals that CTCF sites, key architectural cis-elements, display cell cycle stage-dependent dynamics in factor binding and nucleosome positioning.

Scientific Abstract:
CCCTC-binding factor (CTCF) plays a key role in the formation of topologically associating domains (TADs) and loops in interphase. During mitosis TADs are absent, but how TAD formation is dynamically controlled during the cell cycle is not known. Several contradicting observations have been made regarding CTCF binding to mitotic chromatin using both genomics- and microscopy-based techniques. Here, we have used four different assays to address this debate. First, using 5C, we confirmed that TADs and CTCF loops are readily detected in interphase, but absent during prometaphase. Second, ATAC-seq analysis showed that CTCF sites display greatly reduced accessibility and lose the CTCF footprint in prometaphase, suggesting loss of CTCF binding and rearrangement of the nucleosomal array around the binding motif. In contrast, transcription start sites remain accessible in prometaphase, although adjacent nucleosomes can also become repositioned and occupy at least a subset of start sites during mitosis. Third, loss of site-specific CTCF binding was directly demonstrated using CUT&RUN. Histone modifications and histone variants are maintained in mitosis, suggesting a role in bookmarking of active CTCF sites. Finally, live-cell imaging, fluorescence recovery after photobleaching, and single molecule tracking showed that almost all CTCF chromatin binding is lost in prometaphase. Combined, our results demonstrate loss of CTCF binding to CTCF sites during prometaphase and rearrangement of the chromatin landscape around CTCF motifs. This, combined with loss of cohesin, would contribute to the observed loss of TADs and CTCF loops during mitosis and reveals that CTCF sites, key architectural cis-elements, display cell cycle stage-dependent dynamics in factor binding and nucleosome positioning.