Wt1 Flip-Flops Chromatin in a CTCF Domain

B.V. Gurudatta[1] and Victor G. Corces[1,*]
[1]Department of Biology, Emory University, 1510 Clifton Rd NE, Atlanta, GA 30322
*Correspondence: vcorces@emory.edu
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CTCF plays diverse roles in nuclear organization and transcriptional regulation. In this issue of *Developmental Cell*, Essafi et al. (2011) report a mechanism by which the repressive or active state of chromatin in a domain defined by CTCF can be switched by the Wt1 transcription factor to regulate gene expression.

CTCF is a multifunctional protein that can activate or repress transcription or function as an insulator to regulate promoter-enhancer interactions. To explain these different properties, it has been proposed that CTCF is a "looper," i.e., a protein that simply mediates intra- and interchromosomal interactions between different loci in the genome without actually specifying the consequence of these interactions (Phillips and Corces, 2009). Instead, the functional output of these contacts is determined by the nature of the sequences that are brought together. In some cases, CTCF plays the classical insulator role of interfering with enhancer and promoter communication and may inhibit gene expression (Kurukuti et al., 2006). In other cases, CTCF brings distal enhancers in close proximity to the promoter in order to activate transcription (Xu et al., 2011). Perhaps least understood among CTCF functions in nuclear biology is its possible role in delimiting domains of chromatin structure (Kim et al., 2011). Genome-wide studies of CTCF distribution have uncovered a subset of CTCF sites localized to boundaries between active and repressive chromatin domains marked by histone H2A lysine 5 acetylation (H2AK5Ac) and histone H3 lysine 27 trimethylation (H3K27me3), respectively (Cuddapah et al., 2009). These regions are different between HeLa and CD4+ T cells, suggesting a possible role for CTCF-delimited domains in establishing lineage-specific gene regulation. In this issue, Essafi et al. (2011) shed light on this important problem. The authors demonstrate that the Wilms' tumor 1 (Wt1) transcription factor activates or represses expression of the mouse *Wnt4* gene in a cell-type-specific manner by controlling the state of chromatin in a domain whose boundaries are defined by CTCF (Essafi et al., 2011).

Epithelial-mesenchymal transition during the development of the epicardium, or the reverse process during the development of the kidney, are controlled by Wt1 through the regulation of *Wnt4*. Essafi et al. (2011) now show through chromatin immunoprecipitation experiments that Wt1 binds to the promoter region of *Wnt4* in both tissues but with opposite outcomes in gene expression. In kidney cells, the authors found that Wt1 recruits the CBP/p300 coactivators and switches on transcription of *Wnt4*. In epicardial cells of the heart, however, Wt1 recruits the Basp1 corepressor to silence *Wnt4* expression. These Wt1-induced changes in the transcriptional state of *Wnt4* correlate with changes in chromatin structure as determined by the presence or absence of specific covalent histone modifications. The authors found that kidney cells, where the *Wnt4* gene is active, contain histone H3 that is trimethylated on lysine 4 (H3K4me3) and acetylated on lysine 9 and lysine 14 (H3K9ac and H3K14ac) at the *Wnt4* locus. Epicardial cells, in which *Wnt4* is silenced, lack these modifications and contain H3 trimethylated at lysine 27 instead. The loss of Wt1 in either cell type results in the reversal of the transcriptional state of *Wnt4* and the active/repressive chromatin landscape at the locus. In the kidney mesenchyme, *Wnt4* expression is reduced, and the locus exhibits a decrease in H3K4me3, H3K9ac, and H3K14ac marks and a concomitant enrichment of the H3K27me3 repressive mark (Figure 1).

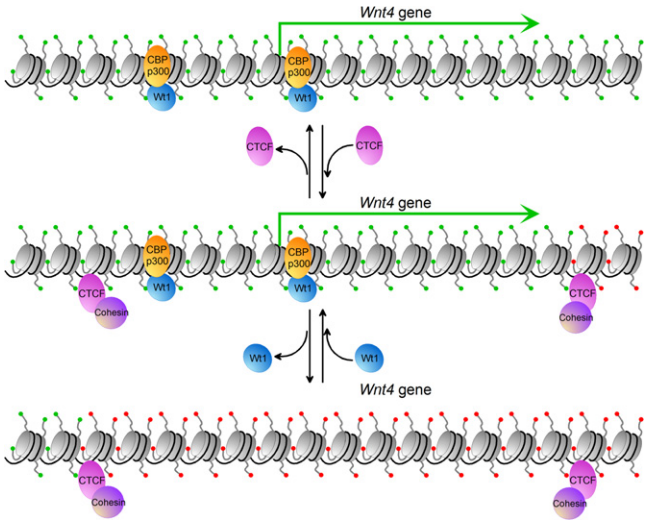

Figure 1. Wt1 Mediates Chromatin Flip-Flop in the CTCF-Delimited *Wnt4* Locus
The *Wnt4* gene is flanked by two CTCF sites (middle row). The gene is active in mesenchymal kidney cells where it is bound by the transcription factor Wt1, which recruits the coactivators CBP/p300. The active histone marks (green) are present in the domain defined by CTCF. Mutation of the Wt1 transcription factor in these cells causes repression of the *Wnt4* gene and enrichment of repressive chromatin marks (red) within the CTCF domain (bottom row). The reverse effect is seen in epicardial cells (not shown). CTCF is critical for the establishment and/or maintenance of the chromatin domain by Wt1 at the *Wnt4* locus. Loss of the CTCF protein causes the spreading of the active chromatin past the boundary (top row) and can result in the transcriptional activation of neighboring genes.

Interestingly, Wt1-dependent changes in chromatin marks at the *Wnt4* locus are confined to a region of the genome delimited by CTCF and the cohesin subunits Rad21 and Sa2. Cohesins have been shown to interact with CTCF and to be required for its function as a chromatin

insulator (Xiao et al., 2011), suggesting that they may contribute to the formation of CTCF-mediated DNA loops (Dorsett, 2011). The formation of such loops enclosing the *Wnt4* locus may be the basis for the functional chromatin domain established by Wt1. Wt1-mediated chromatin- and gene-expression regulation is limited to the region bound by the two CTCF sites and does not affect the neighboring *Zbtb40* and *Cdc42* genes that are located on either side of the domain. Indeed, if the boundaries of the domain are disrupted through the reduction of CTCF and/or cohesin, the transcription of these two neighboring genes is induced (Figure 1). This observation suggests that, in the absence of CTCF, the active chromatin domain created by Wt1 in kidney cells spreads outside of its normal boundaries and alters the transcription of neighboring genes, with perhaps important consequences for the viability or differentiation of the affected cells. The first CTCF insulator analyzed in vertebrates, the chicken HS4 insulator element located in the β-globin locus, shows only enhancer-blocking insulator function (Bell et al., 1999). The findings of Essafi et al. (2011) now convincingly demonstrate that CTCF can also have barrier insulator activity. Analysis of the basis for these two different behaviors of CTCF in different genomic and cell-type contexts should give insights into the mechanisms by which this protein affects nuclear organization and gene expression.

Recently, a high-resolution CTCF chromatin interactome map in mouse embryonic stem cells has identified 1480 *cis*- and 336 *trans*-interacting loci (Handoko et al., 2011). Up to 23% of all loops formed by these CTCF-mediated interactions appear to separate domains of active or repressive chromatin modifications. The nature and function of the genes located in these domains has not yet been analyzed. It will be interesting to examine in future studies whether domain-specific regulation of chromatin structure, as shown by Essafi et al. (2011) for epicardial and kidney mesenchymal cells, is a general mechanism broadly used during cell differentiation. Such mechanism would ensure coregulation of genes present in a specific CTCF-delimited domain.

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Redirecting Traffic in the Nucleus

Kulwant Singh[1] and F. Jeffrey Dilworth[1,2,*]
[1]Sprott Center for Stem Cell Research, Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, ON K1H 8L6, Canada
[2]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada
*Correspondence: jdilworth@ohri.ca
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Nuclear spatial organization of genes has emerged as an important determinant of their transcriptional activity. In this issue, Wang et al. (2011) show that the Msx1 homeoprotein induces a dramatic redistribution of Ezh2 and H3K27me3 to the nuclear periphery of muscle progenitor cells to repress transcription of developmentally regulated genes.

It is now established that mammalian genomes take on a nonrandom spatial organization in the nucleus that is both dynamic and cell-type specific. Chromosomes, segments of the chromatin, or individual gene loci adopt a highly organized structure and move between spatially distinct chromosome territories (Rajapakse and Groudine, 2011). Among these chromosome territories, the nuclear lamina (or nuclear periphery) regroups chromatin enriched for repressed genes and represents ~40% of the genome (Peric-Hupkes et al., 2010), whereas RNA Polymerase II-rich transcription factories in the lumen are associated with highly expressed genes (Eskiw et al., 2010). However, it should be noted that genes on the nuclear periphery are not always repressed nor are genes in the lumen always expressed (Meister et al., 2011).

Like chromatin, the distribution of histone marks has also been observed to change with cell identity. The transcriptionally repressive lysine 27 trimethylation mark on histone H3 (H3K27me3) is established by the Ezh2 subunit of the PRC2