

**The ubiquitous transcription factor CTCF promotes
lineage-specific epigenomic remodeling and
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1 **The ubiquitous transcription factor CTCF promotes lineage-specific epigenomic**
2 **remodeling and establishment of transcriptional networks driving cell differentiation**

3

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22 **Keywords**

23 CCCTC-binding factor (CTCF), Cell differentiation, Cistrome, DNA hydroxymethylation,
24 Epigenome, Enhancer, TET methylcytosine dioxygenase, Transcriptome, Ubiquitous
25 transcription factor

26

27 **Abbreviations**

28 CTCF, CCCTC-binding factor; KLF, Krüppel-like factors; PPARG, Peroxisome proliferator-
29 activated receptor gamma; CEBP, CCAAT/enhancer binding protein; H3K4me1,
30 monomethylation of histone H3 lysine 4; H3K27ac, acetylation of histone H3 lysine 27; TET, Ten-
31 eleven translocation methylcytosine dioxygenase; TF, Transcription factor; 5mC, 5-
32 methylcytosine; 5hmC, 5-hydroxymethylcytosine

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34 **Extra View**

35 A dynamic CTCF chromatin binding landscape promotes DNA hydroxymethylation and
36 transcriptional induction of adipocyte differentiation.

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51 **ABSTRACT**

52 Cell differentiation relies on tissue-specific transcription factors (TFs) that cooperate
53 to establish unique transcriptomes and phenotypes. However, the role of ubiquitous TFs in
54 these processes remains poorly defined. Recently, we have shown that the CCCTC-binding
55 factor (CTCF) is required for adipocyte differentiation through epigenomic remodelling of
56 adipose tissue-specific enhancers and transcriptional activation of Peroxisome proliferator-
57 activated receptor gamma, the main driver of the adipogenic program (PPARG), and its target
58 genes. Here, we discuss how these findings, together with the recent literature, illuminate a
59 functional role for ubiquitous TFs in lineage-determining transcriptional networks.

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76 **Transcriptional control of cell differentiation**

77 Cell differentiation is a highly dynamic process, which allows the establishment of
78 defined phenotypes. This is accomplished through specification of unique transcriptomes, a
79 process that heavily relies on usage of cell type-specific enhancers.^{1,2} Enhancer activation can
80 be achieved through sequential TF recruitment to the chromatin following initial binding of
81 pioneer TFs or involve a concomitant and cooperative binding of multiple TFs.³⁻⁵
82 Cooperative binding may involve assisted loading where TF binding to close or overlapping
83 motifs may facilitate each other's binding because of the very dynamic nature of TF-
84 chromatin interactions.^{6,7} Either way, enhancer activation requires chromatin/epigenomic
85 remodelling, which has now been widely used as a surrogate to monitor enhancer activities
86 during cell differentiation, including adipocyte differentiation.⁸⁻¹⁰ In this context, we have
87 contributed to the identification of DNA methylation as a novel epigenetic mark actively
88 controlled by TFs at enhancers.^{11,12} Moreover, using adipocyte differentiation as a model
89 system, we have shown that loss of DNA methylation at activated enhancers is reciprocally
90 linked to a gain in DNA hydroxymethylation (Fig.1).^{13,14} The **mechanistic** connection
91 between these observations is underlined by Ten-eleven translocation methylcytosine
92 dioxygenase (TET)-mediated DNA hydroxymethylation being an intermediate in the DNA
93 demethylation process.¹⁵ **Importantly, a recent study functionally linked DNA**
94 **hydroxymethylation to DNA demethylation, enhancer activation and gene induction during**
95 **cell differentiation.**¹⁶ However, DNA hydroxymethylation is relatively stable¹⁷ and can also
96 modulate TF or chromatin modifier DNA recognition¹⁸ suggesting that cytosine
97 hydroxymethylation may represent a novel epigenetic mark *per se*.¹⁹ Fujiki et al. subsequently
98 ascribed to the adipocyte lineage-determining TF Peroxisome proliferator-activated receptor
99 gamma (PPARG) the function of promoting DNA hydroxymethylation through chromatin
100 recruitment of TET.²⁰

101 In addition to lineage-determining factors such as PPAR γ , which are expressed in
102 specific tissues, the TF repertoire comprises ubiquitous TFs with similar expression in almost
103 all tissues.²¹ However, with the exception of general TFs involved in the pre-initiation
104 complex (PIC), the role of ubiquitous TFs in cell differentiation has long remained elusive.
105 Here, we discuss the general implications for our most recent findings showing a requirement
106 for ubiquitous CCCTC-binding factor (CTCF) in defining the adipocyte-specific epigenome
107 and transcriptome (Fig.1).²²

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109 **CTCF as a driver of cell type-specific enhancer activities**

110 CTCF, initially identified as a TF binding to insulators,²³ was later revealed to
111 harbour pleiotropic activities including transcriptional activator/repressor, nucleosome
112 positioning and chromatin three-dimensional organizer activities.^{2,23,24} Based on this latter
113 function and on the identification of conserved CTCF binding events across various cell-types
114 and tissues,^{25,27} this TF was proposed to fulfil a general chromatin organization role.²⁸ **At the**
115 **same time**, specific roles for CTCF in neuronal and hematopoietic cell differentiation had
116 been defined.²⁹ In this context, detailed analyses of CTCF ChIP-seq studies revealed the
117 existence of cell type-specific chromatin binding sites.³⁰ **Indeed, thorough comparison of the**
118 **CTCF binding landscape in 19 human cell-types revealed that 64% of identified CTCF**
119 **binding sites are not conserved.**³⁰ **This may have been previously overlooked owing to a more**
120 **limited number of analyzed cell types or to the use of stringent criteria to call bound regions.**
121 **Indeed, conserved binding sites show stronger CTCF recruitment and show less degenerate**
122 **CTCF binding motifs compared to cell type-specific CTCF bound regions.**^{22,30,31} We have
123 now shown that the CTCF ChIP-seq is highly dynamic during the course of adipogenesis **since**
124 **we found that more than half of the CTCF binding sites (>30,000 sites) identified across 4**
125 **stages of the differentiation process were not constitutively bound.**²² Moreover, our study

126 provides evidence that this dynamic CTCF chromatin binding occurs at lineage-specific
127 enhancers, which promote adipocyte differentiation (Fig.1).²² CTCF binding at these
128 enhancers is not detected in preadipocytes and occurs upon differentiation when these
129 regulatory sites are activated. As discussed hereafter, CTCF is necessary for DNA
130 hydroxymethylation²² of enhancers, a process required for cell differentiation.^{16,20}
131 Interestingly, another recent study has uncovered a similar role for another ubiquitous TF, the
132 NF-Y complex, in activation of cell type-specific enhancers.³² How these ubiquitous TFs are
133 targeted to cell type-specific regulatory sites remains to be defined. Regarding CTCF, post-
134 translational modifications and the existence of different modes of DNA recognition might
135 help to modulate target sequence recognition.^{33,34} Alternatively, combinatorial TF binding to
136 enhancers, which now arises as a prevalent mechanism, could shape the CTCF cistrome.
137 Direct CTCF interaction with TFs may, for instance, be involved in this process.^{23,35} Of note,
138 differentially active enhancers in mast cell and multipotent hematopoietic progenitors require
139 TFs which are both specific and shared between the 2 cell-types to regulate transcription,
140 further indicating that commonly expressed TFs are instrumental in regulating cell type-
141 specific enhancer activities.^{27,36}

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143 **Involvement of CTCF in chromatin remodelling at cell type-specific enhancers**

144 Multiple mechanisms may explain the requirement for combinatorial TF binding to
145 enhancers. For instance, combinatorial TF binding to enhancers during adipocyte
146 differentiation allows for cooperative coactivator recruitment and induction of histone
147 acetylation.⁵ In this context, in addition to PPARG, we have found that CTCF promotes TET-
148 mediated DNA hydroxymethylation of enhancers driving adipocyte differentiation.²² In
149 addition to such "quantitative" effects where multiple TFs cooperate to reinforce or amplify a
150 given chromatin modification, individual contributions of different TFs to different enhancer

151 functionalities may occur. TF binding to enhancers is not always simultaneous and can rather
152 involve different kinetics implying a sequential or even a mutually exclusive recruitment.^{5,37}
153 This may in part be driven by the cyclic nature of transcriptional regulatory events that
154 require instructed and sequential TF/cofactor chromatin binding and dismissal.^{38,39} In this
155 context, it will be of great interest to better define whether and how the different
156 functionalities of CTCF **on the chromatin structure** are involved at enhancers and how they
157 relate to those of collaborating TFs. **Indeed, CTCF is able to control local epigenetic**
158 **modifications of DNA and histones.**^{22,40} **It is also able to position surrounding nucleosomes**²⁴
159 **and to act on chromatin three-dimensional folding**⁴¹. Regarding NF-Y, it was hypothesized
160 that this factor promotes chromatin opening at enhancers (potentially through nucleosome
161 displacement) to favor recruitment of master lineage-determining TFs.³²

162 An important aspect of this research area will be to better define the relative influence
163 of DNA methylation/hydroxymethylation on chromatin binding of ubiquitous and cell-
164 specific TF. For instance, CTCF DNA binding can be inhibited by DNA methylation³⁶ while,
165 reciprocally, CTCF is able to promote DNA demethylation suggesting a mutual
166 influence.^{12,35,42} Importantly, this relationship may be strongly linked to the genomic
167 environment as, for example, **conversion of methylated DNA into hydroxymethylated DNA**
168 **correlates with** CTCF binding mostly outside of CpG islands (CGIs).⁴³

169 **Finally, cell-specific CTCF binding has been shown to influence chromatin looping to**
170 **promote interaction between *Ubx* enhancers and promoter to trigger gene transcriptional**
171 **activation in *Drosophila*.**⁴¹

172 Therefore, **CTCF** emerges as a crucial chromatin remodeler at cell-type specific
173 enhancers.

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175 **Ubiquitous TFs and lineage-determining TF networks**

176 We identified the adipose lineage-determining factor PPARG as one main target gene
177 dynamically bound and transcriptionally regulated by CTCF during adipocyte differentiation,
178 (Fig.1).²² Reminiscent of the establishment of specialized transcriptomes in other cell types
179 such as hepatocytes,⁴⁴ adipocyte differentiation involves the build-up of a TF network, which
180 includes, in addition to PPARG, members of the Krüppel-like factor (KLF) and
181 CCAAT/enhancer binding protein (CEBP) families. These TFs show cross-regulation of their
182 expression and cooperate to regulate important adipose-specific target genes.^{45,46} Our study
183 therefore shows that CTCF is part of this network through the regulation of both PPARG
184 expression and activities (Fig.1). These findings are reminiscent of those of Delgado-Olguín
185 et al. regarding myogenesis during which CTCF both regulates the expression of, and
186 cooperates with MyoD⁴⁷. These studies functionally validate predictions made from the
187 analyses of DNaseI footprints from 41 cell and tissue-types indicating that CTCF was
188 surprisingly involved in most cell type-specific TF networks. Interestingly, a similar
189 prediction was made for additional ubiquitous TFs including SP1, NFYA, and MAX
190 suggesting that this could be a general organization scheme of lineage-determining
191 transcriptional networks.⁴⁸

192

193 **Concluding remarks**

194 Our recent study provides an important new contribution to the recent literature
195 describing how ubiquitously expressed TFs functionally contribute to establishing cell-
196 specific transcriptomes and phenotypes. However, important questions still remain to be
197 answered. For instance, CTCF is characterized by an uncommonly large number of potential
198 activities when bound to chromatin, making it central to the regulation of the functional
199 genomic landscape. However, a major challenge arising is to better understand how CTCF's
200 multiple functionalities are specified in space and time. With this aim, it will be instrumental

201 to better understand how cues provided by the local chromatin environment and collaborating
202 TF/cofactors allow for region and cell-specific CTCF binding and functions. This will
203 undoubtedly significantly improve our understanding of the functional connection between
204 ubiquitous and cell type-specific TFs in lineage-determining transcriptional networks.

205

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353

354 **Figure legends**

355 **Fig1. Involvement of ubiquitous TFs in the transcriptional regulation of cell** 356 **differentiation.**

357 The main concepts discussed in the manuscript are summarized (see text for details).

358 The absence or presence of histone modifications that characterize active enhancers (H3K4me1 and
359 H3K27ac) is indicated together with DNA methylation (5mC) and DNA hydroxymethylation (5hmC).
360 H3K4me1, monomethylation of histone H3 lysine 4; H3K27ac, acetylation of histone H3 lysine 27;
361 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine.

362

Fig.1

