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Locus dependent role of CTCF sites in enhancer-promoter transcriptional regulation

Irié Carel[1], Karissa Hansen[2], Annie Adachi[2] and Elphège Nora[2]

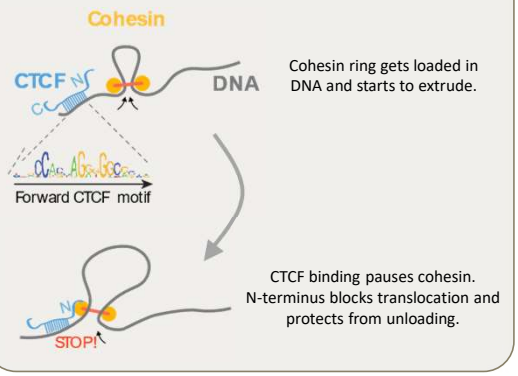
[1]Magistère Européen de Génétique, Université Paris Cité, Paris
[2]University of California San Francisco, San Francisco, USA

INTRODUCTION

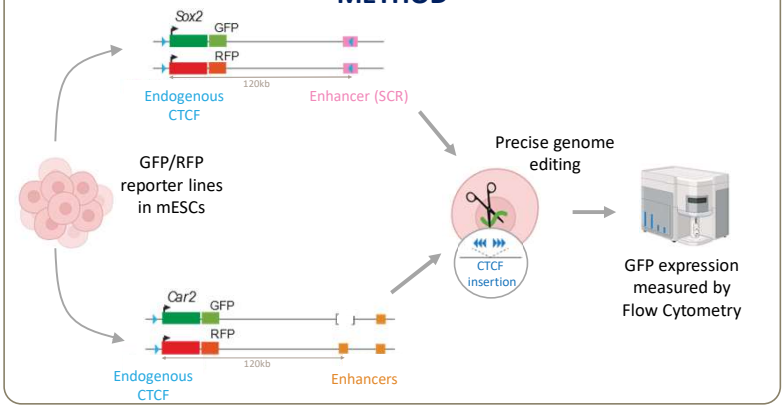
The regulation of communication between enhancers and promoters plays a key role in transcriptional patterning during cell differentiation. When separated by a long distance, the relative 3D positioning of the Cis-Regulatory Elements (CRE) to the promoter relies on the 3D folding of the DNA [1], enabled by the cohesin complex and its cofactors and delimited by CCTC-binding factors (CTCFs) [2]. The role of CTCF sites in gene regulation has only been studied in a handful of loci and its role as an enhancer blocker was recently challenged by a study working on the effect of the CTCF boundary in the *Sox2* locus. Authors reported that the *Sox2* enhancer can bypass artificial insertion of CTCF sites, to regulate the *Sox2* gene [3]. Furthermore, depleting CTCF and the subunits of the cohesin complex (RAD21, WAPL [4]; NIPBL, PDS5 Nora Lab, unpublished) only has a mild effect on *Sox2* expression, indicating loop extrusion is largely dispensable for *Sox2* regulation. In order to identify which genes actually rely on loop extrusion, the Nora lab performed RNA-seq in mouse ES Cells (mESCs) after acute degradation of NIPBL, a protein necessary for the loop extrusion activity but dispensable for the mitotic functions of cohesin (unpublished), and CTCF [5]. Among the down-regulated genes upon depletion, *Car2* has been identified as a CTCF-Cohesin sensitive gene while *Sox2* was only mildly affected (Nora Lab, unpublished).

Following those observations, one of the goals in the Nora Lab is to identify the differences in loop-extrusion sensitiveness between *Car2* and *Sox2* and more generally, to understand how the genomic context may render a given gene sensitive or not to the loop extrusion pathway. During my internship in the Nora Lab, I specifically investigated the role of CTCF sites in the regulation of both *Car2* and *Sox2*.

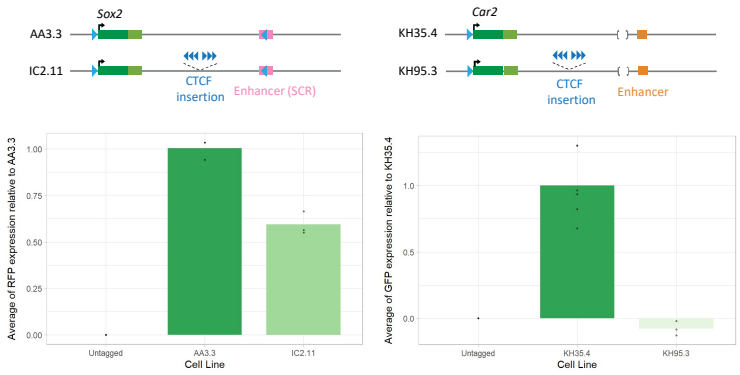
MODEL OF COHESIN-CTCF MEDIATED DNA LOOP EXTRUSION



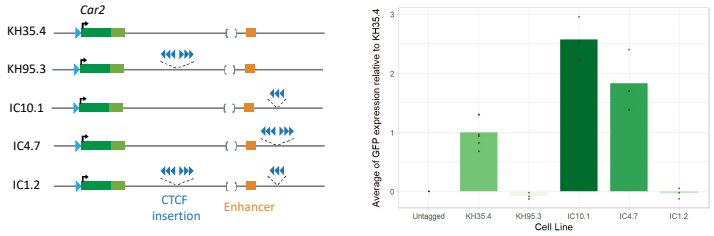
METHOD



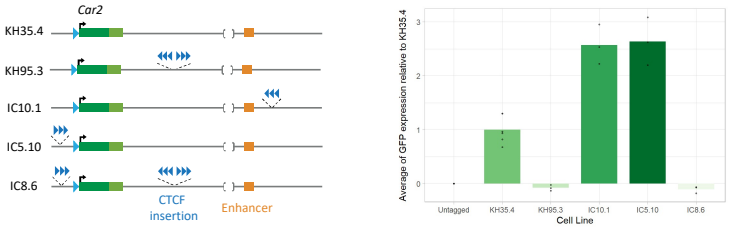
1. CTCF sites insulation strength depends on the genomic context



2. CTCF sites at the enhancer boost *Car2* expression but is not sufficient to bypass intergenic insulation



3. CTCF sites at the promoter boost *Car2* expression but is not sufficient to bypass intergenic insulation



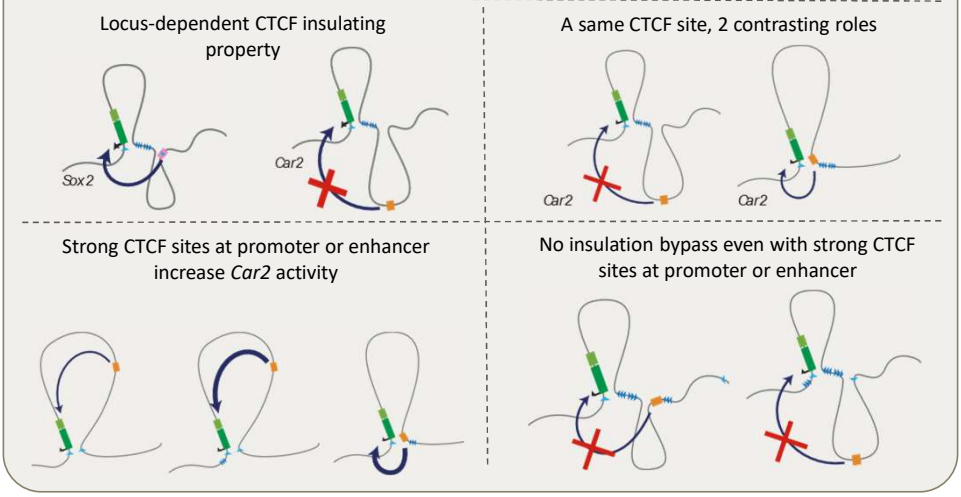
CONCLUSION AND PERSPECTIVES

While I did not discover why *Sox2* and *Car2* have different sensitivity to CTCF/cohesin, these data show that CTCF sites can have contrasting effects depending on the nature of the gene (cohesin sensitive or non-sensitive) but also depending on its relative position to the promoter and the enhancers within a single locus. Moreover, it shows that reinforcing CTCF binding in the *Car2* promoter is sufficient to up-regulate its expression. Furthermore, CTCF insulation bypass needs more than the tethering of enhancer or promoter only. One possible explanation is that we need the combination of both a strong CTCF site at the promoter or at the enhancer in pair to enable the insulation bypass.

In future experiments, it would be interesting to test the above hypothesis by creating a triple CTCF insertion at the promoter, the enhancer and the intergenic loci. Finally to properly explore the hypothesis of loop stacking, the Nora lab will look at the 3D conformation of some of cell lines I have generated with a DNA FISH chromatin tracing approach by microscopy invented by collaborators [6].

Overall, these results provide new insights into the role played by CTCF sites in enhancer-promoter communication and open new sides of transcriptional regulation to be explored.

GRAPHICAL ABSTRACT



References

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[3] Chakraborty, S. *et al.* (2023) 'Enhancer–promoter interactions can bypass CTCF-mediated boundaries and contribute to phenotypic robustness', *Nature Genetics*, 55(2), pp. 280–290. Available at: https://doi.org/10.1038/s41588-022-01295-6.
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