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Dynamic Looping Interactions: Setting the 3D Stage for the Macrophage

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The mechanisms by which 3D chromatin looping interactions mediate cell type-specific gene expression are an active area of investigation. In this issue of *Molecular Cell*, Phanstiel et al. (2017) annotate five classes of loops during macrophage development and predict candidate factors involved in their regulation.

Chromatin engages in long-range looping interactions that bring distal segments of the genome into close spatial proximity (Deng et al., 2014). Looping interactions are critically linked to the regulation of gene expression and have been classified into cell type-specific and constitutive classes during differentiation and reprogramming. Enhancer-promoter interactions are dynamic among cell types and correlate with dynamic occupancy of architectural proteins such as CTCF, Mediator, and cohesin (Beagan et al., 2017; Kagey et al., 2010; Sanyal et al., 2012). By contrast, constitutive loops often play a structural role and are anchored by constitutive occupancy of CTCF and cohesin (Phillips-Cremins et al., 2013; Tang et al., 2015). CTCF-mediated constitutive loops are thought to regulate gene expression by forming insulated neighborhoods that protect genes from aberrant gene activation due to ectopic enhancer looping (Dowen et al., 2014). Recently, over 10,000 loops were identified genome-wide in a transformed human cell line (Rao et al., 2014). The genome-wide landscapes of long-range looping interactions and their dynamic properties across most lineages in the human body remain largely uncharacterized.

In this issue, Phanstiel et al. set out to create genome-wide looping maps during the transition from monocyte precursors to mature, differentiated macrophages (Phanstiel et al., 2017). Using high-resolution *in situ* HiC, the authors report ~16,000 long-range looping interactions in a monocytic leukemia cell line induced to differentiate to macrophages with phorbol myristate acetate. Consistent with previous reports (Rao et al., 2014), the loops anchored by the architectural protein CTCF displayed a strong orientation bias for inward-facing ("convergently oriented") CTCF motifs, thus validating the high quality of their detected loops. Although CTCF is known to be the predominant architectural protein in mammalian cells, Phanstiel et al. show that ~20% of the loops detected in their innate immune system cell types are not enriched for CTCF occupancy. This finding is consistent with a recent report in which a large proportion of loops in mouse neural progenitor cells are not connected by CTCF (Beagan et al., 2017). Together these data open up the possibility that architectural proteins in addition to CTCF might exist to connect key subclasses of long-range looping interactions in cell types of the innate immune system.

A major contribution of Phanstiel et al. is to stratify loops into five distinct classes based on their dynamic structural features and dynamic acetylation of the histone H3 lysine 27 residue (H3K27ac) (Figures 1A–1E). Long-range looping interactions were categorized into: (1) structural loops that are largely constitutive between cell types and show no change in H3K27ac levels at loop anchors (Figure 1A); (2) deactivated, pre-existing loops that are present in both cell types but exhibit a reduction in H3K27ac levels upon macrophage differentiation (Figure 1B); (3) decommissioned loops that are lost and exhibit a correlated loss in H3K27ac upon differentiation (Figure 1C); (4) activated, pre-existing loops that are present in both cell types but acquire increased H3K27ac levels upon macrophage differentiation (Figure 1D); and (5) gained loops that arise *de novo* upon differentiation and correlate with the acquisition of H3K27ac and activated gene expression in the monocyte to macrophage transition (Figure 1E). These results provide a richer understanding of the possible functional roles for different looping interactions, as previous work in understanding looping dynamics has been restricted to loops that are lost, gained, or constitutive between cell types (Figures 1F–1H).

A critical unanswered unknown is whether and/or how the looping classes are connected to gene expression changes during macrophage differentiation. The initial correlation analysis pursued by the authors suggests that expression of genes at the base of both gained (class 5 above) and activated pre-existing (class 4 above) chromatin loops are significantly increased during differentiation compared to static loops (class 1 above). By contrast, the decommissioned loops (class 3 above) did not correlate with reduced gene expression. This observation stands in contrast to literature showing that the decommissioning of pluripotency gene-enhancer loops correlates with loss of enhancer activity and silencing of pluripotency genes upon differentiation (Kagey et al., 2010; Beagan et al., 2017). Thus, it remains to be resolved whether the poor correlation between loop decommissioning and gene expression is unique to the monocyte-macrophage transition, a feature of a comparatively late-stage differentiation paradigm, or simply a function of the low

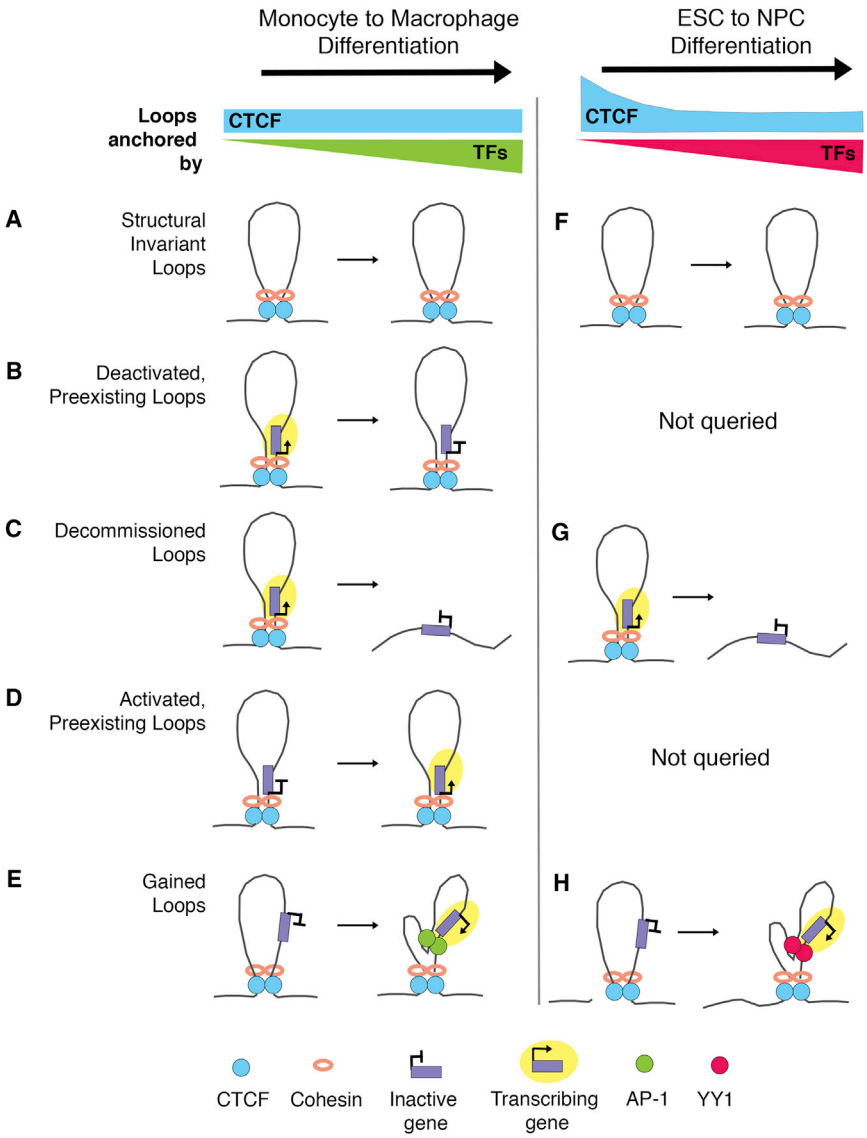

Figure 1. Dynamic Looping Classes Detected during Cell Fate Transitions

(A–E) Looping classes identified by Phanstiel et al. (2017) during monocyte to macrophage differentiation. (A) Structural loops are invariant across cell types and anchored by CTCF and cohesion. (B) Deactivated, pre-existing loops are invariant across cell types but show reduced H3K27ac upon macrophage differentiation. (C) Decommissioned loops are lost upon differentiation and show loss of CTCF occupancy in parallel with loss of the 3D interaction. (D) Activated, pre-existing loops are invariant across cell types but show gained H3K27ac upon macrophage differentiation. (E) Gained loops formed de novo upon differentiation are not enriched for CTCF and are often anchored by transcription factors such as YY1, Fos, Jun, and Klf4.

(F–H) Looping classes identified by Beagan et al. (2017) during embryonic stem cell to neural progenitor cell differentiation. (F) Structural loops are invariant across cell types and anchored by CTCF and cohesion. (G) Decommissioned loops are lost upon differentiation and show loss of CTCF occupancy in parallel with loss of the 3D interaction. (H) Gained loops formed de novo upon differentiation are not enriched for CTCF and are often anchored by transcription factors such as YY1, Fos, Jun, and Klf4.

sample size in this looping class (Kagey et al., 2010). Finally, structural loops (class 1 above) do not exhibit clear trends in the expression of genes at anchors, consistent with the role for these loops in creating insulated neighborhoods that delimit the search space of enhancers for their target genes (Dowen et al., 2014). Some fundamental questions remain regarding the functional role for looping interactions in gene expression regulation. Importantly, the large majority of developmentally regulated genes in the monocyte-to-macrophage transition do not require looping for their upregulation. Future studies should aim to unravel the cause and effect role of structure on genome function and the relative contribution of genomic context (i.e., genetic and epigenetic modifications) and looping to developmentally regulated gene expression.

Another important question is whether and how the differential loop classes use common or distinct organizing principles. Consistent with previous studies, Phanstiel et al. find that decommissioned (class 3 above); deactivated, pre-existing (class 2 above); structural (class 1 above); and activated, pre-existing (class 4 above) loops are all enriched for CTCF at loop anchors. Interestingly, the authors observe strong enrichment for Fos and Jun consensus sequences at gained loops (class 5 above), but not CTCF. This finding is reminiscent of recent work showing that loops gained *de novo* in embryonic stem cell differentiation to neural progenitor cells were not enriched for CTCF and instead anchored by YY1 (Beagan et al., 2017). Similarly, Rubin et al. reported that transcription factors Klf4 and ZNF750, but not CTCF, anchor loops that are gained during epidermal differentiation (Rubin et al., 2017). Functional studies involving transient degradation and then rescue in the absence of any transactivating domain will be needed to dissect whether Fos/Jun serve as bona fide somatic architectural proteins.

Together these studies support a working model in which the large majority of CTCF-mediated looping interactions are set up very early in development, and differentiation involves a progressive pruning back of unneccessary CTCF-mediated enhancer-promoter interactions. By contrast, CTCF-mediated structural loops remain constant throughout development and may assist in assembly of active chromatin hubs that are poised for possible activation. We hypothesize that loops arising *de novo* between lineage-specific genes and their enhancers are not connected by CTCF, but instead a new group of somatic architectural proteins of which YY1 and Fos/Jun might be inaugural members. Future studies will elucidate whether the organizing principles of genome folding discussed here are

pervasive across all stages of mammalian development.

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"Gamete On" for m^{6}A: YTHDF2 Exerts Essential Functions in Female Fertility

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In this issue of *Molecular Cell*, Ivanova et al. (2017) report key functions of the m^{6}A reader YTHDF2 in the regulation of mammalian development during oocyte maturation and early zygotic development.

Sexual reproduction, one of the most fundamental features of life, is also one of the most highly regulated biological processes during an organism's life cycle. It starts with gametogenesis in both parents, with functional gametes forming through meiosis; fertilization then occurs with the merging of oocyte and sperm, which subsequently initiates the development process of the offspring organism. Each of these steps during reproduction is known to be regulated by a series of changes that are highly orchestrated at different levels. As transcription and translation shut down during defined stages of animal gametogenesis and zygotic development, post-transcriptional mechanisms exert critical functions to ensure correct gene regulation and timely progression of reproduction programs. In this issue, a new study by Ivanova et al. (2017) reveals a post-transcriptional mechanism through which RNA modifications and their cognate protein regulators specifically regulate mammalian female oocyte maturation.

Among over 100 different types of known RNA modifications, N^6-methyladenosine (m^{6}A) is the most abundant form present in the internal regions of mammalian mRNA. A series of recent studies have uncovered some of its versatile functions in the regulation of various biological processes in different organisms. m^{6}A can be installed by a methyltransferase complex and reversed by demethylases (Roundtree et al., 2017). The demethylation process mediated by ALKBH5 has been shown previously to critically impact mouse spermatogenesis (Zheng et al., 2013), providing the first hint that this RNA methylation could play important roles in mammalian gametogenesis.

One of the main functions of m^{6}A is to mark groups of mRNAs for accelerated degradation and turnover. This is facilitated by m^{6}A-specific RNA binding proteins (m^{6}A readers) such as YTHDF2 (Roundtree et al., 2017; Wang et al., 2014a). This Ythdf2-mediated decay affects the early development of zebrafish by facilitating the fast clearance of maternal mRNA during maternal-to-zygotic transition (MZT) (Zhao et al., 2017). The m^{6}A-dependent decay of methylated transcripts also significantly impacts mouse embryonic stem cell differentiation and preimplantation development (Batista et al., 2014; Geula et al., 2015; Wang et al., 2014b).

Building on this previous knowledge, Ivanova et al. (2017) hypothesized that the YTHDF2-mediated mRNA decay may play crucial roles in mammalian gametogenesis, as rapid transcriptome turnover could be facilitated by RNA methylation. The authors constructed a transgenic mouse line with epitope-tagged YTHDF2 and confirmed its pervasive expression in both germ and somatic cells during spermatogenesis and folliculogenesis (an oocyte growth phase prior to maturation).