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Spotlight

De novo DNA Methylation: Who's Your DADdy?

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DNA methylation regulates the organization and function of the genome. Yamanaka *et al.* now report that *de novo* methylation of male germ cells of mice involves the transient opening of heterochromatin at megabase-size differentially accessible domains (DADs). This chromatin remodeling likely facilitates *de novo* methylation of the germ cell genome.

Throughout a man's lifetime, the odds of any individual sperm cell he produces influencing the next generation is worse than winning the lottery. However, each of his gametes, fruitful or wasted, represents an epic feat of chromatin reorganization and proper spatiotemporal gene expression. From the start, primordial germ cells (PGCs) are given the colossal task of epigenetic reprogramming involving genome-wide erasure of repressive 5-methylcytosine marks, removing parental imprints, and creating a naïve potency state to prime sex-specific cell differentiation [1]. Following their migration to the embryonic testis, male PGCs (now termed 'gonocytes') undergo mitotic arrest and rapidly re-establish DNA methylation *de novo*, an essential task for differentiation into spermatogonia, the source of sperm. Indeed, absence of *de novo* methylation in mouse male germ cells, such as in DNA methyltransferase (DNMT) 3 family mutants *Dnmt3a*$^{-/-}$ *and Dnmt3l*$^{-/-}$, results in failure to methylate paternal imprinting control regions, activation of transposable elements (TEs), and impaired spermatogenesis [2,3].

Two waves of *de novo* DNA methylation have been reported in mouse male gonocytes between embryonic day (E) 13.5 and postnatal day (P) 2, transitioning around E16.5 [4]. The first wave encompasses *de novo* methylation of most of the genome, while the second uses small Piwi-interacting (pi) RNAs to guide methylation of TEs [4]. One plausible feature distinguishing these waves is the accessibility of DNMTs to the genome to deposit DNA methylation marks. In dividing cells, DNMT recruitment is tightly coupled to DNA replication, taking advantage of locally unwound DNA at replication forks during S phase to gain occupancy of the genome in both euchromatic and heterochromatic regions [5]. However, in cell cycle-arrested gonocytes, the players and strategies for DNMT accessibility and subsequent *de novo* methylation, especially at inaccessible heterochromatic regions, become ambiguous.

Transient Accessibility of Gonocyte Chromatin Reveals DADs

To understand where and when *de novo* DNMTs might be recruited to the genome, Yamanaka *et al.* used pure populations of germ cells to probe the dynamics of genome-wide chromatin accessibility [6]. By performing assay for transposase-accessible chromatin using sequencing (ATAC-seq) to probe chromatin accessibility and NanoCAGE-seq to determine transcriptional start site and transcript abundance, the authors identified specific chromatin regions that acquire greater accessibility between E13.5 and E17.5, and subsequently close by P2. Such timing implicates these gonocyte-specific DADs in the second wave of *de novo* DNA methylation. Using the stringent 1-Mb minimal size cutoff, Yamanaka *et al.* identified a total of 143 DADs on all chromosomes, except the Y chromosome, accounting for ~14% of the mouse genome.

Genomic Features of DADs Highlight TEs

The authors next interrogated the defining features of DADs. Ultimately, 110 of the 143 DADs were found in gene-poor regions of the genome that encode fewer than ten genes per megabase and are instead enriched in TE sequences (70% of E17.5-specific peaks on TEs). Interestingly, a few DADs also included gene clusters, such as those belonging to olfactory receptor or V(D)J recombination, origins of which are based in TEs [7]. Deeper analysis of TE subfamilies and structural elements that overlapped with DADs revealed that 5′ regions of sequences belonging to evolutionarily 'young' LINE-1 subfamilies become accessible, features of full-length elements still with retrotransposition activity. Indeed, upregulation of TE transcripts was observed at E17.5, in correlation with their transiently accessible regions. Given that TE expression is a prerequisite for piRNA production, these data corroborate findings that methylation of young LINE-1 elements during the second wave of *de novo* methylation is piRNA dependent [4].

Epigenetic Regulation of DADs and *Vice Versa*

To understand the players and dynamics of DAD formation that are independent of DNA methylation and DNA replication, the authors interrogated another type of epigenetic regulation, namely, methylation of specific lysine residues in the amino-terminal tail of histone H3. Indeed, DADs corresponded with the reprogramming of active histone mark H3K4me3 and repressive histone marks H3K27me3 and H3K9me3 measured by ChIP-seq. Acquisition of H3K4me3 and complementary loss of H3K27me and H3K9me3 in DADs at

E17.5 resulted in the opening of chromatin and a more euchromatic state, permissive for DNMT binding. DADs gradually returned to a compacted state by P2 with a decrease in H3K4me4 and increase in H3K27me3 and H3K9me3. The plasticity of histone marks exemplified in DADs defined these regions as facultative heterochromatin that can reorganize in response to environmental cues, as opposed to static constitutive heterochromatin regions found at centromeres and telomeres.

The observed histone dynamics were reflected by the dynamic reorganization of chromatin 3D architecture measured by Hi-C. Indeed, the frequencies of long-range intrachromosomal interactions present at E13.5 decreased between E13.5 and E17.5 during DAD formation, signifying decompaction of chromatin structure. By P2, these interactions returned. This momentary decompaction of higher-order chromatin structure represents a transition state responding to DAD formation. Interaction patterns within the transition state, particularly between E17.5 and P0, were significantly rearranged compared with other time points. Specifically, switching between open and closed chromosome conformations based on A/B compartment loci was observed in some DADs [6].

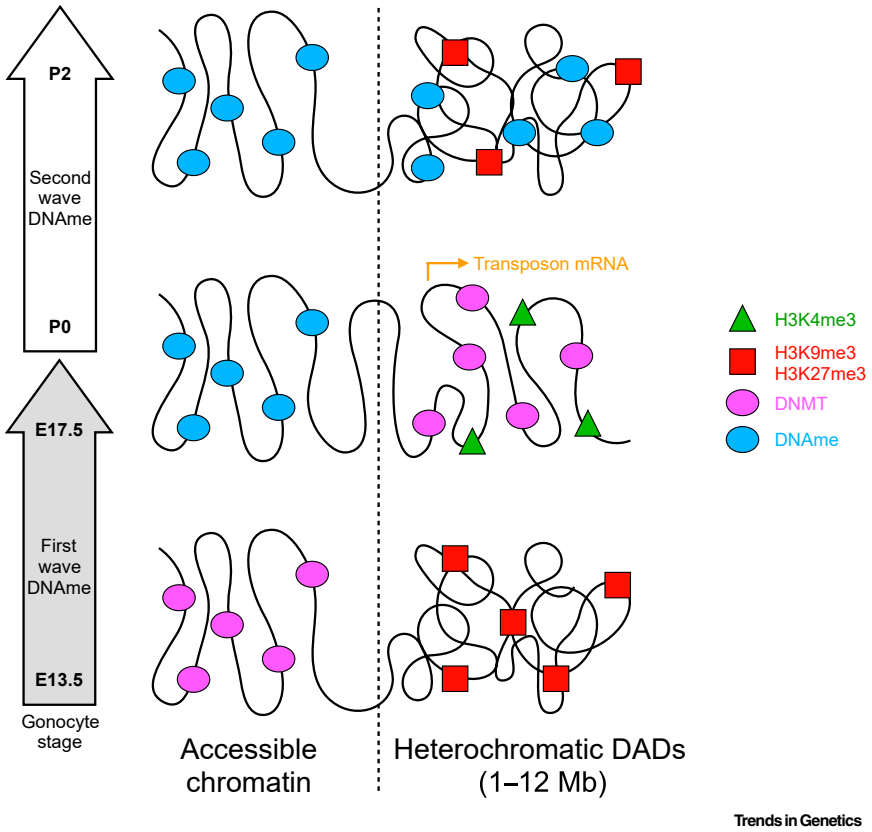

Figure 1. *De novo* DNA Methylation of Heterochromatic Differentially Accessible Domains (DADs).

De novo DNA methylation (DNAme) in male gonocytes occurs in two waves. The first wave targets accessible chromatin regions. The second wave, beginning around embryonic day (E)17.5, targets inaccessible chromatin regions, including gene deserts, gene clusters, and transposons. *De novo* methylation of inaccessible regions is marked by the transient formation of 1–12 Mb DADs, which is accomplished by rewriting active (H3K4me3) and repressive (H3K27me3 and H3K9me3) histone marks. Transient DAD formation permits a burst of transposon expression and allows DNA methyltransferase (DNMT) enzymes access to DNA for *de novo* methylation. Ultimately, DADs resume their compacted chromatin state at the end of gonocyte development. Abbreviation: P, postnatal day.

Cumulatively, Yamanaka *et al.* describe DADs as novel features of facultative heterochromatin that assume a transient relaxed state to permit DNMT recruitment and subsequent deposition of 5-methylcytosine marks for *de novo* methylation (Figure 1). However, the dependency of *de novo* methylation on DADs remains unexplored, for example, upon DNMT overexpression or perturbation of H3K4me3 methyltransferase activity. The mechanisms driving histone remodeling for DAD organization are also unsolved. A tantalizing candidate is the piRNA pathway due to direct relationships with the removal of H3K4me2 and establishment of *de novo* methylation and H3K9me3 patterns on TE sequences [8,9]. The authors speculate that *de novo* methylation related to DADs is likely not sequence specific due to the promiscuous nature of DNMTs, but given the requirement for piRNAs in *de novo* methylation of LINE-1 sequences, further analysis of their overall contribution to DAD organization is warranted [4,6]. Finally, since female germ cells regain methylation postnatally and only after enduring massive LINE-1-driven oocyte death [10], it will be intriguing to determine whether oocytes use the same chromatin-remodeling strategy elucidated by Yamanaka *et al.* in males [6].

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