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Beyond DNA: Programming and Inheritance of Parental Methylomes

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Epigenetic reprogramming of parental genomes following fertilization is important to ensure compatibility for totipotency and development thereafter. New studies by Jiang et al. and Potok et al. now demonstrate how the parental DNA methylomes are reset in zebrafish and reveal striking differences from events in mammals.

Sperm and oocytes are highly distinct and specialized cell types, yet together they generate the totipotent state following fertilization. Significantly, although they make an equivalent genetic contribution to the zygote, their epigenetic states are highly asymmetric due to their diverse origins and are therefore reset soon after fertilization. In vertebrates, this involves global remodeling of the parental DNA methylation (5mC) patterns, which is thought to generate an epigenetic state competent for totipotency (Surani et al., 2007). At the same time, however, the extent to which the inherited parental epigenomes are themselves important for development is unclear. Indeed, resetting of parental epigenomes occurs in the overall context of development, which differs markedly among vertebrates and which may therefore influence the balance between reprogramming and inheritance. In this issue of *Cell*, Jiang et al. (2013) and Potok et al. (2013) now reveal how genome-wide DNA methylation transitions of parental genomes occur during zebrafish development. Notably, whereas the maternal methylome undergoes striking remodeling during early development, the paternal methylome is stably inherited in a remarkably unchanged state. The strategy for reprogramming parental epigenomes is thus fundamentally different between vertebrates (see Figure 1) (Smith et al., 2012).

Zebrafish development proceeds through synchronous cleavage divisions every ~15 min until the midblastula transition (MBT), when major zygotic gene activation (ZGA) commences (~1,000 cells) (Tadros and Lipshitz, 2009). To track DNA methylation transitions during this period, both groups generate whole-genome bisulfite sequencing maps from gametes and early developmental time points flanking the ZGA. Zebrafish oocytes are hypomethylated (75%–80% CpG methylation) relative to sperm (91%–95%), similarly to mice. However, upon fertilization, the paternally derived methylome is stably inherited without significant changes throughout early zebrafish development. In parallel, the maternal methylome is initially stable but subsequently undergoes extensive remodeling that resets its epigenetic state to that of the paternal genome. This occurs through simultaneous DNA demethylation of oocyte-specific hypermethylated regions and de novo methylation of oocyte-specific hypomethylated regions. Thus, by the time of ZGA, the parental genomes reach epigenomic equivalence through selective resetting of the maternal methylome to resemble the stable paternal methylome. At this time, the methylome acquires competence for further development, including primordial germ cell (PGC) specification through the inheritance of preformed germ cell determinants (Figure 1).

The reprogramming strategy in zebrafish contrasts markedly with mice, in which both parental genomes undergo extensive DNA demethylation via active (paternal) and passive (maternal) mechanisms, leading to a shared hypomethylated state that is distinct from both gametic methylomes (Wossidlo et al., 2011; Gu et al., 2011; Inoue and Zhang, 2011; Smith et al., 2012). The different strategies may reflect the underlying developmental programs of mammals and fish; mice activate transcription of the zygotic genome (2 cell) and undergo the first lineage-restricted commitment (~32 cell) relatively early during development, whereas zebrafish rely on maternal factors for ~10 divisions until their ZGA. Thus, mammalian development is under pressure to rapidly generate a methylome that is competent for the switch from a germ cell to a totipotent gene expression program, by demethylation of paternal *Nanog*, for example (Farthing et al., 2008). In contrast, because early development in zebrafish is regulated by maternally inherited factors, the emphasis on rapid epigenomic competence for totipotency may be reduced. Indeed, the greater reliance on maternally inherited determinants may underpin the observed zebrafish oocyte-specific methylation of germline (e.g., *Dazl*, *Piwil1*) and early developmental (e.g., *Hox*, *Pax*) genes, which are presumably methylated to prevent their precocious accumulation as maternal factors in oocytes (which might otherwise skew lineage priming prior to ZGA). The paternal methylome lacking such constraints is apparently already primed for early development at the time of fertilization. It is unclear how DNA demethylation (or de novo methylation) is precisely targeted to

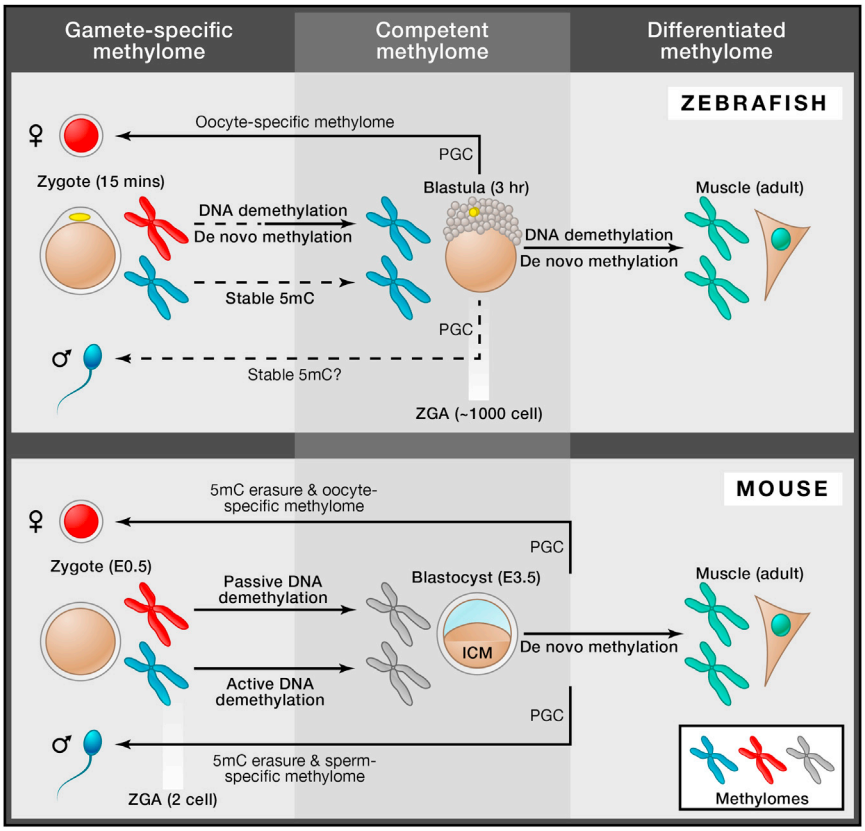

Figure 1. Comparative 5mC Reprogramming in Zebrafish and Mouse

At fertilization, the zygote forms with distinct sperm- and oocyte-specific epigenomes. In zebrafish (top), the paternally inherited methylome (blue) is stably inherited until the midblastula stage, whereas the maternally inherited methylome (red) undergoes programming that includes loss and gain of 5mC and resets the DNA methylation pattern to that of sperm. The midblastula stage methylome is therefore comparable to the sperm methylome and is competent for development of both primordial germ cells (PGC) through the inheritance of germplasm (yellow) and somatic tissues. Somatic differentiation involves further remodeling of DNA methylation (green). In contrast, germline development of sperm may occur with stable inheritance of the blastula methylome, whereas oocyte development establishes an oocyte-specific methylome. In mice (bottom), the parental genomes undergo either passive DNA demethylation (maternal) or active conversion to 5-hydroxymethylcytosine (paternal), which results in a highly hypomethylated epigenome (gray) in the naive epiblast cells of the blastocyst. These cells subsequently undergo de novo remethylation during postimplantation development toward somatic fates. Mammalian PGCs are specified from these methylated somatic-fated cells and therefore undergo a second wave of 5mC reprogramming before establishment of gamete-specific methylomes. ZGA, zygotic gene activation.

specific regions of the maternal genome to progressively reprogram it to the paternal pattern. However, the process appears to be passive and apparently occurs independently of conversion to 5-hydroxymethylcytosine and without involvement of AID/GADD45 activity, which cannot be detected during the time of demethylation (Rai et al., 2008).

The inheritance of the sperm methylome without significant changes until ZGA is a striking observation that raises several questions. Is the inherited sperm methylome important for embryogenesis? How is it recognized and maintained during extensive remodeling of the maternal methylome? Can it be inherited over multiple generations? To evaluate the significance of paternal epigenetic inheritance, Jiang et al. (2013) find that enucleated oocytes can only initiate development following transfer of a sperm nucleus, but not an oocyte nucleus, implying a fundamental epigenetic asymmetry that is consistent with the sperm methylome being in a competent state. However, Potok et al. (2013) find that gynogenetic embryos fertilized with UV-exposed sperm (that carry nonreplicating DNA) apparently develop normally with appropriate remodeling of the maternal methylome. This argues that stable inheritance of the sperm methylome per se does not have a key early developmental role or act as a "template" for maternal reprogramming but rather that sperm may contribute other important factors, perhaps including small RNAs. Further studies are required to reach definitive conclusions concerning the functional role of parentally contributed epigenetic states.

How the paternal methylome is protected from remodeling during development is unclear but could be related to its chromatin state because, unlike mice, zebrafish sperm are not associated with protamines (Wu et al., 2011). Alternatively, the de novo mechanism that establishes the paternal DNA methylation pattern may also maintain it during early development, while also promoting a progressive resetting of the maternal methylome. In any case, the striking similarity between the ZGA-stage methylome and sperm methylome raises the additional intriguing possibility that the paternal DNA methylation pattern may avoid reprogramming throughout the entire zebrafish life cycle. That is, after the paternal methylome is stably maintained until the ZGA stage, when PGC specification occurs, it could subsequently be inherited through germ cell development to mature sperm, as the sperm methylome is near identical to ZGA-stage cells (Figure 1). If so, this suggests a potential route for transgenerational epigenetic inheritance through the paternal germline in zebrafish. However, it remains to be established that germline-fated cells formed through the inheritance of "germplasm" have a comparable methylome to their somatic-fated neighbors at ZGA and that it remains stable through germ cell development.

Overall, the recent studies on zebrafish reveal a distinct strategy of vertebrate epigenetic reprogramming, which does not rely on comprehensive genome-wide DNA demethylation to generate a methylome that is competent to commit to all lineages. This may inform on the functional significance of the process in other vertebrates, in which genome-wide demethylation may be a necessary

requirement for establishing a permissive epigenetic state at just a few key genes. These studies illustrate that the regulation of epigenetic changes should be considered in the context of the diversity of development.

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Dwelling on T Cell Fate Decisions

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Defining determinants of T cell fate is central to understanding adaptive immunity and the design of effective vaccines. Tubo et al. demonstrate that intrinsic properties of T cell receptor signaling dictate whether CD4 T cells adopt predominantly type 1 helper or follicular helper T cell phenotypes in response to bacterial or viral infection.

Naive CD4 T cells are multipotential precursors, each bearing a unique T cell antigen receptor (TCR). TCR recognition of peptide-MHCII complexes (pMHCII) expressed on antigen-presenting cells (APCs) in T cell zones of secondary lymphoid tissues initiates rapid clonal expansion and differentiation of naive precursors into distinct effector subsets specialized for defense against different classes of microbes. A major early bifurcation in CD4 T cell responses determines deployment of alternative types of helper function: commitment to classical effector T cells (such as Th1, Th2, or Th17), which emigrate to nonlymphoid tissues to regulate microbicidal actions of innate immune cells at sites of infection, or to T follicular helper (Tfh) cells, which traffic to B cell follicles where they induce germinal center responses that produce antimicrobial antibodies (Crotty, 2011). In addition to a dominant role for cytokines in specifying these fates, mounting evidence implicates an important role for TCR signal strength. In a tour de force of cellular immunology, Tubo et al. (2013) in this issue of *Cell* follow the fates of individual CD4 T cell clones responding to the same pMHCII ligand during infection and find remarkably divergent contributions to Tfh and non-Tfh effector responses that correlate with intrinsic characteristics of TCR signaling.

The rarity of naive clonal precursors has, until recently, confounded efforts to delineate natural antimicrobial T cell responses. With a frequency of about one in a million for a given antigenic specificity in the CD4 T cell repertoire, or ~100 cells, tracking responses of endogenous T cells to a single peptide antigen has proved challenging. Making the task more daunting is interclonal variation in TCR usage by the few naive T cells that recognize the same pMHCII complex. This raises the possibility that clones activated by the same microbial peptide might display disparate responses that program alternative differentiative fates, even if the averaged population response to that antigen is more stereotypical—albeit distinct for different antigens. In the current report, the authors find that, indeed, individual CD4 T cell clones activated by the same pMHCII complex via distinct TCRs favor disparate programming for Tfh and non-Tfh differentiation (Figure 1). This supports models that predict a component of predestination intrinsic to the mechanics by which a T cell's antigenic receptor engages its ligand and reinvigorates longstanding interests in understanding relationships between TCR signaling thresholds and graded responses.

The findings represent a culmination of two decades of effort by the Jenkins lab to understand CD4 T cell immunity the hard way—not in a culture dish, but in the tissues where they actually occur. Here, they build on their pioneering pMHCII tetramer-based enrichment techniques to enumerate and phenotype rare antigen-specific CD4 T cells (Moon et al.,