The simplest explanation: passive DNA demethylation in PGCs

Zachary D Smith^{1,2,3} and Alexander Meissner^{1,2,3,*}

¹Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA, ²Broad Institute of MIT and Harvard, Cambridge, MA, USA and ³Harvard Stem Cell Institute, Cambridge, MA, USA.

*Correspondence to: alexander_meissner@harvard.edu

The EMBO Journal (2013) 32, 318–321. doi:10.1038/emboj.2012.349; Published online 8 January 2013

Primordial germ cell (PGC) specification is one of two major developmental windows where modifications associated with global heterochromatin maintenance are erased. One of the most intriguing and confounding dynamics to rectify has been the apparent global depletion of cytosine methylation, which has been intensely scrutinized for nearly two decades. While numerous reports have suggested active catalytic removal as the primary mechanism, work by Saitou and colleagues presented in this issue of *The EMBO Journal* provide support for a simpler model whereby the downregulation of essential recruitment factors appears sufficient to erase this mark passively during a phase of rapid proliferation.

During mammalian development, transitions in cellular programs are predominantly accompanied by focal changes to chromatin that reflects local activities of transcriptional activators or repressors. As such, the fraction of the genome that is targeted for remodelling during any given transcription factor driven programming event is comparatively small (Dunham *et al*, 2012). Two exceptions to this general paradigm occur upon specification of the PGC lineage within the developing embryo and upon fertilization within the protamine-compacted paternal genome (Saitou *et al*, 2012). In these contexts, dramatic global epigenetic modification appears to restructure the majority of the genome and does so in a rapid and coordinated fashion (Seki *et al*, 2005). As part of either process, the mechanism behind the apparent global erasure of DNA methylation has posed a particularly vexing problem. In PGCs, this global demethylation is considered essential to erase somatic methylation signatures as well as parental imprints while simultaneously reactivating transcriptional programs associated with pluripotency and gametogenesis.

Until recently, the rapidity of DNA demethylation during PGC specification appeared to support numerous mechanisms that largely centred on an active catalytic step, including strategies employing deamination or oxidation followed by base excision repair (Wu and Zhang, 2010). While these proposed mechanisms appeared robust in certain contexts, they conflict logically with the presumptive energetics, processivity and fidelity such a mechanism would need to safely navigate the millions of CpGs that are normally

methyated in the mouse genome from a strong, self-propagating epigenetic signal to an apparent blank slate.

In this issue, Kagiwada *et al* (2013) have re-investigated the kinetics of demethylation at imprinted regions during PGC migration. By coupling this data to the expression of different regulatory candidates, the global levels of key epigenetic modifications and precise measurements of replication, they present an elegantly refined model. Alternative to a singular catalytic removal, DNA methylation signal is depleted passively over progressive cellular divisions, supporting the seemingly simplest (and most effective) explanation for global erasure (Figure 1).

The PGC progenitor pool is specified early in the proximal epiblast as a population of ~40 cells (Lawson and Hage, 1994). At the onset of their migration to the genital ridge, although difficult to measure experimentally, they likely epigenetically resemble somatic cells and are genomically hypermethylated, including at repetitive elements and promoters essential for gametogenesis (Maatouk *et al*, 2006). PGCs predominantly arrest in G2 prior to epigenetic remodelling, simultaneously downregulating numerous factors that maintain pervasive, genome-spanning heterochromatic modifications. Silenced factors include the essential G9A binding partner, GLP, which directs methylation on Histone 3 Lysine 9, and Uhrf1, which connects the maintenance methyltransferase Dnmt1 to the replication fork, ensuring successful re-establishment of DNA methylation on nascent DNA (Seki *et al*, 2007; Kurimoto *et al*, 2008). While Dnmt1 itself is not substantially downregulated, Kagiwada *et al* (2013) demonstrate that Uhrf1's absence appears sufficient to destabilize Dnmt1's proper recruitment to replicating DNA. To quantitatively demonstrate that the progressive demethylation within PGCs is coupled to replication, BrdU pulse chase experiments indicate that migrating PGCs divide more rapidly than previously assumed (12.6 h rather than 16 h), providing a careful calibration to measurements of DNA methylation during this phase. When combined, the similarity between the rates of DNA methylation decays at target loci and of PGC replication is striking. Moreover, by tying epigenetic events to proliferation, H3K27 methylation, which is concurrently enriched as H3K9 and DNA methylation is erased (Seki *et al*, 2005), appears constant,

Figure 1 Proposed mechanisms for demethylation have suggested either complete catalytic removal of cytosine bases or passive dilution over replication in the absence of maintenance. Several replication-independent models have been posed. Activation Induced Cytidine Deaminase (AICDA) may deaminate methylated cytosines, with the resulting T/G mismatch targeted for Base Excision Repair (BER) through Thymidine DNA Glycosylase (TDG). Alternatively, hydroxymethylation (hme) as mediated by the TET enzymes, plus deamination via AICDA, may result in an atypical hydroxymethyluracil base, which may also be targeted by BER. It has also been suggested that progressive rounds of oxidation may result in carboxylation, which is energetically unstable enough to be removed by a hypothetical carboxylase. In contrast to high energy, multistep processes, cytosine methylation can be oxidized to hydroxymethylcytosine, which may not be heritable over division. This 'pulse' of catalytic activity followed by progressive dilution as cells divide would be similar to the dynamics observed for global H3K9 dimethylation, which may involve histone demethylases (KDMs). Finally, the data presented by Kagiwada and colleagues suggest that division alone may be sufficient for demethylation in the absence of maintenance. In either division dependent model, with or without hydroxymethylation, downregulation of UHRF1 would ensure inefficient recruitment of DNMT1 to DNA during replication. Regulation of global heterochromatin through abrogated recruitment would again be similar to the regulation H3K9 dimethylation, where G9a is prohibited from targeting histones through repression of its cofactor GLP.

fluctuating in tandem with cell-cycle progression. Retention of H3K27 methylation throughout the proliferative, DNA demethylation phase of PGC specification is a careful and logical model, given its equivalent global distribution within the paternal genome after fertilization (Puschendorf *et al*,

2008) or within DNA methylation-free embryonic stem cells (Brinkman *et al*, 2012).

The work presented by Kagiwada *et al* (2013) and others has reshaped our understanding of how global epigenetic reprogramming is coordinated in mammals (Guibert *et al*,

2012; Hackett *et al*, 2012a; Seisenberger *et al*, 2012; Yamaguchi *et al*, 2012). They provide compelling evidence that repressing essential recruitment factors for epigenetic machinery, such as GLP for H3K9 methylation or Uhrf1 for DNA methylation, is a broadly effective way to ensure those modifications are erased within one or several divisions. It remains an outstanding question, however, how certain regions retain their epigenetic signatures and evade this signal, such as strongly repressed repetitive elements of the IAP LTR class or certain imprints, as highlighted in this and other recent studies (Guibert *et al*, 2012; Hackett *et al*, 2012a; Seisenberger *et al*, 2012). In the absence of canonical maintenance mechanisms, continued silencing must rely on alternate recruitment strategies, which may include the epigenetic recognition of local H3K9 trimethylation, a retained modification during this process, or sequence-specific repressors, such as Zfp57, motifs for which are enriched at many protected loci (Seki *et al*, 2005; Guibert *et al*, 2012; Seisenberger *et al*, 2012).

Even more intriguing will be to rectify the potential redundancy or specificity within superficially disparate models, several of which have recently been reported and contribute some supporting evidence to the work presented here (Guibert *et al*, 2012; Hackett *et al*, 2012a; Seisenberger *et al*, 2012; Yamaguchi *et al*, 2012). Hydroxymethylation as mediated by Tet-family dioxygenases has been measured genome-wide during PGC demethylation and shows a strong inverse relationship to diminishing DNA methylation signal (Hackett *et al*, 2012a). Kagiwada *et al* (2013) confirm its expression but do not observe notable upregulation compared to ESCs, which retain predominantly high methylation levels (Hajkova *et al*, 2010; Hackett *et al*, 2012a; Yamaguchi *et al*, 2012). Additionally, Tet1 knockout mice are fertile, albeit less so than wild type (Dawlaty *et al*, 2011), and investigation of global methylation patterns at E13.5 indicates that DNA demethylation is largely unabated in its absence (Yamaguchi *et al*, 2012). It is possible that Tet2 may contribute some functional redundancy, though its expression is less specific and apparently low in most

PGCs, making it difficult to imagine it as exclusively culpable (Hajkova *et al*, 2010; Hackett *et al*, 2012a; Yamaguchi *et al*, 2012). With this data, passive dilution, even if instigated catalytically through oxidation, appears the most likely model.

It is particularly peculiar to consider how these different mechanisms may function when loss of essential recruitment factors would appear to provide an all-encompassing solution. Could it be that hydroxymethylation provides refined targeting to promoter elements that must be expressed early, such as those associated with pluripotency? If this is so, then why does the major effect of Tet1 knockout appear to be the diminished expression of later acting meiosis genes (Yamaguchi *et al*, 2012)? It is possible that different mechanisms target ever more refined loci, with passive methylation providing a global erasure, hydroxymethylation targeting germ-line genes and imprints in tandem with their expression, and base-excision repair, if applicable, relegated to sequences where potentially mutagenic strategies are not deleterious (Hackett *et al*, 2012b). It could also be that hydroxymethylation, which seems a likely player during both PGC specification and fertilization, has other, temporal regulatory roles that function in concert with the myriad of other epigenetic dynamics specific to these developmental windows, before the onset of replication as seen in PGCs and the early embryo (Branco *et al*, 2012). Clarifying the perspective with which DNA demethylation during epigenetic reprogramming is envisioned, from the once prevailing catalytic models to a division dependent, passive model, has provided an incredible framework to investigate these new questions.

Acknowledgements

AM is supported by the Pew Charitable Trusts, NIH grants (U01ES017155 and P01GM099117) and is an NYSCF Robertson Investigator.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Branco MR, Ficiz G, Reik W (2012) Uncovering the role of 5-hydroxymethylcytosine in the epigenome. *Nat Rev Genet* **13**: 7–13
- Brinkman AB, Gu H, Bartels SJ, Zhang Y, Matarese F, Simmer F, Marks H, Bock C, Gnirke A, Meissner A, Stunnenberg HG (2012) Sequential ChIP-bisulfite sequencing enables direct genome-scale investigation of chromatin and DNA methylation cross-talk. *Genome Res* **22**: 1128–1138
- Dawlaty MM, Ganz K, Powell BE, Hu YC, Markoulaki S, Cheng AW, Gao Q, Kim J, Choi SW, Page DC, Jaenisch R (2011) Tet1 is dispensable for maintaining pluripotency and its loss is compatible with embryonic and postnatal development. *Cell Stem Cell* **9**: 166–175
- Dunham I, Kundaje A, Aldred SF, Collins PJ, Davis CA, Doyle F, Epstein CB, Frietze S, Harrow J, Kaul R, Khatun J, Lajoie BR, Landt SG, Lee BK, Pauli F, Rosenbloom KR, Sabo P, Safi A, Sanyal A, Shores N *et al* (2012) An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**: 57–74
- Guibert S, Forne T, Weber M (2012) Global profiling of DNA methylation erasure in mouse primordial germ cells. *Genome Res* **22**: 633–641
- Hackett GA, Sengupta R, Zyllicz JJ, Murakami K, Lee C, Down TA, Surani MA (2012a) Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. *Science* (advance online publication, 12 December 2012; doi:10.1126/science.1229277)
- Hackett JA, Zyllicz JJ, Surani MA (2012b) Parallel mechanisms of epigenetic reprogramming in the germline. *Trends Genet* **28**: 164–174
- Hajkova P, Jeffries SJ, Lee C, Miller N, Jackson SP, Surani MA (2010) Genome-wide reprogramming in the mouse germ line entails the base excision repair pathway. *Science* **329**: 78–82
- Kagiwada S, Kurimoto K, Hirota T, Yamaji M, Saitou M (2013) Replication-coupled passive DNA demethylation for the erasure of genome imprints in mice. *EMBO J* **32**: 340–353
- Kurimoto K, Yabuta Y, Ohinata Y, Shigeta M, Yamanaka K, Saitou M (2008) Complex genome-wide transcription dynamics orchestrated by Blimp1 for the specification of the germ cell lineage in mice. *Genes Dev* **22**: 1617–1635
- Lawson KA, Hage WJ (1994) Clonal analysis of the origin of primordial germ cells in the mouse. *Ciba Found Symp* **182**: 68–84 discussion 84–91

- Maatouk DM, Kellam LD, Mann MR, Lei H, Li E, Bartolomei MS, Resnick JL (2006) DNA methylation is a primary mechanism for silencing postmigratory primordial germ cell genes in both germ cell and somatic cell lineages. *Development* **133**: 3411–3418
- Puschendorf M, Terranova R, Boutsma E, Mao X, Isono K, Brykczynska U, Kolb C, Otte AP, Koseki H, Orkin SH, van Lohuizen M, Peters AH (2008) PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. *Nat Genet* **40**: 411–420
- Saitou M, Kagiwada S, Kurimoto K (2012) Epigenetic reprogramming in mouse pre-implantation development and primordial germ cells. *Development* **139**: 15–31
- Seisenberger S, Andrews S, Krueger F, Arand J, Walter J, Santos F, Popp C, Thienpont B, Dean W, Reik W (2012) The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. *Mol Cell* **48**: 849–862
- Seki Y, Hayashi K, Itoh K, Mizugaki M, Saitou M, Matsui Y (2005) Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. *Dev Biol* **278**: 440–458
- Seki Y, Yamaji M, Yabuta Y, Sano M, Shigeta M, Matsui Y, Saga Y, Tachibana M, Shinkai Y, Saitou M (2007) Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. *Development* **134**: 2627–2638
- Wu SC, Zhang Y (2010) Active DNA demethylation: many roads lead to Rome. *Nat Rev Mol Cell Biol* **11**: 607–620
- Yamaguchi S, Hong K, Liu R, Shen L, Inoue A, Diep D, Zhang K, Zhang Y (2012) Tet1 controls meiosis by regulating meiotic gene expression. *Nature* **492**: 443–447