Reply to Wilkinson: Minor role of programmed methylation and demethylation in mammalian development

Wilkinson (1) does not dispute any of our (2) main claims: that CpG-dense promoters are unmethylated in all tissues and CpG-poor promoters are variably methylated and undergo transcription factor-induced demethylation after activation; that global genome demethylation in cells and tissues does not cause ectopic expression of tissue-specific genes; and that there is little convincing evidence of a biochemical system that performs programmed methylation and demethylation to regulate gene expression during development.

Although we explicitly focus on the role of dynamic DNA methylation in development, Wilkinson (1) objects to our treatment of gametogenesis, even though our only statement on the subject is "such regulation [by dynamic DNA methylation] may occur during gametogenesis. . ." (2). There is evidence that certain classes of genes in the male germ line might be regulated by DNA methylation, but Wilkinson (1) provides no indication as to the mechanism by which specific sequences might be designated for de novo methylation or demethylation. Methylation in male germ cells will result in the irreversible loss of CpG sites because of the high rate of m^5CpG→TpG mutations and loss of the ability to be regulated by methylation. DNA methylation is dispensable for oogenesis; oocytes that lack nearly all methylation of single-copy sequences as a result of deletion of DNA methyltransferase 3-like (*Dnmt3L*) develop normally and have gene-expression patterns indistinguishable from wild-type oocytes (3), but embryos derived from mutant oocytes and wild-type sperm die at midgestation, with abnormal expression of imprinted genes, as a result of a failure to methylate imprinting control regions at a much earlier stage, in the growing oocyte (4).

Wilkinson (1) attaches special importance to a transcription activator-like endonuclease–TET1 fusion protein experiment (see reference 4 within ref. 1), in which DNA methylation is reported to be the sole factor that controls β-globin expression. It is remarkable that very slight demethylation at one short sequence should reactivate β-globin expression in the nonerythroid 293 and HeLa cell lines, given that erythroid-specific transcription factors, such as GATA1, have long been known to be required for expression of globin genes (5). Second, mice that are null for Tet1 are viable and fertile, with no evidence of anemia and with normal male fertility and only slightly reduced female fertility (6). This finding indicates that any TET1-mediated active DNA demethylation is not required for globin gene expression in vivo.

That there is "...already compelling evidence that DNA methylation has critical roles in initiating and maintaining dynamic shifts in gene expression" (1) is a view that has been held with variable conviction and in the absence of compelling evidence over the last 40 y. We question this view, and propose that the essential roles of genomic methylation patterns lie in the monoallelic expression of imprinted genes, the transcriptional silencing of transposons, and the silencing of promoters on the inactive X chromosome in female mammals. All of these regulatory events require the mitotic inheritance of states of gene expression in the presence of all of the factors required for expression of the silenced gene, a property that is largely restricted to organisms that contain methylated genomes.

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The authors declare no conflict of interest.

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