LETTER

Evidence that DNA methylation engenders dynamic gene regulation

Bestor et al. present the thesis that although DNA methylation is important for "irreversible" silencing events (e.g., transposon silencing), it has no role in "dynamic" patterns of gene expression, such as during development (1). Although this report nicely defines this controversial topic, it ignores most of the evidence that DNA methylation does indeed have a causal role in regulating dynamic gene expression events.

Abundant evidence for this notion comes from studies on germ-line genes. The initial hint was the finding that germ-line gene promoters tend to be hypomethylated in the testis and hypermethylated in other tissues (2). Such an association was reinforced by later genome-wide studies that showed, for example, that many germ-line genes become demethylated and transcriptionally induced during primordial germ cell (PGC) development (3). Germ-line genes in later stages of germ cells, including spermatogonia and sperm, are also selectively hypomethylated.

Evidence that DNA methylation has a causal role in regulating germ-line genes includes the following: (*i*) DNA demethylation precedes induction of many germ-line gene transcripts, providing temporal evidence for a causal role; (*ii*) in vitro methylation of germ-line promoters inhibits their transcription in transfected cells; (*iii*) in vitro methylation blocks binding of key transcription factors to germ-line promoters; (*iv*) core germ-line promoter sequences selectively methylated in nontestes tissues are sufficient to confer testis-specific expression in transgenic mice in vivo; and (*v*) loss or inhibition of DNA methyltransferases in somatic cells elicits demethylation of germ-line promoters and their transcriptional induction both in vitro and in vivo (2). The latter finding demonstrates that methylation is the dominant (perhaps the only) mechanism preventing the expression of many germ-line genes in the soma. Recent additional evidence for this comes from a study on ten-eleven translocation methycytosine dioxygenase 1 (TET1), a DNA-binding protein preferentially expressed in germ cells that catalyzes the conversion of methylated cytosine to hydroxymethylated cytosine, an initial step in demethylation. When targeted to a germ-line gene's promoter using a transcription activator-like endonuclease-based approach, TET1 was found to be sufficient to trigger both its demethylation and transcription in somatic cells (4). Perturbation of TET1 in mice causes female meiotic defects and down-regulation of genes involved in meiotic functions in vivo, suggesting physiological relevance.

In what contexts might DNA demethylation regulate gene expression in germ cells? An obvious possibility, as indicated above, is to unleash the expression of the germ-line program during PGC development. In part this may be mediated by transcription factors, such as members of the RHOX homeobox family, which evidence suggests promote PGC differentiation and are encoded by a gene cluster largely controlled by DNA methylation (2, 4, 5). It will be important in the future to determine whether dynamic regulatory roles of DNA methylation extend beyond the germ line. Both correlative and causal evidence indicate that DNA methylation is a good candidate to have roles in other developmental events, including in the transition between the pluripotent/multipotent and differentiated cell states (3). Although additional work remains to be done, there is already compelling evidence that DNA methylation has critical roles in initiating and maintaining dynamic shifts in gene expression.

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