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The *Ciona intestinalis* cleavage clock is independent of DNA methylation

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Highlights

- DNA methylation in early development of *Ciona intestinalis* is static
- Absent of DNA demethylation related to zygotic gene activation
- 5hmC is hardly detected
- Epigenetic polymorphisms are found within the genomes of natural populations

Abstract

The initiation of embryonic gene expression in ascidian embryos appears to be tightly regulated by the number of DNA replication cycles. DNA methylation is thought to contribute to the clock mechanism that counts the rounds of DNA replication. We used mass spectrometry and whole genome bisulfite sequencing to characterize DNA methylation changes that occur in early developmental stages of the ascidian, *Ciona intestinalis*. We found that global DNA methylation in early *Ciona* development was static, and a base-wise comparison between the genomes of consecutive developmental stages

found no DNA demethylation that was related to zygotic gene activation. Additionally, 5hmC was hardly detected by mass spectrometry in the developmental samples, suggesting a lack of demethylation mediated by ten eleven translocation (TET) methylcytosine dioxygenase in *C. intestinalis*. We conclude that DNA methylation is not involved in regulating DNA replication-dependent transcriptional activation.

Previous article

Next article

Abbreviations

AChE, acetylcholinesterase; TET, ten-eleven translocation; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; MS, mass spectrometry; WGBS, whole genome bisulfite sequencing; PBAT, post-bisulfite adaptor tagging; DMR, differentially methylated region; TAO, thousand-and-one amino acids; MBT, mid-blastula transition

Keywords

Ciona intestinalis; Developmental stages; DNA methylation; Whole genome bisulfite sequencing; Zygotic activation