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Regulatory *cis*- and *trans*-elements of mitochondrial D-loop-driven reporter genes in budding tunicates

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ABSTRACT

To unveil the underlying mechanism of mitochondrial gene regulation associated with ageing and budding in the tunicate *Polyandrocarpa misakiensis*, mitochondrial non-coding-region (NCR)-containing reporter genes were constructed. *PmNCR2.3K/GFP* was expressed spatiotemporally in a pattern quite similar to mitochondrial *16S rRNA*. The reporter gene expression was sensitive to high dose of rifampicin similar to mitochondrial genes, suggesting that the transcription indeed occurs in mitochondria. However, the gene expression also occurred *in vivo* in the cell nucleus and *in vitro* in the nuclear extracts. *Mitochondrial transcription factor A* (*PmTFAM*) enhanced reporter gene expression, depending on the NCR length. A budding-specific polypeptide TC14-3 is an epigenetic histone methylation inducer. It heavily enhanced reporter gene expression that was interfered by histone methylation inhibitors and *PmTFAM* RNAi. Our results indicate for the first time that the nuclear histone methylation is involved in mitochondrial gene activity *via TFAM* gene regulation.

1. Introduction

Mitochondria are multifunctional organelles that not only participate in ATP and fatty acid biosynthesis (Albert et al., 2014), but also influence embryonic development and cell death by their components such as large ribosomal RNA (16S rRNA) and cytochrome *c* (Gilbert, 2013). Recently, mitochondria have drawn increased attention regarding organism senescence and mortality, since their activity declines with age (Byrne et al., 1991), and the age-related cumulative mutation of mitochondrial DNA (mtDNA) is implicated in the inevitable and irreversible mitochondrial dysfunction (Hebert et al., 2010).

Human mtDNA has a transcription-controlling unit in the intergenic, non-coding region (NCR) (Tracy and Stern, 1995; Shadel and Clayton, 1997). The mitochondrial NCR, commonly referred as "D-loop," is approximately 1 kb long and contains three transcriptional promoters (Scarpulla, 2008). In the budding tunicate, *Polyandrocarpa misakiensis*, mtDNA is approximately 15 kb long, and the NCR locates between *NADH dehydrogenase subunit 2* (*ND2*) and *12S ribosomal RNA* (*12S rRNA*) genes (Fig. 1A). In *Polyandrocarpa* and other tunicates, however, little is known about the role of NCR in mitochondrial gene regulation.

In *P. misakiensis*, buds grow out from the parental body wall, develop into functional animals, and live for 4–5 months (Fig. 2A1) (Kawamura et al., 2012a). During lifetime, *cytochrome c oxidase subunit 1* (*PmCOX1*) and *PmND1* gene activities gradually attenuate as known in mammals, but this decrease in tunicate mitochondrial activities is reversibly restored during budding, although the neighboring parental somatic tissues continue to age (Kawamura et al., 2012a). Mitochondrial ageing and rejuvenation have been repeated for more than four decades in *Polyandrocarpa* asexual strains that collected in 1970 (Kawamura et al., 2012a).

Several endogenous factors have been identified to regulate *PmCOX1*. TC14-3 is a humoral factor that triggers histone methylation (Kawamura et al., 2012b). It can activate *PmCOX1*, and its effect is blocked by a histone H3 methyltransferase inhibitor GSK343 (Kawamura et al., 2015). The second factor is *Embryonic ectodermal development* (*PmEED*), a component of polycomb group genes. It is strongly expressed during budding, and RNA interference (RNAi) of *PmEED* downregulates *PmCOX1* expression (Kawamura et al., 2012a). The third factor is *Mitochondrial transcription factor A* (*PmTFAM*). Human TFAM specifically binds to sites immediately upstream of the promoters (Rubio-Cosials et al., 2011; Ngo et al., 2011) to initiate *in vitro* transcription (Fisher and Clayton, 1988; Falkenberg et al., 2002; Shi et al., 2012). TFAM also binds non-specifically to mtDNA (Kanki

Abbreviations: COX1, Cytochrome *c* oxidase subunit 1; ISH, *In situ* hybridization; NCR, Non-coding region; RT-PCR, Reverse transcription polymerase chain reaction; TFAM, Mitochondrial transcription factor A

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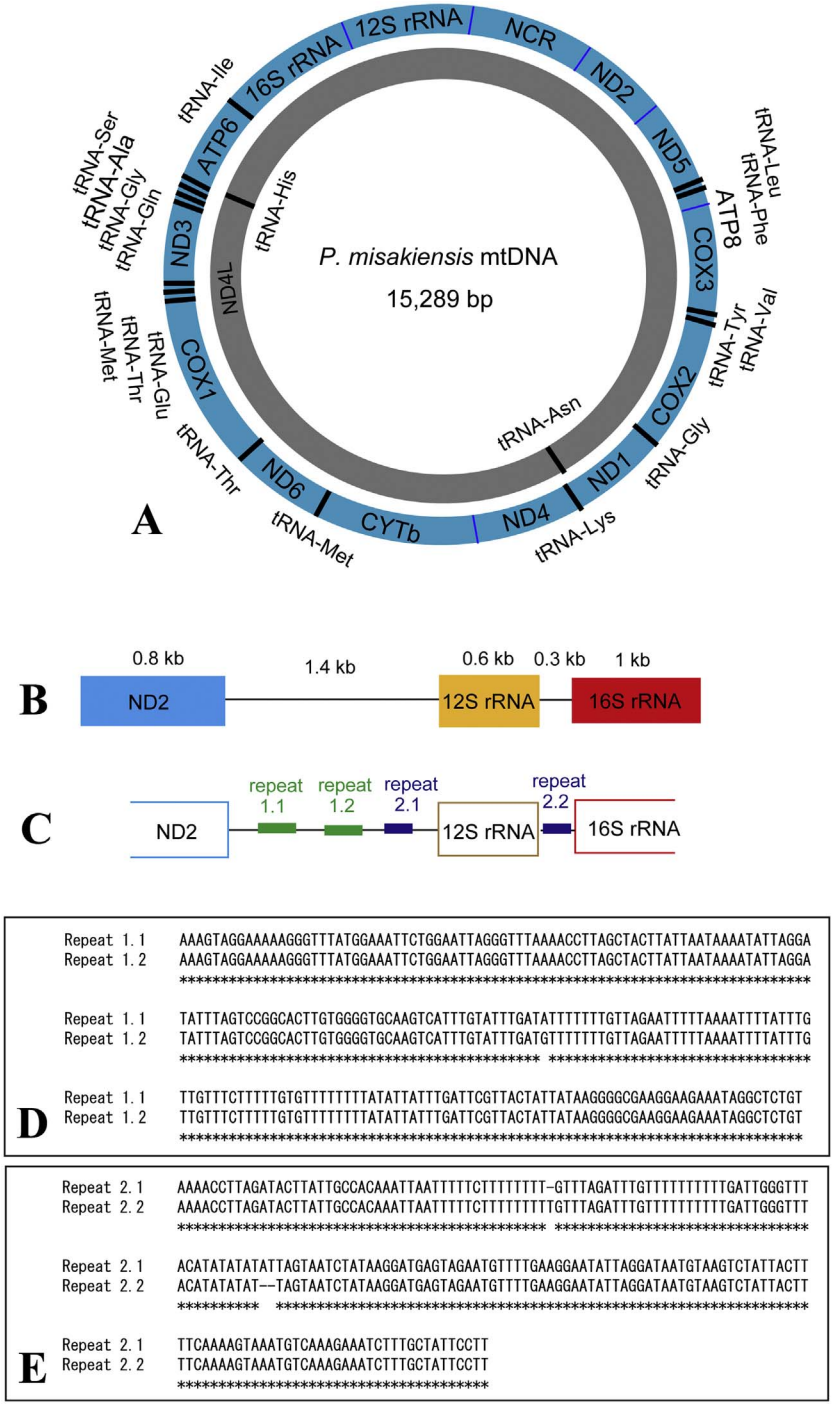

Fig. 1. Mitochondrial genome and *NCR* in *P. misakiensis*. (A) Gene map of 15 kb mtDNA. (B) A 2.3 kb region intervening between *ND2* and *16S rRNA* genes. (C) Tandem repeat units (1.1, 1.2, 2.1, and 2.2) in *PmNCR*. (D) Multiple alignment of repeats 1.1 and 1.2 having 231 nucleotides. (E) Multiple alignment of repeats 2.1 and 2.2 having 191 nucleotides.. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

et al., 2004; Campbell et al., 2012). In *P. misakiensis*, *TFAM* is expressed most abundantly during budding and declines with zooid age. *PmTFAM* mRNA, when introduced *in vivo* into adult animals by electroporation, enhances *PmCOX1* gene expression (Kawamura et al., 2015). It is uncertain at present how *PmEED*–related histone methylation is involved in the activation of mitochondrial genes having no histone proteins and what kinds of relationship, if any, exist among TC14-3, *PmTFAM*, and *PmCOX1*.

In this study, we constructed reporter genes with wild and aberrant NCRs to characterize them with reference to the spatiotemporal expression of reporter gene, intracellular localization of gene expression, and *cis*- and *trans*-elements essential for gene transcription. To get insights into the way how repeatable changes of mitochondrial gene activities are possible during asexual life span, we examined the relationship among histone modification, *TFAM* regulation, and reporter gene expression. As far as we know, this is the first report on the mechanism of *in vivo* mitochondrial gene regulation in invertebrates including tunicates.