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Histone methylation codes involved in stemness, multipotency, and senescence in budding tunicates

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SUMMARY

We examined the dynamics of nuclear histone H3 trimethylation related to cell differentiation and aging in a budding tunicate, *Polyandrocarpa misakiensis*. Throughout zooidal life, multipotent epithelial and coelomic cell nuclei showed strong trimethylation signals at H3 lysine27 (H3K27me3), consistent with the results of western blotting. Epidermal H3K27me3 repeatedly appeared in protruding buds and disappeared in senescent adult zooids. The budding-specific cytostatic factor TC14-3 allowed aging epidermal cells to restore H3K27me3 signals and mitochondrial gene activities via *mitochondrial transcription factor a*, all of which were made ineffective by an H3K27me3 inhibitor. Chromatin immunoprecipitation showed that TC14-3 enhances H3K27me3 of transdifferentiation-related genes and consequently down-regulates the expression of these genes. In contrast, trimethylation signals at H3 lysine4 (H3K4me3) appeared transiently in transdifferentiating bud cells and stably lasted in undifferentiated adult cells without affecting H3K27me3. A transdifferentiation-related gene *external signal-regulated kinase* heavily underwent H3K4me3 in developing buds, which could be reproduced by retinoic acid. These results indicate that in *P. misakiensis*, TC14-3-driven H3K27 trimethylation is a default state of bud and zooid cells, which serves as the histone code for cell longevity. H3K27me3 and H3K4me3 double-positive signals are involved in cell stemness, and absence of signals is the indication of senescence.

1. Introduction

Nucleosomal core histones are subjected to a variety of modifications at their N-terminal "tail." These modifications include methylation, acetylation, phosphorylation, and ubiquitylation of specific amino acid residues; alter the binding of core histones to DNA strands; and regulate the accessibility of trans-acting transcription factors to the enhancer/promoter region of the gene (Iizuka and Smith, 2003). The results are genome-wide gene regulation in embryonic cells (Boyer et al., 2006), tissue-specific stem cells (Konuma et al., 2010), and differentiated cells (Kuzmichev et al., 2005). Interestingly, nucleosomal modification can be transmitted from one cell generation to the next (Turner, 2002), and therefore, the epigenetic messages inscribed on nucleosomal histones are called histone code (Strahl and Allis, 2000).

Lysine 4 and lysine 27 of histone H3 (H3K4 and H3K27) similarly undergo methylation, but with different outcomes: trimethylation of H3K4 (H3K4me3) makes chromatin loose, while H3K27 trimethylation (H3K27me3) makes chromatin condensed. H3K4me3 and H3K27me3 competitively regulate the fate of embryonic stem cells (Rajasekhar and Begemann, 2007) and hematopoietic stem cells (Iwama et al., 2005). H3K4me3 is mediated by Trithorax group that consists of H3K4 methyltransferase (myeloid/lymphoid or mixed-lineage leukemia) and its partners. H3K27me3, on the other hand, is mediated by Polycomb repressive complex 2 (PRC2) of Polycomb group that consists of H3K27 methyltransferase (EZH2) and its partners (EED and SUZ12) (Schuettengruber et al., 2007).

The life span of the budding tunicate, *Polyandrocarpa misakiensis* (Styelidae, Stolidobranchiata), consists of bud formation, bud development, zooidal growth, maturation, and senescence (Fig. 1). The zooids of the budding species possess multipotent atrial epithelium for palleal budding (Kawamura et al., 2008a) and

Abbreviations: ChIP, chromatin immunoprecipitation; COX1, *cytochrome oxidase subunit 1*; ERK, *external signal-regulated kinase*; MET, mesenchymal-epithelial transition; MRC, mitochondrial respiratory complex; PAGE, polyacrylamide gel electrophoresis; PRC2, Polycomb repressive complex 2; RA, retinoic acid; RXR, *retinoid X receptor*; SA-gal, senescence-associated acid β-galactosidase; TFAM, *mitochondrial transcription factor a*.

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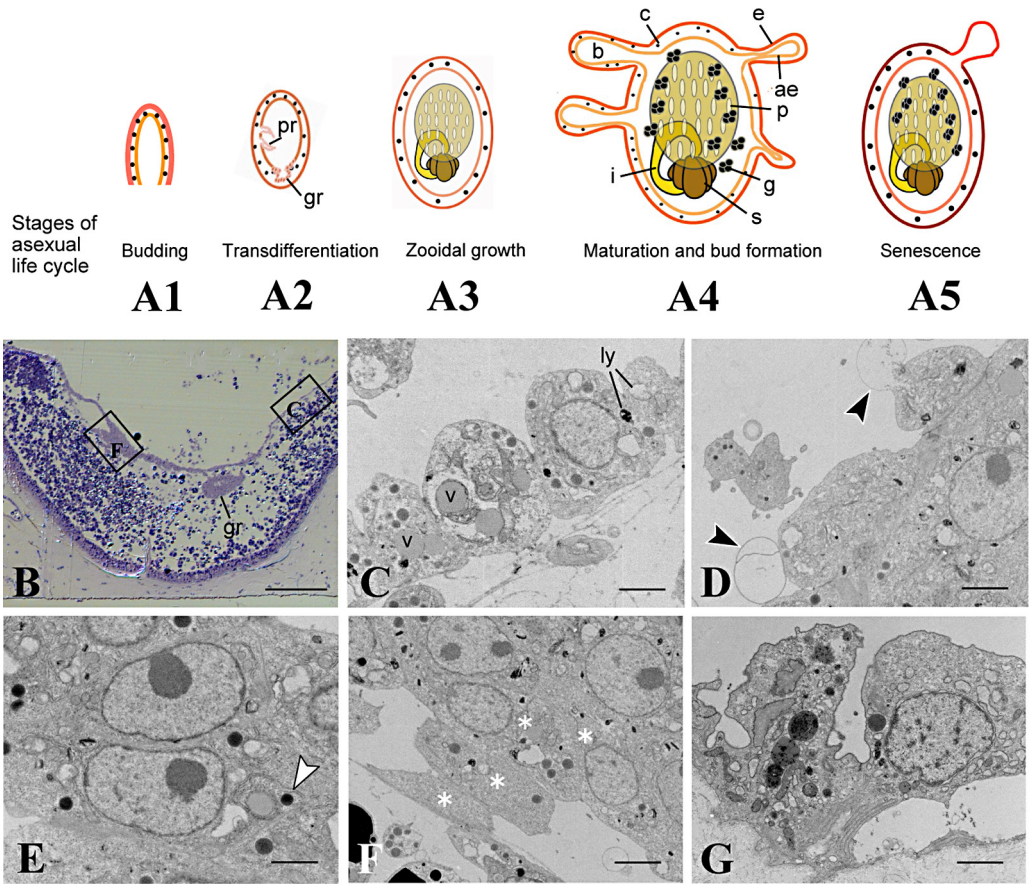

Fig. 1. Major stages of budding life history and the ultrastructure of multipotent cells in the tunicate, *P. misakiensis*. (A) Schematic illustration of buds and zooids. (B) A semi-thin section of 2-day-developing bud, toluidine blue staining. Bar 100 μm. (C) Atrial epithelium in the non-morphogenetic region. Bar 2 μm. (D) Apical surface of the atrial epithelium in the morphogenetic region. Arrowheads show 'bubbles' sprouting from cell surface. Bar 2 μm. (E) Transdifferentiating atrial epithelium. White arrowhead shows an electron-dense granule. Bar 1 μm. (F) Coelomic cells in the process of MET (asterisks). Bar 2 μm. (G) Atrial epithelium of adult zooid. Bar 1 μm. ae, atrial epithelium; b, bud; c, coelomic cell; e, epidermis; g, gonad; gr, gut rudiment; i, intestine; ly, lysosome; m, mitochondria; n, nucleus; p, pharynx; pr, pharyngeal rudiment; s, stomach; v, vacuole.

have pluripotent coelomic cells (Kawamura and Sunanaga, 2010) that give rise to multipotent epithelium (Oka and Watanabe, 1957; Rinkevich et al., 1995; Sabbadin et al., 1975; Tatzuke et al., 2012), body muscle cells (Degasperi et al., 2009; Sugino et al., 2007), cardiac cells (Nunzi et al., 1979), and germline cells (Brown et al., 2009; Sunanaga et al., 2006, 2007). In *P. misakiensis*, bud development involves transdifferentiation of multipotent cells that could be triggered by retinoic acid (RA) (Kawamura and Fujiwara, 1994). Recently, *retinoid X receptor* (*RXR*) was found to serve as a master gene for transdifferentiation to regulate the expression of downstream genes such as *external signal-regulated kinase* (*ERK*) and *β-Catenin* (Kawamura et al., 2013).

The life span of *P. misakiensis* is also interesting from the viewpoint of relationship between budding and senescence. Zooids of this species live for 4–5 months, and before dying, they produce many longer-lived buds, thereby enabling a prolonged lifespan of the asexual strain for more than 4 decades (Kawamura et al., 2012a). The zooidal senescence in *P. misakiensis* involves the attenuation of proliferating cell nuclear antigen and gene activities of *PmEed*, *superoxide dismutase 1*, and mitochondrial respiratory complex (MRC), and inversely it accompanies the increasing enzyme activity of senescence-associated acid β-galactosidase (SA-gal) (Kawamura et al., 2012a; Kawamura and Sunanaga, 2013). These progressive events are indeed stopped by budding and cells resume rejuvenescent condition (Kawamura et al., 2012a). A budding-specific humoral factor TC14-3 could activate both *PmEed* and MRC (Kawamura et al., 2012b), although the signaling pathways remain elusive. These results suggested that histone methylation changes dramatically during zooidal senescence and budding (rejuvenation), which may be related to mitochondrial gene activity. To the best of our knowledge, little is known about how and to what extent tunicate cells are subjected to epigenetic histone modification during the zooidal life.

The purpose of this study was to reveal the trimethylation dynamics of histones H3K27 and H3K4 throughout the lifespan of *P. misakiensis*. The epigenetic histone code of buds and zooids was examined by means of immunohistochemistry, western blotting of purified histones, and chromatin immunoprecipitation (ChIP). Possible core promoter regions of the following genes (Suppl. Fig. 1) were chosen for ChIP analyses: *Mitochondrial transcription factor a* (*TFAM*) and *Prohibitin2* (*PHN2*) are known to be involved in mitochondrial function (Artal-Sanz and Tavernarakis, 2009; Campbell et al., 2012; Kang et al., 2007; Osman et al., 2009). *PmRXR* and *PmERK* as well as *PmPHN2* are transdifferentiation-related genes (Kawamura et al., 2013). *SIRT6* encodes Sirtuin 6, a NAD-dependent histone deacetylase that is involved in the suppression of senescence in mammals (Kugel and Mostoslavsky, 2014; Sharma et al., 2013). *PmSIRT6* was included in this study in relation to zooidal senescence and rejuvenation. TC14-3 and RA were applied *in vivo* to *Polyandrocarpa* cells and tissues to examine whether they could regulate histone modification and gene expression in relation to transdifferentiation and senescence. This study is the first to show the dynamics of histone H3 trimethylation in tunicate life. The results are discussed in the context of the role of H3K27me3 and