Nature Reviews Molecular Cell Biology | Published online 24 Aug 2016; doi:10.1038/nrm.2016.117

POST-TRANSLATIONAL MODIFICATIONS

Extension of the tubulin code

> SETD2 … is a common writer for both histone and tubulin codes

Getty Images/iStockphoto/Thinkstock

Histones harbour various post-translational modifications (PTMs), together known as the 'histone code', which are recognized by many effector and regulatory proteins. A similar code also operates for tubulins that comprise the microtubule cytoskeleton. Notably, however, the proteins that are responsible for establishing both codes (referred to as 'writers' and 'erasers') and for recognizing the modifications (the 'readers') have been considered to be distinct. Park *et al.* now reveal that the methyltransferase SET-domain-containing 2 (SETD2; also known as KMT3A) is a common writer for both histone and tubulin codes, thereby identifying methylation as a novel microtubule PTM and providing evidence that the important role of SETD2 in maintaining genome stability is mediated by remodelling both the chromatin and the cytoskeleton.

Whereas many PTMs are ubiquitous and deposited on many different proteins, protein methylation has been primarily associated with histones. Interestingly, Park *et al.* discovered that SETD2, which is responsible for histone H3 Lys 36 trimethylation (H3K36me3), can be co-immunoprecipitated with α-tubulin — a building block of microtubules. This raised the possibility that α-tubulin can be methylated by SETD2, and analysis of the α-tubulin sequence revealed that Lys40 could potentially serve as a SETD2 substrate. Indeed, a specific antibody raised against an α-tubulin-derived peptide that is trimethylated on Lys40 (α-TubK40me3) immunoprecipitated with native α-tubulin from pre-assembled microtubules; mass spectrometry of α-tubulin derived from a human embryonic kidney cell line further revealed α-TubK40me3 as a microtubule PTM *in vivo*, and *in vitro* methylation assays unequivocally showed that SETD2 is able to introduce this PTM.

Immunostaining of mouse embryonic fibroblasts (MEFs) against α-TubK40me3 demonstrated that microtubule methylation occurs specifically in mitosis; early in mitosis, this PTM was detected in the vicinity of the mitotic spindle poles and on the central spindle, as well as on the midbody in later stages of mitosis and during cytokinesis.

To investigate the role of this microtubule PTM in mitosis, MEFs in which *SETD2* knockout could be induced were generated and visualized by live-cell imaging. Following *SETD2* knockout, these cells exhibited various mitotic defects, including failure of chromosome congression to the metaphase plate, formation of multipolar spindles, lagging chromosomes and cytokinesis failure. In line with this, *SETD2*-knockout cells were frequently polyploid and polynucleated and often featured micronuclei. Importantly, whereas the expression of wild-type SETD2 rescued these defects, the expression of a SETD2 mutant that was shown to be defective in α-tubulin but not histone H3 methylation, was unable to supress mitotic aberrations. This indicated that it is indeed the trimethylation of α-tubulin and not SETD2-mediated modification of chromatin that ensures mitotic fidelity.

SETD2 loss of function and mutations have been associated with various cancers and H3K36me3 has been shown to function in DNA repair, indicating that SETD2 can preserve genomic stability and thereby suppress tumorigenesis through its role in chromatin remodelling. The study by Park *et al.* reveals an alternative mechanism by which SETD2 maintains genomic integrity. How exactly SETD2-mediated α-tubulin trimethylation contributes to microtubule dynamics and mitotic fidelity are interesting questions for future studies. Furthermore, the identification of this novel role of SETD2 also serves as a first demonstration of crosstalk between the histone and tubulin codes. Moving forward, it would be important to study this crosstalk in more detail by investigating other potential common writers, readers and erasers.

Paulina Strzyz

ORIGINAL ARTICLE Park, I. Y. *et al.* Dual chromatin and cytoskeletal remodeling by SETD2. *Cell* **166**, 950–962 (2016)