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PERSPECTIVE

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Microtubule shaft integrity emerges as a crucial determinant of the acetylation pattern

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The dynamic nature of microtubules extends beyond the traditional view of these structures merely growing and shortening at their ends. The concept of shaft dynamics introduces a new perspective, focusing away from the ends. Microtubules can be damaged by dissociation of tubulin dimers along the shaft, which can be repaired by incorporating new tubulin dimers, thus restoring structural integrity. These repair sites can function as rescue sites, allowing depolymerizing microtubules to stop shortening and initiate regrowth, thereby prolonging microtubule lifespan (Andreu-Carbó et al., 2022; Aumeier et al., 2016). While damage can occur spontaneously, it can also be induced locally by mechanical forces and proteins like severing enzymes and motor proteins (Andreu-Carbó et al., 2022; Budaitis et al., 2022; Schaedel et al., 2015, 2019; Triclin et al., 2021; Vemu et al., 2018).

Transient shaft damage provides entry points for proteins to access the microtubule lumen. Indeed, the microtubule lumen can be occupied by several proteins, such as MAP6 and the acetyltransferase αTAT1 (Cuveillier et al., 2020; Szyk et al., 2014). αTAT1 acts in the microtubule lumen by acetylating the lysine 40 residue of α-tubulin (L'Hernaul & Rosenbaum, 1985; Soppina et al., 2012), a post-translational modification (PTM) that affects microtubules' mechanical properties and interactions with molecular motors (Bulinski et al., 1988; Cai et al., 2009; Guardia et al., 2016; Piperno et al., 1987; Reed et al., 2006; Tas et al., 2017; Webster & Borisy, 1989). For this modification, the enzymes responsible for adding or removing an acetyl group must access the lumen. While studies have focused on microtubule acetylation and how αTAT1 enters the lumen, microtubules can also be deacetylated by histone deacetylase 6 (HDAC6), which removes the acetyl group (Hubbert et al., 2002; Skoge & Ziegler, 2016; Zhang et al., 2003). Although the exact mechanism by which HDAC6 accesses the microtubule lumen remains elusive, the discontinuous acetylation pattern in microtubules suggests a coordinated interplay between αTAT1 and HDAC6, implying that HDAC6 might enter the lumen similarly to αTAT1.

In a recent study, we showed that the pattern of microtubule acetylation in cells depends on the presence and distribution of microtubule damage. Specifically, microtubules are deacetylated around these damage sites. This suggests that HDAC6 enters the microtubule lumen through damages along the shaft and locally deacetylates tubulin around damage sites. Artificial increase in shaft damage through overexpression of running kinesin-1 decreases acetylation levels by shortening the acetylated segments along microtubules (Andreu-Carbó et al., 2024). We reasoned that additional entry points to the microtubule lumen enhance HDAC6 accessibility.

αTAT1, which rapidly diffuses through the microtubule lumen (Coombes et al., 2016; Ly et al., 2016; Szyk et al., 2014), is three times smaller than HDAC6 (Howes et al., 2014; Skultetyova et al., 2017). We uncovered that damage formation enhances both acetylation and deacetylation. Kinesin-1, likely by increasing the abundance of damage sites, boosts initial microtubule acetylation of growing microtubules which polymerize from deacetylated tubulin. During the early phase of microtubule acetylation, inhibiting HDAC6 with the specific inhibitor tubacin (Haggarty et al., 2003), led to longer acetylation

segments. This indicates that HDAC6 counteracts αTAT1 during microtubule acetylation.

However, in an established microtubule network, acetylation depends only slightly on microtubule damage and is independent of kinesin-1-induced damage. In contrast, deacetylation of the network relies on HDAC6 entry through damage sites. Thus, damage sites generated by kinesin-1 locally modulate the deacetylation efficiency.

This local deacetylation can explain the characteristic microtubule acetylation pattern (Bulinski et al., 1988; Piperno et al., 1987; Webster & Borisy, 1989). The perinuclear microtubule network is highly acetylated, while the acetylation signal exponentially decays toward the cell edges. We showed that this gradient inversely correlates with the distribution of running kinesin-1 motors, which are more abundant toward the cell periphery. Correspondingly, microtubule damage is more frequent in the cell periphery where acetylated segments are shorter. Changes in kinesin-1 distribution affect not only the level and length of acetylation stretches but also perturb their characteristic distribution. Thus, this indicates that kinesin-1 is an active player in shaping the acetylation pattern through the generation of damage sites.

Our findings highlight that microtubule deacetylation requires the presence of damage sites, emphasizing the role of microtubule shaft integrity in modulating the acetylation pattern. Contrary to the running motor, an immotile kinesin-1 that we had previously shown to cover the microtubule shaft and hinder the formation of damage sites (Andreu-Carbó et al., 2022), hyperacetylates microtubules. Moreover, we demonstrated that the modulation of shaft integrity impacting the acetylation pattern extends beyond the molecular motor kinesin-1. Damage induced by the severing enzyme spastin decreases microtubule acetylation. Conversely, overexpression of the microtubule-associated protein 7 (MAP7) or end-binding protein 3 (EB3), which at high levels cover the microtubule shaft, increases acetylated microtubules. We proposed that controlling microtubule shaft dynamics could serve as a general mechanism to govern the organization of the acetylation pattern within cells.

Our study demonstrated that shaft dynamics regulate not only microtubule length and lifetime (Andreu-Carbó et al., 2022; Schaedel et al., 2015, 2019; Vemu et al., 2018) but also PTM composition. Molecular motors and MAPs can alter the microtubule acetylation pattern by regulating the access of HDAC6 to the microtubule lumen. Thereby, shaft dynamics offer a mechanism to shape the microtubule acetylation pattern without depolymerization of the network. Other mechanisms like active transport of αTAT1 and HDAC6, modulation of the enzyme levels, or local activation, might also be involved (Even et al., 2019; Hubbert et al., 2002; Lafarga et al., 2012; Montagnac et al., 2013).

We propose that motor and MAP distribution is crucial for shaping the microtubule acetylation pattern. In this scenario, the high level of microtubule acetylation around the nucleus results from dense MAP coverage that prevents shaft damage, while peripheral microtubules are more deacetylated due to polymerization from deacetylated tubulin and increased damage. We introduced the concept that molecular motors and MAPs can create an acetylation pattern, that exhibits a gradient scaling with cell dimensions. This gradient may encode positional information within the cell, readable by various proteins. Acetylation is associated with microtubule stabilization and serves as preferred tracks for kinesin-1 motors (Cai et al., 2009; Guardia et al., 2016; Reed et al., 2006), thereby influencing protein localization and transport in the cytoplasm. Proteins that modulate local shaft integrity could control this acetylation gradient, significantly impacting cell physiology.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

There are no new data in this paper.

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