Traffic control inside the cell: microtubule-based regulation of cargo transport

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The cell relies on an intricate system of molecular highways and motors to transport proteins, organelles and other vesicular cargoes to their proper locations. Microtubules, long filaments that form a network throughout the cell, act as highways. The motor proteins kinesin and dynein associate with cargoes and transport them along microtubules. Rather than simply acting as passive tracks, microtubules contain signals that regulate kinesin and dynein to target cargoes to specific locations in the cell. These signals include the organization of the microtubule network, chemical modifications that alter the microtubule surface properties and mechanics, and microtubule-associated proteins that modulate the motility of motor proteins and microtubule polymerization.

How do PTMs control microtubule track stability and direct cargo trafficking?

Microtubules are subject to multiple PTMs including acetylation, tyrosination and polyglutamylation (Figure 1). PTMs confer functional diversity to microtubules by altering their polymerization dynamics and mechanical properties. Microtubules are cylindrical polymers assembled from tubulin dimers. Most microtubule PTMs occur on the carboxy-terminal tail of tubulin that protrudes away from the microtubule surface, with the exception of acetylation that occurs in the lumen (the space inside the hollow cylinder). Importantly, PTMs also affect the electrostatic interactions between positively charged residues on motor proteins and MAPs and the negatively charged carboxy-terminal tails of tubulin. For instance, a positively charged lysine residue present in the TUBB3 isoform of tubulin decreases kinesin-1 processivity, while polyglutamylation, the addition of negatively charged glutamate residues on tubulin tails, increases processivity.

Tubulin PTMs are correlated with the recruitment of specific types of motor proteins (Table 1). Lysosomes transported by kinesin-1 move along stable acetylated microtubules into neuronal axons, while lysosomes transported by kinesin-3 move along peripheral microtubules enriched with tyrosinated α-tubulin. Subcellular compartments such as dendrites and axons in neurons possess microtubules marked by different sets of PTMs (Figure 2). Kinesin-1 localization correlates with stable acetylated and detyrosinated microtubules in fibroblasts and neurons, while kinesin-3's enrichment on tyrosinated microtubules directs cargoes towards the distal (i.e.
outward) ends of dendritic microtubules. Activated dynein complexes preferentially select tyrosinated microtubule tracks, contributing to the initiation of retrograde transport of cargoes from the distal axon towards the cell body of neurons. Further, tubulin PTMs alter the binding of MAPs.

Unlike other tubulin PTMs, acetylation occurs in the lumen of microtubules away from the site of interaction of microtubule-binding proteins on the surface. While microtubule acetylation does not detectably alter the surface conformation of microtubules or directly affect motor protein kinesin binding to the microtubule surface, kinesin-1 moves preferentially along acetylated microtubules in cells. Acetylation is associated with long-lived stable microtubules and is shown to increase the resilience of microtubules against mechanical ageing. Recent work analyzing the motility of purified kinesin motor proteins on isolated microtubule cytoskeletons indicates that tubulin acetylation does not directly affect kinesin-1 motility, but is strongly associated with microtubule bundling which is what enhances kinesin motility. Axonal microtubules in neurons that support rapid long-range cargo transport are highly acetylated and bundled. Taken together, these studies suggest that the cell uses PTMs to establish preferred routes for intracellular transport.

**How do MAPs tune the intracellular transport of cargoes being directed to specific locations in the cell?**

MAPs play important roles in organizing the microtubule cytoskeleton and controlling the motility of motor proteins. Microtubules are highly dynamic structures that constantly grow and shrink. MAPs regulate the polymerization dynamics of microtubules, induce PTMs on specific microtubule filaments, and also regulate the motility of kinesin and dynein.

MAPs regulate transport in a motor-specific manner by inhibiting some motors while enhancing the activity of others (Table 1). Tau, a neuronal MAP, not only stabilizes microtubules by crosslinking them but is also involved in regulating the transport of molecular motors by competing with them for binding sites along the microtubule. Tau inhibits the motility of kinesin-1 more strongly than movement of dynein or kinesin-2. For bidirectional cargoes driven by teams of kinesin-1, kinesin-2 and dynein motors, tau enhances dynein-driven motility by reducing the activity of opposing kinesin motors, indicating that MAPs can control the direction of movement along microtubules. MAP7 (ensconsin) also binds along microtubules and stabilizes them; however in contrast to tau, MAP7 enhances kinesin-1 motility. Further, MAP7 competes with tau for binding along microtubules to regulate kinesin transport.
MAP2 localizes in neuronal dendrites and has been shown to strongly inhibit the motility of both kinesin-1 and dynein. Interestingly, for cargoes driven by teams of kinesin-1 and kinesin-3 motors, MAP2 has been shown to enhance the fast transport of kinesin-3 by inhibiting slow transport of kinesin-1. MAP4, an axonal MAP, regulates the motility of pigment granules in *Xenopus* melanophores driven by dynein and kinesin-2. MAP4 reduces dynein-mediated motility by decreasing the run length of these pigment granules walking towards the microtubule minus-end. Doublecortin, a MAP primarily expressed in developing neurons, promotes neuronal growth and migration. By increasing the binding affinity of kinesin-3 to microtubules, doublecortin enhances the motility of synaptic vesicles transported by kinesin-3. Intracellular cargoes are often driven by teams of kinesin and dynein motors. Employing multiple types of kinesins and dynein on the same cargo, each with different sensitivities to specific MAPs, may enable cargoes to be targeted to specific locations in the cell (Figure 2).

**How does the organization of the microtubule network control trafficking?**

The microtubule cytoskeleton is a dynamic network of filaments that constantly remodel to alter cellular organization. A dramatic example is when the cell reconfigures the microtubule network to form the mitotic spindle for chromosome segregation during cell division. The organization of the microtubule network controls the distribution of motor-driven cargoes. Akin to an ever-changing road map that enables fine-tuning of cargo transport, the polymerization dynamics of microtubule tracks and the packing of several microtubule filaments into bundles contributes to controlling the spatial distribution of cargoes in the cell.

Cells organize the architecture of the microtubule cytoskeleton to control cargo distribution (Figure 2). Microtubules are organized in bundles in neuronal axons with MAPs such as tau serving as spacers between filaments, while MAP-2 decorated microtubule filaments are arranged in an antiparallel orientation in the dendrites. Microtubule bundling is observed not only in neurons, but also in other subcellular compartments such as in primary cilia and flagella, as well as in specialized structures like the mitotic spindle. Interestingly, bundled microtubules are consistently marked by acetylation and often present in stable structures including axons and cilia. Although tau inhibits kinesin-based transport, axonal microtubules that support rapid long-range transport are heavily decorated by tau. The bundling of microtubules in axons may enable efficient transport in the presence of tau by allowing teams of motors to navigate around obstacles and by increasing the number of available binding sites to promote motor binding.

The architecture of the microtubule network targets motor proteins into specific cellular compartments. In axons, the acetylated tau-decorated microtubule bundles are oriented in a parallel manner, with their plus-ends reaching to the synaptic terminal. In dendrites, microtubule bundles are arranged in an antiparallel orientation, with the plus-end of stable acetylated microtubules oriented towards the cell body and the plus-end of dynamic tyrosinated microtubules directed to the cell periphery. Due to this organization, plus-end-directed motor kinesin-1 are targeted to exit dendrites and localize to axons as kinesin preferentially walks on acetylated microtubules. Kinesin-3 prefers tyrosinated microtubules, promoting entry into MAP2-decorated microtubules and the proximal axon.

**Perspective**

A network of interactions between tubulin PTMs, MAPs and motors regulates the organization of the microtubule cytoskeleton and transport along it. These factors allow functional diversity among different populations of microtubules, and enable spatial regulation of transport in the cell. Further, the effects of microtubule signals differ between different motor types, potentially allowing cargo-specific regulation through the set of motor proteins associated with each cargo.
Many open questions remain in understanding how the information encoded in the microtubule network tunes kinesin and dynein motility to target cargoes to specific destinations in space and time. The mechanisms through which microtubule PTMs and associated proteins control transport remain unclear. How do motor proteins recognize specific tubulin post-translational modifications? How does robust kinesin-directed transport occur on microtubules that are heavily decorated by tau in neuronal axons? The combination of the multiple motor proteins involved in intracellular transport and the diverse family of MAPs suggests a vast array of possible interactions, only a few of which have been explored. The answers to these questions are expected to highlight the interdependencies between microtubule network organization, PTMs and MAPs. By studying the interactions of these factors and their combined effects on cargo trafficking, we will better understand how the cell establishes highways and controls transport along them, and how dysregulation results in diseases of the nervous system.

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Further reading

- Janke, C. (2014) The tubulin code: molecular components, readout mechanisms, and functions. J. Cell. Biol. 206, 461–472.
- Sirajuddin, M., Rice, L.M. and Vale, R.D. (2014) Regulation of microtubule motors by tubulin isotypes and post-translational modifications. Nature Cell Biology 16, 335.
- Guardia, C.M., Farias, G.G., Jia, R., Pu, J. and Bonifacino, J.S. (2016) BORC functions upstream of kinesins 1 and 3 to coordinate regional movement of lysosomes along different microtubule tracks. Cell Reports 17, 1950–1961.
- Cai, D., McEwen, D.P., Martens, J.R., Meyhofer, E. and Verhey, K.J. (2009) Single molecule imaging reveals differences in microtubule track selection between kinesin motors. PLoS Biology 7, e1000216.
- Tas, R.P., Chazeau, A., Cloin, B.M.C., Lambers, M.L.A., Hoogenraad, C.C. and Kapitein, L.C. (2017) Differentiation between oppositely oriented microtubules controls polarized neuronal transport. Neuron 96, 1264–1271.
- Kaul, N., Soppina, V. and Verhey, K.J. (2014) Effects of α-tubulin K40 acetylation and detyrosination on kinesin-1 motility in a purified system. Biophys. J. 106, 2636–2643.
- Portran, D., Schaeled, L., Xu, Z., Théry, M. and Nachury, M.V. (2017) Tubulin acetylation protects long-lived microtubules against mechanical ageing. Nature Cell Biology 19, 391.
- Balabanian, L., Berger, C.L. and Hendricks, A.G. (2017) Acetylated microtubules are preferentially bundled leading to enhanced kinesin-1 motility. Biophysical Journal 113, 1551–1560.
- Takemura, R., Okabe, S., Umeyama, T., Kanai, Y., Cowan, N.J. and Hirokawa, N. (1992) Increased microtubule stability and alpha tubulin acetylation in cells transfected with microtubule-associated proteins MAP1B, MAP2 or tau. J. Cell Sci. 103, 953–964.
- Tortosa, E., Kapitein, L.C. and Hoogenraad, C.C. (2016) Microtubule organization and microtubule-associated proteins (MAPs) in Dendrites (Emoto, K., Wong, R., Huang, E. and Hoogenraad, C. eds) pp. 31–75, Springer, Tokyo.
- Chaudhary, A.R., Berger, F., Berger, C.L. and Hendricks, A.G. (2018) Tau directs intracellular trafficking by regulating the forces exerted by kinesin and dynein teams. Traffic 19, 111–121.
- Vershinin, M., Carter, B.C., Razafsky, D.S., King, S.J. and Gross, S.P. (2007) Multiple-motor based transport and its regulation by Tau. Proc. Natl Academy Sci. 104, 87–92.
- Dixit, R., Ross, J.L., Goldman, Y.E. and Holzbaur, E.L. (2008) Differential regulation of dynein and kinesin motor proteins by tau. Science 319, 1086–1089.
- Hoeprich, G.J., Mickolajczyk, K.J., Nelson, S.R., Hancock, W.O. and Berger, C.L. (2017) The axonal transport motor kinesin-2 regulates microtubule obstacle via protofilament switching. Traffic 18, 304–314.
- Barlan, K., Lu, W. and Gelfand, V.I. (2013) The microtubule-binding protein ensconsin is an essential cofactor of kinesin-1. Curr. Biol. 23, 317–322.
- Heins, S., Song, Y.-H., Wille, H., Mandelkow, E. and Mandelkow, E.-M. (1991) Effect of MAP2, MAP2c, and tau on kinesin-dependent microtubule motility. J. Cell Sci. 991, 121–124.
- Gumer, L.F., Katrukha, E.A., Grigoriev, I. et al. (2017) MAP2 defines a pre-axonal filtering zone to regulate KIF1 versus KIF5-dependent cargo transport in sensory neurons. Neuron 94, 347–362 e347.
- Semenova, I., Ikeda, K., Resaul, K., et al. Regulation of microtubule-based transport by MAP4. Mol. Biol. Cell 25, 3119–3132 (2014).
- Liu, J.S., Schubert, C.R., Fu, X., et al. (2012) Molecular basis for specific regulation of neuronal kinesin-3 motors by doublecortin family proteins. Mol. Cell 47, 707–721.
- Chen, J., Kanai, Y., Cowan, N. and Hirokawa, N. (1992) Projection domains of MAP2 and MAP4. Mol. Biol. Cell 3, 707–721.
- Mckenney, R.J., Huynh, W., Vale, R.D. and Sirajuddin, M. (2016) Tyrosination of α-tubulin controls the initiation of processive dynein–dynactin motility. The EMBO J. e201593071.