Review

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Integrated regulation of motor-driven organelle transport by scaffolding proteins

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Intracellular trafficking pathways, including endocytosis, autophagy, and secretion, rely on directed organelle transport driven by the opposing microtubule motor proteins kinesin and dynein. Precise spatial and temporal targeting of vesicles and organelles requires the integrated regulation of these opposing motors, which are often bound simultaneously to the same cargo. Recent progress demonstrates that organelle-associated scaffolding proteins, including Milton/TRAKs (trafficking kinesin-binding protein), JIP1, JIP3 (JNK-interacting proteins), huntingtin, and Hook1, interact with molecular motors to coordinate activity and sustain unidirectional transport. Scaffolding proteins also bind to upstream regulatory proteins, including kinases and GTPases, to modulate transport in the cell. This integration of regulatory control with motor activity allows for cargo-specific changes in the transport or targeting of organelles in response to cues from the complex cellular environment.

Microtubule-based transport in the cell

The spatial complexity of eukaryotic cells relies on the precise regulation of intracellular trafficking. Long-distance organelle transport depends on microtubules, which serve as polarized tracks for motility within the cell. Delivery of diverse cargos along microtubules underlies many essential cellular functions such as protein secretion, growth and injury signaling, protein and organelle degradation, active distribution of mitochondria, and trafficking of RNA granules. Furthermore, cells precisely regulate organelle transport by coordinating motor activity with cellular demands and in response to changes in the cellular environment.

Microtubules are organized into a polarized array, with dynamic plus-ends oriented toward the cell periphery and more stable minus-ends clustered toward the center in most cell types. Anterograde transport, or motility toward plus-ends, is driven by members of the extended kinesin superfamily including kinesin-1, kinesin-2, and kinesin-3; retrograde transport, or motility toward minus-ends, is driven primarily by cytoplasmic dynein acting in concert with its activator dynactin. The biophysical properties of these motors, such as velocity, run length, and stall force, have been studied in detail at the single-molecule level [1]. However, less is known about how these motors are regulated when bound to cargo in order to drive organelle-specific motility.

Recent progress on the roles of adaptor or scaffolding proteins is providing new insights into motor coordination at the cellular level. Scaffolding proteins can effectively regulate the activities of organelle-associated motors to control directionality. Scaffolding proteins also bind to upstream regulatory elements, including kinases, phosphatases, Ca^{2+}-signaling proteins, and G proteins. This allows the integration of diverse signals to yield organelle-specific responses to the local cellular environment. We focus here on new developments in the regulation of organelle-associated motors by scaffolding and adaptor proteins, including Milton/TRAK, Miro (mitochondrial Rho GTPase), RILP (Rab7-interacting lysosomal protein), huntingtin, La, JIP1-4, and Hook. Progress to date has identified some common mechanisms, but also highlights the exquisite selectivity of regulatory control that leads to cargo-specific and region-specific patterns of motility within the cell.

Models for motor regulation during intracellular transport

The simplest model to describe cargo-specific regulation of organelle motility involves the ***selective recruitment*** of either kinesin or dynein motors (Figure 1A). If kinesin is recruited, the organelle will move toward the microtubule plus-end. However, if the bound kinesins dissociate and dynein is recruited, then the cargo will move toward the microtubule minus-end. This model would be predicted to yield unidirectional, highly processive motility.

In contrast to this simple model, increasing evidence from both *in vitro* and cellular studies suggests that opposing kinesin and dynein motors are often bound simultaneously to cargos moving along microtubules [2–7]. Two models have been put forth to describe the interactions of opposing kinesin and dynein motors bound to the same cargo, and the resulting patterns of motility.

Many organelles in the cell exhibit bidirectional motility characterized by brief excursions toward either the microtubule plus- or minus-end punctuated by

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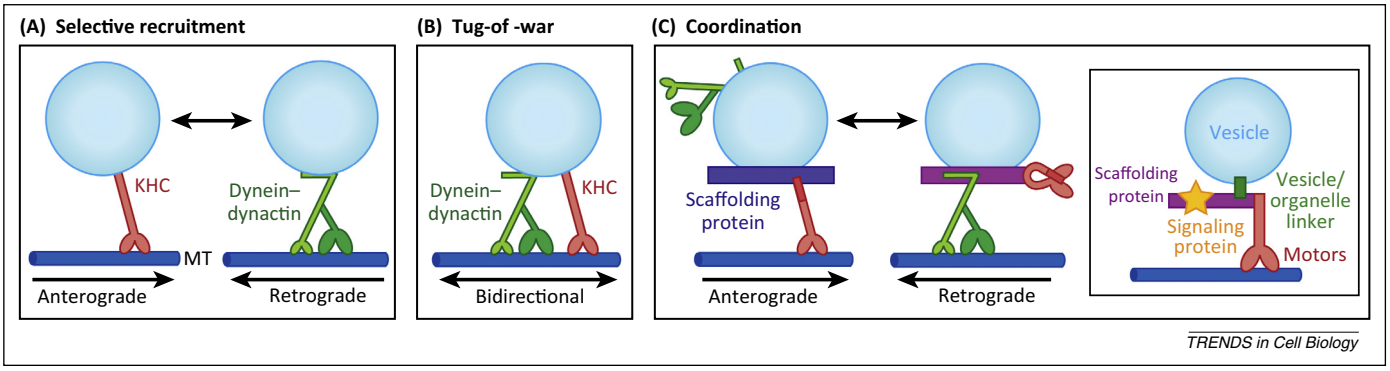

Figure 1. Three current models for the regulation of microtubule motors bound to vesicular and organelle cargo. **(A)** In the **selective recruitment model**, only one type of motor, either kinesin (KHC) or dynein–dynactin, is bound to cargo at a time. If kinesin is bound, the cargo will move unidirectionally in the anterograde direction toward the plus-end of the microtubule. If dynein–dynactin is bound, the cargo will move unidirectionally in the retrograde direction, toward the microtubule minus-end. **(B)** In the **tug-of-war model**, both kinesin and dynein–dynactin motors are bound to the cargo simultaneously. The cargo will move bidirectionally along the microtubule, depending on stochastic variations in the dominant motor type. Note that, for simplification, this figure only illustrates one dynein–dynactin complex per vesicle, but likely 6–8 dynein–dynactin complexes are on each vesicle to reach force balance with one kinesin. **(C)** In the **coordination model**, kinesin and dynein are bound to the cargo simultaneously, but the activities of these motors are governed by a scaffolding protein that coordinates the engagement of dynein–dynactin with the autoinhibition of kinesin. (Inset) Generalized model for the integration of upstream signaling with downstream motility by scaffolding proteins. Scaffolding proteins interact with vesicle/organelle linker proteins, upstream signaling proteins, and molecular motors, forming an integrated regulatory unit. Although scaffolding proteins may also mediate the association of motors with the vesicle or organelle, this is not always the case. KHC, kinesin heavy chain; MT, microtubule.

frequent directional switches. A ***tug-of-war*** mechanism has been proposed to explain these observations. In this scenario, direction of transport is determined by the team of motors, either plus-end-directed or minus-end-directed, which produces the greater force at any given time (Figure 1B). Force-dependent detachment of the opposing motors from the microtubule track prevents unproductive stalling. Stochastic motor release from the track leads to alterations in the dominant motor type over time, resulting in directional switching [8].

Experimental work in several systems supports this model. For example, late endosomes and lysosomes moving along neuronal axons exhibit bidirectional motility, characterized by short run length, either toward or away from the cell body, punctuated by frequent changes in direction. Quantitative immunoblotting of purified vesicles indicates that each organelle binds few (1–2) kinesin motors and a larger team (6–12) of dynein motors [3]. However, because kinesins generally exhibit high unitary stall forces (~5–7 pN) whereas mammalian cytoplasmic dynein has a low unitary stall force (~1 pN) [1], these opposing motors are present near force balance on each organelle. The resulting stochastic tug-of-war between these relatively evenly matched motor teams is predicted to cause frequent directional switches and low net processivity [3].

A third model postulates that opposing motors are simultaneously bound to cargo, but their motility is tightly coordinated by regulatory mechanisms. In this ***coordination*** model both kinesin and dynein motors remain continuously bound to the organelle, but are not constitutively active (Figure 1C). Instead, motor activity may be specifically regulated by post-translational modifications or by adaptor or scaffolding proteins. This model would explain the motility of fast-moving processive cargos with few directional changes, such as autophagosomes moving along neuronal axons. These organelles stably bind both kinesin and dynein motors but undergo primarily unidirectional transport toward the cell soma [4], indicating that the associated kinesin motors are efficiently inhibited during dynein-driven motility [9].

What is the advantage of maintaining simultaneous, stable associations with opposing motors? In the case of the unregulated tug-of-war model, one possibility is that while the stochastic directional changes produce less efficient long-distance motility, these properties may be important for developing and/or maintaining stable distributions of organelles along extended cellular processes such as the axon. In the coordination model, opposing motors may allow quick direction changes, perhaps to avoid roadblocks or traffic jams [10,11]. Another major advantage of the simultaneous binding of opposing motors is to allow for rapid responses to changes in the local cellular environment.

While cargo-specific regulation allows the cell to selectively modulate the motility of different organelle populations, additional mechanisms also contribute to the regulation of trafficking within the cell. There are strong data indicating track-specific regulation of organelle transport via post-translational modification of microtubules [12–15] or through the binding of microtubule-associated proteins (MAPs) that may aid or hinder motor processivity [10,16–18]. Additionally, region-specific regulation of transport may result from differences in the local organization of the cytoskeleton, such as the enrichment of dynamic microtubule plus-ends near the cell cortex, microtubule-microtubule intersections [19], or the formation of dense actin filaments that can induce track switching or impede motion (reviewed in [20]). Finally, given the crowded nature of the cellular environment, there is growing evidence that many of the prominent pauses or directional changes observed during organelle motility result from collisions with other cellular organelles, much like cars colliding at rush hour on crowded highways [21,22]. However, the strikingly different types of motility observed for distinct cargos moving through the same region of the cell indicate that cargo-specific regulation may be the dominant mechanism involved in the control of organelle motility *in vivo*.