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MAPping out distribution routes for kinesin couriers

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ABSTRACT

In the crowded environment of eukaryotic cells, diffusion is an inefficient distribution mechanism for cellular components. Long-distance active transport is required and is performed by molecular motors including kinesins. Furthermore, in highly polarized, compartmentalized and plastic cells such as neurons, regulatory mechanisms are required to ensure appropriate spatio-temporal delivery of neuronal components. The kinesin machinery has diversified into a large number of kinesin motor proteins (Kifs) as well as adaptor proteins that are associated with subsets of cargo. However, many mechanisms contribute to the correct delivery of these cargos to their target domains. One mechanism is through motor recognition of subdomain-specific microtubule (MT) tracks, sign-posted by different tubulin isoforms, tubulin post-translational modifications (PTMs), tubulin GTPase activity and MT associated proteins (MAPs). With neurons as a model system, a critical review of these regulatory mechanisms is presented here, with particular focus on the emerging contribution of compartmentalised MAPs. Overall, we conclude that - especially for axonal cargo - alterations to the MT track can influence transport, although *in vivo*, it is likely that multiple track-based effects act synergistically to ensure accurate cargo distribution.

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3 The size, functional compartmentalisation and polarity of eukaryotic cells present
4 major challenges for sorting and distribution of subcellular components. Transport by the
5 microtubule (MT)-based motors dynein and members of the kinesin superfamily play a
6 significant role in long-range transport and specific distribution of cellular parcels of various
7 sizes, from mRNA particles to mitochondria. Various mechanisms operate to ensure delivery
8 to the correct location, including: 1) cargo-dependent localisation via adaptor proteins; 2)
9 motors with different speeds and directionalities that take particular routes through the
10 cell; 3) MT track-based road blocks and diversions. Discussions of motor-cargo specification
11 have been presented elsewhere (e.g. Akhmanova and Hammer, 2010). A number of recent
12 studies are beginning to provide mechanistic insight into the way in which the kinesin-MT
13 interaction can be modulated - including through the action of MT Associated Proteins
14 (MAPs) - to achieve compartmentalized and polarized cargo distribution, and this review will
15 focus on this subject.
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27 **Introduction: Kinesin family members target specific cargo to particular cellular** 28 **subdomains** 29

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31 In contrast to the paucity of cytoplasmic dynein heavy chains (two genes identified in
32 mice and humans; Pfister et al., 2006), the kinesin repertoire in mammals boasts 45 kinesin
33 heavy chain (KHC) genes, with additional protein variants derived from splice variants (Miki
34 et al., 2003). Kinesin family members are somewhat confusingly referred to as both 'Kifs'
35 and 'kinesins' with different numbering systems. For functional clarity and precision we will
36 use the Kif nomenclature in this review. The well-conserved Kif motor domains define the
37 Kif superfamily, have MT-stimulated ATPase activity and typically operate in the context of
38 homo- or hetero-dimers (Hirokawa et al., 2010; Verhey and Hammond, 2009). The Kif tail
39 domains are divergent and can bind different cargos directly, whilst Kif association with
40 cargo-binding adaptors, including kinesin light chains (KLCs), enables further specific freight
41 interactions.
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51 Kifs use polar, tubular polymers of α/β tubulin heterodimers – MTs – as tracks. MTs
52 are intrinsically dynamic, and although the so-called minus-ends are typically stabilised at
53 the cell's MT organising centre, the opposite plus-ends exhibit phases of polymerization or
54 depolymerisation within the cytoplasm that are precisely regulated *in vivo* (Janke and
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3 Bulinski, 2011; Wade, 2009). Regulation of MT dynamics must necessarily be coordinated
4 with requirements for MT-based transport. In general, whilst transport via dynein is towards
5 the minus-end of MTs (retrograde transport), the Kifs of the subtype N-Kif (with an N-
6 terminal motor domain) are solely responsible for MT-based transport towards the plus-end
7 (anterograde transport; Figure 1). A small minority of Kif family members – the C-Kifs (with
8 C-terminal motor domains) – are minus-end directed and involved in crosslinking and sliding
9 MTs during mitosis, (Peterman and Scholey, 2009; Sharp et al., 2000) and differentiated cells
10 (Bananis et al., 2004; Hanlon et al., 1997; Noda et al., 2001; Saito et al., 1997; Xu et al.,
11 2002; Yang et al., 2001). The remaining Kifs, the M-Kifs, have a central motor domain, act as
12 MT depolymerases and control MT organisation *in vivo* both during cell division and in
13 interphase (Hirokawa et al., 2009; Wordeman, 2005).
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23 The functions of individual Kifs and the cargo they carry have been studied in a wide
24 variety of cell contexts. In the mitotic spindle, for example, the multiple Kifs that operate as
25 part of this complex cellular machinery manoeuvre their cargo in a highly orchestrated
26 manner to ensure accurate segregation of the duplicated chromosomes to each daughter
27 cell (Douglas and Mishima, 2010; Peterman and Scholey, 2009; Sharp et al., 2000). In
28 differentiated cells, the roles of Kifs in carrying different cargos are also emerging (Hirokawa
29 et al., 2009). The existence of different populations of Kif-driven vehicles each with distinct
30 passenger sets could provide a mechanism for restricted and precise distribution of
31 particular cargos in polarized and compartmentalized cells, but this mechanism depends on
32 individual Kifs being dispatched along particular routes to specific destinations.
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42 Below, we will focus primarily on the classic and extensively studied case of neuronal
43 transport. Here, the need for efficient regulatory mechanisms guiding compartment-specific
44 cargo delivery is extreme in many respects. A neuron's large size and high degree of
45 polarization, plasticity, compartmentalization and compartmentalized activity requires
46 direction-restricted transport, often occurring over vast distances and in a manner
47 responsive to discrete spatio-temporal signals. In certain human motor neurons for
48 example, cargos originating in the soma have final destinations more than a metre away in
49 the periphery. Pre- and post-synaptic compartments are defined and dynamically modified
50 by exclusive and on-demand delivery of their specific components, enabling proper
51 neuronal function and plasticity.
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3 The MT array in neurons is very precisely organised, with axonal MTs having their
4 plus-ends located distally, whilst the polarity of MTs in the dendritic compartment is mixed
5 (Baas et al., 1988). Thus, the minus-end (dynein and C-Kifs) and plus-end directed (N-Kifs)
6 motors could by themselves form the basis of a simple system of polarized cargo transport.
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8 However, it is now clear that some plus-end directed Kifs can move preferentially into either
9 axons or dendrites to deliver compartment-specific cargo (Byrd et al., 2001; Huang and
10 Banker, 2011; Jacobson et al., 2006; Nakata and Hirokawa, 2003; Nishimura et al., 2004;
11 Saito et al., 1997; Setou et al., 2000; Shi et al., 2004). The compartment-specific movement
12 of individual Kifs can depend on the experimental context (Table 1) and in particular,
13 whether the motor is constitutively active (i.e. tail-less and typically tagged using GFP) or
14 full-length (i.e. a subset could be autoinhibited and not bound to cargo). Nevertheless, the
15 active guidance of a defined subset of transport Kifs (Silverman et al., 2010) preferentially
16 into axons or dendrites (Hirokawa et al., 2010; Huang and Banker, 2011) represents an
17 intriguing example of transport regulation in this cell type, a phenomenon which appears to
18 both establish and maintain polarized neuronal structure and function (Jacobson et al.,
19 2006; Nakata and Hirokawa, 2007). Furthermore, there appear to be mechanisms for
20 halting/releasing some cargos at specific locations in particular conditions, such as Ca^{2+} -
21 dependent pausing of Kif5-mitochondrial complexes at synapses, or enrichment of Kif5,
22 Kif3A and Kif13B motors in the growth cone of developing axons (Horiguchi et al., 2006;
23 Nadar et al., 2008; Nishimura et al., 2004; Yoshimura et al., 2010). For some kinesins,
24 including Kif5s, the motor domain alone is sufficient to define their physiologically relevant
25 and typically axonal localisation, implying that a track-based guidance system for cargo
26 carried by these motors is in operation. However, some usually dendritic cargos actively
27 define their final destination \square the “cargo steering” hypothesis \square independently of the
28 motors to which they are attached, suggesting that localisation mechanisms not defined by
29 the MT tracks are also in operation (Huang and Banker, 2011; Jenkins et al., 2012; Nakata
30 and Hirokawa, 2003; Setou et al., 2000; Setou et al., 2002).

51 **Are tubulin PTMs road markers for polarized Kif transport?**

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54 The molecular basis for the selective distribution of Kif motors and their cargo is
55 critical in understanding the establishment and maintenance of cell polarity, but we are
56 currently very far from understanding this complex phenomenon. The vast majority of
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3 research on this subject has focused on Kif5 (conventional kinesin/kinesin-1), the founding
4 member of the kinesin superfamily. When Kif5 is truncated at its C-terminus to form a
5 constitutively motile dimer, it preferentially localises to the axonal compartment, implying
6 that the interaction between the Kif5 motor domains and the MT tracks are sufficient to
7 define its polar distribution (Nakata and Hirokawa, 2003; Thorn et al., 2000). One possible
8 mechanism is through compartment-specific post-translational modifications of $\alpha\beta$ -tubulin –
9 typically on MT-incorporated tubulin rather than on the soluble tubulin pool (Figure 1).
10 Most but not all of these modifications are made to the flexible C-terminal tails (CTTs) of α -
11 or β -tubulin on the MT surface (Figure 1), which themselves are known in general terms to
12 contribute to kinesin motility (Thorn et al., 2000). The effects of a number of post-
13 translational modifications (PTMs) of tubulin on Kif5 MT binding, motility, and selectivity
14 have been investigated (for a longer recent review, see also Garnham and Roll-Mecak,
15 2012).

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17 α -tubulin acetylation is a prevalent PTM on stable MTs, particularly in neuronal
18 axons, and has been correlated with enhanced Kif5 binding and motility (Konishi and Setou,
19 2009; Reed et al., 2006). Hyper-acetylation of MTs in all neuronal subcellular compartments
20 via treatment with HDAC inhibitors depolarizes the distribution of Kif5 (Tapia et al., 2010)
21 and its JIP-1 cargo (Reed et al., 2006), at first sight making this modification a compelling
22 candidate for control of polar trafficking. However, the molecular basis for such control is
23 not immediately obvious, since a principal site of α -tubulin acetylation is Lys40, which is
24 located in the lumen of the MT and remote from the kinesin binding site (Nogales et al.,
25 1998; Soppina et al., 2012). In fact, more recent studies suggest that pharmacological
26 perturbation of tubulin acetylation does not affect Kif5 distribution (Hammond et al., 2010;
27 Nakata et al., 2011). Some of these discrepancies may be explained by variations in
28 experimental conditions, including the treatment parameters and age of cultured neurons
29 used, but it should also be noted that HDAC inhibition in the cellular environment can have
30 a number of off target side-effects both on and off the MT, including on MAPs themselves
31 (Cook et al., 2012). Indeed, *in vitro* experiments found no effect of Lys40 acetylation on Kif5
32 binding, run length or velocity, suggesting that this *alone* cannot directly account for
33 selective axonal Kif5 distribution (Soppina et al., 2012; Walter et al., 2012). Although Kif5
34 and associated cargo has been shown to move preferentially on acetylated MTs in COS cells
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3 (Cai et al., 2009), cellular Kif5 and its cargo also move on non-acetylated MTs or those MTs
4 marked by detyrosination. Finally, HDAC6 knockout mice develop normally despite
5 widespread tubulin hyperacetylation (Zhang et al., 2008). In summary, tubulin acetylation
6 alone cannot account for axon specific transport by Kif5.
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11 Similarly, detyrosinated α -tubulin is also found mainly in the neuronal axon and
12 polarised movement of Kif5 and/or its cargos along stable cellular MTs can be selectively
13 disrupted both by antibodies to detyrosinated (but not tyrosinated) tubulin (Gurland and
14 Gundersen, 1995) and by siRNA-mediated knockdown of tubulin tyrosine ligase (Ttl) (Konishi
15 and Setou, 2009). Kif5 binds more tightly to detyrosinated MTs *in vitro* (Dunn et al., 2008;
16 Konishi and Setou, 2009; Kreitzer et al., 1999; Liao and Gundersen, 1998) and recognition of
17 this PTM and related polarized transport depends on Kif5's divergent β 5-L8 region which is
18 part of the kinesin-MT-binding interface (Figure 2). However, although mice deficient in Ttl
19 exhibit abnormal axonal development (Erck et al., 2005; Marcos et al., 2009), ultimately a
20 polarised morphology develops - which likely requires intact polarized Kif5 transport
21 (Hammond et al., 2010; Jacobson et al., 2006; Konishi and Setou, 2009). Indeed, polarized
22 distribution of Kif5 in neuronal populations cannot be fully accounted for by distribution
23 patterns of detyrosinated tubulin and occurs at a developmental stage preceding any
24 polarized distribution of detyrosination (Hammond et al., 2010; Nakata et al., 2011).
25 Furthermore, in COS cells (Cai et al., 2009) much of Kif5's movement occurs on tyrosinated
26 tubulin MTs.
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40 Total but not partial loss of polyglutamylation of at least β -tubulin inhibits Kif5 MT
41 binding, but a similar inhibition also occurs when *increasing* glutamyl chain length past three
42 units (Larcher et al., 1996; Reed et al., 2006). A negative correlation has been reported
43 between tubulin polyglutamylation and the motility of Kif5 cargo adaptor gephyrin when
44 neuronal activity is enhanced in cultures via glycine receptor blockade (Maas et al., 2009).
45 However, as this effect was found to be cargo-specific, with motility of Kif5 cargo adaptor
46 GRIP-1 unaffected by the same treatment, modulation of the interaction between the MT
47 track and the Kif5 motor domain cannot explain this phenomenon. Interestingly, severe loss
48 of the majority of α -tubulin-polyglutamylation with additional partial loss of β -tubulin-
49 polyglutamylation *in vivo* and *ex vivo* has no obvious effect on Kif5 distribution; instead it
50 specifically prevents the normal motility of the Kif1A motor into all neurites (Ikegami et al.,
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3 2007). Knockdown of the β -tubulin glutamylation enzyme TLL7 inhibits outgrowth of all
4 neurites in PC12 cells (Ikegami et al., 2006), and it is possible this could be linked to
5 disruption of kinesin motility. Most brain tubulin is polyglutamylated (Audebert et al., 1994)
6 and this type of PTM is not obviously localized to particular parts of the neuron *per se*,
7 although as the available GT335 glutamylated tubulin antibody does not differentiate
8 between α - and β -tubulin modification, or chain lengths longer than a single unit, it is
9 possible these factors vary between different sub-domains (Bonnet et al., 2001; Hammond
10 et al., 2010; Ikegami et al., 2006). However, in non-proliferating non-neuronal cell lines
11 polyglutamylation levels are very low (Bobinnec et al., 1998), but Kif5 still travels along
12 select MTs (Cai et al., 2009). All considered there is as yet no clear evidence that poly-
13 glutamylation acts as a directional cue for Kifs, but it could be important in providing a
14 suitable general track for some motors such as Kif1A (Ikegami et al., 2007; Ikegami et al.,
15 2006), modulating transport dynamics with age (Audebert et al., 1994), or neuronal activity-
16 dependent regulation of some kinesins (Maas et al., 2009).

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Tubulin PTMs can accumulate cooperatively, such that manipulation of one PTM can
produce off-target effects in others, confounding a straightforward interpretation of
measured phenotypes. For example in the report from Ikegami and colleagues (2007)
genetically targeted loss of α -tubulin polyglutamylated in mice also produces alterations in
tyrosination and β -tubulin polyglutamylated. Although they have received little attention to
date, other tubulin PTMs such as polyglycation, phosphorylation, palmitoylation (Fukushima
et al., 2009) and ADP-ribosylation (Kaslow et al., 1981) could also influence kinesin motility.
While most of the research on MT PTM influence on Kif distribution and localization has
focused on Kif5, the distribution of other kinesin superfamily members may be more or less
influenced by different MT PTMs. For example, although Ikegami and colleagues (2007)
showed that the affinity of Kif5, Kif1A and Kif3A for MTs from mice deficient in α -tubulin
polyglutamylated was reduced, only Kif1A was abnormally localized as a result. In COS cells,
only Kif5 but not Kif17 or Kif1A move preferentially on acetylated and/or detyrosinated MTs
(Cai et al., 2009). An additional source of MT track variation is the range of α - and β - tubulin
isotypes (6 or more genes for each in vertebrates) expressed in different cell types, with
each isotype varying chiefly in the C-terminal region (Fukushima et al., 2009) and therefore
being more or less subject to different combinations of PTMs (Tischfield and Engle, 2010). It

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3 should also be noted that experiments *in vitro* using brain tubulin will typically contain a
4 heterogeneous mixture of tubulin isoforms and PTMs, which is likely to complicate
5 mechanistic interpretations.
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9 Taken together, evidence for a role for tubulin PTMs alone in controlling polarised
10 trafficking is far from conclusive and, at a minimum, it seems very unlikely that a single MT
11 modification can account for all regulated distribution of Kifs. It is more likely that tubulin
12 PTMs act in concert together and with other co-factors (Hammond et al., 2010; Walter et
13 al., 2012). As discussed, diverse mechanisms appear to control differing MT selectivity and
14 cellular distribution of the different Kif family members. Particularly in neurons, structural
15 MAPs are especially abundant and compartmentalised and tubulin PTMs may alter Kif
16 selectivity by altering MT-MAP interactions. Indeed, there are numerous examples of the
17 distribution, or MT interactions, of structural MAPs including MAP1, MAP2 and tau being
18 altered by tubulin PTMs (Bonnet et al., 2001; Boucher et al., 1994; Ikegami et al., 2007;
19 Konishi and Setou, 2009; Larcher et al., 1996; Saragoni et al., 2000; Spiliotis et al., 2008).
20 Conversely, the binding of MAPs to MTs could act upstream to directly or indirectly
21 influence their PTM state; for example, tau binding has been found to positively regulate
22 tubulin acetylation (Perez et al., 2009; Takemura et al., 1992). Compartmentalized MAPs
23 could act indirectly to alter accumulation of MT PTMs and both could thus contribute to
24 selective kinesin distribution. Many MAPs can function to stabilize MTs (Amos and Schlieper,
25 2005) and tubulin PTMs appear to be consequences rather than modulators of increased
26 MT stability (Khawaja et al., 1988; Palazzo et al., 2003; Paturle-Lafanechere et al., 1994).
27 MAPs could thus play a critical role to promote local 'tagging' of MTs with specific PTMs.
28 However, MAPs also contribute directly to the spatio-temporal localization of specific Kif
29 motors during mitosis (Bieling et al., 2010; Ye et al., 2011) and the regulation of Kif
30 distribution by MAPs in neuronal cells independently of tubulin PTMs will be discussed
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50 51 **Regulation of Kifs by the tubulin GTPase cycle**

52 The dynamic properties of MTs arise from the intrinsic GTPase of $\alpha\beta$ -tubulin; in
53 solutions of pure tubulin, MTs undergo stochastic switching between phases of growth \square
54 driven by addition of GTP-bound tubulin \square and shrinkage \square driven by GTP hydrolysis within
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3 the lattice (Desai and Mitchison, 1997). *In vivo*, these dynamics are tightly regulated
4 according to cell activity and have recently been shown to be structurally coupled to the
5 activities of plus-end tracking proteins (+TIPs; Akhmanova and Steinmetz, 2008;
6 Montenegro Gouveia et al., 2010). +TIPs cluster at the plus-ends of growing MTs and control
7 local dynamics and subcellular targeting of MT ends. End Binding proteins (EBs) are the
8 master regulators of these dynamic protein hubs and localise to growing MT ends by
9 recognising a conformation of tubulin that depends on GTP hydrolysis within the MT lattice
10 (Maurer et al., 2011; Maurer et al., 2012).

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18 EBs are involved in setting up MT polarity in neurons, and have also been used as
19 experimental tools to visualise neuronal MT plus ends and, thereby, polarity (e.g. Mattie et
20 al., 2010; Stone et al., 2008). However, it has been broadly assumed that 1) MT tracks are
21 specifically stabilised to support long-range cargo and 2) they are composed of GDP-tubulin
22 because GTP hydrolysis in the lattice has already occurred. The assumption of homogeneity
23 with respect to tubulin-bound nucleotide was challenged during the characterisation of an
24 antibody (hMB11) raised against tubulin bound to the non-hydrolysable GTP analogue
25 GTP γ S (Dimitrov et al., 2008). Using this antibody, small islands along cellular MTs were
26 labelled, suggesting that microtubule GTPase is biochemically heterogeneous in cells, or at
27 least the conformational consequences of tubulin GTPase are (Kueh and Mitchison, 2009).
28 Dimitrov et al initially proposed that such “GTP islands” would promote MT rescue (recovery
29 from MT shrinkage to growth) at functionally important sites in the cell. However, an
30 additional role for these GTP islands has recently been suggested to contribute to Kif5’s
31 polarised axonal localisation (Nakata et al., 2011). hMB11 showed preferential
32 immunolocalisation to the axon and colocalised with Kif5 but not Kif1A motors. In addition,
33 fluorescently tagged Kif5 showed preferential binding to GMPCPP-MTs (a very slowly
34 hydrolysable GTP analogue) as opposed to GDP-MTs *in vitro*, supporting a nucleotide-based
35 mechanism for the *in vivo* binding pattern. Recognition of the underlying MT nucleotide
36 state is also indicated by previous studies that showed that Kif5-driven GMPCPP-MTs move
37 roughly 34% faster than paclitaxel-stabilized GDP-MTs using *in vitro* multi-motor gliding
38 assays, without alteration of Kif’s ATPase rate (Vale et al., 1994), although not all reports
39 support this finding (Moores et al., 2006). The basis of such recognition of – and preferential
40 binding to – “GTP-like” MTs is specified by Kif5’s loop 11 region at its MT binding interface
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3 (Figure 2) (Nakata et al., 2011). A sub-nanometer resolution cryo-EM reconstruction of
4 GMPCPP-MTs (Yajima et al., 2012) suggests that Kif5 loop11 is well positioned to sense
5 nucleotide-dependent conformational changes within the MT (Figure 2). Interestingly, these
6 GTPase-related conformational changes in tubulin are similar to those recognised by EBs,
7 even though the primary MT binding sites for these unrelated families of proteins are very
8 different (Maurer et al., 2012).
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14 The tubulin islands recognised by hMB11 are rather sparsely distributed along axons,
15 suggesting that polarisation of neuronal transport is unlikely to be solely determined by the
16 guanine nucleotide state of MTs. In addition, recent experimental evidence from *in vitro*
17 single molecule imaging shows that Kif5 has *shorter* run lengths and unaltered velocity on
18 GMPCPP-MTs compared to paclitaxel-stabilized GDP-MTs (McVicker et al., 2011). In non-
19 neuronal cells Kif5 travels preferentially on sub-populations of MTs, consistent with the
20 existence of distinctive cues on particular MTs (Cai et al., 2009; Dunn et al., 2008), whereas
21 there are no obvious *inter*-MT differences in GTP-tubulin content, but rather only *intra*-MT
22 discontinuities in GTP-tubulin content along the MT length (Dimitrov et al., 2008). However,
23 structural signals arising within the MT lattice due to GTP islands may be amplified by other
24 factors, including tubulin PTMs (in particular Lys-40 α -tubulin acetylation; Yajima et al.,
25 2012) and selective recruitment of MAPs.
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36 **The Tau/MAP2 family; obstacles to Kifs?**

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39 Tau and MAP2 are homologous MT binding proteins that are abundant in neurons.
40 These MAPs present themselves as particular targets for study in the context of polarised
41 neuronal transport because tau isoforms are predominantly associated with axonal MTs in
42 mature neurons, while MAP2 is restricted to the dendritic compartment (Binder et al., 1985;
43 Deshpande et al., 2008). Tau and MAP2 are very similar in their MT binding domain but
44 differ in their extended “projection domains” which protrude from the MT surface. Tau and
45 MAP2 are highly regulated by phosphorylation at a large number of phosphorylation sites
46 (at least 63 identified; Hanger et al., 2009) inside and outside of the MT binding repeat
47 region, which, amongst other things, can modulate MT association (Figure 3).
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56 Numerous studies have demonstrated that over-expression of tau causes inhibition
57 of Kif5 motility and alters the subcellular distribution of its cargo (Dubey et al., 2008; Ebneth
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3 et al., 1998; Mandelkow et al., 2003; Stamer et al., 2002; Stoothoff et al., 2009; Trinczek et
4 al., 1999). Thus, in primary neuronal cultures or neuronal cell lines, when tau is over-
5 expressed and becomes abnormally distributed, Kif5s and their cargo accumulate in the
6 soma and fail to enter any processes. When added in non-physiological excess to MTs *in*
7 *vitro*, tau - and to a greater extent MAP2 - inhibit the motility of Kif5 motors and associated
8 cargo along MTs (Hagiwara et al., 1994; Heins et al., 1991; Lopez and Sheetz, 1993; Seitz et
9 al., 2002). At sub-saturating concentrations of MAPs, the effects on Kif5 transport is harder
10 to pin down: some constructs exhibit inhibitory effects on motor attachment and run length
11 (Dixit et al., 2008; McVicker et al., 2011; Seitz et al., 2002), whereas others do not inhibit
12 motility at all (Hagiwara et al., 1994; Seitz et al., 2002; von Massow et al., 1989). In fact,
13 enhancements of single molecule Kif5 run length and attachment frequency by some tau
14 constructs has also been reported (Lopez and Sheetz, 1993; Seitz et al., 2002). Whilst there
15 is a general consensus that velocity is not grossly affected once motors are in motion,
16 contrasting evidence has been collected on whether motility inhibition occurs because of
17 shortening of motor run length by tau or reducing motor attachment events (Seitz et al.,
18 2002; Trinczek et al., 1999). The inhibitory effects of tau on long-distance transport may be
19 particularly acute in cargo carried by multiple motors, the most likely physiologically
20 relevant scenario. For example, Vershinin et al demonstrated that, whereas multiple motors
21 can increase the processivity of a cargo along undecorated MTs by an order of magnitude
22 compared to a single Kif5 motor, tau (especially 3R tau) significantly inhibited this
23 processivity enhancement (Vershinin et al., 2007). Even at low tau/tubulin ratios, tau can
24 reduce multi-kinesin-dependent cargo run length by inhibiting the motor reattachment that
25 is required for processivity enhancement. When only single motors were engaged in motor
26 transport, the inhibitory effect of tau on run length was much reduced. Unsurprisingly,
27 therefore, there is no consensus concerning the molecular mechanism(s) for effects of
28 MAP2/tau on kinesin transport, and they have been variably attributed to: 1) direct
29 competition between motor and tau/MAP binding to the MT (Ebnet et al., 1998; Trinczek
30 et al., 1999); 2) resistance to motor movement by the negatively charged projection
31 domains protruding from the MT (Hagiwara et al., 1994; Lopez and Sheetz, 1993); 3)
32 blockage of motility that is independent of high affinity MT binding (Seitz et al., 2002;
33 Vershinin et al., 2007), possibly due to protein aggregation, either non-specific or by
34 filament formation (Ackmann et al., 2000; Duan and Goodson, 2012; Santarella et al., 2004);
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3 4) disruption of the Kif-cargo interaction by tau (Kanaan et al., 2011; LaPointe et al., 2009;
4 Morfini et al., 2007).

7 Two different structural models propose that tau/MAP2 binds either to the luminal
8 surface of MTs (Kar et al., 2003) or the tubulin dimer ridges on the outside of MTs (Al-
9 Bassam et al., 2002). However, the apparently contradictory results can be explained by the
10 experimental conditions of MT assembly used in each study: where paclitaxel is used to
11 stabilize MTs prior to MAP addition, tau binds to the external tubulin ridges of MTs at a site
12 overlapping that of kinesin (Al-Bassam et al., 2002), whereas when MTs are co-assembled
13 with tau without paclitaxel, but rather with the presence of non-hydrolysable GTP analogue
14 GMPCPP, tau binds to the inside lumen of MTs on β -tubulin at a binding site at least partly
15 overlapping that of paclitaxel (Kar et al., 2003). The majority of *in vitro* Kif motility
16 interference studies using tau have utilized pre-formed MTs stabilized with paclitaxel prior
17 to addition of Kif, supporting the interpretation of direct binding competition between Kif
18 and MAP2/tau on the MT surface in these assays, also reinforced by more recent work
19 (McVicker et al., 2011; Peck et al., 2011). One simple conclusion from these findings is that
20 data from experiments relying on MT stabilisation with paclitaxel are artifactual due to
21 paclitaxel blockage of tau's native binding site. However, since GMPCPP-stabilised MTs, are
22 mimics of a GTP-like state, they are also not representative of the majority of cellular MTs
23 which are composed of GDP-tubulin. Instead, it now seems more likely that tau binding to
24 tubulin/MTs is modulated by the nucleotide state of tubulin, which also has an impact on Kif
25 motility. Thus, tau has no effect on Kif transport when paclitaxel and GMPCPP are used
26 together, while it is inhibitory when MTs are stabilized in paclitaxel free, glycerol- and
27 DMSO-containing conditions (McVicker et al., 2011; Peck et al., 2011). That the nucleotide
28 state of tubulin, rather than the presence or absence of bound paclitaxel, modulates the
29 nature of tau binding and Kif motility is consistent with further cryo-EM (Santarella et al.,
30 2004) and binding kinetics (Makrides et al., 2004) studies. Although not yet demonstrated, it
31 is likely that MT binding by other tau family members including MAP2 is also influenced by
32 the nucleotide bound state of the MT, due to high homology in the MT binding domains. In
33 neurons, the MT guanine nucleotide state appears to be somewhat polarized, with higher
34 levels of GTP tubulin in the axon (Nakata et al., 2011); it is possible - though no proven ☐

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3 that this contributes to axonal localisation of tau and that these effects together
4 synergistically contribute to compartmentalized distribution of Kifs.
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8 Whether tau has a major modulatory role in guiding kinesin distribution *in vivo* has
9 been called into question by the observation that tau (Harada et al., 1994) or MAP2 (Teng et
10 al., 2001) knockout mice have no major phenotype. Pathological aggregation and
11 hyperphosphorylation of tau especially in older overexpressing/mutant mice complicates
12 physiological interpretations of tau modulation of motor behaviour (Adams et al., 2009; Duff
13 and Suleman, 2004; Polydoro et al., 2009; Terwel et al., 2005). However, no observable
14 disruption of Kif-based transport in optic axons was reported in tau knockout mice, or in 3-
15 18 month old mice over-expressing human tau (Yuan et al., 2008), or in the *C. elegans*
16 knockout of tau homologue PTL-1 (Tien et al., 2011). It is possible that MAP1b compensates
17 for the absence of tau or MAP2 in these cases because double knockout MAP2/MAP1b or
18 tau/MAP1b mice exhibit abnormal neuronal development, morphology and function (Takei
19 et al., 2000; Teng et al., 2001). However, association of these gross phenotypes to specific
20 alterations of Kif behaviour is premature, especially considering MAP1b and tau share low
21 structural similarity (Amos and Schlieper, 2005) and produce different effects on
22 anterograde transport when depleted from cultured neurons (Jimenez-Mateos et al., 2006).
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35 In studies of the non-neuronal Ltk cell line, over-expression of full-length MAP4 (a
36 non-neuronal MAP related to MAP2/tau) or a truncated construct containing only the MT
37 binding repeats was found to reduce the motility of vesicle transport (Bulinski et al., 1997),
38 and slow the general speed of the cell cycle (Nguyen et al., 1997), consistent with MAP4
39 interference with kinesin motility. Later *in vitro* gliding assays found that high
40 concentrations of the 5, but not 4 or 3 MT binding repeat isoform of MAP4 inhibited MT
41 gliding on Kif5, with increases in MT pausing and decreased MT run lengths. This effect was
42 seen without MAP4 competing with Kif5 for MT binding by co-sedimentation assay.
43 However, particularly high non-physiological saturating concentrations of MAP4 were
44 required in this study for any clear effect on MT motility. Unfortunately, much like the
45 literature on tau and MAP2, it is hard to draw conclusions about any normal *in vivo* role of
46 MAP4 in regulation of Kifs from experiments using non-physiologically high MAP4
47 concentrations *in vitro* and *ex vivo*. However, considering some of the more recent work on
48 tau's sensitivity to tubulin-bound nucleotide (McVicker et al., 2011; Peck et al., 2011) and its
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3 tendency to polymerise when bound to MTs (Duan and Goodson, 2012), more
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5 physiologically orientated experiments on MAP4 are warranted.
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8 In summary, a lack of clear conclusion from the above meta-analysis of *in vitro* and
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10 *ex vivo* work combined with the *in vivo* evidence from transgenic mice suggests that it is
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12 unlikely that MAP2, tau and their relatives have direct, prominent and/or specific
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14 physiological roles in controlling polarized transport of kinesin motors. For example, whilst
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16 polarized transport of Kif5 is one of the initial events in axonal specification, neuronal
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18 polarization of tau and MAP2 localizations occur later in development (Boyne et al., 1995;
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20 Deshpande et al., 2008; Jacobson et al., 2006; Mandell and Banker, 1996; Nakata and
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22 Hirokawa, 2003), and neither are essential for Kif5 polarization (Hammond et al., 2010;
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24 Nakata et al., 2011) or axonal specification (DiTella et al., 1996; Harada et al., 1994; Takei et
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26 al., 2000). Furthermore, Kif5 itself appears to be involved in the polarized transport of tau
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28 (Utton et al., 2005). It is worth noting however that tau family MAPs may act alongside
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30 other MAPs in combinatorial fashion and in response to signals, such that modulation of Kifs
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32 may only become prominent when a number of MAPs collaborate. For example, MARK
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34 kinase activation, which may detach many MAPs from MTs simultaneously (Drewes et al.,
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36 1998; Schaar et al., 2004) has enhancing effects on Kif5 based cargo transport (Mandelkow
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38 et al., 2003; Mandelkow et al., 2004).

39 **DCX/DCLK1 are required for proper distribution of Kif1A and associated cargo**

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41 Doublecortin (DCX) and its close homologue doublecortin-like kinase-1 (DCLK1)
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43 contain tandem MT binding 'DC' domains, named N-DC (N-terminal) and C-DC (C-terminal)
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45 (Sapir et al., 2000; Taylor et al., 2000), as well as a serine/proline-rich C-terminal domain
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47 and, in the case of DCLK1, a kinase domain (Figure 3). The DC domains have a β -grasp-like
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49 fold (Cierpicki et al., 2006; Kim et al., 2003) and thus the MT binding domains of DCX and its
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51 homologues show no similarity to those of the tau/MAP2 family. 11 members of the DCX
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53 family - defined by the presence of at least one DC domain – have now been identified in
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55 humans, along with various splicing isoforms (Dijkmans et al., 2010; Reiner et al., 2006).
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57 Mutations in the *DCX* gene, many of which cluster within the DC domains (Quelin et al.,
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59 2012; Sapir et al., 2000; Taylor et al., 2000), lead to disorders of abnormal brain
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development such as lissencephaly, with disrupted cortical layering associated with

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3 perturbed neuronal migration (des Portes et al., 1998a; des Portes et al., 1998b; Gleeson et
4 al., 1998; Sossey-Alaoui et al., 1998). In mice, DCX knockout causes only subtle brain
5 abnormalities, whereas the additional knockout of DCLK1 produces severe disruption of
6 cortical layering phenotypes resembling the human disease. This is likely due to functional
7 redundancy between the proteins in mice (Corbo et al., 2002; Deuel et al., 2006; Kappeler et
8 al., 2006; Koizumi et al., 2006; Nosten-Bertrand et al., 2008). DCX is expressed
9 predominantly during brain development in migrating neurons, localizing particularly to the
10 extremities of neuronal processes (Tint et al., 2009). Both DCX and DCLK1 bind and stabilise
11 MTs, likely through a shared mechanism that prevents MT depolymerisation; *in vitro*, DCX
12 also promotes MT nucleation independently of the nucleotide bound to tubulin (Bielas et
13 al., 2007; Burgess and Reiner, 2000; Fourniol et al., 2010; Francis et al., 1999; Gleeson et al.,
14 1999; Horesh et al., 1999; Lin et al., 2000; Moores et al., 2004; Moores et al., 2006;
15 Vreugdenhil et al., 2007). The N-DC domain of DCX binds to the pockets between
16 protofilaments at the corners of 4 $\alpha\beta$ -tubulin dimers, enabling the selective stabilization of
17 13 protofilament MTs, which is the main MT architecture *in vivo* (Bechstedt and Brouhard,
18 2012; Fourniol et al., 2010; Liu et al., 2012; Moores et al., 2004; Tilney et al., 1973).

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32 In addition to their roles in MT stabilisation, there is emerging evidence that DCX and
33 DCLK are involved in regulation of Kif-dependent cargo distribution. Deuel and colleagues
34 (2006) described phenotypes in double DCLK and DCX knockout mice that, in addition to
35 stunted neuronal migration and axonal development, showed disrupted distribution of the
36 synaptic vesicle proteins VAMP2 and synaptophysin. In dissociated hippocampal cultures of
37 DCLK^{-/-} neurons RNAi depleted of DCX, VAMP2 and synaptophysin failed to enter axons,
38 instead being retained in the cell body, supporting a defect in synaptic vesicle distribution.
39 Average axon length in either cultured DCLK^{-/-} neurons, or WT neurons treated with DCX
40 RNAi, is shorter than in wild-type non-transfected neurons, while DCLK^{-/-} neurons with DCX
41 RNAi have even shorter axons. As vesicle transport appears to be involved in neurite
42 outgrowth and axonal growth cone-led neuronal migration (Kimura et al., 2003; Shirasu et
43 al., 2000), this study suggested that, in addition to disruption of neuronal MTs themselves,
44 human *DCX*-linked neuronal migration disorders could also result from perturbed cargo
45 distribution.

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3 As part of our efforts to characterise DCX-stabilised MTs *in vitro*, we used an
4 ensemble MT gliding assay with Kif5 to compare the velocity of DCX-stabilized MTs with
5 paclitaxel-stabilized GDP-MTs or GMPCPP-stabilized MTs. Robust motility was seen on all
6 MT populations. However, a small (~16%) but significant reduction in velocity of DCX-MTs
7 was observed (Moore et al., 2006). It is not clear, however, how this finding relates to the
8 *in vivo* observations described above, since although Kif5 may carry some VAMP2-laden
9 vesicles (Song et al., 2009), Kif1A appears primarily responsible for their transport (Liu et al.,
10 2012; Okada and Hirokawa, 1999; Yonekawa et al., 1998). More recent work has further
11 emphasised the differential effects by DCX/DCLK on kinesin motors and their cargo: in DCLK-
12 /- deficient mouse neurons transfected with DCX shRNAi, Kif5 and its mitochondrial cargo
13 show a normal distribution, while VAMP2 positive cargo is mislocalised within the soma (Liu
14 et al., 2012). Time-lapse fluorescence imaging showed that this abnormal localization was
15 coupled with shorter cargo run-lengths and a smaller number of mobile VAMP2 vesicles,
16 although when mobile, they moved at wild-type velocities. No alterations in common
17 tubulin PTMs or the localization of other MAPs were observed, and the abnormal transport
18 and localization of Kif1A/VAMP2 could be rescued by transfection of a shRNAi-resistant WT -
19 but not with lissencephaly-mutant containing - DCX constructs, verifying that DCX/DCLK are
20 likely direct and specific regulators of Kif1A motors. The *in vivo* interaction of Kif1A and DCX
21 was confirmed biochemically in fetal human brain and mouse brain, and in further support
22 of this functional link, in cortical slice cultures Kif1A knockdown resulted in neuronal
23 migration disruptions that phenocopy DCX/DCLK1 deficiency.

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26 From a structural perspective, only cryo-EM reconstructions of DCX-MTs with and
27 without bound Kif5 MD are currently available (Fourniol et al., 2010; Liu et al., 2012), but
28 these structures already provide two major mechanistic insights: 1) the conformation of MT-
29 bound DCX responds to the presence or absence of bound motor, specifically in the linkers
30 on either side of N-DC - the rest of the DCX molecule is not visible in these structures - and
31 2) there is direct interaction between these conformationally variable linkers within DCX
32 and the bound motor, in loop regions of the motor domain which are different between Kif5
33 and Kif1A. Taken together, these data provide clear evidence for facilitation of Kif1A-cargo
34 transport by DCX/DCLK, they suggest molecular mechanisms by which this could occur, and
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3 highlight that in addition to abnormalities due to altered MT dynamics and organisation,
4 DCX-linked pathologies may stem from cellular transport deficits.
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7 **Enscosin/MAP7 promotes proper Kif5 distribution**

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10 In 2008 the *Drosophila* MAP enscosin (mammalian MAP7 or E-MAP-115) was shown
11 to be integral for Kif5 dependent processes in polarized oocytes and neurons, without being
12 required for regulating overall MT architecture (Sung et al., 2008). Fly lines expressing
13 enscosin loss-of-function mutants exhibit abnormal oocyte development which was
14 associated with improper distribution of Kif5, its *oskar* mRNA cargo and associated Stauf
15 protein. In the neurons of these flies, abnormal ectopic distribution specifically of the Kif5
16 cargo synaptotagmin was also observed, diagnostic of its impaired anterograde transport.
17 Using *in vitro* single motor assays in concentrated ovary extracts, the MT association rate
18 and mobility of full length Kif5 were reduced in enscosin mutant extracts; however, this
19 was not the case for the truncated tail-less constitutively active Kif5 dimeric motor. This
20 mechanism is under tight regulation by the MAP and enscosin kinase PAR-1/MARK,
21 indicating that further dynamic control of Kif5 can be achieved through alteration of
22 enscosin phosphorylation state.
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34 More recently, using genetic mutants and RNA interference, enscosin/MAP7 and
35 Kif5B were identified as together being essential for proper nuclear positioning in
36 *Drosophila* and cultured mammalian myotubes during muscle development (Metzger et al.,
37 2012). The related MAPs MAP7D1, MAP7D2 or MAP7D3 on the other hand were not
38 important in this process, indicating the specificity of this regulatory function of
39 MAP7/enscosin. Importantly, a physical interaction of the C-terminal coiled-coil region of
40 MAP7 and the Kif5B tail was observed by co-immunoprecipitation, confirming previous
41 evidence that MAP7/enscosin-mediated regulation of Kif5 occurs at the motor tail. This
42 work and a more recent study confirming the importance of enscosin to Kif5 activation
43 (Barlan et al., 2013) provide an enticing example of a MAP interacting with the tail domain,
44 rather than motor domain, of Kif5. This interaction appears to control Kif5's specific
45 recruitment, activation and thus its transport, likely through modulation of its auto-
46 inhibitory capacity. In fact, Kif5 regulation can occur independently of enscosin MT binding
47 (i.e. its MAP activity). Further investigation of this interaction within a mammalian context is
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3 warranted: although ensconsin/MAP7 may not function as a physiological regulator of MT
4 dynamics, (Faire et al., 1999), its distribution is compartmentalized and somewhat polarized
5 in neurons, and it is modulated by conditional phosphorylation (Bulinski et al., 2001; Sung et
6 al., 2008), all of which are factors that are suggestive of a role for this MAP as a molecular
7 signpost for Kifs. That this regulation may occur via the Kif5 tail also suggests that MAPs
8 could also help manage motor activation and cargo loading to a specific Kif on a particular
9 track.
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15 16 **Diseases associated with Kif distribution defects**

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18 Disrupted kinesin-based cargo transport is strongly implicated in the pathogenesis of
19 a wide variety of conditions, including cancers, familial developmental abnormalities and
20 various forms of neurodegenerative disease (De Vos et al., 2008; Liu et al., 2012; Yu and
21 Feng, 2010). Whereas a number of conditions stem from familial mutations in the Kifs
22 themselves - such as some hereditary spastic paraplegias (Kawaguchi, 2012; Klebe et al.,
23 2012) – some of the remaining conditions may exhibit disrupted Kif-based transport due to
24 perturbed MAP regulation of Kifs. Several of the most prominent examples are discussed
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33 *Alzheimer's Disease and the 'tauopathies'*

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36 Alzheimer's Disease (AD) is a prevalent neurodegenerative condition classically
37 associated with high levels of aggregated A β peptides (resulting from mutations in the
38 familial AD gene *APP* or unknown sporadic reasons) and abnormal phosphorylation,
39 localization and aggregation of tau (Hardy, 2009; Iqbal et al., 2010; Small and Duff, 2008).
40 Similar tau pathology, in the absence of A β pathology, is observed in the related
41 'tauopathies', in some cases due to mutations in the *MAPT* tau encoding gene itself (Hanger
42 et al., 2009; Iqbal et al., 2010). In AD or tauopathy, transgenic mouse models or A β treated
43 cells, disrupted axonal transport and an abnormal distribution of essential cargo such as
44 mitochondria is observed, consistent with axonopathy seen in these model mice and post-
45 mortem brain (De Vos et al., 2008; Stokin et al., 2005). Interestingly, AD models exhibit
46 disrupted Kif5 mediated transport of APP perhaps contributing to the aberrant processing of
47 APP that is associated with A β aggregation (Kamal et al., 2000; Lazarov et al., 2007; Stokin et
48 al., 2005; Thinakaran and Koo, 2008).
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3 Disruption to some of the regulatory mechanisms discussed above could contribute
4 to transport defects in AD and related tauopathies. For example, there is strong evidence
5 that in AD, tau becomes detached from MTs and as a result, is abnormally mislocalized and
6 sequestered in hyperphosphorylated aggregates (Iqbal et al., 2010). Indeed, A β appears to
7 cause the early phosphorylation of tau, causing MT dissociation (Amadoro et al., 2011;
8 Reifert et al., 2011) and redistribution of tau from the axon to the somadendritic
9 compartment in cultured neurons (Zempel et al., 2010). However, there is a continuing lack
10 of consensus concerning the consequences of such tau redistribution for neuronal
11 transport. Evidence that MT-bound tau at *physiological* concentrations facilitates Kif motility
12 (Lopez and Sheetz, 1993; Peck et al., 2011; Seitz et al., 2002) suggests a 'loss of function'
13 hypothesis for abnormal distribution of motors and cargo via tau hyperphosphorylation in
14 AD affected neurons. Alternatively, high concentrations of tau on the MT - perhaps in an
15 aggregated state - could inhibit Kif motility and perturb intracellular transport in AD
16 (Ackmann et al., 2000; Baas and Qiang, 2005; Duan and Goodson, 2012; Mandelkow et al.,
17 2003; Santarella et al., 2004). There is some evidence that total tau levels increase in AD,
18 but this appears to correspond to an increase in clearance-resistant aggregated
19 hyperphosphorylated forms, rather than an increase in newly produced soluble cytosolic
20 tau, as judged by concentrations of mRNA (Hyman et al., 2005; Iqbal et al., 2010; Khatoon et
21 al., 1992). Also in conflict with the 'roadblock' hypothesis: 1) tau extracted from AD brain
22 has poor MT binding capacity and sequesters normal tau and other MAPs (Iqbal et al., 2008;
23 Iqbal et al., 2010), 2) tau phosphorylation at sites detaching it from MTs appear critical for
24 A β -mediated toxicity (Amadoro et al., 2011; Drewes, 2004; Iijima et al., 2010) and, 3) over-
25 expression of tau appears not to affect axonal transport *in vivo* (Yuan et al., 2008).
26 Furthermore, tau is in fact lost from axons and redistributed to the somato-dendritic
27 compartment, implying that other, axonally-localised factors are responsible for the *reduced*
28 axonal transport rates found in AD and the tauopathies (Ittner and Gotz, 2011; Kowall and
29 Kosik, 1987; Zempel et al., 2010).
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51 Despite these contradictions, it is possible that both 'loss of function' and 'roadblock'
52 mechanisms could contribute to AD and the tauopathies, perhaps at different stages. For
53 example, tau phosphorylation could be a protective response to remove an initial
54 'roadblock' insult caused by excessive tau MT binding. Eventually however, aggregation and
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3 hyperphosphorylation of tau may cause its sequestration away from MTs and a 'loss of
4 function' of Kif modulation. Other modulators of neuronal MTs and motors may
5 subsequently be segregated in these aggregates: for example the human growth arrest-
6 specific 7b protein (hGas7b) is highly expressed in brain tissues (Ju et al., 1998) and binds
7 MTs, having independent and co-operative effects with tau on MT dynamics (Hidaka et al.,
8 2012; Uchida et al., 2009). Interestingly, hGas7b binds to tau in a phosphorylation-
9 dependent manner and there is a striking reduction in levels of this protein in AD brain
10 (Akiyama et al., 2009) that could also contribute to abnormal motor/cargo transport and
11 distribution. Even in the same cell, redistribution of tau could lead to simultaneous loss of
12 function and aggregation in different compartments. In addition, transport dysfunction
13 could be a consequence of disruption to the MT track itself due to tau's effects on MT
14 dynamics and stability. Alternatively, transport may be affected by misplaced, aggregated
15 and/or fragmented forms of tau independently of its MT binding as has been suggested, for
16 example through activation of kinase pathways which act on Kifs (Kanaan et al., 2011), or as
17 a downstream consequence of other speculated toxic 'gain-of-function' mechanisms of
18 misplaced (Ittner and Gotz, 2011) or oligomeric tau species (Congdon and Duff, 2008).

31 *Diseases related to DC domain containing proteins*

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35 Mutations in the X-linked *DCX* gene lead to subcortical band heterotopia or 'double
36 cortex' syndrome in females, and lissencephaly or 'smooth brain' syndrome in males,
37 associated with the abnormal migration of neurons leading to disrupted cortical layering
38 and abnormal hippocampal development (des Portes et al., 1998a; des Portes et al., 1998b;
39 Francis et al., 1999; Gleeson et al., 1998). As discussed, recent evidence has suggested that
40 disease causing mutations result in at least in part in the loss of function of DCX as a
41 specific promoter of Kif1A transport, and improper distribution of its synaptic cargos (Deuel
42 et al., 2006; Liu et al., 2012). That DCX-related disruptions to neuronal migration may be
43 linked to improper Kif1A distribution is supported by the importance of DCX, Kif1A and
44 vesicular synaptic cargos such as VAMP2 in neurite outgrowth and nuclear migration
45 processes (Bai et al., 2003; Hepp and Langley, 2001; Kimura et al., 2003; Koizumi et al.,
46 2006; Shirasu et al., 2000; Tsai et al., 2010). Interestingly, DCX deficiencies have recently
47 been implicated in schizophrenia, which may also be associated with abnormal neuronal
48 migration (Eastwood et al., 1995; Fung et al., 2011).

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3 Mutations in the locus of another member of the DCX family, Retinitis Pigmentosa 1
4 (RP1), have been shown to account for roughly 5-10% of all autosomal dominant Retinitis
5 Pigmentosas (Pagon and Daiger, 1993), a form of inherited blindness associated with retinal
6 degeneration. RP1 contains N-terminal tandem DC domains much like DCX and associates
7 with MTs and promotes their assembly and stability. However, it is expressed specifically in
8 the retina where it associates with the MT-rich connecting cilium and outer segment
9 axoneme of photoreceptors, promoting proper outer segment disc structure (Liu et al.,
10 2003; Liu et al., 2002; Liu et al., 2004). It is possible that RP1 has some regulatory role on Kifs
11 here but further work is needed to clarify this (Omori et al., 2010).
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20 The gene locus for DCX family member doublecortin domain containing 2 (DCDC2),
21 which contains tandem DC domains, has been linked to developmental dyslexia specifically
22 affecting reading ability (Meng et al., 2005). Interestingly, dyslexia may result from a subtle
23 defect in neuronal migration and DCDC2, in a manner somewhat similar to DCX, is important
24 for this process in neuronal subpopulations migrating to brain regions important for reading
25 ability (Burbridge et al., 2008; Galaburda et al., 2006; Meng et al., 2005). Importantly,
26 DCDC2 co-localizes with MT rich structures such as the primary cilium in neurons, co-
27 localizes and interacts with Kif3A, and modulates Wnt and Shh signalling possibly through its
28 interaction with Kif3A (Massinen et al., 2011), however a direct effect of DCDC2 on Kif3A
29 movement is yet to be demonstrated.
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38 **A 'MAP-kinesin code'?**

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40 Highly compartmentalized and/or polarized cells, such as neurons, require precise
41 spatio-temporal regulation of motor associated cargo delivery to meet local needs. This
42 could be achieved simply through diverse Kif motors and a variety of cargo-specific adaptor
43 proteins as long as they are under the guidance of specific regulatory mechanisms. It is
44 becoming clear that many selective, regulatory mechanisms exist, as exemplified recently by
45 the demonstration in neurons that selective distribution of different Kifs is apparent at
46 different developmental stages, and shows differential sensitivity to pharmacological
47 treatments such as paclitaxel (Huang and Banker, 2011). In fact, it is likely that many
48 regulatory mechanisms act in concert on a single Kif member to efficiently control both
49 loading onto its MT tracks and its subsequent distribution.
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3 Available evidence is currently overwhelmingly biased towards studies of Kif5-
4 dependent transport in the axon. As mentioned above, some cargos appear to actively steer
5 their own delivery to the dendritic compartment. Nevertheless - and as exemplified by Kif5 -
6 some modulation may occur at the level of the MT itself, where elements in the Kif motor
7 domain may recognize compartmentalized alterations in MT structure conferred by tubulin
8 PTMs, or by tubulin isoform subunit composition. It is also apparent that modulation can
9 occur through motor phosphorylation, cargo association, relief of Kif tail domain-mediated
10 auto-inhibition (see Hirokawa et al., 2010; Verhey and Hammond, 2009) or possibly via
11 direct involvement of motor tails in recognition of specific MTs (Seidel et al., 2012).
12 However, these mechanisms are individually insufficient to account for all aspects of the
13 often highly dynamic spatio-temporal regulation of Kifs.
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23 The literature reviewed here demonstrates that neuronal MAPs make a contribution
24 to the regulated distribution of some Kifs. This regulation may often be restricted to certain
25 Kif family members, as in the case of DCX's specific regulation of Kif1A. Some MAPs may
26 regulate the addition of particular tubulin PTMs to the MT. Some MAPs may alter MT
27 architecture via their MT interaction, thereby allosterically altering the kinesin binding site
28 and indirectly facilitating or hindering its binding and/or movement. Some MAPs may
29 directly interfere with the MT binding of Kifs by competing with their binding sites, while
30 others also trigger signalling pathways independently of MT binding to affect Kif behaviour.
31 Interestingly, in some cases, MAPs may interact with the tail domain of Kifs (exemplified by
32 ensconsin/MAP7), perhaps modulating the auto-inhibitory tail dependent mechanism of Kifs
33 to alter their movement, a possibility often neglected by the common experimental
34 utilisation of truncated constitutively active tail-less motors. The diverse modulatory effects
35 of MAPs on specific Kif family members supports the existence of a 'MAP-Kif code' as
36 suggested by Liu and colleagues (2012), that could work in conjunction with the covalent
37 modifications associated with the 'tubulin code' (Verhey and Gaertig, 2007). The MAP code
38 could provide dynamic spatio-temporal control of the distribution of Kifs and their cargo,
39 and is perhaps disrupted in particular disease states. An important future topic of study of
40 trafficking control is how polarised MAP distribution is initiated and maintained, and how it
41 is coordinated with localisation of tubulin modifying enzymes. In addition, relatively little is
42 known about how MAPs collaborate in transport control, with the apparently mutually
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3 exclusive localisation of DCX and tau providing a particularly intriguing example (Tint et al.,
4 2009). Many known MAPs have not yet been explicitly studied with reference to Kif
5 regulation and with the regular addition of new MAPs to the existing repertoire, there may
6 yet be many more MAPs shown to contribute to the code.
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11 In fact, specific transport is likely the result of multiple interdependent factors \square
12 including the MAP and tubulin codes \square and we are still rather far from a clear idea of how
13 they are integrated. Important insights are yet to come to highlight the fundamental
14 molecular mechanisms that allow these specific factors of the track to activate, recruit or
15 regulate transport of not only kinesin but also dynein motors. Additional levels of
16 coordination are also required with the actin tracks that frequently mediate local, short-
17 range trafficking (e.g. Wagner et al., 2011) and some MAPs may be able to facilitate this MT-
18 actin cross-talk (e.g. Fu et al., 2013). Progress in this field is critical to provide fundamental
19 knowledge on the roadmaps that control trafficking processes that lie at the heart of cellular
20 life.
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LEGENDS

Table 1. Comparison of neuronal human Kifs. The motor core sequence of human neuronal Kifs was compared with Kif5A (residue 8-325) using ClustalW (Larkin et al., 2007). Kifs expressed at moderate and high levels in neuronal cultures are included (relative abundance >1000 according to Silverman et al. (2010). Table references; ¹Charalambous et al. (2013), ²Chu et al. (2006), ³Guillaud et al. (2003), ⁴Hammond et al. (2010), ⁵Hanlon et al. (1997), ⁶Hoang et al. (1999), ⁷Huang and Banker (2011), ⁸Jacobson et al. (2006)⁹(Jenkins et al., 2012), ¹⁰Kanai et al. (2000), ¹¹Kayadjanian et al. (2007), ¹²Kondo et al. (1994), ¹³Konishi and Setou (2009), ¹⁴Lee et al. (2012), ¹⁵Marszalek et al. (1999), ¹⁶Mok et al. (2002), ¹⁷Muresan et al. (1998), ¹⁸Nakata and Hirokawa (2003), ¹⁹Nakata et al. (2011), ²⁰Navone et al. (2001), ²¹Noda et al. (2012), ²²Saito et al. (1997), ²³Setou et al. (2000), ²⁴Song et al. (2009), ²⁵Yamazaki et al. (1995), ²⁶Yang and Goldstein (1998)

Figure 1. N-Kif domain architecture and localisation of major tubulin PTMs. Schematic of a 13 protofilament MT, formed from $\alpha\beta$ -tubulin heterodimers, showing the locations of common tubulin PTMs and dimeric kinesin-1 attachment. The lattice discontinuity called the seam is also indicated. β -tubulin can exist in GTP or GDP bound nucleotide states. Detyrosination of α -tubulin involves the removal of its extreme C-terminal Tyr present in most α -tubulin isoforms; this exposes a Glu at the C-terminus. Polyglutamylation is the addition of one or more additional Glu units to a Glu in the C-terminal tail of α and/or β -tubulin. Unlike other PTMs, acetylation of α -tubulin does not involve the tubulin C-terminal tail but most often occurs in the MT lumen on Lys40. Kinesin motor domains interact with individual $\alpha\beta$ -tubulin heterodimers on the outside of the MT lattice. Below, a schematic of domain organization of a Kif5 dimer with attached cargo. The motor domain, coiled-coil stalk and tail region are encoded for by Kif family proteins, and either bind cargo directly, through associated kinesin light chain (KLC) subunits or other adaptor proteins (not shown).

Figure 2. Structural basis of Kif motor domain interaction with MTs. A) Crystal structure of human Kif5B (1BG2) with regions of sequence insertion and variation amongst neuronal

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3 kinesins highlighted in green and red. These variations are found in the loop regions of the
4 kinesin motor domain while its core structure is well conserved. Specific loop regions (L2, L8,
5 L11) mentioned in the text are labeled in green. B) Front (top) and side (bottom) views of
6 the kinesin- α -tubulin complex (PDB code 2P4N; Sindelar and Downing, 2007). Several of
7 the loop variations are found at the motor-track interface and could thus account for
8 differential motor-track specification. Others may be involved in modifying other aspects of
9 motor function. Arrowheads indicate the location of the tubulin C-terminal tails. An arrow
10 indicates the luminal loop (disordered in this structure) containing the α -tubulin acetylation
11 site α 240. ADP is shown in the Kif5B nucleotide binding pocket, the β -tubulin GTPase site
12 contains GDP while the equivalent site in α -tubulin contains GTP. Figures were prepared
13 using Chimera (Pettersen et al., 2004).
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20 **Figure 3. Domain structures of MAPs proposed to regulate kinesin transport.** Schematic
21 representation of human MAPs proposed to regulate kinesin distribution, divided into
22 homologous subfamilies. Some MAPs, such as DCLK, tau and MAP2 exist as a number of
23 alternatively spliced isoforms; in these cases the longest isoforms are shown. Nascent
24 MAP1b is cleaved into heavy and light chains before recombining to form the full length
25 protein. MAP7 is the mammalian homologue of *Drosophila* ensconsin. Abbreviations: MTBR;
26 microtubule binding repeat (tau family). MTB; microtubule binding region (MAP1 family).
27 EMTB; Ensconsin MT binding domain. Tau and doublecortin family proteins contain
28 unrelated proline-rich regions that are major sites of serine/threonine phosphorylation.
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33 **Figure 4. Microtubule polarity, PTMs and MAPs vary within neuronal subdomains.** A
34 schematic representation of a polarized neuron indicating polarity of MTs, and PTM and
35 MAP density within axons, dendrites and the soma. Axonal MTs are orientated nearly
36 exclusively with +ends (red) distal and -ends (blue) proximal to the cell body, whereas somal
37 and dendritic MTs have mixed polarity. GTP tubulin, Lys40 acetylation and detyrosination
38 are found preferentially in the axonal domain, whereas polyglutamylation occurs
39 throughout the neuron. Tau is found mainly in axons but is absent from the extreme axonal
40 growth cone where DCX and DCLK are highly concentrated. MAP2 is found mainly in
41 dendrites. DCX/DCLK and to a lesser extent MAP1b are found predominantly towards the
42 extremities of growing processes, but also at lower levels throughout the rest of the neuron.
43 There is currently insufficient evidence to attribute a clear sub-cellular distribution to
44 ensconsin/MAP7.
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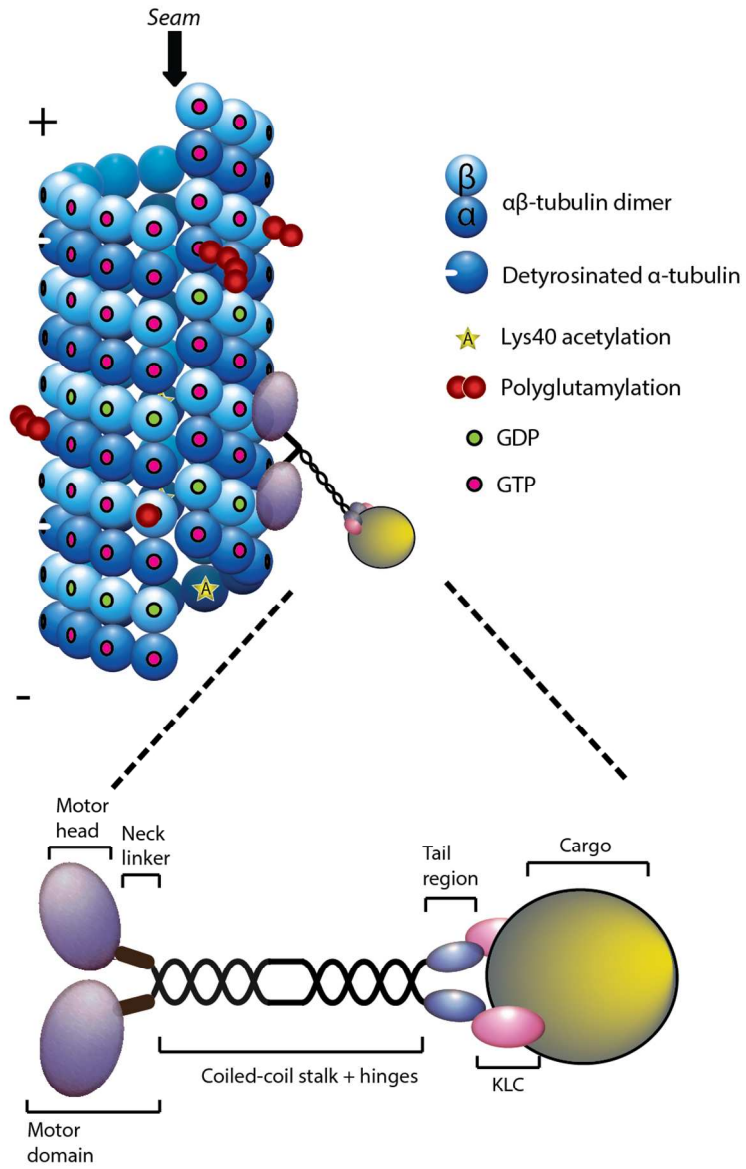
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For Peer Review

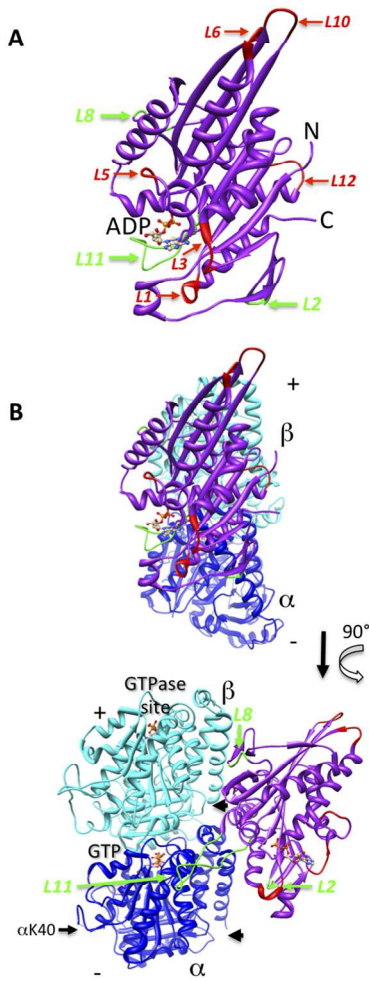
Human Kif	Sub-family	Uniprot	Tail-less motor axon/dendrite preference \geq stage 3 neurons	Full-length preference \geq stage 3 neurons	% motor sequence identity
Kif5A	1	Q12840	Axonal ^{7, 18}	Non-selective ^{e.g. 18,10}	100
Kif5B	1	P33176	Axonal ^{7, 18}	Non-selective ^{e.g. 18}	85
Kif5C	1	O60282	Axonal ^{7, 19, 8, 4, 18}	Non-selective ^{e.g. 18, 10}	91
Kif3A	2	Q9Y496	Axonal as a dimer with Kif3B, weakly axonal as a dimer with Kif3C ⁷	Non-selective ¹⁷ Weakly axonal ¹²	47
Kif3B	2	O15066	See above ⁷	Non-selective ^{17, 25}	44
Kif3C	2	O14782	See above ⁷	Cell type dependent ²⁶ , Non-selective ^{17, 20}	42
Kif17	2	Q9P2E2	Axonal ⁷ Non-selective ^{18, 24}	Dendritic ^{2, 23, 3, 11, 24}	46
Kif1A	3	Q12756	Non-selective ^{7, 19, 8, 13}	Non-selective ⁹	43
Kif1B α	3	O60333-3	Non-selective ⁷	Non-selective ¹⁶	44
Kif1B β	3	O60333-2	Non-selective ⁷	Non-selective ¹	44
Kif13A	3	Q9H1H9	Axonal ⁷	Non-selective ⁹	44
Kif21A	4	Q7Z4S6	Axonal ⁷	Non-selective ¹⁵ Axonal ¹⁴	39
Kif21B	4	O75037	Non-selective ⁷	Dendrites ¹⁵	42
Kif2A	13	O00139	?	Non-selective ²¹	38
KifC2	14	Q96AC6	?	Dendritic ²² , Non-selective ⁵	32
KifC3	14	Q9BVG8	?	Axonal/ <i>synaptic</i> ⁶	37

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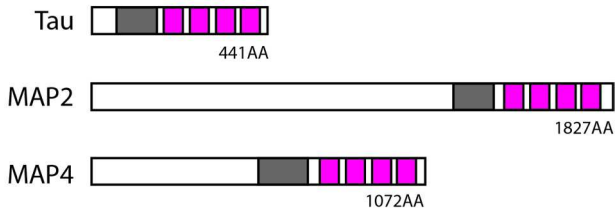
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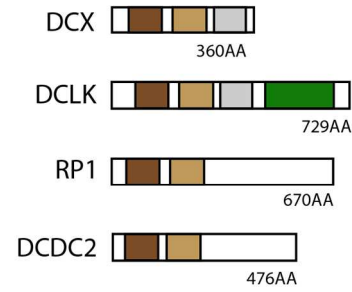
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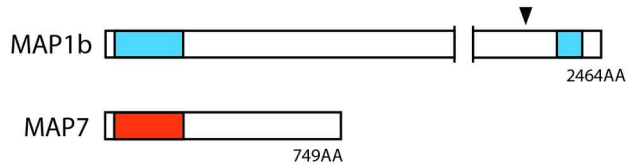
Tau/MAP2 family



Doublecortin family



Other MAPs



Key

MT binding regions

DCX family N-DC domain



DCX family C-DC domain



Tau family MTBR



MAP1 family MTB region



MAP7 family EMTB domain



Other regions

S/T Kinase domain



Serine/Threonine/Proline rich region



Proline rich region

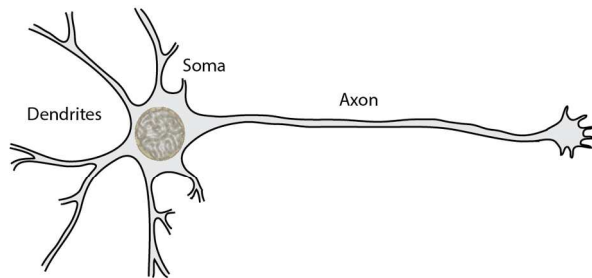


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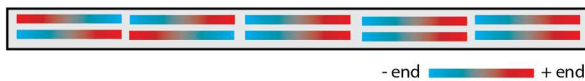


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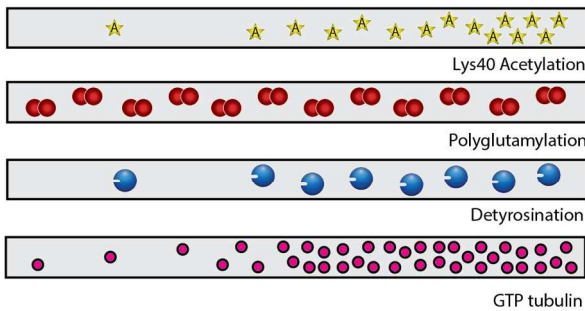
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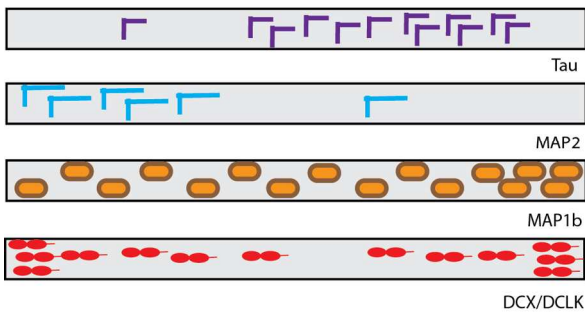
MT polarity



Tubulin PTMs



MAPs



91x185mm (300 x 300 DPI)