Review

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Role of non-motile microtubule-associated proteins in virus trafficking

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Abstract: Viruses are entirely dependent on their ability to infect a host cell in order to replicate. To reach their site of replication as rapidly and efficiently as possible following cell entry, many have evolved elaborate mechanisms to hijack the cellular transport machinery to propel themselves across the cytoplasm. Long-range movements have been shown to involve motor proteins along microtubules (MTs) and direct interactions between viral proteins and dynein and/or kinesin motors have been well described. Although less well-characterized, it is also becoming increasingly clear that non-motile microtubule-associated proteins (MAPs), including structural MAPs of the MAP1 and MAP2 families, and microtubule plus-end tracking proteins (+TIPs), can also promote viral trafficking in infected cells, by mediating interaction of viruses with filaments and/or motor proteins, and modulating filament stability. Here we review our current knowledge on non-motile MAPs, their role in the regulation of cytoskeletal dynamics and in viral trafficking during the early steps of infection.

Keywords: microtubule-associated protein; trafficking; virus.

Introduction

Microtubules (MTs) are cytoskeletal filaments consisting of 13 protofilaments of α- and β-tubulin heterodimers arranged as polymeric cylinders. They are highly dynamic, continuously switching between phases of growth and shrinkage, referred to as dynamic instability (1). MTs are involved in a wide range of cellular functions, including cell morphology, division, motility, and organelle positioning, as well as in disease, such as neurodegenerative disease, infection and immunity. Once the MT filaments are formed by nucleation at the centrosome and elongated from the subunit pool, their stability and mechanical properties are often controlled by a set of proteins that bind along the polymer. These so-called microtubule-associated proteins (MAPs, not to be confused with mitogen-activated proteins) are essential for the regulation of MT dynamics.

Several types of MAPs have been identified in eukaryotes, including MT motors, MT plus-end and minus-end tracking proteins (+TIPs, −TIPs), centrosome-associated proteins and structural MAPs. MTs, motors and non-motile MAPs together constitute a highly efficient and tightly regulated machinery that ensures the directed transport of cargo such as organelles, vesicles, protein complexes and mRNAs within cells. Viruses have evolved multiple ways of hijacking the cellular transport machinery to propel themselves through the cell, from their site of entry to their sites of replication and egress (2–7). In particular, viruses that replicate in the nucleus, such as adenoviruses (8, 9), retroviruses (10, 11), and herpes viruses (12, 13), use the MT network and associated motors to traffic within the cytoplasm either from the cell periphery to the center of the cell (dynein) or in the reverse direction (kinesins). The importance of molecular motors in viral transport is well established and has been reviewed elsewhere (14, 15). More intriguing and less clear is the role of non-motile MAPs in the movement of viruses along microtubular tracks. The aim of this review is to discuss our current understanding of non-motile MAP functions and how they support viral replication.

Structural MAPs

The structural MAPs are non-enzymatic proteins that bind along the length of MTs, enhancing the assembly and the

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stability of the polymer. They can be grouped into two types, based on sequence analysis. Type I MAPs include MAP1A, MAP1B and MAP1S (16, 17). MAP1A and MAP1B are large (>300 kDa), filamentous molecules found predominantly in axons and dendrites, whereas the shorter MAP1S is ubiquitously expressed. All three MAP1 proteins are synthesized as polyprotein precursors that are subsequently cleaved into N-terminal-derived heavy chain and C-terminal-derived light chain to generate multiprotein complexes of one heavy and multiple light chain subunits (18). The light chains generated by MAP1A (LC2) and MAP1B (LC1) are interchangeable and can interact with both MAP1A and MAP1B heavy chains. A third separately encoded light chain 3 subunit (LC3, 16 kDa) is also found in mature MAP1A and MAP1B (19). All three MAP1 light chains as well as the MAP1A and MAP1B heavy chains can bind directly to MTs (Figure 1). In addition to MT-binding activity, MAP1A and MAP1B can also bind to microfilaments (20–22), suggesting that they can regulate both MT and microfilament stability and constitute cross-bridges between both networks.

Type II mammalian MAPs consist of the neuronal proteins MAP2 and Tau, and the ubiquitous MAP4 (23). MAP2 is found only in dendrites, where it forms fibrous cross-bridges between MTs and is involved in organelle trafficking. Tau is abundant in neurons, where it promotes formation of axonal MTs, stabilizes and increases their rigidity and drives neurite outgrowth. Tau can also control intracellular trafficking by interfering with the attachment and detachment cycle of motors, particularly by reducing the attachment of kinesins to MTs (24, 25). Abnormalities in the tau protein are implicated in many neurodegenerative and psychiatric disorders. MAP4 performs many different functions in a range of cells and tissues, including regulation of MT dynamics in interphase, mitosis and meiosis, organization and transport of organelles and vesicles in interphase cells, and regulation of cell shape during differentiation (26, 27).

Microtubule plus-end tracking proteins (+TIPs)

MT plus-end tracking proteins (+TIPs) are a diverse group of evolutionarily conserved cellular factors that accumulate at the ends of growing MTs. They regulate different aspects of cell architecture by controlling MT dynamics, and interaction with cellular structures and proteins (28, 29). The first identified +TIP was cytoplasmic linker protein (CLIP)-170 (officially known as CLIP1) (30). Since then many different families of +TIPs have been identified, that can be classified on the basis of structural elements that mediate interaction with MTs and other +TIPs, with the common characteristic of specifically accumulating at MT plus ends (Figure 2). Minus-end

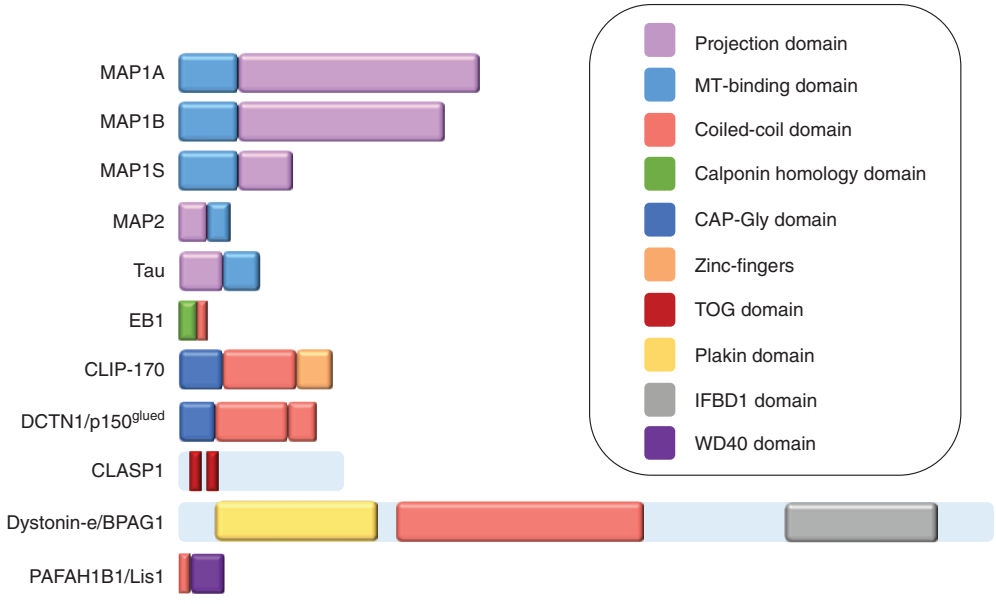

Figure 1: Examples of different MAPs and their functional domains.
Some MAPs, such as MAP2 and tau, exist as multiple alternatively spliced isoforms; in these cases, the longest isoforms are shown. For MAP1 proteins, heavy chains are represented. The projection domains are the residues that extend at the surface of MTs.

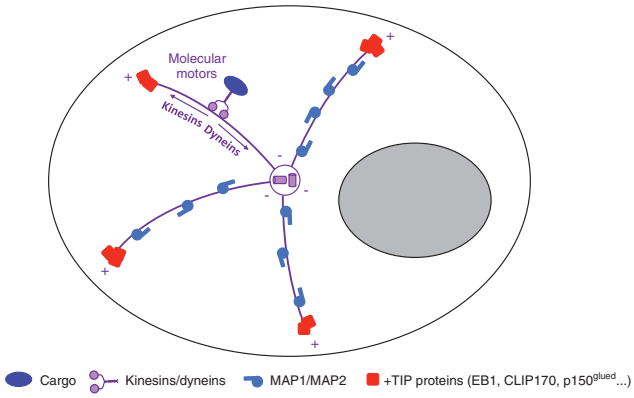

Figure 2: Organization of the MT network and expected localization of MAPs.
MAP1 and MAP2 proteins typically bind along the length of MTs, whereas + TIPs localize at the growing plus end of MTs. Molecular motors move along the length of MTs, with kinesin powering transport towards plus ends, and dynein mediating retrograde transport towards the MTOC.

targeting proteins (– TIPs) have also recently been identified and shown to be involved in the control of minus-end MT dynamics (31).

End-binding proteins (EB) constitute a highly conserved + TIP family, present from yeasts to humans, that localize to spindle and cytoplasmic MTs, and regulate their dynamics and organization (32, 33). In mammalian cells, the three MT end-binding proteins, EB1, EB2 and EB3, share substantial sequence homology, but while EB1 and EB3 promote MT growth by suppressing catastrophe, EB2 does not play a direct role in MT dynamic instability (34, 35). Recent work has proposed that EB2 plays an essential role in the regulation of focal adhesion dynamics and cell migration via its interaction with the kinase MAP4K4 (36). EB3 is expressed preferentially in the central nervous system and muscle, while EB1 is ubiquitously expressed (37). The C-terminal domain of EB proteins promotes several + TIP interactions, and acts as a hub that organizes and maintains + TIP networks (38). In particular, EB1 interacts with components of the dynactin complex, the activator for cytoplasmic dynein: p150^{glued} (DCTN1, described below), p50/dynamitin (DCTN2), and the intermediate chain of dynein (39). EB proteins have also been shown to interact with several kinesin motor proteins, such as kinesin 8 Kif18B to control microtubular length (40) and kinesin Kif17 to stabilize MTs by post-translational acetylation (41).

Cytoskeleton-associated proteins (CAP) rich in glycine residues (CAP-Gly proteins) constitute another + TIP family whose members interact with both MTs and EB proteins. Prominent examples are CLIP proteins and the large subunit of the dynactin complex p150^{glued}. CLIP-170 binds specifically at MT plus ends and modulates MT nucleation, polymerization, and transitions from shrinkage to growth (42). CLIP-170 binds to EB1, α-tubulin, p150^{glued}, and recruits various cargos to MT tips, including the dynein–dynactin complex (43), and vesicles (44). Other CLIP proteins include the brain-specific CLIP-115 and CLIPR-59, which does not localized to MTs at steady-state but is associated with the trans-Golgi network and the plasma membrane where it regulates their dynamics. The large subunit of the dynactin complex, p150^{glued} (DCTN1) is an evolutionarily conserved + TIP protein containing a N-terminal CAP-Gly domain mediating interaction with MT, and two C-terminal coiled-coil regions that are required for dimerization and interaction with the dynein intermediate chain and with dynactin shoulder (45, 46). p150^{glued} promotes MT formation *in vitro* by catalyzing nucleation, increasing polymerization rate, and inhibiting catastrophe (47). The coiled-coil α-helical domain CC1B of p150^{glued} is necessary for its ability to enhance dynein processivity (48). Its overexpression as transdominant uncouples dynein-based transport, and is often used as a tool to demonstrate dependency of viral transport on dynein.

Proteins rich in basic, serine and proline (basic-Ser/Pro) sequences constitute the largest and most diverse family of + TIPs. Prominent examples are the adenomatous polyposis coli (APC) tumor suppressor, and the spectraplakins microtubule-actin crosslinking factor (MACF) and bullous pemphigoid antigen 1 (BPAG1, dystonin). They possess a small four-residue motif, Ser-x-Ile-Pro (SxIP, where x denotes any amino acid), which is specifically recognized by the EBH domain of EB proteins (49). Dystonin is a giant protein that can interact with all three elements of the cytoskeleton, intermediate filaments, actin and MTs, via a N-terminal actin-binding domain and a C-terminal MT-binding domain (50). Four major isoforms (Dystonin a, b, e and n) have been identified to date, with differential tissue expression and resulting from alternative splicing patterns.

Proteins with TOG domains include the CLIP-associated proteins (CLASPs) that were initially characterized through their ability to bind to CLIP-170, CLIP-115 and MTs, and to co-localize with the CLIPs at MT distal ends (51). CLASP1 is ubiquitously expressed, while CLASP2 is more abundant in the brain. CLASP1 and CLASP2 promote MT rescue and inhibit catastrophes (52, 53).

Finally, WD40-repeats proteins are characterized by the presence of repeating units of 44–60 variable residues that end with tryptophan (W) and aspartate (D) dipeptides (54). In the last decade, multiple WD40 protein complexes have been identified, which generally

function as rigid platforms for protein-protein and protein-DNA interactions and are involved in diverse range of cellular processes, such as signal transduction, gene transcriptional regulation, protein modifications, cytoskeleton assembly, vesicular trafficking, DNA damage and repair, cell death and control of cell division (55). Lis1 is a +TIP member of WD40-repeat protein family enriched in neurons whose insufficiency following heterozygous mutations in the *Lis1* gene causes lissencephaly, a severe brain malformation due to abnormal neuronal migration during brain development (56). Lis1 interacts with motors and other +TIP proteins, and regulates MT organization, and dynein/dynactin binding to MTs and cargo (57–59).

Role of non-motile MAPs in intracellular trafficking

Although motors are the molecular complexes that mediate cargo intracellular trafficking, evidence also supports a direct role for MAPs in cargo movement along microtubular tracks. MAPs bind along the length of MTs, differing in their distribution and in the domains that project from the surface of the MT. Post-translational modifications of both MAPs and tubulin are key in regulating their interactions as well as MT dynamics and stability. Phosphorylation of MAPs can either favor binding to assembled MTs, as in the case of MAP1 proteins, or lead to loss of binding, as is seen with MAP2, tau, and MAP4 (60–62). In addition, tubulin undergoes a wide range of post-translation modifications (63, 64), that lead to the existence of different populations of MTs within cells, with detyrosinated and acetylated MTs representing a small subset of stable, non-growing MTs.

It is thought that MAPs can regulate trafficking firstly by serving as adaptor proteins that specify motor and cargo identity within the complex set of proteins that constitute motor-cargo interactions (65). Moreover, depending on their projection domains, MAPs can act as selective obstacles to motor-MT attachment, decreasing both the frequency of productive encounters and the average length of runs, or on the contrary enhance affinity of motors to MTs (66–69). It is thought that the differential regulation of motor attachment to MT by MAPs allows them to modulate movement directionality to achieve compartimentalized and polarized transport (67, 70–72). As the role of MAPs in intracellular transport becomes increasingly clear, so also our understanding of how viruses divert these mechanisms to promote their trafficking within cells.

Promotion of viral trafficking by MAPs

Viruses are obligate parasites that encode a limited number of genes, and require complementing host cell functions for replication and spread. Importantly, once viruses enter cells they require cytoplasmic transport to reach specific subcellular sites for replication and egress. Numerous studies have revealed that retrograde and anterograde transport of animal viruses is supported by MTs and their associated proteins (14, 15, 73, 74), and an increasing number of viral proteins that interact with MTs or MAPs have begun to be identified (Table 1). Virus-MAP interactions, be they functional or physical, can promote virus trafficking in an infected cell, by directly facilitating latching on of virus particles to cytoskeletal tracks, or modifying MT dynamics to optimize movement along the tracks.

MAPs facilitate interaction of viruses with microtubules

HIV-1 has been shown to use actin microfilaments for short-range transport at the cell and nuclear periphery, and MT motors for long-range intracellular movement (10, 75, 76). Confusingly, numerous non-motile tubulin- and actin-associated proteins have been identified by genome-wide screens of proteins thought to contribute to efficient HIV-1 infection (77–79). However, as HIV interacts with the cytoskeleton at multiple points in its replication cycle and remodels the cytoskeleton to promote viral replication, it is unclear which cellular factors are specifically involved in trafficking. Our group reported the interaction of HIV-1 capsids with human MAP1A (see Figure 3) and MAP1S, involving MAP1 light chain LC2. Depletion of MAP1A or MAP1S in primary human macrophages disrupted HIV-1 infection by hampering efficient trafficking of incoming HIV-1 complexes to the nucleus, and reduced the association of HIV-1 capsids with both dynamic and stable MTs, suggesting that MAP1 proteins help tether incoming viral capsids to the microtubular network, thus promoting cytoplasmic trafficking (80). In addition, a role for MAP4 was reported in the early steps of HIV-1 infection. Depletion of MAP4 was shown to impact HIV-1 reverse transcription but not nuclear translocation (81). However, it is currently unknown whether or not MAP4 directly interacts with viral proteins.

Adenovirus (Ad) uses MT-directed transport and dynein to reach the centrosomes and nuclear pores

Table 1: Structural MAPs and +TIPs interact with several different viruses.

MAP family	Examples	Virus interaction	Role of MAP-virus interaction	References
MAP1/2	MAP1A	HIV-1 p24 capsid	Promote HIV-1 capsid transport	(80)
	MAP1B	RSV NS1 and NS2	Contribute to the STAT2-degrading activity of NS2	(104)
	MAP1S	HIV-1 p24 capsid	Promote HIV-1 capsid transport	(80)
	LC3	HIV-1 Vif	Inhibit autophagy	(108)
	MAP4	HIV-1	Promote HIV-1 reverse transcription	(81)
+TIPs	EB1	HIV-1	Induce microtubule stabilization	(91)
		HSV-1	Promote HSV-1 transport	(86)
		HIV-1 Vpr	Alter phagosome movement and maturation	(107)
	CLIP-170	HSV-1	Promote HSV-1 transport	(86)
	$p150^{glued}$	HIV-1	Promote HIV-1 capsid transport	(10)
		Adenovirus	Promote Ad capsid transport	(87)
		HSV	Promote HSV-1 capsid transport	(88, 89)
		HIV-1 Vpr	Alter phagosome movement and maturation	(107)
	CLASP1	HSV-1	Induce microtubule stabilization, virus spread	(97)
	CLASP2	HSV-1	Induce microtubule stabilization, virus spread	(97)
	Lis1	Poliovirus 3A	Membrane protein trafficking	(102)
		HIV-1 Tat	Contribute to microtubule formation	(103)
	Dystonin	HSV-1	Promote HSV-1 capsid transport	(85, 100)

The table lists reported functional and physical interactions between viruses and MAPs, and their effect on viral infection.

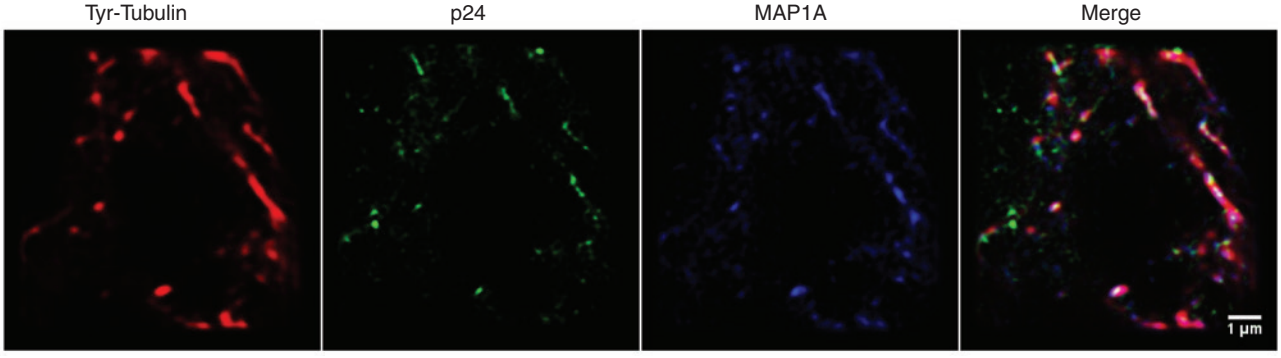

Figure 3: MAP1A interacts with HIV-1 capsid.
Super resolution images of HIV-1 infected HeLa cells were acquired by BioAxial's Conical Diffraction Microscopy (CODIM) (111) at 4 h post-infection. Cells were labeled with anti-tyrosinated (Tyr)-tubulin (red), anti-capsid p24 (green) and anti-MAP1A (blue) antibodies. Scale bar represents 1 µm.

rapidly following receptor-mediated endocytosis. MAPs have been shown to enhance binding of Ad capsid to MTs, although the motor protein dynein seems to be largely responsible for this interaction and for promoting Ad trafficking (82, 83).

In the case of herpes simplex virus type 1 (HSV-1), recent work identifies the MT plus end dystonin/BPAG1 protein as a binding partner of the tegument protein pUL37, which controls the movement of capsids within the cytosol (84). In dystonin-depleted cells, HSV-1 capsids could reach the centrosome of fibroblasts but the transport of capsids away from the centrosome towards the nucleus was significantly blocked, indicating a defect in the polarity switch of viral transport occurring at the centrosome (85). This work was the first to illustrate how non-motile MAPs can regulate the directionality of transport along MTs. Moreover, initiation of capsid transport soon after cell entry requires a +TIP complex comprising CLIP-170, EB1 and dynactin 1. In the absence CLIP-170, viral capsids remained at the cell periphery, unable to reach the nucleus (86).

Finally, the dynein co-factor CAP-gly protein $p150^{glued}$ has been implicated in the infection of several viruses. Overexpression of a transdominant inhibitor of $p150^{glued}$ inhibited HIV-1 and Ad trafficking to the nucleus and inhibited infection (10, 87). Moreover, the herpesvirus

tegument protein VP1/2 interacts with p150^{glued}, and inhibition of dynactin blocks cytoplasmic HSV-1 transport along MTs (88, 89).

MAPs mediate virus-induced microtubule stabilization

Besides interacting with MAPs to latch onto the MT network, viruses can promote their trafficking in infected cells by enhancing MT stability. Several viruses induce MT rearrangements and tubulin acetylation to varying extents, and in some cases, evidence suggests that stable MTs may be important for infection. However, the underlying mechanisms by which many viruses remodel host MT networks and potential roles for MAPs still remain relatively poorly understood. Recent work showed that adenovirus, African swine fever virus (ASFV), influenza A virus, reoviruses, HSV-1, HIV-1, and Ebola virus all induce MT stabilization, often observed with concomitant post-translational modifications such as acetylation and detyrosination in host cells (90–96). Specifically, HSV-1 is thought to promote both its trafficking to the nucleus and its egress by multiple mechanisms, including the stabilization and hyperacetylation of MTs by the tegument protein VP22 (94) and viral kinase Us2 (97), binding of the molecular chaperone Hsp90 (98), disruption of the centrosomal nature of the MT-organizing center (MTOC) and its transfer to the trans-Golgi network (97, 99, 100). In this context, cytoplasmic linker-associated proteins (CLASPs), which are specialized +TIPs that control MT formation at the trans-Golgi network, were found to be specifically required for virus-induced MT stabilization and HSV-1 spread (97). Furthermore, initial engagement of HSV-1 particles onto MTs involves a +TIP complex comprising EB1, CLIP-170, and dynactin-1 (DCTN1) to initiate retrograde transport in infected cells (86).

HIV-1 infection was also shown to induce tubulin post-translational modifications that are associated with the formation of a more stable subset of MTs, thus facilitating translocation of HIV-1 across the cytoplasm (80, 91). Virus-induced MT stabilization, which occurs very rapidly after cell entry (within 1–2 h post-infection), was found to involve EB1 (91) and, albeit to a lesser extent, MAP1A/MAP1S proteins (80). Conversely, HIV-1 infection was impaired by overexpression of moesin, a member of the ezrin, radixin and moesin (ERM) family that negatively regulates the stable MT network (101), thus confirming that MT stabilization favors viral particle trafficking to their site of replication. Together, these findings illustrate how viruses have evolved to target specialized +TIPs and other MAPs to control MT stability and promote early post-entry stages of infection.

Conclusion

For their transport inside infected cells, viruses rely both on cytoskeletal filaments and their associated motor proteins to travel from their site of entry to their site of replication, assembly and egress, with speed and efficiency to avoid rapid overpowering by the cell's innate immunity and degradation machineries. Although some viral proteins have been shown to interact directly with molecular motors, evidence shows that viruses also depend on interactions with actin/tubulin binding proteins for efficient trafficking. The study of MAPs and how they promote viral trafficking is key to better understand the complexities of virus movement within infected cells, in particular to address how directionality of transport and switching between filaments are regulated to reach precise points of destination.

In many published reports, an ambiguity remains whether the interaction of viral proteins with non-motile MAPs can directly affect trafficking or whether motor proteins are inevitably involved. This is particularly true of studies where the implicated MAP is part of a greater motor complex. Moreover, some studies remain inconclusive as to whether virus-MAP interactions that stabilize MTs lead to enhanced infection by specifically affecting viral trafficking, or rather by affecting general transport. For instance, the poliovirus 3A protein was shown to bind to Lis1 and disrupt protein trafficking, but this was found to impact the cell surface expression of short-lived receptors, including those for tumor necrosis factor and interferon (IFN), rather than viral particle trafficking *per se* (102). Moreover, it has been reported that HIV-1 Tat also binds to Lis1 and this interaction might contribute to the effect of Tat on MT formation (103). Further work is undoubtedly warranted to clarify the specific implication of MAPs in viral replication.

It is interesting to note that beyond promoting virus trafficking in the infected cell, interaction of MAPs with viral proteins can impact other cellular functions regulated by MTs such as mitosis, apoptosis, and immunity, and thus contribute to virus-associated pathologies. Human respiratory syncytial virus (RSV), a major cause of severe respiratory diseases, efficiently suppresses cellular innate immunity, using its two unique non-structural proteins, NS1 and NS2. The two NS proteins suppress both type I IFN induction and the IFN response pathways by interfering with various innate immunity actors such as RIG-I and

STAT2. Recently, MAP1B was shown to interact with NS1 and NS2 and to contribute to the STAT2-degrading activity of NS2 (104). In the case of HIV infection, the viral transactivator Tat protein has been shown to contribute to neuronal damage observed in the brain of HIV-1 patients with neurological dysfunctions, by causing rapid proteasome-mediated degradation of MAP2 leading to the collapse of cytoskeletal filaments, as well as aberrant splicing of tau (105, 106). MAP proteins have also been linked to cellular processes such as phagocytosis and autophagy, which are both repressed in HIV-1 target cells, macrophages and T lymphocytes, respectively. Two HIV-1 proteins were recently shown to play an inhibitory role in these processes: Vpr was shown to interact with EB1 and $p150^{glued}$, perturbing their MT plus end localization, thus altering phagosome movement and maturation (107), while Vif was shown to interact directly with LC3B, a major autophagy component (108). In this context, the study of MAP1S will prove an interesting topic, since MAP1S is involved both in autophagy, regulating autophagosomal biogenesis and degradation, and interacting with autophagosome-associated LC3 of MAP1A and MAP1B (109), and in bacterial phagocytosis by macrophages through interaction with MyD88 a key adaptor of Toll-like receptors (110).

The implication of MAPs and MTs in viral disease is a fascinating area of research that is only just emerging and will no doubt generate many interesting studies in the coming years.

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