

# Microtubules and signal transduction

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Although molecular components of signal transduction pathways are rapidly being identified, how elements of these pathways are positioned spatially and how signals traverse the intracellular environment from the cell surface to the nucleus or to other cytoplasmic targets are not well understood. The discovery of signaling molecules that interact with microtubules (MTs), as well as the multiple effects on signaling pathways of drugs that destabilize or hyperstabilize MTs, indicate that MTs are likely to be critical to the spatial organization of signal transduction. MTs themselves are also affected by signaling pathways and this may contribute to the transmission of signals to downstream targets.

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### Abbreviations

<b>APC</b>	adenomatous polyposis coli
<b>CI</b>	cubitus interruptus
<b>ERK</b>	extracellular signal regulated kinase
<b>FAK</b>	focal adhesion kinase
<b>GEF</b>	GDP exchange factor
<b>GPI</b>	glycosylphosphatidylinositol
<b>GRK</b>	G-protein coupled receptor kinase
<b>GSK-3<math>\beta</math></b>	glycogen synthase kinase-3 $\beta$
<b>JNK</b>	Jun amino-terminal kinase
<b>LPA</b>	lysophosphatidic acid
<b>MAP1B</b>	MT associated protein 1B
<b>MAPK</b>	mitogen activated protein kinase
<b>MT</b>	microtubule
<b>MTOC</b>	MT organizing center
<b>PP</b>	protein phosphatase
<b>SPB</b>	spindle pole body

### Introduction

Microtubules (MTs) may be uniquely equipped to transmit signals originating from receptor occupancy because they fill the cytoplasm and usually span the distance from the plasma membrane to the nucleus. In most cells, MTs are organized into a single array with their minus ends associated with a MT organizing center (MTOC). This gives the cell a defined polarity that is based upon the structural polarity inherent in every MT. No other cellular element performs this role. Typically, MT minus ends are located near the nucleus in the center of the cell with their plus ends toward the cell periphery near the plasma membrane. As the motor molecules kinesin and dynein utilize this polarity, by moving to the plus and minus ends of the MT, respectively, MTs provide a system for the directional flow of information.

MTs also present an enormous protein surface in the cell: there is about 1000  $\mu\text{m}^2$  of protein surface on MTs in the

interphase array of the average proliferating fibroblast. This is about the same protein surface area as the plasma membrane and over ten times that of the nuclear envelope. Thus, MTs provide ample surface for protein–protein interactions and new studies suggest that this is used in unexpected ways. MTs themselves are regulated, dynamic polymers that respond to signaling pathways by changing their dynamics and organization in ways that may contribute to the signal transduction process. In this review we examine the literature of the past year and a half and describe some of the findings concerning signal transduction molecules that interact with MTs and the mechanisms by which MTs may contribute to signal transduction. We also consider evidence that signaling pathways induce specific changes in MT dynamics and stability, and discuss how these may contribute to the transmission of signals through the cytoplasm.

### Microtubules and the hedgehog signaling pathway: a new motor-like protein with a new function

One of the best examples of a MT-binding protein involved in signal transduction is Costal 2. Costal 2 was first identified as a gene that represses the hedgehog signaling pathway during *Drosophila* development and subsequent sequence analysis revealed similarity to kinesin heavy chains [1<sup>••</sup>,2<sup>••</sup>]. Costal 2 forms a large multimeric complex with other hedgehog pathway components including Fused, a protein kinase, and Cubitus interruptus (CI), a transcription factor. In the absence of hedgehog, this complex binds to MTs. In extracts prepared from cells treated with hedgehog, the Costal 2 complex shows reduced affinity for the MTs. Thus, this complex may sequester CI in the cytoplasm and, in the presence of hedgehog, release CI so that it can enter the nucleus. It is not yet clear if Costal 2 acts as a motor protein to move the complex, as its putative motor domain motifs are divergent from those typical of kinesin motors.

Another transcription factor that may be regulated by MTs is NF $\kappa$ B. I $\kappa$ B is a negative regulator of NF $\kappa$ B and appears to keep NF $\kappa$ B inactive in the cytoplasm by forming a complex with it. I $\kappa$ B has been shown to interact with dynein light chain and perhaps MTs [3<sup>•</sup>], and these interactions may sequester NF $\kappa$ B or contribute to its delivery, although it is not yet known whether NF $\kappa$ B forms a complex with I $\kappa$ B and the dynein light chain. NF $\kappa$ B has not been shown to be localized on MTs, but MT depolymerization by drugs leads to I $\kappa$ B destruction through a kinase-dependent mechanism, allowing NF $\kappa$ B to bind DNA and stimulate transcription [4].

### Microtubules and the Jun amino-terminal kinase signaling pathway: a moving and complex story

Many protein kinases have been shown to bind to and/or be localized on MTs *in vivo* (see below), and recent studies

suggest novel mechanisms for this association. Components of one mitogen-activated protein kinase (MAPK) pathway, namely the Jun amino-terminal kinase (JNK) pathway, are physically associated with MTs via kinesin-related motor molecules [5•]. Mixed-lineage kinase (MLK)2 — a MAPK kinase kinase-like protein involved in activation of the JNK pathway — interacts with members of the kinesin-like KIF3 family — including a novel member, KIF3X — and with the putative KIF3 targeting component KAP3A [5•]. MLK2 co-immunoprecipitated with KIF3A, KAP3A, 14-3-3 $\epsilon$  and hippocalcin when co-expressed in COS cells, and KIF3A and KAP3A colocalized with MLK2 as punctate structures on MTs, suggesting that KIF3A and KAP3A might form a complex with MLK2 in cells. None of the components caused inhibition or enhancement of MLK2-induced activation of JNK when co-expressed with MLK2; however, co-expressed JNK was shown to partially colocalize with MLK2 on MTs [5•].

Activation of MLK2 may be mediated by the small GTPase Rac, as Rac·GTP binds MLK2 [5•]. Interestingly, Rac can also bind directly to tubulin *in vitro* and there is some evidence for colocalization of Rac and MTs *in vivo* [6]. All this evidence raises the possibility that the MT acts as a scaffold to bring the components of this signaling pathway together, perhaps to increase the efficiency of activation. Motor-signaling complexes may act as intermediaries for transferring activation of upstream components to downstream targets with which the upstream activators do not directly interact. For example, in the JNK pathway, MLK2-KIF3 may relay an activation signal from Rac to JNK. In the plasma membrane, diffusion allows the aggregation of receptors and congregation of adaptors and downstream signaling molecules that is critical for signal transduction. On the MT surface, however, diffusion is restricted and motor molecules may be essential to overcome this diffusional barrier and thus allow ‘aggregation’ on the MT surface. This may be particularly important for the transmission of signals from transiently activated signaling components.

### Microtubules and the extracellular regulated kinase signaling pathway: a tale of sequestration and activation

The extracellular signal regulated kinase (ERK)1 and ERK2 MAPKs interact with MTs *in vitro* [7] and *in vivo* [8,9•,10]. The activated form might be enriched on MTs [8,10], although MAPK translocation to the nucleus triggered during mitogen activation does not appear to change the level of MT-associated MAPK. This suggests that the MT association is not likely to regulate cytoplasmic to nuclear translocation of MAPK but instead, could be important for retaining some activated MAPK in the cytoplasm to regulate cytoplasmic events. Activated MAPK may affect MTs, as MT-associated proteins (MAPs) are phosphorylated extensively by MAPK and this decreases their affinity for MTs [11,12]. Some MAPK mutations which do not affect MT binding, still cause defects in MT organization suggesting that other players are involved [9•]

To date there is no evidence for upstream activators of MAPK interacting with MTs, as there is for JNK.

### The Wnt signaling pathway: is there any more room left on the microtubule?

A number of the components of the Wnt signaling pathway have been shown to interact with MTs. Glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) — which is constitutively active in the absence of Wnt signaling and maintains  $\beta$  catenin in the phosphorylated form causing its degradation — purifies with MTs *in vitro* and phosphorylates a number of MAPs, including tau protein [7]. It has not been shown to be localized to MTs *in vivo* and may only interact transiently with them. GSK-3 $\beta$  phosphorylation of tau and MAP1B has been confirmed *in vivo* [13,14•–16•]. Decreased phosphorylation of MAP1B and tau has opposite effects on their ability to bind to and stabilize MTs, decreasing binding for MAP1B and increasing it for tau [13,14•–16•]. Thus, Wnt signaling results in both destabilizing and stabilizing effects on MTs, yet one effect may predominate locally depending on the actual levels of the MT-associated proteins, the relative activity of phosphatases (tau binds two phosphatases, see below), and other factors. In cortical neuron growth cones for example, MTs appear to be more dynamic after Wnt signaling and this is correlated with increased spreading of the neurons [16•].

The Wnt signaling pathway components adenomatous polyposis coli (APC) and EB1 also interact with MTs. APC is a  $\beta$  catenin binding protein that stimulates MT assembly *in vitro* and is localized on MTs in APC-transfected cells [17,18]. APC constructs lacking the carboxy-terminal region, which is frequently deleted or mutated in colon cancers, do not bind to MTs [17,18]. Endogenous APC in epithelial cells is localized in clusters at peripheral membrane sites near the end of MTs and these clusters are disrupted by MT breakdown [19]. EB1 is an APC binding protein that has recently been localized to cytoplasmic and spindle MTs [20•]. EB1 appears to interact with MTs independently of its interaction with APC, yet it remains unclear whether or not EB1 binds directly to MTs [20•]. Mal3 and Bim1p are EB1 homologues in fission and budding yeast, respectively, and are localized on cytoplasmic and spindle MTs [21•,22•]. Cells lacking these proteins are hypersensitive to MT destabilizing drugs and have defects in spindle function and karyogamy. Taken together, these results point to a physiological role for APC and EB1 in MT function.

But do MTs themselves participate in the Wnt signaling pathway? The localization of APC at peripheral sites suggests that MTs may be involved in positioning APC, yet no motor proteins have been identified that interact with APC, EB1, or other Wnt signaling components. Nonetheless, studies of polarity determination in *Xenopus* suggest that such a motor may be involved in localizing components of the Wnt signaling pathway. Small organelles are moved along subcortical MTs toward the future dorsal side of the embryo where  $\beta$  catenin accumulates [23•] and is seen to colocalize with MTs [23•].

$\beta$  catenin does not possess MT binding sites or activity so it is possible that other components of the Wnt signaling pathway may be moved to the future dorsal site and effect the accumulation of  $\beta$  catenin on the dorsal side of the embryo.

MTs have also been implicated in zebrafish dorsoventral axis specification and this too may involve elements of the Wnt pathway, as disruption of MTs causes defects in axis specification and prevents  $\beta$  catenin from entering nuclei [24\*]. Studies in *Caenorhabditis elegans* have identified components of the Wnt signaling pathway — mutations in some of which result in defective spindle orientation, consistent with a role for the Wnt pathway in cytoskeletal polarization [25\*,26\*]. Many additional aspects of the relationship between the Wnt signaling pathway and MTs remain to be worked out, however, these initial studies seem to suggest that MTs may be involved in localizing or polarizing Wnt signal transduction so that it is active in only part of the cell.

### Microtubules and G-proteins: new interactions and activities

G-protein coupled receptor kinases (GRKs) phosphorylate agonist-activated G-protein coupled receptors generating a docking site for proteins of the arrestin family and down-regulate receptor signaling by promoting receptor endocytosis. Until recently, no other substrates of GRKs were known, but reports from three laboratories now identify tubulin as a second substrate [27\*\*–29\*\*].  $G_{\beta\gamma}$  subunits released by receptor activation may target GRKs to the membrane, and *in vitro* they can also bind to MTs [30] and enhance their phosphorylation by GRKs [27\*\*,29\*\*]. The downstream effects of the tubulin phosphorylation are unknown.  $G_{\alpha}$  subunits also interact with MTs [31,32], and disruption of MTs stimulates or inhibits G-protein coupled receptor signaling depending on the system [33–36].

Because of the diversity of responses to MT breakdown and the multiple interactions of G-protein subunits with tubulin and MTs, it is hard to point to a single mechanism for the modulation of G-protein signaling by MTs. Among the possibilities are: direct nucleotide exchange from tubulin to  $G_{\alpha}$  subunits [31]; MT regulation of availability of  $G_{\alpha}$  or  $G_{\beta\gamma}$  subunits to the receptors or downstream effectors; or MTs providing scaffolding to promote formation of specific complexes. Such complexes have been identified in developing chick neurons, where tubulin,  $G_{i\alpha}$  family members, Fyn kinase, and the glycosylphosphatidylinositol (GPI)-anchored proteins Thy-1 and AvGP50 are found in detergent-resistant complexes [37].

### Microtubules, actin and signaling: signal transduction may integrate cytoskeletal systems

For many cellular processes, the MT and actin cytoskeletons must be coordinated and recent studies suggest that there may be signal transduction systems that integrate the

responses of the two systems. The position of the lamellipodium in many crawling cells is affected by MTs [38,39] and factors released from the MTs may contribute to lamellipodium formation. Acute MT breakdown in fibroblasts leads to the rapid formation of massive protrusions that resemble lamellipodia [40\*] and nM concentrations of nocodazole or taxol — that dampen MT dynamics but do not disrupt MT levels or organization — lead to a proportional reduction in lamellipodial area [40\*,41]. These results suggest that there is a factor associated with MTs which can be released and affect assembly of the actin meshwork. Candidate release factors include Rac, which localizes to MTs *in vitro* [6], and MAPs, which have been shown to substitute for actin binding proteins in peripheral actin assemblies [42\*\*] and induce actin gelation *in vitro* [42\*\*,43,44].

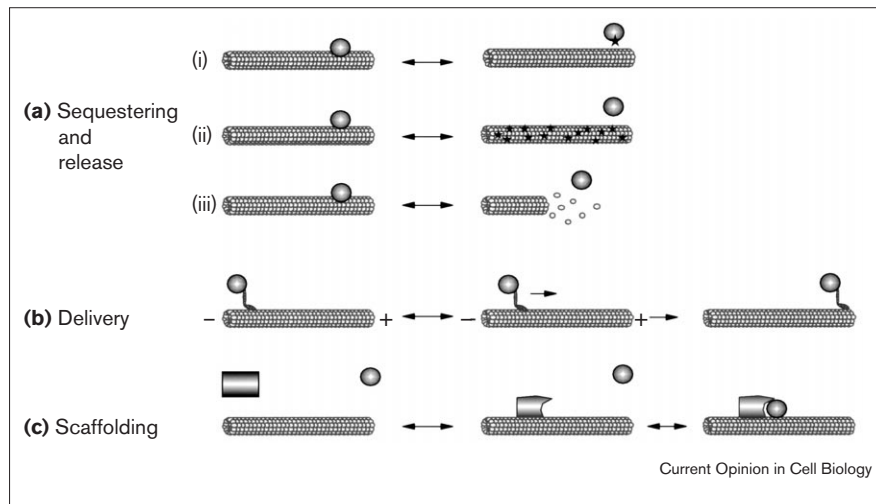
MTs may also modulate actin stress fiber formation. MT breakdown stimulates stress fiber formation in fibroblasts [45] and this is accompanied by assembly of adhesion plaques and tyrosine phosphorylation of the adhesion plaque components focal adhesion kinase (FAK) and paxillin [46]. Tension exerted by MTs on the actin cytoskeleton has been hypothesized to counteract stress fiber formation [46], but the MT-depolymerization induction of stress fibers requires active Rho [47,48] and involves elevated myosin light chain phosphorylation [49], indicating activation of a signaling cascade. Rho has also been shown to be both necessary and sufficient for the generation of stabilized MTs in serum-starved fibroblasts [50\*\*], suggesting coordinate regulation of the actin and MT cytoskeletons. Whether MTs release a factor (e.g., a GTP exchange factor or GEF for Rho) is not currently known and to date no GEFs have been shown to be localized on MTs, although the Rho/Rac GEF, vav, can associate with tubulin in T cells [51]. Taxol is not a strong inhibitor of stress fiber induction by lysophosphatidic acid (LPA) (Gundersen G, Cook TA, unpublished data), so while MTs may harbor a factor that can stimulate stress fiber formation, the factor is not necessary for the formation of stress fibers.

### Microtubules and polarity determination in yeast: genetics identifies new players

Studies in both budding and fission yeast have implicated MTs in signal transduction pathways that regulate cell polarity. MTs in interphase cells of fission yeast are distributed in a small number of long bundles that run the length of the cell. Disruption of these MTs can cause these cells to grow branches or to become bent [52]. The MT-destabilizing drug thiabendazole causes the actin cytoskeleton to become disorganized and the cell growth protein ral3 to be redistributed from the cell tip to the cell middle [53\*] indicating that proteins involved in cell growth and polarity may be positioned by MTs.

Two candidate effectors that may transduce MT positional cues to establish cell polarity are *tea1p* and *pom1p*. Both

Figure 1



Three basic mechanisms for MT-mediated signal transduction are shown. The signaling factor, represented by a sphere, (a) is shown to interact directly with the MT, (b) via a motor protein or (c) via a putative scaffolding factor. (a) For sequestering and release, three possible release mechanisms are envisioned: (i) signal induced modification (represented by a  $\star$ ) of the factor itself, (ii) of the MT or (iii), enhanced MT depolymerization. (c) The scaffolding factor (represented by a cylinder) is shown to undergo a conformational change upon binding to the MT and this generates a binding site for the signaling factor. Other mechanisms (e.g. post-translational modification) may activate the binding of the scaffolding and signaling factors.

proteins show MT-dependent localization to the cell tips, and *tea1* also localizes to one tip of each MT bundle [54••]. Cells with *tea1* mutations form branches and show polarity defects in the presence of an intact MT cytoskeleton [54••]. Cells with *pom1* mutations also have defects in cell polarity, as well as in placement of the medial division plane [55••]. How these proteins affect cell polarity is unknown; *tea1p* may be delivered by MTs to the cell tip where it anchors *pom1p* and possibly other components of the polarizing machinery. Alternatively, these proteins may regulate the dynamics and/or distribution of MTs, thereby affecting the transport of other polarity proteins. Defects in other factors implicated in cell polarity (*Cdc42*, *Rho*, *orb6*, *pak1*) affect the distribution of MTs, but it remains to be seen whether they generate polarity by regulating MTs.

In budding yeast, disruption of MTs does not seem to affect the pathways leading to cell polarity, mating-projection formation, or bud-site selection. Rather, cytoplasmic MTs seem to function primarily in positioning of the nucleus and the spindle pole body (SPB, the yeast MTOC). The SPB does reorient in response to extracellular pheromone during mating and recent work has shown that *kar9*, a novel protein which localizes to the shmoo tip, can regulate the MT cytoskeleton [56••]. *Kar9* may act by capturing the MT and orienting the SPB.

### Signaling roles for traditional microtubule-associated proteins?

There is abundant evidence that traditional MAPs such as tau, MAP1, MAP2, and MAP4, affect MT assembly and stability. MAPs extend a projection domain away from the MT wall which might act as a scaffold for signaling elements on the MT surface. MAP2 binds to protein kinase A (PKA) and MAP4 binds to cyclin B (see Table 1). But tau is perhaps the best example of such a scaffolding protein. Tau binds to protein phosphatase (PP)1 [57•], *fyn*,

*lck* and *src* tyrosine kinases [58•], and PP2A (Bloom G, personal communication). PP1 binds to the amino-terminal projection of tau and tau is capable of binding PP1 to MTs both *in vitro* and *in vivo* ([57•]; Liao H, Gundersen G, unpublished data). *Fyn* also binds to the amino-terminal projection domain on tau although it is not known whether tau directs *fyn* to MTs [57•]. Whether tau can simultaneously bind both PP1 and *fyn* (or one of the other Tyrosine kinases) remains to be tested, although it appears that their binding sites on tau are different. It is possible that the targeting of these proteins to MTs brings them in proximity to MT bound substrates thereby promoting their activity. MAPs may also convey signaling molecules to the actin cytoskeleton where they have been shown to have a functional role [42••]. PP2A binds to the MT binding domain of tau and appears to prevent tau from binding to MTs (Bloom G, personal communication). This suggests that PP2A may regulate tau phosphorylation when it is not on MTs. Other signaling molecules known to interact with MTs (see Table 2) might also turn out that MAPs form a scaffold that supports interactions of other signaling molecules.

The targeting of phosphatases and kinases to MTs by tau and other MAPs suggests that the MT surface is likely to contain a different subset of these enzymes than that in the cytoplasm. Indeed, studies of Alzheimer's disease — in which tau is hyperphosphorylated — have shown that tau is phosphorylated differently when MTs are present than when they are absent [59,60,61•]. Although this has only been shown for tau, it seems likely to apply to other molecules on the MT surface.

### Mechanisms for microtubule 'signal transduction'

The above examples suggest that MTs and the molecules that interact with them contribute to signal

**Table 1****Effects of agents that break down or stabilize microtubules on signal transduction regulated processes.**

Target/process	MT breakdown	MT stabilization	Mechanism	References
<b>Growth factor stimulation of proliferation (DNA synthesis)</b>	Stimulates			[102,103]
	Stimulates		62–78 kDa MAP PO <sub>4</sub>	[104]
	Stimulates		350 kDa MAP PO <sub>4</sub>	[105]
		Inhibits		[106,107]
	Stimulates	Inhibits	MAPK activation	[108]
	Inhibits	Inhibits	pRb (dePO <sub>4</sub> )/cdk2	[109]
	Inhibits			[110,111]
<b>Signaling pathways</b>				
MAPK (ERK)	Stimulates	Inhibits		[108]
Ras, Raf-1, MEK1/2, ERK1/2 and Src	Stimulates			[112*]
		Inhibits		[113]
Raf-1		Stimulates		[114*]
JNK (Ras and ASK1)	Stimulates	Stimulates		[115*]
Gs	Stimulates		Adenylate cyclase	[34,35]
	Inhibits			[116]
EP4 and EP2 receptors	Stimulates			[36]
nAChR	Inhibits			[117]
Apoptosis pathways	Stimulates	Stimulates		(see below)
<b>Gene expression</b>				
IL-8		Stimulates		[118]
IL-1	Stimulates		Ras pathway	[119]
Apoptosis genes (see below)				
<b>Apoptosis</b>	Stimulates	Stimulates	p53 activation	[114,120]
	Stimulates	Stimulates	WAF-1, Rb, myc	[114,121]
	Stimulates	Stimulates	Bcl-2, PKA activation	[122*]
	Stimulates	Stimulates	Bcl-xL	[123*]
		Stimulates	JNK activation/AP-1	[115,124*]
<b>Directed fibroblast locomotion</b>	Inhibits		Ruffles depolarized	[38–40]
	Inhibits (nM)	Inhibits		[40,125]
	Stimulates (μM)	Inhibits	Lamellipodium formation	[39*]
				[39*]
<b>Chemotaxis</b>	Inhibits			[126–128]
		Inhibits		[129]
<b>Neurite extension</b>	Inhibits			[130–132]
		Inhibits		[131,133]
<b>Cell polarization</b>				
Early embryos	Inhibits			[23*,24*]
<i>S. pombe</i>	Inhibits			[52]
Epithelial cells	Inhibits			[134–136]
Osteoclasts	Inhibits			[137]
T cells	Inhibits			[98]
<b>Actin assembly/contractility</b>	Stimulates			[45,46]
	Stimulates		MLC-PO <sub>4</sub>	[49]
	Stimulates		Rho-dependent	[47,48]

transduction by at least three distinct mechanisms: MT sequestering and release, MT delivery and MT scaffolding of signaling molecules (Figure 1). The binding of CI-Costal 2 and NFκB-IκB to the MT surface may be examples of the sequestering and release mechanism. Activation and release of MT-bound signaling factors could be accomplished by: modification of the factor, modification of the MT and/or breakdown of the MT (Figure 1). The latter two release mechanisms are specific for MTs and there is evidence that MT dynamics might be regulated by signal transduction (see below).

Each of these mechanisms might also work in the reverse direction, to sequester inhibitory factors during signal transduction.

MT-mediated delivery could act either by delivering signaling factors to other components on the MT surface or to specific sites in the cell (Figure 1). MLK2-KIF3 may be an example of the former, and an unidentified motor in the Wnt signaling pathway may be an example of the latter. Activation of this mechanism by signal transduction could occur through enhanced motor-cargo interaction,

**Table 2****Candidate microtubule effectors.**

Class	Process affected	Downstream target	References
<b>MAPs</b>			
MAP1A	Actin gelation <i>in vitro</i>	G-actin/F-actin	[44]
MAP2c	Actin gelation <i>in vitro</i> and <i>in vivo</i>	F-actin	[42**]
MAP4	Stress fibers	?	[138]
Tau	Neurite outgrowth	? (membranes)	[139]
	?	fyn/src/lck	[58*]
	?	Protein phosphatase 1	[57*]
	?	Protein phosphatase 2A	(a)
<b>Motors</b>			
Kinesin	Cell locomotion	? (membrane ruffling)	[140]
Costal2	Gene expression	Complex with fused and Cl	[1**,2**]
KIF3A/KIF3X	Signal transduction	Complex with MLK2	[5**]
<b>Kinases</b>			
PKA	Many	MAP2, metabolic enzymes	[141]
Casein kinase II	Many	?	[142,143]
c-mos	Meiotic spindle	Tubulin?	[144]
GSK-3	Signal transduction	MAPs/tubulin?	[7]
ZAP-70 (and vav)	Signal transduction	Tubulin?	[51]
MAPK	Proliferation/signal transduction	MAPs/tubulin?	[7,8,9*,10]
cdk5	?	MAPs?	[145]
GRK2	Signal transduction	tubulin?	[27**--29**]
c-src	Signal transduction/ruffling	?	[146]
fyn	Signal transduction	Tubulin; tau	[147,148]
Ki-Ras (not Ha-Ras)	Signal transduction	?	[149]
PI 3-kinase	Signal transduction	Tubulin?	[150]
<b>Phosphatases</b>			
PP2A	Signal transduction	Tau, MAPs?	[153] (a)
PP1	Signal transduction	Tau, MAPs?	[57*]
<b>Adaptors</b>			
G proteins			
G <sub>i</sub> /G <sub>α</sub>	Signal transduction	?	[31]
G <sub>q</sub>	Signal transduction		[32]
G <sub>β</sub>	Signal transduction		[152]
G <sub>βγ</sub>	Signal transduction		[30]
NF1	Signal transduction	?	[153]
Cyclin B (cdc2)	Cell cycle	MAP4	[154]
Ash/Grb2	Signal transduction	Tubulin	[155]
Tea1p	Cell polarity	Cell cortex?	[54**]
APC	Signal transduction/polarity	Cell cortex?	[18,19]
EB1/mal3	Signal transduction/polarity	APC/MTs?	[20,21]
dis1	Cell polarity	SPB?	[156]
<b>Transcription factors</b>			
c-myc	Transcription	?	[157]
NF-κB	Transcription	?	[4]
	Transcription	Dynein LC	[3*]
Cubitus interruptus (Ci)	Transcription	Costal 2	[1**,2**]
<b>Tubulin PT modification</b>			
Detyrosination	IF recruitment	Kinesin	[65**,92*]
Acetylation	?	?	[76]
Polyglutamylolation	?	Kinesin?	[64]
Polyglycylation	?	?	[158]
Ser phosphorylation	?	?	[159]
Tyr phosphorylation	?	SH2 proteins, fyn/lck	[148,160]
Δ-2 tubulin	?	?	[162]

IF, intermediate filament. (a) G Bloom personal communication.

enhanced motor–MT interaction, or stimulation of the motor itself. Kinesin polypeptides are known to be phosphorylated and this may regulate their interaction with

cargo [62,63\*]. Post-translational modification of tubulin enhances kinesin binding [64,65\*\*], and may contribute to the specific interaction of kinesin with stabilized MTs.

For MT scaffolding we propose that the MT surface provides a scaffold to promote the interaction of two or more factors which would otherwise not interact. It is possible that interaction of one component with the MT induces a binding site for a second factor (Figure 1) or may simply bring low affinity components into proximity so they can interact. Many of the signal transduction components that interact with MTs appear to involve large multimeric complexes and the assembly of these complexes may be promoted by MTs. The large size of these complexes might also limit their diffusion in the cytoplasm and this raises the possibility that without MTs and MT motors to move them, they might not reach their ultimate targets.

### **Microtubule drug studies: is microtubule signal transduction a widespread phenomenon?**

Drugs that breakdown MTs (e.g. colchicine, nocodazole, vinblastine) and drugs that hyperstabilize MTs (e.g. taxol and taxotere) have specific effects on diverse cellular processes involving signal transduction, for example, cell proliferation, gene expression, receptor signaling, apoptosis, and cell polarization (Table 1). These drugs generally bind specifically to tubulin and have relatively few other direct cellular targets, implying that MTs may be involved in at least some of the signaling pathways regulating these processes. For example, MT depolymerizing agents stimulate proliferation, whereas MT stabilizing agents inhibit proliferation, suggesting some sort of MT-mediated sequestering and release mechanism. Yet, these effects depend on cell type and in some cases, MT depolymerizing agents are inhibitory (Table 1). Thus, either distinct mechanisms are involved in the different cell types, or additional effects of the MT active drugs predominate.

For initiation of cell proliferation and many of the other processes shown in Table 1, it remains to be determined whether the contributions of MTs are direct or indirect. If MTs play a direct role in these processes, it should be possible to identify MT binding proteins that are critical for the affected signal transduction pathways; it will also be necessary to show that the MT interaction is critical for the transduction process. There are many candidate 'effectors' for these processes (Table 2) and it is likely that future studies will show that some of these connect MTs to specific signal transduction pathways.

### **Upstream signaling to microtubules**

MT remodeling during differentiation is well known; however, recent studies show that changes in MT dynamics and organization occur rapidly in response to activation of signaling pathways. This raises the possibility that signal-induced changes in MTs may contribute to the regulation of downstream targets. These rapid responses can be grouped into three categories: changes in MT dynamic instability, changes in MT stability and changes in overall MT organization through changes in MT organizing center (MTOC) position (Table 3). Most intriguingly, recent evidence shows that

these responses can be triggered independently of one another, suggesting that separate signal transduction pathways may regulate distinct aspects of MTs.

### **Signal transduction regulation of MT dynamic instability**

MTs are inherently dynamic polymers that exhibit dynamic instability, which is the tendency of MTs to persist in a growing (or shrinking) phase and infrequently switch to the other phase. The transitions are termed catastrophe and rescue. Dynamic instability of MTs reflects intrinsic properties of the subunit  $\alpha\beta$  tubulin heterodimer and is thought to contribute to the rapid turnover of MTs characteristic of many proliferating and undifferentiated cells. Recent reviews deal extensively with dynamic instability of MTs [66,67]. In cells, dynamic instability is clearly regulated by other proteins that interact with tubulin and MTs, for example, MAPs, motors and other molecules, and each of these are potential targets of signal transduction pathways.

MT dynamic instability is activated during the cell cycle when interphase MTs are rearranged to form the mitotic spindle and some of the molecules responsible for this transition have been identified (see Cassimeris this issue pp 134–141). Far fewer studies have examined changes in response to external stimuli, yet two are noteworthy because they are the first to show that external physiological stimuli can activate specific changes in the parameters of MT dynamic instability [68,69]. The latter study showed that MTs in serum-starved fibroblasts have decreased dynamic instability in comparison to those in cells grown in serum or stimulated by the re-addition of serum. Serum re-addition specifically stimulated MT shrinkage rates and to a lesser extent MT growth rates, but did not induced substantial changes in MT transition frequencies. The serum factor responsible was not identified and to date, no one has measured the effect of specific serum growth factors, such as platelet derived growth factor, epidermal growth factor or insulin, on MT dynamics in fibroblasts or other cells. Hepatocyte growth factor (scatter factor) was shown to increase MT dynamics in MDCK cells, with the largest effect on shrinkage rates [68]. The specific signaling pathway leading to the activated dynamics in both these systems remains to be identified. Indirect evidence for alterations in MT dynamic instability parameters by signaling pathways comes from studies of kinases and phosphatase inhibitors [70,71].

The paucity of studies that have shown direct effects of growth factors and/or cytokines on MT dynamic instability, reflects the difficulty in recording MT dynamics in cells rather than lack of evidence for such regulation. Measurements of dynamic instability *in vivo* usually require micro-injection of fluorescent tubulin, low light level imaging of individual cells and relatively sophisticated image analysis. The development of green fluorescent protein (GFP) tagged tubulin, which has been employed in yeast studies [72], may alleviate this problem somewhat, although whether the GFP moiety affects MT

Table 3

## Upstream signaling to microtubules.

Signal	MT response	System	References
<b>Changes in MT dynamics</b>			
Serum	↑ shrinkage/growth rates	Swiss 3T3 cells	[69•]
HGF/scatter factor	↑ shrinkage/growth rates	MDCK cells	[68]
Staurosporine, olomoucine	↓ shrinkage/growth rates	Epithelial cells	[70]
Okadaic acid	↑ catastrophe, shrinkage/growth rates; ↓ rescue	Epithelial cells	[70,71]
<b>Changes in MT stability</b>			
<i>Increased stability</i>			
LPA, serum	↑ stability of a subset; ↑ pausing	NIH-3T3 cells	[49••]
Focal adhesion	↑ stability	Fibroblasts	[82]
Endotoxin	↑ MT number and stability	Monocytes	[81]
TGF-β, serum	↑ stability of a subset	NIH-3T3 cells	[80]
Insulin	↑ stability	Hepatocytes	[163]
NGF (±dbcAMP)	↑ stability and MT bundles	PC12 cells	[87–89]
<i>Decreased stability</i>			
wnt7a	Loss of stable MTs	Neurons	[16•]
Okadaic acid/calyculin	Loss of stable MTs	TC-7/3T3 cells, neurons	[163,164]
Cell–cell contact	Loss of stable MTs	NRK fibroblasts	[90]
<i>Increased MT polymer</i>			
PMA	↑ MT polymer and number	Macrophages	[83,86]
TRH	↑ MT polymer	GH3 cells	[85]
EGF	↑ MT polymer	Adenocarcinoma cells	[83,84]
Thrombin	↑ MT polymer	Fibroblasts	[83]
<b>MT reorganization</b>			
Binding to Ag presenting cell	MTOC orients, MTs polarize	T Cells	[97,165••]
Binding to target cell	MTOC orients, MTs polarize	CTLs, NK cells	[98,99]
Wounding	MTOC orients, polarized stable MTs	3T3/NRK cells	[90,93]
Wounding	MTOC orients	Endothelial cells	[94,95]
Chemoattractant	MTOC orients	Macrophages	[96,126]
Mating factor	Spindle pole body orients	<i>S. cerevisiae</i>	[51]

CTL, cytotoxic T lymphocytes; EGF, epidermal growth factor; HGF, hepatocyte growth factor; NGF, nerve growth factor; PMA, phorbol myristate acetate; TGF, transforming growth factor; TRH, thyrotropin releasing hormone.

dynamics has not been tested and needs to be established. Whether individual parameters of MT dynamic instability are specific targets for regulation by signal transduction needs to be examined further. Yet, the initial results are encouraging and given the number of uncharacterized changes in MTs in response to signal transduction, it is likely that many more will be uncovered.

### Signal transduction induced changes in MT stability

Stable MTs are easily recognized in cells as they show increased resistance to MT antagonists and usually accumulate one of the post-translationally modified forms of tubulin [73–76]. Earlier work has shown that stabilized MTs exhibit a half-life of the order of hours, rather than the minutes typical of usual dynamic MTs [77,78], and that they accumulate in many cell types during differentiation (reviewed in [79]).

MT stabilization is not restricted to differentiating cells, but occurs rapidly and in direct response to specific extracellular growth factors/mitogens and by specific intracellular signaling pathways. Fibroblasts require a serum factor to generate stabilized MTs [80], and in the past year this factor has been identified as the mitogenic lipid LPA [48••]. The LPA stabilization of MTs occurs in as little as 5 minutes and requires

the activity of the small GTPase, Rho. Constitutively active Val14Rho is sufficient to induce stable MT formation in the absence of external stimuli [50••]. Direct measurements of MT dynamics showed that LPA did not significantly alter dynamic instability parameters, but instead increased the length of pause of a subset of MTs suggesting that Rho acts as a switch between dynamic and stabilized states of MTs. Another study confirmed the increase in MT stability in fibroblasts in response to serum, although a specific increase in pause was not detected presumably because serum, rather than the single factor LPA, was used [69•]. Stimulated MT stabilization has also been reported in monocytes in response to endotoxin [81] and in fibroblasts in response to adhesion [82]. A number of earlier studies have shown that MT polymer levels increase rapidly in response to external stimuli [83–86] and during neurite outgrowth [87–89]. These may reflect increased MT stabilization or may be due to other processes.

Interestingly, MT stabilization can be rapidly downregulated. Cell–cell contact rapidly decreases the level of stabilized MTs in migrating fibroblasts through an unknown signaling pathway [90] and the Wnt signaling pathway appears to regulate MT stability in neurons [16•]. Wnt7 triggered the loss

of stabilized MTs and this was correlated with decreased GSK3 $\beta$  phosphorylation and decreased MAP1B phosphorylation and MT binding (see above).

Changes in MT stability may regulate the availability of signaling molecules that interact with MTs by decreasing the rate at which they are released by MT depolymerization. MT stabilization also increases the post-translational modification of tubulin [73–76,80] and there are at least seven post-translational modifications of tubulin that could potentially serve as ‘markers’ of stabilized MTs in cells (see Table 2). Detyrosination of tubulin has been shown directly to increase the binding of conventional kinesin to MTs *in vitro* [65••] and antibodies to polyglutamylated tubulin decrease kinesin binding to tubulin in blot overlays [64]. In fibroblasts, intermediate filaments are preferentially localized on MTs containing detyrosinated tubulin [91] and this appears to be mediated by kinesin [65••,92]. As detyrosinated MTs form preferentially in the leading edge of migrating fibroblasts and are regulated by a LPA/Rho pathway [50••], this is one case where MTs exhibit a polarized response to signaling and induce polarity in a second cell component, that is intermediate filaments. It will be interesting to see whether other organelles are similarly responsive to changes in MT stability and post-translational modification.

#### Regulation of microtubule orientation and microtubule organizing center position

One of the more intriguing responses of the MT cytoskeleton to signal transduction is the reorientation of the MTOC. Fibroblasts and endothelial cells at the edge of a wounded monolayer [93–95] and macrophages [96] reposition their MTOC to a position between the cell nucleus and the leading edge of the cell. An analogous reorientation of the MTOC occurs in T cells in response to antigen presenting cells [97] and in natural killer (NK) and cytotoxic T cells in response to interaction with target cells [98,99]. In these cases, the MTOC reorients toward the site of cell-cell contact. In all systems, the reorientation occurs rapidly (30–60 min) and may involve polarized delivery of components to specific sites since the Golgi accompanies the reoriented MTOC [98]. Although not apparently involving a MTOC, MTs are rearranged in polarized arrays in *Xenopus* [23•] and zebrafish [24•] embryos and these reorientations may deliver signaling components for the establishment of embryonic axes.

The mechanism behind MTOC or MT reorientation in these systems is not well-understood. MT arrays have the capacity for self-centering in the cytoplasm [100]. This would tend to draw the MTOC to the side of the cell that is most extended, i.e., leading lamella of cells at the wound edge or the portion of the T cell facing its target. However, there is reason to believe that more directed mechanism(s) may be involved. Cytochalasin treatment does not inhibit MTOC reorientation [94–96], even though actin-based cell spreading is blocked. More compelling, is evidence that specific signal transduction pathways may be involved

in MTOC orientation. The adhesion molecule CD2 and a novel adaptor protein, CD2AP are required for MTOC reorientation in T cells binding to antigen presenting cells and may be one of the initial molecules stimulating cytoskeletal reorganization [101]. Downstream effectors in this pathway have not been identified, however, MTOC reorientation toward the APC is blocked with dominant negative and constitutively active CDC42 [97], showing that members of the Rho family of small GTPases are involved. It will be interesting to see whether CDC42 mediates MTOC reorientation in other systems.

It is also not known whether underlying changes in MT dynamics or stabilization are involved in MTOC reorientation. Selective stabilization of MTs toward the wound edge accompanies MTOC reorientation in fibroblasts, but in some cells, stable MTs are oriented even when the MTOC is not (93). However, no studies to date have tested whether MT stabilization is necessary for MTOC orientation or whether dynamic MTs may be involved.

#### Conclusion

With the identification of molecules involved in the Hedgehog, Wnt, and JNK and ERK kinase pathways on MTs, it is becoming more clear that MTs play important roles in a number of signal transduction pathways. Particularly exciting is the discovery of MT motor molecules in complexes with signaling components suggesting that assembly or delivery of large signaling complexes may be achieved by moving factors on the MT surface. New evidence suggests that traditional MT-associated proteins may also have roles in signal transduction. MTs themselves respond to signal transduction and this may be an important aspect of the integration and polarization of signaling pathways. Many other cellular processes are affected by drugs that interfere with MT dynamics and stability and one of the future challenges will be to identify the molecular basis for these effects.

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