Trends in Biochemical Sciences

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Review

Microtubule specialization by +TIP networks: from mechanisms to functional implications

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To fulfill their actual cellular role, individual microtubules become functionally specialized through a broad range of mechanisms. The 'search and capture' model posits that microtubule dynamics and functions are specified by cellular targets that they capture (i.e., *a posteriori*), independently of the microtubule-organizing center (MTOC) they emerge from. However, work in budding yeast indicates that MTOCs may impart a functional identity to the microtubules they nucleate, *a priori*. Key effectors in this process are microtubule plus-end tracking proteins (+TIPs), which track microtubule tips to regulate their dynamics and facilitate their targeted interactions. In this review, we discuss potential mechanisms of *a priori* microtubule specialization, focusing on recent findings indicating that +TIP networks may undergo liquid biomolecular condensation in different cell types.

Highlights

Microtubule plus-end tracking protein (+TIP) networks from various species undergo liquid biomolecular condensation *in vitro*.

The mesoscopic size of microtubule tip-localized +TIP networks in cells presents challenges in categorizing them as canonical biomolecular condensates; however, the recent alignment of correlative *in vitro* and *in vivo* evidence, particularly in budding yeast, along with the model's explanatory power, lends substantial support to this notion.

The liquid condensate model of +TIP networks, the '+TIP-body model', offers a promising framework to advance our understanding of microtubule specialization.

A priori specialization of microtubules by +TIPs in asymmetrically dividing cells

To precisely define what we understand under ***a priori/posteriori* microtubule specialization** (see Glossary) of microtubule function (Figure 1A), let us take an example. The spindle of most asymmetrically dividing cells forms between two **MTOCs**: **centrosomes** or **spindle pole bodies (SPBs)**, depending on the organism. Duplication of these MTOCs produces a newly synthesized, young MTOC on the side of the pre-existing, old one. Strikingly, in many cases, these MTOCs segregate non-randomly at mitosis: one daughter cell reproducibly inherits the old MTOC, while the other daughter inherits the new one [1–4]. This process must support some important function since it is observed in fungi, insect, and mammalian stem cells (reviewed in [5–8]). As we elaborate in this review, non-random inheritance of MTOCs may require the distinct and *a priori* specialization of the microtubules emanating from each MTOC. We start by overviewing how **microtubule +TIPs** mediate SPB inheritance in budding yeast, a well-studied example. We cover the signaling pathways that specialize the SPBs and their associated microtubules by controlling +TIP localization. We then review recent data about how these +TIPs form condensates, and discuss how condensation provides a unifying rationale for how they (i) stably associate with microtubules, and (ii) restrict their localization to only selected ones. Lastly, we discuss how conserved these mechanisms might be, taking fission yeast and animal cells as examples.

The prototypical case of budding yeast SPB inheritance

Non-random MTOC inheritance has been extensively studied in the asymmetrically dividing yeast *Saccharomyces cerevisiae*, a simpler model than animal cells because it displays only a few tubulin isotypes and post-translational modifications [9]. In ~95% of yeast budding cycles, the old SPB segregates to the future daughter cell, called the bud in yeast, while the new one remains in the pre-existing cell (see **bud/mother cell)** [10] (Figure 1B). Non-random inheritance is driven by the few **astral microtubules** nucleated at the SPBs, using actin, which is nucleated in the bud, as a guide [11]. Actin–microtubule interaction is mediated by +TIPs, which bind to the plus ends of astral microtubules, and the myosin V motor Myo2, which moves along actin cables towards the bud tip. The crucial factor is the +TIP Kar9 [functionally similar to the mammalian proteins

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adenomatous polyposis coli (APC), microtubule actin crosslinking factor (MACF), and SLAINs [12–15]] (see Figure 1C for list of players and their homologs in other eukaryotes), which recruits Myo2 to microtubule plus ends [16,17]. Kar9 localizes exclusively to the astral microtubules emanating from the old SPB, persisting on them through their polymerization and depolymerization cycles. Kar9 binds microtubules via the +TIPs Bim1 and Bik1. Bim1 belongs to the end-binding (EB) family of proteins, binding preferentially the **GTP cap** of microtubules [18]. Bik1 is orthologous to the cytoplasmic linker protein 170 (CLIP-170) in mammals [19–21]. Exploiting the asymmetry of Kar9 localization, Myo2 moves, aligns, and orients the pre-anaphase spindle such that the old SPB faces the bud (Figure 2A). Thus, Kar9 functionally specializes the astral microtubules emanating from the old SPB, *a priori*.

Thus, *a priori* specialization requires that the localization of the specificity determinant, here Kar9, is restricted to specific microtubules already when they emerge from the MTOC. Kar9 initially decorates microtubules on both sides of the spindle [22], but this symmetry breaks as the spindle reaches its initial resting length of ~1.5 μm [23]. From then on, Kar9 localizes exclusively to growing and shrinking microtubules emanating from the old SPB, until sometime in anaphase when it localizes symmetrically again. Kar9 interaction with Myo2 and the actin cytoskeleton, its phosphorylation, and its sumoylation all contribute to symmetry breaking [24–31]. However, neither one alone, or any combination of them, is essential for this process. Destabilizing actin cables or inactivating Myo2 dampens Kar9 asymmetry which, however, remains biased towards the old SPB [24,32]. Thus, Kar9 specializes the old SPB microtubules *a priori*, indeed, before they encounter actin cables. Myo2 enhances this asymmetry potentially by bringing Kar9 decorated plus ends into the bud [22], *a posteriori*. Both the cyclin-dependent kinase Cdk1/Cdc28 and the SPB-associated LATS kinase Dbf2 phosphorylate Kar9 [26–28,31]. Preventing Kar9 phosphorylation by Cdk1 slows down symmetry breaking but neither prevents it nor randomizes it relative to SPB age [26,31]. Furthermore, since Cdk1 is recruited to microtubule tips by Kar9 itself, its own asymmetry depends on that of Kar9 [28]. Thus, Cdk1 reinforces Kar9 asymmetry but it is not its primary determinant. Preventing Dbf2 from phosphorylating Kar9 randomizes asymmetry relative to SPB age, but does not much decelerate symmetry breaking. The robustness of Kar9 symmetry breaking suggests that it is an autonomous process.

The restriction of Dbf2 activity to the new SPB is enforced by the **SPB inheritance network (SPIN)** prior to anaphase [5,31,33]. Through the activity of two kinases and an acetyltransferase (Figure 1B,C), the SPIN marks the pre-existing SPB as being old [33]. Without this event, new and old SPBs behave interchangeably. The GTPase-activating proteins for Tem1, Bub2 and Bfa1, read the SPIN marks to inhibit Dbf2 on the old SPB. Genetic inactivation of SPIN does not impair Kar9 asymmetry but randomizes it. Interestingly, the SPIN components are conserved in metazoans (Figure 1C), where they localize to centrosomes [34–36]. Furthermore, the SPIN targets – such as Nud1/centriolin [37] and Spc72/centrosomin [38] – are among the few factors conserved between centrosomes and SPBs. Thus, non-random MTOC inheritance might be governed by similar principles from yeast to humans [5,33]. The key role of the Kar9-related protein APC in spindle orientation in male germline stem cells of *Drosophila melanogaster*, where it localizes to only one side of the spindle, supports this notion [39]. Furthermore, APC also localizes to centrosomes in mammals, where it associates preferentially with the mother centriole [40] and contributes to spindle orientation in gut epithelial stem cells [41]. Thus, spindle orientation might rely on *a priori* microtubule specialization in all these systems.

Interestingly, Kar9's binding partners Bim1 and Bik1 never localize fully asymmetrically. Like EBs and CLIP-170 in other organisms, they localize to many if not to all microtubule ends. We refer to them as 'ubiquitous' +TIPs, and those that like Kar9 are restricted to specific microtubules as

Glossary

***A priori*/*posteriori* microtubule specialization:** distinguishes whether a microtubule's function, destination, and interactions are predetermined (i.e., by the MTOC) before it encounters a target (*a priori*) or whether it remains undetermined until – and is thus only specialized when – it encounters a target (*a posteriori*).
Astral microtubules: microtubules emanating from the spindle poles and directed towards the cell periphery.
Biomolecular condensate: inclusive expression for phase-separated structures of a liquid, viscoelastic, or solid nature in a biological context, that is, relatively homogeneous entities formed from many copies of one or a few scaffolding molecules (proteins and/or nucleic acids) and client molecules that partition into the condensate without contributing to its formation.
Bud/mother cell: in asymmetrically dividing budding yeast, a smaller daughter cell – called a 'bud' – buds off of the larger pre-existing mother cell.
Cellular bodies: loosely defined cellular structures identified by microscopy with the aid of fluorescently labelled components, presenting as compact, well-identifiable entities. The term 'body' makes no claims about molecular architecture.
Centrosome: the main MTOC in most proliferating animal cells. It contains a centriole or pair of centrioles surrounded by a pericentriolar matrix.
Comet: +TIPs in metazoans, which track growing but typically not shrinking microtubule ends, appear as comets in time-lapse fluorescence microscopy movies. Comets reflect the presence of the underlying GTP cap in a growing microtubule, and are thus brightest at the microtubule tip with fading intensity along the lattice.
GTP cap: a short region of the microtubule plus end, which has not yet hydrolyzed GTP within β-tubulin.
Microtubule-organizing center (MTOC): a structure which nucleates and anchors microtubules via the γ-tubulin ring complexes, thereby organizing the microtubule cytoskeleton. Examples of MTOCs are SPBs in budding yeast, centrosomes, and the Golgi apparatus in animal cells.
Microtubule plus-end tracking proteins (+TIPs): proteins that bind preferentially to the plus ends of microtubules, either directly or indirectly via other +TIPs.

'patterning' +TIPs [15]. Together, these +TIPs form biochemical protein networks at microtubule plus ends through multiple site-specific, medium- to low-affinity interactions (reviewed in [42,43]). In budding yeast, these interactions assemble Kar9, Bim1, and Bik1, and likely the microtubule polymerase Kip2 [19,20], into a cohesive yet dynamic **+TIP body** of diffraction limited and variable size (Box 1). This body persistently tracks the plus ends of both growing and shrinking microtubules it localizes to, despite Bim1's preference for the GTP cap of growing microtubules. With our current understanding of biochemical protein networks, these observations have introduced two questions. First, what is the biophysical basis for the +TIP body persisting at the plus ends of growing and shrinking microtubules and withstanding and transmitting the forces generated by myosin and microtubule shrinkage? Second, how is the +TIP body autonomously restricting its localization to only one side of the spindle? This second point seems at odds with the first one: it is unintuitive that a persistent structure would stay attached to only some and not to all microtubule tips of the same cytoplasm.

The budding yeast +TIP body as a liquid condensate

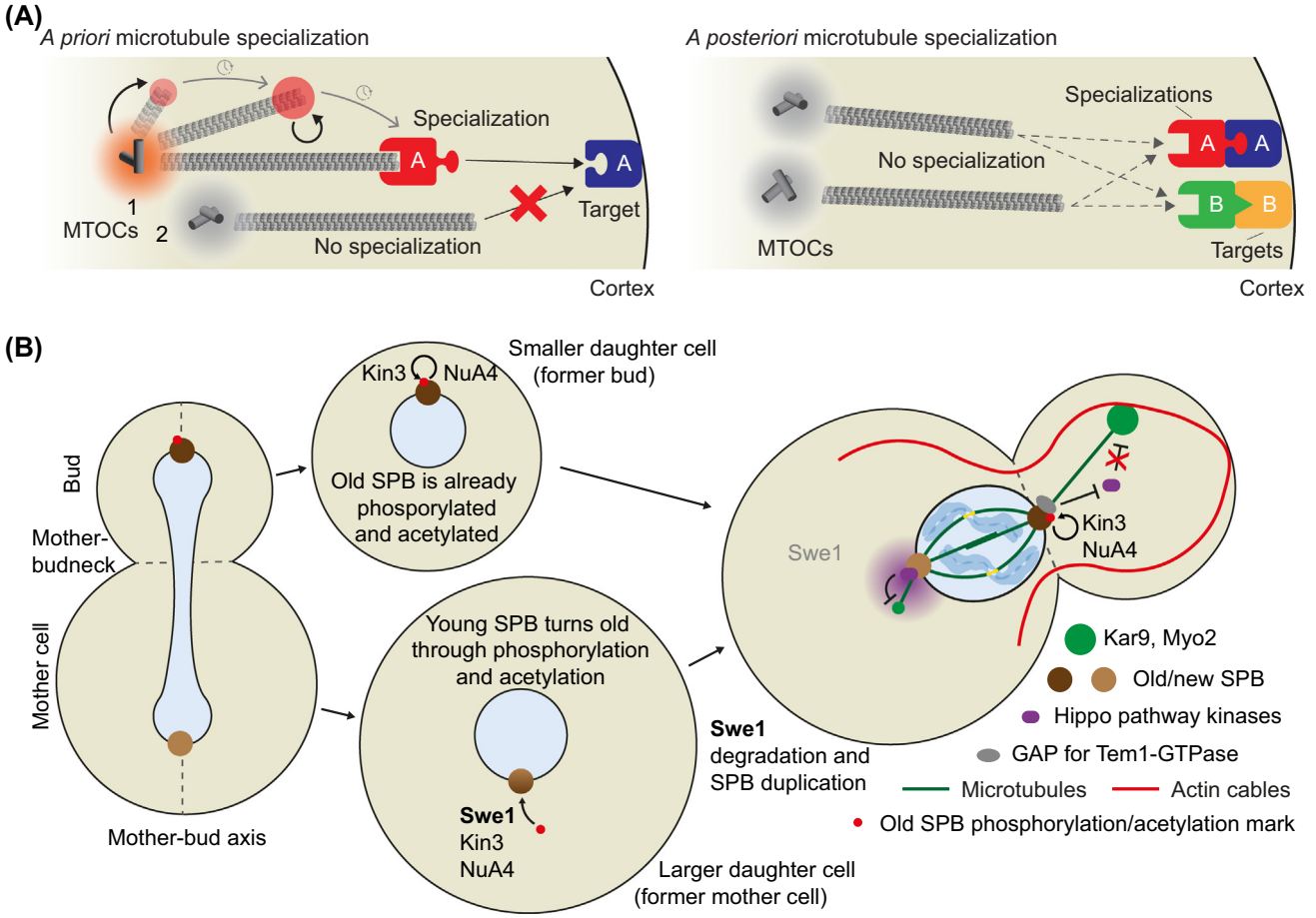
Current progress on the biochemistry, biophysics, and structural biology of Kar9, Bim1, and Bik1 offers a unique framework to address how the body persists at growing and shrinking microtubule plus ends. Recent studies have emphasized that many proteins phase separate *in vitro* into **biomolecular condensates**, and that at least some of them indeed function as such *in vivo* [44–47]. Accumulating hints suggest that this is the case for Kar9, Bim1, and Bik1, as we discuss in the following.

When mixed together *in vitro*, Kar9, Bim1, and Bik1 condense into a viscous liquid at micromolar concentrations, without the need for a crowding agent [48]. The multiple homo- and heterotypic binding interfaces (Figure 3A) linking Kar9 (Figure 3B–D) [15,48], Bim1 (Figure 3E,F) [49], and Bik1 (Figure 3G) [29,50–52] to themselves and to each other form a dense, multivalent network of site-specific, medium- to low-affinity interactions (i.e., dissociation constants in the micromolar to millimolar range) driving condensation *in vitro*. Importantly, these interfaces function redundantly with each other: erasing any of them individually barely affects condensation. However, abrogating at least six of them together substantially does [48]. Thus, multivalency drives a cooperative assembly process most parsimoniously described as condensation, at least *in vitro* (Box 1).

Supporting the idea that these three proteins function as a condensate *in vivo* as well, abrogating the interfaces linking Kar9, Bim1, and Bik1 impairs their cellular functions [48]. Importantly, these interfaces have little impact by themselves on +TIP body formation, its size, localization, and on-spindle positioning and cell viability [48]. By contrast, mutating increasing numbers of interfaces caused the +TIP bodies to become smaller, unstable, and temperature-sensitive. These bodies increasingly detached from shrinking microtubule ends and, ultimately, spindle positioning frequently failed [48]. Introducing artificial binding interfaces corrects many of these defects [15], indicating that the endogenous interfaces are not required for establishing a defined structure but simply for bringing partners together. These observations are in stark contrast with stoichiometric macromolecular complexes where individual interaction interfaces have specific localizations within the complex, fulfilling specific architectural and functional requirements rather than being redundant with each other and replaceable. Thus, the assembly, persistence, and function of the +TIP body at microtubule plus ends *in vivo* (see Box 1 for criteria) may rely on the cohesiveness provided by liquid–liquid phase separation (Figure 3H).

Further observations strengthen the notion that the +TIP body is a condensate *in vivo*. Like liquid droplets, but unlike macromolecular machines, its size is determined under physiological conditions by the amount of material available in the cell. It follows the number of copies of the *KAR9* gene as the cells progress through DNA replication or become diploid [48]. It increases further

Ostwald ripening: a process leading to growth of the biggest condensate at the cost of smaller ones due to the energetically unfavorable higher surface area:volume ratio in smaller condensates. This process is driven by surface tension and is considered to be slow, especially with increasing condensate size.
SPB inheritance network (SPIN): the signaling cascade that writes and maintains the molecular marks encoding SPB age and drives age-dependent SPB inheritance in budding yeast.
Spindle pole body (SPB): MTOCs in budding yeast that are integrated into the nuclear envelope, which stays intact throughout the cell cycle.
+TIP body: a membrane-less structure ('body') formed by +TIPs at the plus end of a microtubule.
Wetting: the process of a liquid spreading on a surface with which it favorably interacts. Depending on the relative energy costs of its own surface and the one at the interface with the underlying surface, wetting can be partial or total.



(C)

Budding Yeast	Fission Yeast	Mammals	Protein Family or Function
Bim1	**Mal3**	**EB1, EB2, EB3**	End binding protein
Bik1	**Tip1**	**CLIP170**	CAP-Gly protein
Kar9		shares features with **APC, MACF, SLAIN1, SLAIN2**	SxIP protein
Myo2	Myo52	MYO5A	Myosin V
Kip2	**Tea2**		Kinesin
Kel1/2	**Tea1**		Kelch domain proteins
Swe1	Wee1	WEE1	Wee1 Ser/Thr kinase
Kin3	Fin1	Nek2	Ser/Thr kinase
NuA4 complex	NuA4 complex	NuA4 complex	Acetylase complex
Nud1	Cdc11	Centriolin	MTOC component
Tem1	Spg1		Ras-type GTPase
Cdc28	Cdc2	CDK1, CDK2, CDK3, CDK4, CDK6	Cyclin dependent kinase
Dbf2	Sid2	LATS1, LATS2	Hippo pathway Ser/Thr kinase

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Figure 1. *A priori* microtubule specialization in budding yeast and in general. (A) *A priori* versus *a posteriori* microtubule specialization. A microtubule is specialized before it encounters its target (*a priori*, left). In this example, only the orange microtubule-organizing center (MTOC) promotes the deposition of a specializing factor (red) for interaction with target A (blue). Over time, this factor accumulates on attached microtubules and specializes them to bind target A, while microtubules of the gray MTOC cannot. If no specialization is provided before and needed for interaction with different targets A or B, microtubules may be specialized upon interaction with a target (*a posteriori*, right). In this case the specializing factors A and B are provided by the targets and all new microtubules are thus equally likely to interact with each target.

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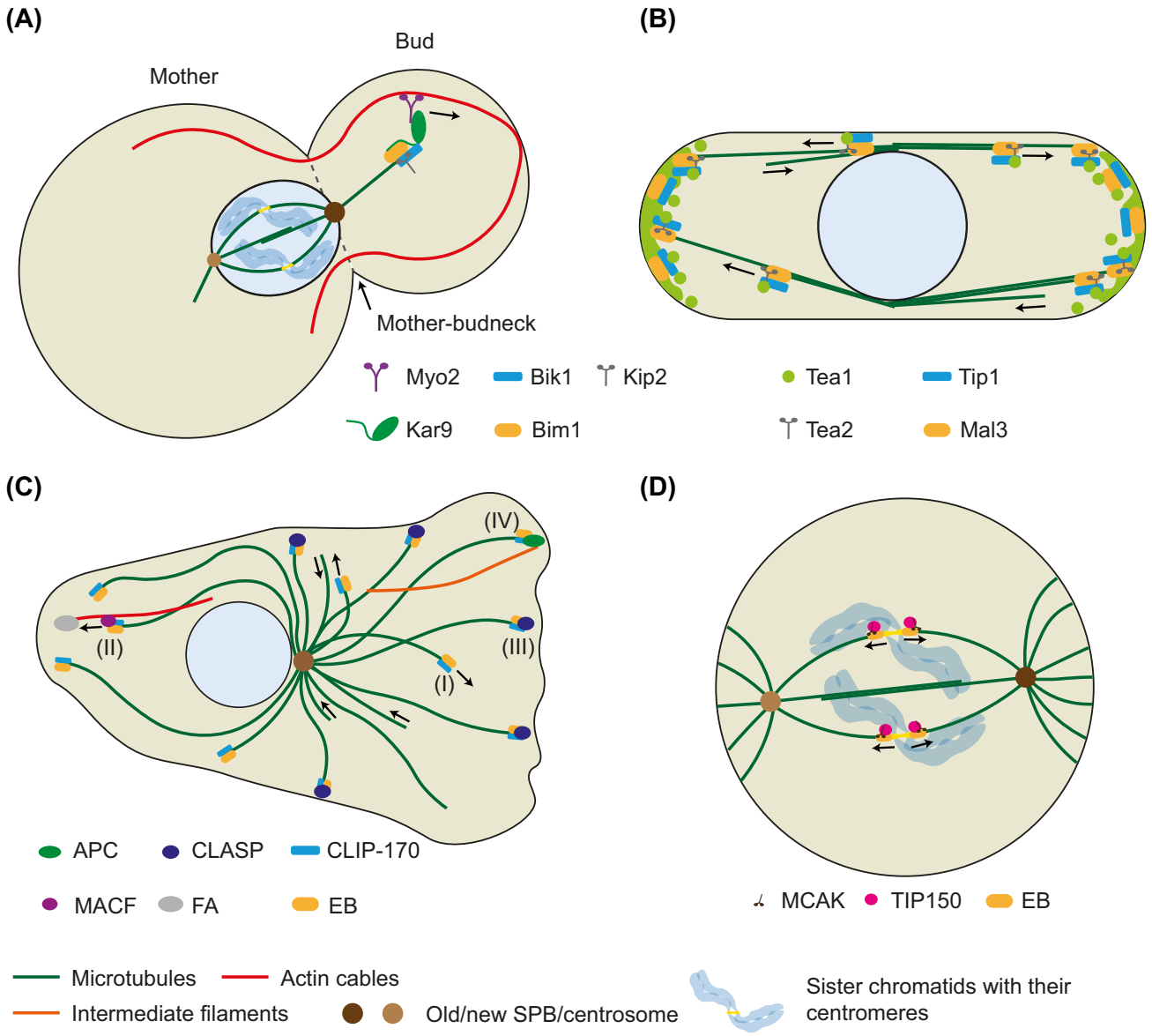

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Figure 2. Plus-end tracking protein (+TIP) networks in different cell types and cellular processes. (A) The budding yeast Kar9-mediated +TIP network involved in spindle positioning and alignment and age-dependent spindle-pole inheritance. (B) The fission yeast +TIP network delivering Tea1 to cell ends to label them as such and locally promote growth. (C) Different +TIP networks in interphase mammalian cells: (I) end-binding (EB)/CLIP-170 promoting microtubule growth towards the cell periphery; (II) microtubule actin crosslinking factor (MACF) guiding a microtubule along actin cables towards a focal adhesion (FA); (III) polarized localization of cytoplasmic linker-associated proteins (CLASPs) to microtubule plus ends at the leading edge; (IV) adenomatous polyposis coli (APC) protein interacting with intermediate filaments at the leading edge. (D) TIP150 involved in building up interkinetochore tension. Abbreviation: SPB, spindle pole body.

(B) The spindle pole body (SPB) inheritance network (SPIN) controls the fate of SPBs during budding yeast cell division. While the newly formed bud generally receives the SPB that is already marked as old by the SPIN, the mother cell receives a young unmodified SPB. After cytokinesis, expression of the Swe1 kinase in G1 of the former mother cell allows initial phosphorylation of the young SPB that is turning old. Swe1 disappears before the newly forming SPB is fully assembled and thus cannot modify it. The downstream kinase Kin3 and the acetylase NuA4 stay active in the former mother and bud and maintain the 'age mark' until the next cell division. During the next division, this mark on the old SPB recruits the GTPase-activating protein (GAP) for the Tem1 GTPase, which locally turns off the downstream Hippo pathway while staying active around the new SPB and therefore inhibiting Kar9 there. (C) The budding yeast proteins involved in astral microtubule specialization and their homologs in fission yeast and mammals.

upon Kar9 overexpression. Moreover, similarly to liquid droplets, +TIP bodies fuse with each other when they meet [48]. However, due to their size around the diffraction limit, it is not possible to establish whether +TIP bodies are *bona fide* liquids using standard microscopy techniques (Box 1). Their small size prevents characterizing internal diffusion and their morphology. Also, there is too little Kar9 in the cytoplasm to measure exchange dynamics with the dispersed phase. Importantly, whether this +TIP body is liquid or not remains an academic question if it has no functional implications [45]. And here, functional considerations that we elaborate on below do make the liquid condensate hypothesis very attractive.

Comparing and contrasting different models of +TIP network architecture

To underline the appeal of the liquid condensate hypothesis, let us consider alternative models of +TIP network architecture. While it is well established that +TIPs assemble via multiple specific interaction sites into dynamic protein networks across eukaryotes (reviewed in [53]), the architecture of these networks is not well understood. Certainly, the strong cooperativity driving +TIP body assembly in budding yeast (described earlier) excludes that stoichiometric Bim1–Bik1–Kar9 complexes bind microtubule ends independently of each other (Figure 4A). Thus, the +TIP body could instead be formed of multimers of a stoichiometric Bim1–Bik1–Kar9 complex: upon crystallization, the N-terminal domain of Kar9 assembles into filaments [15] (Figure 4B). If full-length Kar9 also forms filaments, these could align with the microtubule lattice (Figure 4C) or bend to form a ring around it (Figure 4D), like the Dam1/DASH complex at kinetochores [54]. This could account for its strong affinity for and persistence on both growing and shrinking microtubule ends. However, inactivating any of the involved interfaces would abrogate filament formation and the assembly and function of the +TIP body. Losing several of them simultaneously would not worsen that defect. However, these predictions are at odds with the data. Mutations

Box 1. Bodies and biomolecular condensates

'Cellular bodies' are loosely defined cellular structures, identified by microscopy with the aid of fluorescently labelled components, presenting as compact, well-identifiable entities. The term 'body' makes no claims about molecular architecture. The term biomolecular condensate (short: condensate) was introduced as an inclusive term for phase-separated structures in a biological context where 'condensation' is generally driven by more or less specific interactions among the constituent biomolecules, mediated by multiple medium- to high-affinity interfaces (multivalency) among them. As originally described for homogeneous systems in classical physics, they adopt one of different possible organizational phases: liquid, gel, glass, or solid [46,99]. Such condensates include the nucleolus, germline P granules, or P-bodies, which are micrometer-sized. Liquid condensates of similar composition tend to fuse with each other and exponentially relax to a spherical morphology, indicating their fluid nature and the emergence of mechanical surface tension (Box 2). They accumulate molecules that partition to them and exclude those partitioning out.

Mesoscale condensates include the central pore of the nuclear pore complex, Cajal bodies, chromocenters, and synaptic densities. Their size being close to or below the diffraction limit of light microscopy, they are more difficult to categorize. Therefore, rather than morphology, the characteristics that can be used to distinguish them from other structures are the following: (i) mesoscale condensates are formed by many copies of few scaffolding molecules that locally concentrate in them; (ii) they form by multivalent networks of redundant and replaceable, more or less specific interactions; (iii) the flexible geometry of the involved interaction interfaces relative to each other and their dynamic behavior supports the formation of infinite assemblies; (iv) they lack a fixed stoichiometry, size, and regular structure; this distinguishes them from structured assemblies such as protein complexes and molecular machines (e.g., the ribosome), which are composed of a well-defined, stoichiometric number of copies of each component, and where all the interaction interfaces required for assembly are unique, not interchangeable, and functionally non-redundant; and (v) liquid mesoscale condensates keep the ability to fuse, while fission is unfavorable and less frequent.

Using the following criteria, these characteristics are testable, provided that sufficient structural knowledge is available: (i) fluorescently labelled participating macromolecules form puncta or patches observable by microscopy, (ii) these puncta or patches have a broad intensity distribution and thus variable sizes, (iii) although this might be limited by Pickering mechanisms [100–103], they show the ability to fuse when they encounter each other, (iv) progressively reducing the underlying interaction multivalency (intra- and inter-molecular interactions among scaffold components) using mutagenesis synergistically prevents their formation or reduces their size while increasing the cytoplasmic concentration of the components; multivalency is driven by a multiplicity of site-specific interactions of low to medium affinity and may involve folded as well as disordered domains [45], (v) phenotypic effects due to multivalency reduction can be compensated by introducing synthetic interaction sites, and (vi) their appearance is sensitive to temperature [104–107].

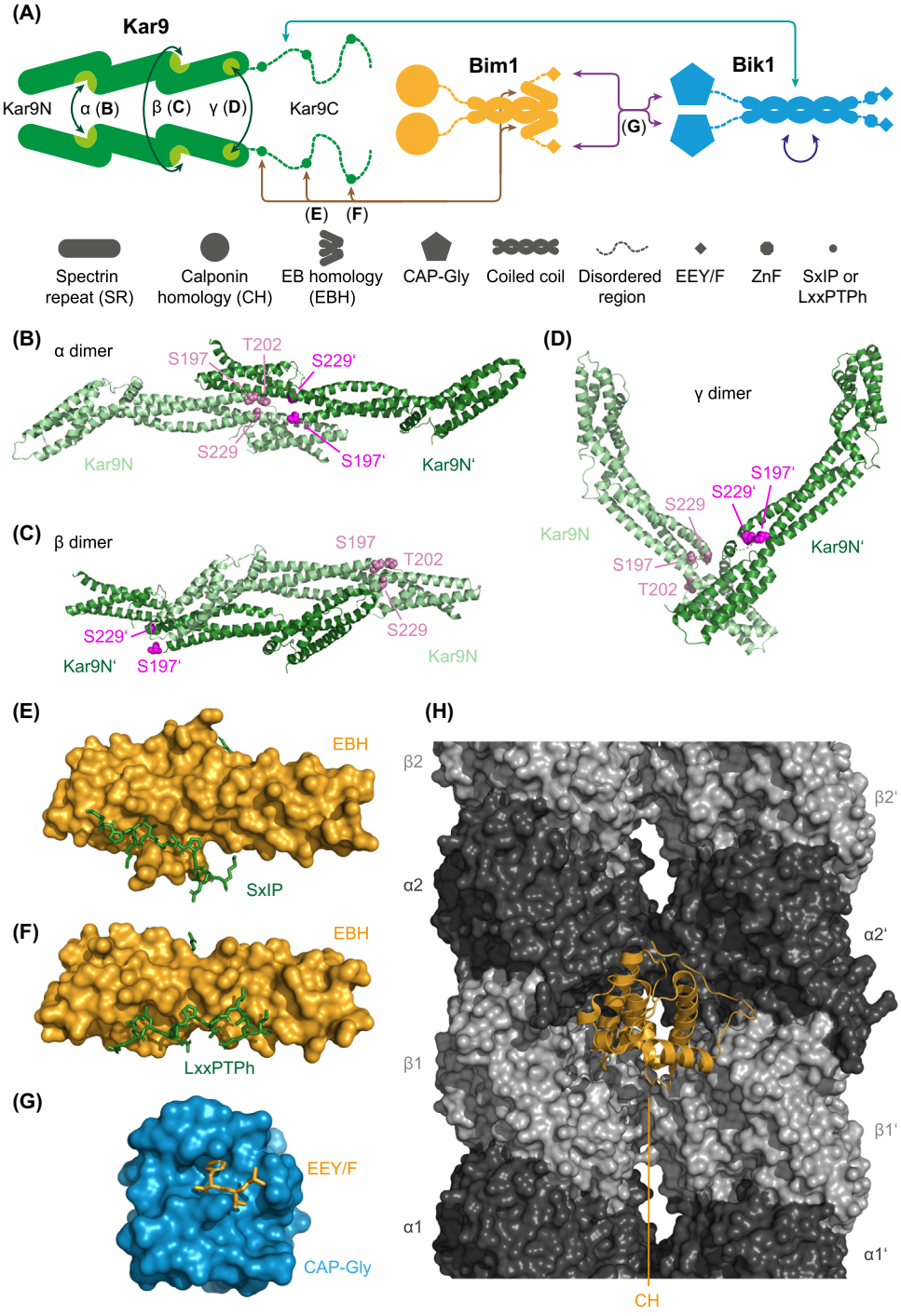

Figure 3. Relevant interactions for plus-end tracking protein (+TIP) network assembly. (A) Schematic drawing of known specific interactions between the core budding yeast +TIP network components Kar9, Bim1, and Bik1. The structural information on the schematized interactions is shown in panels (B–G) (corresponding panel letters are indicated). Adapted from [48]. (B–D) The three crystallographic dimers of the Kar9 N-terminal domain (PDB ID 7AG9) [15,48] with indicated CDK/Dbf2 phosphorylation sites (magenta). (E,F) Linear SxIP and LxxPTPh motifs of microtubule actin crosslinking factor

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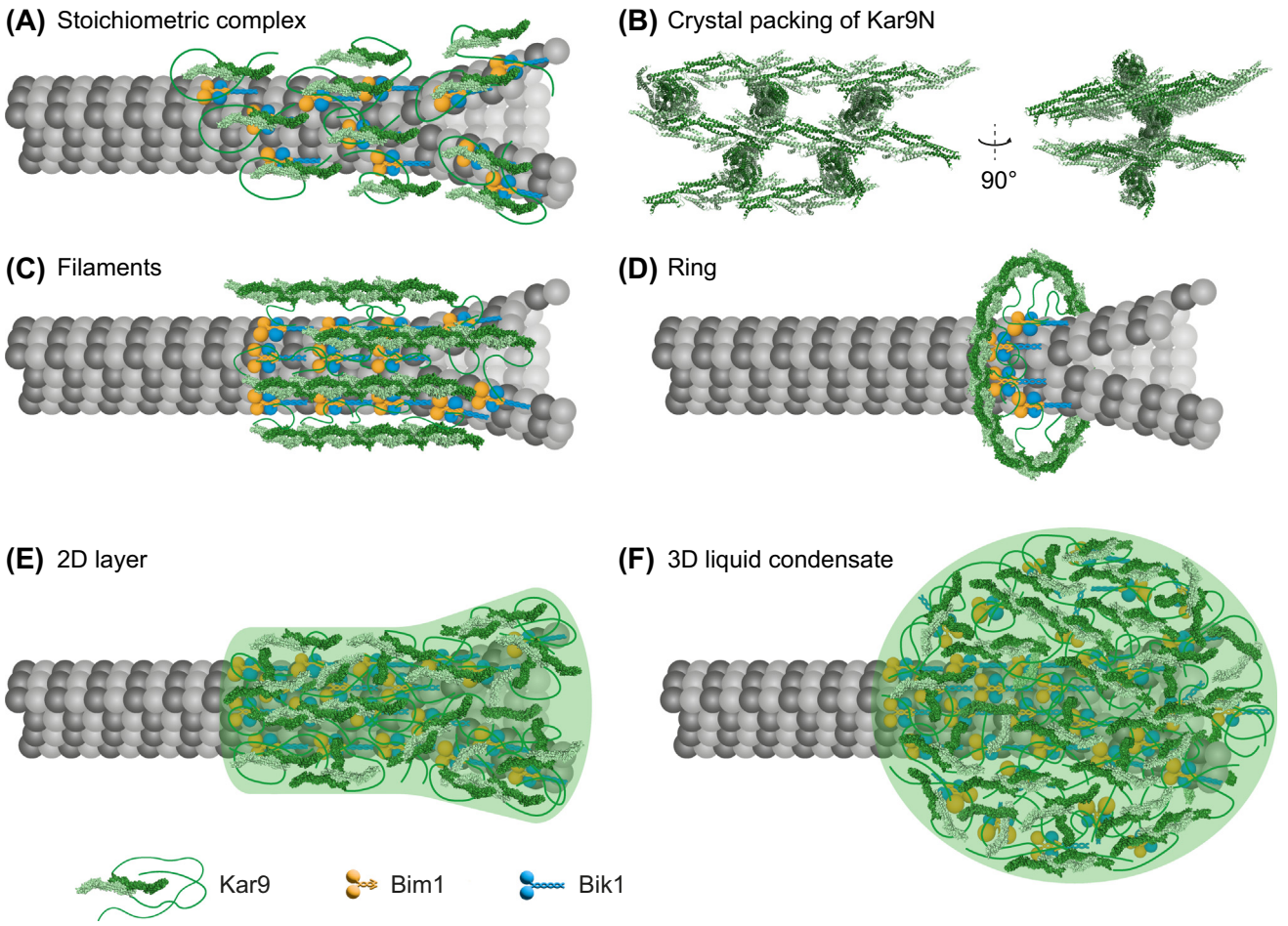

Figure 4. Different models for Kar9 plus-end tracking protein (+TIP) network architecture at microtubule plus ends. (A) Stoichiometric Kar9–Bim1–Bik1 complexes individually associating with the microtubule surface. (B) Crystals formed by the N-terminal domain of Kar9 (Kar9-N) reveal the formation of filaments [48] (PDB ID 7AG9). (C,D) Filaments of Kar9 aligning with the microtubule lattice or bending around it to form a ring. (E) A 2D multivalent layer of +TIPs where each molecule of Kar9 is directly connected to the microtubule via Bim1 and Bik1. (F) A 3D liquid condensate in which Kar9 can be connected to the microtubule lattice indirectly via several multivalent interactions.

affecting these interfaces cause strong phenotypes only when combined [48]. Thus, the formation of filaments and rings seem excluded as well.

As another alternative, the interaction of Kar9 with itself and with microtubule-bound Bim1 and Bik1 could lead to the assembly of a 2D layer on the shaft of microtubules (Figure 4E). Such an architecture would not have a fixed size and stoichiometry and could rely on multivalency. In fact, a 2D layer and a 3D condensate (Figure 4F) are related structures: if the surface tension is higher than the microtubule surface affinity, a condensate would round up and form a 3D structure. If, rather, the surface tension is low, the condensate would totally wet the microtubule surface, forming a 2D layer (Box 2). However, the 2D model has several drawbacks. First, a totally **wetting** condensate

(MACF) (E, green) and Kar9 (F, green), respectively, bound to an end-binding (EB) homology domain (yellow; PDB IDs 3GJO and 5N74, respectively) [108,109]. (G) CAP-Gly domain of Bik1 (blue) bound to the C-terminal EEY/F motif of Bim1 (yellow; PDB ID 6FC6) [50]. (H) Calponin homology (CH) domain of EB (yellow) binding to the microtubule lattice at the longitudinal interdimer interface and between two neighboring protofilaments (α-tubulin dark gray, β-tubulin light gray) (PDB ID 3JAK) [110].

Box 2. Emerging properties of liquid condensates

Why does it matter whether a complex web of physical interactions among various proteins within a biological system (like +TIPs networks at microtubule plus ends, reviewed in [42,43]) form a liquid condensate or some other structure? When macromolecules assemble into liquid condensates, the following new collective properties emerge which are absent in the monomers. As first-order properties, condensates generate a surface and a bulk phase. Both are distinct microenvironments that have the potential to enrich different clients by partitioning. Since favorable interactions between the components of liquid condensates are generally restricted at the surface compared with the bulk, it comes at an energy cost that results in surface tension. The same interactions between condensate components likely slow down their diffusion in the bulk, manifesting in increased viscosity or, depending on the longevity and valency of interactions, in viscoelasticity.

These first-order properties create second-order functional properties. (i) Surface tension promotes minimization of surface area and therefore drives condensates to a spherical shape; this is particularly obvious during the fusion of two condensates of similar composition, and while fusion is thus a favorable process, fission cannot happen spontaneously without involving active processes. (ii) Since larger condensates have less surface area relative to their volume, larger ones are energetically more favorable than smaller ones. Therefore, the saturation concentration [the concentration of the condensing macromolecule(s) in the dilute phase] is a function of the size of the biggest condensate in the system and gradually decreases while that condensate grows. As a result, all smaller condensates that require a higher dilute phase concentration for homeostasis dissolve, while the biggest grows until it is left alone. This process is called Ostwald ripening and has the potential to function as a symmetry breaking mechanism in biological systems. (iii) Condensates can wet other surfaces, either solid or liquid ones. Depending on the relative energetic costs (or surface tensions) of the different surfaces, wetting is absent, partial, or total. In total wetting, a condensate with high affinity for the wetted surface relative to its surface tension spreads into a 2D monolayer. In partial wetting, affinity and surface tension are in balance, and thus surface tension partially maintains the spherical character. In biological systems with many components, their concentrations at different surfaces are likely to vary with their individual affinity for them. (iv) Surface tension and viscoelasticity allow condensates to maintain their shape against mechanical forces that act on them. This, combined with their capacity to wet surfaces, makes them potential 'glues' that could efficiently mediate mechanical coupling.

would require its components to bind the microtubule lattice strongly to support mechanical forces applied on them. Their lateral diffusion would be slow, impeding the body's ability to track growing and shrinking microtubule plus ends, particularly given that Kar9 exchanges little with the cytoplasm [26]. It would also impede the passage of motor proteins towards the microtubule plus end, such as the kinesins Kip3, a microtubule depolymerase [55], and Kip2, a microtubule polymerase [56]. Finally, upon addition of extra material, a 2D layer could grow only by extending along the microtubule shaft, unlike what is actually observed: overexpressed Kar9 remains focused in a punctum of increased size [48]. Thus, the 2D layer model is neither very plausible nor supported by observation.

By contrast, a 3D model solves several biophysical problems, simply drawing from the emerging properties of liquid condensates (Box 2). (i) Droplets offer their bulk for rapid exchanges between the front and the rear of the body as the microtubule grows or shrinks. (ii) Their surface tension provides forces for keeping the body together as it tracks dynamic microtubule ends or as forces are exerted on it. (iii) Their microtubule wetting forces, mediated by Bim1 and Bik1 and their affinity for the microtubule shaft, would prevent them from dropping off shrinking microtubules. (iv) The increased affinity of Bim1 for the GTP cap would help the body to track the plus ends of growing microtubules. Thus, the wetting behavior and surface tension of liquids add attractive and simple biophysical rationales to the list of mechanisms through which +TIPs might track microtubule plus ends during both growth and shrinkage [57]. In conclusion, based on the existing *in vitro* and *in vivo* data, the 'liquid condensate model' currently most parsimoniously explains the persistence of the +TIP body at microtubule ends, which would function as a sophisticated 'bio glue' able to transmit forces between actin and microtubules.

Functional implications of the liquid condensate model

An important strength of the liquid condensate model is, in our opinion, that it can explain symmetry breaking as well. An emerging property of liquid–liquid phase separated condensates is their coarsening by fusion and **Ostwald ripening**, two processes driven by surface tension (Box 2) [44]. This ultimately leads to a reduction in the droplet number over time. Thus, the liquid condensate model

could also explain why the +TIP body decorates only a few microtubules. Supporting this view, reducing the multivalency of interactions in the +TIP body also caused it to decorate more microtubules, on both sides of the spindle, suggesting that condensation indeed contributes to symmetry breaking [48]. This could also explain how phosphorylation promotes and biases Kar9 asymmetry towards the old SPB: the phosphorylation sites are close to the identified dimerization interfaces in Kar9's folded domain and are likely to affect them negatively (Figure 3B–D) [15]. Supporting this notion, phospho-mimicking mutations impair Kar9 self-interactions, increase the cytoplasmic concentration of Kar9, and can even dissolve the +TIP body [15]. Thus, cycles of phosphorylation (CDK/Dbf2) and dephosphorylation (by an unidentified phosphatase) would promote material exchange between coexisting +TIP bodies, speeding up Ostwald ripening and symmetry breaking. Moreover, the higher activity of Dbf2 on the new SPB would bias +TIP body formation to the opposite spindle side, similar to the way in which *Caenorhabditis elegans* oocytes polarize their P granules [58,59].

Thus, the combination of wetting, viscoelasticity, and surface tension offers a simple rationale for both how a cohesive +TIP body transmits forces between actin and microtubules, and how it localizes to only few specific microtubules. On the cohesion side, the body's wetting energy and surface tension would underpin its persistence. On the specificity side, surface tension now promotes symmetry breaking, while its high affinity for the GTP cap ensures that this body forms on microtubule tips. Thus, key emerging properties of liquid condensates could explain many, even sometime seemingly contradictory aspects of the behavior observed with the budding yeast +TIP body.

The liquid condensation of +TIPs is conserved in fission yeast

Interestingly, +TIP networks also seem to operate as liquid condensates in other organisms. Many +TIPs are structurally conserved across eukaryotes [60], and +TIP networks of fission yeast and human cells undergo liquid–liquid phase separation *in vitro.* Several hints suggest that they also do so *in vivo* [61–63]. Strikingly, however, although homologous with the budding yeast +TIP body, these condensates show distinct material properties adapted to their different cellular functions.

The ubiquitous +TIPs of fission yeast Mal3 (EB), Tip1 (CLIP-170), and the kinesin Tea2 (Figure 1C) – by themselves and in combination with each other – form liquid condensates *in vitro* that track the plus ends of reconstituted microtubules [61]. Cryoelectron tomography shows that in the absence of a microtubule, Mal3, Tip1, and Tea2 form a smooth spherical condensate, consistent with a liquid state with a robust surface tension. On microtubules, the condensate spreads along the lattice and extends for tens to hundreds of nanometers from the microtubule surface, consistent with a 3D, microtubule-wetting condensate [61]. However, its surface is rough rather than smooth, indicative of low surface tension or the formation of a gel. A change in condensate composition or its wetting of the microtubule surface might cause this decrease in surface tension. For example, the condensate might now contain tubulin dimers, or Mal3 might be enriched closer to the microtubule surface and depleted from the condensate's surface. Indeed, the condensate appears non-uniform, with a layer of about 25 nm around the microtubule with a more compact appearance than further away from the surface of the microtubule. Such a change in surface tension might be functionally important. Unlike the budding yeast +TIP body, the fission yeast Mal3–Tip1–Tea2 +TIP 'particle' (originally called Tip1 and Tea2 particles [64]) tracks growing but not shrinking microtubules [65]. Moreover, there is no evidence that coarsening confines it to selected microtubules. These behaviors speak for the surface tension of the fission yeast +TIP particle being low (see previously and Box 2).

Furthermore, a key function of fission yeast interphase microtubules is to deliver to the cell ends the cell-polarity protein Tea1, an additional and persistent component of the fission yeast +TIP particle *in vivo*. These interphase microtubules grow from the nuclear surface towards the cell

periphery (Figure 2B) [64–68] where the +TIP particles help to direct their polymerization towards the cell end. The particles use the shaft of pre-existing microtubules as guides and promote both microtubule rescue and growth until the microtubule reaches its destination [68–70]. There, +TIP particles offload from the microtubule, adhere to the cell cortex, and fuse with previously delivered ones [65]. The deposited material subsequently contributes to promoting actin assembly, patterning cell growth and shape. Differences in material properties might explain why fission yeast +TIP particles offload from – while budding yeast +TIP bodies persist on – shrinking microtubules. If their surface tension is indeed low, +TIP particles might better wet growing microtubules but fail to stay together at the plus ends of shrinking ones. Coarsening and persistence at the cortex could be favored if surface tension indeed increases as the particle leaves the microtubule.

Thus in fission yeast, as in budding yeast, the current literature supports that the function and destination of microtubules are already specified as they emerge from their MTOCs, that is, *a priori*, by the presence of a +TIP particle that appears to be a liquid condensate. The main differences between fission and budding yeast +TIP bodies/particles reside in their distinct abilities to track or offload from shrinking microtubules and to singularize individual microtubules. Interestingly, both contribute to symmetry breaking on a cellular level, albeit in different manners: whereas coarsening of the budding yeast +TIP body takes place at microtubule tips [48], the fission yeast +TIP particle coarsens at the cell cortex to break spherical symmetry and polarize cell growth [64–68]. Strikingly, differences in surface tension might suffice to explain the distinct behaviors of these +TIP entities. Thus, the idea that the material properties of +TIP networks contribute to determining their function appears useful for understanding how evolution modulates the same core protein-network machinery for achieving different functional outcomes.

Do +TIP networks phase separate in mammalian cells?

The mammalian ubiquitous +TIPs EB1, EB3, and CLIP-170 also phase separate *in vitro* [62,71]. Upon overexpression, CLIP-170 forms large, tubulin-enriched condensates on microtubules in cells, which depend on the multivalency of CLIP-170 [62]. Thus, mammalian +TIPs might phase separate as their yeast counterparts, raising the question of whether this could be relevant for their cellular function.

In cultured mammalian cells, these +TIPs form so called **comets** that track plus ends of growing (but not shrinking) microtubules, leaving a trace of fading intensity along the lattice [72] (Figure 2CI). Their appearance is thus distinct from the budding yeast +TIP body but reminiscent of the fission yeast +TIP particle. The rapid exchange of EBs with the cytoplasm [73] suggested that the elongated comet shape reflects the distribution of EB-binding sites formed by the GTP cap of growing microtubules [74]. Due to stochastic hydrolysis of GTP in the microtubule lattice, the number of these binding sites decays exponentially with increasing distance from the microtubule plus end [74]. So, why are comets not observed in yeast? In addition to surface tension reducing the spreading of +TIPs along the lattice of yeast microtubules (see earlier), one reason might be the slow growth speed of these microtubules (1–2 μm/min in budding yeast [19], ~3 μm/min in fission yeast [75], and >10 μm/min in mammalian cells [76–80]). Indeed, the length of the comet of mammalian microtubule tips increases with increasing growth rates *in vitro* [81], although the situation is more complicated *in vivo* [73]. Also, the rate of GTP hydrolysis might be different in budding yeast. Both microtubule growth speed and GTP hydrolysis rate could lead to comets in budding yeast being too short to be resolved by conventional fluorescence microscopy.

But do comets really exclude the formation of a liquid, 3D +TIP condensate? Not necessarily. If a liquid condensate following a growing microtubule plus end loses material along the way through fission (like a water drop running down a window), the trailing droplets would rapidly dissolve due

to Ostwald ripening and GTP hydrolysis in the microtubule lattice, also generating a comet-like appearance. Accordingly, EB3 and CLIP-170 comets show a non-uniformly decaying intensity, consistent with such a behavior [62,82]. Characterizing their microstructure using state-of-the-art super-resolution microscopy will be an important step towards determining the material properties and architecture of +TIP comets in mammalian cells. If the trailing foci are condensates, their proposed role in partitioning tubulin could provide a rationale for how CLIP-170 rescues shrinking microtubules [62].

Another question raised by comparing yeast and animal +TIP bodies/comets is whether patterning +TIPs influence the material properties of +TIP networks on selected microtubules in metazoans. Several mammalian +TIPs share similarities with Kar9. For instance, the highly multivalent SLAINs also bind EBs, CLIP-170, and the microtubule polymerase ch-TOG at microtubule plus ends [14]. Other +TIPs such as MACF and APC (also related to Kar9) or CLIP-associated proteins (CLASPs) pattern only selected microtubules [83–86]. MACF guides selected microtubules along actin filaments to focal adhesions in epidermal cells (Figure 2CII). CLASPs localize to MTOCs and microtubule plus ends near the cortex or within the spindle. While CLASPs ubiquitously localize to microtubule plus ends in resting cells, they predominantly decorate those near the leading edge in migrating cells [84] (Figure 2CIII). Similarly, in migrating astrocytes APC localization is restricted to the plus ends of microtubules extending into growing membrane protrusions, where they promote motility (Figure 2CIV) [87,88]. In both cases, inhibition of the kinase GSK-3β by cell polarity factors promotes their interactions with microtubules at the leading edge [84,87]. While in these examples microtubule specialization seems to happen prior to their interaction with the final target, it might happen sometime during microtubule growth, after their nucleation, possibly representing an intermediary mechanism between *a priori* and *a posteriori* specialization. The fact that these cells nucleate most, if not all their microtubules, at a single MTOC, namely the centrosome, would certainly limit the relevance of *a priori* specialization, which is therefore unlikely to be prominent in, for example, interphase fibroblasts. Accordingly, in typically cultured animal cells, most studied +TIPs do not track shrinking microtubule ends [89], implicating an immediate loss of *a priori* specialization once a microtubule undergoes catastrophe. However, a few +TIPs – such as the two EB cargoes tastin and DDA3 [90] – can track both growing and shrinking microtubule ends (reviewed in [57]). It will be valuable to test whether they influence the material behavior of the corresponding comets. Generally speaking, *a priori* specialization might be specifically relevant in cells with multiple MTOCs, such as asymmetrically dividing cells and differentiated cells that nucleate diverse sets of microtubules at distinct MTOCs in addition to centrosomes, such as endosomes and the Golgi apparatus [91,92]. This is the case in neurons [93], as well as epithelial, endothelial [94], and some cancer cells [95]. For example, in addition to the role of APCs in spindle positioning in drosophila stem cells [39], the kinesin-2-dependent guidance of APC-decorated microtubules emanating from the Golgi towards axons in the fly larval neurons [93] is an intriguing candidate for *a priori* microtubule specialization in animals.

Recently, the coacervation of EB1, MCAK, and TIP150 together was linked to their function at kinetochores (Figure 2D) [63]. MCAK is a kinesin-related microtubule depolymerase at kinetochores [96]. TIP150 is a +TIP that localizes to microtubule plus ends relatively uniformly [97]. These proteins certainly do not recapitulate kinetochore complexity, but their *in vitro* characterization nonetheless seems to have predictive power for their function *in vivo*: positively charged residues in the intrinsically disordered region (IDR) of EB1 promote phase separation and microtubule binding *in vitro*, and its own, MCAK, and TIP150 localization to microtubules *in vivo*. They are also required to establish kinetochore tension during mitosis [63]. Certainly, these positive charges might simply mediate microtubule binding. However, replacing the IDR of EB1 with that of the unrelated protein hnRNPA1 restored the phase separation behavior of the chimera,

its ability to track microtubule plus ends *in vitro* and *in vivo*, and EB1 function in mitosis [63]. Using a mutated form of this IDR that undergoes liquid-to-solid transition within hours caused EB1 to lose its function over time as the IDR solidified [63]. While more work is needed to fully understand these effects and their implications for kinetochore function, these observations highlight the functional importance of the material properties that emerge from different +TIP networks.

Concluding remarks

Recent data from distantly related eukaryotes suggest that +TIP networks function as liquid condensates that we propose to uniformly term '+TIP bodies'. The concept of +TIP bodies opens promising research avenues towards understanding how individual microtubules become functionally specialized, either *a priori* or *a posteriori*. Given that there are many more mechanisms contributing to the functional specialization of microtubules, it will be interesting to investigate how +TIP networks influence and are influenced by processes such as tubulin isotypes, tubulin post-translational modifications, and changes in microtubule lattice configurations [9] (see Outstanding questions).

Furthermore, patterning +TIPs deserve particular attention as their impact on the material properties of the host bodies may determine how they specify microtubule function, how these bodies persist or not on shrinking microtubules, promote symmetry breaking, couple forces between microtubules and other structures, or transport and deliver patterning cues to their destinations. Here, their accumulation of passenger factors partitioning to them might be particularly relevant. Thus, if the description of +TIP networks as liquid condensates is accurate, much of their functional diversity might be well explained by differences in their material properties, including wetting behavior, client partitioning, viscosity, and surface tension. Two mechanisms may tune these parameters in evolution: (i) the addition of patterning +TIPs on specific microtubules, and (ii) the selection of ubiquitous +TIPs variants with distinctive condensation behaviors.

Indeed, since a few mutations are generally enough to enable the formation of supramolecular assembly in otherwise soluble proteins [98], changes in material behavior seem easily tunable by evolution. Evolutionary plasticity may thus be a major advantage of condensates, possibly explaining why they are apparently a successful solution for many cellular processes. In summary, progress will now depend on the development of methods for addressing the material behavior of mesoscale +TIP bodies directly *in vivo* and characterize key parameters such as their surface tension and viscosity.

Outstanding questions

What is the accurate architectural description of +TIP networks in cells?

How effectively does the liquid condensate model portray the configuration of +TIP networks while considering potential structural disparities?

Is the postulated condensed nature of +TIP networks primarily aimed at concentrating proteins at microtubule plus ends, or does it go beyond that? Do we have to attribute some of their functional effects to emergent material properties?

How can measurements of +TIP networks' material properties be effectively conducted considering their relatively small dimensions?

What properties of +TIPs networks control their ability to track shrinking microtubule ends?

How common is *a priori* specialization in eukaryotes other than fungi, and where are we most likely to find it? Is it a viable mechanism in cells with hundreds of microtubules emanating from the same place?

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Declaration of interests

No interests are declared.

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