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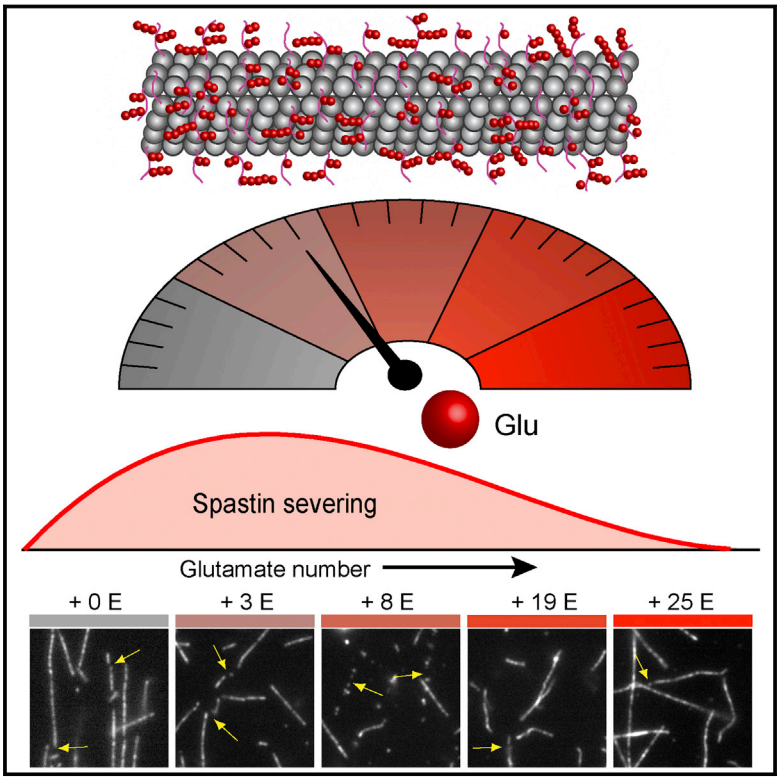
Graded Control of Microtubule Severing by Tubulin Glutamylation

Graphical Abstract



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In Brief

Glutamylation acts as a rheostat that tunes microtubule severing as a function of the number of glutamate molecules added per tubulin to maintain the complex architectures of microtubule arrays.

Highlights

- Glutamylation of intrinsically disordered tubulin tails quantitatively tunes spastin
- Spastin microtubule severing responds biphasically to glutamate number on tubulin
- Localized glutamylation effects propagate to neighboring microtubules
- Control by tubulin glutamylation echoes regulation by multisite phosphorylation

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Graded Control of Microtubule Severing by Tubulin Glutamylation

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SUMMARY

Microtubule-severing enzymes are critical for the biogenesis and maintenance of complex microtubule arrays in axons, spindles, and cilia where tubulin detyrosination, acetylation, and glutamylation are abundant. These modifications exhibit stereotyped patterns suggesting spatial and temporal control of microtubule functions. Using human-engineered and differentially modified microtubules we find that glutamylation is the main regulator of the hereditary spastic paraplegia microtubule severing enzyme spastin. Glutamylation acts as a rheostat and tunes microtubule severing as a function of glutamate number added per tubulin. Unexpectedly, glutamylation is a nonlinear biphasic tuner and becomes inhibitory beyond a threshold. Furthermore, the inhibitory effect of localized glutamylation propagates across neighboring microtubules, modulating severing in *trans*. Our work provides the first quantitative evidence for a graded response to a tubulin posttranslational modification and a biochemical link between tubulin glutamylation and complex architectures of microtubule arrays such as those in neurons where spastin deficiency causes disease.

INTRODUCTION

Microtubule-severing enzymes are critical for the generation and maintenance of microtubule arrays with complex architectures like those in neurons, spindles, and cilia (Roll-Mecak and McNally, 2010). They produce internal breaks in the polymer thereby generating new microtubule ends that can either depolymerize or grow to amplify microtubule numbers (Lindeboom et al., 2013; Ribbeck and Mitchison, 2006; Roll-Mecak and Vale, 2006; Srayko et al., 2006). Spastin is a microtubule severing enzyme critical in neurogenesis (Sherwood et al., 2004; Trotta et al., 2004; Yu et al., 2008), axonal regeneration (Stone et al., 2012), nuclear envelope breakdown (Vietri et al., 2015), mitosis (Zhang et al., 2007), and cytokinesis (Guizetti et al., 2011). Spastin is mutated in 40% of patients with hereditary spastic paraplegias (HSP), neurological disorders characterized by lower extremity weakness (Hazan et al., 1999). HSP spastin mutants are impaired in microtubule severing (Evans et al., 2005; Roll-Mecak and Vale, 2005, 2008) and loss of spastin severing activity leads to disorganized neuronal microtubule arrays and axonopathy (Sherwood et al., 2004; Trotta et al., 2004). Not surprisingly, the microtubule severing activity of these enzymes is stringently regulated and spastin or katanin hyperactivity can be highly deleterious to the cell (Cummings et al., 2009; Lu et al., 2004; Sherwood et al., 2004; Stone et al., 2012). Thus, an important question in understanding the cellular mechanism of microtubule severing enzymes is how their activities are spatially and temporally restricted.

Microtubule severing enzymes act on the intrinsically disordered C-terminal tails of tubulin (McNally and Vale, 1993; Roll-Mecak and Vale, 2005) that decorate the microtubule surface and are hotspots for conserved and chemically diverse posttranslational modifications (reviewed in Garnham and Roll-Mecak, 2012). These modifications vary between cell types and their intracellular distribution patterns are stereotyped suggesting spatial and temporal control of microtubule effectors as the tubulin tails are binding sites for motors and microtubule-associated proteins (MAPs). Thus, these modifications are thought to constitute a cell positioning and navigation system for microtubule effectors or a "tubulin code" (Verhey and Gaertig, 2007; Yu et al., 2015), analogous to the "histone code" (Jenuwein and Allis, 2001). The enzymes that introduce these modifications are important for normal development and alterations in tubulin modifications are linked to cancers, chemotherapy resistance, and neurodegenerative disorders (reviewed in Garnham and Roll-Mecak, 2012). Glutamylation, detyrosination and acetylation are enriched on microtubule subpopulations with long lifetimes that are resistant to drug-induced depolymerization (Gundersen et al., 1984; Schulze et al., 1987; Webster et al., 1987). They are especially abundant in the complex microtubules arrays of spindles, axons, and cilia where the function of microtubule severing enzymes is critical. Kinetochore and interpolar microtubules are detyrosinated, astral microtubules are tyrosinated, midbody and axonal microtubules are detyrosinated, acetylated, and glutamylated, and cilia microtubules have high levels of glutamylation and acetylation (Audebert et al., 1993; Bobinnec et al., 1998; Gundersen et al., 1984; Lacroix et al., 2010; Wolff et al., 1992; Yu et al., 2015).

Glutamylation is the most abundant tubulin modification in the adult mammalian brain where it increases during postnatal neuronal maturation (Audebert et al., 1993; Ikegami et al.,

2006; Redeker, 2010). It involves the reversible addition of glutamates, either singly or sequentially in chains, to the intrinsically disordered tubulin C-terminal tails. The number of glutamates on tubulin tails in neurons is distributed in stereotyped patterns, and disruption of glutamylation levels and patterns leads to neuronal pathologies and defective regeneration of damaged axons (Ghosh-Roy et al., 2012; Lee et al., 2012; Rogowski et al., 2010). While highly dynamic growth cone microtubules are not glutamylated and the soma contains mostly non-glutamylated or small numbers of glutamylated microtubules with short (<2) glutamate chains, the axon is enriched in stable microtubules with longer glutamate chains (Audebert et al., 1993; Eddé et al., 1992; Janke and Kneussel, 2010; Regnard et al., 1999; Wolff et al., 1992). Mass spectrometric analyses of tubulin purified from brain tissue reveals a preponderance of glutamylated tubulin with 3 to 6 glutamates on each tail, with as many as 11 and 7 detected on α- and β-tubulin tails, respectively (Redeker, 2010). Axonemal microtubules are extensively glutamylated with as many as 21 glutamates per tubulin protomer detected (Geimer et al., 1997; Schneider et al., 1998). It is likely that longer glutamate chains are found in vivo but are not detectable due to the difficulty of mass spectrometric analysis of these highly electronegative tubulin tail peptides (Redeker et al., 2005).

Efforts to decipher the effects of tubulin modifications on cellular effectors have been hampered by the unavailability of unmodified microtubules and microtubules with well-defined posttranslational modifications. The overwhelming majority of studies use tubulin purified from brains by repeated cycles of depolymerization and polymerization, which has two consequences: (1) the topographical information originally encoded in the distribution of tubulin posttranslational modifications in cells is lost so that modifications present in the original microtubules become incorporated in random mosaic patterns of tubulin polymerized from them, and (2) the purified tubulin is highly heterogeneous as it is comprised of a randomized mixture of isoforms bearing multiple, chemically distinct, and quantitatively varied posttranslational modifications, including acetylation, phosphorylation, detyrosination, and glutamylation (Sullivan, 1988). While acetylation and detyrosination are monomodifications that can function as simple ON/OFF switches, glutamylation, by virtue of its variable and stereotyped extent (a range of 1 to 21 glutamates have been detected on tubulin tails) (Geimer et al., 1997; Redeker, 2010; Schneider et al., 1998) has the potential for a graded quantitative regulation of microtubule effectors. Tests of this hypothesis require synthesis of microtubules with quantitatively defined extents of glutamylation. In addition to the need for an unmodified microtubule substrate, such efforts have been further hampered by the inherent challenges that stem from the physicochemical properties of glutamylation. This modification is heterogeneous and highly charged, making mass spectrometric analyses challenging. Recent in vitro studies of the tubulin code relied on chimeric *Saccharomyces cerevisiae* tubulin with grafted human tubulin tails. Comparison of multiple tail isoforms, detyrosinated α-tail, and tails with glutamate peptides attached via an unnatural linkage revealed modest effects on kinesin and dynein processivity and speed (Sirajuddin et al., 2014).

Here, we use human-engineered and differentially modified microtubules to investigate the effect of tubulin glutamylation, detyrosination, and acetylation on the activity of the microtubule-severing enzyme spastin. Using preparations of recombinant and differentially modified microtubules, we show that glutamylation is the main modulator of spastin activity and not tubulin acetylation or detyrosination. Using a microtubule series with a range of attached glutamates per tubulin, we unexpectedly find that glutamylation biphasically regulates microtubule severing: spastin activity increases as the number of glutamates per tubulin rises from one to eight, but decreases beyond this glutamylation threshold. We demonstrate that this behavior reflects a linear increase in spastin-microtubule affinity with tubulin glutamylation counteracted by a non-linear decrease in enzyme-specific activity caused by impaired force generation on the tubulin tails. Thus, glutamylation quantitatively tunes severing of a microtubule in response to its precise local modification status, consistent with the variable effects of microtubule-severing enzymes on different microtubule arrays in vivo as well as at different developmental stages (Lee et al., 2009; Sharma et al., 2007; Stewart et al., 2012). The same biphasic response modulates spastin activity in *trans*, because densely glutamylated microtubules act as spastin sinks, thereby propagating the inhibitory effect of glutamylation across a microtubule array. Our results reveal how severing can be precisely controlled spatially and temporally within microtubule arrays and provide quantitative proof for the rheostat-like regulation of microtubule effectors previously hypothesized for tubulin glutamylation, thus furnishing strong support for the tubulin code hypothesis.

RESULTS

Biphasic Regulation by Tubulin Glutamylation

Glutamylation recruits spastin to microtubules in vivo (Zempel et al., 2013), and a qualitative enhancement of spastin severing activity by tubulin glutamylation has been observed in vitro and in vivo (Lacroix et al., 2010). To generate microtubules with defined glutamylation levels, we purified tubulin devoid of posttranslational modifications (Figures 1A and S1) and used tubulin tyrosine ligase-like 7 (TTLL7) glutamylase to modify it in vitro. TTLL7 is the most abundant neuronal tubulin glutamylase (Ikegami et al., 2006; van Dijk et al., 2007). It is critical for neurite outgrowth and is primarily responsible for the dramatic increase in β-tubulin glutamylation observed during neuronal maturation (Ikegami et al., 2006). TTLL7 both initiates and elongates glutamate chains on the C-terminal tubulin tails (Garnham et al., 2015; Mukai et al., 2009). Glutamylation was quantified using reversed-phase liquid chromatography mass spectrometry (LC-MS) (Figures 1 and S1; Experimental Procedures). Proteolytic digest followed by tandem MS analysis confirmed the presence of glutamylation only on the tubulin tails (Supplemental Experimental Procedures; data not shown). Using a microscopy-based assay to quantify spastin severing (Figure 1) we found that spastin activity gradually increases with glutamylation (Figures 1B and 1C), but unexpectedly decreases above a threshold (Figures 1D, 1E, 2A, and S1A; Movie S1). The transition between the stimulatory and inhibitory effects occurs at a mean glutamate number ($<n^E>$) of ~8 (Figure 2A). At $<n^E>$ ~8 severing

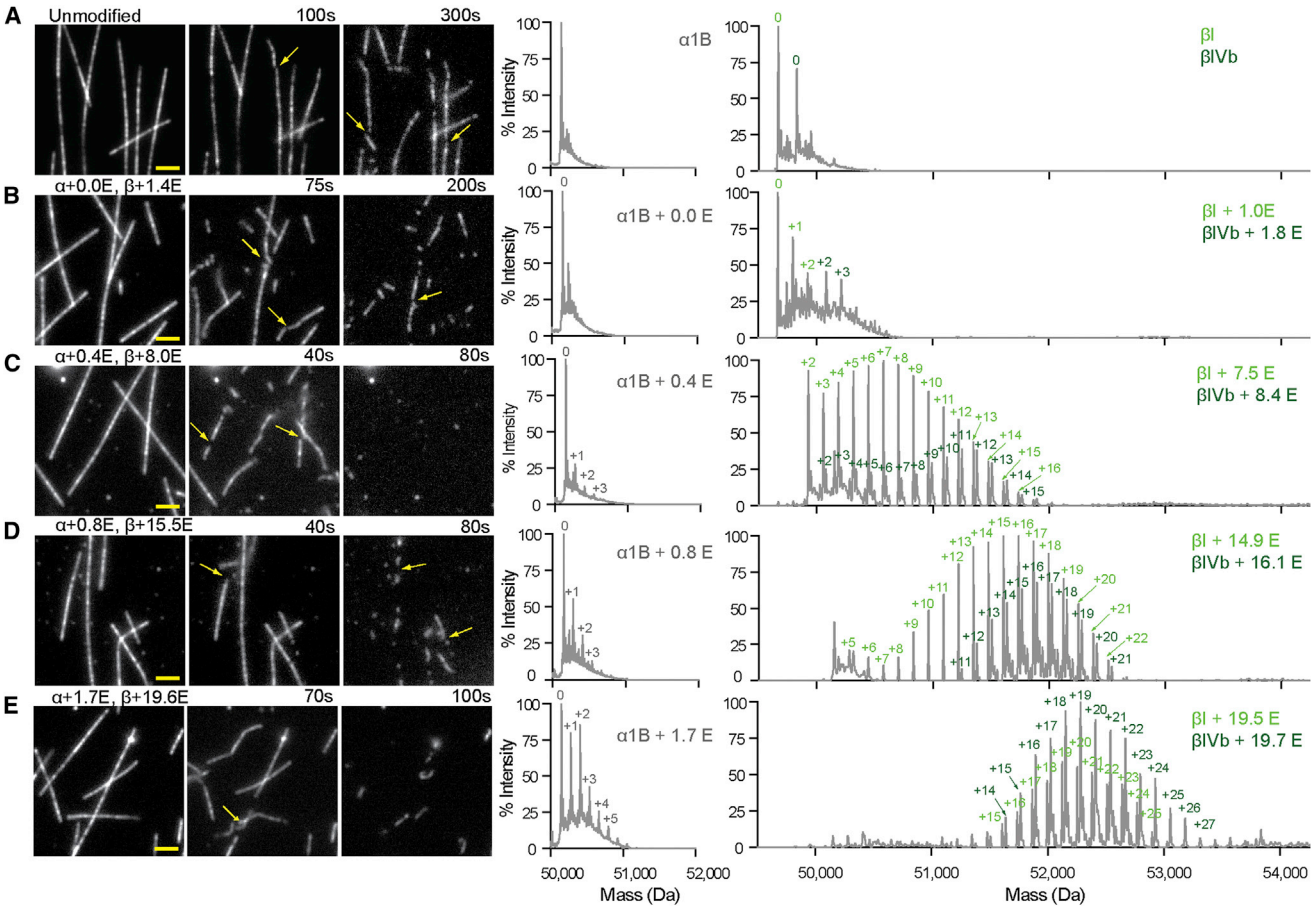

Figure 1. Graded Regulation of Spastin-Catalyzed Microtubule Severing by Tubulin Glutamylation

Left: spastin-catalyzed microtubule severing of unmodified microtubules (A) and microtubules with increasing glutamylation levels (B–E). Scale bar, 2 μm. Right: reversed-phase LC-MS of microtubules used in severing assays shown on the left (Experimental Procedures). There are one detectable α (α1B) and two β (βI and βIVb) isoforms in these preparations. Throughout, numbers of glutamates added to α- and β-tubulin isoforms are indicated in gray and green, respectively. The weighted mean of the number of glutamates ($\langle n^E \rangle$) added to α- and β-tubulin (overall and separately for the two β isoforms) are denoted α + $\langle n^E \rangle$ and, β + $\langle n^E \rangle$, respectively (Experimental Procedures).

See also Figure S1 and Movie S1.

is enhanced 3.8-fold over unmodified microtubules, with a 9-fold increase in maximal rate (Figures 1 and S1A).

The observed stimulatory and inhibitory effects on microtubule severing are mostly due to β-tail glutamylation. Severing of microtubules with essentially unmodified α-tails, but with either 1.4 or 5.1 glutamates on their β-tails, is enhanced by 49% in the latter (Figures 2B and S2). Severing decreases stepwise as $\langle n^E \rangle$ on β-tubulin increases from 18.1 to 19.0 to 19.6 (Figure 2C). Consistent with the dominant role of β-tail glutamylation, microtubules with near identical β-tubulin glutamylation levels ($\langle n^E \rangle$ of 6.7 or 6.8), but a 4-fold difference in α-tubulin glutamylation ($\langle n^E \rangle$ of 0.4 versus 1.6) are severed similarly by spastin (Figure 2D). In contrast, a similar increase in β-tubulin glutamylation from 0 to 1.4 enhances severing 2.4-fold (Figure 2A). Furthermore, constant α-tubulin glutamylation levels ($\langle n^E \rangle$ of 1.6, 1.7, and 1.6) with increasing glutamate numbers on β-tubulin ($\langle n^E \rangle$ of 4.7, 6.8, and 19.6, respectively) recapitulate the biphasic response to glutamylation (Figure 2E). These results indicate that β-tubulin glutamylation suffices to modulate spastin-severing activity.

The α-Tubulin Tail Is Dispensable for Spastin-Mediated Severing

The critical role of the β-tail for spastin function is supported by experiments with engineered human tubulin. We used total internal reflection fluorescence (TIRF) microscopy and analyzed the association of DyLight 488-labeled spastin (the labeled enzyme retains full severing activity) (Figure S3; Experimental Procedures) with engineered microtubules missing either their α- or β-tails (Figures 3 and S3A; Experimental Procedures). Our experiments demonstrate that both tubulin tails make contributions to spastin microtubule binding affinity: removal of the α- or β-tail leads to 69% and 71% reduction in binding, respectively (Figure 3A). However, microtubules without β-tails are resistant to severing, while microtubules without α-tails are still severed, albeit with 63% lower activity (Figure 3A). Microtubules missing

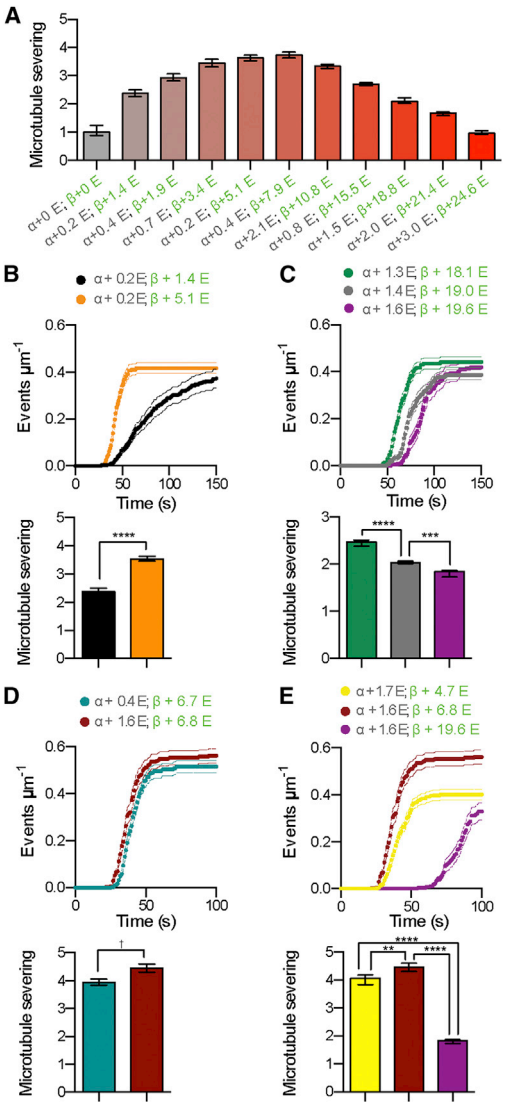

Figure 2. Spastin-Catalyzed Microtubule Severing Displays a Biphasic Response to Tubulin Glutamylation Levels

(A) Spastin-catalyzed microtubule severing varies with the number of glutamates on tubulin tails. $<n^E>$ on α- and β-tubulin, indicated in gray and green, respectively. Error bars, SEM (n > 23 microtubules from multiple chambers). Severing rates normalized to that of unmodified microtubules (Experimental Procedures).

(B and C) Modulation of β-tubulin glutamylation levels is sufficient to increase (B) or decrease (C) microtubule severing activity while α-tubulin glutamylation levels are kept constant (for mass spectra, see Figures S2A and S2B). Error bars, SEM (n = 23 microtubules from multiple chambers for both B and C). ****p < 0.0001; ***p < 0.001. Top panels, progress curves of severing reactions; bottom panels, severing rates as in (A).

(D) Microtubule severing activity is highly sensitive to β-tubulin glutamylation levels (for mass spectra, see Figure S2C). Error bars, SEM (n = 23 microtubules from multiple chambers). †p > 0.01. Top panels, progress curves of severing reactions; bottom panels, severing rates as in (A).

(E) An increase in β-tubulin glutamylation is sufficient to induce a biphasic response in microtubule severing when α-tubulin glutamylation is constant (for mass spectra, see Figure S2D). Error bars, SEM (n = 24 microtubules from multiple chambers). ****p < 0.0001; **p < 0.01. Top panels, progress curves of severing reactions; bottom panels, severing rates as in (A).

See also Figure S1 and Movie S1.

their β-tails are not severed even at spastin concentrations as high as 1 μM (not shown). Thus, the β-tubulin tail is the major regulator for spastin severing. We obtained similar results with microtubules where β-tails were removed by partial proteolysis: removal of ~90% of the β-tails abrogates severing and leads to a 52% reduction in microtubule binding affinity (Figures S3B and S3C). Complete removal of the β-tails leads to a 79% reduction (no binding is detected in the absence of both α- and β-tails under these conditions). Microtubules that retain ~10% of their full-length β-tails are weakly severed by spastin, but only at high spastin concentrations (1 μM versus 50 nM) and at a rate ~100-fold lower than microtubules with intact β-tails (Figures S3B and S3D). Microtubules that retain ~3% of their full-length β-tubulin tails are completely resistant to severing even at these high spastin concentrations (Figure S3D). Thus, while both tails contribute to microtubule binding and severing, the β-tubulin tail is necessary and sufficient for severing.

Tubulin Detyrosination and Acetylation Are Not Major Spastin Modulators

Given the contribution of the α-tail to spastin microtubule binding, we examined whether detyrosination is a spastin modulator. Detyrosination involves the reversible removal of the genetically encoded C-terminal tyrosine of α-tubulin. The tyrosine is added back to soluble tubulin as part of the detyrosination/tyrosination cycle (Raybin and Flavin, 1975; Szyk et al., 2011). The C-terminal tyrosine is an ON/OFF signal for the recruitment of microtubule regulators such as plus-end-binding proteins and motors (Peris et al., 2009; reviewed in Garnham and Roll-Mecak, 2012; Gouveia and Akhmanova, 2010). Experiments with recombinant human tubulin with or without the α-tubulin C-terminal tyrosine show that neither microtubule binding nor severing is significantly affected by detyrosination (Figure 3B). Thus, detyrosination is not a strong modulator of spastin function and the down-regulation in microtubule binding and severing seen with the α-tail deletion is primarily due to interactions with the rest of the α-tail.

We also examined the effects of α-tubulin acetylation on spastin-mediated microtubule severing as the closely related microtubule-severing enzyme katanin was reported to preferentially sever acetylated microtubules in vivo (Sudo and Baas, 2010). Unlike detyrosination and glutamylation that alter the intrinsically disordered C-terminal tubulin tails that decorate the microtubule exterior, α-tubulin acetylation occurs on Lys40 in the microtubule lumen and was proposed to affect the strength of lateral contacts in the microtubule lattice as well as recruit intraluminal MAPs (Cueva et al., 2012). Microtubule severing assays with unmodified and acetylated microtubules generated by in vitro enzymatic modification using tubulin acetyltransferase (Kormendi et al., 2012) show that acetylation has no effect on spastin activity (Figure 4; Supplemental Experimental Procedures), consistent with previous in vivo studies (Sudo and Baas, 2010). It remains to be established whether the effect of acetylation on katanin is direct. Thus, of the three major tubulin modifications enriched in stable microtubules arrays where spastin function is important, glutamylation is the key modulator of spastin-mediated microtubule severing.

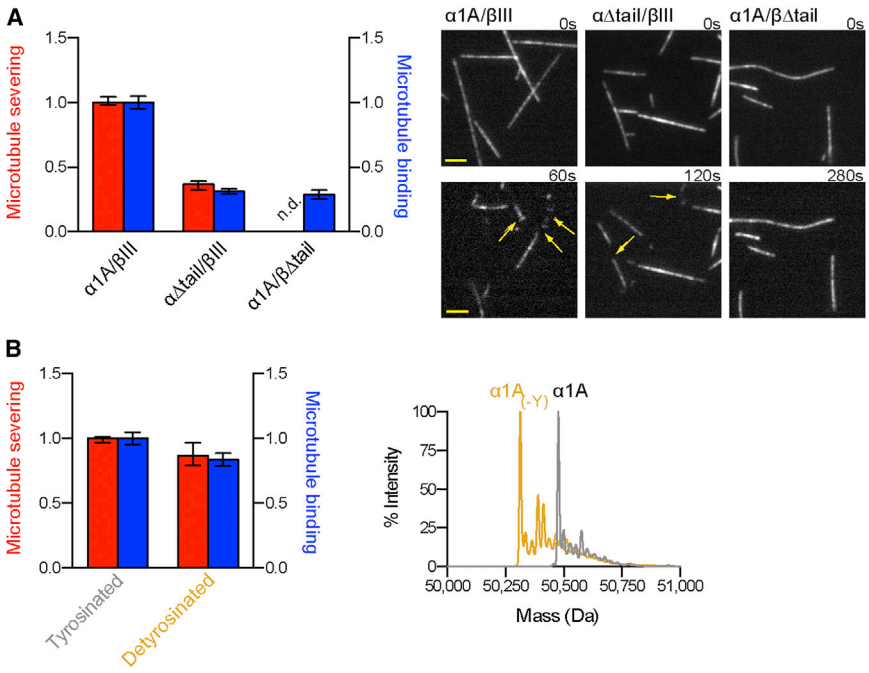

Figure 3. Spastin-Mediated Microtubule Severing Requires the β-, but Not the α-Tubulin C-Terminal Tail

(A) Left: normalized microtubule severing rates (left axis) and binding affinity (right axis) for human recombinant microtubules missing either the α- or β-tail (Figure S3A). Error bars, SEM (n > 34 or 70 microtubules from multiple chambers for severing and binding assays, respectively). Right: engineered human microtubules severed by spastin. Arrows indicate severing sites. Scale bar, 2 μm. See also Figure S3.

(B) Left: normalized microtubule severing rates (left axis) and binding affinity (right axis) for human recombinant tyrosinated and detyrosinated microtubules. Error bars, SEM (n > 15 microtubules for severing assays; n > 96 microtubules for binding assays, from multiple chambers). Right: overlaid reversed-phase LC-MS of tyrosinated (gray) and detyrosinated (yellow) microtubules corresponding to the α-tubulin peak.

Glutamylation Is a Linear Affinity Tuner

To understand the molecular basis for the modulation of spastin activity by tubulin glutamylation, we investigated the effects of this modification on microtubule binding. We found that spastin microtubule binding affinity increases monotonically with glutamylation (Figures 5 and S4). Consistent with this, spastin severing is inhibited by free polyglutamic acid in a manner proportional to its chain length (IC_{50} of 160 ± 1 μM for poly(E) of 6 to 23 glutamates; IC_{50} of 13 ± 1 μM for poly(E) of 23 to 115 glutamates) (Figure 6; Experimental Procedures). Spastin belongs to the family of ATPases associated with various cellular activities (AAA ATPases) and microtubule severing requires ATP hydrolysis (Evans et al., 2005; Roll-Mecak and Vale, 2005). We investigated the effects of soluble polyglutamic acid on spastin catalyzed ATP hydrolysis. We find that spastin ATPase is not inhibited by soluble polyglutamic acid (Figure S5), indicating that the inhibitory effect is not due to ATPase inhibition and most likely to direct competition with the tubulin tails for spastin binding. Remarkably, binding affinity rises linearly with $\langle n^E \rangle$ (slope of ~0.43 per glutamate added to tubulin) indicating that glutamylation acts as a linear affinity tuner (Figure 5B). The strong contribution of electrostatic interactions to spastin microtubule binding is consistent with the salt dependence of microtubule severing (Eckert et al., 2012a). The mean free energy of binding per added glutamate is 0.06 kcal/mol, indicating weak interactions. The linearity for the affinity also indicates a stochastic binding mechanism i.e., the binding affinity is enhanced by increasing local glutamate concentration and not by a cooperative mechanism whereby increasing numbers of glutamates are simultaneously engaged by the enzyme. As motors and MAPs contain positively charged patches critical for microtubule binding, it is likely that glutamylation acts as an affinity tuner for other microtubule regulators (Roll-Mecak, 2015) as suggested by early qualitative experiments with MAP1B, MAP2, and tau (Bonnet et al., 2001; Boucher et al., 1994; Larcher et al., 1996).

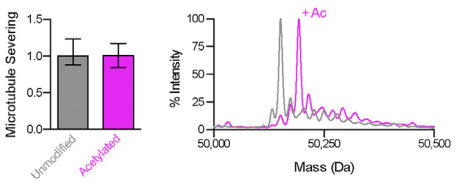

Figure 4. α-Tubulin Acetylation Does Not Affect Spastin-Mediated Microtubule Severing

Left: normalized spastin severing activity with unmodified and acetylated human microtubules. Error bars, SEM (n = 50 and 27 for unmodified and acetylated microtubules, respectively, from multiple chambers). Right: overlaid reversed-phase LC-MS of unmodified (gray) and acetylated (magenta) α-tubulin showing the 42 Da mass shift corresponding to the acetylated species. No mass change is detected in β-tubulin (not shown).

Glutamylation Lowers Spastin Mechanochemical Coupling

Spastin exhibits cooperative severing of microtubules that varies with tubulin glutamylation (Figure 5C). Cooperative assembly on the microtubule was proposed as an activation mechanism for katanin (Hartman and Vale, 1999). Spastin exhibits cooperative severing of unmodified microtubules (Hill coefficient = 3.1; Figure 5C, left panel). Surprisingly, as the glutamate number per tubulin increases, a new behavior emerges wherein the cooperativity peaks at progressively lower spastin concentrations (indicated by shading in Figure 5C) and drops precipitously thereafter. Beyond the peak, severing becomes anti-cooperative. At $\langle n^E \rangle$ of 5.6, maximal severing is observed at 400 nM spastin, while at 12.8, it peaks at 100 nM and drops in half at 400 nM spastin.

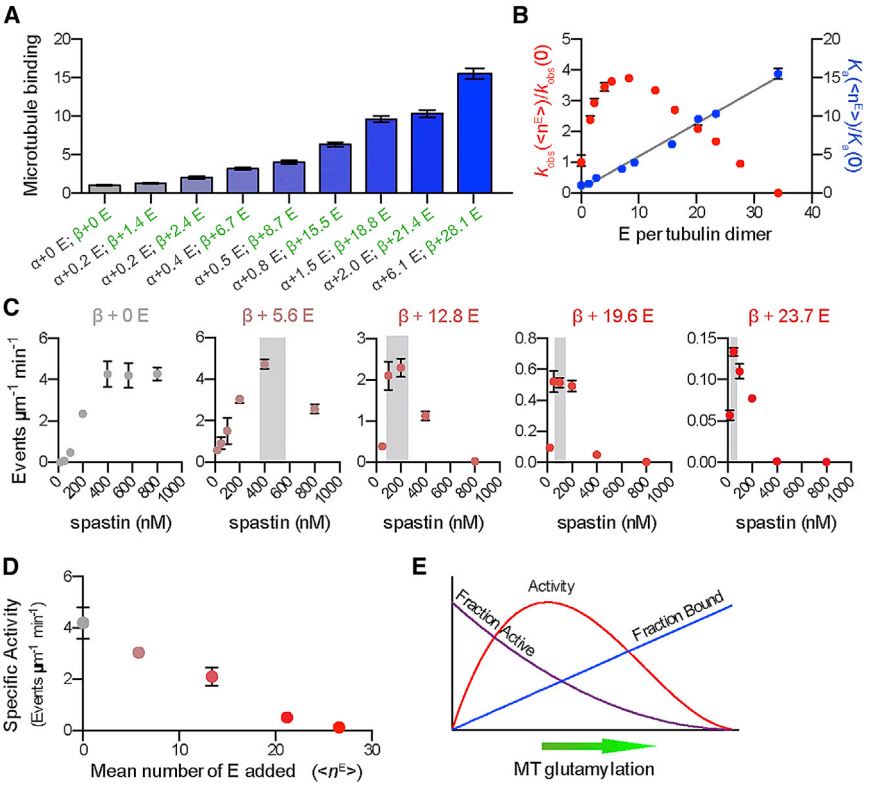

Figure 5. Competition between Affinity and Specific Activity Gives Rise to the Biphasic Regulation of Spastin-Catalyzed Microtubule Severing by Tubulin Glutamylation

(A) Spastin affinity for microtubules increases with the number of glutamates on tubulin tails. Spastin binding on modified microtubules normalized to that for unmodified microtubules). $\langle n^E \rangle$ on α- and β-tubulin are indicated as in Figure 1. Error bars, SEM (n = 50 microtubules from multiple chambers for each $\langle n^E \rangle$).

(B) Spastin microtubule affinity increases linearly with glutamate numbers on tubulin (R^2 = 0.99). Left: microtubule severing rates, k_{obs}. Right: microtubule association constants, K_a for various $\langle n^E \rangle$ normalized to those for unmodified microtubules. Error bars represent SEM.

(C) Microtubule severing rates as a function of spastin concentration for microtubules with various glutamylation levels. Increased glutamylation induces earlier and more abrupt onset of anti-cooperativity of microtubule severing. Error bars, SEM (n > 21 microtubules from multiple chambers for each $\langle n^E \rangle$). Grey shading, region of transition from cooperative to anti-cooperative behavior.

(D) Microtubule severing activities for different glutamylation levels at constant number of bound spastin molecules per tubulin. The specific activity of bound spastin decreases with increased glutamylation. Error bars, SEM (n > 20 microtubules from multiple chambers for each $\langle n^E \rangle$).

(E) Competition between glutamylation-induced increase in microtubule affinity and decrease in enzyme-specific activity yields a biphasic response of spastin severing to microtubule glutamylation levels.

See also Figure S4.

To determine whether tubulin glutamylation alters the specific activity of microtubule-bound spastin, we assayed a series of progressively glutamylated microtubules at concentrations that result in equivalent spastin densities on the microtubule (Figure 5D; Experimental Procedures). We find that the specific activity of microtubule-bound spastin declines steeply and non-linearly with glutamylation (Figure 5D). Since spastin ATPase varies modestly with microtubule glutamylation (Figure S4), it suggests that the mechanochemical coupling of the enzyme (Eckert et al., 2012b) is progressively weakened with increasing glutamylation despite the concomitant increase in microtubule binding affinity; i.e., the enzyme gradually shifts to a non-productive tubulin binding mode with increased glutamylation. Therefore, the combination of the linear increase in affinity with the non-linear decrease in severing specific activity gives rise to the overall biphasic response to microtubule glutamylation (Figures 5B and 5E). Thus, spastin gradually transitions from a microtubule-severing enzyme to a microtubule stabilizing protein as a function of glutamylation. Inactive spastin and katanin mutants crosslink microtubules (McNally and McNally, 2011; Roll-Mecak and Vale, 2008) and katanin crosslinking activity is important for normal spindle morphology (McNally and McNally, 2011). Our work suggests that the glutamylation status of the microtubule could control a switch between these two opposing activities.

Long-Range Effects of Glutamylation

Our work shows that glutamylation gives rise to microtubule-autologous regulation of severing by spastin whereby the precise local glutamylation status of the microtubule itself quantitatively controls spastin activity. The simultaneous divergent increase in spastin microtubule affinity and decrease in severing specific activity in response to glutamylation levels has an important physiological corollary. Because high glutamylation results in a marked increase in spastin microtubule affinity (Figures 5A and 5B), but a decrease in severing activity above a certain threshold (Figures 2A, 5B, and S1A), the modified microtubule can act as a sink for spastin (Figure 7A), thereby inhibiting severing of a less modified microtubule in *trans* in addition to the microtubule-autologous (*cis*) regulation (Figure 7B). Indeed, severing of unmodified microtubules decreases by 41% in the presence of equimolar amounts of microtubules with $\langle n^E \rangle \sim 7$ and progressively decreases in the presence of microtubules with $\langle n^E \rangle$ of 11 or higher (Figure 7B). Because spastin microtubule affinity increases linearly with glutamylation, this effect will be present (in the correspondingly muted form) even at low glutamate numbers.

DISCUSSION

Our work reveals how the fate of a microtubule can be reversibly controlled by posttranslationally modifying an intrinsically

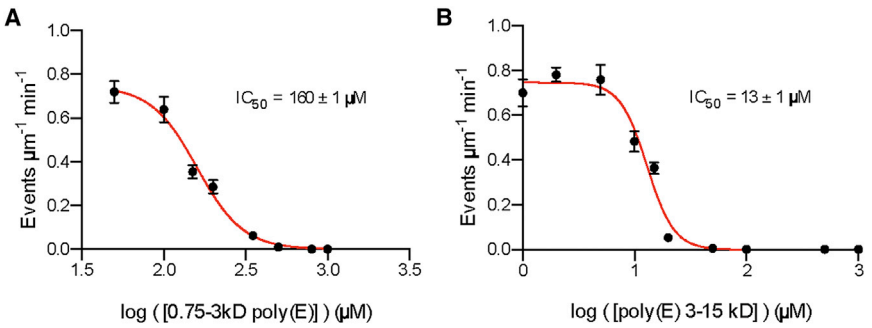

Figure 6. Free Poly-Glutamic Acid Inhibits Spastin Microtubule Severing

(A) Microtubule severing rates in the presence of 0.75–3 kDa poly-glutamic acid (6 to 23 glutamates). Error bars, SEM (n > 24 microtubules from multiple chambers).

(B) Microtubule severing rates in the presence of 3–15 kDa poly-glutamic acid (23 to 115 glutamates). Error bars, SEM (n > 24 microtubules from multiple chambers).

See also Figure S5.

disordered region (IDR) of tubulin not involved in interfaces essential for its polymerization into microtubules. The control of spastin severing by tubulin glutamylation levels enables substrate-regulated, spatially-controlled severing. As glutamylation increases up to $<n^E>$ ~8, microtubule severing is enhanced autologously and repressed in less glutamylated neighboring microtubules because of the linear increase in spastin recruitment with microtubule glutamylation. As $<n^E>$ becomes larger than ~8, severing is progressively inhibited autologously, and this inhibitory effect of high glutamylation levels propagates through spatially adjacent microtubules. This response to glutamylation could be a mechanism to control spastin-mediated midbody microtubule severing during cytokinesis, limit severing of highly glutamylated centriolar microtubules or quantitatively control microtubule severing during neuronal development when β-tubulin glutamylation levels increase progressively. Spastin mutations are responsible for 40% of HSPs, characterized by progressive axonopathy. The graded control of microtubule severing reveals how precise spatial regulation of microtubule severing can be achieved in a neuron where growth cone microtubules are not glutamylated (and thus are poor spastin substrates) and axonal microtubules are enriched in glutamylation and more susceptible to spastin. The differential susceptibility of these microtubule populations could have relevance for HSP patients with spastin mutations.

We propose that the closely related microtubule-severing enzyme katanin exhibits a similar response to glutamylation, consistent with the in vivo observation that katanin severs the glutamylated B-tubule of the axonemal doublet, but leaves the adjacent unmodified A-tubule untouched (Sharma et al., 2007). The response to glutamate numbers could be different for the two enzymes and this could potentially specialize them for microtubule arrays with different glutamylation ranges. Interestingly, glutamylation levels that enhance spastin-induced microtubule severing in cells, lead to recruitment of katanin to these glutamylated microtubules, where it induces mostly bundling and rarely severing (Lacroix et al., 2010).

The biphasic response to glutamylation could be used as a mechanism to initially activate severing enzymes in a microtubule array to equalize microtubule lengths or amplify their numbers (Loughlin et al., 2011; Srayko et al., 2006) and then gradually dampen their activity through the autologous biphasic response as well as the "in *trans*" regulation as the array reaches homeostasis. Newly grown microtubules from severed seeds would have low glutamylation levels and thus be initially protected from severing, allowing the formation of a new dynamic array. The inhibitory effect of higher glutamylation levels could also be a mechanism to locally inactivate and retain a pool of compartmentalized microtubule severing enzymes that are available for rapid activation on microtubules with lower glutamylation levels. This mechanism could be operational at the centrosome (enriched in highly glutamylated microtubules) where spastin and katanin localize and microtubules are released by severing (Zhang et al., 2007).

Spastin forms a hexameric ring when bound to ATP and is thought to destabilize the microtubule by pulling on the tubulin C-terminal tails through a positively charged central pore, by analogy with the mechanism of AAA ATPases such as ClpX and the proteasome (Roll-Mecak and Vale, 2008). Our data suggest that the posttranslationally added glutamates on the C-terminal tails provide additional unproductive binding sites and cause slippage of the tail when engaged by spastin, leading to a reduction in severing efficiency. Thus, even though more spastin molecules are recruited to the microtubule at higher glutamylation, because their specific activity is decreased due to impaired mechanochemical coupling, spastin molecules spend more of their microtubule-bound cycle in a state that is unable to remove tubulin and thus act to stabilize the microtubule, counteracting the action of the molecules in the productive part of their mechanochemical cycles, thus the anticooperative behavior observed at higher spastin densities. As tails with different glutamate numbers coexist on the same microtubule, the biphasic response that we uncovered could also be elicited by having a sharp transition between stimulation and inhibition at a particular glutamylation level. We favor rheostatic control over a threshold model because there is no species common to all the inhibitory microtubule samples ($<n^E>$ above ~8). Future structural studies will shed light on the mechanism of tubulin tail engagement by microtubule severing enzymes.

The intrinsically disordered tubulin tails are hubs of posttranslational modifications. Posttranslational modifications of IDRs are ubiquitously used to assemble complex signaling platforms (Wright and Dyson, 2015). Recent studies revealed involvement of the tubulin tails in diverse cellular networks (Aiken et al., 2014). Here, we show that glutamylation, the most prevalent tubulin tail modification and most abundant in the nervous system, quantitatively tunes the activity of a microtubule regulator. This is reminiscent of the regulatory mechanism by additive phosphorylation on IDRs that can operate both by a rheostatic or threshold response (Wright and Dyson, 2015). For example,

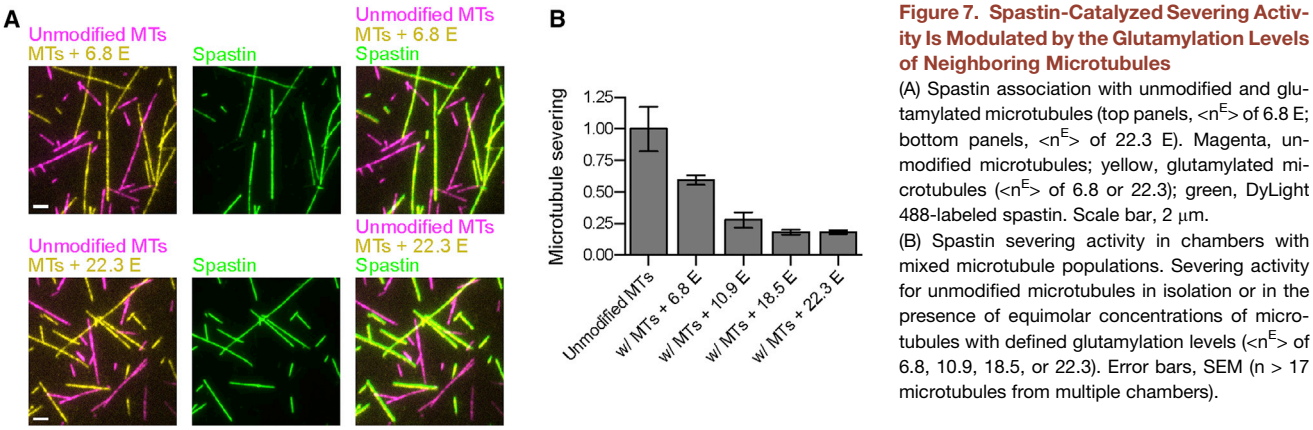

Figure 7. Spastin-Catalyzed Severing Activity Is Modulated by the Glutamylation Levels of Neighboring Microtubules

(A) Spastin association with unmodified and glutamylated microtubules (top panels, $\langle n^E \rangle$ of 6.8 E; bottom panels, $\langle n^E \rangle$ of 22.3 E). Magenta, unmodified microtubules; yellow, glutamylated microtubules ($\langle n^E \rangle$ of 6.8 or 22.3); green, DyLight 488-labeled spastin. Scale bar, 2 μm.

(B) Spastin severing activity in chambers with mixed microtubule populations. Severing activity for unmodified microtubules in isolation or in the presence of equimolar concentrations of microtubules with defined glutamylation levels ($\langle n^E \rangle$ of 6.8, 10.9, 18.5, or 22.3). Error bars, SEM (n > 17 microtubules from multiple chambers).

the unstructured N terminus of the p53 transcription factor is subject to multisite phosphorylation that tunes its affinity for the CREB-binding protein CBP/p300 and recruits it away from other transcription factors in response to genotoxic stress (Lee et al., 2010). Similarly, the DNA binding affinity of the V-Ets Avian Erythroblastosis Virus E26 Oncogene Homolog 1 (Ets-1) transcription factor is tuned by additive phosphorylation of an IDR in its sequence (Pufall et al., 2005). The activation of the cyclin-dependent kinase inhibitor Sic1 is achieved via a sharp threshold response to multisite phosphorylation (Nash et al., 2001). In these cases, as for the tubulin tail, the intrinsically disordered nature of the regulatory region in the protein makes it accessible to modification enzymes as well as readout by cellular effectors, be it another protein or nucleic acid.

Our approach outlines for the first time a general strategy for quantitative, in vitro characterization of the effects of informationally complex tubulin posttranslational modifications such as glutamylation on microtubule regulators. Currently, determination of the glutamylation status of microtubules is limited to immunofluorescence with antibodies that can only distinguish between chains of one versus greater than one or two glutamates (Lacroix et al., 2010; Magiera and Janke, 2013). The graded response of spastin-mediated severing to glutamylation and the switch between opposing biochemical activities as a function of glutamylation underscores the importance of devising new methods to determine the exact glutamylation status of individual microtubules in cells. Our approach for the generation and characterization of human microtubules with differential posttranslational modifications should motivate quantitative studies of the response to tubulin modifications of other microtubule regulators and serve as a quantitative paradigm for deciphering the biological consequences of the astonishing chemical complexity of cellular microtubules. While tubulin acetylation and detyrosination are monomodifications that can generate a binary response, glutamylation, by virtue of the variable number of glutamates added, can elicit a graded response by microtubule effectors. The combinatorial use of these signals can give rise to complex cellular responses of cytoskeletal regulators. Elucidating how differential tubulin posttranslational modifications modulate the activity of microtubule effectors is key to understanding how a single polymer can perform its diverse essential roles in cells. Furthermore, as glutamylation is found on many non-tubulin substrates (van Dijk et al., 2008) e.g., it regulates the histone chaperone activity of nucleosome assembly protein Nap1 (Nap-1) (Miller and Heald, 2015), the precise graded regulation we have uncovered carries general implications for cell signaling via glutamylation.

EXPERIMENTAL PROCEDURES

Generation of Glutamylated Microtubules

Human unmodified tubulin was purified by the TOG affinity method (Widlund et al., 2012) using a TOG1 domain column (Vemu et al., 2014). Taxol-stabilized unmodified microtubules for severing assays were prepared by polymerizing purified unmodified human tubulin with 4% tetramethylrhodamine (TMR)- and 1% biotin-labeled brain tubulin. Microtubules were glutamylated by incubation with *Xenopus tropicalis* tubulin tyrosine ligase-like 7 (TTLL7) at a 1:10 molar ratio of enzyme to tubulin in 20 mM HEPES pH 7.0, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM Glu, 1 mM ATP, 10 μM Taxol at room temperature for various incubation times (10 min for $\langle n^E \rangle$ ~1, 20 min for $\langle n^E \rangle$ ~2, 1 hr for $\langle n^E \rangle$ ~5, 2.5 hr for $\langle n^E \rangle$ ~12, 5 hr for $\langle n^E \rangle$ ~16, 10 hr for $\langle n^E \rangle$ ~25, and 24 hr for $\langle n^E \rangle$ ~35). Control reactions were performed without glutamate. TTLL7 was removed by addition of 0.3 M KCl and sedimentation through a 60% glycerol cushion at 100,000 × *g* for 12 min at 30°C. Microtubules were stored in BRB80 (80 mM PIPES, 1 mM $MgCl_2$, 1 mM EGTA, pH 6.8), 14.3 mM β-mercaptoethanol, 10 μM Taxol. The number of glutamates added to α- and β-tubulin was determined by LC-MS (Supplemental Experimental Procedures). The spectra display the characteristic distribution of masses with peaks separated by 129 Da corresponding to one glutamate (Figure 1). The extent of tubulin glutamylation on α- or β-tubulin was determined by calculating the weighted average of peak intensities for each tubulin species present. Experiments were performed on different days with at least two independent preparations. The mass spectrometric analyses are reproducible within 0.1 $\langle n^E \rangle$ between at least three independent measurements (Figure S1).

Generation of Acetylated Microtubule Substrates and Recombinant Engineered Human Tubulin

A complete description can be found in the Supplemental Experimental Procedures.

Microscopy-Based Severing Assays

Chambers were assembled as in Szyk et al. (2014). Microscopy-based microtubule severing assays were performed as described (Ziółkowska and Roll-Mecak, 2013). A complete description of data collection and analyses can be found in Supplemental Experimental Procedures.

TIRF Microscopy Microtubule-Binding Assays

DyLight 488-labeled spastin was perfused into chambers at a final concentration of 2 or 5 nM in severing reaction buffer (50 mM KCl, 2.5 mM $MgCl_2$, 1 mM ATP, and 1% Pluronic F-127, 10 μM Taxol and oxygen scavengers). Images were acquired using an inverted TIRF microscope. A complete description of image acquisition can be found in the Supplemental Experimental Procedures.

ATPase Assays

ATPase assays were performed as described in the Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2016.01.019.

AUTHOR CONTRIBUTIONS

A.R.M. conceived the project. M.L.V. and A.R.-M. designed experiments, interpreted data. M.L.V. performed all experiments, analyzed all data, and prepared figures. A.R.-M. wrote manuscript with contributions from M.L.V.

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REFERENCES

Aiken, J., Sept, D., Costanzo, M., Boone, C., Cooper, J.A., and Moore, J.K. (2014). Genome-wide analysis reveals novel and discrete functions for tubulin carboxy-terminal tails. Curr. Biol. *24*, 1295–1303.

Audebert, S., Desbruyères, E., Gruszczynski, C., Koulakoff, A., Gros, F., Denoulet, P., and Eddé, B. (1993). Reversible polyglutamylation of alpha- and beta-tubulin and microtubule dynamics in mouse brain neurons. Mol. Biol. Cell *4*, 615–626.

Bobinnec, Y., Moudjou, M., Fouquet, J.P., Desbruyères, E., Eddé, B., and Bornens, M. (1998). Glutamylation of centriole and cytoplasmic tubulin in proliferating non-neuronal cells. Cell Motil. Cytoskeleton *39*, 223–232.

Bonnet, C., Boucher, D., Lazereg, S., Pedrotti, B., Islam, K., Denoulet, P., and Larcher, J.C. (2001). Differential binding regulation of microtubule-associated proteins MAP1A, MAP1B, and MAP2 by tubulin polyglutamylation. J. Biol. Chem. *276*, 12839–12848.

Boucher, D., Larcher, J.C., Gros, F., and Denoulet, P. (1994). Polyglutamylation of tubulin as a progressive regulator of in vitro interactions between the microtubule-associated protein Tau and tubulin. Biochemistry *33*, 12471–12477.

Cueva, J.G., Hsin, J., Huang, K.C., and Goodman, M.B. (2012). Posttranslational acetylation of α-tubulin constrains protofilament number in native microtubules. Curr. Biol. *22*, 1066–1074.

Cummings, C.M., Bentley, C.A., Perdue, S.A., Baas, P.W., and Singer, J.D. (2009). The Cul3/Klhdc5 E3 ligase regulates p60/katanin and is required for normal mitosis in mammalian cells. J. Biol. Chem. *284*, 11663–11675.

Eckert, T., Le, D.T., Link, S., Friedmann, L., and Woehlke, G. (2012a). Spastin's microtubule-binding properties and comparison to katanin. PLoS ONE *7*, e50161.

Eckert, T., Link, S., Le, D.T., Sobczak, J.P., Gieseke, A., Richter, K., and Woehlke, G. (2012b). Subunit Interactions and cooperativity in the microtubule-severing AAA ATPase spastin. J. Biol. Chem. *287*, 26278–26290.

Eddé, B., Rossier, J., Le Caer, J.-P., Promé, J.-C., Desbruyères, E., Gros, F., and Denoulet, P. (1992). Polyglutamylated alpha-tubulin can enter the tyrosination/detyrosination cycle. Biochemistry *31*, 403–410.

Evans, K.J., Gomes, E.R., Reisenweber, S.M., Gundersen, G.G., and Lauring, B.P. (2005). Linking axonal degeneration to microtubule remodeling by Spastin-mediated microtubule severing. J. Cell Biol. *168*, 599–606.

Garnham, C.P., and Roll-Mecak, A. (2012). The chemical complexity of cellular microtubules: tubulin post-translational modification enzymes and their roles in tuning microtubule functions. Cytoskeleton (Hoboken) *69*, 442–463.

Garnham, C.P., Vemu, A., Wilson-Kubalek, E.M., Yu, I., Szyk, A., Lander, G.C., Milligan, R.A., and Roll-Mecak, A. (2015). Multivalent microtubule recognition by tubulin tyrosine ligase-like family glutamylases. Cell *161*, 1112–1123.

Geimer, S., Teltenkötter, A., Plessmann, U., Weber, K., and Lechtreck, K.F. (1997). Purification and characterization of basal apparatuses from a flagellate green alga. Cell Motil. Cytoskeleton *37*, 72–85.

Ghosh-Roy, A., Goncharov, A., Jin, Y., and Chisholm, A.D. (2012). Kinesin-13 and tubulin posttranslational modifications regulate microtubule growth in axon regeneration. Dev. Cell *23*, 716–728.

Gouveia, S.M., and Akhmanova, A. (2010). Cell and molecular biology of microtubule plus end tracking proteins: end binding proteins and their partners. Int. Rev. Cell Mol. Biol. *285*, 1–74.

Guizetti, J., Schermelleh, L., Mäntler, J., Maar, S., Poser, I., Leonhardt, H., Müller-Reichert, T., and Gerlich, D.W. (2011). Cortical constriction during abscission involves helices of ESCRT-III-dependent filaments. Science *331*, 1616–1620.

Gundersen, G.G., Kalnoski, M.H., and Bulinski, J.C. (1984). Distinct populations of microtubules: tyrosinated and nontyrosinated alpha tubulin are distributed differently in vivo. Cell *38*, 779–789.

Hartman, J.J., and Vale, R.D. (1999). Microtubule disassembly by ATP-dependent oligomerization of the AAA enzyme katanin. Science *286*, 782–785.

Hazan, J., Fonknechten, N., Mavel, D., Paternotte, C., Samson, D., Artiguenave, F., Davoine, C.S., Cruaud, C., Dürr, A., Wincker, P., et al. (1999). Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. Nat. Genet. *23*, 296–303.

Ikegami, K., Mukai, M., Tsuchida, J., Heier, R.L., Macgregor, G.R., and Setou, M. (2006). TTLL7 is a mammalian beta-tubulin polyglutamylase required for growth of MAP2-positive neurites. J. Biol. Chem. *281*, 30707–30716.

Janke, C., and Kneussel, M. (2010). Tubulin post-translational modifications: encoding functions on the neuronal microtubule cytoskeleton. Trends Neurosci. *33*, 362–372.

Jenuwein, T., and Allis, C.D. (2001). Translating the histone code. Science *293*, 1074–1080.

Kormendi, V., Szyk, A., Piszczek, G., and Roll-Mecak, A. (2012). Crystal structures of tubulin acetyltransferase reveal a conserved catalytic core and the plasticity of the essential N terminus. J. Biol. Chem. *287*, 41569–41575.

Lacroix, B., van Dijk, J., Gold, N.D., Guizetti, J., Aldrian-Herrada, G., Rogowski, K., Gerlich, D.W., and Janke, C. (2010). Tubulin polyglutamylation stimulates spastin-mediated microtubule severing. J. Cell Biol. *189*, 945–954.

Larcher, J.C., Boucher, D., Lazereg, S., Gros, F., and Denoulet, P. (1996). Interaction of kinesin motor domains with alpha- and beta-tubulin subunits at a tau-independent binding site. Regulation by polyglutamylation. J. Biol. Chem. *271*, 22117–22124.

Lee, H.H., Jan, L.Y., and Jan, Y.N. (2009). Drosophila IKK-related kinase Ik2 and Katanin p60-like 1 regulate dendrite pruning of sensory neuron during metamorphosis. Proc. Natl. Acad. Sci. USA *106*, 6363–6368.

Lee, C.W., Ferreon, J.C., Ferreon, A.C., Arai, M., and Wright, P.E. (2010). Graded enhancement of p53 binding to CREB-binding protein (CBP) by multisite phosphorylation. Proc. Natl. Acad. Sci. USA *107*, 19290–19295.

Lee, J.E., Silhavy, J.L., Zaki, M.S., Schroth, J., Bielas, S.L., Marsh, S.E., Olvera, J., Brancati, F., Iannicelli, M., Ikegami, K., et al. (2012). CEP41 is mutated in Joubert syndrome and is required for tubulin glutamylation at the cilium. Nat. Genet. *44*, 193–199.

Lindeboom, J.J., Nakamura, M., Hibbel, A., Shundyak, K., Gutierrez, R., Ketelaar, T., Emons, A.M., Mulder, B.M., Kirik, V., and Ehrhardt, D.W. (2013). A mechanism for reorientation of cortical microtubule arrays driven by microtubule severing. Science *342*, 1245533.

Loughlin, R., Wilbur, J.D., McNally, F.J., Nédélec, F.J., and Heald, R. (2011). Katanin contributes to interspecies spindle length scaling in Xenopus. Cell *147*, 1397–1407.

Lu, C., Srayko, M., and Mains, P.E. (2004). The Caenorhabditis elegans microtubule-severing complex MEI-1/MEI-2 katanin interacts differently with two superficially redundant beta-tubulin isotypes. Mol. Biol. Cell *15*, 142–150.

Magiera, M.M., and Janke, C. (2013). Investigating tubulin posttranslational modifications with specific antibodies. Methods Cell Biol. *115*, 247–267.

McNally, K.P., and McNally, F.J. (2011). The spindle assembly function of Caenorhabditis elegans katanin does not require microtubule-severing activity. Mol. Biol. Cell *22*, 1550–1560.

McNally, F.J., and Vale, R.D. (1993). Identification of katanin, an ATPase that severs and disassembles stable microtubules. Cell *75*, 419–429.

Miller, K.E., and Heald, R. (2015). Glutamylation of Nap1 modulates histone H1 dynamics and chromosome condensation in Xenopus. J. Cell Biol. *209*, 211–220.

Mukai, M., Ikegami, K., Sugiura, Y., Takeshita, K., Nakagawa, A., and Setou, M. (2009). Recombinant mammalian tubulin polyglutamylase TTLL7 performs both initiation and elongation of polyglutamylation on beta-tubulin through a random sequential pathway. Biochemistry *48*, 1084–1093.

Nash, P., Tang, X., Orlicky, S., Chen, Q., Gertler, F.B., Mendenhall, M.D., Sicheri, F., Pawson, T., and Tyers, M. (2001). Multisite phosphorylation of a CDK inhibitor sets a threshold for the onset of DNA replication. Nature *414*, 514–521.

Peris, L., Wagenbach, M., Lafanechère, L., Brocard, J., Moore, A.T., Kozielski, F., Job, D., Wordeman, L., and Andrieux, A. (2009). Motor-dependent microtubule disassembly driven by tubulin tyrosination. J. Cell Biol. *185*, 1159–1166.

Pufall, M.A., Lee, G.M., Nelson, M.L., Kang, H.S., Velyvis, A., Kay, L.E., McIntosh, L.P., and Graves, B.J. (2005). Variable control of Ets-1 DNA binding by multiple phosphates in an unstructured region. Science *309*, 142–145.

Raybin, D., and Flavin, M. (1975). An enzyme tyrosylating alpha-tubulin and its role in microtubule assembly. Biochem. Biophys. Res. Commun. *65*, 1088–1095.

Redeker, V. (2010). Mass spectrometry analysis of C-terminal posttranslational modifications of tubulins. Methods Cell Biol. *95*, 77–103.

Redeker, V., Levilliers, N., Vinolo, E., Rossier, J., Jaillard, D., Burnette, D., Gaertig, J., and Bré, M.H. (2005). Mutations of tubulin glycylation sites reveal cross-talk between the C termini of alpha- and beta-tubulin and affect the ciliary matrix in Tetrahymena. J. Biol. Chem. *280*, 596–606.

Regnard, C., Desbruyères, E., Denoulet, P., and Eddé, B. (1999). Tubulin polyglutamylase: isozymic variants and regulation during the cell cycle in HeLa cells. J. Cell Sci. *112*, 4281–4289.

Ribbeck, K., and Mitchison, T.J. (2006). Meiotic spindle: sculpted by severing. Curr. Biol. *16*, R923–R925.

Rogowski, K., van Dijk, J., Magiera, M.M., Bosc, C., Deloulme, J.C., Bosson, A., Peris, L., Gold, N.D., Lacroix, B., Bosch Grau, M., et al. (2010). A family of protein-deglutamylating enzymes associated with neurodegeneration. Cell *143*, 564–578.

Roll-Mecak, A. (2015). Intrinsically disordered tubulin tails: complex tuners of microtubule functions? Semin. Cell Dev. Biol. *37*, 11–19.

Roll-Mecak, A., and McNally, F.J. (2010). Microtubule-severing enzymes. Curr. Opin. Cell Biol. *22*, 96–103.

Roll-Mecak, A., and Vale, R.D. (2005). The Drosophila homologue of the hereditary spastic paraplegia protein, spastin, severs and disassembles microtubules. Curr. Biol. *15*, 650–655.

Roll-Mecak, A., and Vale, R.D. (2006). Making more microtubules by severing: a common theme of noncentrosomal microtubule arrays? J. Cell Biol. *175*, 849–851.

Roll-Mecak, A., and Vale, R.D. (2008). Structural basis of microtubule severing by the hereditary spastic paraplegia protein spastin. Nature *451*, 363–367.

Schneider, A., Plessmann, U., Felleisen, R., and Weber, K. (1998). Posttranslational modifications of trichomonad tubulins; identification of multiple glutamylation sites. FEBS Lett. *429*, 399–402.

Schulze, E., Asai, D.J., Bulinski, J.C., and Kirschner, M. (1987). Posttranslational modification and microtubule stability. J. Cell Biol. *105*, 2167–2177.

Sharma, N., Bryant, J., Wloga, D., Donaldson, R., Davis, R.C., Jerka-Dziadosz, M., and Gaertig, J. (2007). Katanin regulates dynamics of microtubules and biogenesis of motile cilia. J. Cell Biol. *178*, 1065–1079.

Sherwood, N.T., Sun, Q., Xue, M., Zhang, B., and Zinn, K. (2004). Drosophila spastin regulates synaptic microtubule networks and is required for normal motor function. PLoS Biol. *2*, e429.

Sirajuddin, M., Rice, L.M., and Vale, R.D. (2014). Regulation of microtubule motors by tubulin isotypes and post-translational modifications. Nat. Cell Biol. *16*, 335–344.

Srayko, M., O'toole, E.T., Hyman, A.A., and Müller-Reichert, T. (2006). Katanin disrupts the microtubule lattice and increases polymer number in C. elegans meiosis. Curr. Biol. *16*, 1944–1949.

Stewart, A., Tsubouchi, A., Rolls, M.M., Tracey, W.D., and Sherwood, N.T. (2012). Katanin p60-like1 promotes microtubule growth and terminal dendrite stability in the larval class IV sensory neurons of Drosophila. J. Neurosci. *32*, 11631–11642.

Stone, M.C., Rao, K., Gheres, K.W., Kim, S., Tao, J., La Rochelle, C., Folker, C.T., Sherwood, N.T., and Rolls, M.M. (2012). Normal spastin gene dosage is specifically required for axon regeneration. Cell Rep. *2*, 1340–1350.

Sudo, H., and Baas, P.W. (2010). Acetylation of microtubules influences their sensitivity to severing by katanin in neurons and fibroblasts. J. Neurosci. *30*, 7215–7226.

Sullivan, K.F. (1988). Structure and utilization of tubulin isotypes. Annu. Rev. Cell Biol. *4*, 687–716.

Szyk, A., Deaconescu, A.M., Piszczek, G., and Roll-Mecak, A. (2011). Tubulin tyrosine ligase structure reveals adaptation of an ancient fold to bind and modify tubulin. Nat. Struct. Mol. Biol. *18*, 1250–1258.

Szyk, A., Deaconescu, A.M., Spector, J., Goodman, B., Valenstein, M.L., Ziolkowska, N.E., Kormendi, V., Grigorieff, N., and Roll-Mecak, A. (2014). Molecular basis for age-dependent microtubule acetylation by tubulin acetyltransferase. Cell *157*, 1405–1415.

Trotta, N., Orso, G., Rossetto, M.G., Daga, A., and Broadie, K. (2004). The hereditary spastic paraplegia gene, spastin, regulates microtubule stability to modulate synaptic structure and function. Curr. Biol. *14*, 1135–1147.

van Dijk, J., Rogowski, K., Miro, J., Lacroix, B., Eddé, B., and Janke, C. (2007). A targeted multienzyme mechanism for selective microtubule polyglutamylation. Mol. Cell *26*, 437–448.

van Dijk, J., Miro, J., Strub, J.M., Lacroix, B., van Dorsselaer, A., Edde, B., and Janke, C. (2008). Polyglutamylation is a post-translational modification with a broad range of substrates. J. Biol. Chem. *283*, 3915–3922.

Vemu, A., Garnham, C.P., Lee, D.Y., and Roll-Mecak, A. (2014). Generation of differentially modified microtubules using in vitro enzymatic approaches. Methods Enzymol. *540*, 149–166.

Verhey, K.J., and Gaertig, J. (2007). The tubulin code. Cell Cycle *6*, 2152–2160.

Vietri, M., Schink, K.O., Campsteijn, C., Wegner, C.S., Schultz, S.W., Christ, L., Thoresen, S.B., Brech, A., Raiborg, C., and Stenmark, H. (2015). Spastin and ESCRT-III coordinate mitotic spindle disassembly and nuclear envelope sealing. Nature *522*, 231–235.

Webster, D.R., Gundersen, G.G., Bulinski, J.C., and Borisy, G.G. (1987). Differential turnover of tyrosinated and detyrosinated microtubules. Proc. Natl. Acad. Sci. USA *84*, 9040–9044.

Widlund, P.O., Podolski, M., Reber, S., Alper, J., Storch, M., Hyman, A.A., Howard, J., and Drechsel, D.N. (2012). One-step purification of assembly-competent tubulin from diverse eukaryotic sources. Mol. Biol. Cell *23*, 4393–4401.

Wolff, A., de Néchaud, B., Chillet, D., Mazarguil, H., Desbruyères, E., Audebert, S., Eddé, B., Gros, F., and Denoulet, P. (1992). Distribution of glutamylated alpha and beta-tubulin in mouse tissues using a specific monoclonal antibody, GT335. Eur. J. Cell Biol. *59*, 425–432.

Wright, P.E., and Dyson, H.J. (2015). Intrinsically disordered proteins in cellular signalling and regulation. Nat. Rev. Mol. Cell Biol. *16*, 18–29.

Yu, W., Qiang, L., Solowska, J.M., Karabay, A., Korulu, S., and Baas, P.W. (2008). The microtubule-severing proteins spastin and katanin participate differently in the formation of axonal branches. Mol. Biol. Cell *19*, 1485–1498.

Yu, I., Garnham, C.P., and Roll-Mecak, A. (2015). Writing and reading the tubulin code. J. Biol. Chem. *290*, 17163–17172.

Zempel, H., Luedtke, J., Kumar, Y., Biernat, J., Dawson, H., Mandelkow, E., and Mandelkow, E.M. (2013). Amyloid-β oligomers induce synaptic damage via Tau-dependent microtubule severing by TTLL6 and spastin. EMBO J. *32*, 2920–2937.

Zhang, D., Rogers, G.C., Buster, D.W., and Sharp, D.J. (2007). Three microtubule severing enzymes contribute to the "Pacman-flux" machinery that moves chromosomes. J. Cell Biol. *177*, 231–242.

Ziółkowska, N.E., and Roll-Mecak, A. (2013). In vitro microtubule severing assays. Methods Mol. Biol. *1046*, 323–334.