Experience from other apparently intractable issues, such as negotiating the end to armed conflict in Colombia and apartheid in South Africa (*13*) and international climate change negotiations (*14*), suggests that such frequent, iterative interactions among a small group of key parties are more likely to engender trust and agreement than an international vote open to the media and campaigning pressures. For example, the success of the 2016 Paris climate agreement built on a prior bilateral agreement between the United States and China. This agreement stemmed from a working group that met several times outside of the public's view over more than 2 years (*14*). Experience from the African Elephant Range States Dialogues also suggests that concordance on ivory policy may best be found outside the public and adversarial environment of CITES CoPs (*9*).

The next CITES CoP is less than 2 years away. We recognize that the politics around ivory policy are challenging, but urge range states to begin a structured process to negotiate the diverse perspectives in this contentious debate as soon as possible, supported by organizations committed to elephant conservation. Successful navigation of different mental models and the associated values, and the trade-offs they imply, will not only enable greater collective action on elephant conservation, but also provide an example of how to enhance the structured use of evidence in CITES decision-making on other iconic species. ■

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SUPPLEMENTARY MATERIALS

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CELL BIOLOGY

Closing the tubulin detyrosination cycle

Enzymes that detyrosinate the microtubule cytoskeleton are identified

By **Anna Akhmanova**[1] *and* **Helder Maiato**[2,3,4]

Microtubules are cytoskeletal filaments that drive chromosome segregation during cell division, control cell shape and motility, and serve as rails for motor protein–based intracellular transport. Microtubules are polymers built of highly conserved subunits, α- and β-tubulin, which contain a globular core and more variable C-terminal tails that are exposed at the microtubule surface. Although microtubules are structurally uniform, they display functional specialization due to the combination of different tubulin isoforms and multiple posttranslational modifications (*1*). Many of these modifications occur within the C-terminal tails and affect microtubule interactions with motor proteins or regulatory factors. The first tubulin modifications were discovered more than 40 years ago and consist of the catalytic removal and reincorporation of the C-terminal tyrosine, an amino acid residue that is present in most α-tubulin isotypes (see the figure) (*2*–*4*). Whereas retyrosination of soluble tubulin is known to be mediated by tubulin-tyrosine ligase (*5*), α-tubulin detyrosination, which occurs preferentially on microtubules, is mediated by an unknown carboxypeptidase activity. On pages 1448 and 1453 of this issue, Aillaud *et al.* (*6*) and Nieuwenhuis *et al.* (*7*) report the identification and characterization of vasohibins as long-sought tubulin carboxypeptidases.

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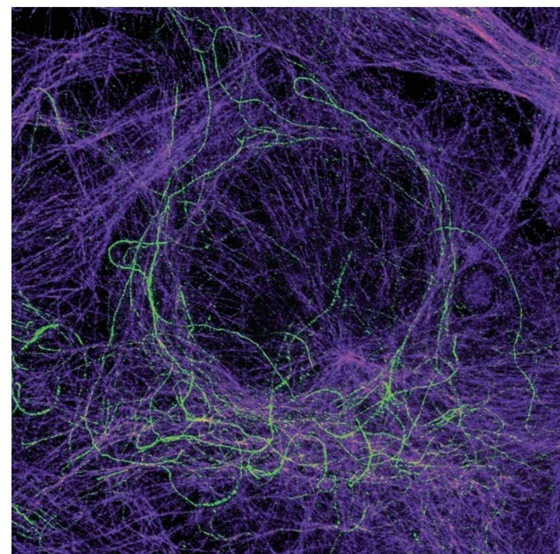
Vasohibins were originally identified as regulators of new blood vessel formation (angiogenesis) (*8*) and have a predicted protease fold with a noncanonical catalytic triad consisting of cysteine, histidine, and serine or threonine (*9*). Mammalian genomes encode two vasohibin paralogs, vasohibin-1 and vasohibin-2, but their proteolytic activity and molecular function had never been explored. Importantly, vasohibins form a complex with the chaperone-like peptide small vasohibin binding protein (SVBP), which is required for vasohibin stability and function (*10*). This might explain why previous attempts to identify the tubulin carboxypeptidase have failed, because standard purification assays could result in dissociation of SVBP from vasohibins, compromising their catalytic activity.



Stable detyrosinated microtubules are highlighted in green, revealing microtubule diversity in human cells.

The two groups seeking the elusive tubulin carboxypeptidase converged on vasohibins using different unbiased approaches. Aillaud *et al.* used chemical proteomics; they developed a potent irreversible inhibitor of tubulin carboxypeptidase and combined it with mass spectrometry–based analysis of fractionated mouse brain lysates to identify vasohibin-1 as the stron-

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gest candidate for the target of their inhibitor (*6*). Conversely, Nieuwenhuis *et al.* performed a genetic screen for regulators of tubulin detyrosination using gene-trapping mutagenesis in a haploid human cell line. Reassuringly, they not only identified tubulin-tyrosine ligase as the strongest negative regulator of tubulin detyrosination but also isolated SVBP as the most prominent positive regulator of this process (*7*).

Both groups biochemically and functionally characterized the vasohibin-SVBP complexes. They found that SVBP binds to vasohibins and enhances their tubulin carboxypeptidase activity both in cells and in assays with purified proteins (*6*, *7*). As expected, the cysteine residues in the predicted catalytic triad of vasohibins were indeed important for the activity (*6*, *7*). Consistent with previous studies of microtubule detyrosination, vasohibin-SVBP complexes displayed higher carboxypeptidase activity toward α-tubulin present in microtubules compared with its unpolymerized form. Loss-of-function studies in cultured neurons, cell lines, and mouse embryos revealed a redundant role of the two vasohibins in tubulin detyrosination. Importantly, some detyrosinated tubulin was still present when both vasohibins were depleted. This could not be attributed to partial depletion of vasohibins or the expression of the α-tubulin isoform TUBA4A, which lacks the C-terminal tyrosine (*7*). Thus, cells contain an additional tubulin-detyrosinating activity that remains to be identified.

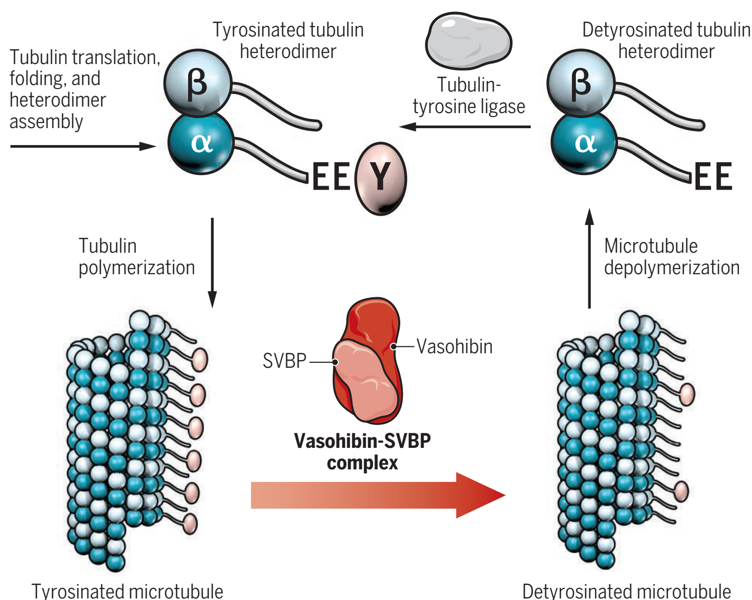
The tubulin detyrosination-retyrosination cycle is intimately linked to microtubule dynamics; freshly polymerized microtubules are typically tyrosinated, whereas more stable, long-lived microtubules can be preferentially detyrosinated (*11*). Furthermore, the affinity of some regulators of microtubule stability is modulated by detyrosination (*1*). The tyrosination state of microtubules can also affect the binding of motor proteins: Some kinesins bind better to detyrosinated microtubules compared with tyrosinated ones; conversely, cytoplasmic dynein with its cofactors prefer tyrosinated microtubules (*1*). As a result, intracellular cargoes linked to particular motor proteins can either preferentially use or avoid detyrosinated microtubules, resulting in differential cargo transport along particular microtubule tracks. Through such mechanisms, tubulin detyrosination plays a role in vital cellular functions, including cell division (*12*), cardiomyocyte contraction (*13*), and neuronal physiology (*14*).

In neurons, the depletion of both vasohibins or SVBP, which reduced but did not abolish the levels of detyrosinated α-tubulin, delayed neuronal differentiation, induced morphological abnormalities, and affected neuronal migration in mouse embryos (*6*). These results are consistent with previous work showing that tubulin-tyrosine ligase (which performs the reverse reaction) is required for neuronal function (*14*). Curiously, contrary to other experimental perturbations that attenuate tubulin detyrosination during mitosis (*12*), vasohibin deficiency did not seem to compromise chromosome congression (in which chromosomes are aligned on the mitotic microtubule spindle), despite a clear reduction in microtubule detyrosination (*7*). This discrepancy might be explained by functional redundancy with unidentified tubulin carboxypeptidases that could ensure sufficient tubulin detyrosination or by redundancy in the process of chromosome congression. Future work is necessary to address this issue, and the availability of powerful new tools, such as potent and specific tubulin carboxypeptidase inhibitors and deficient cell lines developed by Aillaud *et al.* and Nieuwenhuis *et al.*, will certainly play an important role.

The discovery of vasohibins as major tubulin detyrosinating enzymes will help to understand how cells deploy and use tubulin modifications to control cytoskeletal organization and transport. This discovery also raises several exciting questions. Previous work on vasohibins indicated that they might be secreted through an unconventional mechanism (*8*, *10*) and that they can differentially affect angiogenesis and might also have a role in cancer (*8*). It would be interesting to know whether secreted vasohibins maintain their tubulin detyrosinating activity and whether they can participate in intercellular communication mechanisms affecting cytoskeletal organization and trafficking. Another important question is whether tubulin is the only substrate of these enzymes. Aillaud *et al.* showed that vasohibin-2, but not vasohibin-1, could remove the C-terminal tyrosine from the microtubule-regulating protein EB1, although the functional implication of this is unclear. Future structural studies of vasohibins bound to SVBP will be important to address how these enzymes interact with their substrates such as microtubules. Moreover, mice lacking vasohibin-1 or vasohibin-2 have relatively mild phenotypes (*15*), and more detailed analysis of these mice as well as the generation of animals completely lacking the vasohibin-SVBP complexes are needed to address the role of these proteins and tubulin detyrosination in the context of the whole organism. ■

The tubulin detyrosination-retyrosination cycle

Newly translated α-tubulin contains a C-terminal tyrosine (Y). α-Tubulin and β-tubulin form a heterodimer, which assembles into microtubules. The vasohibin-SVBP complex removes C-terminal tyrosines from microtubules. When these microtubules disassemble, detyrosinated α-tubulin can be tyrosinated by tubulin-tyrosine ligase. E, glutamic acid.



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