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MEETING REPORT

Meeting report – the ever-fascinating world of septins

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ABSTRACT

Septins are GTP-binding proteins that assemble into hetero-oligomers. They can interact with each other end-to-end to form filaments, making them the fourth element of the cytoskeleton. To update the current knowledge on the ever-increasing implications of these fascinating proteins in cellular functions, a hundred expert scientists from across the globe gathered from 12 to 15 October 2021 in Berlin for the first hybrid-format (on site and virtual) EMBO workshop Molecular and Cell Biology of Septins.

Introduction

The septin family consists of a myriad of evolutionarily conserved paralogs and isoforms, which, in a regulated manner, organize into oligomeric complexes and higher-order structures, such as filaments, rings or gauzes. On this golden (50th) anniversary of their discovery as critical components in cell division, our understanding of their involvement in additional cellular processes continues to grow, as revealed during this very successful EMBO workshop organized by Helge Ewers (Freie Universität Berlin, Germany), Serge Mostowy (London School of Hygiene and Tropical Medicine, UK) and Aurélie Bertin (Institut Curie, Paris, France). From *in vitro* experiments with purified proteins to *in vivo* studies in mice, the meeting explored the roles of septins and their interaction partners across length and time scales. As imaging technologies continue to advance, genetic perturbations become easier and recombinant septin complexes become more available, the community is well placed to continue discovering the many exciting and novel functions of septins. Here, we recapitulate new findings and themes that emerged from the meeting, highlighting the growing importance of these proteins in health and disease.

Hybrid format

With the pandemic still hindering gatherings across the globe, it was decided to host the meeting in a hybrid format, a first for EMBO workshops. Half the attendees were able to attend in person at the Max Planck Society's Harnack House in Berlin, while the rest joined virtually. Presenters, regardless of their actual location, easily shared their talks via Zoom with just a few hiccups. Questions following presentations were fielded from both virtual and physical attendees. Those in the audience used a microphone to ensure that everyone could hear, while those online unmuted themselves to ask their question face to face. The chat window offered another easy way to ask questions and these were raised by the session chair. The seamless switching between virtual and physical attendees made for a lively, inclusive meeting that fostered the collegiality the community is known for. The poster sessions, often the most difficult to replicate experience of physical meetings, were entirely virtual. Each presenter had a dedicated breakout room and attendees could freely move between them, just like at an in-person meeting, making these sessions an overall success. The greatest challenge of the hybrid format was undoubtedly the scheduling. On this front, the dedication of the septin community truly shone with those joining from the US West coast up in the early morning hours with an extra strong cup of coffee, while others based in Asia or Australia stayed up very late to participate. Overall, the success of the meeting showed that hybrid meetings can be a fruitful experience with proper planning.

Evolutionary relationships

Following an opening evening keynote, the meeting kicked off its first full day with an excellent global summary of septin evolution. The prevalence of septin-encoding genes in extant organisms implies that the ancestral septin appeared long ago, but the absence of septins from land plants and the variation in the number of septin genes even within the animal kingdom indicates that septin evolution has been dynamic. Michelle Momany (University of Georgia, Athens, USA) showed how evolutionary conservation in the five septin groups of fungi, animals and protists illuminates important functional and structural features, including monomer interactions in heteropolymers (Auxier et al., 2019; Pan et al., 2007). While land plants lack septins, they do have septin-related proteins from the translocon of the outer membrane of the chloroplast (Toc) family. Samed Delic (Duke University, Durham, USA) explored the single septin encoded by the genome of the green alga *Chlamydomonas reinhardtii*. Inspired by published structural analysis from the Garratt and Araújo labs (Pinto et al., 2017), Samed found that Sep1 interacts with the Toc proteins at the chloroplast membrane via an ancient oligomerization interface. Based on this evidence, it will be interesting to see what future studies reveal about the co-assembly of septins and their distant cousins into functional complexes.

Specific inter-subunit and higher-order septin assembly

Deciphering the rules that govern the subtle molecular recognition between septin subgroups to achieve correct subunit assembly remains an ongoing area of intensive and cooperative research, comprising data from yeast and mammalian cells. Recent breakthroughs from the Garratt and Araújo, and Trimble laboratories revealed that instead of being localized at the ends of the hetero-oligomeric complexes, SEPT7 or SEPT9 form the central homodimer of either the hexamer or the octamer, respectively (Mendonça et al., 2019; Soroor et al., 2021). Importantly, this reconciles the observations in the human system with the octamer model of budding yeast where Cdc10, the yeast septin most similar to SEPT9, is at the center of the octamer (Bertin et al., 2008). Based

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on crystallographic analysis of the molecular architecture of heterodimeric complexes, Richard Garratt (São Carlos Institute of Physics, Brazil) discussed that specificity between septin subunits partly arises from their switch I and II regions at the G-interface. This would only allow members of the SEPT2 and SEPT6 groups to generate contacts, whereas it would only permit the formation of a SEPT7 homodimer (Rosa et al., 2020). Using cryo-electron microscopy, his group also found that large internal cavities, sheltering the two α_0 helices that bear the polybasic regions, are recovered at the NC-interface between septin group members. This spacing, which allows the flexing of monomers relative to one another (Mendonça et al., 2021), would expose the α_0 helix upon GTP-dependent closure of this NC-interface, thus rendering the oligomer competent for membrane interaction in a curvature-dependent manner. From the same lab, Italo Cavini (São Carlos Institute of Physics, Brazil) further showed that homodimeric coiled-coils of members of the SEPT2 and SEPT6 groups exhibit an orientational duality, with both antiparallel and parallel arrangements. In a physiological context, the hydrophilic antiparallel form would be more prone to membrane association, whereas the hydrophobic parallel structure would occur in stacked filaments, similar to what is observed in cytoskeletal networks (Leonardo et al., 2021).

Using *in vitro* assays with purified septin complexes from different organisms, the talks from Aurélie Bertin (Institut Curie, CNRS, Paris, France) and Gijsje Koenderink (Delft University of Technology, Netherlands) shared findings on the higher-order septin organization obtained through a variety of advanced imaging techniques. Bertin demonstrated how purified yeast septin complexes restrict diffusion within, and cause deformation and even disruption of, synthetic membranes (Vial et al., 2021), as well as how the use of lipid-coated wavy surfaces reveal distinct curvature preferences of certain septins (Beber et al., 2019). Furthermore, the use of purified human octamers, generated in collaboration with the Mavrakis laboratory (Iv et al., 2021), revealed some properties that are shared with yeast septins, but also some key differences, hinting that curvature preference can be 'tuned' by layers of filaments upon other filaments. In her talk, Koenderink showed that purified fly and human hexamers and octamers have the ability to form bundles and/or ring/wheel-like structures; these properties were modified by the addition of synthetic bilayers enriched with specific lipids (Szuba et al., 2020). In a related presentation, Michael McMurray (University of Colorado School of Medicine, USA) discussed that not only do many cytosolic chaperones interact with yeast septins (Denney et al., 2021), but several of these are crucial for correct septin folding. Indeed, one class of chaperones may specifically act co-translationally to prevent ribosome collisions during septin translation, suggesting that chaperones could thus represent novel regulators of hetero-oligomer assembly.

Even though previous studies had suggested that septins can be organized into filaments in cells, the ultimate proof that septins form linear continuous structures in mammalian cells is still missing. Unpublished results presented by Paul Markus Müller (Freie Universität Berlin, Germany), explored this subject using their endogenously tagged SEPT2–GFP cell line (Banko et al., 2019) to measure septin complex dynamics and exchange in cellular filaments via quantitative single-molecule microscopy. Similarly, using tripartite split-GFP complementation assays, Carla Martins (Institut Fresnel, CNRS, Marseille, and CRCT, Toulouse, France) presented evidence that actin-associated septins in mammalian cells polymerize into filaments that are primarily composed of octamers with a small population of septin hexamers.

Cytokinesis

Despite having been first identified as critical factors for cytokinesis (Hartwell, 1971), there is still much to learn about septins at the cleavage plane. In particular, the transition from a single-ring to a double-ring structure prior to abscission was highlighted by multiple researchers. Erfei Bi (University of Pennsylvania, Philadelphia, USA) showed that, in budding yeast, a striking zonal architecture for the septin 'hourglass' pattern in telophase cells, whereby the RhoGEF Bud3 and the anillin-like protein Bud4 localize to the outer regions, and the myosin-II filaments to the middle zone; here, Bud3 and Bud4 play distinct and essential roles in controlling septin filament organization during the hourglass-to-double-ring transition at the onset of cytokinesis (Chen et al., 2020). Specifically, Bud3 stabilizes single filaments, whereas Bud4 strengthens interactions between filaments at the outer regions, allowing the septin filaments in the middle zone to undergo cell-cycle-triggered disassembly, which enables the myosin-II filaments to access the plasma membrane and initiate actomyosin ring constriction. This nicely complements the presentation of Simonetta Piatti (CRBM, CNRS, Montpellier, France) who showed that the mitotic exit network signaling promotes septin double-ring formation through the activity of the phosphatase Cdc14 (Tamborrini et al., 2018). Their follow-up studies explore the downstream targets of Cdc14 and their impact on ring splitting.

Continuing the theme of how septins interact with the cytokinetic machinery, Michael Krauss (FMP, Berlin, Germany) showed that the PIP2-synthesizing kinase PIPK1γ, and specifically two of its isoforms, is required for the concentration of septins at the intercellular bridge. Thanks to stunning super-resolution images of the intercellular bridge using expansion microscopy (courtesy of the Ewers laboratory), he showed that depletion of these variants leads to impaired microtubule interdigitation at the midbody and poor septin localization at the intercellular bridge.

Septin compartmentalization and related functions

The more we learn about septin proteins, the more we realize that their subcellular localization is key to regulating a large variety of cellular processes. Understanding how septins bind to actin and membranes is still a subject of intensive research. Amy Gladfelter (University of North Carolina, Chapel Hill, USA), in a terrific keynote lecture, presented a historical overview of the discovery of septin–membrane associations and her own laboratory's work on how nanometer-sized oligomers can sense micron-scale changes in membrane geometry. Specifically, her group showed that the presence of a conserved amphipathic helix (AH) domain in the C-terminal extension of budding yeast Cdc12 (Cannon et al., 2019) is necessary and sufficient for membrane-curvature sensing. In addition, the Cdc12 AH domain is involved in septin filament bundling, showing that AH domains may provide another layer of control of higher-order septin assemblies and membrane binding (Woods et al., 2021).

More recently, the association of septins with the microtubule cytoskeleton, not only in dividing cells but also in interphase, has attracted much attention. In his opening keynote lecture, Elias Spiliotis (Drexel University, Philadelphia, USA), captivated the audience with the recent advances his group has made in understanding how septins interact with microtubules, as well as regulate their dynamics and microtubule motor-driven transport. In particular, he showed that SEPT2–SEPT6–SEPT7 complexes promote microtubule growth (Nakos et al., 2019). Interestingly, microtubule-associated SEPT9 differentially regulates kinesin-1 and -3 motility, providing a spatial bias in cargo selection for

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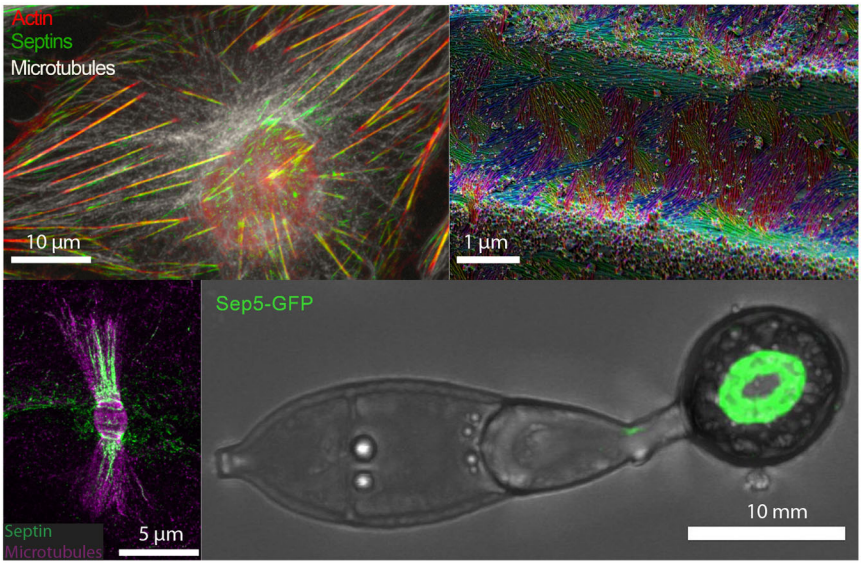

Examples of amazing septin organizations. Top left, immunofluorescence image of human immortalized hepatocytes showing septins primarily coalign with actin stress fibers under normal conditions. Image courtesy of Anita Baillet and Christian Poüs (Université Paris-Saclay, INSERM, France). Top right, SEM image of a wavy substrate incubated with purified septins. Septins orient differently depending on whether the curvature is positive or negative. Image courtesy of Aurélie Bertin (Institut Curie, CNRS, Paris, France). Bottom left, ultrastructure expansion microscopy visualization of SEPT2 and SEPT7 and microtubules at the midbody in mitotic HeLaM cells. Image represents maximum intensity projection of a 3D volume. Image courtesy of Nadja Hümpfer and Helge Ewers (Freie Universität Berlin, Germany). Bottom right, septin ring formation during appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*. Micrograph of *M. oryzae* expressing Septin5–GFP, provided by Lauren Ryder and Nick Talbot (The Sainsbury Laboratory, Cambridge, UK).

dendrite entry (Karasmanis et al., 2018). Also, SEPT9, alone or in complex with SEPT2–SEPT6–SEPT7, directly and specifically interacts with dynein–dynactin to trigger retrograde trafficking (Kesisova et al., 2020). Spiliotis concluded by positing that microtubule-associated septins, by excluding or localizing motors at specific locations, resemble the barrier or scaffolding functions of membrane-associated septins, thereby promoting asymmetry in the cytoplasm.

Several studies also highlighted the role of partner proteins or post-translational modifications (PTMs) in the spatio-temporal distribution of septins. Anita Baillet (Université Paris-Saclay, INSERM, France) found that the interplay between septins, actin and microtubules can be regulated by the nucleotide state of Cdc42 and its downstream effectors of the BORG family. Indeed, Taxol®-induced Cdc42 inactivation triggers the release of BORG2 from stress fibers and its subsequent proteasome-mediated degradation, resulting in the striking relocalization of septins from actin to microtubules (Salameh et al., 2021). Folma Buss (University of Cambridge, UK) spoke about their work that identified LRCH3 not only as a novel adaptor protein linking the actin-based motor MYO6 and the RhoGEF DOCK7 and forming the septin DISP complex, but also as a novel regulator of septin organization (O'Loughlin et al., 2018). Indeed, overexpression of the DISP complex results in the formation of septin rings, indicating a displacement of septins from the actin cytoskeleton. Arnaud Echard (Institut Pasteur, CNRS, Paris, France) showed that SUMOylation of the C-terminal region of septins prevents their bundling within the intercellular bridge in the late steps of cytokinesis (Ribet et al., 2017), which is supported by *in vitro* data obtained together with Aurélie Bertin. Based on their proteomic analysis of purified midbodies (Addi et al., 2020), he also proposed that septin clearance during cytokinesis might be mediated by septin ubiquitylation. Addressing another PTM, Smita Yadav (University of Washington, Seattle, USA) showed that, in hippocampal neurons, SEPT7 phosphorylation at the evolutionarily conserved T426 residue, which is mediated by the autism-related TAOK2 kinase (Yadav et al., 2017), induces its translocation from the base of the dendritic spine to the spine head, where it interacts with distinct isoforms of the 14-3-3 protein family, known to be important for synaptic function. Finally, the relationship between septins and lipids is not restricted to interactions of septins with membrane phosphoinositides. Ulrike Eggert (King's College London, UK) described specific lipid changes that they observed in the lipidomes of cells depleted of SEPT7 or SEPT9, and showed that SEPT2 is associated with specific lipid species, including one enriched at midbodies.

Septin neuronal signaling

It is well established that septins play key roles in neuronal development (Ageta-Ishihara and Kinoshita, 2020). While septins are fundamental to a number of processes in development of neuronal morphology and function, a common theme emerged at this meeting, linking them to signaling. Makoto Kinoshita (Nagoya University, Japan) showed that while SEPT3 knockdown had no effect on dendritogenesis or spine density in hippocampal neurons, it did result in reduced rates of ER extension into dendritic spines. This is specific to the SEPT3 subunit and results from a Ca^{2+}-mediated interaction with the motor protein Myo5a. Following on the theme of Ca^{2+}-mediated signaling, Gaiti Hasan (National Centre for Biological Sciences, Bengaluru, India) showed that SEPT7 negatively regulates Ca^{2+} entry into the endoplasmic reticulum (ER) in Purkinje neurons (Deb et al., 2020). She then reported that knockout (KO) of SEPT7 could surprisingly rescue defects associated with poor store-operated Ca^{2+} entry, making SEPT7 a potential therapeutic target.

Building upon this topic, Hauke Werner (Max Planck Institute for Experimental Medicine, Göttingen, Germany) showed beautiful scanning electron microscopy (SEM) images of myelin sheaths surrounding axons. Using proteomics, his group identified a number of septins that are involved in the formation of these sheaths that are essential for rapid conduction of electrical signals along axons (Patzig et al., 2016). When they knocked out SEPT8, all other septins were lost from the myelin sheath and large myelin outfoldings formed, reducing nerve conduction (Erwig et al., 2019). This suggests that SEPT8 plays an important role in stabilizing the sheaths. On a similar note, Matthew Rasband (Baylor College of Medicine, Houston, USA) used BioID (proximity-dependent biotin identification) to identify septins in the axon initial segment (AIS) (Hamdan et al., 2020), which is responsible for generating the action potential and maintaining neuronal polarity. Strikingly, his lab found that SEPT5 and SEPT6 did not only associate with other known AIS proteins such as Ankyrin G, but play a critical role in

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both AIS assembly and stabilization. This role as a stabilizer of neuronal components is an intriguing parallel to septins functions in non-neuronal cells and hints at essential roles in maintaining specific structures within cells of all types.

Septins and mechanical signals

As we learn more about mammalian septin functions, it is becoming increasingly clear that septins may also have pivotal roles in mechanotranduction, the conversion of mechanical signals into biochemical cues, which is often associated with the actomyosin cytoskeleton. Whereas the association of septins with the actomyosin contractile ring during cytokinesis is well documented, a number of speakers highlighted other potential mechanical functions. John Cooper (Washington University, St Louis, USA) showed that septins can be found at the base of cell–cell junctions in endothelial cells (Kim and Cooper, 2020). He speculated that septins play a mechanical role in stabilizing the junctions by binding to regions of positive curvature created by actin-filled protrusions. Indeed, loss of SEPT2 in these junctions results in the destabilization of the rest of the adherens and tight junction proteins, impairing overall junction integrity. Similarly, Thomas Gronemeyer (Universität Ulm, Germany) highlighted their work, which identified a number of adhesion-related proteins by mass spectroscopy that directly interact with SEPT9 (Hecht et al., 2019). He also showed that changes in SEPT9 expression levels, both knockdown (KD) and overexpression, dramatically impact cell motility, actin organization and focal adhesion morphology. Interestingly, SEPT9 KD could be rescued in part by expression of EPLIN, a focal adhesion protein that binds to SEPT9 via its LIM domain.

In her talk, Celeste Nelson (Princeton University, USA) demonstrated that downstream of the adhesion itself, signaling through β1 integrins present at the adhesion results in increased expression of SEPT6 on stiff substrates (Rabie et al., 2021). This process is important during epithelial-to-mesenchymal transition and metastasis of breast cancer. Increased SEPT6 expression in cells on a stiff substrate leads to a reinforcement of the midbody complex, failed abscission and ultimately more multinucleated cells. Looking upstream of adhesion signaling and at RhoA specifically, Patrick Oakes (Loyola University Chicago, USA) showed that SEPT2 relocalizes from actin filaments to the membrane under Rho kinase inhibition, but that elevated RhoA activity on its own is insufficient to recruit additional SEPT2 to actin stress fibers. Rachel Shannon (University of Toronto, Canada) presented work illustrating that SEPT9 and its interactor, the RhoA GEF ARHGEF18, are important for mitochondrial fission, potentially to generate localized myosin contractility at sites of mitochondrial fission, and leading to the recruitment of the fission factor Drp1 (also known as DNM1L). This work builds upon previous findings that septins interact with Drp1 (Pagliuso et al., 2016; Sirianni et al., 2016) and suggests that the septin–RhoA interaction is more significant that currently appreciated.

There were also intriguing developments regarding a potential relationship between septins and myosins. Vladimir Ugorets (Freie Universität Berlin, Germany) presented observations that actin-decorating septin polymers in proliferating myoblasts are reorganized into shorter rod-like structures and rings during myogenesis. He further showed that Sept9 depletion enhances the myogenic potential of mouse myoblasts, suggesting it is acting as a gatekeeper to prevent premature differentiation.

Finally, Andrew Weems (University of Texas Southwestern, USA) shared exciting new data showing that in non-adherent melanoma cells, septins are recruited to the plasma membrane by a bleb-generated membrane curvature (Weems et al., 2021 preprint). Such blebbing is typically seen when cells detach from extracellular matrix substrates, a stressed condition that can lead to programmed cell death due to a lack of adhesion-driven signaling. In these melanoma cells, however, bleb-recruited septins act as signaling scaffolds, essentially replacing signaling typically mediated through adhesions, leading to the upregulation of ERK and PI3K signaling and ultimately cell survival. Taken together, these presentations highlighted a number of interesting ways in which septins are integrated into mechanical signaling, and pointed to a number of promising new research avenues to pursue in the future.

Septins and disease

Septins are also relevant to disease and not only when they are dysfunctional. Barbara Zieger (University of Freiburg Medical Center, Germany) spoke about their studies using fresh platelets from *Sept8* KO mice to explore in greater detail functional roles for septins in platelets, which was originally suggested by a bleeding disorder in a patient with a septin gene deletion. Defects in specific platelet functions were observed that provide new insights into septin roles (Neubauer et al., 2021). Matthias Gaestel (Hannover Medical School, Germany) also shared phenotypes of tissue-specific deletion of *Sept7* in mice, including a surprising effect on delaying tumorigenesis that is induced by oncogenic activation, suggesting that septin inhibition could be an anti-tumor strategy for solid tumors.

Septins can also protect against infectious diseases. Pox viruses like vaccinia virus exploit the host actin cytoskeleton to spread from cell to cell, and septins assemble around membrane-coated virions attached to the cell surface, inhibiting virion release (Pfanzelter et al., 2018). Michael Way (The Francis Crick Institute, London, UK) shared cryo-electron tomography images revealing that septin filaments are found at a fixed distance from the membrane via an unknown ‘spacer’. Septin complexes similarly assemble into ‘cages’ around some intracellular pathogenic bacteria that also rely on actin polymerization for cell-to-cell spread. Damián Lobato-Márquez (London School of Hygiene and Tropical Medicine, London, UK) described the reconstitution of this process *in vitro* using purified septins, which provides a system to precisely dissect the requirements for septin caging of actively-dividing bacteria. Strikingly, he observed a ∼16-nm spacing of recombinant septin complexes away from the bacterial surface (Lobato-Márquez et al., 2021), which is quite similar to the spacing in the case of vaccinia. Moreover, Bertin reminded us during the discussion that she also had previously seen such spacing at the yeast bud neck (Bertin et al., 2012). The identity of the spacer molecule(s) – perhaps the septin C-terminal extensions? – will be an intriguing subject of future research.

Finally, illustrating their diverse function, septins also help fungi cause disease, such as rice blast caused by the fungus *Magnaporthe oryzae*; here, a septin ring helps the fungus penetrate the plant surface. Nick Talbot (The Sainsbury Laboratory, Cambridge, UK) presented their efforts in exploiting a dependence of septins on very-long-chain fatty acids for their functions in infection in order to identify chemical inhibitors that act as potent fungicides to protect various plants (and even insects!) without any adverse host effects (He et al., 2020).

Future perspectives

Thanks to *in vitro* reconstitution experiments, improved microscopy technologies and sub-atomic resolution of crystal structures, elucidating the timeline underlying the assembly of septin subunits for correct filamentation is well underway. Future functional research will now address whether and how partner

proteins or distinct septin paralogs dynamically contribute to the specific association of septin filaments with membranes or other cytoskeletal networks. In that respect, resolving atomic structures of various septin–partner interactions should uncover new mechanistic insights into higher-order septin assemblies and subcellular partitioning. In addition, emphasis will need to be directed towards the role of PTMs, which have been recently uncovered as an additional layer of regulation of septin localization and function in both dividing and non-dividing cells. Deciphering septin functions *in vivo* will obviously require efforts across systems and scales. The prospect of a number of soon to be publicly available endogenously labeled septin and septin *flox* mice, as revealed by Roberto Weigert (NIH, Bethesda, USA), is exciting, and no doubt these tools will aid this endeavor. Additionally, the discovery of specific chemical tools would be very useful, as use of forchlorfenuron, an inhibitor of septin assembly, is marred by off-target effects and mechanistic uncertainty. Currently, the search for new septin inhibitors remains a neglected area that warrants further efforts. The main goal of the ongoing intensive multidisciplinary research will ultimately tell us why dysregulation of septin localization or function can cause neurodegenerative disorders or participate in the emergence of infections or cancers, and whether septins could be feasible targets to treat such diseases.

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Competing interests
The authors declare no competing or financial interests.

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