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Cytoskeletal Filament Systems

Assembly, Regulation, and Interplay in Mammalian Cells

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In memory of my father

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LIST OF PUBLICATIONS

Publications included in the thesis

This thesis is based on the following published papers and manuscripts, which will be referred to by their roman numerals (I-IV). Published papers are reproduced with permission from the journal publishers.

I **Sellin ME**, Holmfeldt P, Stenmark S, Gullberg M (2008). Op18/Stathmin Counteracts the Activity of Overexpressed Tubulin-Disrupting Proteins in a Human Leukemia Cell Line.
Experimental Cell Research 314(6):1367-77.

II **Sellin ME**, Holmfeldt P, Stenmark S, Gullberg M (2008). Global Regulation of the Interphase Microtubule System by Abundantly Expressed Op18/Stathmin.
Molecular Biology of the Cell 19(7):2897-906.

III **Sellin ME**, Sandblad L, Stenmark S, Gullberg M (2011). Deciphering the Rules Governing Assembly Order of Mammalian Septin Complexes.
Molecular Biology of the Cell 22(17):3152-64.

IV **Sellin ME**, Holmfeldt P, Stenmark S, Gullberg M (2011). Microtubules Support a Disc-like Septin Arrangement at the Plasma Membrane of Mammalian Cells.*
Molecular Biology of the Cell, Accepted.
*Chosen by the editorial board as highlighted article

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Holmfeldt P, Brännström K, **Sellin ME**, Segerman B, Carlsson SR, Gullberg M (2007). The *Schistosoma mansoni* Protein Sm16/SmSLP/SmSPO-1 is a Membrane-Binding Protein that Lacks the Proposed Microtubule-Regulatory Activity. ***Molecular and Biochemical Parasitology*** 156(2):225-34.

Brännström K, **Sellin ME**, Holmfeldt P, Brattsand M, Gullberg M (2009). The *Schistosoma mansoni* Protein Sm16/SmSLP/SmSPO-1 Assembles into a Nine-Subunit Oligomer with Potential to Inhibit Toll-Like Receptor Signaling. ***Infection and Immunity*** 77(3):1144-54.

Holmfeldt P, **Sellin ME**, Gullberg M (2009). Predominant Regulators of Tubulin Monomer-Polymer Partitioning and their Implication for Cell Polarization. ***Cell and Molecular Life Sciences*** 66(20):3263-76.

Holmfeldt P, **Sellin ME**, Gullberg M (2010). Upregulated Op18/Stathmin Activity Causes Chromosomal Instability through a Mechanism that Evades the Spindle Assembly Checkpoint. ***Experimental Cell Research*** 316(12): 2017-26.

Thorslund SE, Edgren T, Pettersson J, Nordfelth R, **Sellin ME**, Ivanova E, Francis MS, Isaksson EL, Wolf-Watz H, Fällman M (2011). The RACK1 Signaling Scaffold Protein Selectively Interacts with *Yersinia pseudotuberculosis* Virulence Function. ***PLOS One*** 6(2):e16784.

ABSTRACT

The cell represents the basic unit of structure and function for all life. The interior of a eukaryotic cell is organized by an extensive array of protein filaments – collectively referred to as the cytoskeleton. These filaments serve diverse essential functions, e.g. to provide mechanical resilience, facilitate intracellular transport, and enable cell polarization, locomotion and division. Here I have explored the mechanisms that regulate synthesis and assembly of two cytoskeletal filament systems – microtubules and septins – and how these interact in human cells. The present thesis is based on three principal discoveries. Firstly, we have found that the microtubule-destabilizing protein Op18/Stathmin also regulates synthesis of tubulin heterodimers, which are the building blocks for microtubules. Secondly, we have unraveled the general rules that govern assembly of mammalian septins into native polymerization-competent heterooligomers. Finally, our combined results point to a non-reciprocal interplay whereby interphase microtubules support a disc-like arrangement of septin filaments, which delineate static plasma membrane regions. I here discuss the physiological significance and implications of these findings.

ABBREVIATIONS

AcGFP	*Aequorea coerulescens* green fluorescent protein
APC	Adenomatous polyposis coli
CaMKIV	Calcium/calmodulin-dependent protein kinase IV
CCT	Chaperonin containing t-complex
Cdc	Cell division cycle
CDK1	Cyclin-dependent kinase 1
CLASP	CLIP-associated protein
CLIP	Cytoplasmic linker protein
EB	End-binding protein
E-like	Tubulin binding cofactor E-like protein
γTuRC	Gamma-tubulin ring complex
IF	Intermediate filament
MAP	Microtubule-associated protein
MAPK	Mitogen-activated protein kinase
MARK	Microtubule affinity regulating kinase
MCAK	Mitotic centromere-associated kinesin (a.k.a. XKCM1)
MDCK	Madin-darby canine kidney cells
MT	Microtubule
MTOC	Microtubule organizing center
Op18	Oncoprotein of 18 kilodalton (a.k.a. Stathmin)
PDB	Protein data bank
PIP_2	Phosphatidylinositol-4,5-bisphosphate
Plx	*Xenopus* polo-like kinase
PKA	Protein kinase A
RB3	Rat homolog of the *Xenopus* gene XB3
SCG10	Superior cervical ganglion 10
SEPT	Septin
shRNA	short hairpin ribonucleic acid
Sm16	*Schistosoma mansoni* protein of 16 kilodalton
T2S	Tubulin 2 – stathmin 1 (Op18) complex
TBC	Tubulin binding cofactor
TOGp	Tumor overexpressed gene protein (a.k.a. XMAP215)
UNC	Uncoordinated

1. INTRODUCTION

1.1 The functions of intracellular filament systems collectively denoted the cytoskeleton

In order for your body to withstand collapsing while you pick up this book, to adjust the posture while sliding into the chair, to transport nutrients to your brain and eyes for reading and to convey information that fine-tune the movements required to turn the page, an extensive infrastructure system is needed. Evolution has solved these demands through the development of bones for structural integrity, muscles for movement, blood vessels for transport, and nerves for signaling between distant parts of your body.

On the level of a single cell, the demands are much the same. To function as an independent living organism or as a unit of a multi-cellular metazoan such as yourself, cells need structural support to counter pressure, machinery for movement and division, internal scaffolding to determine direction, and transport systems to deliver goods between different regions. These demands all rely on a diverse set of filament forming proteins that for historic reasons are collectively referred to as the cytoskeleton. However, in contrast to what the term implies cytoskeletal filaments display a dynamic behavior and capability of rapid remodeling in response to regulatory signals.

This thesis focuses on the basic questions of (i) how the building blocks for filament formation are synthesized and assembled, ii) how they polymerize into higher-order structures and (iii) how different filament systems interact to enable function. Specifically, this thesis deals with how two types of cytoskeletal filaments, microtubules and septin filaments, are assembled and interplay in human cells.

1.2 Common properties of cytoskeletal filament systems

The mammalian cytoskeleton is composed of a rich network of protein filaments that extends throughout the cell cytoplasm and in association with cellular membranes. Filaments are formed by the regulated polymerization of stable building blocks, which here will be referred to as protomers. Protomers may either comprise individual folded proteins, or heteromeric complexes of several proteins combined into a stable arrangement. During the assembly process protomers in some cases incorporate nucleotide bases, such as adenosine-tri-phosphate (ATP) or guanosine-tri-phosphate (GTP). Nucleotide incorporation has structural consequences for the configuration of protomers, and cycles of GTP or ATP hydrolysis are often linked to the dynamics of polymerization.

Polymerization into higher-order filamentous structures includes formation of both longitudinal and lateral bonds between adjacent protomers (Figure 1). This process can involve a nucleation step, whereby several protomers come together to form a seed, followed by filament elongation.

Polymerization may yield intermediate forms, protofilaments, which subsequently combine into the final polymer. The intrinsic molecular configuration of the protomer building-blocks and how they face each other during polymerization confer polarity or apolarity to the filament. This property is of functional importance, since polar arrangements enable the cell to sense direction and work as guidance cues for polarized transport of cargos, while apolar arrangements result in structural symmetry.

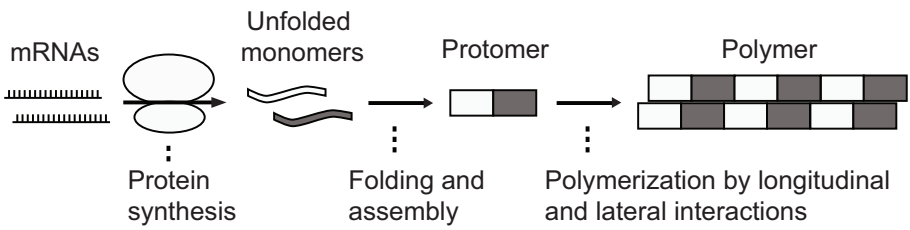

Figure 1. Schematic representation of cytoskeletal filament assembly.

Polymerization of protomers is a reversible process, tightly coupled to the physiological requirements of the cell. Some types of filaments are used for transport, others generate mechanical pushing or pulling forces, while still others maintain cell architecture, and this diversity in function is reflected in the intrinsic dynamicity of the respective filament systems. Combination of longitudinal and lateral bonds and a high degree of compaction help to stabilize filaments, while at the same time making them less capable of remodeling. In addition to the intrinsic differences between filament types, multiple regulatory mechanisms, including binding of accessory proteins or posttranslational modifications, have evolved to co-ordinate polymerization and depolymerization of cytoskeletal systems with cell signaling events.

1.3 Diverse cytoskeletal filament systems and their functions in mammalian cells

Traditionally the cell has been viewed to contain three distinct cytoskeletal systems; intermediate filaments, actin and microtubules (Alberts et al., 2008). In this thesis I have chosen to include also the septins as an emerging cytoskeletal filament system. Although debatable, I find this classification valid, since similarly to the three traditional systems, septins polymerize into extended filaments *in vitro* and *in vivo* and function as internal scaffolds for the structural organization of the cell (reviewed by Weirich et al., 2008). The principal features that distinguish the four cytoskeletal filament systems are summarized in Table 1.

Table 1. A comparison of cytoskeletal filament systems.

	Protomer unit	**Bound nucleotide**	**Filament structure**	**Polar**	**Principal function**
IF filaments	Staggered IF tetramer	None	ø~10 nm bundled filaments	No	Mechanical resilience
Actin filaments	G-actin monomer	ATP/ADP	ø~8 nm twisted paired filaments	Yes	Many
Micro-tubules	αβ-tubulin hetero-dimer	GTP/GDP	ø~25 nm, 13 protofilament hollow tubes	Yes	Many
Septin filaments	Septin heteromer*	GTP/GDP	Paired filaments**	No	Organization of cell cortex***

*See section 1.14 and 2.6, **See section 1.15, ***See section 1.16-1.17 and 2.8-2.9

The direct analogy with a skeleton really only applies to one of the cytoskeletal systems; the intermediate filaments (IFs). IFs comprise a wide variety of ~10nm thick filamentous structures that provide mechanical resilience to cells and to sub-cellular organelles, such as the nucleus. Filaments are formed by coiled-coil dimerization of IF proteins through alpha-helical rod domains, followed by several levels of higher-order polymerization into bundled filaments with a high degree of compaction (reviewed by Fuchs and Cleveland, 1998). In mammals the IF family includes more than 60 different gene products, which are expressed in a cell type and tissue specific manner, conferring differential rigidity between tissues. Their importance for mechanical resilience is highlighted by the frequent involvement of IF gene mutations in tissue fragility and blistering diseases (reviewed by Omary et al., 2004).

In contrast to the relatively static intermediate filaments, the actin system generally displays a high degree of dynamicity. Actin monomers polymerize into twisted paired filaments that may adopt branched, bundled or meshwork arrangements depending on the local signaling environment and adaptor protein interactions. Actin filaments sculpt cell morphology and enable formation of cellular protrusions through local polymerization. Together with myosin, aligned actin filaments also exert contractile forces critical for cell movement, cytokinesis and muscle cell contraction (reviewed by Ridley, 2006; Small et al., 1999).

Microtubules and septins are the main focus of this thesis and will be described in detail in subsequent sections. Briefly, microtubules are built from αβ-tubulin heterodimers that polymerize into hollow tubes used by the cell for intracellular transport, polarity establishment, cell shape remodeling and segregation of chromosomes during cell division (reviewed by Desai and Mitchison, 1997). The protomer units and higher-order filament arrangements of mammalian septins are less well defined and is one of the

specific topics of this thesis (Sections 2.6-2.9 and Paper III-IV). Septin filaments interact with phospholipids in the plasma membrane and appear predominantly involved in organization of the cell cortex (reviewed by Caudron and Barral, 2009; McMurray and Thorner, 2009b).

Cells grow and divide by an ordered chain of events, the cell cycle, which involves an interphase for growth and biomolecule synthesis and a mitosis phase for chromosome separation and cell splitting. Upon functional maturation many cell types leave the cell cycle to adopt a terminally differentiated state. Cell cycle transitions result in dramatic remodeling of cytoskeletal systems to account for their phase-dependent functions (Figure 2).

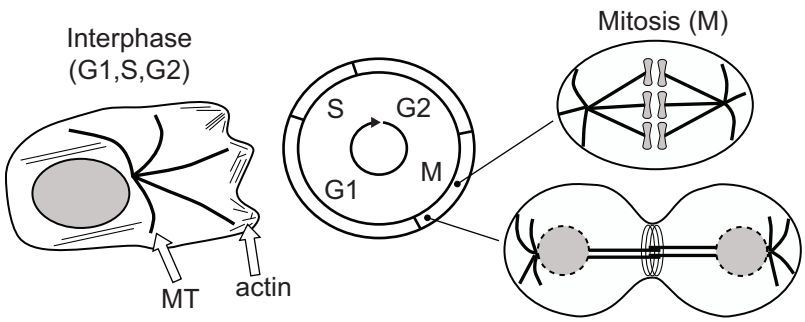

Figure 2. Cell cycle progression and rearrangement of cytoskeletal filaments, exemplified by microtubules (MT, thick lines) and actin filaments (narrow lines).

1.4 Our interest in the microtubule system

The activities of our lab over the last two decades were originally founded on the discovery of a cytosolic protein, called Oncoprotein18/Stathmin (Op18), which is upregulated in a wide variety of human cancers and represents a potent phosphorylation target downstream of the activated T-cell receptor. A seminal breakthrough in the mechanistic understanding of this protein was reached in 1996, when our lab and the lab of Tim Mitchison by independent approaches identified Op18 as a microtubule destabilizing factor (Belmont and Mitchison, 1996; Marklund et al., 1996). This discovery marked the start for an intensive era of investigation into the role(s) of Op18 in microtubule system regulation. Along the way, the intricate regulatory signaling cascades and counteractive factors that interplay with Op18 have been unraveled (Figure 3). Paper I and II of this thesis represent discoveries made along this line of research. Additionally, the experimental approaches developed have been of key importance also for the findings presented in Paper III and IV.

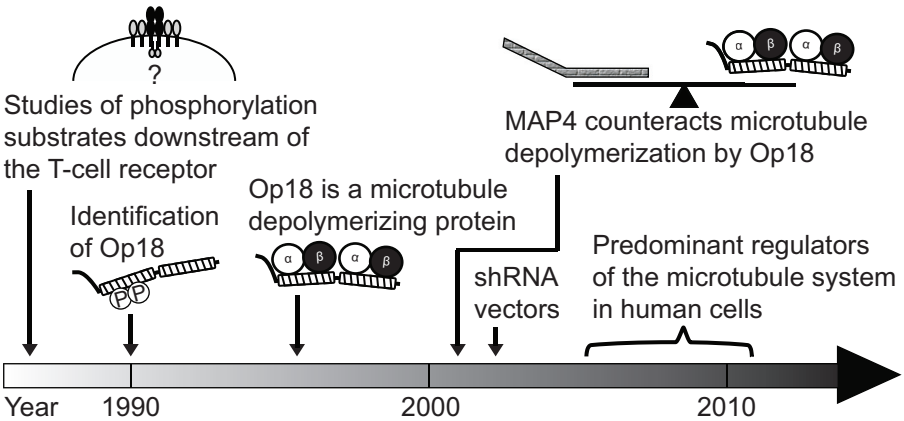

Figure 3. Timeline of the activities in the research group up to present day.

1.5 Discovery of microtubules and their building blocks

After the realization that the cell is the basic unit of all living organisms (Schleiden, 1838; Schwann, 1839), a great mystery of 19th century cell biology was how new cells could arise. The theory of binary fission from pre-existing cells finally prevailed over the idea that animal cells were continuously formed de novo. Detailed examination of the fission process showed that the nuclear material was reorganized into threadlike structures and subsequently separated before cell splitting; a process from then on termed mitosis (mitos=thread in Greek) (Flemming, 1882). Thin fibers had been observed that appeared to connect to the threads of nuclear material, but whether these fibers represented authentic structures or fixation artifacts remained a matter of hot debate (reviewed by Paweletz, 2001).

Ultra-structural work by several research groups in the 1950s and 60s showed that the spindle apparatus indeed was no artifact, but consisted of highly organized tubules built from laterally stacked thinner filaments (reviewed by Brinkley, 1997). The link between microtubules and the protein(s) subsequently named tubulin was revealed by biochemical identification of the cellular binding partner of the anti-mitotic drug colchicine (Borisy and Taylor, 1967; Weisenberg et al., 1968). Since the days of these seminal discoveries, an impressive body of literature has accumulated, delineating various roles of the microtubule system in normal cell physiology, as well as involvement in disease states or as a target for treatment strategies. These topics are amply discussed in contemporary undergraduate textbooks. In the subsequent sections I have chosen to focus on aspects of central importance for the thesis.

1.6 Synthesis and assembly of tubulin heterodimers

The protomer unit of the microtubule system, the αβ-tubulin heterodimer, is a stable ~110kDa protein complex, composed of two subunits with ~40% polypeptide sequence identity and a high degree of conservation between species. The roughly spherical subunits are assembled in tandem with the front of α- facing the back of β-tubulin, resulting in a polar peanut-like arrangement (Figure 4) (Nogales et al., 1998).

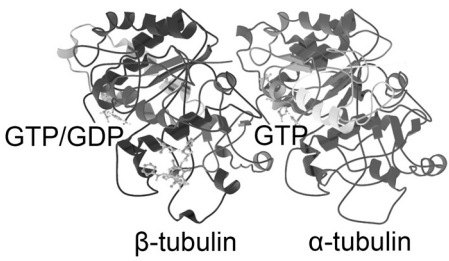

Figure 4. Structure of the αβ-tubulin heterodimer, with bound nucleotide in the N-site of α- and the E-site of β-tubulin indicated. PDB ID: 1TUB.

The α- and β-subunit of the tubulin heterodimer do not exist as free individual proteins, but are promptly co-assembled upon synthesis. Tubulin folding and heterodimer assembly require the sequential action of the cytosolic chaperonin CCT and five tubulin binding cofactors (TBC A-E), present across kingdoms (reviewed by Lopez-Fanarraga et al., 2001; Szymanski, 2002). Newly synthesized tubulin polypeptides are recruited by prefoldin (Vainberg et al., 1998), and adopt a quasi-folded state within the environment of the barrel-shaped CCT (reviewed by Lewis et al., 1996). Upon release, α-tubulin becomes transiently bound to TBCB and then TBCE, while β-tubulin interacts with TBCA and then TBCD. Assembly of the heterodimer ensues through formation of a TBCE-α-TBCD-β- super complex (Tian et al., 1999; Tian et al., 1996). During assembly, GTP is incorporated in the N-site (Non-exchangeable) of α-tubulin and in the E-site (Exchangeable) of β-tubulin. Binding of TBCC and E-site GTP hydrolysis result in release of the mature tubulin heterodimer from the cofactor super complex into the cytosolic pool (Figure 5) (Fontalba et al., 1993; Tian et al., 1999).

In addition to assisting in the folding process, tubulin cofactors, in particular TBCD and TBCE, are capable of interacting with cytosolic tubulin heterodimers resulting in their selective disruption (Figure 5) (Bhamidipati et al., 2000; Kortazar et al., 2007; Martin et al., 2000 and Paper I). While the physiological significance of these disruptive activities remains unclear, they may provide a means for the cell to exert quality control over the native tubulin pool (reviewed by Lundin et al., 2010). The discovery of a TBCE-

like protein that possesses tubulin degrading activity (Bartolini et al., 2005), but does not take part in the folding process, further hints towards this possibility. In Paper I, we examine the consequences of elevated tubulin-disrupting activities in intact human cells. Our results reveal a cellular mechanism with potential to balance tubulin-disrupting activities of assembly cofactors and related proteins (Section 2.2 and Paper I).

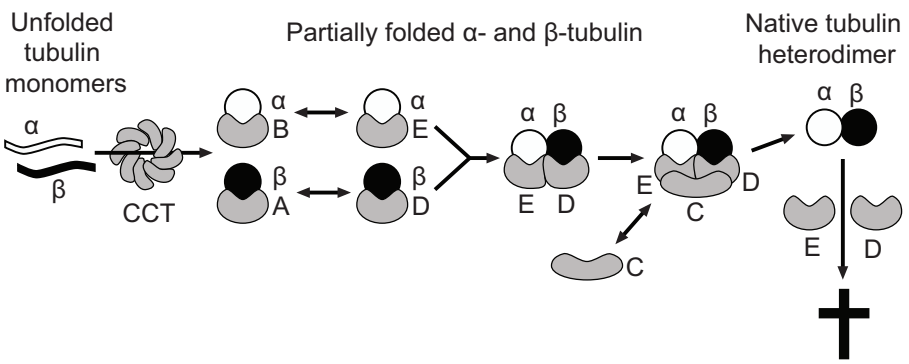

Figure 5. Folding, assembly and disruption of tubulin heterodimers.

Tubulins are encoded by several genes resulting in isotypes that differ primarily in their C-terminal tail regions. The number of isotypes vary significantly between species with mammals expressing at least 6 α- and 7 β-tubulin variants in tissue specific combinations (reviewed by Luduena, 1998). Whether or not tubulin isotype composition is of importance for microtubule function has been a long-standing matter of debate. Cell types with a specialized microtubule system, such as neurons, express typical αβ-tubulin combinations that may confer increased stability or facilitate transport (reviewed by Katsetos et al., 2003). Also, differences in microtubule polymerization kinetics and responses to microtubule poisoning drugs have been noted between isotypes (e.g. Bhattacharya et al., 2011 and refs therein). However, it is evident that heterodimers of all αβ-isotype combinations are capable of co-polymerizing *in vitro* and in intact cells (reviewed by Luduena, 1998), suggesting that differences may indeed be subtle.

Tubulin heterodimers represent ~2% of total cytosolic protein content in a typical proliferating mammalian cell (Paper II). In order to control tubulin synthesis, the cell appears to have evolved a refined system for autoregulatory feedback, whereby the levels of cytosolic tubulin heterodimers determine the stability of tubulin mRNAs (Ben-Ze'ev et al., 1979; Caron et al., 1985a; Caron et al., 1985b; Cleveland et al., 1981). Only ribosome-bound and actively translated tubulin mRNAs are susceptible to

autoregulation and the mechanism depends on the first four amino acids of the emerging nascent polypeptide, combined with a so far unknown way of sensing tubulin heterodimer content (Figure 6) (Gay et al., 1989; Gay et al., 1987; Pachter et al., 1987; Theodorakis and Cleveland, 1992; Yen et al., 1988).

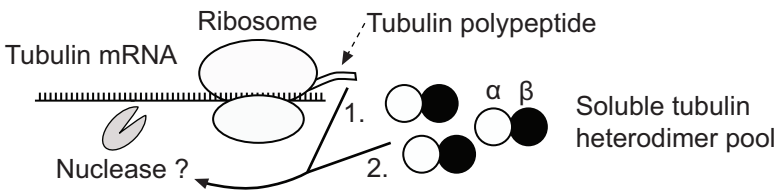

Figure 6. A model for autoregulation of tubulin mRNA stability. Adapted from (Cleveland, 1989).

The original model for autoregulation of tubulin synthesis is based on experiments using microtubule-poisoning drugs and it has been argued that the system does not respond to more physiological changes (Barlow et al., 2002; Boggs and Cabral, 1987; Wang et al., 2006). In paper II, we describe how depleting human T-lymphocytes of the microtubule regulator Op18/Stathmin results in lowered total tubulin levels mediated by the autoregulatory feedback mechanism. This lends support to a physiological relevance of tubulin synthesis autoregulation and is, to my knowledge, the first report of an endogenous MT regulator that modulates tubulin synthesis directly through this mechanism (Section 2.3 and Paper II).

1.7 Microtubule polymerization and functions

Tubulin heterodimers polymerize by longitudinal and lateral interactions to form hollow ~25nm wide tubes (Figure 7). The intrinsic polarity of the heterodimer confers polarity to microtubules, which expose α-tubulin at one end, denoted the minus end, and β-tubulin at the other, denoted the plus end (reviewed by Desai and Mitchison, 1997). Tubulin polymerization under *in vitro* conditions results in tubules consisting of a variable number of laterally stacked protofilaments (Chretien and Wade, 1991) or even tubulin sheets (Larsson et al., 1976; Nogales et al., 1995), while microtubules in intact cells as a rule comprise 13 protofilaments, as a consequence of templated nucleation (Evans et al., 1985).

Nucleation is the rate-limiting step of microtubule polymerization (reviewed by Desai and Mitchison, 1997). This step is facilitated *in vitro* by microtubule-stabilizing drugs, glycerol, or seeds that provide a polymerization template. In intact cells, microtubule organizing centers (MTOCs), such as the centrosome and basal body of animal cells and the

spindle pole body of fungi, represent the primary sites for microtubule nucleation. The centrosome of animal cells consists of a pair of perpendicularly oriented centrioles surrounded by a cloud of pericentriolar material containing several hundred different proteins (reviewed by Bettencourt-Dias and Glover, 2007). Among these, a lock-washer-shaped "γ-tubulin ring complex" (γTuRC) has a key role in the nucleation process. According to the prevailing model γTuRC provides a template that scaffolds polymerization and remains attached to the minus end of the growing microtubule (Oakley et al., 1990; Zheng et al., 1995).

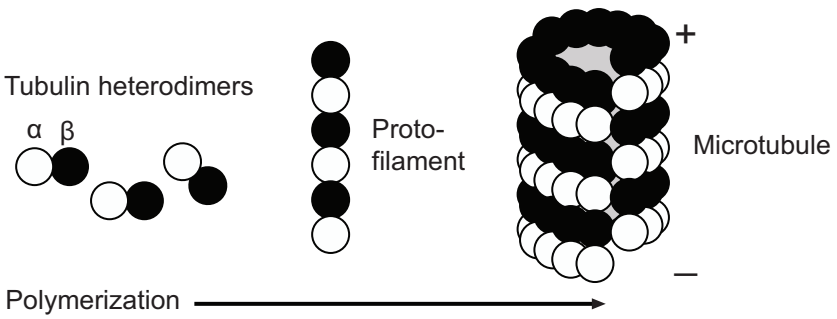

Figure 7. Tubulin heterodimer polymerization.

Microtubule polymerization is a dynamic and reversible process that involves phases of growth and shrinkage separated by transitions between these two states, denoted catastrophes and rescues (Figure 8) (reviewed by Desai and Mitchison, 1997). Although the total microtubule mass within a cell is in equilibrium with the soluble tubulin heterodimer pool, individual microtubules still go through phases of growth and shrinkage (Cassimeris et al., 1988), a phenomenon termed dynamic instability (Mitchison and Kirschner, 1984). GTP-binding and hydrolysis by the E-site of β-tubulin plays a key role in regulating the dynamic behavior of microtubules. Only GTP-bound tubulin is fully polymerization competent, and incorporation of a new layer of heterodimers at the microtubule tip induces GTP hydrolysis in the former layer. GTP hydrolysis induces a straight-to-curved conformational shift in the tubulin heterodimer and it has been postulated that a thin layer of GTP-bound tubulin forms a cap at the plus tip, thus hindering the microtubule lattice from fraying apart due to the intrinsic curving propensity (reviewed by Nogales and Wang, 2006).

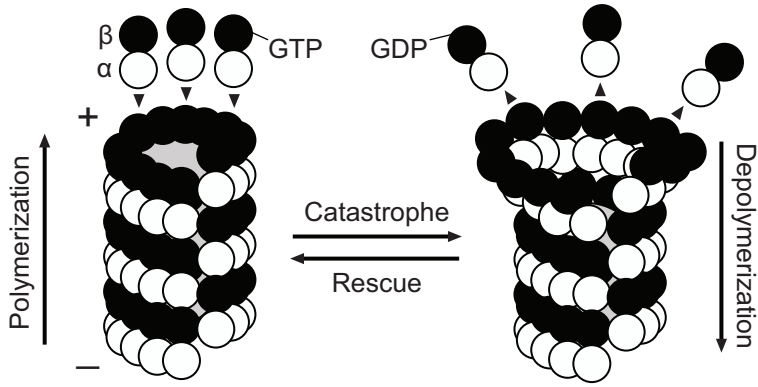

Figure 8. Dynamic parameters of microtubule polymerization.

The dynamic behavior of microtubules can be modulated by microtubule regulatory proteins (Section 1.8) or by post-translational modification of the C-terminal tails of α and β-tubulin. Post-translational modifications, including acetylation, detyrosination and polyglutamylation, are believed to specify distinct sets of microtubules for motor protein-dependent transport and/or confer increased stability (reviewed by Wloga and Gaertig, 2010).

Microtubules are required for fundamental processes both in interphase cells and in mitosis. During interphase, microtubules function as tracks for intracellular transport, stabilize cellular attachments and organize cytoplasmic organelles. In addition, MTs form the inner core structures of cellular appendages, such as cilia and flagella, and enable their motor protein-dependent movement (reviewed by Lindemann and Lesich, 2010). Upon transition into mitosis the interphase microtubule array transforms into a bipolar spindle (Figure 2 above), a transition accompanied by a decrease in mean microtubule length and increased catastrophe frequency (Belmont et al., 1990). The mitotic spindle organizes the condensed chromosomes at the metaphase plate in mid mitosis, and subsequently segregates them equally into the two forming daughter cells upon satisfaction of the spindle assembly checkpoint (reviewed by Rieder, 2011). The functions of the microtubule system depends on the ability of individual microtubules to constantly probe the cytoplasm for stabilizing points of contact at the cell cortex or on mitotic chromosomes. This property relies on dynamic instability and has been denoted “search-and-capture” (Kirschner and Mitchison, 1986).

1.8 Microtubule regulatory proteins

Although purified tubulin heterodimers can polymerize into microtubules in a test tube, recent screens have revealed >250 microtubule interacting proteins present in tissue lysates (Hughes et al., 2008). While the specificity of these candidates in many cases remains uncertain, the sheer number of

hits has been interpreted to suggest a staggering complexity in the microtubule regulatory circuits of intact cells. Proteins that regulate microtubule polymer status can be broadly categorized as either microtubule stabilizing or destabilizing factors. The levels of these counteractive factors, as well as phosphorylation-dependent regulation of their activities, dictate the global partitioning of tubulin heterodimers between soluble and polymeric pools (Fig 9) (reviewed by Holmfeldt et al., 2009). In addition, local effects on individual microtubules at the cell cortex or in the vicinity of mitotic chromosomes represent another layer of regulation.

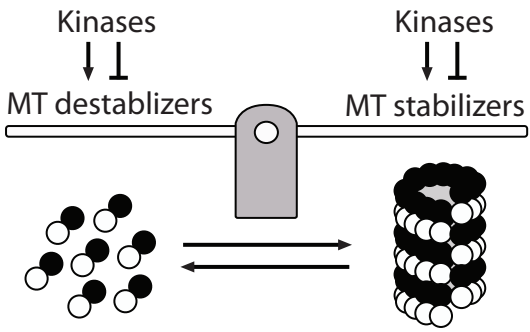

Figure 9. Counteractive and phosphorylation-dependent regulation of tubulin heterodimer partitioning. Adapted from (Holmfeldt et al., 2009).

Microtubule-stabilizing proteins act by two principal mechanisms; either by binding along the microtubule lattice or by associating with exposed plus tips. The classical microtubule associated proteins (MAPs), exemplified by the ubiquitous MAP4 and the neuronally enriched MAP2 and Tau, stabilize microtubules by direct binding to the sides of microtubules, thereby bridging adjacent tubulin heterodimers through multiple MT-binding domains (reviewed by Mandelkow and Mandelkow, 1995). The +TIP class of proteins consists of more than 20 structurally diverse members with the ability to track growing microtubule ends (reviewed by Akhmanova and Steinmetz, 2008). This class includes XMAP215/TOGp, which facilitates tubulin heterodimer incorporation (Brouhard et al., 2008), CLIP170, which promotes rescue events (Komarova et al., 2002) and APC and CLASP proteins, which stabilize microtubule ends at cortical locations (reviewed by Akhmanova and Steinmetz, 2008). The prototypical microtubule end-binding (EB) proteins appear essential for the correct localization of many of the +TIP proteins carrying a signature SxIP sequence motif (Honnappa et al., 2009).

Microtubule destabilizing proteins may carry out their functions through at least three principal mechanisms, namely (i) by induction of microtubule catastrophes at plus tips, as exemplified by XKCM1/MCAK (Kline-Smith and Walczak, 2002), (ii) by severing of microtubules by e.g.

Katanin (McNally and Vale, 1993), or (iii) by sequestration of soluble tubulin heterodimers, as has been proposed for Op18/Stathmin (Section 1.9). Also other means of interfering with polymerization may exist, e.g. by inhibition of templated nucleation. However, due to the interdependencies of dynamic parameters of tubulin polymerization, it has in most cases been exceedingly difficult to pinpoint physiologically relevant modes of action for microtubule regulatory proteins, as further expanded upon below (Section 1.9).

In addition to proteins regulating microtubule dynamics and polymer content, a battery of motor proteins traffic microtubules in both directions (reviewed by Vale, 2003). These include plus end directed kinesins and minus end directed dynein that move minute protein and mRNA cargos, larger vesicles, or even entire organelles between different regions of the cell. Motor proteins can also bind to anti-parallel microtubules and slide them relative to each other or position the microtubule array relative to the cell cortex.

1.9 Op18 – a major microtubule destabilizing factor

Oncoprotein18/Stathmin (Op18) originally gained attention for its frequent up regulation in human cancer tissues (hence the term oncoprotein) and phosphorylation downstream of a variety of cell signaling pathways (Hanash et al., 1988; Sobel and Tashjian, 1983). Op18, a protein highly conserved among vertebrates and also present in invertebrates such as *Drosophila melanogaster* (Lachkar et al., 2010; Ozon et al., 2002), is the founding member of a protein family that includes the Op18-like RB3, SCG10, and SCLIP proteins (reviewed by Cassimeris, 2002). Op18 is ubiquitously expressed, cytosolic, and abundant in most proliferating cells as well as some post-mitotic cells of neural origin. In contrast, other family members are expressed at low levels (Bieche et al., 2003) in a cell type restricted manner, and localize to cellular membranes through an N-terminal targeting sequence (reviewed by Cassimeris, 2002). Hence, membrane bound Op18 family members seem likely to carry out tissue-specific functions different from the cytosolic Op18 protein.

The first clues to the molecular function of Op18 came from observations that progression into mitosis and formation of the mitotic spindle require multi-site Op18 phosphorylation by cell cycle regulatory kinases (Brattsand et al., 1994; Larsson et al., 1995; Marklund et al., 1994b). Subsequent biochemical experiments in *Xenopus* egg lysates and ectopic expression in human cells established Op18 as a potent microtubule destabilizing protein (Belmont and Mitchison, 1996; Marklund et al., 1996), and the physiological relevance of this activity has later been supported by studies depleting the endogenous protein by RNA interference or gene knockout approaches (Holmfeldt et al., 2007b; Ringhoff and Cassimeris, 2009 and Paper II). Op18 contains an unstructured N-terminus and a C-

terminal region with alpha-helical propensity, capable of binding two tubulin heterodimers aligned in tandem in a T2S complex (Figure 10) (Gigant et al., 2000; Steinmetz et al., 2000).

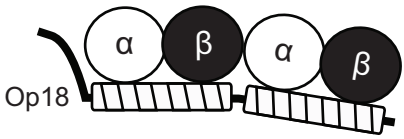

Figure 10. The T2S complex formed by two tubulin heterodimers bound to Op18. Based on PDB ID: 1FFX.

The principal mechanism by which Op18 exerts microtubule destabilization in intact cells has remained controversial despite intense investigation. Originally, microtubule destabilization was attributed to a catastrophe promoting activity at microtubule tips (Belmont and Mitchison, 1996), but this was thereafter challenged by claims that catastrophe promotion was merely a consequence of a tubulin heterodimer sequestering activity resulting in lowered soluble tubulin concentration (Curmi et al., 1997; Jourdain et al., 1997). Subsequent molecular dissection identified regions of Op18 and experimental conditions that illustrated both of these activities. Catastrophe promotion *in vitro* was shown to require the N-terminal of Op18 and was more pronounced at pH>7, while the tubulin sequestering activity appeared independent of the N-terminal and could be potentiated by a buffer of pH<7 (Howell et al., 1999). In addition, in mouse fibroblasts that contain too low levels of Op18 to exert tubulin sequestration, Op18 gene deletion was recently shown to result in an increased rate of microtubule nucleation suggesting a possible third mode of action (Ringhoff and Cassimeris, 2009). While the issues of specific tubulin/MT-directed Op18 activities remain unresolved, the proposed mechanisms are not mutually exclusive and may simply reflect activities that depend on the cellular context. Furthermore, the results presented in this thesis reveal an additional role for Op18 in regulating total tubulin content and possibly also in safeguarding soluble tubulin heterodimers against tubulin-disruptive activities (Sections 2.2-2.3 and Paper I-II).

Op18 is phosphorylated on four serine residues in response to a wide variety of cell surface receptor stimuli or at mitotic entry (Figure 11). Phosphorylation decreases the binding affinity for tubulin heterodimers and results in a rapid increase in the microtubule content of intact cells (Holmfeldt et al., 2009). In interphase, Op18 phosphorylation occurs in response to cell signaling pathways activating e.g. CaMKIV, MAPK and PKA (Gradin et al., 1998; Marklund et al., 1993; Marklund et al., 1994a). It has been shown that phosphorylation-inactivation of Op18 is sufficient to

explain the rapid increase in microtubule content noted upon stimulation of the T-cell receptor (Holmfeldt et al., 2007b). The consequence of this effect is an increased interface between the microtubule array and the cell cortex, which may facilitate T-cell polarization towards an antigen-presenting cell.

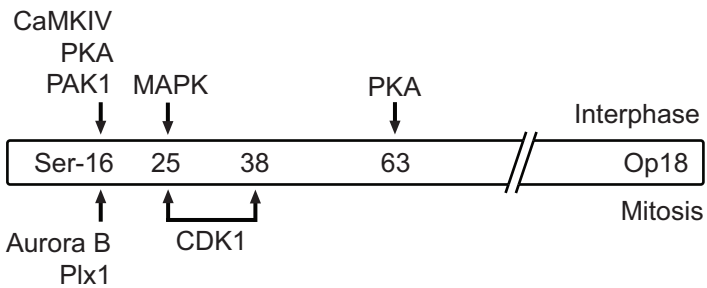

Figure 11. Op18 phosphorylation by signal transduction and cell cycle-regulated kinases.

Upon entry into mitosis, Op18 is phosphorylated to completion on serine-25 and -38 by the master regulatory kinase CDK1, and to high stochiometry on serine-16 and -63 by other less well defined kinase systems (Larsson et al., 1997; Larsson et al., 1995; Marklund et al., 1996). Multisite phosphorylation inactivates Op18 until the metaphase checkpoint has been satisfied, which results in a sudden kinase activity drop in late mitosis. Complete Op18 inactivation upon mitotic entry agrees well with the finding that extensive shRNA-mediated Op18 depletion in human cells is not associated with growth restriction or aberrant progression through mitosis (Holmfeldt et al., 2007b and Paper II). However, elevated Op18 activity, caused by upregulation of expression or a tumor-associated Q18E mutation, interferes with spindle assembly, leads to chromosome mis-segregation, and may hence be a driver of aneuploidy (Holmfeldt et al., 2006; Holmfeldt et al., 2010).

Based on experiments in *Xenopus* egg extracts, the microtubule-stabilizing protein XMAP215/TOGp and the depolymerizing kinesin XKCM1/MCAK were originally proposed as the predominant counteractive regulators of the interphase microtubule array (Tournebize et al., 2000). However, in mammalian cells the physiological functions of these proteins appear primarily of relevance for mitosis and do not include global regulation of microtubule content (reviewed by Holmfeldt et al., 2009). Instead, a regulatory module composed of Op18, the microtubule-stabilizing protein MAP4, and their respective regulatory kinases, govern the microtubule status of interphase cells (Figure 12). Depletion of either of these factors has dramatic effects on tubulin heterodimer partitioning, while co-depletion results in similar microtubule content as in normal cells, but

renders the microtubule system inert to regulatory signals (Holmfeldt et al., 2007b).

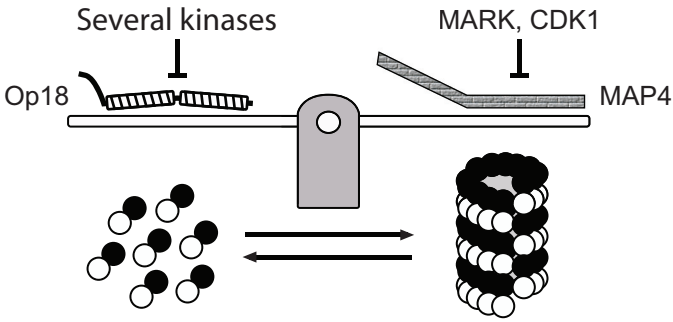

Figure 12. Regulation of tubulin heterodimer partitioning by the counteractive activities of Op18 and MAP4. Adapted from (Holmfeldt et al., 2009).

1.10 Our interest in the septins

As delineated above, our search for proteins capable of counteracting microtubule destabilization by Op18 revealed a yin-yang relationship between Op18 and the ubiquitous microtubule-stabilizing protein MAP4. Our initial interest in the septins stemmed from a report implicating these filament forming proteins in the regulation of MAP4 activity (Kremer et al., 2005). This opened up the possibility that septins could govern the polymerization status of the microtubule system. During the course of our investigations we have, however, refuted any functional or physical links between septins and MAP4, and we have found no support for a model whereby septins would act as regulators of tubulin heterodimer partitioning. On the contrary, our analysis reveals a non-reciprocal mechanism whereby the interphase microtubule system supports a disc-like septin arrangement at the plasma membrane of non-adherent cells (Paper IV). Additionally, our results point to a generic model for how the multitude of septins expressed in mammalian cells assemble into a pool of six- to eight-subunit protomers that comprise the building-blocks for higher-order septin structures (Paper III).

The structure and function of septins represented an entirely new field for our research group. Although I am poorly acquainted with the experimental approaches used in fungal model systems, I have found the literature based on experiments in fungi to provide a fairly coherent picture. In contrast, studies in mammalian cell models have not arrived at a consensus view regarding the basic architecture and functions of septins, which is especially obvious for proposed functions in interphase. As a consequence, subsequent sections focus on properties of the septin system that are supported by experimental evidence across kingdoms, while omitting some of the more provocative claims of recent high-impact papers.

1.11 Discovery of the septins – an emerging cytoskeletal filament system

The septins were first discovered in screens for temperature-sensitive budding yeast mutants defective in cell cycle progression, named cell division cycle (cdc) mutants (Hartwell, 1971; Hartwell et al., 1970). Among the mutants isolated, four (denoted cdc3, cdc10, cdc11 and cdc12) displayed defects in cytokinesis at the restrictive temperature, resulting in elongated buds, polyploidisation and failure in mother cell-bud separation (Hartwell, 1971). These mutants lacked the membrane-proximal bud neck filamentous structures that had recently been discovered by electron microscopy (Byers and Goetsch, 1976a, b). Subsequent immuno-staining approaches confirmed that the individual Cdc3, Cdc10, Cdc11 and Cdc12 proteins indeed all localized to the bud neck (Ford and Pringle, 1991; Haarer and Pringle, 1987; Kim et al., 1991) although their ultrastructural arrangement would continue to cause debate for the decades to come (reviewed by McMurray and Thorner, 2009b). Meanwhile, cloning and sequence comparison indicated that the four cdc-genes encoded highly homologous proteins representing a novel protein family. To account for their role in the septation process the proteins would thereafter be referred to as "septins" (Figure 13)(reviewed by Sanders and Field, 1994).

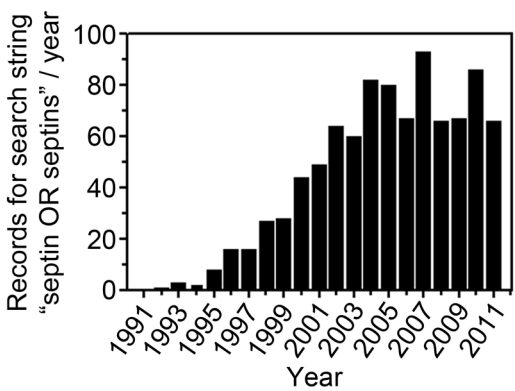

Figure 13. Records in the ISI Web of Knowledge database containing the word "septin" or "septins" by year (as of October 2011).

It took until the mid-1990s for septins to be recognized also in metazoans. A key discovery opening this venue was made in a screen for genes involved in *Drosophila melanogaster* eye development (Neufeld and Rubin, 1994). As a by-product, the screen produced a mutant fly that displayed defects in embryonic cell proliferation caused by failed cytokinesis. The gene product involved, aptly named Pnut because of the peanut resemblance of mutant cells, turned out to be homologous to the yeast septins, and was found to localize to the cleavage furrow of dividing cells.

In mammals, several sequences with homology to yeast septins were identified in screens with unrelated aims (H5, now SEPT4 Kato, 1990; Nedd5, now SEPT2 Kumar et al., 1992; hCDC10, now SEPT7 Nakatsuru et al., 1994; DIFF6, now SEPT1 Nottenburg et al., 1990). Following up on one of these screens, functional characterization of the putative septin Nedd5 (SEPT2) showed localization to the contractile ring and involvement in cytokinesis (Kinoshita et al., 1997). Hence, based on experiments in several model organisms, it was at the mid-90s evident that septins comprise an evolutionary conserved protein family of importance for cytokinesis. In addition, several studies indicated diverse functional roles also in interphase or post-mitotic cells (reviewed by Longtine et al., 1996 and discussed further below).

1.12 Septin domains and sequence motifs

Septins belong to the P-loop GTPase superfamily, which also encompasses Ras-like proteins, and is more distantly related to the myosin and kinesin type of ATPases (Leipe et al., 2002). P-loop GTPases share a conserved G-domain architecture characterized by the phosphate binding motif GxxxxGKS/T and additional motifs that yield guanine base specificity (reviewed by Saraste et al., 1990; Wittinghofer and Vetter, 2011). Within this superfamily, septins are characterized by a variable N-terminal extension (NTE) and in most cases a C-terminal coiled coil extension (CTE) flanking the centrally located G-domain (Figure 14) (reviewed by Weirich et al., 2008). Septins can dimerize through their G-domains (Sirajuddin et al., 2007), while coiled-coil elements in the C-terminal stabilize septin-septin interactions (Bertin et al., 2008; Bertin et al., 2010). In addition, a polybasic alpha helix directly N-terminal of the G-domain appears important for membrane lipid interactions (Bertin et al., 2010; Casamayor and Snyder, 2003; Zhang et al., 1999).

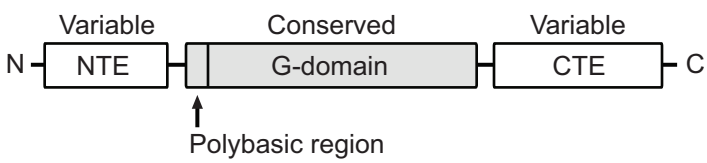

Figure 14. Septin primary sequence elements.

1.13 Evolution and classification of septins

Septins are found in divergent organisms representing fungi as well as non-chordate and chordate animals, but are notably absent in plants (Pan et al., 2007). Sequenced fungal genomes harbor 4-8 septin genes (Pan et al., 2007), while vertebrate genomes contain 9-17 (Cao et al., 2007). Early phylogenetic analysis of fungal septins (Momany et al., 2001) or sequence similarity analysis of animal septins (Kartmann and Roth, 2001) in both cases resulted

in clustering into distinct groups. However, direct comparisons did not reveal obvious counterparts between fungal and animal septins (Kinoshita, 2003b). Large scale phylogenetic analysis of septins across kingdoms clustered all sequences into 5 groups of which animal septins were found exclusively in group 1 or 2 (Pan et al., 2007). Thus, orthologous relationships between septins from different kingdoms cannot be readily established by analysis of primary sequences. This likely reflects no more than a single duplication of an ancestral septin gene before the splitting of fungi and animal branches, followed by subsequent duplications and divergence within the respective lineages (Pan et al., 2007).

The 13 mammalian septins were initially given whimsical names connected to their mode of discovery, but have later been renamed SEPT1-12 and SEPT14 (Macara et al., 2002). A SEPT13 gene was initially proposed (Hall et al., 2005), but this represents a pseudogene (HGNC:32339). Mammalian septins cluster into 4 homology-based subgroups that are named by the founding members SEPT2, SEPT3, SEPT6 and SEPT7 (Figure 15) (Cao et al., 2007; Kinoshita, 2003a). However, invertebrate animals such as *C. elegans* and *D. melanogaster* harbor members of only 2 or 3 of these subgroups and the functional significance of this classification system has remained unclear.

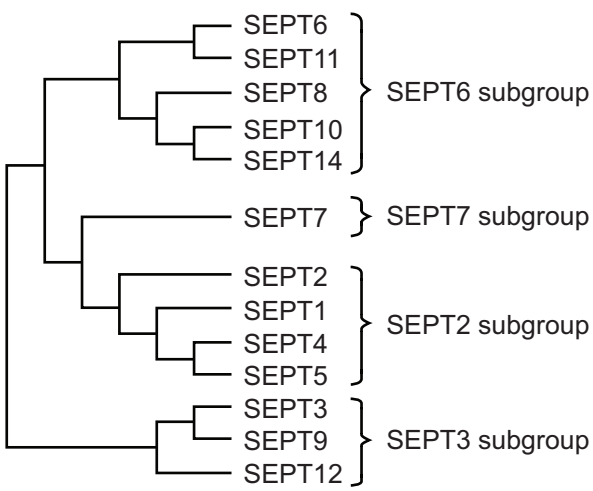

Figure 15. Phylogenetic relationships between human septins. Adapted from (Kinoshita, 2003a).

In addition to the multitude of septin genes, most animal septin transcripts are alternatively spliced to encode several isoforms (reviewed by Russell and Hall, 2011). The most extreme case characterized so far, the human SEPT9 gene, displays a staggering complexity in its transcriptional

regulation and splicing, giving rise to up to 18 transcripts (McIlhatton et al., 2001), that encode at least 5 distinct proteins (reviewed by Russell and Hall, 2011). Septin isoforms generally vary in the length of N- and C-terminal extensions, while retaining the central G-domain.

1.14 Hetero-oligomerization of septins into protomer units

A single cell expresses multiple septin family members, in mammalian cells commonly amounting to 7 or more co-expressed proteins (Paper III). Expressed septins associate with each other to form hetero-oligomeric complexes that can be purified biochemically (Field et al., 1996; Frazier et al., 1998; Hsu et al., 1998). High-resolution imaging of septin complexes purified from endogenous sources or after recombinant co-expression has revealed elongated rod-shaped particles corresponding to tetramers, hexamers, or octamers depending on the organism (Bertin et al., 2008; Field et al., 1996; John et al., 2007; Lukoyanova et al., 2008; Sirajuddin et al., 2007). The subunit arrangement within rods of co-expressed recombinant septins is nonrandom and results in an apolar palindrome comprising two copies of each polypeptide (Figure 16) (Bertin et al., 2008; John et al., 2007; Sirajuddin et al., 2007).

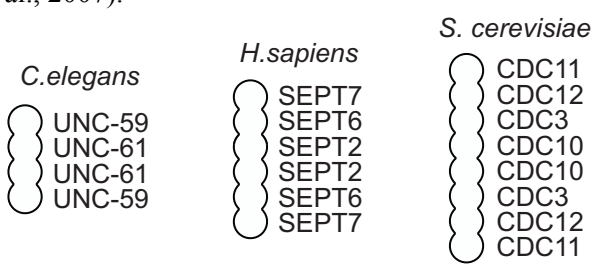

Figure 16. Septin complexes formed upon recombinant co-expression of the indicated septins from *C. elegans*, *H. sapiens* and *S. cerevisiae*.

Most septins contain a C-terminal coiled-coil extension (Figure 14 above) and it was initially believed that these extensions constitute the primary mediators of septin-septin interactions. However, crystallopraphic studies have shown that G-domain interfaces mediate both homotypic and heterotypic subunit interactions within complexes (Sirajuddin et al., 2007). Septin monomers fold with the nucleotide binding motifs of the G-domain proximal to one surface (denoted the G-surface) and the N- and C-terminal extensions protruding from the opposite side (the NC-surface). The unifying principle for both septin hetero-oligomer and higher-order filament formation lies in alternating G- and NC-interface interactions between adjacent subunits aligned as beads-on-a-string (Figure 17) (Sirajuddin et al., 2007). In addition, coiled-coil interactions may further stabilize hetero-oligomeric septin complexes and are of importance for their higher-order filament arrangement (Bertin et al., 2008; Bertin et al., 2010).

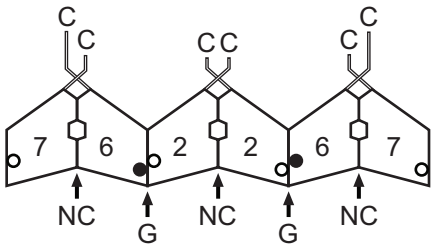

Figure 17. Structural representation of the recombinantly expressed human SEPT2/6/7 complex. Open circles indicate bound GDP and closed circles indicate GTP. Based on PDB ID: 2QAG.

The fact that septins form stable heteromeric complexes combined with the multitude of differentially expressed septin genes in animal cells (Cao et al., 2007; Hall et al., 2005), results in a staggering amount of potential complex arrangements. This has raised questions regarding the degree of combinatorial diversity and whether biochemical rules exist that direct the assembly process. Mammalian cells express minimally one member of each of the four homology subgroups (Figure 15 above) (Cao et al., 2007). It has been postulated that septin complexes may be formed by combining members from three different subgroups (Kinoshita, 2003a). Based on co-immunostaining, biochemical purification, yeast-two hybrid approaches or recombinant co-expression, a multitude of different complexes have so far been proposed (Hsu et al., 1998; Kinoshita et al., 2002; Lukoyanova et al., 2008; Nagata et al., 2004; Nakahira et al., 2010; Sandrock et al., 2011). However, evidence supporting these claims are in many cases indirect and septin pairing during ectopic/recombinant expression is fairly promiscuous (Sheffield et al., 2003).

On the biochemical level, GTPase activity appears important for the paring of subunits within septin complexes, with mixed GTP-GDP interfaces favoring stability (Sirajuddin et al., 2009). This is consistent with alternating GTPase activity between neighboring subunits in the *S. cerevisiae* octamer as well as the recombinant human SEPT2/6/7 hexamer. Notably the SEPT6 subgroup is GTPase incompetent, while members of the SEPT2, SEPT3 and SEPT7 are predictably GTPase proficient. However, as evident from the tetrameric complex formed by the *C.elegans* septins UNC-59 and UNC-61 (John et al., 2007), which are both predictably GTPase proficient (Sirajuddin et al., 2009), alternating GTPase activity does not represent a strict assembly rule.

In summary, septins in mammals as well as other species assemble into heteromeric complexes, but the rules governing the assembly process are far from clear. This especially applies to the oligomeric context of SEPT3 subgroup members, including the ubiquitously expressed SEPT9.

The results presented in this thesis establish a functional significance of the classification of mammalian septins into the four homology-based subgroups, which correspond to four distinct subunit positions during hetero-oligomer assembly. In addition, we conclude that mammalian septins exist exclusively in the context of six- to eight-subunit heteromers with free subunit pools being virtually nonexistent (Section 2.6 and Paper III). The "core" hetero-oligomeric complexes described in this section represent the protomers of septin filaments and will for the remainder of the thesis be referred to as septin "heteromers".

1.15 Higher-order septin filament formation

The link between genetic ablation of septins and disappearance of the bud neck filaments in *S. cerevisiae* (Byers and Goetsch, 1976a, b; Frazier et al., 1998) hinted that septins may form filamentous structures similarly to the three classical cytoskeletal systems. This notion was substantiated by studies showing that all mitotically expressed septins localized to the neck filaments (Ford and Pringle, 1991; Frazier et al., 1998; Haarer and Pringle, 1987; Kim et al., 1991) and that purified or recombinant yeast septins assemble into filaments in vitro (Farkasovsky et al., 2005; Frazier et al., 1998; Versele et al., 2004). Initially, cdc10 or cdc11 mutants were reported that seemingly lacked neck filaments, but were still viable, which argued that septin filament formation was dispensable for essential functions in this organism (Frazier et al., 1998). However, a recent study has shown that these Cdc10- or Cdc11-less cells indeed form rudimentary filaments by atypical interactions of end-exposed subunits, while genetically disabling polymerization results in lethality (McMurray et al., 2011). Hence, the essential functions of budding yeast septins strictly depend on polymerization of heteromers into higher-order filamentous structures.

Purified or recombinant animal septin heteromers form filaments similar to the yeast counterparts (Field et al., 1996; Kinoshita et al., 2002), but whether filament formation is a strict requirement for function in metazoans remains unclear. A wide array of filamentous septin assemblies have been reported in animal cells, but frequently these co-align with cellular structures such as actin bundles (e.g. Kinoshita et al., 2002; Schmidt and Nichols, 2004b) and may thus be a consequence of templating rather than constituting independent filaments. We have in the course of our studies developed tools for validated imaging of higher-order septin assemblies in human cells and these issues are further discussed below (Section 2.7-2.8 and Paper IV).

The formation of septin higher-order filaments involve longitudinal joining of adjacent heteromers through the G-domains of end-exposed subunits (Figure 18) (reviewed by Weirich et al., 2008). Octameric yeast heteromers polymerize via end exposed NC-interfaces, while recombinant human hexamers or *C. elegans* tetramers utilize G-interfaces at ends (Bertin

et al., 2008; John et al., 2007; Sirajuddin et al., 2007). The plasticity of polymerization is further illustrated by atypical filament formation of yeast heteromers in the absence of the normally end-located Cdc11 or centrally located Cdc10 subunits (McMurray et al., 2011). Individual animal septins may even form filaments by homotypic interactions if artificially expressed at high levels (Mendoza et al., 2002; Sirajuddin et al., 2009 and Paper IV). Thus, septin filament formation can be achieved with a high degree of flexibility.

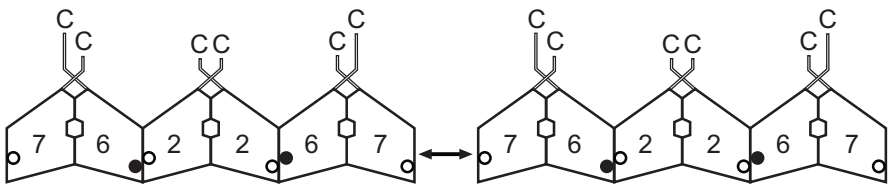

Figure 18. Septin polymerization, as exemplified by the human SEPT2/6/7 heteromer.

In vitro, septin heteromers polymerize upon dialysis into a low ionic strength buffer, while filaments can be disassembled into heteromers by high salt treatment (Farkasovsky et al., 2005; Frazier et al., 1998; Versele et al., 2004 and Paper III). This suggests that salt sensitive interactions are critical for filament formation, but not sufficient to explain assembly into stable heteromers. Polymerization appears not to require nucleation factors or depend on rapid GTP hydrolysis cycles (Vrabioiu et al., 2004), which contrasts to polymerization of tubulin heterodimers into microtubules (Section 1.7). However, association to lipid surfaces containing the phosphoinositide PIP_2 can promote heteromer polymerization, even under otherwise non-ideal conditions, such as high salt buffers or for incomplete heteromers (Bertin et al., 2010; Tanaka-Takiguchi et al., 2009). Additionally, templating along actin bundles may represent another polymerization-promoting mechanism in intact cells (Kinoshita et al., 2002).

The filaments observed after low salt dialysis of septin heteromers frequently display a paired arrangement, resembling rail-road tracks (Bertin et al., 2008; Bertin et al., 2010; Frazier et al., 1998). The distance between parallel filaments is consistent with association through C-terminal extensions (Bertin et al., 2008). In the presence of PIP_2 containing lipid surfaces, however, also heteromers devoid of C-terminal extensions may adopt a paired arrangement via lateral G-domain interactions (Bertin et al., 2010). Recently, a polarized microscopy study has suggested that paired filaments are prominent also in intact cells of diverse origin (DeMay et al., 2011). Hence, septin heteromers polymerized into paired filaments may represent a common functional arrangement across kingdoms (Figure 19).

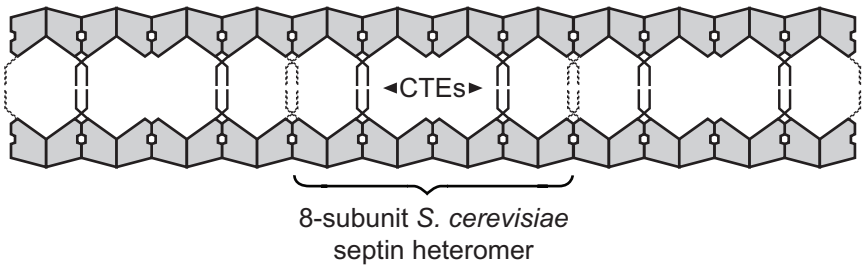

Figure 19. Paired septin filaments, as exemplified by polymerization of the budding yeast octameric heteromer. Adapted from (Bertin et al., 2008).

1.16 The budding yeast paradigm – organization of the cell cortex by septin filaments

The functions and organization of the septin system have been most thoroughly examined in budding yeast where, in my mind, a relatively coherent picture has emerged. In this organism, a cortical Cdc42-organized septin patch assembles in late G1 to mark the incipient bud site. Upon bud emergence, the septin patch rearranges into an hourglass collar structure, followed by further rearrangement into two split rings that enclose the actomyosin machinery in cytokinesis (Figure 20) (reviewed by McMurray and Thorner, 2009b; Oh and Bi, 2011). Septin function is important for early bud site selection, cell cycle coordination, recruitment of polarity factors that promote polarized growth (reviewed by Oh and Bi, 2011) and for positioning of the mitotic spindle relative to the bud (Kusch et al., 2002). In addition, septins are required for both processes leading to completion of cytokinesis, namely the assembly of the actomyosin contractile ring and the deposition of primary septum material (reviewed by Bi, 2001).

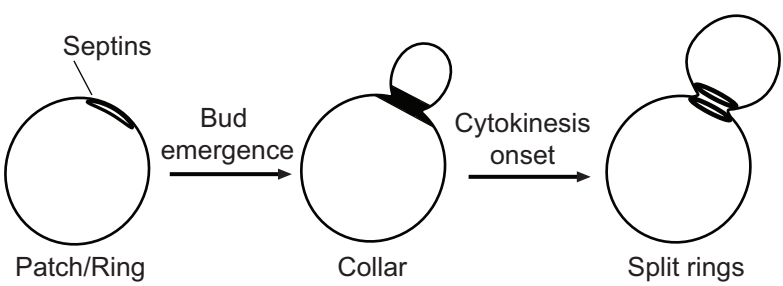

Figure 20. Septin structural rearrangements at the bud neck of *S. cerevisiae*.

Two models have been proposed to explain septin function in yeast. According to the scaffolding model septins tether factors, such as the contractile myosin myo-1, to their correct cortical location (Bi et al., 1998; Lippincott and Li, 1998; Longtine et al., 1996), while according to the diffusion barrier model a septin-dependent fence segregates membrane-bound factors into distinct cortical compartments (Barral et al., 2000; Takizawa et al., 2000). The scaffolding and diffusion barrier models are not mutually exclusive and seem rather to reflect septin properties important for distinct cell cycle events.

1.17 Septin structure and function in mammalian cells

In contrast to the fairly coherent picture of the budding yeast septin system, animal septins have been ascribed a myriad of arrangements and functions (reviewed by Cao et al., 2009; Kinoshita, 2006; Weirich et al., 2008), as well as involvement in various disease states (reviewed by Peterson and Petty, 2010). Similar to localization at the yeast bud neck, septins localize at the cleavage furrow in mammalian, as well as in *C. elegans* and *Drosophila* cells (Kinoshita et al., 1997; Neufeld and Rubin, 1994; Nguyen et al., 2000 and Paper IV). However, the consequences of septin ablation for cytokinesis progression vary considerably between cells, ranging from severe to undetectable (Estey et al., 2010; Kinoshita et al., 1997; Tooley et al., 2009 and Paper III). I find it conceivable that these differences may be attributed to differential stringency requirements for cytokinesis in adherent and polarized cells, as compared to suspension-growing nonpolarized cell types. For example, adherent cells undergo dramatic actin cytoskeleton rearrangements between interphase and late stages of mitosis, which may be accompanied with an increased dependency on accessory components at the cytokinetic furrow.

The mechanism for septin function in mammalian cytokinesis has been proposed to include both scaffolding of key factors for actomyosin ring contraction (Joo et al., 2007) and formation of a membrane-proximal diffusion barrier during cytokinesis (Schmidt and Nichols, 2004a). Notably, also involvement in chromosome congression and separation earlier in mitosis has been postulated (Spiliotis et al., 2005; Zhu et al., 2008); a mechanism which would not have an evolutionary counterpart in fungal systems.

Compartmentalization of the cell cortex by filamentous septin structures appears to be a common theme also in interphase and post-mitotic mammalian cells (reviewed by Caudron and Barral, 2009). A septin ring compatible with a diffusion barrier (Figure 21) has been identified separating the mid and principle piece of spermatozoa (Ihara et al., 2005; Kwitny et al., 2010; Steels et al., 2007), at the base of the primary cilium (Hu et al., 2010; Kim et al., 2010), and possibly also at the base of dendritic spines of neurons (Tada et al., 2007; Xie et al., 2007).

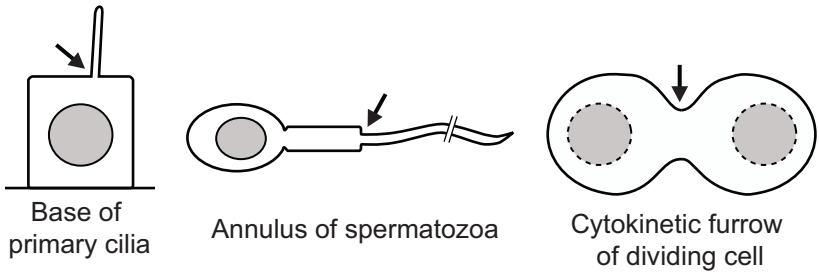

Figure 21. Examples of putative septin-dependent diffusion barriers in animal cells.

Another consistently observed localization of septins in interphase cells is co-alignment along actin stress fibers (Joo et al., 2007; Kinoshita et al., 2002; Kinoshita et al., 1997; Schmidt and Nichols, 2004b). These contractile actin bundles are predominant in fibroblasts and display similarities with the actomyosin machinery in cytokinesis, such as presence of myosin-II and RhoA-dependent regulation. Septin filaments and actin-bundles appear interdependent with elimination of one resulting in the concomitant elimination of the other (Kinoshita et al., 2002; Schmidt and Nichols, 2004b).

In addition to assemblies at the cytokinetic furrow, the base of appendages and associated with contractile actin bundles, septins have also been ascribed a wide variety of functions both at cortical locations (e.g. Hsu et al., 1998; Mostowy et al., 2009), cytosolic locations (e.g. Kremer et al., 2007; Kremer et al., 2005; Mostowy et al., 2010), or associated with the microtubule system (e.g. Bowen et al., 2011; Nagata et al., 2003; Spiliotis et al., 2008; Surka et al., 2002). While the diversity of reported septin functions and arrangements may indeed reflect true diversity, several outstanding issues make it difficult to synthesize a coherent picture. Due to the complexity of expression patterns most reports have focused on individual septins and it is often unclear if postulated functions can be attributed to a single septin, a heteromer containing several septins, or higher-order structures comprising the whole array of expressed septins within a particular cell. This issue is further complicated by the fact that native assembly states of mammalian septins have remained poorly defined. In addition, the tools for visualization, such as fluorescent reporter constructs, are highly artifact prone as a result of the innate propensity of septins to form promiscuous assemblies at elevated levels (Section 2.7 and Paper IV).

The work presented in this thesis shed light on several of these fundamental properties of mammalian septins. In Paper III we define the native assembly states of endogenous mammalian septin heteromers and the rules that dictate their subunit arrangement. In Paper IV we describe the caveats related to visualization of authentic higher-order septin structures and present tools for imaging of an unperturbed system, which have enabled

us to describe a non-reciprocal relationship between cortical septins and the microtubule system.

1.18 Interplay between microtubules and septins

The budding yeast septin collar interacts with astral microtubules and is of importance for spindle positioning relative to the division plane (Kammerer et al., 2010; Kusch et al., 2002). In addition, at sporulation or when exposed to starvation conditions yeast septins re-localize to the microtubule array (Pablo-Hernando et al., 2008). Apart from these two observations, other examples of microtubule – septin interplay have to my knowledge not been demonstrated in this organism.

An early screen for microtubule-interacting proteins in *Drosophila melanogaster* yielded the septins Sep1, Sep2 and Pnut (Sisson et al., 2000) and later screens using both fly and mammalian cell lysates have confirmed this link (Hughes et al., 2008; Sakamoto et al., 2008). Septin-microtubule co-localization has also been observed in intact mammalian cells by imunofluorescence microscopy of endogenous proteins or using fluorescent reporter constructs (Bowen et al., 2011; Martinez et al., 2006; Nagata et al., 2003; Spiliotis et al., 2008; Spiliotis et al., 2005; Surka et al., 2002; Xie et al., 2007), but the extent of co-localization appears highly cell-type specific (Hanai et al., 2004). Studies on septin-microtubule interaction have focused on individual septins, in particular SEPT2 (Bowen et al., 2011; Spiliotis et al., 2008; Spiliotis et al., 2005) and isoforms of SEPT9 (Nagata et al., 2003; Surka et al., 2002). Despite that most septin family members have at some point been proposed to interact with microtubules (see refs in Silverman-Gavrila and Silverman-Gavrila, 2008), the general impression is that this property is exclusive to individual or subsets of expressed septins. In contrast to this notion, our analysis in Paper IV reveals equal contribution of all expressed septins to microtubule supported cortical septin discs, and we have failed to identify any individual septin subunit essential for the interaction with microtubules (Section 2.8 and Paper IV).

Three models have been proposed for how mammalian septins may influence the status and function of the interphase microtubule array. These posit that (i) cytosolic septin heteromers or filaments sequester the microtubule-stabilizing protein MAP4 through direct interaction (Kremer et al., 2005), that (ii) septins bind to and define a specialized subset of microtubules for polarized transport (Spiliotis et al., 2008) or that (iii) cytosolic septin fibers act as guidance tracks for the outgrowth of microtubules in polarizing cells (Bowen et al., 2011). These three models are difficult to reconcile with each other, with our current data (Paper IV), as well as with observations from an earlier study in the MDCK cell model used to posit models (ii) and (iii) (Joberty et al., 2001). The interplay between septins and microtubules is a key focus of this thesis and is discussed in detail in section 2.4, 2.5 and 2.8.

2. RESULTS AND DISCUSSION

2.1 Origin, development, and aims of the current thesis project

When I applied to start in the research group I was appealed by the model system and genetic tools that had been developed through the efforts of earlier group members. The model system is based on human suspension-growing cell lines of hematopoietic origin and a stringently replicating Epstein Barr virus-based vector system. Vectors and conditions have been optimized to enable induced or constitutive ectopic expression of proteins at graded levels, as well as shRNA-mediated depletion of up to three gene products simultaneously, and even complementation strategies using shRNA-immune constructs. The stringent replication control of the vector allows for expansion of large cell cultures for bulk biochemical assays, which can be easily combined with analysis of single cells by fluorescence microscopy or flow cytometry. Prior to my arrival, this powerful experimental system had enabled the group to tease apart some of the intricate regulatory mechanisms that govern the microtubule status of human cells (reviewed by Holmfeldt et al., 2009).

As a candidate I initially worked on a parasite-derived secreted protein, named Sm16, which had been proposed as a homologue to the Op18/Stathmin family of microtubule destabilizers. This project would turn out to be anything but straight-forward; for one thing the proposal that Sm16 was an exogenous microtubule regulator in human cells turned out to be incorrect. However, experiences made while working on this project taught me valuable lessons about the pitfalls of experimental approaches, introduced techniques that would build the core of the current thesis, and led me to part-take in the evacuation of an American state. Our results from the Sm16 project so far are summarized elsewhere (Brannstrom et al., 2009; Holmfeldt et al., 2007a).

As the Sm16 project diverged away into the field of innate immunity, I chose to refocus on the microtubule regulatory circuits of human cells, where I felt our experimental tools could be put to a more productive use. Per Holmfeldt, a senior Ph D student, had successfully optimized the transfection system also for the Jurkat T-lymphocyte cell line and one of the initial discoveries made was that Op18 depletion led to a decrease in the total pool of tubulin heterodimers. This led us to examine i) whether the microtubule regulatory activity of Op18 also includes regulation of total tubulin content, and if so, ii) by which mechanism this level of regulation is exerted (Paper I and II)?

Parallel work in the lab had established Op18 and MAP4 as the major counteractive regulators of tubulin heterodimer partitioning in interphase cells (Holmfeldt et al., 2007b). This finding, together with proposals by others (Kremer et al., 2005), sparked our initial interest in the septins.

Consequently, we embarked on a study to dissect the molecular interplay between microtubules and septins. Along the way we realized that several basic aspects of mammalian septin biology remained uncharacterized, which hampered our ability to properly interpret our data. We therefore expanded our study to include i) analysis of the native assembly state(s) of mammalian septins and ii) methods for visualization of authentic septin structures in live cells (Paper III and IV).

Below I discuss the major findings of the thesis in light of this project development. Since the individual papers are accompanied by separate discussion sections (Papers I-IV), I here focus on describing the progression of the project and expand on matters not detailed elsewhere.

2.2 Op18 has potential to protect tubulin heterodimers from disruptive activities

Since the initial discovery of the microtubule-directed action of Op18 (Belmont and Mitchison, 1996; Marklund et al., 1996), several studies have shown that Op18 is the major microtubule destabilizing protein of interphase mammalian cells (reviewed by Holmfeldt et al., 2009). The results presented in paper I and II, stem from the surprising observation that depletion of Op18 in the Jurkat T-lymphocyte cell line not only resulted in the expected effect on tubulin partitioning, but also a notable lowering of total tubulin content (Paper II, Fig 3). In principal, such an effect could be explained by a role for Op18 either i) in the synthesis and/or assembly of new tubulin heterodimers or ii) in the turnover of the pre-existing tubulin heterodimer pool.

Purified tubulin heterodimers decay within a few hours in solution (Prasad et al., 1986 and our unpublished observation), while in intact cells tubulin turnover is slow with a half-life of >48 hours (Caron et al., 1985a). Given that the endogenous Op18 levels in our cell model systems are sufficient for complex formation with the entire soluble tubulin heterodimer pool (Paper II, Fig 1), we reasoned that Op18 may exert a tubulin-protective effect in intact cells. Indeed, we found that addition of stochiometric amounts of Op18 counteracted the spontaneous decay of purified tubulin heterodimers stored *in vitro* (our unpublished observation).

To examine if Op18 also exerts tubulin protection in cells we exploited the finding that ectopic expression of the tubulin assembly cofactor TBCE or a TBCE-like (E-like) protein causes tubulin disruption (Bartolini et al., 2005; Bhamidipati et al., 2000). This provided us with experimental tools to dramatically increase tubulin turnover in intact cells, which facilitated analysis of Op18-mediated protection. Our results revealed that induced expression of TBCE or E-like leads to immediate destruction of soluble, but not polymeric, tubulin (Paper I, Fig 1), suggesting that these proteins exert their effect through direct interaction with soluble tubulin heterodimers. Most significantly, simultaneous depletion of Op18 augmented tubulin

disruption by TBCE or E-like, while artificially increased Op18 levels were protective (Paper I, Fig 4-6). Ectopic expression of an Op18 family member with higher affinity for tubulin further enhanced the protective effect, supporting that Op18-mediated protection is a consequence of direct tubulin heterodimer binding (Paper I, Fig 6). Hence, Op18 has potential to protect tubulin from spontaneous decay *in vitro* as well as from tubulin-disruptive activities in intact cells (Figure 22).

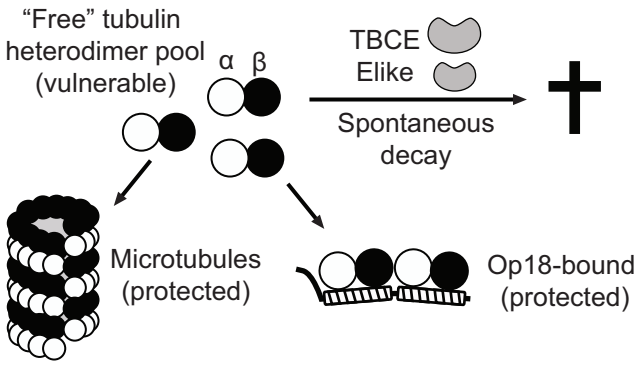

Figure 22. A model for protection of tubulin heterodimers against disruptive activities.

To address the physiological significance of tubulin protection by Op18 in intact cells is not trivial, since alteration of Op18 levels also results in dramatic effects on tubulin heterodimer partitioning. Upon Op18 depletion the majority of all tubulin heterodimers become incorporated into microtubules (Paper I, Fig 3), which according to our data represent a protected state (Paper I, Fig 1). The effect on partitioning can be compensated for by incubation with microtubule depolymerizing drugs (e.g. as done in Paper I, Fig 5-6). However, upon prolonged incubation (>5h) this treatment results in accumulation of cells blocked in mitosis, which hampers analysis of tubulin protein turnover in cells not expressing ectopic tubulin-disrupting proteins. Still, we have failed to detect that Op18 depletion is accompanied by pronounced effects on endogenous tubulin turnover in the absence of ectopic tubulin-disruptive activities (our unpublished data). Thus, while the results in Paper I provide proof-of-principle that Op18 has potential to exert tubulin protection, the physiological significance of this activity remains unclear. In any case, a tubulin protective role is clearly insufficient to explain the degree of lowered tubulin content observed in Op18-depleted Jurkat cells.

2.3 Op18 functions as a global regulator of the microtubule system in mammalian cells

As outlined in the introduction (Section 1.6 and Figure 6 above), a post-transcriptional mechanism for regulation of tubulin synthesis has been proposed based on the effects of drugs that alter tubulin heterodimer partitioning. According to the proposed model, tubulin mRNA:s are degraded in response to increased soluble tubulin heterodimer content (reviewed by Cleveland, 1989). Involvement of Op18 in regulation of tubulin synthesis provides an alternative explanation to the observed link between Op18 and total tubulin protein content, which prompted us to investigate this possibility.

In both K562 and Jurkat cells, Op18 depletion results in increased microtubule polymer levels (Paper II, Fig 2), while ectopic Op18 overexpression causes depolymerization (Paper II, Fig 4). However, the microtubule system of K562 cells appears much more responsive to alterations in Op18 content as compared to in Jurkat cells. This seems likely to reflect differential composition of additional microtubule regulators in these two cell types. Importantly, while both depletion and ectopic expression revealed a positive relationship between Op18 and tubulin protein content in Jurkat cells, this was not observed in K562 cells (Paper II, Fig 3-4).

To explore the differences between Jurkat and K562 cells, we investigated the effects of microtubule-directed drugs on tubulin mRNA levels in various cell types. As expected, treatment with the microtubule-stabilizing drug taxol increased the levels of α- and β-tubulin mRNA in both Jurkat cells and normal T-lymhocytes, while the depolymerizing drug colchicine had the opposite effect (Paper II, Fig 5). The levels of primary unspliced β-tubulin transcript appeared in all cases largely unaffected, suggesting that Jurkat cells and normal T-lymphocytes can regulate tubulin mRNA stability through the previously described autoregulatory mechanism. In contrast, this mechanism appears non-operational in K562 cells, since neither taxol nor colchicine had any effect on tubulin mRNA levels in this cell line (Paper II, Fig 5). Since tubulin mRNA autoregulation has been observed in divergent cell types across species (reviewed by Cleveland, 1989), this finding likely reflects an inactivation of some autoregulatory circuit component(s) during transformation of the K562 cell line.

To directly address if Op18 regulates tubulin mRNA stability, we quantified α- and β-tubulin mRNA and primary β-tubulin transcript in Jurkat cells after either shRNA-mediated Op18 depletion or artificial overexpression. The results revealed that Op18 mediates positive and reversible regulation of tubulin mRNA levels without detectable alterations in transcription (Paper II, Fig 6). Furthermore, excessive Op18 levels abolish tubulin mRNA regulation by microtubule-directed drugs (Paper II, Fig 7). Hence, in addition to determining tubulin heterodimer partitioning, Op18

positively regulates total tubulin content through autoregulation of tubulin mRNA stability in cells where this system is operational (Figure 23).

The precise mechanism behind tubulin mRNA autoregulation remains poorly understood. Based on the actions of microtubule-directed drugs, it was originally proposed that the cell by some means senses the levels of soluble (non-polymeric) tubulin heterodimers and that an increase in soluble tubulin represents the signal for mRNA degradation (Ben-Ze'ev et al., 1979; Cleveland et al., 1981). Our data show that elevated Op18 expression results in a dramatic increase in the level of soluble tubulin heterodimers, combined with increased tubulin mRNA stability, i.e. opposite to what would be expected by the proposed model. In my view, this finding refutes the proposal that tubulin mRNA stability is governed directly in response to the polymerization state of the microtubule system. Rather, it is conceivable that the pool of soluble and non-Op18-bound tubulin heterodimers is sensed by the cell and dictates tubulin mRNA stability (Figure 23). However, given that alterations in the levels of these truly "free" heterodimers can be expected to be balanced by polymerization into microtubules, this model also has weaknesses (expanded upon in the discussion section of Paper II).

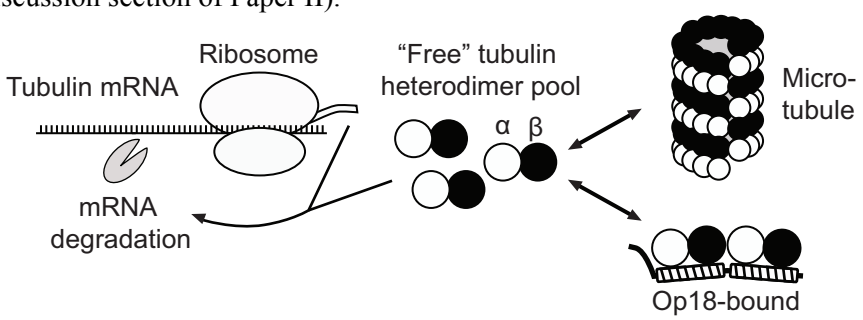

Figure 23. A model for involvement of Op18 in autoregulation of tubulin mRNA stability.

In summary, we have found that Op18 modulates total tubulin content through autoregulation of tubulin mRNA stability, which indicates a physiological significance of this previously proposed regulatory mechanism. The finding that Op18 modulates tubulin synthesis, combined with a potential tubulin-protective role, as well as potent regulation of tubulin heterodimer-polymer partitioning, led us to propose that Op18 acts as a global regulator of the interphase microtubule system. Originally, we suggested that global effects of Op18 may be confined to cells in which this protein is abundantly expressed (Paper II). A recent study, however, has shown that also the modest Op18 levels observed in mouse embryonic fibroblasts (~6-fold less than proliferating T lymphocytes) has profound impact on tubulin heterodimer-polymer partitioning (Ringhoff and

Cassimeris, 2009). Significantly, this effect was shown to depend on an additional role of Op18 in regulating microtubule nucleation. Hence, Op18 interacts with the interphase microtubule system on several different levels, depending on the cellular context and Op18 content.

Scooped on false premises – a note on Fletcher and Rorth, Curr Biol 2007

While we worked on the link between Op18 and the total tubulin content in human cells, a paper was published in Current Biology (Fletcher and Rorth, 2007), reporting that a loss-of-function mutation in the Op18 homologue of *Drosophila* resulted in dramatically lowered tubulin levels and a collapse of the microtubule system. This paper was, however, subsequently retracted after it was found that the fly stock contained additional genetic lesions. It is stated in the retraction that the authors "cannot cleanly attribute the originally observed effects" to the *Drosophila* Op18 homologue, but whether more subtle effects on total tubulin content were still observed in a clean knockout is to me unclear.

2.4 Previously proposed models on interplay between septins and MAP4 appear mutually exclusive

Microtubule destabilization by Op18 can be counteracted by microtubule associated proteins that bind and stabilize the microtubule polymer lattice. Data from our lab indicate that Op18 and the ubiquitously expressed microtubule associated protein MAP4 constitute the predominant and counteractive regulators of tubulin heterodimer partitioning during the interphase of our human cell model systems (Holmfeldt et al., 2007b). MAP4 is subject to multi-site phosphorylation by CDK1 upon mitotic entry (Ookata et al., 1997) or by MARK family kinases in interphase (Drewes et al., 1997), which abolishes MAP4-microtubule association and thereby promotes microtubule depolymerization.

Phosphorylation represents the only well-characterized mechanism for regulation of MAP4 activity. However, in 2005 it was reported that a septin heteromer composed of SEPT2, 6, and 7 binds directly to the proline-rich region of MAP4 and thereby blocks its association to microtubules in HeLa cells (Kremer et al., 2005). Septin depletion was shown to result in increased interphase microtubule stability and a large fraction of cells that failed at some undefined step of mitosis. Both of these effects were reversed by simultaneous co-depletion of MAP4. Based on these results, the authors proposed that septins exert indirect regulation of microtubule stability through sequestration of MAP4 in the cytosol (Kremer et al., 2005).

An independent research group (Spiliotis et al., 2008) subsequently proposed a superficially similar interplay between septins and MAP4 based on studies of another epithelial cell line (MDCK). However, the mechanistic details of their model appear to my mind mutually exclusive with the conclusions by Kremer et al. Thus, instead of a direct septin-MAP4 interaction in the cytosol, Spiliotis et al proposed that SEPT2 competes with MAP4 for binding to a subset of microtubules, thereby specifying them for

polarized transport. In addition, while the original model (Kremer et al., 2005) implied global effects on the entire interphase microtubule array, the subsequent model (Spiliotis et al., 2008) included only local effects.

2.5 Results from the present thesis project refute a functional link between septins and MAP4

During the course of previous studies we have refined methods for quantitative analysis of tubulin heterodimer-polymer partitioning. Our main strategy relies on gentle permeabilization of cells in a buffer that preserves microtubules, but releases soluble tubulin heterodimers, followed by immuno-staining and quantification of microtubule content by flow cytometry. This method readily detects alterations in tubulin partitioning that accompany depletion of endogenous microtubule regulators, such as Op18 and MAP4 (e.g. Paper II, Fig 2 and Paper IV, Fig S6). Using this method we have failed to detect any effect on tubulin heterodimer partitioning or microtubule stability after depletion of individual septins, such as SEPT2, 5, 6 and 9 (our unpublished data) or after extensive depletion of the entire septin system (~95% depletion of SEPT7, Paper IV, Fig 5 and S6). Furthermore, while overexpression of Op18 or MAP4 causes dramatic and opposite effects on tubulin partitioning, overexpressed SEPT2, which at elevated levels forms homo-polymers, has no effect (Paper IV, Fig S7). Finally, using an immuno-precipitation strategy that readily detects dynamic protein interactions, we have been unable to detect any interaction between septins and MAP4. These experiments involved both the endogenous proteins and various levels of artificial overexpression (our unpublished data).

In summary, our combined data do not support any functional or physical link between septins and MAP4, and septins appear not to exert global regulation of the interphase microtubule system. I can only speculate on the reasons for this apparent conflict with previous proposals, but one aspect is worth pointing out. The original study (Kremer et al., 2005) concluded that septin depletion results in increased microtubule stability in HeLa cells, as judged by increased levels of acetylated tubulin (Kremer et al., 2005, Fig 1). However, septin depletion in HeLa cells causes failed progression through cytokinesis (e.g. Estey et al., 2010), which was indeed observed, but not explicitly stated (Kremer et al., 2005, Fig 9, note that cytokinetic failure is referred to as “mitotic defects” or “abnormal nuclei”). Hence, a normal cycling cell population was compared with a septin-depleted population comprising mainly binuclear/polyploid cells. Given these premises, it appears likely to me that the apparent increase in microtubule stability is an indirect consequence of compromised cell cycle progression of septin-depleted HeLa cells. It should be noted in this context that septins are not essential for cytokinesis of suspension-growing cells of hematopoietic origin (Tooley et al., 2009 and Paper III). Thus, our functional

studies of septin-depleted K562 and Jurkat cells have not been complicated by significant accumulation of binucleated/polyploid cells.

Based on the present data, we cannot exclude a local interplay between SEPT2 and MAP4 in polarized epithelial cells, as previously proposed (Spiliotis et al., 2008). However, the results from our study do not support that septins and MAP4 compete for microtubule binding sites (our unpublished data). In addition, we have found that while septins in their native heteromeric context localize exclusively at the plasma membrane of our cell models, ectopic SEPT2-reporters are prone to form artificial cytosolic filaments that localize to the perinuclear region (Paper IV, Fig S2).

2.6 Mammalian septins assemble into stable six- to eight-subunit heteromers arranged according to homology-based subgroups

Studies of mammalian septins have focused on individual family members or a heteromer comprising SEPT2/6/7. However, mammalian cells express various combinations of the 13 septin genes in a tissue specific manner (Paper III, Fig 1). While it is clear that septins assemble into heteromers, the pairing rules and native assembly states of the multitude of mammalian septins have remained elusive (Section 1.14). After a period of initial confusion, it became evident to us that meaningful studies of the septin system required knowledge of native assembly states.

During earlier studies of the parasite-derived Sm16 protein we utilized a method for analysis of the hydrodynamic parameters of protein complexes in crude samples (Brannstrom et al., 2009; Siegel and Monty, 1966). This method is based on western blot detection of proteins in fractions after separation by size exclusion chromatography and gradient centrifugation. Comparison with standard proteins allows for estimation of the molecular weight of protein complexes with a precision of +/- ~10%. By this mean, the size and shape of septin heteromers could be determined in crude lysates from cells lacking or overexpressing individual septins (Paper III, Fig 3-8). Estimates based on hydrodynamic parameters were further corroborated by single particle electron microscopy analysis of purified septin heteromers (Paper III, Fig 8-9).

The conclusions from our analyses are summarized in a model for the ordered assembly of individual septins into heteromers (Paper III, Fig 10). The model illustrates that individual septins are unstable outside the heteromeric context, but become progressively stabilized upon co-assembly into six- to eight-subunit heteromers that comprise the protomers for higher-order septin structures. Our combined data, interpreted in the light of a previous crystallographic study (Sirajuddin et al., 2007), indicate that subunit positions within heteromers coincide with the four previously described homology subgroups of mammalian septins (Figure 15 above). It should be noted that all cell types appear to express at least one member of each subgroup (Cao et al., 2007).

Our finding that endogenous septins exist exclusively in the context of six- to eight-subunit heteromers (free septins were estimated to <0.5% of the total septin content) has implications for the interpretation of previously proposed septin functions. Since our study includes human cells representing myeloid, lymphoid, and epithelial cell types, the conclusions seem likely to be applicable across diverse cell model system. It follows that the present study refutes the notion that individual mammalian septins function autonomously.

The final part of our model delineates formation of octameric septin heteromers by SEPT7-dependent incorporation of SEPT9 isoforms at ends of hexamers (Paper III, Fig 10). Octamers are depicted with a SEPT9 subunit at each end, which makes them similar to budding yeast heteromers (Bertin et al., 2008) both with respect to subunit number and polymerization interfaces at ends. However, it remains equally possible that SEPT9 subunits instead dimerize at one end of the hexamer (Figure 24). Although SEPT9 does not appear to homodimerize in the absence of oligomerization partners (Paper III, Fig 5), several lines of evidence point to that a dimer at one end is the likely arrangement. Observations in support of this arrangement include:

i) Despite substochiometric expression levels of SEPT9, octamers appear favored over heptamers (Paper III, Fig 9), which suggests co-operativity in the incorporation of SEPT9 into heteromers. It seems unlikely that a co-operative effect could be transmitted across all six central subunits to favor incorporation of the second SEPT9 at opposite end of the heteromer.

ii) Our data suggest that SEPT9 and other septins are equally represented in insoluble higher-order septin structures (Paper III, Fig 2, Paper IV, Fig 7, and our unpublished data). This could be interpreted to suggest that SEPT9-containing octamers co-polymerize with hexamers into a common polymer system. According to current structural understanding, an ability to co-polymerize with hexamers implies G-interfaces at both ends of SEPT9-containing octamers.

iii) A SEPT9 protein carrying a C-terminal AcGFP- or Flag-tag does not assemble into heteromers (Paper IV, Fig 2 and our unpublished data). If monomeric SEPT9 is incorporated at each end of a hexamer though G-interface interactions with SEPT7, it is difficult to envisage how a C-terminal SEPT9 tag would interfere with assembly. However, if SEPT9 subunits form an assembly induced dimer at one end of a hexamer through a homotypic NC-interaction, this observation could be readily explained by the fact that SEPT9 isoforms lack a C-terminal extension.

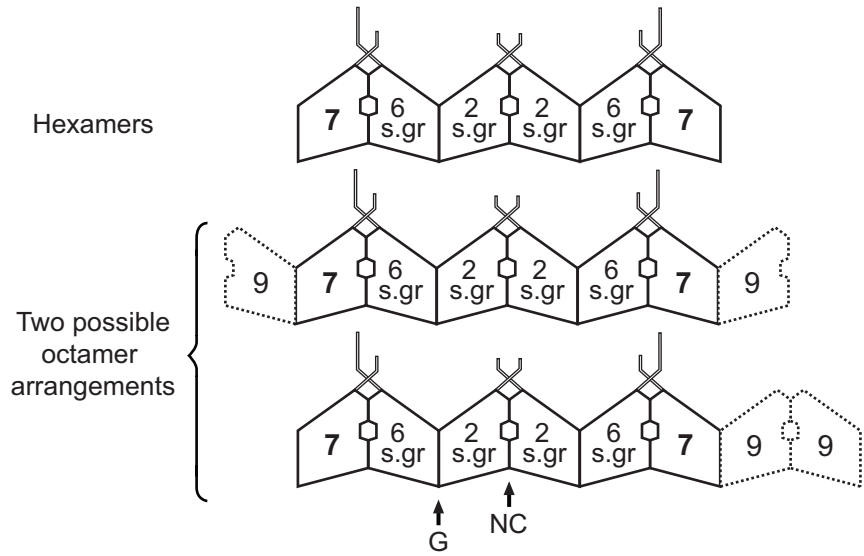

Figure 24. Models for the subunit arrangement of hexameric and octameric mammalian septin heteromers.

2.7 A systematic evaluation of approaches for live visualization of the septin system

Contemporary strategies for visualization of proteins in intact cells rely on expression of reporter constructs, which encode fusion derivatives of a protein of interest and a detectable tag. Fluorescent fusion reporters based on tubulins or EB-family proteins are commonly used to track individual microtubules and measure their dynamic behavior in live cells (reviewed by Gierke et al., 2010). In a similar fashion, fusions of actin or actin binding proteins to fluorescent proteins are used for visualization of actin filaments (Riedl et al., 2008). Previous studies of the septin system have involved an array of reporter constructs encoding individual septins fused at either the N- and C-terminus to fluorescent proteins or epitope tags.

Visualization by means of reporter constructs is subject to several pitfalls, which include mis-localization that depends on excessive expression levels or interference by N- or C-terminal tags. In addition, correct visualization of multi-subunit complexes, such as septin heteromers, requires that the reporter incorporates into the endogenous system without altering its structure or function.

In budding yeast, fluorescent reporters have successfully been used to unravel many of the septin rearrangements that accompany cell cycle and developmental transitions (Cid et al., 2001; McMurray and Thorner, 2008; Vrabioiu and Mitchison, 2006). Live visualization of septins in budding yeast has been aided by two important advantages of this model system. Firstly, experimental tools for replacement of chromosomal septin genes by

sequences encoding fusion reporter counterparts eliminate the risk of non-physiological expression levels. Secondly, the presence of essential septin assemblies with a defined localization at the bud neck provides a stringent criterion for reporter evaluation. In contrast, studies in mammalian cell models have commonly employed fusion derivatives expressed from plasmids at unphysiological levels, and there are reports on a multitude of septin localizations during both interphase and mitosis. It appears to me that many of these reports are flawed by artifacts caused by unbalanced septin expression.

Our results presented in paper III show that individual mammalian septins exist exclusively as subunits of heteromers, which in turn reversibly polymerize into higher-order structures. To analyze if individual subunits are exchangeable within preformed heteromers, we used our genetic tools for induced ectopic expression of tagged septins. Analysis of N-terminally His-tagged SEPT7 (Paper IV, Fig 1) or C-terminally Flag-tagged SEPT2, 6, or 7 (our unpublished data), revealed no incorporation into the pre-existing heteromer pool. However, incorporation into heteromers increased progressively over a 4 day period following induced expression (Paper IV, Fig 1). Given a ~24h doubling time of the cell line, our data indicate that septins assemble co-translationally into stable heteromers that do not exchange their subunits. It follows that septin reporters can be expected only to become incorporated during *de novo* hetero-oligomerization. This finding has implications for strategies relying on transient expression of septin reporters, since expression over short time periods will predictably result in visualization of artificial structures.

Purified septin heteromers are stable *in vitro* even when stored in buffers of high ionic strength (Paper III and reviewed by McMurray and Thorner, 2009a). However, an elegant set of experiments in budding yeast has surprisingly revealed that heteromers readily exchange their subunits during cell cycle and developmental transitions in this organism (McMurray and Thorner, 2008). This was interpreted by the authors to suggest the presence of undefined cellular factor(s) that facilitate subunit reshuffling *in vivo*. This finding contrasts to our analysis of heteromer stability in human cells and may reflect a difference in the properties of septins and/or accessory proteins between fungi and mammalian cells.

We next evaluated reporter-derivatives representing all four homology-based subgroups of mammalian septins under optimal conditions, i.e. low level expression over multiple cell division cycles. Notably, even under these conditions, aimed at facilitating incorporation during *de novo* heteromer synthesis, most reporters displayed a propensity to persist in a non-heteromeric state (Paper IV, Fig 2, and expanded upon in the supplementary data section). Only SEPT7 fused to a C-terminal AcGFP-tag fulfilled all criteria of a specific reporter of native septin assemblies, namely i) competition with the cognate endogenous septin without detectable effects

on other septins, ii) complete incorporation into heteromers, iii) an interphase localization consistent with results from immuno-staining of endogenous proteins, and iiii) consistent localization to the cytokinetic furrow in late mitosis (Paper IV, Fig 2-3).

2.8 Microtubule-supported cortical discs are predominant higher-order septin structures in interphase mammalian cells lacking external cues

Our cell model systems comprise suspension-growing cell lines, which in the absence of substrate adhesion are shaped as spheres. This provides spatial separation over the entire cell cortex and facilitates imaging of both cross sections and along the plasma membrane. Live observation of K562 or Jurkat cells expressing the SEPT7-AcGFP derivative revealed an exclusively cortical localization of all detectable septin structures. These appeared all as punctuate assemblies and in most cases as discs attached flat against the membrane (Paper IV, Fig 3 and 8). These cortical discs are the predominant septin structures observed in interphase cells, but rearrange into assemblies at the cleavage furrow in late stages of mitosis (Paper IV, Fig 3).

As outlined above, our initial interest in septins stemmed from proposals that septins regulate microtubules through binding of MAP4 in the cytosol or by other mechanisms (reviewed by Spiliotis, 2010). However, we did not detect septins in the cytosol or co-localized with the radial interphase microtubule array (Paper IV, Fig 3-4, and our unpublished data). Most significantly, depletion or overexpression of septins had no effect on tubulin heterodimer partitioning or microtubule stability (Paper IV, Fig 5, S6 and S7).

To examine a potentially inverse dependency, i.e. a microtubule-dependence for the assembly of septins into cortical discs, microtubules were eliminated by addition of nocodazole, storage on ice, or induced expression of the tubulin-disruptive TBCE. Irrespective of the method used, we consistently found that microtubule depolymerization caused disintegration of cortical septin discs into diffuse, but still cortical, structures (Paper IV, Fig 4-5). Thus, the results from this study suggest a non-reciprocal interplay, whereby the dense interphase microtubule array of non-adhered cells supports a cortical disc-like septin arrangement (Paper IV, Fig 11).

Several earlier studies of adhesion substrate-dependent cell types have reported a varying degree of co-localization between septins and microtubules (Section 1.18), which contrasts to the exclusively cortical localization observed in K562, Jurkat cells (Paper IV), or mouse T-lymphocytes (Tooley et al., 2009). This apparent discrepancy may in part be explained by visualization of ectopic structures of tagged septins in previous studies, as outlined in section 2.7. However, it also appears likely that differences in the structural arrangement of microtubules between cell types

may have consequences for septin-microtubule interactions. We and others have found that artificial bundling of microtubules upon prolonged Taxol treatment causes accumulation of septins onto microtubule-associated filamentous structures (Paper IV, Fig 6 and Bowen et al., 2011). It remains possible that physiological microtubule bundling in e.g. platelets and polarizing epithelial cells (Bowen et al., 2011; Martinez et al., 2006) is sufficient to explain septin-microtubule co-localization in these cell-types. Such structural selectivity finds precedence in the observed co-localization of septins with actin bundles in fibroblasts, but not with other types of more dynamic actin arrangements (Kinoshita et al., 2002; Schmidt and Nichols, 2004b and Paper IV, Fig 8-9). Thus, cell-type specific septin localizations may be determined by multivalent binding to PIP_2 or other components of the plasma membrane, combined with low affinity interactions with actin filaments and/or microtubules (or cognate adaptor proteins), that can be expected to gain in avidity in cases of filament bundling (Figure 25).

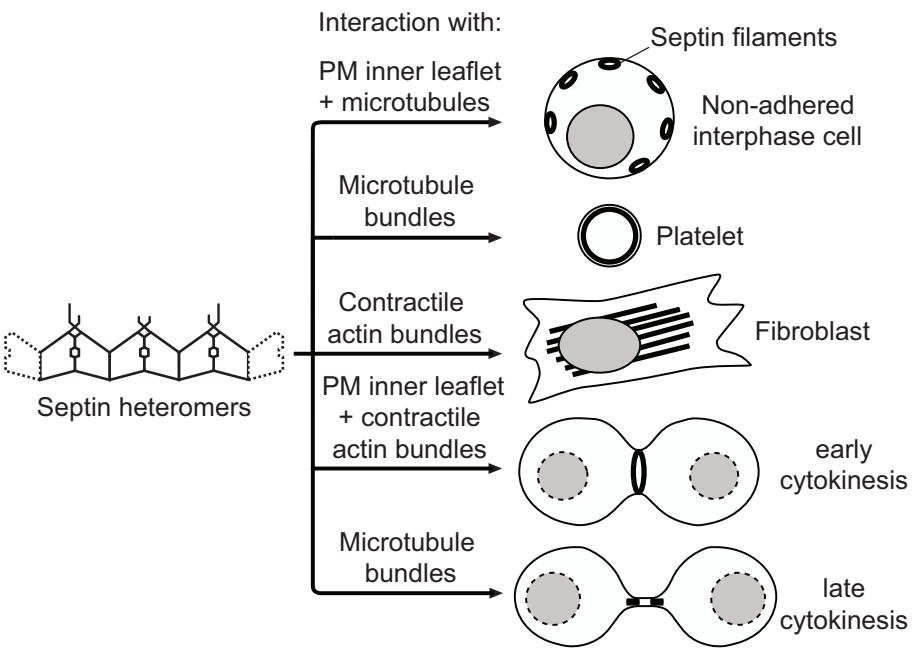

Figure 25. A model for how multivalent interactions with the plasma membrane, contractile actin bundles and/or microtubules dictate cell type and cell-cycle phase specific septin localizations.

Following cell permeabilization in a physiological buffer all septins are released into the supernatant, while permeabilization at low ionic strength results in retention of septins in cell-associated insoluble structures (Paper III, Fig 2). Significantly, microtubule stabilization also results in

preservation of insoluble septin structures upon cell permeabilization (Paper IV, Fig 7). A detailed analysis has revealed identical partitioning of individual septins between soluble and insoluble states at all conditions tested (Paper III, Fig 2, S1, Paper IV, Fig 7, S10, and our unpublished data). Hence, the combined data from Paper III and IV indicate that all expressed septins contribute equally to higher-order structures, such as the microtubule-supported cortical septin discs of non-adherent cells.

2.9 Higher-order septin assemblies define static cortical regions

Mammalian septins have been ascribed diverse functions that in some cases imply involvement in dynamic membrane remodeling events, e.g. exocytosis and phagocytosis (see refs in Peterson and Petty, 2010). However, our data presented in paper IV suggest that septins define regions of the plasma membrane with static properties. The microtubule-supported sub-membraneous septin discs observed in non-adherent cells appear remarkably non-motile when imaged over time (our unpublished observation). In contrast, simultaneous visualization of clathrin-mediated endocytosis, internalization induced by cross linking of cell surface receptors (Paper IV, Fig 10), or endocytosis through non-clathrin-mediated pathways (e.g. cholera toxin subunit B, our unpublished data) in all cases revealed the expected rapid accumulation of markers in the endomembrane system. Notably, septins were completely exluded from endocytic vesicles at all conditions tested.

Jurkat T-lymphocytes plated on an integrin-activating substrate migrate by an amoeboid mode of locomotion, with a dynamic pseudopod and a trailing uropod. We found that septin discs (and/or other punctuate septin assemblies) all accumulate in the uropod during amoeboid migration, while simultaneous actin filament visualization revealed the expected enrichment in the highly dynamic pseudopod (Paper IV, Fig 8). Plating of the K562-KA8 cell line on an adhesion substrate does not result in motility, but cells attach and spread through formation of dynamic filopodia. Cell spreading was accompanied by transformation of uniform septins discs into heterogeneous punctuate structures and continuous assemblies at the cell margin. Importantly, also in this cell model we found that septins were completely absent from dynamic protrusions (Paper IV, Fig 9). Hence, our combined results indicate that structurally diverse cortical septin assemblies are all mutually exclusive with plasma membrane regions undergoing extensive remodeling.

Septins clearly have a role in polarized exocytosis during bud formation in *S. cerevisiae*. Involvement in this dynamic process seems at first glance difficult to reconcile with static properties. However, the initially diffuse septin ring formed at the presumptive bud site is rearranged into a collar that appears static by FRAP analysis and that delineates the region of active bud growth, rather than being included in it (reviewed by Oh and Bi,

2011). Thereafter, septins at this location may serve as a diffusion barrier, which appears consistent with static properties. Moreover, in diverse types of mammalian cells septins appear localized to the bases of, but not within, protrusions (Kinoshita et al., 1997; Tada et al., 2007; Xie et al., 2007), cilia or flagella (Caudron and Barral, 2009; Hu et al., 2010). These findings seem generally consistent with our observations of static septin assemblies.

Whether septins directly influence dynamic properties of the plasma membrane of interphase cells, or are passively recruited to static regions remains unclear. We have found that septin-depleted K562-KA8 cells remain capable of attaching and spreading on an adhesion substrate, but may display subtle alterations in the distribution, length and/or width of dynamic protrusions (our unpublished observation). Septin depletion in HeLa cells has been reported to cause dramatic effects on cell shape and spreading (Kremer et al., 2007). However, based on the presented images it appears evident that the increased size and altered shape of septin-depleted HeLa can be fully explained by the fact that binuclear/polyploid cells were included in the analysis (Kremer et al., 2007, Fig 1-2, note that septin depletion in HeLa causes failure in cytokinesis). Another recent study has reported decreased cortical rigidity in septin-depleted T-lymphocytes (Tooley et al., 2009). This report is in general agreement with our findings on localization of septins to the uropod of migrating T-lymphocytes (Paper IV, Fig 8), but at this location we found only punctuate septin assemblies. Thus, our data do not support the proposed presence of a rigidifying filamentous septin corset at the uropod.

In summary, the data presented in Paper IV indicate that microtubule-supported septin discs and other diverse higher-order septin assemblies localize exclusively at the plasma membrane and define static cortical regions. Our estimates suggest that as much as 5-10% of the plasma membrane inner leaflet is occupied by septins. It appears reasonable to assume that this localization is at least partly linked to the ability of septins to bind the compartmentalized phosphoinositide PIP_2 (Bertin et al., 2010; Tanaka-Takiguchi et al., 2009; Zhang et al., 1999). I find it conceivable that such multivalent interactions between higher-order septin assemblies and the inner leaflet may result in macro domains with altered membrane fluidity, local deformability and/or lipid composition. It remains to be tested if septins by this mean contribute to compartmentalization of the cell cortex beyond their established roles as molecular scaffolds and in diffusion barrier formation.

MAIN FINDINGS OF THE THESIS IN SUMMARY

- Op18 may serve as a global regulator of the interphase microtubule system

- Mammalian septins exist exclusively as components of stable six- to eight-subunit heteromers that assemble according to homology-based subgroups

- Native septin heteromers are generally not arranged as perfect palindromes

- Incorporation of SEPT9 isoforms at end(s) of hexamers is the distinguishing feature of octameric septin heteromers

- Microtubules support cortical septin discs at the plasma membrane of non-adhered cells

- Higher-order septin assemblies appear mutually exclusive with dynamic regions of the cell cortex

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"Science is a collaborative enterprise, spanning the generations. We remember those who prepared the way, seeing for them also." – Carl Sagan

Being part of a research enterprise is a peculiar thing to experience. You spend your waking hours studying the unknowns around you, but at the end of the day what you have learnt most about is the inner workings of your own mind. These past years have offered a ride of exhilarating highs, but also of occasional humbling lows, and I wouldn´t have missed it for the world. I have been fortunate to share the ride with an outstanding group of people, who I owe my deepest gratitude.

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REFERENCES

Akhmanova, A., and Steinmetz, M.O. (2008). Tracking the ends: a dynamic protein network controls the fate of microtubule tips. Nat Rev Mol Cell Biol *9*, 309-322.

Alberts, B., Wilson, J.H., and Hunt, T. (2008). Molecular biology of the cell, 5th edn (New York, Garland Science).

Barlow, S.B., Gonzalez-Garay, M.L., and Cabral, F. (2002). Paclitaxel-dependent mutants have severely reduced microtubule assembly and reduced tubulin synthesis. Journal of Cell Science *115*, 3469-3478.

Barral, Y., Mermall, V., Mooseker, M.S., and Snyder, M. (2000). Compartmentalization of the cell cortex by septins is required for maintenance of cell polarity in yeast. Mol Cell *5*, 841-851.

Bartolini, F., Tian, G., Piehl, M., Cassimeris, L., Lewis, S.A., and Cowan, N.J. (2005). Identification of a novel tubulin-destabilizing protein related to the chaperone cofactor E. J Cell Sci *118*, 1197-1207.

Belmont, L.D., Hyman, A.A., Sawin, K.E., and Mitchison, T.J. (1990). Real-time visualization of cell cycle-dependent changes in microtubule dynamics in cytoplasmic extracts. Cell *62*, 579-589.

Belmont, L.D., and Mitchison, T.J. (1996). Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. Cell *84*, 623-631.

Ben-Ze'ev, A., Farmer, S.R., and Penman, S. (1979). Mechanisms of regulating tubulin synthesis in cultured mammalian cells. Cell *17*, 319-325.

Bertin, A., McMurray, M.A., Grob, P., Park, S.S., Garcia, G., 3rd, Patanwala, I., Ng, H.L., Alber, T., Thorner, J., and Nogales, E. (2008). Saccharomyces cerevisiae septins: supramolecular organization of heterooligomers and the mechanism of filament assembly. Proc Natl Acad Sci U S A *105*, 8274-8279.

Bertin, A., McMurray, M.A., Thai, L., Garcia, G., 3rd, Votin, V., Grob, P., Allyn, T., Thorner, J., and Nogales, E. (2010). Phosphatidylinositol-4,5-bisphosphate promotes budding yeast septin filament assembly and organization. J Mol Biol *404*, 711-731.

Bettencourt-Dias, M., and Glover, D.M. (2007). Centrosome biogenesis and function: centrosomics brings new understanding. Nat Rev Mol Cell Biol *8*, 451-463.

Bhamidipati, A., Lewis, S.A., and Cowan, N.J. (2000). ADP ribosylation factor-like protein 2 (Arl2) regulates the interaction of tubulin-folding cofactor D with native tubulin. J Cell Biol *149*, 1087-1096.

Bhattacharya, R., Yang, H., and Cabral, F. (2011). Class V beta-tubulin alters dynamic instability and stimulates microtubule detachment from centrosomes. Mol Biol Cell *22*, 1025-1034.

Bi, E. (2001). Cytokinesis in budding yeast: the relationship between actomyosin ring function and septum formation. Cell Struct Funct *26*, 529-537.

Bi, E., Maddox, P., Lew, D.J., Salmon, E.D., McMillan, J.N., Yeh, E., and Pringle, J.R. (1998). Involvement of an actomyosin contractile ring in Saccharomyces cerevisiae cytokinesis. J Cell Biol *142*, 1301-1312.

Bieche, I., Maucuer, A., Laurendeau, I., Lachkar, S., Spano, A.J., Frankfurter, A., Levy, P., Manceau, V., Sobel, A., Vidaud, M.*, et al.* (2003). Expression of stathmin family genes in human tissues: non-neural-restricted expression for SCLIP. Genomics *81*, 400-410.

Boggs, B., and Cabral, F. (1987). Mutations affecting assembly and stability of tubulin: evidence for a nonessential beta-tubulin in CHO cells. Mol Cell Biol *7*, 2700-2707.

Borisy, G.G., and Taylor, E.W. (1967). The mechanism of action of colchicine. Binding of colchincine-3H to cellular protein. J Cell Biol *34*, 525-533.

Bowen, J.R., Hwang, D., Bai, X., Roy, D., and Spiliotis, E.T. (2011). Septin GTPases spatially guide microtubule organization and plus end dynamics in polarizing epithelia. J Cell Biol *194*, 187-197.

Brannstrom, K., Sellin, M.E., Holmfeldt, P., Brattsand, M., and Gullberg, M. (2009). The Schistosoma mansoni protein Sm16/SmSLP/SmSPO-1 assembles into a nine-subunit oligomer with potential to inhibit Toll-like receptor signaling. Infect Immun *77*, 1144-1154.

Brattsand, G., Marklund, U., Nylander, K., Roos, G., and Gullberg, M. (1994). Cell-cycle-regulated phosphorylation of oncoprotein 18 on Ser16, Ser25 and Ser38. Eur J Biochem *220*, 359-368.

Brinkley, W. (1997). Microtubules: a brief historical perspective. J Struct Biol *118*, 84-86.

Brouhard, G.J., Stear, J.H., Noetzel, T.L., Al-Bassam, J., Kinoshita, K., Harrison, S.C., Howard, J., and Hyman, A.A. (2008). XMAP215 is a processive microtubule polymerase. Cell *132*, 79-88.

Byers, B., and Goetsch, L. (1976a). A highly ordered ring of membrane-associated filaments in budding yeast. J Cell Biol *69*, 717-721.

Byers, B., and Goetsch, L. (1976b). Loss of Filamentous Ring in Cytokinesis-Defective Mutants of Budding Yeast. Journal of Cell Biology *70*, A35-A35.

Cao, L., Ding, X., Yu, W., Yang, X., Shen, S., and Yu, L. (2007). Phylogenetic and evolutionary analysis of the septin protein family in metazoan. FEBS Lett *581*, 5526-5532.

Cao, L., Yu, W., Wu, Y., and Yu, L. (2009). The evolution, complex structures and function of septin proteins. Cell Mol Life Sci *66*, 3309-3323.

Caron, J.M., Jones, A.L., and Kirschner, M.W. (1985a). Autoregulation of tubulin synthesis in hepatocytes and fibroblasts. J Cell Biol *101*, 1763-1772.

Caron, J.M., Jones, A.L., Rall, L.B., and Kirschner, M.W. (1985b). Autoregulation of tubulin synthesis in enucleated cells. Nature *317*, 648-651.

Casamayor, A., and Snyder, M. (2003). Molecular dissection of a yeast septin: distinct domains are required for septin interaction, localization, and function. Mol Cell Biol *23*, 2762-2777.

Cassimeris, L. (2002). The oncoprotein 18/stathmin family of microtubule destabilizers. Curr Opin Cell Biol *14*, 18-24.

Cassimeris, L., Pryer, N.K., and Salmon, E.D. (1988). Real-time observations of microtubule dynamic instability in living cells. J Cell Biol *107*, 2223-2231.

Caudron, F., and Barral, Y. (2009). Septins and the lateral compartmentalization of eukaryotic membranes. Dev Cell *16*, 493-506.

Chretien, D., and Wade, R.H. (1991). New data on the microtubule surface lattice. Biol Cell *71*, 161-174.

Cid, V.J., Adamikova, L., Sanchez, M., Molina, M., and Nombela, C. (2001). Cell cycle control of septin ring dynamics in the budding yeast. Microbiology *147*, 1437-1450.

Cleveland, D.W. (1989). Autoregulated control of tubulin synthesis in animal cells. Curr Opin Cell Biol *1*, 10-14.

Cleveland, D.W., Lopata, M.A., Sherline, P., and Kirschner, M.W. (1981). Unpolymerized tubulin modulates the level of tubulin mRNAs. Cell *25*, 537-546.

Curmi, P.A., Andersen, S.S., Lachkar, S., Gavet, O., Karsenti, E., Knossow, M., and Sobel, A. (1997). The stathmin/tubulin interaction in vitro. J Biol Chem *272*, 25029-25036.

DeMay, B.S., Bai, X., Howard, L., Occhipinti, P., Meseroll, R.A., Spiliotis, E.T., Oldenbourg, R., and Gladfelter, A.S. (2011). Septin filaments exhibit a dynamic, paired organization that is conserved from yeast to mammals. J Cell Biol *193*, 1065-1081.

Desai, A., and Mitchison, T.J. (1997). Microtubule polymerization dynamics. Annu Rev Cell Dev Biol *13*, 83-117.

Drewes, G., Ebneth, A., Preuss, U., Mandelkow, E.M., and Mandelkow, E. (1997). MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. Cell *89*, 297-308.

Estey, M.P., Di Ciano-Oliveira, C., Froese, C.D., Bejide, M.T., and Trimble, W.S. (2010). Distinct roles of septins in cytokinesis: SEPT9 mediates midbody abscission. J Cell Biol *191*, 741-749.

Evans, L., Mitchison, T., and Kirschner, M. (1985). Influence of the centrosome on the structure of nucleated microtubules. J Cell Biol *100*, 1185-1191.

Farkasovsky, M., Herter, P., Voss, B., and Wittinghofer, A. (2005). Nucleotide binding and filament assembly of recombinant yeast septin complexes. Biol Chem *386*, 643-656.

Field, C.M., al-Awar, O., Rosenblatt, J., Wong, M.L., Alberts, B., and Mitchison, T.J. (1996). A purified Drosophila septin complex forms filaments and exhibits GTPase activity. J Cell Biol *133*, 605-616.

Flemming, W., ed. (1882). Zellsubstanz, Kern und Zelltheilung (Leipzig, F.C.W. Vogel).

Fletcher, G., and Rorth, P. (2007). Drosophila stathmin is required to maintain tubulin pools. Curr Biol *17*, 1067-1071.

Fontalba, A., Paciucci, R., Avila, J., and Zabala, J.C. (1993). Incorporation of Tubulin Subunits into Dimers Requires Gtp Hydrolysis. Journal of Cell Science *106*, 627-632.

Ford, S.K., and Pringle, J.R. (1991). Cellular Morphogenesis in the Saccharomyces-Cerevisiae Cell-Cycle - Localization of the Cdc11 Gene-Product and the Timing of Events at the Budding Site. Dev Genet *12*, 281-292.

Frazier, J.A., Wong, M.L., Longtine, M.S., Pringle, J.R., Mann, M., Mitchison, T.J., and Field, C. (1998). Polymerization of purified yeast septins: Evidence that organized filament arrays may not be required for septin function. Journal of Cell Biology *143*, 737-749.

Fuchs, E., and Cleveland, D.W. (1998). A structural scaffolding of intermediate filaments in health and disease. Science *279*, 514-519.

Gay, D.A., Sisodia, S.S., and Cleveland, D.W. (1989). Autoregulatory control of beta-tubulin mRNA stability is linked to translation elongation. Proc Natl Acad Sci U S A *86*, 5763-5767.

Gay, D.A., Yen, T.J., Lau, J.T., and Cleveland, D.W. (1987). Sequences that confer beta-tubulin autoregulation through modulated mRNA stability reside within exon 1 of a beta-tubulin mRNA. Cell *50*, 671-679.

Gierke, S., Kumar, P., and Wittmann, T. (2010). Analysis of microtubule polymerization dynamics in live cells. Methods Cell Biol *97*, 15-33.

Gigant, B., Curmi, P.A., Martin-Barbey, C., Charbaut, E., Lachkar, S., Lebeau, L., Siavoshian, S., Sobel, A., and Knossow, M. (2000). The 4 A X-ray structure of a tubulin:stathmin-like domain complex. Cell *102*, 809-816.

Gradin, H.M., Larsson, N., Marklund, U., and Gullberg, M. (1998). Regulation of microtubule dynamics by extracellular signals: cAMP-dependent protein kinase switches off the activity of oncoprotein 18 in intact cells. J Cell Biol *140*, 131-141.

Haarer, B.K., and Pringle, J.R. (1987). Immunofluorescence localization of the Saccharomyces cerevisiae CDC12 gene product to the vicinity of the 10-nm filaments in the mother-bud neck. Mol Cell Biol *7*, 3678-3687.

Hall, P.A., Jung, K., Hillan, K.J., and Russell, S.E. (2005). Expression profiling the human septin gene family. J Pathol *206*, 269-278.

Hanai, N., Nagata, K., Kawajiri, A., Shiromizu, T., Saitoh, N., Hasegawa, Y., Murakami, S., and Inagaki, M. (2004). Biochemical and cell biological characterization of a mammalian septin, Sept11. FEBS Lett *568*, 83-88.

Hanash, S.M., Strahler, J.R., Kuick, R., Chu, E.H.Y., and Nichols, D. (1988). Identification of a Polypeptide Associated with the Malignant Phenotype in Acute-Leukemia. Journal of Biological Chemistry *263*, 12813-12815.

Hartwell, L.H. (1971). Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. Exp Cell Res *69*, 265-276.

Hartwell, L.H., Culotti, J., and Reid, B. (1970). Genetic control of the cell-division cycle in yeast. I. Detection of mutants. Proc Natl Acad Sci U S A *66*, 352-359.

Holmfeldt, P., Brannstrom, K., Sellin, M.E., Segerman, B., Carlsson, S.R., and Gullberg, M. (2007a). The Schistosoma mansoni protein Sm16/SmSLP/SmSPO-1 is

a membrane-binding protein that lacks the proposed microtubule-regulatory activity. Mol Biochem Parasitol *156*, 225-234.

Holmfeldt, P., Brannstrom, K., Stenmark, S., and Gullberg, M. (2006). Aneugenic activity of Op18/stathmin is potentiated by the somatic Q18-->e mutation in leukemic cells. Mol Biol Cell *17*, 2921-2930.

Holmfeldt, P., Sellin, M.E., and Gullberg, M. (2009). Predominant regulators of tubulin monomer-polymer partitioning and their implication for cell polarization. Cell Mol Life Sci *66*, 3263-3276.

Holmfeldt, P., Sellin, M.E., and Gullberg, M. (2010). Upregulated Op18/stathmin activity causes chromosomal instability through a mechanism that evades the spindle assembly checkpoint. Exp Cell Res *316*, 2017-2026.

Holmfeldt, P., Stenmark, S., and Gullberg, M. (2007b). Interphase-specific phosphorylation-mediated regulation of tubulin dimer partitioning in human cells. Mol Biol Cell *18*, 1909-1917.

Honnappa, S., Gouveia, S.M., Weisbrich, A., Damberger, F.F., Bhavesh, N.S., Jawhari, H., Grigoriev, I., van Rijssel, F.J.A., Buey, R.M., Lawera, A.*, et al.* (2009). An EB1-Binding Motif Acts as a Microtubule Tip Localization Signal. Cell *138*, 366-376.

Howell, B., Larsson, N., Gullberg, M., and Cassimeris, L. (1999). Dissociation of the tubulin-sequestering and microtubule catastrophe-promoting activities of oncoprotein 18/stathmin. Mol Biol Cell *10*, 105-118.

Hsu, S.C., Hazuka, C.D., Roth, R., Foletti, D.L., Heuser, J., and Scheller, R.H. (1998). Subunit composition, protein interactions, and structures of the mammalian brain sec6/8 complex and septin filaments. Neuron *20*, 1111-1122.

Hu, Q., Milenkovic, L., Jin, H., Scott, M.P., Nachury, M.V., Spiliotis, E.T., and Nelson, W.J. (2010). A septin diffusion barrier at the base of the primary cilium maintains ciliary membrane protein distribution. Science *329*, 436-439.

Hughes, J.R., Meireles, A.M., Fisher, K.H., Garcia, A., Antrobus, P.R., Wainman, A., Zitzmann, N., Deane, C., Ohkura, H., and Wakefield, J.G. (2008). A microtubule interactome: complexes with roles in cell cycle and mitosis. PLoS Biol *6*, e98.

Ihara, M., Kinoshita, A., Yamada, S., Tanaka, H., Tanigaki, A., Kitano, A., Goto, M., Okubo, K., Nishiyama, H., Ogawa, O.*, et al.* (2005). Cortical organization by the septin cytoskeleton is essential for structural and mechanical integrity of mammalian spermatozoa. Dev Cell *8*, 343-352.

Joberty, G., Perlungher, R.R., Sheffield, P.J., Kinoshita, M., Noda, M., Haystead, T., and Macara, I.G. (2001). Borg proteins control septin organization and are negatively regulated by Cdc42. Nat Cell Biol *3*, 861-866.

John, C.M., Hite, R.K., Weirich, C.S., Fitzgerald, D.J., Jawhari, H., Faty, M., Schlapfer, D., Kroschewski, R., Winkler, F.K., Walz, T.*, et al.* (2007). The Caenorhabditis elegans septin complex is nonpolar. EMBO J *26*, 3296-3307.

Joo, E., Surka, M.C., and Trimble, W.S. (2007). Mammalian SEPT2 is required for scaffolding nonmuscle myosin II and its kinases. Dev Cell *13*, 677-690.

Jourdain, L., Curmi, P., Sobel, A., Pantaloni, D., and Carlier, M.F. (1997). Stathmin: a tubulin-sequestering protein which forms a ternary T2S complex with two tubulin molecules. Biochemistry *36*, 10817-10821.

Kammerer, D., Stevermann, L., and Liakopoulos, D. (2010). Ubiquitylation regulates interactions of astral microtubules with the cleavage apparatus. Curr Biol *20*, 1233-1243.

Kartmann, B., and Roth, D. (2001). Novel roles for mammalian septins: from vesicle trafficking to oncogenesis. J Cell Sci *114*, 839-844.

Kato, K. (1990). A Collection of cDNA Clones with Specific Expression Patterns in Mouse Brain. Eur J Neurosci *2*, 704-711.

Katsetos, C.D., Herman, M.M., and Mork, S.J. (2003). Class III beta-tubulin in human development and cancer. Cell Motil Cytoskeleton *55*, 77-96.

Kim, H.B., Haarer, B.K., and Pringle, J.R. (1991). Cellular Morphogenesis in the Saccharomyces-Cerevisiae Cell-Cycle - Localization of the Cdc3 Gene-Product and the Timing of Events at the Budding Site. Journal of Cell Biology *112*, 535-544.

Kim, S.K., Shindo, A., Park, T.J., Oh, E.C., Ghosh, S., Gray, R.S., Lewis, R.A., Johnson, C.A., Attie-Bittach, T., Katsanis, N.*, et al.* (2010). Planar cell polarity acts through septins to control collective cell movement and ciliogenesis. Science *329*, 1337-1340.

Kinoshita, M. (2003a). Assembly of mammalian septins. J Biochem *134*, 491-496.

Kinoshita, M. (2003b). The septins. Genome Biol *4*, 236.

Kinoshita, M. (2006). Diversity of septin scaffolds. Curr Opin Cell Biol *18*, 54-60.

Kinoshita, M., Field, C.M., Coughlin, M.L., Straight, A.F., and Mitchison, T.J. (2002). Self- and actin-templated assembly of Mammalian septins. Dev Cell *3*, 791-802.

Kinoshita, M., Kumar, S., Mizoguchi, A., Ide, C., Kinoshita, A., Haraguchi, T., Hiraoka, Y., and Noda, M. (1997). Nedd5, a mammalian septin, is a novel cytoskeletal component interacting with actin-based structures. Gene Dev *11*, 1535-1547.

Kirschner, M., and Mitchison, T. (1986). Beyond self-assembly: from microtubules to morphogenesis. Cell *45*, 329-342.

Kline-Smith, S.L., and Walczak, C.E. (2002). The microtubule-destabilizing kinesin XKCM1 regulates microtubule dynamic instability in cells. Mol Biol Cell *13*, 2718-2731.

Komarova, Y.A., Akhmanova, A.S., Kojima, S., Galjart, N., and Borisy, G.G. (2002). Cytoplasmic linker proteins promote microtubule rescue in vivo. J Cell Biol *159*, 589-599.

Kortazar, D., Fanarraga, M.L., Carranza, G., Bellido, J., Villegas, J.C., Avila, J., and Zabala, J.C. (2007). Role of cofactors B (TBCB) and E (TBCE) in tubulin heterodimer dissociation. Exp Cell Res *313*, 425-436.

Kremer, B.E., Adang, L.A., and Macara, I.G. (2007). Septins regulate actin organization and cell-cycle arrest through nuclear accumulation of NCK mediated by SOCS7. Cell *130*, 837-850.

Kremer, B.E., Haystead, T., and Macara, I.G. (2005). Mammalian septins regulate microtubule stability through interaction with the microtubule-binding protein MAP4. Mol Biol Cell *16*, 4648-4659.

Kumar, S., Tomooka, Y., and Noda, M. (1992). Identification of a set of genes with developmentally down-regulated expression in the mouse brain. Biochem Biophys Res Commun *185*, 1155-1161.

Kusch, J., Meyer, A., Snyder, M.P., and Barral, Y. (2002). Microtubule capture by the cleavage apparatus is required for proper spindle positioning in yeast. Genes Dev *16*, 1627-1639.

Kwitny, S., Klaus, A.V., and Hunnicutt, G.R. (2010). The Annulus of the Mouse Sperm Tail Is Required to Establish a Membrane Diffusion Barrier That Is Engaged During the Late Steps of Spermiogenesis. Biol Reprod *82*, 669-678.

Lachkar, S., Lebois, M., Steinmetz, M.O., Guichet, A., Lal, N., Curmi, P.A., Sobel, A., and Ozon, S. (2010). Drosophila stathmins bind tubulin heterodimers with high and variable stoichiometries. J Biol Chem *285*, 11667-11680.

Larsson, H., Wallin, M., and Edstrom, A. (1976). Induction of a sheet polymer of tubulin by Zn2+. Exp Cell Res *100*, 104-110.

Larsson, N., Marklund, U., Gradin, H.M., Brattsand, G., and Gullberg, M. (1997). Control of microtubule dynamics by oncoprotein 18: dissection of the regulatory role of multisite phosphorylation during mitosis. Mol Cell Biol *17*, 5530-5539.

Larsson, N., Melander, H., Marklund, U., Osterman, O., and Gullberg, M. (1995). G2/M transition requires multisite phosphorylation of oncoprotein 18 by two distinct protein kinase systems. J Biol Chem *270*, 14175-14183.

Leipe, D.D., Wolf, Y.I., Koonin, E.V., and Aravind, L. (2002). Classification and evolution of P-loop GTPases and related ATPases. J Mol Biol *317*, 41-72.

Lewis, S.A., Tian, G., Vainberg, I.E., and Cowan, N.J. (1996). Chaperonin-mediated folding of actin and tubulin. J Cell Biol *132*, 1-4.

Lindemann, C.B., and Lesich, K.A. (2010). Flagellar and ciliary beating: the proven and the possible. J Cell Sci *123*, 519-528.

Lippincott, J., and Li, R. (1998). Sequential assembly of myosin II, an IQGAP-like protein, and filamentous actin to a ring structure involved in budding yeast cytokinesis. J Cell Biol *140*, 355-366.

Longtine, M.S., DeMarini, D.J., Valencik, M.L., Al-Awar, O.S., Fares, H., De Virgilio, C., and Pringle, J.R. (1996). The septins: roles in cytokinesis and other processes. Curr Opin Cell Biol *8*, 106-119.

Lopez-Fanarraga, M., Avila, J., Guasch, A., Coll, M., and Zabala, J.C. (2001). Review: postchaperonin tubulin folding cofactors and their role in microtubule dynamics. J Struct Biol *135*, 219-229.

Luduena, R.F. (1998). Multiple forms of tubulin: different gene products and covalent modifications. Int Rev Cytol *178*, 207-275.

Lukoyanova, N., Baldwin, S.A., and Trinick, J. (2008). 3D reconstruction of mammalian septin filaments. J Mol Biol *376*, 1-7.

Lundin, V.F., Leroux, M.R., and Stirling, P.C. (2010). Quality control of cytoskeletal proteins and human disease. Trends Biochem Sci *35*, 288-297.

Macara, I.G., Baldarelli, R., Field, C.M., Glotzer, M., Hayashi, Y., Hsu, S.C., Kennedy, M.B., Kinoshita, M., Longtine, M., Low, C.*, et al.* (2002). Mammalian septins nomenclature. Mol Biol Cell *13*, 4111-4113.

Mandelkow, E., and Mandelkow, E.M. (1995). Microtubules and microtubule-associated proteins. Curr Opin Cell Biol *7*, 72-81.

Marklund, U., Brattsand, G., Shingler, V., and Gullberg, M. (1993). Serine 25 of oncoprotein 18 is a major cytosolic target for the mitogen-activated protein kinase. J Biol Chem *268*, 15039-15047.

Marklund, U., Larsson, N., Brattsand, G., Osterman, O., Chatila, T.A., and Gullberg, M. (1994a). Serine 16 of oncoprotein 18 is a major cytosolic target for the Ca2+/calmodulin-dependent kinase-Gr. Eur J Biochem *225*, 53-60.

Marklund, U., Larsson, N., Gradin, H.M., Brattsand, G., and Gullberg, M. (1996). Oncoprotein 18 is a phosphorylation-responsive regulator of microtubule dynamics. EMBO J *15*, 5290-5298.

Marklund, U., Osterman, O., Melander, H., Bergh, A., and Gullberg, M. (1994b). The phenotype of a "Cdc2 kinase target site-deficient" mutant of oncoprotein 18 reveals a role of this protein in cell cycle control. J Biol Chem *269*, 30626-30635.

Martin, L., Fanarraga, M.L., Aloria, K., and Zabala, J.C. (2000). Tubulin folding cofactor D is a microtubule destabilizing protein. Febs Letters *470*, 93-95.

Martinez, C., Corral, J., Dent, J.A., Sesma, L., Vicente, V., and Ware, J. (2006). Platelet septin complexes form rings and associate with the microtubular network. J Thromb Haemost *4*, 1388-1395.

McIlhatton, M.A., Burrows, J.F., Donaghy, P.G., Chanduloy, S., Johnston, P.G., and Russell, S.E. (2001). Genomic organization, complex splicing pattern and expression of a human septin gene on chromosome 17q25.3. Oncogene *20*, 5930-5939.

McMurray, M.A., Bertin, A., Garcia, G., 3rd, Lam, L., Nogales, E., and Thorner, J. (2011). Septin filament formation is essential in budding yeast. Dev Cell *20*, 540-549.

McMurray, M.A., and Thorner, J. (2008). Septin stability and recycling during dynamic structural transitions in cell division and development. Curr Biol *18*, 1203-1208.

McMurray, M.A., and Thorner, J. (2009a). Reuse, replace, recycle. Specificity in subunit inheritance and assembly of higher-order septin structures during mitotic and meiotic division in budding yeast. Cell Cycle *8*, 195-203.

McMurray, M.A., and Thorner, J. (2009b). Septins: molecular partitioning and the generation of cellular asymmetry. Cell Div *4*, 18.

McNally, F.J., and Vale, R.D. (1993). Identification of Katanin, an Atpase That Severs and Disassembles Stable Microtubules. Cell *75*, 419-429.

Mendoza, M., Hyman, A.A., and Glotzer, M. (2002). GTP binding induces filament assembly of a recombinant septin. Curr Biol *12*, 1858-1863.

Mitchison, T., and Kirschner, M. (1984). Dynamic instability of microtubule growth. Nature *312*, 237-242.

Momany, M., Zhao, J., Lindsey, R., and Westfall, P.J. (2001). Characterization of the Aspergillus nidulans septin (asp) gene family. Genetics *157*, 969-977.

Mostowy, S., Bonazzi, M., Hamon, M.A., Tham, T.N., Mallet, A., Lelek, M., Gouin, E., Demangel, C., Brosch, R., Zimmer, C.*, et al.* (2010). Entrapment of intracytosolic bacteria by septin cage-like structures. Cell Host Microbe *8*, 433-444.

Mostowy, S., Tham, T.N., Danckaert, A., Guadagnini, S., Boisson-Dupuis, S., Pizarro-Cerda, J., and Cossart, P. (2009). Septins Regulate Bacterial Entry into Host Cells. PLoS One *4*.

Nagata, K., Asano, T., Nozawa, Y., and Inagaki, M. (2004). Biochemical and cell biological analyses of a mammalian septin complex, Sept7/9b/11. J Biol Chem *279*, 55895-55904.

Nagata, K., Kawajiri, A., Matsui, S., Takagishi, M., Shiromizu, T., Saitoh, N., Izawa, I., Kiyono, T., Itoh, T.J., Hotani, H.*, et al.* (2003). Filament formation of MSF-A, a mammalian septin, in human mammary epithelial cells depends on interactions with microtubules. J Biol Chem *278*, 18538-18543.

Nakahira, M., Macedo, J.N., Seraphim, T.V., Cavalcante, N., Souza, T.A., Damalio, J.C., Reyes, L.F., Assmann, E.M., Alborghetti, M.R., Garratt, R.C.*, et al.* (2010). A draft of the human septin interactome. PLoS One *5*, e13799.

Nakatsuru, S., Sudo, K., and Nakamura, Y. (1994). Molecular cloning of a novel human cDNA homologous to CDC10 in Saccharomyces cerevisiae. Biochem Biophys Res Commun *202*, 82-87.

Neufeld, T.P., and Rubin, G.M. (1994). The Drosophila Peanut Gene Is Required for Cytokinesis and Encodes a Protein Similar to Yeast Putative Bud Neck Filament Proteins. Cell *77*, 371-379.

Nguyen, T.Q., Sawa, H., Okano, H., and White, J.G. (2000). The C-elegans septin genes, unc-59 and unc-61, are required for normal postembryonic cytokineses and morphogenesis but have no essential function in embryogenesis. Journal of Cell Science *113*, 3825-3837.

Nogales, E., and Wang, H.W. (2006). Structural intermediates in microtubule assembly and disassembly: how and why? Curr Opin Cell Biol *18*, 179-184.

Nogales, E., Wolf, S.G., and Downing, K.H. (1998). Structure of the alpha beta tubulin dimer by electron crystallography. Nature *391*, 199-203.

Nogales, E., Wolf, S.G., Khan, I.A., Luduena, R.F., and Downing, K.H. (1995). Structure of tubulin at 6.5 A and location of the taxol-binding site. Nature *375*, 424-427.

Nottenburg, C., Gallatin, W.M., and St John, T. (1990). Lymphocyte HEV adhesion variants differ in the expression of multiple gene sequences. Gene *95*, 279-284.

Oakley, B.R., Oakley, C.E., Yoon, Y.S., and Jung, M.K. (1990). Gamma-Tubulin Is a Component of the Spindle Pole Body That Is Essential for Microtubule Function in Aspergillus-Nidulans. Cell *61*, 1289-1301.

Oh, Y., and Bi, E. (2011). Septin structure and function in yeast and beyond. Trends Cell Biol *21*, 141-148.

Omary, M.B., Coulombe, P.A., and McLean, W.H.I. (2004). Mechanisms of disease: Intermediate filament proteins and their associated diseases. New Engl J Med *351*, 2087-2100.

Ookata, K., Hisanaga, S., Sugita, M., Okuyama, A., Murofushi, H., Kitazawa, H., Chari, S., Bulinski, J.C., and Kishimoto, T. (1997). MAP4 is the in vivo substrate for CDC2 kinase in HeLa cells: identification of an M-phase specific and a cell cycle-independent phosphorylation site in MAP4. Biochemistry *36*, 15873-15883.

Ozon, S., Guichet, A., Gavet, O., Roth, S., and Sobel, A. (2002). Drosophila stathmin: a microtubule-destabilizing factor involved in nervous system formation. Mol Biol Cell *13*, 698-710.

Pablo-Hernando, M.E., Arnaiz-Pita, Y., Tachikawa, H., del Rey, F., Neiman, A.M., and Vazquez de Aldana, C.R. (2008). Septins localize to microtubules during nutritional limitation in Saccharomyces cerevisiae. BMC Cell Biol *9*, 55.

Pachter, J.S., Yen, T.J., and Cleveland, D.W. (1987). Autoregulation of tubulin expression is achieved through specific degradation of polysomal tubulin mRNAs. Cell *51*, 283-292.

Pan, F., Malmberg, R.L., and Momany, M. (2007). Analysis of septins across kingdoms reveals orthology and new motifs. BMC Evol Biol *7*, 103.

Paweletz, N. (2001). Walther Flemming: pioneer of mitosis research. Nat Rev Mol Cell Biol *2*, 72-75.

Peterson, E.A., and Petty, E.M. (2010). Conquering the complex world of human septins: implications for health and disease. Clin Genet *77*, 511-524.

Prasad, A.R.S., Luduena, R.F., and Horowitz, P.M. (1986). Bis(8-Anilinonaphthalene-1-Sulfonate) as a Probe for Tubulin Decay. Biochemistry *25*, 739-742.

Ridley, A.J. (2006). Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. Trends Cell Biol *16*, 522-529.

Rieder, C. L. (2011). Mitosis in vertebrates: the G2/M and M/A transitions and their associated checkpoints. Chromosome Res *19*, 291-306.

Riedl, J., Crevenna, A.H., Kessenbrock, K., Yu, J.H., Neukirchen, D., Bista, M., Bradke, F., Jenne, D., Holak, T.A., Werb, Z*., et al.* (2008). Lifeact: a versatile marker to visualize F-actin. Nat Methods *5*, 605-607.

Ringhoff, D.N., and Cassimeris, L. (2009). Stathmin regulates centrosomal nucleation of microtubules and tubulin dimer/polymer partitioning Mol Biol Cell *(in press)*.

Russell, S.E., and Hall, P.A. (2011). Septin genomics: a road less travelled. Biol Chem *392*, 763-767.

Sakamoto, T., Uezu, A., Kawauchi, S., Kuramoto, T., Makino, K., Umeda, K., Araki, N., Baba, H., and Nakanishi, H. (2008). Mass spectrometric analysis of microtubule co-sedimented proteins from rat brain. Genes Cells *13*, 295-312.

Sanders, S.L., and Field, C.M. (1994). Cell division. Septins in common? Curr Biol *4*, 907-910.

Sandrock, K., Bartsch, I., Blaser, S., Busse, A., Busse, E., and Zieger, B. (2011). Characterization of human septin interactions. Biol Chem *392*, 751-761.

Saraste, M., Sibbald, P.R., and Wittinghofer, A. (1990). The P-loop--a common motif in ATP- and GTP-binding proteins. Trends Biochem Sci *15*, 430-434.

Schleiden, M.J. (1838). Beiträge zur Phytogenesis. Arch Anat Physiol Wiss Med 136-176.

Schmidt, K., and Nichols, B.J. (2004a). A barrier to lateral diffusion in the cleavage furrow of dividing mammalian cells. Curr Biol *14*, 1002-1006.

Schmidt, K., and Nichols, B.J. (2004b). Functional interdependence between septin and actin cytoskeleton. BMC Cell Biol *5*, 43.

Schwann, T., ed. (1839). Mikroskopische untersuchungen über die uebereinstimmung in der struktur und dem wachstum der thiere und pflanzen (Berlin, Sanderschen Buchhandlung).

Sellin, M.E., Holmfeldt, P., Stenmark, S., and Gullberg, M. (2008a). Global Regulation of the Interphase Microtubule System by Abundantly Expressed Op18/Stathmin. Mol Biol Cell *19*, 2897-2906.

Sellin, M.E., Holmfeldt, P., Stenmark, S., and Gullberg, M. (2008b). Op18/Stathmin counteracts the activity of overexpressed tubulin-disrupting proteins in a human leukemia cell line. Exp Cell Res *314*, 1367-1377.

Sellin, M.E., Sandblad, L., Stenmark, S., and Gullberg, M. (2011). Deciphering the rules governing assembly order of mammalian septin complexes. Mol Biol Cell *22*, 3152-3164.

Sheffield, P.J., Oliver, C.J., Kremer, B.E., Sheng, S., Shao, Z., and Macara, I.G. (2003). Borg/septin interactions and the assembly of mammalian septin heterodimers, trimers, and filaments. J Biol Chem *278*, 3483-3488.

Siegel, L.M., and Monty, K.J. (1966). Determination of molecular weights and frictional ratios of proteins in impure systems by use of gel filtration and density gradient centrifugation. Application to crude preparations of sulfite and hydroxylamine reductases. Biochim Biophys Acta *112*, 346-362.

Silverman-Gavrila, R.V., and Silverman-Gavrila, L.B. (2008). Septins: new microtubule interacting partners. ScientificWorldJournal *8*, 611-620.

Sirajuddin, M., Farkasovsky, M., Hauer, F., Kuhlmann, D., Macara, I.G., Weyand, M., Stark, H., and Wittinghofer, A. (2007). Structural insight into filament formation by mammalian septins. Nature *449*, 311-315.

Sirajuddin, M., Farkasovsky, M., Zent, E., and Wittinghofer, A. (2009). GTP-induced conformational changes in septins and implications for function. Proc Natl Acad Sci U S A *106*, 16592-16597.

Sisson, J.C., Field, C., Ventura, R., Royou, A., and Sullivan, W. (2000). Lava lamp, a novel peripheral Golgi protein, is required for Drosophila melanogaster cellularization. Journal of Cell Biology *151*, 905-917.

Small, J.V., Rottner, K., and Kaverina, I. (1999). Functional design in the actin cytoskeleton. Curr Opin Cell Biol *11*, 54-60.

Sobel, A., and Tashjian, A.H., Jr. (1983). Distinct patterns of cytoplasmic protein phosphorylation related to regulation of synthesis and release of prolactin by GH cells. J Biol Chem *258*, 10312-10324.

Spiliotis, E.T. (2010). Regulation of microtubule organization and functions by septin GTPases. Cytoskeleton (Hoboken) *67*, 339-345.

Spiliotis, E.T., Hunt, S.J., Hu, Q., Kinoshita, M., and Nelson, W.J. (2008). Epithelial polarity requires septin coupling of vesicle transport to polyglutamylated microtubules. J Cell Biol *180*, 295-303.

Spiliotis, E.T., Kinoshita, M., and Nelson, W.J. (2005). A mitotic septin scaffold required for Mammalian chromosome congression and segregation. Science *307*, 1781-1785.

Steels, J.D., Estey, M.R., Froese, C.D., Reynaud, D., Pace-Asciak, C., and Trimble, W.S. (2007). Sept12 is a component of the mammalian sperm tail annulus. Cell Motil Cytoskel *64*, 794-807.

Steinmetz, M.O., Kammerer, R.A., Jahnke, W., Goldie, K.N., Lustig, A., and van Oostrum, J. (2000). Op18/stathmin caps a kinked protofilament-like tubulin tetramer. EMBO J *19*, 572-580.

Surka, M.C., Tsang, C.W., and Trimble, W.S. (2002). The mammalian septin MSF localizes with microtubules and is required for completion of cytokinesis. Mol Biol Cell *13*, 3532-3545.

Szymanski, D. (2002). Tubulin folding cofactors: half a dozen for a dimer. Curr Biol *12*, R767-769.

Tada, T., Simonetta, A., Batterton, M., Kinoshita, M., Edbauer, D., and Sheng, M. (2007). Role of Septin cytoskeleton in spine morphogenesis and dendrite development in neurons. Curr Biol *17*, 1752-1758.

Takizawa, P.A., DeRisi, J.L., Wilhelm, J.E., and Vale, R.D. (2000). Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. Science *290*, 341-344.

Tanaka-Takiguchi, Y., Kinoshita, M., and Takiguchi, K. (2009). Septin-mediated uniform bracing of phospholipid membranes. Curr Biol *19*, 140-145.

Theodorakis, N.G., and Cleveland, D.W. (1992). Physical evidence for cotranslational regulation of beta-tubulin mRNA degradation. Mol Cell Biol *12*, 791-799.

Thorslund, S.E., Edgren, T., Pettersson, J., Nordfelth, R., Sellin, M.E., Ivanova, E., Francis, M.S., Isaksson, E.L., Wolf-Watz, H., and Fallman, M. (2011). The RACK1 signaling scaffold protein selectively interacts with Yersinia pseudotuberculosis virulence function. PLoS One *6*, e16784.

Tian, G., Bhamidipati, A., Cowan, N.J., and Lewis, S.A. (1999). Tubulin folding cofactors as GTPase-activating proteins. GTP hydrolysis and the assembly of the alpha/beta-tubulin heterodimer. J Biol Chem *274*, 24054-24058.

Tian, G., Huang, Y., Rommelaere, H., Vandekerckhove, J., Ampe, C., and Cowan, N.J. (1996). Pathway leading to correctly folded beta-tubulin. Cell *86*, 287-296.

Tooley, A.J., Gilden, J., Jacobelli, J., Beemiller, P., Trimble, W.S., Kinoshita, M., and Krummel, M.F. (2009). Amoeboid T lymphocytes require the septin cytoskeleton for cortical integrity and persistent motility. Nat Cell Biol *11*, 17-26.

Tournebize, R., Popov, A., Kinoshita, K., Ashford, A.J., Rybina, S., Pozniakovsky, A., Mayer, T.U., Walczak, C.E., Karsenti, E., and Hyman, A.A. (2000). Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in Xenopus egg extracts. Nat Cell Biol *2*, 13-19.

Vainberg, I.E., Lewis, S.A., Rommelaere, H., Ampe, C., Vandekerckhove, J., Klein, H.L., and Cowan, N.J. (1998). Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin. Cell *93*, 863-873.

Vale, R.D. (2003). The molecular motor toolbox for intracellular transport. Cell *112*, 467-480.

Wang, Y., Tian, G., Cowan, N.J., and Cabral, F. (2006). Mutations affecting beta-tubulin folding and degradation. J Biol Chem *281*, 13628-13635.

Weirich, C.S., Erzberger, J.P., and Barral, Y. (2008). The septin family of GTPases: architecture and dynamics. Nat Rev Mol Cell Biol *9*, 478-489.

Weisenberg, R.C., Borisy, G.G., and Taylor, E.W. (1968). The colchicine-binding protein of mammalian brain and its relation to microtubules. Biochemistry *7*, 4466-4479.

Versele, M., Gullbrand, B., Shulewitz, M.J., Cid, V.J., Bahmanyar, S., Chen, R.E., Barth, P., Alber, T., and Thorner, J. (2004). Protein-protein interactions governing septin heteropentamer assembly and septin filament organization in Saccharomyces cerevisiae. Mol Biol Cell *15*, 4568-4583.

Wittinghofer, A., and Vetter, I.R. (2011). Structure-function relationships of the G domain, a canonical switch motif. Annu Rev Biochem *80*, 943-971.

Wloga, D., and Gaertig, J. (2010). Post-translational modifications of microtubules. J Cell Sci *123*, 3447-3455.

Vrabioiu, A.M., Gerber, S.A., Gygi, S.P., Field, C.M., and Mitchison, T.J. (2004). The majority of the Saccharomyces cerevisiae septin complexes do not exchange guanine nucleotides. J Biol Chem *279*, 3111-3118.

Vrabioiu, A.M., and Mitchison, T.J. (2006). Structural insights into yeast septin organization from polarized fluorescence microscopy. Nature *443*, 466-469.

Xie, Y., Vessey, J.P., Konecna, A., Dahm, R., Macchi, P., and Kiebler, M.A. (2007). The GTP-binding protein Septin 7 is critical for dendrite branching and dendritic-spine morphology. Curr Biol *17*, 1746-1751.

Yen, T.J., Gay, D.A., Pachter, J.S., and Cleveland, D.W. (1988). Autoregulated changes in stability of polyribosome-bound beta-tubulin mRNAs are specified by the first 13 translated nucleotides. Mol Cell Biol *8*, 1224-1235.

Zhang, J., Kong, C., Xie, H., McPherson, P.S., Grinstein, S., and Trimble, W.S. (1999). Phosphatidylinositol polyphosphate binding to the mammalian septin H5 is modulated by GTP. Curr Biol *9*, 1458-1467.

Zheng, Y., Wong, M.L., Alberts, B., and Mitchison, T. (1995). Nucleation of microtubule assembly by a gamma-tubulin-containing ring complex. Nature *378*, 578-583.

Zhu, M., Wang, F., Yan, F., Yao, P.Y., Du, J., Gao, X., Wang, X., Wu, Q., Ward, T., Li, J*., et al.* (2008). Septin 7 interacts with centromere-associated protein E and is required for its kinetochore localization. J Biol Chem *283*, 18916-18925.