SYMPOSIUM

Mammalian Septin Function in Hemostasis and Beyond

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Interest in the biology of mammalian septin proteins has undergone a birth in recent years. Originally identified as critical for yeast budding throughout the 1970s, the septin family is now recognized to extend from yeast to humans and is associated with a variety of events ranging from cytokinesis to vesicle trafficking. An emerging theme for septins is their presence at sites where active membrane or cytoplasmic partitioning is occurring. Here, we briefly review the mammalian septin protein family and focus on a prototypic human and mouse septin, termed SEPT5, that is expressed in the brain, heart, and megakaryocytes. Work from neurobiology laboratories has linked SEPT5 to the exocytic complex of neurons, with implications that SEPT5 regulates neurotransmitter release. Striking similarities exist between neurotransmitter release and the platelet-release reaction, which is a critical step in platelet response to vascular injury. Work from our laboratory has characterized the platelet phenotype from mice containing a targeted deletion of SEPT5. Most strikingly, platelets from $SEPT5^{null}$ animals aggregate and release granular contents in response to subthreshold levels of agonists. Thus, the characterization of a SEPT5-deficient mouse has linked SEPT5 to the platelet exocytic process and, as such, illustrates it as an important protein for regulating platelet function. Recent data suggest that platelets contain a wide repertoire of different septin proteins and assemble to form macromolecular septin complexes. The mouse platelet provides an experimental framework to define septin function in hemostasis, with implications for neurobiology and beyond. Exp Biol Med 229:1111–1119, 2004

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The Septins of Yeast

Septins are a family of cytoplasmic proteins originally discovered in budding yeast *(Saccharomyces cerevisiae)* as essential proteins of cytokinesis (1). The term *septin* was chosen after it was observed that four related gene products localize to the division plane in budding yeast and, if mutated, block the budding process (Fig. 1). There was speculation that septin proteins were actually yeast neck filaments, which have been long recognized as necessary for budding, because purified septins polymerize *in vitro* and appear filamentous (2). However, the molecular mechanism whereby septins contribute to the yeast-budding process has remained elusive. Septins also play a major role in septation/cytokinesis events in the fission yeast *Schizosaccharomyces pombe*, although they are dispensable for cell viability. To date, seven septins have been characterized in *S. pombe* (four are found in the division plane and three participate in the sporulation of meiotic cells; Ref. 3).

Recent studies suggest that yeast septins contribute to the cytoskeletal architecture by creating diffusion barriers within the dividing cytoplasm (2, 4–6). In addition to this function, septins are related to many other important yeast division processes such as bud-site selection, chitin deposition, and spindle orientation (7, 8). Thus, partitioning the cytoplasm during the growth of the developing bud becomes a controlled, and critical, process in the yeast life cycle.

Septin Genes in Higher Eukaryotic Cells

The presence of septins in higher eukaryotic cells was dismissed for a number of years because asymmetric cell division represents a process that is unique to yeast (9). However, this changed in 1994 when Neufeld and Rubin

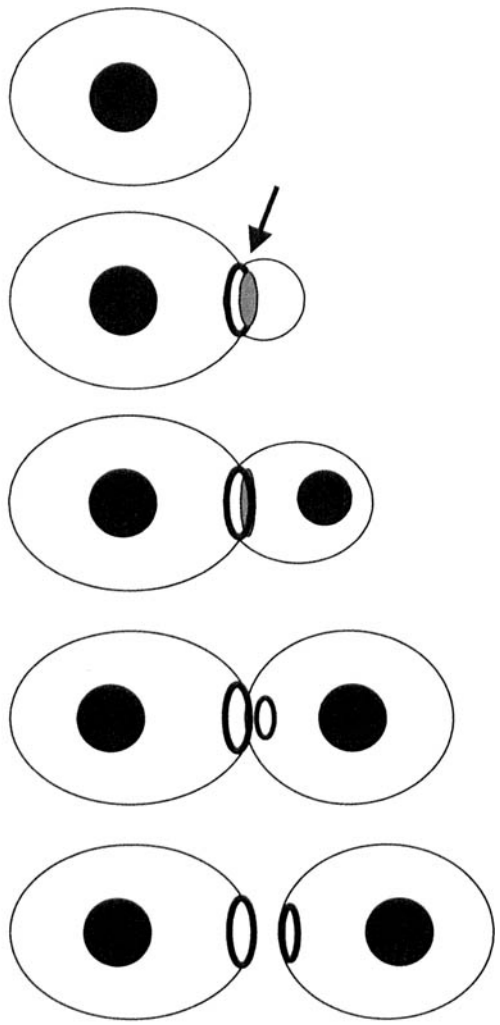

Figure 1. Yeast budding. A septin ring forms near the cleavage plane between budding mother and daughter yeast cells (arrow). The formation of a septin ring signifies the commitment of the cell to undergo asymmetric cell division with the cleavage plane identified by the location of the septin ring. Ultimately, the septin ring separates and remains after the completion of the division marking the membrane region where cell division has occurred.

(10) identified a *Drosophila* protein, designated PNUT, that was localized to the cleavage furrow in dividing cells and displayed a sequence similar to yeast septins. Mutations in pnut blocked cytokinesis and resulted in embryonic lethality. Gene databases have expanded since the characterization of pnut, and it is clear that a septin family, though missing in plants, is found in most, if not all, animal cells (9). Some investigators have speculated, on the basis of similarities between mammalian septins and their yeast counterparts, that these septins form a scaffolding matrix on which other proteins assemble to perform unique jobs for particular cell types (11).

In organisms with relatively complete genetic databases (e.g., mice, humans), 12 septin genes have been identified (12) and unifying nomenclature has been adopted (Table 1; Ref. 13). An analysis of expressed sequence tags, along with data from an increasing number of reports, shows a common practice of alternative 5′ mRNA processing in mammalian septin genes (14–19). Thus, each single gene can express multiple isoforms, a characteristic that expands the functional diversity of septin proteins, although this possibility is just beginning to be explored (18).

Our interest in septins was initiated by characterizing the human locus containing the platelet receptor glycoprotein Ibβ gene (14). We identified an uncharacterized gene residing 250 nucleotides 5′ to the platelet glycoprotein Ibβ gene. This gene displayed a striking sequence similarity to the *Drosophila Pnut* gene. We designated the gene *cell division control related-1* (CDC*rel*-1) in accordance with its sequence similarity to yeast septins. We would later realize that this nomenclature was less than perfect because we observed the highest levels of CDC*rel*-1 mRNA in cells (i.e., neurons, megakaryocytes) that were no longer undergoing active cytokinesis (20). Nevertheless, the identification and chromosome localization of CDC*rel*-1 (chromosome 22q11.2) led to our identification of a second human septin, CDC*rel*-2, which has a high degree of sequence similarity to CDC*rel*-1 and resides on human

Table 1. Mammalian Septin Nomenclature[a]

Adopted septin nomenclature		
Mouse (*gene*/protein)	Human (*gene*/protein)	Alternative names
Sept1/SEPT1	*SEPT1*/SEPT1	Diff6, Pnutl3
Sept2/SEPT2	*SEPT2*/SEPT2	Nedd5, mKIAA0158
Sept3/SEPT3_v1–2	*SEPT3*/SEPT3_v1–3	Sep3, B530002E20Rik, SEP3, bK250D10.3
Sept4/SEPT4_v1–6	*SEPT4*/SEPT4_v1–6	Bh5, PNUTL2, H5, ARTS, Bradeion, SEPT4, hcdc*rel*-2
Sept5/SEPT5	*SEPT5*/SEPT5	PNUTL1, CDC*rel*-1, hcdc*rel*-1
Sept6/SEPT6	*SEPT6*/SEPT6_v1–6	Sep6, 2810035H17Rik, SEP2, KIAA0128
Sept7/SEPT7	*SEPT7*/SEPT7	Cdc10, E430034N22, Hcdc10
Sept8/SEPT8	*SEPT8*/SEPT8	Sepl, mKIAA0202, SEP2, KIAA0202
Sept9/SEPT9_v1–5	*SEPT9*/SEPT9_v1–5	Msf, MSF1, Sint1, PNUTL4, MSF1, SINT1, PNUTL4, AF17q25, KIAA0991
Sept10/SEPT10	*SEPT10*/SEPT10_v1–2	FLJ11619
Sept11/SEPT11	*SEPT11*/SEPT11	FLJ10849
Sept12/SEPT12	*SEPT12*/SEPT12	4933413b09Rik, FLJ25410

[a] Adapted from Ref. 13. ARTS, apoptotic-related protein in the TGF-β signaling; CDC*rel*-1, cell division control related-1.

chromosome 17 (18). This characterization demonstrates that human septin genes, like their yeast counterparts, are a family of related gene sequences with differing tissue-specific expression patterns. A standardized nomenclature for mammalian septin genes and proteins has recently been adopted, with CDC*rel*-1 and CDC*rel*-2 now referred to as SEPT5 and SEPT4, respectively (13). Prior to the standardization of the nomenclature, alternative names for SEPT4 and SEPT5 included H5, CDC*rel*-1, CDC*rel*-2, PNUTL1, PNUTL2, and apoptotic-related protein in the TGF-β signaling pathway (ARTS; Table 1).

Mammalian Septin Structure

The primary structure of most mammalian septins predicts an M_r range of 40,000 to 60,000. The most striking feature of the primary sequence is a central core domain (~300 amino acids) sharing a minimum of 35% sequence similarity to yeast septins (Fig. 2). Septins of a single species display a minimum of 75% sequence identity within their central core domains (21). The sequence similarity among central core domains is contrasted by the NH_2 termini, which are unique to each septin and presumably contribute to the functional specificity of the protein. As mentioned, alternative processing at the 5′ ends of septin genes may also contribute to functional specificity with translation of cell-specific NH_2 termini. The most common example of this is the COOH-terminal domain, which displays features of a coiled-coil structure, suggesting some involvement in protein-protein interaction.

Most septins possess a P-loop motif, a characteristic of GTP-/ATP-binding proteins, in their conserved central domain (22). GTP binding and GTPase activity have been demonstrated *in vitro* for some septins and, as such, the septins represent a novel group of GTPases that are distinct from the small *ras*-like GTPases or any of the other known GTPase proteins (2, 11). In addition, some septins contain a conserved polybasic region that binds phosphatidylinositol 4,5 biphosphate [PtdIns(4,5)P_2] (23). The binding of PtdIns(4,5)P_2 and GTP to septins is mutually exclusive, which implies that the binding of PtdIns(4,5)P_2 at a membrane surface only occurs in the GDP-bound conformation, whereas the GTP-bound form is prevented from localizing to a plasma membrane (23). The presence of guanine nucleotide and PtdIns(4,5)P_2-binding motifs provides septins with structural features that are capable of regulating function.

Mammalian septins can be found *in vivo* as hetero-oligomeric complexes assembled from different members of the septin family, yet little is known about how these filaments assemble or how their assembly is spatially and temporally regulated. Septin heterotypic assembly has been documented among different species where three to four different septin polypeptides associate (10, 24, 25) and *in vitro* where heterotypic septin complexes polymerize into filaments (2, 26). A recent report by Blaser *et al.* (27) shows

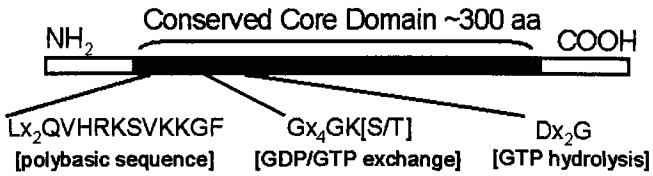

Figure 2. Mammalian septin domain organization. Mammalian septins range from 350 to 500 amino acids in length. Most septins contain a conserved central core domain of ~300 residues. Within the core conserved domain is a polybasic sequence proposed to interact with phosphatidylinositol 4,5 biphosphate [PtdIns(4,5)P_2] and sequences comprising a P-loop motif characteristic of GTP-/ATP-binding proteins. (Reprinted with permission from Ref. 39).

that human septin SEPT5 binds to septin SEPT8 (KIAA0202) with a remarkably high affinity. Our results demonstrate that the heterotypic interaction between SEPT5 and SEPT8 is a prerequisite for targeting both proteins to a vesicle location in the cytosol (28). Indeed, SEPT5 expression in the absence of SEPT8 results in SEPT5 having a diffuse localization throughout the cytoplasm of transfected cells (Fig. 3). However, a concomitant expression of both SEPT5 and SEPT8 causes a redistribution and colocalization of both proteins to a vesicular location in the cytoplasm (Fig. 3). Septin complexes immunoprecipitated from mouse brain contain at least eight different septin monomers, but the exact composition of a single complex has yet to be defined (29). Thus, understanding how the formation of septin macromolecular complexes is temporally regulated during human septin assembly becomes an important step in understanding septin function.

Based on sequence similarity, mammalian septins can be classified into four groups (21) or, by phylogeny, into four closely related subfamilies (Fig. 4; Refs. 12, 30). Data suggest that septins from one group will interact preferentially with members of a different group. Multiple reports have shown that SEPT9 (Group 1), SEPT6 (Group 2), SEPT2 (Group 3), and SEPT7 (Group 4) are co-immunoprecipitated from NIH3T3 cell lysates (25) or HeLa cell lysates (29, 31). Sheffield *et al.* (32) have further studied the interaction between SEPT2, SEPT6, and SEPT7, and their study reports that these three septins form a heterotrimer or, if taken in pairs, heterodimers. In *Drosophila*, four septins have been described: Pnut, Sep1, Sep2, and Sep5. *Drosophila* Sep2 and Sep5 share sequence similarities with members of the mammalian Group 2, Sep1 shares similarities with Group 3, and Pnut shares similarities with Group 4 (21). Sep1, Sep2, and Pnut colocalize at the sites of embryo cellularization. Future studies should be directed at an understanding of how septin expression is regulated and how the loss of expression of a single septin can be compensated by the overexpression, or underexpression, of a different septin (33). Perhaps septin loss in a specific cell type can be counterbalanced by a different septin from the same group. Indeed, additional studies are needed to understand if this compensatory process occurs for all septins or if it is unique to a few situations.

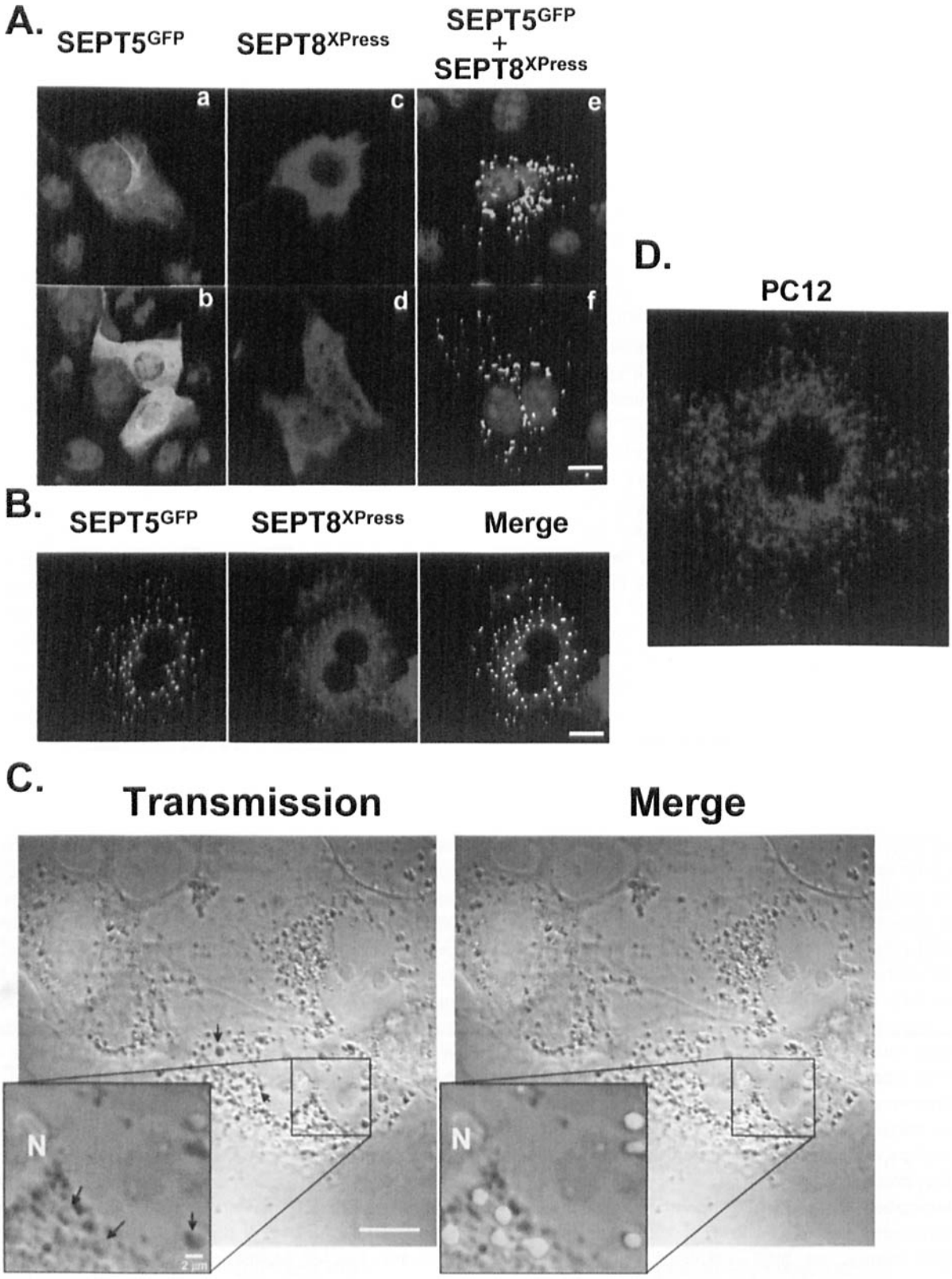

Figure 3. SEPT5 and SEPT8 organized into macromolecular complexes around cytosolic vesicle-like organelles. (A) COS-7 cells were transfected with *SEPT5*/pEGFP-N1 and/or *SEPT8*/pcDNA3.1-His cDNA. Transfection of a SEPT5 cDNA alone (a and b) revealed a diffuse cytoplasmic location of SEPT5 protein as detected by a GFP tag. In these images, nuclei were stained with propidium iodide (red). Transfection of a SEPT8 cDNA alone (c and d) revealed a similar diffuse cytoplasmic location as detected by the presence of a C-terminal Xpress epitope and immunofluorescence produced by anti-Xpress antibodies (red). COS-7 cells were cotransfected with both *SEPT5*/pEGFP-N1 and *SEPT8*/pcDNA3.1-His cDNA, nuclei were stained with propidium iodide, and SEPT5 was detected by green fluorescence (e and f). (B) COS-7 cells

Mammalian Septin Function

The characterization of the *Drosophila* PNUT protein was followed shortly thereafter by the characterization of a mouse septin, SEPT2 (also referred to as Nedd5; Ref. 34). SEPT2 is widely expressed and concentrates at the cleavage furrow of dividing cells. Mutations or antibodies that prevent SEPT2 from binding GTP disrupts the fibrous appearance of SEPT2 and prevents cytokinesis, resulting in the accumulation of polyploid cells.

Although the functional characterizations of PNUT and SEPT2 reveal an interesting parallel to their yeast homologs, it is becoming clear that septin function in mammalian cells goes well beyond participation in cytokinesis (22, 35). As previously mentioned, the human septin SEPT5 is the most abundant in cells that are not undergoing cell division. Several reports provide insights into the functions of SEPT4 and SEPT5 proteins. For example, Hsu *et al.* (36) purified protein(s) with the SEPT4 and/or SEPT5 sequences as part of the brain sec6/sec8 complex, which is a critical complex for neurotransmitter release. Whether the identity of the protein was SEPT4, SEPT5, or both, was impossible to discern because the peptide sequence obtained from the purified protein was from the protein's central core domain, a region where SEPT4 and SEPT5 are almost identical. Shortly thereafter, Caltagarone *et al.* (37) cofractionated SEPT5 with SNAP25-labeled membranes and synaptophysin-marked synaptic vesicles from neurons. The link to neurotransmitter release became stronger when Beites *et al.* (38) demonstrated a direct interaction between SEPT5 and the protein syntaxin. Moreover, Beites *et al.* (38) suggest that the interaction of SEPT5 with syntaxins might inhibit exocytosis in neurons and might be a regulatory step in the control of neurotransmitter release (38).

Work from our laboratory characterized the platelet phenotype in a SEPT5^{null} animal (39). This work provided physiologic evidence linking SEPT5 to the platelet exocytic process and suggested that SEPT5 is a negative regulator of the platelet-secretion response. Normal platelet function requires the ability of platelets to secrete effector molecules from storage granules (40). The physiologic relevance of platelet secretion is best exemplified in humans with congenital-release problems that result in a bleeding phenotype (41). Platelets contain three types of storage granules: the unique α-granules, dense (or δ) granules, and lysosomal granules. The coordinated release of storage-granule components supports hemostasis, thrombosis, and tissue repair at the site of vascular injury. Soluble adhesion molecules, protease inhibitors, and growth factors are

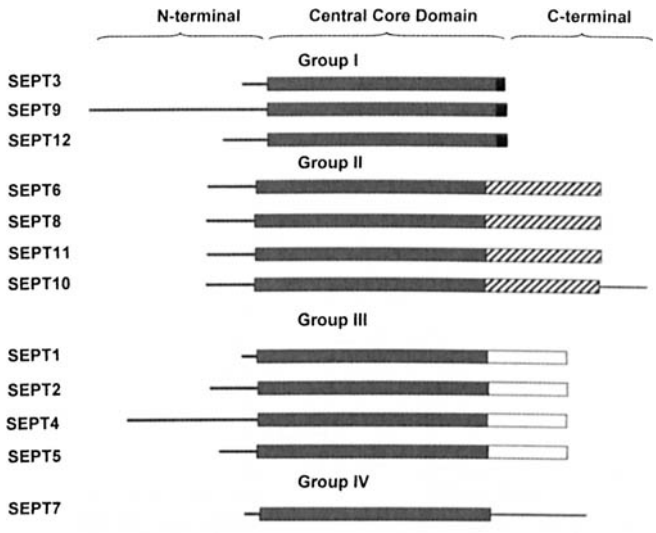

Figure 4. Mammalian septin groups. Based on a primary sequence similarity, the individual septins can be categorized into four different groups. Evidence suggests that macromolecular septin complexes are composed of a septin protein from each individual group (12, 21).

secreted by α-granules. Dense granules secrete small molecule agonists such as ADP, calcium, and magnesium. The role of SEPT5 in this platelet secretion is based on (i) the genetic and protein identity of platelet SEPT5, (ii) the demonstration of an interaction between SEPT5 and syntaxin 4, (iii) immunogold localization of SEPT5 to membranous areas surrounding α-granules, and (iv) characterization of the enhanced platelet-secretion response in the platelets from SEPT5-deficient animals (39). The enhanced platelet-secretory response was evident in the aggregation of platelets using subthreshold levels of an agonist (e.g., fibrillar collagen; Fig. 5) that activate platelets, causing the release of stored adhesive ligands and subsequent platelet aggregation. An involvement of SEPT5 in platelet secretion parallels some of the molecular mechanisms that control neurotransmitter release. In an unexpected finding, we observed that the overexpression of SEPT5 in mouse platelets coincides with an increase in the size of platelet α-granules, suggesting a related role for SEPT5 in the maintenance of normal α-granule morphology (42).

There is a growing appreciation for molecular similarities between platelet secretion and the mechanisms that control neurotransmitter release in neurons (40, 43, 44). Platelet biologists are fortunate that the release of neurotransmitters is one of the most intensively studied aspects of neurobiology (45, 46). From studies initiated on neurons, a hallmark molecular mechanism among secretory cells was

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were cotransfected with both *SEPT5*/pEGFP-N1 and *SEPT8*/pcDNA3.1-His cDNA. SEPT5 and SEPT8 were detected as previously described and the images were merged to reveal colocalization. (C) COS-7 cells were cotransfected with both *SEPT5*/pEGFP-N1 and *SEPT8*/pcDNA3.1-His. SEPT5 was detected as previously described. Cells were observed by differential interference contrast microscopy and SEPT5 epifluorescence images were overlaid. Arrows indicate an example of colocalization between SEPT5/SEPT8 complexes and vesicle-like organelles. (D) Rat pheochromocytoma cells (PC12) were labeled with the anti-SEPT5 monoclonal antibody LJ-33. The bound monoclonal was detected with an Alexa Fluor 568 goat–anti-mouse polyclonal antibody. Bars = 20 μm. (Reprinted with permission from Ref. 28).

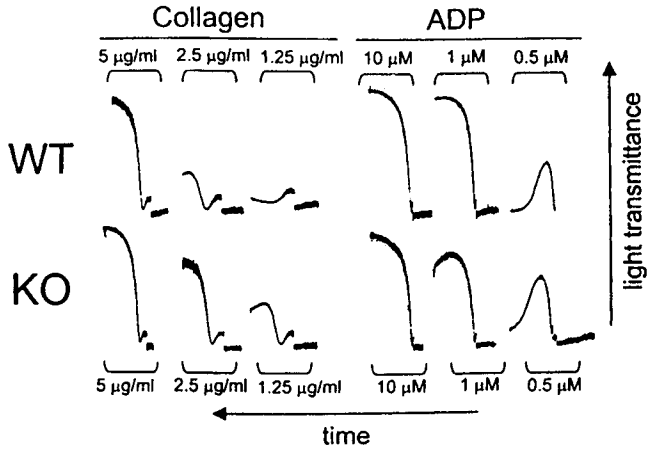

Figure 5. Hyperactivity of SEPT5^{null} platelets. Blood was drawn from wild-type and SEPT5^{null} littermates generated from SEPT5+/– (x) and SEPT5+/– (crosses). Stirred platelet-aggregation profiles using insoluble fibrillar collagen (Type I) or ADP at the indicated concentrations are shown. Platelet aggregation is noted by an increase in light transmittance. The results are representative of aggregation experiments performed with mouse platelet–rich plasma obtained from pooled blood that was isolated from littermates of the same genotype. SEPT5^{null} platelets aggregate more than wild-type platelets using subthreshold levels of agonists. (Reprinted with permission from Ref. 39).

described: the soluble NSF attachment protein receptor (SNARE) hypothesis. According to this hypothesis, SNARE molecules form a vesicle-fusion apparatus that targets the granule to the plasma membrane and facilitates vesicle docking and fusion between the vesicle and target membranes. Some proteins are associated with the vesicle and are referred to as v-SNARE components. Proteins associated with the target membrane are referred to as t-SNARE components. Soluble cytoplasmic proteins presumably modulate the process and are referred to as s-SNARE components. A number of individual platelet proteins have been identified in SNARE complexes (e.g., syntaxin 2, syntaxin 4, SNAP-23, synaptobrevin, a platelet homolog of the neuron Sec1 protein; Refs. 43, 47–50). Each of these proteins has a role in neuron exocytosis as well, but there are distinctive differences between neurotransmitter release and platelet secretion (40). One major difference is the triggering mechanism for secretion. For platelets, secretion occurs after receptor activation or stimulation with a chemical agonist. Neurons are triggered by membrane depolarization and Ca^{2+} influx. Also striking is the time of release for platelets (2–5 secs) and neurons (within 200 μsecs). Thus, the molecular basis of neurotransmitter and platelet release shares common components (e.g., SEPT5, syntaxins), but there must be distinctive features unique to the specific cell type.

Platelets and Neuron Function

A wealth of literature exists associating variant platelet phenotypes to differing forms of neuron dysfunction (51–53). As previously discussed, there is a growing appreciation for the basic similarities and dissimilarities that exist between neurotransmitter release and the platelet-release reaction. Thus, as has been speculated for decades, the platelet may be viewed as a circulating blood marker of abnormal events occurring in the nervous system. As such, platelet dysfunction does not directly contribute to neuronal imbalance, but it is an easily assayed marker of events that are also occurring during neurotransmitter release. The link of SEPT5 to abnormalities in platelet function, and the possibility that it links to an abnormal neural function, is intriguing. Indeed, the SEPT5 gene resides in a locus that is recognized as containing genes associated with schizophrenia (i.e., 22q11.2; Ref. 54). Recent evidence has implicated the relevance of SEPT5 in the pathogenesis of schizophrenia (55). In addition, gene profiling has revealed that levels of SEPT5 mRNA become undetectable in the cerebral cortex following the treatment of mice with 3,4-methylenedioxymethamphetamine (i.e., Ecstasy; Ref. 56). Thus, the potential exists to define the association between platelet and abnormal-brain phenotypes at the molecular level. As previously mentioned, the assembly of individual septins into macromolecular complexes provides another level of regulating *in vivo* septin function that may also contribute to the reported results. Future studies should provide new insights and directions for the clinical management of both platelet and neuron dysfunction.

Mammalian Septin Function Beyond Platelets and Neurons

As previously suggested, the role of septins is not limited to cell division and cell secretion events. Septins have been shown to be associated with different types of cancer (16, 57, 58). In particular, four septins (i.e., SEPT5, SEPT6, SEPT9, SEPT11) have been identified as in-frame fusions with the mixed lineage leukemia (MLL) proto-oncogene in patients with acute myeloid leukemia (57, 59–61). At first, these fusion proteins suggested a role of septins and, in particular for SEPT9 (previously called MLL septin-like fusion), in tumorigenesis. Montagna *et al.* (62) show that SEPT9 is overexpressed in different mouse mammary gland adenocarcinomas and in cell lines established from human breast tumors. However, other studies (16, 63) show that the SEPT9 gene, located on chromosome 17q25, maps to a region commonly lost in ovarian and breast carcinomas, which implies that it may act as a potential tumor repressor and contradicts the Montagna *et al.* study. The tumor-suppressor properties of SEPT9 also conflict with a recent study that demonstrates that SEPT9 plays a decisive role in cytokinesis through its association with microtubules (31). Interestingly, SEPT9 levels are increased in brain tissue from Down syndrome fetuses (64). Based on the pro-tumorigenesis activity of SEPT9, the authors suggest that SEPT9 levels may explain the fact that children with Down syndrome have increased incidences of acute leukemia (64). On the other hand, knowing whether SEPT9 acts as a tumor

repressor could provide a better understanding of why patients with Down syndrome have lower incidences of brain tumors. Future studies should focus on the role of SEPT9, a topic that is complicated by the fact that SEPT9 is alternatively spliced, which produces more than 15 different isoforms (65).

Septins have also been implicated in apoptosis. ARTS is an alternative transcript of the SEPT4 gene that relocates to the nucleus on induction of apoptosis by TGF-β (66). Studies have show that overexpression of ARTS increases TGF-β–induced apoptosis. Interestingly, and as it happens with other apoptotic-regulating proteins such as Apaf-1 and CED-4, the apoptotic function of ARTS is suppressed when a mutation is introduced in its P-loop consensus sequence (66). Recently, more insights about the apoptotic role of ARTS have been provided by Gottfried *et al.* (67) and show that ARTS interacts with an inhibitors of apoptosis protein and induces a de-repression of caspases to promote apoptosis. This work highlights the degree of specialization that each alternative variant can display and raises several questions about the roles of the different domains of each septin.

The protein encoded by a full-length SEPT4 mRNA has been implicated in neurodegenerative diseases, detected in cytoplasmic inclusions and Lewy bodies, and associated with α-synuclein, a protein that causes the development of Parkinson's disease when mutated (e.g., A53T, A30P; Refs. 68–70). Of the septins, only SEPT4 is found in Lewy bodies, and its association with α-synuclein and α-synphilin-1 probably induces cell apoptosis by forming toxic soluble complexes (68). Other reports show that SEPT4 (and SEPT5) is a target of parkin, an E3 ubiquitin protein ligase (71, 72). The loss of parkin activity is the cause of familial autosomal-recessive Parkinson's disease. Other neurodegenerative diseases have also been linked to septins. SEPT1, SEPT2, and SEPT4 are found in the neurofibrillary tangles and glial fibrils of Alzheimer's disease (73). Further studies are needed to clarify to what extent the accumulation of septins contributes to the pathology of Parkinsonism or Alzheimer's disease.

Concluding Remarks

A plethora of different functions has been associated with septin proteins since their first recognition in higher eukaryotes. A number of reports associate septins with cellular events where active membrane movement and cytoplasmic partitioning is occurring. Indeed, our work with the SEPT5^{null} platelet established a direct link between SEPT5 and the platelet secretory response. For our studies, we will use a platelet model of septin function to better define the platelet-secretion process and other aspects of megakaryocyte/platelet biology. Indeed, defining the role of platelet septins may also help in the understanding of molecular interactions that are important in neuronal cells. Similar, but not identical, roles of a given protein in different tissues may also illuminate why estimates on the number of human genes consistently yields figures more than 3-fold greater than the actual number found in the genome.

As previously summarized, septins are also being associated with a range of pathologic states from neurodegenerative diseases (e.g., synucleinopathies such as Parkinsonism and Alzheimer's disease) and schizophrenia to cancer. Given the impact and importance of these diseases to public health, it will be of outstanding interest to address the molecular mechanisms by which septins participate in the development of these diseases. Individual septin functions are being defined, but little is known about the formation, regulation, and role of septin filaments. Future studies on the biochemical properties of septin filaments and their interactions with other proteins (e.g., SNARES, actin, α-synuclein) should provide the basic science to appreciate the formation and function of macromolecular septin complexes.

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