Biochemical and Cell Biological Analyses of a Mammalian Septin Complex, Sept7/9b/11*

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Septins are members of a conserved family of cytoskeletal GTPases present in organisms as diverse as yeast and mammals. Unlike lower eukaryotic cells, the physiological significance of mammalian septin complexes is largely unknown. Using specific antibodies, we found at least five septins, Sept2, Sept7, Sept8, Sept9b, and Sept11, in septin complexes affinity-purified with anti-Sept7 antibody-conjugated column from rat embryonic fibroblast REF52 cells. Immunofluorescence studies revealed co-localization of Sept7, Sept9b, and Sept11 along stress fibers in REF52 cells. Biochemical and immunoprecipitation analyses revealed that the three septins directly bind with each other through their N- or C-terminal divergent regions. These septins per se formed distinct and characteristic filament structures when transiently expressed in COS7 cells. When two of the three septins were co-expressed in COS7 cells, combination-dependent filament elongation, bundling, or disruption was observed. Taken together, our results suggest that septin filament structures may be affected by interactions with other septins included in the complex.

Septins, a family of cytoskeletal filament-forming proteins, were first identified in yeast and have since been identified in most eukaryotic organisms, with the exception of plants (for a review, see Refs. 1–6). Although septins have a 25% or closer identity over their entire length, sequence similarity is greatest in the central domain, which contains guanine nucleotide interactive motifs homologous to those of ras-related small GTPases. In addition to the conserved central domain, each septin has a divergent N terminus and, with a few exceptions, a C terminus predicted to form a coiled-coil region.

The septins were originally identified in Saccharomyces cerevisiae as a group of cell division cycle regulatory genes (7). Thereafter, many septins have been identified and extensively studied with genetic analyses using lower eukaryotes such as yeast, Drosophila melanogaster, and Caenorhabditis elegans. Some septins in these organisms play an essential role in cytokinesis (for a review, see Refs. 1, 3, and 8). On the other hand, accumulating genetic and cell biological observations on yeast septins indicate that they are also required for localized chitin deposition, bud site selection, cell cycle control, and plasma membrane compartmentalization and for regulating some kinases (for a review, see Refs. 2, 3, and 9).

As for mammalian cells, 12 septin genes have been identified, mainly by cDNA cloning or data base analyses, and several of these have been characterized. Some septin transcripts are likely to undergo complex splicing, showing the presence of numerous numbers of mammalian septin family proteins (for a review, see Refs. 6 and 10). Sept2 and Sept9 are thought to play a role during cytokinesis (11–13). Sept5, Sept6, and Sept9 appear to be involved in tumorogenesis (14–19). The presence of a variety of septins in mammalian cells, even in nondividing cells such as neurons and platelets, indicates that septins are likely to be involved in yet unidentified various cellular processes such as vesicle fusion processes (20–22), apoptosis (23), and neurodegeneration (24, 25).

To better understand the physiological functions of septin complexes, it is required to elucidate septin complex structures at the molecular level. Septins form filament structures, which are most likely to be essential to their function. In yeast and fruit fly, isolated septin polypeptides are found in tight complexes with defined stoichiometries (26, 27). In mammalian cells, a recent study revealed that Sept2/6/7 is predominant in septin complexes affinity-purified from brain tissues or HeLa cells with anti-Sept2 antibody (28). Moreover, the recombinant Sept2/6/7 complex resembles endogenous septin complexes in their morphological and biochemical properties (28). The multiplicity of mammalian septin genes and their splicing patterns suggests that septin complexes with a different composition from Sept2/6/7 are likely to exist.

We now report identification of Sept2, Sept7, Sept8, Sept9b, and Sept11 in septin complexes immunopurified from rat embryonic fibroblast REF52 cells with anti-Sept7 antibody. Among these septins, we focused on three members with different characters, Sept7, Sept9b, and Sept11 for the following reasons. First, Sept7 is a component of the well characterized Sept2/6/7 complex and may not be replaceable with other septins in the complex (6), suggesting an important role in the septin filament framework. Second, Sept9b appears to lack a predicted coiled-coil region and thus to have a unique character in septin complexes. In addition, Sept9b is most likely to be involved in cell signaling by interacting with a guanine nucleo-
otide exchange factor for a small GTPase Rho, SA-RhoGEF. Finally, Sept11 is homologous to Sept6 and thus possibly replaceable with Sept6 in the septin complexes in REF52 that do not contain Sept6 (6). Based on the in vivo and in vitro results obtained in this study, we provide evidence for a septin complex similar to but distinct from Sept2/6/7, and we propose the possibility that septin complex structures may be influenced by the combination of septin components included in the complex.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—Expression plasmids of human Sept7, Sept9b, and Sept11 were produced as described (28, 29). The cDNA fragments of Sept9b-N1 (aa1–258), Sept9b-N2 (aa1–146), Sept9b-N3 (aa 147–258), Sept9b-Cent (aa 259–543), Sept9b-C (aa 544–568), Sept7-N (aa 1–31), Sept7-Cent (aa 32–298), Sept7-C1 (aa 244–418), Sept7-C2 (aa 318–418), Sept11-N (aa 1–147), Sept11-Cent (aa 148–329), Sept11-C1 (aa 290–429), and Sept11-C2 (aa 364–429), obtained using PCR, were subcloned into pGEX-4T3 or pMAL (see Fig. 5). All constructs were verified by DNA sequencing.

**Preparation and Characterization of Antibodies**—The GST-fused Sept9b fragment (aa 1–258), Sept7 fragment (aa 244–418), and Sept8 fragment (aa 291–508) expressed in E. coli served as antigens. Rabbit polyclonal antibodies anti-Sept7, anti-Sept8, and anti-Sept9-N, specific for Sept7, Sept8, and Sept9a/b, respectively, were generated and affinity-purified. Anti-Sept11 antibody was kindly provided by Dr. M. Kinoshita (Kyoto University) (11). Western blot analysis was done, and immunoreactive bands were visualized as described (13). To confirm specificity of the purified antibodies, they were preabsorbed by respective recombinant proteins used as antigens.

**Cell Culture, Transfection, and Immunofluorescence**—REF52, COS7, and HeLa cells were grown as described (29). Transient transfection was carried out using the Lipofectamine method (Invitrogen). Immunofluorescence analysis was done as described (13). To detect the septins, affinity-purified anti-septin antibodies were used as the primary antibody. To visualize Myc tag, Flag tag, actin, or tubulin, cells were reacted with 9E10, M2 (Eastman Kodak Co.), rhodamine-phalloidin (Molecular Probes, Inc., Eugene, OR) or anti-tubulin monoclonal antibody (Sigma), respectively. Polyclonal anti-FLAG antibody was used where indicated. Alexa 488- or Alexa 350-labeled IgG or FluoroLink Cy3-linked IgG (Molecular Probes) was used as a secondary antibody. When analyzing the cells, we used a confocal microscope (LSM-G2B00; Olympus).

**Septin Complex Isolation from REF52 Cells**—REF52 cells were harvested with the extraction buffer (50 mM Hepes, pH 7.6, 0.5% Triton X-100, 0.5% Chaps, 1 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, 1 mM chymostatin, and 1 mM pepstatin) and incubated on ice for 15 min. The cell supernatant was obtained by centrifuging this extract at 16,000 × g for 40 min at 4 °C. Affinity-purified anti-Sept7 antibody was adsorbed to Affi-Gel Hz beads (Bio-Rad) according to the manufacturer’s instructions. The beads were then added to the cell supernatant and incubated with gentle agitation for 3 h at 4 °C. The beads were sedimented and washed four times with 20 volumes of the extraction buffer. The beads were then poured into a column and drained by gravity. One column volume of elution buffer, 0.1 M glycine (pH 2.5), was then added. The column was then allowed to drain by gravity and eluted with seven more column volumes of the elution buffer followed by neutralization. The majority of the septin complex was found in fractions 2–4. The contents of individual septins were estimated by SDS-PAGE followed by Western blotting using the specific antibodies.

**Expression and Purification of Recombinant Proteins**—Sept7, Sept9b, and Sept11 and their truncated mutants were expressed in *Escherichia coli* as GST or MBP fusion. Protein concentration was determined by the method of Bradford (Bio-Rad), and purity of the protein preparations was confirmed on Coomassie Blue-stained SDS-polyacrylamide gels.

In **Vitro Binding Analyses of Purified Sept7, Sept9b, and Sept11**—Binding of a GST- or MBP-fused septin or its truncated mutants with other septins was determined in a pull-down assay. Each septin or its mutants (3 μg) were incubated with another septin, GST, or MBP (3 μg), in 50 mM Hepes (pH 8.0) containing 0.1% Chaps and 0.1% Triton-X

1 K. Nagata and M. Inagaki, submitted for publication.
2 The abbreviations used are: aa, amino acids; Chaps, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; GST, glutathione S-transferase; MBP, maltose-binding protein.

**RESULTS**

**Production of Antibodies Specific for Sept7, Sept8, and Sept9**—To characterize mammalian septins, we first prepared rabbit polyclonal antibodies against septins, including Sept7, Sept8, and Sept9a/b, and affinity-purified them on a column to which respective antigens had been conjugated. Specificity of the antibodies was confirmed with COS7 cell lysates in which various septin proteins were overexpressed. As shown in Fig. 1, A and B, the anti-Sept7 and anti-Sept8 antibodies specifically recognized Sept7 and Sept8, respectively, in Western blot analyses. Anti-Sept9-N antibody specifically recognized Sept9a and Sept9b in Western blot analyses, since the polypeptide used as
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mediated signal transduction pathways (31).

In Vivo Interaction of Sept9b, Sept7, and Sept11 Expressed in COS7 Cells—The possibility of physiological interaction among Sept7, Sept9b, and Sept11 was tested. We first asked whether Sept7 forms a complex with Sept9b and Sept11 in COS7 cells. When Myc-Sept9b or -Sept11 was expressed with FLAG-Sept7 into COS7 cells, Myc-Sept9b and -Sept11 were efficiently co-immunoprecipitated with FLAG-Sept7 (Fig. 4, A and B). Myc-Sept11 was also co-precipitated with FLAG-Sept9b with a good stoichiometry in the same analysis as above (Fig. 4C). These results suggest the possible in vivo interaction among Sept7, Sept9b, and Sept11.

In Vitro Association of Sept9, Sept7, and Sept11 with Each Other—Complex formation and co-localization of Sept7, Sept9b, and Sept11 in REF52 cells (Fig. 2) and in vivo interaction among them in COS7 cells (Fig. 3) implies direct interaction among these three septins. We thus tested whether Sept7, Sept9b, and Sept11 associate directly with each other by a pull-down assay using GST- and MBP-fused proteins. As shown in Fig. 5A, Sept9b interacted with Sept7 (left) and Sept11 (middle) in vitro. Sept7 also formed an in vitro complex with Sept11 (Fig. 5A, right). These results suggest that Sept7, Sept9b, and Sept11 can form a heterocomplex through direct association with each other, although competition for a common binding site of a septin by other two may occur. Under our conditions, recombinant purified septin did not interact with purified GST and MBP proteins without fused polypeptides (data not shown). As a next set of experiments, molecular dissection analyses were done to determine the region in each septin essential for direct interaction with other septins. The
Coomassie Blue staining pattern of the purified septin fragments used were shown in Fig. 5B. As shown in Fig. 5, C and F, Sept9b bound with Sept7 and Sept11 through its N-terminal extension. It is notable that the length of the N terminus of Sept9b required for interactions with Sept7 and Sept11 is different; a shorter length of Sept9b-N terminus was sufficient for binding to Sept11. Fig. 5, D and G, show that the C-terminal coiled-coil region of Sept7 was required for binding with Sept9b and Sept11. For the binding with Sept11, a shorter length of Sept7-C terminus was sufficient compared with the case of Sept9b. On the other hand, Sept11 bound with Sept7 and Sept9b through its C-terminal coiled-coil region. There was also a difference in length of the C terminus required for interactions with Sept9b and Sept7, and a shorter C-terminal region of Sept11 is sufficient to associate with Sept7 (Fig. 5, E and H). A summary of the mapping analyses of interactive regions of Sept7, Sept9b, and Sept11 is depicted in Fig. 5I.

**Septin-Septin Interaction Alters the Filament Structures in COS7 Cells**—Since any two septins among Sept7, Sept9b, and Sept11 formed an *in vivo* immunocomplex when expressed in COS7 cells, it is likely that these co-expressed septins are also co-localized in COS7 cells. We thus tested the possibility. First of all, we analyzed localization of endogenous Sept7, Sept9b, and Sept11 in COS7 cells. As depicted in Fig. 1F, COS7 cells...
FIG. 5. Biochemical interaction among Sept7, Sept9b, and Sept11 and domain analyses of the interaction. A, pull-down experiments of GST-Sept9b with MBP-Sept7 (left) or MBP-Sept11 (center) and of GST-Sept11 with MBP-Sept7 (right). GST-fused septin and/or MBP-fused septin were incubated, and pull-down analyses were done using glutathione-Sepharose beads. Upper panels show the input in each experiment. The precipitates were analyzed by SDS-PAGE, followed by staining with Coomassie Blue (lower panels). B, proteins used in the domain analyses were separated by SDS-PAGE (10%) and stained with Coomassie Blue. In addition to the intact proteins as indicated (*), partly degraded proteins were seen in the purified samples. C, GST-fused Sept9b and its deletion mutants were incubated with MBP-Sept7 or -Sept11, and pull-down analyses were done. The precipitates were subjected to SDS-PAGE (10%) followed by Western blotting with anti-MBP antibody (New England Biolabs). D, MBP-fused Sept7 and its deletion mutants were incubated with GST-Sept9b or GST-Sept11 followed by pull-down assays. Protein binding was analyzed as in C. E–H, schematic representations of the binding regions of Sept9b (F), Sept7 (G), and Sept11 (H) with other septins used. The plus and minus signs summarize the positive and negative interactions observed in C–E, respectively. I, possible model of interaction among Sept7, Sept9b, and Sept11. The N-terminal variable region of Sept9b interacts with the C-terminal coiled-coil regions of Sept7 and Sept11. Sept7 and Sept11 interact with each other through their C-terminal coiled-coil regions.
FIG. 6. Interactions between two septins expressed in COS7 cells affect the filament structures. A, COS7 cells were double-stained with anti-Sept9-N (a, d, and g) and rhodamine-phalloidin (b, e, and h). The merged image is also shown (c, f, and i). The cells were also stained with anti-Sept7 (j) or anti-Sept11 (k). Bar, 10 μm. B, Myc-Sept7 was transiently expressed in COS7 cells with FLAG tag (a), FLAG-Sept9b (b and c), or FLAG-Sept11 (d and e). C, Myc-Sept9b was transiently expressed with FLAG tag (a), FLAG-Sept7 (b and c), or FLAG-Sept11 (d and e). D, Myc-Sept11 was transiently expressed with FLAG tag (a), FLAG-Sept7 (b and c), or FLAG-Sept9b (d and e). B–D, after 24 h of transfection, cells were fixed and stained with 9E10 (a) or double-stained with 9E10 (b and d) and M2 (c and e). Bar, 10 μm. E, Western blots showing protein analyses of a mammalian septin complex.
express Sept9b predominantly as is the case of REF52 cells. When immunofluorescent double-staining was done, these septins were present in very similar short filamentous structures in the cells (Fig. 6A, a, d, g, j, and k). It is notable that Sept9-containing septin filaments in COS7 cells did not show obvious co-localization with actin filaments (Fig. 6A, a–c), microtubules (Fig. 6A, d–f) or vimentin filaments (Fig. 6A, g–i).

We then explored expression patterns of Sept7, Sept9b, and Sep11, and further examined the localization patterns of respective septins when they are expressed in various combinations in COS7 cells. As shown in Fig. 6B (a), Myc-Sept7 per se forms thin and short filaments in COS7 cells. Co-expression of Sept9b made the filaments straight and longer (Fig. 6B, b). FLAG-Sept9b was incorporated into the filaments as determined by immunofluorescent analyses (Fig. 6B, b and c). On the other hand, co-expression of FLAG-Sept11 with Myc-Sept7 disrupted the short filaments of Myc-Sept7 (Fig. 6B, d). It should be noted that FLAG-Sept11 co-localized with Myc-Sept7 in an immunofluorescent analysis despite the filament disruption (Fig. 6B, d and e).

Myc-Sept9b per se forms thin and long filaments in COS7 cells (Fig. 6C (a)). As shown in Fig. 6C, b and c, Myc-Sept9b becomes straight when FLAG-Sept7 is co-expressed (see also Fig. 6B, b and c). The different Sept7/9b-filament density in Fig. 6, B (b and c) and C (b and c), was due to the difference of the expressed protein levels in each experiment. It is notable that the basic characters of Sept7/9b-filaments are very similar as long as the ratio of Sept7 to Sept9b is equal. Co-expression of FLAG-Sept11 facilitated the bundling of Myc-Sept9b-filaments, perhaps due to lateral association of the filaments (Fig. 6C, b–e). Immunofluorescent double-staining revealed that co-expressed Sept7 and Sept11 were co-localized with Sept9b, suggesting interactions between the co-expressed proteins (Fig. 6C, b–e).

Finally, the effects of Sept7 and Sept9b on the Sept11 filament structure were examined. Myc-Sept11 formed thin and long filaments, which were disrupted by co-expression of Sept7 (Fig. 6D, a–c). The phenotype was similar to that shown in Fig. 6B, d and e. Despite filament disruption, Sept7 was also co-localized with Sept11 in the cells (Fig. 6D, b and c). Co-expres-

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expression levels in transfected cells with a titrated mixture of M2 and 9E10. F, Myc-Sept9b was transiently expressed with FLAG-Sept7 (a–f) or FLAG-Sept11 (g–l). Myc-Sept7 was transiently expressed with FLAG-Sept11 (m–r). After 24 h of transfection, cells were fixed. Myc tag and FLAG tag were stained with 9E10/Alexa-488 (a, d, g, j, m, and p) and anti-FLAG polyclonal antibody/Alexa-350 (data not shown), respectively. F-actin and microtubules were co-stained with rhodamine-phalloidin (b, h, and n) and anti-tubulin monoclonal antibody (e, k, and q), respectively. The merged images were also shown (c, f, i, l, o, and r). Bar, 10 μm.
sion of Sept9b made the Sept11 filaments thicker by facilitating the bundling as is the case in Fig. 6C (Fig. 6D, d–f). As shown in Fig. 6E, the expression levels of respective septins expressed in Fig. 6, B–D, were estimated to be comparable in Western blot analyses.

Since septin filaments are highly related to actin and microtubule filaments (11–13, 28, 29, 31), dynamic septin structural alteration observed in Fig. 6, B–D, raises the possibility of cytoskeletal reorganization by expression of two septin molecules. Indeed, the filaments produced by Sept7/9b and Sept9b/11 resembled actin stress fibers and microtubules, respectively. We thus asked if co-expression of two septins in various combinations induces cytoskeletal reorganization and if the produced septin filaments align with actin and/or microtubule filaments. Consequently, the filaments produced by Sept7/9b and Sept9b/11 were co-localized with neither actin filaments nor microtubules (Fig. 6F, a–I). It is also notable that any combination of the three septins had no effects on actin and microtubule filament structures in COS7 cells (Fig. 6F). Sept7, Sept9b, or Sept11 per se had little effect on the structures of actin filaments and microtubules in COS7 cells (data not shown).

We next analyzed the effects of exogenously expressed septins on endogenous septin filaments and actins in cells by monitoring the distribution of Sept9b. We used REF52 cells for this set of experiments, since Sept7, Sept9b, and Sept11 are distributed along stress fibers in the cells, and it is easy to detect the change of alterations of endogenous septin structures. Since Sept7, Sept9b, and Sept11 are localized along with stress fibers in REF52 cells, the effects of expression of these septins on actin filaments were also monitored.

When Myc-Sept9b was expressed in REF52 cells, the molecule appeared to form thin and straight filaments, and endogenous Sept9b was incorporated into the filaments (Fig. 7A, a–c). Expression of Myc-Sept7 or -Sept11 induced a drastic change in endogenous septin filaments. These septins formed characteristic filaments similar to those observed in COS7 cells, and endogenous Sept9b was incorporated into the filaments (Fig. 7A, d–i). Interestingly, despite the drastic change of septin filaments in the cells, no obvious change was observed in stress fiber structures (Fig. 7B), suggesting that actin filament organization might be independent of the septin filament structures in REF52 cells.

DISCUSSION

Given their high degree of conservation, ubiquitous expression, and proven role in cytokinesis, septins are certain to be important players in regulating cell architecture and function. Despite the recent progress on the mammalian septin complex

![Fig. 7. Effects of exogenously expressed septins on endogenous septin and actin filament structures in REF52 cells.](image-url)
structure, much less information is available regarding function of mammalian septins as compared with lower eukaryotic cells. The important aspects of the present work are to demonstrate for the first time that 1) at least five mammalian septins, Sept2, Sept7, Sept8, Sept9b, and Sept11 are components of in vivo septin complexes isolated from REF52 cells, although the precise modes of mutual interaction among these septins remain to be determined; 2) Sept7, Sept9b, and Sept11 are distributed as a filamentous pattern along actin stress fibers in REF52 cells in an actin filament-dependent manner; 3) the three septins interact with each other in vitro and in vivo, and intramolecular regions required for in vitro interaction among these septins are determined; and 4) septin filament structures may be influenced by mutual interaction of the components in the complexes.

The basic structure of septin complexes from yeast, fruit fly, and mammals are thought to be conserved; the complexes purified from these organisms appear as filaments 7–9 nm wide and of variable length with a periodicity of 25–32 nm (20, 26, 27). The septin complexes from yeast and Drosophila are composed of four and three polypeptides, respectively. Recently, a septin complex was identified in mammalian tissues as hetero-oligomers composed of Sept2/6/7 (28, 33). Biochemical analyses with reconstituted Sept2/6/7 revealed physiological significance of the complex. On the other hand, the fact that purified mammalian septin complexes contained far more than the three septins with an obscure stoichiometry implies the presence of multiple mammalian septin complexes other than Sept2/6/7. In this context, it is possible that Sept7/9b/11 is one such septin complex in REF52 cells, although in vitro reconstitution study is required to confirm it. On the other hand, the presence of many septin genes and far more splicing variants therefore suggest redundancy and interchangeable of components in septin complexes. Since Sept11 is a member of the Sept6 group and widely expressed in mammalian tissues compared with Sept6 (data not shown), it may be a substitute for Sept6 in a possible Sept7/9b/11 complex.

Although the higher ordered structural similarity among septin complexes in different species is striking, how these complexes are assembled from different numbers of divergent septins is largely unknown. Whereas the importance of coiled-coil regions in septins for filament formation has been proposed (26, 30, 31), it is notable that Sept9b, which lacks a predicted coiled-coil region, binds to C termini of both Sept7 and Sept11 through its long N-terminal extension, which does not contain any predicted domain structure so far categorized. The results suggest that septin isoforms with long N-terminal regions may utilize the regions as well as coiled-coil domains to interact with other septins.

It is probable that the cell type-specific septin expression determines the subunit composition of septin complexes and their higher architectures. Alternatively, septins might form complexes composed of different molecules within different types of cells even if same septin molecules are expressed there. In such a case, the ratio of each septin to others might influence septin polymer structures and functions. It is also possible that subunit composition of a septin complex in cells may change dynamically in a spatiotemporal manner and that septins may form more than one type of filament. The determinants of structures, localization, properties, and perhaps functions of septin complexes might be the combination of septin components included in the complexes. Based on this hypothesis, large septin complexes containing different polypeptides may be localized in different intracellular places and are assembled to accomplish different biological processes, perhaps recruiting different proteins. The physiological significance of GTP/GDP binding and/or GTP hydrolysis activity of septins has not been elucidated. It is likely to have implications in their structural organization and functions. It is, however, tempting to speculate that yet unidentified septin-associated proteins may modify septin structures and functions. It seems that the structures and functions of mammalian septins are much more complicated compared with lower eukaryotic cells.

In the present study, we found that Sept7, Sept9b, and Sept11 are expressed in REF52 cells, distributed along with actin stress fibers in the cells. As is the case of Sept2- or Sept4-containing septin filaments (11, 34), the septin filament structure containing Sept7, Sept9b, and Sept11 depends on the integrity of actin filament in REF52 cells. Although one must clarify how septin complexes interact with actin filaments, a nuclear protein, anillin, was recently identified as a septin protein that can bind to both actin and septins (28). Since anillin is largely confined in the nucleus in interphase cells, it is most likely to function in mitotic phase, and septins may be anchored by an unknown adaptor protein to interphase actin filaments.

Although septin complexes have been purified from mammalian tissues, and in some cases the components were identified, mutual effects of septins in the complex have not been studied. In the present study, we analyzed the effect of a septin on another one by a transient transfection method using COS7 cells. When overexpressed in COS7 cells, Sept7, Sept9b, and Sept11 per se are distributed as characteristic filaments. Although the septin filaments observed here did not necessarily reflect their physiological intracellular distribution, effects of a septin on another septin structure can be analyzed. If septin filaments formed by overexpression of a single molecule are disrupted by co-expression of another septin, we assess that the co-expressed septin has a negative effect on the filament forming ability of the other. On the contrary, if filaments by overexpressed septin become elongated and/or thicker by co-expression of another septin, we assume that the co-expressed septin has a positive effect on the other. The present results should suggest that septin filament morphology is affected by combination of the components in the filaments.

Extensive cytological and biochemical investigations are required to clarify whether interactions among various septins are essential for septin structures and functions in mammalian tissues.

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