Septin Organization in *Saccharomyces cerevisiae*: Interactions Between Septin Subunits and Complexes

Abstract

Septins belong to a conserved family of GTP-binding proteins that assemble filaments at the mother bud neck in *S. cerevisiae* cells (Byers & Goetsch, 1976; Mostowy & Cossart, 2012). Not much is known about how septins form these filament structures, however, recent studies have shed some light on the structural organization of these structures. One such study, carried out by Bertin et al., supports a model wherein septin subunits form octamer rod complexes that then assemble into filaments (2008). Here, we investigate how septins organize in *S. cerevisiae*, using the model defined by Bertin et al. as the basis for our hypothesis that septins form octamers that associate with each other to form filaments. To examine the interactions between septins that form the octamer as well as the interactions between rod complexes, a directed two-hybrid approach was chosen. Our results showed a strong association between Cdc11 and Cdc12—subunits that make up the septin core. Furthermore, Cdc11 was observed to interact with itself, albeit to a lesser amount, suggesting that rod complexes associate with each other as well. These results were consistent with the model characterized by Bertin et al. Taking both of these results into account, we conclude that octamer interactions are stronger than rod interactions. This finding builds off of previous models, providing a better understanding of the mechanisms involved with septin organization in yeast cells.

Introduction

Septins were first discovered in *S. cerevisiae* using mutagenic screens to investigate genes necessary for cytokinesis. Four genes were found to cause defects in cell division when mutated; the genes identified from this study were consequently named cell division cycle genes–Cdc3, Cdc10, Cdc11, and Cdc12 (Hartwell, 1971). Septins are a relatively new field of research, but there are a few things that are known about their functions and interactions. Septins have been shown to associate closely with the plasma membrane in budding yeast; using EM, septins have been observed to assemble filament structures, about 10 nm wide, that form rings at the bud neck region during bud emergence and disassemble at the onset of cytokinesis (Byers & Goetsch, 1976). In yeast cells, septins have three important domains (Figure 1A)—a polybasic region that is involved in association with membranes, a GTP-binding domain, and a C-terminal extension/coiled-coiled domain. Septin interactions occur through these three domains. In many organisms including yeast, septins are known to form symmetric oligomers, which then interact to form filaments, cages, or ring structures (Mostowy & Cossart, 2012). A recent study by Bertin et. al showed that septins in yeast are organized into rod-shaped octamers. Septins within the octamer were found to assemble in a particular order: Cdc11-Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12-Cdc11. These rods lack polarity and interact with each other to form a filament (2008). In this study, we investigate how septins organize in *S. cerevisiae*, using the model defined by Bertin et al. as a starting point. From our results, we conclude that interactions between septin subunits within the octamer are stronger than that of those between rod structures that assemble into filaments.

Materials and Methods

Pairwise septin interactions were screened using a yeast two-hybrid assay. Yeast grown to log phase were provided by Ken Kaplan. Log phase yeast were transformed with Gal4 activating domain (AD) and binding domain (BD) vectors according to standard protocol. Cultures of yeast cells containing plasmids encoding either BD/Cdc11-AD, Cdc11-BD/Cdc11-347-415-AD, Cdc11-101-217-BD/Cdc11-AD, or Cdc12-BD/Cdc11Δ347-415-AD were plated on -Leu -Ura media at 30° C for approximately 2 days. Single colonies from each strain were selected and grown on test plates deficient in adenine or histidine (-Ade -Leu -Ura and -His Leu -Ura) at 30° C for approximately 2 days.

Results

Cdc11 interacts with Cdc12

The first question we set out to address was how septin subunits interact within the octamer complex. To investigate this question, we specifically looked at the interactions between Cdc11 and Cdc12—two septins that were shown to associate as part of the septin core in the model put forth by Bertin et al. We observed the interactions between the two using a directed yeast two-hybrid approach. A yeast strain with BD bait and Cdc11-AD prey was used as a negative control to ensure the specificity of the interactions tested in our yeast-two hybrid system. The results from the yeast two-hybrid analysis can be seen in Figure 1B. Yeast cells with plasmids encoding Cdc12-BD/Cdc11Δ347-415-AD showed substantial growth on both test plates, suggesting a high affinity interaction. From this result, we concluded that Cdc11 interacts with Cdc12. Furthermore, Cdc11's C-terminal extension is not necessary for this interaction to occur.

Cdc11 interacts with itself

We next addressed how septin rod complexes interact to form filaments. Once again, a yeast two-hybrid system was utilized to observe protein interactions. This time, we looked at how Cdc11 interacts with itself—an association involved in filament formation as predicted by the Bertin model. Two different strains were prepared with plasmids encoding either Cdc11-101-217-BD/Cdc11-AD or Cdc11-BD/Cdc11-347-415-AD. The logic behind this was approach was to distinguish the necessary domains for this interaction to occur. The results of this assay can be seen in Figure 1B. Yeast cells with plasmids encoding Cdc11-101-217-BD/Cdc11-AD showed little to no growth in -Ade -Leu -Ura media, but substantial growth in -His -Leu -Ura media. We decided to score this as a moderate affinity interaction as a result. However, this test should be repeated in order to make this conclusion with certainty. From this result, we concluded that Cdc11 interacts with itself via its G-domain. On the other hand, yeast cells with plasmids encoding Cdc11-BD/Cdc11-347-415-AD showed no growth. Thus, we concluded that the C-terminal extension of Cdc11 is not sufficient for this interaction to occur.

Octamer interactions are stronger than rod interactions

Using a yeast two-hybrid assay, we found that Cdc11 and Cdc12 interact with strong affinity. We also observed that Cdc11 interacts with itself, though possibly with less affinity. Taking both of these results into account, we conclude that octamer interactions are stronger than rod interactions.

Discussion

In this study, we provide evidence that octamer interactions are stronger than rod interactions. Using a yeast two-hybrid assay,

we have shown that Cdc11 interacts with Cdc12 and that Cdc11 interacts with itself. This result is consistent with the previous work done by Bertin et al, which was the foundation for our hypothesis that septins assemble into octamer complexes that then interact with each other to form filaments. In addition to these previously reported interactions, we go beyond this model by suggesting that octamer interactions are stronger than rod interactions. There are several caveats to the yeast two-hybrid system that was utilized in this study to make this conclusion. First, septins are known to assemble in the cytosol or at the plasma membrane (Mostowy & Cossart, 2012). In a yeast two-hybrid assay, protein interactions occur in the nucleus where septins do not usually associate with each other. Second, with a yeast two-hybrid approach, protein interactions are observed inside the yeast cell alongside thousands of other proteins. It is possible that any of these other proteins could affect the interactions between bait and prey proteins that are observed. For example, these unrelated proteins facilitate the interactions we observe and yield false positives. Experiments with purified septin subunits, like the assays performed in the study by Bertin et al., could provide better insight into the presence and interactions of such adapter proteins. All in all, two-hybrid analysis is good at identifying pairwise protein interactions; it only provides a qualitative measurement of the affinity between two interacting proteins. However, that is not to say that the results found here are at all insignificant. The interactions observed in this study are a great starting point for further study into the organization of septins. Though many recent studies have provided models for septin organization, there are still many unanswered questions regarding the molecular mechanisms or factors responsible for septin assembly. To observe interactions between septins and other proteins, it is worth noting that we attempted to look at the interactions between Bem4 and the C-terminal extension of Cdc11 in our two-hybrid assay. This transformant yielded no growth, so we did not discuss this result in our paper. However, we may want to repeat this test or pick another protein of interest in order to observe the interactions between non-septin proteins and the CTE of Cdc11. In general, further research is required to expand our understanding of the mechanisms involved in septin interactions and assembly.

A

Polybasic region	GTP binding domain	Coiled-coiled domain
12-19	101-217	360-415

B

Bait / Prey	-Ade -Leu -Ura	-His -Leu -Ura	Interaction
BD / Cdc11-AD			-
Cdc12-BD / Cdc11Δ347-415-AD			++
Cdc11-101-217-BD / Cdc11-AD			+
Cdc11-BD / Cdc11-347-415-AD			-

Figure 1. Pairwise Interactions Between Septin Subunits

(A) Schematic showing Cdc11. Septins in yeast consist of three conserved domains.

(B) Yeast grown to log phase were transformed with Gal4 activating domain (AD) and binding domain (BD) vectors (far left column) and plated on -Leu -Ura media at 30° C for approximately 48 hours. Individual colonies from each strain were then grown on test plates (-Ade -Leu -Ura and -His -Leu -Ura) at 30° C for approximately 72 hours. Growth for each strain was assessed by comparing the colony sizes of different strains. For each transformant, substantial growth was scored a (++), intermediate growth was scored a (+), and little to no growth was scored a (-). The affinity between bait and prey proteins (far right column) was interpreted from the amount of growth on each test plate.

Image in (A) is modified from Fig. 1, Mostowy & Cossart (2012).