DOI: 10.1017/S1431927605501399
Microsc Microanal 11(Suppl 2), 2005

Towards an understanding of yeast septins' organization *in vivo*

Alina M. Vrabioiu* and Timothy J. Mitchison *

* Department of Systems Biology, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115

Septins are a family of proteins important for cell division in most eukaryotic organisms. Septins form filaments *in vitro* and associate with guanine nucleotides [1]. These are trademarks of the dynamic cytoskeleton. However, nucleotide binding does not regulate polymer dynamics (as for actin and microtubules), but rather plays a structural role in the assembly of the septin complex [2], considered to be the unit of the septin filament. Our research aims at providing further understanding of septins' organization *in vivo* and at grasping how their three dimensional organization relates to their function.

We conducted our investigation in the yeast *Saccharomyces cerevisiae*. There are five septin polypeptides vegetatively expressed in this organism, two of which are required for viability. All of them share the same bud-neck localization and undergo dynamic reorganization as a function of cell cycle [3]. Just before the yeast cell buds, septins localize as a cap to the site of the future bud. As the bud continues to grow septins form an hourglass shaped structure at the bud-neck (Fig. 1). Towards the end of cytokinesis, the hourglass structure splits into two rings (Fig.1) that persist on the two resulting cells [3].

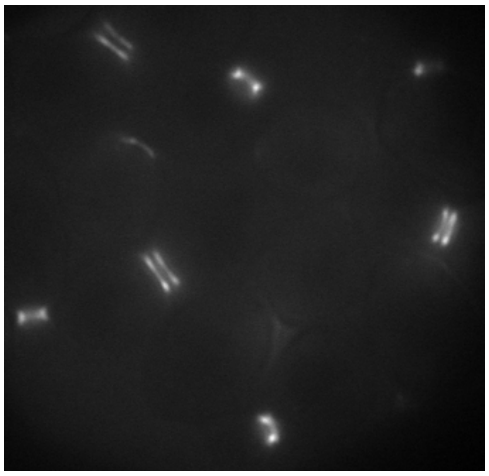
To probe the nature of the higher order organization of these dynamic structures, we analyzed GFP-tagged septin strains via fluorescence polarization microscopy. We reasoned that if septins form filaments *in vivo*, and if those filaments assemble into ordered structures, we could detect preferential absorption/emission of a particular orientation of polarized light.

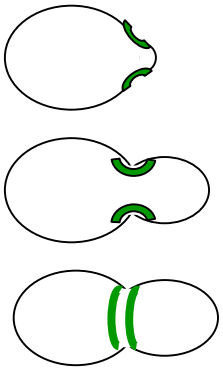
The setup we used was a Nikon TE300 inverted microscope with two filter wheels, one in the excitation, and the other in the emission. Each filter wheel contained two film polarizers perpendicular to each other and arranged such that the excitation and emission setups were parallel relative to the light path. Assigning the letters H and V to horizontal and vertical orientations of the polarizers, relative to the light path, we collected fluorescence images under the following illuminations: HH, VV, HV, VH (the first letter designates the orientation of the excitation polarizer, while the latter designates the orientation of the emission polarizer). We obtained two measurements from this experiment. One was HH-VV, which is a measure of differential absorption of polarized light as a function of bud-neck orientation, the other was r = (HH-HV)/(HH+2HV), the anisotropy of GFP in the particular analyzed structure.

We determined that within the hourglass organization, the GFP dipoles have preferred orientations (Fig. 2). By combining polarization and anisotropy determinations we were able to make a model about septin filaments' orientation within this structure. In addition, we characterized each of the other structures: caps, hourglass shaped, and rings. Based on this data, we derived a model for septins' structural rearrangements during cell division.

References

[1] C.M. Field et al., *J. Cell Biol.* 133 (1996) 605.
[2] A.M. Vrabioiu et al., *J. Biol. Chem.* 279 (2004) 3111.
[3] V.J. Cid et al., *Microbiology* 147 (2001) 1437.





1A **1C**

FIG. 1. Widefield fluorescence images of GFP septin strains. 1A contains hourglass structures and ring structures; 1B is a diagram of *S. cerevisiae* division, starting with early budding stage, and finishing with the late stage of cell division.

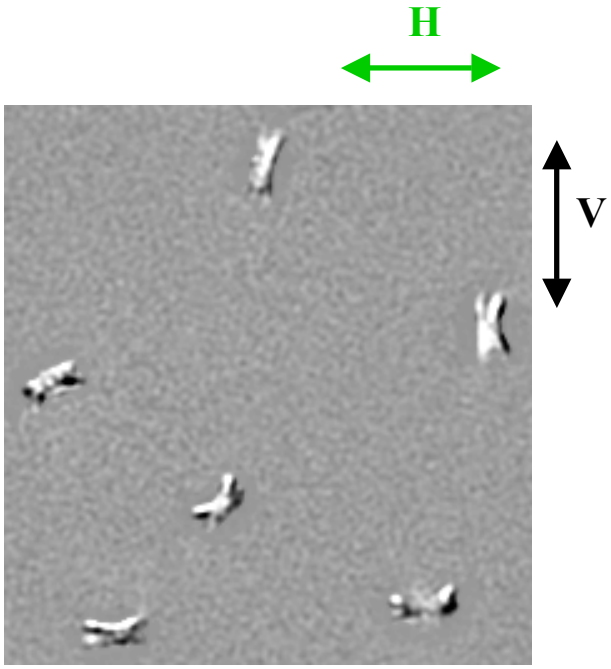


FIG. 2. A representative subtraction image (HH-VV) for the hourglass structures. The images were intensity corrected and aligned before subtraction. The H and V arrows indicate the orientations of the horizontal and vertical polarizers relative to the sample, respectively.

https://doi.org/10.1017/S1431927605501399 Published online by Cambridge University Press