

DYNAMIC ARCHITECTURE OF YEAST SEPTIN FILAMENTS STUDIED BY POLARIZED FLUORESCENCE RECOVERY AFTER PHOTBLEACHING

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Septin, the fourth type of cytoskeleton, plays a significant role in the yeast budding process. Previous studies showed that septin filaments rotate 90° during the budding using polarization fluorescence microscopy (FPM) [1], and recent research based on electron microscopy (EM) modeled the filaments disassembly and reassembly during hourglass-to-double ring conversion [2]. However, there are conflicts between the FPM and EM results and the underlying rearrangements and dynamics of septin during the cell cycle remains elusive. Here, we further studied the septin dynamics with POLarized Fluorescence Recovery After Photobleaching (PolFRAP) and super-resolution imaging based on structured illumination.

Firstly, we observed four states of septin architecture during cell division using our polarized structured illumination microscopy (pSIM) [3] system: patch, hourglass, hourglass-to-double ring transition, and split rings. With dipole orientation mapping and high time-spatial resolution, we first see the dynamic orientation change of septin from parallel to perpendicular along the mother-bud axis.

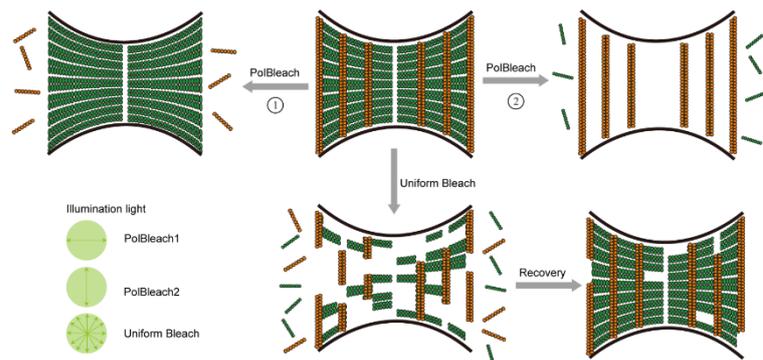


Figure: The principle of PolFRAP.

Secondly, to better understand the filament rearrangement during this process, we used the method of PolFRAP to calculate the content of mutually perpendicular filaments during the different cell cycle, respectively. Our data show that the fluorescence of two kinds of filaments recovery after bleaching at different rates except for the telophase of cell division. Filaments perpendicular to the mother-bud axis are not recovered at the telophase state. The results demonstrate that there is no disassembly during hourglass-to-double ring transition, and different assembly rates of two kinds of filaments may result in a 90° polarization rotation.

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