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doi:10.1017/S1431927619007438
Microsc. Microanal. 25 (Suppl 2), 2019

Interconnection of Actin-binding Proteins and Septins in Asymmetric Cell Division

AV Vakhrusheva[1*], TB Stanishneva-Konovalova[1] and OS Sokolova[1]

[1.] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russian Federation.
* Corresponding author: vakhrusheva.ann@yandex.ru

The process of asymmetric division is necessary in the early stages of organism development, also to maintain a pool of stem cells and tissue homeostasis [1]. Cytokinesis occurs due to contraction of the actomyosin ring and the formation of a septum. Septins are collected at the site of cell division, and at later stages of mitosis are involved in the formation of the actomyosin ring, they also surround it playing the role of a barrier [2]. HOF1 is also a major contributor to the contractile ring and septum formation [3]. But interconnections between HOF1, septin and actin are not known yet.

For the expression and subsequent purification of the full-length HOF1 protein in *E. coli* bacteria (Rosetta 2 strain), the plasmid pMALc2, carrying the MBP-Hof1-6xHis sequence, was used. For the expression and subsequent purification of the four yeast septins Cdc11, Cdc12, Cdc3 and Cdc10 in *E. coli* cells (strain BL21), two bicistronic vectors were used, containing the following Cdc sequences with the tags: Cdc12-6xHis, Cdc10-SNAP, Cdc11-S and Cdc3-Flag. Plasmids were provided by our colleagues from Brandeis University, Waltham, USA. During our work expression conditions were optimized and protein purification was carried out using nickel affinity and ion exchange chromatography. For electron microscopy experiments grids with purified protein samples were prepared and stained with 2% uranyl acetate solution. Several tens of micrograph pairs were taken from these grids in a JEOL2100 transmission electron microscope with an accelerating voltage of 200 kV.

HOF1 is known to participate in the actomyosin ring and septin barrier formations. Using electron microscopy, it was shown that HOF1 interacts with formin Bnr1, which is involved in the formation of actin filaments. The HOF1-Bnr1 complex binds to actin filaments (Fig. 1, Left) and inhibits actin polymerization. In addition, HOF1 can also interact directly with actin (Fig. 1, Right).

Besides it was shown that HOF1 can interact in different ways with septin filaments: either via Bnr1 or directly (Fig. 2). Comparing the binding of HOF1 protein to actin and septin filaments, differences were found. On actin filaments HOF1 is localized chaotically, from different sides of actin and by different parts. On the contrary, a certain orderliness can be traced on septin filaments: HOF1, both alone and in combination with Bnr1 protein, is always located just across the septin filaments, as if "intercepting" their bundles, as well as alternating every 100 +/- 20 A°. Thus, septins most likely have a specific site of interaction with HOF1 protein, unlike actin filaments. Microscopy of HOF1-Bnr1 protein was also performed with actin and septin filaments at the same time and a scheme of their simultaneous interaction was proposed (Fig. 3) [4].

References:

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[4] The study was carried out with the financial support of the RFBR in the framework of Scientific Project No. 18-34-00347.

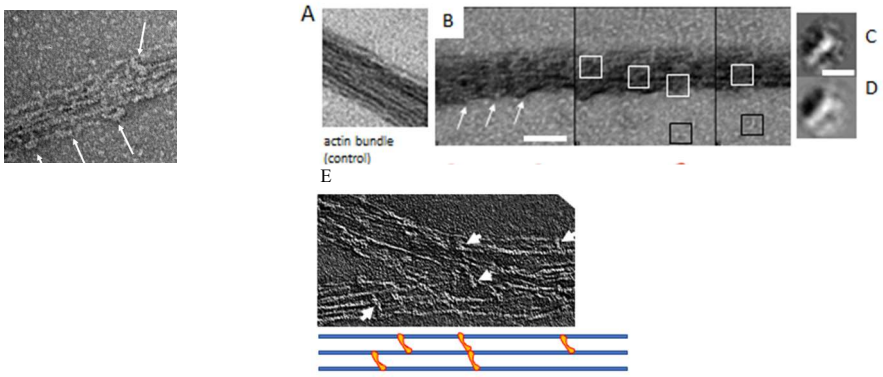

Figure 1. Left) Electron microscopy of the HOF1-Bnr1-actin complex. The arrows indicate the disordered arrangement of the HOF1 protein. Right) A - an actin bundle (without HOF1); B - electron microscopy of the binding of HOF1 with actin, scale bar - 5 nm. White squares - HOF1 on actin, black - free HOF1 on the carbon substrate; C - class sums of free HOF1, scale bar - 10 nm; D - HOF1 class sums with actin; E - tomogram of actin filaments with F-BAR domain of HOF1 and their binding scheme.

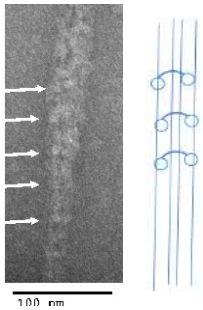

Figure 2. Electron microscopy and a putative HOF1-Bnr1-septin interaction scheme (left). The arrows indicate the location of the HOF1.

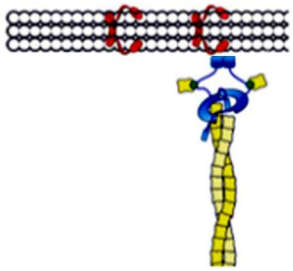

Figure 3. A scheme showing possible interactions of actin (yellow squares) with septin strands (white circles) formed by interaction with HOF1 (red) through the formin Bnr1 (blue).

https://doi.org/10.1017/S1431927619007438 Published online by Cambridge University Press