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Analysis of the interactions between ABP1 and ARP2/3 complex in two binding sites by molecular dynamics simulation

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The relevance of the study is associated with the problems of actin filaments branching during changing the shape, movement and morphogenesis of cells. Violations in these processes are observed in a number of diseases (myopathy, hearing impairment, cancer).

The Arp2/3 complex plays a key role in nucleating actin filaments branching. The actin nucleator Arp2/3 complex consist of seven subunits, two of them closely resemble actin monomers. Arp2/3 complex is inactive in the cytoplasm, but to prime it for nucleation is should be activated by nucleation-promoting factors (NPFs). NPFs could be classified into two types: type I and type II. Type I NPFs include WASP, WAVE/SCAR, WASH. Type II NPFs including Abp1 and Cortactin, bind to f-actin. Yet it was shown that Abp1 can protect branches from Gmf-initiated debranching. Abp1 contains an N-terminal actin depolymerization factor (ADF)-homology domain that possesses high level of homology with the ADFH domains from Cofilin and Gmf (the glia maturation factor). Thus Abp1 may bind to Arp2/3 complex at the same place, as Gmf. Additionally, the point mutation in ADFH domain of Abp1 has been identified, that interferes with its function. We have recently used electron microscopy (EM) and single-particle analysis to determine the 3D structure of Abp1-bound Arp2/3 complex. In this study, we demonstrated that Abp1 binds to Arp2/3 complex with a 2:1 molar ratio and successfully protects the branches from Gmf action. We demonstrated that Abp1 can dimerise through interactions of its C-terminal parts and while the ADF-homology domain of one Abp1 molecule in the dimer may bind Arp2/3 complex, the ADF-homology domain of the other molecule is available to bind F-actin. Here we performed the molecular modelling of the interactions of ADFH domains with Arp2/3 complex in two binding sites, found previously by the combined EM-MD approach (Popinako, 2018). We have predicted the specific Arp2/3 complex surface residues that are involved in interactions with ADFH domain of Abp1 (wild type (WT) and bearing the point mutation (1-5)), and established the background of stabilization the branch junctions by Abp1. Using molecular dynamics simulations we demonstrated the quantitative and qualitative changes in hydrogen bonds upon binding the Abp1. We identified the specific amino acid residues in the Abp1 and Arp2/3 complex

that stabilize the interactions. Phylogenetic and structural analyses of the recently defined Abp1 binding site on Arp3 subunit indicate a new mechanism for Arp2/3 complex activation that involves the interactions between the Arp2/3 complex and Abp1 at two binding sites. The MD studies were supported by RFBR (16-34-60252 to A.P.). The MD research has been carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University.This work structural analysis has been carried out using computing resources of the federal collective usage center Complex for Simulation and Data Processing for Mega-science Facilities at NRC “Kurchatov Institute” (ministry subvention under agreement RFMEFI62117X0016), http://ckp.nrcki.ru/.