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Actin Nucleation During Plasma Membrane Blebbing of Adhering Cells

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Plasma membrane (PM) blebs are highly dynamic spherical protrusions emanating from the cell surface. They have been observed during diverse processes such as cytokinesis, adhesion, cell motility or virus entry. As a common feature among these different types of blebbing, actin polymerization controls the dynamics of blebbing. However, the precise molecular machines governing actin polymerization during blebbing remain to be characterized.
Here, we employed HeLa cell adhesion blebbing as a model system to study the role of the 21 known human actin nucleators in blebbing. Using a small siRNA screen, we identified several actin nucleators that were indispensable for bleb formation. Among these, silencing of DIAPH3 and Spir-1 resulted in the most robust reduction of blebbing. The involvement of these two nucleators was therefore studied in more detail.
DIAPH3 downregulation resulted in reduced blebbing efficiencies and increased cell spreading on uncoated surfaces. This phenotype closely resembled the behavior of cells adhering to integrin ligand-coated surfaces. DIAPH3 belongs to a formin group of actin nucleators known to generate unbranched actin filaments. Several different DIAPH3 isoforms were identified on both mRNA and protein level in HeLa cells. Intriguingly, only DIAPH3 isoform 1 induced blebbing, whereas an active version of isoform 7 led to the formation of filopodia. Both types of protrusions depended on actin nucleation activity of the respective formin isoform. Moreover, the N-terminal region comprising GTPase-binding domain (GBD) served as a determinant governing formation of isoform-specific protrusions and subcellular localization of the respective isoform.
On the other hand, Spir-1 overexpression resulted in inhibition of adhesion blebbing. This effect was independent of Spir-1´s actin nucleation activity, but required the C-terminus (CT) implicated in the localization of Spir proteins. Previously reported block of vesicle transport processes upon overexpression of Spir-1 CT suggests that Spir-1 exerts its role in adhesion

blebbing via general effects on the anterograde transport, which might contribute to regulation of blebbing during adhesion.

In summary, adhesion blebbing is regulated by the specific cooperation of distinct actin nucleators. The specificity of the actin nucleator isoforms for distinct functions suggests that generation of isoforms increases the capability of cells to control a variety of fundamentally different actin-based processes. Identification of proteins that selectively interfere with formation of PM blebs is a crucial step towards understanding of and interfering with the function of these protrusions e.g. in tumor cell invasion.