Communicative & Integrative Biology 7:5, e970972; October 1, 2014; © 2014 Taylor & Francis Group, LLC

Genetic links between bacterial dynamin and flotillin proteins

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Dynamin is a membrane-associated GTPase that confers motor-like functions in membrane dynamics, such as endocytosis, in eukaryotic cells. Flotillin (reggie) proteins are also a widely conserved class of membrane proteins, associated with the formation of protein assemblies within the membrane, and with endocytotic processes. Bacterial dynamin has been shown to bind to membranes in vitro and to mediate membrane fusion. *Bacillus subtilis* DynA localizes to the cell division septum, and it was recently shown that it indeed plays a role in cell division. Interestingly, dynamin shows a genetic interaction with flotillin proteins in this prokaryotic model organism and the absence of both proteins results in a cell division and cell shape defect. Here, we show that in addition to the morphological phenotypes, a dynamin/flotillin double deletion strain shows a synthetic defect in cell motility, much stronger than that of flotillin single mutant cells. While the contribution of altered cell shape and slower growth of the double deletion strain on motility cannot be clearly assessed, our data emphasize the fact that dynamin and flotillin proteins play tightly connected functions in a wide range of aspects in membrane processes in bacteria.

Keywords: Dynamin, membrane curvature, flotillin, lipid microdomains, organization of membrane proteins

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Submitted: 05/13/2014

Revised: 06/12/2014

Accepted: 06/13/2014

http://dx.doi.org/10.4161/cib.29578

Dempwolff F, Wischhusen HM, Specht M, Graumann PL. The deletion of bacterial dynamin and flotillin genes results in pleiotrophic effects on cell division, cell growth and in cell shape maintenance. BMC Microbiol 2012; 12:298; PMID:23249255; http://dx.doi.org/10.1186/1471-2180-12-298

Dynamin has first been discovered and described in eukaryotic cells.[1] Due to the fact that it is also conserved in many bacterial species,[2] dynamin must have evolved early in evolution. The protein is a GTPase that can perform motor-like tasks in the context of membrane-bending and fusion. It can tubulate on membranes, and undergoes GTP-driven conformational changes. One of its major functions is the pinching off of clathrin-coated vesicles, by constricting the neck of these structures, and it has additional functions, such as in organelle division.[3-5] No such processes are known to occur in bacteria, so the presence of dynamin in many bacterial genomes has been puzzling. Through cell biological, biochemical, and genetic experiments, it has become clear that bacterial dynamin is involved in cell division, a fundamental process in which membrane bending and fusion must occur.[6,7] DynA in *Bacillus subtilis* appears to be expressed in low amounts and localizes to the cell membrane, with an enrichment at the division septum.[6,7] Midcell localization occurs after the initial formation of the Z ring by FtsZ (**Fig. 1**), the bacterial tubulin ortholog.[8] Deletion of *dynA* has no considerable phenotype, but exacerbates the division defect of two other genes, whose products play a role in cell division.[7] Purified DynA binds to membrane vesicles and promotes their fusion,[6] indicating that the protein may be involved in the final step of cell division, the generation of two separate membranes from the invaginating cell membrane.

Additional evidence for an involvement of dynamin in division comes from its link to flotillin proteins, which belong to a class of proteins conserved from bacteria to human, existing in many different domain architectures, in which the SPFH domain (for "**s**tomatin **p**rohibitin, **f**lotillin, and **H**fl**C**/**K**") is the conserved part.[9,10] Flotillins are membrane associated, either through a single N-terminal membrane span, as in many bacteria, or through a membrane anchor, as in eukaryotic cells. The N-terminus is followed by the SPFH domain, and an additional domain, the flotillin domain (not present in, e.g., prohibitins or stomatins), composed of mostly heptad rich repeats.

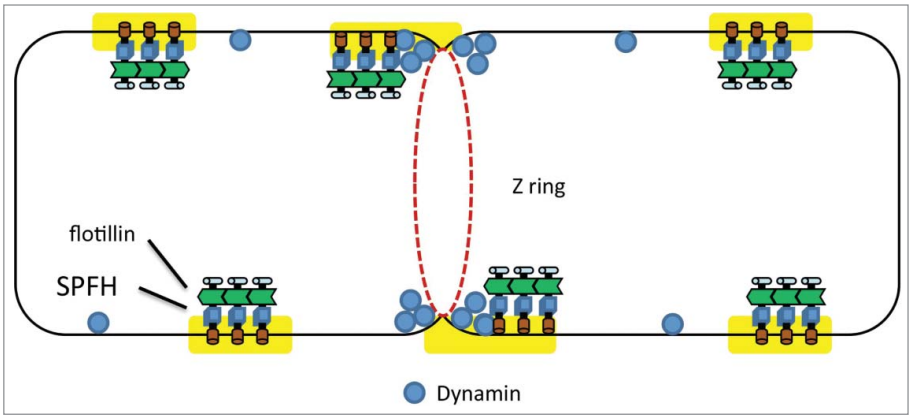

Figure 1. Model for the function(s) of bacterial dynamin and flotillin (reggie) proteins. The FtsZ polymer is shown as a dashed oval, and putative specific lipids associated with flotillin assemblies are depicted in yellow. The main domains in bacterial flotillins are named. FloT from *B. subtilis* has an additional C-terminal domain of unknown function.

B. subtilis flotillin T has a further domain at its C terminus, which is also predicted to form a short coiled coil. Flotillins play a role in the nerve recovery after severing in fish (where they were first discovered and termed "reggie" proteins,[11]) clathrin-independent endocytosis,[12] cytoskeletal rearrangements,[13] cell-cell adhesion,[14] as well as nutrient uptake,[15] and thus in a wide variety of membrane dynamics. Recently, it was shown that flotillins in bacteria also play important roles in membrane processes, including the timing of the differentiation process of sporulation,[16] cell shape maintenance, and cell division.[17]

Interestingly, a deletion of the gene encoding flotillin T protein in *B. subtilis* and of *dynA* results in cell filamentation.[7] A flotillin double mutant (*floT* and *floA*) in *B. subtilis* also shows a cell filamentation defect, and overproduction of flotillin T results in the considerable shortening of cells,[18] supporting the idea that flotillins are directly involved in the division process.

However, the activity of DynA does not appear to be restricted to cell division. A *floT dynA* double deletion strain has an additional defect in cell shape maintenance, and the deletion of *dynA* in addition to the major player in shape maintenance, MreB, exacerbates the severe *mreB* defect. MreB is an actin-like protein that forms extended filaments underneath the bacterial cell membrane,[19] which move in a seconds time frame along various angles relative to the longitudinal axis of the cell. The absence of DynA in *mreB* mutant cells leads to cell filamentation and most pronounced to an exacerbated loss of maintaining the correct cell width, generating huge oval-shaped cells.

During our analysis of *dynA floT* double mutant cells, we noted an additional phenotype: when inoculated on soft agar plates, mutant cells spread across the surface much slower than wild type cells, and also considerably slower than *floT* single mutant cells (**Fig. 2**). These results show that the loss of *dynA* in *floT* mutant cells exacerbates the motility defect. This could be due to several non-exclusive reasons: 1) the defect in cell morphology could decrease swimming efficiency, or 2) double mutant cells grow considerably slower than wild type cells, which may also affect the speed of swimming and/or the assembly of flagella due to energetic constraints. Thus, at this stage, it is impossible to state which is the main reason for the severe motility defect in *dynA/floT* double mutant cells. However, the synthetic effect on motility underscores the genetic link between bacterial dynamin and flotillin.

Thus, the functions of flotillins and of dynamin converge at the process of cell division, where membrane binding as well as membrane fusion are important events. It is not yet clear which of the two stages are affected by the loss of dynamin and flotillins, or if even both may be compromised. What is known for flotillins is that they are part of membrane structures also containing NfeD proteins,[17] and additional proteins such as KinC,[20] for which biochemical and cell biological evidence has been presented, and likely several other proteins, such as cell wall metabolism component Pbp5, secretory protein SecY, membrane transporters like FhuD, as well as energy metabolism-related protein AtpDG.[21] It has recently been shown

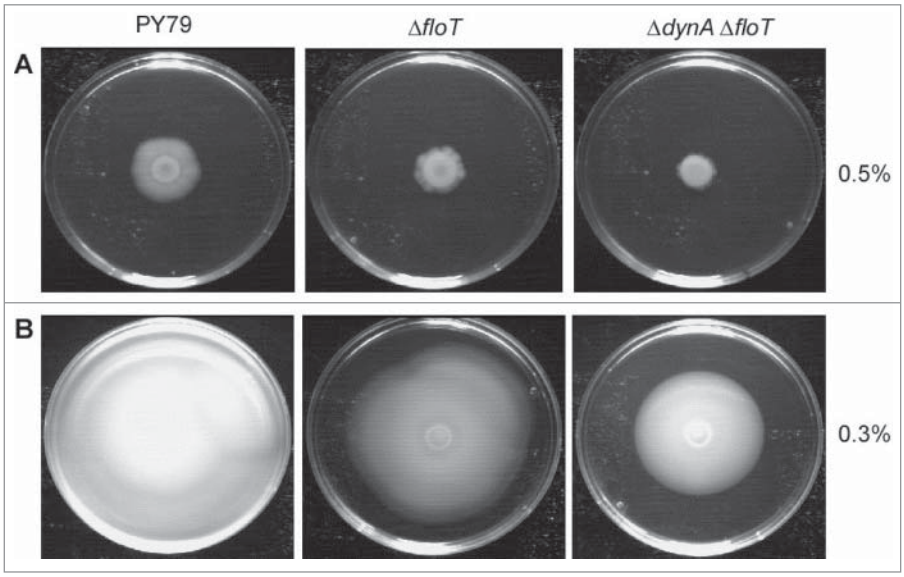

Figure 2. Motility assays. (**A**) swarming on 0.5% agar plates, (**B**) swimming on 0.3% agar plates. Wild type: *Bacillus subtilis* PY79, ΔfloT: strain HiHO 114 (in frame deletion of *yuaG/floT*), ΔdynA ΔfloT: strain FD 249 (*ypbR::tet,* in frame deletion of *yuaG/floT*).

that the overproduction of flotillins increases the stability of a protease, FtsH, within the membrane, which in turn affects cell division and other membrane-associated processes.[18]

How might flotillins and dynamin cooperate? The absence of flotillin or their overproduction has been shown to affect membrane fluidity,[21,22] and some lipids are known to facilitate membrane bending, such as cardiolipin. It is well conceivable that flotillins are associated with specific lipids such as cardiolipin, which would facilitate membrane bending, or membrane fusion, performed by dynamin; both processes are crucial steps during bacterial cell division (**Fig. 1**). Indeed, flotillin T has been co-isolated with negatively charged phospholipids, such as phosphatidylglycerol or cardiolipin[16] and it has been proposed that induction of positive membrane curvature at the septum is achieved by insertion of the paddle domain of dynamin; interactions between dynamin dimers from opposite sides of the closing septum may initiate fusion of the two membranes.[23] It will be exciting to elucidate this possible mechanism at a molecular scale.

Intriguingly, dynamin mutations are found in neural disorders,[24] and dynamin plays a role in memory formation,[25] which is interesting in light of the fact that flotillins/reggies have been identified to be essential for nerve regeneration[11] and axon growth.[26] Although this connection may be far fetched, the tight connection of flotillin and dynamin-like proteins in bacteria indicates that their eukaryotic counterparts could also be closely connected in their activities. First evidence of an interplay of both protein families comes from the observation that dynamin is necessary for flotillin turnover at eukaryotic membranes.[27]

In summary, bacterial cells provide a new avenue to study the function of dynamin and flotillin proteins at a molecular level. Given their implication in an exceedingly broad range of membrane-associated cellular processes, it will be important to continue to investigate the two classes of proteins in all cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft and by the LOEWE Center for Synthetic Microbiology, SYNMIKRO.

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