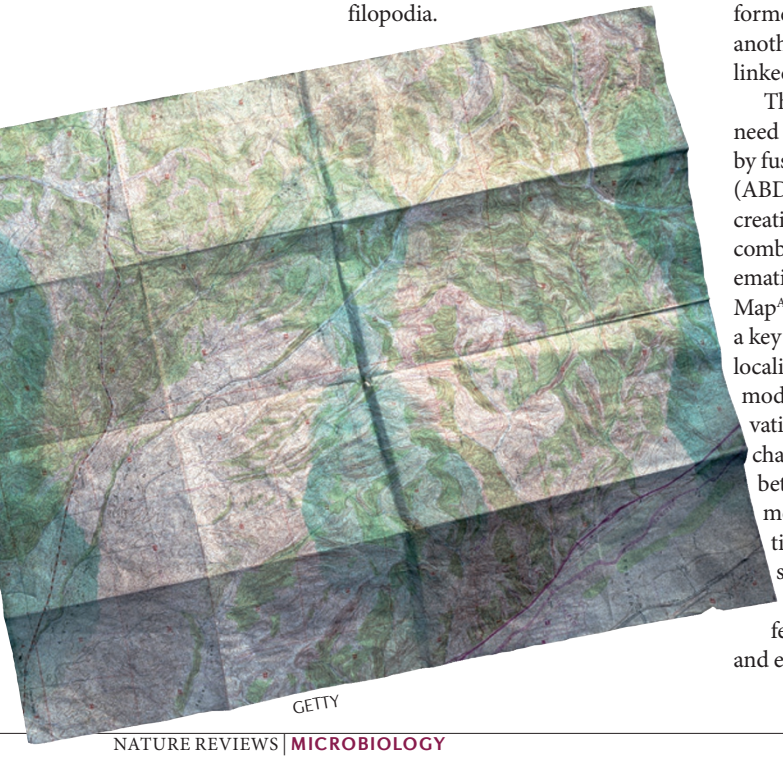
CELLULAR MICROBIOLOGY

EPEC puts actin on the Map

> actin nucleation locally amplifies CDC42 signal transduction through a Map-dependent positive feedback loop

A recent *Cell* paper provides detailed insights into the interaction between the enteropathogenic *Escherichia coli* (EPEC) effector mitochondrial-associated protein (Map) and the host protein cell division cycle 42 (CDC42) and, in the process, reveals a new role for actin as a spatial and temporal regulator of eukaryotic signal transduction.

RHO family GTPases, which regulate the cell cytoskeleton, are key constituents of many eukaryotic cell signalling pathways and, as such, are major targets for pathogenic bacteria. The EPEC type III-secreted effector Map is a guanine nucleotide exchange factor (GEF) that is known to activate the RHO family GTPase CDC42. Following its injection into the host cell, Map contacts the host protein EBP50 (also known as NHERF1), and the Map–EBP50 complex activates CDC42 at the cell membrane, leading to the localized formation of filopodia.



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Robert Orchard, Neal Alto and colleagues wanted to go beyond this basic understanding of the Map signalling network to probe the precise circuitry involved in activating CDC42 at the site of bacterial adhesion on the host cell membrane. They used live-cell imaging to assess the effects of Map on F-actin dynamics and CDC42 activation and found that, in the absence of external cues, Map polarized CDC42 on the cell surface, resulting in the formation of spatially restricted clusters of actin-rich membrane protrusions. In addition to a GEF domain, Map contains a carboxy-terminal PDZ domain-binding motif that interacts with EBP50, and both domains were required to polarize CDC42 activity on the cell membrane. Further *in vitro* analysis of the Map–CDC42 signalling cascade revealed that EBP50 did not target Map to cell membrane receptors. Instead, EBP50 formed a scaffolding complex with another host cell protein, ezrin, that linked Map to the actin cytoskeleton.

The authors circumvented the need for this scaffolding complex by fusing the actin-binding domain (ABD) of ezrin directly to Map, creating Map^{ABD}. *In vitro* analysis, combined with structural and mathematical modelling of the minimal Map^{ABD} signalling network, revealed a key role for actin dynamics in the localization of Map and CDC42. The models suggested that CDC42 activation occurs as a result of the stochastic cycling of Map and F-actin between the cytosol and the plasma membrane, and that actin nucleation locally amplifies CDC42 signal transduction through a Map-dependent positive feedback loop. Computational and experimental analysis confirmed that CDC42 could spontaneously polarize to the plasma membrane in this manner. However, during *E. coli* infection *in vivo*, the polarization of CDC42 is not random but instead is specific for the bacterium–host interface. To reconcile these opposing views, the authors looked at the effects of outside-in stimulation using fibronectin-coated beads to stimulate actin polymerization at defined cell surface sites. They found that, in cells expressing wild-type Map or Map^{ABD}, bursts of actin polymerization were associated with the areas of fibronectin bead stimulation, and these areas were enriched in CDC42 activity. By contrast, in cells expressing Map mutants that do not interact with actin, no actin polymerization or CDC42 accumulation was observed. Finally, time-lapse microscopy using an enhanced GFP (eGFP)-tagged Map^{ABD} construct showed that Map was recruited to the area of fibronectin bead stimulation just before the burst of actin polymerization.

The authors present a model in which, through an as-yet-unidentified signal, the adhesion of EPEC to the host cell surface stimulates a local actin rearrangement at the cell membrane, creating what the authors refer to as an actin 'landmark'. Map interacts with the EBP50–ezrin scaffolding complex and recognizes this landmark, establishing a positive feedback loop that nucleates CDC42 at the cell membrane and leads to localized actin polymerization and sustained CDC42 activity at the adhesion site.

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ORIGINAL RESEARCH PAPER Orchard, R. C. *et al.* Identification of F actin as the dynamic hub in a microbially induced GTPase polarity circuit. *Cell* **148**, 803–815 (2012)