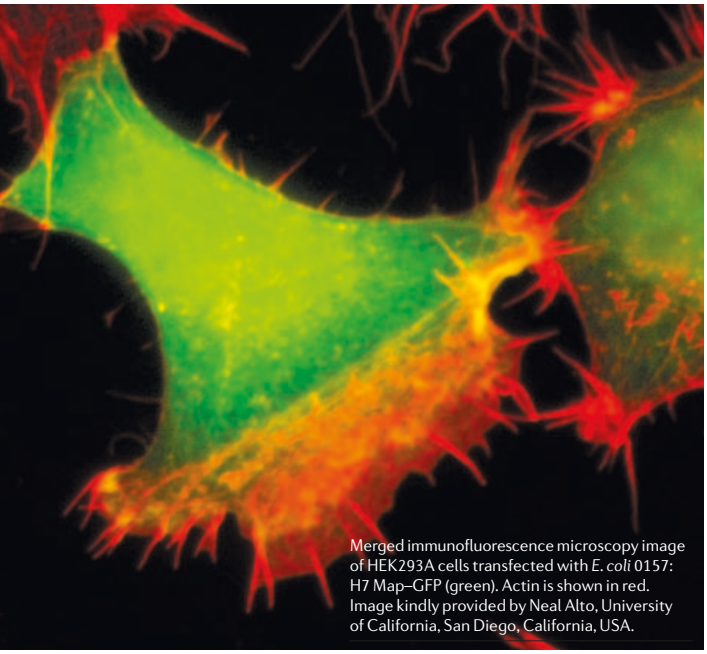
TYPE III SECRETION

Expert mimics



Merged immunofluorescence microscopy image of HEK293A cells transfected with *E. coli* 0157:H7 Map–GFP (green). Actin is shown in red. Image kindly provided by Neal Alto, University of California, San Diego, California, USA.

New work by Jack Dixon and collaborators has identified a large family of bacterial type III secreted effectors that mimic the functions of members of the Rho family of small GTPases.

Many Gram-negative bacterial pathogens use a type III secretion system to secrete effector proteins directly into host cells. Each pathogen secretes a distinct set of effectors with activities that are pertinent to its virulence strategy. The functions of the effector proteins can therefore vary but generally involve subverting the functions of the host cell in some way. The Rho GTPases, including RhoA, Rac1, Cdc42 and Rab, are referred to as 'molecular switches' and are important cellular regulators. As such, their activities are the targets for many bacterial virulence factors and cytotoxins; however, until now, no bacterial mimics of these proteins were known.

Dixon and co-workers used one particular effector protein, the Map protein from enteropathogenic *Escherichia coli* (EPEC), as their starting point. A BLAST search with *E. coli* 0157:H7 Map as the reference sequence identified a number of homologues, from which the conserved motif WXXXE was derived. Further analysis of the search results using this motif identified a 24-member family, in six different classes across 11 different Gram-negative pathogens, including Map, *Shigella flexneri* IpgB1 and IpgB2, and *Salmonella* spp. SifA and SifB.

Transfection experiments revealed that IpgB1-, IpgB2- and Map-containing green fluorescent protein (GFP)-fusion proteins induced distinct phenotypes in a human embryonic kidney cell line. IpgB2–GFP induced the formation of actin stress fibres, IpgB1–GFP induced the formation of actin-rich dorsal membrane ruffles, and Map–GFP induced the formation of filopodia; these three actin-based activities are reminiscent of the activities of the small GTPases RhoA, Rac1 and Cdc42, respectively. Transfection with fusion proteins in which the Trp or Glu residues in the WXXXE motif were mutated to alanine had no effect, as did transfection with GFP alone.

Alto *et al.* went on to carry out a detailed characterization of the cellular effects of *S. flexneri* IpgB2. Given that the formation of actin stress fibres is associated with RhoA, was this IpgB2-dependent activity being mediated through RhoA? Using inhibitors to disrupt RhoA signalling, it was found that the actin stress fibres observed were formed by a novel mechanism independent of RhoA activity. Immunoprecipitation and yeast two-hybrid work revealed that both the Rho-associated kinase ROCK and the formin homology protein mDia were targets of IpgB2, and that contact with these downstream effectors of RhoA occurs through the Rho-binding domain (GBD).

Analysis of the downstream effects of Map showed that the filopodia observed were generated independently of Cdc42 activity, and analysis of a Map-deletion EPEC strain proved that Map was required for filopodia formation. Additionally, a conserved eukaryotic targeting sequence, the PDZ ligand motif, was identified in the C-terminal domain of Map, and again yeast two-hybrid and immunoprecipitation assays were used to identify the binding ligand, which proved to Ebp50, a cell-surface protein that localizes target proteins to the apical surface of epithelial cells.

Together, these results suggest not only that this family of secreted effectors are functional mimics of Rho GTPases, but also that the use of eukaryotic targeting sequences can direct some of these effectors to specific cellular locations for maximum effect.

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ORIGINAL RESEARCH PAPER Alto, N. M. *et al.* Identification of a bacterial type III effector family with G protein mimicry functions. Cell **124**, 133–145 (2006)

Links:

Escherichia coli
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj&cmd=Retrieve&dopt=Overview&list_uids=12319

Map
http://us.expasy.org/uniprot/Q5WMD0

Shigella flexneri
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj&cmd=Retrieve&dopt=Overview&list_uids=12334

IpgB1
http://us.expasy.org/uniprot/Q6XVY7

IpgB2
http://us.expasy.org/uniprot/Q6XW05