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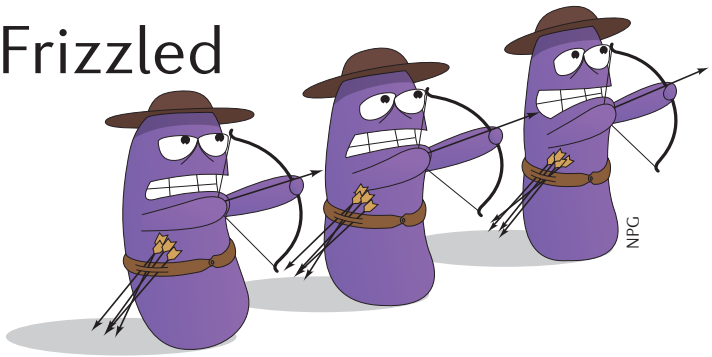
BACTERIAL TOXINS

TcdB aims for Frizzled

> TcdB binds to two different receptors, CSPG4 and FZD, in a cell type-dependent manner



The opportunistic Gram-positive bacterium *Clostridium difficile* causes diarrhoeal disease in individuals following antibiotic treatment and is the leading cause of gastroenteritis-associated death in developed countries. The major virulence factors of *C. difficile* are the exotoxins *C. difficile* toxin A (TcdA; also known as ToxA) and toxin B (TcdB), both of which inactivate RHO GTPases to disrupt the actin cytoskeleton and initiate cell rounding and intestinal epithelial cell death. Although these toxins are known to enter host cells through receptor-mediated endocytosis, how TcdB targets the colonic epithelium is not well understood.

Previous reports implied that chondroitin sulfate proteoglycan 4 (CSPG4) is a TcdB receptor in cell lines, but its role *in vivo* remained to be established. Here, using a genome-wide screen, Tao *et al.* identified members of the WNT receptor Frizzled family (FZD) as physiologically relevant receptors for TcdB in the colonic epithelium.

TcdB contains several potential receptor-binding domains, including combined repetitive oligopeptide repeat (CROP) domains that bind to carbohydrates. The authors carried out two CRISPR–Cas9 mediated screens with full-length TcdB or a truncated version that lacked the CROP domain, respectively, to find host cell factors that are involved in TcdB activity. They identified CSPG4 from the screen with full-length TcdB, which is in agreement with previous reports. For the truncated version of TcdB, they identified the plasma membrane protein FZD2. The authors confirmed their screening result by generating knockout cells; CSPG4-deficient cells showed enhanced resistance to the full-length toxin, but similar sensitivity to the truncated version, whereas *FZD2*$^{-/-}$ cells were less sensitive to the truncated version. Consistently, CSPG4 is only recognized by the full-length toxin, whereas FZD2 is recognized by both the full-length toxin and the truncated toxin that lacks the CROP domain. These findings suggest that CSPG4 and FZD2 are CROP-dependent and CROP-independent receptors for TcdB, respectively.

The authors went on to show that TcdB binds not only to the extracellular domain of FZD2 (known as the cysteine-rich domain (CRD)), but also to the homologous CRDs of FZD1 and FZD7, with similar nanomolar levels of binding affinity. Consistently, triple knockout cells that lacked FZD1, FZD2 and FZD7 were highly resistant to truncated TcdB and also less sensitive to full-length TcdB. The authors further demonstrated that recombinant FZD2–CRD blocked entry of full-length TcdB into *CSPG4*$^{-/-}$ cells, which indicates that FZD receptors mediate the entry of TcdB into cells independent of CSPG4.

Importantly, immunohistochemistry analysis showed that FZD2 and FZD7 are expressed in the human and mouse colonic epithelium, whereas CSPG4 is localized to subepithelial myofibroblasts. Thus, FZD receptors are likely to be the dominant TcdB receptors in the colonic epithelium. Indeed, colonic organoids that were cultured from *Fzd7*$^{-/-}$ mice were more resistant to TcdB than wild-type colonic organoids, and this effect was enhanced in *Fzd7*$^{-/-}$ colonic organoids in which *Fzd1* and *Fzd2* were also knocked down. Moreover, TcdB competed with WNT for binding to FZD receptors, which blocked downstream WNT signalling and led to organoid death.

Finally, to gain *in vivo* insights into the role of FZD receptors in TcdB binding, the authors injected TcdB directly into the lumen of colon segments in mice and showed TcdB binding to and entering the colonic epithelium, whereas co-injection of TcdB with recombinant FZD2–CRD abolished TcdB binding. In addition, fluid accumulation and tissue damage in the colonic epithelium was significantly reduced in *Fzd7*$^{-/-}$ mice compared with wild-type litter mates when challenged with TcdB.

In summary, the findings suggest that TcdB binds to two different receptors, CSPG4 and FZD, in a cell type-dependent manner. Further studies are required to elucidate the role of inhibiting WNT signalling by TcdB binding to FZD receptors in *C. difficile* pathogenesis and whether modulating WNT signalling may provide therapeutic benefit for the treatment of diseases that are associated with *C. difficile* infection.

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ORIGINAL ARTICLE Tao, L. *et al.* Frizzled proteins are colonic epithelial receptors for *C. difficile* toxin B. *Nature* http://dx.doi.org/10.1038/nature19799 (2016)