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Novel receptors for bacterial protein toxins

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While bacterial effectors are often directly introduced into eukaryotic target cells by various types of injection machines, toxins enter the cytosol of host cells from endosomal compartments or after retrograde transport via Golgi from the ER. A first crucial step of toxin–host interaction is receptor binding. Using optimized protocols and new methods novel toxin receptors have been identified, including metalloprotease ADAM 10 for *Staphylococcus aureus* α-toxin, laminin receptor Lu/BCAM for *Escherichia coli* cytotoxic necrotizing factor CNF1, lipolysis stimulated lipoprotein receptor (LSR) for *Clostridium difficile* transferase CDT and low-density lipoprotein receptor-related protein (LRP) 1 for *Clostridium perfringens* TpeL toxin.

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Introduction

During host–pathogen interaction, many pathogenic bacteria produce a cocktail of protein toxins and effectors, which manipulate the eukaryotic host cell behavior for the benefit of the pathogen. Thereby, the pathogens are able to evade host defense mechanisms. This is frequently achieved by covalent modification and/or modulation of regulatory molecules of host cells crucially involved in cell signaling and/or control of innate and acquired immunity defense mechanisms. As most of the host target molecules of toxins and effectors are located inside cells, different mechanisms evolved for entering this cellular compartment.

Direct injection of bacterial effectors into target cells

Some Gram-negative bacteria, like *Salmonella*, *Shigella*, *Pseudomonas* or *Yersinia* directly inject toxic proteins, named effectors or outer proteins, into the cytosol of mammalian cells (for review see [1–3]). This process usually requires a direct contact between bacterium and eukaryotic cell. Injection of the proteins is mediated by a syringe-like apparatus, the type III secretion (T3S) system, which is assembled of several proteins by the pathogen. For example the *Shigella* needle complex with a molecular weight of more than 3 MDa protrudes into the extra-bacterial space and forms a hollow structure with an inner diameter of approximately 2–3 nm [2,4]. This allows the passage of partly folded proteins through this needle into the cytosol of mammalian cells. The injectisome of *Yersinia* is made of 27 Yop secretion (Ysc) proteins, forming a long hollow needle similar to the injectisomes from *Salmonella* and *Shigella* [5–7]. Direct injection of the effector proteins enables them to reach the cytosolic target molecules without any exposure to the body fluid or interaction with the outer cell surface. Therefore, the proteins are protected from the attack of several proteases.

The type IV secretion (T4S) system, which resembles in some aspects the T3S system consists of 12 (e.g. in *Agrobacterium tumefaciens*) to 27 essential proteins (e.g. in *Legionella*), and is used by *Agrobacterium*, *Helicobacter*, *Brucella*, *Bordetella* and *Legionella* to inject effectors into host cells or to release toxin (pertussis toxin) into the environment [8,9]. Moreover, some T4S systems participate in conjugative transfer of plasmids between bacteria. Finally, the most recently deciphered system for delivery of effectors is the type VI secretion system, which is comprised of ~13 conserved proteins and a variable complement of accessory elements. Initially it was identified as a system to target eukaryotic cells but later turned out to be more important for inter-bacterial injections [10].

Uptake of toxins by receptor-mediated endocytosis

Other toxins are secreted by the bacteria into the surrounding body fluid. Some of these toxins directly insert into the plasma membrane of mammalian cells forming a pore (e.g. cholesterol dependent cytolysins [11]). However, most secreted protein toxins act intracellularly and are AB toxins, consisting of a biologically active part (A part) and a part involved in target cell binding and up-take (B part). AB toxins enter the cytosol usually by receptor-mediated endocytosis. The two main pathways that have been described for the uptake of such toxins into the cytosol are discussed below.

Short trip toxins

'Short trip toxins' bind to a cellular receptor, are taken up via endocytosis and are released from acidified endosomes

into the cytosol [12]. Acidification is required for insertion of a hydrophobic part of the toxins into the endosomal membrane and to form a pore allowing transport of the unfolded catalytic part of the toxin into the cytosol. However, pore formation could not be proven for all toxins by now. Probably best understood is the pore-formation by the protective antigen (PA), the binding component of anthrax toxin [13], which is a tripartite toxin. PA is proteolytically activated and then forms a heptameric prepore, which binds up to three molecules of edema factor (EF) or lethal factor (LF), the biologically active components of anthrax toxin. The toxin complexes bind to anthrax toxin receptors (ANTXR1 and ANTXR2) and are taken up by endocytosis. In low pH endosomes, the prepore undergoes a conformational change with membrane insertion and formation of a pore by the mushroom-like structure with a ~10 nm-long stem that spans the membrane. The pore serves as an active transporter and a charge state-dependent Brownian ratchet mechanism is suggested to be responsible for translocation of unfolded EF and LF through the PA-pore [14]. Cellular chaperones may be required for refolding the toxins. Inhibition of common chaperones like HSP90 by geldanamycin and cyclophilins by cyclosporins has been shown to block the uptake/activity especially of ADP-ribosylating toxins [15–17]. For some toxins it has been shown, that only the catalytic part is released into the cytosol. This requires the cleavage of these single chain toxins. The protease activity can be also part of the toxin as shown for RTX toxins and *Clostridium difficile* toxins A and B, which harbor a cysteine protease domain for autocleavage [18,19]. The protease is allosterically activated by inositol hexakisphosphate, which is present at high concentrations in the cytosol [20,21].

Long trip toxins

'Long trip toxins' also bind to a cellular receptor and are taken up by endocytosis. In contrast to short trip toxins they are not released from the endosome into the cytosol but transported backwards to Golgi and ER from which the proteins are released into the cytosol. A prominent example for a long trip toxin is shiga toxin from *Shigella dysenteriae* and certain strains of *Escherichia coli* [22,23]. Shiga toxin is an AB5 toxin, which binds with its pentameric B-subunits to its receptor globotriaosylceramide (Gb3). The toxin complex is taken up by clathrin-dependent and clathrin-independent endocytosis and then retrogradly sorted to the Golgi apparatus. This is a complicated travel, depending on vesicle membrane lipids, and numerous proteins, including p38 and protein kinase Cδ, retromer components, clathrin and dynamin, Cdc42, Rab proteins and several others. The intra-Golgi transport depends on various Rab proteins including Rab6, Rab33b and Rab43. Also the transport from Golgi to ER is variable and may occur via COPI-dependent and COPI-independent routes, depending on Cdc42, microtubules and actin. Eventually, the A component of shiga toxin leaves the ER and reaches the cytosol using the sec61 translocon of the ER-associated protein degradation (ERAD) system [24].

Considering the above mentioned up-take mechanisms, it is obvious that cell targeting and receptor binding is the first pivotal step for the action of the intracellularly acting toxins. In the following, we will describe new findings about toxin receptors.

Methods to identify receptors for bacterial toxins

A crucial task for understanding the pathophysiological action of secreted bacterial toxins is the identification of their cellular receptors. Attempts to identify the toxin receptors by affinity purification from isolated cell membranes often fail, probably, because of miss-orientation of the membrane proteins. However, two of the recently identified receptors for bacterial toxins (ADAM10 for *Staphylococcus aureus* α-hemolysin and LU/BCAM for *E. coli* CNF1) were identified by a classical affinity based co-precipitation assay with a simple but crucial modification of the protocol: the toxin was incubated with living cells [25••,26•]. This guarantees an intact membrane potential and correct folding and functionality of the receptor. The limiting factor of this method is the stability/affinity of the receptor–ligand interaction which has to endure cell lysis and precipitation.

Other assays for identification of toxin receptors are based on the fact that many bacterial toxins lead to cell rounding and detachment or killing of cells. Therefore, genetic screens can be performed by selecting attaching/surviving cell clones, following random gene knockout. This survival assay has been developed using haploid cells and virus-based random gene insertion by the group of Brummelkamp [27•]. Limiting factor of this method is the requirement of the protein randomly knocked out to be not essential for the viability of the cells. Moreover, expression of functional isoforms or redundancy for toxin binding may limit the success of this assay. However, several receptors for bacterial proteins toxins, including LSR as receptor for the *C. difficile* transferase CDT and LRP1 as receptor for *Clostridium perfringens* TpeL have been identified using this method.

New methods have been developed, which allow the knockout of genes also in diploid cells, circumventing the restriction to work with haploid cell lines. The clustered regularly interspaced short palindromic repeat (CRISPR) Cas (CRISPR associated) system constitutes the adaptive immune system present in many bacteria and most archaea for their protection against foreign DNA. Short CRISPR-RNA sequences guide effector complexes formed with Cas proteins for the cleavage of the foreign DNA or RNA (for review see [28•]). The system was further developed that eukaryotic genes can be targeted. Sequence libraries resembling the eukaryotic genome are

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