We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 4

Roles of Cellular Redox Factors in Pathogen and Toxin Entry in the Endocytic Pathways

Jianjun Sun

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/50087

1. Introduction

1.1. Host-pathogen interaction: A process of co-evolution

In the mind of most human beings, microbial pathogens, including viruses, bacteria, and parasites, are the foreign invaders that cause diseases, and sometimes death. Thus, prevention and treatment of infectious diseases, by controlling and eradicating microbial pathogens, has become one of the major tasks of modern medicine. Based on current evolutionary theory, however, most of the pathogens we've seen nowadays are the species that have evolved through a close interaction with their hosts (e.g. humans and other animals). In an even broader sense, the hosts and the pathogens co-evolved through a mutual interaction. During this co-evolution, hosts and pathogens develop specific, intricate systems to either defend or invade, which in turn presents us with an intriguing picture of host-pathogen interaction. Host cells have the ability to defend themselves against the invasion of microbial pathogens. On one hand, host cells use the plasma membrane as a physical barrier that prevents pathogens from entering the cytoplasm, leaving pathogens in the harsh environment of the extracellular milieu, where pathogens are exposed to anti-microbial elements, such as antibodies, cytokines, and complement factors. On the other hand, professional phagocytic cells can engulf microbial pathogens into the phagosomes that later fuse with lysosomes, where reactive oxygen species (ROS), low pHs, and proteases can inactivate and kill the pathogens. Through the course of evolution, however, pathogens have developed effective strategies against such host defense systems. Viruses and obligate intracellular bacterial and protozoan pathogens gain access into phagocytic and non-phagocytic cells through membrane remodeling events, such as phagocytosis and macropinocytosis. In many cases, these membrane-remodeling events are controlled by cytoskeletal rearrangement mediated by pathogen-produced effector proteins (e.g. toxins) [1-3]. Within the phagosomes, pathogens have evolved a variety of mechanisms to protect

themselves from damage by the hostile environment. For instance, pathogens can produce effector proteins to antagonize ROS effects [4], inhibit phagosome maturation, block phagosome-lysosome fusion, and escape from phagosomes [5]. In contrast, extracellular pathogens need to protect themselves from being engulfed by professional phagocytic cells. Such pathogens usually produce toxins to disarm the host cell defense, resulting in inhibition of phagocysis, or in cell killing [6]. Some bacterial toxins are directly translocated into the host cytoplasm through sophisticated 'molecular syringes', such as type III or type IV secretion systems [7-9], which are multi-subunit molecular machines that span the bacterial and host membranes and translocate effectors directly into host cells. Other toxins (e.g. AB toxins) are secreted by bacteria in the vicinity of the host cell and these toxins bind to specific receptors and are taken up by endocytosis [10-13]. Once internalized, bacterial toxins usually take advantage of the hostile environment in endosomes/lysosomes, and they hijack host factors, which enables translocation into the cytosol. For instance, many bacterial toxins utilize endosomal acidification (low-pH) as a trigger for conformational conversion, which activates toxins and/or facilitates release of toxins into the cytoplasm [14]. Moreover, some pathogens and toxins hijack cellular redox factors, thus allowing them enter into the host cells, which will be discussed in detail in this review.

1.2. Endocytic pathways: The portals of entry for microbial pathogens and toxins

Endocytosis is a physiological process of invagination and pinching-off pieces of the plasma membranes, and IT serves as a ubiquitous mechanism that facilitate the internationalization of various particles and molecules from the extracellular milieu into the host cytoplasm. Endocytosis plays a vital role in a diverse range of physiological processes, including maintenance of cellular homeostasis, cell polarity, and uptake of nutrients. Thus, it is not surprising that a great variety of microbial pathogens and toxins have evolved to exploit aspects of this internalization process as portals of entry into host cells. Based on the nature (e.g. size) of the extracellular substrates, endocytosis has been categorized into phagocytosis and pinocytosis. Phagocytosis is involved in engulfment of large particles (e.g. cell debris and bacterial pathogens) by professional phagocytic cells, such as macrophages, monocytes and neutrophils. Pinocytosis, on the other hand, is typically involved in uptake of small particles, such as viruses and bacterial toxins, by non-phagocytic cells. Based on the proteins involved in membrane vesiculation, endocytosis is defined by several types of mechanisms, including clathrin-mediated, caveolin-mediated, lipid raft-dependent, and macropinocytosis, etc. These different mechanisms have been described in recent excellent reviews [6,15-18].

Despite the diversity of membrane vesiculation, upon endocytosis most pathogens and toxins follow one of the two intracellular trafficking pathways (**Figure 1**). In pathway 1, pathogens and toxins travel to the early and late endosomes where some pathogens (e.g. HIV, *Chlamydia*, *Leishmania*) and toxins (e.g. anthrax toxin, diphtheria toxin, botulinum toxin) translocate to the cytosol; while others (e.g. *Listeria monocytogenes*) travel to the lysosomes and are then released into the cytosol. In pathway 2, instead of going through the endo-lysosomal pathway, pathogens and toxins (e.g. SV40, cholera toxin, shiga toxin, exotoxin A) traffic to the Golgi, and from the Golgi to the endoplasmic reticulum (ER), a

pathway in reverse of the classical secretion pathway, called retrograde transport. An ER retention signal (e.g. KDEL) is usually required for this transport to occur [10,13,15,16,18,19].

To date, substantial evidence has suggested that cellular redox factors play an essential role in pathogen and toxin entry through endocytosis. These redox factors include protein disulfide bond isomerase (PDI), γ-interferon inducible lysosomal thiol reductase (GILT), NADPH oxidases (Nox) and some ER-chaperones. These redox factors function at various sites in the endocytic pathways that facilitate pathogen and toxin entry into the cells (**Figure 1**). These events will be discussed in detail in the later sections of this review.

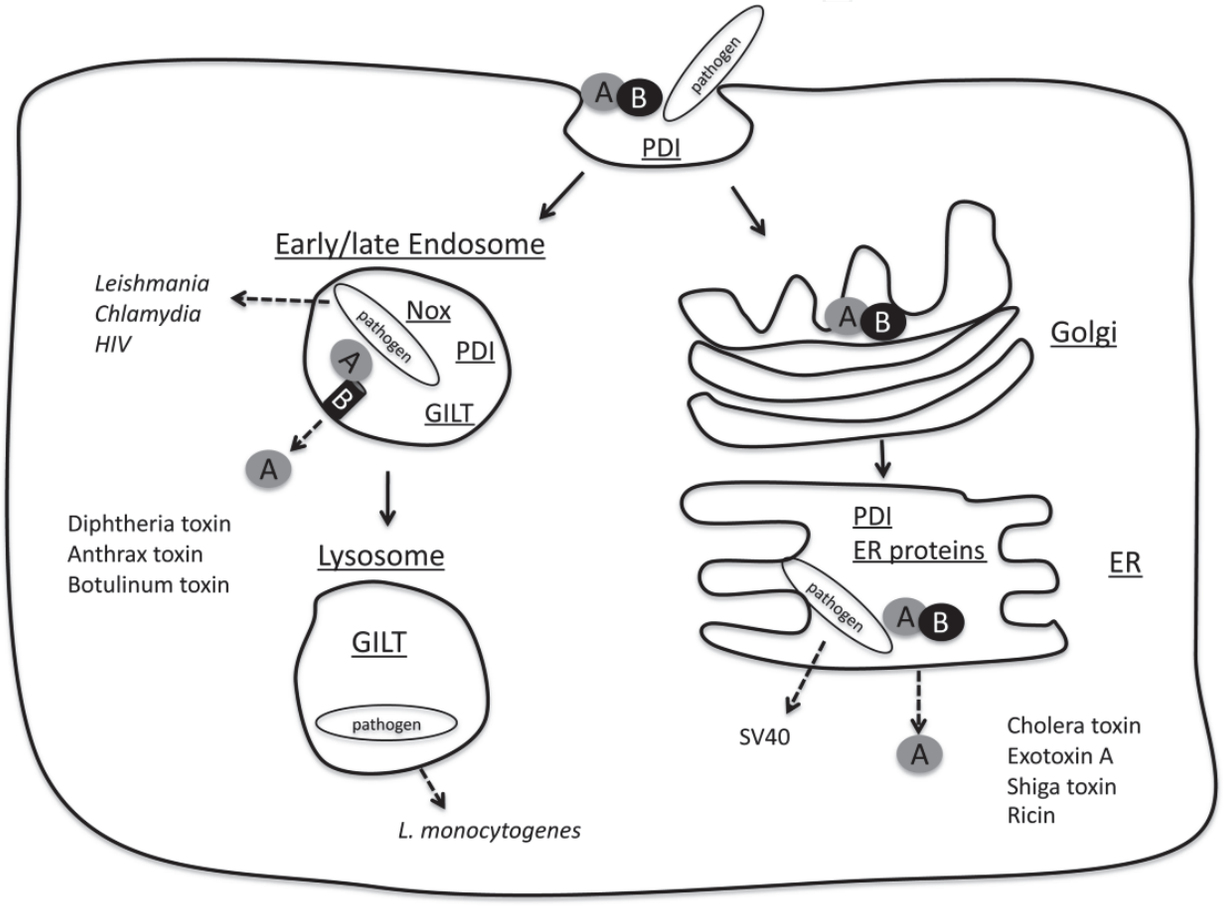

Figure 1. Interaction of cellular redox factors with microbial pathogens and toxins in the endocytic pathways. Microbial pathogens and toxins are internalized into the host cells through endocytosis. Pathway 1 (left): The pathogens and toxins travel to endosomes and/or lysosomes where they translocate to the cytosol. Pathway 2 (right): the pathogens and toxins undergo retrograde transport through Golgi to ER where they are released into the cytosol. In this cartoon, HIV, SV40, Chlamydia, Listeria monocytogenes, Leishmania, are presented as the representatives of microbial pathogens that are discussed in the review. A group of AB toxins are also shown in this cartoon, and some of these toxins are discussed in this review. The cellular redox factors, such as PDI, GILT, NADPH oxidase (Nox), are placed into the various locations of the endocytic pathways according to the current literature. Due to space limitation, the cartoon only depicts a simplified illustration. Solid arrows: intracellular trafficking; dashed arrows: translocation across the membranes.

1.3. Disulfide bond: A redox-controlled switch for pathogen and toxin entry

Disulfide bond, a covalent link between a pair of cysteine residues, plays important roles in protein structure and function. Disulfide bond has a significant impact on thermodynamics of protein folding, as it can stabilize the native conformation by disfavoring the unfolded form. In a naturally folded protein, disulfide bond maintains the protein's integrity by protecting the protein from damage by oxidants and proteolytic enzymes [20-23]. More importantly, in mature, folded proteins, some disulfide bonds can function as molecular switches that can turn "on" or "off" certain protein functions. This is usually accomplished via conformational changes induced by breaking, forming, or the isomerization of the disulfide bonds [24-26]. These diverse, reversible features of disulfide bonds can be readily manipulated by redox factors ranging from small molecular reagents (e.g. reduced/oxidized glutathione) to macromolecular redox enzymes (e.g. oxidoreductases). Moreover, these redox factors are ubiquitously present, yet un-equally distributed in the sub-cellular compartments of eukaryotic cells, which offers a distinct spatial regulation of the thiol/dithiol equilibrium [27,28]. In the course of evolution, microbial infection has become a highly regulated process. Thus, activation at the right time and at the right location is an important factor for pathogens and toxins to successfully cause an infection. Thus, a readily controlled disulfide bond "on/off" switch is evolutionarily favored. It is not surprising that pathogens and toxins use disulfide bonds as redox-controlled switches for invasion.

Increasing evidence has shown that cellular redox factors play pivotal roles in pathogen and toxin entry into the endocytic pathways, particularly through modulating the thiol-dithiol states of pathogen- and/or host-factors. For instance, cellular entry of certain bacterial toxins (e.g. diphtheria toxin [29-33], cholera toxin [34], botulinum neurotoxins [35,36], anthrax toxin [37]) are apparently dependent on the redox states (either reduced or oxidized) of the specific disulfides of either the toxin molecules, or the host receptors. At the same time, protein disulfide isomerase (PDI) [38] and other redox factors, such as gamma-interferon-inducible lysosomal thiol reductase (GILT) [39]) and NADPH oxidase [40-42], have been implicated in regulating the redox states of the disulfides. Similarly, PDI and others are also involved in the entry of numerous pathogenic bacteria (e.g. *Chlamydia* [43,44], *Listeria* [39]), viruses (e. g. HIV [45] and SV40 (46,47]) and parasites (e. g. *Leishmania* [40]) through endocytosis. This review will present the current major findings on the roles of cellular redox factors in pathogen and toxin entry with an attempt to outline the strategies and mechanisms that microbial pathogens and toxins utilize to hijack the cellular redox factors within the endocytic pathways.

2. Cellular redox factors in endocytic pathways

2.1. Redox potentials of endocytic pathways: Oxidizing or reducing?

Eukaryotic cell is organized into several distinct sub-cellular compartments, each of which maintains a distinct redox potential [28]. Relative to the extracellular milieu, which is oxidizing, it is generally believed that endocytic pathways are reducing. This notion is based on the primary function of endocytosis: i.e., the uptake and degradation of foreign and self-

particles including proteins, for which a reducing potential facilitates protein unfolding. This concept has been well supported by the evidence that uptake and activation of some bacterial toxins, such as diphtheria toxin, cholera toxin and *Pseudomonas* exotoxin A, through the endocytic pathways involves reduction of disulfides. The dynamics of disulfide reduction in endocytic pathways have recently been studied with fluorescence resonance energy transfer (FRET) using a fluorescent folate conjugate, in which folate-BODIPY and Rhodamine is linked with a disulfide bond [48]. Reduction of this disulfide bond changes fluorescence from red to green, which allows real-time fluorescence imaging of the reduction in cells. Reduction was observed to occur in endosomes, with a half-life of 6 hours post-endocytosis. Using this experimental setup, reduction did not depend significantly on extracellular surface thiols or redox machinery within lysosomes or Golgi. The yielded products were sorted into different endosomes and trafficked in different directions. This excellent FRET design ensures an accurate assessment of disulfide bond reduction during normal vesicle trafficking in living cells and demonstrates that reduction occurs in the endocytic pathways. In fact, the presence of distinct redox potentials between the oxidizing extracellular space and the reducing endocytic pathways has created interest in disulfide bonds as a potential tool for drug delivery. For example, the disulfide-based bioconjugation approach has become a popular conjugation method applied in a variety of cellular drug delivery systems. Successful applications of thiol-based conjugation resulted in targeted delivery and enhanced cytosolic delivery, improved pharmacokinetics, and increased stability of the drugs [49-52].

While it is generally accepted that endocytic pathways are reducing, there is also evidence suggesting the contrary. In another independent study, a disulfide linker cleavage assay was developed whereby rhodamine red was linked to an anti-HER2 antibody through a peptide linker containing a disulfide bond [53]. Cleavage of the disulfide bond would release self-quenching of the fluorophore. In breast carcinoma SKBr3 cells, no linker cleavage was observed, as detected by fluorescence dequenching upon internalization. In contrast, the conjugate did display fluorescence dequenching when it was diverted to the lysosomal pathways, which could be an effect partly due to proteolytic degradation rather than disulfide reduction. More convincingly, the redox potentials of endocytic compartments were measured directly by expressing a redox-sensitive variant of GFP fused to various endocytic proteins. The results showed that recycling endosomes, late endosomes, and lysosomes were not reducing, but rather oxidizing and to a level comparable with conditions in the ER.

In summary, the redox potentials in the endocytic pathways appear to vary accordingly to different ligands, different cell types, and different physiological/pathological conditions. For instance, NADPH oxidase, the major enzyme that catalyzes the production of reactive oxygen species (ROS), is regulated by hormone or growth factors in normal cells, but it is constitutively activated in cancerous cells such as HeLa and hepatoma cells [54]). It is well known that in professional phagocytic cells NADPH oxidase is activated upon pathogen infection and that it produces ROS within the phagosomes, a process called oxidative burst that is described below.

2.2. Oxidative burst and NADPH oxidase in professional phagocytic cells

When professional phagocytes recognize pathogen-associated molecular patterns that are located on microbial pathogens, the phagocytes will internalize them through phagocytosis and activate a strong bacterial killing mechanism, called oxidative or respiratory burst, which is marked as an abrupt increase of superoxide formation within the phagosomes [55]. This process is mainly catalyzed by NADPH oxidase, a membrane-associated enzyme complex that is located on the phagosome membrane and generates superoxide (O_2^-) by the one-electron reduction of oxygen, using NADPH as the electron donor. Assembly and activation of NADPH oxidase requires phosphorylation of its subunits and translocation of cytosolic components to the plasma membrane [56,57]. The superoxide anion generated is enzymatically converted to hydrogen peroxide by superoxide dismutase (SOD). The generated hydrogen peroxide can serve as a precursor for hydroxyl radical (•OH) generation via a Fenton-like reaction. Hydrogen peroxide then enters cells and forms hydroxyl radical that can kill many microorganisms by reacting with different macromolecules, including proteins and DNA. Except for killing microorganisms directly, ROS can also work as secondary messengers in many signaling pathways within phagocytic cells, which promote actions of other antibacterial agents and stimulate inflammation. However, chronic inflammation induced by ROS may damage the host tissue and induce apoptosis of the phagocytic cells [56-58].

2.3. Protein disulfide isomerizes and other oxidoreductases (e.g. GILT)

2.3.1. Protein disulfide isomerase (PDI)

Enzymatic activities and sub-cellular localization:

PDI is a ubiquitous dithiol/disulfide oxidoreductase chaperone belonging to the thioredoxin oxireductase superfamily. There are around 20 PDI homologues, and the structure and function of eukaryotic PDIs have been covered in recent excellent reviews [59,60]. The prototypic PDI contains 5 domains ordered as a-b-b'-a'-c, in which two thioredoxin-like motifs (CXXC) are located in the domains a and a', respectively. The primary function of PDI is to promote protein oxidative folding in the ER. The PDI redox-domains catalyze three redox reactions: reduction (breaking disulfide bond), oxidation (forming disulfide bond), and isomerization (exchanging disulfide bond). Independently of its redox activity, PDI also functions as a chaperone, which requires its ATPase and Ca^{2+} activities [60-62]. PDI contains a KDEL sequence at the domain c, which facilitates its retention in the ER lumen, and PDI cycles between ER and cis-Golgi through the KDEL receptor. Despite its KDEL sequence and ER retention mechanism, PDI is also involved diverse intracellular trafficking processes and is even secreted outside cell and can be found at the cell surface [59]. The cell-surface PDI is thought to localize on the plasma membrane by attachment to lipids, glycans and integral membrane proteins [59,63]. Unlike other members of thioredoxin family, PDI is not normally found in the cytosol. In addition to catalyzing protein oxidative folding in the ER, PDI has been shown to be actively involved in many other processes, such as ER-associated

degradation, trafficking, calcium homeostasis, antigen presentation and host-pathogen interaction [60].

ER-located PDI:

The ER-located PDI plays an important role in host-pathogen interactions, particularly in antigen presentation and ER-mediated phagocysis of intracellular pathogens. Antigen presentation occurs through two pathways: the exogenous pathway and the endogenous pathway. In the exogenous pathway, the antigens from extracellular pathogens (e.g. fungi, bacteria, and parasites) are captured and processed in the phagosome/lysosome compartments within the long-lived antigen presenting cells (e.g. macrophages and dendritic cells) and then form complexes with MHC class II. The antigen complexed with MHC-II is then presented on the cell surface and subsequently recognized by helper CD4+ T cells. In endogenous pathway, self cell antigens and viruses synthesized within cells are degraded by the proteasome and then form complexes with MHC class I, which are presented on the cell surface and recognized by cytotoxic CD8+ T cells. The two pathways sometimes overlap and antigens can be presented by both MHC class I and class II. These include some intracellular bacterial and parasite pathogens that pass or live in the phagosomes, such as *salmonella typhimurium*, *Mycobacterium tuberculosis*, *Leishmania spp* and *Trypanosoma cruzi*. As a part of the protein folding machinery in the ER, PDI has been shown to directly regulate antigen processing of the MHC class I complex [64,65] .

Phagocysis is the main mechanism for the professional phagocytes to internalize large pathogens. A recent study has found that fusion of the ER with the plasmalemma underneath phagocytic cups is a source of membranes for the phagosome formation within macrophages. The ER-associated chaperones, including PDI, are involved in this intense membrane remodeling process [66,67]. Of particular interest to this review article is the ER-associated PDI that is involved in the translocation of the toxins (e.g. cholera toxin) and pathogens (e.g. SV40 and *Leishmania*) from the ER to the cytosol.

Secreted and cell-surface associated PDI:

While PDI enzymes are predominantly located in the ER where they act as chaperones and facilitate protein folding, they also can be secreted extracellularly and located on the cell surface. The secreted PDI and the cell surface PDI can be identified by the use of antibodies or specific ligands. Since PDI is a soluble protein, PDI is associated with the cell surface probably through electrostatic interaction with other surface-located proteins, peptides or lipids. The thioredoxin sites of PDI appear to be involved in the reducing activity of the cell exterior where protein disulfide bonds are reduced or reshuffled. Recent research has shown that the level of cell surface thiols positively correlates to the amount of cell surface PDI (68). Consistent with the fact that the thiol groups of cell surface proteins are involved in cell adhesion, PDI thioredoxin activity plays an important role in cell adhesion. In leukocyte adhesion, PDI reducing activity maintains the adhesion protein L-selectin in a particular conformation (disulfide breaking) on the cell membrane that is not accessible to proteolytic enzymes. Inhibition of PDI leads to a conformational change in L-selectin (disulfide forming), and the subsequent cleavage of L-selectin, which results in lose of cell adhesion

[22]. PDI is also involved in the integrin-mediated platelet adhesion. Integrin receptors contain "open" and "close" conformations that represent the "on" and "off" states of ligand binding, respectively. It has been suggested that PDI regulates the open and close conformations through reduction and/or reshuffling of the disulfide bonds in integrins [23,69,70]. The cell-surface PDI not only plays important roles in physiological processes, it is also involved in pathological events, particularly in the entry of pathogens and toxins into host cells as discussed in detail in this review.

PDI regulation of NADPH oxidase:

NADPH oxidase is not only the main source of ROS production during oxidative burst, it also has been described as another cell surface-associated protein with disulfide–thiol interchange activity [54,71]. NADPH oxidase proteins on the mammalian cell surface exhibit two different activities, oxidation of hydroquinones (or NADH) and protein disulfide-thiol interchange. Protein thiols on the membranes were measured by reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB; Ellman's reagent) and the results suggested that protein disulfides may be the natural electron acceptors for NADH oxidation within plasma membrane vesicles. Protein disulfides of the membranes were reduced, with a concomitant stoichiometric increase in protein thiols in the presence of NADH, while the increase in protein thiols was inhibited in parallel to the inhibition of NADH oxidation.

It is not clear how NAD(P)H oxidase catalyzes disulfide-thiol exchange. Interestingly, PDI has been shown to regulate NAD(P)H oxidase activity. In rabbit aortic smooth muscle cells PDI was found to be co-localized and co-immunoprecipitated with the oxidase subunits p22, Nox1, and Nox4. Inhibition of PDI using PDI antagonism, such as bacitracin, scrambled RNase, neutralizing antibody, or antisense oligonucleotide, resulted in inhibition of the oxidase activity, which suggests that PDI closely associates with NAD(P)H oxidase, and acts as a novel redox regulator of the oxidase [72]. Later, the PDI-mediated regulation of NADPH oxidase activity was confirmed, showing that PDI plays a role in organizing NADPH oxidase activation in a variety of physiological/pathological events [41,42,73], of which PDI association with NADPH oxidase is required for phagocytosis of Leishmania chagasi promastigotes in macrophages as is discussed later in this review.

2.3.2. Gamma-interferon (IFN-γ)-inducible lysosomal thiol reductase, GILT

Proteins internalized via the endocytic pathways are usually degraded in lysosomes, where proteolysis is facilitated by protein denaturation induced by acidic condition and by reduction of inter- and intra-molecular disulfide bonds. While high concentration of cysteines was claimed to be the physiological reducing agent in lysosomes, this small molecular reducing agent alone appears inefficient in disulfide reduction in an acidic environment, since disulfide reduction requires deprotonantion of thiols, which is not favored by acidic environments. Thus, the presence of redox enzymes within the acidic cellular compartments had been postulated for a long time [74,75]. Known redox enzymes, such as thioredoxin reductase and glutathione reductase, normally function in neutral pH environments, so they are obviously not the likely candidates. The enzymes that catalyze the

reduction within acidic compartments had been elusive until recently when GILT was identified as the first thiol reductase optimally active at low pH (4.0 – 5.5) [76,77]. GILT is expressed constitutively in antigen-presenting cells, in which it is synthesized as a 35-kDa glycoprotein precursor containing a mannose-6-phosphate signal sequence and is co-localized with early endosomes. The amino- and carboxyl-terminal propeptides are cleaved in the early endosomes and the 30-kDa mature enzyme is delivered by the mannose 6-phosphate receptors through the endocytic pathways to late endosomes and lysosomes. The mature enzyme is found in MHC class II-containing compartments, where it catalyzes disulfide bond reduction to facilitate antigen processing [78]. GILT can also facilitate the transfer of disulfide-containing antigens into the cytosol, enhancing their cross-presentation by MHC class I [79]. Compared to other members of the thioredoxin family, GILT possesses seminar yet distinctive enzymatic characteristics. GILT has a similar catalytic active site (-C-X-X-C-), but does not have the common motif (-C-G-H/P-C-) that is shared by the members of the thioredoxin family. GILT shows optimal activity at pH 4.0-5.5, while the other members of the family function optimally at neutral pH. Moreover, GILT requires a reducing agent, such as DTT or cysteine (but not glutathione) to regenerate and retain its activity in vitro [76], which is consistent with the potential function of cysteine in the acidic compartments for disulfide reduction.

In addition to being constitutively expressed in antigen presenting cells, GILT is induced and up-regulated by interferon-r (IFN-γ) in other cell types via signal transducer and activator of transcription 1 [80]. GILT has been found to accumulate in macrophage phagosomes as they mature into phagolysosomes [81]. Most interestingly, GILT is a critical host factor for Listeria monocytogenes infection [39], as is discussed later in this review.

3. Roles of redox factors in entry of bacterial toxins through endocytosis

3.1. AB toxins and interchain disulfide bond

A number of proteins produced by bacterial pathogens are highly toxic to mammalian cells due to their ability to enter the cytosol and attack essential cellular metabolic and/or signal transduction pathways. These toxic proteins mostly belong to AB toxin family (82). AB toxins contain two structurally and functionally distinctive moieties: an enzymatically active A moiety that normally modifies a cellular target upon entry into the cytosol, leading to cell death or other pathological effects; and a binding/translocation B moiety that binds to cell surface receptors and translocates the A moiety into the cytosol. Commonly, an AB toxin is synthesized and secreted from the pathogen as an inactive form. This inactive precursor is activated through a proteolytic cleavage performed by either a host or a pathogen protease at a region between two cysteine residues. The cleavage results in a di-chain toxin molecule with the A moiety and the B moiety linked by a disulfide bond (**Figure 2**). AB toxin-mediated intoxication of the host cells starts with B moiety binding to the cell surface receptors, followed by receptor-mediated endocytosis. Some toxins, such as anthrax toxin, diphtheria toxins, and Clostridial neurotoxins, traffic to endosomes, where acidification triggers conformational change on B moiety that forms a protein conductive channel/pore

on the endosomal membranes and translocates A moiety into the cytosol. Other toxins, including shiga toxin, cholera toxin, exotoxin A, will travel through a retrograde transport pathway to arrive at the ER. There, A moiety is released into the cytosol (**Figure 1 and 2**). In either of these two intracellular trafficking schemes, it is presumed that the interchain disulfide that links A and B moieties must be cleaved prior to translocation of A moiety into the cytosol. While the mechanism of disulfide reduction-dependent translocation is not fully understood, and may be toxin-specific, current research has provided evidence that cellular redox factors play essential roles in toxin translocation by mediating reduction of the interchain disulfide.

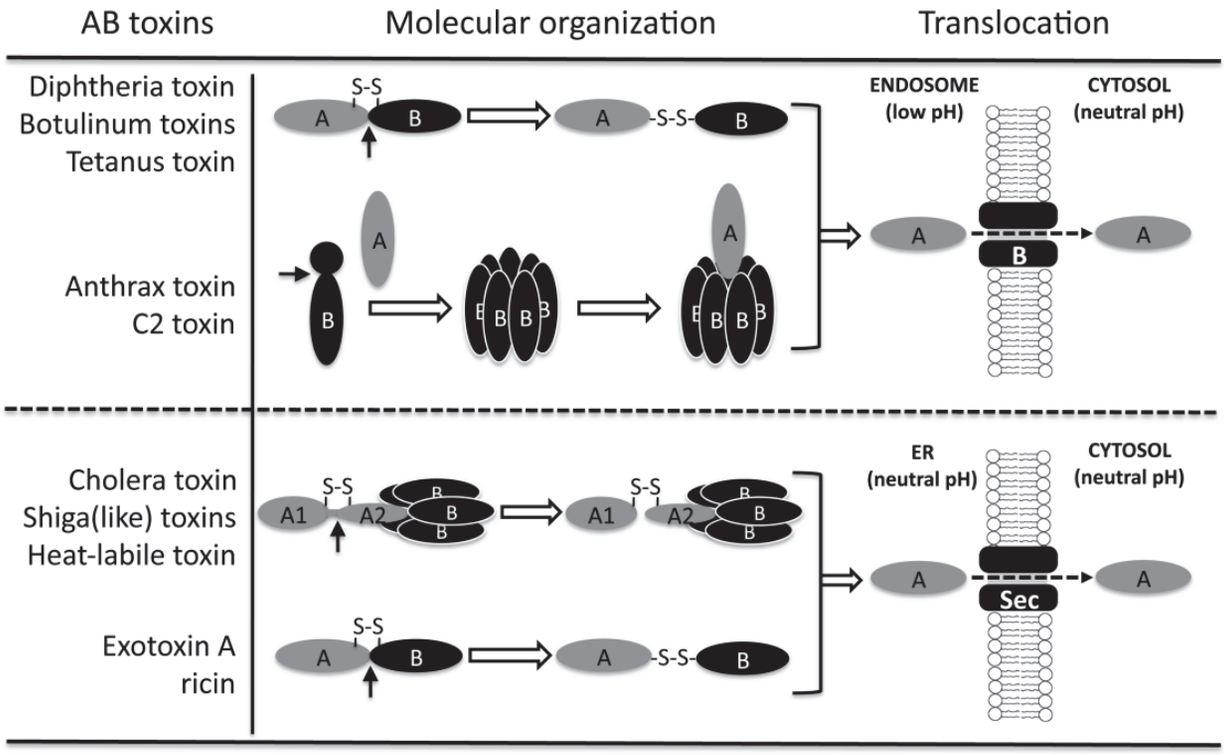

Figure 2. Molecular organization and translocation of AB toxins. Based on molecular organization (presence or absence of interchain disulfide bond) and sites of membrane translocation (endosome or ER), AB toxins are divided into four groups as indicated in this cartoon, with the representative toxins listed in each group. Group 1: the toxins (e.g. diphtheria toxin) are produced as a single-polypeptide precursor. Activation requires a proteolytic cleavage to generate a dichain molecule that is linked by an interchain disulfide bond. The toxins travel to the endosomes where the B moiety forms a pore on the endosomal membranes and translocates the A moiety into the cytosol. Group 2: the A and B moieties of the toxins (e.g. anthrax toxin and C2 toxin) are produced as separate proteins. The B moiety is activated by proteolytic cleavage and assembles into a heptameric complex that recruits the A moiety. Within the endosomes, the B moiety forms a pore on the endosomal membranes and translocates the A moiety into the cytosol. While there is no interchain disulfide bond, the disulfide bonds of anthrax toxin receptor are required for the toxin translocation, which is discussed in this review. Group 3: The proteolytic cleavage occurs in the A moiety of the toxins (e.g. Cholera toxin), resulting in two fragments, A1 and A2, that are linked by a disulfide bond. In the ER, A1 is translocated with the assistance of the ER machinery (e.g. Sec) to the cytosol, which requires the reduction of the disulfide bond. Group 4: the toxins (e.g. exotoxin A) share a similar structural organization with the toxins in Group 1, but translocation occurs in the ER, instead of the endosomes.

3.1.1. Diphtheria toxin and cell-surface PDI

Diphtheria toxin (DT) is secreted as a single polypeptide chain of 535 residues (58 kDa) from toxigenic strains of Corynebacterium diphtheriae [83]. DT is activated by a proteolytic cleavage that is catalyzed by the cellular protease furin, which results in two protomers (DT-A, 21 kDa; DT-B, 37 kDa) that are linked by a disulfide bridge (**Figure 2**). DT-A is an ADP-ribosyltransferase that ADP-ribosylates elongation factor 2 (EF-2) in the cytosol. DT-B is responsible for cell binding and translocation of DT-A to the cytosol. The receptor-binding domain at the C-terminal half of DT-B binds to the cell surface receptor (heparin-binding EGF-like growth factor) and enters the cell through the receptor-mediated, clathrin-dependent endocytosis. Within the endosomes, the acidic pH triggers a conformational change on DT-B, leading to the exposure of the hydrophobic domains and an increased tendency to interact with the membrane lipids. Thus, DT-B inserts into the membranes and forms a cation-selective channel that translocates DT-A into the cytosol, where DT-A inhibits protein synthesis by ADP-ribosylation of EF-2 [84].

An earlier study showed that membrane-impermeant sulfhydryl inhibitors (DNTB and pCMBS) markedly inhibited DT cytotoxicity, an effect that was not due to inactivation of unbound DT, inhibition of endocytosis, or impairment of endosomal acidification [32]. This indicated that the reductive cleavage of DT's interchain disulfide bond mediated by the cell surface sulfhydryls is required for the DT cytotoxicity. A later independent study of DT-mediated Vero cell intoxication showed that reduction of the single interchain disulfide bond is the rate-limiting step of the entire intoxication process, and this reduction occurred only after the toxin had passed through a low pH triggered structural change on DT-B in an early endosome [30]. Together, these studies suggested that the reductive activation of DT is catalyzed by sulfhydryls that are originally present at the cell surface and through vesiculations, become situated at the inner face of nascent endosomes. Sulfhydryl groups blocked at the cell surface will remain blocked in primary endosomes whose fluid volume still contains inhibitors. More interestingly, specific PDI inhibitors, bacitracin and anti-PDI antibodies, effectively inhibited DT-mediated cytotoxicity [33], suggesting that cell-surface PDI is involved in the translocation of DT through the reduction of the interchain disulfide bond. The PDI-catalyzed reduction appears to be specifically restricted to the site of interchain disulfide bond, but not others. In an earlier study, intramolecular disulfide bonds were generated in the DT-A domain by introducing double cysteine residues [29]. During endocytosis, the interchain disulfide bond was found to be reduced, while the engineered intramolecular disulfide bonds remained intact, which inhibited DT-A unfolding and membrane translocation. This target-specific reduction of the interchain disulfide bond implicates the existence of enzymatic specificity.

More recently, however, an assay of measuring the in vitro delivery of DT-A from the lumen of purified early endosomes to the external milieu has shown that cellular thioredoxin reductase activity plays an essential role in the cytosolic release of the DT-A, suggesting that other cellular redox enzymes, except for PDI, may also be involved in DT translocation [85].

3.1.2. Cholera toxin and ER-located PDI

Cholera toxin (CT), produced by the bacterium *Vibrio cholerae*, acts on intestinal epithelial cells in mammals to induce massive salt and water secretion, causing severe diarrhea [86,87]. CT consists of one A subunit, an ADP-ribosyltransferase that targets heterotrimeric Gs proteins, and five B subunits that bind to the cell surface receptor, ganglioside GM1. The A subunit is cleaved by a bacterial endoprotease to form two fragments, A1 and A2, that are linked by a disulfide bond (**Figure 2**). Reduction of the disulfide bond is required for translocation of A1 fragment from ER to the cytosol, where it ADP-ribosylates Gs. ADP-ribosylation of Gs results in a constitutive activation of adenylyl cyclase and an increase of cAMP levels. The A2 fragment bores through the center of the B subunit ring with a C-terminal KDEL (Lys-Asp-Glu-Leu) sequence that protrudes outwards. CT enters cells through clathrin-independent endocytic pathways, primarily through the cholesterol-rich plasma membrane domain caveolae. The ER retrieval KDEL sequence located on the A2 fragment leads the toxin through the retrograde transport pathway from Golgi network to the ER. Within the ER the A1 fragment is unfolded, released from the rest of the toxin, and translocated across the ER membrane to the cytosol [88]. It has been reported that translocation occurs through the Sec61 channel and utilizes ER-associated degradation (ERAD), which is a physiological process for retro-translocation of mis-folded proteins into the cytosol.

Although the mechanism of A1 translocation from ER into the cytosol is not clear, it is presumed that the toxin in the ER must undergo the following events: subunits must be disassembled, the disulfide bond must be reduced, and the A1 fragment must be unfolded in order to be translocated through the Sec61 channel. However, the ER is the compartment where proteins are folded, assembled and disulfide bonds are formed. This apparent paradox has raised the question of how the disassembly and unfolding of the toxin occur in such an unfavorable environment. Because of the reported role of PDI in the reduction of the interchain disulfide bond of DT, inhibitors of cell-surface PDI, such as bacitracin, DTNB, and anti-PDI antibodies, were tested [89,90]. In contrast to DT, these inhibitors had no effect on CT cytotoxicity in intact cells, suggesting that the reduction of CT does not occur at the cell surface or in the early endocytic pathways. In the presence of Triton X-100, however, these inhibitors significantly inhibited CT activity. Further study revealed that the A1 fragment is co-localized with PDI in the ER-derived membrane fraction, suggesting that PDI is the redox factor that catalyzes the CT reduction at the ER [90]. Subsequently, an excellent study showed that protein disulfide isomerase (PDI) in the ER lumen functions to disassemble and unfold the toxin once the A chain is cleaved [34]. In this reaction PDI acts as a redox-driven chaperone: that is, in the reduced state, it binds to the A chain and unfolds it, while in the oxidized state it releases the substrate. Moreover, the PDI-mediated CT translocation in the ER appears to be coordinated with a series of ER proteins, such as Ero1, Erp72, and Derlin-1 [34,91]. Together, these studies have revealed a highly coordinately operations exploited by PDI and other ER chaperone proteins in CT translocation.

3.2. Membrane translocation of Botulinum neurotoxins and anthrax toxin: two stories without cellular redox enzymes?

3.2.1. Botulinum neurotoxins: Interchain disulfide bridge remains intact throughout the translocation

Botulinum neurotoxins (BoNTs), produced by various strains of the spore-forming bacteria (e.g. *Clostridium botulinum, C. butyricum, and C. barati*), are known as the most poisonous toxins in nature [11,92]. To date, seven antigenically distinguishable BoNTs (designated from A to G) have been identified in research. The seven serotypes of BoNTs in combination with another neurotoxin of clostridia, tetanus neurotoxin (TeNT) from *C. tetani*, make up the clostridial neurotoxin family. BoNTs cause flaccid paralysis by targeting to peripheral motorneuron, while TeNT causes opposite symptoms by acting in inhibitory interneurons [82]. Despite their extreme cytotoxicity, when applied with an appropriate dose BoNTs could act as effective drugs because of their powerful neuroparalytic activity. In fact, BoNT serotype A is the first biological toxin that has received FDA approval for treatment of human diseases, such as cervical torticollis, strabismus, and dystonias.

Each BoNT is synthesized as a ~150 kDa single chain protein. This single chain precursor is subsequently cleaved into a di-chain molecule, in which the ~50 kDa light chain (LC) and the ~100 kDa heavy chain (HC) remain linked via a single disulfide bond [93,94] (**Figure 2**). The HC is composed of an N-terminal translocation domain and a C-terminal receptor-binding domain. The receptor-binding domain binds to both gangliosides and protein receptors on the cell membrane and the toxin is internalized through a receptor-mediated endocytosis [95-97]. Within the acidic endosomes, the translocation domain undergoes a conformational change to form a pore on the endosomal membranes and translocates LC to the cytosol. In the cytosol, LC, a zinc endoprotease, specifically cleaves SNARE proteins, resulting in inhibition of synaptic exocytosis [13,87,92,98].

Apparently BoNTs share similar features with DT in terms of molecular organization (e. g. The A and B moieties are linked by an interchain disulfide bond) and the mode of entry into the host cells (e.g. receptor-mediated endocytosis, low-pH-induced pore formation and translocation). Despite of these apparent similarities, however, the interchain disulfide bridge of BoNTs appears to dictate a different mechanism in translocation, relative to DT. The dynamics of the toxin translocation was elegantly examined in a single channel/single molecule assay using patch clamp recording on the cell membranes [36,99]. The disulfide bond needs to remain intact throughout LC translocation, and premature reduction of the disulfide bond even after channel formation or within the lipid bilayer arrests translocation. Consistent with this result, addition of the reducing agent TCEP before the toxin endocytosis inhibited the proteolytic activity of BoNT/B in human neuronal SHSY-5Ycells (35). It is hypothesized that the disulfide bridge between LC and HC is intact in the low pH, oxidizing environment of the endosomal lumen. Once LC is translocated across the membranes, the disulfide bridge is reduced in the neutral pH, reducing cytoplasm, which results in LC release [92]. Moreover, in an in vitro planar lipid bilayer system, BoNT can conduct the translocation of LC into the trans compartment without the presence of

additional cellular factors [10] Together, these results strongly support a model that HC-LC complex embedded in the membrane is a transmembrane chaperone. The HC chaperone activity driven by a pH gradient across the endosome prevents aggregation of the LC in the acidic vesicle interior, maintains the LC in a unfolded conformation during translocation, and releases it after it refolds at the neutral-pH cytosol. In this model, the reduction of interchain disulfide bond only occurs after the LC translocation to the cytosol [36]. Although in planar lipid bilayer, reduction of the disulfide bond occurs without additional factors. Presently, it is not clear if any cytosolic redox enzymes facilitate this process in vivo.

3.2.2. Anthrax toxin: Pore formation and translocation require intact disulfide bonds of the receptors

Anthrax toxin, produced by *Bacillus anthracis*, is responsible for the major symptoms of anthrax disease [14]. Anthrax toxin is a tripartite AB toxin consisting of two A moieties, lethal factor (LF, 90 kDa) and edema factor (EF, 90 kDa), and one B moiety, protective antigen (PA, 83 kDa). Anthrax toxin-mediated intoxication of host cells starts with PA binding to the cell surface receptors. Currently, two receptors for PA have been identified: anthrax toxin receptor 1 (ANTXR1; or, tumor endothelial marker 8, TEM8) and anthrax toxin receptor 2 (ANTXR2; or, capillary morphogenesis protein 2, CMG2) [101-103]. The extracellular domains of the two receptors share over 60% of sequence homology. Both contain a conserved von willebrand factor A (VWA) domain, which binds to PA (104), and a newly defined immunoglobulin-like (Ig-like) domain [37]. Upon binding to the cell surface receptors, PA83 is cleaved by the cellular protease furin into PA63 and PA20. The PA63 self-assembles into a heptameric or an octameric complex, called prepore, to which LF and EF bind. Endocytosis of anthrax toxin is a highly regulated event, in which S-palmitoylation of the receptor cytoplasmic tail plays a role to prevent constitutive endocytosis of the toxin. The toxin-receptor complex is redistributed on the plasma membrane from the glycerophospholipidic regions to the specialized domains of lipid rafts, where receptor ubiquitination triggers endocytosis. The toxin-receptor complex is supposedly internalized into the cell through the clathrin-mediated endocytosis [12,105,106]. Within the endosomes, acidification triggers conformational change on PA and converts the prepore into a pore on the endosomal membranes, through which LF and EF are translocated to the cytosol. There, LF, a zinc-dependent protease, cleaves MAP kinase kinases, which results in lethality of the host cells; EF, an adenylate cyclase, increases cellular cAMP level, which causes edema.

Unlike many other AB toxins that are produced as a single polypeptide chain and require proteolytic cleavage to generate A and B moieties linked by an interchain disulfide bond, anthrax toxin is produced as the three separate polypeptides: PA, LF and EF (**Figure 2**). Most interestingly, anthrax toxin has no cysteine residue out of the total 2373 residues in the three proteins. This unique "cysteine-free" feature appears to exclude the possibility of exploiting redox-controlled "thiol-dithiol exchange" as a potential mechanism that regulates anthrax toxin translocation. However, our recent study showed that the disulfide bonds in the Ig-like domain of ANTXR2 were required for anthrax toxin pore formation and membrane translocation [37]. Reduction of the disulfide bonds significantly blocked anthrax

toxin pore formation on the liposomal membrane and plasma membranes evidenced by the release of K+ ion, and it also blocked translocation of a model substrate across the cell membranes. More recently, purified PDI was shown to facilitate the refolding of the recombinant extracellular domain of ANTXR2 (Sun, unpublished data), indicating that the receptor disulfide bonds may be subjected to redox regulation by PDI or PDI-like oxidoreductases. The mechanism of anthrax inhibition induced by reduction of the receptor disulfide bonds is not yet fully understood.

Based on the available data, one can hypothesize that anthrax toxin translocation must require an oxidative environment or factors that favor the receptor disulfide bond formation, instead of reducing it as of DT, CT and other toxins. But how might the endosomes maintain a disulfide bond favorable environment? In the early stage of anthrax infection, macrophages are activated by the components of *Bacillus anthracis* and launch a strong oxidative burst within the phagosomes for bacterial killing immediately after phagocytosis of bacteria [4]. In addition, it is reported that anthrax is expressed and plays an essential role in several stages of infection, including the very early stage, in the newly germinated spores within macrophages [107,108]. An earlier study has also shown that anthrax lethal toxin stimulated an oxidative burst in macrophages and induced cytolysis [109]. The toxin-induced macrophage lysis was dependent on the ability of the macrophages to mount an oxidative burst and was inhibited by exogenous antioxidants. Based on the above evidence and given the fact that anthrax toxin requires a redox environment that favors receptor disulfide bond formation for translocation, an intriguing hypothesis is that the bacterium and the toxin stimulate an oxidative burst within the host cells so as to ensure anthrax toxin translocation. Consistent with this hypothesis, *B. anthracis* has evolved mechanisms defending itself against oxidative stress, including superoxide dismutases, peroxidases, and catalases, all of which suppress the damaging Fenton reaction catalyzed by reactive oxygen or nitrogen species [4,110,111]. Moreover, as mentioned above, the unusual "cysteine-free" feature of anthrax toxin might have been selected through evolution permitting the toxin to be exempt from the damaging thiol-modifications caused by oxidative stress. In summary, Bacillus anthracis and anthrax toxin may have evolved the ability to subvert oxidative burst, the host defense mechanism, for their own benefit. Instead of being damaged by oxidative burst, anthrax toxin takes advantage of its oxidizing power that maintains integrity of the receptor disulfide bonds for toxin translocation.

3.3. Thiol-activated cytolysins and GILT: Reduction of undecapeptide cysteine

Thiol-activated cytolysins are a group of pore-forming toxins that are secreted by taxonomically diverse species of gram-positive bacteria, responsible for life-threatening infections [112]. Currently, over 20 family members have been identified, including listeriolysin O (LLO) from *Listeria monocytogenes* that causes meningitis and abortion; perfringolysin O (PFO) from *Clostridium perfringens* that causes gas gangrene; and pneumolysin (PLY) from *Streptococcus pneumoniae* that causes pneumonia and meningitis. Each of these toxins is produced as a single polypeptide chain with molecular weight ranging from 50 – 80 kDa and shares high degree of sequence similarity ranging from 40 –

80%, suggesting a close structural and functional relation between them. In deed, the toxins share a common mode of action. All of the thiol-activated cytolysins are produced as water-soluble monomers and use cholesterol as cell surface receptors. Upon binding to the cholesterol, these toxins undergo cholesterol-dependent oligomerization and membrane insertion, leading to membrane damage. Thus, they are also referred to as cholesterol-dependent cytolysins, CDCs. The diameters of the ring-shaped pores can exceed 150 Å, making these toxins a widely used tool as membrane-permeabilizing agents in cell biology. Not surprisingly, all the available crystal structures of the toxins share an elongated, four-domain structure. Upon pore formation, the toxins undergo dramatic domain rearrangements that have been recently revealed by excellent fluorescence measurements and by cryo-electronic microscopy study [113-115].

Thiol-activated cytolysins share another critical common feature, that is, the toxins are activated by reducing agents and suppressed by oxidation [116,117]. The requirement of thiol-reduction for the toxin activation appears to rely on a single cysteine residue located in a highly conserved undecapeptide (also known as tryptophan-rich region) in domain 4 of the toxins [118,119]. The undecapeptide cysteine is the only cysteine present in the primary structure of the secreted toxins. Irreversible oxidation of this cysteine inhibited cytolytic activity, suggesting that this cysteine plays a central role in the cytolytic mechanism. Recently, GILT was found to be a critical host factor for *Listeria monocytogenes* infection [39]. As an intracellular pathogen, *L. monocytogenes* is internalized into the phagosomes, where it secrets LLO to form pores on the endosomal membranes that facilitate bacterial escape from the phagosomes to the cytosol. Since LLO activation requires reducing activity, the authors speculated that GILT, the only known thiol oxidoreductase present in the phagosomes, might activate LLO in vivo. The results confirmed the authors' hypothesis, showing that mice lacking GILT are resistant to *L. monocytogenes* infection; GILT activates LLO within the phagosomes by the thiol reductase activity and purified GILT activates recombinant LLO in vitro.

While the thiol(s) targeted by GILT was not directly identified, the highly conserved, undecapeptide cysteine is obviously the potential target. Besides LLO, GILT also activates streptolysin O (SLO), produced by *Streptococcus pyogenes*, as measured by the haemolysis of sheep red blood cells. But GILT failed to activate the SLO mutant that lacked the undecapeptide cysteine residue. Thus, GILT presumably targets to the characteristic cysteine residue and GILT-mediated exposure of this critical cysteine may result in a conformational change that allows the formation of the pre-pore complex and full activation.

4. Cellular entry of pathogenic bacteria, viruses, and parasites that require cellular redox factors

Available evidence has indicated that cellular redox factors are widely involved in entry of numerous microbial pathogens, ranging from bacteria, viruses and parasites. Here, we briefly review the best-characterized examples from each category.

4.1. Pathogenic bacteria: Chlamydia entry

Chlamydia trachomatis is the leading bacterial agent responsible for sexually transmitted diseases. Two biovariants of *C. trachomatis*, trachoma and lymphogranuloma venereum, cause 90 million new sexually transmitted infections per annum and 400 – 600 million cases of trachoma worldwide. As an obligatory intracellular pathogen, it requires host invasion for survival and growth. However, little is known about the molecular mechanism of Chlamydia entry into host cells. Serovar E, an adhesion molecule from *C. trachomatis*, is known to be required for invasion of genital epithelial cells, but the host factor(s) required for the pathogen entry was not known until PDI was identified as an potential mediator [43]. PDI was detected in an earlier immunoprecipitation experiment [120]., in which a biotinylated apical membrane protein receptor attached to elementary body (EB) was stripped off the surface of HE-1B cells and immunoprecipitated with anti-EB antibodies, followed by 2D SDS-PAGE and MALDI MS analysis. During EB attachment, exposure of HEC-1B cells to three different inhibitors of PDI reductive activity (DTNB, bacitracin, and anti-PDI antibodies) resulted in reduced chlamydial infection. Subsequently, a proteomic study of CHO6 cell line [121], a mutagenized cell line resistant to attachment and infection by Chlamydia, showed that CHO6 has a defect in processing of the leader sequence of PDI, which results in altered cellular distribution of PDI. PDI is abundantly localized in the ER, and surface localization is predominantly sequestered to large patches compared to the punctate pattern in the wild type CHOK1 cells. Complementation by expression of full-length PDI restored *C. trachomatis* binding and infectivity in the CHO6 mutant cell line. These data directly demonstrate that native PDI at the cell surface is required for effective chlamydial attachment and infection. Most recently, RNA interference was used to confirm that cellular PDI is essential for Chlamydial attachment to cells [44]. More precisely, the role of PDI in the process of chlamydial infection was further dissected using genetic complementation and PDI-specific inhibitors, showing that PDI has two essential and independent roles in the process of chlamydial infection. It is structurally required for chlamydial attachment, and the thiol-mediated oxido-reductive function is necessary for entry. While PDI is required for chlamydial attachment, it does not function as a receptor for the pathogen. Other host factor(s) that structurally associate with PDI may be required for chlamydial attachment.

4.2. Viral entry

As obligatory intracellular parasites, viruses can only replicate within host cells. Most viruses that infect vertebrate and insect cells exploit the endocytic pathways to enter the host cells, particularly through macropinocytosis [15,16]. Entry of enveloped virus to the host cells normally requires binding of virus to the cell surface and fusion of the viral membrane with the host cell membrane. These processes are accomplished through a coordinated interaction between viral envelope glycoproteins and host cell surface receptors, during which conformational changes of the proteins involved play an essential role in virus binding and/or membrane fusion. Increasing evidence suggests that the conformational changes are largely triggered by isomerization or reduction of the disulfide

bonds catalyzed by either viral- or host- redox factors. The requirement of redox factors for viral entry is exemplified with human immunodeficiency virus (HIV), Newcastle disease virus [122-124], Sindbis virus [125] and avian leukosis virus [126], etc. For the non-enveloped virus SV40, the virus is internalized through endocytosis and retrograde transported to ER, where it makes use of the thiol-disulfide oxidoreductases, ERp57 and PDI as well as the retrotranslocation proteins, to move to the cytosol. The HIV and SV40 viruses are discussed below.

4.2.1. HIV

Infection of human immunodeficiency virus (HIV) starts with viral binding to attachment factors, such as mannose binding C-type lectin receptor and intracellular adhesion molecule on the surface of CD4+ lymphocytes. The HIV envelope glycoprotein gp120 binds to CD4 protein, the primary receptor of HIV-1, and undergoes conformational changes, which allows the virus to interact with its co-receptors, CXCR4 or CCR5. Subsequently, these interactions stimulate downstream conversion of HIV gp41 envelope subunit to a competent fusion conformation [45]. Initially, inspired by the finding that the cell-surface PDI reductive activity is required for DT entry and cytotoxicity through reduction of the interchain disulfide bond, the roles of cell-surface PDI in HIV entry into human lymphoid cells were tested with PDI inhibitors, DTNB, bacitracin and anti-PDI antibodies [127]. The result showed that HIV infection was markedly inhibited by those inhibitors, suggesting that HIV and its target cell engage in the PDI-mediated thiol-disulfide interchange and that the reduction of critical disulfides in viral envelope glycoproteins may be the initial event that triggers conformational changes required for HIV entry and cell infection. This finding revealed a novel direction in the study of HIV entry. A series of experiments have been performed to define the roles of PDI or PDI-like redox enzymes in viral entry and membrane fusion, particularly in the aspect of thiol-disulfide interchange on viral- and host-factors. PDI was first found clustered at the CD4+ lymphocyte surface in the vicinity of CD4-enriched regions and later PDI was co-precipitated with both soluble and cellular CD4 [128]. Moreover, anti-PDI antibodies and the inhibitors of its catalytic function altered HIV envelope-mediated membrane fusion, which suggests that PDI catalytic activity functions in the HIV envelope-mediated cell-cell fusion in a post-CD4 binding step [129]. It is believed that PDI-CD4 interaction at the cell surface enables PDI to reach CD4-bound viral glycoproteins. HIV gp120 is a highly disulfide-bonded molecule that attaches HIV to CD4 and co-receptor CXCR4 or CCR5, thus it becomes a potential target of cell-surface PDI. It has been shown that soluble PDI cleaved disulfide bonds in the recombinant gp120 in vitro and the gp120 bound to the CD4 on the cell surface undergoes a disulfide reduction that is prevented by the PDI inhibitors [130]. Furthermore, additional studies showed that on average two of the nine disulfides of gp120 are reduced during interaction with the lymphocyte surface after CXCR4 binding prior to fusion and that the cell surface PDI catalyzes this process. Thus, the PDI-mediated disulfide restructuring within the HIV envelope constitutes the molecular basis of the post-receptor binding conformational changes that induce fusion competence. Due to the essential role of PDI in HIV entry, PDI is

regarded as a potential drug target. Most recently, a high-throughput screening of PDI-specific inhibitors identified the natural compound juniferdin as the most potent inhibitor of PDI. And derivatives of juniferdin were synthesized and used to carry out further studies, of these, compound 13 showed comparable inhibitory activity but reduced cytotoxicity, compared to juniferdin [131].

Interestingly, PDI knockdown by siRNA in U373 and HeLa cells had little effect on HIV infection as compared to the effect mediated by general thiol inhibitors [132]. This discrepancy raised the question whether the reductive activity of PDI is coupled to other redox enzymes that could enhance the redox-dependent viral membrane fusion and entry. This hypothesis has been supported by several recent studies. The extracellular portion of CD4 contains four immunoglobulin-like domains, D1 to D4. The D2 disulfide bond appeared redox-active and regulated by thioredoxin that is secreted by CD4(+) T cells. Locking the CD4 and the thioredoxin active-site dithiols in the reduced state with a hydrophilic trivalent arsenical blocked entry of HIV-1 into host cells [133]. More recently, human glutaredoxin-1 (Grx1) has been shown to efficiently catalyze gp120 and CD4 disulfide reduction in vitro [134]. Grx1 catalyzes the reduction of two disulfide bridges in gp120 in a similar manner to that of PDI. Anti-Grx1 antibodies inhibited the Grx1 activity and block HIV-1 replication in cultured peripheral blood mononuclear cells. The polyanion PRO2000, previously shown to prevent HIV entry, inhibited the Grx1- and PDI-dependent reduction of gp120 disulfides. Thus, other redox enzymes other than PDI may also be involved in HIV entry. Studies that further dissect the specific roles of PDI and other redox enzymes in HIV entry are needed to uncover the mechanism of HIV entry.

4.2.2. SV40

Simian virus 40 (SV40) is a simple, non-enveloped DNA virus that belongs to the polyoma virus family. It uses ganglioside GM1 as receptor, and enters host cells through a unique endocytic pathway, caveolae/lipid raft-mediated endocytosis [15,47,135]. After internalization, instead of trafficking to endosome/lysosome compartments, it traffics into a pH-neutral, caveolin-containing endocytic organelle, called caveosome. From there the virus moves in noncaveolar vesicles along microtubules to the ER through retrograde transport. In the ER, SV40 manages to translocate into the cytosol, and from there it enters the nucleus via nuclear pore complexes for viral replication. SV40 capsids are composed of homopentamers of the major capsid protein VP1, and VP1 is associated with one of the minor structural proteins VP2 or VP3. The virus has icosahedral symmetry and contains 72 pentamers, of which 12 are five-coordinated and the rest of 60 are six-coordinated. The pentamers are linked to each other by the interchain disulfide bonds between the residues Cys104. Isomerization of the disulfide bonds in the ER is crucial for the viral uncoating process [136-138]. Recent data has shown that SV40 makes use of the protein folding and quality control machinery in the ER for initial uncoating and membrane translocation [46]. Among all the ER-resident proteins, ERp57 and PDI more specifically regulate SV40 infection through isomerization of the disulfide bonds. Silencing of ERp57 and PDI substantially decreases SV40 infection. In addition, these ERp57 and PDI cooperate with the

ER-associated degradation (ERAD) proteins, Derlin-1 and Sel1L, facilitating a Ca^{2+}-dependent retrotranslocation from the ER to the cytosol.

4.3. Parasite entry

4.3.1. PDI, NADPH oxidase and Leishmania entry

Leishmania, a family of obligate intracellular parasites, causes leishmaniases in millions of individuals worldwide [139]. The parasites are transmitted by a variety of species of sand flies from two major genera Phlebotomus and Lutzomyia, respectively. The life cycle of Leishmania starts with a motile promastigote form in the insect host, in which they attach to the midgut wall to avoid being expelled with the blood meal. They later migrate to the digestive tract and differentiate into a non-dividing metacyclic form. The metacyclic promastimgotes are injected into the mammalian host, enter macrophages and differentiate into non-flagellated amastigotes that replicate and persist intracellularly, which provides a reservoir for transmission. Entry of promastimgotes into macrophages through phagocytosis is a critical step for leishmania infection. Inside the phagosome and/or phagolysosome vesicles, the parasites are exposed to enzymes, antimicrobial peptides, and ROS generated by NADPH oxidase activation [140]. Surprisingly, the promastimgotes are able to survive in such a stressful environment and differentiate into amastigotes, progressing to an active infectious disease. While the mechanism of pathogenesis is still elusive, current research has greatly advanced our understanding of the process. It is well known that Nox2 oxidase, a prototypic member of the NADPH oxidase family, is activated during phagocytosis of Leishmania, and uptake of the parasites is inhibited by antioxidants (e. g. catalase) [141]. This suggests that the oxidative stress induced by NADPH oxidase activation may have a favorable effect, instead of the expected anti-microbial effect, with regard to parasite infectivity. Recently, PDI has been shown to be involved in phagocytosis of Leishmania chagasi through regulation of NADPH oxidase, in which PDI was found to closely associate with the NADPH oxidase, and inhibitors of PDI (bacitracin, phenylarsine oxide, anti-PDI antibody) significantly blocked promastigote phagocytosis (40). These results correlate well with, and are supported by, the previous findings that proteomic study of macrophages showed that PDI is involved in the formation of the phagosomes during phagocytosis of some parasites, including Leishmania (66,81). And PDI is closely associated with NADPH oxidase and plays an organizer role in NADPH oxidase activation [41,72].

5. Conclusions and future perspectives

Current studies have revealed excitingly novel features of host-pathogen interaction. Microbial pathogens (bacteria, viruses, and parasites) and bacterial toxins exploit different aspects of the endocytic pathways, and hijack cellular redox factors to accomplish entry and invasion. Despite the fact that the pathogens and toxins are very diverse, the mechanism involved in the infectivity could be readily narrowed down to any of the simple redox reactions: reduction, oxidation, or isomerization of the thiol/dithiol groups on proteins from either pathogens or hosts. Therefore, these redox reactions could potentially be part of a

general mechanism for pathogen and toxin invasion, and this redox-dependent entry mechanism can be an attractive target for anti-microbial and anti-toxin drug development. The specificity of regulation is supposed to heavily rely on specific interaction of the cellular redox factors with the pathogens in a timely and spatial manner in various endocytic pathways. Thus far, PDI seems to be involved in many of the pathogen and toxin entry events, which is, at least in part, due to its diverse cellular distribution, ranging from the cell surface to the ER. Further studies are needed to address this question if other members of the PDI family and other ER-residing folding machinery are involved in pathogen and toxin entry. It is exciting that GILT was identified as the first oxidoreductase active optimally in the acidic compartments. The roles of GILT in entry of pathogens or toxins other than *L. monocytogenes*/LLO warrant further investigation. Finally, some pathogens or toxins can survive through the oxidative burst within the phagosomes and even can take advantage of this host defense mechanism for invasion. Thus, more studies are needed to look into the new insights concerning the role of oxidative burst in host-pathogen interactions.

Author details

Jianjun Sun
Department of Biological Sciences, University of Texas at El Paso, El Paso, TX, USA

Acknowledgement

I apologize to those individuals whose work was not discussed in this article due to space limitations. I thank Dr. Giulio Francia and Caroline Chen for proofreading the article. The author's work is supported by grants from the NIH 5SC1GM095475, G12MD007592 and 5G12RR008124.

6. References

[1] Barbieri JT, Riese MJ, Aktories K. Bacterial toxins that modify the actin cytoskeleton. Annu Rev Cell Dev Biol. 2002;18:315–344.

[2] Aktories K, Barbieri JT. Bacterial cytotoxins: targeting eukaryotic switches. Nat Rev Micro. 2005 May 1;3(5):397–410.

[3] Ham H, Sreelatha A, Orth K. Manipulation of host membranes by bacterial effectors. Nat Rev Micro. 2011 Sep.;9(9):635–646.

[4] Shatalin K, Gusarov I, Avetissova E, Shatalina Y, McQuade LE, Lippard SJ, et al. Bacillus anthracis-derived nitric oxide is essential for pathogen virulence and survival in macrophages. Proc Natl Acad Sci USA. 2008 Jan. 22;105(3):1009–1013.

[5] van der Wel N, Hava D, Houben D, Fluitsma D, van Zon M, Pierson J, et al. M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. Cell. 2007 Jun. 29;129(7):1287–1298.

[6] Ernst JD. Bacterial inhibition of phagocytosis. Cell Microbiol. 2000 Oct.;2(5):379–386.

[7] Enninga J, Rosenshine I. Imaging the assembly, structure and activity of type III secretion systems. Cell Microbiol. 2009 Oct. 1;11(10):1462–1470.
[8] Juhas M, Crook DW, Hood DW. Type IV secretion systems: tools of bacterial horizontal gene transfer and virulence. Cell Microbiol. 2008 Dec. 1;10(12):2377–2386.
[9] Llosa M, Roy C, Dehio C. Bacterial type IV secretion systems in human disease. Mol Microbiol. 2009 Jul. 1;73(2):141–151.
[10] Lord JM, Smith DC, Roberts LM. Toxin entry: how bacterial proteins get into mammalian cells. Cell Microbiol. 1999 Sep.;1(2):85–91.
[11] Binz T, Rummel A. Cell entry strategy of clostridial neurotoxins. J. Neurochem. 2009 Jun.;109(6):1584–1595.
[12] van der Goot G, Young JAT. Receptors of anthrax toxin and cell entry. Mol Aspects Med. 2009 Dec.;30(6):406–412.
[13] Geny B, Popoff MR. Bacterial protein toxins and lipids: pore formation or toxin entry into cells. Biol. Cell. 2006 Nov.;98(11):667–678.
[14] Collier RJ, Young JAT. Anthrax toxin. Annu Rev Cell Dev Biol. 2003;19:45–70.
[15] Mercer J, Helenius A. Virus entry by macropinocytosis. Nature. 2009 May;11(5):510–520.
[16] Carrasco L. Entry of animal viruses and macromolecules into cells. FEBS Lett. 1994 Aug. 22;350(2-3):151–154.
[17] Gruenheid S, Finlay BB. Microbial pathogenesis and cytoskeletal function. Nature. 2003 Apr. 17;422(6933):775–781.
[18] Pizarro-Cerdá J, Cossart P. Bacterial adhesion and entry into host cells. Cell. 2006 Feb. 24;124(4):715–727.
[19] Falnes PO, Sandvig K. Penetration of protein toxins into cells. Curr. Opin. Cell Biol. 2000 Aug.;12(4):407–413.
[20] Mamathambika BS, Bardwell JC. Disulfide-linked protein folding pathways. Annu Rev Cell Dev Biol. 2008;24:211–235.
[21] Welker E, Wedemeyer WJ, Narayan M, Scheraga HA. Coupling of conformational folding and disulfide-bond reactions in oxidative folding of proteins. Biochemistry. 2001 Aug. 7;40(31):9059–9064.
[22] Chen VM, Hogg PJ. Allosteric disulfide bonds in thrombosis and thrombolysis. J Thromb Haemost. 2006 Dec. 1;4(12):2533–2541.
[23] Hogg PJ. Contribution of allosteric disulfide bonds to regulation of hemostasis. J Thromb Haemost. 2009 Jul. 1;7 Suppl 1:13–16.
[24] Hogg PJ. Disulfide bonds as switches for protein function. Trends Biochem Sci. 2003 Apr. 1;28(4):210–214.
[25] Schmidt B, Ho L, Hogg PJ. Allosteric disulfide bonds. Biochemistry. 2006 Jun. 20;45(24):7429–7433.
[26] Hogg PJ. Biological regulation through protein disulfide bond cleavage. Redox Rep. 2002;7(2):71–77.
[27] Halvey PJ, Watson WH, Hansen JM, Go Y-M, Samali A, Jones DP. Compartmental oxidation of thiol-disulphide redox couples during epidermal growth factor signalling. Biochem J. 2005 Mar. 1;386(Pt 2):215–219.

[28] Go Y-M, Jones DP. Redox compartmentalization in eukaryotic cells. Biochim Biophys Acta. 2008 Nov.;1780(11):1273–1290.

[29] Falnes PO, Choe S, Madshus IH, Wilson BA, Olsnes S. Inhibition of membrane translocation of diphtheria toxin A-fragment by internal disulfide bridges. J Biol Chem. 1994 Mar. 18;269(11):8402–8407.

[30] Papini E, Rappuoli R, Murgia M, Montecucco C. Cell penetration of diphtheria toxin. Reduction of the interchain disulfide bridge is the rate-limiting step of translocation in the cytosol. J Biol Chem. 1993 Jan. 25;268(3):1567–1574.

[31] Ryser HJ, Mandel R, Ghani F. Cell surface sulfhydryls are required for the cytotoxicity of diphtheria toxin but not of ricin in Chinese hamster ovary cells. J Biol Chem. 1991 Oct. 5;266(28):18439–18442.

[32] Feener EP, Shen WC, Ryser HJ. Cleavage of disulfide bonds in endocytosed macromolecules. A processing not associated with lysosomes or endosomes. J Biol Chem. 1990 Nov. 5;265(31):18780–18785.

[33] Mandel R, Ryser HJ, Ghani F, Wu M, Peak D. Inhibition of a reductive function of the plasma membrane by bacitracin and antibodies against protein disulfide-isomerase. Proc Natl Acad Sci USA. 1993 May 1;90(9):4112–4116.

[34] Tsai B, Rodighiero C, Lencer WI, Rapoport TA. Protein disulfide isomerase acts as a redox-dependent chaperone to unfold cholera toxin. Cell. 2001 Mar. 23;104(6):937–948.

[35] Shi X, Garcia GE, Neill RJ, Gordon RK. TCEP treatment reduces proteolytic activity of BoNT/B in human neuronal SHSY-5Y cells. J. Cell. Biochem. 2009 Aug. 1;107(5):1021–1030.

[36] Fischer A, Montal M. Crucial role of the disulfide bridge between botulinum neurotoxin light and heavy chains in protease translocation across membranes. J Biol Chem. 2007 Oct. 5;282(40):29604–29611.

[37] Sun J, Collier RJ. Disulfide bonds in the ectodomain of anthrax toxin receptor 2 are required for the receptor-bound protective-antigen pore to function. PLoS ONE. 2010;5(5):e10553.

[38] Stolf BS, Ioannis S, Lopes LR, Vendramin A, Goto H, Laurindo FRM, et al. Protein disulfide isomerase and host-pathogen interaction. ScientificWorldJournal. 2011;11:1749–1761.

[39] Singh R, Jamieson A, Cresswell P. GILT is a critical host factor for Listeria monocytogenes infection. Nature. 2008 Oct. 30;455(7217):1244–1247.

[40] Santos CXC, Stolf BS, Takemoto PVA, Amanso AM, Lopes LR, Souza EB, et al. Protein disulfide isomerase (PDI) associates with NADPH oxidase and is required for phagocytosis of Leishmania chagasi promastigotes by macrophages. Journal of Leukocyte Biology. 2009 Oct.;86(4):989–998.

[41] Laurindo FRM, Fernandes DC, Amanso AM, Lopes LR, Santos CXC. Novel role of protein disulfide isomerase in the regulation of NADPH oxidase activity: pathophysiological implications in vascular diseases. Antioxid Redox Signal. 2008 Jun.;10(6):1101–1113.

[42] Fernandes DC, Manoel AHO, Wosniak J, Laurindo FR. Protein disulfide isomerase overexpression in vascular smooth muscle cells induces spontaneous preemptive NADPH oxidase activation and Nox1 mRNA expression: effects of nitrosothiol exposure. Arch Biochem Biophys. 2009 Apr. 15;484(2):197–204.
[43] Dautry-Varsat A, Subtil A, Hackstadt T. Recent insights into the mechanisms of Chlamydia entry. Cell Microbiol. 2005 Dec.;7(12):1714–1722.
[44] Abromaitis S, Stephens RS. Attachment and entry of Chlamydia have distinct requirements for host protein disulfide isomerase. PLoS Pathog. 2009 Apr.;5(4):e1000357.
[45] Ryser HJ-P, Flückiger R. Progress in targeting HIV-1 entry. Drug Discov Today. 2005 Aug. 15;10(16):1085–1094.
[46] Schelhaas M, Malmström J, Pelkmans L, Haugstetter J, Ellgaard L, Grünewald K, et al. Simian Virus 40 depends on ER protein folding and quality control factors for entry into host cells. Cell. 2007 Nov. 2;131(3):516–529.
[47] Medina-Kauwe LK. "Alternative" endocytic mechanisms exploited by pathogens: new avenues for therapeutic delivery? Adv Drug Deliv Rev. 2007 Aug. 10;59(8):798–809.
[48] Yang J, Chen H, Vlahov IR, Cheng J-X, Low PS. Evaluation of disulfide reduction during receptor-mediated endocytosis by using FRET imaging. Proc Natl Acad Sci USA. 2006 Sep. 12;103(37):13872–13877.
[49] Saito G, Swanson JA, Lee K-D. Drug delivery strategy utilizing conjugation via reversible disulfide linkages: role and site of cellular reducing activities. Adv Drug Deliv Rev. 2003 Feb. 10;55(2):199–215.
[50] Bareford LM, Swaan PW. Endocytic mechanisms for targeted drug delivery. Adv Drug Deliv Rev. 2007 Aug. 10;59(8):748–758.
[51] Rajendran L, Knölker H-J, Simons K. Subcellular targeting strategies for drug design and delivery. Nat Rev Drug Discov. 2010;9(1):29–42.
[52] Jones AT, Gumbleton M, Duncan R. Understanding endocytic pathways and intracellular trafficking: a prerequisite for effective design of advanced drug delivery systems. Adv Drug Deliv Rev. 2003 Nov. 14;55(11):1353–1357.
[53] Austin CD, Wen X, Gazzard L, Nelson C, Scheller RH, Scales SJ. Oxidizing potential of endosomes and lysosomes limits intracellular cleavage of disulfide-based antibody-drug conjugates. Proc Natl Acad Sci USA. 2005 Dec. 13;102(50):17987–17992.
[54] Morré DJ, Chueh PJ, Lawler J, Morré DM. The sulfonylurea-inhibited NADH oxidase activity of HeLa cell plasma membranes has properties of a protein disulfide-thiol oxidoreductase with protein disulfide-thiol interchange activity. J. Bioenerg. Biomembr. 1998 Oct.;30(5):477–487.
[55] Halliwell B. Biochemistry of oxidative stress. Biochem Soc Trans. 2007 Nov. 1;35(Pt 5):1147–1150.
[56] Iles KE, Forman HJ. Macrophage signaling and respiratory burst. Immunol Res. 2002;26(1-3):95–105.
[57] Gwinn MR, Vallyathan V. Respiratory burst: role in signal transduction in alveolar macrophages. J Toxicol Environ Health B Crit Rev. 2006;9(1):27–39.

[58] Forman HJ, Torres M. Reactive oxygen species and cell signaling: respiratory burst in macrophage signaling. American Journal of Respiratory and Critical Care Medicine. 2002 Dec. 15;166(12 Pt 2):S4–8.

[59] Turano C, Coppari S, Altieri F, Ferraro A. Proteins of the PDI family: unpredicted non-ER locations and functions. J. Cell. Physiol. 2002 Nov.;193(2):154–163.

[60] Appenzeller-Herzog C, Ellgaard L. The human PDI family: versatility packed into a single fold. Biochim Biophys Acta. 2008 Apr. 1;1783(4):535–548.

[61] Ellgaard L, Ruddock LW. The human protein disulphide isomerase family: substrate interactions and functional properties. EMBO Rep. 2005;6(1):28–32.

[62] Kersteen EA, Raines RT. Catalysis of protein folding by protein disulfide isomerase and small-molecule mimics. Antioxid Redox Signal. 2003 Aug. 1;5(4):413–424.

[63] Terada K, Manchikalapudi P, Noiva R, Jauregui HO, Stockert RJ, Schilsky ML. Secretion, surface localization, turnover, and steady state expression of protein disulfide isomerase in rat hepatocytes. J Biol Chem. 1995 Sep. 1;270(35):20410–20416.

[64] Kim Y, Kang K, Kim I, Lee YJ, Oh C, Ryoo J, et al. Molecular mechanisms of MHC class I-antigen processing: redox considerations. Antioxid Redox Signal. 2009 Apr.;11(4):907–936.

[65] Park B, Lee S, Kim E, Cho K, Riddell SR, Cho S, et al. Redox regulation facilitates optimal peptide selection by MHC class I during antigen processing. Cell. 2006 Oct. 20;127(2):369–382.

[66] Gagnon E, Duclos S, Rondeau C, Chevet E, Cameron PH, Steele-Mortimer O, et al. Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. Cell. 2002 Jul. 12;110(1):119–131.

[67] Tsai B, Walczak CP, Bernardi KM. Endoplasmic reticulum-dependent redox reactions control ER-associated degradation and pathogen entry. Antioxid Redox Signal. 2011 Dec. 5.

[68] Jiang XM, Fitzgerald M, Grant CM, Hogg PJ. Redox control of exofacial protein thiols/disulfides by protein disulfide isomerase. J Biol Chem. 1999 Jan. 22;274(4):2416–2423.

[69] Lahav J, Gofer-Dadosh N, Luboshitz J, Hess O, Shaklai M. Protein disulfide isomerase mediates integrin-dependent adhesion. FEBS Lett. 2000 Jun. 16;475(2):89–92.

[70] Jordan PA, Gibbins JM. Extracellular disulfide exchange and the regulation of cellular function. Antioxid Redox Signal. 2006 Feb.;8(3-4):312–324.

[71] Chueh P-J, Morré DM, Morré DJ. A site-directed mutagenesis analysis of tNOX functional domains. Biochim Biophys Acta. 2002 Jan. 31;1594(1):74–83.

[72] Janiszewski M, Lopes LR, Carmo AO, Pedro MA, Brandes RP, Santos CXC, et al. Regulation of NAD(P)H oxidase by associated protein disulfide isomerase in vascular smooth muscle cells. J Biol Chem. 2005 Dec. 9;280(49):40813–40819.

[73] de A Paes AM, Veríssimo-Filho S, Guimarães LL, Silva ACB, Takiuti JT, Santos CXC, et al. Protein disulfide isomerase redox-dependent association with p47(phox): evidence for an organizer role in leukocyte NADPH oxidase activation. Journal of Leukocyte Biology. 2011 Oct.;90(4):799–810.

[74] Gainey D, Short S, McCoy KL. Intracellular location of cysteine transport activity correlates with productive processing of antigen disulfide. J. Cell. Physiol. 1996 Aug.;168(2):248–254.
[75] Creighton TE, Zapun A, Darby NJ. Mechanisms and catalysts of disulfide bond formation in proteins. Trends Biotechnol. 1995 Jan.;13(1):18–23.
[76] Arunachalam B, Phan UT, Geuze HJ, Cresswell P. Enzymatic reduction of disulfide bonds in lysosomes: characterization of a gamma-interferon-inducible lysosomal thiol reductase (GILT). Proc Natl Acad Sci USA. 2000 Jan. 18;97(2):745–750.
[77] Phan UT, Arunachalam B, Cresswell P. Gamma-interferon-inducible lysosomal thiol reductase (GILT). Maturation, activity, and mechanism of action. J Biol Chem. 2000 Aug. 25;275(34):25907–25914.
[78] Glickman JN, Morton PA, Slot JW, Kornfeld S, Geuze HJ. The biogenesis of the MHC class II compartment in human I-cell disease B lymphoblasts. J Cell Biol. 1996 Mar.;132(5):769–785.
[79] Singh R, Cresswell P. Defective cross-presentation of viral antigens in GILT-free mice. Science. 2010 Jun. 11;328(5984):1394–1398.
[80] Luster AD, Weinshank RL, Feinman R, Ravetch JV. Molecular and biochemical characterization of a novel gamma-interferon-inducible protein. J Biol Chem. 1988 Aug. 25;263(24):12036–12043.
[81] Garin J, Diez R, Kieffer S, Dermine JF, Duclos S, Gagnon E, et al. The phagosome proteome: insight into phagosome functions. J Cell Biol. 2001 Jan. 8;152(1):165–180.
[82] Barth H, Aktories K, Popoff MR, Stiles BG. Binary bacterial toxins: biochemistry, biology, and applications of common Clostridium and Bacillus proteins. Microbiol Mol Biol Rev. 2004 Sep. 1;68(3):373–402, table of contents.
[83] Collier RJ. Diphtheria toxin: mode of action and structure. Bacteriological reviews. 1975 Mar. 1;39(1):54–85.
[84] Collier RJ. Understanding the mode of action of diphtheria toxin: a perspective on progress during the 20th century. Toxicon. 2001 Nov. 1;39(11):1793–1803.
[85] Ratts R, Zeng H, Berg EA, Blue C, McComb ME, Costello CE, et al. The cytosolic entry of diphtheria toxin catalytic domain requires a host cell cytosolic translocation factor complex. J Cell Biol. 2003 Mar. 31;160(7):1139–1150.
[86] Sears CL, Kaper JB. Enteric bacterial toxins: mechanisms of action and linkage to intestinal secretion. Microbiol. Rev. 1996 Mar.;60(1):167–215.
[87] Fujinaga Y. Transport of bacterial toxins into target cells: pathways followed by cholera toxin and botulinum progenitor toxin. J Biochem. 2006 Aug.;140(2):155–160.
[88] Lencer WI, Constable C, Moe S, Jobling MG, Webb HM, Ruston S, et al. Targeting of cholera toxin and Escherichia coli heat labile toxin in polarized epithelia: role of COOH-terminal KDEL. J Cell Biol. 1995 Nov.;131(4):951–962.
[89] Orlandi PA. Protein-disulfide isomerase-mediated reduction of the A subunit of cholera toxin in a human intestinal cell line. J Biol Chem. 1997 Feb. 14;272(7):4591–4599.

[90] Majoul I, Ferrari D, Söling HD. Reduction of protein disulfide bonds in an oxidizing environment. The disulfide bridge of cholera toxin A-subunit is reduced in the endoplasmic reticulum. FEBS Lett. 1997 Jan. 20;401(2-3):104–108.
[91] Bernardi KM, Forster ML, Lencer WI, Tsai B. Derlin-1 facilitates the retro-translocation of cholera toxin. Mol Biol Cell. 2008 Mar.;19(3):877–884.
[92] Montal M. Translocation of botulinum neurotoxin light chain protease by the heavy chain protein-conducting channel. Toxicon. 2009 Oct.;54(5):565–569.
[93] Lacy DB, Tepp W, Cohen AC, DasGupta BR, Stevens RC. Crystal structure of botulinum neurotoxin type A and implications for toxicity. Nat Struct Biol. 1998 Oct.;5(10):898–902.
[94] Lacy DB, Stevens RC. Sequence homology and structural analysis of the clostridial neurotoxins. J Mol Biol. 1999 Sep. 3;291(5):1091–1104.
[95] Dong M, Liu H, Tepp WH, Johnson EA, Janz R, Chapman ER. Glycosylated SV2A and SV2B mediate the entry of botulinum neurotoxin E into neurons. Mol Biol Cell. 2008 Dec.;19(12):5226–5237.
[96] Dong M, Tepp WH, Liu H, Johnson EA, Chapman ER. Mechanism of botulinum neurotoxin B and G entry into hippocampal neurons. J Cell Biol. 2007 Dec. 31;179(7):1511–1522.
[97] Chen C, Fu Z, Kim J-JP, Barbieri JT, Baldwin MR. Gangliosides as high affinity receptors for tetanus neurotoxin. J Biol Chem. 2009 Sep. 25;284(39):26569–26577.
[98] Yeh FL, Dong M, Yao J, Tepp WH, Lin G, Johnson EA, et al. SV2 mediates entry of tetanus neurotoxin into central neurons. PLoS Pathog. 2010;6(11):e1001207.
[99] Fischer A, Montal M. Single molecule detection of intermediates during botulinum neurotoxin translocation across membranes. Proc Natl Acad Sci USA. 2007 Jun. 19;104(25):10447–10452.
[100] Koriazova LK, Montal M. Translocation of botulinum neurotoxin light chain protease through the heavy chain channel. Nat Struct Biol. 2003 Jan.;10(1):13–18.
[101] Bradley KA, Mogridge J, Mourez M, Collier RJ, Young JA. Identification of the cellular receptor for anthrax toxin. Nature. 2001 Nov. 8;414(6860):225–229.
[102] Scobie HM, Rainey GJA, Bradley KA, Young JAT. Human capillary morphogenesis protein 2 functions as an anthrax toxin receptor. Proc Natl Acad Sci USA. 2003 Apr. 29;100(9):5170–5174.
[103] Liu S, Crown D, Miller-Randolph S, Moayeri M, Wang H, Hu H, et al. Capillary morphogenesis protein-2 is the major receptor mediating lethality of anthrax toxin in vivo. Proc Natl Acad Sci USA. 2009 Jul. 28;106(30):12424–12429.
[104] Lacy DB, Wigelsworth DJ, Scobie HM, Young JAT, Collier RJ. Crystal structure of the von Willebrand factor A domain of human capillary morphogenesis protein 2: an anthrax toxin receptor. Proc Natl Acad Sci USA. 2004 Apr. 27;101(17):6367–6372.
[105] Abrami L, Leppla SH, van der Goot FG. Receptor palmitoylation and ubiquitination regulate anthrax toxin endocytosis. J Cell Biol. 2006 Jan. 16;172(2):309–320.
[106] Abrami L, Liu S, Cosson P, Leppla SH, van der Goot FG. Anthrax toxin triggers endocytosis of its receptor via a lipid raft-mediated clathrin-dependent process. J Cell Biol. 2003 Feb. 3;160(3):321–328.

[107] Guidi-Rontani C, Weber-Levy M, Labruyère E, Mock M. Germination of Bacillus anthracis spores within alveolar macrophages. Mol Microbiol. 1999;31(1):9–17.
[108] Guidi-Rontani C, Levy M, Ohayon H, Mock M. Fate of germinated Bacillus anthracis spores in primary murine macrophages. Mol Microbiol. 2001 Nov. 1;42(4):931–938.
[109] Hanna PC, Kruskal BA, Ezekowitz RA, Bloom BR, Collier RJ. Role of macrophage oxidative burst in the action of anthrax lethal toxin. Mol Med. 1994 Nov. 1;1(1):7–18.
[110] Gusarov I, Nudler E. NO-mediated cytoprotection: instant adaptation to oxidative stress in bacteria. Proc Natl Acad Sci USA. 2005 Sep. 27;102(39):13855–13860.
[111] Cybulski RJ, Sanz P, Alem F, Stibitz S, Bull RL, O'Brien AD. Four superoxide dismutases contribute to Bacillus anthracis virulence and provide spores with redundant protection from oxidative stress. Infect Immun. 2009;77(1):274–285.
[112] Rossjohn J, Feil SC, McKinstry WJ, Tweten RK, Parker MW. Structure of a cholesterol-binding, thiol-activated cytolysin and a model of its membrane form. Cell. 1997 May 30;89(5):685–692.
[113] Ramachandran R, Tweten RK, Johnson AE. The domains of a cholesterol-dependent cytolysin undergo a major FRET-detected rearrangement during pore formation. Proc Natl Acad Sci USA. 2005 May 17;102(20):7139–7144.
[114] Johnson AE. Fluorescence approaches for determining protein conformations, interactions and mechanisms at membranes. Traffic. 2005 Dec. 1;6(12):1078–1092.
[115] Tilley SJ, Orlova EV, Gilbert RJC, Andrew PW, Saibil HR. Structural basis of pore formation by the bacterial toxin pneumolysin. Cell. 2005 Apr. 22;121(2):247–256.
[116] Geoffroy C, Gaillard JL, Alouf JE, Berche P. Purification, characterization, and toxicity of the sulfhydryl-activated hemolysin listeriolysin O from Listeria monocytogenes. Infect Immun. 1987 Jul.;55(7):1641–1646.
[117] Portnoy DA, Chakraborty T, Goebel W, Cossart P. Molecular determinants of Listeria monocytogenes pathogenesis. Infect Immun. 1992 Apr.;60(4):1263–1267.
[118] Soltani CE, Hotze EM, Johnson AE, Tweten RK. Specific protein-membrane contacts are required for prepore and pore assembly by a cholesterol-dependent cytolysin. J Biol Chem. 2007 May 25;282(21):15709–15716.
[119] Soltani CE, Hotze EM, Johnson AE, Tweten RK. Structural elements of the cholesterol-dependent cytolysins that are responsible for their cholesterol-sensitive membrane interactions. Proc Natl Acad Sci USA. 2007 Dec. 18;104(51):20226–20231.
[120] Davis CH, Raulston JE, Wyrick PB. Protein disulfide isomerase, a component of the estrogen receptor complex, is associated with Chlamydia trachomatis serovar E attached to human endometrial epithelial cells. Infect Immun. 2002 Jul.;70(7):3413–3418.
[121] Conant CG, Stephens RS. Chlamydia attachment to mammalian cells requires protein disulfide isomerase. Cell Microbiol. 2007 Jan.;9(1):222–232.
[122] Jain S, McGinnes LW, Morrison TG. Overexpression of thiol/disulfide isomerases enhances membrane fusion directed by the Newcastle disease virus fusion protein. J Virol. 2008 Dec.;82(24):12039–12048.
[123] Jain S, McGinnes LW, Morrison TG. Role of thiol/disulfide exchange in newcastle disease virus entry. J Virol. 2009 Jan.;83(1):241–249.

[124] Jain S, McGinnes LW, Morrison TG. Thiol/disulfide exchange is required for membrane fusion directed by the Newcastle disease virus fusion protein. J Virol. 2007 Mar.;81(5):2328–2339.

[125] Abell BA, Brown DT. Sindbis virus membrane fusion is mediated by reduction of glycoprotein disulfide bridges at the cell surface. J Virol. 1993 Sep.;67(9):5496–5501.

[126] Smith JG, Cunningham JM. Receptor-induced thiolate couples Env activation to retrovirus fusion and infection. PLoS Pathog. 2007 Dec. 21;3(12):e198.

[127] Ryser HJ, Levy EM, Mandel R, DiSciullo GJ. Inhibition of human immunodeficiency virus infection by agents that interfere with thiol-disulfide interchange upon virus-receptor interaction. Proc Natl Acad Sci USA. 1994 May 10;91(10):4559–4563.

[128] Fenouillet E, Barbouche R, Courageot J, Miquelis R. The catalytic activity of protein disulfide isomerase is involved in human immunodeficiency virus envelope-mediated membrane fusion after CD4 cell binding. J Infect Dis. 2001 Mar. 1;183(5):744–752.

[129] Gallina A, Hanley TM, Mandel R, Trahey M, Broder CC, Viglianti GA, et al. Inhibitors of protein-disulfide isomerase prevent cleavage of disulfide bonds in receptor-bound glycoprotein 120 and prevent HIV-1 entry. J Biol Chem. 2002 Dec. 27;277(52):50579–50588.

[130] Barbouche R, Miquelis R, Jones IM, Fenouillet E. Protein-disulfide isomerase-mediated reduction of two disulfide bonds of HIV envelope glycoprotein 120 occurs post-CXCR4 binding and is required for fusion. J Biol Chem. 2003 Jan. 31;278(5):3131–3136.

[131] Khan MMG, Simizu S, Lai NS, Kawatani M, Shimizu T, Osada H. Discovery of a small molecule PDI inhibitor that inhibits reduction of HIV-1 envelope glycoprotein gp120. ACS chemical biology. 2011 Mar. 18;6(3):245–251.

[132] Ou W, Silver J. Role of protein disulfide isomerase and other thiol-reactive proteins in HIV-1 envelope protein-mediated fusion. Virology. 2006 Jul. 5;350(2):406–417.

[133] Matthias LJ, Yam PTW, Jiang X-M, Vandegraaff N, Li P, Poumbourios P, et al. Disulfide exchange in domain 2 of CD4 is required for entry of HIV-1. Nat Immunol. 2002 Aug. 1;3(8):727–732.

[134] Auwerx J, Isacsson O, Söderlund J, Balzarini J, Johansson M, Lundberg M. Human glutaredoxin-1 catalyzes the reduction of HIV-1 gp120 and CD4 disulfides and its inhibition reduces HIV-1 replication. Int J Biochem Cell Biol. 2009 Jun. 1;41(6):1269–1275.

[135] Lin AE-J, Guttman JA. Hijacking the endocytic machinery by microbial pathogens. Protoplasma. 2010 Aug.;244(1-4):75–90.

[136] Li PP, Nakanishi A, Clark SW, Kasamatsu H. Formation of transitory intrachain and interchain disulfide bonds accompanies the folding and oligomerization of simian virus 40 Vp1 in the cytoplasm. Proc Natl Acad Sci USA. 2002 Feb. 5;99(3):1353–1358.

[137] Liddington RC, Yan Y, Moulai J, Sahli R, Benjamin TL, Harrison SC. Structure of simian virus 40 at 3.8-A resolution. Nature. 1991 Nov. 28;354(6351):278–284.

[138] Li PP, Nakanishi A, Tran MA, Salazar AM, Liddington RC, Kasamatsu H. Role of simian virus 40 Vp1 cysteines in virion infectivity. J Virol. 2000 Dec.;74(23):11388–11393.

[139] Sibley LD. Invasion and intracellular survival by protozoan parasites. Immunol Rev. 2011 Mar.;240(1):72–91.

[140] Beattie L, Kaye PM. Leishmania-host interactions: what has imaging taught us? Cell Microbiol. 2011 Nov.;13(11):1659–1667.

[141] Van Assche T, Deschacht M, da Luz RAI, Maes L, Cos P. Leishmania-macrophage interactions: insights into the redox biology. Free Radic Biol Med. 2011 Jul. 15;51(2):337–351.

IntechOpen