Review

Targeting membrane trafficking in infection prophylaxis: dynamin inhibitors

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Many pathogens hijack existing endocytic trafficking pathways to exert toxic effects in cells. Dynamin controls various steps of the intoxication process used by numerous pathogenic bacteria, viruses, and toxins. Targeting dynamin with pharmaceutical compounds may therefore have prophylactic potential. Here we review the growing number of pathogens requiring dynamin-dependent trafficking to intoxicate cells, outline the mode of internalization that leads to their pathogenicity, and highlight the protective effect of pharmacological and genetic approaches targeting dynamin function. We also assess the methodologies used to investigate the role of dynamin in the intoxication process and discuss the validity and potential pitfalls of using dynamin inhibitors (DIs) as therapeutics.

Targeting intracellular membrane trafficking to combat pathogens

The evolution of the microbial world has produced impressive molecular strategies to gain entry to and incapacitate specific target cells in the host. The existence of various cellular endocytic pathways provides an array of opportunities for microbial pathogens and viruses to access and infect our cells [1]. Once inside cells, many pathogens further hijack intracellular trafficking mechanisms to find or generate sheltered compartments to prepare for replication and release. Endocytic pathways range from clathrin-mediated endocytosis (CME) to macropinocytosis, enabling cells to internalize essential proteins, fluids, lipids, and other signals or nutrients. These pathways require the spatiotemporal coordination of many different proteins and lipids. One protein, dynamin, is a common denominator for more of these pathways than any other enzyme [2,3]. Dynamin is a GTPase that oligomerizes around the neck of nascent endocytic compartments to pinch them off from the plasma membrane and may act in a similar manner in other initial endocytic events (Box 1). It also has other key regulatory roles in trafficking that are less well-characterized, such as vesicle formation from the trans-Golgi network and regulation of actin dynamics [2,4].

Recent developments have led to the design of compounds targeting key or converging points of these membrane trafficking pathways to prevent infection. Multiple endocytic entry points and subsequent trafficking events can be targeted to prevent either internalization or trafficking of pathogens, thereby precluding their pathogenic activities. Such drug design is distinct from compounds that directly target, for example, viral replication or bacterial cell walls. This therapeutic approach is likely to have a broader spectrum of applications and avoid major pitfalls of other currently available treatments, such as the development of resistance, and may ultimately prove to work in synergy with more traditional approaches. Several recent studies have explored these novel options and some have successfully extended their investigations to animal models (Box 2).

The development of a large number of small-molecule DIs, combined with the growing number of studies documenting the dynamin dependency of pathogenicity, paves the way for new therapeutic strategies aimed at blocking the uptake and trafficking of a broad range of pathogens (Box 2). One such study highlighted the successful use of small-molecule DIs to block the internalization of botulinum neurotoxin type-A (BoNT/A) and delay the onset of botulism in mice [5]. This was the first translation of the cell-based use of DIs to an animal model of botulism. Although a growing number of studies point toward a prophylactic effect of interfering with vesicular trafficking as a promising therapeutic strategy, it is unclear whether these pathways are suitable targets *in vivo*. Trafficking events have important physiological functions and it is not yet known whether targeting them in long-term therapy might lead to undesirable side effects. The following sections review the current literature to determine what is known about dynamin-dependent trafficking pathways and the pathogens that utilize them. Current limitations of the studies and conflicting results are discussed to determine whether it is possible to move forward in this field and develop DIs as therapeutic compounds.

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Box 1. Dynamin

Dynamin is a GTPase protein with several functional domains. Its best understood role is in the fission of endocytic vesicles from the plasma membrane, but it is also involved in other cellular functions [2]. The protein comprises a GTPase domain, followed by a middle domain, a pleckstrin homology (PH) domain, a GTPase effector domain (GED), and, finally, a proline-rich domain (PRD) at the C terminus. Dynamin assembles into a tetramer, with the middle and GED forming a stalk that connects the PH domain and the GTPase domain. The PRD is located near the GTPase domain. The three isoforms of dynamin (I–III) are all expressed by mammalian tissues [2]. Whereas dynamin II is widely expressed in all tissues, dynamin I is found only in neuronal or neurosecretory cells and dynamin III is expressed in neurons, testis, and megakaryocytes [2]. The specific roles of the isoforms remains unclear. Dynamin may play slightly different roles in different endocytic pathways, but is consistently involved in the budding off of endocytic vesicles. It has been established that multiple dynamin dimers/tetramers oligomerize around the neck of invaginating vesicles and, by constricting and/or stretching the neck, cause scission of the vesicle from the plasma membrane [2]. The role of dynamin in CME is the best studied and many proteins that recruit and bind to dynamin have been identified [92,93]. However, dynamin function has also been attributed to other clathrin-independent endocytic pathways, such as presynaptic bulk endocytosis, caveolae, and IL2R endocytosis, but the mechanisms involved in these pathways are less well described [14]. In CME, dynamin's mechanical action occurs via oligomerization-dependent GTP hydrolysis, and this is likely to be the case for other vesicular endocytic pathways. Dephosphorylation of dynamin I or II is required for its function in bulk endocytosis and cytokinesis, respectively [12,93]. Dynamin is also likely to have a similar role in vesicular budding during other stages of membrane trafficking, such as at the Golgi complex, and its inhibition is able to block the viral budding of hepatitis B [64].

Canonical endocytic pathways requiring dynamin function

Since dynamin was discovered [6,7], it has been found to play a role in various endocytic pathways, as well as other cellular functions (Figure 1 and Box 1) [2]. Dynamin acts in the initial stages of CME by catalyzing the fission of endocytic vesicles from the plasma membrane through the formation and subsequent constriction of a collar or helix around the neck of nascent endocytic vesicles [2]. GTP hydrolysis drives both the vesicle fission step and disassembly of the oligomerized dynamin. Dynamin assists in the release of vesicles from the plasma membrane in other types of endocytic pathway, but how this occurs is not well-characterized. Dynamin is also involved in endocytosis-independent functions, including vesicle formation from the trans-Golgi network, the cytokinesis stage of mitosis [8], the sperm acrosome reaction [9], regulation of exocytosis [10] and actin dynamics [4].

A distinct characteristic of CME is the formation of clathrin-coated vesicles at the plasma membrane, a process that involves the assembly of a range of accessory and adaptor proteins, such as AP2, and is assisted by BAR domain-containing proteins that promote membrane curvature and recruit dynamin [3]. Dynamin operates in at least two endocytic modes of synaptic vesicle recycling in neurons [11], being required for both CME and for the activity-dependent bulk endocytosis, which involves rapid retrieval of a large portion of the plasma membrane in a clathrin-independent manner [12]. Dephosphorylation of dynamin I by calcineurin triggers the latter process [13].

Box 2. Development of small-molecule inhibitors including DIs to target pathogen intoxication pathways

A large number of viruses, toxins, and pathogens are able to gain entry to cells via their endocytic pathways. Numerous small-molecule inhibitors have now been developed that can block key steps along these pathways and hence interfere with the intoxication process of the pathogen. HIV-1 has been shown to enter cells through CME and this internalization step can be prevented by blocking the clathrin step of endocytosis using Pitstop, or by blocking the dynamin-dependent vesicle entry point with dynasore or MiTMAB [17,49,50]. Trafficking of Shiga toxin [94] and ricin [95] from early endosomes to the Golgi complex has also been targeted. Shiga toxin and ricin both bypass degradation and the late endosomal system by trafficking from early endosomes directly to the Golgi apparatus. Manganese specifically blocks the retrograde trafficking of Shiga toxin by binding to GPP130, a protein involved in endosome-to-Golgi trafficking [94]. High-throughput screening has identified the small-molecule inhibitors Retro-1 and -2, which also block the trafficking of Shiga toxin and ricin from early endosomes to the trans-Golgi network [95]. The target of these compounds is currently unknown [95]. Another example of altering the trafficking pathway of pathogens is the blockade of *Salmonella*. The bacterium is endocytosed within a *Salmonella*-containing vesicle that eventually fuses with late endosomes to mature and begin replication. Inhibition of the lipid kinase PIKfyve, which is involved in endosomal trafficking, prevents this fusion from occurring, thereby blocking replication of the bacterium [96]. Trafficking of the neurotoxin BoNT/A can be blocked at multiple time points. The DI Dyngo-4a is able to prevent the internalization of the toxin into recycling synaptic vesicles [5], whereas the compound toosendanin delays the toxin's paralytic effect by preventing the release of the enzymatically active light chain into the cytosol [97]. These studies suggest that infection prophylaxis can be achieved *in vitro* at multiple points in vesicle-trafficking pathways, featuring both the initial point of pathogen entry and the point at which it escapes an intracellular organelle into the cytoplasm. The next stage is to develop these compounds into therapeutic inhibitors.

Dynamin plays an essential role in other modes of endocytosis. Caveolae-mediated endocytosis is defined by uncoated, flask-shaped invaginations from the plasma membrane that require the protein caveolin [14]. Although caveolae do not use the same adaptor and accessory proteins as CME, dynamin is required for the scission of caveolae from the plasma membrane [15]. Dynamin is involved in macro- and micropinocytosis-like endocytic pathways [16–19] and in the phagocytosis of large particles such as bacteria or viruses [19]. In the latter instance, dynamin acts at an earlier point in the pathway that is likely to control actin dynamics shaping the phagocytic cup [20]. Dynamin has also been implicated in other endocytic pathways, such as Arf6-dependent endocytosis [21], and endocytosis of the interleukin-2 receptor (IL2R) [22,23]. Despite the incomplete molecular understanding of some of these pathways, the common requirement for dynamin GTPase activity advocates for the potential use of DIs in a broad range of prophylactic treatments.

Tools to study dynamin function

All research tools have strengths and limitations that must be carefully assessed to avoid pitfalls in data interpretation. The available tools to determine the cellular roles of dynamin fall into three main categories: (i) genetic modifications (transgenic animals, RNAi, overexpression of mutant or wild-type plasmids); (ii) introduction of