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Chapter 3

Lysosomes: How Plasma Membrane Repair Route Can Be Hijacked by Parasites?

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Abstract

Lysosomes are acidic organelles that are not only involved in degradation processes but also participated in other cellular functions, such as specialized secretion and plasma membrane (PM) resealing. When the PM is ruptured, Ca^{2+} flows from the extracellular milieu toward the cytoplasm potentially triggering cell death. In order to escape from the apoptotic route, cells developed an elegant mechanism in which lysosomes are recruited to the sites of injuries in a Ca^{2+}-dependent fashion. Lysosomes, fuse with the PM releasing their enzymatic content. Acid sphingomyelinase (ASM), one of the secreted enzymes, cleaves sphingomyelin into ceramide, inducing compensatory endocytosis and internalization of the membrane-damaged site. *Trypanosoma cruzi*, the etiological agent of Chagas disease, relies heavily on lysosomes to successfully invade mammalian cells. By mechanically injuring the host PM, *T. cruzi* evokes lysosome exocytosis, and subsequently, compensatory endocytosis. The latter drives the parasite into the host cell, where it can replicate. This early association with lysosomes prevents *T. cruzi* evasion from the host cells allowing colonization of host intracellular milieu. This review chapter will summarize the main contributions in the field exploring the crosstalk between PM repair and *T. cruzi* invasion and how the understanding of these mechanisms evolved throughout the years.

Keywords: plasma membrane repair, lysosomes, exocytosis, compensatory endocytosis, *Trypanosoma cruzi*

1. Introduction

The word lysosome is derived from the Greek words *lysis* (loosening, breaking) and *soma* (body) and literally means 'digestive body'. Those acidic organelles were identified primarily by the biochemist Christian de Duve, in 1955, when he was studying the carbohydrate metabolism, the mechanism of insulin in the liver and the role of an enzyme, known at that time,

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as hexose phosphate (and later denominated glucose-6-phosphatase) [1]. By doing sucrose gradient centrifugal fractionation, de Duve identified four main fractions on the liver homogenate: nuclear, large granules (mostly composed by mitochondria), small granules (microsomes) and a supernatant. The glucose-6-phosphate enzyme was identified in the microsome fraction [2]. Based on biochemistry enzymatic analysis, de Duve and his group postulated that acid phosphatase must be enclosed within membranous vesicles in such a way that the enzyme could not leak out, and the substrate could not get in [3]. The first morphological observation of a lysosome was performed in 1956, when it was seen under an electron microscope by Novikoff, who later developed the acid phosphatase staining for identifying lysosomes morphologically [4].

Up to this date, more than 50 different enzymes were identified within lysosomes. Those membrane-delimited organelles are present in most nucleated mammalian cells. Lysosomes are mostly scattered across the cytoplasm but can become more concentrated around the perinuclear region upon stimuli [5]. Lysosome intracellular movement is required for its proper functioning and has shown to be tightly regulated in the cell [5]. Given their acidic interior, mostly composed by hydrolases, lysosomes are pivotal in intracellular degradation processes [6] such as intracellular digestion and autophagy [7, 8]. In order to digest endocytic cargo (membrane-bound vesicles resultant from pinocytosis or phagocytosis events) or autophagosomes, lysosomes have to fuse with those vesicles so their enzymes can have access to their content [9–11].

Besides being pivotal for intracellular degradation processes, lysosomes are also important for a plethora of physiological processes inside the cell, such as bone matrix resorption by osteoclasts [12], m-TOR-dependent antigen presentation by macrophages and dendritic cells [13], cholesterol transport [14], Ca^{2+}-regulated PM resealing upon injury [15] and cell death [16], just to cite a few examples. Perturbations in lysosomal homeostasis, such as dysfunction of lysosomal hydrolases, impairment in lysosomal traffic and biogenesis might induce lysosomal storage disorders due to accumulation of unprocessed substrata inside this organelle. There are more than 50 different types of lysosomal storage diseases that were already identified [17].

As mentioned before, lysosomes play an important role in membrane resealing upon injury, and they are a fundamental part of the endocytic pathway. The endocytic pathway is basically composed by early and late endosomes and lysosomes. Internalized particles are delivered to early endosomes and are either recycled back to the membrane or transported to late endosomes. When they reach the late endosomes, the endocytosed material can be sorted by the Golgi apparatus and transported to the membrane or fuse with lysosomes to be degraded [18].

There is no doubt that the endocytic pathway is fundamental for nutrient uptake, cell signalling [19], and migration [20]. A summary of the diverse cellular functions that the lysosomes are involved in is depicted in **Figure 1**. Intriguingly, the endocytic route is also explored by pathogens in order to successfully invade their host cells [21]. Some of these pathogens evolved in order to develop mechanisms to evade lysosomal fusion in order to protect them from being degraded from lysosomal enzymes. However, in some cases, the pathogen drives itself to encounter lysosomes in order to guarantee intracellular survival. The gram-positive bacteria, *Coxiella burnetti*, causative agent of Q fever [22], requires fusion with lysosomes in order to mature its parasitophorous vacuole [PV] and to replicate [23–27]. This pathogen also

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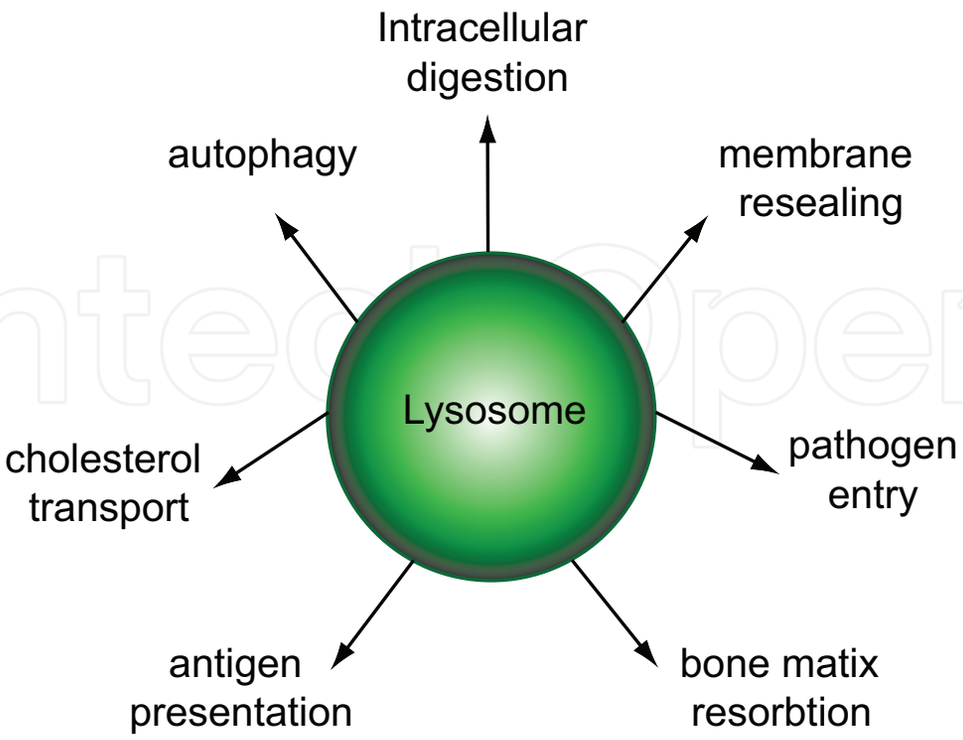


Figure 1. The multitask lysosome. Besides being involved in intracellular digestion, lysosomes also participate in other important cellular functions, such as autophagy, cholesterol transport, antigen presentation, bone matrix resorption, plasma membrane resealing upon injury and pathogen entry in host cells.

requires two lysosomal membrane proteins: lysosomal associated membrane protein 1 and 2 (LAMP-1 and LAMP-2) [28] in order to have normal PV size and bacterial replication rate [29]. Another example is the protozoan parasite *Leishmania donovani* that causes visceral leishmaniasis in humans [30]. By examining the infection of bone marrow–derived macrophages by *L. donovani* metacyclic-derived promastigotes, Forestier and colleagues (2011) demonstrated that the early invasion process is constituted by four phases: (1) contact between highly motile and polarized promastigotes and the PM preceding phagocytosis, (2) formation of the PV, differentiation into amastigote form and intracellular orientation of the parasite, (3) movement of the parasite towards the cell membrane leading to local wounding and (4) PV translocates to the perinuclear region of the host cell. They identified LAMP-1 positive tight PVs as early as 30 min post-infection and demonstrated that the parasites remained viable at those harsh and acidic conditions. Interestingly, at phase 3, when the parasite moves back towards the membrane, it causes membrane rupture evoking lysosomal exocytosis to reseal the membrane. They also found LAMP-1 decorating the parasite's flagellum facing the PM wounded area [31]. The gram-negative bacteria, *Neisseria meningitidis* and *Neisseria gonorrhoeae*, causative agents of meningitis and gonorrhoea, respectively, have a very interesting mechanism of invading mucosal cells. The invasion process can be divided into 4 different steps: (1) attachment, (2) phagocytosis of the bacteria by the host cells at the apical portion, (3) transport of those phagocytosed bacteria to the basal part of the cell and (4) exocytosis of the bacteria-containing vesicles to subepithelial tissues [32]. *Neisseria* secretes an immunoglobulin called IgA1. This immunoglobulin is able to cleave LAMP-1 when this protein is at the host cell

PM. In order to do that this bacteria induces Ca^{2+}-dependent lysosomal exocytosis, and when LAMP-1 is exposed at the surface, it is cleaved by IgA1 [33]. By cleaving LAMP-1, the bacterium also alters other lysosomal constituents, such as LAMP-2, lysosomal acid phosphatase and CD63 [34], which is thought to improve the bacteria intracellular survival.

One of the most interesting pathogens that interact with lysosomes in order to successfully invade host cells is the protozoan parasite *Trypanosoma cruzi. T. cruzi* is the causative agent of Chagas's disease [35], a tropical neglected disease that has no effective vaccine or cure and still affects about 6–7 million people worldwide [36, 37]. Virtually, *T. cruzi* can infect basically all nucleated cells from its mammalian host. The early entry process is complex and involves a plethora of receptors and proteins that are secreted in order to orchestrate parasite attachment and invasion [38]. One step that is pivotal for the parasite entry and infection is its early association with lysosomes [39]. *T. cruzi* subverts the PM wound healing route in order to get access to the intracellular milieu [40]. In fact, the understanding of the intertwined PM repair and *T. cruzi* entry processes evolved in parallel and was elegantly explored by Dr. Norma Andrews' group since the 90s. This chapter will try to explore how the understanding of those mechanisms evolved through time and which are the key players in both membrane healing and *T. cruzi* entry process.

2. From membrane resealing to *Trypanosoma cruzi* invasion: what is the role played by lysosomes?

2.1. Plasma membrane injury and resealing: lysosomes save the day

It has been known since the early 90s that professional secretory cells, such as hepatocytes [41, 42], activated platelets [43, 44], pancreatic acinar cells [45, 46], macrophages [47, 48], osteoclasts [49, 50] and neutrophils [51, 52] are able to undergo regulated lysosomal secretion. However, until the mid-90s, it was not known whether non-professional secretory cells had the capability of performing lysosomal exocytosis. In 1995, Miyake and McNeil demonstrated for the first time that endothelial cells were able to accumulate vesicles near PM injured sites, and those vesicles underwent Ca^{2+} mediated exocytosis in order to seal those wounds [53]. In 1996, Coorssen and colleagues have shown that epithelial cells enlarged their surface area by ~20–30% due to exocytosis promoted by increase in intracellular Ca^{2+}. However, back then, they just hypothesized that the increase in area was probably due to secretion of endosomes or lysosomes [54]. In 1997, Rodriguez and collaborators demonstrated that non-secretory cells, such as fibroblasts, myoblasts and epithelial cells, were able to trigger lysosomal exocytosis upon increase in intracellular Ca^{2+} levels. By performing enzymatic assays, they measured the presence of lysosomal enzymes, such as β-hexosaminidase and cathepsin D, in the supernatant of stimulated cells. In parallel, they also showed the presence of a lysosomal glycoprotein, Igp120, at the PM, corroborating the lysosomal exocytosis hypothesis [55].

Cells have evolved throughout time in order to develop a mechanism by which injuries in the PM could be quickly sealed in order to prevent cytoplasm leakage and cell death. Collagen matrix contraction assays for mimicking tissue morphogenesis and wound healing show that, upon contraction, fibroblasts can uptake extracellular dyes due to the formation of small

pores in the membrane. Those small wounds are sealed within 5 s in the presence of Ca^{2+} [56]. Tissues that are under mechanical stress, such as skeletal muscle [57], heart [58], gut [59] and skin [60] also have the ability to reseal their torn membranes and depend on this process for proper functioning. Impairment in sarcolemma resealing upon injury, for example, might cause muscular dystrophy [61].

2.2. Membrane resealing mechanism: from the patch hypothesis to acid sphingomyelinase-mediated compensatory endocytosis

The mechanism by which lysosomes reseal damaged plasma membranes was first proposed by Reddy and collaborators in 2001 [62]. Using non-professional secretory cells, such as epithelial cells, myoblasts and fibroblasts, they showed that membrane injury upon scratching is able to trigger lysosomal exocytosis in a Ca^{2+}-regulated manner. Similarly to neuronal synaptic vesicles that have a Ca^{2+}-sensor protein called synaptotagmin I (syt-I) [63], lysosomes have an isoform of synaptotagmin named syt-VII [64, 65]. Synaptotagmins are proteins that have a short ectodomain (N terminus lumenal domain), a transmembrane region and two cytoplasmic domains C2A and C2B that are Ca^{2+}-sensor domains. Reddy and colleagues demonstrated that the C2A domain is the one responsible for regulating Ca^{2+}-dependent lysosomal exocytosis [62]. Since then, it had been shown that lysosomes are able to undergo exocytosis in order to reseal PM injuries generated by different sources, such as pathogens [31] and pore-forming toxins [66], other than mechanical wounding. The most accepted model for PM repair in nucleated cells was proposed in the early 2000s and was called 'The Patch Hypothesis'. According to that model, right underneath the injured site lysosomes underwent chaotic fusion events in which they either fused directly with the PM or with one another in a homotypical fusion manner. Those abnormally enlarged vesicles ended up fusing with the injured PM donating membrane to seal the wounded region [67, 68]. However, the patch model failed to explain the repair caused by pore-forming toxins, which stably binds to the membrane. Later, it was shown that the wounding caused by pore-forming toxins led to the formation of intracellular vesicles.

Wound healing experiments performed in the presence of gold-BSA, added prior to injury, demonstrated that those vesicles have an endocytic origin given that they retained gold-BSA in their lumen [15, 69]. Nonetheless, lysosomes play a pivotal role in the endocytosis-mediated plasma membrane resealing model. Following membrane lesion and increase in intracellular Ca^{2+}, those organelles undergo exocytosis and secrete their enzymes into the extracellular medium. Acid sphingomyelinase (ASM) is one of the enzymes that remain active extracellularly after secretion, generating ceramide as a product of sphingomyelin hydrolysis [70, 71]. Ceramide coalesces at the membrane forming highly ordered domains excluding other lipids, such as glycerophospholipids, from those patches [72]. Those domains induce membrane curvature and budding [71, 73, 74] dragging the injured region inward, in a processes called compensatory endocytosis, closing the wound. Cells either deficient in ASM or pharmacologically inhibited fail to undergo compensatory endocytosis but still trigger lysosomal exocytosis. Addition of recombinant ASM to the extracellular medium is able to restore compensatory endocytosis in those cells [15]. Other lysosomal enzymes are also important to regulate the process. It has been proposed that cysteine proteases, cathepsins B and L,

released during lysosomal exocytosis may contribute to facilitate ASM access to PM [75]. Additionally, cathepsin D, another lysosomal enzyme released upon exocytosis, becomes active only later after its release and is responsible for negatively modulating ASM activity, closing the wounding cycle [75]. **Figure 2** depicts a timeline illustrating the evolution of the experimental models that explains how Ca^{2+}-dependent membrane resealing upon lysosomal exocytosis is regulated within cells.

2.3. *Trypanosoma cruzi*: how this parasite can take advantage of intracellular endocytic route to perpetuate its intracellular cycle: the essential role of lysosomes in the process

2.3.1. Trypanosoma cruzi and Chagas disease

T. cruzi is an obligatory flagellated intracellular parasite that causes Chagas disease in human hosts. This pathogen was first identified by the Brazilian doctor Carlos Chagas, in 1909, who not only identified the parasite but also unravelled its life cycle, the invertebrate host, the domestic reservoirs and the symptoms of the disease [35]. *T. cruzi* has a complex life cycle that consists of colonizing the midgut of an invertebrate host (a reduviid bug, also known as 'kissing bug') and several tissues from vertebrate hosts [76, 77]. The cycle on the invertebrate host begins when the reduviid bug takes a blood meal from a mammalian host, containing the trypomastigote forms of the parasite. The bloodstream trypomastigotes are the parasite infective form on vertebrate hosts. Once inside the insect midgut, the parasite differentiates into the epimastigote form, capable of replicating in the invertebrate host. Epimastigotes attach to the waxy walls of the insect hindgut where they differentiate into the metacyclic form in a process known as metacyclogenesis [78].

During a blood meal, the insect excretes, together with the urine and faeces, the metacyclic trypomastigotes, which are capable of infecting the vertebrate host. These released trypomastigotes reach the mammalian host bloodstream either via the wound site or through mucous membranes. Once inside the vertebrate host the metacyclic trypomastigotes can infect a plethora of nucleated cells. When the parasite invades the host cell, it can differentiate into the amastigote form, which is the replicative form on the mammalian host. After several rounds of replication, the amastigotes differentiate into the trypomastigote form and the cells, crowded with parasites, burst open. Extracellular trypomastigotes are now free to perpetuate their cycle and infect new cells and tissues. The process that comprises from intracellular invasion to intracellular multiplication, and cell rupture takes about 4–5 days [79, 80].

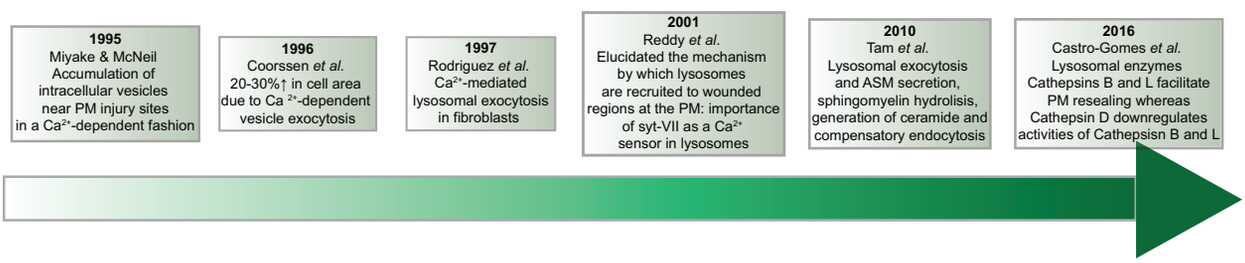


Figure 2. How Ca^{2+}-dependent lysosomal recruitment for plasma membrane resealing models evolved with time.

Recent statistics provided by World Health Organization (WHO) website shows that about 6–7 million people are estimated to be infected with *T. cruzi*, mostly in Latin America [37]. Chagas disease can be transmitted via several routes, being the vectorial route (through the contaminated insect) the canonical one. Along with the vectorial transmission, there are other primary routes of infection, being oral, placental and blood transfusion, the main ones, especially in non-endemic countries where the vector is not present. Those routes are responsible for the worldwide dissemination of Chagas disease [81, 82]. There are other less common ways of acquiring Chagas disease, such as laboratory accidents, dealing with infected animals and organ donation from deceased patients who had Chagas disease [83].

Chagas disease has two phases: acute and chronic. The acute phase lasts from 4 to 8 weeks, and it is usually asymptomatic. However, mild symptoms like fever, for example, might happen 1–2 weeks after infection from the insect vector bite or a month later in other cases of transmission. Only 5–10% of the symptomatic cases might lead to death [84]. The patients who survive from the acute phase will enter chronic phase, which lasts for the patient's lifespan. The majority of the individuals that enter the chronic phase have the indeterminate form of the disease. However, 30–40% of the patients will potentially develop cardiomyopathy, 10% will develop megaesophagus, megacolon, cardiodigestive or neurological problems [37, 85, 86]. Until this day, there is no vaccine available or 100% effective cure for Chagas disease, especially if the disease is diagnosed during the chronic phase. There are two drugs, benznidazole and nifurtimox, which have proven to be effective for some cases during the acute phase. However, their use is limited due to low availability and severe side effects [87].

2.3.2. Trypanosoma cruzi entry in host mammalian cells

As mentioned, *T. cruzi* is able to infect most nucleated cells, ranging from professional to non-professional phagocytic cells, the latter being the main focus of the parasite. In order to colonize host cells, *T. cruzi* has to go through four main steps: cell contact and/or attachment to the host cell membrane, intracellular signalling, internalization and intracellular multiplication. Therefore, the events that happen at the plasma membrane are paramount in order to guarantee a successful infection. In order to attach to the host PM, *T. cruzi* uses a variety of proteins that trigger intracellular signalling and parasite entry. Metacyclic trypomastigotes and cell-released trypomastigotes have different repertoires of redundant glycoproteins that have the ability to bind to the extracellular matrix or to specific receptors at the host PM helping in the parasite internalization process (for excellent reviews, please read [38, 88–90]).

Once the parasite gets in contact with host cells, the internalization odyssey takes place. Among them, Ca^{2+} signalling as well as lysosomal recruitment and fusion with the parasitophorous vacuole have been shown to be pivotal for a successful invasion [39, 40] Those two components are also fundamental for modulating PM repair in nucleated mammalian cells, as already described in Section 2.2. We are going to explore on the next subsection, how *T. cruzi* subverts this strategy, used for cells to mend their torn membranes, in order to invade host cells.

2.3.3. Trypanosoma cruzi and lysosomes: importance during parasite entry, maturation and intracellular multiplication

The first evidence showing that *T. cruzi* relies on lysosomes for cell entry was published in 1992 by Tardieux and collaborators [91]. They were motivated by previous work that suggested that *T. cruzi* entry mechanism differed from other pathogens, since actin disruption did not prevent cell invasion in non-professional phagocytic cells [92]. Therefore, *T. cruzi* host cell internalization process was distinct from a phagocytosis-mediated event. If the actomyosin cytoskeleton was not providing the force to drive the parasite towards the host cell cytoplasm, which component of the host cell would be playing this role? It had been shown that when *T cruzi* invades cells it resides temporarily in an acidic vacuole from lysosomal origin [93]. Thus, they decided to investigate how lysosomes participate in that process. They identified lysosomal accumulation near the parasite attachment site at host cell plasma membrane, during the first steps of parasite invasion. By doing perturbations in the microtubule cytoskeleton, in which lysosomes migrate on, they verified changes in *T. cruzi* infection rates. Drug treatments that promoted an outward motion of lysosomes (from the perinuclear region to near the PM) enhanced invasion, whereas blockage of lysosome migration towards the PM inhibited *T. cruzi* entry. Loading lysosomes with sucrose also decreased invasion rate. These authors also showed that cytochalasin D-mediated actin depolymerisation increased invasion by changing lysosomal distribution within the host cell [91]. This work was fundamental for the field since it demonstrated that lysosomes were important for the first stages of parasite invasion, donating membrane for the formation of the parasitophorous vacuole. However, they did not know back then what triggered lysosome secretion during *T. cruzi* invasion.

Two years later, Tardieux and colleagues demonstrated that by exposing NRK cells either to trypomastigotes or to membranes isolated from trypomastigotes, Ca^{2+} transients were elicited in the host cell cytoplasm after only 200 s of exposure, which is faster than the invasion process *per se*, which lasts about 10 min [94]. The same experiment using epimastigotes or epimastigote-isolated membranes, the non-infective form, did not lead to host cell Ca^{2+} transients. Interestingly, they also challenged the cells with *Trypanosoma brucei*, the causative agent of African sleeping sickness, and did not see Ca^{2+} response either. In addition to that, they demonstrated that by blocking intracellular Ca^{2+} transients, before *T. cruzi* exposure, invasion rates decreased. Treatment of host cells for 4 h with Pertussis toxin, known to uncouple $G_{\alpha i}$ and $G_{\alpha 0}$ from their receptors, impairing intracellular signalling cascade [95], halted intracellular Ca^{2+} transients generated upon *T. cruzi* stimulation, suggesting that the parasite-induced Ca^{2+} signalling was likely linked to phospholipase C (PLC) activation and IP_3-mediated Ca^{2+} release from intracellular stores [96, 97]. These two works were primordial since they linked Ca^{2+}-mediated signalling, evoked by *T. cruzi*, and host cell membrane interaction, to lysosomal recruitment and parasite invasion.

In 1995, two other papers from Dr. Norma Andrews' group demonstrated that a Trypomastigote soluble peptidase (also referred to as Proteolytically Generated Trypomastigote Factor—PGTF) was able to generate Ca^{2+} transients in NRK cells [98]. They also proved that PGTF is an agonist of PLC/IP_3 generating Ca^{2+} transients, ultimately leading to actin cytoskeleton remodelling which facilitates *T. cruzi* invasion [99].

Years later, in 2001, Wilkowski and collaborators showed that incubation of phagocytic and non-professional phagocytic cells with phosphatidylinositol 3-kinase (PI3K) inhibitors, prior to *T. cruzi* exposure, reduced the invasion rates in those cells [100]. In addition to that, they also demonstrated that *T. cruzi* trypomastigotes or purified trypomastigote membranes elicited high activation of PI3K and PKB/Akt (protein kinase B) on host cells, which was not detected when cells were incubated with epimastigotes or their isolated membranes [100].

Two years later, Woolsey and collaborators demonstrated that even though lysosomes were important for *T. cruzi* invasion, there was a population of parasites that entered cells via a tight parasitophourous vacuole that were devoid of lysosomal markers and formed exclusively by host cell PM markers [101]. These data indicated that *T. cruzi* would also be able to enter host cells via PM invagination and only later fuse with lysosomes. This invasion process was also shown to be independent of host cell actin and to involve PI3K activation [101].

In 2004, Andrade and Andrews demonstrated that parasites that entered the host cell via PM-invagination mechanism gradually escape cells if they do not associate with lysosomal markers, demonstrating that association with lysosomes was pivotal for a successful invasion [39]. Therefore, in the early 2000s, there were two convergent accepted models for *T. cruzi* cell invasion: one that was mediated by host cell PM invagination with later association with lysosomal markers, and another one that was dependent on early lysosomal association, in which lysosomes fused with the PM donating membrane for parasitophorous vacuole formation.

The fact that *T. cruzi* entry was dependent on Ca^{2+} signalling and lysosomal exocytosis, similarly to the lysosomal-mediated plasma membrane repair (explored in Section 2.2) [15], inspired Fernandes and colleagues to investigate whether the parasite would subvert this process to gain access to the host cell. First, they demonstrated that extracellular Ca^{2+} chelation inhibited *T. cruzi* invasion significantly, showing that intracellular Ca^{2+} stores were not the only source during parasite invasion. They also showed that *T. cruzi* causes host cell PM injuries that are rapidly sealed in the presence of Ca^{2+} [40]. Additionally, the concomitant incubation of streptolysin O (SLO), a pore-forming toxin that binds to cholesterol-enriched domains at the PM [102], and *T. cruzi* increased invasion rate, reinforcing the role of extracellular Ca^{2+} in parasite entry process [40]. Finally, pharmacological inhibition or siRNA for ASM (the enzyme responsible for compensatory endocytosis and membrane resealing) reduced trypomastigote invasion, while addition of purified ASM to the extracellular media in ASM depleted cell cultures restored *T. cruzi* invasion. The latter strongly suggested that *T. cruzi* depends on compensatory endocytosis for entering host cells. The proof that compensatory endocytosis was in fact the route of invasion came from their findings showing *T. cruzi* parasitophorous vacuole decorated with ceramide markers. As we already mentioned in Section 2.2, ceramide is generated by ASM-mediated cleavage of sphingomyelin, and it is responsible for the endocytic-directed events following lysosomal fusion. This ceramide containing vacuole was shown to fuse later with lysosomes, providing the anchoring force to retain the parasites inside the host cell [40]. This work set in stone the fact that *T. cruzi* subverts the physiological process by which lysosomes fuse with the PM upon injury in order to successfully invade cells.

Lysosome fusion with plasma membrane induced upon membrane injury is a tightly regulated process and dependent on PM cholesterol content [103]. In 2012, Hissa and collaborators

demonstrated that cholesterol depletion of cardiomyocytes prior to exposure to trypomastigotes changed the distribution of lysosomes within the host cell and evoked a massive lysosomal exocytosis near the cell cortex, even in the absence of extracellular Ca^{2+} [104]. These critical lysosomal exocytic events led to a decrease in parasite internalization and lysosomal association for parasitophorous vacuole maturation [104]. One year later, Hissa and colleagues proposed a mechanism by which cholesterol depletion triggered intracellular Ca^{2+}-independent lysosomal secretion. Using methyl-beta cyclodextrin (MβCD) to chelate cholesterol from PM, they showed, by measuring mechanical properties of cell cortices, that cholesterol-depleted cells become more rigid with less membrane fluctuations [105]. This work corroborated previous studies done in cholesterol-depleted endothelial cells [106]. In line with that, cholesterol depletion induced Rho activation, which in turn led to actin polymerization enhancing cortical rigidity. Most importantly, the authors showed that lysosomal exocytosis triggered upon cholesterol depletion was not only Ca^{2+} but also Syt-VII independent, pointing out to a non-regulated secretion of those organelles. They suggested that actin polymerization induced by cholesterol depletion was responsible for the secretion of a lysosomal pool near the cell cortex. Based on these results, one can conclude that cells should have at least two different pools of these organelles, one located closer to the cell cortex, and most likely to be involved with membrane resealing events, and the second located closer to the cell nuclei and probably related to intracellular digestion. For the first pool, actin polymerization could work as an exocytic driving force, whereas for the second, it would present as a barrier for fusion with the PM. In fact, treatment of cells with Latrunculin-A, an actin filament-disrupting drug, induced the secretion of a more internally localized lysosomal pool [105]. In 2015, Hissa and Andrade demonstrated that *T. cruzi* preferentially uses cortical, cholesterol depletion-sensitive lysosomal pool as opposed to the more internally localized, Latrunculin-A sensitive lysosomal reservoir, linking the cortical pool of lysosomes with plasma membrane repair [107].

Regarding intracellular development, *T. cruzi* association with lysosomes remains crucial. In order to replicate in the host cytosol, trypomastigotes need to escape from the lysosomal-enriched parasitophorous vacuole and differentiate into amastigote form. In the late 80s and the early 90s, it was shown that *T. cruzi* secretes a hemolysin factor, active in low pH (5.5), which was capable of lysing erythrocytes isolated from different animal species by forming a large pore in their membranes [108]. If the acidic nature of the parasitophorous vacuole was altered, by raising its pH, parasites were unable to escape to the cytosol. These data corroborated the existence of a hemolysin protein secreted by the parasite, identified as Tc-Tox, which would form a pore at the vacuolar membrane allowing *T. cruzi* to exit and fall into the host cell cytosol [109].

Lysosomal membrane proteins are also important for *T. cruzi* entry and intracellular development, multiplication and release. By using LAMP1/2 knockout cells (LAMP-1/2 KO), Albertti and collaborators showed that LAMP-1 and 2 were important for parasite invasion. Absence of LAMP led to a decrease in parasite ability to invade host cells. Moreover, they showed that, even though parasite entry was reduced, intracellular multiplication was faster in those LAMP-1/2 KO cells, and more trypomastigotes were released after 96 h of infection [110].

Those results point out to the importance of these highly sialilated lysosomal proteins for parasite invasion and intracellular development.

As exposed here, opposite to other pathogens, *T. cruzi* takes advantage of lysosomes to infect and perpetuate its life cycle in the vertebrate hosts. It hijacks lysosomes and the physiological route that cells use to repair their torn plasma membranes in order to successfully invade them. Later, it uses lysosomal membrane and acidic environment to gain access to host cell cytosol and colonize it. Any perturbations that prevent lysosomal association with the parasitophorous vacuole or lysosomal distribution, such as host cell PM cholesterol content, culminate with parasite escape and consequently less invasion. Besides, lysosomal content and membrane alterations may also interfere with parasite intracellular development. Therefore, host cell lysosomes control and/or interfere with parasite entry, development, and extracellular release.

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