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Secretory organelle trafficking in *Toxoplasma gondii*: A long story for a short travel

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ABSTRACT

Toxoplasma gondii (T. gondii) possesses a highly polarized secretory system, which efficiently assembles *de novo* micronemes (MIC) and rhoptries (ROP) during parasite replication. Pioneer works have studied the sorting motifs within MIC and ROP proteins, required for their trafficking towards their final destination. These studies led to the conclusion that protein processing and protein sorting are inter-dependent activities. More recent works have revealed the trafficking routes taken by the MIC and ROP proteins by examining the functions of the endo-exocytic compartments and identified key molecules involved in protein sorting and transport. These recent findings have suggested that *T. gondii* has repurposed the evolutionarily conserved regulators of the endosomal system to the secretory pathway. This review reports the pioneer as well as the most recent findings on the molecular mechanisms regulating apical organelle and dense granule biogenesis and portrays the parasite as a remarkable secretory machine that has efficiently remodeled its trafficking system to adapt to an intracellular lifestyle.

1. Introduction

Parasites belonging to the phylum Apicomplexa exhibit a highly polarized organization of their subcellular structures. In particular, the secretory system contains apically oriented unique organelles: the micronemes and the rhoptries, as well as, the more dispersed cytosolic dense granules, which are essential for parasite virulence. These organelles contribute to a plethora of functions ranging from host cell attachment and invasion, to establishment and maintenance of the parasitophorous vacuole (PV) and to parasite replication and survival. Microneme proteins regulate parasite motility and host cell adhesion / invasion. In particular, the microneme protein AMA1 and RON proteins (Rhoptry Neck proteins) are first secreted upon parasite adhesion and form a transitory ring shaped adhesive structure called the moving junction (MJ), which aids propulsion of the parasite into the host cell (Alexander et al., 2005; Lebrun et al., 2005; Bargieri et al., 2014). While the RON proteins favor invasion, ROP (Rhoptry bulb proteins) and dense granule (GRA) proteins play an essential role in parasite survival by modulating host immune and metabolic responses (Hakimi et al., 2017). Owing to the essential roles of these virulent factors, elucidating the molecular mechanisms by which the secretory organelles are newly formed during parasite replication has been an intense focus of research over the last three decades. To this end, the highly polarized organization of the parasite secretory system, together with the numerous genetic tools recently developed, has facilitated the use of *T. gondii* as a powerful model to study protein trafficking and organelle biogenesis in apicomplexan parasites. Micronemes and rhoptries are formed *de novo* during daughter cell assembly by budding of vesicles containing newly synthesized ROP and MIC proteins from the Golgi apparatus. Therefore, the synthesis, sorting and transport of MIC and ROP proteins are tightly linked to the biogenesis of the secretory organelles. Morphologically, *T. gondii* possesses an endoplasmic reticulum (ER) that is contiguous with the nuclear envelope and a Golgi apparatus consisting of a single stack of three to five cisternae (Pelletier et al., 2002). Genome sequencing indicates that the molecular machinery regulating vesicular transport in Apicomplexa is partially conserved compared to other Eukaryotes. Components of the vesicle budding, transport and fusion machinery have been identified, including *N*-ethylmaleimide-sensitive fusion (NSF) factor (Chaturvedi et al., 1999), a reduced set of Rab proteins (Kremer et al., 2013), Soluble *N*-ethylmaleimide-sensitive-factor Attachment protein Receptor proteins (SNAREs) (Jackson et al., 2013), subunits of the coatomer (Pfluger et al., 2005), clathrin adaptor complexes (Ngo et al., 2003) and Vacuolar Protein Sorting (VPS) proteins (Morlon-Guyot et al., 2015; Sangare et al., 2016). In addition,

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evolutionarily conserved sorting motifs functioning in the transport of proteins to micronemes and rhoptries have been characterized (Di Cristina et al., 2000) (Hoppe et al., 2000). Strikingly, *T. gondii* and possibly other apicomplexan parasites use their endolysosomal system as an intermediate track to transport proteins destined for regulated secretion and secretory organelle biogenesis. Likely, merging the endocytic and exocytic pathways allows convenient access to proteases necessary for the maturation of secretory proteins.

2. Cell division and the tight relationship with secretory organelle biogenesis

Unlike the conventional process of cell division involving mitosis employed by most Eukaryotes, *T. gondii* goes through a special mode of replication called endodyogeny. Each cycle of DNA replication is followed by the assembly of daughter cell scaffolds, which promotes nuclear mitosis and cytokinesis. Therefore, *T. gondii* daughter cells do not arise by fission of the mother cell, but instead bud within the mother cell. Unlike *Saccharomyces*, the model eukaryote (which initiates budding at G1/S), budding in the *T. gondii* tachyzoite begins in late S phase, immediately prior to the entry into mitosis. These observations partly led to the important finding that the usual G2 gap that separates the S phase from mitosis is likely missing in the asexual stages of this parasite (Gubbels et al., 2008).

During cell replication, micronemes and rhoptries are assembled *de novo* and positioned at the apical pole of each daughter cell (Nishi et al., 2008). The assembly is aided by the highly polarized organization of the secretory pathway. Proteins flow in a sequential fashion from the endoplasmic reticulum (ER) to the Golgi apparatus, and from there to endosomal-like compartments, to eventually reach intermediate compartments, precursors of mature rhoptries and micronemes (Tomavo et al., 2013). Importantly, the ER exit site is restricted to a small region of the nuclear envelope that is close to the centrosome (Hager et al., 1999). As mitosis occurs, the Golgi apparatus start dividing by lateral extension and fission. The medial fission of the Golgi stacks occurs in close juxtaposition with the duplicated centrosomes and appears to be complete prior to nuclear division, highlighting the essential role of the secretory pathway in establishing cell polarity. Of note, centrosomes display an unusual pattern of migration during cell replication. The mother centrosome transiently dissociates from the Golgi apparatus and the apicoplast to migrate to the basal end of the nucleus, divide and move back to the apical end to re-associate with the duplicated Golgi (Hartmann et al., 2006). This centrosome migration may be involved in defining the apical-basal polarity of daughter cells and subcellular structures. Division and segregation of the apicoplast, ER and nucleus occur next and are dependent on the elongation of the daughter scaffolds, thus are inhibited by microtubule depolymerizing drugs. Immature rhoptries and micronemes then begin to form *de novo* by vesicular budding from the Golgi, within the developing parasites after daughter scaffolds have started to elongate (Nishi et al., 2008). Strikingly, in *T. gondii,* endosomal-like compartments are found in intimate proximity to the trans-Golgi network (TGN) (detailed in the following section). This physical association presumably optimizes the transport and processing of newly synthesized proteins towards the apical pole where rhoptries and micronemes are positioned for an efficient invasion process.

Interestingly, it has been observed that ROP maturation from pre-ROP compartments to mature club-shaped organelles is dependent on completion of cytokinesis (Dubremetz, 2007). Similarly, targeted depletion of the protein ROP2 results in an arrest of cytokinesis (Nakaar et al., 2003). Therefore a functional relationship exists between cytokinesis and the assembly and positioning of ROP and MIC organelles during cell division, however, direct molecular links have not been identified so far.

3. The *T. gondii* plant-like endosomal system: the Rab GTPases

T. gondii displays a stripped down version of the more complicated trafficking system of mammalian cells and other unicellular Eukaryotes. For instance, Apicomplexa possess only a basic set of Rabs (Kremer et al., 2013). In addition, several highly conserved endocytic factors are missing. Indeed, *T. gondii* and the other Apicomplexa lack nearly all of the components of the Endosomal Sorting Complexes Required for Transport (ESCRT) (Tomavo et al., 2013). *T. gondii* also lacks the Golgi-associated, Gamma adaptin ear containing, Arf binding coat proteins (GGAs) that regulate the anterograde protein transport between the TGN and the endo-lysosomal compartments (Tomavo et al., 2013).

The spatial organization and the functions of the endosomal and exocytic compartments in *T. gondii* have for long remained unclear. In mammalian cells, the early endosome (EE) compartment is involved in regulating the transport of endocytosed material to the sorting endosomes for recycling purpose or to the late endosomes for degradation (Mellman, 1996). Early studies indicated that in *T. gondii*, TgRab5A, which classically defines EE, localizes to a tubulo-vesicular compartment closely associated to but distinct from the Golgi, which can be dispersed by Brefeldin A (BFA) and to large, lucent vesicles containing internal membranes (Robibaro et al., 2002). In a similar way, TgRab7, a marker of late endosomes (LE) in mammalian cells, was detected in *T. gondii* in a restricted compartment adjacent to but distinct from the Golgi apparatus (Parussini et al., 2010). These early observations suggested that *T. gondii* has diverted the endosomal system activity to a secretory purpose. This hypothesis was confirmed by an overexpression screen of the Rabs expressed in *T. gondii*, showing that TgRab5A and TgRab5C function as important regulators of the traffic to micronemes and rhoptries (further developed in the next chapters). Of note, conventional EEs have not been identified in plants (Contento and Bassham, 2012; Dettmer et al., 2006). There are strong evidences that plants rather possess a hybrid TGN/EE compartment serving as a sorting platform for protein trafficking to the different subcellular compartments. This hybrid TGN/EE is also the first site for receiving the endocytosed material (Contento and Bassham, 2012; Dettmer et al., 2006). Thus, *T. gondii* seems to possess an endosomal-like system more similar to plants than fungi or vertebrates. Importantly, so far, neither clathrin-dependent nor caveolae-regulated endocytic activities have been demonstrated in *T. gondii*. M. Meissner and co-workers examined this aspect by characterizing the function of clathrin (Pieperhoff et al., 2013). They found that the clathrin heavy chain 1 (CHC1) localized to the Golgi apparatus, but not at the parasite surface. In addition, functional ablation of CHC1 resulted in Golgi aberrations, a block in the biogenesis of the microneme and rhoptry organelles, and of the pellicle, suggesting a major role for clathrin in the secretory pathway but not in endocytosis. However, due to the lack of traceable endocytic markers, this conclusion has not yet been clearly ascertained and requires further investigation. Interestingly, V. Carruthers and colleagues have observed the uptake of large proteins from the host cell into intracellular parasites after depletion of the cathepsin L protease (Dou et al., 2014). Thus, this could represent a useful tool to test the function of clathrin and the clathrin adaptor complex AP2 in endocytosis.

In addition, the function and localization of TgRab11A and TgRab11B, which are important regulators of the recycling and secretory activities in other Eukaryotes, were characterized *in T. gondii*. Overexpression of TgRab11 A mutated in its GTPase domain leads to a cytokinesis defect due to a block in the Inner Membrane Complex (IMC) formation and to a defect in the secretion of plasma membrane proteins, suggesting that TgRab11 A regulates constitutive secretion (Agop-Nersesian et al., 2009). TgRab11B localizes to the Golgi at the initial phase of cell division and accumulates to the nascent IMC of daughter parasites. Overexpression of TgRab11B mutated in its GTPase domain impaired the trafficking of newly synthesized IMC proteins from the Golgi to the daughter cell buds and thereby daughter cell budding (Agop-Nersesian et al., 2010). These two studies indicate that the