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What's the role of autophagy in trypanosomes?

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Trypanosomes are protozoa that diverged early in the eukaryotic lineage exhibiting unusual molecular and biochemical features. For example, trypanosomes use a glutathione-spermidine conjugate named trypanothione to regenerate thiols [1], decode their mitochondrial transcripts through extensive RNA editing [2], and possess a compartmentalized glycolysis in unusual peroxisomes called glycosomes [3]. *Trypanosoma brucei*, the causative agent of sleeping sickness in humans, experiences complex environmental changes during its complicated life cycle, in which it undergoes differentiation processes in both a vertebrate and an invertebrate host. In the vertebrate host, the medically relevant bloodstream forms (BSF) exist as two distinct stages that are morphologically and metabolically different: (i) Long slender forms, which rapidly proliferate and (ii) short stumpy forms that are cell cycle-arrested but adapted to survive in the tsetse fly. When taken up by the invertebrate host (the tsetse fly), the parasites transform into procyclic forms (PCF). The molecular mechanisms regulating the conversion of long slender forms to short stumpy forms are not clear. It is known that proliferating long slender forms live in a glucose-rich milieu and consequently possess a simple metabolism based on obtaining ATP from the breakdown of glucose [4]. In contrast, PCF have a functional mitochondrion, glycolysis is coupled to oxidative phosphorylation, and their metabolism depends largely on amino acids [5]. Short stumpy forms contain a better developed mitochondrion than long slender parasites, which allows them to survive upon uptake by the fly [6]. Under these circumstances parasite organelle turnover during its life cycle is crucial.

The main cellular pathway involved in the degradation of cytoplasmic organelles and long-lived proteins is autophagy [7]. Autophagy may occur as a non-selective or a selective process. Depending on the nature of the material to be surrounded, it can be defined as macroautophagy, when fractions of the cytoplasm, containing bulk cytosol and organelles, become surrounded by a double-membrane structure to form the phagophore; or microautophagy, when part of cytoplasmic components are directly taken up by lysosomal membrane engulfment. Sorting of redundant or damaged organelles for their degradation occur throughout various autophagy-related processes such as pexophagy, mitophagy, ER-phagy, ribophagy, and micronucleophagy (reviewed in 8). In this scenario, *T. brucei* must also possess mechanisms for the breakdown and recycling of obsolete cellular components in order to guarantee homeostasis during its differentiation and/or development. Indeed, orthologues of yeast autophagy-related (Atg) proteins have been identified in *T. brucei* by bioinformatic analyses [9].

Most of the understanding on the autophagy process is due in large part to investigations using *Saccharomyces cerevisiae*. The high tractability of these cells' genetics, in combination with intensive cellular and physiological research, has established *S. cerevisiae* as a model of autophagy. Hence, research groups have taken advantage of the knowledge on yeast autophagy and have extrapolated this information to other single-cell organisms by comparing and analyzing orthologous genes. In *T. brucei*, this can be performed by knocking down genes using RNAi [10]. The paper by Proto *et al*. in this issue of Microbial Cell [11], and that of Li *et al.* (2012) [12], take advantage of the RNAi method to develop reporter cell lines for the autophagy pathway in *T. brucei*. In the future, this method will allow a detailed and systematic evaluation of all orthologous autophagy-related proteins and help piecing together the puzzle of the *T. brucei* autophagy pathway.

Proto *et al.* genetically engineered PCF and BSF of a *T. brucei* cell line well suited for RNAi, to express fluorescently-tagged chimeric proteins such as YFP-Atg8.1 and YFP-Atg8.2 serving as autophagy-specific reporters. The system was validated by subjecting the parasite to starvation and following the re-localization of the fusion proteins from the

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cytosol to punctate structures by using fluorescence microscopy [11]. Their results on starvation reproduced those previously reported by Li *et al* (2012). Li and coworkers further characterized these punctate structures by electron microscopy and immunogold labeling, observing the typical feature of autophagosomes. Using this model, Proto and colleagues decreased the expression of specific Atg proteins by RNAi to disturb the autophagy process in both BSF and PCF of *T. brucei* and then evaluated how this disruption affected the parasite differentiation and cell death. Autophagy-defective mutants of PCF showed an altered growth in normal media while BSF mutants grew normally. The authors suggest that autophagy does not appear to be required for the differentiation of BSF to PCF [11]. Still, *T. brucei* stages drastically differ metabolically as well as in the organization of their subcellular structures and autophagy is normally involved in general cell architecture remodeling [7]. Moreover, it has been demonstrated in *Leishmania*, a closely related parasite, that autophagy is a key step in differentiation [13]. Thus, the question arises of what specific alternative pathway is responsible for *T. brucei* differentiation? The answer remains to be resolved but it may involve one or more of at least three options: i) the simplest explanation is that another protein not homologous to Atg8 is the one responsible. This would enlighten why autophagy is not detected in some cases. It has been described in trypanosomatids that some Atg proteins carry out different functions than those of their counterparts in *S. cerevisiae*, e.g., an Atg8-homologous protein in *Leishmania* is a truly functional Atg12 [14]. ii) Although most of the protein components are shared, perhaps just a part of the autophagy pathway is divergent between *T. brucei* and yeast. These new parasite components have not been identified yet in the database. An example of this is the cytoplasm-to-vacuole targeting (Cvt) [15], which is known to be unique in yeast and some filamentous fungi. The Cvt pathway, closely related to degradative autophagy, uses most of the Atg proteins. However, Cvt is not directly involved in the autophagy process; conversely, it participates in a biosynthetic pathway [16]. iii) Another possibility is that the whole dynamic rearrangement of the parasite structure relies not only on a major macroautophagy process, but on a sum of different and selective kinds of microautophagy processes (which would also be divergent) and maybe on other types of still unknown pathways. An example is the micropexophagy of glycosomes. Its importance has been clearly demonstrated in the glycosome turnover during the transition from BSF to PCF. Under this situation and similar to what it is known to happen in other organisms, a quick rise of Atg8 expression would be predicted in order to cope with the demanding situation. In contrast, the level of *T. brucei* Atg8 and its transcripts were low and there were no significant differences between the various differentiation stages [17].

Another question addressed in the article of Proto and colleagues was the possible function of autophagy in programmed cell death (PCD). Two experiments were evaluated: i) dihydroxyacetone (DHA) treatment and ii) starvation. DHA causes cell cycle arrest and is able to kill *T. brucei* at a concentration that is innocuous to humans [18, 19]. Proto and colleagues treated ATG5 RNAi-induced mutant trypanosomes with DHA and found similar DHA effects compared to uninduced control cells [11]. Unexpectedly, the parasites only showed a slight increase of punctate structures (Atg8 reporter protein) even at 6 mM. Interestingly, autophagy-defective mutants of BSF (*ATG*5-knockdown) showed a similar IC_{50} (concentration that inhibits the 50% of parasite growth), suggesting that autophagy is not required for cell death. Moreover, genetically engineered PCF in which *ATG*3 or *ATG*7 genes were knocked down by RNAi were significantly affected in acute nutrient starvation, suggesting that autophagy is required to survive under those circumstances. Conversely, Li *et al.* (2012) using a Tb*ATG*8.1/8.2 double knock-down or an *ATG*3-RNAi mutant observed improved cell viability under nutrient-limited conditions. Although these results are apparently contradictory they could be compatible. Under acute starvation, autophagy is probably working as a survival strategy. But under nutrient-limited conditions, the pathway could be inducing cell death in a part of the parasite population, allowing the use of the limited food source for few parasites. A similar statement has been suggested in yeast [20].

It is important to keep in mind that only half of the Atg proteins present in yeast apparently have orthologous in trypanosomatids. These protozoa branched off early in the eukaryotic evolution tree and display extensive specializations in their biochemical routes when compared to other eukaryotes. These parasites present enormous divergences; for example, the glycolysis route is highly conserved from bacteria to mammalian cells and 100% of the glycolytic enzymes are also present in trypanosomes. However, in these parasites the pathway is contained in a unique organelle, the glycosome. The glycolytic enzymes show traits of plant/algal-like proteins and the control of the pathway is exceptional and completely unexpected [3]. Thus, similar to glycolysis, the autophagy pathway could also be different in trypanosomatids.

Future studies will further contribute to unravel the mysteries of the autophagy pathway in *T. brucei*, for instance using genetic tools based on genome-scale RNA interference [21]. Taken together, Proto *et al.* have started to shed light on the molecular mechanism of autophagy in African trypanosomes by generating reporter BSF-cell lines. This article will motivate the development of new and more refined mutant parasites as tools to define the roles of the autophagy pathway in *T. brucei*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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