Target of rapamycin (TOR) kinase in Trypanosoma brucei: an extended family

Manuel Saldivia*, Antonio Barquilla*, Jean-Mathieu Bart*,†, Rosario Diaz-González*, Michael N. Hall‡ and Miguel Navarro*†

*Consejo Superior de Investigaciones Científicas, IPBLN, 18100 Granada, Spain, †Centro Nacional de Medicina Tropical, ISCIII, 28019 Madrid, Spain, and ‡Biozentrum, University of Basel, CH-4056 Basel, Switzerland

Abstract

The complex life cycle of Trypanosoma brucei provides an excellent model system to understand signalling pathways that regulate development. We described previously the classical functions of TOR (target of rapamycin) 1 and TOR2 in T. brucei. In a more recent study, we described a novel TOR kinase, named TOR4, which regulates differentiation from the proliferative infective form to the quiescent form. In contrast with TOR1 loss-of-function, down-regulation of TOR4 triggers an irreversible differentiation process through the development of the insect pre-adapted quiescent form. TOR4 governs a signalling pathway distinct from those controlled by the conventional TOR complexes TORC1 and TORC2. Depletion of TOR4 induces all well-known characteristics of the quiescent developmental stage in trypanosomes, including expression of the PAD (proteins associated with differentiation) surface proteins and transcriptional down-regulation of the VSG (variant surface glycoprotein) gene. TOR4 kinase forms a structurally and functionally distinct complex named TORC4. TOR4 associates with LST8 (lethal with sec-13 protein 8) and other factors including an armadillo-domain-containing protein and the major vault protein, which probably serves as a scaffold for this kinase. Research in T. brucei, a protozoan parasite that diverged from the eukaryotic tree early in evolution, may help to uncover new functions of TOR kinases.

Introduction

Since its discovery in 1991 [1], TOR (target of rapamycin) has emerged as a multi-tasking kinase involved in a broad range of processes in eukaryotes. TOR protein kinase is of interest for many reasons. First, it is highly conserved throughout eukaryotic evolution from yeast to humans [2]. Secondly, TOR acts as a ‘master switch’ of cellular anabolic and catabolic processes in response to the quantity and quality of nutrients/energy [3]. Finally, TOR is currently a major drug target for the pharmaceutical industry given its key role in aging, cancer and neurological diseases.

The TOR protein kinase is an atypical serine/threonine kinase that belongs to the PIKK (phosphoinositide 3 kinase-like kinase) family [4]. At first, TOR inhibition seemed simple: once inside the cell, rapamycin binds to FKBP12 (FK506-binding protein 12) and this binary complex acquires high affinity for the FRB (FKBP12/rapamycin-binding) domain of TOR, to interfere with the function of the kinase. However, rapamycin only inhibits a subset of functions performed by TOR [5]. For that reason, current efforts are directed to identify compounds with the ability to inhibit overall mTOR (mammalian TOR) function, indirectly such as metformin [6] or directly such as ATP-competitive inhibitors that target the mTOR catalytic domain [7].

TOR protein kinase works in two broad multiprotein complexes named TORC1 and TORC2, which integrate different external and internal stimuli to preserve cell fitness. Although the two complexes trigger distinct processes in response to different cues [8–10], they co-operate to coordinate cell growth [11].

Considering all the implications of TOR in the maintenance of the cell growth and proliferation, the biological network of TOR kinases is expanding each year. New studies are focused not only on novel TOR functions in mammals and yeast, but also on describing the role of this kinase in other eukaryotes.

The protozoan parasite Trypanosoma brucei, the aetiological agent of human African trypanosomiasis, undergoes a life cycle that alternates between the tsetse fly and a mammalian host. These adaptations involve particular changes in gene expression to complete metabolic and morphological changes at each stage [12,13]. In addition, during the course of mammal infection, the parasite must differentiate from the proliferative (long slender) to quiescent (short stumpy) bloodstream form, to adapt cell metabolism to the distinct nutrient and energetic conditions encountered during the transition from the mammalian host to the insect host and, importantly, to avoid exhaustion of the mammalian
host [14,15]. In the present article, we summarize the roles of TOR protein kinase in the biology of T. brucei.

**The T. brucei TOR network**

Most eukaryotes co-ordinate cell growth via a single TOR. However, micro-organisms such as trypanosomatids express additional TOR paralogues that retain the classical structure of mTOR kinase domains [16]. Interestingly, trypanosome cell growth is regulated by four distinct TOR kinases, which constitutes the most complex TOR network described so far in a eukaryote [16–19]. Tb (T. brucei) TOR1 and TbTOR2 present the typical TOR domain architecture, whereas TbTOR3 (previously known as TOR-like 1) has an extra PDZ domain and TbTOR4 (previously known as TOR-like 2) lacks a conserved FRB domain [16]. Moreover, every single TB protein binds a different subset of proteins, comprising four distinct TORCs [16–19]. The presence of additional TOR proteins, of four distinct TOR-containing complexes, and domain architecture variations within the TORs themselves suggest that these novel members of the TOR family could have acquired additional roles compared with those carried out by TORC1 or TORC2, new roles possibly related to the complex life cycle performed by these parasitic protozoa.

The first two sensu stricto TORs described in trypanosomes, TbTOR1 and TbTOR2, control distinct aspects of cell growth by signalling through distinct TbTORCs. TbTOR1 localizes to the nucleus and regulates temporal aspects of cell growth, typically controlled by nutrients, such as protein synthesis. TbTOR1 binds to Tbraptor (Raptor is regulatory associated protein of TOR) and TBLST8 (LST8 is lethal with sec-13 protein 8) and constitutes TbTORC1. Depletion of TbTOR1 protein led to delocalization of RNA pol (polymerase) I from the nucleolus [16], indicating that this kinase positively controls rDNA (ribosomal DNA) transcription and protein translation. Furthermore, the absence of TORC1 activity results in reduced cell size, arrest in G1-phase of the cell cycle and appearance of autophagy-like vesicles [20], as occurs in other eukaryotes [21].

TbTOR2 plays a key role in polarization of the actin cytoskeleton, which is required for the proper functioning of secretory and endocytic processes, cell division and cytokinesis in trypanosome [17,20]; this is in line with the role of TORC2 in other eukaryotes [22]. TbTOR2 binds exclusively to TaAVO3 (adheres voraciously (to TOR2) 3) in TbTOR2 and associates with endoplasmic reticulum and mitochondrial membranes.

Although the function of these classical TORC1 and TORC2 complexes seems to be conserved in T. brucei, rapamycin potently impairs parasite proliferation by inhibiting TORC2 signalling exclusively, in contrast with what occurs in yeast and mammals. Consistently, the residues in the TOR FRB domain involved in rapamycin binding in yeast and mammals are not found in the TbTOR1 FRB domain, thus preventing binding of rapamycin to TbTOR1 [16,17,20]. In T. brucei, TORC2 disruption occurs upon prolonged rapamycin treatment whereas TORC1 function remains unaffected, discounting long-term inhibition of TORC1 signalling as the cause of TORC2 disruption [11,16,23].

TOR enzymatic activity can also be inhibited via ATP competition, and most of the new PI3K/mTOR catalytic inhibitors target the protein ATP-binding pocket [24], in some cases developed as human anti-cancer agents [5]. PI3K/mTOR inhibitors might have an effect against pathogens such as T. brucei, since parasite and human kinases share a conserved ATP-binding/kinase domain. Indeed, a recent study investigated the effect of some of these drugs against trypanosomatid parasite cell growth and proliferation [25]. Among these inhibitors, NVP-BEZ235 has demonstrated to be a potent trypanocide in cell culture and in a mouse model of the human pathogen T. b. rhodesiense. These results suggest that NVP-BEZ235 is a good starting point for anti-protozoan drug discovery by further chemical optimization. Thus we consider the tripanosomatid TOR kinase family a promising class of targets because all of these proteins are essential for proliferation of the parasites and no single kinase can substitute for any other TbTOR [16,18,19].

TOR overall readouts seem to be conserved in T. brucei. However, genes coding for upstream regulatory signalling described in other eukaryotes, such as the insulin growth factor receptors [26] and TSC (tuberous sclerosis complex)/Rheb are not found encoded in the parasite genome. Likewise, S6K (ribosomal S6 kinase), an extensively studied effector of TORC1 [27], cannot be identified by sequence homology [28]. AGC kinases are poorly represented in trypanosomatid genomes as compared with humans.

It has been suggested that the ancestral eukaryote ancestor might possess a single TOR kinase, but the gain or loss of TOR members happened across the Eukarya domain as independent events. Most likely, TOR duplication occurred in Saccharomyces cerevisiae and Saccharomyces pombe, like in Trypanosomatids, as independent events [29]. Conversely, microsporidians, Plasmodium falciparum, Cryptosporidium parvum and Theileria parva genomes lack the entire mTOR pathway, indicating additional independent loss events for TOR signalling in eukaryotes [2,29,30].

Surprisingly, Trypanosomatids are the only eukaryotes whose genomes encode two additional TOR kinase paralogues, namely TbTOR3 and TbTOR4 [16]. Both kinases conserve the classical PIKK backbone and display new features not described in other TORs to date.

TbTOR3 kinase is found in cytosolic granules dispersed through the cytosol, which specifically relocalizes towards the cell periphery under hyperosmotic conditions. This protein possesses a unique protein–protein interaction PDZ domain and is implicated in the osmotic responses through control of polyphosphate levels and acidocalcisome size [18]. Ablation of TbTOR3 causes a progressive inhibition of cell proliferation producing enlarged parasites accumulating in the S/G2-phase of the cell cycle. TbTOR3 kinase is also found in Leishmania, where null mutants also show defects in osmoregulation. Interestingly, this mutant was unable to replicate in macrophages in vitro, or establish infections in vivo [31].
However, TbTOR3 does not bind to the canonical proteins TbLST8, TbRaptor or TbAVO3 [16,19], suggesting that this kinase is an unusual TOR kinase that does not assemble into a conventional TORC.

TbTOR4 plays a crucial role in T. brucei biology [19]. TbTOR4 is a peripheral membrane protein that maintains the characteristic TOR kinase structure, but lacks the FRB domain, therefore it is not inhibited by rapamycin treatment. Similarly, TbTOR1 and TbTOR3 present a poorly conserved FRB domain and cannot bind to the TbFKP12–rapamycin complex [16].

TbTOR4 maintains proliferation of T. brucei. TbTOR4 loss-of-function triggers differentiation of the proliferating bloodstream form to the quiescent form in monomorphic trypanosomes, a laboratory-adapted strain in which the mechanism that regulates the quiescence differentiation process is impaired. Similar to TbTOR1 and TbTOR2, TbTOR4 binds TbLST8 and constitutes the novel TORC named TbTORC4. Other TbTORC4 subunits are the Armadillo domain-containing protein TbArmtor and the TbTORC4 scaffold.

TbTOR4 loss-of-function results in down-regulation of the bloodstream form-specific VSG (variant surface glycoprotein) and ESAGs (expression site-associated genes) and up-regulation of the PAD (proteins associated with glycoprotein) and ESAGs (expression site-associated genes) specific of the quiescent form, and thus remodelled expression of surface proteins [19]. In addition, loss of TbTOR4 promotes activation of mitochondria and resistance to mild acidic pH, features that pre-adapt to bloodstream trypanosomes for the environment they would eventually encounter in the insect midgut. As part of the cell differentiation process, TbTOR4 depletion halts cell cycle progression at G1 phase irreversibly (G0). The irreversibility of this process is important to prevent the proliferative form from exhausting and prematurely killing the mammalian host.

Implications of TOR kinases in the life cycle of T. brucei
Multiple TOR kinases might have arisen in Kinetoplastidae through duplication followed by functional diversification of these early–branched eukaryotes. Novel TORs may provide new functions and confer selective advantages on complex life cycles. The alternation between distinct host and distinct compartments within hosts requires a fine adaptation of the parasite to constant variation in carbon and metabolically accessible nitrogen sources, temperature, pH and osmotic pressure, among others. This adaptation is achieved through switching between anabolic and catabolic processes to face sequential changes that occur through the life cycle of this obligate parasite. This involves changes in pivotal processes for cell survival such as protein synthesis, autophagy and cytokinesis, all regulated by TOR kinases. It is important to understand the distinct developmental stimuli that may regulate TOR function in the parasite.

After the ingestion of trypanosomes by the tsetse fly during a blood meal, the parasite undergoes drastic remodelling of cellular and biochemical processes for 20–30 days to reach maturation, giving rise to metacyclic trypomastigotes, the pre-adapted/infective form for the mammalian host [32]. In the related parasite Trypanosoma cruzi, metacyclogenesis is triggered by starvation [33], which involves extensive activation of autophagic processes [34], suggesting that the inhibition of TOR1 function (TORC1) might play an important role in this process. Indeed, we reported previously that TbTOR1 inactivation promotes survival under conditions of nutritional stress [20]. Autophagy is also activated in other cell differentiation processes in Leishmania [35], suggesting that the extensive cellular differentiation requires a potent recycling machinery to degrade ‘old’ organelles to obtain precursors for the ‘new’ cellular components [36].

Once in the mammal host, trypanosomes proliferate rapidly and reach high densities that can compromise host viability. In this amplification period, it is conceivable that all TOR kinases are active to support growth, since RNAi (RNA interference) experiments showed that depletion of any single TOR kinase has deleterious effects on cell growth and proliferation.

Furthermore, trypanosomes live extracellularly in the bloodstream and undergo antigenic variation to stay ahead of the lytic immunoglobulin response raised to successive antigenic types. Antigenic variation occurs through the switching of VSG gene expression, transcribed by RNA pol I in a nuclear compartment named the ESB (expression site body) [37]. TbTOR1 and TbTOR4 loss-of-function reduced RNA pol I transcription and subnuclear localization [16,19]. The fact that both TbTOR1 and TbTOR4 depletion affect RNA pol I activity and trigger a G2/G1 blockade in the cell cycle suggests that TbTOR4 might have evolved from TbTOR1 to acquire new functions involved in programmed cell differentiation within the mammalian host.

The production of stumpy forms represents a form of quorum sensing triggered upon high parasitaemia, which is, in turn, a threat to parasite survival in the mammal. Moreover, stumpy forms are a pre-adaptation of the bloodstream parasite for survival in the tsetse fly midgut. TbTOR4 represents a global regulator of this differentiation process. However, the involvement of other TORs in the process is unknown. Herman et al. [38] identified increased glycosome turnover involving autophagy in the stumpy form, suggesting that reduced TbTOR1 activity might play a role in this process.

Once the tsetse fly ingests bloodstream trypanosomes during the blood meal, parasites differentiate to the procyclic form, which is adapted to survive and proliferate in the tsetse fly midgut. This differentiation is accompanied by the release of the VSG and the acquisition of the procyclin surface glycoprotein. TbTOR4 down-regulation seems to be important for this process to occur rapidly [19]. Conversely, TbTOR1 depletion inhibited the bloodstream form from procyclic differentiation and blocks the appearance of...
proliferation to quiescence transitions may provide insights into uncovering new function of TOR kinases. In particular, TbTOR4, from the eukaryotic tree early in evolution, may help to elucidate differentiation.

It would be interesting to explore the roles of TbTOR4, TbTOR1, mitochondrial inactivation and autophagy in T. brucei differentiation.

Studies in T. brucei, a protozoan parasite that diverged from the eukaryotic tree early in evolution, may help to uncover new function of TOR kinases. In particular, novel TOR functions in adaptive responses that mediate nutrient transitions may provide insights into understanding uncontrolled cell growth in cancer cells.

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