Seminars in Cell & Developmental Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

mTOR and autophagy: A dynamic relationship governed by nutrients and energy

E.A. Dunlop*, A.R. Tee

Institute of Cancer and Genetics, Cardiff University, Heath Park, Cardiff CF14 4XN, UK

ARTICLE INFO

Article history:
Available online xxx

Keywords:
mTOR
Autophagy
Nutrient
ULK1
VPS34
Lysosome

ABSTRACT

Mechanistic target of rapamycin (mTOR) functions as a key homeostatic regulator of cell growth and orchestrates whether anabolic or catabolic reactions are favoured. mTOR complex 1 (mTORC1) manages multiple biosynthetic pathways and promotes cell growth when nutrients are in plentiful supply. Many advances have been made over the last decade on nutrient sensing centred on mTORC1. Recent research reveals that mTORC1 maintains nutrient homeostasis through lysosomal biogenesis and autophagic processes. Cells utilise autophagy to recycle damaged or unwanted organelles and macromolecules and in so doing, generate energy and recovers precursor building blocks necessary for normal growth. It is clear that mTOR and autophagy are closely integrated within cells, where defects in signalling through both pathways are known to drive the onset of a range of human diseases, such as cancer and neurodegenerative disease. This review focuses on the dynamic signalling interplay between mTOR and autophagy, which is governed by a core set of proteins that sense nutrients at lysosomal membranes.

Contents

Abbreviations: Akt1, v-akt murine thymoma viral oncogene homolog 1; AMBRA, Autophagy/Beclin-1 regulator 1; AMPK, AMP-dependent protein kinase; ATG, autophagy-related; BECN1, beclin 1; DEPTOR, DEP domain containing mTOR-interacting protein; EGFR, epidermal growth factor receptors; FIP200, focal adhesion kinase family interacting protein of 200 kDa; PIKFYVE, FYVE finger containing phosphatidylinositol 5-kinase; [Ca^{2+}]I, intracellular pool of Ca^{2+}; LAMTOR1-5, late endosomal/lysosomal adaptor, MAPK and mTOR activator 1-5; GATOR, Rag GTPases and GTRs; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; HEK, human embryonic kidney; IGF, insulin-like growth factor; RNAi, interfering RNA; IBP, interferon regulatory factor-4 binding protein; mTOR, mechanistic target of rapamycin; MAP1LC3A, microtubule-associated protein 1 light chain 3 alpha; mTORC1, mTOR complex 1; mTORC2, mTOR complex2; MEF, mouse embryonic fibroblast; PI, phosphatidylinositol; PIK3CA, PI-4,5-bisphosphate 3-kinase, catalytic subunit alpha; PRAS40, proline-rich Akt substrate of 40 kDa; PX, Phox homology; PAT1, proton-assisted amino acid transporter; Raptor, rapamycin-associated protein of TOR; Rictor, rapamycin insensitive companion of TOR; ROS, reactive oxygen species; SQSTM1, Sequestome 1; TFE3, transcription factor binding to IGHM enhancer 3; TFEB, transcription factor EB; TBC1D7, Tre2-Bub2-Cdc16-1 domain family member 7; TRAF6, TNF receptor-associated factor 6, E3 ubiquitin protein ligase; TSC, Tuberous Sclerosis Complex; ULK1, unc-51 like autophagy activating kinase 1; v-ATPase, vacuolar H^+-ATPase; VPS34, vacuolar protein sorting-34.

* Corresponding author. Tel.: +44 02920 687785.
E-mail address: DunlopEA@cardiff.ac.uk (E.A. Dunlop).

http://dx.doi.org/10.1016/j.semcdb.2014.08.006
1084-9521/

1. Introduction

Careful management of cell growth is coordinated through anabolic and catabolic processes and is in part, regulated through mechanistic target of rapamycin (mTOR). Anabolism consumes energy and simple precursors (i.e., ATP, amino acids, fatty acids and nucleotides) to generate more complex molecules essential for cell growth. To regenerate energy and precursor building blocks during cellular growth, unwanted complex molecules can also be broken down by catabolism. Consequently, anabolic and catabolic processes often work in unison and are tightly controlled through sensing cellular nutrient and energy levels, as well as growth factor and hormonal inputs.mTOR is a serine/threonine protein kinase that is integral to two distinct cellular complexes, termed mTOR complex 1 (mTORC1) and mTORC2, where these complexes sense and integrate a variety of inputs, including growth signals, nutrients and energy status. mTORC1 consists of the core components, mTOR, rapamycin-associated protein of TOR (Raptor) and mLST8 (also known as GbetaL) [1]. In addition, proline-rich Akt substrate of 40 kDa (PRAS40) [2,4] and DEP domain containing mTOR-interacting protein (DEPTOR) [5] can also associate with and negatively regulate mTORC1. mTOR and LST8 are also integral to mTORC2, while components distinctive to mTORC2 include rapamycin insensitive companion of TOR (rictor), SIN1 and Protor (reviewed Ref. [6]). mTORC1 is the better studied of the two complexes and, by managing multiple biosynthetic pathways, plays a key anabolic role in promoting cell growth and proliferation. Through direct phosphorylation and activation of v-akt murine thymoma viral oncogene homolog 1 (Akt1) [7], mTORC2 indirectly promotes mTORC1 activation further down the signalling pathway. Consequently, mTORC2 can be considered upstream of mTORC1. As well as promoting cell growth and proliferation, mTORC2 influences cell morphology through regulation of cytoskeletal organisation.

A cell must first increase its biomass prior to cell division and mTORC1 does this through a number of coordinated mechanisms. mTORC1 builds up cellular protein content through ribosomal biogenesis and enhances initiation and elongation of protein translation (reviewed in Ref. [8]). To enhance protein build-up when nutrients and energy are sufficient, mTORC1 down-regulates macroautophagy (referred to as autophagy hereafter). Autophagy is a catabolic process where macromolecules are sequestered in double membrane bound autophagosomes that fuse with lysosomes to allow their enzymatic break down. In conditions of nutrient and energy sufficiency, mTORC1 is active, and through phosphorylation of early autophagy promoting complexes mTORC1 ensures that autophagy is inhibited. Although autophagy is down-regulated by mTORC1 when nutrients are plentiful, a low basal level of autophagy still occurs to prevent build-up of damaged organelles and aggregated or misfolded macromolecules. Revealing dynamic signalling interplay between autophagy and mTORC1, autophagic signalling switches mTORC1 off when nutrients and energy become limiting.

2. mTORC1 control of autophagy

2.1. Autophagy initiation is controlled through ULK1 and VPS34

Autophagy initiation is coordinated by two kinases, unc-51 like kinase 1 (ULK1, also known as autophagy-related (ATG)-1) and vacuolar protein sorting-34 (VPS34, also known as PIK3C3). ULK1 is a Ser/Thr protein kinase, while VPS34 is a class III phosphoinositol 3-kinase. As part of larger protein complex, ULK1 is activated following nutrient depletion and is considered upstream of VPS34 [9,10]. Activation of both ULK1 and VPS34 drives the recruitment of additional ATG proteins to phagophore membranes and promotes autophagosomal maturation. As well as regulating autophagy, ULK1 and VPS34 are known to influence signal transduction through mTORC1 (discussed in detail below).

2.2. Autophagy is governed by opposing kinase activities from mTORC1, ULK1 and AMPK

ULK1 functions in a complex with ATG13, and focal adhesion kinase family interacting protein of 200 kDa (FIP200, also known as RB1-inducible coiled-coil 1) [11,12]. Nutrient withdrawal stimulates this ULK1-ATG13-FIP200 complex and initiates autophagy via ULK1 autophosphorylation and phosphorylation of the binding partners, ATG13 and FIP200 [11,12]. mTORC1 plays a central role in the regulation ULK1 and autophagy initiation, where inhibition of mTORC1 upon rapamycin treatment enhances the kinase activity of ULK1, while mTORC1 activation through Rheb overexpression potently represses ULK1 [11].mTORC1 inhibits ULK1 through at least two mechanisms, with the first involving direct protein phosphorylation. Historically, it was known from yeast studies that TOR was an upstream kinase of Atg1 (the yeast homologue of ULK1) and that phosphorylation of Atg13 by TOR reduced the affinity of Atg13 for Atg1. The Atg1-Atg13 association and subsequent activation of Atg1 are required for autophagy induction in yeast, so TOR-mediated dissociation of the Atg1 complex prevents autophagy when nutrients are sufficient [13]. Several studies published in 2009 demonstrated that mTORC1 could negatively regulate the mammalian ULK1-ATG13-FIP200 complex in a comparable manner [11,12,14]. Although the integrity of the ULK1-ATG13-FIP200 complex is not regulated in response to nutrients (unlike the yeast Atg1-Atg13 complex), mTORC1 directly phosphorylates Atg13 and ULK1 in vitro [11,14]. This occurs through association of the mTORC1 component, Raptor, with ULK1 under nutrient-rich conditions [14]. Serine 758 was identified as the major mTORC1-mediated phosphorylation site on ULK1 and is considered to inhibit ULK1 [15,16].

In addition to ULK1 phosphorylation, mTORC1 indirectly destabilises ULK1 and impairs autophagy through phosphorylation of Autophagy/Beclin-1 regulator 1 (AMBRA1) [17]. Phosphorylation of AMBRA1 at Ser52 by mTORC1 prevents Lys-63-linked ubiquitination of ULK1 by TNF receptor-associated factor 6, E3 ubiquitin protein ligase (TRAF6). Lys-63-linked ubiquitination of ULK1 causes self-association, which enhances stability and through trans-autophosphorylation promotes ULK1 activation. During conditions that favour mTORC1 activation, AMBRA1 is kept in an inactive state by mTORC1 phosphorylation and is tethered to intracellular vesicles as part of a dynein motor complex. However, upon conditions when mTORC1 is inactivated, autophagy is promoted through rapid Lys-63-ubiquitination of ULK1 by the AMBRA1-TRAF6 complex, causing ULK1 self-association and enhancement of its kinase activity.

AMP-dependent protein kinase (AMPK, also known as PRKAA2) plays an important homeostatic role in the regulation of ULK1 and mTORC1 and is dependent on the energy status of the cell. In conditions of glucose starvation when cellular energy levels are low, AMPK binds to and activates ULK1 through phosphorylation [15,16,18–20]. Revealing a feedback mechanism, phosphorylation of ULK1 by mTORC1 was shown to impede the ability of AMPK to activate ULK1 [15,16]. ULK1 activation by AMPK is also further amplified through several signalling mechanisms that lead to mTORC1 inhibition. Firstly, AMPK phosphorylates Raptor, which consequently leads to association of 14-3-3 with the phosphorylated Raptor protein and disruption of mTORC1 signal transduction [21]. Secondly, AMPK activates the upstream negative mTORC1 regulator, Tuberous Sclerosis Complex 2 (TSC2) through direct phosphorylation [22]. As a third mechanism, AMPK also indirectly

Please cite this article in press as: Dunlop EA, Tee AR. mTOR and autophagy: A dynamic relationship governed by nutrients and energy. Semin Cell Dev Biol (2014), http://dx.doi.org/10.1016/j.semcdb.2014.08.006