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The mTOR pathway: Implications for DNA replication

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ABSTRACT

DNA replication plays a central role in genome health. Deleterious alteration of replication dynamics, or "replication stress", is a key driver of genome instability and oncogenesis. The replication stress response is regulated by the ATR kinase, which functions to mitigate replication abnormalities through coordinated efforts that arrest the cell cycle and repair damaged replication forks. mTOR kinase regulates signaling networks that control cell growth and metabolism in response to environmental cues and cell stress. In this review, we discuss interconnectivity between the ATR and mTOR pathways, and provide putative mechanisms for mTOR engagement in DNA replication and the replication stress response. Finally, we describe how connectivity between mTOR and replication stress may be exploited for cancer therapy.

Contents

1. Introduction

DNA replication is a highly regulated process with profound implications for genome stability. "Replication stress" is a general term referring to any condition that alters DNA replication rates

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[reviewed in (Giannattasio and Branzei, 2017; Zeman and Cimprich, 2014; Zhang et al., 2018)]. There are many sources of replication stress, including endogenous stress from shortages of histones or deoxyribonucleotide triphosphates (dNTPs), hard to replicate DNA sequences, and collisions between transcription and replication complexes [reviewed in (Giannattasio and Branzei, 2017; Zeman and Cimprich, 2014; Zhang et al., 2018)]. Exogenous factors, such as chemotherapeutics that negatively impact DNA replication, also induce replication stress. Notably, oncogene expression leads to replication stress early in cancer development through diverse mechanisms that include depletion of the tightly regulated dNTP pool (Bester et al., 2011). Genome instability is one of the canonical hallmarks of cancer, and replication stress is emerging as a major driver of oncogenic genome alteration (Burrell et al., 2013; Hanahan and Weinberg, 2011).

The replication stress response is triggered by the presence of stalled replication forks. Once active, the replication stress response evokes several physiological processes including: cell cycle arrest to prevent mitosis; inhibition of the de novo firing of replication origins and activation of dormant origins in replicons where replication was already initiated; activation of pathways to increase the cellular pool of dNTPs; and the stabilization, repair, and restart of stalled replication forks (Branzei and Foiani, 2010; Zeman and Cimprich, 2014; Zhang et al., 2018). ATR (ataxia telangiectasia and rad3 related) is the main regulator of the replication stress response, and through its effector CHK1 (checkpoint kinase 1), controls these aforementioned processes [reviewed in (Saldivar et al., 2017)]. The ATR-CHK1 signaling axis is highly conserved, with Mec1-Rad53 in *S. cerevisiae* and Rad3-Cds1 in *S. pombe* sharing many similarities with mammalian ATR-CHK1 both structurally and functionally [reviewed in (Saldivar et al., 2017)].

The mechanistic target of rapamycin (mTOR) is an atypical serine/threonine kinase belonging to the phosphatidylinositol 3-kinase (PI3K)-related kinase (PIKK) family. The PIKK family includes ATR and the other major regulators of the DNA damage response (ATM and DNA-PK), suggesting a common evolutionary origin. Canonical mTOR function is to regulate cellular responses to a wide range of environmental stresses, including nutrient starvation, growth factor deprivation and hypoxia [reviewed in (Saxton and Sabatini, 2017)]. However, recent evidence supports an expanded role for mTOR in the DNA damage and replication stress responses. In this review, we summarize evidence linking mTOR to DNA replication and the replication stress response and discuss how this link might be exploited for cancer therapy.

2. The canonical mTOR pathway

2.1. Upstream and downstream targets of mTOR

mTOR is the catalytic subunit of two protein complexes, mTOR complex 1 (mTORC1) and 2 (mTORC2) (Loewith et al., 2002; Sarbassov et al., 2005a). The mTORC1 and mTORC2 complexes differ in their accessory subunits which mediate substrate specificity (Fig. 1). mTORC1 is activated in response to growth factors and mitogen-dependent signaling pathways through repression of the inhibitory tuberous sclerosis complex (TSC). TSC is a heterotrimeric complex consisting of TSC1, TSC2 and TBC1D7, which is converged upon by several activating pathways upstream of mTOR (Dibble et al., 2012). For example, the IGF-1 (insulin/insulin-like growth factor-1) pathway induces production of PIP3 (phosphatidylinositol (3,4,5) trisphosphates) by PI3K (phosphoinositide 3-kinase), which in turn triggers AKT-dependent phosphorylation of TSC2 that releases mTOR1 repression (Huang and Manning, 2008) (Fig. 1). Similarly, RTK (receptor tyrosine kinase)-dependent Ras signaling also phosphorylates TSC2 to release and activate mTORC1 (Huang and Manning, 2008). Other mTORC1 regulators include the Wnt signaling pathway (Inoki et al., 2006), the inflammatory cytokine TNFα (Lee et al., 2007), the stress responsive metabolic regulator (AMPK) (Inoki et al., 2003), and regulated in development and DNA damage response 1 (REDD1) (Brugarolas et al., 2004). In contrast, the upstream regulation of mTORC2 is poorly characterized (Cybulski and Hall, 2009). As with mTORC1, mTORC2 is responsive to growth factors (e.g. IGF-1/PI3K) (Liu et al., 2015) and to the ribosome maturation factor Nip7, although the mechanistic basis for mTORC2 activation remains unclear (Zinzalla et al., 2011).

mTORC1 maintains the balance of anabolic processes including ribosome biogenesis and production of nucleotides, lipids, and proteins, with catabolic processes that include cell cycle arrest, cell death, and autophagy. Nucleotide production induced by mTORC1 activation includes the de novo synthesis of both pyrimidines and purines through phosphorylation of p70S6K and ATF4. Specifically, p70S6K activates CAD (Carbamoyl-Phosphate Synthetase 2, Aspartate Transcarbamylase, and Dihydroorotase) an essential component of the de novo pyrimidine synthesis pathway (Ben-Sahra et al., 2013; Robitaille et al., 2013), whereas ATF4 activates MTHFD2, to provide one-carbon units for purine synthesis (Ben-Sahra et al., 2016). mTOR-dependent p70S6K activation also induces lipid production and glucose metabolism [reviewed thoroughly elsewhere (Saxton and Sabatini, 2017)].

Another mTORC1 anabolic role is the coordinated induction of rRNA synthesis and ribosomal protein production to promote ribosome biogenesis. rRNA synthesis requires transcription, which is regulated thorough an mTORC1 effect on Pol I and III (Kantidakis et al., 2010; Mayer et al., 2004). However, the effect of mTORC1 on transcription is not restricted to rRNA synthesis only. Instead mTORC1 inhibition effects global gene transcription via the regulation of specific transcription factors [reviewed in (Laplante and Sabatini, 2013)]. In addition to global effect on gene transcription, mTORC1 globally controls protein production, including ribosomal proteins. This is achieved through phosphorylation of two direct targets: p70S6K and 4E-BP1(a member of the eukaryotic initiation factor 4E binding protein family). p70S6K phosphorylation is important for translation initiation (Holz et al., 2005), while phosphorylation of 4E-BP1 proteins enables dissociation of the translation initiation factor eIF4E and assembly of the mature 80S ribosome necessary for 5' cap-dependent mRNA translation (Gingras et al., 1999). In addition, mTORC1 also promotes cell growth by suppressing autophagy and protein turnover through inhibition of several key protein targets that include: ULK1, a regulator of autophagosome formation (Kim et al., 2011); TFEB, a transcription factor involved in expression of the autophagy machinery (Settembre et al., 2012); and ERK5, which regulates proteasome-dependent proteolysis (Rousseau and Bertolotti, 2016).

mTORC2 mainly controls cell proliferation, cell survival, and cytoskeleton rearrangements through three distinct signaling pathways. First, mTORC2 regulates the AGC (PKA/PKG/PKC) family of protein kinases including PKCα (Jacinto et al., 2004; Sarbassov et al., 2004), PKCδ (Gan et al., 2012) and PKCγ (Thomanetz et al., 2013), all of which drive actin cytoskeleton remodeling and cell migration. Second, mTORC2 activates the PI3K/AKT signaling pathway that controls cell survival and proliferation through several key substrates including the mTORC1 inhibitor TSC2 (Sarbassov et al., 2005b). Finally, mTORC2 activates SGK1 which is also a member of the AGC family and important to the cellular stress response through its role in ion transport (Garcia-Martinez and Alessi, 2008) (Fig. 1).

Notably, an in-depth understanding of the direct targets of mTORC1 and 2 were enabled by the identification of Rapamycin, a compound with antifungal, immunosuppressive and antitumor characteristics (Martel et al., 1977; Vezina et al., 1975). Rapamycin,

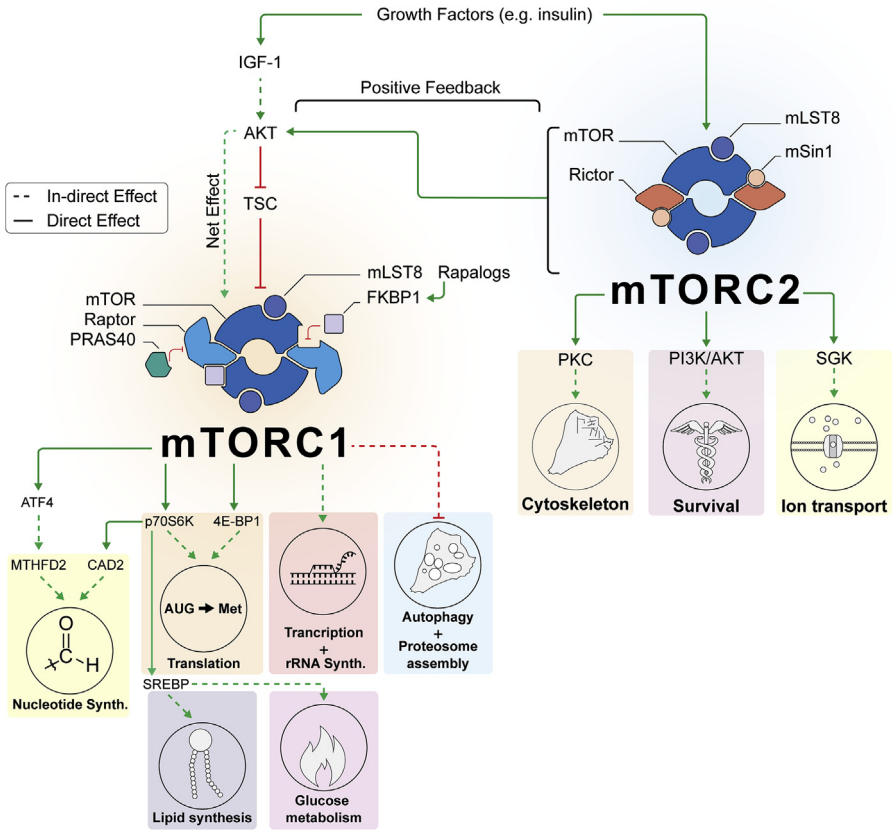

Fig. 1. The mTORC1 and mTORC2 signaling pathways. The mTORC1 and 2 complexes are illustrated with accessory proteins, based on the 5.9 Å cryo-EM structure of mTORC1 and 2 associated with the FKBP12-rapamycin complex (Yang et al., 2013). Both mTORC1 and 2 are activated in response to growth factors. AKT activation stimulates mTORC1 activation by inhibiting the TSC complex, leading to mTORC1 derepression. As a substrate of mTORC2, AKT also functions to establish a feedback loop between the two TORC complexes. Major pathways downstream of mTORC1 and 2 are illustrated.

and the corresponding family of derivatives that function similarly (i.e. "rapalogs"), primarily inhibit mTORC1 but will also inhibit mTORC2 under chronic exposure (Sarbassov et al., 2006). Rapamycin and the rapalogs form a complex with the FK506 binding protein-12 (FKBP-12) that binds mTOR to block mTORC1 activity (Chung et al., 1992). This inhibits mTORC1-dependent cell cycle progression, cell survival, and angiogenesis. The second generation of mTOR inhibitors much as PP242, BEZ235 and INK128 block the catalytic activity of mTOR and therefore inhibit both mTORC1 and 2 [reviewed in (Zheng and Jiang, 2015)]. To date, no specific mTORC2 inhibitor has been identified.

2.2. Evolutionary conservation of the TOR pathway

The role of the TOR pathway in proliferation and growth regulation is evolutionary conserved in most eukaryotes including yeast [reviewed in (Loewith and Hall, 2011)], flies (Zhang et al., 2000), plants (Xiong and Sheen, 2014) and mammals [reviewed in (Saxton and Sabatini, 2017)]. The "two branches-two complexes" mode of TORC substrate specificity is also conserved across eukaryotic species [reviewed in (Wullschleger et al., 2006)]. In budding yeast there are two genes encoding TOR catalytic subunits. Yeast Tor1 can function as the catalytic subunit in either TORC1 or TORC2, whereas Tor2 serves only in the TORC2 complex, [reviewed in (Loewith and Hall, 2011)). Like the mammalian complex, yeast TORC1 responds to diverse environmental cues and controls anabolic processes, and yeast TORC2 induces actin cytoskeleton rearrangements. In *S. cerevisiae* TORC2 also has a direct role in regulating lipid synthesis [reviewed in (Roelants et al., 2017)].

2.3. mTOR localization

mTOR protein has been found at several cellular locations [reviewed in (Betz and Hall, 2013)]. There is a broad consensus that TORC1 is found at the lysosome in diverse species from yeast to humans, though it has also been reported in the ER, Golgi, cell membrane and mitochondria as well [reviewed in (Betz and Hall, 2013)]. Components of the mTORC1 signaling pathway, including mTOR itself, Raptor, and p70S6K, have been detected in the nucleus, however it is not clear if they form a functional nuclear complex (Audet-Walsh et al., 2017; Rosner and Hengstschlager, 2008). TORC2 was documented at the plasma membrane (Ebner et al., 2017), endosomal vesicles (Ebner et al., 2017), mitochondria-associated ER membrane (Boulbes et al., 2011) and in the mitochondria (Desai et al., 2002). However, several papers identified that mTORC2 shuttles between the nucleoplasm and the cytoplasm but its specific role inside the nucleus is unknown (Rosner and Hengstschlager, 2008, 2012). How the distribution of TORC1 and 2 to various locations is regulated, and how this localization effects complex function, is a matter for future studies.

2.4. Cross talk between mTOR complexes

A series of complex and context-dependent feedback loops exist between the mTORC complexes [reviewed in (Xie and Proud, 2013)]. Both mTOR complexes are activated by insulin

and insulin-like growth factors (IGFs). The insulin/PI3K pathway activates AKT by stimulating its phosphorylation at AKT-Thr308 (Williams et al., 2000). AKT then phosphorylates and represses TSC2 to activate mTORC1 (Inoki et al., 2002) and the mTORC2 subunit Sin1 on T86 in response to insulin (Humphrey et al., 2013). However, AKT is also an mTORC2 substrate, as mTORC2 phosphorylates AKT on Ser 473 (Guertin et al., 2006). However, AKT-pSer473 was not found to be important for mTORC1 activation. Thus, while AKT is upstream of mTORC1 and downstream of mTORC2, it is not clear whether it mediates crosstalk between the two mTORC complexes [reviewed in (Huang and Manning, 2009)]. mTORC1 can impair mTORC2 activation, as well as inhibit its own activity, thorough a negative autoregulatory loop by either phosphorylating insulin receptor substrate-1 (IRS-1) (Um et al., 2004) or Grb10 (Hsu et al., 2011). Both IRS-1 and Grb10 are involved in signaling events upstream of PI3K, hence their inhibition suppresses both mTOR complexes. A further inhibitory link from mTORC1 to mTORC2 involves p70S6K. mTORC1-dependent p70S6K phosphorylation of the mTORC2 subunit Sin1 in response to IGF1/insulin was shown to dissociate Sin1 from the mTORC2 complex and inhibit mTORC2 activity (Liu et al., 2013). However, an independent study identified that Sin1 phosphorylation in response to IGF1/insulin augments mTORC2 activity, suggesting instead a positive feedback loop (Humphrey et al., 2013). As these studies were done in different cell types, it is possible that the crosstalk between mTORC complexes is regulated in a cell type specific manner.

2.5. mTOR and cell cycle progression

Cell cycle progression demands a large supply of nutrients to ensure adequate energy and protein synthesis to support cell growth. As the main coordinator of nutrient availability and metabolism, mTORC1 can regulate cell cycle progression through its functions controlling energy homeostasis [reviewed in (Cuyas et al., 2014)]. However, beyond its control of cellular metabolism, mTORC1 can also directly affect the cyclin dependent kinases (CDKs), their obligate cyclin binding partners, and the CDK inhibitors which regulate cell cycle progression (Fig. 2).

Inhibiting mTOR activity, by rapamycin or nutrient starvation, induces G1-phase cell cycle arrest in p53 competent cells (Foster et al., 2010). Both the 4E-BP1 and p70S6K downstream arms of the TORC1 signaling cascade are independently required to mediate mTOR-dependent progression from G1-phase by transcriptionally and translationally regulating the G1/S transition cyclins (D-type and E-type cyclins) (Averous et al., 2008; Fingar et al., 2004; Oka et al., 2013). Additionally, the CDK inhibitor $p27^{kip1}$ is phosphorylated by AKT or SGK1 (Hong et al., 2008). Phosphorylation of $p27^{kip1}$ sequesters this protein in the cytoplasm to block its nuclear function as a CDK inhibitor, thus promoting stabilization of the cyclin D–CDK4/6 complex and G1/S transition (Medema et al., 2000).

Furthermore, in response to DNA damage or replication stress, p53 negatively regulates mTORC1 while simultaneously inducing cell cycle arrest via induction of $p21^{CIP1/WAF1}$ (Feng et al., 2007). Notably, there is a bi-directional crosstalk between mTORC1 and p53, as mTORC1 activates p53 in response to DNA damage via the p70S6K pathway (Lai et al., 2010). The p53-mTORC axis can be pro-survival or pro-cell death depending on many factors which include the cell type, duration of stimuli, and the amount of damage [reviewed in (Ma et al., 2018)]. In recent years several studies have also shown that different members in the TOR pathway also regulate mitotic progress including mTOR itself, its regulators TSC and raptor, and the mTORC1 target p70S6K [reviewed in (Cuyas et al., 2014)].

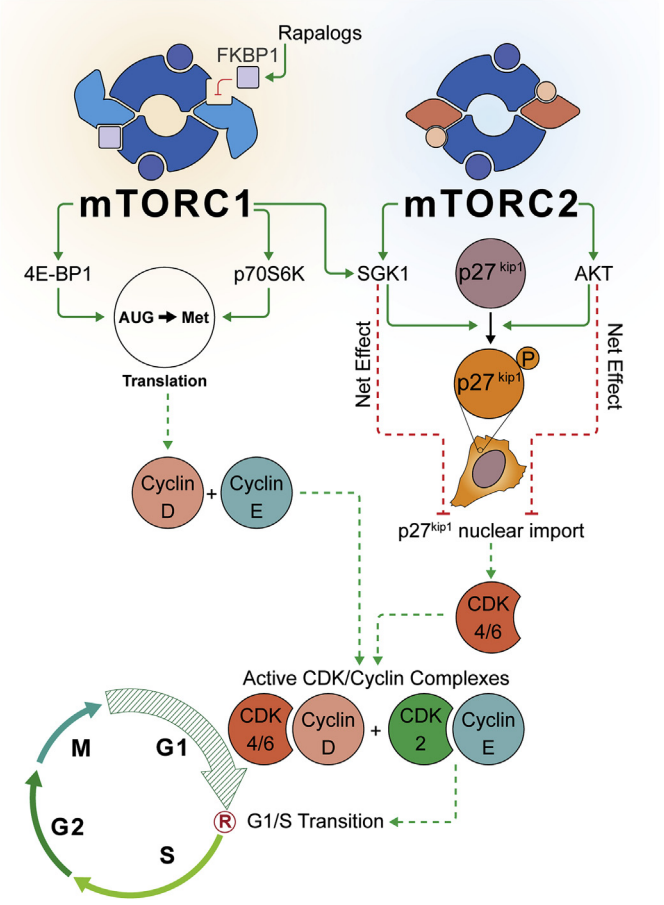

Fig. 2. mTORC1 and 2 regulate the G1/S transition. mTORC1 upregulates cyclins D and E through regulation of transcription and translation. mTORC1 and 2 also regulate phosphorylation of the CDK inhibitor $p27^{kip1}$, blocking $p27^{kip1}$ nuclear localization. The net effect of these activities promotes stabilization of the Cyclin D–CDK4/6 complex and CyclinE-CDK2 required for G1/S transition.

3. mTOR and DNA replication

Many lines of evidence link mTOR regulation with replication dynamics (Fig. 3). First, inhibition of mTOR in p53-deficient mouse embryo fibroblasts and cancer cells does not induce G1-arrest as observed in p53-proficient cell lines. Instead inhibiting mTOR in p53-compromised tissues induces apoptosis specifically during S-phase as the DNA is replicated (Huang et al., 2001, 2003). This suggests mTOR activity is required to effectively complete DNA replication.

Second, treating budding yeast with Rapamycin dramatically increased cell lethality, specifically during S-phase, in response to the alkylating agent methyl-methane sulfonate (MMS) (Shen et al., 2007). The MMS mechanism of action includes replication stress induction, and the observed S-phase lethality suggested a role for TORC in promoting S-phase progression in the presence of DNA damage. Furthermore, direct analysis of replication fork progression in MMS treated cells revealed that TOR function includes promoting replication fork stability (Shen et al., 2007). Interestingly, cells from Tuberous Sclerosis (TSC) patients, which are characterized by exorbitant mTORC1 signaling due to the loss of function of the mTOR inhibitory TSC complex, display replication stress and asymmetric fork progression, aberrant S-phase progression, and hypersensitivity to genotoxic stress (Pai et al., 2016). Together, these data suggest that disrupting mTOR signaling, by

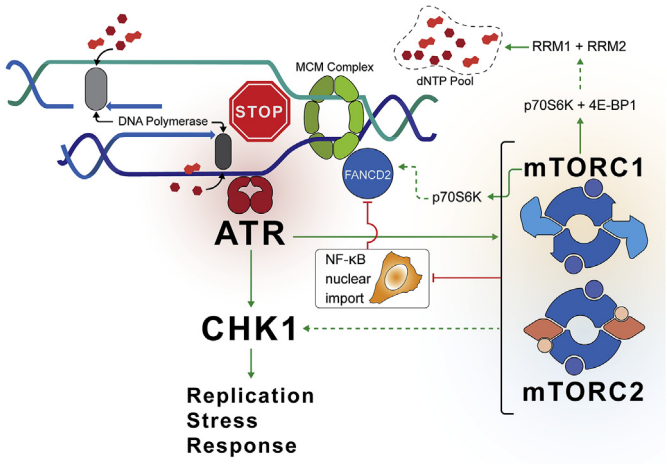

Fig. 3. Potential mTOR functions in DNA replication. A stalled replication fork is illustrated with the leading and lagging strand DNA polymerases, the mini-chromosome maintenance (MCM) 2–7 helicase, the FANCD2 repair factor, and ATR kinase. ATR kinase regulates the replication stress response and directly phosphorylates mTOR. mTORC1 and 2 participate in the replication stress through multiple pathways. mTORC1 and 2 upregulate the ATR effector CHK1 to promote the replication stress response. mTORC 1 and 2 also upregulate FANCD2 transcription by blocking the nuclear localization of NF-κB, which suppresses FANCD2 expression. Additionally, mTORC1 and 2 also upregulate RNR complex members RRM1 and 2, which catalyze dNTP production during the replication stress response.

either inhibition or upregulation, disrupts the DNA replication program.

A third line of evidence comes from several yeast mutants with hypomorphic alleles of the replication initiation complex genes, *CDC45* and *DPB11*, which exhibit perturbations in DNA replication and an increased sensitivity to TOR inhibitors (Shen et al., 2007). Additionally, mutant fission yeast cells defective for TORC2 or the downstream AGC-like kinase, Gad8, are highly sensitive to chronic replication stress but are insensitive to ionizing radiation (Schonbrun et al., 2013).

Lastly, in an unbiased screen that analyzed the translatome and transcriptome changes induced by mTORC1/2 in response to DNA damage in breast cancer cells, the most differentially expressed genes were DNA replication proteins (Silvera et al., 2017). This includes members of the DNA polymerase family including Pol-E2, Pol-q, PolG2 and PolA2, as well as licensing and replication factors such as MCM3, MCM6, ORC6, CLSPN, PCNA and GINS2. These genes were dependent on mTORC1/2 regulation at both the mRNA and protein level. Furthermore, mTORC1/2 inhibition induced persistent replication stress, evidenced by S-phase arrest, sustained phosphorylation of CHK1, ATM and CHK2, and γ-H2AX foci formation (Silvera et al., 2017). Interestingly, the global transcriptional/translational response to DNA damage was dependent on both mTORC1 and 2, as independent inhibition of mTORC1 or 2 did not impair the coordinated response to DNA damage nor did it induce replication stress (Silvera et al., 2017).

3.1. Potential functions for mTOR in DNA replication

3.1.1. ATR-CHK1

Several lines of evidence are congruent with bi-directional feedback between the mTOR and the ATR-CHK1 signaling axes. In a large-scale proteomic analysis to identify ATM and ATR substrates in human 293T cells, several proteins from the IGF1-AKT-mTOR pathway were identified, including: TSC1, AKT, 4E-BP1 and p70S6K. Suggesting these proteins are directly phosphorylated by ATM or ATR under DNA damage conditions (Matsuoka et al., 2007). In addition, mTOR itself is transiently phosphorylated following DNA damage in an ATR-dependent manner (Selvarajah et al., 2015; Shen et al., 2007).

Conversely, evidence supporting mTORC1/2 regulation of ATR-CHK1 signaling induced by replication stress was found in several cancer cell lines (Selvarajah et al., 2015; Zhou et al., 2017). Zhou et al. identified in rhabdomyosarcoma xenografts and cultured rhabdomyosarcoma cells that mTORC1 suppressed spontaneous DNA damage and replication stress by elevating CHK1 protein levels (Zhou et al., 2017). The effect was not mediated by 4E-BP1 and cap-dependent protein translation, but rather by p70S6K-dependent upregulation of the G1/S cyclin-dependent kinases (CDK4 and 6) required to induce *CHK1* transcription. Furthermore, in this context the mTORC1-p70S6K axis was essential for increases in *CHK1* transcription, whereas the mTORC2-AKT axis promoted this effect but was not essential (Zhou et al., 2017). In breast and colorectal cancer cells, mTORC2 was found to be essential for CHK1 upregulation in response to DNA damage during S-phase. Ablating mTORC2 prevented DNA damage-induced S and G2/M cell cycle arrest, as well as total CHK1 protein upregulation and phosphorylation, while mTORC1 was dispensable in these cell lines. Crosstalk between the ATR and mTOR complexes thus appears to be regulated with cell type specificity (Selvarajah et al., 2015).

Cumulatively, the data reveal reciprocal regulation of mTOR and ATR-CHK1 signaling in response to S-phase DNA damage. This interaction is essential for promoting cell cycle arrest, repair, and survival under replication stress conditions. Moreover, both mTORC1 and 2 affect the ATR-CHK1 pathway independently and through different modes of regulation. Further studies are required to characterize the complicated nature of those feedback loops and the unique roles that mTORC1 and 2 play in response to impaired S-phase progression.

3.1.2. Ribonucleotide reductase

Tight regulation of dNTP pools is essential to maintain replication fork speed in eukaryotic cells, and elevation of the dNTP pool is a physiological outcome of the replication stress response (Zhao et al., 2001). Replication stressed cells elevate their dNTP pool by ATR–CHK1 dependent upregulation of RRM2 (Ribonucleoside-diphosphate Reductase subunit M2), a subunit of the Ribonucleotide Reductase (RNR) complex that catalyzes dNTP production from ribonucleotides (NTPs) (Buisson et al., 2015). Consistent with a protective capacity in the replication stress response, high levels of RRM2 were shown to suppress different phenotypes associated with ATR dysfunction and insufficiency (Chabes et al., 2003). A similar outcome occurs in yeast, where the replication checkpoint (Mec-Rad53 in *S. cerevisiae* and Rad3-Cds1 in *S. pombe*) leads to upregulation of RNR through nucleus-to-cytoplasm redistribution of RNR subunits and increased transcription (Bondar et al., 2004; Lee and Elledge, 2006).

TORC1 facilitates NTP biosynthesis. This role was traditionally assumed to be part of the TORC1 program to stimulate ribosome biogenesis, a process that requires a large NTP pool necessary to support elevated rRNA transcription. However, TORC1 also modulates RNR activity, to facilitate dNTP synthesis from the cellular NTP pool (Shen et al., 2007). In *S. cerevisiae,* TORC1 induces transcription of *RNR1* and *3* following replication stress in a Rad53-dependent manner to promote cell survival at the cost of increased mutation rates (Shen et al., 2007). In mammals, mTORC1 positively controls both RRM1 (ribonucleotide reductase large subunit 1) and RRM2 in various cancer cell lines and mouse tumor xenografts. This occurs through a transcriptional mechanism dependent on p70S6K upregulation of CDK4/6 activity, and eIF-4E cap-dependent protein translation (He et al., 2017). Thus, mTORC signaling plays a role in regulating the nucleotide pool in cancer cells and in response to

replication stress.

In *S. pombe*, TORC2 and its mediator Gad8 also regulate RNR expression through interaction with the *Mlu*I cell cycle box-binding factor (MBF) transcription complex. In response to replication stress, TORC2 and Gad8 relocate to the nucleus where Gad8 binds the MBF transcription complex inducing the transcription of several genes that participate in the G1/S transition and the replication stress response. This includes, *cdt2*+, an adaptor subunit of an E3 ubiquitin ligase essential for initiating DNA replication; *cdc18*+, a MCM complex loader; and *cdc22*+, which encodes for the large subunit of the RNR complex (Cohen et al., 2016).

3.1.3. FANCD2

FANCD2 belongs to the Fanconi Anaemia Pathway required for repair of double strand breaks (DSBs) and stalled replication forks [reviewed in (Federico et al., 2018)]. In the replication stress response, ATR mediates the transient association of FANDC2 with the MCM helicase complex at stalled replication forks to control replisome function (Lossaint et al., 2013). FANCD2 also recruits FAN1 (Fanconi Anemia associated Nuclease 1), a 5′ flap endonuclease to stalled replication forks to re-start DNA replication and to prevent chromosome abnormalities (Lachaud et al., 2016).

mTORC1 regulates FANCD2 through at least two different mechanisms. In paediatric rhabdomyosarcoma cells and mouse xenografts, mTORC1 was shown to regulate FANCD2 transcription by the p70S6K signaling arm that promotes CDK4/6 activity (Shen et al., 2013). Inhibiting TORC1, or knockdown of mTOR by siRNA, decreased FANCD2 protein levels both *in vivo* and *in vitro*. A different mechanism was shown in hematopoietic stem and progenitor cells where mTOR deficiency increases phosphorylation of NF-κB and promotes its translocation to the nucleus. There, NF-κB binds to FANCD2 promotor and suppresses FANCD2 expression. Interestingly, for the NF-κB-dependent reduction of FANCD2 expression both TORC1 and 2 complexes appear to be required (Guo et al., 2013).

3.1.4. Actin cytoskeleton

One of the main functions of TORC2 is to regulate the polymerization of monomeric or globular actin to filamentous actin (F-actin). This is mediated through TORC2 regulation of the AGC-family of kinases (the PKC proteins in mammals and Ypk1 and Ypk2 in yeast) (Kamada et al., 2005). Several studies in yeast have demonstrated that TORC2 impacts replication integrity and genome stability through an undefined role in actin dynamics. A chemical genetic screen performed in *S. cerevisiae* identified a role for TORC2 in mediating the survival of cells exposed to replication stress, oxidative damage, or break-inducing agents like Zeocin or ionizing radiation (Shimada et al., 2013). Further characterization identified that the functional outcomes of inhibiting TORC2 could be recapitulated by ablating Ypk1 and/or 2, or by preventing actin polymerization (Shimada et al., 2013). These results indicated that TORC2-dependent F-actin regulation participated in the cellular response to low levels of DNA damage. Similar results were obtained from studies in *S. pombe* where disruption of Gad8, a mediator of TORC2-dpendent actin remodeling (Ikai et al., 2011), resulted in hypersensitivity to DNA-damaging agents (Schonbrun et al., 2009). A follow up study suggested that the TORC2 complex was specifically required for cell survival under chronic DNA replication stress induced by hydroxyurea (HU), MMS, or camptothecin (Schonbrun et al., 2013). Furthermore, synthetic lethal interactions were identified between *Tor2* or *gad8* mutants, and deletions of DNA repair or replication fork re-start genes, such as *mus81*, *mms1*, or *mms22*, even in the absence of DNA damage-inducing agents (Schonbrun et al., 2013).

It is currently unknown whether mammalian mTORC2 plays role in DNA replication and genome stability thorough an effect on actin remodeling. However, recent studies identified that nuclear F-actin participates in *drosophila* and mammalian DSB repair, though no link to mTORC function was explored (Caridi et al., 2018; Schrank et al., 2018). Additionally, during G1-phase, F-actin plays role in replication initiation through transcription regulation (Parisis et al., 2017). Importantly, under nucleotide depletion induced by HU, the levels of nuclear actin and two factors that stimulate actin polymerization, IQGAP1(IQ Motif Containing GTPase Activating Protein 1) and Rac1 GTPase (Rac family small GTPase), are significantly increased (Johnson et al., 2013). IQGAP1 is a master regulator of actin dynamics and is a substrate of the mTOR2 effector PKCε (Grohmanova et al., 2004), and the Rac1 GTPase regulates both mTORC1 and 2 and is regulated by mTORC2 (Saci et al., 2011). However, whether mTOR induces actin polymerization in response to replication stress, and what role actin dynamics play in the replication stress response, is a matter for further study.

4. Targeting replication stress through mTOR

Aberrant mTOR activation contributes to the pathogenesis of many tumor types [reviewed in (Kim et al., 2017; Meric-Bernstam and Gonzalez-Angulo, 2009; Mossmann et al., 2018)]. mTOR itself is rarely mutated, but rather is affected by modulation of its upstream regulators or downstream effectors. Oncogenic PI3K/AKT signaling, which occurs upstream of mTOR, is altered by mutation, overexpression, or deletion in many types of cancer including: breast, endometrial thyroid, prostate, melanoma and glioblastoma [reviewed in (Meric-Bernstam and Gonzalez-Angulo, 2009)). p53 and LKB1, negative regulators of mTOR, are commonly mutated across a variety of tumor types [reviewed in (Saxton and Sabatini, 2017)]. Since these oncogenic pathways converge on mTOR, it is not surprising that an immense research effort has focused on the development of mTOR inhibitors for cancer therapy. The first generation “rapalogs” were poorly tolerated, and therefore have limited clinical utility. However, second generation catalytic inhibitors demonstrated important clinical benefits in several cancer types. Unfortunately, the success of single-agent therapy in the mTOR pathway was limited and mainly resulted in disease stabilization rather than regression [reviewed in (Meric-Bernstam and Gonzalez-Angulo, 2009)].

As outlined here, our assertion is that mTOR plays an important role in DNA replication and the replication stress response. Notably, combining classic chemotherapies that interfere with DNA replication, such as cisplatin, melphalan, and etoposide, with mTOR inhibitors proved to be highly lethal in both cell line and mouse models (Mondesire et al., 2004; Shen et al., 2013; Silvera et al., 2017; Steelman et al., 2008; Zhou et al., 2017). For example, RAD001 (everolimus), a rapamycin derivative, dramatically enhances cisplatin-induced apoptosis in wild-type p53 human non-small cell lung carcinoma, but not mutant p53 tumor cells (Beuvink et al., 2005). Rapamycin was also shown to enhance cisplatin-induced apoptosis in human promyelocytic leukemia cell line HL-60 and the human ovarian cancer cell line SKOV3 (Shi et al., 1995). Treatment with the mTOR inhibitor AZD8055 together with melphalan showed synthetic lethality in cultured RH30 cells (Shen et al., 2013). Combinatorial mTOR inhibition with rapamycin, along with the genotoxic chemotherapeutic Paclitaxel, enhanced anti-tumor efficacy *in vivo* in nude mice bearing breast cancer xenografts (Mondesire et al., 2004). Considering the evidence described in this review, a compelling model for the enhanced lethality of genotoxic stress combined with mTOR inhibition results from a diminished capacity in the treated cells to tolerate replication stress.

In the last two decades, significant efforts were deployed to develop therapies, such as ATR and CHK1 inhibitors, that target the replication stress response [reviewed in (Forment and O'Connor, 2018)]. Interestingly, combinatorial CHK1 and mTOR inhibition showed promising results. This specific treatment induced synergistic cytotoxicity in p53 mutant colon cancer cell lines (Massey et al., 2016) and in Ewing sarcoma cell lines (Koppenhafer et al., 2018). Thus, combining mTOR inhibitors with factors required to repair replication stress-induced damage may be a promising approach for cancer therapy.

5. Conclusions and future perspectives

Our collective knowledge of the replication stress response has grown dramatically in recent years, exposing the complex and intricate signaling mechanisms that protect genome stability. Replication stress presents a clear challenge to human health through its prominent role driving oncogenic transformation. However, endogenous replication stress in tumors presents therapeutic opportunities via synthetically lethal challenges to cancer cells. While mTOR's primary activities related to governing cellular metabolism are better understood, here we highlighted the evidence supporting functions for mTOR in DNA replication and the replication stress response.

We interpret the published data to suggest that both ATR and mTOR function in regulating cellular homeostasis between anabolic and catabolic pathways. For mTOR, this includes regulating the anabolic production of cellular building blocks (i.e. proteins, lipids and nucleotides) with catabolic programs such as autophagy and cell death. Similarly, in the event of replication stress, ATR intervenes to ensure faithful anabolic production of nascent DNA, and if necessary, signals catabolic growth arrest or cell death should replication stress become excessive. Both the ATR and mTOR signaling cascades balance pro-death and pro-survival outcomes. As self-renewal of an accurate genetic code is essential for life, it is not surprising that cells muster their full regulatory capacity to ensure accurate replication and passage of their genetic material to daughter cells.

While we are intrigued with the connectivity between mTOR and DNA replication, much work is needed to elucidate the underlying mechanistic details. In particular, because of the central role that mTOR plays in cell metabolism, down-regulating or inhibiting mTOR may lead to consequences that are not directly due to the targeting of this kinase. Additional work is necessary to identify direct versus indirect outcomes. Furthermore, as PIKKs have overlapping consensus sequences for substrate phosphorylation, it may be difficult to ascribe phosphorylation events specifically to mTOR, ATR, DNA-PKcs or ATM. Thus, it is of central importance to gain a better understanding of how the “repair or death” decision is made at the molecular level in response to replication stress, and how the subsequent signaling is controlled specifically through ATR and/or mTOR signaling. Additionally, it is critical to understand how mTOR and ATR signaling is spatiotemporally regulated, and how signals between these pathways may cross from the nucleus to the cytoplasm. Additionally, further studies are required to understand the different roles that mTORC 1 and 2 play in the replication stress response. Finally, we look forward to understanding if targeting mTOR enhances the efficacy of genotoxic chemotherapeutics and if this has benefit to cancer patients.

Declaration of interest

The authors declare they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbiomolbio.2019.04.002.

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