mTOR Signalling in Health and Disease

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Abstract
The TOR (target of rapamycin) proteins are found in all eukaryotes. TOR has a protein kinase domain, as well as other domains through which it interacts with partner proteins to form at least two types of multiprotein complex, TORC1 and TORC2 (TOR complexes 1 and 2). Rapamycin, an antibiotic and immunosuppressant, inhibits functions of TORC1. Use of this drug has revealed roles for TORC1 and its mammalian counterpart, mTORC1, in promoting many anabolic processes. mTORC1 signalling is activated by growth factors and nutrients. It is highly active in many cancers and plays a role in tumorigenesis and in other diseases. Much less is known so far about the functions and regulation of (m)TORC2. The goal of this meeting was to bring together researchers studying the roles of mTORC1/2 in normal cell and animal physiology in diverse systems, as well as scientists exploring the therapeutic value of inhibiting mTOR (mammalian TOR) signalling.

Introduction
The TOR (target of rapamycin) genes were first discovered about 20 years ago by virtue of the fact that mutations in them rendered yeast cells insensitive to the growth inhibitory effects of rapamycin. Rapamycin is a polyketide compound first isolated from the bacterium Streptomyces hygroscopicus, which in turn was found in a soil sample taken on Easter Island. The subsequent two decades, especially the second one, have witnessed rapid advances in our knowledge of the cellular roles of the TOR protein kinases and their orthologues in other eukaryotic organisms, and of their involvement in human disease. The goal of the ‘mTOR Signalling in Health and Disease’ meeting was to discuss recent advances both in the biology of TOR, especially mTOR (mammalian TOR), and of strategies to inhibit signalling through mTOR to treat human diseases. The present article aims to highlight some of the major topics and discoveries that were discussed, especially those described in the accompanying articles in this issue of Biochemical Society Transactions; sadly, space considerations preclude a detailed discussion of every poster and oral presentation at this meeting. This article focuses mainly on those contributions which are linked to articles in this issue of Biochemical Society Transactions. I apologize to all those contributors whose findings have not been discussed here.

mTORC (mTOR complex) 1 and 2
mTOR is a large (almost 300 kDa) multidomain protein which possesses a kinase domain similar to lipid kinase of the PI3K (phosphoinositide 3-kinase) family, but which phosphorylates proteins on serine or threonine residues rather than lipids. It also contains multiple HEAT repeats and a domain which binds to rapamycin, when that compound is associated with the immunophilin FKBP12 (FK506-binding protein 12). It should be noted that rapamycin–FKBP12 does not interact with the kinase domain of mTOR or directly
**Figure 1 | Composition of mTORC1 and mTORC2**

The upstream control of mTORC1 of mTORC1 by amino acids and by insulin and growth factors is indicated, as is the possible regulation of mTORC2 by PI3K. Known substrates for mTORC1 and mTORC2 are shown (only selected substrates in the case of mTORC1). mTOR kinase inhibitors block functions of both types of complex, whereas rapamycin only impairs some of the functions of mTORC1. The double line from raptor to mTORC1 substrates indicates raptor’s role in recruiting substrates to mTORC1. PRAS40, proline-rich Akt substrate of 40 kDa, TSC, tuberous sclerosis complex. See the text for more details.

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inhibit its kinase activity; indeed the precise mechanism by which rapamycin interferes with (m)TOR function still remains obscure.

mTOR (and, where known, its orthologues in other species) forms two types of multiprotein complex, termed mTORC1 and mTORC2 (Figure 1), which differ markedly in terms of their regulation and their substrate specificity. Short-term treatment with rapamycin only interferes with functions of mTORC1, and even then only with a subset of those functions (Figure 1). Hence, our understanding of mTORC1 is much more advanced than that of mTORC2, for which there is so far no specific inhibitor. mTORC1 contains the proteins mTOR, raptor (regulatory associated protein of mTOR) and mLst8, while mTORC2 contains rictor (rapamycin-insensitive companion of mTOR) in place of raptor plus mLst8, Sin1 and protor (protein observed with rictor). Deptor binds to mTOR and is a negative regulator of mTORC1 and mTORC2 (not shown in Figure 1) [1]. Raptor appears to confer substrate specificity on mTORC1 by interacting with short motifs (TOR signalling or TOS motifs) in its substrates; the role of rictor in mTORC2 is not well understood.

Recently, several academic or industrial groups have developed inhibitors of the kinase activity of mTOR, which impair the functions of mTORC2 and mTORC1, including rapamycin-insensitive functions of the latter. These provide a potentially valuable tool for studying the cellular roles of mTOR, and may help in the development of mTOR kinase inhibitors as anticancer agents.

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**mTORC1 and protein synthesis**

The best understood targets of mTORC1 are proteins which phosphorylate or otherwise regulate proteins involved in mRNA translation. They include the ribosomal protein S6Ks (S6 kinases), which phosphorylate several proteins linked to mRNA translation such as S6 itself, eIF (eukaryotic initiation factor) 4B and eEF2 (eukaryotic elongation factor 2) kinase, and 4E-BP1/2 (eIF4E-binding proteins 1 and 2), which bind to and inhibit eIF4E when in their hypophosphorylated forms. However, the contribution of these various downstream targets of mTORC1 signalling to the control of protein synthesis remains unclear.

Joseph Avruch (Harvard Medical School, U.S.A.) [2] described the discovery of a novel mechanism by which mTOR can control the translation of certain mRNAs. These proteins [IMP (IGF2 mRNA-binding protein) 1–3] interact with the 5′-untranslated region of the mRNA for IGF2 (insulin-like growth factor 2). Translation of this mRNA is driven by an IRES (internal ribosome-entry segment) and is independent of eIF4E, but is nonetheless inhibited by rapamycin. Overexpressing IMP2 enhanced the translation of a reporter mRNA containing the IGF2 5′-untranslated region. Avruch and colleagues showed that IMP2 interacts directly with mTOR (rather than with raptor) and is phosphorylated by mTOR, enhancing its ability to promote IRES-driven translation. Thus the RNA-binding protein is a novel mTOR-activated enhancer of IRES function. This is a novel example of translational control where mTOR promotes IRES-directed translation of a specific mRNA.
Chris Proud (Southampton) [3] studied the effects of rapamycin or mTOR kinase inhibitors on protein synthesis in human (HeLa) cells, and reported that, although rapamycin has only a very small effect on protein synthesis rates in the short term (up to 6 h), the latter compounds exert stronger inhibition (by approx. 30%). Rapamycin did not impair the formation of initiation factor complexes involving eIF4E, but mTOR kinase inhibitors did do so; however, this difference does not appear to explain their ability to more strongly inhibit protein synthesis. In heart muscle cells, cardiomyocytes, agents that induce heart growth (hypertrophy) activate protein synthesis, and this effect is inhibited by rapamycin, but again does not appear to be due to inhibition of eIF4E function. Thus the mechanisms by which mTORC1/mTOR regulates translation in the short term still remain to be uncovered. This group also used a new stable-isotope labelling technique to quantify the effects of rapamycin and mTOR kinase inhibitors on the synthesis of individual proteins; this revealed a wide spectrum of effects. In particular, rapamycin (as expected) inhibited the synthesis of proteins encoded by mRNAs that contain a 5′-TOP (terminal oligopyrimidine tract), but mTOR kinase inhibitors inhibited much more strongly, indicating that both rapamycin-sensitive and -insensitive effects of mTOR are involved in controlling the translation of such mRNAs.

Thus, even though mRNA translation is considered to be the process whose control by mTOR(C1) is best understood, many questions remain to be addressed concerning the mechanisms by which mTOR signalling controls protein synthesis in the short term. In the longer term, mTOR can also regulate ribosome production and thus the cellular capacity for protein synthesis (see below).

Other cellular processes controlled by mTORC1

It has been known for some years that mTORC1 regulates the synthesis of rRNA and the synthesis of ribosomal proteins (which are encoded by members of the 5′-TOP family of mRNAs mentioned above). The large rRNAs are made by Pol III (RNA polymerase III), which was shown some time ago to be controlled by mTORC1 [4]. Recent studies indicate that mTORC1 also controls Pol III, which makes the 5S rRNA. In yeast, this involves the phosphorylation of Maf1p, an inhibitor of Pol III, which is excluded from the nucleus upon phosphorylation by Sch9 (the yeast orthologue of the S6Ks), thus alleviating the inhibition of Pol III. AnnemiekeMichels (Lausanne) [5] reported that mammalian MAF1 is also regulated by mTORC1, although a quite different mechanism appears to operate to control Pol III in mammals, since mTORC1 does not seem to affect its nucleoplasmic localization and MAF1 is not phosphorylated by S6K1. Instead, MAF1 appears to be a direct substrate for mTORC1. Furthermore, rapamycin still impairs Pol III transcription in cells expressing a MAF1 mutant in which the known phosphorylation sites have been mutated, suggesting that mTORC1 also affects Pol III through additional mechanisms.

mTORC1 promotes several anabolic processes (e.g. protein synthesis or ribosome biogenesis); recent work indicates that these include lipogenesis, which is likely to be important, for example, for the construction of additional membranes during cell growth and proliferation. The SREBP1s (serum-response-element-binding proteins) have been shown to be activated in an mTORC1-dependent manner [6]. Caroline Lewis (Cancer Research UK) [7] described recent work aimed at understanding how mTORC1 does this. mTORC1 modulates both the levels of the mRNA for SREBP1 and the processing of the SREBP1 polypeptide to yield its active nuclear form.

(m)TORC1 signalling represses autophagy, a degradative process that is activated in nutrient-deprived cells. Kun-Liang Guan (University of California San Diego) [8] described recent work directed at understanding the control of autophagy focusing on ULK1, a mammalian homologue of the yeast protein kinase Atg1, which plays a key role in regulating autophagy. AMPK (AMP-activated protein kinase) plays a role, together with mTORC1, in regulating Atg1/ULK1. They act in opposing ways. AMPK phosphorylates ULK1; since AMPK is activated under conditions of nutrient starvation, this may provide a mechanism by which autophagy is stimulated in response to starvation.

Cellular functions of the S6Ks

The ribosomal protein S6Ks were the first targets (substrates) of mTORC1 to be discovered, on the basis of the ability of rapamycin to inhibit their activation. However, their physiological roles remain largely unclear. Ivan Gout (University College London) [9] described the ability of one S6K isoform, S6K2, to interact with DNA via an ‘AT-hook’ domain in its C-terminus, and that this increases its activity. It will be important to study whether S6K2 affects gene expression, e.g. by modulating gene transcription.

Mario Pende (Paris Descartes University) [10] reported that, despite the fact that S6Ks phosphorylate several proteins involved in mRNA translation, there was no apparent role for these kinases in the translational control of specific mRNAs. In contrast, they do regulate the expression of several proteins involved in rRNA processing and/or ribosome assembly. This is of substantial interest given the known role of the mTORC1 pathway in regulating the transcription of rRNAs; control of ribosome biogenesis may underlie, at least in part, the reductions in cell size observed in animals in which S6Ks have been genetically knocked out.

mTORC1 in health and disease

Numerous studies in various diverse species reveal a role for TOR/mTOR signalling in the control of lifespan: various manipulations that impair TOR/mTOR signalling increase lifespan. Ivana Bjedov (University College London) [11] described several approaches to understanding the role of TOR in lifespan in the fruitfly, Drosophila. A key

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question is: which downstream processes link TOR/mTOR to the modulation of lifespan? Candidates include mRNA translation, autophagy and resistance to oxidative damage, each of which may contribute to the lifespan extension seen in response to impairment of TOR/mTOR signalling. It is interesting to note that nutrients activate (m)TORC1, while caloric restriction is well known to favour longevity.

mTOR plays many roles in mammalian development: knocking out either mTOR or raptor causes lethality very early in embryonic development. Laetitia Mazelin (Lyon) [16] described a key role for mTOR in early postnatal cardiac development in mice, while Romana Tomasoni (San Raffaele University, Italy) [13] reported that conditional loss of TSC1 (tuberous sclerosis complex 1), an upstream inhibitor of mTORC1 signalling, perturbs the development of the thymus and of T-cells. Further studies in such conditional knockout animals will reveal additional roles for mTORC1 in normal development.

One of the first observed effects of rapamycin in mammalian systems was its ability to inhibit the proliferation of T-cells; Gwyn Williams (Keele) [14] described an essential role for the non-coding RNA GAS5 (growth arrest-specific transcript 5) in the effects of rapamycin on T-cell proliferation, although its mechanism of action remains an interesting unresolved question.

**Targeting mTOR for therapy**

Factors such as the discoveries that mTORC1 is regulated by oncogenic signalling [e.g. is activated in cells lacking the tumour suppressor PTEN (phosphatase and tensin homologue deleted on chromosome 10)] and that the proliferation of such cells is strongly inhibited by rapamycin have led to a rapid growth of interest in developing anticanter therapies based on mTOR inhibition. Several speakers at this meeting described the results from animal models of cancer or clinical trials with cancer patients, and readers are referred to their articles in this issue for detailed information.

In brief, Sylvie Guichard (AstraZeneca, U.K.) [15] described data from work using the mTOR kinase inhibitor AZD8055, which generally has greater effects than rapamycin on the induction of autophagy or inhibition of cell proliferation in cancer cell lines. One concern with rapamycin is its ability to (re)activate signalling through PI3K and PKB (protein kinase B, also known as Akt), by interrupting the feedback loop from mTORC1 via S6K1 to inhibition of PI3K signalling. Since cancer cells frequently show activation of more than one signalling pathway, it may be beneficial to target additional pathways; indeed, use of the MEK (mitogen-activated protein kinase kinase kinase 3) by amino acids and its role in the control of mTORC1. This involves the regulation by amino acids of its phosphorylation, and hence its activity, via PP2A (protein phosphatase 2A) (specifically, PP2A complexed with a regulatory protein, PR61c).

Other recent work has identified a role for the Rag family of small GTPases in the control of mTORC1 by amino acids [20, 21], and it will be important to elucidate how MAP4K3 and the Rags operate (together?) to control mTORC1. It seems that one simple model, where MAP4K3 and the Rags operate (together?) to control mTORC1, but also in their inhibitory action on mTORC2 and thus, potentially, on the PKB/Akt arm of PI3K signalling (Figure 1). However, further clinical studies are required to demonstrate clear clinical benefits.

**Control of (m)TORC1**

Important questions remain about the control of mTORC1, in particular its regulation by amino acids (which promote mTORC1 signalling). Richard Lamb (Cross Cancer Institute, Canada) [19] described recent work on the regulation of the protein kinase MAP4K3 (mitogen-activated protein kinase kinase kinase 3) by amino acids and its role in the control of mTORC1. This involves the regulation by amino acids of its phosphorylation, and hence its activity, via PP2A (protein phosphatase 2A) (specifically, PP2A complexed with a regulatory protein, PR61c).

Anna Melone (Basel) [22] presented evidence that nutrient regulation of yeast TOR requires PtdIns3P, but that the class III PI3K Vps34 is not involved. (Its mammalian orthologue, hVps34, has previously been implicated in controlling mTORC1.)

**Control and functions of mTORC2**

In contrast with the rapid advances in understanding the regulation and role of mTORC1, our knowledge of mTORC2 is much more rudimentary. In part, this reflects the lack of a specific tool (inhibitor) to study its roles in cellular regulation.

Mike Hall (Basel) [23] reported that TORC2 in yeast interacts with the ribosome. Interestingly, another group has reported that mTORC2 interacts with mammalian ribosomes [24]. He also discussed the key question ‘(how) is mTORC2 regulated?’ mTORC2 phosphorylates certain protein kinases.
TOR in other organisms

All eukaryotes appear to contain homologues of TOR. Robbie Loewith (Geneva) [27] described work in yeast which aims to identify the ‘targets’ controlled by TORC1 and TORC2. For TORC2, the best-characterized effectors are, to date, S6K, a protein kinase, a likely homologue of the S6Ks, and Tap42, a regulator of protein phosphatase activity. In this study, chemical genetic techniques were applied in combination with label-free proteomic methods to try to identify proteins whose phosphorylation is altered in response to manipulations that perturb TORC1 and or TORC2 signalling. The data revealed numerous proteins whose phosphorylation is controlled by TOR signalling. Related studies show that TORC1 has a prominent role in regulating proteins implicated in ribosome biogenesis.

Work on plant TOR has been hindered by the fact that rapamycin does not impair TOR signalling in plant cells. Christian Meyer (Institut Jean Pierre Bourgin, France) [28] gave a comprehensive overview of current knowledge of TOR signalling in plant systems, where, in line with its effects in other systems, TOR promotes growth and increased biomass. As in other organisms, plant TOR can form distinct complexes (TORC1 and TORC2), and some TOR targets, known from other systems, have been identified in plants (e.g. S6K and the Pol III regulator MAF1). This area, the regulation and physiological roles of plant TOR, appears to be an important topic for future work, given, for example, the importance of understanding the control of plant growth for the agriculture industry.

Janni Petersen (Manchester) [29] discussed the role of TOR in controlling cell division in Schizosaccharomyces pombe, where TORC1 regulates the timing of cell division in response to changes in nutritional conditions [30]. Impaired nutrient availability advances the onset of mitosis, as does the TORC1 inhibitor rapamycin. Such effects appear to be independent of TORC2, and may be mediated (at least in part) through Gad8, a possible orthologue of S6K.

Concluding comments

The last decade has seen truly remarkable progress in understanding mTOR/TOR, its partner proteins, its control, and its roles in cell and organismal physiology. Further progress in the last of these will doubtless stem from the development and use of animals with conditional and/or tissue-specific knockouts of components of mTORCs or their downstream targets. This will be especially important for components of mTORC2, since we lack a specific inhibitor of this complex with which to study its roles. Although work using rapamycin has revealed many functions for mTORC1, for example, major questions remain about how mTORC2 controls cellular processes such as ribosome production, gene transcription and metabolism. Although deregulated mTORC1 signalling does seem to play an important role in many cancers, substantial further work is needed to understand how to target this pathway, including its downstream targets, for effective disease therapy.

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