TOR signalling regulates mitotic commitment through stress-activated MAPK and Polo kinase in response to nutrient stress

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Abstract
Cell growth and cell division are coupled to control cell size and this co-ordination is often modulated by the availability of nutrients. In many eukaryotes, TOR (target of rapamycin) signalling is involved in coupling nutrient sensing to cell growth and division controls. Nutrient stress inhibits TOR signalling to advance the timing of cell division and thus leads to continued cell division at reduced cell size. Most changes in the environment stimulate stress-activated MAPK (mitogen-activated protein kinase) signalling pathways. Several MAPKs also have a general role in the control of mitotic onset and cell division. In the present paper, I discuss the interplay between two major signalling pathways, the TOR and the stress MAPK signalling pathways, in controlling mitotic commitment, with the main focus being on fission yeast (Schizosaccharomyces pombe).

The environment modulates cell proliferation: implications for understanding cancer progression
The tight coupling between cell growth and cell-cycle progression enables eukaryotic cells to divide at a constant size during continuous proliferation and generate organs and organisms of specific sizes. The size at division is often modulated by changes in nutrient availability [1–5]. TOR (target of rapamycin) signalling pathways co-ordinate this coupling between cell growth and cell-cycle progression in response to a variety of environmental cues including stress and nutritional availability [6]. Inappropriate cell growth and proliferation is a hallmark of cancer. Cancer cells generate abnormally sized and shaped tumour masses and they migrate to inappropriately colonize parts of the body. Cancer cells therefore undergo proliferation in environments that are markedly different from their normal context and so are likely to have an altered nutrient and oxygen supply. Thus they are regularly more stressed than normal tissues. Therefore, not surprisingly, alterations in mTOR (mammalian TOR) and stress-response MAPK (mitogen-activated protein kinase) signalling have both been linked to cancer. Furthermore, several signalling components upstream and downstream of the mTOR kinase are frequently altered in human tumours [6]. Thus an increased understanding of environmental control of cell division is likely to clarify how control of proliferation in cancer cells differs from that in their untransformed progenitor cells. The fission yeast Schizosaccharomyces pombe is an ideal model organism for studying G2/M controls, as these can be easily investigated by measuring cell length at septation. This is because the rod-shaped cell grows by elongation and undergoes mitosis and division at a defined length under steady-state growth conditions [1] (Figure 1A). Furthermore, the controls regulating the timing of mitotic commitment in fission yeast are thought to be directly comparable with controls found in mammalian cells [7].

Nutrient stress inhibits TOR signalling to advance cell division
TOR kinases are part of a major signalling network in eukaryotic cells that are involved in nutrient sensing [8]. Inhibition of TOR signalling in fission yeast, via either small-molecule inhibition with rapamycin or by simply shifting cells from a rich to a poor nitrogen source (nutrient stress), advanced the timing of cell division [5,9] and led to continued cell divisions at reduced cell size (Figure 1A). Similar observations have been reported in Drosophila, where an increase in cell number was observed after rapamycin treatment of cell cultures and wings [10]. This conservation extends to humans, as rapamycin inhibition of mTOR in proliferating mammalian cells similarly reduced cell size at division [11]. In addition, mammalian HEK (human embryonic kidney)-293 cells advance cell division following modest starvation owing to confluent growth. The subsequent addition of fresh serum to release this starvation delays mitosis and increases cell size at division [2]. This behaviour is completely mimicked in the response of fission yeast to modest changes in available nutrients [5]. Therefore a role for TOR signalling in nutrient control of cell size at division appears to be generally conserved.

Nutrient stress advances mitotic entry
Activation of MPP (maturation-promoting factor) [Cdc2 (cell division cycle 2 kinase)–cyclin B complex] is a prerequisite for mitotic entry [7]. Activation of MPP at

Key words: mitogen-activated protein kinase (MAPK), mitotic commitment, nutrient stress, Polo kinase, target of rapamycin (TOR), yeast.

Abbreviations used: ATF1, activating transcription factor 1; Cdc2, cell division cycle 2 kinase; ERK, extracellular-signal-regulated kinase; MAPK, mitogen-activated protein kinase; MPF, maturation-promoting factor; mTOR, mammalian target of rapamycin; SAMRP, stress-activated MAPK-response pathway; SPB, spindle pole body; TOR, target of rapamycin; TORC, TOR complex; TSC, tuberous sclerosis complex.

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Figure 1 | Fission yeast cells undergo division at a defined length under steady-state growth conditions

(A) Wild-type cells grown in glutamate medium (good nitrogen source) are longer at cell division than cells grown in proline medium (poor nitrogen source). (B) sty1Δ delays mitotic onset, resulting in division at an increased size, whereas constitutive activation of the MAPKK Wis1 (wis1.DD) advances mitotic commitment, and cells divide at reduced cell size compared with wild-type cells.

any point in the G2-phase of the cell cycle will promote mitotic entry and result in viable division at reduced cell size. Exposure of populations of wild-type fission yeast cells that have been synchronized with respect to cell cycle progression to nutrient stress or rapamycin advanced the timing of commitment to mitosis and so reduced the size at which cells divided [9]. The Cdc2 subunit of MPF is normally inhibited in G2 by phosphorylation of Tyr15 [12]. Removal of this phosphate by the activating phosphatase Cdc25 promotes mitosis. Thus the timing of mitotic commitment is determined by the balance in the activity of Cdc25 and the inhibitory kinase Wee1 [7] that phosphorylates Cdc2 on Tyr15 (Figure 2). Both Cdc25 and Wee1 are required for the advancement of mitotic onset induced by rapamycin or nutrient stress in fission yeast [9].

MAPK signalling controls mitotic onset in fission yeast

In all eukaryotes, SAMRPs (stress-activated MAPK-response pathways) play an important role in modulating intercellular signalling to entrain a range of processes with changes in the environment. Higher eukaryotes and budding yeast have several such SAMRPs, each of which responds to different stresses. In contrast, in fission yeast, a single major SAMRP is activated in response to a variety of outside stimuli (Figure 2). This main stress-response pathway also has a general role in mitotic onset. A win1.1 mutation in the MAPKKK (MAPK kinase kinase) or a deletion of the MAPK sty1Δ delays mitotic onset, resulting in division at an increased size, whereas constitutive activation of the MAPKK (MAPK kinase) Wis1 (wis1.DD) advances mitotic commitment to have the opposite effect of promoting division at reduced cell size [14–16] (Figure 1B). Recruitment of the Polo kinase Plo1 to the SPBs (spindle pole bodies) modulates Cdc25/Wee1-controlled Cdc2 activity [17–20]. Spc1/Sty1 MAPK signalling controls phosphorylation of Ser402 of Plo1, which drives the recruitment of the kinase to SPBs [9,18]. Therefore MAPK regulation of Ser402-dependent Plo1 localization to the SPBs, provides a way by which cells can either advance or delay mitotic commitment. This control of Polo kinase function through an MAPK-dependent phosphorylation is conserved from fission yeast to mammalian cells. A recent study demonstrated that Ser326 of Plk1 (Plo1 homologue) regulates its function during mitotic progression and a Plk1-S326E mutant, which mimics phosphorylation, can rescue MAPK-depletion-induced mitotic defects [21].

MAPK control of mitotic onset in humans and Xenopus

The control of mitotic commitment through stress MAPK signalling in fission yeast is conserved in mammalian cells and Xenopus oocytes. A block to ERK1 (extracellular-signal-regulated kinase 1) MAPK activation delays mitotic onset, indicating that ERK1 activity is required for mitotic entry in both systems [22,23]. When mammalian cells are exposed to severe stress, another MAPK, p38, is activated. The function of p38 in this context is to block mitotic onset until the stress has been dealt with [24]. Thus, in higher eukaryotes,
one type of MAPK signalling promotes mitosis, while another type, in response to severe stress, delays it. The main fission yeast MAPK Spc1/Sty1 mimics both these responses because, in addition to its role in promoting mitosis in response to nutrient stress, toxic stress induces a high level of Spc1/Sty1 signalling that will block mitosis until the stress has been dealt with. Thus Spc1/Sty1 behaves as a functional homologue of both ERK1 and p38 MAPKs.

**Nutrient stress promotes MAPK-dependent recruitment of Plo1 to SPBs, not because it elicits a transcriptional response**

The Spc1/Sty1 MAPK signalling pathway is modulated by a large variety of stresses (Figure 2), to entrain a range of cellular processes with the changes in the environment. A key and well-documented response is the transcriptional response in which Sty1/Spc1 phosphorylation of ATF1 (activating transcription factor 1) invokes a number of changes in the proteome to enable cells to adjust to the changes in the environment. MAPK signalling is also essential for nutrient-stress- or rapamycin-induced acceleration of mitosis, because cells depleted in the MAPK sty1Δ or the MAPKK wist1Δ failed to advance mitotic onset following the respective manipulations [9]. In contrast, cells deficient in ATF1, which as a consequence fail to mount the transcriptional response, still advance mitotic commitment. Thus this Sty1/Spc1 control over mitotic commitment is independent of the transcriptional response. It is, however, blocked by mutating Ser402 of Plo1 to alanine to block the recruitment of this cell-cycle regulator to the SPBs [9], demonstrating that it is by modulating the recruitment of Plo1 to SPBs that cells respond to nutrient stress.

**TOR signalling controls MAPK activation**

Mammals contain one TOR kinase, whereas budding and fission yeasts contain two. In fission yeast, only Tor2 performs an essential role [25–28]. Following nutrient stress, fission yeast Tor1 kinase activity is reduced [9]. In contrast with wild-type cells, tor1Δ cells did not advance mitotic commitment in response to nutrient stress and maintained cell length upon shifts into medium containing a poor nitrogen source. Importantly tor1Δ mutants were still able to accelerate mitosis when exposed to acute removal of the nitrogen source (‘nutrient starvation’). Therefore Tor1 specifically regulates mitotic commitment in response to the modest change in nitrogen quality of nutrient stress and is not necessary for the acute response of nitrogen starvation. The inhibition of Tor1 signalling following nutrient stress or addition of rapamycin activates Sty1/Spc1 [9], consistent with the essential role of the MAPK in the rapamycin or nutrient-stress-induced acceleration of mitosis. This TOR-dependent MAPK activation is mediated via a down-regulation of the PyP2 MAPK phosphatase [9]. The reduction in PyP2 activity boosts the basal level of Spc1/Sty1 signalling to promote Plo1 recruitment to the SPBs (Figure 2). Interestingly, a recent study has demonstrated a post-translational regulation of the ERK phosphatase DUSP6 (dual-specificity phosphatase 6)/MKP3 (MAPK phosphatase 3) by the mTOR pathway [29]. Therefore the TOR-dependent control of MAPK signalling through regulation of an MAPK phosphatase observed in fission yeast may also be conserved in human cells.

**TORCs (TOR complexes) in fission yeast**

In all cell types studied to date, two distinct TORCs have been identified: TORC1 and TORC2 [30]. The composition of the fission yeast TORCs have mainly been studied in cells grown in rich medium, where rapamycin has no effect [31]. Fission yeast Tor1 was shown to be part of the rapamycin-insensitive TORC2, and Tor2 was shown to be part of TORC1 [25,28,32]. TSC (tuberous sclerosis complex) 1 and TSC2 are two upstream regulators of TOR signalling [6]. Interestingly, fission yeast cells deleted for tsc1Δ and tsc2Δ appear to be hypersensitive to their environment, as they are specifically unable to grow in minimal media [27]. Importantly, it is defined minimal medium [5] that most closely mimics the limited conditions cells encounter in their natural environment. Together with the observation that rapamycin treatment only affects fission yeast cells grown in minimal medium [9,33], this indicates that TOR signalling in rich and minimal media are fundamentally different. It would therefore be interesting to compare the composition and status of TORCs from cells grown in minimal glutamate medium with the data already accumulated on complexes isolated from rich medium [25,28].

In budding yeast (*Saccharomyces cerevisiae*), two distinct TORC1 subcomplexes, including Tor1 or Tor2 respectively, have been described [34]. As the TOR signalling pathway is highly conserved in eukaryotes, an attractive possibility would be if Tor1 turned out to be a TORC1 component in minimal medium. If Tor1 is exclusively a TORC2 component, fission yeast would be the only organism characterized to date in which TORC2 is sensitive to rapamycin.

**Budding yeast and mammalian TOR blocks mitotic entry following nutrient starvation**

Two recent studies in budding yeast and mammalian cells reported an mTOR-dependent block of mitotic commitment in response to changes in nutrient availability; however, the cells in these studies were exposed to an acute withdrawal of nutrients [35,36], which is best characterized as a nutrient-starvation-response. This response differs from the modest ‘nutrient stress’ response of a change in nitrogen quality that promotes mitosis in fission yeast. Nutrient stress is most likely to represent the chronic fluctuations in nutrient supply that are experienced by cells in their natural settings. Importantly, acute nutrient starvation is likely to severely stress the cells and so promote the strong activation of MAPK signalling that transiently blocks mitotic commitment. Such a block in mitotic entry avoids segregation of chromosomes and organelles that could have been damaged by the imposed stress. It is unclear whether nutrient starvation affected MAPK signalling in budding yeast and mammalian cells. However, if the studies in fission yeast do reflect a general
eukaryotic response, monitoring the amplitude with which MAPK signalling fluctuates in response to different nutrient manipulations might be as important as monitoring whether it changes at all.

**Perspectives**

Rapamycin inhibition of TOR signalling activates MAPK to promote cell division in fission yeast [9]. Interestingly, a recent study demonstrated that rapamycin inhibition of mTORC1 (mammalian TORC1) in human cancer cells also stimulates MAPK signalling [37]. Importantly, the anti-tumour activity of rapamycin was enhanced when the drug was combined with compounds that inhibited MAPK signalling. Therefore extending our knowledge of environmental control of cell division in simple model systems such as yeasts may help to refine strategies for the application of drugs such as rapamycin and highlight new opportunities for novel compounds and combination therapies for treatment of cancer.

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**References**


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