Response to leucine in *Schizosaccharomyces pombe* (fission yeast)

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**Abstract**

Leucine (Leu) is one of the branched-chain, essential amino acids in animals, including humans. Fungi, including the fission yeast *Schizosaccharomyces pombe*, can biosynthesize Leu, but deletion of any of the genes in this biosynthesis leads to Leu auxotrophy. In this yeast, although a mutation in the Leu biosynthetic pathway, *leu1-32*, is clearly inconvenient for this species, it has increased its usefulness as a model organism in laboratories worldwide. Leu auxotrophy produces intracellular responses and phenotypes different from those of the prototrophic strains, depending on the growing environment, which necessitates a certain degree of caution in the analysis and interpretation of the experimental results. Under amino acid starvation, the amino acid-auxotrophic yeast induces cellular responses, which are conserved in higher organisms without the ability of synthesizing amino acids. This mini-review focuses on the roles of Leu in *S. pombe* and discusses biosynthetic pathways, contribution to experimental convenience using a plasmid specific for Leu auxotrophic yeast, signaling pathways, and phenotypes caused by Leu starvation. An accurate understanding of the intracellular responses brought about by Leu auxotrophy can contribute to research in various fields using this model organism and to the understanding of intracellular responses in higher organisms that cannot synthesize Leu.

**Keywords:** fission yeast, *Schizosaccharomyces pombe*, leucine, *leu1-32*, general amino acid control, TORC1

**Introduction**

Leucine (Leu) is one of the branched-chain amino acids (BCAAs) like isoleucine (Ile) and valine (Val). It is also one of the essential amino acids in animals, including nematodes, silkworms, and humans (Oda 2007, Le Couteur et al. 2020). It has important physiological functions in protein synthesis, metabolism, nutrient uptake, and control of aging (Le Couteur et al. 2020). As the most bioactive BCAA, Leu contributes to the maintenance of the level of cellular acetyl coenzyme A and the acetylation of cytoplasmic proteins during nutrient deprivation (Mariño et al. 2014, Son et al. 2020). Since BCAA biosynthetic pathways exist in plant, fungi, archaea, and bacteria, but not in animals, these pathways may also be targets for herbicides and antimicrobial compounds (Liu et al. 2016). Various studies have reported a relationship between the lifespan of organisms and the presence of essential amino acids including Leu (Fontana and Partridge 2015, Ohtsuka et al. 2019, Le Couteur et al. 2020), and an accurate understanding of this phenomenon will contribute to a wide range of life science fields, including aging.

Amino acid or protein restriction causes activation of general amino acid control and suppression of the target of rapamycin (TOR) pathway, leading to improved metabolic fitness, increased stress tolerance, and extended lifespan (Gallinetti et al. 2013). Restrictions on methionine and tryptophan have been shown to reduce the activity of nutrient-sensing pathways and extend the lifespan in mice (Fontana and Partridge 2015). In yeasts such as the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, cells can synthesize all amino acids, but a defect in the amino acid biosynthetic pathway leads to the loss of synthetic ability, resulting in activation of the general amino acid control and suppression of the TOR pathway, depending on the growth environment (Zaborske et al. 2009, Kamada 2017, Ohtsuka et al. 2019, Fukuda et al. 2021). In *S. cerevisiae*, restriction of methionine, asparagine, or glutamate (Glu) has been reported to extend the chronological lifespan, which is defined as the survival of a cell population after the stationary phase (Powers et al. 2006, Dilova et al. 2007, Ruckenstuhl et al. 2014, Fontana and Partridge 2015). Starvation of Leu, one of the BCAAs, has also been found to activate the general amino acid control and extend the chronological lifespan in *S. pombe* (Ohtsuka et al. 2019). This chronological lifespan extension by Leu starvation occurs in Leu auxotrophic cells but not in prototrophic cells (Ohtsuka et al. 2019).

In research using the model organism *S. pombe*, Leu auxotrophs have been used since the latter half of the twentieth century (Kohli et al. 1977, Beach and Nurse 1981). These auxotrophs have made it convenient to study this organism as described later in the text and made significant contributions to research in various fields (Seike et al. 2019, Imai et al. 2020, Fukuda et al. 2021). However, as Leu auxotrophy can cause intracellular responses differ-
ent from those of prototrophic cells, caution is needed when evaluating the results of studies using auxotrophic cells. For example, Leu starvation results in completely different chronological lifespan depending on whether cells are Leu auxotrophs or not (Ohtsuka et al. 2019). Additionally, the supplementation of Leu to the medium affects not only the growth of Leu auxotrophic cells but also the length of prototrophic cells (Petersen and Russell 2016). Notably, autophagy defects lead to a significantly reduced survival rate under nitrogen depletion in Leu auxotrophic cells, but not in arginine (Arg) or lysine (Lys) auxotrophic cells (Kohda et al. 2007), indicating that the phenotype differs dependent on the type of amino acid auxotrophy. Although Leu has had a strong influence on research using S. pombe, there are not many reports summarizing the relationships involved. This mini-review summarizes the Leu utilization and starvation responses that have been clarified to date in S. pombe. A clear understanding of the relationship between this model organism and Leu is expected to facilitate more accurate interpretations of the results of future studies using this organism.

**Leu biosynthesis in S. pombe**

Schizosaccharomyces pombe can biosynthesize Leu, as do other fungi, and half of Leu synthesis occurs by the same pathway as the other BCAAs Ile and Val (Fig. 1) (McDonald et al. 1974). Because all the enzymes that act in Ile and Val biosynthesis are also required for Leu biosynthesis, there are Leu auxotrophic strains but not auxotrophic strains that require only Ile or Val.

BCAAs including Leu are synthesized from pyruvate (Kondo et al. 2012, Liu et al. 2016). The enzyme acetolactate synthase (ALS), also known as acetyl-CoA synthase, which catalyzes the first reaction of the BCAA biosynthetic pathway, converts two pyruvate molecules to (2S)-2-acetolactate ([2S]-2-hydroxy-2-methyl-3-oxobutanoate), which is required for Leu and Val synthesis, while converting pyruvate and 2-oxobutanoate (2-ketobutyrate) to (S)-2-aceto-2-hydroxybutanoate, which is required for Ile synthesis (Fig. 1) (Liu et al. 2016). ALS consists of a large subunit that acts as a catalytic core and a small subunit that acts as a regulatory subunit (Liu et al. 2016). In S. pombe, the former is encoded by ilv1+, and the latter is predicted to be encoded by ilv6+ as identified through sequence similarity (McDonald et al. 1974, Bekkaoui et al. 1993). ALS activity is strongly inhibited by feedback from the final product, Val, and is also less potently inhibited by Ile; however, the inhibitory activity of Leu is small (McDonald et al. 1973, 1974). It has also been reported that mercury reversibly weakens the inhibition of ALS activity by Val (McDonald et al. 1974). Full activity of this ALS requires flavin adenine dinucleotide (McDonald et al. 1974). Some biochemical pathways are thought to be derived from ancestral enzymes, which have broader substrate specificity than current enzymes, and the biosynthetic pathway from 3-methyl-2-oxobutanoate to Leu is similar to part of the biosynthetic pathway from 2-oxoglutarate to Lys (Fondi et al. 2007, Larson and Idris 2010). The final step in Leu biosynthesis, i.e. the transamination reaction, is catalyzed by BCAT Eca39, which is respectively, by acetoxyacid reductoisomerase, which is predicted to be encoded by ilv5+ (Fig. 1) (McDonald et al. 1974, Ryan and Kohlhaw 1974). Acetoxyacid reductoisomerase is also known as Mg-dependent isomeroreductase, and it has been confirmed that at least the reaction using (S)-2-aceto-2-hydroxybutanoate as a substrate requires Mg2+ in S. pombe (McDonald et al. 1974, Ryan and Kohlhaw 1974). The ortholog of this enzyme in S. cerevisiae is Ilv5, which uses NADPH as the redox cofactor for its catalytic reaction (Wess et al. 2019). Like ALS, the protein encoded by ilv5+ is localized in the mitochondria (Matsuyama et al. 2006).

(2R)-2,3-dihydroxy-3-methylbutanoate and (2R,3R)-2,3-dihydroxy-3-methylpentanoate are then converted by dihydroxy-acid dehydratase to 3-methyl-2-oxobutanoate and (S)-3-methyl-2-oxopentanoate, respectively (McDonald et al. 1974). The full activity of these reactions requires Mg2+ or Mn2+ (McDonald et al. 1974). In S. pombe, this enzyme is predicted to be encoded by SPAC17G8.06c, but this prediction has not been confirmed. Therefore, we investigated whether S. cerevisiae ILV3 encoding dihydroxy-acid dehydratase can complement this gene (Fig. 2). The deletion mutant of SPAC17G8.06c can grow in yeast extract complete medium but cannot grow in Edinburgh minimal medium (EMM) with Leu. This is because this gene is involved in the synthesis of Leu as well as Ile and Val. However, even if Leu, Ile, and Val are added, this mutant cannot grow in EMM, whereas it can surprisingly grow in synthetic dextrose medium with these BCAAs. This observation suggests that this enzyme is involved in the biosynthesis of Leu, Ile, and Val and is also essential for the growth of S. pombe in EMM. The defective growth of ∆SPAC17G8.06c mutant was complemented not only by the expression of S. pombe SPAC17G8.06c itself but also by the S. cerevisiae ILV3. This finding suggests that S. pombe SPAC17G8.06c is a functional ortholog of S. cerevisiae ILV3 and encodes a dihydroxy-acid dehydratase. On this basis, we called SPAC17G8.06c ilv3+.

Like ALS and Ilv5, Ilv3 is also localized in the mitochondria (Matsuyama et al. 2006).

At this stage, Ile and Val are produced by BCAAs aminotransferase (BCAT), encoded by eca39+, from (S)-3-methyl-2-oxopentanoate and 3-methyl-2-oxobutanoate, respectively (Fig. 1) (Eden and Benvenisty 1998). BCAT catalyzes the transamination of the amino group of Glu to Leu, Ile, and Val stereoselectively, with cofactor pyridoxal 5'-phosphate (PLP) (Buzsudnova et al. 2017). These reactions are reversible and not only act as the last steps in BCAA biosynthesis but also as the first steps in the BCAA catabolic pathways, which are important reactions in BCAA metabolism in all organisms (Eden and Benvenisty 1998, Buzsudnova et al. 2017).

As in Anheuser-Busch baker’s yeast, BCAT Eca39 has been confirmed to be present in various parts of S. pombe cells, including cytoplasm and mitochondria (Ryan and Kohlhaw 1974, Eden and Benvenisty 1998, Matsuyama et al. 2006). In S. cerevisiae, there are two BCATs, Bat1, which acts in mitochondria, and Bat2, which acts in cytosol (Wess et al. 2019). Humans also have two BCATs, BCAT1, which acts in cytosol, and BCAT2, which acts in mitochondria (Bledsoe et al. 1997).

Leu is synthesized from 3-methyl-2-oxobutanoate, which is also used in Val synthesis, through four step reactions (McDonald et al. 1974). Some biochemical pathways are thought to be derived from ancestral enzymes, which have broader substrate specificity than current enzymes, and the biosynthetic pathway from 3-methyl-2-oxobutanoate to Leu is similar to part of the biosynthetic pathway from 2-oxoglutarate to Lys (Fondi et al. 2007, Larson and Idris 2010). The final step in Leu biosynthesis, i.e. the transamination reaction, is catalyzed by BCAT Eca39, which is
Figure 1. Leucine biosynthesis pathway in fission yeast, Schizosaccharomyces pombe. The details of each process are described in the text. TPP, thiamine pyrophosphate; FAD, flavin adenine dinucleotide; PLP, pyridoxal phosphate.
Figure 2. *Schizosaccharomyces pombe* SPAC17G8.06c (*ilv3*) encodes a dihydroxy-acid dehydratase. (A) Growth of ED668 (h<sup>+</sup> ade6-M216 leu1-32 ura4-D18) and ΔSPAC17G8.06c (from Bioneer) strains in complete (YE), synthetic dextrose (SD), and Edinburgh minimal (EMM) media with supplements (Ade, 40 μg/mL adenine; Leu, 60 μg/mL leucine; Ura, 20 μg/mL uracil; Ile, 40 μg/mL isoleucine; Val, 40 μg/mL valine; 5 × Ile, 200 μg/mL isoleucine; 5 × Val, 200 μg/mL valine). (B) Growth of ΔSPAC17G8.06c cells carrying pREP41-Sp. *ilv3*<sup>+</sup> (*S. pombe* SPAC17G8.06c inserted in pREP41) or pREP41-Sc. *ILV3* (*S. cerevisiae* *ILV3* inserted in pREP41) in EMM with supplements (Ade, 40 μg/mL adenine; Ura, 20 μg/mL uracil; 5 μg/mL thiamine). To make pREP41-Sp.*ilv3*<sup>+</sup> and pREP41-Sc.*ILV3*, DNA fragments of the *ilv3*<sup>+</sup> and *ILV3* were amplified from each genome of *S. pombe* and *S. cerevisiae* using following primers, respectively: TTAGCATATGATGTTCTGCAAGCTTCTCC and CCAGGATCCTATGCGCGCTTATAAAAGCATTG for *ilv3*<sup>+</sup>, AGTACATATGGGCTTGTTAACGAAAGTTGC and ATAGGATCCTCGATTGGGGCCTATAATGC for *ILV3*. The amplified DNA fragments were digested with NdeI and BamHI and then cloned into the plasmid pREP41. The composition of SD medium is 0.67% yeast nitrogen base without amino acids (Difco) and 2% glucose. The composition of EMM is as previously described (Moreno et al. 1991).
the same enzyme as in Ile and Val syntheses, and the other three reactions are catalyzed by the enzymes encoded by leu1+, leu2+, and leu3+ (Fig 1) (Kikuchi et al. 1988, Eden and Benvenisty 1998, Larson and Idnurm 2010). A loss-of-function mutation of any of leu1+, leu2+, or leu3+ leads to Leu auxotrophy (Kohli et al. 1977). The gene leu3+ encodes 2-isopropylmalate synthase, which uses acetyl-CoA to acetate 3-methyl-2-oxobutanoate to produce (2S)-2-isopropylmalate, and is regulated by the general amino acid control (McDonald et al. 1974, Larson and Idnurm 2010, Tarumoto et al. 2013). Leu allosterically inhibits 2-isopropylmalate synthase (McDonald et al. 1974, Larson and Idnurm 2010), and this interaction can contribute to the maintenance of Leu homeostasis. Leu3 is the only known 2-isopropylmalate synthase, in S. cerevisiae, Leu4 and Leu9 are paralogs with 2-isopropylmalate synthase activity (Wess et al. 2019). Among these paralogs, Leu4 accounts for about 80% of the total synthase activity (Kohlihaw 2003).

The enzyme 3-isopropylmalate dehydratase (isopropylmalate isomerase) converts (2S)-2-isopropylmalate to (2R,3S)-3-isopropylmalate (Kohlihaw 2003). In S. pombe, this enzyme is predicted to be encoded by leu2+, which is regulated by the transcription factor Fil1 acting on the general amino acid control (Duncan et al. 2018). In S. cerevisiae, this enzyme is encoded by LEU1 and is localized in cytosol (Hammer et al. 2020).

(2R,3S)-3-isopropylmalate is converted to 4-methyl-2-oxopentanoate by 3-isopropylmalate dehydrogenase (Kohlihaw 2003). This catalytic reaction requires NAD+ and divalent cations (Mn2+ or Mg2+) (Gräczer et al. 2011). In S. pombe, 3-isopropylmalate dehydrogenase is encoded by leu1+, and S. pombe leu1+ can complement the Escherichia coli leuB mutation and S. cerevisiae leu2 mutation (Kikuchi et al. 1988). Leu1 is localized in the cytosol (Kikuchi et al. 1988, Matsuyma et al. 2006). The 4-methyl-2-oxopentanoate produced by Leu1 is reversibly converted to Leu by Eca39 (Eden and Benvenisty 1998).

The intracellular localizations of the enzymes involved in BCAA biosynthesis suggest that in S. pombe Ile and Val biosynthesis is completed in the mitochondria, but Leu biosynthesis involves reactions in both the cytosol and the mitochondria (Kikuchi et al. 1988, Kohlihaw 2003, Matsuyma et al. 2006).

Utilization of Leu auxotrophy in S. pombe

Schizosaccharomyces pombe Leu auxotrophs have been used in numerous studies. Among them, the Leu auxotroph created by the mutation of leu1+ encoding 3-isopropylmalate dehydrogenase acting on Leu biosynthesis, mentioned above, is widely used. In addition, various plasmid vectors targeting Leu auxotrophy have been developed and used in research (Table 1).

The leu1-32 mutation producing Leu auxotrophy has been widely used in research (Kiryia et al. 2017, Kurauchi et al. 2017, Fukuda et al. 2021, Jiménez-Saucedo et al. 2021). In the leu1-32 mutation, guanine 137 is changed to adenine, which changes the amino acid sequence from glycine (Gly) 46 to Glu. The Gly of the leu1-32 mutation is conserved in E. coli LeuB and S. cerevisiae Leu2 (Fig. 3A). The Gly46 is also conserved in a number of bacterial species including the hyperthermophilic bacterium Thermus thermophilus and the torula yeast Pseudobacter aeriodeniae (Candida utilis) (Wallon et al. 1997). This mutation site is relatively distant from the active center of the enzyme but is a helix-capping residue, which reportedly increases the stability of the adjacent α-helix (Fig. 3B) (Wallon et al. 1997, Aurora and Rose 1998). Therefore, the leu1-32 mutation may make it difficult to maintain the conformation required for Leu1 to have proper catalytic activity, resulting in the loss of enzymatic activity and Leu auxotrophy.

In the latter half of the twentieth century, the Leu auxotroph of the leu1-32 mutant was shown to be complemented by a plasmid carrying S. cerevisiae LEU2 encoding 3-isopropylmalate dehydrogenase (Beach and Nurse 1981). This method has since been used, and various plasmids targeting Leu auxotrophic strains have been created (Table 1). Because LEU2 complements the leu1 mutant with high copy number, the number of copies of the plasmids carrying LEU2 tends to be high (Siam et al. 2004, Kiriya et al. 2017). These plasmids, which can be used in Leu auxotrophic strains, are diverse; some are stably retained in cells, some aim to integrate the target sequence into the chromosome, some incorporate a promoter whose expression level can be adjusted, and some can be fused with epitope tags or fluorescent protein to target proteins.

Response to Leu starvation and uptake in S. pombe

Leu auxotrophic strains facilitate artificial genetic manipulation and are a powerful tool in various fields of research while simultaneously causing the Leu starvation response under some culture environments (Ohtsuka et al. 2019). Leu-starved cells activate the general amino acid control, suppress the TORC1 pathway, arrest the cell cycle at G1, reduce translational activity, and extend the chronological lifespan (Ohtsuka et al. 2019, Fukuda et al. 2021) (Fig. 4).

Activation of general amino acid control

The general amino acid control is important for cell survival in amino acid starvation conditions, and its activation stimulates the selective expression of genes involved in stress response and the biosynthesis of amino acids (Tarumoto et al. 2013). The general amino acid control is conserved from yeast to mammals and is called the amino acid response in mammals (Kilberg et al. 2012). Under amino acid starvation, uncharged tRNA binds to and activates protein kinase Gcn2 (general control nonrepressible 2), and then activated Gcn2 phosphorylates the α-subunit of eIF2 (eukaryotic initiation factor 2), resulting in the suppression of eIF2 and general repression of mRNA translation (Zaborske et al. 2009, Anda et al. 2017, González and Hall 2017). At this time, the translational activities of certain genes, including fil1+ encoding a transcription factor required for amino acid starvation response, increase; this translational regulation is mediated by a 5′-leader sequence that contains multiple upstream ORFs (Duncan et al. 2018, Jiménez-Saucedo et al. 2021). The S. pombe Fil1 is a functional homolog of S. cerevisiae Gcn4 and ATF4 in mammals (Duncan et al. 2018). Thus, amino acid starvation activates the general amino acid control, and Leu starvation also activates the general amino acid control in S. pombe (Anda et al. 2017, Ohtsuka et al. 2019, 2021).

Induction of Ecl1 family proteins

In S. pombe, amino acid starvation increases Fil1 translation and induces significant expression of ecl1+ and weaker expression of ecl2+ (Ohtsuka et al. 2019). The proteins encoded by ecl1+ and ecl2+ are two of the three Ecl1 family proteins of S. pombe, and Ecl1 family proteins are conserved in fungi (Azuma et al. 2012, Ohtsuka and Alba 2017). In S. pombe, Ecl1 is a zinc-binding protein, whose expression is induced by various stresses, including sulfur, amino acid, and magnesium starvation, and oxidative stress (Miwa et al. 2011, Shimasaki et al. 2014, 2017, 2020, Ohtsuka et al. 2021). This protein acts on several cellular responses, including autophagy, cell cycle, translation, sexual differentiation, and the regulation
Figure 3. (A) Schizosaccharomyces pombe Leu1 (Sp_Leu1), S. cerevisiae Leu2 (Sc_Leu2), and E. coli LeuB (Ec_LeuB) amino acid sequences. The Gly 46 position corresponding to the S. pombe leu1-32 mutation and the corresponding Sc_Leu2 and Ec_LeuB positions are shown in red. In the leu1-32 mutation, this Gly is changed to Glu.

(B) The three-dimensional structure of the 3-isopropylmalate dehydrogenase homodimer of E. coli (Ec_LeuB) shown from two different viewpoints (www.uniprot.org/uniprot/P30125). The mutation points of Gly corresponding to the S. pombe leu1-32 mutation are shown as red dots. This enzyme binds NADH to the active site with a divalent cation (Mn$^{2+}$ takes precedence over Mg$^{2+}$ or other divalent cations) (Wallon et al. 1997, Gráczer et al. 2011). In the catalytic cycle, this enzyme performs domain closure, which is required for interactions between Mn$^{2+}$ and the substrate (2R,3S)-3-isopropylmalate (Gráczer et al. 2011). Structural analysis using 3-isopropylmalate dehydrogenase of the bacterium Thermus thermophilus showed that Tyr139 and Lys185 are important for the catalytic function, which correspond to Tyr145 and Lys195 in E. coli LeuB and Tyr142 and Lys191 in S. pombe Leu1, respectively (Miyazaki and Oshima 1993, Palló et al. 2014). Structural analysis using T. thermophilus also showed that Mn$^{2+}$ binds Asp217 (second subunit of the dimeric enzyme), Asp241, and Asp245, which correspond to Asp227, Asp251, and Asp255 in E. coli LeuB and Asp224, Asp249, and Asp253 in S. pombe Leu1, respectively (Palló et al. 2014). Furthermore, the substrate, (2R,3S)-3-isopropylmalate, binds T. thermophilus LeuB via the lysi94, lysi104, lysi132, Tyr139, Asp217 (second subunit of the dimeric enzyme via Mn$^{2+}$), and Asp241 (via Mn$^{2+}$), which correspond to lysi99, lysi109, lysi138, Tyr145, Asp227, and Asp251 in E. coli LeuB and lysi96, lysi106, lysi135, Tyr142, Asp224, and Asp249 in S. pombe Leu1, respectively (Palló et al. 2014).
of the chronological lifespan (Ohtsuka and Aiba 2017, Shimasaki et al. 2020, Ohtsuka et al. 2021). Currently, the details of the relationships are not clear, but the cellular response evoked by the induction of Ecl1 family proteins is similar to that caused by the inhibition of TORC1 signaling.

### Inhibition of TORC1 complex

TOR is a serine/threonine kinase which is highly conserved among eukaryotes and which regulates various AGC kinases and controls intracellular responses such as growth, autophagy, cell cycle, translation, sexual differentiation, and the maintenance of telomere length (Hidayat et al. 2003, Urban et al. 2007, Jacinto and Lorberg 2008, Kupiec and Weisman 2012, Otsubo et al. 2020). Two TOR complexes have been identified, namely, TORC1 and TORC2. In S. pombe, Tor1 is a component of rapamycin-insensitive TORC2, and Tor2 is a component of TORC1 (Petersen 2009). Tor1 can also reportedly act as TORC1 (Hartmuth and Petersen 2009). TORC1 normally comprises Mip1, Pop3, Toc89, Toc1, and Tor2, whereas TORC2 consists of Bi51, Pop3, Sin1, Ste20, and Tor1 (Hayashi et al. 2007, Otsubo and Yamamoto 2008, Yanagida 2009). In mammals, Leu has been reported to regulate autophagy via acetylation of Rheb (Kariya et al. 2017). In S. pombe, TORC1 is regulated primarily by three signaling pathways, namely, the tuberous sclerosis complex (TSC) complex, GAP activity toward the Rag GTPase 1 (GATOR1), and the general amino acid control pathway (Fukuda et al. 2021). Although Leu starvation activates TORC1 via the general amino acid control pathway, some perturbations in the other two signaling pathways can change the TORC1 activity and also affect the cellular phenotypes against Leu (van Slegtenhorst et al. 2005, Ma et al. 2013). In humans, inactivation of tuberous sclerosis protein TSC1 or TSC2 causes tuberous sclerosis (Matsumoto et al. 2002). In S. pombe, the TSC complex, which negatively regulates Rheb GTPase, consists of Tsc1 and Tsc2 (Matsumoto et al. 2002, Davie et al. 2015). In S. pombe, Rheb is encoded by rbb1 and interacting with TORC1 activity (Weisman et al. 2007, Murai et al. 2009, Fukuda et al. 2021). Each deletion of tsc1 or tsc2 or rbb1 mutant decreases Leu import (Matsumoto et al. 2002, van Slegtenhorst et al. 2005, Weisman et al. 2007, Murai et al. 2009, Ma et al. 2013). GATOR1, consisting of Iml1, Npr2, and Npr3, suppresses TORC1 via Rag GTPase Gtr1 and Gtr2 (Chia et al. 2017). The deletion of npr2 also decreases Leu import (Ma et al. 2013). The general amino acid control pathway transmits the amino acid starvation signal to TORC1 (Fukuda et al. 2021). It has been reported that a decrease in the precursors of tRNA, which

### Table 1. Plasmid vectors using leucine auxotrophy in S. pombe.

| Plasmid     | Replication origin | Marker | Promoter | Chromosome integration | Tag       | References                     |
|-------------|--------------------|--------|----------|------------------------|-----------|--------------------------------|
| pALSK        | ars1               | ScLEU2 | –        | –                      | –         | (Tanaka et al. 2000)           |
| pART1        | ars1               | ScLEU2 | adh1     | –                      | –         | (McLeod et al. 1987)           |
| pcl-X        | ars1               | ScLEU2 | SV40     | –                      | –         | (Tanaka et al. 2000)           |
| pCMVL        | ars1               | ScLEU2 | CMV      | –                      | –         | (Igarashi et al. 1991)         |
| pDB248X Sc 2αm| –                   | ScLEU2 | –        | –                      | –         | (Nischt et al. 1986)           |
| pDUAL        | ars1               | leu1   | –        | –                      | –         |                                |
| (pDUAL2)     | –                  | ura4+  | nmt1+    | –                      | FLAG GFP  | (Matsuyama et al. 2004)        |
| pIL2         | –                  | ScLEU2 | –        | –                      | o         | (Nakamura et al. 2001)         |
| pIRT2        | ars1               | ScLEU2 | –        | –                      | –         | (Hindley et al. 1987)          |
| pJK13        | –                  | leu1   | –        | –                      | o         | (Keeney and Boeke 1994)        |
| pJK148       | –                  | –      | –        | –                      | –         | (Hindley et al. 1987)          |
| pLB-Dblet    | ars3002            | ScLEU2 | –        | –                      | –         | (Yamada et al. 1997)           |
| pREP1        | –                  | –      | –        | –                      | –         |                                |
| pREP3        | –                  | –      | –        | –                      | –         |                                |
| pREP41       | ars1               | ScLEU2 | nmt1+    | –                      | Myc GFP   | (Craven et al. 1998)           |
| pREP81       | –                  | –      | ura4+    | –                      | o         | (Maundrell 1993)               |
| pRGG         | ars1               | Kan    | nmt1+    | –                      | –         | (Maundrell 1993)               |
| pRIP1/s      | –                  | ScLEU2 | nmt1+    | –                      | mCherry CFP|                                |
| pRIP3/s      | –                  | –      | sup3-5   | nmt1+                  | o         | (Maundrell 1993)               |
Figure 4. Response to leucine starvation in the fission yeast *Schizosaccharomyces pombe*. The details of each process are described in the text. AAT, amino acid transporter.

also affects the general amino acid control pathway, may suppress TORC1 activity (Otsubo et al. 2018, Otsubo et al. 2020). Thus, the Leu starvation signal suppresses TORC1 activity via the general amino acid control pathway, and appropriate intracellular responses appear to be caused through the various factors regulated TORC1, such as the AGC kinases Psk1, Sck1, and Sck2 (Nakashima et al. 2012, Otsubo et al. 2017).

**Regulation of amino acid transporters**

In *S. pombe*, TORC1 and TORC2 regulate the expression of amino acid transporters (AATs), in different directions (Ma et al. 2015). That is, suppression of TORC1 increases the expression of some AATs, whereas suppression of TORC2 decreases the expression of the AATs (Weisman et al. 2007, Krzyzanowski et al. 2012, Ma et al. 2015). In *S. pombe*, these reverse effects of TORC1 and TORC2 are
also observed in other phenotypes. Inactivation of TORC1 causes cell shortening and cell cycle arrest in G1, whereas deletion mutants of tor1+ or its substrate gad8+ cause cell elongation and inability to arrest in G1 (Martin and Lopez-Aviles 2018). Deletion mutants of tsc1+ or tsc2+ increase TORC1 activity and decrease the expression of AATs, including isp5+, resulting in a decrease in the uptake of Arg and Leu (van Slegtenhorst et al. 2004, van Slegtenhorst et al. 2005, Weisman et al. 2007). The growth of Δtsc2 or Δspr2 mutants is strongly influenced by Leu auxotrophy (Ma et al. 2013), possibly due to poor Leu uptake resulting from increased TORC1 activity. However, some AATs are controlled in the opposite direction to the transporters described above. The loss of Tor1 decreases the expression of AAT genes including isp5+, per1+, put4+, and SPFB2B2.01 but increases the expression of cat1+, whereas inhibition of Tor2 increases isp5+, per1+, put4+, and SPFB2B2.01 but decreases cat1+ (Ma et al. 2015). It has been reported that the GATA transcription factor Gaf1 is involved in the regulation of expression of AAT by TORC1 (Ma et al. 2015).

The suppression of TORC1 by amino acid starvation regulates the localization of AATs, including the cationic amino acid transporter Cat1, to the cell membrane surface (Ma et al. 2013). Conversely, the upregulation of TORC1 activity by deletion of tsc2+ causes mis-localization of Cat1 (Aspuria and Tamanoi 2008, Takahashi et al. 2012). Thus, in conditions of amino acid starvation, TORC1 is considered to regulate the amino acid uptake by regulating the transcription and localization of AATs.

The Spt-Ada-Gcn5 acetyltransferase (SAGA) complex is a multi-protein complex that modifies chromatin (Soffers and Workman 2020) and is thought to control AATs and regulate Leu uptake in S. pombe (Takahashi et al. 2012). Deletion of gcn5+, which encodes a component of the SAGA complex, reduces the uptake of Leu, which depends on the amino acid permease Agp3 (Takahashi et al. 2012). gcn5+ genotypically interacts with tor2+ and too89+, encoding TORC1 subunits, and with tor1+, encoding a TORC2 subunit (Ryan et al. 2012, Laboucarié et al. 2017). nlg1+ and sg29+, encoding other subunits of the SAGA complex, also genotypically interact with too89+ (Ryan et al. 2012), and taf12+, encoding another subunit of the SAGA complex, interacts with tor1+ and tor2+ (Laboucarié et al. 2017). TORC1 and TORC2 respond to starvation and regulate the phosphorylation status of Taf12 (Laboucarié et al. 2017).

In S. pombe, high-quality nitrogen sources such as NH4+ suppress the uptake of poor nitrogen sources such as amino acids (Takahashi et al. 2012). HECT-type ubiquitin-protein ligase E3, Pub1, negatively regulates Leu uptake by preventing the localization of AATs to the cell membrane, via the ubiquitination of AATs in the presence of NH4+ (Karagiannis et al. 1999, Nakase et al. 2012, Takahashi et al. 2012). AATs on the cell membrane are transported to the vacuole by endosomal sorting complex required for transport (Nakase et al. 2012). Endocytosis of the AAT Cat1 is regulated by An1, which is an arrestin-related trafficking adaptor (Nakase et al. 2013, Nakashima et al. 2014). Pub1 interacts with and ubiquitinates An1, leading to AAT endocytosis under the regulation of TSC-Rheb pathway (Nakase et al. 2013, Nakashima et al. 2014).

In addition, the AATs involved in Leu uptake in S. pombe may also be affected by pH. Leu auxotrophic leu1-32 cells grow poorly under neutral to basic pH conditions, suggesting that the pH of the extracellular environment is important for Leu uptake (Arndt and Atkins 1996).

Regulation of the cell cycle, chronological lifespan, and sexual development

Leu starvation arrests the cell cycle at G1 in a manner dependent on the Ecl1 family proteins (Ohtsuka et al. 2019). This response may occur via TORC1 because both factors are associated with general amino acid control (Ohtsuka et al. 2019, Fukuda et al. 2021); however, the details are not known. Treatment with both rapamycin and caffeine has been shown to suppress TORC1 but does not induce G1 arrest (Takahara and Maeda 2012). Furthermore, sulfur depletion causes cell cycle arrest in an Ecl1 family protein-dependent manner, but the cell cycle arrest occurs at G2 phase (Ohtsuka et al. 2017, Ohtsuka et al. 2021a). Meanwhile, although the detailed relationship between Leu and the cell cycle is unknown, it has been reported that the deletion of G1 cyclin Pas1 decreases the uptake of Arg and Leu independently of the effect of TORC1 activation (van Slegtenhorst et al. 2005).

The chronological lifespan is assessed by the survival of cells entering the stationary phase (Takuma et al. 2013, Hibi et al. 2018, Legon and Rallis 2021). More than 100 genes have been reported to be involved in the lifespan extension, more than 30 drugs extend the lifespan, and starvation of various types of nutrients extend the lifespan in S. pombe (Matsui et al. 2021, Ohtsuka et al. 2021b, c, Romilia et al. 2021). Leu starvation extends the chronological lifespan of S. pombe Leu auxotrophic cells but not prototrophic cells, in an Ecl1 family protein-dependent manner (Ohtsuka et al. 2019). Leu starvation also results in a decrease in intracellular ribosome levels, which has been suggested to be important for regulation of the chronological lifespan (Ohtsuka et al. 2021). Leu starvation induces autophagy, which is required for maintaining the chronological lifespan during Leu starvation (Corral-Ramos et al. 2021). Meanwhile, lifespan studies have been conducted not only on Leu auxotrophic cells, but also on other amino acid-auxotrophic cells. The restriction of Lys, one of the essential amino acids for mammals, in Lys-auxotrophic cells extends the chronological lifespan, similar to the restriction of Leu, which also extends the chronological lifespan (Ohtsuka et al. 2019). Although a strain with the leu1-32 mutation that leads to Leu auxotroph tends to have a longer lifespan than a prototrophic strain (Ohtsuka et al. 2017, Hibi et al. 2018, Matsui et al. 2021), it is likely to be the same for Lys: the deletion of lys7+, which is involved in Lys biosynthesis, reportedly extends the lifespan (Rallis et al. 2014, Ohtsuka et al. 2021b).

The restriction of Arg, which is an essential amino acid for chicken or salmon, but not for humans (Oda 2007), in Arg-auxotrophic cells also extends the chronological lifespan (Ohtsuka et al. 2019). Although Leu restriction significantly affects the lifespan in an autophagy mutant with Leu auxotrophy, Arg or Lys restriction has no significant effect on the corresponding mutants with Arg or Lys auxotrophy (Corral-Ramos et al. 2021). However, in histidine (His)-auxotrophic strains, His starvation has been shown to shorten the lifespan (Ohtsuka et al. 2019).

Unlike nitrogen starvation, Leu starvation does not induce sexual development (Ohtsuka et al. 2019). It is not known whether the inability to synthesize Leu causes a deficiency in the synthesis of new proteins required for sexual development or whether Leu starvation induces intracellular signaling pathways that suppress the sexual development response.

Conclusions

Like other fungi, S. pombe can synthesize Leu, but mutations in the Leu biosynthetic pathway can cause a Leu auxotrophic phenotype, sometimes resulting in different phenotypes of the prototrophic cells. However, Leu auxotrophy has markedly increased the usefulness of this organism as a model organism, and many plasmids that can only be used in Leu auxotrophic strains have been designed. Although the study on the Leu biosynthesis pathway in S. pombe is not as advanced as that in S. cerevisiae, it is sug-
gested that the protein encoded by SPAC17G8.06c is a functional ortholog of S. cerevisiae Ilv3 (Fig. 2). Among the Leu biosynthetic pathway, a mutation of leu1+, leu1-32, is widely used, which causes Leu auxotrophy. The leu1-32 mutation is a mutation that causes an amino acid substitution at the helix-capping residue, suggesting that this mutation cannot maintain a proper structure and loses its enzymatic activity.

Leu starvation leads to the induction of autophagy, a decrease in the number of ribosomes, and extension of the chronological lifespan through the induction of the general amino acid control and suppression of TORC1. Because some intracellular responses due to amino acid starvation are also observed in higher organisms, amino acid-auxotrophic yeast may be a useful model in studies analyzing the effects of essential amino acids, including Leu, on organisms contributes to an accurate understanding of not only Leu auxotrophic cells of Schizosaccharomyces pombe. In Leu auxotrophic cells of S. pombe, the cellular response activity to Leu starvation can be easily regulated by adjusting the amount of Leu in the medium. Changes in the amount of Leu supplementation significantly alter the expression of ecl1+ that responds to Leu starvation. Moreover, Leu removal causes a variety of responses, such as increasing the activity of general amino acid control, suppressing TORC1 activity, extending the lifespan, and arresting G1 (Ohtsuka et al. 2019, Corral-Ramos et al. 2021, Fukuda et al. 2021, 2021). To study Leu starvation, the findings obtained from studies using Leu auxotrophic cells of S. pombe are expected to contribute to the understanding of not only S. pombe itself but also higher organisms that cannot synthesize Leu, because the factors involved in signal transduction pathway respond to Leu starvation, such as TORC1 signaling or general amino acid control, are conserved from yeast to mammals.

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**References**

Anda S, Zach R, Grallert B. Activation of Gcn2 in response to different stresses. Mata J (ed). PLoS One 2017;12 e0182143.

Arndt GM, Atkins D. pH sensitivity of Schizosaccharomyces pombe: effect on the cellular phenotype associated with lacZ gene expression. Curr Genet 1996;29 457–61.

Aspuria P-J, Tamano F. The Tsc/Rheb signaling pathway controls basic amino acid uptake via the Cat1 permease in fission yeast. Mol Genet Genomics 2008; 279 441–50.

Aurora R, Rose GD. Helix capping. Protein Sci 1998;7 21–38.

Avruch J, Long X, Ortiz-Vega S et al. Amino acid regulation of TOR complex 1. Am J Physiol Endocrinol Metab 2009; 296 E592–602.

Azuma K, Ohtsuka H, Murakami H et al. Extension of chronological lifespan by Scel1 depends on mitochondria in Saccharomyces cerevisiae. Biosci Biotechnol Biochem 2012; 76:1938–42.

Beach D, Nurse P. High-frequency transformation of the fission yeast Schizosaccharomyces pombe. Nature 1981; 290 140–2.

Bekkaoui F, Nadin-Davis SA, Crosby WL. Isolation and structure of an acetolactate synthase gene from Schizosaccharomyces pombe and complementation of the ilv2 mutation in Saccharomyces cerevisiae. Curr Genet 1993;24 544–7.

Bezusnovova EY, Boyko KM, Popov VO. Properties of bacterial and archaeal branched-chain amino acid aminotransferases. Biochem 2017;82 1572–91.

Bledsoe RK, Dawson PA, Hutson SM. Cloning of the rat and human mitochondrial branched chain aminotransferases (BCATm). Biochimica Et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology 1997;1339 9–13.

Chia KH, Fukuda T, Sofyantoro F et al. Regulator and GATOR1 complexes promote fission yeast growth by attenuating TOR complex 1 through Rag GTPases. Elife 2017;6 1–21.

Corral-Ramos C, Barrios R, Ayté J et al. TOR and MAP kinase pathways synergistically regulate autophagy in response to nutrient depletion in fission yeast. Autophagy 2021;1:1–16.

Davie E, Forte GMA, Petersen J. Nitrogen regulates AMPK to control TORC1 signaling. Curr Biol 2015;25 445–54.

Dilova I, Easlon E, Lin SJ. Calorie restriction and the nutrient sensing signaling pathways. Cell Mol Life Sci 2007;64 752–67.

Duncan CDS, Rodriguez-López M, Ruis P et al. General amino acid control in fission yeast is regulated by a nonconserved transcription factor, with functions analogous to Gcn4/Atf4. Proc Natl Acad Sci 2018;115 E1829–38.

Eden A, Benvenisty N. Characterization of a branched-chain amino acid aminotransferase from Schizosaccharomyces pombe. Yeast 1998;14 189–94.

Fondi M, Brilli M, Emiliani G et al. The primordial metabolism: an ancestral interconnection between leucine, arginine, and lysine biosynthesis. BMC Evol Biol 2007;7 Suppl 2 S3.

Fontana L, Patridge L. Promoting health and longevity through diet: from model organisms to humans. Cell 2015;161 105–18.

Fukuda T, Sofyantoro F, Tai YT et al. Tripartite suppression of fission yeast TORC1 signaling by the GATOR1-Sea3 complex, the TSC complex, and Gcn2 kinase. Elife 2021;10 1–22.
Gallinetti J, Harputlugil E, Mitchell JR. Amino acid sensing in dietary restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR. Biochem J 2013;449:1–10.

González A, Hall MN. Nutrient sensing and TOR signaling in yeast and mammals. EMBO J 2017;36:397–408.

Grácer É, Merli A, Singh RK et al. Atomic level description of the domain closure in a dimeric enzyme: Thermus thermophilus 3-isopropylmalate dehydrogenase. Mol Biosyst 2011;7:1646–59.

Hammer SK, Zhang Y, Avalos JL. Mitochondrial compartmentalization confers specificity to the 2-ketoacid recursive pathway: increasing isopentenol production in Saccharomyces cerevisiae. ACS Synthetic Biology 2020;9:546–55.

Hartmuth S, Petersen J. Fission yeast Tor1 functions as part of TORC1 to control mitotic entry through the stress MAPK pathway following nutrient stress. J Cell Sci 2009;122:1737–46.

Hayashi T, Hatanaka M, Nagao K et al. Rapamycin sensitivity of the Schizosaccharomyces pombe tor2 mutant and organization of two highly phosphorylated TOR complexes by specific and common subunits. Genes Cells 2007;12:1357–70.

Hibi T, Ohutsuka H, Shimasaki T et al. Identification of a novel protein kinase that affects the chronological lifespan in fission yeast. FEMS Microbiol Lett 2017;364:e02527.

Hijazat S, Tschimganine and its derivatives extend the chronological lifespan of yeast. Yeast. 1988;4:294–308.

Hodgson A, Hall MN. Nutrient sensing and TOR signaling in yeast. Genes Cells 2007;12:1357–70.

Igarashi M, Nagata A, Jinno S et al. ue1+–like gene in human cells. Natl Microbiol Lett 1990;353:80–3.

Imai Y, Shimasaki T, Enokimura C et al. Inhibition of amino acid–mTOR signaling by a leucine derivative induces G1 arrest in Jurkat cells. Biochem Biophys Res Commun 2003;301:417–23.

Hindley J, Phear G, Stein M et al. sul+ encodes a predicted 13-kilodalton protein that is essential for cell viability and is directly involved in the division cycle of Schizosaccharomyces pombe. Mol Cell Biol 1987;7:504–11.

Jiménez-Saucedo T, Berlanga JJ, Rodríguez-Gabriel M. Translational regulation of AGC kinases in yeast and mammals. Biochem J 2015;468:457–68.

Kohda TA, Tanaka K, Konomi M et al. Fission yeast autophagy induced by nitrogen starvation generates a nitrogen source that drives adaptation processes. Genes Cells 2007;12:155–70.

Kohlihaw Gb. Leucine biosynthesis in fungi: entering metabolism through the back door. Microbiol Mol Biol Rev 2003;67:1–15, table of contents.

Kohli J, Hottinger H, Munz P et al. Genomic mapping in Schizosaccharomyces pombe by mitotic and meiotic analysis and induced haploidization. Genetics 1977;87:471–89.

Kondo T, Tezuka H, Ishii J et al. Genetic engineering to enhance the Ehrlich pathway and alter carbon flux for increased isobutanol production from glucose by Saccharomyces cerevisiae. J Biotechnol 2012;159:32–7.

Krzynówek MK, Koziłowska E, Koziłowski P. Identification and functional analysis of the eth1+ gene encoding enhancer of rudimentary homolog from the fission yeast Schizosaccharomyces pombe. PLoS One 2012;7:e49059.

Kupiec M, Weisman R. TOR links starvation responses to telomere length maintenance. Cell Cycle 2012;11:2268–71.

Kurauchi T, Hashizume A, Imai Y et al. Identification of a novel protein kinase that affects the chronological lifespan in fission yeast. BMC Genomics 2017;21:135–47.

Le Couteur DG, Solon-Biet SM, Cogger VC et al. Branched chain amino acids, aging and age-related health. Ageing Res Rev 2020;64:101198.

Lego N, Rallis C. Genome-wide screens in yeast models towards understanding chronological lifespan regulation. Brief Funct Genomics 2021;1–9.

Liu Y, Li Y, Wang X. Acetoxyhydroxycyacin synthases: evolution, structure, and function. Appl Microbiol Biotechnol 2016;100:8633–49.

Ma N, Liu Q, Zhang L et al. TORC1 signaling is governed by two negative regulators in fission yeast. Genetics 2013;195:457–68.

Ma Y, Ma N, Liu Q et al. Tor signaling regulates transcription of amino acid permeases through a GATA transcription factor Gaf1 in fission yeast. PLoS One 2015;10:e0144677.

Maríño G, Pietrocola F, Eisenberg T et al. Regulation of autophagy by cytosolic acetyl-coenzyme A. Mol Cell 2014;53:710–25.

Martin R, Lopez-Aviles S. Express yourself: how PP2A-B55ab helps TORC1 talk to TORC2. Curr Genet 2018;64:43–51.

Matsui K, Okamoto K, Hasegawa T et al. Identification of ksg1 mutant and organization of two recombination subunits. Gene 1993;122:299–306.

McDonald RA, Satyanarayana T, Kaplan JG. Biosynthesis of branched-chain amino acids in Schizosaccharomyces pombe: properties of acetohydroxyacid synthetase. J Bacteriol 1973;114:332–40.

McDonald RA, Satyanarayana T, Kaplan JG. Biosynthesis of branched-chain amino acids in Schizosaccharomyces pombe:}
regulation of the enzymes involved in isoleucine, valine, and leucine synthesis. J Biol Chem 1974;52:51–9.

McLeod M, Stein M, Beach D. The product of the mei3+ gene, expressed under control of the mating-type locus, induces meiosis and sporulation in fission yeast. EMBO J 1987;6:729–36.

Møller B. The location of nitrite reductase and other enzymes related to amino acid biosynthesis in the plastids of root and leaves. Plant Physiol 1974;54:550–5.

Miwa Y, Ohtsuka H, Naito C et al. Ecl1, a regulator of the chronological lifespan of Schizosaccharomyces pombe, is induced upon nitrogen starvation. Biosci Biotechnol Biochem 2011;75:279–83.

Murai T, Nakase Y, Fukuda K et al. Distinctive responses to nitrogen starvation in the dominant active mutants of the fission yeast Schizosaccharomyces pombe. Genet Res 2009;83:517–27.

Nakamura T, Nakamura-Kubo M, Hirata A et al. The Schizosaccharomyces pombe spo5+ gene is required for assembly of the forespore membrane and genetically interacts with psy1+–encoding syntaxin-like protein. Mol Biol Cell 2001, 12:3955–72.

Nakase M, Nakase Y, Chardwiriyapreecha S et al. Intracellular trafficking and ubiquitination of the Schizosaccharomyces pombe amino acid permease Aat1p. Microbiology 2012;158:659–73.

Nakase Y, Nakase M, Kashiwazaki J et al. The fission yeast β-arrestin-like protein Any1 is involved in TSC-Rheb signaling and the regulation of amino acid transporters. J Cell Sci 2013;126:3972–81.

Nakashima A, Kamada S, Tanamoni F et al. Fission yeast arrestin-related trafficking adaptor, Arn1/Any1, is ubiquitinated by Puβ13 ligase and regulates endocytosis of Cat1 amino acid transporter. Biology Open 2014;3:542–52.

Nakashima A, Otsubo Y, Yamashita A et al. Psk1, an AGC kinase family member in fission yeast, is directly phosphorylated and controlled by TORC1 and functions as S6 kinase. J Cell Sci 2012;125:5840–9.

Nakashima A, Sato T, Tanamoni F. Fission yeast TORC1 regulates phosphorylation of ribosomal S6 proteins in response to nutrients and its activity is inhibited by rapamycin. J Cell Sci 2010;123:777–86.

Nischt R, Thirouff E, Käufer NF. Molecular cloning of a ribosomal protein gene from the fission yeast Schizosaccharomyces pombe. Curr Genet 1986;10:365–70.

Oda H. Essential amino acids and nonessential amino acids in evolution. Nippon Eiyo Shokuryo Gakkaishi 2007;60:137–49.

Ohtsuka H, Aiba H. Factors extending the chronological lifespan of yeast: Ecl1 family genes. FEMS Yeast Res 2017;17:fox066.

Ohtsuka H, Kato T, Sato T et al. Leucine depletion extends the lifespan of leucine-auxotrophic fission yeast strain by inducing Ecl1 family genes via the transcription factor Fil1. Mol Genet Genomics 2019;294:1499–509.

Ohtsuka H, Kobayashi M, Shimasaki T et al. Magnesium depletion extends fission yeast lifespan via general amino acid control activation. Microbiolopen 2021;10:e1176.

Ohtsuka H, Shimasaki T, Aiba H. Extension of chronological lifespan in Schizosaccharomyces pombe. Genes Cells 2021;26:459–73.

Ohtsuka H, Shimasaki T, Aiba H. Genes affecting the extension of chronological lifespan in Schizosaccharomyces pombe (fission yeast). Mol Microbiol 2021;115:623–42.

Ohtsuka H, Shimasaki T, Aiba H. Response to sulfur in Schizosaccharomyces pombe. FEMS Yeast Res 2021a;21:1–9.

Ohtsuka H, Takinami M, Shimasaki T et al. Sulfur restriction extends fission yeast chronological lifespan through Ecl1 family genes by downregulation of ribosome. Mol Microbiol 2017;105:84–97.

Otsubo Y, Kamada Y, Yamashita A. Novel links between TORC1 and traditional non-coding RNA, trNA. Genes 2020;11:956.

Otsubo Y, Matsuo T, Nishimura A et al. RNA production links nutrient conditions to the onset of sexual differentiation through the TORC1 pathway. EMBO Rep 2018;19:1–12.

Otsubo Y, Nakashima A, Yamamoto M et al. TORC1-dependent phosphorylation targets in fission yeast. Biomolecules 2017;7:50.

Otsubo Y, Yamamoto M. TOR signaling in fission yeast. Crit Rev Biochem Mol Bioi 2008;43:277–83.

Petersen J, Russell P. Growth and the environment of Schizosaccharomyces pombe. Cold Spring Harb Protoc 2016;2016 pdb.top079764.

Petersen J. TOR signalling regulates mitotic commitment through stress-activated MAPK and Polo kinase in response to nutrient stress. Biochem Soc Trans 2009;37:773–7.

Powers RW, Kaeberlein M, Caldwell SD et al. Extension of chronological life span in yeast by decreased TOR pathway signaling. Genes Dev 2006;20:174–84.

Rallis C, López-Maury L, Georgescu T et al. Systematic screen for mutants resistant to TORC1 inhibition in fission yeast reveals genes involved in cellular ageing and growth. Biology Open 2014;3:161–71.

Romila CA, Townsend S, Malecki M et al. Barcode sequencing and a high-throughput assay for chronological lifespan uncover ageing-associated genes in fission yeast. Microbiol Cell (Graz, Austria) 2021;8:146–60.

Ruckenstuhl C, Netzerberger C, Entfellner I et al. Lifespan extension by methionine restriction requires autophagy-dependent vacuolar acidification. PLoS Genet 2014;10:e1004347.

Ryan CJ, Roquev A, Patrick K et al. Hierarchical modularity and the evolution of genetic interactomes across species. Mol Cell 2012;46:691–704.

Ryan ED, Kohlhab GB. Subcellular localization of isoleucine-valine biosynthetic enzymes in yeast. J Bacteriol 1974;120:631–7.

Sei Kea, Maekawa H, Nakamura T et al. The asymmetric chemical structures of two mating pheromones reflect their differential roles in mating of fission yeast. J Cell Sci 2019;132:1–8.

Shimasaki T, Ohtsuka H, Naito C et al. Ecl1 is a zinc-binding protein involved in the zinc-limitation-dependent extension of chronological life span in fission yeast. Mol Genet Genomics 2017;292:475–81.

Shimasaki T, Ohtsuka H, Naito C et al. Ecl1 is activated by the transcription factor Atf1 in response to H2O2 stress in Schizosaccharomyces pombe. Mol Genet Genomics 2014;289:655–95.

Shimasaki T, Okamoto K, Ohtsuka H et al. Sulphur deprivation induces autophagy through Ecl1 family genes in fission yeast. Genes Cells 2020;25:825–30.

Siam R, Dolan WP, Forsburg SL. Choosing and using Schizosaccharomyces pombe plasmids. Methods 2004;33:189–98.

Sickmann A, Reinders J, Wagner Y et al. The proteome of Saccharomyces cerevisiae mitochondria. Proc Natl Acad Sci 2003;100:13207–12.

Soffers JHM, Workman JL. The SAGA chromatin-modifying complex: the sum of its parts is greater than the whole. Genes Dev 2020;34:1287–303.

Som SN, Park SJ, Stamatakou E et al. Leucine regulates autophagy via acetylation of the mTORC1 component raptor. Nat Commun 2020;11:1–3.

Takahara T, Maeda T. TORC1 of fission yeast is rapamycin-sensitive. Genes Cells 2012;17:698–708.

Takahashi H, Sun X, Hamamoto M et al. The SAGA histone acetyltransferase complex regulates leucine uptake through the Agp3 permease in fission yeast. J Biol Chem 2012;287:38158–67.

Takuma K, Ohtsuka H, Azuma K et al. The fission yeast pln2 mutant displays a lengthened chronological lifespan. Biosci Biotechnol Biochem 2013;77:1548–55.
Tanaka K, Yonekawa T, Kawasaki Y et al. Fission yeast Eso1p is required for establishing sister chromatid cohesion during S phase. Mol Cell Biol 2000;20:3459–69.

Tarumoto Y, Kanoh J, Ishikawa F. Receptor for activated C-kinase (RACK1) homolog Cpc2 facilitates the general amino acid control response through Gcn2 kinase in fission yeast. J Biol Chem 2013;288:19260–8.

Urban J, Soulard A, Huber A et al. Sch9 is a major target of TORC1 in Saccharomyces cerevisiae. Mol Cell 2007;26:663–74.

van Slegtenhorst M, Carr E, Stoyanova R et al. tsc1+ and tsc2+ regulate arginine uptake and metabolism in Schizosaccharomyces pombe. J Biol Chem 2004;279:12706–13.

van Slegtenhorst M, Mustafa A, Henske EP. Pas1, a G1 cyclin, regulates amino acid uptake and rescues a delay in G1 arrest in Tsc1 and Tsc2 mutants in Schizosaccharomyces pombe. Hum Mol Genet 2005;14:2851–8.

Wallon G, Kryger G, Lovett ST et al. Crystal structures of Escherichia coli and Salmonella typhimurium 3-isopropylmalate dehydrogenase and comparison with their thermophilic counterpart from Thermus thermophilus. J Mol Biol 1997;266:1016–31.

Weisman R, Roitburg I, Nahari T et al. Regulation of leucine uptake by tor1+ in Schizosaccharomyces pombe is sensitive to rapamycin. Genetics 2005;169:539–50.

Weisman R, Roitburg I, Schonbrun M et al. Opposite effects of tor1 and tor2 on nitrogen starvation responses in fission yeast. Genetics 2007;175:1153–62.

Wess J, Brinek M, Boles E. Improving isobutanol production with the yeast Saccharomyces cerevisiae by successively blocking competing metabolic pathways as well as ethanol and glycerol formation. Biotechnol Biofuels 2019;12:1–15.

Yamada H, Ohmiya R, Yamamoto E et al. Characterization of multicopy suppressor genes that complement a defect in the Wis1-Sty1 MAP kinase cascade involved in stress responses in Schizosaccharomyces pombe. J Gen Appl Microbiol 1997;43:209–15.

Yanagida M. Cellular quiescence: are controlling genes conserved? Trends Cell Biol 2009;19:705–15.

Zaborske JM, Narasimhan J, Jiang I et al. Genome-wide analysis of tRNA charging and activation of the eIF2 kinase Gcn2p. J Biol Chem 2009;284:25254–67.