FEMS Yeast Research, 21, 2021, foab041

https://doi.org/10.1093/femsyr/foab041
Advance Access Publication Date: 19 July 2021
Minireview

MINIREVIEW

Response to sulfur in *Schizosaccharomyces pombe*

Hokuto Ohtsuka*,†, Takafumi Shimasaki and Hirofumi Aiba‡

Laboratory of Molecular Microbiology, Department of Basic Medicinal Sciences, Graduate School of Pharmaceutical Sciences, Nagoya University, Chikusa-ku, Nagoya 464–8601, Japan

*Corresponding author: Laboratory of Molecular Microbiology, Department of Basic Medicinal Sciences, Graduate School of Pharmaceutical Sciences, Nagoya University, Chikusa-ku, Nagoya 464-8601, Japan. Tel: +81-52-747-6805; E-mail: hokuto_ohtsuka@ps.nagoya-u.ac.jp

One sentence summary: This mini review summarizes the latest sulfur metabolism mechanism and starvation response along with sulfate transporters identification in the fission yeast *Schizosaccharomyces. pombe*.

Editor: John Morrissey
†Hokuto Ohtsuka, http://orcid.org/0000-0001-7843-2602
‡Hirofumi Aiba, http://orcid.org/0000-0002-7775-0446

ABSTRACT

Sulfur is an essential component of various biologically important molecules, including methionine, cysteine and glutathione, and it is also involved in coping with oxidative and heavy metal stress. Studies using model organisms, including budding yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*), have contributed not only to understanding various cellular processes but also to understanding the utilization and response mechanisms of each nutrient, including sulfur. Although fission yeast can use sulfate as a sulfur source, its sulfur metabolism pathway is slightly different from that of budding yeast because it does not have a trans-sulfuration pathway. In recent years, it has been found that sulfur starvation causes various cellular responses in *S. pombe*, including sporulation, cell cycle arrest at G_2, chronological lifespan extension, autophagy induction and reduced translation. This MiniReview identifies two sulfate transporters in *S. pombe*, Sul1 (encoded by SPBC3H7.02) and Sul2 (encoded by SPAC869.05c), and summarizes the metabolic pathways of sulfur assimilation and cellular response to sulfur starvation. Understanding these responses, including metabolism and adaptation, will contribute to a better understanding of the various stress and nutrient starvation responses and chronological lifespan regulation caused by sulfur starvation.

Keywords: fission yeast; *Schizosaccharomyces pombe*; sulfate; sulfur; *sul1*$^+$; *sul2*$^+$

INTRODUCTION

Sulfur is an essential element for proteins, lipids and various metabolites, and it functions in the binding of metal ions and proteins (Zhang *et al.* 2004; Koprivova and Kopriva 2016). Various sulfur compounds, including cysteine (Cys), methionine (Met) and glutathione (GSH), are essential for cell growth, and the sulfur metabolism network regulates their synthesis (Marzluf 1997; Lee *et al.* 2010; Huang *et al.* 2017). In addition to its importance as a nutrient in cells, sulfur metabolism and responses are also studied from an applied point of view because sulfur-containing products are generally unfavorable for foods and beverages and considerably affect the flavor (Holt *et al.* 2011; Huang *et al.* 2017).

Although sulfur is present in biomolecules as reduced forms, such as thiols or sulfides, many available forms in nature are oxidized sulfates (SO_4^{2-}), which plants, fungi and many bacteria can take up, reduce and incorporate into biomolecules (Olson 2012; Koprivova and Kopriva 2016). In contrast, animals, including humans, cannot take up sulfate to synthesize Cys and Met. Therefore, they must consume foodstuffs that contain sulfur-containing amino acids and proteins (Maruyama-Nakashita *et al.* 2006; Koprivova and Kopriva 2016; Ward and

Received: 1 April 2021; **Accepted:** 13 July 2021

DeNicola 2019). Fission yeast (*Schizosaccharomyces pombe*), which is a model organism of unicellular eukaryotes, is considered to have diverged from the budding yeast *Saccharomyces cerevisiae* hundreds of million years ago (Sipiczki 2000; Hedges 2002; Hayles and Nurse 2018). Studies using this yeast have contributed significantly to the understanding of various cellular processes, including not only cell cycle, cellular morphology, sexual development, chronological lifespan (CLS; the viability of a cell population during the stationary phase) but also nutrient responses (Hayles and Nurse 2018; MacKenzie and Lacefield 2020; Otsubo, Kamada and Yamashita 2020; Ohtsuka, Shimasaki and Aiba 2021a). *Schizosaccharomyces pombe* can sufficiently assimilate and utilize sulfate, thiosulfate, Cys and GSH as sulfur sources; its ability to use sulfite and Met is weak but observable (Bánszky, Simonics and Maráz 2003). Met is not a sufficient source of sulfur because *S. pombe*, unlike *S. cerevisiae*, which can synthesize Cys from Met, does not have a transsulfuration pathway that converts Met or homocysteine to Cys (Brzywczy *et al.* 2002; Hébert, Casaregola and Beckerich 2011; Takagi and Ohtsu 2017; Fig. 1). Furthermore, *S. pombe* has little ability to assimilate the sulfur compounds methane sulfonate, taurine, isethionate, heptane sulfonate, 3-morpholinopropane-1-sulfonic acid, benzenesulfonate, dimethyl sulfone, diethyl sulfone, dibutyl sulfone, sulfolane, dimethyl sulfoxide, methyl phenyl sulfoxide, sulfamate, methyl sulfate, sodium dodecyl sulfate and 4-nitrobenzenesulfate (Linder 2012).

Although *S. pombe* is a useful model organism for understanding various cellular processes, details regarding its sulfur response have not been reviewed for more than a decade. This MiniReview summarizes the latest findings on the sulfur metabolic pathways and sulfur starvation response.

SULFATE TRANSPORT IN *S. POMBE*

Each sulfate ion (SO_4^{2-}) is transported along with three H^+ into cells in an energy-dependent manner (Mendoza-Cózatl *et al.* 2005). In *S. cerevisiae*, *SUL1*, *SUL2* and *SOA1* are genes encoding sulfate transporters (Cherest *et al.* 1997; Huang *et al.* 2017). Although there is no apparent homolog of Soa1 in *S. pombe*, the products of SPBC3H7.02 and SPAC869.05c in *S. pombe*, are considered to be homologs of Sul1p (Sc_Sul1) and Sul2p (Sc_Sul2) in *S. cerevisiae* due to the similarity of their amino acid sequences (Figure S1a, Supporting Information). However, these have not yet been identified. To contribute to the understanding of these genes, we conducted several experiments using SPBC3H7.02- and SPAC869.05c-deficient strains.

First, when grown in Edinburgh minimum medium with sulfate as the only sulfur source, growth inhibition was not obvious in either single-deletion mutant, indicating that both have the ability to take up sufficient sulfate in Edinburgh minimum medium (Figure S1b, Supporting Information). However, the double-deletion mutant did not grow in Edinburgh minimum medium, suggesting that the products of SPAC869.05c and SPBC3H7.02 are the only transporters involved in the uptake of sulfate in *S. pombe*.

Compared to SPAC869.05c, SPBC3H7.02 has a higher abundance of mRNA and is induced by sulfur starvation (Ohtsuka *et al.* 2017; Figure S1c, Supporting Information). Therefore, SPBC3H7.02 is called *sul1*$^+$, and SPAC869.05c is called *sul2*$^+$. However, the deletion of *sul1*$^+$ or *sul2*$^+$ did not cause growth defects. Thus, the amount of sulfate that decreased in the cultures of these mutants was quantified. Sulfate was slightly reduced in the cells with a single deletion of *sul1*$^+$ or *sul2*$^+$ compared to the wild-type cells (Figure S1d, Supporting Information). On the other hand, the amount of the double-deletion strain was found to be significantly reduced, suggesting that these gene products were essential for sulfur uptake although only one of them has the capacity sufficiently. Finally, we investigated whether the growth of these mutant strains could be restored by Cys or Met supplementation (Figure S1e, Supporting Information). Cys supplementation restored the growth of the double mutant in our study. On the other hand, Met supplementation restored the growth only slightly, suggesting that Met may not prove to be an effective sulfur source in *S. pombe*. Future analysis will reveal additional features of *sul1*$^+$ and *sul2*$^+$ in *S. pombe*.

SULFUR METABOLISM IN *S. POMBE*

Studies of the sulfur assimilation pathway in *S. pombe*, such as those using the growth inhibitor selenate (SeO_4^{2-}), which has the same metabolic pathway as sulfate (SO_4^{2-}), have revealed several metabolic mechanisms for synthesizing Cys, Met and GSH from sulfate (Simonics, Bánszky and Maráz 2002; Fig. 2).

Sulfate, an inorganic sulfur source, is taken up into *S. pombe* cells by the sulfate permeases Sul1 and Sul2 (Simonics, Bánszky and Maráz 2002; Fig. 2). Sulfate adenylyltransferase (ATP sulfurylase) is encoded by *sua1*$^+$ in *S. pombe*; it activates and adenylates sulfate to produce adenosine-5′-phosphosulfate (APS; Simonics and Maráz 2008). APS is phosphorylated to produce 3′-phosphoadenosine-5′-phosphosulfate (PAPS) by adenylyl-sulfate kinase (APS kinase; Koprivova and Kopriva 2016), which is encoded by *met14*$^+$ in *S. pombe* (Fujita *et al.* 2006). PAPS reductase, which is encoded by *met16*$^+$ (Fujita *et al.* 2006), acts on reduced thioredoxin and PAPS to produce free sulfite (SO_3^{2-}; Mendoza-Cózatl *et al.* 2005). It has been suggested that thioredoxin (Trx1) acts as a primary electron donor for PAPS reductase at this point (Song and Roe 2008).

Because sulfite has antimicrobial and antioxidant activities, it is used as a regulated food additive (Park and Bakalinsky 2000), while *S. pombe* can use sulfite as the sulfur source (Bánszky, Simonics and Maráz 2003). In terms of homologous genes of the sulfite efflux transporter encoded by *SSU1* in *S. cerevisiae* (Park and Bakalinsky 2000; Huang *et al.* 2017), *S. pombe* has four tandem duplication genes, SPBPB10D8.04c, SPBPB10D8.05c, SPBPB10D8.06c and SPBPB10D8.07c. These gene products may be involved in sulfite transport.

Intracellular sulfite is reduced to H_2S and sulfide (S^{2-}) by sulfite reductase using three NADPH molecules (Vande Weghe and Ow 1999; Mendoza-Cózatl *et al.* 2005). Sulfite reductase has two types of structures; α, β and γ heteromeric structure and $\alpha2\beta2$ heterotetrameric structure (Brânzanic, Ryde and Silaghi-Dumitrescu 2020). The sulfite reductase complex of *S. pombe* is expected to be the $\alpha2\beta2$ type. Sir1 is the β subunit in *S. pombe* (Miki *et al.* 2008). Additionally, the sulfite reductase from *S. cerevisiae* is also considered to be the $\alpha2\beta2$ type, which has an α subunit Met10 protein (Hansen, Cherest and Kielland-Brandt 1994). Owing to sequence similarity, it is expected that *S. pombe* also has Met10 as the α subunit ortholog.

H_2S is a toxic and biologically active gas that is reportedly involved in lifespan extension in *S. cerevisiae* and nematodes, and it has been shown to be involved in aging in mammals (Huang *et al.* 2017; Sokolov *et al.* 2021). At physiological solutions, approximately 80% of H_2S exists in dissociated form, so hydrosulfide anion (HS^-), 20% of H_2S and vanishingly little S^{2-} (Olson 2012; Sokolov *et al.* 2021). In *S. pombe*, H_2S and sulfide are thought to have three possible fates. The first is that sulfide-quinone oxidoreductase Hmt2 and FAD in mitochondria oxidize sulfide through ubiquinone reduction (Vande Weghe and

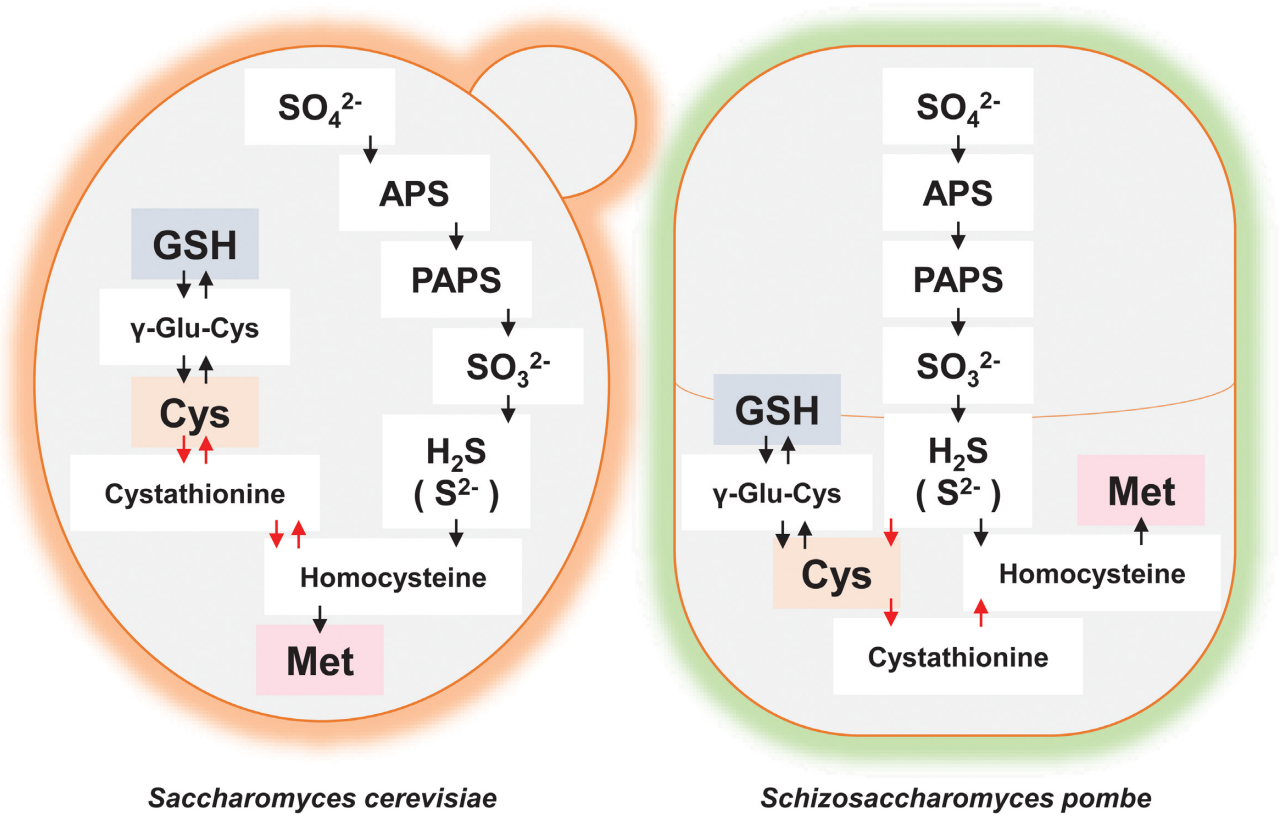

Figure 1. Sulfur metabolism in budding yeast *Saccharomyces cerevisiae* and in fission yeast *Schizosaccharomyces pombe*. Sulfate is used to synthesize cysteine (Cys), methionine (Met) and glutathione (GSH). In the reduction process, sulfate is converted to adenosine 5′-phosphosulfate (adenylyl sulfate; APS), 3′-phosphoadenosine-5′-phosphosulfate (3′-phosphoadenylyl sulfate; PAPS), sulfite (SO_3^{2-}) and hydrogen sulfide (H_2S) or sulfide (S^{2-}). Cys is used for the synthesis of GSH and Met via γ-Glutamyl-cysteine (γ-Glu-Cys).

Ow 1999, 2001). In not only the gutless clam, lugworms, ribbed mussels and chicken mitochondria but also mammalian cells, it has been reported that this mitochondrial electron transport also contributes to ATP production when the sulfide is proper level (Olson 2012; Módis *et al.* 2013; Quinzii *et al.* 2017), suggesting that Hmt2 may also contribute the energy production in *S. pombe*. Meanwhile, because sulfide is toxic to cells when it is excess, it is thought that this reaction by Hmt2 controls cellular sulfide levels (Zhang *et al.* 2008; Kawamukai 2009). Consistent with this idea, ubiquinone-deficient mutants and respiration-deficient mutants have been reported to accumulate sulfide (Miki *et al.* 2008; Kawamukai 2009). Although sulfide can be oxidized to sulfate in mammals via thiosulfate reductase, sulfur dioxygenase and sulfite oxidase in mitochondria (Quinzii *et al.* 2017), the details of the mechanism after oxidation by Hmt2 are unknown in *S. pombe*. Deficiency of *S. pombe hmt2*$^+$ has been shown to cause sulfide accumulation, hypersensitivity to heavy metals that interact with GSH, such as cadmium, and decreased phytochelatin, a peptide that chelates and sequesters heavy metals (Vande Weghe and Ow 2001; Mendoza-Cózatl *et al.* 2005).

The second possible fate is in Met synthesis. *met17*$^+$ encodes homocysteine synthase, which receives inhibitory feedback from Met and catalyzes homocysteine formation from O-acetylhomoserine and sulfides, such as H_2S (Yamagata 1984; Brzywczy *et al.* 2007). Met17 can also react with O-succinylhomoserine and L-homoserine to directly synthesize homocysteine (Yamagata 1984). Homocysteine is then converted to Met by the methionine synthase encoded by *met26*$^+$ (Fujita *et al.* 2006). The third possible fate is in Cys biosynthesis. There are two pathways for *de novo* Cys biosynthesis: the cystathionine pathway and the O-acetylserine pathway. In *S. cerevisiae* the cystathionine pathway is predominant, whereas in *S. pombe* only the O-acetylserine pathway is used (Fujita and Takegawa 2004). The cysteine synthase encoded by *cys11*$^+$ produces Cys from sulfide and O-acetylserine in *S. pombe* (Fujita and Takegawa 2004; Brzywczy *et al.* 2007). As described above, the sulfur reduced to sulfide is used to produce the sulfur-containing amino acids Cys and Met, and the sulfide level is strictly controlled by Hmt2.

Because *S. pombe* does not have a trans-sulfuration pathway, it cannot directly synthesize Cys from Met (Brzywczy *et al.* 2002; Hébert, Casaregola and Beckerich 2011), but it can still convert Cys to Met. *met3*$^+$ is a gene involved in Met auxotrophy (Kohli *et al.* 1977), which is thought to encode the cystathionine γ-synthase, which catalyzes the first step in the pathway to produce Met from Cys by converting Cys to cystathionine. Similarly, it has been suggested that SPAC23A1.14c also encodes cystathionine γ-synthase (Harrison *et al.* 2005). Cystathionine β-lyase encoded by SPCC11E10.01 converts cystathionine to homocysteine (Ejim *et al.* 2004; Holt *et al.* 2011). Although SPCC11E10.01 was reported as *str3*$^+$ in 2004, SPAC1F8.03c encoding a heme transporter was already identified using the same name in 2003 (Pelletier *et al.* 2003; Plante and Labbé 2019). Therefore, to avoid confusion, we refer to SPCC11E10.01 as *cbl1*$^+$ because it encodes cystathionine β-lyase (Fig. 2). The homocysteine produced here can be converted to Met by Met26 as described above.

GSH (γ-Glu-Cys-Gly) is present at high concentrations in the cells of many organisms. It is not only involved in the detoxification of xenobiotics and oxidative stress response but is also

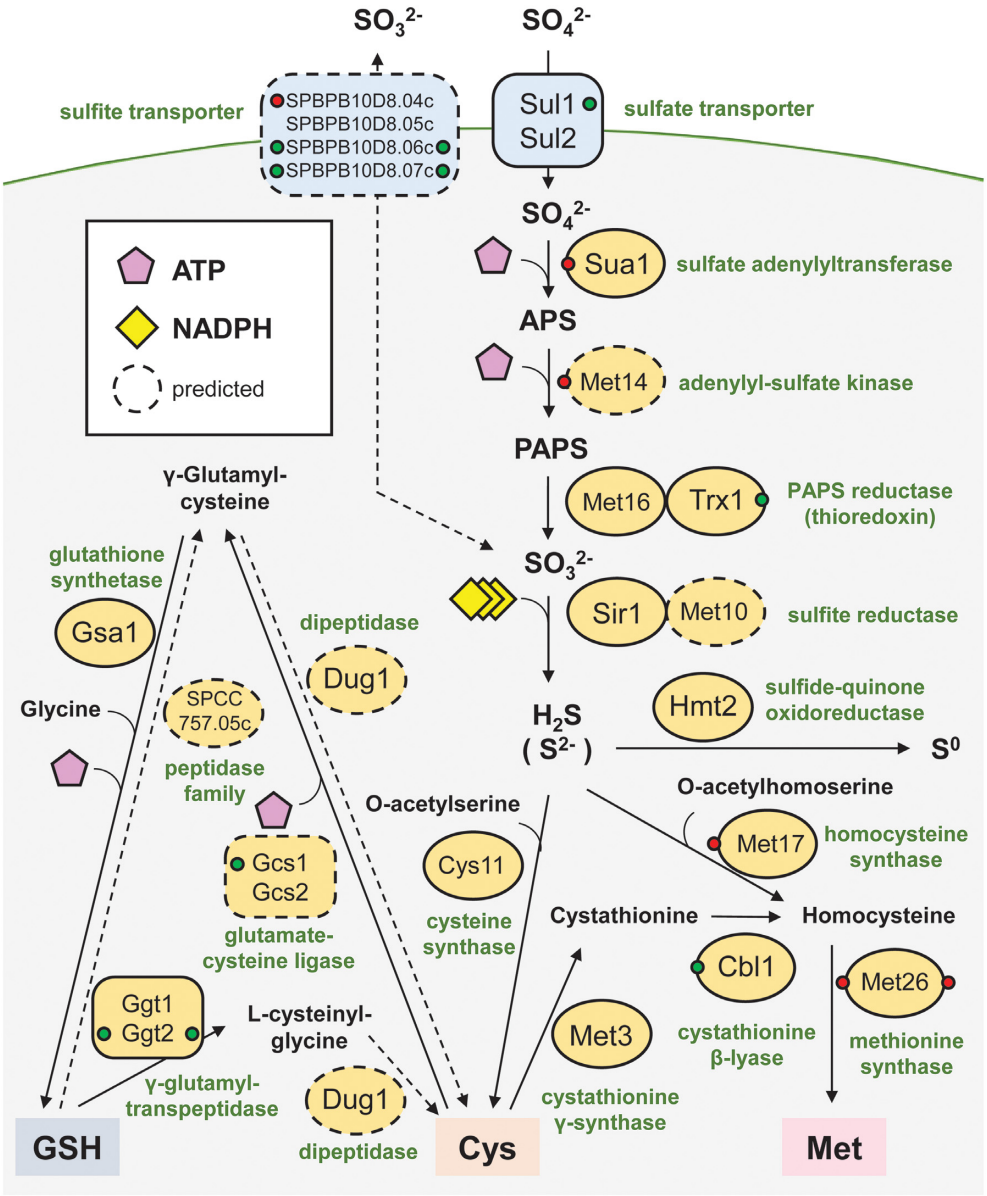

Figure 2. Sulfur metabolism in fission yeast, *Schizosaccharomyces pombe*. Sulfate taken up by sulfate transporters is used to synthesize cysteine (Cys), methionine (Met) and glutathione (GSH). In the reduction process, sulfate is converted to adenosine 5′-phosphosulfate (adenylyl sulfate; APS), 3′-phosphoadenosine-5′-phosphosulfate (3′-phosphoadenylyl sulfate; PAPS), sulfite (SO_3^{2-}) and hydrogen sulfide (H_2S) or sulfide (S^{2-}). Cys is used for the synthesis of GSH via γ-Glutamyl-cysteine (γ-Glu-Cys) and for the synthesis of Met via cystathionine and homocysteine. The SPBC3H7.02 protein is denoted as Sul1, and the SPAC869.05c protein is denoted as Sul2. The cystathionine β-lyase encoded by SPCC11E10.01 is referred to here as Cbl1. The at least doubled expression after 3 or 6 h of sulfur starvation is marked with a green circle on the left or right side of each factor, respectively (Ohtsuka *et al.* 2017). The expression that was suppressed to less than half after 3 or 6 h of sulfur starvation is marked with a red circle on the left or right side of each factor, respectively (Ohtsuka *et al.* 2017). It has been confirmed that Cys11 and Hmt2 is localized in the mitochondria (Vande Weghe and Ow 1999; Matsuyama *et al.* 2006). The broken line indicates expectations. The details of each process are described in the text.

a major reservoir of non-protein reduced sulfur (Mutoh, Nakagawa and Hayashi 1995; Mendoza-Cózatl *et al.* 2005; Lushchak 2010). GSH is synthesized from Cys in a two-step reaction (Coblenz and Wolf 1995; Huang *et al.* 2017). Conversely, it is considered that GSH can be converted to Cys in two two-step reactions. Cys is converted to the dipeptide γ-glutamylcysteine (γ-Glu-Cys) in an ATP-dependent manner by the glutamate-cysteine ligase encoded by *gcs1*$^+$ (γ-glutamylcysteine synthetase) (Coblenz and Wolf 1995; Mutoh, Nakagawa and Hayashi 1995; Chaudhuri, Ingavale and Bachhawat 1997). This reaction is the rate-limiting step in GSH biosynthesis (Chaudhuri, Ingavale and Bachhawat 1997). Gcs2 is a homolog of GCLM, human glutamate-cysteine ligase modifier subunit (Gipp, Bailey and Mulcahy 1995; Vilella *et al.* 2009). Gcs2 is expected to be a regulatory subunit of glutamate-cysteine ligase and may also be involved in regulating the catalytic reaction. Subsequently, glutathione synthetase encoded by *gsa1*$^+$ synthesizes GSH from γ-Glu-Cys and glycine (Gly) (Mutoh *et al.* 1991; Wang and Oliver 1996).

In *S. cerevisiae*, there are two pathways for the degradation of GSH to Cys. The first is that GSH is decomposed into γ-Glu-Cys via the amidotransferase Dug2-Dug3 complex and then Cys is produced by the dipeptidase Dug1; the second is that GSH is decomposed to L-cysteinyl-glycine by γ-glutamyltranspeptidase

Ecm38 and then Cys is produced by Dug1, which also cleaves γ-Glu-Cys (Mehdi, Thierie and Penninckx 2001; Kaur, Ganguli and Bachhawat 2012). Because *S. pombe* can assimilate GSH as a sulfur source (Bánszky, Simonics and Maráz 2003), it likely retains the decomposition mechanism for GSH. *S. pombe* has *dug1*$^+$ as a homolog of *DUG1* and SPCC757.05c as a homolog of *DUG2*. In addition, it has been confirmed that Ggt1 and Ggt2 have glutathione hydrolase activity as homologs of Ecm38 (Park *et al.* 2004).

In addition, the mutant with deletion of *sua1*$^+$, which encodes a sulfate adenylyltransferase functioning in the first step of sulfur assimilation, has limited growth in glycerol-based media in which cells mainly perform respiration, which has the potential to produce reactive oxygen species, rather than fermentation for energy production (Zuin *et al.* 2008; Malecki *et al.* 2016). The limited growth of Δ*sua1* cells in glycerol medium may be related to oxidative stress because sulfur assimilation is also involved in the production of GSH, which is involved in the oxidative stress response. Correspondingly, deficiencies of other factors involved in GSH synthesis from assimilated sulfur, namely *met14*$^+$, *met16*$^+$, *sir1*$^+$, *cys11*$^+$, *gcs1*$^+$ and *gsa1*$^+$, also prevent normal growth on glycerol medium (Zuin *et al.* 2008; Malecki *et al.* 2016).

Schizosaccharomyces pombe sufficiently assimilates thiosulfate, which possesses more reduced sulfur atom than sulfate and so it is energetically-favored over sulfate, in addition to sulfate, Cys and GSH (Bánszky, Simonics and Maráz 2003; Funahashi *et al.* 2015). In *S. cerevisiae*, which can also assimilate thiosulfate, a comparative analysis of metabolism has been performed when using sulfate and thiosulfate as sulfur sources (Funahashi *et al.* 2015). Recently, in *Escherichia coli*, structural analysis of the membrane protein YeeE, which engages in thiosulfate uptake, was performed (Tanaka *et al.* 2020). However, the detailed mechanism of uptake and utilization of thiosulfate in *S. pombe* remains obscure.

In this way, it is considered that *S. pombe* metabolizes sulfate as a sulfur source and synthesizes sulfur-containing amino acids and GSH, which can be used for sulfur storage and countermeasures against oxidative and heavy metal stresses, while maintaining a mechanism that limits excessive levels of the toxic intermediate products, sulfite and sulfide.

RESPONSE TO SULFATE STARVATION IN *S. POMBE*

Schizosaccharomyces pombe is considered to have various mechanisms for adjusting intracellular sulfur concentrations through the above-mentioned metabolism. Conversely, during sulfur starvation, cells perform not only these metabolic responses but also the intracellular responses necessary for environmental adaptation. Sulfur starvation leads to cell cycle arrest at G_2, translational repression, autophagy induction, sporulation and CLS extension; all of these processes depend on the Ecl1 family genes (Ohtsuka *et al.* 2017; Shimasaki *et al.* 2020; Ohtsuka, Shimasaki and Aiba 2021a; Fig. 3).

In *S. pombe*, there are three homologous Ecl1 family genes, *ecl1*$^+$, *ecl2*$^+$ and *ecl3*$^+$, which play a central role in the sulfur starvation response. Because overexpression of each extends CLS, they were named Ecl genes (extender of chronological lifespan; Ohtsuka et al. 2008, 2009; Ohtsuka and Aiba 2017). These genes are induced weakly by nitrogen starvation (*ecl1*$^+$) and strongly by nutrient starvation (amino acids, sulfur and magnesium) (*ecl1*$^+$); they are also induced by oxidative stress (*ecl1*$^+$) and heat shock

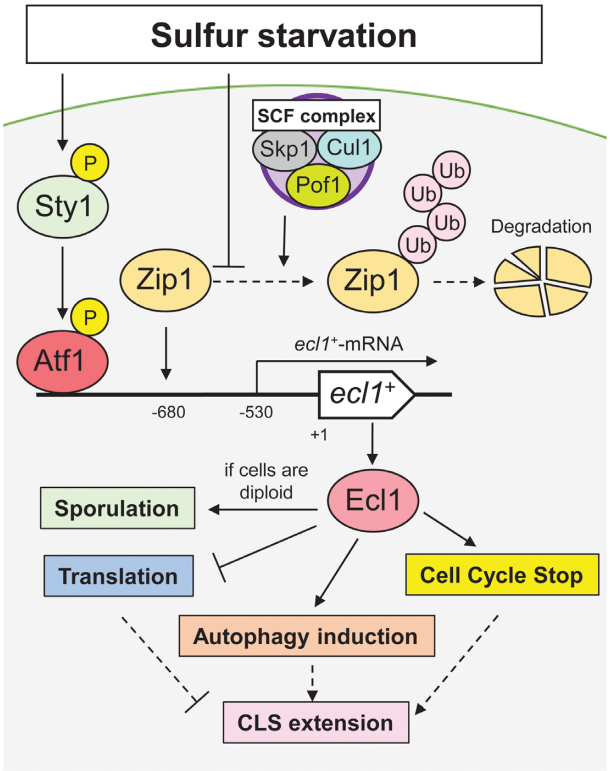

Figure 3. *Schizosaccharomyces pombe* responds to sulfur starvation, causing sporulation induction, G_2 arrest, autophagy induction, translational repression and chronological lifespan (CLS) extension.

(*ecl2*$^+$) (Miwa *et al.* 2011; Ohtsuka *et al.* 2011, 2019, 2021; Shimasaki *et al.* 2014). Therefore, they are thought to act not only in response to sulfur starvation but also as part of the cellular responses to these various environmental changes. These factors are also required for the starvation response for trace metals such as iron and zinc, and they lead sexual development and CLS extension (Ohtsuka *et al.* 2015; Shimasaki *et al.* 2017), suggesting that Ecl1 family genes act in response to various types of nutrient starvation. Ecl1 family genes are induced by multiple transcription factors, including Atf1, Fil1, Hsf1 and Zip1, which correspond to various individual stimuli (Ohtsuka et al. 2019, 2021).

Under sulfur starvation, *ecl1*$^+$ is induced by the transcription factor Zip1 (Ohtsuka *et al.* 2017). During the vegetative growth phase, Zip1 binds to the F-box protein Pof1 of the E3 ubiquitin ligase complex, Skp, Cullin and F-box containing complex (SCF complex) and is degraded through ubiquitination (Harrison *et al.* 2005). Conversely, under sulfur depletion, Zip1 stabilizes, undergoes nuclear translocation and regulates the transcription of target genes (Ohtsuka *et al.* 2017). *Schizosaccharomyces pombe* Zip1 is the ortholog of *S. cerevisiae* Met4, which negatively regulate the genes coding for sulfate adenylyltransferase (*MET3*, *S. pombe sua1*$^+$), adenylyl-sulfate kinase (*MET14*, *S. pombe met14*$^+$) and PAPS reductase (*MET16*, *S. pombe met16*$^+$; Wu *et al.* 2009). In *S. pombe*, Zip1 regulates the expression of *ecl1*$^+$ and also sulfur metabolism-related genes, such as *sua1*$^+$, *sul2*$^+$ (SPAC869.05c), *sir1*$^+$, *met10*$^+$ and SPBPB10D8.04c (Harrison *et al.* 2005; Guo *et al.* 2012). This suggests that both Zip1 and its ortholog Met4 act in the sulfur starvation response.

Sulfur starvation also induces Sty1 phosphorylation (Zuin *et al.* 2010), which is a stress-activated protein kinase and

homolog of human p38 and *S. cerevisiae* Hog1 (Gaits *et al.* 1998). Although the mechanism how the starvation activates Sty1 is unclear, Styl activation phosphorylates and activates the transcription factor Atf1, which also regulates *ecl1*$^+$ (Shimasaki *et al.* 2014). Therefore, in addition to Zip1, the Sty1 pathway might also contribute to the induction of *ecl1*$^+$ expression under sulfur starvation. Thus, in *S. pombe*, sulfur starvation results in *ecl1*$^+$ induction, while in *S. cerevisiae*, Ecl1 family genes might be involved as well. *S. cerevisiae* has one Ecl1 gene, *ECL1* (Azuma *et al.* 2012), which is also induced by sulfur starvation (Saldanha, Brauer and Botstein 2004).

Under sulfur starvation, *S. pombe* cells do not die immediately because of their adaptive response, and they can even survive for a considerable period of time. In haploid cells, sulfur starvation leads to CLS extension via Ecl1 family genes, which maintain viability (Ohtsuka *et al.* 2017). At this time, unlike in nitrogen starvation, sulfur starvation does not cause arrest at the G_1 phase, but at G_2; this does not lead to haploid conjugation (Ohtsuka *et al.* 2017). Conversely, in diploid cells, sporulation occurs in an Ecl1 family gene-dependent manner (Ohtsuka *et al.* 2017). Consistent with this, sulfur starvation induces genes related to sexual development, including *ste11*$^+$ and *mei2*$^+$, in an Ecl1 family gene-dependent manner (Ohtsuka *et al.* 2017). Through such a response, *S. pombe* is considered to survive even under sulfur starvation.

CLS is defined as the viability of a cell population in the stationary phase and is considered to be a model for non-dividing cellular lifespan in higher organisms (Takuma *et al.* 2013; Lin and Austriaco 2014; Hibi *et al.* 2018; Banerjee, Joshi and Nagotu 2020; Ohtsuka, Shimasaki and Aiba 2021b). There are several mechanisms of CLS extension by sulfur starvation. Under sulfur depletion, autophagy induction and translational repression occur in Ecl1 family gene-dependent manner (Ohtsuka *et al.* 2017; Shimasaki *et al.* 2020). In autophagy-deficient cells, CLS extension was significantly reduced under sulfur starvation, so autophagy induction is considered to be one cause of CLS extension (Shimasaki *et al.* 2020). In addition, under this condition, the levels of ribosomal factors also decrease in Ecl1 family gene-dependent manner (Ohtsuka et al. 2017, 2021). Similar to sulfur starvation, leucine starvation leads to *ecl1*$^+$ induction, decreasing ribosome and CLS extension (Ohtsuka *et al.* 2019, 2021). Using ribozinoindole-1, which inhibits ribosome biogenesis (Kawashima *et al.* 2016), artificial suppression of ribosomes in the Δ*ecl1/2/3* triple mutant, which does not have suppressed ribosomes and has short CLS under starvation, restores cell viability and extends CLS. This suggests that the suppression of ribosome levels is necessary to maintain cell survival under leucine starvation (Ohtsuka *et al.* 2021). Similarly, it is considered that appropriately reducing the level of ribosomes, which can suppress metabolism and energy consumption under nutrient starvation, also contributes to the maintenance of cell survival under sulfur starvation.

CONCLUSION

There have been several reports of fission yeast responses to sulfur, but very few have reviewed them for more than a decade. In this MiniReview, the sulfur response reported so far has been summarized. In addition to the identification of the sulfate transporters, sulfur metabolism pathway and starvation response of fission yeast have mainly been described.

Schizosaccharomyces pombe can sufficiently use sulfate as a sulfur source, and its uptake depends on two transporters, Sul1 and Sul2, which are similar to Sul1 and Sul2 in *S. cerevisiae*. If these are both deleted, growth does not occur when sulfate is the only sulfur source, so it is considered that there are only these two sulfate transporters in *S. pombe*.

Unlike *S. cerevisiae*, *S. pombe* does not have a trans-sulfuration pathway, so there is no simple conversion from Met to Cys. For this reason, Met does not appear to be a useful sulfur source for this yeast. Because sulfur is also important for GSH synthesis, the sulfur source is not only a nutrient source but also affects oxidative stress and heavy metal stress responses.

In *S. pombe*, the sulfur starvation response has similarities and differences with other types of nutrient starvation response. The similarities include induction of sporulation, decreased translation, autophagy induction and CLS extension, whereas the differences include cell cycle arrest at G_2 instead of G_1, and no mating response. Understanding the molecular mechanism of each nutrient depletion response by comparing these similarities and differences is expected to contribute to further understanding of the diversity of cellular responses and the cellular mechanism itself.

CONSENT TO PARTICIPATE

Not applicable

AVAILABILITY OF DATA AND MATERIAL

All the data have been presented in the manuscript.

ETHICS APPROVAL

Not applicable

CONSENT FOR PUBLICATION

Not applicable

AUTHOR CONTRIBUTIONS

HO has made major contributions to this study and toward writing the manuscript. TS performed experiments for characterization of sulfur transporters. TS and HA have contributed to the factual and logical confirmation, and revision of this manuscript.

ACKNOWLEDGMENTS

We are grateful to the scientists whose work provided the basis for this review and would like to thank T. Hasegawa and K. Suzuki for the technical assistance and Enago (www.enago.jp) for the English language review. FY23945 strain was provided by the National Bio-Resource Project (NBRP) (YGRC) of the MEXT, Japan.

FUNDING

This work was supported by a Grant-in-Aid for Young Scientists from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to HO) [JP19K15730] and a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to HA) [JP17H03792] and [JP20H02898].

Conflicts of interest. None declared.

REFERENCES

Azuma K, Ohtsuka H, Murakami H *et al.* Extension of chronological lifespan by ScEcL1 depends on mitochondria in *Saccharomyces cerevisiae*. *Biosci Biotechnol Biochem* 2012;**76**:1938–42.

Banerjee R, Joshi N, Nagotu S. Cell organelles and yeast longevity: an intertwined regulation. *Curr Genet* 2020;**66**:15–41.

Bánszky L, Simonics T, Maráz A. Sulphate metabolism of selenate-resistant *Schizosaccharomyces pombe* mutants. *J Gen Appl Microbiol* 2003;**49**:271–8.

Brânzanic AMV, Ryde U, Silaghi-Dumitrescu R. Importance of the iron–sulfur component and of the siroheme modification in the resting state of sulfite reductase. *J Inorg Biochem* 2020;**203**:110928.

Brzywczy J, Natorff R, Sieńko M *et al.* Multiple fungal enzymes possess cysteine synthase activity in vitro. *Res Microbiol* 2007;**158**:428–36.

Brzywczy J, Sieńko M, Kucharska A *et al.* Sulphur amino acid synthesis in *Schizosaccharomyces pombe* represents a specific variant of sulphur metabolism in fungi. *Yeast* 2002;**19**:29–35.

Chaudhuri B, Ingavale S, Bachhawat AK. *apd1*$^{+}$, a gene required for red pigment formation in *ade6* mutants of *Schizosaccharomyces pombe*, encodes an enzyme required for glutathione biosynthesis: a role for glutathione and a glutathione-conjugate pump. *Genetics* 1997;**145**:75–83.

Cherest H, Davidian JC, Thomas D *et al.* Molecular characterization of two high affinity sulfate transporters in *Saccharomyces cerevisiae*. *Genetics* 1997;**145**:627–35.

Coblenz A, Wolf K. Gcs1, a gene encoding gamma-glutamylcysteine synthetase in the fission yeast *Schizosaccharomyces pombe*. *Yeast* 1995;**11**:1171–7.

Ejim LJ, D'Costa VM, Elowe NH *et al.* Cystathionine β-lyase is important for virulence of *Salmonella enterica* serovar Typhimurium. *Infect Immun* 2004;**72**:3310–4.

Fujita Y, Takegawa K. Characterization of two genes encoding putative cysteine synthase required for cysteine biosynthesis in *Schizosaccharomyces pombe*. *Biosci Biotechnol Biochem* 2004;**68**:306–11.

Fujita Y, Ukena E, Iefuji H *et al.* Homocysteine accumulation causes a defect in purine biosynthesis: further characterization of *Schizosaccharomyces pombe* methionine auxotrophs. *Microbiology* 2006;**152**:397–404.

Funahashi E, Saiki K, Honda K *et al.* Finding of thiosulfate pathway for synthesis of organic sulfur compounds in *Saccharomyces cerevisiae* and improvement of ethanol production. *J Biosci Bioeng* 2015;**120**:666–9.

Gaits F, Degols G, Shiozaki K *et al.* Phosphorylation and association with the transcription factor Atf1 regulate localization of Spc1/Sty1 stress-activated kinase in fission yeast. *Genes Dev* 1998;**12**:1464–73.

Gipp JJ, Bailey HH, Mulcahy RT. Cloning and sequencing of the cDNA for the light subunit of human liver γ-glutamylcysteine synthetase and relative RNA levels for heavy and light subunits in human normal tissues. *Biochem Biophys Res Commun* 1995;**206**:584–9.

Guo L, Ghassemian M, Komives EA *et al.* Cadmium-induced proteome remodeling regulated by Spc1/Sty1 and Zip1 in fission yeast. *Toxicol Sci* 2012;**129**:200–12.

Hansen J, Cherest H, Kielland-Brandt MC. Two divergent *MET10* genes, one from *Saccharomyces cerevisiae* and one from *Saccharomyces carlsbergensis*, encode the alpha subunit of sulfite reductase and specify potential binding sites for FAD and NADPH. *J Bacteriol* 1994;**176**:6050–8.

Harrison C, Katayama S, Dhut S *et al.* SCF(Pof1)-ubiquitin and its target Zip1 transcription factor mediate cadmium response in fission yeast. *EMBO J* 2005;**24**:599–610.

Hayles J, Nurse P. Introduction to fission yeast as a model system. *Cold Spring Harb Protoc* 2018;**2018**. DOI: 10.1101/pdb.top079749.

Hébert A, Casaregola S, Beckerich JM. Biodiversity in sulfur metabolism in hemiascomycetous yeasts. *FEMS Yeast Res* 2011;**11**:366–78.

Hedges SB. The origin and evolution of model organisms. *Nat Rev Genet* 2002;**3**:838–49.

Hibi T, Ohtsuka H, Shimasaki T *et al.* Tschimganine and its derivatives extend the chronological life span of yeast via activation of the Sty1 pathway. *Genes Cells* 2018;**23**:620–37.

Holt S, Cordente AG, Williams SJ *et al.* Engineering *Saccharomyces cerevisiae* to release 3-mercaptohexan-1-ol during fermentation through overexpression of an *S. cerevisiae* gene, *STR3*, for improvement of wine aroma. *Appl Environ Microbiol* 2011;**77**:3626–32.

Huang C-W, Walker ME, Fedrizzi B *et al.* Hydrogen sulfide and its roles in *Saccharomyces cerevisiae* in a winemaking context. *FEMS Yeast Res* 2017;**17**:1–10.

Kaur H, Ganguli D, Bachhawat AK. Glutathione degradation by the alternative pathway (DUG pathway) in *Saccharomyces cerevisiae* is initiated by $(Dug2p\text{-}Dug3p)_2$ complex, a novel glutamine amidotransferase (GATase) enzyme acting on glutathione. *J Biol Chem* 2012;**287**:8920–31.

Kawamukai M. Biosynthesis and bioproduction of coenzyme Q_{10} by yeasts and other organisms. *Biotechnol Appl Biochem* 2009;**53**:217–26.

Kawashima SA, Chen Z, Aoi Y *et al.* Potent, reversible, and specific chemical inhibitors of eukaryotic ribosome biogenesis. *Cell* 2016;**167**:512–24.

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

Koprivova A, Kopriva S. Sulfation pathways in plants. *Chem Biol Interact* 2016;**259**:23–30.

Lee TA, Jorgensen P, Bognar AL *et al.* Dissection of combinatorial control by the Met4 transcriptional complex. *Mol Biol Cell* 2010;**21**:456–69.

Lin S-J, Austriaco N. Aging and cell death in the other yeasts, *Schizosaccharomyces pombe* and *Candida albicans*. *FEMS Yeast Res* 2014;**14**:119–35.

Linder T. Genomics of alternative sulfur utilization in ascomycetous yeasts. *Microbiology* 2012;**158**:2585–97.

Lushchak VI. Oxidative stress in yeast. *Biochemistry (Moscow)* 2010;**75**:281–96.

MacKenzie AM, Lacefield S. CDK regulation of meiosis: lessons from *S. cerevisiae* and *S. pombe*. *Genes* 2020;**11**:1–27.

Malecki M, Bitton DA, Rodríguez-López M *et al.* Functional and regulatory profiling of energy metabolism in fission yeast. *Genome Biol* 2016;**17**:1–18.

Maruyama-Nakashita A, Nakamura Y, Tohge T *et al.* *Arabidopsis* SLIM1 is a central transcriptional regulator of plant sulfur response and metabolism. *Plant Cell* 2006;**18**:3235–51.

Marzluf GA. Molecular genetics of sulfur assimilation in filamentous fungi and yeast. *Annu Rev Microbiol* 1997;**51**:73–96.

Mehdi K, Thierie J, Penninckx MJ. γ-Glutamyl transpeptidase in the yeast *Saccharomyces cerevisiae* and its role in the vacuolar transport and metabolism of glutathione. *Biochem J* 2001;**359**:631–7.

Mendoza-Cózatl D, Loza-Tavera H, Hernández-Navarro A *et al.* Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. *FEMS Microbiol Rev* 2005;**29**:653–71.

Miki R, Saiki R, Ozoe Y *et al.* Comparison of a *coq7* deletion mutant with other respiration-defective mutants in fission yeast. *FEBS J* 2008;**275**:5309–24.

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.

Módis K, Coletta C, Erdélyi K *et al.* Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. *FASEB J* 2013;**27**:601–11.

Mutoh N, Nakagawa CW, Ando S *et al.* Cloning and sequencing of the gene encoding the large subunit of glutathione synthetase of *Schizosaccharomyces pombe*. *Biochem Biophys Res Commun* 1991;**181**:430–6.

Mutoh N, Nakagawa CW, Hayashi Y. Molecular cloning and nucleotide sequencing of the γ-glutamylcysteine synthetase gene of the fission yeast *Schizosaccharomyces pombe*. *J Biochem (Tokyo)* 1995;**117**:283–8.

Ohtsuka H, Aiba H. Factors extending the chronological lifespan of yeast: ecl1 family genes. *FEMS Yeast Res* 2017;**17**:fox066.

Ohtsuka H, Azuma K, Murakami H *et al.* *hsf1*$^+$ extends chronological lifespan through Ecl1 family genes in fission yeast. *Mol Genet Genomics* 2011;**285**:67–77.

Ohtsuka H, Ishida M, Naito C *et al.* Sexual development of *Schizosaccharomyces pombe* is induced by zinc or iron limitation through Ecl1 family genes. *Mol Genet Genomics* 2015;**290**:173–85.

Ohtsuka H, Kato T, Sato T *et al.* Leucine depletion extends the lifespans of leucine-auxotrophic fission yeast 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. *Microbiologyopen* 2021;**10**:e1176.

Ohtsuka H, Mita S, Ogawa Y *et al.* A novel gene, *ecl1*$^+$, extends the chronological lifespan in fission yeast. *FEMS Yeast Res* 2008;**8**:520–30.

Ohtsuka H, Ogawa Y, Mizuno H *et al.* Identification of Ecl family genes that extend chronological lifespan in fission yeast. *Biosci Biotechnol Biochem* 2009;**73**:885–9.

Ohtsuka H, Shimasaki T, Aiba H. Extension of chronological lifespan in *Schizosaccharomyces pombe*. *Genes Cells* 2021b;**26**:459–473. DOI: 10.1111/gtc.12854.

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

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.

Olson KR. Mitochondrial adaptations to utilize hydrogen sulfide for energy and signaling. *J Compar Physiol B* 2012;**182**:881–97.

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

Park H-J, Lim H-W, Kim K *et al.* Characterization and regulation of the γ-glutamyl transpeptidase gene from the fission yeast *Schizosaccharomyces pombe*. *Can J Microbiol* 2004;**50**:61–6.

Park H, Bakalinsky AT. *SSU1* mediates sulphite efflux in *Saccharomyces cerevisiae*. *Yeast* 2000;**16**:881–8.

Pelletier B, Beaudoin J, Philpott CC *et al.* Fep1 represses expression of the fission yeast *Schizosaccharomyces pombe* siderophore-iron transport system. *Nucleic Acids Res* 2003;**31**:4332–44.

Plante S, Labbé S. Spore germination requires ferrichrome biosynthesis and the siderophore transporter Str1 in *Schizosaccharomyces pombe*. *Genetics* 2019;**211**:893–911.

Quinzii CM, Luna-Sanchez M, Ziosi M *et al.* The role of sulfide oxidation impairment in the pathogenesis of primary CoQ deficiency. *Front Physiol* 2017;**8**:1–8.

Saldanha AJ, Brauer MJ, Botstein D. Nutritional homeostasis in batch and steady-state culture of yeast. *Mol Biol Cell* 2004;**15**:4089–104.

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 H_2O_2 stress in *Schizosaccharomyces pombe*. *Mol Genet Genomics* 2014;**289**:685–93.

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

Simonics T, Bánszky L, Maráz A. Genetics of sulphate assimilation in *Schizosaccharomyces pombe*. *Acta Microbiol Immunol Hung* 2002;**49**:279–83.

Simonics T, Maráz A. Cloning of the ATP sulphurylase gene of *Schizosaccharomyces pombe* by functional complementation. *Can J Microbiol* 2008;**54**:71–4.

Sipiczki M. Where does fission yeast sit on the tree of life? *Genome Biol* 2000;**1**:REVIEWS1011.

Sokolov AS, Nekrasov PV, Shaposhnikov MV *et al.* Hydrogen sulfide in longevity and pathologies: inconsistency is malodorous. *Ageing Res Rev* 2021;**67**:101262.

Song JY, Roe JH. The role and regulation of Trxl, a cytosolic thioredoxin in *Schizosaccharomyces pombe*. *J Microbiol* 2008;**46**:408–14.

Takagi H, Ohtsu I. L-cysteine metabolism and fermentation in microorganisms. *Adv Biochem Eng Biotechnol* 2017;**159**:129–51.

Takuma K, Ohtsuka H, Azuma K *et al.* The fission yeast *php2* mutant displays a lengthened chronological lifespan. *Biosci Biotechnol Biochem* 2013;**77**:1548–55.

Tanaka Y, Yoshikaie K, Takeuchi A *et al.* Crystal structure of a YeeE/YedE family protein engaged in thiosulfate uptake. *Sci Adv* 2020;**6**:eaba7637.

Vande Weghe JG, Ow DW. A fission yeast gene for mitochondrial sulfide oxidation. *J Biol Chem* 1999;**274**:13250–7.

Vande Weghe JG, Ow DW. Accumulation of metal-binding peptides in fission yeast requires *hmt2*$^+$. *Mol Microbiol* 2001;**42**:29–36.

Vilella AJ, Severin J, Ureta-Vidal A *et al.* EnsemblCompara GeneTrees: complete, duplication-aware phylogenetic trees in vertebrates. *Genome Res* 2009;**19**:327–35.

Wang CL, Oliver DJ. Cloning of the cDNA and genomic clones for glutathione synthetase from *Arabidopsis thaliana* and complementation of a *gsh2* mutant in fission yeast. *Plant Mol Biol* 1996;**31**:1093–104.

Ward NP, DeNicola GM. Sulfur metabolism and its contribution to malignancy. *Int Rev Cell Mol Biol* 2019;**347**:39–103.

Wu CY, Roje S, Sandoval FJ *et al.* Repression of sulfate assimilation is an adaptive response of yeast to the oxidative stress of zinc deficiency. *J Biol Chem* 2009;**284**:27544–56.

Yamagata S. O-acetylhomoserine sulfhydrylase of the fission yeast *Schizosaccharomyces pombe*: partial purification, characterization, and its probable role in homocysteine biosynthesis. *J Biochem* 1984;**96**:1511–23.

Zhang M, Wakitani S, Hayashi K *et al*. High production of sulfide in coenzyme Q deficient fission yeast. *Biofactors* 2008;**32**:91–8.

Zhang Z, Shrager J, Jain M *et al*. Insights into the survival of *Chlamydomonas reinhardtii* during sulfur starvation based on microarray analysis of gene expression. *Eukar Cell* 2004;**3**:1331–48.

Zuin A, Castellano-Esteve D, Ayté J *et al*. Living on the edge: stress and activation of stress responses promote lifespan extension. *Aging* 2010;**2**:231–7.

Zuin A, Gabrielli N, Calvo IA *et al*. Mitochondrial dysfunction increases oxidative stress and decreases chronological life span in fission yeast. Wölfl S (ed.). *PLoS ONE* 2008;**3**:e2842.