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Minireview

Yca1 metacaspase: diverse functions determine how yeast live and let die

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Abstract

The Yca1 metacaspase was discovered due to its role in the regulation of apoptosis in *Saccharomyces cerevisiae*. However, the mechanisms that drive apoptosis in yeast remain poorly understood. Additionally, Yca1 and other metacaspase proteins have recently been recognized for their involvement in other cellular processes, including cellular proteostasis and cell cycle regulation. In this minireview, we outline recent findings on Yca1 that will enable the further study of metacaspase multifunctionality and novel apoptosis pathways in yeast and other nonmetazoans. In addition, we discuss advancements in high-throughput screening technologies that can be applied to answer complex questions surrounding the apoptotic and nonapoptotic functions of metacaspase proteins across a diverse range of species.

Keywords: Yca1, metacaspase, yeast, apoptosis

Introduction

Apoptosis is a regulated cell death (RCD) program (Carmona-Gutierrez et al. 2018), associated with unique morphological hallmarks that distinguish it from other types of cell death (Kerr et al. 1972). Though initially studied in animal cell populations, apoptosis is now recognized as a well-conserved cellular process that also occurs in bacteria and single-celled eukaryotes (reviewed in Bayles 2014 and Kaczanowski 2016). The study of apoptosis in unicellular species has been gaining interest over the past few years because of its pharmacological applications (reviewed in Vandana et al. 2019) and unique evolutionary implications (reviewed in Galimov et al. 2019).

Scientists have studied apoptosis in unicellular species for decades (reviewed in Ameisen 2002). Yeast has proven to be a useful model organism for the study of apoptosis (Büttner et al. 2013; reviewed in Priault et al. 2003). This is because all major apoptotic hallmarks observed in mammalian cells are conserved in the budding yeast *Saccharomyces cerevisiae* (reviewed in Carmona-Gutierrez et al. 2010) (Table 1). In addition, recent phylogenetic studies have determined that *S. cerevisiae* encodes protein homologs for all core apoptotic machinery identified in metazoans (Kaczanowski 2016, Klim et al. 2018) (Table 1). However, the mechanisms that drive apoptosis in yeast are distinct from metazoan apoptosis and poorly understood. Metazoan apoptosis is mediated by the intrinsic and extrinsic apoptosis pathways, both of which include multiple caspase family proteins (reviewed in D'Arcy 2019). By contrast, the apoptosis pathway(s) in *S. cerevisiae* remain unidentified, and apoptosis can occur both dependent and independent of its sole caspase-like protein, Yca1 (Madeo et al. 2002, Mitsui et al. 2005, Büttner et al. 2007).

Yca1 is a metacaspase protein (Madeo et al. 2002), belonging to a family of protease enzymes that share homology with the metazoan caspase proteins (Uren et al. 2000). Like caspases, metacaspases advance apoptotic cascades through the proteolytic cleavage of target substrates. Unlike caspases, however, metacaspases cleave after alternative residues (Arg and Lys, instead of Asp) (Vercammen et al. 2004) and are exclusively identified in nonmetazoans (Uren et al. 2000). Metacaspases are conserved across all domains of life (Minina et al. 2017) and effect apoptosis in a diverse range of species including bacteria, fungi, and plants (Tsiatsiani et al. 2011, Asplund-Samuelsson et al. 2012, Bayles 2014). In single-celled eukaryotes, examples of metacaspases with additional functions are continually being discovered (Bouvier et al. 2018, Shrestha et al. 2019, Fernandez et al. 2021). In this review, we discuss recent findings in *S. cerevisiae* that may enable researchers to fully characterize a pathway for Yca1-dependent apoptosis, as well as current studies on the involvement of Yca1 in additional cellular processes.

Yca1 structural overview

In *S. cerevisiae*, metacaspase activation is accomplished through the catalytic processing of full-length Yca1 (Wong et al. 2012). Yca1 is processed in at least two steps: first, Yca1 is cleaved at the carboxyl ends of Lys331 and Lys334, yielding a large and a small subunit, then the large subunit is further cleaved at the carboxyl ends of Arg72 and Lys86, yielding two smaller ∼36-kDa fragments (Wong et al. 2012) (Fig. 1). Only the fragment containing the catalytic residue Cys276 is required for Yca1 proteolytic activity (Wong et al. 2012).

The *YCA1* locus also encodes a 22-codon N-terminal pro-domain with two alternative start sites, but it is unknown whether the pro-domain is translated and later cleaved (thus presenting an additional processing step) or subject to upstream transcrip-

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Table 1. Conservation of apoptotic hallmarks between humans and budding yeast. All proteins listed are homologous. Regulatory pathways are diverged between metazoans and protozoans. All apoptotic phenotypes observed in humans are present in budding yeast.

	Apoptotic hallmark	*H. sapiens*	*S. cerevisiae*
Core apoptotic machinery	Apoptotic induction factor, mitochondrial	AIFM2	Ndi1, Aif1
	Apoptotic DNase, mitochondrial	ENDOG	Nuc1
	Caspase/metacaspase, cytoplasmic	CASP1-CASP12	Yca1
	Apoptotic protease, cytoplasmic	HTRA/OMI	Nma111
Regulatory pathways	**Evolutionarily diverged between metazoans and yeast*	External, internal	*No canonical pathways*
Phenotypes	Loss of mitochondrial membrane potential	ü	ü
	Increased cytochrome c release	ü	ü
	Increased ROS abundance	ü	ü
	PS externalization	ü	ü
	Chromatin condensation	ü	ü
	Genomic DNA fragmentation	ü	ü

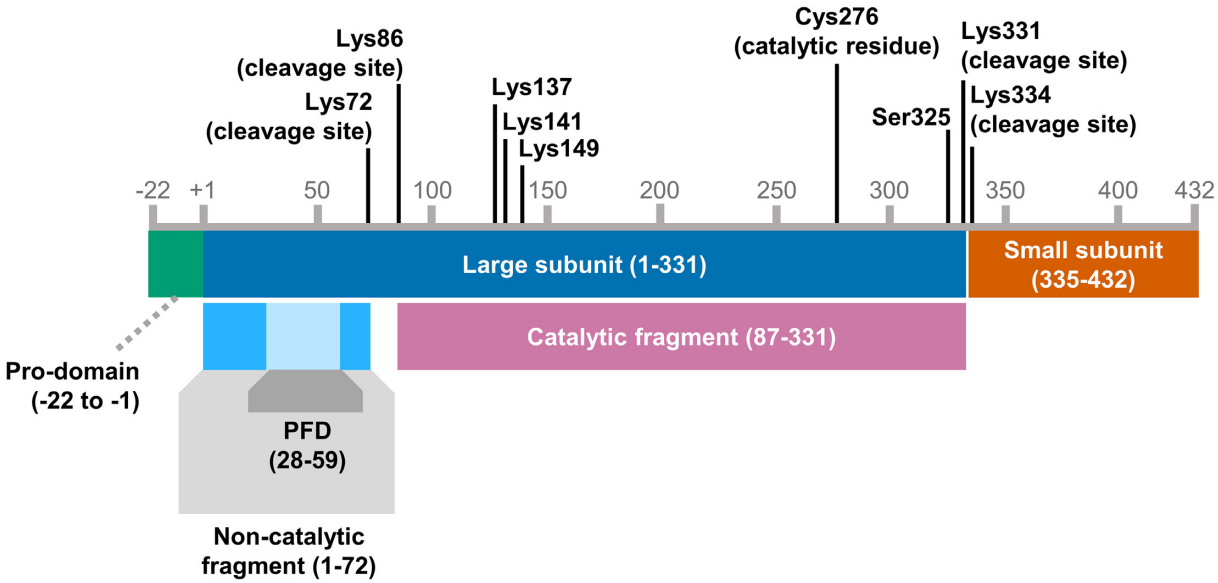

Figure 1. Annotated Yca1 protein sequence. (Above) Yca1 residues that undergo post-translational modification. (Below) Yca1 protein structural units. Prion-forming domain abbreviated to PFD.

tional/translational regulation (Erhardt et al. 2010). Longer Yca1 isoforms containing complete or partial pro-domain sequence (Yca1 $_{453}$ and Yca1 $_{451}$) are capable of forming insoluble aggregates, but Yca1 lacking the pro-domain (Yca1 $_{432}$) is the predominant form expressed under normal physiological conditions (Erhardt et al. 2010).

The mechanism underlying the first step of Yca1 processing is also unknown: while cleavage of the large subunit into two product fragments depends on a calcium-dependent autoproteolytic mechanism, cleavage of full-length Yca1 into the large and small subunits is calcium-independent and thought to be driven by another, as yet unidentified protease (Wong et al. 2012).

The mechanism by which calcium activates Yca1 proteolytic activity is also unknown. In *Trypanosoma brucei*, the TbMCA2 metacaspase undergoes reversible structural changes upon calcium binding (Machado et al. 2013), suggesting a similar mechanism may be present in *S. cerevisiae*. With a recently developed method for performing cross-linking mass spectrometry on refolded metacaspases (De Lima et al. 2022), it is now possible to observe whether Yca1 and other metacaspases undergo conformational changes driven by binding interactions with calcium and other cell signaling molecules.

It is worth noting that the catalytic and noncatalytic product fragments of the Yca1 large subunit have the same apparent observed size (~36 kDa) despite being significantly different in amino acid sequence length (72 AAs vs. 246 AAs, respectively) and predicted molecular weight (8 kDa vs. 27 kDa, respectively) (Wong et al. 2012). While the discrepancy between the observed and expected molecular weights of the C-terminal large subunit fragment can be explained by recently identified post-translational modifications (ubiquitination on the lysine residues K137, K141, K149, K331, K334, and K363, as well as phosphorylation on the S325 residue) (Shrestha et al. 2019), it is unclear why the N-terminal large subunit fragment appears significantly larger than predicted.

While the Yca1 structural features and processing steps are known, much remains to be discovered about Yca1-dependent apoptosis, such as how Yca1 activation is regulated, which Yca1

substrate(s) is processed to effect apoptosis, and how the use of Yca1-dependent apoptosis to effect RCD is determined in specific contexts.

Yca1 and apoptotic RCD

In *S. cerevisiae*, apoptosis can occur in a completely Yca1-dependent or -independent manner, depending on the apoptosis trigger to which cells are exposed (Xu et al. 2010, Scariot et al. 2016). Thus, it will be important to establish methods for determining the degree of Yca1 involvement in future studies of yeast apoptosis. In addition, the identification of apoptosis triggers that activate single or multiple apoptotic responses may be leveraged to identify the currently uncharacterized apoptosis pathway(s) in yeast.

Methods for assessing Yca1-dependent apoptosis

A set of guidelines for characterizing apoptotic phenotypes in yeast suggested that a study on apoptosis in yeast should employ (1) at least two apoptosis-specific methods (one of which must be Annexin V staining) and (2) at least one viability assay to adequately evaluate any potential apoptotic response (Table 2; Carmona-Gutierrez et al. 2018).

To study the involvement of Yca1 in a specific apoptotic response, these guidelines should be followed in both wild type and Yca1-impaired backgrounds (e.g. *yca1*Δ or wild type treated with Z-VAD-FMK caspase inhibitor). By observing whether the apoptotic phenotypes measured are partially or completely abrogated, one can determine the degree of Yca1 involvement in the apoptotic response.

Completely Yca1-dependent apoptosis

The following apoptotic triggers induce completely Yca1-dependent apoptosis (i.e. apoptosis-specific phenotypes and inviability were eliminated in *yca1*Δ): valproic acid, cadmium, H_2O_2 (in a *bre1*Δ genetic background), miltefosine (MI), and Mancozeb (MZ) (Table 2). Citral and geraniol also appear to induce Yca1-dependent apoptosis, but have yet to be sufficiently characterized (Carmona-Gutierrez et al. 2018, Scariot et al. 2021).

It should be noted that the phenotypes exhibited by cells undergoing completely Yca1-mediated apoptosis do not look identical and vary between studies. For example, ROS production is a feature of MI-induced apoptosis (Biswas et al. 2014), but does not appear to function as a major effector of apoptosis in the same way it does in the completely Yca1-dependent apoptosis triggered by Mancozeb, valproic acid, and cadmium (Mitsui et al. 2005, Nargund et al. 2008, Scariot et al. 2016). Whether this difference is the result of different apoptotic triggers eliciting distinct subsets of apoptotic phenotypes in specific contexts or because of the use of different strain backgrounds is unclear. While apoptotic triggers and phenotypes are well-characterized, this issue highlights the limitations of studying yeast apoptosis while the upstream signaling pathways and downstream cascade components remain unidentified.

Partially Yca1-dependent and Yca1-independent apoptosis

In *S. cerevisiae*, many apoptotic triggers appear to induce partially Yca1-dependent or Yca1-independent apoptosis, in which the deletion of *YCA1* results in partially reduced or unaffected apoptotic phenotypes (Fig. 2). Proteins and other cellular factors that have been observed regulating apoptosis alongside Yca1

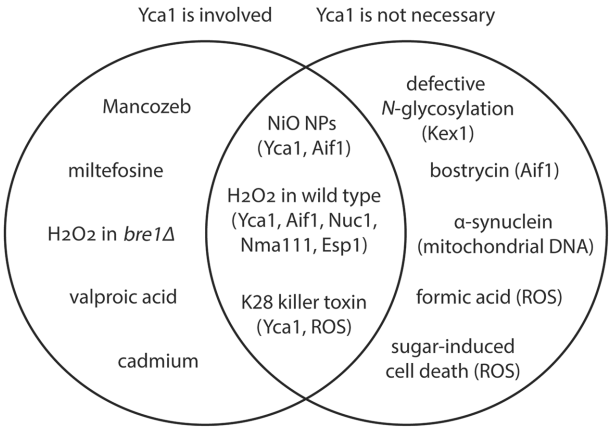

Figure 2. Apoptotic triggers that induce apoptosis with varying degrees of Yca1 involvement. (Left) Apoptotic triggers that induce apoptosis for which the Yca1 pathway is necessary. (Middle) Apoptotic triggers that induce apoptosis for which deletion of *YCA1* results in partial loss of apoptotic phenotype. All apoptotic factors involved are listed in parentheticals. (Right) Apoptotic triggers that induce apoptosis for which the Yca1 pathway is not necessary. Necessary apoptotic factors are listed in parentheticals.

include: Aif1 (Wang et al. 2014, Sousa et al. 2019), Nuc1 (Wang et al. 2014), Nma111 (Wang et al. 2014), Yno1 (Rinnerthaler et al. 2012), Esp1 (Yang et al. 2008), an unidentified alternative protease (Ivanovska and Hardwick 2005), ROS (Reiter et al. 2005), cytochrome c (de Castro et al. 2011), and mitochondrial DNA (Du et al. 2007). Proteins and other cellular factors that have been reported to participate in Yca1-independent apoptosis include: Aif1 (Xu et al. 2010), Nuc1 (Büttner et al. 2007), Kex1 (Hauptmann and Lehle 2008), Ybh3 (Büttner et al. 2011), Rho5 (Singh et al. 2019), ROS (Hoeberichts et al. 2010), and mitochondrial DNA (Büttner et al. 2008). These observations suggest that multiple pathways regulate apoptosis in yeast.

Recent phylogenetic analysis suggests that the following five proteins constitute an evolutionarily conserved 'core apoptotic machinery': Yca1, Aif1, Nuc1, Ndi1, and Nma111 (Klim et al. 2018). This is consistent with a previously proposed model from the same group, in which the five 'core' proteins participate in a single apoptotic cascade (Kaczanowski 2016). However, experimental evidence demonstrates that Yca1 and Aif1 are able to induce apoptosis completely independently from one another, which suggests that apoptotic regulation may be more complex than initially assumed (Xu et al. 2010, Scariot et al. 2016). This highlights the need to understand how the use of specific apoptosis pathways (and more broadly, apoptosis instead of alternative cell death programs such as necrosis and autophagy) to effect RCD is determined.

Yca1 and age-driven apoptotic programmed cell death (PCD)

Yeast are a widely used model organism for the study of aging (reviewed in Denoth Lippuner et al. 2014). *Saccharomyces cerevisiae* exhibits two distinct forms of aging: chronological aging and replicative aging (Mortimer and Johnston 1959, Longo 1997; see Longo and Fabrizio 2012 for review). The chronological life span (CLS) of a yeast cell is the amount of time a nondividing cell can survive, while the replicative life span (RLS) is defined as the number of replicative events a mother cell can perform before undergoing PCD. Age-driven PCD via apoptosis in *S. cerevisiae* has been observed to occur as a function of both CLS and RLS

Table 2. Assays for evaluating apoptotic phenotypes. ∗Assays not described in '*Guidelines and recommendations on yeast cell death nomenclature*', but used in previous apoptotic studies.

Assay	Phenotype assessed	Example
Annexin V/PI costaining	Annexin V: PS externalization PI: necrosis	Büttner et al. (2007)
Annexin V/7AAD costaining∗	Annexin V: PS externalization 7AAD: necrosis (indirect)	Scariot et al. (2016)
FLICA staining∗	Metacaspase activation	Biswas et al. (2014)
D2R staining∗	Metacaspase activation	Acosta-Zaldívar et al. (2016)
DAPI staining	Chromatin condensation	Madeo et al. (1997, 1999)
TUNEL test	Genomic DNA fragmentation	Madeo et al. (1997, 1999)
Single cell gel electrophoresis (or 'comet' assay)	Genomic DNA fragmentation	Carmona-Gutierrez et al. (2013)
DHE staining	ROS production	Acosta-Zaldívar et al. (2016)
NAC rescue∗	ROS production	Acosta-Zaldívar et al. (2016)
Cytochrome c immunodetection	Cytochrome c release	Acosta-Zaldívar et al. (2016)
*cyc3*Δ viability rescue∗	Cytochrome c release	Acosta-Zaldívar et al. (2016)
RH123 staining	Mitochondrial membrane potential	Nargund et al. (2008)
CFU counting	Viability (via clonogenicity)	Biswas et al. (2014)
Yeast pinning	Viability (via clonogenicity)	Scariot et al. (2016)

(Lin and Austriaco 2014) and there is evidence suggesting that Yca1 plays a role in mediating CLS-driven apoptosis (Laun et al. 2001).

Yca1 was first implicated in CLS-driven apoptosis when it was observed that Yca1 overexpression strains undergo apoptosis after 44 hours in liquid culture without the introduction of any external triggers (Madeo et al. 2002). This was later supported by the finding that wild type strains also undergo CLS-driven apoptosis, and that the percentage of surviving cells in prolonged cultures (1–35 days) is significantly increased in *yca1*Δ populations compared to wild type (Herker et al. 2004). Further support was found with the observation that Yca1 processing (via 12-kDa fragment production) is increased in chronologically aged cultures, suggesting that Yca1 plays a role in mediating CLS-driven apoptosis (Hill et al. 2014).

While apoptosis has been observed to occur more frequently in replicatively aged cells (Laun et al. 2001), the degree to which Yca1 participates in RLS-driven apoptosis is currently unknown.

Role of Yca1 in nonapoptotic cellular processes

Yca1 has also been shown to have roles in several nonapoptotic processes.

Proteostasis

Protein aggregation is associated with cellular aging and apoptosis in both humans (reviewed in Trigo et al. 2019; reviewed in Reiss et al. 2018) and yeast (Sanada et al. 2011, Saarikangas and Barral 2015). Yca1 participates in two distinct proteostasis mechanisms. A role for Yca1 in proteostasis was first suggested by the observation that deletion of Yca1 leads to significant protein abundance changes in both the 19S and 20S subunits of the 26S proteasome, as well as total ubiquitinated protein (Khan 2005). It was subsequently discovered that deletion of Yca1 (*yca1*Δ) and expression of catalytically inactivated Yca1 (Yca1 ^{C276A}) both result in increased but distinct levels of protein aggregation (Lee et al. 2010), suggesting that Yca1 simultaneously mediates aggregate clearance through both catalytic and noncatalytic mechanisms. Later work presented two models for Yca1 proteostasis pathways: catalytically driven Yca1 regulation of the ubiquitin cycle and noncatalytic Yca1 coordination of HSP family proteins (Lee et al. 2010, Shrestha et al. 2019).

Yca1 regulates the ubiquitin cycle through the cleavage of the ubiquitin precursor Rsp31 (Shrestha et al. 2019). Rsp31 cleavage is dependent on Yca1 catalytic residue C276 and other Yca1 post-translational modifications. Yca1 is phosphorylated on the S325 residue, which enables the ubiquitination of multiple Yca1 lysine residues by the phosphoserine-targeting E3 ligase Rsp5 (Fig. 1). It is thought that Yca1 phosphorylation and ubiquitination are required for Rsp31 cleavage, because loss of these PTMs results in increased aggregate abundance, but this has not been directly observed. Nevertheless, Shrestha et al. (2019) propose a model in which upon stress, an candidate kinase phosphorylates Yca1, targeting Yca1 for ubiquitination and binding to Rsp5, which drives Rsp31 cleavage and resultant production of free ubiquitin for use by the ubiquitin–proteasome system (UPS).

In addition to participation in the ubiquitin cycle, Yca1 is thought to regulate proteostasis by recruiting HSP family members to cytosolic protein aggregates (Lee et al. 2010). In eukaryotes, misfolded proteins are sequestered into tightly regulated, cytoprotective inclusion bodies, such as juxtanuclear quality control (JUNQ) and insoluble protein deposit (IPOD) inclusions (Kaganovich et al. 2008). Yca1 localizes to JUNQ and IPOD inclusions during heat stress and aging, and cells lacking Yca1 display an increased number of inclusions (Hill et al. 2014). Localization to JUNQ inclusions is not mediated by the Yca1 catalytic core (Hill et al. 2014) or Yca1 ubiquitination (Shrestha et al. 2019), but instead appears to be mediated by the Yca1 prion-forming domain (PFD) (Erhardt et al. 2010, Lee et al. 2010). At the same time, Yca1 has been shown through both mass spectrometry and protein copurification to interact with HSP family members including Hsp40, Hsp70, and Hsp104, which form a protein aggregate-remodelling complex. As such, Lee et al. (2010) propose a model in which Yca1 participates in proteostasis by targeting the Hsp40/Hsp70/Hsp104 complex to cytosolic protein aggregates such as JUNQ and IPOD inclusions.

Distinguishing between the catalytic and noncatalytic Yca1 proteostasis mechanisms will allow us to revisit and reinterpret previous studies. One example is the negative interaction between

Yca1 deletion and Ydj1 deletion, which results in decreased cell viability and irregular cell and aggregate morphologies (Hill et al. 2014). At the time this interaction was published, Ydj1 was only known as a homolog for human Hsp40. However, Ydj1 was also identified as an adaptor for Rsp5 later that same year (Fang et al. 2014). Thus, it is unclear whether this interaction results from the role of Yca1 in the ubiquitin cycle, HSP family recruitment to cytosolic aggregates, or both. This distinction could also be applied to the Yca1-mediated asymmetrical clearance of aggregates from daughter cells during cell division (Hill et al. 2014), for which the underlying mechanism(s) is currently unknown.

There is a growing interest in the crosstalk between apoptosis and proteostasis, as a number of recent studies have identified multifunctional proteins that mediate the two cellular functions in evolutionarily distant species. Alongside Yca1, the mitochondrial NADH dehydrogenase Nde1 exhibits apoptosis-inducing activity in *S. cerevisiae*. This apoptotic activity is regulated by the UPS as well as a mitochondrial protein quality control (PQC) i-AAA protease complex, demonstrating additional links between apoptosis and proteostasis in budding yeast (Saladi et al. 2020). In the distantly related fungal species *Magnaporthe oryzae* and *Ustilago maydis* (Morel et al. 2015), metacaspase family proteins demonstrate both apoptosis-mediating activity and a role in proteostasis, as evidenced by the increased abundance of protein aggregates in metacaspase knockout strains (Mukherjee et al. 2017, Fernandez et al. 2021). This relationship is even observed in the holoarchaeon *Haloferax volcanii*, in which caspase activity is a core component of the unfolded protein response i-AAA complex (Seth-Pasricha et al. 2019), suggesting that cross-talk between apoptosis and proteostasis is conserved between *Archaea* and *Eukarya*. Together, these studies provide early evidence for a conserved relationship between the evolution of apoptosis and proteostasis that has yet to be fully uncovered.

Cell cycle regulation

Yca1 also plays a role in cell cycle regulation, though the mechanism is unknown. Both Yca1 deletion and catalytic inactivation result in a defective G2/M checkpoint and a prolonged G1/S transition (Lee et al. 2010, Bouvier et al. 2018). These cell cycle phenotypes are unaccompanied by changes in ROS production or 20S proteasome activity (Lee et al. 2010), suggesting that Yca1 cell cycle regulation is distinct from the function of Yca1 in apoptosis and proteostasis.

Ddi1 is a ubiquitin-dependent aspartyl protease (Yip et al. 2020, Krylov and Koonin 2001), involved in cell cycle progression through the Pds1 mitotic checkpoint (Diaz-Martinez et al. 2006). It is speculated that Ddi1-dependent cell cycle control is accomplished through its protease activity (Krylov and Koonin 2001). Recently, Ddi1 was identified as a proteolytic substrate itself, being cleaved by Yca1 both *in vivo* and *in vitro* (Bouvier et al. 2018). Thus, it seems likely that Yca1 affects cell cycle regulation through its proteolytic interaction with Ddi1, though this has yet to be demonstrated.

The role of metacaspases in cell cycle regulation is conserved in other species. In the protozoan parasites *Trypanosoma cruzi* and *Leishmania donovani*, metacaspases are essential for cell cycle progression (Lee et al. 2007a, Laverriere et al. 2012). For this reason, alongside the absence of metacaspases in mammalian hosts, metacaspases have become a promising pharmacological target for recent drug discovery studies in various pathogenic organisms (as discussed in Eyssen and Coetzer 2020).

Other cellular processes

Differential proteomic studies comparing the proteomes of wild type and *yca1*Δ cells have been performed in both physiological growth conditions (Lee et al. 2010, Ždralević et al. 2015) and apoptosis-inducing conditions (Longo et al. 2015). These studies found that deletion of YCA1 significantly alters the expression of proteins involved in carbon, amino acid, and nucleotide metabolism, protein biosynthesis and transport/folding, and stress response. Functional studies validating Yca1 involvement in the described cellular processes have yet to be performed.

Genetic screens and transcriptomic profiling of cells undergoing apoptosis have also been carried out, but the conclusions drawn from these experiments are limited compared to those from proteomic studies. To identify candidate genes involved in apoptosis, both available genetic screens identify strains from the yeast deletion library that exhibit increased viability in apoptosis-inducing conditions (Teng et al. 2011, Sousa et al. 2013). However, the genes identified in these experiments are not specific to the Yca1 pathway, as the apoptotic triggers used, heat stress and acetic acid, do not exclusively induce Yca1-dependent apoptosis (Lee et al. 2007b, Longo et al. 2015). Transcriptomic profiling experiments in cells undergoing apoptosis are unable to control for the chromatin condensation that occurs during apoptosis (Dong et al. 2017), which makes it difficult to distinguish whether a given differentially expressed gene is a cause or effect of apoptosis.

Conclusions

The Yca1 metacaspase is a multifunctional protein with many roles in *S. cerevisiae* (Fig. 3). While Yca1 activity has been identified as contributing to apoptosis, proteostasis, and cell cycle regulation, broad questions remain about how each is achieved. Regarding the Yca1-dependent apoptosis, which apoptosis triggers induce pathway activation, which upstream signals are induced by these triggers, and which substrate(s) and downstream cascade components are required for effecting Yca1-dependent apoptosis are unknown. Regarding the Yca1 protein itself, how calcium and other cell signaling molecules activate Yca1 metacaspase activity and whether this activity can be inhibited by other cellular factors have yet to be determined. More broadly, how the use of Yca1-dependent apoptosis to effect RCD is determined in specific contexts is also poorly understood. In addition, much work remains to be done on the following Yca1 functions, including: distinguishing the catalytic and noncatalytic Yca1 proteostasis mechanisms, identifying how exactly Yca1 regulates cell cycle progression, and determining whether Yca1 is directly or indirectly involved in other cellular processes, including metabolism, protein production, and stress response.

Recent advancements in high-throughput screening technologies hold the promise to answer the complex questions surrounding Yca1. Cross-linking mass spectrometry on refolded metacaspases will allow us to identify novel binding interactions between cell signaling molecules and metacaspases from various species (De Lima et al. 2022). The recent development of a genome-wide CRISPRi library in *S. cerevisiae* will enable us to perform powerful screens across experimental conditions such as different apoptosis triggers to identify novel genetic interactions pathways (Momen-Roknabadi et al. 2020). Image-enabled cell sorting can now be used to measure changes in Yca1 localization and interactions relevant to each of its multiple functions at the single-cell level (Schraivogel et al. 2022).

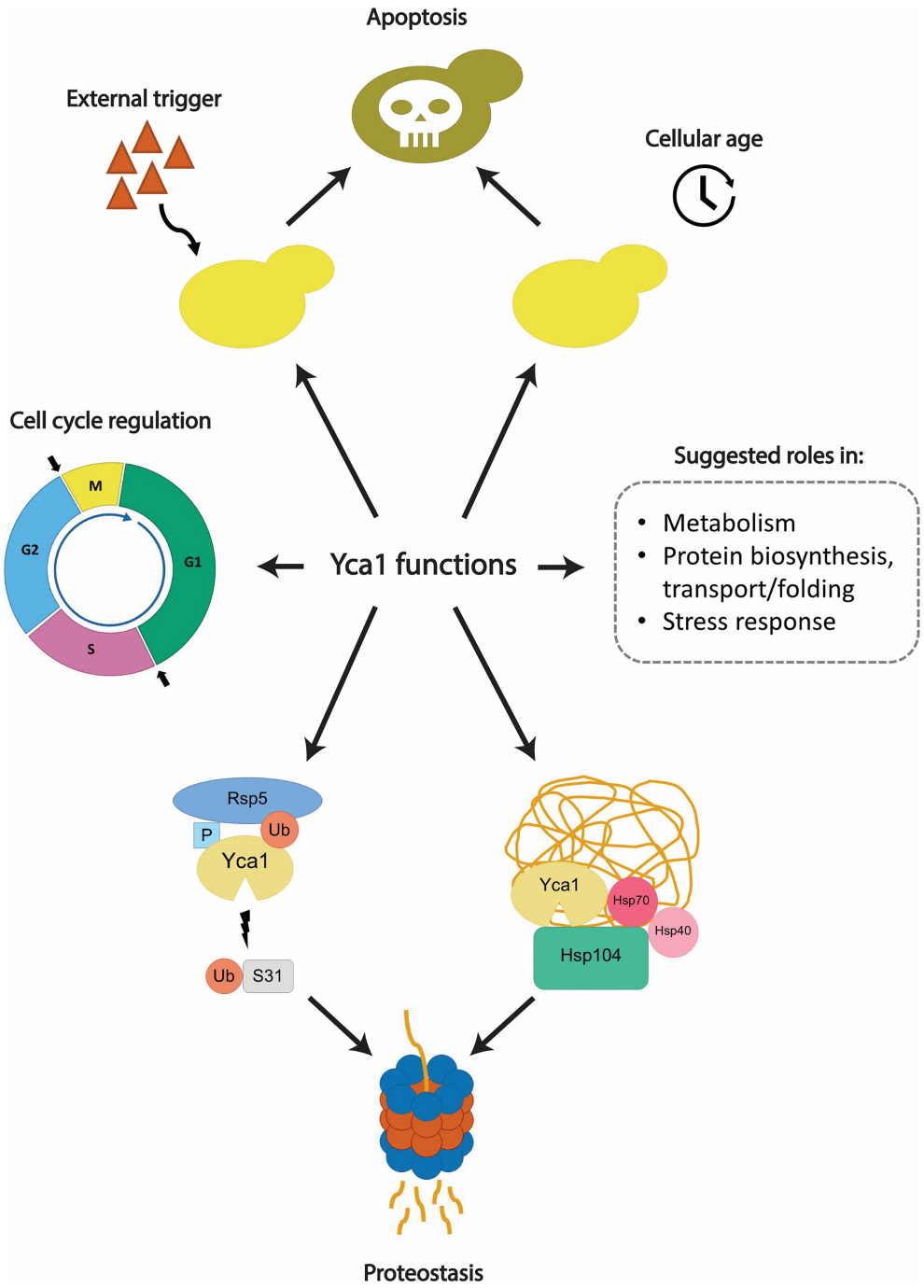

Figure 3. Yca1 apoptotic and nonapoptotic functions. (Top) Yca1 effects apoptosis as a form of RCD, shown being induced by an external apoptosis trigger (red triangles), as well as programmed cell death (PCD), shown being induced by cellular aging (clock). (Left) Yca1 regulates progression through the G1/S and G2/M transitions (black arrowheads) in the *S. cerevisiae* cell cycle. (Right) Cellular processes that Yca1 is suggested to participate in, based on multiple proteomic studies. (Bottom) Yca1 regulates proteostasis through the *de novo* ubiquitin synthesis pathway (left arrowhead) and targeting of the Hsp40/Hsp70/Hsp104 complex to cytosolic protein aggregates (right arrowhead).

Our understanding of how Yca1 performs its diverse set of functions in yeast will serve as the foundation for studying metacaspases across species.

Conflict of interest. None declared.

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