Review

Autophagy, P-Body Assembly, Mitochondrial Hyper-Fission, and Cell Death: Unexpected Secondary Roles of the Mediator Kinase Module Following Stress

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Autophagy, P-Body Assembly, Mitochondrial Hyper-Fission, and Cell Death: Unexpected Secondary Roles of the Mediator Kinase Module Following Stress

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Abstract: Following unfavorable environmental cues, cells reprogram pathways that govern transcription, translation, and protein degradation systems. This reprogramming is essential to restore homeostasis or commit to cell death. This review focuses on the secondary roles of two nuclear transcriptional regulators, cyclin C and Med13, that play key roles in this decision processing. Both proteins are members of the Mediator kinase module (MKM) of the Mediator complex, which, under normal physiological conditions, positively and negatively regulates a subset of stress response genes. However, cyclin C and Med13 translocate to the cytoplasm following cell death and cell survival cue, respectively, interacting with a host of cell death and cell survival proteins. In the cytoplasm, cyclin C is required for stress-induced mitochondrial hyper-fission and promotes regulated cell death pathways. Cytoplasmic Med13 promotes stress-induced assembly of processing bodies (P-bodies) and is required for the autophagic degradation of a subset of P-body assembly factors by cargo hitchhiking autophagy. This review will focus on these secondary, a.k.a. "nigh" jobs" of "cyclin C and Med13, outlining the importance of these secondary functions in maintaining cellular homeostasis following stress.

Keywords: Mediator Kinase Module; cyclin C; Med13; mitochondrial hyper-fission; cell death; P-body; cargo hitchhiking autophagy

1. Introduction

Under normal physiological conditions, cells attain homeostasis through complex regulatory events. This includes balancing the transcription and translation of proteins with their degradation, predominantly by the ubiquitin-proteasome system (UPS) and many macroautophagy (hereafter autophagy) pathways. Understanding the molecular details of how cells balance these events is crucial, as the misregulation of these pathways results in an imbalance in homeostasis. Moreover, as the efficiency of degradation pathways deteriorates with age, such imbalances are linked with age-related pathologies, including cancer and proteinopathies (Figure 1) [1–7]. Failing degradative pathways allow misfolded proteins to accumulate that, over time, can aggregate into larger structures, which are characteristic of proteinopathies, including Alzheimer's disease and Amyloid Lateral Sclerosis (ALS) [8,9].

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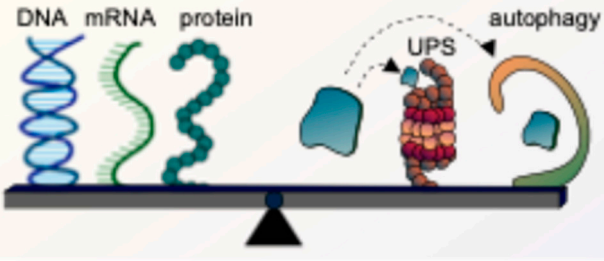

Figure 1. Transcription and translation must be balanced with protein degradation mechanisms, namely the ubiquitin proteasome system (UPS) and/or autophagy to maintain homeostasis in both physiological and stress conditions.

Cells are constantly subjected to environmental or physiological stress, including osmotic, salt, thermal, and nutritional stress [10]. After exposure, cells face a critical decision: they must either adapt and survive or commit to regulated cell death pathways. Adaptation involves the reprogramming of transcription, translation, and protein degradation pathways. Due to the high degree of conservation among these processes, the budding yeast *Saccharomyces cerevisiae* is an excellent model for studying the molecular stress response. Insights from this single-celled eukaryote have provided valuable information for understanding mammalian biology. For instance, autophagy was first identified in yeast and later found to be highly conserved across species. Furthermore, yeast has been instrumental in characterizing genetic pathways linked to neurodegenerative diseases such as ALS, Parkinson's, or Alzheimer's [11–16].

In addition to guiding studies in higher eukaryotes, studying the stress response in yeast is important in its own right. In the US, the wine industry generates billions of dollars of revenue annually, and craft beer brewing alone has recently emerged as a multibillion-dollar industry [17,18]. *S. cerevisiae* encounters different stresses during brewing, such as hyperosmotic, ethanol, and thermal stresses [19,20]. In addition, in fed-batch-operated industrial bioreactors, pockets of yeast can face glucose starvation, resulting in negative consequences for production performance [21]. Understanding and manipulating the yeast stress response could spur industrial advancements and increase fermentation yield efficiency [22–24].

This review focuses on the Mediator kinase module (MKM – formally Cdk8 kinase module) of the Mediator complex. This highly conserved complex consists of 4 proteins: cyclin C, its cognate kinase Cdk8, and two structural proteins, Med12 and Med13. In mammals, the MKM contains paralogues of its members except cyclin C, namely MED12L, MED13L, and CDK19 [25]. Unlike other better-known members of the cyclin-dependent kinase family, cyclin C-Cdk8 does not mediate cell cycle progression [26,27]. In yeast and mammals alike, the cyclin C-Cdk8 both positively and negatively regulates a subset of stress response genes [28–34]. In addition, cyclin C and Med13 have secondary cytoplasmic roles following cell death and cell survival cues, respectively (Figure 2). In the cytoplasm, they interact with a host of cell death and cell survival proteins, including the newly defined cargo hitching autophagy pathways [35]. This review outlines the importance of these secondary functions of cyclin C and Med13, maintaining cellular homeostasis following stress.

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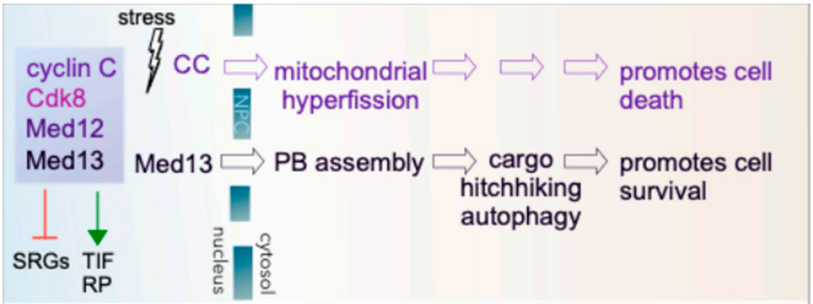

Figure 2. Cytoplasmic roles of cyclin C (CC) and Med13 following stress in yeast. The Mediator Kinase M odule (MKM) predominantly represses stress response genes (SRGs) in physiological conditions. It also positively regulates a subset of genes encoding for translation initiation factors (TIF) and ribosomal proteins (RP). Following ROS stress, cyclin C translocate s to the cytoplasm, which is required for mitochondrial hyperfission. Before destruction by the UPS, cyclin C also promotes regulated cell death by unknown mechanisms. Following starvation stress, Med13 translocates to the cytoplasm where it is required for P-Body assembly. It is also required for the autophagic degradation of a subset of PB assembly factors by cargo hitchhiking autophagy. The translocation of Med13 following starvation stress is necessary for cell survival.

2. Structure of the CKM

The eukaryotic Mediator comprises of a large core 26-subunit Mediator complex (cMED) and a dissociable Mediator Kinase Module (MKM), which functions as a critical coregulator of RNA polymerase II (RNAPII) transcription (Figure 3) [27,36–40]. As such, the mediator complex exists as two distinct entities, depending on whether it is bound to the MKM. Structural and biochemical studies identified the MKM–Mediator association as reversible, leading to the model that the MKM sterically inhibits the Mediator from interacting with RNA Pol II at promoters [27,36,41–48]. Unbound MKM also exists and likely functions to regulate transcription independently of the cMed [42,49,50]. Additional roles for MKM-Mediator in regulating RNA Pol II elongation rate have been described [51,52]. However, it remains unclear if this function is mediated by the MKM-Mediator complex or the MKM acting as an independent entity [51–53]. In yeast, the MKM predominantly represses stress response genes, while in mammalian cells, the MKM both negatively and positively regulates a wider array of genes [54,55]. A recent cryo-electron microscopy structure of the yeast MKM also revealed that Med13's bilobal architecture resembles that of argonaute proteins [56]. While this would imply a new role of the MKM in interacting with nucleic acid duplexes, the lack of several critical conser ved catalytic residues found in other argonaute proteins adds complexity to how exactly Med13 regulates transcription [56].

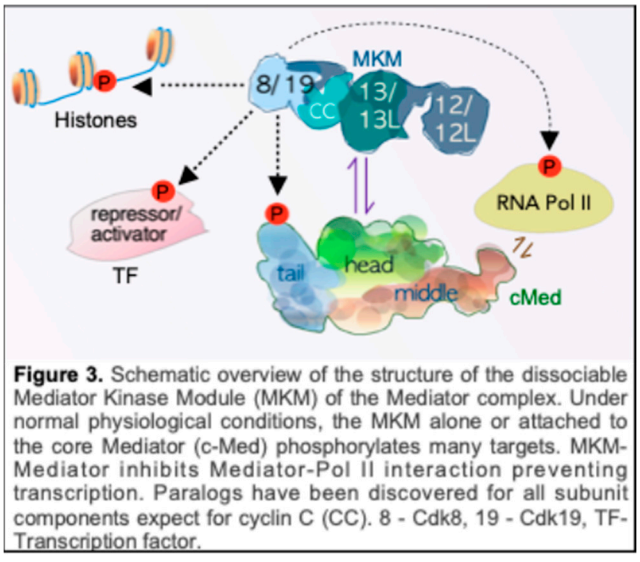

Figure 3. Schematic overview of the structure of the dissociable Mediator Kinase Module (MKM) of the Mediator complex. Under normal physiological conditions, the MKM alone or attached to the core Mediator (c-Med) phosphorylates many targets. MKM-Mediator inhibits Mediator-Pol II interaction preventing transcription. Paralogs have been discovered for all subunit components expect for cyclin C (CC). 8 - Cdk8, 19 - Cdk19, TF-Transcription factor.

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3. Transcriptional Reprogramming by the MKM Following Stress

A. *Overview of MKM regulated gene expression.*

Precise mechanistic details of how the MKM-Mediator regulates gene expression are still emerging [57]. Typically, cyclin binding activates CDKs, which are stabilized by CDK-activating kinase phosphorylation in the CDK T-loop [58]. Cdk8 lacks this canonical phosphorylation residue, with Med12 possibly stabilizing the cyclin C-CDK8/CDK19 interaction in humans [42,59]. Cyclin C and its cognate kinase are constitutively active in normal unstressed conditions, where they phosphorylate a multitude of factors to both positively and negatively regulate transcription [28,29,33,51–53,60–69]. Key among these targets are many transcription factors (TF) [28–34]. In addition, the MKM and MKM-Mediator regulate different targets, including histones [42] and RNA polymerase II tail, providing additional control of gene transcription [70,71]. In mammals, phosphorylation of TFs by the MKM-Mediator may be transmitted directly through enhancer regulatory elements to either repress or activate transcription. [53,72]. In summary, in all eukaryotes, the MKM influences important transcriptional outcomes that play a role in maintaining cellular and developmental homeostasis.

B. The MKM predominantly negatively regulates SRGs in yeast.

In yeast, the MKM predominantly negatively regulates a subset of stress response genes, including those that encode for antioxidants, molecular chaperones, autophagy, and development [33,37,49,54,73–82]. Activating these genes requires disassociating the MKM from cMed and disassembling the MKM. However, whether these actions co-occur remains unknown as the molecular mechanisms controlling these processes are poorly understood. Studies from our group have revealed that the mechanism used to disassemble the MKM depends on the nature of the stress signal and involves the translocation of either cyclin C or Med13 to the cytoplasm (Figure 2). This change in subcellular address contributes to stress-induced transcriptional reprogramming and is also required for cyclin C and Med13 to perform their secondary cytoplasmic functions. In short, cyclin C, but not other MKM members, relocates to the cytoplasm, following cell death cues (oxidative stress -ROS) [83]. Cytoplasmic cyclin C is necessary and sufficient for ROS-induced mitochondrial hyperfission and promotes regulated cell death [84]. This response is conserved [85–88]. Med13, but not cyclin C, crosses the nuclear pore complex following cell survival cues (nitrogen starvation). In the cytoplasm, Med13 promotes the assembly of a subset of ribonucleoproteins (RNPs) into mRNA processing bodies (P-bodies) [89]. Med13 also directs the autophagic degradation of a subset of P-Body assembly factors, including the conserved decapping protein Edc3, by a newly discovered hybrid autophagy mechanism called cargo hitchhiking autophagy [35,82,90]. The subcellular address of Med13 and Med13L in humans following stress remains unknown.

C. The MKM positively regulates a subset of genes required for translation.

Although the MKM primarily represses stress response genes (SRGs), we recently discovered that it also positively regulates a subset of genes encoding ribosomal proteins (RPs) and translation initiation factors (TIFs) under normal physiological conditions [91]. This mechanism demonstrates the conserved nature of MKM activity, as it also occurs in mouse embryonic fibroblasts (MEFs) and human cell lines [91]. The repression of this subset of translation-associated genes occurs in response to stress and is dependent on the disassembly of the MKM [91]. This process makes sense because ribosome biogenesis consumes over 60% of cellular energy and resources [92]. Reprogramming ribosome biosynthesis helps reduce intracellular energy consumption and represents a conserved mechanism for cellular survival during stress [93]

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4. Mechanisms of CKM Disassembly

A. MKM disassembly following cell death cues triggered by ROS

In response to oxidative stress, cyclin C, but not Cdk8, translocates to the cytoplasm, resulting in MKM disassembly [83]. This role is conserved [69,85]. In yeast, the molecular details of MKM disassembly in response to ROS involve an intertwined network of phosphorylation and ubiquitination events. The stress signal is transmitted by the conserved MAPK of the Cell Wall Integrity (CWI) pathway to Slt2 (Mpk1, (ERK ortholog) [94] that directly phosphorylates cyclin C and Med13 [95–98]. Intriguingly, Kdx1 (Mlp1), the pseudokinase of the CWI pathway, is also required for cyclin C release [97]. Kdx1 also interacts with the transcription factor Ask10 and likely induces cyclin C translocation to the cytoplasm indirectly [97,99]. CWI triggered phosphorylation of Med13 results in its destruction by UPS mediated by the E3 ligase complex SCF^{Grr1} [98,99]. In addition, cyclin C is directly phosphorylated at a single site (S266). The net outcome is MKM disassembly and cyclin C nuclear release [83,84] In yeast, the UPS destroys cytoplasmic cyclin C following its role in mitochondrial hyper-fission [54,74,84].

Genetic studies showed that Med13 destruction is required for cyclin C nuclear release. In addition, Med13 destruction requires a priming event mediated by Cdk8 in unstressed cells [98]. Thus, recognition of the Med13 degron uses two phosphorylation marks, one to prime the degron, the second for its recognition by ubiquitin ligases [100,101]. This ensures that releasing cyclin C to the cytoplasm is the correct response to the environmental input. Additionally, direct phosphorylation of Med13 by Snf1, a highly conserved adenosine monophosphate-activated protein kinase (AMPK) that is activated in response to a variety of stresses is required for its translocation [102,103].

Taken together, the studies discussed above suggests that Med13 acts as a physical tether, keeping cyclin C in the nucleus. Consistent with this, in the absence of Med13, cyclin C is aberrantly released into the cytoplasm where it induces mitochondrial fission in the absence of stress [99]. Furthermore, deletion of the holoenzyme associating domain (HAD), the domain on cyclin C that interacts with Med13 [71] also results in the precocious release of cyclin C from the MKM into the cytoplasm and mitochondrial fission in the absence of stress [99]. The HAD is highly conserved, being required for the cyclin C-Med13 interaction in mammalian cells [87]. Based upon these studies we designed a peptide mimetic of this domain (S-HAD) which stimulates cyclin C nuclear release in mammalian cells in the absence of stress, highlighting the conservation of both the domain and function [87].

B. MKM disassembly following cell survival cues triggered by nitrogen starvation

In the yeast model system, nitrogen starvation activates both bulk and cargo hitchhiking autophagy pathways. Bulk autophagy is essential for recycling cellular components, producing energy, and ensuring survival [104]. In contrast, cargo hitchhiking autophagy is a selective pathway that degrades specific substrates, including Med13, certain ribosomal proteins, and components involved in PB assembly [35]. Given that the MKM represses many crucial autophagy genes, it is not surprising that effective induction of autophagy requires its disassembly [82,105]. Following nitrogen starvation cyclin C does not translocate to the cytoplasm. Instead, the ubiquitin-proteasome system (UPS) targets and degrades cyclin C in the nucleus via an unidentified E3 ligase [35,82]. This response is vital for survival, as it prevents cyclin C from causing mitochondrial hyper-fission and regulated cell death [105]. Thus mitochondria remain hyperfused, a morphology associated with maximal ATP production [4,106]. MKM disassembly following nitrogen starvation also results in Med13 translocating to the cytoplasm [82,90]. Here it is required for P-Body assembly, and the autophagic degradation of a subset of P-Body assembly factors by cargo hitchhiking autophagy [35,89]

How the stress signal is transmitted to the MKM following nitrogen starvation remains unknown. However, it is known that in yeast the activity of Slt2 is activated in response to the Target of Rapamycin Complex 1 (TORC1) inhibition [107]. However, phosphorylation of cyclin C by Slt2 is not required for its degradation following nitrogen starvation [105]. This suggests that a different

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protein, possibly Med13, is the target of Slt2 activity following starvation stress. These studies highlight how the MKM responds to cell death and cell survival cues, despite the fact that the CWI is activated in both cases. Interpretation of the input signal is critical as expressing a fusion protein in which cyclin C is tethered to the outer mitochondrial membrane results mitochondrial fission and execution cell death pathways following cell survival cues [105].

5.. The Roles of Cytoplasmic Cyclin C

Cytoplasmic cyclin C is required for stress induced hyper-fission in both yeast and mammalian cells, indicating that this response is conserved [108]. Cytoplasmic cyclin C also promotes regulated cell death pathways in both yeast and mammals. However, whilst we discovered that cyclin C interacts with the BAX, a BCL-2 family pro-apoptotic member, in mammalian cells, yeast cells do not harbor a BAX ortholog [109]. Although ROS exposure leads to the same outcome in both yeast and mammals, different mechanisms are employed to direct cells into regulated cell death pathways.

A. Cyclin C and stress-induced mitochondrial hyperfission in yeast.

Mitochondrial fission occurs in two distinct scenarios: firstly, during cell division under normal physiological conditions and mitophagy, and secondly, as a response to stress that results in cell death The key difference between these pathways is the presence of cytoplasmic cyclin C in the latter (see Figure 4). In yeast, following ROS cyclin C translocates to the cytoplasm where it interacts with the yeast mitochondrial fission machinery adaptor protein called Mdv1 (Figure 5A) [84]. Mdv1, and another adaptor protein called Caf4, recruits Dnm1 to sites of mitochondrial fission by interacting with the conserved mitochondrial outer membrane (OMM) protein called Fis1 [110,111]. Dnm1 is a GTPase, that works by constricting and dividing mitochondria in a GTP-hydrolysis dependent manner [112,113]. Here Dnm1 forms a multimeric ring complex around mitochondria which initiates separation of the inner and outer mitochondrial membranes [114,115]. Following GTP hydrolysis the complexes constrict, resulting in mitochondrial fission [116]. Exactly, how the cyclin Dnm1-fission complex reduces mitochondrial hyper-fission is unclear in yeast.

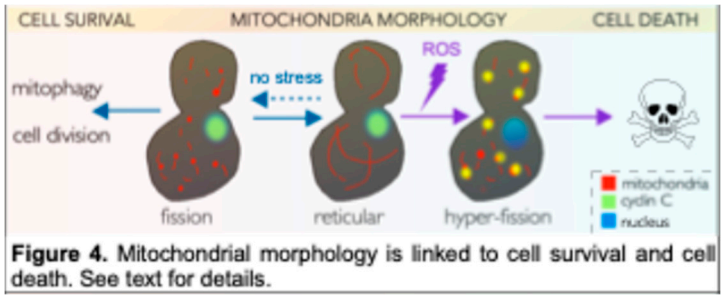

Figure 4. Mitochondrial morphology is linked to cell survival and cell death. See text for details.

This same fission mechanism occurs during regular cell division or during homeostasis to control mitochondrial quality except that cyclin C is not present [117,118]. This has led to a model in which cyclin C localization to the OMM stimulates hyper-fission, a phenotype required for initiating cell death pathways [84,119,120]. In support of this model, the absence of cyclin C, or in cis and trans mutants that keep cyclin C in the nucleus, stress-induced hyper-fission and cell death is significantly reduced [84]. While the exact mechanism by which cyclin C helps induce stress-induced mitochondrial fragmentation is still unclear, cyclin C most likely acts early in the stress response to promote the formation of productive Fis1-Mdv1-Dnm1 complexes, leading to fission [84]. Other observations strongly support this model: firstly, artificially placing cyclin C at the mitochondria via fusion of the OMM-associating domain of Fis1 to cyclin C's C-terminus is enough to induce fission even in unstressed cells [105]. Secondly, cyclin C mediated hyperfission role of cyclin C is not dependent upon its transcriptional duties [83,84]. Lastly, as mentioned above, retaining cyclin C in the nucleus following ROS stress significantly reduces hyperfission.

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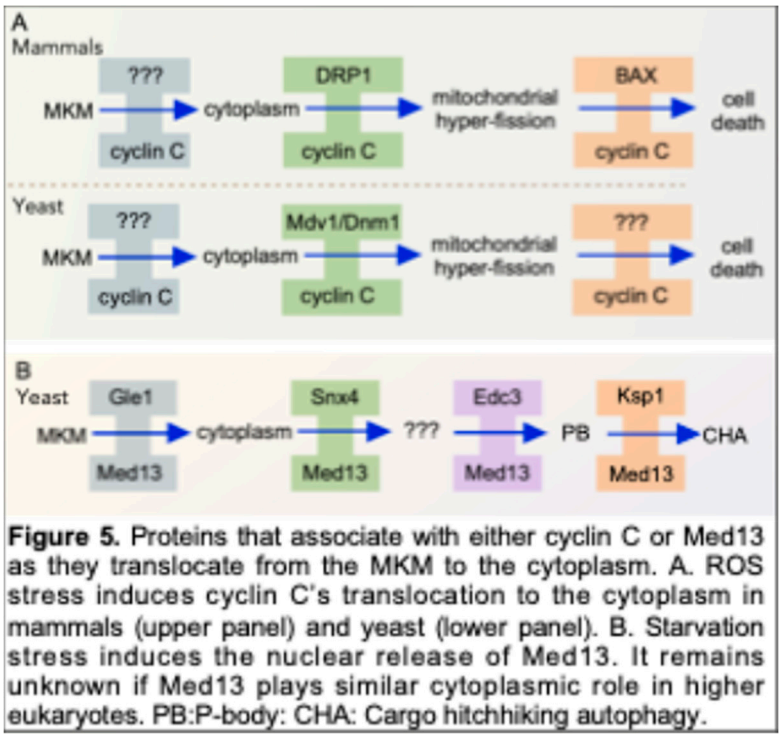

Figure 5. Proteins that associate with either cyclin C or Med13 as they translocate from the MKM to the cytoplasm. A. ROS stress induces cyclin C's translocation to the cytoplasm in mammals (upper panel) and yeast (lower panel). B. Starvation stress induces the nuclear release of Med13. It remains unknown if Med13 plays similar cytoplasmic role in higher eukaryotes. PB:P-body: CHA: Cargo hitchhiking autophagy.

B. Cyclin C and mitochondrial hyper-fission in mammals.

In mammals, DRP1-dependent mitochondrial fission following oxidative stress is also part of the regulated cell death pathway (RCD, Figure 5A) [108]. Just as in yeast, stress-induced translocation of cyclin C from the nucleus to the cytosol is responsible for the mitochondrial hyper-fission [85]. Outlining the conserved nature of this response, *Escherichia coli* purified yeast GST-cyclin C can induce mitochondrial fission in mouse embryonic fibroblasts [85]. Cyclin C directly interacts with DRP1 GTPase domain to stimulate it via its second cyclin box domain (CB2) [86]. In this context, the interaction between cyclin C and DRP1 induces structural changes in DRP1, transforming it from oligomers with low GTPase activity to dimers capable of forming high-GTPase activity filaments [86]. The conservation of the cyclin C–Dnm1/DRP1 association could help in future studies examining DRP1 related diseases including neurodegenerative disorders, ischemia-reperfusion injury and optic atrophy [121,122].

Many Drp1 associated diseases are associated with point mutations in the Drp1 gene, which can then be generated in yeast and used to study mitochondrial morphology and protein function [121,123]. Additionally, DRP1 inhibitors are being tested in clinical trials to treat both cancers and cardiovascular diseases [124–127]. Due to the important role DRP1 plays in cell division in healthy cells, inhibiting it this broadly can often have negative consequences. Since many DRP1 related diseases involve a disease-mediated increase in ROS causing DRP1 dependent mitochondrial hyper-fission, specifically targeting the ROS dependent cyclin C – DRP1 interaction could help overcome negative side effects from general DRP1 inhibition [128–130]

C. Cyclin C and cell death in yeast.

Numerous key features of RCD observed in mammalian cells are also conserved in yeast [131]. However, the mechanisms driving RCD in yeast are distinct from those of metazoan apoptosis and remain poorly understood. For instance, RCD can occur in yeast both in a manner dependent on and independent of its sole caspase-like protein, Yca1 [132–135]. Interestingly, cells lacking cyclin C display remarkable resistance to stress-induced cell death, a phenomenon observed in both yeast and mammalian systems [85,136]. Although the precise role of cyclin C in this context is not fully understood, simply enhancing cyclin C-mediated mitochondrial fission through overexpression alone is insufficient to trigger programmed cell death (PCD) [84]. This suggests that an additional signal(s) is necessary to initiate RCD, which might come from another protein localized to the

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mitochondria during the stress response or through a stress response pathway that modifies cyclin C post-translationally. This important distinction sets cyclin C apart from other known inducers of mitochondrial RCD. For example, ectopic targeting of p53 or the BH3 family member Bax to the mitochondria can effectively induce cell death in non-stressed mammalian cells. In contrast, there is conflicting evidence for the role of Ybh3, the sole BH3 protein in yeast, in inducing regulated cell death [137–140]. In conclusion, while cyclin C plays an integral role in RCD in yeast, the exact mechanisms underlying its function are still not fully elucidated.

D. Cyclin C and cell death in metazoans.

BAX and other members of the BCL-2 family are important regulators of the intrinsic pathway of apoptosis in mammalian cells [141]. Upon activation, BAX oligomerizes at the OMM resulting in mitochondrial outer membrane permeabilization (MOMP) [142]. In yeast there are no apparent homologs of the Bcl-2 family proteins. Cytoplasmic cyclin C is required for both the activation and mitochondrial localization of BAX in mammalian cells [87]. It directly interacts with active Bax and is dependent on the presence of DRP1 [87]. Later research also confirmed that BAX and DRP1 directly interact [143]. This suggests a model where cyclin C, BAX, and DRP1 all together form a complex essential for apoptosis. Artificially stimulating cyclin C nuclear release in the absence of stress still leads to BAX association, however, BAX does not oligomerize [87]. This supports the model in which release of cyclin C from the nucleus is insufficient to promote cell death pathways.

S. cerevisiae does not encode for any known homologs of BCL-2 family. However, murine BAX expression induces cell death in yeast [144]. This toxicity can be rescued by co-expressing antiapoptotic members of the BCL-2 family, such as BCL-2 or BCL-XL [144]. How BAX kills yeast remains elusive. Deletion of the mitochondrial inner membrane protein Uth1 results in strengthened cell wall that contributes to resistance to BAX-mediated toxicity, [145,146]. Also, overexpression Rgl1, a pro-survival protein that plays a role in iron metabolism, suppresses BAX toxicity [147]. However, the relationship between these two events remains unclear, and has not led to a mechanism on how BAX mediates cell death in yeast. Likewise, despite identifying a yeast homolog of the BAX inhibitor protein 1 (Bxl1), this protein cannot counter the toxic effects of Bax expression in yeast [140,148,149]. Lastly, it is known that the expression of BAX results in cytochrome c release from the mitochondria, confirming that BAX permeabilizes mitochondrial membranes [150]. This is supported by the finding that the F0F1-ATPase proton pump is essential for BAX-induced cell death in yeast and mammalian cells [151,152]. This suggests that the permeability pore operates through a conserved mechanism. The cytochrome c release is partially dependent on ER-mitochondrial association; however, cytochrome c is not required for cell death in yeast [153,154]. Cyclin C's role in BAX-dependent cell death in yeast is unclear, but its mitochondrial association and involvement in RCD make it a protein of interest.

6. Cytoplasmic Roles of Med13

In the yeast model system, Med13 translocates to the cytoplasm following TOR1 inhibition triggered by either nitrogen starvation or treatment with rapamycin [82,90]. Here it is required for processing body (P-body) assembly and the autophagic degradation of a subset of P-body assembly factors (Figure 5B) [89]. It remains unknown if these roles are conserved, but given the conservation of cyclin Cs secondary job, it is highly likely that Med13 will follow suit.

A. Med13's role in P-body assembly.

Inhibiting mRNA translation is a critical strategy that cells employ to manage protein levels effectively. Under stress, cells strategically downshift the production of proteins vital for growth while ramping up those necessary for adaptation. A pivotal mechanism for blocking mRNA translation is the formation of P-bodies. These highly conserved, membrane-less structures within the cytoplasm are reservoirs that contain non-translating mRNA-protein complexes (mRNPs) [155,156]. Initially, it was believed that mRNAs within P-bodies were simply stored for future

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translation or subjected to degradation [157–160]. However, subsequent studies have revealed that P-bodies are not crucial for mRNA degradation [161]. Instead, they are increasingly recognized as crucial temporary storage sites for translationally repressed mRNAs that remain ready for activation when conditions improve [162].

The assembly of P-bodies occurs through a process known as liquid-liquid phase separation (LLPS), creating dynamic droplets that are distinctly separate from the surrounding cytoplasm [163]. Their assembly benefits cells, as P-body formation occurs more rapidly than changes in the transcriptional or translational programs. Moreover, the rapid disassembly of P-bodies after the stress abates, rapidly provides cells with ready-to-go translation components. The proteins that reside within P-bodies are predominantly RNA-binding proteins or factors with low sequence complexity that contain prion-like domains (PLDs). These PLDs possess the remarkable ability to undergo spontaneous conversion into aggregated states and serve as templates for the recruitment of additional proteins.

In yeast, P-bodies are consistently present under normal physiological conditions, but their size and number notably increase in response to various stressors, such as nitrogen and carbon starvation [163]. The assembly of P-bodies is fundamentally dependent on several conserved decapping proteins, including Dcp1/Dcp2, Edc3, Dhh1, along with the Pat1–Lsm1–7 complex and Xrn1 [158]. Edc3 serves as a crucial scaffold for P-body assembly, by leveraging its multivalent interactions [164–168]. Med13 is essential for P-body assembly following nitrogen starvation; the stark reduction of Edc3 foci in *med13Δ* cells underscores its importance [89]. The mechanistic details of why Med13 is required for P-body formation remain to be completely elucidated; however, it is clear that the PLD domain of Med13 is indispensable for this process [89]. The exact function of these polyQ/N tracts is not well understood, but it has been suggested that the domain can interact with itself or with other polyQ/N regions to promote the aggregation of mRNPs [168].

These studies indicates that Med13 plays a significant role in promoting the liquid-liquid phase separation (LLPS) of P-body proteins. Furthermore, Med13 contains two motifs strongly linked with LLPS: an RNA Recognition Motif (RRM) and a large intrinsically disordered region (IDR). IDRs are recognized as key drivers of LLPS, as they can simultaneously create multiple interactions with other components [169]. Additionally, the amino acids within IDRs are more exposed and accessible to post-translational modifications, which are critical regulators of biomolecular phase separation [170–172]. This suggests that Med13 itself may undergo LLPS, a direction future studies should pursue.

B. Med13's role in cargo-hitchhiking autophagy (CHA)

Med13 is also required for the autophagic degradation of both Edc3 and Dhh1 following nitrogen starvation. Since Med13 does not direct the autophagic degradation of Xrn1, this suggests that Med13 serves as an exclusivity factor that differentiates among various P-body components [89]. A new hybrid autophagy mechanism implements the autophagy of these decapping proteins called cargo-hitchhiking autophagy, which uses components from both selective and bulk autophagy mechanisms [35].

i) Snx4 promotes CHA

CHA was previously named Snx4-assisted autophagy, as this conserved sorting nexin is required for the degradation of all identified CHA cargos to date [35,82,173–175]. Sorting nexins are a family of conserved phosphoinositide-binding proteins that play fundamental roles in orchestrating cargo sorting through the endosomal network. As such, human SNX4 is implicated in a variety of synaptic processes [176,177]. Significantly, SNX4 has already been linked with the etiology of AD, with SNX4 protein levels being decreased by 70% in brains of severe Alzheimer's disease (AD) cases [178].

Sorting nexins are recruited to endosomal membrane domains by a phox homology (PX) domain that recognizes phosphatidylinositol-3-phosphate (PtdIns3P) [175]. Snx4 also contains two Bin–Amphiphysin– Rvs (BAR) domains that sense membrane curvature and tubulate vesicles [179]. The molecular role of Snx4 (Atg24) in autophagy is also related to membrane bending as it stabilizes and

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drives the opening of the inner membrane rim of non-selective phagophores in yeast [180]. The net effect is a phagophore membrane with a wide enough opening to accommodate large cargos, including ribosome and proteasome subunits [180–183].

ii) CHA uses phagophores built by the Atg17 scaffold complex

In CHA, specific cargo, including Med13, are recruited to growing phagophores primarily used to degrade random cytosolic contents by non-selective (a. k. a. bulk) autophagy. These phagophores are triggered by starvation stress. Their formation is dependent on the Atg17 scaffold complex, which consists of the Atg17 scaffold and two regulatory proteins, Atg29 and Atg31 [184]. This process differs from selective autophagy, where phagophores are constructed using the Atg11 scaffold [185]. Selective autophagy pathways play crucial roles in maintaining homeostasis under physiological conditions. They are responsible for removing dysfunctional proteins (such as in mitophagy, ER-phagy, and ribophagy), protein aggregates (aggrephagy), and pathogens like viruses and bacteria (xenophagy) in higher eukaryotes [186]. Likely, Med13-Edc3 is not the only cargo captured in Atg17-built phagophores. Instead, these phagophores probably contain random cytosolic contents alongside other cargo that may hitch a ride, including a subset of ribosomal proteins [174].

The formation of the phagophore assembly site (PAS) for both selective and non-selective autophagy depends on liquid-liquid phase separation (LLPS) [187,188]. During starvation, the Atg1 complex—comprised of Atg1, Atg13, and a trimeric Atg17 scaffold undergoes LLPS [189]. This fluidity is vital for recruiting downstream Atg proteins necessary for autophagosome formation. As mentioned above P-Body assembly occurs via LLPS. How these two biomolecular condensates (BMC) interact remains unknown. One intriguing possibility is that Med13 could play a role, possibly acting as a conduit between these two BMCs.

iii) CHA uses selective autophagy receptor proteins to recognize cargo

Although CHA uses phagophores built for the degradation of random cytoplasmic contents, cargo recognition is dependent upon an autophagic receptor protein [35]. The autophagic receptor for the selective degradation of Med13 is Ksp1. Typical of autophagic receptors, Ksp1 interacts with the phagophore-bound protein Atg8 via its conserved Atg8 interaction motif (AIM) [90,190]. Ksp1 is a casein-like kinase that negatively regulates autophagy in replete media [191–195]. However, its role as a receptor protein is **kinase-independent,** illustrating its dual and opposing roles in autophagy. Ksp1 also associates with ribosomal and PB proteins [196,197]. Interestingly, Ksp1 does not directly interact with Edc3. Instead, Med13 performs this role, suggesting it may act as a conduit between Edc3 and Ksp1 [90].

iv) The nucleoporin Gle1 is required for CHA of Med13.

Cyclin C is a small 33 kDa protein that can diffuse through the nuclear pore complex (NPC) without the assistance of active transport by exportins. In contrast, Med13 is a larger scaffold protein, approximately 150 kDa in size, which requires active transport to cross the nuclear-cytoplasmic barrier. However, our studies have shown that β karyopherin exportins are not needed for Med13's transit through the NPC. Similarly, Dpb5 and the Mex67-Mtr2 heterodimer, which can transport cargo across the NPC, do not play a role in this process. Instead, Gle1, a conserved component of cytoplasmic NPC fibrils, is essential for Med13's passage across the NPC [82].

GLE1 is a mobile nucleoporin in mammalian cells [198], suggesting that it could play a similar role in the nuclear export of Med13. Interestingly, in yeast Med13 remains in the nucleus following nitrogen starvation in *snx4Δ* mutants [82]. This observation implies that Snx4 may communicate with the NPC, potentially by interacting with Gle1 during the transfer of Med13 as it exits the NPC. Future experiments will be necessary to investigate this idea further.

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7. Diseases Associated with the MKM

A. Diseases associated with cyclin C-CDK8/CDK19

i) Cyclin C tumor suppressor roles

Deletions of the genomic locus containing the cyclin C encoding gene (6q21) are found in blood cancers and solid tumors, hinting at a tumor suppressor role for cyclin C [88,199,200]. However, the extent to which the nuclear and mitochondrial roles play in tumor suppression is unknown.

The cyclin C gene is heterozygously deleted in a significant majority of T-cell acute lymphoblastic leukemia (T-ALL) cases [199]. In vivo, the cyclin C-CDK8 kinase phosphorylates ICN1, resulting in Notch1 repression, a single-pass transmembrane receptor [201]. Cyclin C heterozygosity or its deletion derepresses ICN1 levels, promoting T-ALL progression [199]. Notch activation is present in many cancers, including several where 6q21 deletions have been observed (acute lymphoblastic leukemia, non-Hodgkin's Lymphoma, prostate cancer, amongst others), likely due to this specific suppressive role involving ICN1 [202–206]. In other cancers, such as human squamous cell carcinoma, Notch1 functions as a tumor suppressor. Investigating cyclin C deletion or activation in those tumors could reveal whether it plays a role in those tumors as an oncogene instead [199,207,208].

Cyclin C also plays a tumor suppressor role in anaplastic thyroid tumors [209]. Deletion of Cyclin C alone in the thyroid only stimulates a modest increase in hyperplastic growth. However, in combination with ablation of the *PTEN* tumor suppressor [210], the thyroid size increases dramatically, eventually killing the animal [209]. Despite the increase in ROS production throughout thyroid cancer development, it is not the mitochondrial role of cyclin C that is involved, but rather alterations in the Jak-Stat signaling pathway that destabilize tumor suppressors p53 and p21 [87,211]. Given the high degree of conservation between yeast and mammalian cyclin C, yeast cyclin C is an ideal model for understanding its role in carcinogenesis in mammals.

ii) Cdk8 is an oncogene

CDK8 was first reported as a putative oncogene in colorectal cancer in 2012 [212]. In this context, CDK8 mediates the aberrant activation of the Wnt/ β—catenin signaling pathway. Additionally, the phosphorylation of various transcription factors, such as Smads, STAT1, and NFκB, by CDK8 is frequently dysregulated in many other types of cancer [28,64,213]. This suggests that cancers associated with CDK8 are predominantly driven by transcriptional dysregulation [214]. Currently, mutations in both CDK8 and CDK19 have been linked to over 100 malignancies, including cancers of the colon [212,214], breast [215], prostate [216], pancreas [217], melanoma [218] and leukemias [219]. It comes as no surprise then that modulating the transcriptional activity of the MKM has emerged as an attractive therapeutic strategy for treating cancer. Consequently, specific inhibitors targeting CDK8/CDK19 are currently being tested in various clinical trials [213].

iii) Cyclin C and TDP-43 mediated cell death

Transactive Response Binding Protein 43 (TDP-43) is a ubiquitously expressed protein involved in mRNA regulation [220]. It is the primary aggregate protein found in many age-related proteinopathies such as Amyotrophic Lateral Sclerosis (ALS) and Frontal Tubular Dementia (FTD) [221]. TDP-43 toxicity has been associated with autophagy impairment, mitochondrial dysfunction, and the generation of reactive oxidative species [222–226].

Increased levels of ROS are observed in several neurodegenerative diseases, including ALS [227]. Moreover, mitochondrial hyper-fission has also been observed in the post-mortem neurons of patients diagnosed with ALS and other proteinopathies as well as in cell culture [228–232]. Additionally, the hallmark of neurodegeneration is the aberrant premature dying of neurons [233]. This has led to a model in which the expression of mutant TDP-43 results in the formation of aberrant cytoplasmic aggregates of TDP-43.

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Using the established yeast model of TDP-43 pathology, it has been shown that cyclin C, but not Med13, promotes TDP-43-mediated cell death [234]. Likewise, the deletion of Dnm1 rescues TDP-43 toxicity, pointing to the potential mitochondrial fission role of cyclin C in TDP-43 toxicity. Furthermore, cyclin C translocates to the cytoplasm following TDP-43 overexpression. The free radical scavenger, N-acetyl-cysteine (NAC), inhibits cyclin C cytoplasmic relocalization. These studies suggest the possibility that cytoplasmic cyclin C may promote mitochondrial hyper-fission and cell death upon expression of mutant TDP-43. This is exciting as cyclin C is a new player in TDP-43 biology and could represent a new target for drug therapies. This is important as currently, there are no effective cures for ALS, and patient survival upon diagnosis is around 3-5 years [235].

B. Diseases associated with Med13 biology.

In mammals, the MKM contains paralogues of its members except cyclin C, namely MED12L, MED13L, and CDK19 [25]. The biological roles of these paralogues remain poorly understood, but they appear to be functionally distinct [236]. Many mutations in human MED13L result in MED13L syndrome, a disease characterized by a range of symptoms that vary in severity. These include intellectual disability, facial dysmorphism, hypotonia, congenital heart disease, as well as speech and motor delay [237–240]. In MED13L, mutant fibroblasts cyclin C is aberrantly released into the cytoplasm, leading to mitochondrial fragmentation and increased mitochondrial dysfunction [241]. Variants in CDK8, *MED13,* MED12, and MED12L are also associated with neurodevelopmental disorders [242–247], with cyclin C's role here being unknown. In addition, mutations in Med12 are linked with uterine leiomyomas [248]. Interestingly, all the MKM neurodevelopmental disorders are phenotypically similar, indicating a probable overlap in pathogenic mechanisms.

8. Outlook

The MKM has evolved in the last 20 years from being a transcriptional regulator to mediating mitochondrial hyper-fission, regulated cell death, PB assembly, and cargo hitchhiking autophagy. These secondary roles depend upon the nuclear release of either cyclin C or Med13, following ROS or starvation stress, respectively. These studies support the emerging concept that proteins can perform two distinct functions, referred to as "day and night jobs" [249,250], which various external or intrinsic stimuli can trigger. As mentioned above, Ksp1 has two opposing roles in autophagy, which are dependent and independent of its kinase function [90,191–195]. Likewise, Cdk8 does not translocate to the cytoplasm following stress in either yeast or mammals [84,85], indicating that the cytoplasmic role of cyclin C is kinase independent.

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Abbreviations: Adenosine Monophophate-activated Protein Kinase:AMPK ; Amyloid Lateral Sclerosis:ALS ; ATG8 Interaction Motif:AIM ; Bin–Amphiphysin– Rvs:BAR ; Biomolecular Condensates:BMC ; Cell Wall Integrity pathway:CWI ; Core-Mediator Complex:cMED ; Holoenzyme-associating Domain:HAD ; Inner Mitochondrial Membrane:IMM ; Intrinsically Disordered Region:IDR ; Liquid-liquid Phase Separation:LLPS ; Mediator Kinase Module:MKM ; Outer Mitochondrial Membrane:OMM ; Mitochondrial Outer Membrane permeabilization MOMP: Mouse Embryonic Fibroblasts:MEFs ; N-acetyl-cysteine:NAC ; Non-translating mRNA-protein complexes:mRNPs ; Nuclear Pore Complex:NPC ; Phagophore Assembly Site:PAS ; Phosphatidylinositol-3-phosphate:PI(3)P ; Phox Homology:PX ; Prion-like Domains:PLDs ; Processing Body:P-bodies ; Regulated Cell Death:RCD ; Ribonucleoproteins:RNPs ; Ribosomal Proteins:RPs ; RNA Polymerase II:RNAPII ; RNA Recognition Motif:RRM ; Stress Response Genes:SRGs ; T-Cell Acute Lymphoblastic Leukemia:T-ALL ; Transcription Factors:TFs ; Translation Initiation Factors:TIFs ; Ubiquitin Proteasome System:UPS.

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