The *TP53* signaling network in mammals and worms

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Abstract

The nematode worm *Caenorhabditis elegans* has been an invaluable model organism for studying the molecular mechanisms that govern cell fate, from fundamental aspects of multicellular development to programmed cell death (apoptosis). The transparency of this organism permits visualization of cells in living animals at high resolution. The powerful genetics and functional genomics tools available in *C. elegans* allow for detailed analysis of gene function, including genes that are frequently deregulated in human diseases such as cancer. The *TP53* protein is a critical suppressor of tumor formation in vertebrates, and the *TP53* gene is mutated in over 50% of human cancers. *TP53* suppresses malignancy by integrating a variety of cellular stresses that direct it to activate transcription of genes that help to repair the damage or trigger apoptotic death if the damage is beyond repair. The *TP53* paralogs, *TP63* and *TP73*, have distinct roles in development as well as overlapping functions with *TP53* in apoptosis and repair, which complicates their analysis in vertebrates. *C. elegans* contains a single *TP53* family member, *cep-1*, that shares properties of all three vertebrate genes and thus offers a simple system in which to study the biological functions of this important gene family. This review summarizes major advances in our understanding of the *TP53* family using *C. elegans* as a model organism.

Keywords: **TP53** *family;* C. elegans*; apoptosis; DNA repair; cell cycle; cancer*

INTRODUCTION

The *TP53* gene family is best known for its roles in protecting the integrity of the genome by inducing cell cycle arrest, apoptosis and DNA repair in cells after genotoxic stress [1]. Humans and other mammals contain three *TP53* family paralogs, *TP53*, *TP63* and *TP73*, which have overlapping and specific functions [2]. Because *TP53* is mutated in about one-half of all human cancers, and *TP63* and *TP73* have also been shown to possess tumor suppressor function [3–6], an understanding of how this protein family controls genome stability is of primary medical and biological interest.

Initially thought to be confined to vertebrate lineages, the *TP53* family is now considered to be much more ancient. The most ancestral *TP53* gene reported is found in the unicellular protozoan *Entamoeba histolytica* [7], but this has been disputed based on weak sequence homology [8]. However, it was a similar cryptic sequence homology of the *Caenorhabditis elegans TP53* family member CEP-1 that prevented its detection by standard homology search programs for several years after the sequence was available to the public [9, 10]. The approximately 15% sequence homology between the DNA binding domain of CEP-1 and mammalian *TP53* would have been disregarded were it not for the conservation of amino acids required for binding *TP53* DNA consensus sequences [9, 10]. Functional analysis revealed biological roles for CEP-1 conserved across the vertebrate *TP53* family, including DNA damage-induced apoptosis, cell cycle control

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The Hospital for Sick Children (SickKids) is recognized as one of the world's foremost pediatric health-care institutions and is Canada's leading center dedicated to advancing children's health through the integration of patient care, research and education.

and aging [9–13]. Thus, despite weak sequence similarity CEP-1 has ancestral functions in a variety of biological processes required for maintaining genome stability.

In this review, we discuss how studies of CEP-1 have provided insight into the mammalian *TP53* family and what other questions can be addressed using the powerful genetics and *in vivo* methods of *C. elegans*.

THE BIOLOGICAL FUNCTIONS OF CEP-1

After recognizing CEP-1 as a potential *C. elegans TP53* family member, work began to functionally characterize cep-1 loss-of-function (*lf*) phenotypes using mutant alleles and gene knockdown by RNA interference (RNAi). It was found that cep-1 was dispensable for normal development; cep-1 loss of function mutation or ablation by RNAi yields worms with no gross morphological defects and normal fertility [9, 10]. This is in contrast to mutants in mammalian *TP63* and *TP73*, which are required for development in mice [14–20]. Like cep-1, mammalian *TP53* is essentially dispensable for normal development; however, mice lacking *TP53* develop various types of malignancies and females often exhibit anencephaly [21–23]. cep-1 loss-of-function does cause some subtle phenotypes, including a low but appreciable rate of embryonic lethality and a mild high incidence of males ('Him') phenotype [9]. In *C. elegans*, males are produced by non-disjunction of the sex chromosome, leading to animals that have only one X chromosome instead of two. These nondisjunction events are rare in wild-type, such that males are produced at a frequency of approximately 1 in 500 animals [24]. Ablation of cep-1 by RNAi leads to increased nondisjunction of the X chromosome that results in approximately 2% male offspring [9], which is also recapitulated in strains carrying strong loss-of-function mutations in cep-1, such as the *lg12501* allele (A.K. Jolliffe *et al.*, manuscript in preparation). This meiotic nondisjunction phenotype suggests an ancestral role for the *TP53* family in maintaining genome stability. The mild embryonic lethality associated with cep-1 mutation is likely a result of autosomal nondisjunction, but this remains to be determined [9].

Apoptosis

Despite its modest effects on normal *C. elegans* development, cep-1 mutation was found to have profound effects on the germline in response to genotoxic stress. Various types of DNA damage induce apoptotic cell death in the late pachytene region of the meiotic germline (but not the soma) by engaging specific upstream sensors that recognize and transmit damage signals through a conserved checkpoint pathway [25] to activate CEP-1 [9, 10] (Figure 1). Simultaneously, DNA damage also transiently arrests division of mitotically proliferating germline stem cells and activates repair pathways that can presumably override apoptotic signals at lower levels of damage [25–28]. The resistance of somatic lineages to apoptotic cell death induced by genotoxic stress is due to the inability of these cells to activate checkpoint kinases such as the ataxia–telangiectasia-mutated (ATM) homolog ATM-1, which transduces damage signals in the germline to activate CEP-1 [29, 30]. Although CEP-1 is required for increased germline apoptosis after genotoxic stress it is dispensable for physiological germ cell death, which culls approximately 50% of meiotic germ cells, possibly to supply nutrients to the limited number of oocytes that will be fertilized [9, 10, 31]. cep-1 is also dispensable for developmentally programmed cell death in the soma, which occurs in a stereotypical fashion during the course of normal development by the activation of lineage-specific transcription factors [9, 10, 32]. These transcription factors, like CEP-1 in the germline, control the expression of the BH3-only protein EGL-1 to activate the core apoptosis pathway [33] (Figure 1). This requirement for the sole *C. elegans TP53* family member in DNA damage-induced apoptosis mirrors the role of *TP53* in mammalian cells challenged with genotoxic stress. Although mammalian *TP53* proteins can activate a number of pro-apoptotic proteins, including the BH3-only proteins NOXA and Puma [34, 35], CEP-1 seems to promote apoptosis exclusively through transactivation of EGL-1, although it can also activate expression of the EGL-1 paralog CED-13 [36]. The mammalian *TP53* paralogs *TP63* and *TP73* can also activate apoptosis in response to various forms of DNA damage, in some cases by transactivation of PUMA and Noxa [37–41]. Moreover, the induction of apoptosis in response to genotoxic agents is one of the most ancient and conserved roles of the *TP53* family and critical to their function as genome guardians [8]. However, in mammals the situation is complex because *TP63* and *TP73* cooperate to promote *TP53*-dependent apoptosis in some cell types [37] but not others

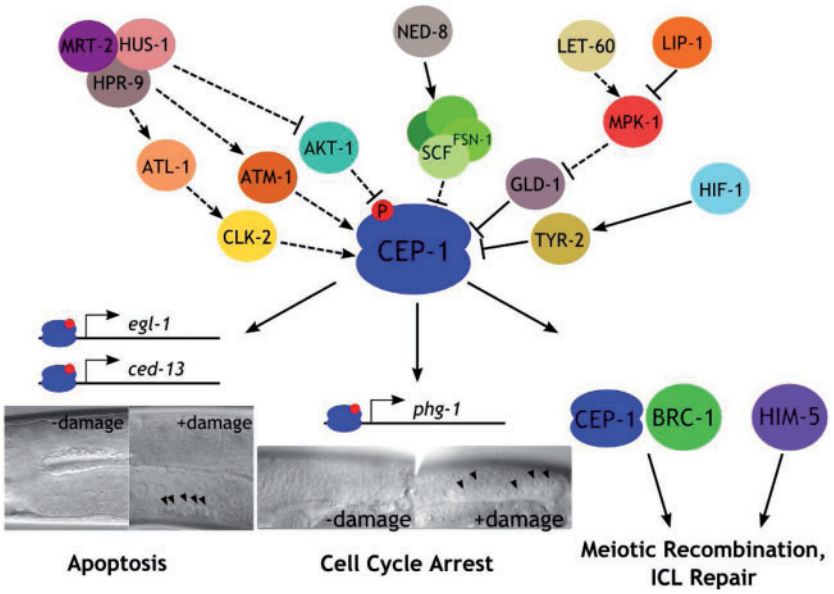

Figure 1: The CEP-1 signaling network in *C. elegans*. CEP-1 is activated by genotoxic stress through the DNA damage checkpoint consisting of the 9-1-1 sensor complex (HPR-9, MRT-2 and HUS-1), which transduces signals via the DNA damage response kinases ATL-1 and ATM-1 to activate CEP-1 by phosphorylation. CEP-1 integrates these signals through a variety of positive and negative regulators (described in the text) to activate apoptosis and cell cycle arrest. Direct interactions with CEP-1 are shown with solid lines and indirect interactions are indicated with broken lines. In the absence of exogenous stress, CEP-1 cooperates with the BRCA1 homolog BRC-1 and the meiotic protein HIM-5 to repair programmed DNA double-strand breaks generated during meiosis. The effects of CEP-1 in the *C. elegans* germline are shown in the images below. Arrowheads show apoptotic corpses in the pachytene region (left panel) and enlarged nuclei undergoing cell cycle arrest (middle panel) in the mitotic region of the wild-type (N2) germlines treated with UV irradiation. Not shown in this figure are parallel signaling pathways that cooperate with CEP-1 to promote apoptosis.

[42]. Given that *C. elegans* contains a single *TP53* family member that embodies properties of all three vertebrate proteins, analysis is greatly simplified in this organism.

Cell cycle regulation

Of equal importance in maintaining genome stability is the ability to sense and repair damage below thresholds that favor elimination of the damaged cell by apoptosis. Mammalian *TP53* can also induce cell cycle arrest or even long-term senescence in response to DNA damage, which was recognized before its apoptotic function was discovered. It was the cell cycle function of *TP53* that earned it the title 'guardian of the genome' [43, 44]. In *C. elegans*, DNA damage causes a transient cell cycle arrest in the mitotic (proliferating) region of the germline through a conserved checkpoint pathway [25]. At first, it was thought that cep-1 played no role in cell cycle arrest because ionizing radiation (IR)-induced cell cycle arrest is intact in cep-1 mutants [9, 10]. However, it was later realized that cep-1 was required for cell cycle arrest in the mitotic germline in response to ultraviolet (UV) irradiation, highlighting the importance of using other forms of genotoxic stress to understand the *in vivo* functions of this gene [12, 28]. Thus, CEP-1 can be thought of as "guardian of the *C. elegans* germline" similar to how *TP53* is regarded as guardian of the genome. In fact, *TP63* has a similar role as CEP-1 in protecting the female germline of mice from genotoxic insult [45].

In addition to a role for UV-induced cell cycle arrest in the mitotic germline, CEP-1 also appears to be capable of slowing the cycling of somatic cells during *C. elegans* development. Animals with mutations in the clk-2 checkpoint gene have drastically slower developmental rates during both embryogenesis and subsequent larval stages [46–48]. This developmental delay can be at least partially rescued by a mutation in cep-1, indicating the worm *TP53* family gene has a role in halting the cell cycle during somatic development, probably

due to endogenous replication stress caused by mutation of clk-2 [12, 49]. Because the rescue of developmental rate is not complete when cep-1 is ablated, there are likely other genes that function in parallel. *TP63* has been proposed to predate tumor suppressor *TP53* [2, 50] and can also induce cellular senescence, suggesting this may be another ancient function of the *TP53* family. However, it appears that senescence is a somewhat less conserved role for the *TP53* family than apoptosis [8, 51] and so far, no cellular senescence functions have been ascribed to cep-1 in *C. elegans*.

DNA repair

There is also some evidence that cep-1 plays a more direct role in DNA repair. Animals with mutation in cep-1 are hypersensitive to replication stress induced by hydroxyurea (HU) and damage by the alkylating agent *N*-ethyl-*N*-nitrosourea (ENU) [12, 52]. Though it is intriguing that cep-1 might promote DNA repair in response to these genotoxic agents, it remains possible that the protection conferred by cep-1 to HU and ENU is secondary to its roles in apoptosis. Because cep-1 mutants do not have elevated rates of spontaneous mutation (the mutator phenotype), nor are their offspring hypersensitive to IR-induced lethality, it is likely that the role of CEP-1 in DNA repair is specific to particular types of damage and/or cooperative functions with other DNA repair proteins. Genetic screens in *C. elegans* should therefore identify cooperating genes and pathways that cause synthetic lethality (or hypersensitivity to genotoxic agents) when ablated in combination with cep-1.

Using this approach, we recently discovered a role for cep-1 in meiotic homologous recombination (HR) and interstrand cross-link (ICL) repair that is independent of its apoptotic or cell cycle functions (A.K. Jolliffe *et al*., manuscript in preparation). In these DNA repair roles, cep-1 may have a similar function as the *C. elegans* homolog of the mammalian breast cancer type 1 susceptibility protein BRCA1. The BRCA1 tumor suppressor has known roles in promoting DNA repair, especially in HR downstream of DNA double-strand breaks and crosslinking agents in mammals [53, 54]. In *C. elegans*, the BRCA1 homolog BRC-1 promotes HR repair during meiotic recombination and in response to ICL reagents [55, 56]. Mammalian *TP53* and BRCA1 interact and cooperate in the transcriptional regulation of various DNA damage-responsive genes [57]. The similarity of cep-1 and brc-1 loss-of-function mutants, including a mild non-disjunction phenotype and hypersensitivity to ICL reagents, suggests that the *TP53* family may have ancient roles in repair as well as apoptosis.

EVOLUTION AND CONSERVATION OF CEP-1

As noted earlier, identification of the *C. elegans TP53*-like transcription factor CEP-1 went unrecognized for several years after its sequence became available. Despite low sequence homology to *TP53*, CEP-1 retains several key residues known as ''hot spots'' in human *TP53* because they are targeted for mutation in >50% of human tumors [9, 10]. Most of the hot spot mutations render the *TP53* protein transcriptionally inactive or generate neomorphic gain-of-function variants [58]. The crystal structure of the CEP-1 DNA binding domain revealed a remarkably well-conserved three-dimensional structure, indicating that sequence does not necessarily predict structural homology [59]. In support of a high degree of structural homology, CEP-1 was shown to bind *TP53* consensus sites on DNA in a yeast 1-hybrid assay and *in vitro* with recombinant protein [10, 59]. There are some key differences between CEP-1 and mammalian *TP53*, including highly conserved residues in CEP-1 that do not directly contact DNA [59]. Although mammalian *TP53* and its paralog *TP63* function as tetramers, CEP-1 appears instead to bind DNA as a dimer [60–63]. However, the mammalian *TP53* family can be thought of as a dimer of dimers that assemble into a tetrameric structure on DNA. The primary dimerization interface is well conserved between CEP-1 and *TP53* but the second interface that allows binding of the two dimers is absent in the worm protein [63]. Importantly, when the structure of the C-terminus of CEP-1 was solved it revealed a previously undetected sterile alpha motif domain, indicating that CEP-1 is structurally more similar to *TP63* and *TP73* than *TP53* [63]. This is in accordance with hypotheses about the evolutionary origin of the *TP53* gene family; the earliest *TP53* genes identified thus far are more similar to *TP63*/*TP73*, with the shorter *TP53* paralog appearing later in evolution and specific to vertebrates [2].

The recent identification of a *TP53* gene in the planarian flatworm *Schmidtea mediterranea* has revealed an important role in stem cell proliferation and

self-renewal that is not present in CEP-1 [64]. Ablation of *TP53* by RNAi in planarians causes their stem cell progeny to remain in an undifferentiated state, where they are proliferative [64]. Mammalian *TP53* regulates quiescence and self-renewal in a variety of mammalian stem cells [65–67], suggesting that the function of *TP53* in stem cell homeostasis is ancestral and lost in *C. elegans* and *Drosophila*. However, because *TP53* promotes compensatory proliferation in *Drosophila* [68], it is possible that Ecdysozoa have harnessed *TP53* to control self-renewal by mechanisms distinct from mammals and planarians to sculpt and repair damaged tissue. Although cep-1 is dispensable for the normal proliferation of germline stem cells (the only self-renewing cells in *C. elegans* adults), in mutants where their proliferation and differentiation are perturbed causing tumor-like growth, cep-1 appears to control some aspects of homeostasis [69, 70]. Organisms that contain a single member of the *TP53* family therefore offer key advantages for studying these mechanisms in a simplified genetic setting.

REGULATION OF CEP-1 ACTIVITY

Positive regulation

For effective genome integrity and growth, cells must keep *TP53* family proteins at low basal levels activating them only when genotoxic or other stresses are encountered. The most characterized positive regulators of CEP-1 function are the DNA damage checkpoint genes, particularly the 9-1-1 complex encoded by the homolog of *Schizosaccharomyces pombe* rad9$^+$gene hpr-9 [25, 71], the rad1$^+$ homolog mrt-2 (*mor*tal germline *2*) [72], and the *h*ydroxy*u*rea-*s*ensitive *1* (hus1$^+$) gene hus-1 [25, 71]. In parallel to the 9-1-1 checkpoint is clk-2, a homolog of human CLK2 [73, 74], which is also required to promote DNA damage-induced cell cycle arrest and cep-1-dependent apoptosis [25, 46]. In *C. elegans*, mutational inactivation of the 9-1-1 complex leads to both a mutator phenotype and complete abrogation of CEP-1-dependent apoptosis after DNA damage [71, 75]. Loss of clk-2 gives very similar phenotypes to *hus-1* or *mrt-2* loss-of-function [75]. However, CEP-1 must be able to be activated in the absence of CLK-2 because loss-of-function mutations in cep-1 partially rescue the slow development rate of clk-2 mutants [12, 49]. The exact mechanism by which either of these pathways signal to CEP-1 remains unclear but activation of CEP-1 by genotoxic stress is accompanied by increased phosphorylation and stabilization of endogenous protein, similar to its mammalian counterparts [52, 76–80].

One *TP53* family activation pathway that is conserved from worms to mammals involves the ataxia–telangiectasia and Rad3-related (ATR) kinase, called ATL-1 in *C. elegans*. ATL-1 is recruited to stalled replication forks after DNA damage and is required upstream of CLK-2 to promote both DNA repair and CEP-1-dependent apoptosis [30]. In mammalian systems, the ATR kinase is required for phosphorylation and activation of *TP53* in response to UV damage [81]. The related kinase ATM-1 (homologous to mammalian ATM) also promotes CEP-1-dependent apoptosis but only at low doses of UV [28]. Generally, the role for ATM-1 in activating CEP-1 appears to be less important compared with the activation of mammalian *TP53* by ATM. However, *C. elegans* atm-1 mutants have a mutator phenotype and a high incidence of spontaneous chromosomal fusions in germ cells, which are hallmark features of genomic instability [82]. Although genotoxic stress induces ATM-1/ATL-1 activity in the germline, these kinases do not appear to get activated (based on immunoreactive pS/TQ consensus sites) in somatic cells, which are resistant to IR-induced apoptosis [29]. Although most DNA damage response proteins are not detectably expressed in the soma, this tissue does express some genes involved in nonhomologous end joining and is capable of repairing damaged DNA [29]. Overall, there appears to be a selective silencing of the DNA damage response and cep-1-dependent apoptosis, but not repair, in the soma of *C. elegans*. It is currently unknown whether ATM-1 and/or ATL-1 directly phosphorylate CEP-1, which would be expected if their mechanisms of action were conserved from nematodes to mammals.

The first protein shown to affect CEP-1 phosphorylation was AKT-1, one of the *C. elegans* homologs of the Akt/PKB kinase, which functions downstream of the 9-1-1 checkpoint to dampen signaling to CEP-1 [77]. AKT-1 is a central component of the insulin/phosphoinositide 3-kinase (PI3K) signaling pathway, which has important roles in diverse biological processes, such as larval development, stress resistance, cell proliferation and organism lifespan [83, 84]. Interestingly, the insulin pathway promotes damage-induced germline apoptosis through AKT-2 and DAF-16/FOXO by modulating levels of Ras signaling downstream or independently of

CEP-1 [85]. Therefore, AKT-1 modulates checkpoint signaling to CEP-1 independent of its canonical role in the insulin/PI3K pathway. In the future, it will be important to identify which proteins AKT-1 normally phosphorylates to affect CEP-1 phosphorylation and dampen stress signals form the DNA damage checkpoint.

Negative regulation

Given their profound ability to induce cell death and cell cycle arrest, *TP53* family proteins must be kept under strict regulatory control for cell growth and proliferation to be maintained. Mammalian *TP53*, for example is under tight negative control by the E3 ubiquitin ligase Mdm2, which sequesters *TP53* and promotes its degradation through the proteasome [86, 87]. The importance of Mdm2 in the negative regulation of *TP53* is perhaps best exemplified by the rescue of embryonic lethality of $Mdm2^{-/-}$ mice by the simultaneous deletion of *TP53* [88, 89]. *TP63* and *TP73* are negatively regulated in similar as well as mechanistically distinct ways by ubiquitin ligases, including Mdm2 and Itch [90–92]. The *C. elegans* genome does not appear to contain an Mdm2 homolog required to keep CEP-1 levels in check [77]. Many ubiquitin ligases regulate the *TP53* family of proteins in mammals, and emerging evidence indicates that CEP-1-dependent apoptosis is also influenced by some of the same ligases in worms, but likely by different mechanisms. For example, the E3 ubiquitin ligase SCF^{FSN-1} negatively regulates the levels of phosphorylated CEP-1, but this appears to function through an indirect mechanism [52]. Interestingly, the mammalian counterpart of this ligase, SCF^{FBXO45}, negatively regulates *TP73* by polyubiquitin-mediated degradation [93].

Except for ubiquitous expression during normal embryonic development, the CEP-1 protein has generally low abundance, and appears to be restricted to a few pharyngeal cells in the soma and the germline of adult animals [9, 94]. It is interesting to note that CEP-1 is also expressed in male germ cells [52], which do not undergo physiological or stress-induced apoptosis [25, 31]. CEP-1 is activated in the male germline based on increased expression of egl-1 and ced-13, but the CED-3 caspase is not activated [95]. Perhaps CEP-1 has other functions in the male germline, such as promoting HR and/or DNA repair. As mentioned earlier, AKT-1 negatively regulates CEP-1 downstream of the 9-1-1 checkpoint by a mechanism that does not require activation of the canonical insulin/PI3K pathway [77]. Mammalian Akt also negatively regulates *TP53* and *TP73* by indirectly inhibiting their ability to activate apoptosis [96–98]. For example, Akt/PKB can phosphorylate and inhibit Bax, a direct transcriptional target of *TP53* [99, 100]. Akt/PKB can also phosphorylate and activate Mdm2, which increases the rate of *TP53* turnover [97, 101].

Another kinase that functions to keep CEP-1 in check is VRK-1, the *C. elegans* homolog of the vaccinia-related kinase [102]. Animals carrying mutations in vrk-1 show germline depletion that can be substantially rescued by mutation of cep-1, suggesting that the VRK-1 kinase inhibits the ability of CEP-1 to arrest cell cycle/proliferation in the absence of DNA damage. Homologs of VRK-1 have similar roles in maintaining proliferation in other systems, and mammalian Vrk1 can directly phosphorylate *TP53*, though this appears to stabilize the protein, an event that is usually associated with activation rather than repression of *TP53* family proteins [103, 104]. It will be important to sort out the complex regulation of CEP-1 by phosphorylation and determine which kinases directly control its *in vivo* activity in the future.

CEP-1 levels are also tightly regulated by translational repression through the RNA binding protein GLD-1, which represses the translation of numerous germline transcripts in *C. elegans* by directly binding their 3'-untranslated regions (UTRs) [94, 105]. gld-1 mutants have higher levels of endogenous CEP-1 protein and their germ cells undergo massive apoptosis that is rescued by a cep-1 mutation [94]. Recent reports have implicated post-transcriptional, 3'-UTR-dependent regulation of *TP53* in mammalian cells, suggesting this type of negative regulation may be conserved [106, 107]. Activation of MPK-1/extracellular-regulated kinase (ERK) in *C. elegans* by either LET-60/Ras or by loss of the LIP-1 phosphatase restricts GLD-1 levels in the germline, leading to CEP-1 activation and increased apoptosis after DNA damage [108]. Oncogenic Ras creates genotoxic stress that can activate *TP53*, and Ras activation coupled with *TP53* mutation commonly leads to malignancy [109]. Interestingly, hyperactivation of LET-60/Ras signaling requires the DAF-2 insulin-like receptor [85, 110]. Using *C. elegans* to study the relationship between oncogenes such as Ras (let-60) and the *TP53* family (cep-1) provides unique opportunities for exploring the cooperative roles of these important genes in a genetically tractable organism.

CEP-1 is also negatively regulated indirectly by HIF-1, the *C. elegans* homolog of the mammalian HIF transcription factor [80]. HIF is induced by conditions of low oxygen and regulates various cellular responses to this type of stress [111]. In the worm, HIF-1 upregulates the TYR-2 tyrosinase, which is secreted from head sensory neurons and antagonizes CEP-1 in the germline [80]. In mammals, HIF and the *TP53* family have a complex interrelationship, with HIF activation sometimes activating and sometimes repressing *TP53* activity, depending on cell type and perhaps on differential expression of various *TP53* family isoforms in different cell types [112–117]. The mammalian homolog of the TYR-2 tyrosinase, TRP2 has been shown to have anti-apoptotic activity in human melanomas, perhaps by antagonizing one of the mammalian *TP53* family members by a similar mechanism to that seen in *C. elegans* [118].

The inhibitor of apoptosis stimulating protein of *TP53* (iASPP) APE-1 physically interacts with CEP-1 and inhibits cep-1-dependent apoptosis in the germline, demonstrating a conserved function between worms and mammals [119]. More recently, iASPP has been shown to regulate *TP63* through a feedback loop involving microRNAs that control *TP63* translation during skin development [120]. Various other genes also negatively regulate CEP-1, though the mechanisms of action of these factors are less clear. One of these is the c-Abl homolog ABL-1, which negatively regulates cep-1-dependent apoptosis in response to IR but not alkylating reagents [121]. Interestingly, c-Abl regulates the apoptotic activity of *TP73* in mammalian cells, including the DNA cross-linking reagent cisplatin [122, 123]. Collectively, studies of CEP-1 regulation in *C. elegans* suggest that this ancestral *TP53*-like protein has properties similar to all three vertebrate family members.

DOWNSTREAM TARGETS

Like its family members in mammals, CEP-1 was shown to function as a transcription factor [10, 59, 63]. In accordance with its structural similarity to the *TP53* DNA binding domain, CEP-1 can bind and activate transcription from *TP53* DNA binding sites, where CEP-1 and *TP53* have nearly identical preferences for individual nucleotides in their consensus binding sequences [10, 59]. Moreover, CEP-1 likely performs its biological functions by regulating the expression of various genes, and to date there have been no non-transcriptional roles for CEP-1 reported.

In an effort to detect CEP-1 responsive genes, two microarray studies were carried out in cep-1 mutant animals challenged with different forms of genotoxic stress. Transcriptional profiling of genes regulated by UV irradiation revealed a large number of putative CEP-1 targets that overlap with mammalian *TP53*, *TP63* and *TP73* transcriptional targets, suggesting again that CEP-1 has similarities to all three *TP53* family members [12]. In contrast, another study using IR as a source of stress revealed only a few CEP-1 transcriptional targets [124]. It is likely that there are more than a few transcriptional targets of CEP-1 given the hundreds of genes identified for mammalian *TP53* family members. More direct methods such as ChIP or deep sequencing should help to resolve these apparent contradictions. In addition, different forms of stress will likely direct CEP-1 to distinct sets of target genes, similar to what has been reported for the vertebrate *TP53* family of proteins [125–127].

Some direct targets of CEP-1-dependent transcriptional regulation are known. The *C. elegans* core apoptotic pathway begins with EGL-1, a BH3-only containing protein that is required for most apoptotic events in this organism, including those induced by DNA damage in the germline [25, 33, 128, 129]. CEP-1 function is required for egl-1 mRNA upregulation after genotoxic stress, mirroring the requirement for both CEP-1 and EGL-1 in DNA damage-induced apoptosis [71]. Although EGL-1 accounts for the vast majority of cell death regulation by this transcription factor, CEP-1 can also regulate expression of the other *C. elegans* BH3-only domain gene, ced-13 [36]. This is similar to the mammalian pathway, where all three *TP53* family members can transcriptionally regulate the expression of BH3-only domain proteins NOXA and PUMA in various tissues to induce apoptosis in response to various cellular stresses or developmental cues [34, 35, 41, 130]. Transcriptional activation of BH3-only domain proteins therefore appears to be a highly conserved mechanism for promoting cell death over a wide diversity of organisms.

The function of CEP-1 in regulating UV-dependent cell cycle arrest in the mitotic germline may be less well conserved. In mammalian cells, *TP53* halts the cell cycle mainly by transcriptional activation of the cyclin-dependent kinase inhibitor (CKI) p21/Cip1/Waf1 [131–133]. *C. elegans* contains

two CKIs, cki-1 and cki-2, both with roles in developmental cell cycle regulation in specific cell types, but neither are required for the transient arrest of mitotically proliferating germ cells in response to genotoxic stress, and neither appear to be transcriptionally regulated by CEP-1 [12, 71, 124, 134, 135]. One gene that is required for mitotic germ cell arrest after DNA damage is phg-1, the *C. elegans* homolog of the mammalian Gas1 cell cycle regulator [12, 136]. phg-1 contains *TP53* responsive elements in both its promoter and intronic sequences, and is upregulated in a CEP-1-dependent manner after UV stress [12]. The slow growth of clk-2 mutants can be partially rescued by ablation of cep-1 or phg-1, suggesting that PHG-1 may mediate the function of CEP-1 in somatic cell cycle regulation as well [12]. Gas1 is regulated in a *TP53*-dependent manner, but this is apparently independent of *TP53*'s transactivation function, suggesting the *TP53*-Gas1 pathway of cell cycle arrest induction may be functionally but not mechanistically conserved [137]. However, inspection of the Gas1 promoter and introns reveals several putative *TP53*-binding sites (W.B. Derry, unpublished data).

Given that no transcriptional targets have been identified for CEP-1 that account for its other functions, such as DNA repair and organismal aging, more work remains to be done to elucidate how CEP-1 controls these biological processes. *TP53* family members in mammals have also been shown to have direct, non-transcriptional roles in maintaining genomic stability and even promoting apoptosis, and it remains possible that CEP-1 has similar transcription-independent roles [138–140]. Transcription-dead mutants, either created by random mutagenesis or by expression of mutated transgenes in strong cep-1 loss-of-function backgrounds, would certainly help to answer these questions.

EMERGING ROLES FOR CEP-1

CEP-1 has been linked to other biological processes in *C. elegans*, including germline tumor proliferation and innate immunity [69, 141]. Mutations in the KH domain containing RNA binding protein GLD-1 cause meiotic germ cells to re-enter mitosis and develop a hyperplastic 'tumor' phenotype [105]. Although no genomic instability has been reported with these tumorous germlines, such as gross chromosomal rearrangements, the hyperplastic phenotype has been shown to be suppressed by mutations in components of the insulin signaling pathway that increase lifespan [69]. It was argued that hypomorphic mutations in the DAF-2 insulin receptor that extend lifespan by increasing expression of DAF-16/FOXO target genes directly or indirectly promote CEP-1-dependent apoptosis [69]. However, recent evidence indicates that neither DAF-16 nor DAF-2 affect physiological apoptosis in the germline [77, 85, 142]. In contrast, DAF-2 is required to promote DNA-damage-induced apoptosis through DAF-16 but independent (or downstream) of CEP-1 [85]. Reduction of DAF-2 signaling by RNAi or mutation does not prevent germ cells from exiting meiosis, but it does suppress phosphorylation of the ERK, known as MPK-1 in the worm, by downregulation of Ras signaling [85]. Thus, it is possible that the effects of *daf-2* mutation on germ cell proliferation and apoptosis could be evident only in the context of tumorous gemlines.

Other longevity-associated genes have also been shown to regulate germline apoptosis independently of cep-1. For example, the sirtuin SIR-2.1, a histone deacetylase, was also reported to promote damage-induced germline apoptosis independently of CEP-1 transcriptional activation, possibly through an effect on CED-4/Apaf-1 [142]. However, SIR-2.1 does not appear to function through the insulin signaling pathway in this regard (M. Hall and W.B. Derry, unpublished data). The inhibitor of growth protein ING-3, a member of a protein family that binds histone modifying enzymes in mammals to regulate *TP53* [143–145], was shown to promote IR-induced germline apoptosis through the CEP-1 pathway, but it was not reported whether it affected the transcriptional activity of CEP-1 [146]. In addition, the kri-1 gene (an ortholog of human CCM-1/Krit1) is required to promote increased lifespan of worms sterilized by germline ablation by controlling the nuclear localization of DAF-16 [147]. kri-1 is also required to promote damage-induced germline apoptosis (but not physiological germline apoptosis) by a non-cell autonomous mechanism that is independent of CEP-1, SIR-2.1 and DAF-16 [148]. The retinoblastoma complex promotes physiological and damage-induced germline apoptosis downstream of, or in parallel to, CEP-1 by controlling the transcription of core apoptosis genes [149]. The EEL-1 ubiquitin ligase, a homolog of the mammalian *TP53*-directed ligase Huwe1, is also required to promote IR-induced germline apoptosis by a mechanism

that is also independent of CEP-1 [150]. The model emerging from these studies is that CEP-1 promotes germline apoptosis in cooperation with a network of proteins that operate in parallel signaling pathways to influence the core apoptosis machinery in the germline.

CEP-1: WHAT THE WORM CAN TEACH US ABOUT THE *TP53* FAMILY

Since its discovery, CEP-1 has led to important insights about *TP53* family function throughout evolution and has provided an important model for *TP53* family function in mammals. Sometimes, understanding of CEP-1 has confirmed what we already know from mammals, supporting the notion that DNA damage response is well conserved and that *C. elegans* can be an important model for these processes. At other times, knowledge of CEP-1 regulation and function has driven research in other systems, leading to new understanding of the *TP53* family in mammals [93]. *C. elegans* allows us to study *TP53* family function in the context of a multicellular organism, using all of the tools developed by the worm community over the last 40 years. Greater understanding of CEP-1, its roles and its regulation will hopefully lead to insights which generate hypotheses to be tested in the more complex mammalian *TP53* family landscape, with its multiple paralogs, cell types and disease states.

Key Points

- The nematode worm *C. elegans* contains a single *TP53*-like gene, cep-1, that is activated in response to genotoxic stress by a conserved DNA damage checkpoint signaling pathway.
- The CEP-1 protein shares structural homology with the more ancestral vertebrate paralogs, *TP63* and *TP73*, but controls biological processes in common with all three vertebrate proteins.
- Activation of CEP-1-dependent apoptosis requires parallel signaling from autonomous and nonautonomous pathways.
- Emerging studies implicate roles for CEP-1 in lifespan control and developmental timing.

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