Cdt1 degradation to prevent DNA re-replication: conserved and non-conserved pathways
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Abstract
In eukaryotes, DNA replication is strictly regulated so that it occurs only once per cell cycle. The mechanisms that prevent excessive DNA replication are focused on preventing replication origins from being reused within the same cell cycle. This regulation involves the temporal separation of the formation of the pre-replicative complex (pre-RC) from the initiation of DNA replication. The replication licensing factors Cdt1 and Cdc6 recruit the presumptive replicative helicase, the Mcm2-7 complex, to replication origins in late M or G1 phase to form pre-RCs. In fission yeast and metazoa, the Cdt1 licensing factor is degraded at the start of S phase by ubiquitin-mediated proteolysis to prevent the reassembly of pre-RCs. In humans, two E3 complexes, CUL4-DDB1CDT2 and SCFSkp2, are redundantly required for Cdt1 degradation. The two E3 complexes use distinct mechanisms to target Cdt1 ubiquitination. Current data suggests that CUL4-DDB1CDT2-mediated degradation of Cdt1 is S-phase specific, while SCFSkp2-mediated Cdt1 degradation occurs throughout the cell cycle. The degradation of Cdt1 by the CUL4-DDB1CDT2 E3 complex is an evolutionarily ancient pathway that is active in fungi and metazoa. In contrast, SCFSkp2-mediated Cdt1 degradation appears to have arisen relatively recently. A role for Skp2 in Cdt1 degradation has only been demonstrated in humans, and the pathway is not conserved in yeast, invertebrates, or even among other vertebrates.

Cdt1 degradation and the control of DNA replication
To maintain genome integrity, DNA replication must be strictly regulated to occur only once per cell cycle. Replication is, therefore, tightly regulated to prevent the re-initiation of DNA replication within the same S phase. A failure to restrict DNA replication results in 're-replication', in which the genome is over-replicated within the same cell cycle via origin re-firing. In eukaryotes, the extent of DNA replication is controlled by temporally restricting the assembly of the pre-replicative complex (pre-RC) through a process termed 'replication licensing' (reviewed in [1,2]). Pre-RCs form on replication origins through the sequential binding of DNA replication proteins during late mitosis or G1 phase. The six-member origin recognition complex (ORC) binds replication origins on newly-synthesized chromatin. During late mitosis or G1 phase, the replication licensing factors Cdt1 and Cdc6 are recruited to the origin. Cdt1 and Cdc6 together load the presumptive replicative helicase, the Mcm2-7 complex, onto the origin to complete pre-RC formation. During S phase, pre-RCs are activated by phosphorylation via CDK and DDK (Dbf4-dependent kinase) activity. This phosphorylation allows the recruitment of essential replication
In humans, two distinct E3 complexes, CUL4-DDB1 and SCFSkp2, have been reported to target Cdt1 for ubiquitin-mediated degradation. Both of these E3s are members of the cullin-RING ligase (CRL) class of ubiquitin ligases. The two E3 complexes utilize distinct mechanisms for targeting Cdt1 ubiquitination. In this review, we will focus on the regulation of Cdt1 degradation in different species and explore the conservation of pathway components and mechanisms across species and phyla.

### The CUL4-DDB1 complex targets Cdt1 for degradation

Studies in *C. elegans* first suggested the involvement of CUL4 in Cdt1 degradation. The inactivation of the *C. elegans* *cul-4* gene by RNAi causes proliferating cells to arrest in S phase and undergo massive levels of DNA reaplication [17]. The DNA content of the re-replicating cells increases up to 100 C (where 2 C is the normal diploid DNA content). In *C. elegans*, as in vertebrates and fission yeast, CDT-1 is degraded as cells enter S phase [17]. However, when *cul-4* is inactivated, CDT-1 is not degraded in S phase, but instead accumulates in the re-replicating cells [17]. Reduction of CDT-1 levels by half abolishes the re-appearance in *cul-4* RNAi cells, indicating that CDT-1 accumulation is a critical factor in causing the re-replication. This work showed that CUL-4 negatively regulates CDT-1 levels, but did not address whether CDT-1 is a direct target of the *CUL-4* complex. It was subsequently shown in humans, *Xenopus*, fission yeast, and *C. elegans* that the CUL4 ubiquitin ligase directly mediates Cdt1 degradation during S phase [13,16,21,22].

In humans and *Drosophila*, Cdt1 is rapidly degraded in response to DNA damage induced by UV- or γ-irradiation, presumably to prevent DNA replication until the DNA damage can be repaired [23]. CUL4 is also required for this Cdt1 degradation pathway [23,24]. The CUL4-mediated degradation of Cdt1 upon DNA damage occurs independently of DNA replication or the classic DNA damage pathway that includes the ATM/ATR and CHK1/CHK2 kinases [23]. The CUL4-dependent Cdt1 degradation in response to DNA damage can occur throughout the cell cycle (in G1, S, and G2 phases) [13,22-24]. Given the cell cycle-independent nature of the degradation, it is fair to ask whether the degradation is simply to prevent DNA replication in S phase or whether there is an additional cell cycle-independent role.

### The modular structure of CUL4-DDB1 ubiquitin ligase complex

The cullin-RING ubiquitin ligase (CRL) complexes represent the largest super-family of multisubunit E3s in eukaryotes [25]. The prototype of the CRL is the SCF complex, which comprises: the cullin CUL1 (which forms a rigid scaffold); the RING-H2 finger protein Roc1/Rbx1/Hrt1 (which is bound to the C-terminus of CUL1 and facilitates ubiquitin conjugating enzyme loading and activation); the adaptor protein Skp1 (which is bound to the
Two distinct molecular pathways for Cdt1 degradation. (A and B) CUL4-DDB1

Figure 1

CUL4-DDB1 and SCF

CDT2

CUL4

DDB1

CDT2

SCF

Skp2

Cyclin

CDK

Cdt1

PCNA

DNA

CDT2 and PCNA are required for Cdt1 degradation

The DCAF family is predicted to contain ~90 members in mammals, of which at least 49 have been shown to physically interact with CUL4 or DDB1, mainly by co-expression/co-IP in mammalian cells [27,33-35]. Five members of the DCAF family are known to function as SRSs, and the functions of their respective complexes are as follows. CUL4-DDB1CSA targets the degradation of the nucleotide excision repair protein CSB [36]. CUL4-DDB1WD40CSA, which includes a dimeric SRS consisting of hDET1 and hCOP1, targets the degradation of the transcription factor c-jun [31]. CUL4-DDB1VprBp is known to be hijacked by the Vpr protein of the human immunodeficiency virus (HIV) to induce cell cycle arrest [37]. CUL4-DDB1DD2 mediates stable ubiquitin modifications of histones H2A, H3 and H4 and the nucleotide excision repair protein XPC [38-40]. Finally, CUL4-DDB1CDT2 has been implicated in the degradation of mammalian p53, fission yeast Spd1, and Cdt1 (described below) [22,26,29,33,41,42]. It is likely that there will be additional CUL4-DDB1 complexes containing different DCAF proteins that function in a wide-range of molecular and physiological processes.
fied human Cdt1 can bind directly to purified DDB1 [24]. Further, in vitro translated C. elegans DDB-1 made with a wheat germ extract binds to bacterially-produced recombinant GST-CDT-1 [16]. The C. elegans experiments, however, do not rule out the possibility that a plant protein from the wheat germ extract bridges the interaction between DDB-1 and CDT-1. Additional experiments will be required to clarify exactly how the CUL4-DDB1CDT2 complex binds to Cdt1.

In Xenopus egg extract, the degradation of Cdt1 by CUL4-DDB1CDT2 in S phase requires the interaction between Cdt1 and PCNA [18,33] (Fig. 1A). Cdt1 binds PCNA through a PCNA-interacting protein (PIP) box motif in the Cdt1 N-terminus [18]. The PIP box is also required for the CUL4-DDB1-mediated degradation of Cdt1 during S phase in humans and C. elegans [13,16,43]. The degradation of Cdt1 in response to UV irradiation has similarly been shown to require the association of Cdt1 with PCNA in humans, Drosophila, and fission yeast [13,18,26,43,44]. This suggests that the two distinct Cdt1 degradation events, occurring in response to DNA damage or S-phase entry, are triggered by the same molecular signal: Cdt1 binding to chromatin-associated PCNA. PCNA forms a trimeric ring structure that is loaded onto DNA during both DNA replication and DNA repair [45,46]. One can hypothesize that chromatin-loaded PCNA (potentially in conjunction with other factors) is sufficient to promote Cdt1 binding and its subsequent degradation.

**SCFSkp2 functions redundantly with CUL4-DDB1CDT2 to degrade Cdt1 in humans**

In humans, the SCFSkp2 E3 complex also targets Cdt1 for degradation. Human Cdt1 is phosphorylated by cyclin-CDK complexes, and the phosphorylation is dependent on a cyclin-binding (Cy) motif within Cdt1 [47,48]. The phosphorylation on threonine 29 within the N-terminus of Cdt1 is required for its interaction with Skp2 [49]. Mutating the N-terminal CDK-phosphorylation sites of Cdt1 increases its half-life in asynchronous human cells [47]. Similarly, siRNA depletion of Skp2 increases the level of Cdt1 in asynchronous human cells [13,50]. These results indicate that SCFSkp2 regulates Cdt1 levels in response to CDK-phosphorylation (Fig. 1B).

There have been differing reports on the effect of inactivating the SCFSkp2 pathway on Cdt1 levels in human S-phase cells. One study indicated that Skp2 was required to allow S-phase degradation of Cdt1 [50]. A second study indicated that mutation of the cyclin-binding motif of Cdt1 (which prevents Skp2 binding) does not block the majority of Cdt1 degradation in S phase, although higher residual levels of Cdt1 protein are observed in S-phase cells [48]. Finally, three other reports indicate that inactivation of the SCFSkp2-mediated Cdt1 degradation pathway does not stabilize Cdt1 during S phase [13,43,49]. Recent work has clarified these apparent contradictions by showing that in human cells both SCFSkp2 and CUL4-DDB1CDT2 pathways redundantly target Cdt1 for degradation during S phase [13,43].

Analysis of published results suggests that SCFSkp2 mediates Cdt1 degradation throughout the cell cycle. This conclusion is based on the observation that Skp2 siRNA depletion in asynchronous cells leads to a three-fold increase in Cdt1 levels, even though Skp2 siRNA treatment does not affect S- or G2-phase levels of Cdt1 (because Cdt1 is still targeted for degradation by CUL4-DDB1CDT2) [13]. This implies that Cdt1 levels must increase in non-S- or G2-phase cells upon Skp2 siRNA treatment (presumably G1 phase cells). As described above, Skp2 redundantly targets Cdt1 for degradation during S and G2 phases. Therefore, it can be concluded that Skp2 targets Cdt1 degradation throughout the cell cycle. In contrast, CUL4-DDB1-mediated Cdt1 degradation is S-phase specific [13].

**Cdt1 degradation in other metazoans and yeast**

The roles of Skp2 and CUL4 in degrading Cdt1 have also been explicitly compared in C. elegans. Inactivation of C. elegans cul-4 or ddb-1 fully stabilizes CDT-1 during S phase [16,17]. In contrast, the C. elegans Skp2 homolog, skpt-1, does not contribute to CDT-1 degradation or re-division even in a sensitized ddb-1 mutant background [16]. skpt-1 null mutant homozygotes are completely viable and appear overtly wild-type with the exception of a low-penetration gonad migration defect, indicating that the gene is not required for any essential functions [16].

In Xenopus egg extract, CDK-phosphorylation of Cdt1 is not required for Cdt1 degradation [21]. This implies that SCFSkp2 is not required for Cdt1 degradation because CDK-phosphorylation of Cdt1 is a prerequisite for recognition by human Skp2 [47,48], and most SCFSkp2 substrates must be phosphorylated to be recognized [25]. In contrast, CUL4-DDB1 is essential for Cdt1 degradation in Xenopus egg extract, with DDB1 depletion blocking Cdt1 degradation during S phase [18]. These results suggest that CUL4-DDB1CDT2 is the predominant E3 for Cdt1 degradation in Xenopus, and that SCFSkp2 either has no role or has only a minor, subservient role in Cdt1 degradation.

In Drosophila, mutation of all of the N-terminal CDK-phosphorylation sites of Cdt1 is not able to block S-phase degradation, although it does provide a limited increase in overall stability [51]. This indicates that a phosphorylation-dependent pathway (and by implication SCFSkp2) either is not involved or is redundant for Cdt1 degradation during S phase in Drosophila. There are currently no reports on the function of the fly Skp2 homolog.
Fission yeast does not have a recognizable Skp2 homolog, but does express the CUL4-DDB1 \textit{CDT2} complex. Fission yeast CUL4-DDB1 \textit{CDT2} is essential for the degradation of Cdt1 during S phase and in response to DNA damage, indicating that it is the dominant pathway for regulating Cdt1 levels [22]. Taken together, these studies suggest that SCFSkp2-mediated degradation of Cdt1 is not conserved in non-mammalian species (Table 1).

**Is the SCFSkp2-dependent Cdt1 degradation pathway conserved in mice?**

The studies described above suggest that SCFSkp2-mediated degradation of Cdt1 is not conserved in yeast, invertebrates, or even the vertebrate Xenopus laevis. It is therefore valid to ask whether SCFSkp2-mediated Cdt1 degradation is conserved among mammals; and in fact, there is evidence that casts doubt on the conservation of the pathway in mice. Inactivation of Skp2 by siRNA treatment in human cells leads to an approximately three-fold increase in the steady state level of Cdt1 [13,50]. However, Skp2 \textsuperscript{-/-} knockout mice or Skp2 \textsuperscript{-/-} MEFs (mouse embryonic fibroblasts) do not have elevated levels of Cdt1 [13,52]. In contrast, DDB1 \textsuperscript{-/-} knockout mice have elevated Cdt1 levels in proliferating tissues [53]. Further, Cdt1 protein level is stabilized after UV-irradiation in DDB1 \textsuperscript{-/-} MEFs [53]. These results indicate that in mice, the CUL4-DDB1 complex is required non-redundantly for proper Cdt1 degradation during normal cell cycle progression and in response to DNA damage; in contrast, loss of Skp2 does not perturb these processes.

It is interesting that Skp2 \textsuperscript{-/-} knock-out mice are completely viable and fertile [54]. This is particularly striking in light of the long list of human Skp2 substrates, including important cell cycle and transcriptional regulators: Cdt1, Orc1, p27\textsuperscript{Kip1}, p21\textsuperscript{Cip1}, cyclin E, cyclin D, cyclin A, c-Myc, b-Myb, p130/pRB2, E2F-1, p57\textsuperscript{Kip2}, MKP-1, RAG-2, FOXO1, and Cdk9 [47,48,50,54-73]. Although Skp2 \textsuperscript{-/-} mice are viable, they exhibit a minor defect of polyploidy and extra centrosomes in the cells of a few tissues [54]. Both of these defects arise as secondary consequences of a failure of these cells to enter mitosis, with the affected cells subsequently re-entering the next cell cycle and duplicating their DNA and centrosomes [52]. Significantly, the mitotic defect is suppressed by co-inactivation of p27\textsuperscript{Kip1}, suggesting that the inability to degrade p27\textsuperscript{Kip1} causes the defect [52]. The lack of phenotypes associated with a failure to degrade other potential substrates suggests either that they are not substrates in mice, that their degradation is not important for development, or that they are under redundant control with other degradation pathways. Taken together, the available evidence suggests that CUL4-DDB1 \textit{CDT2} is the predominant ubiquitin ligase to mediate Cdt1 degradation in mice, and that SCFSkp2 either does not target Cdt1 for degradation or does so only as a minor pathway that cannot compensate for loss of CUL4-DDB1 \textit{CDT2}.

**When did genes for the two Cdt1-degradation pathways arise during evolution?**

To determine when the genes for the CUL4-DDB1 \textit{CDT2} and SCFSkp2 complexes arose during evolution, we analyzed divergent species using reciprocal BLAST searches [74]. We limited our analysis to those organisms in which the whole genome had been sequenced, so that a failure to detect a gene would be meaningful. Cullin genes were not found in bacteria or archaea, but at least two cullins were found in all of the eukaryotic genomes that we examined (Table 2). The observation of cullins in protists suggests that the cullin gene family arose early in the eukaryotic lineage (Table 2, Fig. 2). All eukaryotic species examined contain cullins that were most similar to metazoan CUL1 and CUL4 in reciprocal BLAST analysis, with the exception of budding yeast (which lacks a CUL4-like gene) (Table 2). This suggests that an ancestral duplication that gave rise to CUL1-like and CUL4-like genes occurred early in eukaryotic evolution. This result matches a phylogenetic analysis of cullins, in which the first branch point of the cullin phylogeny creates two clades, with the first clade giving rise to CUL1, CUL2 and CUL5, and the second clade giving rise to CUL3 and CUL4 [75]. The adaptor proteins Skp1 and DDB1 are present whenever CUL1-like and CUL4-like genes are observed, suggesting that the association between the cullins and their adaptor proteins is ancient (Table 2, Fig. 2).

The substrate-specific components CDT2 and Skp2 appear to have arisen at different points in eukaryotic evolution. CDT2 is observed in all animals analyzed, and a majority of fungi and plants, but is not observed in protists (Table 2, Fig. 2). This suggests that CDT2 arose in the main ancestral eukaryote lineage after the protist lineages were divergent species using reciprocal BLAST searches [74]. We limited our analysis to those organisms in which the whole genome had been sequenced, so that a failure to detect a gene would be meaningful. Cullin genes were not found in bacteria or archaea, but at least two cullins were found in all of the eukaryotic genomes that we examined (Table 2). The observation of cullins in protists suggests that the cullin gene family arose early in the eukaryotic lineage (Table 2, Fig. 2). All eukaryotic species examined contain cullins that were most similar to metazoan CUL1 and CUL4 in reciprocal BLAST analysis, with the exception of budding yeast (which lacks a CUL4-like gene) (Table 2). This suggests that an ancestral duplication that gave rise to CUL1-like and CUL4-like genes occurred early in eukaryotic evolution. This result matches a phylogenetic analysis of cullins, in which the first branch point of the cullin phylogeny creates two clades, with the first clade giving rise to CUL1, CUL2 and CUL5, and the second clade giving rise to CUL3 and CUL4 [75]. The adaptor proteins Skp1 and DDB1 are present whenever CUL1-like and CUL4-like genes are observed, suggesting that the association between the cullins and their adaptor proteins is ancient (Table 2, Fig. 2).

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**Table 1: Cdt1 degradation directed by CUL4-DDB1 \textit{CDT2} and SCFSkp2 in different species.**

| Species          | CUL4-DDB1 \textit{CDT2} | SCFSkp2 |
|------------------|--------------------------|---------|
| Human            | Yes                      | Yes     |
| Mice             | Yes                      | (No)\textsuperscript{a} |
| Frogs            | Yes                      | No      |
| Flies            | Yes                      | ?\textsuperscript{b} |
| Nematodes        | Yes                      | No      |
| Fission yeast    | Yes                      | No\textsuperscript{c} |

\textsuperscript{a} Available evidence suggests that SCFSkp2 does not directly target Cdt1 degradation in mice, however direct experiments have not been performed (see text).

\textsuperscript{b} No published studies have addressed the role of Skp2 in Drosophila.

\textsuperscript{c} Fission yeast lack a recognizable Skp2 homolog. See text for references and discussion.
diverged, but prior to the generation of plants. In contrast, Skp2 apparently arose later in evolution. Skp2 is present in animals, but is not detected in fungi or plants, suggesting that it arose after the branching of fungi from the main eukaryotic lineage but prior to the genesis of metazoa (Table 2, Fig. 2). This analysis implies that CDT2, and by extension the CUL4-DDB1CDT2 complex, is more ancient than Skp2 and the SCF<sup>Skp2</sup> complex.

The finding that the CUL4-DDB1<sup>CDT2</sup> complex targets Cdt1 for degradation in fission yeast and C. elegans, while SCF<sup>Skp2</sup> does not, suggests that the CUL4-DDB1<sup>CDT2</sup> pathway is the ancient, conserved pathway for controlling the extent of DNA replication via Cdt1 degradation. A prediction of this hypothesis is that yeast or metazoan species that have lost genes for the CUL4-DDB1CDT2 complex would have to employ a different strategy to restrict Cdt1 activity during S phase. In this regard, it should be noted that budding yeast (unlike other fungi) does not contain CUL4, DDB1, or CDT2 (Table 2). Strikingly, budding yeast employ a strategy for regulating Cdt1 that has not been observed in any other species: Cdt1 is exported from the nucleus with the Mcm2-7 complex rather than being degraded [3]. The fungal ancestor of budding yeast must have originally had the genes for the CUL4-DDB1CDT2 complex and then lost them, because the genes are found diverged, but prior to the genesis of plants. In contrast, Skp2 apparently arose later in evolution. Skp2 is present in animals, but is not detected in fungi or plants, suggesting that it arose after the branching of fungi from the main eukaryotic lineage but prior to the genesis of metazoa (Table 2, Fig. 2). This analysis implies that CDT2, and by extension the CUL4-DDB1CDT2 complex, is more ancient than Skp2 and the SCF<sup>Skp2</sup> complex.

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Table 2: Conservation of CUL4-DDB1<sup>CDT2</sup> and SCF<sup>Skp2</sup> components in prokaryotic and eukaryotic species.

| Group      | Phylum or Division | Species                     | Cullins* | DDB1 | CDT2 | Skp1 | Skp2 |
|------------|--------------------|-----------------------------|----------|------|------|------|------|
| Eubacteria |                    |                             |          |      |      |      |      |
| Fimbriae   |                    |                             |          |      |      |      |      |
| Firmicutes |                    |                             |          |      |      |      |      |
| Proteobacteria |                |                             |          |      |      |      |      |
| Archaea    |                    |                             |          |      |      |      |      |
| Crenarchaeota |                |                             |          |      |      |      |      |
| Euryarchaeota |               |                             |          |      |      |      |      |
| Protist    |                    |                             |          |      |      |      |      |
| Apicomplexa|                    |                             |          |      |      |      |      |
| Euglenozoa |                    |                             |          |      |      |      |      |
| Slime Mold |                    |                             |          |      |      |      |      |
| Amoebozoa  |                    |                             |          |      |      |      |      |
| Plant      |                    |                             |          |      |      |      |      |
| Magnoliophyta |                |                             |          |      |      |      |      |
| Fungi      |                    |                             |          |      |      |      |      |
| Ascomycota |                    |                             |          |      |      |      |      |
| Basidiomycota |               |                             |          |      |      |      |      |
| Invertebrate|                    |                             |          |      |      |      |      |
| Nematoda   |                    |                             |          |      |      |      |      |
| Arthropoda |                    |                             |          |      |      |      |      |
| Vertebrate |                    |                             |          |      |      |      |      |
| Chordata   |                    |                             |          |      |      |      |      |

A recognizable homolog in the species is designated by a check mark, and the absence of a homolog by a dash.

* The number of cullins is recorded in each organism. The metazoan cullin (CUL1-CUL5) for which each cullin is most related (by reciprocal BLAST analysis) is indicated in parentheses (e.g., 2 (1, 4) = two cullins that are most related to CUL1 and CUL4, respectively). The divergent CUL7 is listed separately for humans.

**For Danio rerio (zebrafish), genes that were predicted to encode cullin proteins of less than 100 amino acids were not included.
Figure 2
The genesis of CUL4-DDB1\textsuperscript{CDT2} and SCFSkp2 E3 components. CUL4-DDB\textsuperscript{CDT2} and SCFSkp2 complex components were examined in representative organisms of diverse phyla (Table 2). A phylogenetic tree of the taxa analyzed, from euubacteria to mammals, is presented. Note that distances between branches are not to scale. Species and major classifications are color-coordinated, and the temporal locations of the presumed origins of E3 component genes are in red. CUL1-like and CUL4-like, as well as their adaptor proteins DDB1 and Skp1, respectively, appear to have arisen early in eukaryotes, as they are absent from archaea and bacteria but are found in the eukaryotes examined. CDT2, the SRS for a CUL4-DDB1 E3 complex, appears to have arisen prior to the generation of green plants. Skp2, the SRS for a CUL1 E3 complex, appears to have arisen after the genesis of fungi but prior to the generation of metazoa. The branching order is based on a phylogenetic analysis using rRNA [76]. Note that other phylogenies, based on protein sequences, reverse the order of plants and slime molds [77]. Combining our genomic data with this alternative branching of phyla (not shown) would imply that CDT2 was created prior to plants in the main eukaryotic lineage but then lost within the slime mold lineage.

in plants and other fungi (Fig. 2). It is possible that the loss of these genes put pressure on budding yeast to develop a novel strategy to regulate Cdt1 during S phase. Alternatively, the nuclear-export strategy may have developed and co-existed with the CUL4-DDB1\textsuperscript{CDT2} pathway, but the redundancy between the two pathways subsequently allowed the loss of the CUL4-DDB1\textsuperscript{CDT2} genes.

Conclusion
In humans, both CUL4-DDB1\textsuperscript{CDT2} and SCFSkp2 redundantly target Cdt1 for degradation. SCFSkp2-mediated degradation of Cdt1 is not restricted to S phase in humans, but instead occurs throughout the cell cycle. In contrast, CUL4-DDB1\textsuperscript{CDT2}-mediated degradation of Cdt1 is S-phase specific. The current evidence suggests that in fission yeast, \textit{C. elegans}, \textit{Xenopus}, and potentially even in mice, SCFSkp2 does not contribute significantly to Cdt1 regulation, while the CUL4-DDB1\textsuperscript{CDT2} complex is a major regulator of Cdt1 degradation in these species. The extent to which SCFSkp2-mediated Cdt1 degradation is conserved in mammals other than humans is not yet clear. Genome comparisons suggest that the CUL4-DDB1\textsuperscript{CDT2} complex arose earlier in evolution than SCFSkp2 based on the finding that a CDT2 ortholog is present in plants and fungi, while a Skp2 homolog is absent in these organisms. We propose that CUL4-DDB1\textsuperscript{CDT2} is the ancient and paradigm ubiquitin ligase for the degradation of Cdt1 in response to S-phase entry and DNA damage. Further experiments will be required to address the interesting question of when during early eukaryotic evolution the CUL4-DDB1 complex first began to regulate DNA replication.

Competing interests
The author(s) declare that they have no competing interests.

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