Loss of Csn5 causes both loss of deneddylation activity and loss of CRL-associated deubiquitination activity mediated by the deubiquitinating enzyme Ubp12 [6]. Ubiquitination and turnover of CRL substrate receptor proteins is suppressed by Ubp12 [13], and thus it is possible that the loss of F-box proteins that we observed in Csn5-depleted cells was due to loss of either deneddylation by Csn5 and/or F-box protein deubiquitination by Ubp12. However, expression of Csn5 carrying point mutations in the JAMM motif did not restore cyclin F or Skp2 protein levels in cells depleted of endogenous Csn5 (Figure 3B), even though human CSN complexes bearing a mutated JAMM domain possess associated deubiquitinating activity equivalent to that of wild type CSN [4]. Thus, we propose that loss of CSN-dependent deneddylation is sufficient to bring about a reduction of steady-state F-box protein levels in human cells.

The exact mechanism by which the substrate adaptors are turned over in Csn5-depleted cells is unknown. However, the stimulatory effect of neddylation on SCF activity *in vitro* [3-6] and the restoration of cyclin F levels by dominant-negative Cul1 in Csn5-depleted cells suggests an autocatalytic mechanism wherein an F-box protein is ubiquitinated by the neddylated Cul1-Hrt1 catalytic module present in the same SCF complex. We propose that once an SCF ubiquitin ligase exhausts its pool of available substrate, deneddylation of the Cul1 subunit by CSN brings about disassembly of the complex, with the end result being that the Skp1-F-box heterodimer is released and Cul1-Hrt1 are sequestered into an inactive assemblage with CAND1. In Csn5-depleted cells, the inactivation process is not initiated and therefore the F-box protein remains constitutively associated with a highly active, neddylated cullin-RING catalytic core, resulting in a high rate of F-box protein ubiquitination and degradation.

Interestingly, whereas most F-box proteins that we examined (e.g. cyclin F) exhibited a significant decline in levels upon depletion of Csn5, others (β-TrCP, Emi1) were not affected (G.A.C., unpublished data). We do not understand the basis for this difference, but it is unlikely to reflect whether a given F-box protein is turned over in normal cells, because β-TrCP levels were not reduced in Csn5-depleted cells even though transfected β-TrCP is turned over with a reasonably brisk half-life of ~120 minutes [29]. Clearly, more work is needed to determine why accumulation of only some F-box proteins depends upon CSN.

Given the substantial reduction in the steady-state levels of multiple F-box proteins upon depletion of Csn5, it is surprising that depleted cells continued to proliferate with normal cell cycle kinetics and did not exhibit a dramatic phenotype. For example, overexpression of cyclin E [28] or deletion of Fbw7 [30] causes chromosomal instability, but we were unable to detect any measurable defect in chromosome status in Csn5-depleted cells (data not shown). Given that depletion of Csn5 can affect so many different F-box proteins and presumably SOCS/BC box and BTB domain proteins as well, it is possible that opposing pathways were perturbed in such a way as to prevent the onset of chromosome instability. A similarly mild growth effect of depleting Csn5 was seen in a previous study of the normal diploid human fiboroblast BJ1 cell line [4]. As we have noted previously, the function of Csn5 may be more critical in a multicellular context, or in the face of differentiation signals that evoke a major change in the repertoire of cellular proteins [15].

While this manuscript was in preparation, it was reported that loss of function mutations in CSN components in *S. pombe* and *Neurospora* renders the substrate-recruiting subunits of both Cul1 and Cul3-based CRL complexes unstable, resulting in accumulation of their respective targets [13,31]. In addition, deneddylation of cullins mediated by CSN is required for the maintenance of cullin protein levels in *Drosophila* and for Cul1 stability in *Neurospora* [31,32]. In our original studies in *S. pombe* [3], we observed normal levels of Cul1 in a *csn1*Δ mutant, and this result has been confirmed and extended to other cullins in *S. pombe* [6,13] as well as *Arabidopsis thaliana* [33]. The results reported here indicate that accumulation of human cullins does not depend on CSN-dependent deneddylation. It is unclear why human, fission yeast, and *Arabidopsis* cullins behave differently from those of *Drosophila* and *Neurospora*.

Conclusion

We have shown that CSN can stabilize F-box proteins, thus acting positively on SCF. CSN has been shown to participate in several different processes, including development, transcription, and cell cycle progression [reviewed in [15]]. We propose that loss of deneddylation and a consequent decline in F-box protein abundance underlies these phenotypes.

Methods

Cell culture, cell lines and plasmids

HEK293 cell lines were obtained from ATCC. Cell Lines were grown in Minimum Essential Media (MEM) supplemented with 10% FBS and grown at 37C with 5% CO_2. For G1 cell cycle arrest, cells were grown in MEM media without Serum for 24 hours. For G2 cell cycle arrest, cells were treated with 330 nM of Nocodazole (Sigma) for 24 hours. All transfections were preformed using the calcium phosphate method. Briefly, cells were grown to a density of 1.2–2.0 × 10^6 cells per 6 cm plate. 5 ug of DNA was mixed to a volume of 450 uL and 50 uL of 2.5 M $CaCl_2$