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Targeted silencing of Jab1/Csn5 in human cells downregulates SCF activity through reduction of F-box protein levels

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Abstract

Background: SCF ubiquitin ligases target numerous proteins for ubiquitin dependent proteolysis, including p27 and cyclin E. SCF and other cullin-RING ligases (CRLs) are regulated by the ubiquitin-like protein Nedd8 that covalently modifies the cullin subunit. The removal of Nedd8 is catalyzed by the Jab1/MPN domain metalloenzyme (JAMM) motif within the Csn5 subunit of the Cop9 Signalosome.

Results: Here, we conditionally knock down Csn5 expression in HEK293 human cells using a doxycycline-inducible shRNA system. Cullin levels were not altered in CSN-deficient human cells, but the levels of multiple F-box proteins were decreased. Molecular analysis indicates that this decrease was due to increased Cul1- and proteasome-dependent turnover. Diminished F-box levels resulted in reduced SCF activity, as evidenced by accumulation of two substrates of the F-box protein Fbw7, cyclin E and c-myc, in Csn5-depleted cells.

Conclusion: We propose that deneddylation of Cul1 is required to sustain optimal activity of SCF ubiquitin ligases by repressing 'autoubiquitination' of F-box proteins within SCF complexes, thereby rescuing them from premature degradation.

Background

Proteins are marked for degradation by the 26S proteasome via the covalent attachment of chains of the 76-amino acid protein ubiquitin [reviewed in [1]]. This process involves three discreet steps. First, ubiquitin is activated by the ubiquitin conjugating enzyme (E1) through the hydrolysis of ATP to AMP to yield a high energy thioester intermediate between the C-terminal glycine of ubiquitin and the catalytic cysteine of the E1.

Subsequently, ubiquitin is transferred onto the catalytic cysteine of one of many ubiquitin conjugating enzymes (E2) which, in turn, transfer their cargo onto substrates with the help of ubiquitin ligase enzymes (E3).

One of the best-studied E3 ubiquitin ligase enzymes is the four subunit complex SCF [reviewed in [2]]. SCF consists of two activities: the first, contained within the Cul1 and RING domain Hrt1/Roc1/Rbx1 proteins, is the ability to recruit and activate the E2 to facilitate ubiquitin transfer from the E2 onto substrate; the second resides within the variable F-box proteins, which are linked to Cul1 via Skp1 and are thought to recruit substrates for ubiquitination by the Cul1/Hrt1 sub-complex. The large number of different F-box proteins gives SCF the opportunity to access a wide