Roles of SCF and VHL Ubiquitin Ligases in Regulation of Cell Growth

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1 Introduction

Over the past few years, a growing body of evidence has brought to light critical roles for ubiquitin-dependent protein degradation in controlling the cellular levels of a large variety of proteins such as cyclins, cyclin-dependent kinase inhibitors, oncogenes, and tumor suppressors, which play integral roles in regulation of cell growth. Ubiquitin-dependent protein degradation is a complex, multistep process that proceeds with the tagging of target proteins with a poly-ubiquitin chain and culminates with the processive, ubiquitin-dependent degradation of tagged proteins by the 26S proteasome (Hershko et al. 1983; Hochstrasser 1995, 1996; Hershko and Ciechanover 1998). In the first step, the C-terminus of ubiquitin is covalently linked through a thioester bond to the active site cysteine residue of an E1 ubiquitin-activating enzyme. Ubiquitin is then transferred from the E1 via a thioester linkage to an active site cysteine residue in one of a number of E2 ubiquitin-conjugating enzymes. Ubiquitin is then either (1) conjugated directly via an isopeptide bond to the ε-amino group of a lysine in the target protein, (2) conjugated via an isopeptide bond to another ubiquitin moiety on the target protein as part of synthesis of the poly-ubiquitin tag, or (3) transferred from the E2 via a thioester bond to an active site cysteine residue in one of a growing family of E3 ubiquitin ligases, which then conjugate ubiquitin to specific target proteins.

The E3 components of the ubiquitin cascade are responsible for recognizing, binding specifically to, and recruiting target proteins for poly-ubiquitylation. E3s fall into two functional classes (Hershko and Ciechanover 1998; Joazeiro and Weissman 2000). One class includes the HECT (homologous

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to E6AP carboxyl terminus) domain proteins, which accept ubiquitin through a thioester bond and transfer it to target proteins. The other class includes a diverse collection of proteins that do not accept ubiquitin, but function exclusively as target recognition modules that bind to both the E2 ubiquitin-conjugating enzyme and the target protein, bringing them into close proximity and, in so doing, dramatically stimulating poly-ubiquitylation of target proteins. Among members of this class of E3s are the recently discovered multiprotein SCF (Skp1-Cul1/Cdc53-F-box protein) and von Hippel–Lindau (VHL) tumor-suppressor complexes, which are now known to play crucial roles in a variety of processes including the control of cell growth (Deshaies 1999; Tyers and Jorgenson 2000). Below, we discuss recent progress in research on the SCF and VHL E3 ubiquitin ligases.

2
Architectures of the SCF and VHL E3 Ubiquitin Ligases

The SCF and VHL tumor-suppressor complexes are members of two structurally related families of E3 ubiquitin ligases that use a heterodimeric module composed of a member of the Cullin protein family and the RING-H2 finger protein Rbx1 (also referred to as ROC1 or Hrt1) to activate ubiquitylation of target proteins by the E2 ubiquitin-conjugating enzymes Cdc34 and Ubc5, respectively. SCF complexes include a member of the F-box family of proteins, which recognize, bind specifically to, and recruit target proteins for ubiquitylation. F-box proteins are linked to a Cul1(Cdc53)/Rbx1 module by the Skp1 adaptor protein, which binds to the degenerate, ~40 amino acid sequence motif called the F-box, which is present in F-box proteins (Bai et al. 1996; Patton et al. 1998a). F-box proteins are modular and contain, in addition to an F-box, a protein–protein interaction domain that is responsible for binding selectively to target proteins (Feldman et al. 1997; Skowyra et al. 1997). For example, the F-box proteins Cdc4 and β-TRCP bind target proteins through C-terminal WD40 repeats (Feldman et al. 1997; Skowyra et al. 1997; Yaron et al. 1998; Spencer et al. 1999; Winston et al. 1999), and Grr1 and Skp2 bind their target proteins through C-terminal leucine-rich repeats (Skowyra et al. 1997; Carrano et al. 1999; Marti et al. 1999; Sutterluty et al. 1999). Evidence accumulated to date indicates that the interaction of F-box proteins to their cognate targets depends upon prior phosphorylation of the target proteins. Although the complete repertoire of F-box proteins expressed in eukaryotic cells has not been defined, estimates are that *Saccharomyces cerevisiae* may contain more than 10 such proteins, and mammalian cells more than 100 (Patton et al. 1998b).

As a component of the VHL ubiquitin ligase, the VHL tumor-suppressor protein functions analogously to F-box proteins in the SCF complex to recruit target proteins for ubiquitylation (Maxwell et al. 1999; Cockman et al. 2000; Kamura et al. 2000; Ohh et al. 2000; Tanimoto et al. 2000). The VHL protein is linked to a Cul2/Rbx1 module by the ubiquitin-like Elongin B and Skp1-like Elongin C adaptor proteins. Elongins B and C form a stable subcomplex that

binds to a short BC-box motif present in the VHL protein (Duan et al. 1995; Kibel et al. 1995; Kishida et al. 1995). Based on the work of Pavletich and co-workers, who recently reported the high resolution crystal structure of the VHL–Elongin BC complex, binding of Elongin BC to the BC-box in the VHL protein is governed by interaction of a leucine residue at the N-terminus of the BC-box with a hydrophobic pocket created by residues in the C-terminus of Elongin C (Stebbins et al. 1999). Although the VHL complex is presently the only proven E3 ubiquitin ligase containing the Elongin BC complex, a large family of BC-box proteins have now been characterized, and it is likely that some of these proteins will turn out to have roles in ubiquitin-dependent protein degradation. Among these BC-box proteins are Elongin A and the SOCS-box protein family, which includes the SH2 domain-containing suppressor of cytokine signalling-1 (SOCS-1) protein, as well as more than 20 additional proteins containing either SH2, *ras*-like, WD-40 repeat, SPRY, or ankyrin repeat domains (Hilton et al. 1998; Kamura et al. 1998; Zhang et al. 1999).

2.1 The Cullin Proteins

To date, the Cullin protein family includes at least three members in *S. cerevisiae*, five members in *Caenorhabditis elegans*, and six members in mammalian cells (Kipreos et al. 1996). Biochemical and genetic studies implicate the Cullins in a variety of cellular processes including signal transduction, transcriptional regulation, and the control of cell growth. The founding Cullin family member, Cul5 (also referred to as the vasopressin-activated, calcium-moblizing-1 (VACM-1) protein), was identified as a cytoplasmic arginine vasopressin receptor (Burnatowska-Hledin et al. 1995). Although VACM-1 has been shown to function in signal transduction through its ability to mobilize calcium, stimulate D-myo-inositol 1,4,5-triphosphate production, and inhibit cAMP production (Burnatowska-Hledin et al. 2000), its mechanism of action in these processes is not known. Mutations of the *C. elegans* Cul1 and Cul2 genes result in cell cycle defects. *C. elegans* Cul1 mutants exhibit hyperplasia of many cell types, suggesting that it is required for transition of *C. elegans* cells from G1 to G0 or from G1 to the apoptotic pathway during development (Kipreos et al. 1996). *C. elegans* Cul2 is required for normal mitotic chromosome condensation and for transition of proliferating *C. elegans* cells from G1 to S (Feng et al. 1999). Mice lacking either the Cul1 or Cul3 genes exhibit early embryonic cell cycle defects correlating with misregulation of the cellular levels of cyclin E (Dealy et al. 1999; Singer et al. 1999). In addition, as discussed in more detail below, mammalian and *S. cerevisiae* Cul1/Cdc53 have been shown to play crucial roles in the regulation of both transcription and cell cycle progression as components of the SCF ubiquitin ligase, and mammalian Cul2 functions as a component of the VHL ubiquitin ligase in regulation of hypoxia-inducible transcription.

2.2
The RING Finger Protein Rbx1

Biochemical purification of the SCF and VHL tumor suppressor complexes led to identification of the RING finger protein Rbx1 (also referred to as ROC1 and Hrt1; Kamura et al. 1999a; Seol et al. 1999; Skowyra et al. 1999; Tan et al. 1999). Rbx1 was found to be an integral subunit of both SCF and VHL ubiquitin ligases and to be essential for ubiquitylation of target proteins. Rbx1 interacts specifically with the C-terminal portion of Cullin proteins to form a heterodimeric module (Wu et al. 2000b). The Cullin–Rbx1 module is sufficient to promote non-specific poly-ubiquitin formation by the E2 ubiquitin conjugating enzymes Cdc34 and Ubc5 (Ohta et al. 1999; Seol et al. 1999). Notably, a complex containing Rbx1 and the yeast Cul1 ortholog Cdc53 promote auto-ubiquitylation of Cdc34, and it has been proposed that this complex directly activates E2 ubiquitylation activity (Seol et al. 1999; Skowyra et al. 1999).

Recently, a number of additional RING finger proteins have been shown to activate ubiquitylation by a wide range of E2s, suggesting that the RING motif may play a general role in ubiquitin-dependent protein degradation (Lorick et al. 1999; Joazeiro and Weissman 2000). Among RING finger proteins shown to have E2-stimulatory activity are (1) the E3 Ubr1, which targets proteins of the N-end rule pathway for ubiquitylation (Xie and Varshavsky 1999), (2) the proto-oncogene c-Cbl (Joazeiro et al. 1999), which promotes ubiquitylation of activated receptor tyrosine kinases, (3) the Mdm2 protein (Fang et al. 2000), which targets the p53 tumor suppressor protein for ubiquitylation, and (4) the Apc11 component of the anaphase-promoting complex (APC; Zachariae et al. 1998; Gmachl et al. 2000; Leverson et al. 2000).

3
Targets of the SCF Ubiquitin Ligase

3.1
The *S. cerevisiae* SCF^{Cdc4} Complex

A role for the SCF ubiquitin ligase in control of cell growth was originally brought to light by studies indicating that the SCF complex is responsible for phosphorylation-dependent targeting of *S. cerevisiae* cyclin-dependent kinase inhibitor Sic1 for ubiquitylation and destruction by the proteasome. The Sic1 protein is synthesized in late mitosis and blocks the transition of yeast cells from G1 to S by inhibiting the activities of Clb/Cdc28 kinases (Nugroho and Mendenhall 1994; Schwob et al. 1994). Sic1 must be destroyed to allow yeast cells to enter S phase and begin DNA synthesis (Donovan et al. 1994). Sic1 is ubiquitylated by the SCF^{Cdc4} ubiquitin ligase, which includes the WD-repeat-containing F-box protein Cdc4 (Feldman et al. 1997; Skowyra et al.

1997). Phosphorylation of Sic1 by the Cdc28 kinase is a prerequisite for its binding to the Cdc4 WD-repeat domain and subsequent ubiquitylation by the SCF^{Cdc4} complex.

The SCF^{Cdc4} complex is also responsible for phosphorylation-dependent targeting of *S. cerevisiae* cyclin-dependent kinase inhibitor Far1 and DNA replication factor Cdc6 for ubiquitylation and destruction by the proteasome (Drury et al. 1997; Henchoz et al. 1997). The Far1 protein promotes yeast cell cycle arrest in response to the alpha factor mating pheromone by inhibiting Cln/Cdc28 kinases. The Cdc6 protein is required for the initiation of DNA synthesis and functions in this process by promoting formation and maintenance of the pre-replicative complex at origins of DNA replication. In *S. pombe*, the Pop1 protein and its homologue Pop2 exhibit sequence similarity to *S. cerevisiae* Cdc4 and are required for phosphorylation-dependent targeting of the cyclin-dependent kinase inhibitor Rum1 and the DNA replication factor Cdc18 for ubiquitylation and destruction by the proteasome (Kominami and Toda 1997; Kominami et al. 2000).

In addition to its roles in cell cycle control, the SCF^{Cdc4} complex participates in transcriptional regulation by promoting phosphorylation-dependent ubiquitylation of *S. cerevisiae* DNA binding transcriptional activator GCN4 (Meimoun et al. 2000). The GCN4 protein is a member of the basic leucine zipper (bZIP) family of transcription factors and positively regulates expression of genes required for biosynthesis of amino acids and purines. When yeast are grown in rich media, GCN4 is rapidly phosphorylated by the Pcl/Pho85 kinase, ubiquitylated by the SCF^{Cdc4} complex, and destroyed by the proteasome; when yeast are grown under conditions of limiting amino acids, the Pcl/Pho85 kinase is inactivated, GCN4 is stabilized, and activates expression of its target genes.

3.2 The *S. cerevisiae* SCF^{Grr1} Complex

The *S. cerevisiae* G1 cyclins Cln1 and Cln2 promote efficient transition of yeast cells into S phase. Cln1 and Cln2 are ubiquitylated by the SCF^{Grr1} ubiquitin ligase, which includes the leucine-rich repeat (LRR)-containing F-box protein Grr1 {1706, 2447, 1361, 1650}. Phosphorylation of Cln1 and Cln2 by the Cdc28 kinase is a prerequisite for their ubiquitylation by the SCF^{Grr1} complex. Phosphorylated Cln1 and Cln2 are is recruited to the SCF^{Grr1} complex through their specific binding to the Grr1 leucine-rich repeats.

The SCF^{Grr1} ubiquitin ligase is also responsible for phosphorylation-dependent targeting of *S. cerevisiae* Cdc42 effector proteins Gic1 and Gic2 for ubiquitylation and degradation by the proteasome {2423}. Cdc42 is a member of the rho family of GTPases and has roles in yeast cell cycle progression, organization of the actin cytoskeleton, and bud site selection and bud emergence. Gic1 and Gic2 positively regulate Cdc42 activity. Interaction of Gic1 and Gic2