abundant amounts of hydrogen peroxide in the peroxisome that must be metabolized by the light-sensitive enzyme catalase. The Karpinski work, in keeping with other studies (*10*, *11*), also points to a crucial role for information derived directly from the plastoquinone pool in the transmission of long-distance signals that allow adaptive protection of the photosynthetic machinery. Although details of the mechanisms affording this protection at remote sites are not yet clear, Karpinski *et al.* provide the first evidence of a systemic regulatory system that leads to adaptation to adverse conditions.

Their work was conducted in *Arabidopsis*, a shade-loving species that has become the paradigm for plant genetics research. In nature, a broad gamut of habitats dictate a variety of strategies to cope with varying light availability. Some plants are shade-loving whereas others prefer brighter light. Many plants are able to inhabit both environments by having leaves adapted to intense light ("sun" leaves) and other leaves adapted to lower light ("shade" leaves). The observations of Karpinski and coworkers should stimulate much research in crop species and other plants. Such future research will establish whether remote sensing of excess irradiation really is a general phenomenon that allows leaves distal from the destructive light environment to develop preemptive sunscreens against the threat of excess light.

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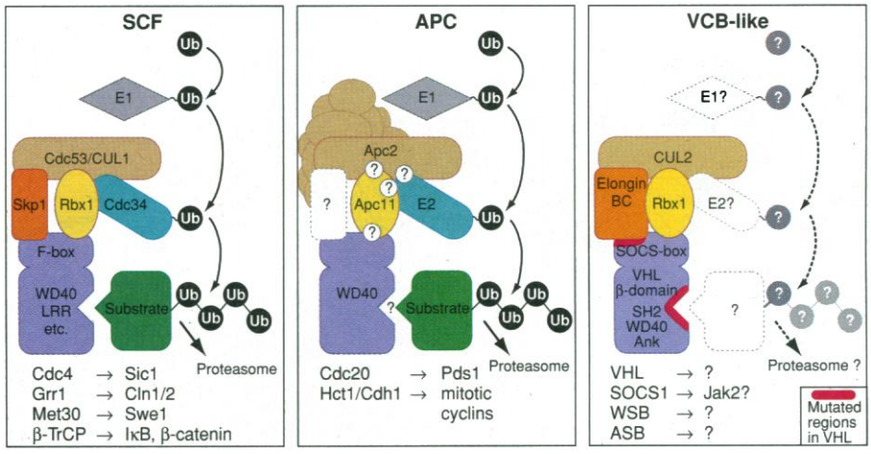
PERSPECTIVES: BIOCHEMISTRY

One Ring to Rule a Superfamily of E3 Ubiquitin Ligases

Mike Tyers and Andrew R. Willems

Lately, the ubiquitin system of intracellular protein degradation seems to have taken cellular regulation by storm. In a recurrent theme, the stability (and hence abundance) of critical regulatory proteins in the cell is often dynamically controlled in response to external or internal stimuli. In most instances, proteins are marked for rapid degradation by conjugation to ubiquitin, a small, highly conserved protein. The enzymatic pathways of ubiquitin modification are complex, but in essence entail recognition of a substrate protein by the ubiquitination machinery, attachment of a poly-ubiquitin chain to the substrate, and capture of the ubiquitinated substrate by a protease complex, the 26*S* proteasome (*1*). Because protein degradation must be highly selective in order for the cell not to cannibalize itself, the substrate recognition step mediated by enzymes called E3 ubiquitin ligases is crucial (see the figure). Not surprisingly, given the multitude of different substrates, E3 ligases are a highly diverse group. One route by which the cell achieves such diversity is by conscripting numerous substrate-specific adapter proteins that recruit protein substrates to core ubiquitination complexes. Two reports on pages 657 and 662 in this week's *Science* (*2*, *3*), and one in last week's issue (*4*), provide structural and functional insights into what may turn out to be a superfamily of E3 ubiquitin ligase complexes—the SCF (Skp1–Cdc53/CUL1–F-box protein) family, the APC (Anaphase-Promoting Complex) family, and the VCB (VHL–Elongin C/Elongin B) family.

The SCF family is the exemplar for combinatorial control of E3 ligase specificity. SCF complexes contain adapter subunits called F-box proteins that recognize different substrates through specific protein-protein interaction domains (*5*). F-box proteins link up to a core catalytic complex—composed of Skp1, Cdc53 (called CUL1 in metazoans), and the E2 ubiquitin-conjugating enzyme, Cdc34—through the F-box motif, which is a binding site for Skp1 (see the figure). The preponderance of F-box proteins in sequence databases (now in the hundreds) fueled



An E3 ubiquitin ligase superfamily. A common architecture may underlie three different E3 ubiquitin ligase complexes that mediate the targeted degradation of many cellular proteins. In targeting substrate proteins for degradation, ubiquitin is passed from an E1 ubiquitin-activating enzyme to an E2 ubiquitin-conjugating enzyme to the protein substrate, with the final step (ligating ubiquitin to the substrate) catalyzed by an E3 ubiquitin ligase. The SCF and APC complexes are known to be E3 ligases, whereas the VCB-like complexes are only inferred to be E3 ligases on the basis of their similar overall architecture to the APC and SCF families. Each complex interacts with a set of adapter proteins that recruit different binding partners through specific protein-protein interaction domains such as WD40 repeats and leucine-rich repeats (LRR). Representative examples are shown below each complex. The newly discovered subunit Rbx1 and its homolog Apc11 may play an integral role in tethering components to each other and in activating the E2 enzyme. Question marks indicate speculative components or interactions.

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speculation that a host of proteins may be targeted for degradation by SCF pathways (*5*). This prediction has played out in spectacular fashion as known targets of SCF complexes now include cell cycle regulatory proteins such as cyclins and CDK inhibitors and transcriptional regulators such as IκB and β-catenin, among many others (*6*, *7*).

The APC is a second E3 ligase that uses different adapters to target different substrates, which include mitotic cyclins and other proteins that regulate mitosis (*8*). In a surprise finding reported in *Science* a year ago, the Apc2 subunit of the APC turned out to be a homolog of Cdc53, hinting at a possible distant relationship between the APC and SCF complexes (*9*, *10*). A third complex, composed of the von Hippel–Lindau tumor suppressor protein (VHL) and its associated subunits Elongin B, Elongin C, and CUL2, has a tenuous connection to E3 ligases because of sequence similarity between Skp1 and Elongin C, ubiquitin and Elongin B, and Cdc53 and CUL2 (*11*). VHL is mutated in many types of cancers, particularly renal cell carcinomas, but its biochemical function is unknown (*11*). Although there is no direct evidence to suggest that the VCB complex is an E3 ligase, the combinatorial theme is recapitulated because the Elongin BC subcomplex also interacts with proteins that contain a motif termed the SOCS-box (after the suppressor of cytokine signaling family of proteins), which is similar to the Elongin C binding region in VHL (*4*, *12*). Like F-box proteins, SOCS-box proteins thus contain a common docking site coupled to different protein-protein interaction domains. The fact that SOCS-box proteins are implicated in signal attenuation (*13*) is also consistent with a possible role in proteolysis.

A discovery spearheaded by the Conaway group at the Oklahoma Medical Research Foundation now suggests that the SCF, APC, and VCB complexes share a much closer overall architecture than previously anticipated (*2*). Kamura *et al.* identified a protein called Rbx1 as a stoichiometric component of the mammalian VCB complex and, prompted by the finding that Rbx1 interacts directly with the Cdc53 homolog CUL2, determined that Rbx1 is also an integral component of the human and yeast SCF complexes (*2*). Furthermore, Rbx1 is a close homolog of the APC subunit Apc11, which together with Rbx1 defines a distinct subclass of RING finger proteins. The RING finger is a small, metal-binding domain often found in subunits of multiprotein complexes (*14*). Genetic and biochemical analysis of Rbx1 function in yeast revealed that it is required for SCF-mediated ubiquitination of the CDK inhibitor Sic1 (*2*). In parallel, the Harper and Elledge groups at Baylor College of Medicine were pursuing an activity that stimulated the ubiquitination of yeast G_1 cyclins by recombinant SCF complexes (*15*). Skowyra *et al.* were well into the arduous task of purifying the missing activity, which upon a direct test, turned out to be Rbx1 (*3*).

What does Rbx1 do within the VCB and SCF complexes? For one, it interacts with a remarkable number of other subunits. In the VCB complex, Rbx1 independently binds VHL, the Elongin BC subcomplex, and CUL2 (*2*). In the SCF complex, Rbx1 interacts with Cdc53/CUL1, Cdc34, and at least three different F-box proteins, but does not interact with Skp1 (*2*, *3*). As the F-box protein partners of Rbx1 share only the F-box motif, Rbx1 probably binds to at least part of the F-box site, perhaps competing with Skp1. One pivotal function of Rbx1 is to recruit Cdc34 into the SCF complex by bridging or stabilizing the Cdc34-Cdc53 interaction (*3*). An unanticipated finding by Skowyra *et al.* is that the Rbx1-SCF holocomplex greatly stimulates the catalytic activity of Cdc34 (*3*). This mechanism may limit E2 activity to the context of the fully assembled protein substrate–E3 ligase complex, thus preventing nonspecific ubiquitination. Finally, as other E3 ligases participate in the transfer of ubiquitin to the substrate through ubiquitin-thioester intermediates on catalytic cysteine residues (*1*), it is possible that one of the many cysteines in Rbx1 could fulfill this role in SCF complexes.

The structure of the VCB complex reported last week by the Pavletich group at the Memorial Sloan-Kettering Cancer Center provides insight into both VHL function and SCF structure (*4*). VHL has a bipartite structure, consisting of an α-helical domain linked to a β-sheet domain. Two extensive interfaces on opposite sides of Elongin C interact independently with VHL and Elongin B. A concave hydrophobic pocket on Elongin C meshes with VHL to form an intermolecular four-helix bundle, with three helices donated by VHL and one by Elongin C. The other side of Elongin C is tightly interwoven with Elongin B through an intermolecular β-sheet structure. As expected from its sequence, the core structure of Elongin B is highly similar to that of ubiquitin.

The structure of VHL allows sense to be made of the rich database of known VHL mutations in tumors (*4*). One group of mutations clusters in the α-helical domain, and probably disrupts the interaction with Elongin C, whereas another group clusters in the β-sheet domain. Most intriguingly, a subset of the β-sheet domain mutations appears to define a binding surface for an as yet unidentified protein suggesting that, like F-box proteins, VHL may recruit one or more binding partners into a core complex.

The VHL–Elongin C interface is instructive in two ways. First, as suspected, it reveals that the SOCS-box motif in VHL forms key contacts with Elongin C (*4*). Thus it is likely that other SOCS-box proteins will interact with Elongin BC in a similar manner to VHL. Second, as Skp1 can be precisely modeled on the Elongin C structure, Stebbins *et al.* venture to suggest that the VHL–Elongin C interface is a useful template on which to model the F-box protein–Skp1 interaction (*4*).

The three papers raise many tantalizing issues. First and foremost, is the VCB complex a bona fide E3 ligase? There is as yet no direct evidence that this complex mediates conjugation of ubiquitin, Elongin B, or other ubiquitin-related proteins (*16*). Moreover, an E2 ubiquitin-conjugating enzyme has not been detected in association with VHL, although Cdc34 is a reasonable candidate given its interaction with Rbx1. While expectations are high that VHL will be an E3 ligase, prudence is still warranted, particularly as the SCF component Skp1 also plays a key structural role in the CBF3 kinetochore complex, which is not an E3 ligase (*6*). If the VCB complex is an E3 ligase, what are its substrates and how do they stimulate cell proliferation in cancer cells that lack proper VHL function? To elaborate on the combinatorial theme, might the dozens of known SOCS-box proteins (*12*, *13*) also recruit substrates for ubiquitination by VCB-like complexes, thereby placing myriad signal transduction pathways under direct proteolytic control?

With respect to the APC, it remains to be seen if Apc11 plays an analogous role to Rbx1, perhaps in tethering Apc2 to its cognate E2, or in stimulating the ubiquitination reaction. As for enzymatic mechanism, it must be determined how the fully assembled SCF complex stimulates the intrinsic activity of Cdc34. From a structural perspective, how will Rbx1 fit into the already complex VHL–Elongin C interface, and what subtle variations will explain the specificity of Skp1 for F-box proteins and Elongin C for SOCS-box proteins? To generalize the theme in another direction, will Rbx1 or Apc11 form E3 ligase complexes with any of the half dozen or so other Cdc53/CUL1-like proteins of unknown function? If so, then Rbx1 and Apc11 may lay claim to a truly prodigious number of degradation pathways. Finally, although it is clear that Rbx1 and Apc11 define a distinct subclass of RING finger proteins, Elledge and Harper also note that

a number of other E3 ligases contain similar RING finger domains, which they designate the R-box (*3*). Might the R-box play a more universal role in ubiquitin conjugation, or is it just coincidence that a common structural element occurs in a variety of ubiquitination complexes? Regardless, the role of the RING finger domain in protein destruction has eerily fulfilled the portent of another famous RING trilogy: "One Ring to rule them all, One Ring to find them, One Ring to bring them all and in the darkness bind them." (*The Lord of the Rings*, J. R. R. Tolkien).

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PERSPECTIVES: GEOSCIENCE

Giant Lava Flows, Mass Extinctions, and Mantle Plumes

Paul E. Olsen

What are the consequences and origins of the largest volcanic events known on Earth? These include the so-called large igneous provinces (or LIPs) that comprise enormous edifices of basaltic lava and associated igneous rocks formed over a relatively brief time interval (*1*). Two of the largest LIPs, the Siberian Traps (~2.5 × 10^6 km^3) and Deccan Traps (~2.6 × 10^6 km^3), were extruded onto the land surface (*2*) and are often termed continental flood basalts. Each is also associated with a mass extinction, the Siberian Traps with the extinction at the end of the Permian (250 million years ago) and the Deccan Traps with the extinction at the end of the Cretaceous (65 million years ago). In recent years, a third giant continental LIP associated with a mass extinction has been identified in the long-studied Triassic-Jurassic (~201 million years ago) lavas and igneous intrusions that mark the rifting of the supercontinent of Pangea and the formation of the Atlantic Ocean. As reported by Marzoli *et al.* on page 616 of this issue (*3*), this Central Atlantic Magmatic Province (CAMP) may be the largest LIP of all, at least in area. Before the formation of the Atlantic Ocean, it extended over 7 million km^2, from France to southern Brazil, covering substantial portions of four tectonic plates (see the figure). And yet this igneous activity probably occurred over less than a few million years. The origin of this LIP bears on the mechanisms of mass extinction, continental breakup, and the motive force behind continental drift itself.

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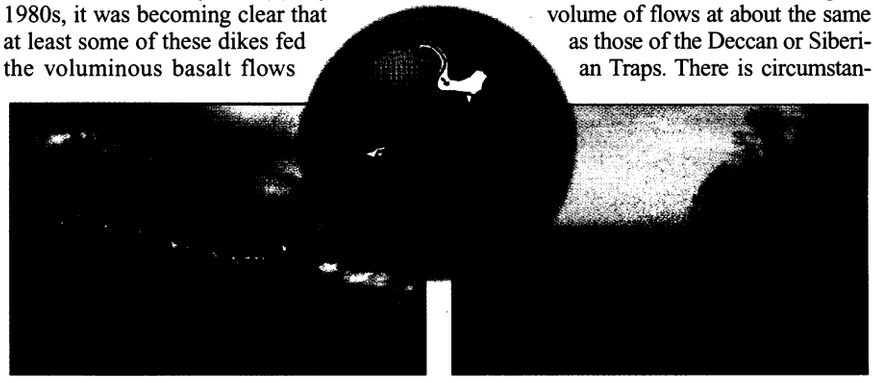
Recognition of this Triassic-Jurassic LIP has been long in coming, perhaps delayed by the fact it was dismembered during formation of the Atlantic Ocean, either deep eroded or deeply buried, and is difficult to precisely date. However, as long ago as 1971, May (*4*) showed that the numerous linear dikes of basaltic composition in eastern North America, Africa, southwestern Europe, and South America made up a giant radiating dike swarm when placed in their predrift positions, their focus being near Florida. This is in fact the largest radiating dike swarm known in the solar system (*5*). By the 1980s, it was becoming clear that at least some of these dikes fed the voluminous basalt flows and sills in the Triassic-Jurassic rift basins on the Atlantic Margin (*6*) and that the age of this dike system is about 201 million years (*7*). Paleontological data established that the flows were very close in time to the Triassic-Jurassic boundary and hence to its purported mass extinction event and that the flows in fact date the boundary (*8*). As was true for the events at the end of the Permian and the Cretaceous, the temporal association of the extensive basalt flows and a mass extinction has led to speculation that the eruption of the lavas triggered ecologically catastrophic climate change through massive input of volatiles into the atmosphere (*9*).

As with the other two events, however, the proposed links between the CAMP LIP and mass extinction remain very controversial, with substantial volume, timing, and mechanism problems. Although the preerosional extent of the Deccan and Siberian lavas probably exceeded 2.0 × 10^6 km^3, the present volume of CAMP lavas in the rift basins is more than an order of magnitude smaller. However, all of the exposed rifts are deeply eroded to depths of several kilometers (*10*). Assuming that the distribution of dikes and other intrusions is a guide to the preerosion extent of the associated lavas, Marzoli *et al.* (*3*) estimate the original volume of flows at about the same as those of the Deccan or Siberian Traps. There is circumstantial evidence (*11*) that the recently recognized seaward-dipping reflectors (*12*) off the eastern United States may also be part of the CAMP, although their age is very poorly constrained. The volume of these basaltic, purportedly terrestrial, flows would raise the total to about 4 million km^3. This excludes the volume of intrusive CAMP rocks, which could add about the same amount again.



CREDIT: P. E. OLSON

Big lava. (Left) Basalt flow (brown) in the CAMP on top of the Triassic-Jurassic boundary (white) on Triassic rift lake sediments (reddish brown). **(Center)** Pangea during the Late Triassic–Early Jurassic with four terrestrial LIPs (north to south: Siberian Traps, CAMP basalts, Deccan Traps, and Karroo lavas). **(Right)** The Palisades Sill, an intrusive part of the CAMP event, exposed along the shores of the Hudson River, near New York City.