stimulated termination completely. They suggest that Q may act in part by blocking hairpin formation close to RNAP (see the figure). In other words, Q could block oligonucleotide access close to RNAP but allow some pull-out if pairing occurred further upstream; thus, Q would inhibit hairpin formation until after RNAP passes the uracil-rich sequence required for termination. However, a recent report showing that RNAP does not move forward when a termination sequence breaks mRNA's contacts with the exit channel and the DNA template (at low ionic strength) favors the alternative collapse model (*5*) (see the figure).

Mutant RNAPs that increase or decrease termination and that may affect contacts to the RNA:DNA hybrid also shift the "oligonucleotide-release" window downstream or upstream, respectively. Thus, like the Q protein, hybrid stability affects where the oligonucleotides can pair to trigger termination. This leads Yarnell and Roberts to suggest that Q could act by stabilizing the hybrid (see the figure). Stabilization would shift the "oligonucleotide-release" window because more base pairs are required to compete with the stabilized hybrid. This hypothesis is attractive because it explains how Q could block both hairpin formation and backtracking, and thus simultaneously inhibit pausing, arrest, and termination. It also is consistent with the finding that antisense oligonucleotides must pair to mRNA within eight nucleotides of its 3′ end to dissociate a paused, hairpin-stabilized RNAP containing a more stable RNA:DNA hybrid (*7*).

How can these ideas be applied to understanding human gene regulation? RNAPII must switch from hesitant to overdrive transcription to transcribe through the pause signals, arrest signals, and nucleosomes in human genes (typically 10^4 to 10^6 base pairs in length) that would otherwise halt RNAPII completely (*8*). Abnormal regulation of this efficiency switch has been implicated in several human diseases including myeloid leukemia, malignant transformation of several types of human cells, and growth of HIV (*9*). Flipping the efficiency switch to "on" requires phosphorylation of multiple sites on RNAPII's largest subunit. Phosphorylated RNAPII may recruit factors that stimulate mRNA elongation and transcription through nucleosomes (perhaps in a similar fashion to Q). Once RNAPII passes the polyadenylation site in DNA, dephosphorylation of the large subunit releases the transcription factors, restores RNAPII's intrinsic sensitivity to pause and termination signals, and quickly stops transcription (*10*).

The central idea that instability of the RNA:DNA hybrid leads to rearrangement of the transcription complex (see the figure) appears to explain how RNAPII pauses and arrests (*11*), and probably how it becomes a target for termination factors. Like the proposed antitermination action of Q, RNAPII's switch to efficient transcription could involve stabilization of the RNA:DNA hybrid (and possibly, inhibition of RNA hairpin formation) so that it never becomes susceptible to pausing, arrest, or termination.

From one perspective then, pausing, arrest, termination, and antitermination can all be explained by protein or nucleic acid interactions that affect both positioning of the RNA 3′ end in the active site and lateral sliding by a relatively rigid RNAP (*4*). However, strong evidence exists that the actual switch between inefficient and efficient transcription must involve a conformational change in RNAP. Removing a small subunit or promoting the rapid elongation of mRNA with high concentrations of nucleotides in vitro causes RNAP to switch to a state that resists pausing or termination (*12*). The mutant RNAPs of Yarnell and Roberts behave as if this switch were sticky, locking the enzyme in either the overdrive or hesitant state. Perhaps proteins like Q stabilize RNAP in an overdrive conformation that optimizes RNA contacts with the DNA template and active site; the change from this conformation to one that tolerates misplacement of RNA could be a feature built into the RNAP itself. The next hurdles to be overcome are determining whether a rigid RNAP or a conformational change best explains RNAP's overdrive switch and whether termination occurs by pull-out or collapse.

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PERSPECTIVES: PLANT BIOLOGY

Leaves in the Dark See the Light

Christine H. Foyer and Graham Noctor

Photosynthesis—the process by which plants harness the sun's energy to generate simple carbon compounds—supports all life on Earth. It is a complex process of successive reduction-oxidation (redox) reactions that use up carbon dioxide and water and produce energy-rich carbohydrate and oxygen as the end products. The evolution of oxygen-giving photosynthesis radically altered Earth's atmosphere and enabled the development of aerobic life. Oxygen-consuming organisms, although able to exploit the powerful oxidizing properties of oxygen, are also condemned to exist in the unstable tinderbox atmosphere of 21% O_2.

Although photosynthesis cannot proceed in the absence of light, excess light is potentially dangerous to the plant because it can cause persistent decreases in the rates of photosynthesis (photoinhibition) (*1*). Leaves have evolved various mechanisms to deal with excess light energy, enabling plants to function optimally over a relatively broad window of light intensities. At low irradiance, harvesting of light predominates, but as the light intensity increases, effective dissipation of energy becomes progressively more important in preventing photoinhibition and is essential for plant survival (*2*). If the protective processes are overwhelmed, photoinhibition will decrease the efficiency and capacity of photosynthesis and cause leaf damage that is comparable to human sunburn. Now on page 654, Karpinski and colleagues report the intriguing finding that exposure of plants to high-intensity light activates a systemic signaling system that "warns" regions of the plant not exposed to bright light of an impending dangerous stimulus (*3*). The investigators exposed one-third of *Arabidopsis* leaves to high-intensity light—which is believed to result in the production of damag-

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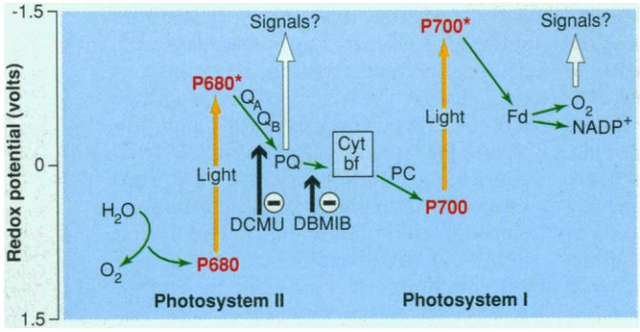
ing active oxygen species—and demonstrated expression of a protective antioxidant gene in leaves that were kept in the shade. They propose that a systemic messenger (possibly hydrogen peroxide) produced in the exposed leaves was able to travel to different parts of the plant and switch on adaptive gene expression.

In leaves, the photosynthetic process occurs in a discrete organelle, the chloroplast (*4*). This organelle contains thylakoid membranes that transduce light energy into chemical energy, producing a reduced product (reductant) and adenosine 5′-triphosphate (ATP), which together drive the assimilation of inorganic elements into cellular matter. Light energy is transduced by two pigment protein complexes operating in series. Numbered according to the historical order in which they were discovered, these are the photosystems (PS) I and II. Excitation of PSII produces a strong oxidant capable of splitting water; operation of PSI leads to formation of a reductant that is powerful enough to reduce $NADP^+$ (nicotinamide adenine dinucleotide phosphate) (see the figure). Analysis of the yield of chlorophyll fluorescence emitted from the thylakoid membrane provides a stethoscope that can probe the function and regulation of photosynthetic electron transport (*5*). Similarly, inhibitors (such as DCMU and DBMIB) that act at specific sites in the electron transport chain (see the figure) allow us to evaluate the importance of its individual components, particularly plastoquinone.



Electron chain-gang. Components of the photosynthetic electron transport chain in plants are positioned according to approximate redox potential. As in respiration, the net flow of electrons is from components of low potential to those with higher potential. In photosynthesis, however, charge separation occurs when the photosystem reaction centers (P680 and P700) are excited by light (to P680* and P700*, respectively). This enables relatively high-potential components to transfer electrons to lower potential components. In this way, light energy is converted into chemical energy. PQ, plastoquinone; Q_A, Q_B, quinone acceptors of photosystem II; Cyt bf, the cytochrome b_6f complex, containing several redox-active components; PC, plastocyanin; Fd, ferredoxin. The reduction and oxidation of the PQ pool is inhibited by DCMU and DBMIB, respectively.

The photodamage to leaves exposed to excess light is partly attributable to the production of unstable intermediates by the photosynthetic electron transport system—if these intermediates are formed faster than they can be used up, damaging side reactions result (*6*). The most important of these side reactions is the interaction of the unstable intermediates with oxygen to produce partially reduced oxygen species (superoxide, hydrogen peroxide, hydroxyl radicals) and highly reactive singlet oxygen. For a long time these active oxygen species have been considered solely in a negative light. Only more recently has it been appreciated that they form an indispensable part of the redox balance that is perceived by the cell nucleus and that evokes adaptive changes in gene expression (*7*).

As with any effective information-transducing mechanism, powerful signals must be controlled. The chemical reactivity of active oxygen species also requires rapid and effective processing to ensure appropriate cellular redox poise and to prevent leaf damage. In plants, as in animals, this control is furnished by a battery of antioxidants (*8*) that include low molecular weight compounds (vitamin C, vitamin E, glutathione, carotenoids) and enzyme components such as superoxide dismutase, catalase, and ascorbate peroxidase. It is interesting to note that whereas plants, like animals, rely on catalases and peroxidases to remove hydrogen peroxide, vitamin C (ascorbic acid) replaces glutathione as the major sacrificial reductant for peroxidase action.

During evolution, plants embraced the energetic potential of interactions between oxygen and the antioxidant system, such that the formation and destruction of active oxygen species is an integral part of the regulation of photosynthesis. Two important examples of this are the high rates of hydrogen peroxide formation during photorespiration and the dismutation of superoxide and its subsequent metabolism. Although the reaction that metabolizes superoxide has frequently been considered as an overflow sink for electrons, it is coupled to ATP formation and is therefore subject to the same regulation as $NADP^+$ reduction (*9*). As with NADPH formation, the reduction of oxygen will tend to increase with light intensity. This means that in excess light, the potential for active oxygen species production increases.

Until recently the signal transduction pathways of photosynthesis were largely unexplored. The most attractive candidate signaling molecules are products and components of electron transport, as these are the links between the photosynthetic light reactions and metabolism. The central position of the plastoquinone pool in key processes such as photosynthetic control and thylakoid protein phosphorylation is well established (*2*). Hence, it is logical to assume that vital signal-transducing elements will respond to the redox state of the plastoquinone pool (see the figure). Indeed, several authors have reported local control of gene expression by electron transport components in the plastoquinone region of the chain (*10, 11*).

In their study, Karpinski *et al.* go beyond the local concept of gene control and suggest that signals arising from photosynthesis provoke changes in gene expression in remote parts of the plant that have not experienced the primary eliciting stimulus. Exposure of *Arabidopsis* leaves to high light intensities induced the antioxidant gene, ascorbate peroxidase (*APX2*), at a remote site in the plant that had not been exposed to the bright light. Furthermore, the photosynthetic system as a whole appeared better able to cope with the threat of high light intensity as a result of exposure of just one small part of the plant to the offending stimulus.

The very existence of a systemic response to excess light is a remarkable finding. It is, however, reminiscent of the systemic acquired resistance that plants develop following wounding, pathogen attack, or other environmental challenge (*7*). Plants subject to these traumas often develop resistance in undamaged or uninfected regions. Various diffusible signal molecules, including hydrogen peroxide, have been implicated in spreading the news of attack to unharmed parts of the plant, thereby arming the whole plant against subsequent challenge. Systemic acquired resistance can be viewed as broadly analogous to the effect of vaccination in mammals. Drawing parallels with systemic acquired resistance, Karpinski *et al.* conclude that hydrogen peroxide, a diffusible, relatively long-lived active oxygen species, is a player in the systemic adaptation of the plant to excess light. If so, this hydrogen peroxide could originate in the chloroplasts of exposed leaves, as suggested by the authors. However, we cannot discount a contribution from hydrogen peroxide produced by other processes associated with photosynthesis, such as photorespiration. This depends on photosynthetic electron transport and produces

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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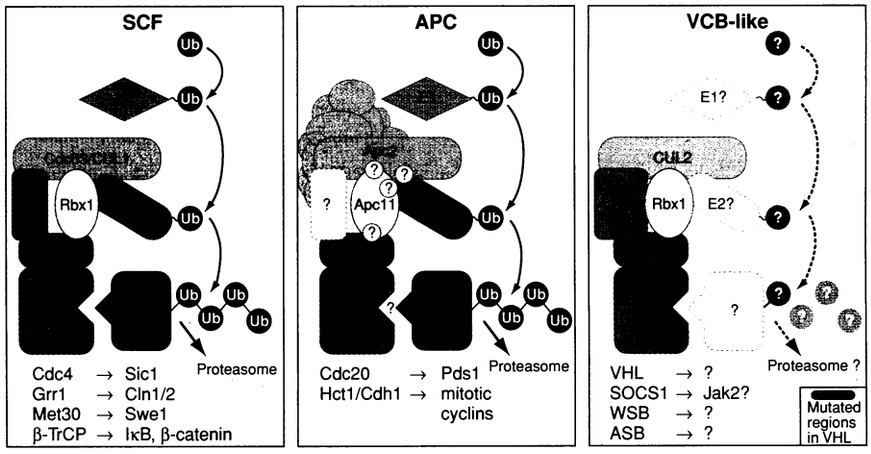
PERSPECTIVES: BIOCHEMISTRY

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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