

THE DIVERSE ROLES OF UBIQUITIN AND THE 26S PROTEASOME IN THE LIFE OF PLANTS

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A tightly regulated and highly specific system for the degradation of individual proteins is essential for the survival of all organisms. In eukaryotes, this is achieved by the tagging of proteins with ubiquitin and their subsequent recognition and degradation by the 26S proteasome. In plants, genetic analysis has identified many genes that regulate developmental pathways. Subsequent analysis of these genes has implicated ubiquitin and the 26S proteasome in the control of diverse developmental processes, and indicates that proteolysis is a crucial regulatory step throughout the life cycle of plants.

PROTO-ONCOGENE

A gene that has the potential to change into an active cancer-causing oncogene.

Regulated proteolysis has an essential role in the development of all organisms. A protein might be degraded because it no longer functions correctly or has failed to fold properly following translation; other proteins are degraded to release nitrogen and carbon in the form of amino acids. However, the proteolysis of some proteins is triggered by distinct environmental and developmental cues to control the abundance of crucial cellular regulators. In all these cases the mechanism of proteolysis must be very specific and tightly controlled. Degradation of the wrong protein or an error in the timing of proteolysis could prove catastrophic for an organism. This is probably best illustrated by the many short-lived PROTO-ONCOGENE products that are regulated by proteolysis, and the numerous types of cancer that develop when proteolysis of these proto-oncogenes is disrupted^{1,2}.

The ability to switch from one developmental programme to another in response to an environmental signal is crucial for the survival of all organisms. This is particularly true in plants, in which a high degree of developmental plasticity is required to cope with their sessile lifestyle. This means that plants, unlike most animals, are unable to move away from an unfavourable environment. They must instead cope with the stress *in situ*, by producing protective compounds, by altering their morphology to adjust to the stress and, in some more extreme cases, by accelerating their life cycle to

survive in the form of seeds. The switch from one developmental state to another requires the removal of pre-existing regulatory networks and the assembly of new ones, a process that often depends on proteolysis³.

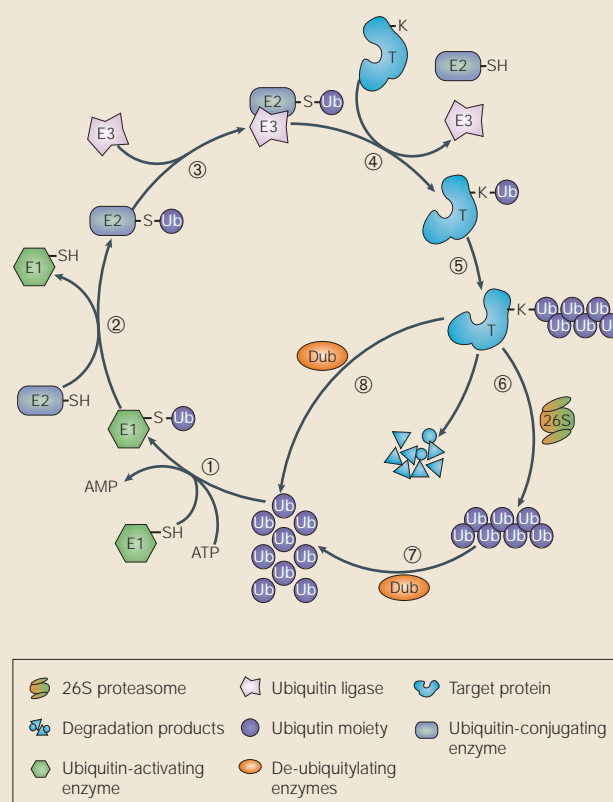
In this review, we discuss the ubiquitin/26S proteasome system in plants and outline its widespread role in many plant developmental processes. In recent years, the number of developmental pathways in plants that have been shown to involve ubiquitin has grown enormously. In particular, recent advances in hormone signalling and plant responses to pathogens have confirmed the importance of ubiquitin and the 26S proteasome as crucial regulators of plant development and their response to the environment.

The ubiquitin/26S proteasome system in plants. One of the most widely studied, and arguably the most important, proteolysis systems in eukaryotes is the ubiquitin/26S proteasome system (Ub/26S)²⁻⁷. Ubiquitin is a 76-amino-acid globular protein, which is able to attach to the target protein through a carboxy-terminal extension⁷. A sequential cascade of enzymic reactions results in a covalent linkage between ubiquitin and a lysine residue in the target protein (see BOX 1 for more details). The subsequent addition of ubiquitin moieties through the K48 residue in ubiquitin results in the formation of polyubiquitin chains on the target

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Box 1 | The ubiquitin/26S proteasome cycle

Ubiquitin is a highly conserved eukaryotic protein that consists of 76 amino acids⁷. Although originally identified as a covalent modifier responsible for tagging proteins for degradation by the 26S proteasome, it is now known that protein modification by ubiquitin is also necessary for many other functions, including the regulation of ENDOCYTOSIS, transcriptional activity and the control of DNA repair¹²⁷. The ubiquitylation of a target protein (T) involves a sequential cascade of enzymatic activities that results in the formation of an ISOPEPTIDE BOND between the G76 residue of ubiquitin and the ϵ -amino group of a lysine residue in the target protein. This process starts with the ubiquitin-activating enzyme (E1), which forms a THIOESTER LINKAGE with the G76 residue of ubiquitin (1). This process is not thought to have any discriminatory or regulatory role and generally eukaryotic organisms only contain a few E1 isoforms. In the next step (2), the ubiquitin is passed to a ubiquitin-conjugating enzyme (E2) again through a thioester linkage. The E2 carries the activated ubiquitin to the ubiquitin ligase (E3), which facilitates the transfer of the ubiquitin from the E2 to a lysine residue in the target protein, often by forming an intermediate complex with the E2 and the target (3 and 4). The E3 is generally considered to be most important in controlling target specificity⁴ because it is responsible for recruiting the target protein and positioning it for optimal transfer of the ubiquitin moiety from the E2. Subsequent rounds of CONJUGATION (5) add polyubiquitin chains to the target, with the type of chain formed determining the fate of the target protein. In general, polyubiquitin chains that are formed through the K48 residue of ubiquitin are destined for degradation through the 26S proteasome (6), where the target protein is subsequently degraded and the ubiquitin monomers are reclaimed by the action of de-ubiquitylating enzymes (7). Monomeric ubiquitylation and polyubiquitylation through the K29 and K63 residues of ubiquitin is also possible and it is thought that these alternative ubiquitylation events lead to regulatory processes other than degradation¹²⁷. Again, the action of de-ubiquitylating enzymes (8) is responsible for recycling the ubiquitin back into the pool of free ubiquitin within the cell.



protein. These polyubiquitylated proteins are subsequently recognized by the 26S proteasome and degraded (see BOX 2 for more details).

In plants, the Ub/26S system is involved in degrading a wide range of proteins in both the nucleus and cytoplasm. Indeed, it has been said that it is difficult to find a biological process in plants that does not have some connection to ubiquitylation⁸. The importance of the Ub/26S system in plants is also reflected in the large number of genes in the *Arabidopsis thaliana* genome that encode Ub/26S components — more than 1,300 such genes (~ 5% of the total genome) have been identified⁴.

Although ubiquitin was first identified on the basis of its role in proteolysis, it has become increasingly clear that ubiquitylation is involved in processes other than simple targeting to the proteasome. It is now known that both mono-ubiquitylation and non-canonical polyubiquitylation (through the K63 residue in ubiquitin) are involved in protein trafficking, DNA repair and the regulation of transcription and translation (for reviews, see REFS 9–11). Although, at present, these alternative roles for ubiquitylation have only been demonstrated in yeast and mammals, given the great similarity between ubiquitylation in plants and other eukaryotes, it seems likely that they will eventually be found in plants.

The COP9 signalosome (CSN) is a multi-protein complex that is intimately related to the Ub/26S system. Although the CSN was originally identified as a repressor of photomorphogenesis in plants (see below for

more details), it is highly conserved in mammals, *Drosophila* and the fission yeast *Schizosaccharomyces pombe* (see REFS 12–14 for reviews). The CSN shows remarkable homology to the lid subcomplex of the 26S proteasome (TABLE 1 and BOX 2), and, recently, it has been shown that the CSN and the 26S proteasome physically associate *in vivo*¹⁵. This finding indicates that the CSN has a direct role in protein degradation, possibly as the lid for a specialized CSN proteasome¹⁴. The CSN can interact with stem-cell factor (SCF)-type E3 ligases through both the cullin and RBX1 subunits^{16–18} (BOX 3). Although the exact role of the CSN is unknown, it is thought to function in part by regulating SCF-type E3 ligases through mediating the removal of a ubiquitin-like conjugate NEDD8/RUB from the cullin subunit of the E3, or through its associated de-ubiquitylation activity^{16–18}. The addition of NEDD8/RUB to target proteins involves a cascade of enzymic reactions that are very similar to those involved in ubiquitylation. An important enzyme in this process in plants is AXR1, which was identified as a positive regulator of auxin response¹⁹, and is a component of the NEDD8/RUB activating enzyme that mediates the first step in the conjugation to the cullin subunit of the SCF-type E3 ligase (REF. 20). Analysis of *Arabidopsis axr1* mutants has shown that the CSN and AXR1 are involved in the regulation of many pathways controlled by E3-mediated events²¹. The regulation of SCF activity by NEDD8/RUB modification is complex and it is thought that both the NEDD8/RUB

ENDOCYTOSIS

The uptake of extracellular materials within membrane-bound vesicles by cells.

ISOPEPTIDE BOND

The covalent linkage that joins amino-acid residues through an amide bond.

THIOESTER LINKAGE

A non-covalent chemical bond between two proteins formed through thioester.

CONJUGATION

The addition of ubiquitin moieties to a growing polyubiquitin chain.

modification state and the association/disassociation of NEDD8/RUB from the cullin subunit of the SCF are important in controlling E3 activity^{16–18}. CSN influences ubiquitylation and subsequent proteolysis in many aspects of plant development by regulating SCF-type E3 ligases (see below for a more detailed discussion).

There are many other types of proteolysis system found in plants that do not involve ubiquitin or the 26S proteasome. For example, the photosynthetic organelle, the chloroplast, contains an extensive proteolysis system for removing damaged components of the photosynthetic machinery²². Chloroplast proteases resemble prokaryotic protein-degradation systems,

probably owing to the endosymbiotic origin of plant organelles^{22,23}. There are also exo- and endoproteolytic activities associated with plant peroxisomes that have extensive roles in response to oxidative stress²⁴ (see REFS 22,25 for further discussion of these topics).

Roles of ubiquitin and the proteasome in plants
In the past few years, there has been a marked increase in the number of plant developmental processes that have been shown to involve ubiquitin. A variety of genetic screens for mutants in many developmental pathways have isolated components of putative E3 ligases, thereby indicating a role for ubiquitylation and/or

Box 2 | The 26S proteasome and the COP9 signalosome (CSN)

The 26S proteasome is often the last step in the life cycle of proteins. The 26S proteasome is a large complex of approximately 700 kDa found in the nucleus and cytoplasm of both plants and animals³. The 26S proteasome is essential in a myriad of cellular processes that control the degradation of proteins that have been tagged with ubiquitin (see BOX 1). In the absence of ATP, the 26S proteasome dissociates into two subcomplexes. The 20S core protease complex consists of a hollow cylinder formed from four stacked rings of seven α and β subunits (a, yellow and blue subunits, respectively), and contains the central ATP-independent catalytic domain³. Bound to the 20S proteasome at one or both ends is the 19S regulatory particle that can be further divided into lid and base subcomplexes (a). The base subcomplex consists of six ATPases and three non-ATPase subunits, whereas the lid subcomplex is formed from eight non-ATPase subunits. During the process of degradation, a polyubiquitylated protein is first recognized by the 19S regulatory particle (b1). The protein is then fed through the base complex, which is thought to act as a 'reverse chaperone', unfolding the protein and translocating it to the 20S core particle¹³⁴ (b2). It is within the 20S core particle that the protein is subjected to the five types of protease activity associated with the β subunits, including trypsin-like, chymotrypsin-like and peptidyl-glutamyl bond-hydrolyzing activity³ (b3). Finally, short peptides are released from the 26S proteasome to be scavenged back by the cell to form new proteins (b4).

The COP9 signalosome (CSN) is a highly conserved multiprotein complex that was initially identified as a repressor of light-dependent development in plants¹²³. It is now known that the CSN is important in many development pathways, from the regulation of floral development to controlling responses to hormones¹². The CSN consists of eight subunits (c) and when the genes that encode these proteins were identified in both plants and animals, it was realized that the CSN shows remarkable similarity to the lid subcomplex of the 19S regulatory particle¹³⁵. Both complexes show a similar internal structure, being composed of eight subunits with each of the CSN and lid subunits being paralogous to each other, showing one-to-one complementarity (TABLE 1). Given these observations, it has been suggested that the CSN can replace the lid subcomplex to form a specialized 'CSN-proteasome'¹⁴. The recent observation that the CSN associates with the proteasome, as well as several E3-ligase complexes, supports this hypothesis and indicates that the CSN might provide an essential link between the regulation of E3 activity and the 26S proteasome¹⁵.

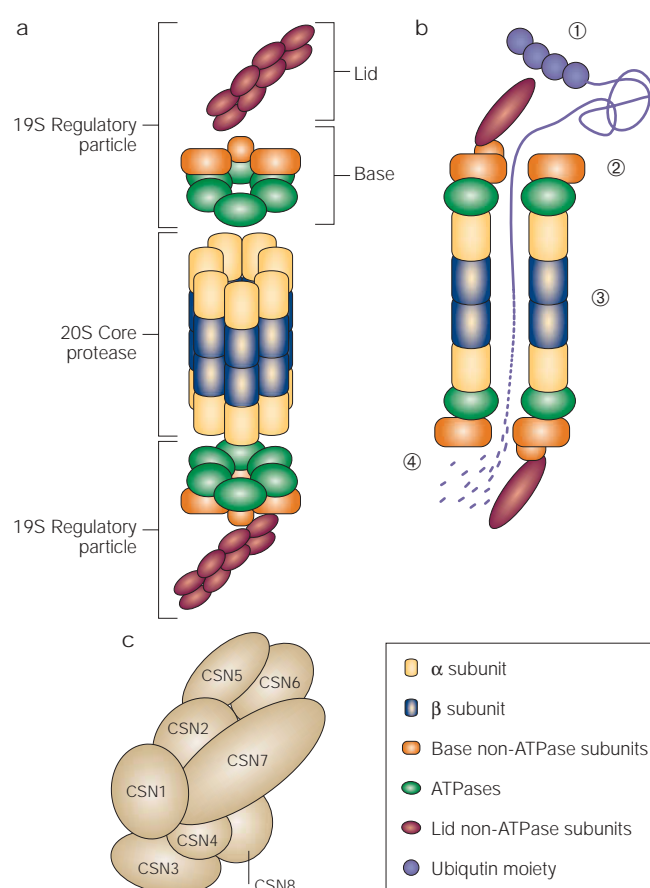


Table 1 | The subunits of the COP9 signalosome

COP9 signalosome subunit	Other name in <i>Arabidopsis</i>	Lid paralogue	Homology to lid subunit (amino-acid identity)	References
CSN1	COP11/FUS6	RPN7	22%	128
CSN2	FUS12/COP12	RPN6	21%	129
CSN3	FUS11/COP13	RPN3	20%	130
CSN4	COP8/FUS4	RPN5	19%	131
CSN5	AJH1 AJH2	RPN11	28%	132
CSN6	CSN6a CSN6b	RPN8	22%	51
CSN7	FUS5/COP15	RPN9	15%	133
CSN8	COP9/FUS7/FUS8	RPN12	18%	111

proteolysis in regulating that pathway. Many of these screens have identified proteins that contain an F-box or RING-finger motif (BOX 3). Examples include ORE9, an F-box protein that was isolated in a genetic screen for mutants with altered *SENESCENCE*²⁶, also identified as the MAX2 protein in a screen for lateral branching mutants in *Arabidopsis*²⁷; the F-box proteins LKP2 and ZTL, which were identified in *Arabidopsis* mutants with altered circadian regulation^{28,29}; and the F-box protein

SON1, which was isolated in a screen for suppressors of mutations in the *R* gene *NIM1* that confers resistance to *Peronospora parasitica*³⁰. F-box proteins, such as the AtGRH1, that are involved in regulating repression of gene expression in response to glucose, have also been isolated from cDNA library screens in yeast³¹.

Genetic screens in other plant species have also discovered F-box and RING proteins, such as the F-box-containing FIMBRIATA, which regulates homeotic genes and cell division in *Antirrhinum*³² and MsRH2-1 — a RING-H2 protein that is implicated in a wide range of developmental processes in alfalfa³³. The recent identification of ARC1, an E3 ligase that promotes protein degradation during the rejection of self-incompatible *Brassica* pollen, further demonstrates the role of ubiquitin and the 26S proteasome in the regulation of self-pollination³⁴. HECT-type E3 ligases (BOX 3) are also important during plant development. For example, the HECT E3 UPL3 has recently been shown to function in the development of trichomes (leaf hairs)³⁵. Mutations in *UPL3* cause a defect in trichome formation in *Arabidopsis* (REF. 35). Clearly, the identification of a protein that contains a signature motif such as F-box or RING finger does not necessarily establish a link to the Ub/26S system. It will be interesting to see how many of the approximately 700 proteins in the *Arabidopsis*

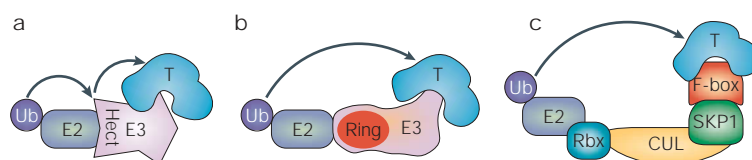
Box 3 | Ubiquitin E3 ligases

As the final step in the ubiquitin cascade (see BOX 1), the E3 ubiquitin ligation represents a crucial step in the control of the ubiquitylation process. The E3 recognizes the ubiquitylation signal in the

target protein and coordinates the transfer of the ubiquitin moiety from the E2 to a lysine residue in the target protein. The E3 binds directly to the target protein and is therefore generally considered to provide the specificity to the ubiquitination cascade. This control of specificity is reflected in the large number of E3 genes found in eukaryotes. For example, in *Arabidopsis* there are only 2 E1 genes, 46 E2 and E2-like genes, but more than 1,200 genes that encode components of ubiquitin E3s⁷. E3s have been classified into groups on the basis of their subunit composition⁷. HECT (homology to E6 carboxyl terminus) domain E3s (a) consists of a single subunit. During the ubiquitylation event, HECT E3s form an intermediate thioester linkage with ubiquitin before transfer to the lysine residue in the target protein. HECT E3s are typically >100 kDa, with the protein-interaction domains amino-terminal to the HECT domain. Although many HECT domain E3s have been identified in humans (>50), only a relatively small number have been found in yeast (5) and *Arabidopsis* (7) (REF. 4).

The RING/U-box family is also a class of single polypeptide E3s that contain a motif known as a RING finger (b). In the RING E3s, this motif is maintained by the arrangement of eight cysteines and histidines that CHELATE two zinc ions. By contrast, the U-box E3s use intramolecular interactions other than zinc chelation to maintain the RING finger¹³⁷. Unlike HECT E3s, RING/U-box E3s do not participate directly in the transfer of ubiquitin to the target and instead provide a scaffold for the correct interaction between the E2 and target. Approximately 400 potential RING E3s have been identified in *Arabidopsis*, along with at least 37 U-box-containing proteins^{4,138}. The final class of E3s is the complex E3s, which include the SCF-type E3 (c), the related VBC-Cul2 E3, other cullin-based E3s and the anaphase promoting complex (APC). The SCF E3 consists of four polypeptides: SKP1, Cullin, an F-box protein and RBX1. Similar to the RING/U-box E3, the SCF acts as a scaffold to facilitate the transfer of the ubiquitin from the E2 to the target protein. Cullin and SKP1 provide a core scaffold, RBX1 contains a RING finger that binds the E2, whereas the specificity of the SCF complex is conferred by the F-box protein that contains the protein-interaction domains that are responsible for capture of the substrate.

A recent analysis of the *Arabidopsis* genome identified ~700 genes that encoded proteins containing the 40-amino-acid F-box motif¹³⁹. These proteins can be classified into 19 groups on the basis of their domain structure, with some F-box proteins showing low-level constitutive expression, whereas others show some degree of tissue specificity in their expression patterns¹³⁴. The *Arabidopsis* genome also contains 10 cullin genes and 21 SKP1-like genes, allowing the formation of an almost limitless array of potential SCF subtypes¹⁴⁰.



SENESCENCE

Regulated death of an organ or a cell after its normal physiological function.

CHELATION

The process by which an organic chemical bonds with metal ions and thereby removes them from solutions.

genome that contain an F-box can ultimately be linked to the activity of an SCF-type E3 ligase.

Flower development and the Ub/26S system
Flower pattern formation is a well-characterized process that involves ubiquitin and possibly the proteasome. Early genetic and molecular studies in *Arabidopsis* and *Antirrhinum* led to the ABC model of floral development (FIG. 1). According to this model, organ fate is encoded by three classes of organ identity genes (A, B and C). Expression of these genes in overlapping domains of the floral primordia results in the determination of organ identity^{36–39}. The A genes function alone in the outer WHORLS to produce SEPALS, A and B genes function together in the next whorl to specify petals, B and C genes produce STAMENS in the adjacent whorl and C genes act alone in the central whorl to produce CARPELS. The B genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) encode transcription factors, which act as a heterodimer to activate the genes that are necessary to specify petals and stamens^{39–41}. Loss of either of these genes results in the loss of petals and stamens, whereas ectopic expression results in the ectopic production of petals and stamens^{40–43}. The abundance of *AP3* and *PI* is controlled by *UNUSUAL FLORAL ORGANS* (*UFO*) and *LEAFY* (*LFY*), functional loss of which results in the loss of petal and stamen identity^{45–47}. The first indication of a role for the Ub/26S system in floral development came from the cloning of *UFO* and the realization that *UFO* encoded an F-box protein that activates or maintains the transcriptional activity of *AP3* (but not *PI*)^{48,49}. The possibility that *UFO* forms an SCF-type ubiquitin E3 ligase (BOX 3) was also supported by the fact that *UFO* interacts with the *Arabidopsis* homologues of SKP1 (ASK1 and ASK2) (BOX 3) both genetically and biochemically^{48,49}. Using immunoprecipitation, it has been shown more recently that *UFO* forms a complex with ASK1 and CULLIN1 (CUL1), supporting the prediction that an E3 ligase, SCF^{UFO}, mediates *AP3* activation⁵⁰.

Interestingly, examination of partial loss-of-function mutants revealed a role for the CSN in floral development, associated with a decrease in *AP3* expression⁵¹. *In situ* hybridization showed that the CSN is highly enriched in flowers, particularly in the inner whorls⁵⁰. Furthermore, immunoprecipitation of cell extracts showed that the CSN associates with *UFO* *in vivo*, and is required for the gain-of-function activity of *UFO* in *AP3* activation and floral organ TRANSFORMATION⁵⁰. No biochemical target for SCF^{UFO} has been identified so far, possibly because SCF^{UFO} targets a direct repressor of *AP3* for degradation. Alternatively, SCF^{UFO} might target a repressor of the upstream factor, *LFY*, which in turn affects *AP3* expression. Which (if any) of these possibilities is correct remains to be determined, but it is clear that both ubiquitin and the CSN control floral B genes.

It should be noted that some defects in floral development in CSN mutants cannot be explained by defects in *AP3* expression alone⁵⁰. It is therefore likely that the CSN can regulate factors other than *UFO* that modulate floral development¹².

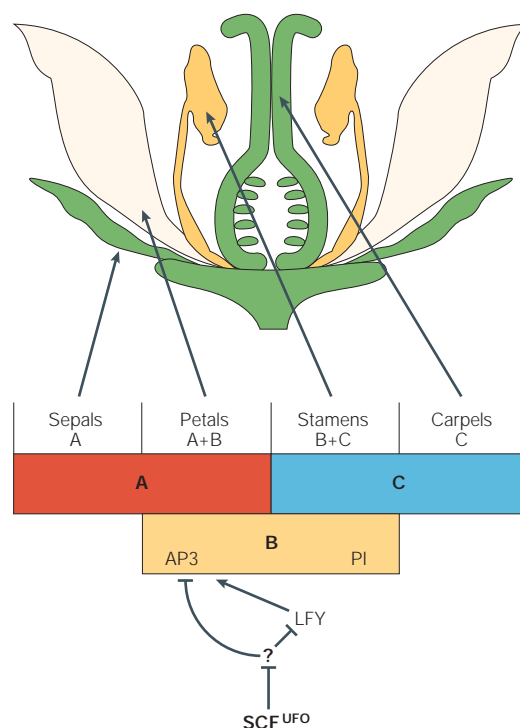


Figure 1 | The ABC model of floral organ development and the role of SCF^{UFO} in regulating the B-type gene AP3. In the 'ABC' model of floral organ development, organ identity is controlled by the expression of three classes of genes (A, B and C) in overlapping domains. In the first whorl, A-type genes are expressed alone and produce sepals. In the second whorl, the co-expression of A- and B-type genes results in the production of petals. In the third whorl, the co-expression of B- and C-type genes produces stamens, whereas the expression of C-type genes in the central whorl produces carpels. The SCF^{UFO} has a key role in controlling the activation of the B-class genes, such as *AP3* (but not *PI*), in the second and third whorls. However, so far, neither the ubiquitylation activity nor any substrate has been identified, although a role for the downstream regulator *LFY* has been indicated. *AP3*, *APETALA3*; *LFY*, *LEAFY*; *PI*, *PISTILLATA*; *UFO*, *UNUSUAL FLORAL ORGANS*.

WHORLS

A specific layer of flower organs that is generated through floral meristem activity.

SEPALS

The protective outer layer of flower.

STAMENS

The third layer of flower that bears the male gametophyte that produces pollen.

CARPELS

The fourth whorl of flower that bears the female gametophyte.

TRANSFORMATION

The change from one developmental pattern to another.

Ubiquitin and plant hormones

Classic plant hormones (phytohormones) are small non-protein molecules that control and integrate a wide variety of developmental processes. Auxin was the first phytohormone to be discovered, followed by gibberellins, cytokinins, abscisic acid, ethylene, jasmonates and brassinosteroids⁵². It has been known for some years that protein degradation through the Ub/26S system is important in regulating responses to phytohormones. The most widely studied system is probably the degradation of the AUX/IAA family of transcription repressors in response to auxin, which has been extensively reviewed (see REFS 5,6,53–56). Here, we summarize recent advances in understanding the role of the Ub/26S system in gibberellin-, ethylene-, brassinosteroid- and abscisic-acid-regulated processes. The role of proteolysis in jasmonate signalling is also discussed below in the context of its role in plant responses to pathogens.

Gibberellin-regulated processes. Gibberellins (GAs) are a family of tetracyclic diterpenoid growth factors, more than a hundred of which have so far been identified in plants⁵⁷. The analysis of mutants that are defective in the biosynthesis of GAs has shown the wide range of developmental processes that are regulated by GAs. For example, the *Arabidopsis ga1-3* mutant contains a deletion that removes the activity of *ent*-kaurene synthase, an early step in the biosynthesis of GAs^{58,59}. In contrast to wild-type plants, *ga1-3* plants are dwarfed and show reduced APICAL DOMINANCE, with plants showing increased numbers of lateral branches, reduced ability to germinate, having delayed flowering phenotype and being male sterile⁵⁸. GAs have also been implicated in programmed cell death in barley ALEURONE CELLS⁶⁰.

Genetic screens in *Arabidopsis* have identified many components of the GA perception and downstream signalling systems⁵⁷. Among the downstream signalling genes is *RGA*, which acts as a repressor of GA signalling, as mutations in this gene partially suppress the loss-of-function *ga1-3* mutant⁶². *RGA* belongs to a class of nuclear-localized transcription regulators, the DELLA family, which mediate the GA signal⁵⁷. The first indication of a role of proteolysis in GA signalling came from the observation that addition of exogenous GA to *Arabidopsis* caused a rapid reduction in *RGA* abundance⁶². A similar observation was also made with another member of the DELLA family of GA signalling repressors, *SLENDER RICE1* (*SLR1*)⁶³. Subsequently, it was shown that the GA-induced destabilization of *SLENDER* (*SLN*), the barley homologue of *SLR1*, could be prevented with proteasome inhibitors⁶⁴. Recently, a GA-insensitive dwarf rice mutant, *gid2*, was cloned and identified as an F-box protein⁶⁵ (BOX 3). It was shown that the DELLA repressor *SLR1* accumulated to high levels in *gid2* mutants, and that the *GID2* protein was associated with the rice SKP1 homologue⁶⁵. The identification of *SLEEPY1* (*SLY1*)⁶⁶, a positive regulator of GA signalling and the *Arabidopsis* counterpart of rice *GID2*, further substantiates the hypothesis that SCF^{GID2} in rice and SCF^{SLY1} in *Arabidopsis* are involved in the degradation of members of the DELLA-family repressor proteins in response to GA.

Ethylene-regulated processes. Ethylene is a gaseous plant hormone that is implicated in the regulation of many plant developmental processes, including fruit ripening, seed germination, leaf and petal abscission, organ senescence and responses to stress⁶⁷. The ethylene signalling pathway is perhaps the best characterized among the classical plant hormones⁶⁷. Regulated protein degradation has been implicated in both ethylene biosynthesis and in downstream signalling events^{67,68}. It has been suggested that the abundance of the key ethylene biosynthetic enzyme, ACC synthase, is regulated by ethylene and other stimuli, and that this regulation might involve proteasome-mediated degradation⁶⁷. It has recently been shown that EIN3, a downstream signalling component of the ethylene pathway, is primarily regulated by the Ub/26S pathway at the level of protein degradation⁶⁸.

Interestingly, EIN3 also serves as a convergence point for both ethylene signalling and regulatory responses to glucose⁶⁸.

Brassinosteroid-regulated processes. Plants, like animals, use steroids to regulate growth and development. However, in contrast to animals, brassinosteroids (BRs) are detected at the plasma membrane by a membrane-localized receptor kinase BRI1 (REF. 69). As with other phytohormones, BRs have a wide range of roles during development. Mutants defective in BR biosynthesis or signalling show defects such as dwarfism, sterility and light-grown development in darkness⁷⁰. Phenotypes of BR-deficient and BR-signalling mutants can be suppressed by a dominant mutant of *Arabidopsis bZR1-1D*. *BZR1*, which acts as a positive regulator of BR signalling, accumulates in the nucleus and is stabilized in response to BRs⁷¹. Recent data indicate that BRs induce dephosphorylation and accumulation of *BZR1* and that degradation of the phosphorylated form of *BZR1* can be prevented with proteasome inhibitors⁷². It therefore seems likely that the Ub/26S system is important in BR signalling, although the precise mechanism for this control remains to be identified.

ABA-regulated processes. One of the roles of the phytohormone abscisic acid (ABA) is to protect the developing plant from drought stress. During the early stages of germination, if a seedling is faced with a lack of water, it can arrest its growth until that stress has been removed. This stress-induced growth arrest is mediated by ABA and can be PHENOCOPIED by the addition of exogenous ABA during the early stages of germination⁷³. This drought-induced growth arrest depends on the expression of the transcription factor ABI5, which in turn accumulates in response to ABA⁷³. Recently, it has been shown that in the absence of ABA, ABI5 is ubiquitinated and subsequently degraded by the 26S proteasome⁷⁴. This degradation is enhanced by the ABI5 binding protein AFP, although the precise role of AFP remains to be determined⁷⁴.

Plant pathogens and SCF E3 ligases and the CSN. In natural environments, plants are exposed to a wide range of pathogens, including bacteria, viruses, fungi and insects. Plants resist such attacks by sophisticated defence mechanisms, including localized cell death, often called the hypersensitive response (HR), which produces a burst of reactive oxygen species at the site of infection and induces systemic immunity throughout the plant (for reviews, see REFS 75–79). The HR is triggered by a product of a plant disease resistance (*R*) gene that directly or indirectly recognizes a pathogen determinant, usually an effector protein (E) encoded by a pathogen avirulence (*avr*) gene (FIG. 2).

Numerous *R* genes have been identified from several plant species, and the structural similarity between *R* gene products that confer resistance to a wide range of pathogens indicates the existence of common signalling pathways downstream of pathogen perception^{78,80,81}. One of these common components is the *RAR1* gene

APICAL DOMINANCE

Concentration of growth at the tip of a plant shoot, where a terminal bud exerts partial inhibition of auxiliary bud growth.

ALEURONE CELLS

A cell type in cereal kernels that undergoes highly regulated cell death to release stores of minerals and nutrients to the developing embryo.

PHENOCOPY

The production of a phenotype, which closely resembles a phenotype that normally results from specific gene expression or from gene mutation.

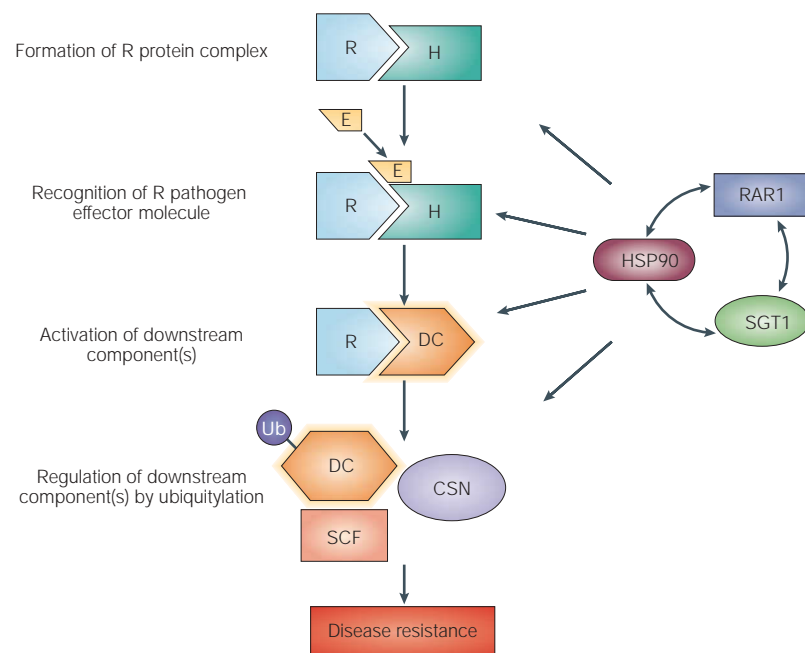


Figure 2 | A model for R protein-dependent disease-resistance signalling and the role of SCF and the COP9 signalosome. Resistance (R) proteins often complex with host proteins (H). R protein complexes might recognize a pathogen-derived effector protein (E) directly or detect a conformational change of H caused by E. The activated R protein then signals to presumed downstream components (DCs). Regulation of downstream signalling components is thought to be controlled by ubiquitylation that is mediated by SCF and CSN complexes. RAR1 and SGT1 function closely with HSP90, which potentially operates several steps of the signalling pathway. CSN, COP9 signalosome; SGT1, suppressor of the G2 allele of *skp1-4*.

POWDERY MILDEW

A group of plant diseases that are caused by the growth of fungal mycelium and the production of spores on the surface of plant tissues.

DENEDDYLYATION

The removal of the ubiquitin-like modifier NEDD8 (also called RUB) from a protein.

HISTONE DEACETYLASE

The enzyme that removes acetyl groups from lysine residues in the DNA-binding histone group of proteins.

SKOTOMORPHOGENIC

Developmental pattern followed by seedlings in the absence of light.

HYPOCOTYLS

The stem of a seedling.

COTYLEDONS

The leaves of a seedling formed during embryonic development.

PHOTOMORPHOGENIC

Developmental pattern followed by seedlings in the presence of light.

that is required for resistance to POWDERY MILDEW conferred by the *R* genes *Mla6* and *Mla12* (REF. 82). RAR1 is highly conserved in eukaryotes and contains two CHORD domains (cysteine- and histidine-rich domains) that probably form a new zinc-finger structure⁸². RAR1 interacts with plant orthologues of yeast SGT1 (SUPPRESSOR OF THE G2 ALLELE OF *skp1-4*), and plants with reduced amounts of SGT1 have reduced resistance to pathogens^{83–86}. Both RAR1 and SGT1 interact with HSP90, which is also required for disease resistance, indicating that RAR1 and SGT1 might function in the resistance pathway as co-chaperones^{86,87}. In yeast, SGT1 serves several functions, probably together with HSP90, in the regulation of the cell cycle as well as the cyclic AMP pathway^{88,89}. During yeast cell-cycle control, SGT1 regulates the activity of SCF-type E3 ligases through its interaction with SKP1 (REF. 90). The *Arabidopsis* homologues of *SGT1* (*SGT1a* and *SGT1b*) can complement a yeast *sgt1* cell-cycle mutant and SGT1 associates with the core SCF subunits in barley and *Nicotiana benthamina*, strongly indicating that SGT1 performs similar biochemical functions in plants and yeast^{83,89}.

The importance of SCF-type E3 ligases in pathogen responses is also strongly supported by the observation that *N. benthamina* plants with reduced amounts of SKP1 are compromised for *R* gene-mediated resistance to tobacco mosaic virus (TMV)⁸⁹. It therefore seems very likely that SCF-type E3 ligases are important for *R* gene-mediated resistance to pathogens. Unfortunately,

it is not clear which proteins are targeted for ubiquitylation during the defence response, or indeed whether the targets are degraded or activated by monoubiquitylation or non-canonical polyubiquitylation.

Interestingly, as seen with other SCF-mediated processes, the CSN also seems to have a role in *R* gene-mediated resistance. This is based on the observation that RAR1 associates with the CSN in both cell extracts from barley and *N. benthamina*^{83,89}. Furthermore, silencing of the CSN in *N. benthamina* also compromises *R* gene-mediated resistance to TMV in a similar manner to that seen with silencing of *SGT1* and *SKP1* (REF. 89). Again, whether the CSN DENEDDYLYATES the cullin subunit, and thereby regulates an SCF E3 ligase mediating disease resistance, remains to be determined. Interestingly, *Arabidopsis* SGT1b is required for correct SCF^{TIR1}-mediated auxin responses⁹¹, which further indicates that SGT1 might be a regulator of SCF-type E3 ligases that are not specifically associated with disease resistance.

Jasmonate-regulated processes. Another link between proteolysis and responses to pathogens came from the investigation of the *Arabidopsis coronatine insensitive1* (*coi1*) mutant. The jasmonate (JA) family of phytohormones regulates pollen development, inhibition of plant growth, and wound- and pathogen-induced defence responses⁹². The *coi1* mutant was identified in a genetic screen for insensitivity to the bacterial toxin coronatine, which is similar in structure to jasmonates⁹³. *coi1* mutants are unable to express protective pathogen-induced genes, making them susceptible to insect and pathogen attack^{94,95}. *COI1* encodes an F-box protein that can associate with both SKP1 and cullin subunits to assemble into an SCF-type E3 ligase, SCF^{COI1} (REFS 96–98). Interestingly, COI1 binds to HISTONE DEACETYLASE (HDAC), and targeted ubiquitylation by SCF^{COI1} of HDAC might be involved in jasmonate-regulated gene expression⁹⁶. It has recently been shown that the CSN associates with SCF^{COI1} and that plants with reduced CSN function show reduced responses to JA⁹⁹. The observation that the modification of the CUL1 subunit in SCF^{COI1}, probably through AXR1-dependent neddylation, has a crucial role in the control of JA signalling⁹⁷ also strongly supports a role for the CSN in ubiquitylation mediated by SCF^{COI1}.

Proteolysis and light-regulated development

Light has pronounced effects on growth throughout the life cycle of plants (for a recent review, see REF. 100). The effect of light on the period between seed germination and the formation of the first true leaves has been studied extensively¹⁰¹. After germination, seedlings follow one of two developmental patterns. In darkness, seedlings follow SKOTOMORPHOGENIC (etiolated) development, having long HYPOCOTYLS, and closed, unexpanded COTYLEDONS that are protected by an apical hook (FIG. 3). By contrast, growth in the light results in PHOTOMORPHOGENIC (de-etiolated) development, which is characterized by short hypocotyls and open and expanded cotyledons that can photosynthesize (FIG. 3). The switch between

dark- and light-grown development, which starts with the perception of light by the photoreceptors, phytochrome and cryptochrome, ultimately involves genome-wide changes in gene expression brought about by transcriptional cascades^{102–104}.

Mutant screens in *Arabidopsis* have identified many components of the signalling pathways that are downstream of photoreceptors (for recent reviews, see REFS 101–104). Among the genes that are important for de-etiolation are the pleiotropic *COP/DET/FUS* genes, which are required for the repression of photomorphogenesis in darkness¹⁰⁷. The *COP/DET/FUS* genes seem to define four biochemical entities: COP1, DET1, COP10 and the CSN^{107,109–111}. COP1 functions as a light-inactivatable repressor of photomorphogenesis, is predominantly localized in the nucleus in the dark but not in light, and interacts with transcription factors such as HY5, HYH and LAF1, which promote photomorphogenesis^{112–116}. The first indication of a role for protein degradation in photomorphogenesis came from the discovery that HY5 abundance was directly correlated with the extent of photomorphogenesis, and that the degradation of HY5 in darkness involved the 26S proteasome and COP1 (REF. 117). COP1 contains a RING domain¹⁰⁸, which is found in many ubiquitin E3 ligases (BOX 3), and it was proposed that COP1 mediates the ubiquitylation and subsequent degradation of HY5 in darkness¹¹⁷. However, it was not until very recently that the ubiquitylation activity of COP1 on the transcription factors HY5 and LAF1 was demonstrated *in vitro*^{118,119}.

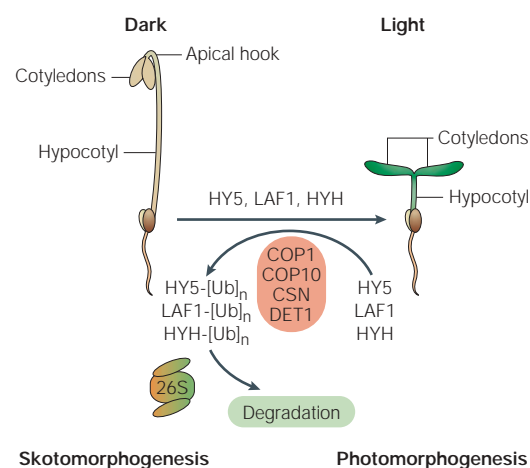


Figure 3 | The effect of light on *Arabidopsis* seedling development and the ubiquitylation and protein degradation conferred by the COP/DET/FUS group of proteins. After germination, *Arabidopsis* seedlings follow one of two developmental pathways: skotomorphogenesis in the dark and photomorphogenesis in the light. The switch from dark- to light-grown development requires the positive action of transcription factors, including HY5, LAF1 and HYH. In the dark, these transcription factors are degraded by the Ub/26S system through the action of COP1, COP10, the CSN and DET1, thereby causing the repression of photomorphogenesis. CSN, COP9 signalosome; Ub/26S, ubiquitin/26S proteasome system.

Interestingly, *COP10* encodes a protein that acts with COP1 and the CSN to repress photomorphogenesis and shows amino-acid similarity to ubiquitin conjugating (E2) variant (UEV) proteins¹¹⁰. UEVs have been shown to function in yeast and mammalian systems in the production of non-canonical polyubiquitin chains¹²⁰. Consequently, it has been suggested that COP10 might act as an E2, with the E3 activity of COP1. Alternatively, COP10 might regulate COP1 activity by mediating the formation of unusual polyubiquitin chains on COP1 and thereby positively regulating its activity¹¹⁰. So far, no apparent biochemical link between DET1 and ubiquitylation has been discovered, although it is required for COP1-mediated HY5 degradation in darkness¹¹⁷. However, it has recently been shown that DET1 interacts with DAMAGE DNA BINDING PROTEIN1 (DDB1) and can bind histone H2B, indicating a role for chromatin remodelling in the regulation of photomorphogenesis^{121,122}.

The remaining *COP/DET/FUS* genes encode subunits of the CSN (BOX 2). The role of the CSN in the regulation of SCF-type E3 ligases through deneddylation of the cullin subunit has already been mentioned above. It should be noted that genetic studies have shown that both COP1 and the CSN are required for repression of photomorphogenesis in darkness^{114,117}. Although a direct physical link between the CSN and COP1 has not been established, the genetic data seem to indicate that the CSN might regulate the E3 activity of COP1 (REF. 13). One possible mechanism for this regulation could be that the CSN act as a lid for a specialized proteasome for degradation of COP1 substrate. Another possible regulatory mechanism is implied by the observation that the nucleocytoplasmic partitioning of COP1 is altered in mutants that lack the CSN¹²³. Whether the CSN controls the localization of COP1 through a neddylation/deneddylation process or through some as yet unidentified activity remains to be determined. However, it is clear that the CSN has some role in regulating non-SCF-type E3 ligases such as COP1.

Conclusions and perspectives

As in all eukaryotes, highly regulated proteolysis is essential to plant life. This is reflected, at least in part, by the large portion of the *Arabidopsis* genome that encodes proteins that are potentially involved in the Ub/26S system. To cope with their static lifestyle, plants have evolved a considerable degree of developmental plasticity. This flexibility allows plants to alter their developmental programme to best suit the changing environmental conditions and stresses that are found in nature. There is now growing evidence that targeted protein degradation, and specifically the Ub/26S system, is important in conferring this plasticity of plant development. In this review, we have outlined some of the developmental processes in which the Ub/26S system is thought to be involved. The list of processes that involve the Ub/26S system is by no means complete and it is very likely that in the coming years many more developmental pathways will be linked to ubiquitin and/or regulated proteolysis.

In many of the examples described here, the role for the Ub/26S system is not yet fully understood and in some cases is yet to be conclusively established. In several cases, a crucial role for an E3 has been found but the specific target (or targets) remains to be identified. In degradation, ubiquitin acts together with 26S proteasome but because it functions in regulatory processes other than degradation, the discovery of its role in a developmental pathway does not necessarily imply the involvement of 26S proteasome in these processes. Nevertheless, it seems likely that ubiquitin and/or the 26S proteasome will be found to have some function in most (if not all) of the developmental pathways in plants.

Control of proteolysis is an area of the Ub/26S system in plants that requires more study. In many of the examples described above, the initial event leading to a change in development is known, downstream signalling components might have been identified and the role of an E3 ligase established. However, an understanding of how the E3 ligase activity is regulated is yet to be achieved. Take, for example, the role of proteolysis in photomorphogenesis. Physiological, biochemical and genetic studies over many years have identified the photoreceptor molecules in plants that are responsible for photomorphogenesis (for reviews, see REFS 101,102,105,106,124). The identification of mutants with defects in photomorphogenesis has also allowed many downstream components of light signalling to be identified, central to which seems to be the E3 ligase COP1. It has recently been shown that SPA1, a specific regulator of phyA signalling, can modify the activity of COP1 *in vitro*^{118,119}, although its role in the light signalling pathway remains to be determined.

A direct physical association between COP1 and CRY1 and CRY2 (the blue light photoreceptors) has now been shown^{125,126}. As mentioned above, the nuclear localization of COP1, and the role of the CSN in that localization, seems to be crucial for COP1 function^{116,123}. It is therefore likely that the E3 activity of COP1 is controlled in many ways, through both activation/deactivation and changes in localization that involve the CSN, upstream signalling components and, in some cases, direct interaction with the photoreceptors themselves. If the regulation of other E3 ligase activities is as complicated as that of COP1, it is likely that extensive studies will be required before the regulation of ubiquitin-mediated proteolysis in plants is fully understood. The CSN also seems to be crucial for the regulation of SCF-type and non-SCF E3-ligase activities. Just how widely the CSN is used as an E3 regulator is unknown. What is clear is that any insights into how proteolysis is regulated will require a much better understanding of the CSN.

It is only in recent years that the importance of regulated proteolysis, and more specifically of the Ub/26S system, in controlling plant development has been recognized. The Ub/26S system has been implicated in several plant development pathways from flowering to pathogen attack, and it is likely that its role in many more developmental processes will eventually be discovered. In the coming years, a greater understanding of the Ub/26S system will inevitably lead to a better understanding of plant development. We can therefore say with certainty that there are exciting times ahead for ubiquitin and the 26S proteasome in plants.

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Competing interests statement

The authors declare that they have no competing financial interests.

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