CellPress
Current Biology
Magazine

in PSK-mediated cell expansion, receptors of RGF1 (RGFRs) in root meristem development, extra microsporocytes 1 (EMS1) in tapetum development, ERECTAs in stomatal patterning, HAESEs in floral organ abscission, and FLS2, EFR and PEPRs in plant immunity. In addition, SERK3 dimerizes with receptor-like protein (RLP) immune receptors, such as RLP23. Some RLPs constitutively complex with another LRR-RLK, SOBIR1, and subsequently recruit SERK3 upon ligand perception, forming a tripartite SERK3–RLK–RLP complex. Therefore, SERK interactions with a broad array of receptors modulate the interconnected architecture of the cellular signaling networks, and yet they disseminate diverse biological outcomes.

How do SERKs achieve specificity in interconnected signaling networks? SERKs redundantly yet unequally contribute to different physiological processes. For instance, SERK3 plays a major role in plant immunity, SERK4 additively contributes to immunity only in the absence of SERK3, whereas SERK1 and SERK2 have no contribution to immunity. Differential affinities with the cognate receptors may contribute to their functional specificity. *SERKs* also exhibit differential expression patterns and levels. *SERK3* is abundantly expressed in most tissues, whereas *SERK4* is only expressed in mature leaves, and *SERK1* and *SERK2* in flowers and seeds. SERKs phosphorylate different substrates. In plant immune signaling, SERK3 phosphorylates BIK1 family receptor-like cytoplasmic kinases (RLCKs), and sugar transport protein 13 (STP13) to regulate sugar uptake activity. SERK3 and SERK4 phosphorylate cyclic nucleotide-gated channel 19 (CNGC19) and CNGC20 in regulating cellular homeostasis and cell death containment. Furthermore, the phosphorylation status of SERK3 and its microdomain at the plasma membrane contribute to its specific involvement in immunity and BR signaling.

What remains to be explored? Although only a small portion of LRR-RLKs have been assigned a biological function, strikingly, many of them function in concert with SERKs.

It is tempting to speculate additional roles of SERKs being continuously revealed with the studies of RLKs. Recent studies have suggested SERK3 may be able to associate with non-LRR RLKs, such as malectin-like domain RLKs. Do SERKs also function as co-receptors of non-LRR-RLKs? Additionally, so far, only a few SERK substrates have been identified. Additional downstream substrates transmitting different cellular signaling events await to be discovered. Also, it remains a central mystery as to why and how diverse receptors repetitively use SERKs as co-receptors. How are SERKs evolved to bear so many functions? Can we manipulate SERKs in crop plants to achieve high yield with improved resistance? Nevertheless, the study of SERKs presents a conundrum to dissect the functional plasticity of key shared signaling modules.

Where can I find out more?

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Primer

Brassinosteroid signalling

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Steroids are the only hormones shared between mammals and plants. In spite of the divergence between plants and animals more than 1 billion years ago, it is remarkable that similar steroid hormones are still used to control many of the same developmental and physiological processes. Unlike mammals, plants have no glands for steroid production, and individual cells are able to generate hormones. Mammalian cells typically respond to steroids by means of nuclear receptors, whereas in plants, the receptor kinases are anchored to cell membranes with steroid-binding domains exposed to the cell exterior. To date, only one type of steroid hormone, named brassinosteroids (BRs), has been found in plants. Research has shown that BRs are involved in acclimation to environmental stresses, cell elongation, and resistance to pathogens, thus increasing plant growth and crop yield. Over the last decades, mostly based on studies in the model plant *Arabidopsis thaliana*, tremendous progress has been made in the identification of BR signalling components — from the cell surface receptors to the core transcription factors and their target genes in the cell nucleus. Hence, in plants, the BR signalling pathway has become one of the best understood signal transduction pathways and serves as a paradigm for receptor kinase-mediated cellular signalling. Here, we provide an overview of the core BR signalling components in *Arabidopsis* that have been well characterized by genetic, proteomic, and genomic studies.

The discovery of brassinosteroids

BRs were first discovered in 1979 in extracts of oilseed rape (*Brassica napus*) pollen that exhibited a growth-promoting activity, and consequently were named ‘brassins’. Since the identification of the most active BR, brassinolide (BL; Figure 1A), at least 69 chemically different BRs have been found throughout the plant kingdom, including land plants and green algae, indicating that BRs arose early during plant evolution. BRs have

been recognized as plant hormones since the identification of BR-deficient mutants in *Arabidopsis*. Mutations in BR biosynthetic genes lead to distinct growth defects in *Arabidopsis*, such as dwarfism, reduced cell elongation, dark-green and thickened round-shaped leaves, reduced apical dominance, delayed flowering and senescence, male sterility, and de-etiolation in darkness (Figure 1B,C). These phenotypes are rescued by exogenous BL, suggesting that BRs are essential growth-promoting hormones. Endogenous amounts of BRs in plant tissues are extremely low when compared with the other plant hormones, and these levels are tightly controlled by BR signalling.

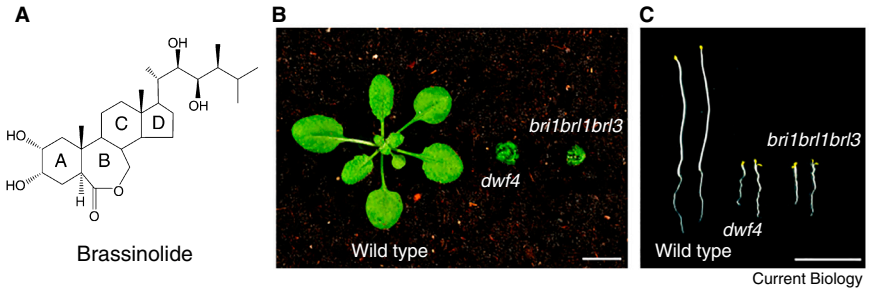

Figure 1. BRs are essential growth-promoting hormones.
(A) Chemical structure of the most active BR, brassinolide (BL), with the steroid rings labelled as A, B, C, and D. (B) Phenotypes of 21-day-old *Arabidopsis* wild-type plant, the BR biosynthetic mutant *dwf4* and the BR receptor mutant *bri1 brl1 brl3*. The *dwf4* and *bri1 brl1 brl3* mutants display severe dwarfism, including small, round and dark-green leaves. (C) When grown in complete darkness for 5 days, *dwf4* and *bri1 brl1 brl3* show de-etiolation phenotypes with open and expanded cotyledons and short hypocotyls. Scale bars, 1 cm.

Receptors for BRs at the cell surface

BRs are perceived outside the cell by a plasma membrane-localized receptor, designated BRASSINOSTEROID-INSENSITIVE1 (BRI1). Genetic screens for BR resistance with respect to root growth identified the first BR-insensitive mutant (*bri1*) in *Arabidopsis*. The *bri1* mutant displayed phenotypes that resembled the BR biosynthetic mutants (Figure 1B,C), but could not be rescued by exogenous BRs. Positional cloning revealed that the *BRI1* gene encodes a protein with similarity to the mammalian Toll-like receptors, consisting of an extracellular leucine-rich repeat (LRR) domain, a single transmembrane domain, and a cytoplasmic serine/threonine kinase. BRI1 is highly conserved across different plant species. In *Arabidopsis*, BRI1 has three homologs, BRI1-LIKE1 (BRL1), BRL2, and BRL3. Early binding studies with a biotin-tagged photoaffinity labeled active BL precursor revealed that BRI1, BRL1, and BRL3, but not BRL2, bind BRs with a high affinity. Moreover, only BRL1 and BRL3 can rescue the phenotypic defects in the *bri1* mutant when expressed under the BRI1 promoter, indicating that BRL1 and BRL3 are functional BR receptors. Whereas *BRI1* is widely expressed, *BRL1* and *BRL3* are mainly expressed in vascular tissues and display weak phenotypes when knocked out. BRI1 associates with a smaller LRR receptor kinase, BRI1-ASSOCIATED KINASE1 (BAK1). BAK1 is also known as SOMATIC EMBRYOGENESIS RECEPTOR KINASE3 (SERK3), which belongs to a family of five LRR receptor kinases in *Arabidopsis*. BAK1 was identified as a BRI1-interacting protein by means of a yeast two-hybrid screen and independently identified as a suppressor of *bri1*. The *bak1* loss-of-function mutant resembles weak *bri1* mutants and BAK1 overexpression suppresses the *bri1* phenotype.

BAK1 function in BR signalling is redundant with SERK1 and SERK4. The *serk1 bak1 serk4* triple mutant phenocopies a null *bri1* mutant, establishing an indispensable role for SERKs in BR signalling. In 2011, the crystal structure of the BRI1 extracellular domain was solved, and despite a sequence similarity to the mammalian Toll-like receptors, the ectodomain of BRI1 was found not to adopt the anticipated horseshoe structure, but to form a right-handed superhelix composed of 25 LRRs. BL binds to the hydrophobic groove of the extracellular domain of BRI1 created between the inner surface of the helical LRR and the 70-amino acid 'island domain' located between LRRs 21 and 22. This mechanism is preserved for BRL1 and likely for BRL3, given the high conservation of the BL-binding groove among the three BR receptors. Subsequently, the crystal structures of the ternary complexes between the extracellular domains of BRI1 with either BAK1 or SERK1, and BL were identified. These structural data demonstrated that BL induces heterodimerization of BRI1 and BAK1 or SERK1, and that BAK1 and SERK1 are directly involved in BL recognition. These results revealed that BAK1 and SERK1 function as co-receptors of BRI1 and that this interaction is required for BRI1 activation, thus resembling the ligand-induced activation of the mammalian receptor tyrosine kinases.

BR-induced downstream signalling network

In the absence of BRs, BRI1 remains in an inactive state through multiple mechanisms, including auto-inhibitory carboxyl terminus, auto-phosphorylation and PROTEIN PHOSPHATASE 2A (PP2A)-mediated dephosphorylation of the kinase domain, and interaction with the inhibitory protein BRI1 KINASE INHIBITOR1 (BKI1) (Figure 2). Upon BR binding to the BRI1 receptor, BRI1 phosphorylates BKI1, leading to its dissociation from BRI1 and full activation of BRI1 through *trans*-phosphorylation between the cytoplasmic kinase domains of BRI1 and BAK1 or other SERK members. Once fully activated, BRI1 phosphorylates the BR SIGNALING KINASEs (BSKs) and the CONSTITUTIVE DIFFERENTIAL GROWTH1 (CDG1) proteins, both belonging to the receptor-like cytoplasmic kinase (RLCK) superfamily.

The RLCKs share homology with the kinase domains of the LRR receptor kinases, but lack an extracellular domain. They localize to the plasma membrane via lipid modifications (such as amino-terminal myristoylation or palmitoylation) and this localization is required for their function. BSKs and CDG1 belong to two distinct RLCK subfamilies, XII and VII, respectively, but they both function in parallel as positive regulators of the BR signalling. BSKs were discovered through proteomics studies as BRI1-interacting proteins. They function as

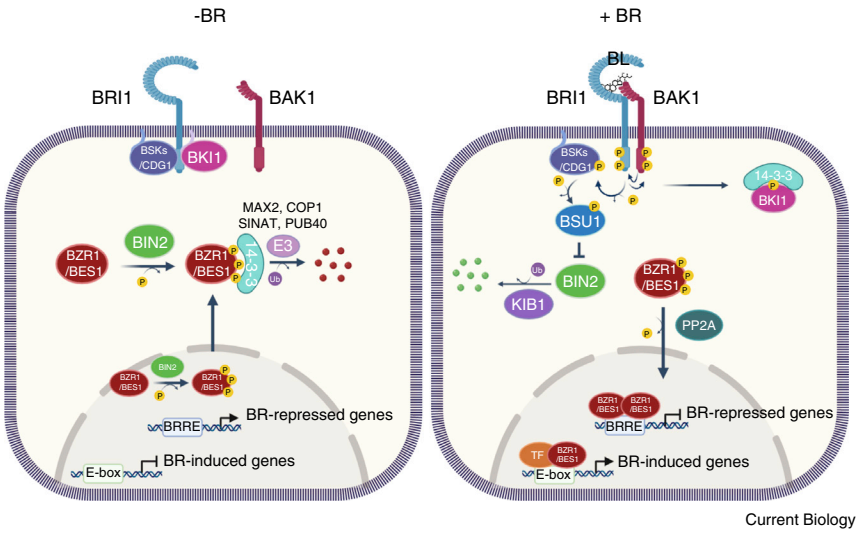

Figure 2. The BR signalling pathway in *Arabidopsis*.
Left, in the absence of BRs, association of BRI1 with BKI1 keeps BRI1 inactive. Inactivation of BRI1 allows BIN2 to be constitutively active and to phosphorylate BZR1 and BES1 transcription factors. In turn, BZR1 and BES1 phosphorylation causes their cytoplasmic retention by interaction with 14-3-3 and abolishes their DNA-binding activity. BZR1 and BES1 degradation is mediated by different E3 ligases (*e.g.,* MAX2, COP1, SINAT, and PUB40). Right, when BRs are present, BR binding to BRI1 and the co-receptor BAK1 leads to the dissociation of BKI1 from BRI1 and triggers *trans*-phosphorylation between BRI1 and BAK1. Activated BRI1–BAK1 receptor complex transduces its signal to BSKs and CDG1 through direct phosphorylation. BSKs or CDG1 activate the BSU1 phosphatase. BSU1 then inactivates BIN2 by dephosphorylation, and the E3 ligase KIB1 mediates the BIN2 degradation. Meanwhile, PP2A activates BZR1 and BES1 via dephosphorylation and allows the transcription factors to enter the nucleus and to bind DNA for BR-responsive gene regulation. The figure is created with BioRender.

scaffolds and BRI1 phosphorylation promotes their association with a family of phosphatases, called BRI1 SUPPRESSOR1/BSU-LIKEs (BSU1/BSLs), to activate them. On the other hand, CDG1 was identified as a dominant activation-tagged mutant displaying constitutive BR responses. BRI1 activates the CDG1 kinase through phosphorylation, and CDG1 in turn phosphorylates BSU1/BSLs to activate them.

BSU1 and BSLs belong to the unique kelch-like domain family of protein phosphatases. BSU1 was identified as a positive regulator of BR signalling in a mutant screen for suppressors of the weak *bri1-5* mutant. The quadruple mutant for BSU1 and BSLs displays severe BR-related phenotypes. Activated BSU1 or BSLs can, in turn, dephosphorylate a conserved tyrosine residue in the constitutively active kinase BRASSINOSTEROID INSENSITIVE2 (BIN2), a key negative regulator of the BR signalling, to inactivate its kinase activity. BIN2 was first found as a BR-insensitive gain-of-function mutant, *bin2-1*, that showed a severe dwarf phenotype similar to that of the *bri1* null mutant.

BIN2 and its homologs belong to the glycogen synthase kinase3 (GSK3)-like family of serine/threonine kinases in *Arabidopsis* that are highly conserved in all eukaryotes. Like the mammalian GSK3, plant GSK3-like kinases have diverse substrates and function as hubs in crosstalks with other signalling pathways. Of the 10 *Arabidopsis* GSK3-like kinases, BIN2 and its close homologs BIN2-LIKE1 (BIL1) and BIL2 act redundantly as negative regulators of BR signalling, so that the triple *bin2-3 bil1 bil2* loss-of-function mutant exhibits constitutive BR responses.

However, other GSK3-like family members might also function in BR signalling. Besides BR-induced dephosphorylation, BIN2 activity is regulated by the interaction with the F-box E3 ubiquitin ligase KINK SUPPRESSED IN BZR1-1D (KIB1), leading to proteasomal degradation of BIN2 in the presence of BRs. In addition to regulation via degradation, BIN2 activity is regulated through interactions with scaffolding proteins that induce changes in BIN2 localization. For example, OCTOPUS and POLAR sequester BIN2 to the plasma membrane in the phloem and in the stomatal lineage, respectively. Besides BIN2, scaffolding of other BR signalling components to the plasma membrane through interactions with membrane-associated TETRATRICOPEPTIDE THIOREDOXIN-LIKE has recently been implicated in the regulation of BR signalling.

BR-regulated gene expression

The *Arabidopsis* BRASSINAZOLE RESISTANT1 (BZR1) and BRI1 EMS SUPPRESSOR1 (BES1), also known as BZR2, are the two key transcription factors in BR signalling that directly regulate the expression of many target genes. These transcription factors have a plant-specific and highly conserved DNA-binding domain with a basic helix-loop-helix (bHLH)-like motif. The gain-of-function *bes1-D* and *bzr1-1D* mutants were isolated in forward genetic and chemical genetic screens, respectively. Both *bes1-D* and *bzr1-1D* suppress the dwarf phenotypes of weak *bri1* and *bin2-1* mutants, accumulate dephosphorylated BZR1 and BES1 proteins in the nucleus independently of BL, and display constitutive BR responses, suggesting that they act downstream of BIN2 as positive regulators of BR signalling.

Although single loss-of-function BES1 or BZR1 mutants do not show BR-related defects, phylogenetic analysis indicates that BES1 and BZR1 belong to a six-member family, including BES1, BZR1, BES1/BZR1 HOMOLOG1 (BEH1), BEH2, BEH3, and BEH4, hinting at functional redundancy. Indeed, the hextuple mutant (*bzr-h*) displayed growth phenotypes that were almost identical to those of the null *bri1* mutant. The activities of BZR1 and BES1 are tightly regulated via phosphorylation. In the absence of BRs, the constitutively active BIN2 phosphorylates BZR1 and BES1 to inhibit their activity by multiple distinct mechanisms. Phosphorylation of BZR1 and BES1 abolishes their DNA-binding activity, promotes their cytoplasmic retention by interaction with either 14-3-3 proteins or BRZ-SENSITIVE-SHORT HYPOCOTYL1 (BSS1), and targets them for degradation. In the presence of BRs, BIN2 is inactivated and a PP2A phosphatase dephosphorylates BZR1

and BES1, which then become active in the nucleus to control BR-responsive gene expression.

To maintain growth and development and to ensure responses to specific environmental or hormonal cues, the stability of BZR1 and BES1 is precisely regulated by multiple E3 ubiquitin ligases that control their degradation through the 26S proteasome or selective autophagy. For example, BES1 degradation is promoted by strigolactones through the activation of the shoot branching-inhibiting factor, the F-box protein MORE AXILLARY GROWTH LOCUS2 (MAX2). BES1 interacts with MAX2 and acts as its substrate for degradation. Phosphorylated BZR1 and dephosphorylated BES1 are degraded by the E3 ligases, CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) under dark conditions and by SEVEN IN ABSENTIA (SINA) of *Arabidopsis thaliana* (SINAT) under light conditions, respectively. In addition, BZR1 degradation is mediated by PLANT U-BOX40 (PUB40) in a root-specific manner.

Genome-wide microarray assays in *Arabidopsis* have revealed that BRs regulate the expression of thousands of genes. Active BZR1 and BES1 can both bind the conserved BR-response elements (BRREs) (CGTG$^{T}/_{C}$G, G-box motif variants) and E-box elements (CANNTG) mostly in the promoters of BR-repressed and BR-induced genes, respectively. The BRREs are enriched in BR biosynthetic gene promoters and in the presence of BRs, BZR1 and BES1 bind to these elements as homodimers to repress the BR biosynthetic gene expression, thus forming a negative feedback loop to preclude BR signalling through inhibition of BR production. BZR1 and BES1 bind to E-box elements by generating heterodimers with other bHLH transcription factors to activate the BR-induced gene expression. Crystal structure analysis of the DNA-binding domain of BZR1 in a DNA-containing G-box complex supports these binding models and identified specific residues in BZR1, determining the binding specificities, binding affinities, and dimer formation.

Evolution of BR signalling

Plants and mammals use different mechanisms to perceive the steroid hormones that remarkably exert analogous functions to regulate a wide range of developmental and physiological responses. The canonical BR signal transduction pathway has emerged relatively late during evolution (Figure 3). Although the family of the LRR receptor kinases is found in lineages from liverworts to angiosperms, receptor kinases with an island domain in their LRR extracellular domains that is crucial for BL binding occur only in gymnosperms and angiosperms. By contrast, most of the remaining BR signalling proteins (*e.g.* BSK1, CDG1, BSU1, BIN2, BZR1, PP2A, and 14-3-3), except BKI1, are present in liverworts and some even in green algae, indicating that certain cytosolic BR signalling modules arose before the divergence of the land plants. These proteins are well conserved among the plant kingdom, but they might have been involved in other pathways before acquiring BR-related functions.

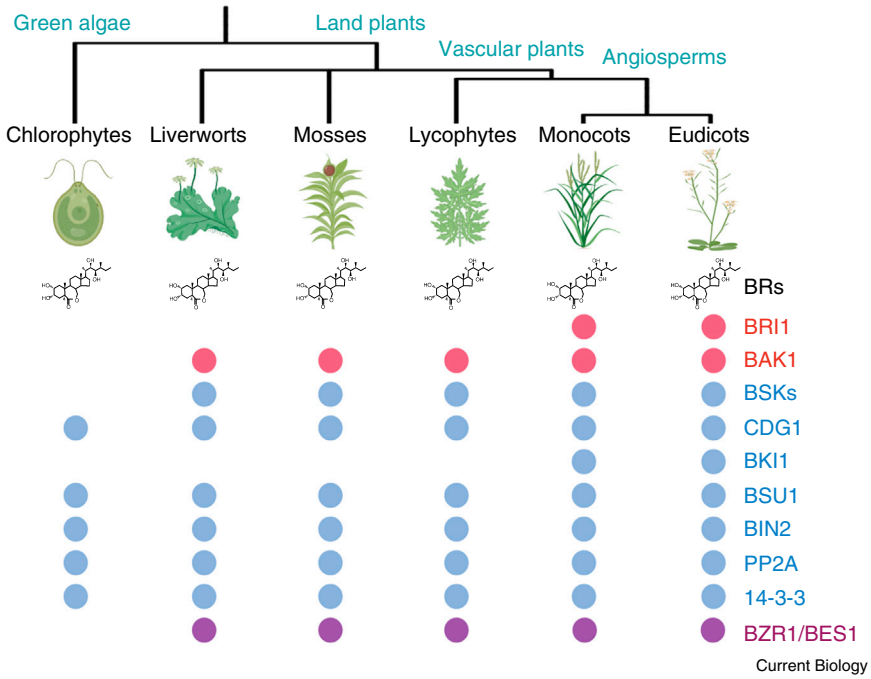

Figure 3. Evolution of BR signalling.
Putative functional orthologs of BR receptors (red), BR signalling components (blue), and BR-regulated transcription factors (purple) were identified from the different clades of the plant kingdom. The genomes of *Chlamydomonas reinhardtii* (chlorophyte, green algae), *Marchantia polymorpha* (liverwort), *Physcomitrella patens* (moss), *Selaginella moellendorffii* (lycophyte), *Oryza sativa* (monocot), and *Arabidopsis thaliana* (eudicot) were used as a representative of each clade. The phylogenetic tree was generated with phyloT, a phylogenetic tree generator based on NCBI taxonomy. The BR structure denotes the BR detection and the solid circles indicate the presence of orthologs. The figure was created with BioRender.

Together, these data indicate that the canonical BR perception appeared before the divergence of angiosperms, but that the BR signalling components emerged much earlier. Bearing in mind that BRs are found in green algae and all land plants, if BR signalling operates in lower plants, they may use yet unknown nuclear or cytoplasmic BR receptors.

Perspectives

Since the discovery of BRs almost 40 years ago, a tremendous amount of research employing classical genetics, proteomics, genomics, and molecular approaches contributed to the identification of key BR biosynthetic enzymes and the determination of the BR perception and downstream signalling mechanisms. Consequently, the BR signal transduction pathway was elucidated from the plasma membrane-localized receptor kinases to the transcriptional networks in the nucleus. However, it is becoming increasingly apparent that this linear pathway is not a free-standing entity, but that

it interacts with other hormonal and stress-response pathways. For example, BRs regulate diverse physiological and developmental processes through interaction with nearly all plant hormones.

Although some genetic and biochemical data that hint at possible mechanisms for these interactions are available, a future challenge will be to get a better insight into the hormonal crosstalk in plant growth and development, not only under optimal conditions, but also under different adverse environmental conditions. Along these lines, more efforts are needed to unravel the mechanisms by which BR signalling regulates plant adaptation to biotic and abiotic stresses. Moreover, because many BR signalling components are shared with other receptor-mediated signal transduction pathways, it is very important to further elucidate these interactions and to understand how these diverse signalling pathways are integrated in similar cellular contexts.

Although the BRI1 signal transduction pathway is generally considered to operate in every cell type and other BRI1 receptor homologs to be simply redundant, new evidence challenges this hypothesis and suggests that other functional BR receptors can probably play different signalling roles in specific cell types by using downstream signalling components that might differ from the canonical BRI1-initiated signalling. Other receptor kinases or receptor-like proteins may also affect BR signalling in a non-canonical way by interacting with BRI1. For example, the RECEPTOR-LIKE PROTEIN44 (RLP44) binds to both BRI1 and BAK1, thus acting as a scaffold to promote their association. RLP44 is sufficient to activate BR signalling in response to cell wall cues, at least partially independent of BRs. Therefore, the identification of non-canonical BR signalling components and new molecular mechanisms of activation is crucial. Future BR signalling research on whether or how the canonical BR signalling integrates the non-canonical BR signalling will provide a new dimension to the BR signalling pathway. Altogether, by fully understanding BR signalling we will be able to develop new effective strategies for designing improved crops.

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Traffic accident increase attributed to Daylight Saving Time doubled after Energy Policy Act

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The impact of Daylight Saving Time (DST) transitions on the human circadian system and everyday life is currently subject to close analysis. In their recent paper, Fritz *et al*. [1] studied large scale United States (US) registry data (1996 to 2017) on fatal motor vehicle accidents (MVA) and reported the incidence rate ratio. The authors report results for data before 2006, for data after 2007, and for the whole observation period. They also report morning, afternoon and whole-day results. The discussion and conclusions are derived mainly from the whole-day figures and those from the entire 1996 to 2017 period. Yet the breakdown illustrates a most interesting fact: the amendments in the Uniform Time Act made by the Energy Policy Act doubled the increase of fatal morning MVA attributed to the spring transition.

Figure 1 shows a simplistic scenario at 40°N latitude (the latitude of New York City and Madrid) in which the yearly evolution of the solar altitude z at the latest sunrise time — here after the H-hour — is plotted (black line). Solar altitude is appropriate for properly addressing a key parameter related to the rate of traffic accidents: illumination conditions (see *photoperiod* in Figure 1B in [1]). The H-hour is a function of latitude only when expressed as a mean solar time or as a distance to solar noon. It impacts human social life since an activity starting at or after the H-hour would certainly occur in the photoperiod irrespective of calendar date. People at 40°N latitude would see the sun crossing the horizon on January 5th at the H-hour and would see the sun as high as $z = 30°$ above the horizon in June at the same hour of the day, if nothing else changes. As the sun gets higher in the sky during spring/summer people naturally