Review

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The BBX family of plant transcription factors

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The B-box (BBX) proteins are a class of zinc-finger transcription factors containing a B-box domain with one or two B-box motifs, and sometimes also feature a CCT (CONSTANS, CO-like, and TOC1) domain. BBX proteins are key factors in regulatory networks controlling growth and developmental processes that include seedling photomorphogenesis, photoperiodic regulation of flowering, shade avoidance, and responses to biotic and abiotic stresses. In this review we discuss the functions of BBX proteins and the role of B-box motif in mediating transcriptional regulation and protein–protein interaction in plant signaling. In addition, we provide novel insights into the molecular mechanisms of their action and the evolutionary significance of their functional divergence.

BBX proteins

The *Arabidopsis* (*Arabidopsis thaliana*) genome encodes around 1500 transcription factors, 40% of which are specific to plants [1]. Zinc-finger transcription factors are a relatively large family of transcription factors in plants (circa 15% of the total), and these play a central role in plant growth and development [1–3]. Zinc-finger proteins contain zinc-finger domains that are stabilized by metal ions including zinc and that have the property to interact with DNA, RNA, or proteins [2]. A subgroup of zinc-finger proteins, which contain one or two B-Box motifs predicted to be involved in protein–protein interactions, are known as BBX proteins. BBX proteins belong to a functionally diverse family encoded by genes that are highly conserved across all multicellular species including blue-green algae and mosses [2,4–7]. In animals, the B-box domain is often associated with proteins that contain RING (really interesting new gene) and coiled-coil domains, which are referred to as RBCC/TRIM (for RING, B-box, coiled-coil/TRIPARTITE MOTIF) [8,9]. The RBCC/TRIM proteins play important roles in diverse cellular processes including ubiquitination, protein trafficking, and transcriptional regulation [10,11]. By contrast, in plants, the B-box domain is either found alone or together with the CCT domain [2,6]. These B-box-containing proteins interact with the coiled-coil domain of other proteins to create a functional equivalent to RBCC/TRIM [12,13]. For example, CONSTANS (CO/BBX1) directly interacts with coiled-coil domain-containing protein, SUPPRESSOR OF PHYA1 (SPA1) [12]. In addition, CO and other BBX proteins interact directly with CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1), another coiled-coil domain-containing protein [13–15]. Research on the physiological functions of BBX proteins and their mechanisms of action have progressed substantially since the first review was published [2], which was mostly focused on the nomenclature of BBX proteins. Here we highlight the importance of the B-box motif in the regulation of transcription and in mediating protein–protein interaction, and overview the functions and molecular mechanisms of BBX proteins in fine-tuning plant growth and development.

Evolution and structural domains of BBX proteins

In *Arabidopsis*, BBX proteins are grouped into five structure groups depending on the presence of at least one B-box domain and a CCT domain. The B-box domain contains one or two B-box motifs of ~40 residues in length. The B-box can be divided into two types, B-box1 and B-box2, based on their consensus sequence and the spacing of zinc-binding residues [7,16–18]. In *Arabidopsis*, 21 of the 32 BBX proteins (BBX1–13 and BBX18–25) contain two B-boxes in tandem, whereas 11 BBX proteins (BBX14-BBX17 and BBX26-BBX32) contain one B-box (Figure 1A). Similarly, in rice (*Oryza sativa*), 17 of the 30 BBX proteins contain tandem B-boxes in their N termini [6]. The presence of B-box1 and B-box2 sequences in both *Arabidopsis* and rice suggests that, in plants, the B-box domain is largely conserved (Figure 1B). The conserved residues in the B-box motifs have been shown to be crucial in mediating protein–protein interactions and transcriptional regulation [15,19–22]. Furthermore, a phylogenetic study with 214 BBX proteins belonging to 12 plant species from green algae to dicots showed that the B-box consensus sequences of each structure group retained a common and conserved domain topology [7]. In addition, comparative analysis of plant genomes suggests that the B-box1 and B-box2 motifs likely originated

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(A)

AGI number	BBX name	Domain structure	Protein length	Structure group	Interacting partners	Refs
		B-box1 B-box2 CCT VP motif				
AT5G15840	BBX1		373	I	COP1, SPA1, HOS1	[12,13,54]
AT5G15850	BBX2		355	I	ND	
AT3G02380	BBX3		347	I	ND	
AT2G24790	BBX4		294	I	COP1	
AT5G24930	BBX5		406	I	*AT5G26710*	[95]
AT5G57660	BBX6		355	I	ND	
AT3G07650	BBX7		372	II	*RCD1*	[43]
AT5G48250	BBX8		373	II	*RCD1*	[43]
AT4G15250	BBX9		330	II	ND	
AT3G21880	BBX10		364	II	ND	
AT2G47890	BBX11		332	II	*SERK1*	[96]
AT2G33500	BBX12		402	II	ND	
AT1G28050	BBX13		433	II	ND	
AT1G68520	BBX14		406	III	ND	
AT1G25440	BBX15		417	III	ND	
AT1G73870	BBX16		392	III	ND	
AT1G49130	BBX17		326	III	ND	
AT2G21320	BBX18		172	IV	ND	
AT4G38960	BBX19		226	IV	ND	
AT4G39070	BBX20		242	IV	COP1	
AT1G75540	BBX21		331	IV	HY5, BBX32	[19,38]
AT1G78600	BBX22		319	IV	HY5, HYH, COP1	[20]
AT4G10240	BBX23		162	IV	ND	
AT1G06040	BBX24		248	IV	HY5, COP1, HYH, RCD1, HPPBF-1	[15, 22,29,42,43,69]
AT2G31380	BBX25		238	IV	HY5, COP1, HYH	[15,29]
AT1G60250	BBX26		251	V	ND	
AT1G68190	BBX27		356	V	ND	
AT4G27310	BBX28		223	V	ND	
AT5G54470	BBX29		215	V	ND	
AT4G15248	BBX30		117	V	ND	
AT3G21890	BBX31		121	V	ND	
AT3G21150	BBX32		225	V	BBX21, EMF1, GmBBX64	[21,38,60]

(B)

Arabidopsis

B-box1: C-X_2-C-X_{7-8}-C-X_2-D-X-A-X-L-C-X_2-C-D-X_3-H

B-box2: C-X_2-C-X_3-P-X_4-C-X_2-D-X_3-L-C-X_2-C-D-X_3-H

Rice

B-box1: C-X_2-C-X_8-C-$X_?$-C-X_2-C-X_4-H-X_8-H

B-box2: C-X_2-C-X_8-C-X_7-C-X_2-C-X_4-H-X_8-H

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Figure 1. Structural domains of B-box (BBX) proteins and their interacting partners. **(A)** Subfamily of 32 *Arabidopsis* BBX proteins showing domain organizations, protein length, the structural group they belong to, and their interacting partners. Interacting partners in italics indicate that the functional relevance of the interaction has not yet been demonstrated. **(B)** Consensus sequences of B-box1 and B-box2 motifs in *Arabidopsis* and rice. Conserved Cys (C) and His (H) residues involved in protein–protein zinc ligation are indicated. Abbreviations: AGI, *Arabidopsis* Genome Initiative; CCT, CONSTANS, CO-LIKE and TOC1 motif; COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; EMF1, EMBRYONIC FLOWER 1; GmBBX64, *Glycine max* BBX64; HOS1, HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE1; HPPBF1, H-PROTEIN PROMOTER BINDING FACTOR 1; HYH, HY5 HOMOLOG; HY5, ELONGATED HYPOCOTYL 5; ND, not determined. RCD1, RADICAL-INDUCED CELL DEATH1; SERK1, SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE 1; SPA1, SUPPRESSOR OF PHYA-105; VP, valine-proline motif. See Refs. [95,96].

from segmental duplications and internal deletion events [7].

The CCT domain is a basic motif of 42–43 amino acids with functional roles in some BBX proteins [12,23,24]. Sequence alignments of BBX proteins suggest that the CCT domain is also highly conserved [7]. In *Arabidopsis*, 17 of the 32 BBX proteins (BBX1–17) have a CCT domain close to their C termini [25]. Structure groups I (BBX1–6) and II (BBX7–13) have two B-boxes and a CCT domain, whereas proteins of structure group III (BBX14–17) have one B-box and a CCT domain (Figure 1A) [2,7]. Similarly, in rice, 17 of the 30 BBX proteins contain the CCT domain [6]. The CCT domain has important functions in transcriptional regulation and nuclear protein transport [12,13,26–28]. An

example of this is the CCT domain of CO, which has been shown to be crucial in mediating the expression of *FLOWERING LOCUS T* (*FT*) by directly binding to its promoter [24]. Furthermore, nuclear localization signals (NLSs) consisting of a short amino acid sequence as part of the CCT domain play a central role in the BBX protein localization to the nucleus [13,23,27,28]. In addition to the B-box and CCT domains, some BBX proteins contain a valine-proline (VP) motif of six amino acids, with the consensus sequence G-I/V-V-P-S/T-F in their C termini (Figure 1A). The VP motif is very close to the CCT domain, separated by 16–20 amino acids, and is important for the interaction with COP1 [23,29]. It has been suggested that the evolution of BBX proteins was constrained by the conservation of amino acid sequences in the two B-boxes, but has radiated variation into NLSs, VP, and other novel motifs [7,30].

The presence of BBX genes in the genome of different species from algae to monocots and dicots clearly suggests an ancient origin [7,31]. Most green algae have a single B-box motif. However, the presence of two B-box motifs in the unicellular green alga *Chlamydomonas* suggests that the B-box duplication event has taken place much before land colonization of plants, at least 450 million years ago, probably in the upper Silurian period [7,32]. The rapid expansion of BBX proteins during the course of evolution, and the fact that they are highly conserved across the plant kingdom, suggest that BBX proteins might have played crucial roles in the adaptation of land plants [7].

Functions of the B-box domain

Although the functions of the B-box domain in animals were established some time ago [8–11], in plants they have only now begun to be unraveled. Recent studies suggest that the B-box domain plays a crucial role in the regulation of transcription, and in mediating heterodimer formation both within and outside the BBX protein family [15,19–22]. At least four BBX proteins (BBX21, 22, 24, 25) physically interact with transcription factor ELONGATED HYPOCOTYL 5 (HY5) [15,19–21], and three (BBX22, 24, 25) interact with HYH (HOMOLOG OF HY5) transcription factors [20,22]. The fact that site-directed mutations in the B-box motifs converting aspartic acid to alanine completely impede the interaction with HY5 suggests a crucial role of this motif in mediating BBX interaction with HY5, and also with HYH – as documented for BBX24 [15,19–21]. Very recent studies suggest that BBX proteins also interact within other family members as seen for BBX32–BBX21 and BBX32–GmBBX62 [21,33]. Interestingly, site-directed mutations in the B-box motif together with computational approaches suggest that the B-box motif of BBX32, and conserved cysteines and aspartic acids residues outside but close to B-box domain, are necessary for the interaction with GmBBX62 [21]. Furthermore, point mutations in the B-box domain of BBX21 also reduce the transcriptional activation of *CHI* (*CHALCONE SYNTHASE*) promoter [19], whereas mutations in the BBX22 B-box motif reduce the activation of both *CHI* and *CAB1* promoters, as demonstrated in transient expression studies [20]. Interestingly, a point mutation on the B-box domain of BBX25 increases HY5-mediated transcriptional activation of the *BBX22* promoter suggesting an indirect and negative action of BBX25 on the expression of BBX22 through the physical interaction with HY5 [15]. Similarly, BBX32 indirectly reduces HY5 transcriptional activity through a protein–protein interaction with BBX21 [33]. Collectively, these lines of evidence suggest that B-box domains play crucial roles in mediating protein–protein interactions and in the regulation of transcription.

BBX proteins in seedling photomorphogenesis

BBX proteins are involved in seedling de-etiolation, controlling hypocotyl growth, anthocyanin production, chlorophyll accumulation, lateral root growth, and cotyledon unfolding (Figure 2A; Table 1). Specifically BBX4, BBX20, BBX21, and BBX22 promote photomorphogenesis [19,20,23,34,35] whereas BBX18, BB19, BBX24, BBX25, and BBX32 suppress photomorphogenesis [15,33,36–38]. *bbx4* mutant seedlings show long hypocotyls only in red light [23], whereas *bbx20* mutant seedlings show long hypocotyls in red and blue light [35], and *bbx21* and *bbx22* mutant seedlings show long hypocotyls under red, far-red, and blue light [19,20]. These results suggest that BBX proteins act in photomorphogenesis downstream of the phytochrome and cryptochrome pathways. By contrast, *bbx24*, *bbx25*, and *bbx32* mutant seedlings develop short hypocotyls in red, far-red, and blue light, suggesting that they suppress photomorphogenesis irrespective of the photoreceptor type [15,33,37]. Furthermore, BBX18- and BBX19-overexpressing lines have longer hypocotyls than wild type plants under red and far-red continuous light, whereas *bbx18* and *bbx19* mutant seedlings develop hypocotyls similar to those of wild type plants, suggesting that they play redundant functions during de-etiolation [38]. Also, MISREGULATED IN DARK10 (BBX23/MIDA10), a member of structure group IV, represses apical hook unfolding in dark-grown seedlings [39]. Using a micro-based approach and functional characterization of *mida* mutants, it was demonstrated that BBX23 is involved in one of the PIF3 branches of signaling that inhibit photomorphogenesis in the dark [39].

BBX proteins are involved in both cooperative and antagonistic interactions for the regulation of seedling photomorphogenesis. By genetic analysis, it was demonstrated that BBX21 enhances the functions of both BBX20 and BBX22, and suppresses the function of BBX32 [19,33,35]. BBX32 physically interacts with BBX21 and reduces HY5-mediated transcriptional activity [33]. Interestingly, BBX21 and BBX22 directly interact with HY5, and enhance its activity [19,20,34]. Furthermore, the epistatic interaction between BBX24 and BBX25 suggests that they enhance each other's function, but also that they can work independently to regulate seedling photomorphogenesis [15]. Both BBX24 and BBX25 suppress HY5 function by forming inactive heterodimers with HY5, thereby reducing the transcriptional activity of HY5 on target genes such as *CHI* and *CHS* [15]. This clearly indicates that BBX24 and BBX25 act as transcription corepressors of HY5 [15], and likely of HYH [22]. All these findings suggest that BBX proteins play opposite functions in the same physiological process: whereas BBX21 and BBX22 are transcriptional coactivators, BBX24 and BBX25 are corepressors of the action of HY5. Epistatic analyses between BBX proteins and COP1

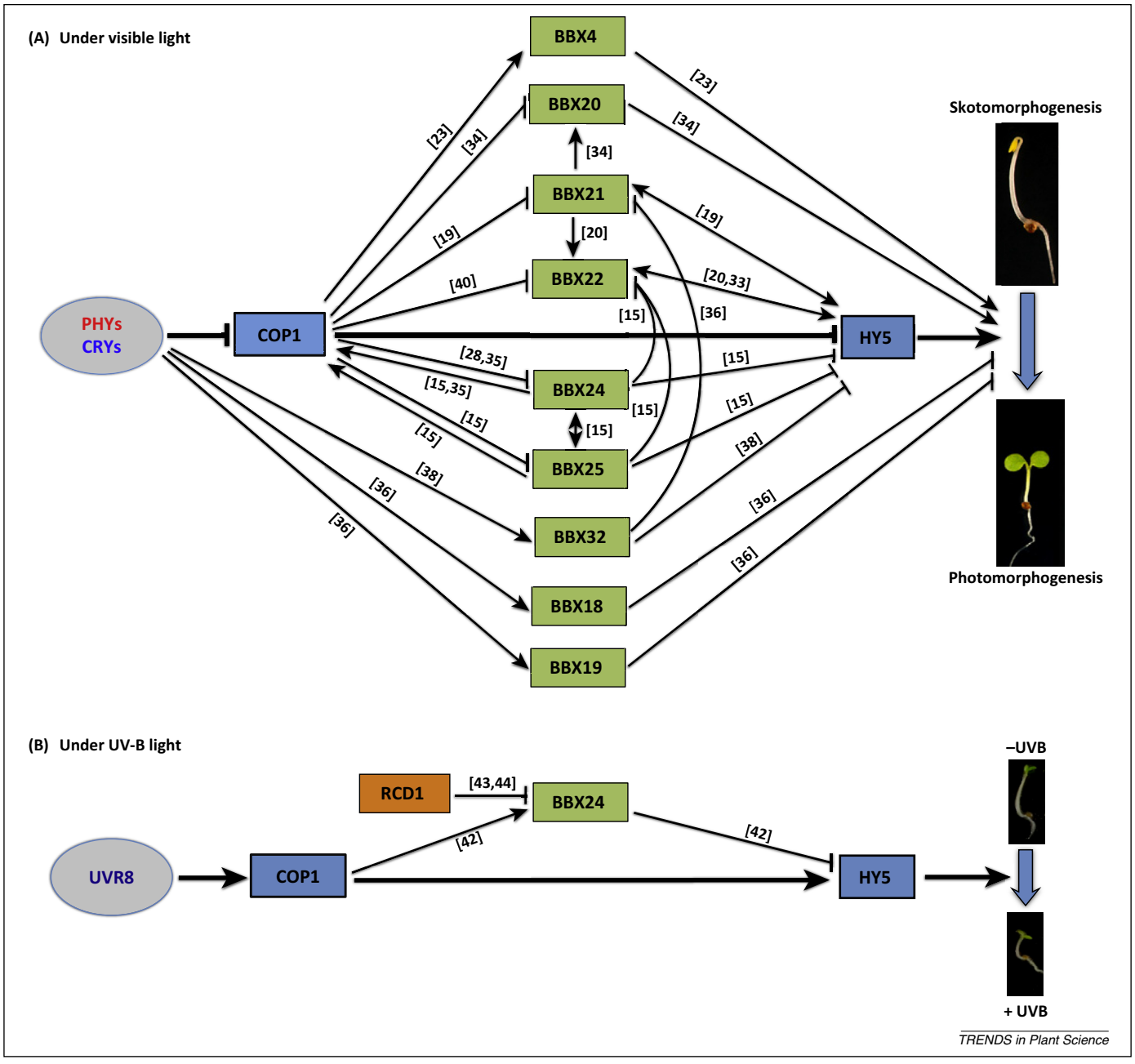

Figure 2. B-box (BBX) proteins are involved in seedling photomorphogenesis. **(A)** BBX proteins modulate seedling development by integrating light signals perceived by phytochrome and cryptochrome photoreceptors through the COP1 and HY5 signaling pathway. BBX4 integrates red light signals, BBX20 integrates red and blue light signals, and the other BBX proteins integrate red, far-red and blue light signals. BBX4, BBX20, BBX21, and BBX22 promote photomorphogenesis by suppressing COP1 function. BBX4 and BBX20 directly interact with COP1, whereas BBX21 and BBX22 colocalize with COP1 in nuclear speckles. BBX21 and BBX22 directly interact with HY5 and enhance its functions, inhibiting hypocotyl growth and increasing pigment accumulation. At the same time, HY5 enhances the functions of BBX21 and BBX22 (double arrows). Furthermore, BBX21 enhances the functions of both BBX20 and BBX22 to inhibit hypocotyl growth. By contrast, BBX18, BBX19, BBX24, BBX25, and BBX32 inhibit seedling photomorphogenesis. BBX24 and BBX25 directly interact with HY5 and COP1, suppressing HY5 function and enhancing COP1 action. By a negative feedback mechanism, COP1 degrades both BBX24 and BBX25. BBX32 directly interacts with BBX21, forming inactive heterodimers and reducing HY5 transcriptional activity, thus showing antagonistic functions with HY5. **(B)** Under UV-B light, the UVR-8 photoreceptor absorbs UV-B light and activates COP1, which in turn modulates the expression of many UV-B-responsive genes through HY5-dependent and -independent pathways. BBX24 is part of a negative feedback mechanism of the UV-B pathway. UV-B increases *BBX24* expression in a COP1-dependent manner, and BBX24 directly interacts with HY5, reducing the transcriptional activity of HY5. Furthermore, RCD1 negatively regulates BBX24 action. Numbers in parentheses indicate relevant references. Abbreviations: COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; CRYs, CRYPTOCHROMES; HY5, ELONGATED HYPOCOTYL 5; PHYs, PHYTOCHROMES; RCD1, RADICAL-INDUCED CELL DEATH1.UV-B, ULTRAVIOLET-B radiation; UVR8, UV RESISTANCE LOCUS 8.

have shown that BBX4, BBX20, BBX21, and BBX22 repress COP1 function, whereas BBX24 and BBX25 enhance COP1 function [15,19,20,23,35,36]. Interestingly, BBX4, BBX20, BBX24, and BBX25 directly interact with COP1 [23,29,35], whereas BBX21 and BBX22 are recruited by COP1 into nuclear speckles [19,20]. In addition, COP1 ubiquitinates and degrades BBX22 in dark conditions [20,40]. The stability of BBX proteins appears to be transient. In fact, BBX22 has a half-life of 20 minutes in the dark and 60 minutes in the light [40]. Very recently it has been shown that BBX20 undergoes COP1-mediated degradation in the dark, suggesting that it is also a downstream target of COP1 [35]. Further, both BBX24 and BBX25 are degraded by COP1 as part of a feedback regulatory mechanism [15,36]. In addition, the stability and accumulation of BBX proteins depend on the activity of the circadian clock [37]. In fact, light and

Table 1. Functions of BBX proteins in *Arabidopsis* and crop species

Plant species	AGI Number	BBX name	Other names	Input signal	Physiological role	Mode of regulation	Refs
Arabidopsis (*Arabidopsis thaliana*)	AT5G15840	BBX1	CO	Light	Flowering in LD	Positive	[51]
				Light	Stomatal opening	Positive	[80,81]
	AT5G15850	BBX2	COL1	Light	Circadian clock	Positive	[59]
				Cold	Abiotic stress response	ND	[82][a]
				ABA, cADPR	Abiotic stress response	ND	[71][a]
	AT3G02380	BBX3	COL2	Light	Circadian clock	Positive	[59]
				ABA, cADPR	Abiotic stress response	ND	[71][a]
	AT2G24790	BBX4	COL3	Light	Photomorphogenesis	Positive	[23]
				Light	Flowering in LD and SD	Negative	[23]
				Light	Shoot branching	Positive	[23]
				Light	Lateral root development	Positive	[23]
	AT5G57660	BBX6	COL5	Light	Flowering in SD	Positive	[58]
				JA	Flower development	ND	[83][a]
				Cold	Abiotic stress response	ND	[72][a]
	AT3G07650	BBX7	COL9	Light	Flowering in LD	Negative	[57]
				Fungi pathogen	Biotic stress response	ND	[84][a]
				Cold	Abiotic stress response	ND	[72][a]
	AT4G15250	BBX9		JA	Flower development	ND	[83][a]
	AT2G47890	BBX11	COL13	OPDA	Biotic stress response	ND	[73][a]
				ABA, cADPR	Abiotic stress response	ND	[71][a]
				Cold	Abiotic stress response	ND	[72][a]
	AT1G28050	BBX13		ABA, cADPR	Abiotic stress response	ND	[71][a]
	AT1G25440	BBX15		Cold	Abiotic stress response	ND	[72][a]
	AT1G73870	BBX16	COL7	Low R:FR	Shoot branching	Negative	[66]
				Low R:FR	SAR	Positive	[66]
				ABA, cADPR	Abiotic stress response	ND	[71][a]
	AT2G21320	BBX18	DBB1a	Light	Photomorphogenesis	Negative	[37]
					Flower development	Positive	[85]
				GA	Photomorphogenesis	Positive	[77]
				ABA, cADPR	Abiotic stress response	ND	[73][a]
	AT4G38960	BBX19	DBB1b	Light	Photomorphogenesis	Negative	[37]
				ABA, cADPR	Abiotic stress response	ND	[71][a]
	AT4G39070	BBX20	BZS1	Light	Photomorphogenesis	Positive	[35]
				Brassinosteriods	Photomorphogenesis	Negative	[35,75]
				Chitin	Biotic stress response	ND	[74][a]
	AT1G75540	BBX21	STH2	Light	Photomorphogenesis	Positive	[19]
				Low R:FR	SAR	Negative	[14]
	AT1G78600	BBX22	STH3/LZF1	Light	Photomorphogenesis	Positive	[20]
				Light	Chloroplast development	Positive	[38]
				Low R:FR	SAR	Negative	[14]
				ABA, cADPR	Abiotic stress response	ND	[71][a]
	AT4G10240	BBX23	MIDA10	Dark	Skotomorphogenesis	Positive	[39]
	AT1G06040	BBX24	STO	Light	Photomorphogenesis	Negative	[15,36,37]
				Salt	Abiotic stress response	Positive	[69]
				UV-B	Hypocotyl inhibition	Negative	[42]
				Low R:FR	SAR	Positive	[14,15]
				Cold	Abiotic stress response	ND	[72][a]
	AT2G31380	BBX25	STH	Light	Photomorphogenesis	Negative	[15,38]
				Low R:FR	SAR	Positive	[15]
	AT5G54470	BBX29		Light, Cold	Abiotic stress response	ND	[86][a]
	AT3G21150	BBX32	EIP6	Light	Photomorphogenesis	Negative	[38,33]
				Light	Flowering in LD	Negative	[60]
				OPDA	Biotic stress response	ND	[73][a]
				Chitin	Biotic stress response	ND	[74][a]
Rice (*Oryza sativa*)	Os06g0275000	OsBBX18	Hd1	Light	Flowering in LD	Negative	[61]
				Light	Flowering in SD	Positive	[61]
	Os09g0240200	OsBBX27	OsCO3	Light	Flowering in SD	Negative	[62]
	Os02g0610500	OsBBX5	OsCOL4	Light	Flowering in LD and SD	Negative	[63]
Soybean (*Glycine max* L.)		BBX32		Overexpression in soybean	Grain yield	Positive	[79]
Barley (*Hordeum vulgare*)		HvCO1		Circadian clock	Flowering in LD and SD	Positive	[87]

Table 1 (*Continued*)

Plant species	AGI Number	BBX name	Other names	Input signal	Physiological role	Mode of regulation	Refs
Banana (*Musa sapientum*)		MaCOL1		Chilling	Abiotic stress response	Positive	[88][b]
				Fungi pathogen	Biotic stress response	Positive	[88][b]
				Ethylene	Fruit ripening	Positive	[88][b]
Chrysanthemum (variety Zhongshanzigui)		CgZFP1		Overexpression in *Arabidopsis*	Abiotic stress response	Positive	[89]
Beetroot (*Beta vulgaris*)		BvCOL1		Overexpression in *Arabidopsis*	Flowering in LD	Positive	[90]
Grape (*Vitis vinifera* L.)		VvCO		Light	Flowering	Positive	[91][b]
		VvCOL1		Light	Bud dormancy	Positive	[91][b]
		VvZFPL		Overexpression in *Arabidopsis*	Abiotic stress response	Positive	[92]
				Overexpression in *Arabidopsis*	Photomorphogenesis	Negative	[92]
Potato (*Solanum tuberosum*)		StCO		Overexpression in potato	Tuber formation	Negative	[93]
		BBX1		Overexpression in potato	Tuber formation	Negative	[94]

[a]Data collected from microarray experiments; BBX functional characterization needs to be confirmed.

[b]Data collected from expression experiments.

Abbreviations: ABA, abscisic acid; AGI, *Arabidopsis* Genome Initiative; BBX, B-box; Bv, *Beta vulgaris*; BZS1, *bzr1–1D* suppressor1-dominant (bzs1–D); cADPR, cyclic ADP-ribose; Cg, *Chrysanthemum grandiflorum*; CO, CONSTANS; COL, CONSTANS LIKE; COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; CRYs, CRYPTOCHROMES; DBB1a and DBB1b, double B-box 1a and double B-box 1b; EIP6, EMF1 interacting protein 6; EMF1, embryonic flower 1; Hd1, heading date 1; HY5, ELONGATED HYPOCOTYL 5; Hv, *Hordeum vulgare*; JA, jasmonic acid; LD, long day photoperiod; LZF1, LIGHT REGULATED ZINC-FINGER 1; Ma, *Musa acuminata*; MIDA10, MIS-REGULATED IN DARK 10; ND, no data (microarray data); OPDA, 12-oxo-phytodienoic; Os, *Oryza sativa*; PHYs, PHYTOCHROMES; R:FR, red-light to far-red-light ratio; SAR, shade avoidance response; SD, short day photoperiod; St, *Solanum tuberosum*. STH, salt tolerant-homolog; STO, salt tolerant; UV-B, ULTRAVIOLET-B radiation; Vv, *Vitis vinifera*; ZFPL, zinc-finger protein like; ZFP1, zinc-finger protein 1.

the circadian clock tightly regulate BBX18, BBX19, BBX22, BBX24, and BBX25 [37].

The low fluence rate of UV-B radiation induces photomorphogenic responses through the action of the ULTRAVIOLET RESISTANCE LOCUS 8 (UVR8) photoreceptor [41]. Upon UV-B irradiation, UVR8 protein accumulates in the nucleus and activates COP1, which in turn modulates the expression of many UV-B-responsive genes both in HY5-dependent and -independent manners, thereby inhibiting hypocotyl growth in *Arabidopsis* seedlings. The *bbx24* mutant is hypersensitive to UV-B and displays a dwarfed phenotype [42]. BBX24 is involved in the negative regulation of UV-B signaling, attenuating HY5 accumulation and suppressing transcriptional activity, probably by forming inactive heterodimers with HY5 [42]. Interestingly, BBX24 physically interacts with RADICAL-INDUCED CELL DEATH1 (RCD1), another regulator of UV-B signaling that inhibits the expression of *BBX24* [43,44]. These results suggest that BBX24 together with RCD1 are involved in fine-tuning UV-B photomorphogenic responses through a negative feedback mechanism [Figure 1B]. Furthermore, in a transcriptome study of COP1-regulated genes under low UV-B irradiation in *Arabidopsis* seedlings, it has been found that *BBX5* and *BBX18* are promoted, whereas *BBX7* and *BBX8* are repressed by COP1, suggesting that other BBX proteins could be working in opposite directions within the UV-B signaling pathway [45].

BBX proteins in flowering

Flowering is under the control of different signaling pathways that converge to create a robust seasonal response [46]. Some BBX proteins are involved in the photoperiod pathway of flowering (Figure 3, Table 1). In *Arabidopsis*, flowering is significantly delayed in *co* mutant plants, and CO-overexpression lines flower earlier than wild type plants grown under long day conditions (LD) [47–49]. Under short day conditions (SD), *co* mutants flower at the same time as wild type plants, whereas CO-overexpression transgenic lines flower early even in SD, suggesting that the *CO* dosage is a limiting factor [47]. CO is a central coordinator of light and clock inputs, triggering the expression of *FT* [50,51]. CO promotes the expression of *FT* by the binding of its CCT domain with the *FT* promoter on the CO-responsive elements (CORE) and CCAAT-box elements [24,52,53]. Furthermore, CO directly interacts with COP1 and SPA1 to SPA4 proteins through its CCT domain [12,13]. SPA1 specifically targets CO in SD [12], whereas COP1 targets CO both in SD and LD [13,54]. Full-length CO also interacts with HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES1 (HOS1), which further undergoes proteasome-mediated degradation [55]. HOS1 targets CO during the photoperiod, probably in a phyB-dependent manner [55]. These observations are further supported by the fact that *cop1* and *hos1* mutants flower early both in SD and LD, whereas *spa1* mutants flower much earlier than wild type plants only in SD [12]. The fact that HOS1 targets CO early in the day, and COP1 and SPA1 during the night, demonstrates the existence of multiple signaling pathways for ubiquitin ligases that regulate CO protein abundance. Furthermore, two basic helix-loop-helix (bHLH) transcription factors, FLOWERING BHLH 1 (FBH1) and FBH2, bind to the *CO* promoter through G- and E-box sequences and activate its expression in both LD and SD [56].

At least a further three CO-LIKE (COL) proteins, BBX4, BBX6, and BBX7, regulate flowering [23,57,58]. *bbx4* mutant plants flower early under both SD and LD, suggesting that the role of BBX4 in flowering is opposite to

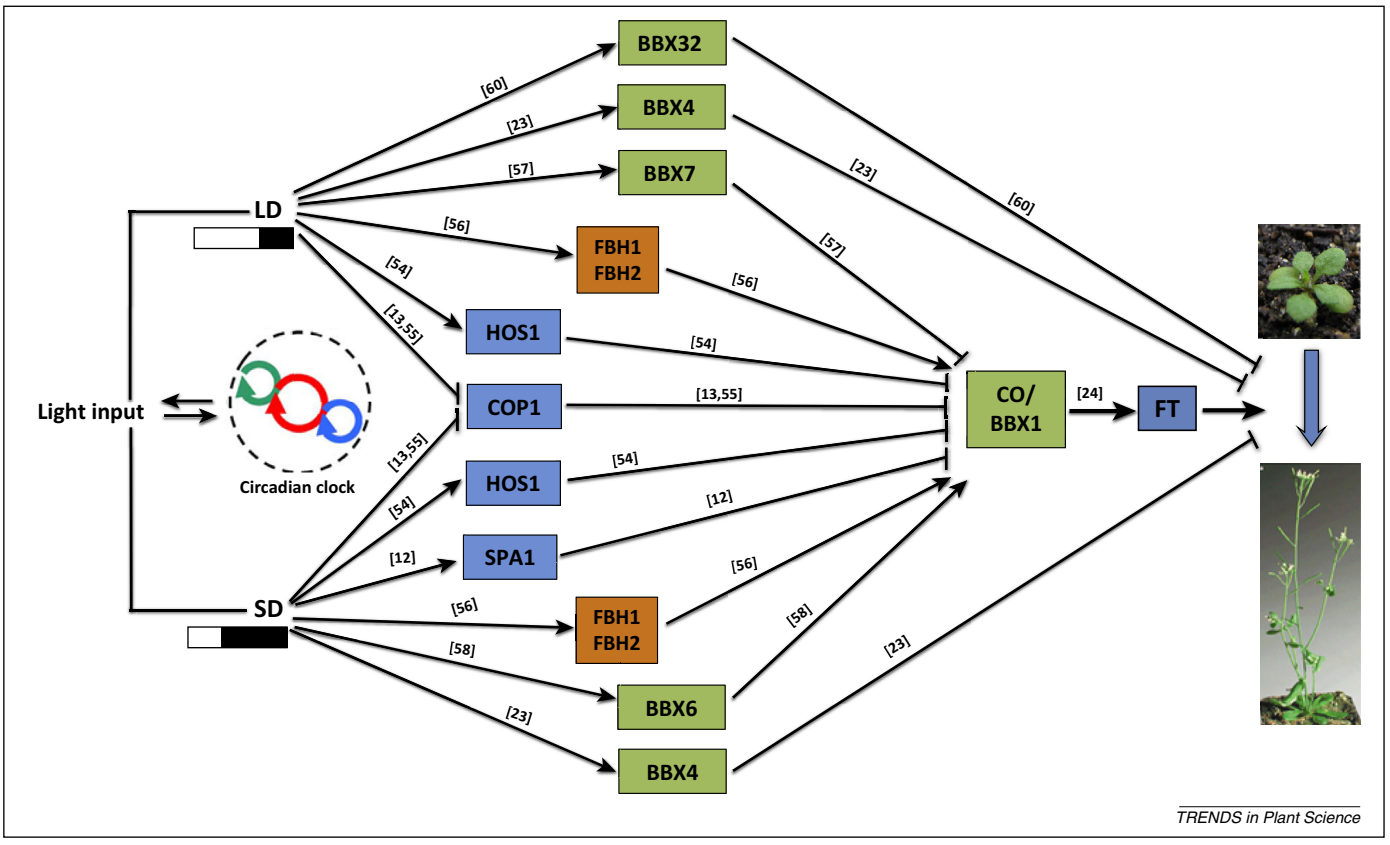

Figure 3. B-box (BBX) proteins regulate the photoperiodic pathway of flowering. Light and the circadian clock both coordinate CO/BBX1 activity, which triggers *FT* expression, thereby promoting flowering under LD in *Arabidopsis* plants. CO protein abundance is controlled by COP1, HOS1, and SPA1, which are components of the E3 ubiquitin ligase complex. COP1 and HOS1 target CO for degradation in both LD and SD, whereas SPA1 targets CO for degradation in SD. Two bHLH proteins, FBH1 and FBH2, promote flowering by activating *CO* expression under both LD and SD. Furthermore, additional BBX proteins act to modulate flowering through CO-dependent or -independent pathways. BBX6 induces flowering by enhancing *CO* expression under SD, whereas BBX7 suppresses flowering under LD by negatively regulating *CO* expression. However, BBX32 negatively regulates flowering under LD probably in a CO-independent manner. BBX4 suppresses flowering in both LD and SD. CO involvement in the circadian clock as an integrator of light inputs is not represented in the figure to gain in clarity. The numbers in parentheses indicate references. Abbreviations: bHLH, basic helix-loop-helix; CO, CONSTANS; COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; FBH1 and FBH2, FLOWERING BHLH 1 and 2; FT, FLOWERING LOCUS T; HOS1, HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE1; LD, long day; SD, short day; SPA1, SUPPRESSOR OF PHYA-105.

that of CO [23]. *bbx7* mutants also flower earlier than wild type plants, whereas BBX7-overexpression lines delay flowering in LD [57], suggesting that BBX7 represses flowering probably by reducing the expression of *CO* and *FT* [57]. By contrast, *bbx6* mutant plants flower normally under SD, but BBX6 overexpression induces early flowering by promoting *FT* expression [58]. These results indicate that BBX6 function is redundant with other flowering regulators [58]. However, there are other COL proteins with high homology to CO, such as BBX2 and BBX3, which have no clear roles in flowering but which have been reported to be important for the circadian clock function [59]. In addition to COL proteins, BBX32 overexpression suppresses flowering in LD [40]. The fact that *bbx32* mutant plants respond to photoperiod in a similar manner to wild type plants suggests that BBX32 negatively regulates flowering in a dose-dependent manner [60].

Similarly, *Heading date1 (Hd1)*, the CO ortholog in rice, promotes flowering in SD but inhibits it in LD [61]. Two additional COL proteins, OsBBX27/OsCO3 and OsBBX5/OsCOL4, are involved in the photoperiod pathway [62,63]. Whereas OsBBX27 represses flowering in SD, OsBBX5 inhibits flowering in both LD and SD [62,63]. Although COL proteins have both distinct and overlapping functions, their functions are highly conserved in the flowering pathways of *Arabidopsis*, rice, and probably other crop plants such as beetroot (*Beta vulgaris*), grape (*Vitis vinifera* L.), and tomato (*Solanum lycopersicum*) (Table 1).

BBX proteins in shade-avoidance responses

The intimate connection between the photoreceptors pathways and shade-avoidance responses has been thoroughly reviewed recently [64]. Reduction of the red/far-red (R/FR) ratio by neighboring plants is a signal of future competition, and individuals respond early by increasing the length of their vegetative structures to reach light for photosynthesis. BBX proteins mediate cell elongation in shaded environments [14,15,65,66]. Screening for mutants with long hypocotyls under simulated canopy has shown that BBX21/LHUS represses elongation growth specifically under shade [14]. Several BBX members of structure group IV are involved in shade avoidance but with opposite roles: BBX19, BBX21, and BBX22 inhibit, whereas BBX18, BBX24, and BBX25 promote, hypocotyl elongation under a low R/FR ratio [14,15,65]. BBX21 positively regulates the expression of early shade-response genes such as *PAR1*, *HFR1*, *PIL1*, and *ATHB2* in the first hour of shade, but later inhibits elongation growth. These results suggest that BBX21 could be a component of a negative feedback

loop to avoid exaggerated elongation responses such as those occurring with HFR1 and PAR1 [14]. BBX21 and BBX22 are involved in the COP1 signaling pathway because *bbx21* and *bbx22* mutants partially restore the shade-avoidance response in the *cop1* background [14]. Furthermore, *bbx24* mutant plants develop significantly shorter hypocotyls under shade, and the *bbx25* mutant further enhances the *bbx24* phenotype in a partially redundant manner [15]. The short hypocotyl phenotype of the *bbx24 bbx25* double mutant under shade is completely COP1-dependent because the *bbx24 bbx25 cop1* triple mutant resembles the *cop1* phenotype [15], suggesting that BBX proteins act in the COP1 signaling pathway under shade. Similarly, BBX16 also promotes hypocotyl growth under shade, probably acting as a positive transcriptional regulator of *PIL1* [65]. In addition, the overexpression of BBX16 dramatically enhances the number of primary rosette branches under high R/FR ratio [65].

BBX proteins in abiotic and biotic stresses

In addition to the functions of BBX proteins in growth and development, some studies suggest that they are also involved in signaling pathways induced by abiotic and biotic stresses. For example, *Arabidopsis* BBX18 subexpressing lines have increased thermotolerance, whereas overexpression lines have reduced thermotolerance [67]. The *BBX18* expression was induced in plants exposed to a 2 h heat treatment at 42 °C [67]. Furthermore, BBX18 negatively regulates the expression of heat-responsive genes such as *DGD1*, *Hsp70*, *Hsp101*, and *APX2*, thereby reducing germination and seedling survival after the heat treatment [67].

BBX24 is involved in salt stress signaling [68,69]. In fact, BBX24 was originally isolated as a SALT-TOLERANT (STO) protein in a screen aimed to identify *Arabidopsis* cDNA clones that confer increased salt tolerance in yeast (*Saccharomyces cerevisiae*) salt-sensitive calcineurin mutants [68]. BBX24/STO cDNA complements the yeast calcineurin-deficient mutant phenotype and enhances the salt-tolerance capacity of wild type yeast [68]. Further, the overexpression of BBX24 in *Arabidopsis* confers salt tolerance compared to wild type plants [69]. BBX24 transgenic plants exposed to a medium supplemented with 50 and 100 mM NaCl show a significant increase in root length compared to wild type plants [69]. However, *BBX24* expression is not inducible by salt, suggesting that the effects caused by BBX24 are likely to be indirect. Interestingly, BBX24 interacts directly with H-protein promoter binding factor1 (HPPBF-1), a salt-responsive MYB transcription factor [69].

In addition, genome-wide expression analyses suggest the probable involvement of BBX proteins in other stress signaling responses. Absicic acid (ABA) phytohormone is activated when plants are exposed to different stresses [70]. Large-scale microarray studies show that BBX genes are differentially expressed in response to ABA, cyclic ADP-ribose (cADPR), and low temperatures [71,72]. Previously it was shown that cADPR is involved in an early ABA signaling event [70]. However, the direct involvement of BBXs in abiotic stress signaling pathways has to be demonstrated.

BBX proteins also participate in wounding and defense responses (Table 1). In a microarray study comparing the effects on wounding response in *Arabidopsis* plants treated with jasmonic acid (JA), methyl jasmonate (MeJA), or the cyclopentenone precursor of JA, 12-oxo-phytodienoic acid (OPDA), it was found that *BBX32* expression is upregulated by OPDA, but not by JA or MeJA [73]. Another study showed that BBX32 expression is also increased after a short treatment with chitin, a substance found in the cell walls of fungi and the exoskeleton of insects and nematodes [74]. Chitin-responsive transcription factors are key elements in the ability of chitin to modify gene expression as part of the plant defense reaction. In light of these observations, BBX32 seems to be involved in plant defense pathways.

BBX proteins and hormonal signaling networks

Evidence for the role of BBX proteins in hormonal signaling pathways is scarce. BZS1/BBX20 integrates signals from brassinosteroids (BR) and light pathways [35]. BRASSINAZOLE RESISTANT 1 (BZR1), a positive transcription factor, promotes hypocotyl growth by directly binding to *BBX20* and repressing its expression [75]. Interestingly, a GATA-binding zinc-finger protein (GATA2) also inhibits hypocotyl growth by repressing BR signaling action [76]. Therefore, it can be hypothesized that BBX20 collaborates with GATA2 in mediating light and BR crosstalk.

BBX18 is involved in the gibberellin (GA) signaling pathway [77]. Molecular and phenotypic investigations demonstrate that BBX18 promotes hypocotyl growth by increasing bioactive GA levels. Indeed, BBX18 increases the expression of *GA3ox1* and *GA20ox1* metabolic genes, and suppresses the expression of *GA2ox1* and *GA2ox8* catabolic genes under light [77]. The antagonistic regulation of light and GA in seedling de-etiolation and the involvement of BBX proteins in the COP/HY5 signaling pathway [78] suggest that BBX18 may act as an integrator of both GA and COP1/HY5 pathways.

Furthermore, a microarray database obtained from rice plants exposed to auxin, GA, and cytokinin treatments showed that 11 *BBX* transcripts responded differentially to the addition of the phytohormone, and most of them harbor hormone-responsive *cis*-acting elements in their promoters. These observations suggest the probable involvement of OsBBX proteins in hormone signaling as transcriptional regulators [6]. However, further investigations are necessary to demonstrate clearly their role in hormone signaling pathways.

Concluding remarks and future perspectives

Although significant progress has been made in understanding the functions of many BBX proteins in different developmental responses in *Arabidopsis*, the roles of BBX proteins have only now begun to be unraveled in other plant species (Table 1). Our knowledge of the function of BBX proteins is probably limited by the complexity and modularity of the system and the relatively modest amount of functional information available to date. However, this review clearly establishes that BBX proteins constitute a group of transcription factors whose members have

opposite functions in the regulation of the same physiological process. This feature, which is not common in other transcription factor families, opens up new avenues of research to learn how plants integrate endogenous and environmental signals for fine-tuning their growth and development. In the coming years, understanding the molecular mechanisms of each individual BBX protein will be an important task.

Furthermore, the involvement of BBX proteins in flowering and biotic and abiotic stresses argues in favor of their use in transgenic crops to obtain desirable agronomic characters. For example, manipulating the expression of *CO* and *COL* and their orthologs in crops could be a fruitful strategy to design plants with early or late flowering time depending on production requirements or local climatic limitations. For example, early flowering may be a desirable trait in crop plants where seeds are the harvested product, but late flowering could be an advantage when total biomass is the objective of the production, as is the case for green leafy vegetables, bioethanol, or fodder crops. Very recently it has been shown that the heterologous overexpression of *Arabidopsis* BBX32 protein in soybean plants increases grain yield under field conditions [79]. These results suggest that BBX protein manipulation in crops might be a strategy to increase food production.

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