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Transcriptional networks orchestrating programmed cell death during plant development

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Abstract

Transcriptional gene regulation is a fundamental biological principle in the development of eukaryotes. It does not only control cell proliferation, specification, and differentiation, but also cell death processes as an integral feature of an organism's developmental program. As in animals, developmentally regulated cell death in plants occurs in numerous contexts and is of vital importance for plant vegetative and reproductive development. In comparison with the information available on the molecular regulation of programmed cell death (PCD) in animals, however, our knowledge on plant PCD still remains scarce.

Here, we discuss the functions of different classes of transcription factors that have been implicated in the control of developmentally regulated cell death. Though doubtlessly representing but a first layer of PCD regulation, information on PCD-regulating transcription factors and their targets represents a promising strategy to understand the complex machinery that ensures the precise and failsafe execution of PCD processes in plant development.

Keywords

programmed cell death; development; differentiation; transcription factors; gene regulation; plants

3 Introduction

Many aspects of eukaryote development are determined by differential gene expression, which is in turn controlled by complex gene regulatory networks. Protein levels are regulated at multiple steps from gene transcription over *de novo* protein translation to protein degradation. Transcription factors (TFs) are defined as proteins that show sequence-specific DNA binding and are capable of activating and/or repressing gene transcription. In this context, TFs control differential gene expression and thus can act as central switches to coordinate cellular differentiation and development by modulating expression of individual or groups of genes (Riechmann et al., 2000).

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Just as cell division, growth, and differentiation, also cell death is a fundamental biological process of cellular life. A multitude of causes can bring about cellular death in eukaryotes. Instantaneous and catastrophic death of cells by severe physical, chemical, or mechanical insults is referred to as ‘accidental cell death’, while ‘regulated cell death’ (RCD) describes different forms of a genetically encoded, actively controlled demise of cells (Galluzzi et al., 2018). Historically, metazoan cell death processes have been divided according to morphological characteristics into type I or apoptosis, type II or autophagy, and type III or necrosis. To date, a dozen major RCD routines are recognized, which can display morphological features ranging from fully necrotic to fully apoptotic and from pro-inflammatory to anti-inflammatory. These different RCD processes can be triggered by diverse kinds of stresses which are too severe or prolonged for an adaptive response. However, RCD occurs also in the absence of stresses as an inherent part of regular physiological or developmental programs. These cases of cell death are generally referred to as ‘programmed cell death’ (PCD) (Galluzzi et al., 2018).

Different forms of PCD have crucial functions in the development and the physiology of animals and plants. In comparison to metazoans, plants have a set of unique cellular components. The presence of cell walls, chloroplasts and large central vacuoles necessitates and offers independent solutions for the regulation and execution of plant PCD (Van Durme & Nowack, 2016). Furthermore, central apoptotic regulators, including those homologous to Bcl-2 family proteins or caspases, are not encoded in plant genomes, suggesting that the genetics of plant PCD differs substantially from classical animal PCD modes (van Doorn, 2011). As for metazoan PCD, there have been attempts to classify plant PCD into different categories, e.g. based on morphology (van Doorn, 2011) or biological context (Daneva et al., 2016). Morphologically, vacuolar cell death has been described as depending on a combination of autophagy-like processes and the release of lytic enzymes from the disintegrating central vacuole. In contrast, plant necrosis has been characterized by the absence of vacuolar cell death features, early rupture of the plasma membrane, and protoplast shrinkage (van Doorn, 2011). Regarding the biological context, environmental PCD (ePCD) triggered by biotic and abiotic stresses has been contrasted with developmentally controlled PCD (dPCD) that occurs in the absence of environmental perturbation (Daneva et al., 2016). Of note, the transcriptional regulation of several genes has been found to be positively associated with different cases of dPCD, whereas the same genes have not been found to be regulated in ePCD contexts (Olvera-Carrillo et al., 2015).

In comparison with the information available about the molecular regulation of animal PCD, our knowledge of plant PCD still remains fragmented. In animals, evidence suggests that the core of the PCD machinery is largely regulated post-translationally, i.e. on the level of protein localization, activation, or destruction (Fuchs & Steller, 2015). Nevertheless, especially in the context of cancer and development, also the regulation of gene transcription has been shown to be crucial for animal PCD initiation and execution. The tumor suppressor p53, for instance, is a TF regulating the expression of over 500 genes, among them many pro-apoptotic regulators (Aubrey et al., 2017). Mutations in p53 are commonly found in human cancer cells, particularly in the DNA-binding domain (Olivier et al., 2002), documenting the importance of transcriptional control of PCD in the prevention of cancer. Similarly, the tumor-suppressing action of the retinoblastoma protein pRB occurs largely via

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the TF E2F1, which controls the transcription of pro-apoptotic genes (Polager & Ginsberg, 2009). In *Drosophila melanogaster*, the *reaper, head involution defective* and *grim* genes (encoding the inhibitor-of-apoptosis antagonizing RHG proteins) are transcriptionally regulated during fly embryogenesis. Whereas deletion of *RHG* genes blocks apoptosis, inducible expression is sufficient to cause wide-spread ectopic apoptosis (White et al., 1996). Finally, the pro-apoptotic gene *EGG-LAYING ABNORMAL-1* (*EGL-1*) is transcriptionally controlled, repressed by CELL DEATH SPECIFICATION-1 (CES-1) and activated by a basic HELIX-LOOP-HELIX (bHLH) TF complex, to regulate apoptosis in *Caenorhabditis elegans* neuronal cell death (Thellmann et al., 2003)

Also in plants, the regulation of gene transcription has been identified to be an important regulatory principle for different types of PCD. In this chapter, we will give an overview on the involvement of major plant TF classes in different developmentally controlled PCD processes.

4 NAC transcription factor family

Transcription factors belonging to the NAC [for NO APICAL MERISTEM (NAM), ARABIDOPSIS TRANSCRIPTION ACTIVATION FACTOR (ATAF), CUP-SHAPED COTYLEDON (CUC)] family comprise one of the largest groups of plant-specific transcriptional regulators. NAC TFs are involved in a multitude of biological processes, from plant development to stress responses (Fan et al., 2018). Interestingly, NAC TFs have been implicated in different types of cell death, including ePCD and dPCD processes. In *Brassica napus* (rapeseed), several NAC TFs modulate the reaction to environmental stresses by promoting accumulation of reactive oxygen species and a hypersensitive response like ePCD (Chen et al., 2017; Yan et al., 2018). Also in dPCD, NAC TFs play a prominent role, as will be discussed in the following paragraphs.

The evolution of efficient long-distance water and solute transport systems was crucial for the successful colonization of terrestrial habitats by plants (Ohtani et al., 2017). PCD and PCD-like processes play an important role during the development of plant vascular tissues, particularly in the xylem tracheary elements and the phloem sieve elements (see recent review by Heo et al., 2017). The xylem in vascular plants is the transport network for water and extends from the root to the shoot tissues. In order to achieve efficient long-distance transport through cell lumina, conductive xylem cells undergo a PCD process characterized by total cytoplasmic clearance and preservation of the reinforced secondary cell wall (SCW) (Escamez & Tuominen, 2014). Over the last decades, the developmental program of xylem development has been elucidated in quite some detail, including xylem specification, patterning, differentiation, and PCD.

Many aspects of xylem differentiation have been discovered in xylogenic cell cultures of *Zinnia elegans* and *Arabidopsis thaliana*, in which xylem differentiation can be induced by hormones (Demura et al., 2002; Kubo et al., 2005). Downstream of hormonal signaling, numerous TFs have been implicated in the different processes of xylem differentiation, including PCD as its final step (Fig. 1) (Fukuda, 2004). In *A. thaliana*, a microarray analysis revealed that 1705 genes were differentially expressed upon induction of xylem formation

(Kubo et al., 2005). Among the up-regulated genes, there were several NAC TFs with similarity to the xylem-expressed Zinnia TF Z567, and the seven members of the corresponding NAC clade in *A. thaliana* were designated *VASCULAR-RELATED NAC-DOMAIN1* (*VND1*) to *VND7* (Kubo et al., 2005). While recessive loss-of-function approaches failed to produce specific phenotypes, overexpression of *VND6* and *VND7* caused ectopic formation of xylem-like cells, which resembled protoxylem in the case of *VND7* and metaxylem in the case of *VND6*. Accordingly, *pVND6::YFP-NLS* reporters were expressed in the differentiating metaxylem, whereas *pVND7::YFP-NLS* reporters showed expression in the protoxylem. Ultimately, overexpression of both *VND6* and *VND7* leads to ectopic cell death, suggesting that these TFs control both SCW formation and PCD (Kubo et al., 2005). Since the initial discovery of the VNDs, many other elements have been identified (Fig. 1). Of over 80 TFs expressed during xylem vessel differentiation, 14 were found to activate the expression of *VND7* in transient expression assays, including *VND1-6* and *VND7* itself. These putative *VND7* regulators were found bind to a specific motif in the *cis*-regulatory region of *VND7* (Endo et al., 2015). Inducible overexpression of *VND1-5* and *ANAC075* triggered ectopic differentiation of xylem vessels. Surprisingly, however, whereas VND7 targets were upregulated after overexpression of these TFs, *VND7* mRNA levels themselves were not markedly raised, indicating a complex regulation of VND7 activity *in planta* (Endo et al., 2015). Next to NAC TFs, also LATERAL ORGAN BOUNDARIES DOMAIN/ASYMMETRIC LEAVES2-LIKE (LBD/ASL) and GATA-type zinc-finger TF family members were identified as putative *VND7* regulators (Fig. 1) (Endo et al., 2015).

More recently, *VND1*, *VND2*, and *VND3* have been identified to contribute to xylem vessel differentiation in cotyledons independent of *VND6* and *VND7* (Tan et al., 2018). The formation of ectopic xylem in detached hormone-treated cotyledons was found to depend on the presence of functional *VND1*, *VND2*, and *VND3*. Furthermore, a *vnd1 vnd2 vnd3* triple mutant showed inhibited xylem vessel formation of secondary veins in cotyledons under dark conditions (Tan et al., 2018). These findings suggest that *VND1* to *VND3* have specific functions and might be linking light conditions to vessel formation (Tan et al., 2018).

Next to TFs promoting vessel formation, also differentiation-inhibiting NAC TFs have been identified in controlling correct xylem differentiation. For instance, VND-INTERACTING2 (VNI2)/ANAC083 represses xylem vessel differentiation and PCD through interacting with VND7, thereby repressing VND7 target genes (Yamaguchi et al., 2010). The presence of a C-terminal PEST degradation motif suggests that VNI2 is post-translationally controlled to fine-tune VND7 activity, and C-terminally truncated VNI2 dominantly inhibited the development of xylem vessels (Yamaguchi et al., 2010). The xylem-expressed *XYLEM NAC DOMAIN1* (*XND1*)/*ANAC104* has also been shown to negatively regulate xylem differentiation and PCD (Fig. 1). *XND1* overexpression generates dwarf plants in which viable xylem parenchyma cells lacking fortified SCWs replaced functional xylem vessels (Zhao et al., 2008). Interestingly, XND1 has recently been shown to interact with the cell cycle and differentiation regulator RETINOBLASTOMA-RELATED (RBR) via highly conserved C-terminal motifs. Deletion of these motifs severely compromise the effects of *XND1* overexpression, possibly linking *RBR*-controlled processes with xylem differentiation and PCD control (Zhao et al., 2017).

Taken together, NAC TFs play a prominent role in the developmental program of xylem formation, controlling early decisions as well as the late differentiation steps of SCW formation and PCD (Fig. 1). *XYLEM CYSTEINE PROTEASE1* (*XCP1*) and *XCP2*, for instance, have been shown to contribute to cellular autolysis before PCD (micro-autolysis) and after PCD (macro-autolysis). As a consequence, the *xcp1* and *xcp1 xcp2* mutants show a delay in cytoplasmic clearance of dying xylem vessels (Fig. 1) (Avci et al., 2008). Similarly, *METACASPASE9* (*MC9*) has been shown to participate in the cytoplasmic clearing after tonoplast rupture (Fig. 1) (Bollhöner et al., 2013; Escamez et al., 2016). The cysteine protease ZCP4 has been shown to specifically function during xylem vessel PCD in Zinnia (Fukuda, 2004). It degrades the chromatin structure inside the nucleus, helping a rapid nuclear degradation triggered by vacuole rupture during PCD in *Z. elegans* (Obara et al., 2001). Next to these proteases, also other PCD-associated hydrolytic enzymes have been identified as being regulated by VND7, including *RIBONUCLEASE3* (*RNS3*), *BIFUNCTIONAL NUCLEASE1* (*BFN1*), and *PUTATIVE ASPARTIC PROTEINASE3* (*PASPA3*) (Zhong et al., 2010), although to date the specific functions of most of them remain unclear.

In contrast to the xylem vessels, the mature phloem sieve elements remain alive. However, to optimize nutrient transport, also sieve elements reinforce their cell walls and get cleared of most organelles, including the nucleus, in a process that is reminiscent of cellular clearance in PCD (Geldner, 2014). Acting downstream of the MYB TF ALTERED PHLOEM DEVELOPMENT (APL), ANAC045 and ANAC086 have been shown to redundantly regulate the phloem enucleation process by controlling the expression of *NEN4*, encoding a phloem-specific nuclease (Furuta et al., 2014).

Outside the vascular bundle, dPCD processes also play important roles in plant development. In the root cap of *A. thaliana*, for instance, PCD processes have been shown to regulate organ size. The root cap consists of two tissues, the columella root cap at the very tip of the root, and the lateral root cap (LRC), which flanks the root meristem (Kumpf & Nowack, 2015). Although there is continuous root cap cell production by specific stem cells, root cap organ size does not increase accordingly, implying a disposal of mature cells to compensate for the production of new cells. While columella cells get shed off the root in regular intervals as packets of living cells, expression of numerous dPCD-associated genes in the LRC, including *RNS3*, *BFN1*, *PASPA3*, and *MC9*, hinted to a PCD process occurring in the root cap (Olvera-Carrillo et al., 2015). Upstream of *PASPA3* and *BFN1*, the NAC TF SOMBRERO (SMB/ANAC033) has been identified as a master regulator of root cap PCD (Fendrych et al., 2014). *SMB* was first implicated in root cap maturation based on the observation that *smb* mutants show prolonged root cap division activity and delayed root cap differentiation (Bennett et al., 2010; Willemsen et al., 2008). This delay of maturation also affects expression of PCD-associated genes, and causes a delay of PCD execution, which in *smb* mutants occurs further up in the root elongation zone as compared to the wild type. In this zone, *smb* root cap cells die in an aberrant way without post-mortem corpse clearance, which leads to the root proper being covered in unprocessed LRC cell corpses (Fendrych et al., 2014). Recently, two additional NAC TFs, ANAC087 and ANAC046, have been identified to regulate distinct aspects of root cap PCD (Huysmans et al., 2018). *ANAC087* is

expressed in the entire root cap, whereas *ANAC046* expression is restricted to the columella root cap. *ANAC087* indirectly promotes expression of *BFN1* to control nuclear degradation after root cap cell death in the root elongation zone. ANAC046, however, is a direct regulator of *BFN1* and other PCD-associated genes and, redundantly with *ANAC087*, controls PCD onset in the columella root cap during and after shedding of these cells into the rhizosphere (Huysmans et al., 2018). Downstream of these NAC TFs, *BFN1* has been implicated in chromatin degradation in the root cap, as a *bfn1* loss-of-function mutant shows a delay of nuclear degradation (Fendrych et al., 2014). The *Z. elegans BFN1* homolog, *ZINNIA ENDONUCLEASE1* (*ZEN1*), has been shown to have a similar loss-of-function phenotype in xylogenic cell cultures of *Z. elegans* (Ito & Fukuda, 2002), although to date, no *bfn1* phenotype has been described in xylem vessels of *A thaliana*.

NAC TFs have also been implicated in senescence processes and age-induced PCD (Daneva et al., 2016). Ethylene-regulated TFs control the onset of leaf senescence (Koyama, 2014). ETHYLENE-INSENSITIVE3 (EIN3) directly activates expression of *ORESARA1* (*ORE1)/ ANAC092/AtNAC2*, encoding a central positive regulator of leaf senescence (Kim et al., 2014). Also, the NAC TF gene *AtNAP* is under the direct control of EIN3, whereas the *ORE1* paralog *ORS1* (Balazadeh et al., 2011) and the *ANAC019* and *ANAC055* genes are activated during leaf senescence in an EIN3-independent manner (Hickman et al., 2013). In all cases, NAC TFs positively regulate leaf senescence (Koyama, 2014). More recently, it has been shown that the *BnaANAC087* gene is induced by multiple stress and hormone treatments and is highly expressed in senescent leaves (Yan et al., 2018).

Also senescence of the ephemeral floral organs has been shown to be controlled by ethylene and NAC TFs (Shibuya, 2018). Floral organ senescence can be initiated by successful pollination, but also lastingly unpollinated flowers will start to senesce in a species-specific ageing process (Rogers, 2013). During the age-induced senescence of unpollinated flowers, ORE1 and a novel flower-specific NAC TF, KIRA1 (KIR1/ANAC074), have been identified as partially redundantly operating regulators of age-induced stigma degeneration (Gao et al., 2018). In dominant-negative single mutants and *ore1 kir1* double mutants, the stigma life span is substantially prolonged. Interestingly, the stigma's function supporting pollen tube growth towards the ovules is not extended accordingly, suggesting that *ORE1* and *KIR1* control only the PCD aspect during floral senescence but not the time span of stigma receptivity. Nevertheless, misexpression of *KIR1* was sufficient to cause ectopic cell death in tobacco and *A. thaliana*, and early expression of *KIR1* in the stigma caused precocious cell death and loss of floral receptivity, suggesting that NAC-controlled PCD is sufficient to restrict floral receptivity in time (Gao et al., 2018).

5 MYB transcription factor family

The first TF cloned from plants was COLORED1 (C1) of maize (*Zea mays*), a MYB TF with high structural homology to the vertebrate proto-oncogene *c-MYB* (myeloblastosis) (Dubos et al., 2010). In contrast to the plant-specific NACs, MYB TFs are represented in all eukaryotes. Yet, just like NAC TFs, MYB TFs occur in large gene families and regulate a great diversity of processes in development, differentiation, metabolism, and responses to environmental stresses and PCD processes (Ambawat et al., 2013).

A prominent role for *MYB* genes is controlling aspects of dPCD during tapetum development. The tapetal cell layer borders the anther locules and is crucial for microsporogenesis and pollen maturation. In particular, tapetal PCD plays a vital secretory role in the development of pollen grains by contributing metabolites and enzymes that are important for pollen wall synthesis and pollen coat deposition (Liu & Fan, 2013). Manipulating tapetal PCD invariably affects male fertility, indicating its importance for pollen development and coat formation (Gómez et al., 2015). The most upstream TF in tapetal development is GAMYB, a MYB TF important in gibberellic acid (GA) signaling in cereal seeds and during anther development (Fig. 2a,b) (Aya et al., 2009). In rice (*Oryza sativa*), GA-deficient and GA-insensitive mutants exhibit similar defects in tapetal development and PCD as do *gamyb* mutants (Aya et al., 2009). Interestingly, *GAMYB* function is also conserved in dicots, for instance in *A. thaliana* (Plackett et al., 2011). GAMYBs are post-transcriptionally repressed by the microRNA *mirR159*, which is GA-regulated in *A. thaliana*, but GA-independent in rice (Fig. 2a,b) (Alonso-Peral et al., 2010; He et al., 2015).

Downstream of GAMYB, a network of MYB, bHLH, and PLANT HOMEODOMAIN (PHD)-finger TFs coordinates tapetum development. Among the MYB TFs in *A. thaliana* and rice, *TAPETAL DEVELOPMENT AND FUNCTION1* (*TDF1/MYB35*) and *MALE STERILE188* (*MS188/MYB80*) occupy a central position in the tapetum control network together with bHLH TFs (Gu et al., 2014) (Fig. 2). The *A. thaliana tdf1* mutant develops overproliferating and enlarged tapetal cells that fail to maintain tapetum fate and typical degeneration patterns (Zhu et al., 2008). Rice *tdf1* mutants show a similar loss-of-function phenotype as *A. thaliana* mutants, and rice *TDF1* is able to cross-complement the *tdf1* mutant in *A. thaliana* (Cai et al., 2015). Further downstream, *MS188/MYB80* plays a central role in controlling the timing of tapetal PCD. The *ms188/myb80* mutants show premature tapetal degeneration, characterized by an early onset of vacuolation and chromatin degradation in tapetal cells, as shown with a terminal deoxynucleotidyl transferase Dutp nick end labeling (TUNEL) assay (Phan et al., 2011). Interestingly, *MYB80* homologs in the crop plants wheat (*Triticum aestivum*), rice, rapeseed, and cotton (*Gossypium hirsutum*) (cotton) have similar expression patterns and functions as *MS188/MYB80* in *A. thaliana* (Phan et al., 2012). Moreover, the crop MYB80 homologs could restore the wild-type phenotype in the *A. thaliana ms188/myb80* mutant. These data show that the regulation of tapetal PCD has a high degree of evolutionary conservation (Fig. 2a,b).

Downstream of MS188/MYB80, TFs including the PHD-finger TF MALE STERILITY1 (MS1), as well as the protease UNDEAD (Vizcay-Barrena & Wilson, 2006) have been identified (Fig. 2a). UNDEAD is an aspartic protease that prevents the early onset of PCD, since *undead* knock-down lines show precocious PCD similar to *ms188/myb80* mutants. As UNDEAD shows a mitochondrial target sequence, it might act by hydrolyzing (a) still unknown apoptosis-inducing target protein(s) in mitochondria (Phan et al., 2011).

CYSTEINE ENDOPEPTIDASE1 (*CEP1*), encoding a papain-like cysteine protease promoting tapetal PCD, is indirectly regulated by *MS188/MYB80* (Fig. 2a). *CEP1* overexpression causes premature tapetal PCD and male sterility, whereas *cep1* mutants show an interrupted tapetum PCD associated with a decrease of pollen fertility (Zhang et al.,

2014). These results demonstrate the importance of the precise timing of tapetal PCD for full male fertility. MS188/MYB80 also regulates the expression of *RESPIRATORY-BRUST OXIDASE HOMOLOG E* (*RBOHE*), implicating the transcriptional control of reactive oxygen species production in tapetal PCD (Xie et al., 2014).

Besides controlling PCD processes in the tapetum, MYB TFs are also playing important roles in cell death events that occur during plant fertilization. When a growing pollen tube reaches the female gametophyte, both the pollen tube cell and the receptive synergid cell undergo coordinated disintegration to facilitate gamete fusion (Dresselhaus et al., 2016). The three pollen tube-expressed, redundantly acting MYB TFs MYB97, MYB101 and MYB120 have been shown to regulate this process on the male side. Triple mutant pollen tubes do not show the rupture of the pollen tube cell to release the sperm cells, and fail to trigger synergid cell degeneration (Leydon et al., 2013; Liang et al., 2013). Of several pollen tube-expressed target genes (Leydon et al., 2013), a secreted cysteine-rich peptide might be involved in the communication processes required for successful fertilization. Cell-to-cell signaling depending on the receptor-like kinase FERONIA and specific calcium signatures in both synergid and pollen tube cells have been shown to orchestrate the synchronized degeneration of synergid and pollen tube cells (Ngo et al., 2014; Zhou & Dresselhaus, 2019) (see Zhou & Dresselhaus, this issue).

6 bHLH transcription factor family

The bHLH TFs are present in all eukaryotes (Riechmann et al., 2000). In *A. thaliana*, the bHLH family represents the second largest class of TFs comprising more than 150 genes (Feller et al., 2011). In plants, bHLH TFs have prominent roles in developmental patterning processes, fruit dehiscence, secondary metabolite biosynthesis, hormone signaling, and stress responses (Feller et al., 2011; Goossens et al., 2017; Ohashi-Ito & Fukuda, 2016; Ran et al., 2013),, often interacting with MYB TFs in heteromeric protein complexes to fine-tune these processes (Feller et al., 2011). Although MYB and bHLH TFs have been implicated in dPCD regulation, no such interaction has been shown so far in this particular context.

Alongside MYB TFs, several bHLH TFs regulate tapetal differentiation and PCD (Fig. 2a). The bHLH TF-encoding gene *DISFUNCTIONAL TAPETUM1* (*DYT1*) is strongly expressed in the tapetum and developing microspores (Zhang et al., 2006), and is in turn controlled by the MADS-domain TF SPOROCYTELESS/NOZZLE (Zhang et al., 2006). Interestingly, a functional *pDYT1::DYT1-GFP* fusion protein was localized exclusively to the tapetum and could not be detected in microspores, suggesting that the critical function of *DYT1* takes place in the tapetum (Gu et al., 2014). Loss of *DYT1* function results in a precocious vacuolation of tapetum cells, the down-regulation of many tapetum-expressed genes, and male sterility (Zhang et al., 2006). Likewise, *A. thaliana* plants expressing a dominant-repressive *DYT1-SRDX* version display impaired male fertility, suggesting that *DYT1* acts as a transcriptional activator (Feng et al., 2012). DYT1 was found to directly promote expression of *TDF1/MYB35* by binding to its promotor region *in vivo* (Fig. 2a). Expression of *TDF1* under the control of the *DYT1* promoter is able to restore the expression of downstream TFs including *MS188*/*MYB80* and the PHD-finger TF-encoding gene *MS1* (Fig. 2a). In this *trans*-complementation line, tapetum development was partially

restored, although male fertility was not (Gu et al., 2014). Thus, *DYT1* is indirectly required for the expression of *MS188/MYB80* (Feng et al., 2012), the bHLH TF-encoding gene *ABORTED MICROSPORES* (Galluzzi et al.) (Sorensen et al., 2003), and *MS1* (Vizcay-Barrena & Wilson, 2006), indicating that *DYT1* is acting upstream of *TDF1/MYB35*, *AMS*, *MS188/MYB80*, and *MS1* (Fig. 2a). AMS is a regulator of tapetal PCD and, as such, important for pollen wall formation, as explained in the previous section (Fig. 2a) (Xu et al., 2010). *AMS* is expressed in the tapetum of *A. thaliana*, and loss of *AMS* function causes premature tapetum cell death (Fig. 2a) (Sorensen et al., 2003). AMS probably regulates lipid transfer proteins, which are involved in pollen coat formation, and E3 ubiquitin ligases to degrade particular target proteins (Li et al., 2017). Consistent with the model of a transcriptional cascade, *AMS* and *MS1* are expressed in the tapetum later than *DYT1* during anther development (Fig. 2a) (Li et al., 2017).

In the tapetum of rice, several bHLH TFs have been functionally characterized, some of them acting homologously to *A. thaliana* tapetum bHLH TFs (Fig. 2b). *TAPETUM DEGENERATION RETARDATION* (*TDR*)/*bHLH5* is a putative *AMS* ortholog, although male sterility in rice *tdr* mutants has been associated with delayed rather than precocious tapetal degeneration (Li et al., 2006). *DYT1* has a putative homolog in rice, *UNDEVELOPED TAPETUM1* (*UDT1/bHLH164)*, which causes aberrant tapetum differentiation and male sterility when mutated (Jung et al., 2005). Additionally, *TDR INTERACTING PROTEIN2* (*TIP2/bHLH142*) expression has been found to be essential for timely tapetal PCD and pollen development in rice. *TIP2* deletion causes male sterility by blocking tapetal PCD (Ko et al., 2017). Interestingly, *TIP2* misexpression under an ubiquitous promoter triggers premature tapetum PCD, which equally leads to male sterility (Ko et al., 2017), again highlighting the importance of the accurate control of tapetum PCD for male fertility. TIP2 has been shown to interact with TDR to modulate the expression of yet another bHLH TF, ETERNAL TAPETUM1 (EAT1)/DTD/bHLH141) (Ko et al., 2017). Similar to *tip2* mutants, the *eat1* mutant exhibits delayed tapetal PCD. Notably, the EAT1 TF has been shown to directly regulate the expression of two aspartic proteases, *OsAP37* and *OsAP25*, which in turn are sufficient to execute PCD when misexpressed (Niu et al., 2013). In addition, bHLH142/TIP2 cooperates with TDR through protein-protein interaction to modulate the transcriptional activity of *EAT1* (Fig. 2b) (Ko et al., 2017). Taken together, both in rice and *A. thaliana*, there is a largely conserved transcriptional network to control tapetum differentiation and PCD (Cai et al., 2015).

Similar to the tapetum surrounding and supporting developing microspores, the endosperm tissue in the developing seed supports the growing embryo. During early seed development in *A. thaliana*, the endosperm proliferates vigorously, but later ceases growth and subsequently gets almost completely degraded, providing space and nutrients to the expanding embryo (Ingram, 2010). *RETARDED GROWTH OF EMBRYO1* (*RGE1*)/*ZHOUPI* (*ZOU*) encodes an endosperm-specific bHLH TF, and *rge1*/*zou* loss-of-function mutants develop an endosperm that fails to degenerate after cellularization, persisting throughout mid-seed development. As a consequence, embryo growth is severely impeded and mature seeds show a collapsed seed phenotype at maturity (Kondou et al., 2008; Yang et al., 2008). Interestingly, the dwarfed embryos are viable and can develop into healthy plants if germination is supported by the addition of sucrose (Yang et al., 2008). Loss-of-function

of the related *INDUCER OF CBP EXPRESSION1* (*ICE1*) causes a similar endosperm persistence phenotype as *rge1*/*zou* (Denay et al., 2014). *RGE1/ZOU* and *ICE1* are co-expressed in the endosperm and interact in yeast via their bHLH domains, which is important for their role in endosperm breakdown (Denay et al., 2014). Despite the fact that maize has a largely persistent endosperm, knock-down of the single maize ZOU homolog, ZmZOU, causes slightly reduced embryo size. Interestingly, not only the embryo surrounding region, but also the embryonic suspensor show retarded breakdown, suggesting partly conserved and partly divergent roles of ZOU in regulating tissue degeneration in monocots and dicots (Grimault et al., 2015).

Endosperm degradation in *A. thaliana* is characterized by expression of dPCD-associated genes (Olvera-Carrillo et al., 2015), and of these at least *PASPA3* and *BFN1* have been shown to be strongly downregulated in *rge1/zou* mutants (Fourquin et al., 2016). Quantification of the mechanical properties of developing seeds revealed an increased apparent stiffness, indicating an increased turgor of the persistent endosperm (Fourquin et al., 2016). Furthermore, endosperm cell wall breakdown was reduced, suggesting that *RGE1*/*ZOU* primarily promotes cell wall degradation. Cell wall breakdown would allow the growing embryo to exert mechanical stress on endosperm cells (Fourquin et al., 2016). The questions as to whether this mechanical stress simply causes a passive physical disruption of endosperm cells, thus representing a form of accidental cell death, or whether embryo growth and development provide mechanical or other cues to cause an active endosperm PCD process remain to be elucidated.

7 Other transcription factor families involved in PCD

Besides TFs of the large NAC, MYB, and bHLH classes, members of several other TF families have been implicated in dPCD control.

During xylem vessel differentiation, TFs of the LBD/ASL, GATA, and class III HD-Zip families are acting as upstream regulators of *VND7* (Fig. 1) (Soyano et al., 2008). LBD15/ASL11, LBD30/ASL19, GATA5, and GATA12 are able to induce *VND7* promoter activity in a dual luciferase (LUC) assay (Endo et al., 2015). In *35S::LBD15-SRDX* expressing cotyledons, vein formation was incomplete causing a discontinuity of the xylem network (Ohashi-Ito et al., 2018). Inducible *LBD30* expression triggered trans-differentiation of cells from nonvascular tissues into xylem vessel-like cells (Soyano et al., 2008). Similar cells were formed in transgenic plants overexpressing *GATA12*, but not *GATA5* (Endo et al., 2015). Still, *pGATA5::YFP-NLS* and *pGATA12::YFP-NLS* signals were overlapping with those of *pVND7::YFP-NLS* in the differentiation zone of the root stele (Endo et al., 2015).

As mentioned in the sections covering MYB and bHLH TFs above, *MS1* encodes a PHD-finger TF that is controlled by MS188/MYB80 in the A. thaliana tapetal PCD network (Fig. 2a) (Vizcay-Barrena & Wilson, 2006). *PTC1*, the rice homolog of *A. thaliana MS1* (Li et al., 2011), is also involved in tapetal PCD (Fig. 2b). The PTC1 TF controls tapetal PCD and pollen development acting downstream of GAMYB and bHLH142 (Fig. 2b) (Li et al., 2011). The rice *ptc1* mutant shows phenotypic similarity to *A. thaliana ms1* lacking tapetal DNA fragmentation, delayed tapetal degeneration, abnormal pollen wall formation, and

aborted pollen development (Li et al., 2011). PTC1 together with TDR regulates the expression of the PCD-promoting *OsCP1* gene, encoding a cysteine protease involved in protein degradation (Li et al., 2011; Li et al., 2006). The *oscp1* mutant confers pollen degradation as a result of functional defects in the tapetum (Lee et al., 2004).

During fertilization, both the pollen tube and the receptive synergid degenerate. While *MYB97*, *MYB101*, and *MYB120* regulate this process on the pollen tube side, a heterodimer of the REPRODUCTIVE MERISTEM (REM) TFs VALKYRIE (Galluzzi et al.) and VERDANDI (VDD) has been implicated in controlling these cell death processes on the female side in the receptive synergid cell (Mendes et al., 2016). Although *vdd-1* and *val* loss-of-function ovules are still able to attract pollen tubes, their synergid cells do not degenerate, and the attracted pollen tube does not rupture to release its sperm cells (Mendes et al., 2016).

After fertilization, the nucellus degenerates in wild-type seeds of *A. thaliana*, likely representing yet another dPCD process (Xu et al., 2016). The *A. thaliana* MADS-domain TF-encoding gene *AGAMOUS-LIKE62* (*AGL62*) is expressed specifically in the endosperm and initiates PCD signaling in the nucellus. *AGL62* acts through inhibiting the function of *Polycomb* group proteins, which repress nucellus degeneration before fertilization (Xu et al., 2016). Further downstream in this pathway, another two MADS-domain TFs, TRANSPARENT TESTA16 (TT16) and GORDITA (GOA), redundantly promote nucellus PCD. In the *tt16-1* mutant, and even more so in the *tt16 goa* double mutant, nucellar degradation is delayed (Xu et al., 2016). In rice, the MADS-domain TF MADS29 has been shown to promote nucellus PCD, possibly by regulating the expression of cysteine proteases (Yin & Xue, 2012).

8 Conclusion

Complex transcriptional networks coordinate cellular specification and differentiation. For several plant cell types, including xylem vessels, root cap cells, the tapetum layer, or the endosperm, degenerative processes represent the ultimate step of cellular differentiation. Not surprisingly, cell death events have to be tightly controlled in both the spatial and the temporal dimension, as demonstrated by severe developmental defects in mutants affected in PCD processes.

In the developmental context, different forms of PCD are intricately interconnected with earlier steps of the cellular differentiation process. Interestingly, to date there are only few TFs known to exclusively regulate cell death; rather, most TFs regulate several features of cellular differentiation with PCD as its final stage.

Although our understanding of transcriptional PCD regulation remains fragmented, evidence so far suggests that there is a transcriptionally controlled core machinery of developmental PCD in plants. Intriguingly, there seems to be a gradient from tissue-specific transcriptional regulation to process-specific gene expression patterns (Fig. 3). Most TFs identified to date are tissue-specific, for instance VNDs in the xylem, SMB in the root cap, or DYT1 in the tapetum. These TFs likely act as master differentiation regulators of specific cell types,

integrating developmental signals to ensure their proper cellular differentiation, of which dPCD represents the ultimate step. Downstream of these key TFs exist complex gene regulatory networks and TF cascades. Some of the TFs in these networks are less tissue-specific, and are commonly expressed in several cell types preparing for dPCD. Although we still know few such intermediate TFs, recent examples include ANAC087 and ANAC046, which are expressed in both the root cap and xylem vessels, and ORE1 that regulates gene expression both in senescent leaves and floral organs. Finally, a plethora of genes, most of them encoding hydrolytic enzymes like BFN1, RNS3, PASPA3, has been found to be commonly expressed prior to almost all dPCD processes, independent of the tissue context (Fig. 3). These dPCD-associated genes thus tend to be not specific for a certain cell type, but rather for the particular process of dPCD.

Dedicated approaches combining both genetics and network modeling approaches are required to unravel the complex transcriptional networks that ensure the failsafe integration of PCD processes in the differentiation programs of specific plant cells, tissues, or organs. Although transcriptional modulation undoubtedly represents only one facet of PCD control in the developmental context, knowledge about the TFs and their target genes will be an important way forward for a comprehensive understanding of PCD regulation in plants.

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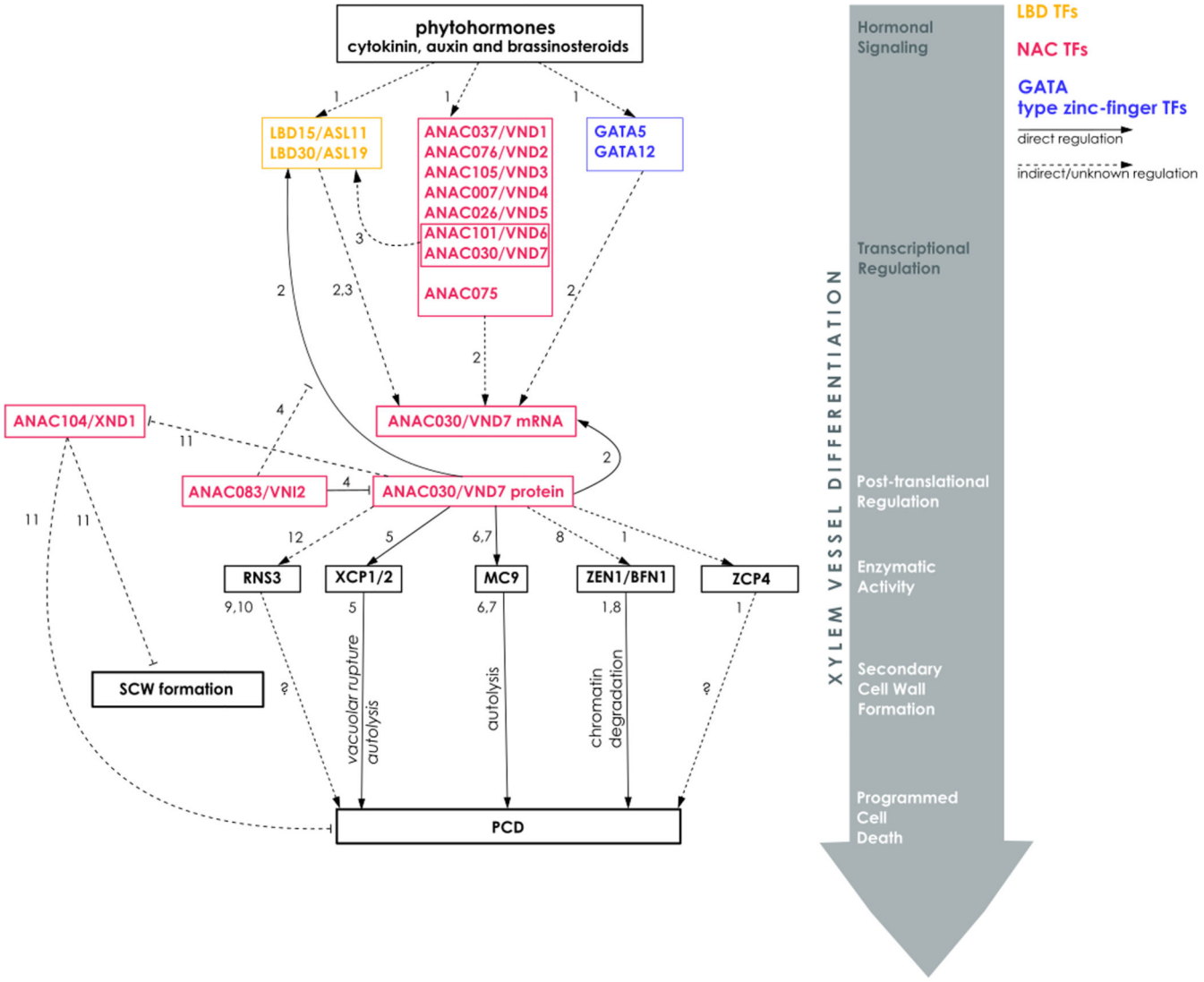

Figure 1. Transcriptional regulation of xylem vessel differentiation and PCD.

LBD TFs are indicated in yellow; NAC TFs in magenta; GATA type zinc-finger TFs in blue. Non-TF proteins are indicated in gray. Solid lines indicate direct regulation; dotted lines indicate indirect regulation. Arrow-headed lines represent positive regulation, and bar-headed lines negative regulation of targets.

Corresponding references: 1: Fukuda, (2004); 2: Endo et al., (2015); 3: Soyano et al., (2008); 4: Yamaguchi et al., (2010); 5: Avci et al., (2008); 6: Bollhöner et al., (2013); 7: Escamez et al., (2016); 8: Ito & Fukuda (2002); 9: Bariola et al., (1994); 10: Olvera-Carrillo et al., (2015); 11: Zhao et al., (2008); 12: Zhong et al., (2010).

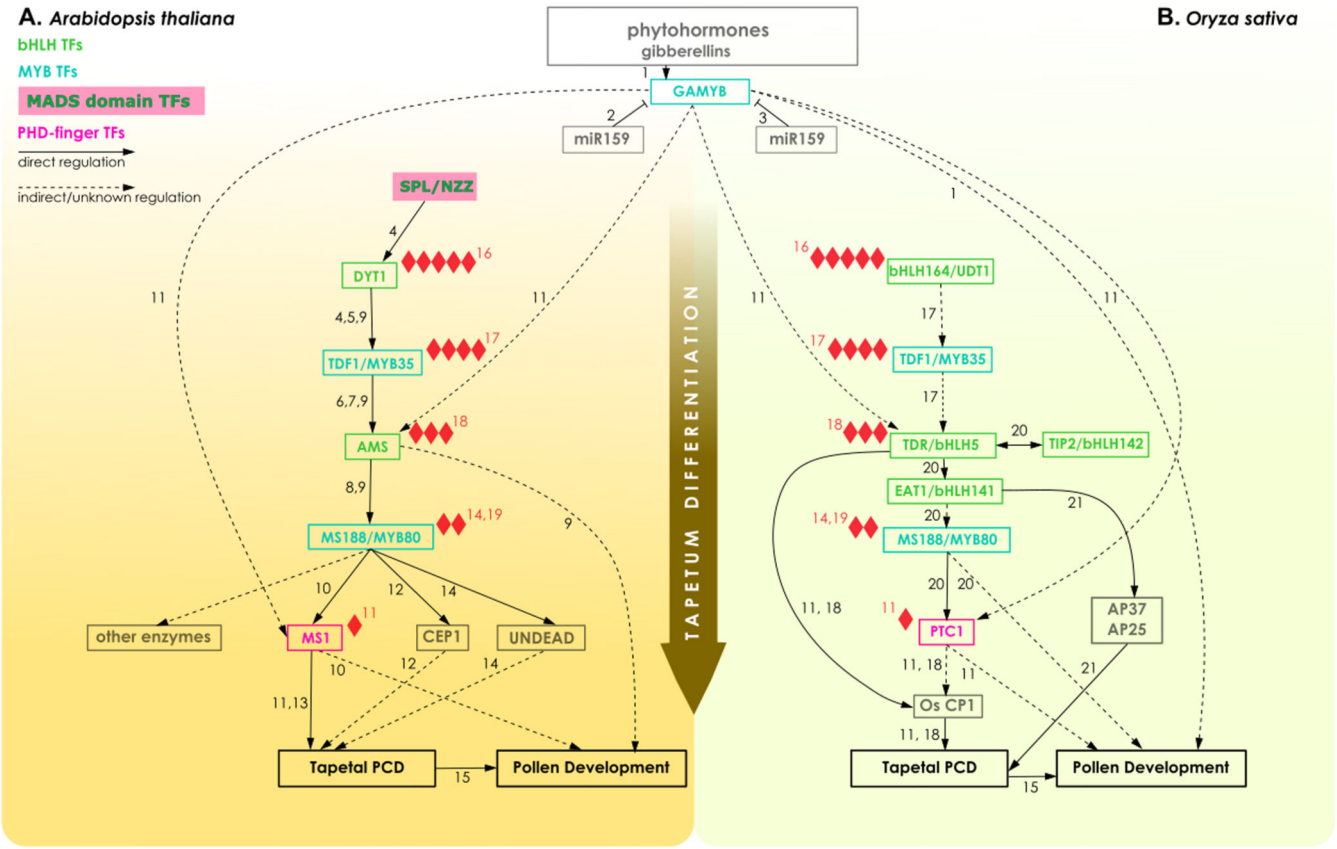

Figure 2. The MYB/bHLH network: transcriptional regulation of tapetal differentiation and PCD in A. thaliana (A) and in O. sativa (rice) (B).

MYB TFs are indicated in turquoise, bHLH TFs in green, MADS-domain TFs in green-squared pink, and PHD-finger TFs in magenta. Non-TF proteins are indicated in gray. Solid lines indicate direct regulation; dotted lines indirect or unknown nature of regulation. Arrow-headed lines represent positive regulation, and bar-headed lines negative regulation of targets. Red rhombi indicate homology between *A. thaliana* and *O. sativa*. Corresponding references: 1: Aya et al., (2009); 2: Alonso-Peral et al., (2010); 3: He et al., (2015); 4: Zhang et al., (2006); 5: Feng et al., (2012); 6: Xu et al., (2010); 7: Zhu et al., (2008); 8: Sorensen et al., (2003); 9: Li et al., (2017); 10: Zhang et al., (2007); 11: Li et al., (2011); 12: Zhang et al., (2014); 13: Vizcay-Barrena & Wilson (2006); 14: Phan et al., (2011); 15: Gómez et al., (2015); 16: Jung et al., (2005); 17: Cai et al., (2015); 18: Li et al., (2006); 19: Phan et al., (2012); 20: Ko et al., (2017); 21: Niu et al., (2013).

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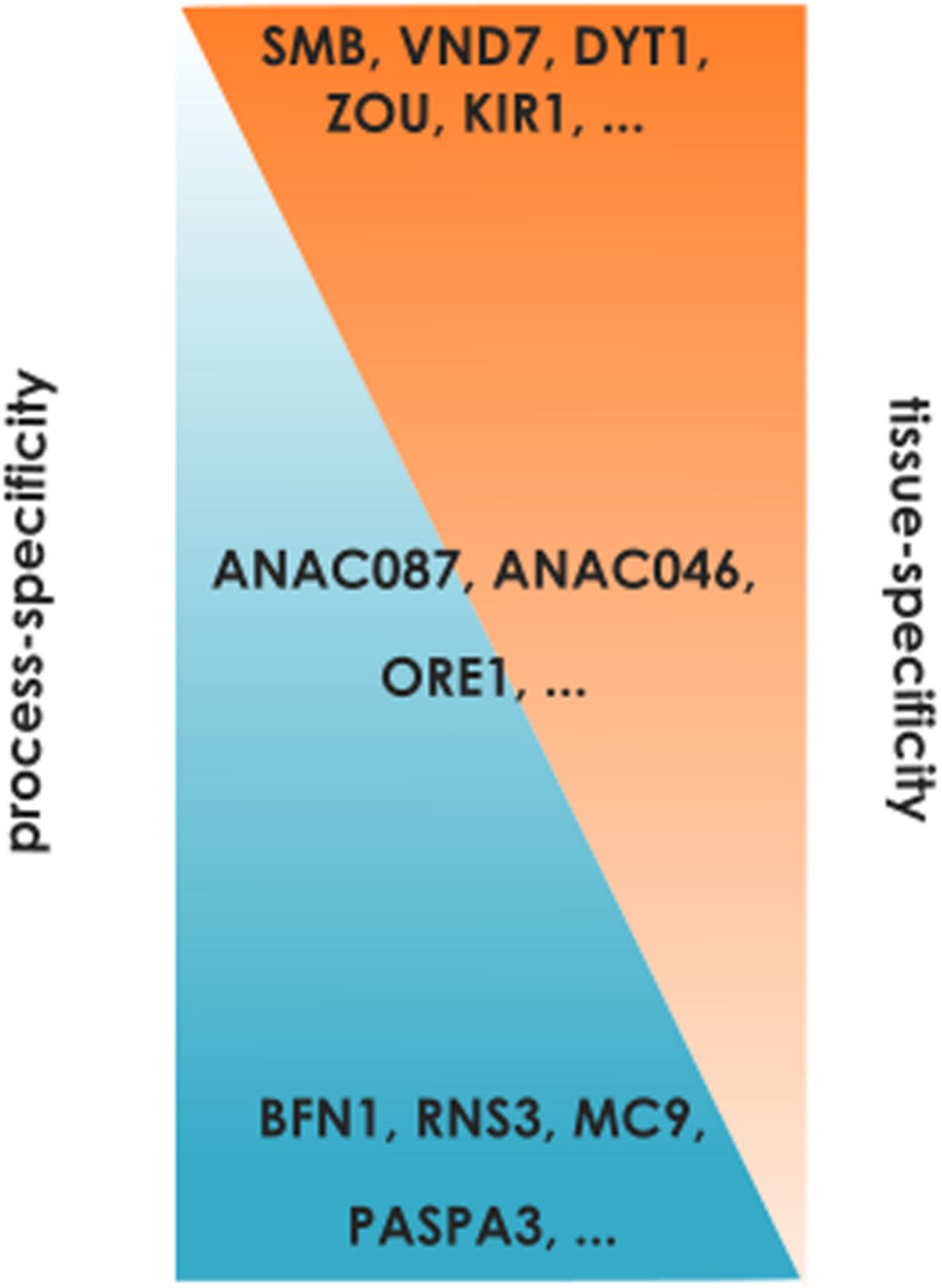

Figure 3. PCD gene regulatory networks show a trade-off between tissue-and process-specificity.
Many upstream TFs are tissue-or cell type-specific (e.g. SMB in the root cap; VND7 in protoxylem; DYT1 in tapetum differentiation; RGE1/ZOU in endosperm degeneration; KIR1 in the degenerating floral stigma) while a few intermediate TFs are commonly regulated in several cell types preparing for PCD (e.g. *ANAC087* and *ANAC046* are commonly expressed in xylem and root cap cells; *ORE1* is expressed in stigma and senescent leaves). PCD-associated downstream target genes (e.g. *BFN1*, *RNS3*, *PASPA3*,

MC9, and others) are not tissue-specific, but commonly expressed in many cell types or tissues undergoing dPCD.