Journal of Experimental Botany, Vol. 75, No. 5 pp. 1205–1209, 2024
https://doi.org/10.1093/jxb/erae008

eXtra Botany

Insight

Waking up Sleeping Beauty: DNA damage activates dormant stem cell division by enhancing brassinosteroid signaling

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This article comments on:

Takahashi N, Suita K, Koike T, Ogita N, Zhang Y, Umeda M. 2024. DNA double-strand breaks enhance brassinosteroid signaling to activate quiescent center cell division in Arabidopsis. Journal of Experimental Botany **75**, 1364–1375.

Stem cells are generally described as cells that exist in an undifferentiated or only partially differentiated state. When they divide, they give rise to two daughter cells, one of which retains its stem cell-like properties, while the other sibling generally differentiates and adopts the cell fate of the surrounding tissue. By selectively killing stem cells using radiomimetic drugs, Takahashi *et al.* (2024) uncovered a signaling module in the *Arabidopsis thaliana* root that awakens dormant stem cells to help replace stem cells lost through DNA damage.

Stem cells are generally thought of as blank slates with the potential to become virtually any cell type within the host's body. A key feature is their ability to self-renew through the process of cell division. When stem cells divide, one cell usually retains its stem cell-like properties, while the other daughter cell eventually differentiates to take on the fate of its surrounding cells or tissue. In both plants and mammals, these stem cells can be found in microenvironments, commonly referred to as stem cell niches (SCN). In the root of *A. thaliana*, the SCN is located at the extreme end of the tip. Within this SCN, two types of stem cells can be found: actively dividing stem cells that encircle other stem cells that appear to be dormant, hence the name quiescent center (QC) cells (Clowes, 1954).

Sacrifices made for the greater good

All the information needed for a plant to turn into a plant is stored in its DNA. Every cell in the plant's body contains an exact copy of this genetic blueprint. Mistakes in this genetic manual can lead to impaired development, defective offspring, or even death of the whole organism. To avoid this from happening, eukaryotes have evolved mechanisms that allow them to detect and adequately respond to such damage. DNA double-strand breaks are the most severe type of DNA damage, and the presence of such breaks can lead to the activation of the ATAXIA TELANGIECTASIA MUTATED (ATM) kinase, which acts as a DNA damage sensor (Pedroza-Garcia *et al.*, 2022). In plants, an activated ATM subsequently phosphorylates and thereby activates the SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1) transcription factor, which functions as the counterpart to the mammalian p53 tumor suppressor, inducing the expression of a plethora of genes that facilitate DNA repair, impose a cell cycle arrest, or execute a cell death program (Yoshiyama *et al.*, 2013; Bourbousse *et al.*, 2018; Ogita *et al.*, 2018). The latter represents an efficient way to eliminate stem cells that contain too many DNA lesions to repair, preventing them from being retained within the organism and, as such, sacrificing them for the greater good (Fulcher and Sablowski, 2009). However, to maintain a fully functional SCN, stem cells lost through cell death need to be replaced by new ones, a phenomenon called regeneration.

Under laboratory conditions, DNA damage-induced cell death can be achieved by exposing plants to medium containing drugs capable of compromising genomic integrity. Zeocin and bleomycin are two such radiomimetic drugs, being antibiotic glycopeptides produced by *Streptomyces verticillus* and commonly used as chemotherapeutics due to their capacity

to intercalate into DNA, resulting in its cleavage. Arabidopsis root vascular stem cells are particularly sensitive to these drugs, and they undergo cell death upon exposure to either of these compounds (Box 1). This active cell death program is no longer observed in mutants that are defective for *ATM* or *SOG1*, indicating that these sensors are involved in activating this 'sacrifice-for-survival' approach (Fulcher and Sablowski, 2009; Yoshiyama *et al.*, 2013). Although the loss of a significant proportion of the vascular stem cell population is expected to result in a root tip collapse, plants prevent this from happening by initiating a regenerative response. In this response, cells adjacent to the dying ones are stimulated to divide, replenishing the recently deceased cells (Heyman *et al.*, 2013; Canher *et al.*, 2020). Being in direct contact with the dead vascular stem cells, this also applies for the otherwise proliferation-dormant QC cells (Cruz-Ramírez *et al.*, 2013; Heyman *et al.*, 2013) (Fig. 1). Takahashi *et al.* (2024) found that at 18 h after exposure of Arabidopsis roots to zeocin, 15% of the seedlings showed signs of activated QC cell division, increasing to 50% within the following 6 h. The percentage of plants showing dividing QC cells following zeocin exposure was drastically reduced in an *ATM*-deficient background and was completely absent in seedlings mutated for *SOG1*, indicating the need for active DNA damage signaling to initiate QC cell division.

Box 1. The Arabidopsis stem cell niche and its response to DNA damage

In *A. thaliana*, the stem cell niche (SCN) is located at the base of the root meristem. The SCN contains the different types of stem cells, i.e. the columella stem cells, the cortex–endodermal initial cells, and the vascular stem cells, which give rise to all respective tissues present within the root. At the center of the SCN are the quiescent center (QC) cells that are required to maintain the undifferentiated status of its surrounding stem cells (van den Berg *et al.*, 1997). In contrast to the surrounding stem cells that show a high proliferation activity, QC cells divide only sporadically. DNA labeling experiments and time-lapse imaging of an Arabidopsis root showed that it can take a QC cell from 3 d up to 7 d to engage in a round of cell division, whereas the division frequency of its surrounding stem cells is at least twice as fast (Dolan *et al.*, 1993; Rahni and Birnbaum, 2019). Possibly because of this, vascular and columella stem cells are particularly susceptible to compromised genome integrity, for example upon exposure to DNA damage-inducing compounds, including zeocin. This susceptibility presents itself by activating a cell death program within these vascular and columella stem cells, as opposed to the QC cells that display a notable resistance towards DNA integrity-compromising compounds. Therefore, when the integrity of the SCN is compromised by, in the worst case, a cell death event, cell division in the QC is activated to help repair the damage inflicted. The occurrence of even a single dead cell is sufficient to fire up this 'regenerative' cell division program, and is often preceded by transcriptional activation of the *ETHYLENE RESPONSE TRANSCRIPTION FACTOR 115* (*ERF115*) transcription factor-encoding gene, a key instigator of this regenerative response (Heyman *et al.*, 2013, 2016).

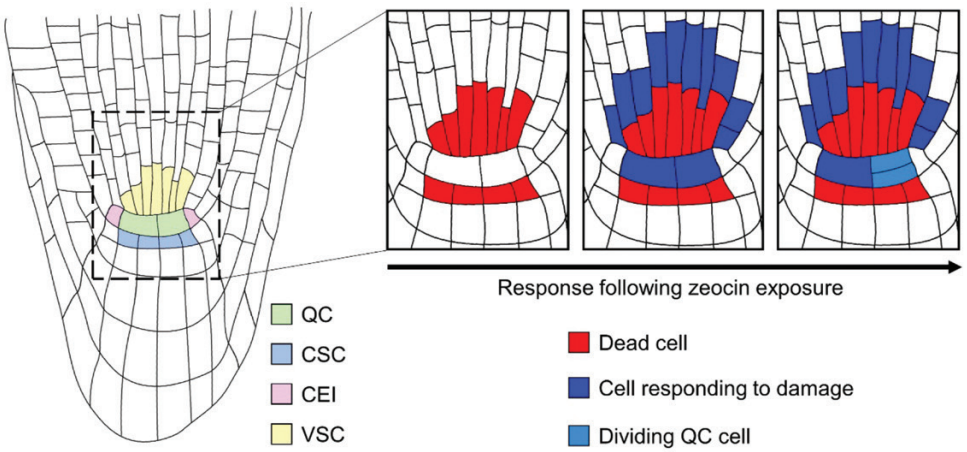

Fig. 1. Schematic representation of the *A. thaliana* root tip, including the stem cell niche (SCN). On the left, the organization of the different stem cells is shown; on the right, a close-up of the SCN response to zeocin treatment. QC, quiescent center; CSC, columella stem cells; CEI, cortex–endodermal initial; VSC, vascular stem cell.

Communication is key

Communication between cells and across tissues is essential and often facilitated by signaling molecules, including hormones. Being such plant hormones, brassinosteroids (BRs) play a role in a variety of developmental processes, including seed and pollen development, flowering time, lateral root initiation, and vascular tissue formation (Nolan *et al.*, 2020) (Box 2). In the root tip, application of the BR brassinolide activates QC cell division (González-García *et al.*, 2011). In addition, DNA damage-induced vascular stem cell death results in nuclear accumulation of BRASSINAZOLE RESISTANT1 (BZR1) in the neighboring cells, indicative for activated BR signaling (Canher *et al.*, 2022). Likewise, Takahashi *et al.* (2024) demonstrated the activation of the fluorescent BZR1–yellow fluorescent protein (YFP) reporter within QC cells following zeocin treatment, prior to the onset of cell division. In contrast, no BZR1–YFP activation could be observed in *sog1* mutant roots, which fail to initiate QC cell division following zeocin treatment, suggesting a role for a SOG1-mediated BR signaling in driving QC cell division (Takahashi *et al.*, 2024).

By investigating the factors that might activate BZR1 and thus BR signaling in response to DNA damage, Takahashi *et al.* (2024) found increased expression of the plasma membrane-localized BR receptor-encoding gene *BRASSINOSTEROID INSENSITIVE 1-LIKE 3* (*BRL3*). This response appears to be specific for *BRL3* because no difference was observed for the other BR receptor-encoding genes *BRI1* and *BRL1*. Moreover, using a chromatin enrichment assay, SOG1 was found to bind directly to the *BRL3* locus upon DNA damage, further supporting the hypothesis that SOG1-dependent DNA damage-induced QC cell division occurs via BRL3. Indeed, plants mutated for *BRL3* showed a drastic reduction in QC cell division following zeocin treatment compared with wild-type plants, whereas *bri1* and *brl1* mutants were only mildly affected in QC cell division activation, further supporting a predominant role for *BRL3*. Correspondingly, QC cell-specific overexpression of *BRL3*, thus artificially increasing BR signaling, results in spontaneous QC cell divisions even in the absence of DNA damage.

Although Takahashi *et al.* (2024) revealed the molecular cascade initiated by DNA damage that feeds into BR signaling to activate QC cell division, the downstream effector that initiates stem cell division remained elusive. Previously, it was shown that expression of the *ETHYLENE RESPONSE FACTOR 115* (*ERF115*) transcription factor-encoding gene is rapidly activated in response to DNA damage-induced cell death to initiate a stem cell division program required for the recovery process, and its expression was found to be BR dependent (Heyman *et al.*, 2013, 2016). In line with this observation, *brl3* mutants that are compromised in QC cell division activation following zeocin exposure showed a strong reduction in the occurrence of *ERF115*-positive QC cells compared with the wild type, suggesting that ERF115 acts as the QC cell division instigator at the end of the signaling cascade (Takahashi *et al.*, 2024) (Fig. 2).

Where to go from here

Takahashi *et al.* (2024) unraveled the signal transduction cascade by which increased BR signaling stimulates cell division in otherwise dormant QC cells via SOG1-dependent DNA damage signaling. Although their work makes a significant contribution to understanding DNA damage signaling and the plant's response to call upon its reserve stem cell pool to come to the rescue, some outstanding questions remain.

First, Takahashi *et al.* (2024) showed a remarkable role for BR in the downstream activation of QC cell division following DNA damage-induced stem cell death, but is it really that simple? Besides BR, other plant hormones, including auxin and the known wounding-related hormone jasmonate (JA), have been found to activate QC cell division (Zhou *et al.*, 2019). The presence of dead stem cells, such as those selectively generated using a high-power laser, has been shown to cause JA accumulation within the SCN (Zhou *et al.*, 2019). In addition, the plant hormone auxin is actively produced and transported in the root tip, in that way establishing the highest concentration within the QC cells, required for them to maintain their quiescent state (Sabatini *et al.*, 1999). The presence of dead cells within the SCN disrupts this auxin flux, thereby altering auxin homeostasis within the QC cells (Canher *et al.*, 2020). Do all these hormonal inputs act on the same downstream effectors to activate QC cell division? Is there a hormonal crosstalk that regulates QC cell division and do they share similar signaling components through which these pathways converge at some point, or do all these signaling cascades operate independently from one another?

Secondly, when it finally comes down to the activation of QC cell division, a key question that remains is whether the initial trigger is instigated by DNA damage signaling, or is the occurrence of dead cells by the apoptotic program that follows. Indeed, upon mutation of *SOG1*, no QC cell division activity is detected after treatment with zeocin; however, this generally coincides with a lack of stem cell death. It remains technically challenging to uncouple one from the other, namely preserving a functional DNA damage signaling network that does not activate an apoptotic program upon DNA damage-inducing conditions. Vice versa, can you generate dead cells within specific cell types without compromising genome integrity? It is possible to selectively eliminate cells using a high-power laser; however, can any collateral DNA damage in neighboring cells be really excluded? Exposure of seedlings to near-freezing temperatures also activates an SCN apoptotic program, but this is also dependent on DNA damage signaling (Hong *et al.*, 2017). It is perfectly possible to mechanically remove larger portions of the root using a fine needle or razor blade. Although it is unlikely, but again not impossible, that

Box 2. Brassinosteroid signaling and QC cell maintenance

Activated brassinosteroid (BR) hormone signaling entails a signal transduction cascade involving membrane-bound receptors, phosphorylation relays, and transcription factor activation. In the absence of BR, the BRASSINOSTEROID-INSENSITIVE 2 (BIN2) kinase phosphorylates the BRASSINAZOLE RESISTANT 1 (BZR1) and BRASSINOSTEROID INSENSITIVE 1-EMS-SUPPRESSOR 1 (BES1) transcription factors, resulting in their cytoplasmic localization and thereby rendering them inactive. However, the presence of BR triggers association of its membrane-bound receptor BRASSINOSTEROID INSENSITIVE 1 (BRI1) with the BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) co-receptor. This BRI1–BAK1 active heterodimer can initiate an intracellular phosphorylation cascade, resulting in the activation and stabilization of the BZR1 and BES1 transcription factors, allowing them to initiate transcription of a variety of BR-responsive genes. Based on sequence similarity, three other members of the BRI1 receptor family have been identified in Arabidopsis, called BRASSINOSTEROID INSENSITIVE 1-LIKE (BRL) 1–3. Although *BRL2* was found not to encode a functional receptor, BRL1 and BRL3 are able to transduce the BR signal alongside BRI1 (Caño-Delgado *et al.*, 2004).

BR signaling contributes to many aspects of plant growth and development, including cell elongation, vascular differentiation, cell cycle progression, and maintenance of meristem size (Nolan *et al.*, 2020). Within the SCN, BRs are also known to control QC cell maintenance. Under standard growth conditions, the vascular stem cell- and QC cell-specific expression of the *BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTER* (*BRAVO*) transcription factor-encoding gene represses QC cell division (Vilarrasa-Blasi *et al.*, 2014). Treatment with brassinolide (BL; an active form of BR) results in rapid BRAVO degradation, allowing activation of QC cell division, which is reflected by a remarkable expansion of the expression domain of the QC-specific transcription factor-encoding gene *WUSCHEL-RELATED HOMEOBOX 5* (*WOX5*) (González-García *et al.*, 2011; Vilarrasa-Blasi *et al.*, 2014). In addition, BL treatment activates the expression of the *ETHYLENE RESPONSE FACTOR 115* (*ERF115*) transcription factor-encoding gene, a driver of stem cell division, in the QC cells (Heyman *et al.*, 2013), in that way showing a dual mode of action by which BR regulates QC cell division.

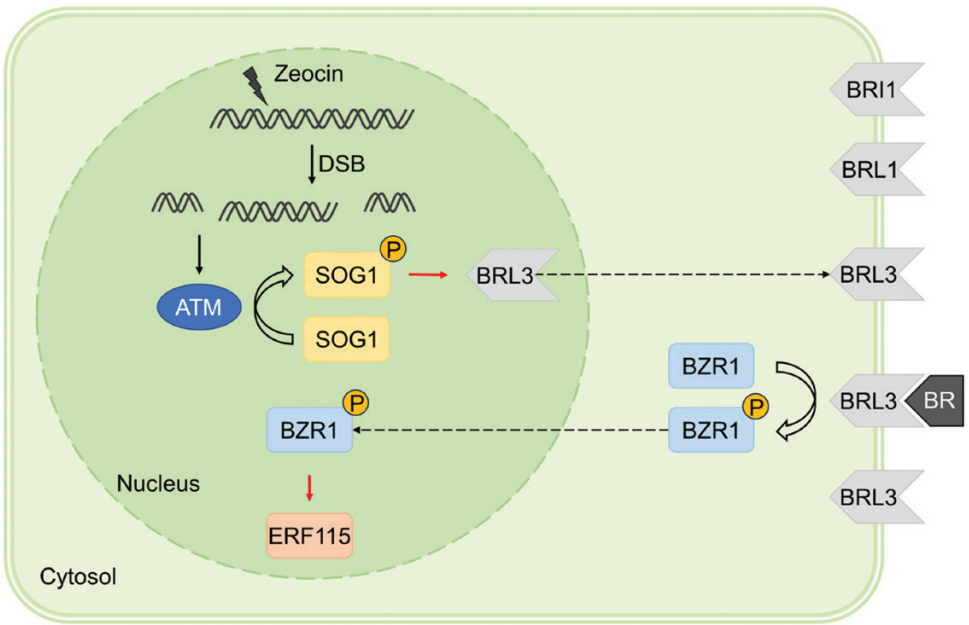

Fig. 2. Signaling cascade initiating QC cell division in response to DNA damage. The presence of zeocin results in DNA double-strand breaks (DSB), leading to the activation of the DNA damage sensor ATM. Subsequently, the ATM kinase phosphorylates and activates the SOG1 transcription factor, which increases *BRL3* expression. Increased numbers of the membrane-exposed BRL3 receptor enhance BR signaling by promoting nuclear localization of the BZR1 transcription factor, resulting in the expression of *ERF115*, followed by QC cell division. Red lines indicate transcriptional activation, solid black lines indicate activation, and dashed lines indicate protein translocation. An orange encircled P indicates phosphorylation.

some DNA damage signaling might be at work, this method lacks finesse and therefore a cell- or tissue-specific resolution is lost. Indeed, an ERF115-dependent regenerative response is still observed in remaining root stumps following tip excision of *SOG1*-deficient seedlings (Johnson *et al.*, 2018).

Finally, although Takahashi *et al.* (2024) have described a mechanism by which QC cells respond to loss of genome integrity, this mechanism may not apply to other stem cells or tissues, as seen by the decreased but still sustained transcriptional activation of *ERF115* in the *brl3* mutant. This leaves the question about the nature of other signals capable of activating an ERF115-dependent regenerative response.

Acknowledgements

The authors thank Annick Bleys for help with preparing the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

Funding

The authors are supported by the European Union (ERC-Adv, UNROPO, 101053972).

Keywords: DNA damage, hormone signaling, stem cells.

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