OPINION PAPER

Border cells versus border-like cells: are they alike?

Azeddine Driouich*, Caroline Durand, Marc-Antoine Cannesan, Giuseppe Percoco and Maité Vicré-Gibouin

Laboratoire Glyco-MEV, IFRMP 23, Plate-forme de Recherche en Imagerie Cellulaire de Haute Normandie, Université de Rouen, F-76821 Mont Saint Aignan, France

* To whom correspondence should be addressed: E-mail: azeddine.driouich@univ-rouen.fr

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Abstract

Roots of many plants are known to produce large numbers of ‘border’ cells that play a central role in root protection and the interaction of the root with the rhizosphere. Unlike border cells, border-like cells were described only recently in the model plant Arabidopsis thaliana and other Brassicaceae species and very little is known about the functional properties of border-like cells as compared with ‘classical’ border cells. To stimulate discussion and future research on this topic, the function of border cells and the way border-like cells are organized, maintained, and possibly involved in plant protection is discussed here.

Key words: Arabinogalactan protein, border cells, border-like cells, cell wall, homogalacturonan, mucilage, plant defence, plant root cap, xylogalacturonan.

What are border cells?

The current definition of border cells as put by M. Hawes and co-workers is as follows: ‘Border cells are those cells that separate from the root tips of higher plants and disperse individually into suspension immediately after their contact with water’. (Hawes et al., 2000, 2003). The number of border cells produced by a root in a given period of time by a given family is conserved (from a few hundreds to several thousands) and the cells remain viable for weeks after their detachment (Fig. 1A).

Based on the above definition, it was assumed that Arabidopsis thaliana, a well-studied species in plant biology, does not produce border cells (i.e. dispersed cells when placed in water). However, it has been possible to show that root tips of A. thaliana seedlings produce sheets of attached cells that remain associated together after their release (Vicré et al., 2005). They never become dispersed individually as single cells when put into water as happens, for example, to pea border cells. A similar border-like cell phenotype was found in other Brassicaceae, including rapeseed (Brassica napus), Brussels sprout (Brassica oleraceae), and mustard (Sinapis alba) (Driouich et al., 2007). These cells do not seem to fit the definition of ‘border cells’ as cited above and, therefore, to emphasize their unusual organization pattern, they were named border-like cells (Fig. 1B, C).

Function of border cells

For many years, border cells were considered of little or no interest but it has become clear that these cells play a major role in the interaction of plant roots with the rhizosphere (Hawes et al., 2000). A wide range of studies has demonstrated that border cells have an impact on plant health and survival by protecting the root meristem from pathogenic infection. For instance, border cells of pea, the most widely used plant model for border cell studies, are capable of inhibiting growth of the fungus Nectria haematococca in vitro (Gunawardena and Hawes, 2002). They are also capable of repulsing the fungus, thereby preventing infection of the root tip (Gunawardena et al., 2005). They seem to do so by encasing the hyphae in a kind of mantle, and once the mantle is removed the root tip remains free of infection. Interestingly, such a parasite-expulsion strategy has also been observed in intestinal mammalian cells, which are under continuous renewal and were shown to repel the invading nematode parasite, Trichuris trichuria from colonizing the gut (Cliffe et al., 2005).

Border cells can also repel pathogenic bacteria by means of their secreted mucilage. In addition, it has been shown that border cells of legumes export a large number of...
proteins (the secretome) during their detachment from the root cap (Wen et al., 2007). The secretome is a fundamental protective component of the root cap that contains many antimicrobial enzymes, including chitinase, peptidase, and glucanase (Wen et al., 2007; De la Peña et al., 2008).

One of the most interesting recent findings related to border cell function is that, in pea, they secrete extracellular DNA that is involved in root tip resistance to fungal infection (Wen et al., 2009). It is notable that extracellular DNA plays an analogous role in several other systems. Human white blood cells form an extracellular structure called the neutrophil extracellular trap (NET) that contains DNA along with antimicrobial peptides and enzymes. The NET is formed by activated neutrophils in response to microbial invasion and traps and kills the pathogens (Wartha et al., 2007). Extracellular DNA is also known to be present in bacterial secretions that form biofilms and to be essential for biofilm stability (Arundhati and Paul, 2008). The role of the DNA in these cellular fortifications remains to be elucidated.

Border cells were also shown to be involved in the response to abiotic stress. For instance, exposure of border cells to aluminium induces secretion of an important layer of mucilage that chelates aluminium and prevents it from penetrating the root tip (Miyasaka and Hawes, 2001). Another function of border cells and their secreted mucilage is lubrication that helps root penetration into compact soil. The total number of border cells released from the root tip of maize was shown to increase significantly in compacted sandy soil compared with loose sand (Ijima et al., 2004). Also, the release of border cells is dependent on the water status of the soil with fewer cells being produced in dry sandy soil compared with wet soil (Ijima et al., 2003).

Although border-like cells differ from border cells in their pattern of release, it is likely that they function similarly. However, a direct involvement of border-like cells in defence awaits further investigation. One of the approaches to evaluate the role of border-like cells in defence is to assess the resistance of mutants lacking such cells to infection with soil-borne pathogens. One such mutant is \textit{fez} that produces fewer border-like cells than does the wild type (Willemsen et al., 2008; C Durand, A Driouich, unpublished results; Fig. 2A). \textit{FEZ} is a NAC-domain transcription factor that is required for root cap development. It is active in root cap stem cells and allows the production of cap tissues via the control of the orientation of cell division. The activity of \textit{FEZ} in the epidermal/lateral root cap cell initials is able to promote the formation of root cap cells including border-like cells (Willemsen et al., 2008). It is predicted that \textit{fez} roots will be readily succumbing to pathogen attack.

The organization pattern of border-like cells is dependent on homogalacturonan

The unusual, adherent, habit of border-like cells when compared with border cells raised the question of the components responsible for cell adhesion. Using immunofluorescence imaging, it has been possible to show that border-like cells of \textit{Arabidopsis} secrete abundant arabinogalactan protein and homogalacturonan epitopes (Vicrè et al., 2005). However, they do not secrete as much mucilage as

![Fig. 1. Border cells (BC) are released by \textit{P. sativum} root tips as isolated cells (A). Morphological phenotypes of root tips showing border-like cells (BLC) of the wild-type \textit{Arabidopsis} (B, C). In (C) BLC are stained with Calcofluor. Bars, 50 \textmu m (A) or 20 \textmu m (B, C).](image-url)
pea border cells do. By examining a series of selected Arabidopsis mutants deficient in the biosynthesis of xylglucan, cellulose, and pectin, Durand et al. (2009) showed that homogalacturonan is a fundamental component of border-like cell organization. The quasimodo1-1 (qua1-1) mutant, which is deficient in homogalacturonan biosynthesis (Bouton et al., 2002), releases border-like cells that fully disperse in the surrounding environment, a phenotype that resembles that of border cells in pea. This observation is consistent with the role of homogalacturonan in the control of cell attachment and adhesion. Separation of the border-like cells in qua1-1 is accompanied by concomitant production and secretion of a mucilaginous matrix (Fig. 2B), not usually seen in the wild type. The mucilage is abundant and contains mainly the LM8 xylogalacturonan (XGA) epitope and arabinogalactan protein epitopes as revealed by probing with specific antibodies (Durand et al., 2009).

It is interesting to note that the sheaths of abundant mucilage enclosing border cells resembles biofilm bacterial secretions, which aid in cell-to-cell aggregation, protection from desiccation, and resistance against harmful substances (Davey and O’Toole, 2000). Such a border cell ‘biofilm’ formation in qua1-1 root is perhaps a key factor for their survival, stability, and thus their defence activity.

Does qua1-1 make functional border cells?

As described above, the major difference between the border cells of many plants and the border-like cells of the Brassicaceae is the latter’s persistent adhesion. Therefore, qua1-1 provides the ideal material to test the relative performance of border cells when they are kept adherent or allowed to separate. The mucilage produced by qua1-1 seems to retain the separated cells close to each other and close to the root tip, thereby preventing them from moving away in the presence of water. It is currently not known whether the retention of cells in the vicinity of root tips via the mucilage in qua1-1 mutant or as a block of attached cells in the wild-type is important for their function. But unity makes strength or ‘l’union fait la force’!

Mucilage has been implicated in the defensive function of border cells in pea (Hawes et al., 2000). It is also possible that the mucilage produced by qua1-1 has a protective activity.

Interestingly, the mucilage secreted in qua1-1 mutants was found to be enriched in the XGA epitope detected by LM8 antibodies (Durand et al., 2009). The same observation was made in qua2-1 (Fig. 3), a mutant deficient in a putative methyl transferase (Mouille et al., 2007). XGA is an α-(1→4)-linked D-galacturonic acid chain highly substituted with β-D-xylose (Zandleven et al., 2005). Although the precise function of XGA is not clearly established, it was speculated that XGA could be involved in the resistance to pathogen attack (Willats et al., 2004; Jensen et al., 2008). This hypothesis was based on the fact that substitution of galacturonans with xylose makes XGA more resistant to digestion by endopolygalacturonases. As pathogens invade plant tissues, they synthesize and secrete a set of enzymes including endopolygalacturonase in order to degrade the plant cell walls. It is thus possible that the presence of XGA in the mucilage of the qua mutants would restrain the progression of pathogens and provide root protection against infection.

It will be interesting to analyse to what extent qua1-1 appears to have either gained or lost putative border cell functionality. In our laboratory, the current focus is on comparing the response of qua1-1 to the wild type when challenged with various pathogens and abiotic stresses, thereby improving our understanding of the unusual adherent border cell phenotype of A. thaliana and its relatives.

Arabinogalactan proteins might control binding of micro-organisms to border cells

Unlike homogalacturonan, arabinogalactan proteins do not seem to be involved in the attachment and organization of

Fig. 2. Morphological phenotypes of root tips showing border-like cells (BLC) of the mutants fez (A) and quasimodo1-1 (B). Note the abundant mucilage (M) that encloses the BLC of quasimodo1-1. fez mutants produce almost no BLC. Bars, 10 μm (A) or 50 μm (B).
border-like cells in the wild type, although these proteins (especially fasciclin-like arabinogalactan protein) were suggested to be important for cell adhesion (Johnson et al., 2003; Driouich and Baskin, 2008). A careful examination of all fasciclin-like arabinogalactan protein mutants did not reveal any alteration of border-like cells pattern (C Durand, A Driouich, unpublished data). Separated cells were not observed.

Arabinogalactan proteins may fulfill other functions at the cell surface of border-like cells. The alteration of arabinogalactan protein biosynthesis or incorporation within the cell wall induces a significant reduction in the binding of rhizobacteria to border-like cells and the root cap in Arabidopsis (Vicré et al., 2005). Consistent with the implication of cell wall arabinogalactan proteins in the attachment of soil-borne bacteria is the observation that the rat1 AtAGP17 mutant, containing a T-DNA insertion in the promoter region of a gene encoding an arabinogalactan protein, is resistant to infection and transformation by Agrobacterium tumefaciens. Resistance to transformation was shown to be correlated to a deficiency of A. tumefaciens in binding root cells (Nam et al., 1999; Gaspar et al., 2004). Also, incubation of Arabidopsis roots with β-glucosyl Yariv, an agent known to bind arabinogalactan proteins, inhibits attachment and transformation by the same bacteria. Therefore, it appears that, while homogalacturonan produced by border-like cells is involved in their attachment to each other, arabinogalactan proteins seem to function in binding and, possibly, in recognition of micro-organisms.

**Conclusion and prospects**

Border cells of the legume, pea, are clearly involved in defence against fungal pathogens. But what about the border-like cells of Arabidopsis and other Brassicaceae? One of the most important questions with regard to the function of border-like cells is related to their role in defence. Are their formation and release stimulated in the presence of specific pathogens? Do they produce specific anti-microbial molecules in response to biotic stress, and also in response to abiotic stress? How is their adherent phenotype adaptive? Is the abundant mucilage secreted by the cells of the mutant qua1-1 related to their role in defence?

We should now move forward and search for the components required for the function of border-like cells of the Brassicaceae (some of which are agriculturally important crops). The use of large-scale transcriptomic or metabolomic profiling, for instance, would be a powerful approach for the identification of novel defensive molecules produced by border-like cells in response to specific microbe infections.

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References


