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DVL genes play a role in the coordination of socket cell recruitment and differentiation

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Abstract

Specialized plant cells arise from undifferentiated cells through a series of developmental steps. The decision to enter into a certain differentiation pathway depends in many cases on signals from neighbouring cells. The ability of cells to engage in short-range intercellular communication permits the coordination of cell actions necessary in many developmental processes. Overexpression of genes from the *DEVIL/ROTUNDIFOLIA (DVL/ROT)* family results in severe developmental alterations, but very little is known about their mechanism of action. This work presents evidence that suggests a role for these genes in local signalling, specifically in the coordination of socket cell recruitment and differentiation. Overexpression of different *DVL* genes results in protuberances at the base of the trichomes surrounded by several rows of elongated epidermal cells, morphologically similar to socket cells. Localized overexpression of *DVL4* in trichomes and socket cells during early developmental stages activates expression of socket cell markers in additional cells, farther away from the trichome. The same phenomenon is observed in an activation tagged line of *DVL1*, which also shows an increase in the number of socket cells in contact with the trichome. The roles of individual *DVL* genes have been difficult to discover since their overexpression phenotypes are quite similar. In *gl1* leaves that lack trichomes and socket cells *DVL1* expression shows a 69% reduction, suggesting that this gene could be involved in the coordination of socket cell development in wild-type plants.

Key words: Development, *DVL/ROT*, peptides, *Arabidopsis thaliana*.

Introduction

Cellular communication is crucial for determining developmental fates in all multicellular organisms. Cell-to-cell signalling is part of a complex system of communication that regulates basic cellular activities and coordinates cell actions. Plant cell-to-cell communication makes use of small peptide signals and specific receptors. The first functional plant peptide to be discovered was systemin from tomato (Pearce *et al.*, 1991). Since then, over 10 families of peptides have been found to play a role in plant development (Farrokhi *et al.*, 2008). A number of these peptides have been shown to take part in the initiation of proliferation and/or differentiation of mature tissues (Matsubayashi and Sakagami, 1996); the regulation of the shoot apical meristem (Clark *et al.*, 1997; Fletcher *et al.*, 1999); root growth and development (Pearce *et al.*, 2001); floral abscission (Butenko *et al.*, 2003; Stenvik *et al.*, 2006); and reproduction (Schopfer *et al.*, 1999).

Among the least understood plant peptides are those in the DEVIL (DVL) or ROT-FOUR-LIKE (RTFL) family. Its role in development was discovered through the characterization of *devil1-1 dominant (dvl1-1D)*, an activation-tagged line (Wen *et al.*, 2004). This dominant mutant shows pleiotropic phenotypes: shortened stature, rounder rosette

leaves, clustered inflorescences, shortened pedicels, and siliques with horned tips. There are more than 20 *DVL* genes in *Arabidopsis* and at least 24 in rice encoding small proteins of around 50 amino acids.

Overexpression of *ROTUNDIFOLIA4* (*ROT4/DVL16*) results in a phenotype similar to *dvl1-1D*; short, rounded leaves, short floral organs, and short inflorescence stems (Narita *et al.*, 2004). Shorter leaves are caused by a reduction in the number of cells in the longitudinal axis, but the size does not appear to be altered. This phenotype suggests that *ROT4* controls polarized cell proliferation. A more recent study revealed that *ROT4* overexpression reduced the meristematic zone size within the leaf blade (Ikeuchi *et al.*, 2011). In addition, *ROT4/DVL16* overexpression results in a protrusion of the main inflorescence stem at the base of pedicels. It was proposed that *ROT4* expression provides a positional cue that helps to establish organ boundaries by regulating cell proliferation along the longitudinal axis.

In *Medicago truncatula*, *MtDVL1* is induced during nodule formation and its overexpression results in reduced nodulation (Combier *et al.*, 2008). It is possible that this phenotype is also linked to the regulation of cell proliferation since cell-cycle reactivation in response to infection is a key step in the nodulation process. No loss-of-function phenotypes have yet been found in either knock-out mutants of *Arabidopsis* and rice or through silencing constructs, suggesting a high degree of functional redundancy (Narita *et al.*, 2004; Wen *et al.*, 2004).

Constitutive expression of 10 out of 11 *DVL* genes tested, including *DVL1* and *ROT4/DVL16*, resulted in similar phenotypes in rosettes, inflorescences, and siliques, pointing to a common mechanism of action (Wen *et al.*, 2004; Wen and Walker, 2006). The DVL proteins have a conserved region 32 amino acids long, located towards the C-terminus that seems to be sufficient to produce the phenotype (Ikeuchi *et al.*, 2011). This region contains several strictly conserved amino acids that are required for proper function (Wen *et al.*, 2004). DVL proteins do not have a signal peptide and overexpression of *DVL1* has no effect when the protein is directed towards the secretory pathway (Wen *et al.*, 2004). No evidence of post-translational processing or secretion has yet been found and ROT4–green fluorescent protein (GFP) fusions localize to the plasma membrane (Ikeuchi *et al.*, 2011). The mechanism of action of the DVL peptides is still unknown, but there is some information on downstream events. Overexpression of several *Arabidopsis DVL* genes results in downregulation of *FUL/AGL8*, a MADS-box gene involved in valve differentiation (Wen *et al.*, 2004). It has also been found that inducible overexpression of *DVL4* alters the expression levels of at least 41 genes, including many transcription factors (Larue *et al.*, 2010).

One of the phenotypes caused by *DVL/RFTL* overexpressing lines is the formation of a stalk-like protrusion on the base of trichomes (Wen and Walker, 2006; Ikeuchi *et al.*, 2011). Trichomes are unicellular hairs originating from epidermal cells that have provided a convenient model for the study of developmental regulation at a cellular level,

as well as cell-to-cell communication (Schellmann and Hulskamp, 2005). Trichome formation is a complex process that has been divided into six stages: (1) radial expansion, (2) emergence of the stalk, (3) initial branching, (4) elongation of the branches, (5) final expansion, and (6) development of papillae (Szymanski and Marks, 1998). The epidermal cells that surround the trichome are called socket cells and they have a characteristic morphology, different from the typical epidermal cells or pavement cells. Socket cells become clearly visible when the trichome cell initiates branching (Hulskamp and Schnittger, 1998; Szymanski and Marks, 1998). A complex pattern of interactions between developing epidermal cells determines which cells become committed to trichome development (Pesch and Hulskamp, 2009). In addition, it has been proposed that socket cells are recruited from the surrounding epidermal cells by a signal produced by the trichome (Larkin *et al.*, 1996).

The trichome phenotype of *DVL* overexpression provides a convenient system to explore the mechanism of action of these small proteins. One of the advantages of this approach is the large number of mutants affected in different stages of trichome development that have been characterized in detail (Marks, 1997; Hulskamp *et al.*, 1999; Larkin *et al.*, 2003). This work provides evidence of the role of DVL proteins and DVL1 in particular in trichome development, specifically in the recruitment and differentiation of socket cells through a mechanism of short-range communication.

Materials and methods

Plant materials and growth

Columbia ecotype (Col-0) of *Arabidopsis thaliana* was used as wild type and is the background ecotype for all mutants used in this study. *gl1*(CS225) and *gl3* (CS66) alleles were obtained from the *Arabidopsis* Biological Resources Center (Ohio State University, Columbus, OH). The *gl1* and *gl3* plants were crossed with *dvl1-1D* to make *dvl1-1D/gl1* and *dvl1-1D/gl3* double mutants and confirmed by segregation. Selected GAL4-GFP enhancer-trap lines were ordered from http://www.enhancertraps.bio.upenn.edu. Plants were grown at 22°C on a 16/8 light/dark cycle. To induce DVL4 overexpression in line *GVG DVL4*, plants in the rosette stage were sprayed with 30 μM dexamethasone in water.

Environmental scanning electron microscopy

To examine trichome development, leaves from plants at 4-leaf and 6-leaf stages were collected, fixed in 2.5% gluteraldehyde in 0.1 M cacodylate buffer and then rinsed four times in sterile water. The samples were viewed using a FEI Quanta FEG 600 Scanning Electron Microscope (FEI Company, Hillsboro, Oregon, USA) at Hv 10.0kv.

Analysis of GAL4-GFP enhancer-trap lines

Fresh leaf tissue was examined using a Leica DMI4000B inverted microscope with a GFP filter (Leica L5). Photographs were taken using a 1.4-megapixel, 12-bit, cooled QImaging CCD camera.

Expression of dvl1-1D by quantitative real-time PCR

The expression of *dvl1-1D* was assayed using leaf tissue from Col-0, *dvl1-1D*, *gl1*, and *dvl1-1D/gl1* plants. RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's

recommendations. To remove any contaminating DNA, samples were treated using Turbo DNA-free DNase treatment (Ambion). One microgram of total RNA was reverse transcribed using an oligo-dT primer and the Omniscript RT kit (Qiagen). For real-time PCR, Absolute QPCR SYBR green mix (ABgene) was used, and PCR was performed using DNA Engine Opticon 2 (MJ Research). The Col-0 vs. *gl1* comparisons were normalized by testing three reference genes (At2g28390, At4g26410, At4g34270) in both lines (Czechowski *et al.*, 2005). Transcript abundance for each reference gene and for *dvl1-1D* was normalized by the $2^{\Delta\Delta}$ Ct method and the reference gene stability was assessed by Genorm at an M value < 0.5 for the two most stable genes, At2g28390 and At4g26410 (Vandesompele *et al.*, 2002). A normalized expression level for each sample was derived by dividing the $2^{\Delta\Delta}$ Ct expression by the normalization factor generated by Genorm. The *dvl1-1D* vs. *gl1 dvl1-1D* comparisons were normalized by using the two most stable reference genes (At2g28390, At4g26410) from the first assay. Transcript abundance was calculated in the same manner as above. Reference gene stability was assessed by Genorm to be at M < 0.58. The primer sequences were: At2g28390, 5′-AAC TCT ATG CAG CAT TTG ATC CAC T-3′, 3′-TGA TTG CAT ATC TTT ATC GCC ATC-5′; At4g26410, 5′-GAG CTG AAG TGG CTT CCA TGA C-3′, 3′-GGT CCG ACA TAC CCA TGA TCC-5′; At4g34270, 5′-GTG AAA ACT GTT GGA GAG AAG CAA-3′, 3′-TCA ACT GGA TAC CCT TTC GCA-5′; DVL1, 5′-CAA TGC CTC TAA ATG TAT TA-3′, 3′-CCA AAC TAF CAC TAA TCT TTC C-5′.

Results and discussion

Overexpression of different DVL genes causes protuberances in the base of trichomes

The trichome phenotype of seven lines overexpressing different *DVL* genes was characterized. Line *dvl1-1D* was isolated in an activation tag screen (Wen *et al.*, 2004). Constitutive overexpression constructs for *DVL8*, *9*, *15*, *16*, and *20* have already been described (Wen and Walker, 2006). The final line *GVG DVL4* is a dexamethasone-inducible construct (Larue *et al.*, 2010). All of the overexpressing lines exhibit a protuberance at the base of the trichome (Fig. 1). The protuberance ranges from a bump (*dvl1-1D*) to a long stalk (*35S::DVL8*). It is filled by tightly packed cells morphologically similar to the palisade cells of the mesophyll and surrounded by elongated epidermal cells, several rows of them in the lines with a strong phenotype (Fig. 1C–E). In many cases, small and rounded socket cells are observed at the top of the protuberance (Fig. 1C–E).

The fact that the cells surrounding the trichome can respond to all these different proteins to produce a similar phenotype does not imply that all of them are involved in the normal development of trichomes and neighbouring cells. An alternative explanation is that they have specialized functions in different organs or cell types but work through a common mechanism and can substitute for each other. It is therefore possible that only one or a few of the *DVL* genes play a role in trichome development.

Protuberances form at late stages of trichome development

To understand how the protuberance at the base of the trichome is formed the adaxial surfaces of Col-0 and *dvl1-1D*

Col-0

dvl1-D

35S::DVL20
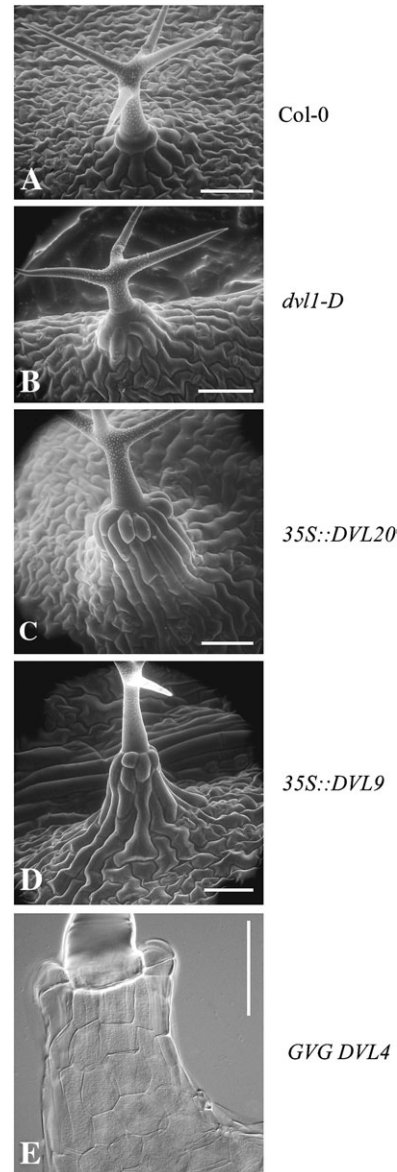
35S::DVL9

GVG DVL4

Fig. 1. Leaf trichomes from four lines overexpressing *DVL* genes. (A) Col-0; (B) *dvl1-1D*; (C) *35S::DVL20*; (D) *35S::DVL9*; (E) *GVG DVL4*. Bars = 100 µm.

leaves were examined using environmental scanning electron microscopy. For this analysis, the second pair of leaves was examined in plants at 4-leaf and 6-leaf stages. At the 4-leaf stage there were still trichomes in stage 1 (trichome initiation) and at the 6-leaf stage mature trichomes could be observed.

Examination of Col-0 at 4-leaf stage showed evenly spaced trichomes representing all six stages of trichome development (Fig. 2A). Inspection of *dvl1-1D* leaves of the same age suggests there are fewer trichomes and the trichomes that are present are at younger developmental stages compared with Col-0 (Fig. 2B). However, 6 days later, the *dvl1-1D* trichomes resemble those of Col-0. This is likely caused by delayed leaf development in *dvl1-1D*. No difference was observed in the number of trichomes per leaf in *dvl1-1D* compared with Col-0, suggesting that *DVL1* is not involved in trichome patterning (data not shown).

As trichomes reach maturity, there appear to be no major morphological differences between trichomes from Col-0 and *dvl1-1D* (Fig. 2C). It is after the *dvl1-1D* trichomes branch and their socket cells differentiate that surrounding epidermis cells begin to elongate and a protuberance at the base of the trichome arises, progressively becoming more pronounced (Fig. 1B). The same pattern of events was observed in the other overexpression lines.
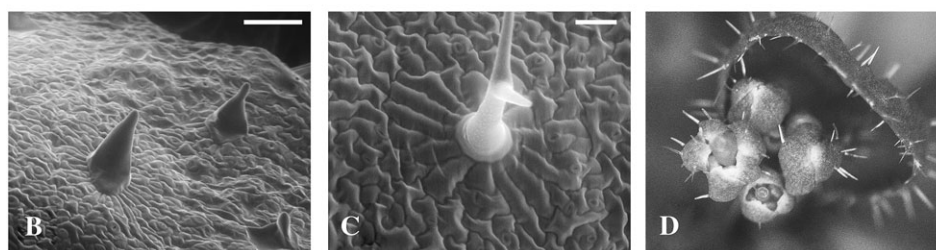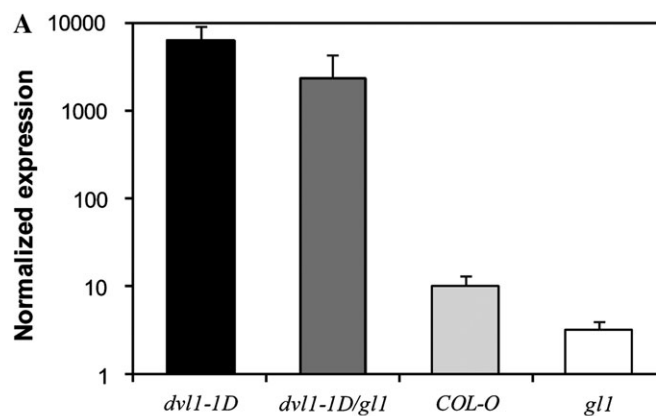
Genetic interactions

To determine the relationship between the pathway activated by *DVL1* overexpression and the course of trichome development, *dvl1-1D* was crossed with a series of mutants with alterations in different stages of this process. One of the selected mutants was *glabra1* (*gl1*), characterized by the absence of trichomes (Herman and Marks, 1989). Mutations in *GLABRA3* (*GL3*) have two effects on trichome
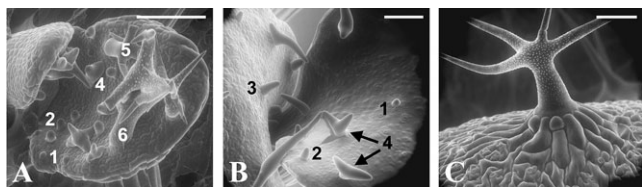
Fig. 2. Trichome development in Col-0, *dvl1-1D*, and *GVG DVL4*. (A) Col-0 at 4-leaf stage; (B) *dvl1-1D* at 4-leaf stage. Note the difference in stages of trichome development between Col-0 and *dvl1-1D*. (C) *dvl-1D* mature trichome before the protuberance forms. 1–6 = stages of trichome development. Bars=100 μm.

development (Koornneeff *et al.*, 1982). On early leaves, there is a decrease of trichome initiation. On later leaves, trichome initiation is more uniform, but the trichomes tend to be less branched and undergo fewer rounds of endoreduplication than Col-0. Other mutants that were crossed with *dvl1-1D* were: *stichel* (*sti*), with trichome development arrested before the first branching and with no socket cell formation (Ilgenfritz *et al.*, 2003); *zwichel* (*zwi*), with trichomes that branch only once (Oppenheimer *et al.*, 1997); and finally *kaktus* (*kak*), which produces large trichomes with up to five branches (Hulskamp *et al.*, 1994).

Leaves from *dvl1-1D/gl1* plants had no trichomes or socket cells, and no protuberances in their surfaces. On the other hand, the flower buds, individual flowers, and siliques retained the *dvl1-1D* phenotype (clustered inflorescences, reduced sepals, horned siliques). To determine if *DVL1* expression depends on the presence of trichomes, qPCR was performed in wild-type and *gl1* leaves (Fig. 3A). Expression of *DVL1* in *gl1* is 31% of the value in Col-0 and this difference is significant ($P = 0.020$). A reduction of similar magnitude in *DVL1* expression was observed in *dvl1-1D/gl1* compared with *dvl1-1D* ($P = 0.048$). It seems likely that the majority of *DVL1* expression in wild-type leaves takes place in trichomes or socket cells, or in response to a signal produced by them. In the case of *dvl1-1D*, the activity of the 35S promoter appears to be modulated by the same mechanisms that regulate expression in Col-0. *DVL1* promoter–reporter constructs have been generated in the study laboratory, but reporter expression was undetectable, possibly due to low levels of native gene expression (unpublished data).

Fig. 3. Genetic interactions. (A) Analysis of *gl1* and *dvl1-1D* single and double mutant lines by real-time PCR. Average transcript abundance of *DVL1* in Col-0, *dvl1-1D*, *gl1*, and *dvl1-1D/gl1*. Error bars represent standard deviation. (B–D) *DVL1* overexpression in trichome mutant background: (B) trichome from *dvl1-1D/gl3*; (C) trichome from *dvl1-1D/gl1*; (D) flower bud and cauline leaf from *dvl1-1D/sti*. Bars = 50 μm (A and B).

As for *dvl1-1D/gl3*, on both the second and fourth leaves, trichome emergence and growth resembled that of the *gl3* single mutant (Fig. 3B). Socket cells were identified at the base of the unbranched trichomes on the fourth leaves of both *gl3* and *dvl1-1D/gl3*. However, the protuberance was not present (Fig. 3C). In the *dvl1-1D/sti* and *dvl1-1D/zwi* double mutant lines, the leaf phenotype resembled that of *sti* and *zwi*, respectively, with no socket cells or protrusion at the base of the trichomes (Fig. 3D). As with *dvl1-1D/gl1*, other organs retained the *dvl1-1D* phenotype in the double mutants. Finally in *dvl1-1D/kak* double mutants, trichomes were similar to those of *kak* plants, but with a protuberance at the base as in *dvl1-1D*.

The lack of protuberance in *dvl1-1D/gl3*, *dvl1-1D/sti*, and *dvl1-1D/zwi* could be caused by a lower expression of *DVL1* due to modulation of the expression level by the native promoter. However, when *gl3* and *zwi* were crossed with *GVG DVL4*, induction of *DVL4* expression with dexamethasone also failed to induce the formation of protuberance (data not shown). All these results suggest that *DVL1* overexpression on its own is not sufficient to cause the formation of protuberances at the base of trichomes. It appears that an additional signal is required. This signal could be produced in the trichomes or socket cells at a late stage in maturation. The *gl3*, *sti*, and *zwi* mutations would block the production of this signal by arresting trichome development at an earlier stage.

Development of the socket cells in dvl1-1D

Socket cells in *dvl1-1D* appear smaller and there are a larger number of them around each trichome than in Col-0 (Fig. 4A). To analyse socket cell development in detail, *dvl1-1D* was crossed with E254, an enhancer-trap line with *GFP* expression limited to trichomes and socket cells (Fig. 4). At stage 1, as the trichome cell starts to expand, the E254 line shows fluorescence in the trichome and the surrounding epidermal cells which will later become socket cells (Fig. 4B). In *dvl1-1D/E254*, the number of fluorescent cells is increased (Fig. 4E). This is also evident at later stages, with the fluorescence extending several cells away from the trichome (Fig. 4F). However, when the bulge forms, the fluorescence is limited to the socket cells in contact with the trichome, disappearing from the elongated epidermal cells that surround them (Fig. 4G).

Thus it seems that *DVL1* overexpression is enough to activate some socket cell-specific promoters in epidermal cells that would not normally differentiate into socket cells. Some of the cells that respond to *DVL1* overexpression might become additional socket cells, explaining the larger number of these observed in mature *dvl1-1D* trichomes. The cells that form the sides of the protuberance could also be the result of the activation of part of the socket cell developmental pathway. These cells elongate in a radial orientation with respect to the trichome, as do mature socket cells in Col-0 (Fig. 1). They also lack the pronounced lateral bulges of typical pavement cells. However, *GFP* expression in *dvl1-1D/E254* disappears from these cells, suggesting that they do not
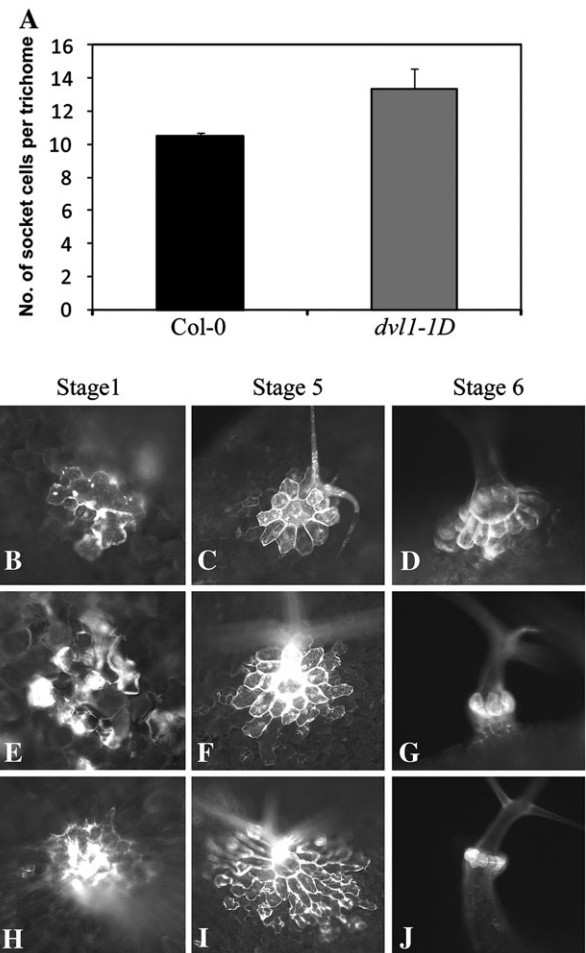
Fig. 4. Socket cell development in *DVL* overexpressors. (A) Number of socket cells in Col-0 and *dvl1-1D*. Error bars represent standard deviation. (B–J) Analysis of socket cell development using crosses to line E254, a GAL4-GFP enhancer-trap with expression limited to trichome and socket cells. GFP expression was followed at different stages of trichome development in line E254 (B–D), *dvl1-1D/E254* (E–G), and *GVG DVL4/E254* (H–J).

maintain the same developmental programme as normal socket cells. It is likely that other factors, in addition to the pathway activated by *DVL1*, are necessary for full socket cell differentiation, possibly involving direct contact with the trichome.

DVL overexpression in trichomes and socket cells is sufficient to induce a protuberance

This study took advantage of a previously characterized *GVG DVL4* transgenic line (Larue *et al.*, 2010) to obtain cell-specific *DVL* overexpression, by crossing it with GAL4-GFP enhancer-trap lines. These lines express a GAL4-VP16 transcriptional activator in a cell-specific manner. This activator can bind the GAL4 UAS promoter in the *GVG DVL4* construct, driving expression of *DVL4* independent of dexamethasone application. Several enhancer-trap lines were crossed with *GVG DVL4* to study the results of localized overexpression and the progeny was examined during various

stages of trichome development. In addition to line E254 that has already been described, the three other lines selected were E2763 (expression in trichome, socket cells, and scattered epidermis), E1388 (expression in trichome, socket cells, and epidermis) and E4150 (expression in trichome).

The *GVG DVL4*/E254 line had a *GFP* expression pattern similar to *dvl1-1D*/E254. In early stages of trichome development, the fluorescence extended a few cells from the trichome, but no GFP was detectable at later stages in the cells that form the sides of the protrusion (Fig. 4H–J). This result shows that *DVL4* and *DVL1* can activate similar pathways. Furthermore, the extension of *GFP* expression to additional cells in *GVG DVL4*/E254 compared with E254 can only be explained if *DVL4* either moves or creates a moving signal that is capable of reaching neighbouring cells activating the enhancer trap.

DVL4 expression in *GVG DVL4*/E254 line was enough to induce the formation of protrusions that are very similar to those induced in *GVG DVL4* by the application of dexamethasone (Fig. 4J). During the formation of the stalk the main location of GFP and therefore *DVL4* expression is in the true socket cells, with weaker expression in the trichomes. Protuberances are also formed in *GVG DVL4*/E2763 and *GVG DVL4*/E1388 which show a similar expression pattern (Fig. 5A, B). On the other hand, protuberances do not appear in line *GVG DVL4*/E4150 (Fig. 5C, D) where GFP and *DVL4* expression are limited to trichome cells. Fluorescence in this line starts after trichome branching, but before elongation is complete. It is possible that overexpression of *DVL* genes in the socket cells is required for the phenotype to appear or that the surrounding cells can only to respond to the signal generated by *DVL* overexpression at early stages of differentiation.

Concluding remarks

DVL genes code for small proteins that seem to play a role in many different developmental processes, but whose
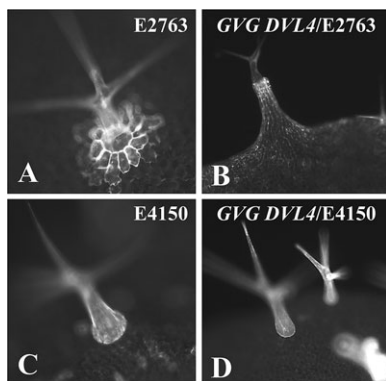
mechanism of action is currently unknown. Our results indicate that *DVL1* expression in leaves depends in large part on the formation of trichomes. Overexpression of *DVL1* increases the number of socket cells around trichomes and extends the field of expression of a socket cell-specific promoter. A similarly expanded expression of socket cell markers was observed in lines where *DVL4* is specifically overexpressed in trichomes and socket cells. This suggests that *DVL* genes can generate a short-range mobile signal that activates promoter elements characteristic of socket cells in developing epidermal cells where these promoters are not normally active. Additional signals, possibly originating in the trichome, seem necessary to maintain socket cell identity as shown by the reduced marker expression in later stages of development.

On the basis of these results, it is proposed that *DVL1* is likely to be expressed in wild-type trichomes or socket cells where it plays a role in coordinating the recruitment and development of the ring of socket cells through cell-cell communication. Overexpression of *DVL1* or other *DVL* genes capable of stimulating the same signalling pathway would increase the level of this signal and result in parts of the socket cell programme turning on neighbouring epidermal cells. Recent studies have found some differences between the transcriptome of socket cells and pavement cells (Lieckfeldt *et al.*, 2008; Schliep *et al.*, 2010). It would be interesting to study what role *DVL1* has in creating or maintaining these differences.

The formation of protuberances can be explained in this hypothesis as the result of elongation towards the trichome of epidermal cells in which *DVL* overexpression activates parts of the socket cell programme. In addition, the protuberances appear to involve the proliferation of mesophyll cells (Fig. 1E). This could be another response to short-range signals produced by the trichome or socket cells or it could be a reaction to mechanical stresses created by the epidermis. In any case, the formation of protuberances seems to require additional signals from mature trichomes, which are not produced in *gl3*, *sti* or *zwi* plants.

There is clearly a complex network of interactions among developing trichomes and neighbouring cells and these results strongly suggest that *DVL1* could play an important role in this process. A role for *DVL* genes in the local coordination of development could also lie beneath other phenotypes, such as the shorter leaves of *ROT4* overexpression lines (Ikeuchi *et al.*, 2011). In these plants, the front of cell cycle arrest is displaced towards the base of the leaf, with a consequent reduction in the size of the meristematic zone. This result could be explained by the involvement of *DVL* genes in the coordination of the transition from division to differentiation along the width of the leaf, which would be necessary in order to maintain its shape. The alterations caused by *DVL* overexpression in silique shape could also result from lack of developmental coordination among the different cell tissues within this organ. Further investigation of this hypothesis, possibly with the help of localized and/or inducible overexpression systems, could help to clarify the mechanism of action of *DVL* genes.

Fig. 5. Localized overexpression of *DVL4*. (A) E254, *GFP* expression in line E763, limited to trichomes, socket cell and scattered epidermis. (B) Protuberances in line *DVL4 GVG*/E2763. (C) GFP expression in line E4150, limited to trichomes. (D) Absence of protuberances in line *DVL4 GVG*/E4150.

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