RESEARCH PAPER

The SERK1 gene is expressed in procambium and immature vascular cells

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Abstract

The SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 (SERK1) gene is expressed in the procambium of the vascular bundles in roots, hypocotyls, and inflorescence stems. In younger parts of roots and hypocotyls, SERK1 expression was less restricted and was also observed in protoxylem cells, immature metaxylem cells and phloem companion cells. In roots, SERK1 expression was first detected in root vascular stem cells and was notably absent from the QC. In general, the SERK1 protein level as visualized by expression of a SERK1-YFP fusion protein closely followed the pattern of gene expression. In hypocotyls, prolonged application of 2,4-D resulted in extensive unorganized proliferation of SERK1 expressing cells originating from the procambium and pericycle. In roots, 2,4-D treatment results in an increase in SERK1 transcription that results in a moderate increase in the amount of SERK1-YFP fusion protein. The restricted vascular pattern of SERK1 expression in roots remains unaffected after 2,4-D treatment.

Key words: 2,4-D, procambium, SERK1, somatic embryogenesis, stem cells, transit amplifying (TA) cells, vascular tissue.

Introduction

In plants, somatic embryos can develop from a single competent cell (Nomura and Komamine, 1985; Toonen et al., 1994), but the origin of these competent cells remains uncertain. In general, competent cells arise from explants cultured in media supplemented with strong synthetic auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D) (Mordhorst et al., 1997, 1998). 2,4-D may have several roles in this process, acting as an auxin directly or modifying intracellular IAA metabolism and/or as a ‘stressor’ (Feher et al., 2002, 2003). Other plant hormones, such as cytokinin and abscisic acid and several non-hormonal, stress-inducing factors can also induce the formation of cells finally capable of somatic embryogenesis (reviewed by Feher et al., 2003). Once somatic embryos have been formed, they employ the same developmental mechanisms as zygotic embryos (Mordhorst et al., 1997, 2002).

Two groups of genes involved in the acquisition of embryogenic competence in Arabidopsis have been identified. Negative regulators show an increased embryogenic competence upon mutation. Examples are primordia timing (pt), clavata (clv) 1 and 3 (Mordhorst et al., 1998), and pickle (pkl) (Ogas et al., 1997, 1999; Dean Rider et al., 2003). Positive regulators show an increased embryogenic competence after ectopic expression. Examples are BABY BOOM (BBM) (Boutilier et al., 2002), WUSCHEL (WUS) (Zuo et al., 2002), and LEAFY COTYLEDON 1 and 2 (LEC1 and 2) (Lotan et al., 1998; Stone et al., 2001; Gaj et al., 2005). These positive regulators show the formation of somatic embryos or embryo-like structures originating from different organ and cell types without the application of external hormones.

The acquisition of embryogenic competence is marked by an increase in expression of the LRR receptor-like kinase SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 (SERK1) gene in Arabidopsis (Hecht et al., 2001), and its orthologues in carrot (Schmidt et al., 1997), Daucus (Somleva et al., 2003), sunflower (Thomas et al., 2004), and rice (Hu et al., 2005). When ectopically expressed, SERK1 increases embryogenic competence in culture (Hecht et al., 2001). Ectopic expression of the MADS-box transcription factor AGAMOUS-LIKE15 (AGL15) also results in increased embryogenic...
competence in tissue culture. AGL15 is expressed in early stage zygotic embryos and is relocalized from the cytoplasm to the nucleus at the onset of embryonic development (Perry et al., 1996, 1999). Ectopic expression of AGL15 resulted in an increase in SERK1 expression (Harding et al., 2003). Recently, AGL15 and SERK1 were shown to be present together in complexes that include components of the brassinosteroid signalling pathway such as BRASSINOSTEROID-INSENSITIVE 1 (BRI1) and its co-receptor BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1)/SERK3 (Karlova et al., 2006). These results suggest that SERK1 may be involved in brassinolide signalling as well as in the acquisition of embryonic competence. In plants, SERK1 and its closest homologue SERK2 together regulate specification of the tapetal cell layer in anthers (Albrecht et al., 2005; Colcombet et al., 2005). The SERK1 protein is found during megasporogenesis, in developing ovules from stage I–II in ovule development onwards and in all cells of the embryo sac (Albrecht et al., 2005; Colcombet et al., 2005; Kwaaitaal et al., 2005). In seedlings, the SERK1 protein is found in the vascular bundles of roots, stems, leaves, and lateral roots (Hecht et al., 2001; Kwaaitaal et al., 2005). Collectively, these observations suggest that SERK1-mediated signalling is part of multiple developmental processes, most likely involving brassinosteroids and certainly including embryogenic cell formation.

In Medicago, both auxin and cytokinin are needed to obtain competent cells. The observed increase in MiSERK1 expression in response to auxin and cytokinin was, however, not restricted to cells that acquired embryogenic competence, suggesting a more general effect (Nolan et al., 2003). In Arabidopsis SERK1 expression increases in response to auxin at the adaxial side of cotyledons, the shoot apical meristem, and the vascular tissue of seedlings (Hecht et al., 2001). In Daucus carota explants, the vascular tissue is the origin of competent cells. After auxin treatment, cell proliferation is initiated in provascular cells (Guzzo et al., 1994). Pro cambial or provascular cells are vascular stem cells that originate from the apical meristem and generate xylem and phloem precursor cells that, in turn, differentiate into xylem and phloem, the two basic elements of the plant vascular tissue (Fukuda, 2004). In Dactylis glomerata L., cells giving rise to somatic embryos originate close to the vascular bundles of leaf explants and a subset of these cells express SERK1 (Somleva et al., 2003).

This paper focuses on Arabidopsis SERK1 expression as observed in vascular tissue of the primary root, stem, and hypocotyl. The results show that SERK1 is expressed in cells of the immature xylem and the procambium. Later in vascular tissue development SERK1 becomes restricted to procambial cells in root, stem, and hypocotyl. As embryogenic competence in Arabidopsis explants is usually initiated by exposure to 2,4-D, the effect of 2,4-D on SERK1 transcription and protein localization in the vascular bundles of hypocotyls and roots was also investigated. The results show that prolonged 2,4-D exposure results in proliferation of mainly procambium cells, and to a lesser extent, pericycle cells that already expressed SERK1 and continue to express SERK1, which suggested that competent cells in Arabidopsis tissue culture are mainly derived from SERK1-expressing procambial cells in planta.

Materials and methods

Plant lines and growth conditions

Seeds were surface-sterilized and transferred to half-strength MS medium (Murashige and Skoog, 1962; Duchefa Biochemie, Haarlem, the Netherlands) agar plates containing 1% (w/v) sucrose, pH 5.8. After 2 d at 4°C, the plates were transferred to a growth chamber under fluorescent light at 22°C with a 16/8 h light/dark cycle. After 1 week, seedlings were transferred to soil and grown to maturity under the same conditions. The SERK1 promoter–GUS (pSERK1::GUS) line was previously described by Hecht et al. (2001), the SERK1 promoter–SERK1–YFP, (pSERK1::SERK1:: YFP) line by Kwaaitaal et al. (2005), the DR5 auxin responsive element–GFP (DR5–GFP) line by Benkova et al. (2003), and the HB8 promoter–GUS (pHB8::GUS) line by Baima et al. (1995).

GUS staining

The samples were immersed in a 1 mg ml⁻¹ X-Gluc (5-bromo-4-chloro-3-indolyl-β-d-glucuronic acid; Duchefa Biochemie, Haarlem, the Netherlands), 0.1% (v/v) Triton X100, 10 mM EDTA, 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide in 100 mM Na-phosphate, pH 7.2, and placed for 30 min under vacuum. Seedlings were incubated overnight at 37°C. Stem sections just above the first cauline leaf, below the second cauline leaf, and from the base of the stem of 4-week-old plants were cut to a suitable size before immersion and incubated at 37°C for 2 d. Seedlings were mounted in chloral hydrate:glycerol:water (8:1:2 by vol.) and imaged with Nikon Optiphot-2 microscope with Normarski optics (Tokyo, Japan). Photographs were taken with a Nikon Coolpix 990 digital camera. Alternatively, seedlings were embedded and sectioned as described below.

Plastic embedding and sectioning

For root and hypocotyl sectioning, seedlings were first imbedded in 1% (w/v) agarose in 100 mM Na-phosphate solution pH 7.2 and cut to the right size with a razor blade. Samples were vacuum-infiltrated for 15 min in 5% (w/v) glutaraldehyde in 100 mM phosphate pH 7.2 and fixed overnight at 4°C. The material was washed twice with 100 mM phosphate buffer pH 7.2 and once with water for 15 min at room temperature. The samples were dehydrated through an ethanol range (10/30/50/70/96/100%, v/v). Next, the samples were infiltrated with Technovit 7100 (Hereaus Kulzer, Wehrheim, Germany) in the following range: 3:1, 1:1, and 1:3 ethanol: Technovit solutions were prepared and the plastic polymerization was done according to manufacturer’s protocol. With a microtome (Biocut, Reickert and Jung, Leica, Rijswijk, the Netherlands) 7 µm sections were cut and dried on slides. Sections were stained in 0.05% (w/v) Ruthenium Red in water for 10 min, dried, and imaged using a Zeiss Axioplan 2 microscope (Jena, Germany).
Agar embedding and sectioning
Plant tissue was embedded in 1.5% (w/v) agarose in 50 mM Na-phosphate at pH 7.2 and 2–3 mm sections were cut manually with a razor blade, placed in a drop of 50 mM Na-phosphate at pH 7.2 on a cover slip and imaged directly as described below.

Treatment of seedlings with 2,4-dichlorophenoxyacetic acid (2,4-D) or 1-N-naphthylphthalamic acid (NPA)
Seedlings were germinated and grown on a plate according to the conditions described above, but the plates were oriented vertically. Four days after germination (DAG) the seedlings were transferred to plates containing 0.1 μM or 5 μM 2,4-D (Sigma) or 10 μM or 50 μM NPA (Duchefa Biochemie, Haarlem, the Netherlands). Seedlings expressing the described reporter constructs were followed and harvested at different time points for GUS staining, fluorescence microscopy or sectioning, and subsequent microscopy.

Fluorescence microscopy
Seedlings or plants expressing fluorescent reporter constructs were imaged with a confocal laser scanning microscope (Zeiss Confocor 2-LSM 510 combination setup, Jena, Germany). A ×40 Plan-Neofluar oil immersion objective with a numerical aperture of 1.3 was used. GFP was excited with a 458 nm argon laser line controlled by an acousto-optical-tunable filter (AOTF). The excitation and emission light was separated by a dichroic beam splitter (HFT 458/514). The CFP fluorescence was filtered through a 475–525 nm band pass filter. GFP was excited with the 488 nm argon laser line controlled by the AOTF, and excitation and emission light were separated through a HTF488 beam splitter. The GFP fluorescence was filtered through a 505–550 nm band pass filter. YFP was excited with the 514 nm argon laser line controlled by the AOTF, and excitation and emission light were separated through a HTF514 beam splitter. The YFP fluorescence was filtered through a 530–600 nm band pass filter. Chlorophyll and red autofluorescence were detected using a NFT635 filter combined with a 650 nm long pass filter. For comparison of treatments, microscope settings were kept the same for all samples.

Results
SERK1 is expressed in the procambial cells of the vascular bundle in all organs studied
SERK1 is expressed in the reproductive tissues and the vascular bundles of seedlings (Albrecht et al., 2005; Kwaaitaal et al., 2005). Because the provascular tissue is an origin of embryogenic competent cells (Guzzo et al., 1994; Schmidt et al., 1997; Somleva et al., 2000; Raghavan, 2004) SERK1 expression as observed in the vascular bundle was investigated in more detail. This detailed investigation will make it the possible to determine the relationship between SERK1 as a marker for single competent cells in culture and its vascular localization. Arabidopsis plants expressing either a SERK1 promoter–GUS fusion (pSERK1::GUS) (Hecht et al., 2001) construct or a SERK1 promoter SERK1 cDNA–Yellow Fluorescent Protein cDNA (YFP) (pSERK1::SERK1::YFP) construct (Kwaaitaal et al., 2005) were used to investigate the vascular localization of SERK1 in seedlings and inflorescence stems. Figure 1A shows a longitudinal view of the primary root of an Arabidopsis seedling expressing pSERK1::GUS. GUS staining was observed in the vascular bundle, commencing directly proximal to the quiescent centre (QC) in cells referred to as stem cells (Xu et al., 2006) or vascular initials (Kwaaitaal et al., 2005). Figure 1B to E show transverse sections through the root tip of a pSERK1::GUS-expressing plant at the positions marked in Fig. 1A. Figure 1B shows a section at 250 μm from the tip. To help assign the SERK1-expressing cells in Fig. 1B, the different cell types in the same section as shown in this figure are assigned according to Mahonen et al. (2000) and colour-coded (Fig. 1C). GUS staining was observed in the procambium, protoxylem, metaxylem, and phloem companion cells and was also discernible in metaxylem cells. About 60 μm from the quiescent centre (Fig. 1D), most cells of the immature vascular bundle show GUS staining. GUS staining seemed higher in the metaxylem and was not observed of the protophloem initial cells. Immediately proximal to the QC, one of the vascular stem cells showed GUS staining (Fig. 1E). The SERK1 protein was first visible in the vascular stem cells. YFP fluorescence was higher in the first vascular descendants of the stem cells and became reduced in more mature parts of the vascular bundle (Fig. 1F). In cross-sections at ~250 μm from the QC in the pSERK1::SERK1::YFP line, low YFP fluorescence was detected in the complete vascular bundle except for the protophloem (Fig. 1G). Some yellow autofluorescence was also observed in the metaxylem in cross-sections through wild-type roots, suggesting that most of the YFP signal in the root metaxylem of the pSERK1::SERK1::YFP line is caused by autofluorescence (data not shown). In cross-sections through the immature vascular bundle of the pSERK1::SERK1::YFP line, ~60 μm proximal to the QC, the YFP fluorescence increased and was found in membranes of the metaxylem, protoxylem, and procambium (Fig. 1H). The YFP fluorescence seemed higher in the metaxylem and appeared absent from the protophloem initial cells.

To summarize, SERK1 expression in roots is initially seen in stem cells of the root vasculature and most cells of the immature vascular bundle. Later SERK1 expression becomes restricted to the procambium, protoxylem, and phloem companion cells. The SERK1 protein localization pattern closely followed the gene expression pattern.

Next, SERK1 expression in the hypocotyl vasculature was investigated. Figure 2A shows GUS staining in a cross-section about 70 μm below the apical meristem of a hypocotyl from a 4-d-old seedling containing the pSERK1::GUS construct. Cell and tissue types were assigned according to Busse and Evert (1999a, b). SERK1 expression was confined to the procambium cells (Fig. 2A). In a younger part of the hypocotyl at 50 μm from the apical meristem (Fig. 2B) SERK1 procambium expression increased, was higher and remained visible in the adjacent
vascular cells of the metaxylem and phloem and the pericycle. A hand-cut section through the hypocotyl of seedlings containing the pSERK1::SERK1::YFP construct showed a pattern similar to the GUS staining (Fig. 2D); YFP fluorescence was observed in the procambium cells. Figure 2E shows the yellow autofluorescence in a comparable section of a wild-type hypocotyl. Autofluorescence was visible in the metaxylem and in a few procambial cells. The YFP signal in the wild-type sections suggests that the remaining signal in the metaxylem seen in Fig. 2D is caused by autofluorescence. In the hypocotyl SERK1 expression appears to be more restricted to the procambium and also here the protein localization follows the gene expression pattern closely. The HD-ZIPIII transcription factor HB8 has been reported as a regulator of procambium proliferation as well as a marker for procambium development (Baima et al., 1995, 2001). Figure 2C shows that HB8 expression is indistinguishable from that of SERK1 and also marks the procambium and metaxylem cells.

Subsequently, different positions of the inflorescence stem, representing a range of developmental stages of the...
inflorescence stem vascular tissue were studied. Sections were made of GUS-stained and plastic-embedded inflorescence stems of 4-week-old Arabidopsis plants containing the pSERK1::GUS fusion. Figure 3A shows a section through the vascular bundle at the base of the stem. The protoxylem, the metaxylem; procambium, and phloem tissues can be distinguished. Procambium cells are seen here as an arch of two to three cell layers between the xylem and phloem. Individual cells in the procambium seem to have an increased SERK1 expression based on the GUS staining intensity. A section through the stem just below the second cauline leaf showed a similar pattern (Fig. 3B). Also here a limited amount of cells within the procambial layer showed GUS staining. Sections just above the first cauline leaf showed GUS staining in all procambial cells and in the immature metaxylem cells (Fig. 3C).

Figure 3D shows a GUS-stained section of the HB8 promoter GUS marker line at the base of the inflorescence stem. HB8 is transcribed in the protoxylem and procambium (Fig. 3D). At position 1 in the inflorescence stem (Fig. 3E), HB8 was expressed in immature metaxylem, procambium, and single phloem cap cells. Figure 3F–H show hand-cut sections through the stems of 4-week-old Arabidopsis plants containing the pSERK1::SERK1::YFP construct. The red fluorescence in all images originates from chlorophyll. Figure 3F shows a section through a vascular bundle at the base of the stem. Similar to the GUS expression data (Fig. 3A), only a subset of procambium cells shows YFP fluorescence. Figure 3G shows a section just below the second cauline leaf. The brightest YFP fluorescence is seen in only a subset of the procambium cells. Yellow fluorescence was also observed in some phloem and phloem companion cells. Figure 3H shows a section just above the first cauline leaf of the pSERK1::::SERK1::YFP line where yellow fluorescence is strongest in all procambial cells and immature metaxylem cells (imx), but also visible in cells in the interfascicular region. Autofluorescence in wild-type plants was restricted mainly to mature xylem elements (data not shown). In inflorescence stems, the SERK1 protein localization pattern closely follows the gene expression pattern. To summarize, SERK1 appears to be expressed in procambium cells in the vasculature in all organs investigated. In roots, SERK1 expression commences in all stem cells of the vascular tissue and continues in the first descendants of these stem cells. In roots, hypocotyls, and inflorescence stems, metaxylem cells in the immature vascular bundle also express SERK1.

Fig. 3. SERK1 expression in the inflorescence stem. (A–C) GUS and Ruthenium red-stained 7 μm cross-sections through the inflorescence stem of a 4-week-old pSERK1::GUS-expressing plant. (A) Base of the stem. (B) Just below the second cauline leaf. (C) Just above the first cauline leaf. Blue arrows mark cells with GUS staining. (D, E) GUS and Ruthenium red-stained 7 μm cross-sections through the inflorescence stem of a 4-week-old pH8::GUS-expressing plant (Baima et al., 1995). (D) At position 4. The arrow marks GUS-stained protoxylem. The arrowhead marks GUS-stained procambium. (E) At position 1. The arrowhead marks a single phloem cap cell with GUS staining. The arrow marks procambium and immature metaxylem with GUS staining. (F–H) YFP fluorescence in hand-cut sections of stems from a pSERK1::SERK1::YFP-expressing plant. (F) Base of the stem. (G) Just below the second cauline leaf. (H) Just above the first cauline leaf. Yellow arrows mark cells with YFP fluorescence. px, Protoxylem; mx, metaxylem; imx, immature metaxylem; pc, procambium; ph, phloem. Scale bar = 50 μm.
Prolonged exposure to 2,4-D induces the proliferation of SERK1 expressing cells

Given the findings that SERK1 marks and promotes the formation of cells competent to initiate somatic embryogenesis and the presence of SERK1 in the procambium, the response of the SERK1 gene under conditions that allow formation of embryogenic cells was investigated. Embryogenic cell formation in Arabidopsis explant tissue requires a prolonged incubation with 2,4-dichlorophenoxyacetic acid (2,4-D) (Mordhorst et al., 1997).

pSERK1::GUS-expressing seedlings were transferred 4 DAG to plates containing 5 μM 2,4-D and were embedded and sectioned 2, 7, and 10 d after transfer. Representative sections are shown taken about 70 μm below the apical meristem. Cell and tissue types were assigned according to Busse and Evert (1999a, b). Figure 4A shows a section of a non-treated hypocotyl after 2 d. Procambium cells flanking the xylem seem to have more GUS staining. After 2 d of 2,4-D treatment there is a clear increase in the number of pericycle cells (compare bracket in Fig. 4A with bracket in Fig. 4D) that also begin to express SERK1 (Fig. 4D). A slight increase in GUS staining was observed in the endodermal cell layer. Figure 4B shows a non-treated hypocotyl after 7 d. The vascular bundle is enlarged, due to an increase in the number of cells in all vascular tissues (e.g. xylem, phloem, pericycle, and procambium). The procambium cells flanking the mature xylem seem to have more GUS staining than the adjacent cells. At 7 d of 2,4-D treatment (Fig. 4E), the first procambium cells begin to proliferate, while the number of pericycle cells outside the phloem poles increased (compare bracket in Fig. 4B with bracket in Fig. 4E). The GUS staining is more even throughout the vascular bundle. The endodermis is flattened due to the enlargement of the vascular bundle. No expression of SERK1 in the endodermis or tissue layers other than the vascular bundle was observed after 7 d of incubation with 2,4-D. Figure 4C shows a non-treated hypocotyl after 10 d. The SERK1 expression pattern was similar to that shown in Fig. 4B; procambium cells flanking the mature xylem seemed to have more GUS staining compared with the surrounding tissues. After 10 d of 2,4-D treatment (Fig. 4F), a disorganized mass of cells is visible in the centre of the vascular bundle expressing SERK1. This mass of cells appears to have originated from the procambium cells rather than from the pericycle, because cells similar to the enlarged pericycle cells (bracket in Fig. 4F) observed after 2 d (bracket in Fig. 4D) and 7 d (bracket in Fig. 4E) of treatment with 2,4-D were still present and surrounding the mass of cells in the centre. The vascular bundle is completely disorganized due to the massive proliferation of cells from a procambial origin. Even after this prolonged incubation, cortical and epidermal cells do not express SERK1 (Fig. 4F).

In roots, 2,4-D induces SERK1 expression only in procambium cells

The response of SERK1 to 2,4-D in roots was investigated next. In roots, the different vascular cell types can more easily be recognized, so observed changes in SERK1...
expression in response to 2,4-D can be better assigned to specific cell types. Figure 5A shows GFP fluorescence in the root tip of the DR5 element–GFP marker line (Benkova et al., 2003). An auxin maximum, visualized by high GFP fluorescence, is present in the QC, the columella initial cells, and the mature columella cells. A 2 d incubation of the same line with 0.1 μM 2,4-D strongly increased the GFP fluorescence in all root tissues (Fig. 5B). Higher concentrations (0.5 μM and 2 μM) of 2,4-D gave a similar response (data not shown). These results confirm that the application of 2,4-D results in an elevated auxin response in all root tissues. Figure 5C shows GUS staining in a non-treated root containing the pSERK1::GUS construct and the root shown in Fig. 5D was treated for 2 d with 0.1 μM 2,4-D. Higher concentrations of 2,4-D gave similar results (data not shown). SERK1 expression gradually increased, but remained localized to cells in the vascular bundle. Figure 5E shows a control root of a line containing a pSERK1::SERK1::YFP construct. Figure 5I shows SERK1–YFP fluorescence after 2 d of incubation with 0.1 μM 2,4-D. SERK1–YFP fluorescence increased slightly and the region with increased SERK1–YFP fluorescence was longer and wider compared to non-treated roots. In addition, the incubation of roots with 2,4-D resulted in an increase in autofluorescence in the epidermis (Fig. 5F) Higher concentrations of 2,4-D showed similar results (data not shown). In response to the 2,4-D treatment, SERK1 transcription was clearly increased, but only resulted in a slight increase of SERK1–YFP protein. No induction of SERK1 was seen in cells outside the vascular tissue. To determine which cells actually showed a change in SERK–YFP protein, cross-sections were made about 60 μm from the QC. Figure 5G shows a section through a non-treated seedling. Figure 5H shows a section of a seedling treated with 0.1 μM 2,4-D for 2 d. YFP fluorescence is elevated in all procambium cells of the 2,4-D-treated seedlings, while the amount of cells present at this stage was similar to non-treated seedlings. Similar experiments in the hypocotyl did not show an increase in SERK1–YFP protein. Taken together these observations suggest that the observed limited increase in SERK1–YFP protein after 2,4-D treatment is due to an increase in transcription of the SERK1 gene in procambium cells and not due to the increased proliferation of cells expressing SERK1. A comparable result was obtained after treatment of roots containing the pSERK1::GUS and pSERK1::SERK::YFP constructs with NPA (data not shown).

Discussion

Earlier observations suggested that the Arabidopsis SERK1 gene is expressed in sporophytic tissues, during lateral root outgrowth and in the vascular bundles (Hecht et al., 2001; Kwaaitaal et al., 2005). Here it is shown that the SERK1 gene is expressed in the procambium of the vascular bundles in roots, hypocotyls, and inflorescence stems. In younger parts of roots and hypocotyls, SERK1 expression was less restricted and was also observed in protoxylem cells, immature metaxylem cells, and phloem companion cells. In roots, SERK1 expression was first detected in root vascular stem cells and was notably

![Fig. 5. SERK1 gene expression and protein level in roots after 2,4-D treatment. (A, B) GFP fluorescence in primary root tips of DR5-element::GFP-expressing seedlings. (A) Non-treated, (B) 2 d of treatment with 0.1 μM 2,4-D. (C) Primary root tip of a pSERK1::GUS-expressing and GUS-stained seedling. (D) Primary root tip of a pSERK1::GUS-expressing seedling treated with 0.1 μM 2,4-D for 2 d and GUS stained. (E) YFP fluorescence in primary root tips of a pSERK1::SERK1::YFP-expressing seedling. (F) YFP fluorescence in primary root tips of a pSERK1::SERK1::YFP-expressing seedling after 2 d of treatment with 0.1 μM 2,4-D. Arrowheads mark 2,4-D induced autofluorescence in the epidermis. (G, H) YFP fluorescence in hand-cut sections ~60 μm from the QC of pSERK1::SERK1::YFP-expressing seedlings. (G) Non-treated, (H) treated for 2 d with 0.1 μM 2,4-D. The dashed red line marks the xylem plate and the arrowheads in (G) and (H) mark the phloem poles. Scale bar = 20 μm.](http://jxb.oxfordjournals.org)
Embryos are reported as origins of somatic embryos from the SAM and at the base of the cotyledons (Hecht et al., 2001). However, neither the meristem itself nor the stem cells present in the meristem appear to be required because mutants lacking a functional SAM like shoot meristemless (stm), waschel (was), and zwille/pinhead (zll/pnh) did not show a reduction in embryogenic competence (Mordhorst et al., 2002). In a cytological study where Arabidopsis embryos were followed over time in the presence of 2,4-D, it was found that the shoot apical meristem and the hypocotyl did not participate in somatic embryogenesis as long as cells in the cotyledons were actively dividing. Instead, cell divisions were initiated in procambial cells eventually leading to the formation of somatic embryos on the cotyledons (Raghavan, 2004). Also in carrot (Guzzo et al., 1994, 1995) and in Dactylis glomerata L., cells close to the vascular bundles of leaves are thought to be the origin of somatic embryos (Somleva et al., 2000). So, apparently the property to form embryogenic cells in tissue culture is restricted to the vascular stem cell or TA cell population and is not shared with the stem cells in the apical meristem.

In carrot tissue culture, DcSERK marks a single embryogenic competent cell and somatic embryos only originate from DcSERK-expressing cells (Schmidt et al., 1997). However, not all SERK-expressing cells develop into embryos. Medicago SERK1 is induced by 2,4-D in both embryogenic and non-embryogenic lines (Nolan et al., 2003), which also indicates that not all SERK-expressing cells are embryogenically competent. Most likely other factors present in a subpopulation of the SERK1-expressing cells are needed to specify embryogenic competence fully.

**SERK1 as a marker of transit amplifying cells?**

To help answer this question it is essential to determine the identity of the SERK1-expressing cells in planta. Because the SERK1 gene was originally identified as a marker for embryogenically competent single cells in tissue culture (Schmidt et al., 1997), an important remaining question is whether this competence was newly acquired or already present in the organized explant tissue. Procambium is regarded as a primary meristematic tissue with the task to provide xylem and phloem cells (Steeves and Sussex, 1989; Mahonen et al., 2000). Procambium cells fulfill the criteria for being stem cells since they have the capacity for long-term self renewal and being able to differentiate into one or more specialized cell types (Alison et al., 2002; Rippon and Bishop, 2004). Procambium cells can, therefore, be regarded as pluripotent stem cells (Mahonen et al., 2006) and these pluripotent cells express SERK1. Both plant and animal stem cell pools are maintained in niches by signals from surrounding cells. Stem cell divisions are intrinsically asymmetric, due to the ability to generate one cell that remains as a stem cell and another that exits the niche and differentiates to become a Transit Amplifying (TA) cell. The main task of TA cells is to increase the population of cells originating from a single stem cell division. These cells have a limited proliferative capacity and a limited differentiation potential (Stahl and Simon, 2005; Singh and Bhalla, 2006). The first TA cells maintain many of the characteristics of the stem cells and gradually lose these properties with continued division and differentiation. The progressive differentiation of the TA cells, will coincide with activation of genes marking the differentiated state (Potten and Loeffler, 1990). When this TA cell model is superimposed on the plant vasculature, the immature vascular cells proximal to the root stem cells can be regarded as TA cells (Stahl and Simon, 2005). SERK1 is expressed in most immature vascular cells and, upon maturation of the organ, becomes confined to the procambium. It is therefore proposed that SERK1 expression marks the vascular TA cell population.

In Arabidopsis, the SAM and the cotyledon axils of embryos are reported as origins of somatic embryos (Mordhorst et al., 1998, 2002), while SERK1 gene expression was reported to be increased by 2,4-D in both the SAM and at the base of the cotyledons (Hecht et al., 2001). Therefore, the TA cells might be the ones that have become competent for embryogenesis and express the SERK1 gene (Schmidt et al., 1997). Competent enlarged cells undergo an asymmetric division, which is considered to be the earliest event in both zygotic and somatic embryogenesis. From this asymmetric division small, isodiametric cells are generated, which proliferate into so-called pro-embryonic masses that generate somatic embryos after the removal of 2,4-D (Guzzo et al., 1994). One unanswered question in these complex and sequential events in the pathway to
of the SERK1 signalling complex that regulates the acquisition of a competent state or in the absence of their normal ‘vascular’ cues. In this scenario, SERK1 expression marks and SERK1 possibly maintains a pluripotent cell population residing in plant vascular tissues.

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References


Ogas J, Kaufmann S, Henderson J, Somerville C. 1999. PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in Arabidopsis. Proceedings of the National Academy of Sciences, USA 96, 13839–13844.


Raghavan V. 2004. Role of 2,4-dichlorophenoxyacetic acid (2,4-D) in somatic embryogenesis on cultured zygotic embryos of Arabidopsis: cell expansion, cell cycling, and morphogenesis during continuous exposure of embryos to 2,4-D. American Journal of Botany 91, 1743–1756.


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