Pluripotent versus totipotent plant stem cells: dependence versus autonomy?

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Little is known of the mechanisms that induce the dedifferentiation of a single somatic cell into a totipotent embryogenic cell that can either be regenerated or develop into an embryo and subsequently an entire plant. In this Opinion article, we examine the cellular, physiological and molecular similarities and differences between different plant stem cell types. We propose to extend the plant stem cell concept to include single embryogenic cells as a totipotent stem cell based on their capacity to regenerate or develop into an embryo under certain conditions. Our survey suggests that differences in chromatin structure might ensure that meristem-localized stem cells have supervised freedom and are pluripotent, and that embryogenic stem cells are unsupervised, autonomous and, hence, freely totipotent.

The evolving stem cell concept: stem cells grow up and leave the niche

Owing to their capacity to repair incurable lesions and their potential use in regenerative medicine-based therapies, animal stem cells (see Glossary) are in the media spotlight [1]. However, an ongoing ethical controversy exists concerning the use of human embryonic stem cells to develop therapies because of their capacity to regenerate a whole organism. Stem cells are undifferentiated cells that have the unique characteristics of both self-renewal and the capacity to develop into precursors that can form different cell types or tissues. The classical stem cell concept is based on animal stem cells being localized in a specialized cell environment (referred to as the stem cell niche) that controls their developmental fates. However, the recent techniques that have allowed the *in vitro* culture of animal stem cells have extended this concept to cells that are able to self-renew and to differentiate outside of the stem cell niche. These animal stem cell lines can be cultured under *in vitro* conditions that allow proliferation without differentiation for months

to years. Although the ability to proliferate plant embryogenic cells and to control their differentiation *in vitro* is well known and has been exploited for horticulture and

Glossary

Amyloplast: a plastid that forms starch grains.
Chromatin: eukaryotic genetic information that consists of DNA packaged with histone and non-histone proteins into chromatin. The basic unit of chromatin is the nucleosome, in which 146 bp of DNA are wrapped around an octamer of histone proteins. Chromatin structure can influence gene expression by affecting the accessibility of regulatory proteins to their target sites. Chromatin exists in two general forms, the transcriptionally active euchromatin and transcriptionally inactive heterochromatin.
Differentiation: series of changes that occur in cells and tissues during development resulting in their specialization.
Embryogenic callus: an undifferentiated 'unorganized' tissue enriched in embryogenic cells.
Embryogenic cell: a cell that requires no further external stimulus to produce a somatic embryo.
Embryogenic competence: the capacity of a cell to develop into an embryo (see embryogenic cell).
Isodiametric shape: having equal diameters or axes.
Meristem: the undifferentiated plant tissue from which new cells are formed, such as that at the tip of a stem or root.
Nucleolus: the region of the nucleus where ribosomal components are assembled.
Plasmodesmata: cytoplasmic channels that span the plasma membrane and cell wall of plants and form connections between adjacent cells.
Signals: internal and external factors that induce changes in cell structure and function.
Somatic cell: any cell of a plant or an animal other than a germ cell.
Somatic embryogenesis: the production of an embryo from somatic cells of the plant, as opposed to gametophytic cells (zygotic embryogenesis). Somatic embryos develop through stages similar to those reported for zygotic embryos.
Stem cells: any cell that can proliferate in an undifferentiated state and can give rise to differentiated cell or tissue types. Cells with the ability to divide for indefinite periods in culture and can give rise to specialized cells or tissues.
Stem cell developmental potency
Multipotent: ability of a single stem cell to develop into more than one cell type of the (plant) body.
Pluripotent: ability of a single stem cell to give rise to most but not all the various cell types that make up the body.
Totipotent: a totipotent stem cell can give rise to all the cell types that make up the body. Totipotency is the property of a cell whereby it retains the potential of developing into a complete adult organism. The ability of a cell to proceed through all the stages of development and, thus, to produce a whole adult organism.
Unipotent: a stem cell able to develop only one cell or tissue type.
Vacuome: the collective term for all the vacuoles within a cell.

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agriculture applications for many years, little is known about the molecular basis for their developmental abilities. In plant biology, the focus of stem cell research has been on the pluripotent stem cells contained in the root and shoot apices, which corresponds to the classical stem cell concept [2–6]. These pluripotent stem cells give rise to the cells and tissues found in the root and shoot, but do not have the ability to form an embryo (Figure 1). By contrast, under various conditions, a somatic plant cell can dedifferentiate to give rise to a totipotent embryogenic cell that has the ability to proliferate and/or regenerate an embryo [7–9]. The regeneration of a complete embryo from a single totipotent somatic cell via somatic embryogenesis is a remarkable example of the totipotency of plants, which has been known for >40 years [10]. In many cases, somatic embryos appear to originate directly from single somatic cells that are induced by either high doses of auxin or stress-related treatments [7,11]. In a special issue of *Science*, 'What don't we know?', devoted to the 25 most important questions facing scientists of all disciplines, only two questions were raised with regard to the plant kingdom, one of which was: how does a somatic cell become a whole plant [12]? In this Opinion article, we propose to extend the concept of stem cells to include embryogenic stem cells that arise from plant somatic cells. We examine the cellular, physiological and molecular similarities and differences between plant meristematic stem cells and embryogenic stem cells originating directly from single somatic cells. In our view,

embryogenic cells can be considered totipotent stem cells based on their aptitude to regenerate or develop into an embryo under certain conditions. In addition, as is the case with animal stem cells, epigenetic mechanisms might have important roles in determining the developmental capabilities of plant stem cells. Differences in chromatin characteristics might control the dependency and supervised freedom of meristematic stem cells (pluripotent), and the independence and unsupervised, autonomous freedom of single cell-derived Embryogenic stem cells (totipotent).

Physico-chemical and physiological conditions: moderate and reassuring versus extreme and risky cellular environments

Within the shoot primary meristems, meristematic stem cells are fed by phloem sap that first moves through conducting tissues and then enters the cells, preferentially via the symplastic pathway [13]. Although phloem content can vary between plant species, it is usually rich in nitrogen in the form of amino acids (which accounts for the major share of dry weight) and abundant in organic acids and phosphates, whereas growth regulators are found at low concentrations ($<0.1 \mu M$). In this context, the cells adjacent to stem cells provide an environment favourable to the function and maintenance of stem cell activity. Intercellular interactions can generate important positional signals via information-bearing molecules (e.g. proteins and RNA)
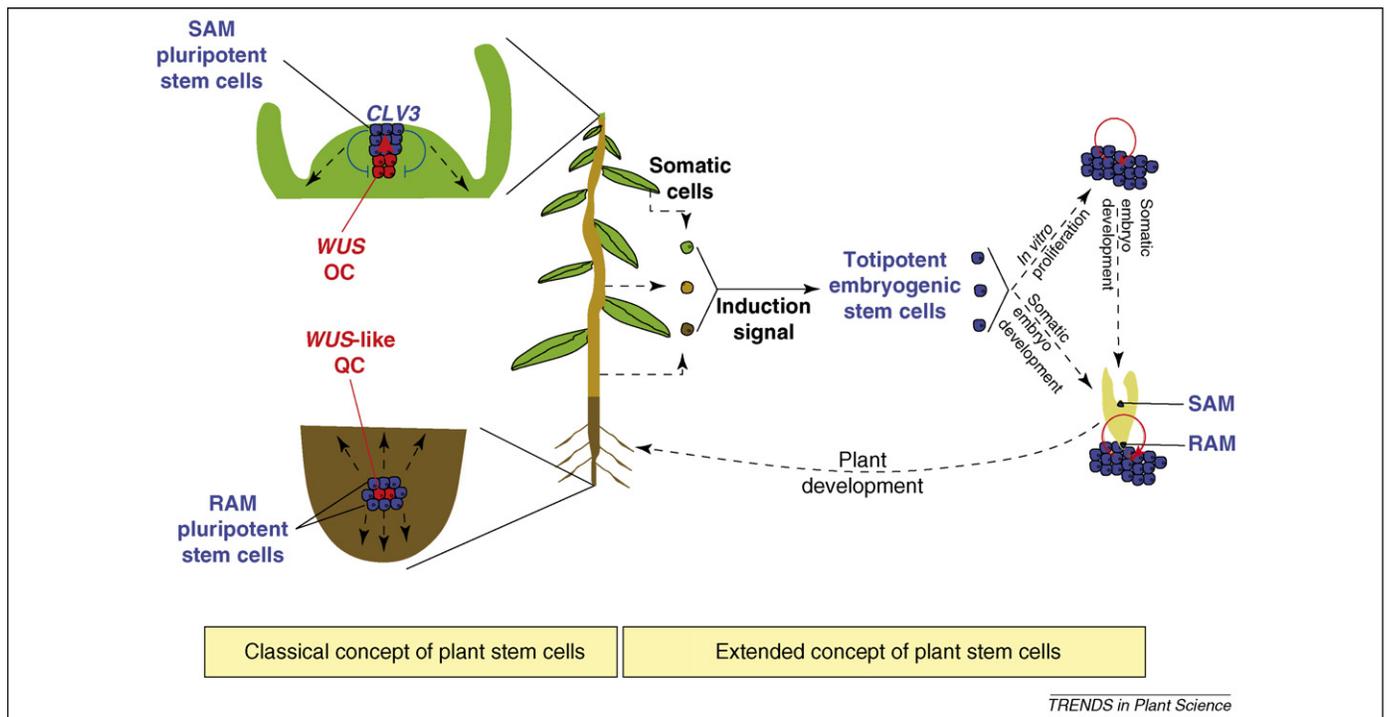
Figure 1. Classical and extended concept of plant stem cells. The plant body is the product of founder cells located in the apical meristems. During zygotic embryogenesis, plants generate the shoot apical meristem (SAM) and the root apical meristem (RAM), which function throughout the life of the plant to provide a continuous source of new cells for organogenesis. The founder cells are stem cells defined both by self-renewal and their potential to differentiate into multiple cell and tissue types. They are pluripotent meristematic stem cells. In the SAM and the RAM, the maintenance of stem cells depends upon the tissue microenvironment, known as the niche, which provides intercellular signals for stem cell regulation. In the SAM, the stem cells (in blue) are located above the WUS-expressing organizing centre (OC, in red), which is surrounded by differentiating and differentiated cells (in green). Signalling from the OC (red arrow) confines a stem cell to the upper zone, which, in turn, feeds back to limit the OC size via CLV signalling (blue lines). In the RAM, stem cells (blue) are maintained by signalling from the quiescent centre (red). Black dotted arrows indicate the differentiation gradient. Somatic cells from different organs can be induced by various signals to form embryogenic stem cells either by stress-related treatments or the ectopic expression of a number of genes encoding transcription factors. Single embryogenic cells can proliferate (self-renewal) *in vitro* and can give rise to the early organization of the plant body that includes the embryo shoot and root meristems and, consequently, all the cell and tissue types of the plant body. Single embryogenic cells are totipotent stem cells.

that pass through the plasmodesmata in a regulated manner [14–16]. Such information might have a role in the maintenance of stem cells and is important for the recruitment of daughter cells to differentiate, form primordia and gain organ identity. By contrast, totipotent embryogenic stem cells often become individualized when the cells are placed under propitious conditions to initiate the somatic embryogenesis pathway [8]. For many species, high levels of 2,4-dichlorophenoxyacetic acid, a synthetic auxin analogue, are used to induce the formation of embryogenic cells, although other induction factors are possible [9]. Indeed, the induction of somatic embryogenesis can be achieved without auxin with a range of other treatments, such as contact with heavy metals, high concentrations of minerals, sugars and osmotic stress [17–21]. All these stress-related responses can promote genomic reprogramming [22] of transcriptional activity required for somatic cells to become embryogenic cells.

Pluripotency and totipotency: organized versus unorganized regulation

Over the past decade, considerable progress has been made towards improving our understanding of the regulation of pluripotent plant stem cell induction and maintenance using *Arabidopsis* as an experimental model. The maintenance of pluripotent stem cells requires cells that are mitotically less active within the organizing centre (OC) and the quiescent centre (QC); these cells are adjacent to stem cells in the shoot and root meristems [2] (Figure 1). In the shoot meristems, it has been shown that the expression of the *WUSCHEL* (*WUS*) gene, which encodes a homeodomain transcription factor [23], is required in the OC to produce an unknown signal that induces the stem cell identity non-cell-autonomously [5]. Ectopic expression of *WUS* is sufficient to induce the expression of the stem cell marker gene *CLAVATA3* (*CLV3*) and ectopic stem cell fate [24]. CLV3 is a secreted protein that is thought to initiate downstream signalling events via interactions with the CLV1–CLV2 receptor complex in underlying cells, which results in the limitation of the *WUS* expression domain in the OC and, therefore, the stem cell population [25,26]. This negative regulatory CLV–WUS feedback loop maintains stem cell number at the population level; an increase in the number of stem cells leads to an increase in CLV3, which feeds back to reduce the *WUS* expression domain and, thus, stem cell proliferation. It appears that CLV–WUS signalling pathways might function to control stem cell activity in the root using different mechanisms. Indeed, recent studies have revealed that the *WUS*-like gene *WOX5* is expressed in the QC of the root meristem in an equivalent manner to *WUS* in the shoot meristem OC [27,28]. In addition, when the *CLV*-like gene *CLE40* is overexpressed, a reduction in meristematic activity in the root meristem is observed [29]. However, in contrast to the response observed in the shoot meristem, the CLV-like signalling restricts the root meristem size without directly interfering with the QC and stem cell specification, instead it initiates the differentiation of stem cell descendents [30,31]. Therefore, similar but distinct *WUS*- and *CLV*-based mechanisms appear to maintain pluripotent stem cell populations in the shoot and root meristems.

Other transcription factors specific to each of the meristems have been identified. The homeobox gene *SHOOT MERISTEMLESS* (*STM*), which is part of the large *KNOX* gene family, is involved in the initiation and organizational maintenance of the shoot meristem [32]. It is first expressed at the late globular stage of embryogenesis in the centre of the apical domain that gives rise to the shoot meristem, then, during vegetative growth, it is expressed throughout the shoot meristem, but not in differentiating cells, leaf primordia or leaves [33]. In contrast to WUS, STM activity appears to be required to prevent the differentiation of stem cell daughter cells that proliferate before adopting an organ-specific cell fate. In the root meristem, the transcription factors *SHORT ROOT* (*SHR*) and *SCARECROW* (*SCR*) of the *GRAS* gene family are required to maintain the stem cell population and control their differentiation capacity [34,35]. *SHR* is expressed in the stele tissue above the stem cells but the SHR protein moves out of these cells into the QC cells where it induces *SCR* expression, which is necessary for the maintenance of the QC non-cell-autonomously. The activity of two other transcription factors PLETHORA (PLT) 1 and PLT2 are also necessary to establish the root stem cell population during embryogenesis and for their maintenance after germination [34,36]. The *PLT* genes function in distinct but overlapping pathways to that of *SHR* and *SCR*. The two *PLT* genes are expressed in the stem cells and the QC, which is specified by the intersection of the *SCR*/*PLT* gene expression domains. In the absence of PLTs, SHR initiates the differentiation of the stem cell daughters. The *PLT* genes are also induced by auxin and regulate the expression of three of the *PIN-FORMED* (*PIN*) genes that encode auxin efflux facilitators involved in polar auxin transport [37]. It is thought that auxin initiates embryonic root development and the position of the stem cell population through the regulation of *PLT* gene expression, which feeds back to increase *PIN* expression required for stable polar auxin flow and proper stem cell placement in the meristem. Although the transcriptional regulatory networks and signal transduction pathways that control stem cell population and maintenance appear to differ in the shoot and root meristems, it is clear that the surrounding tissues are integrated in the tight control of stem cell functional identity, and therefore, their aptitude for pluripotency.

In addition to transcription factors and signalling proteins, chromatin remodelling factors have also been implicated in the regulation of meristem activity [38]. Chromatin remodelling factors include histone chaperones, histone modification enzymes and ATP-dependent chromatin remodelling enzymes, which all have important roles in plant development through the establishment of gene expression patterns that can be maintained during cell division [39]. The *Arabidopsis fasciata* (*fas*) mutants *fas1* and *fas2* have an expanded *WUS* expression domain in the shoot meristem, a perturbed *SCR* expression in the root meristem and, as a result, have altered root and shoot meristem organization [40]. The *FAS1* and *FAS2* genes encode subunits of the chromatin assembly factor-1 (CAF-1), which is similar to the yeast histone chaperone complex involved in the inheritance of heterochromatin

through mitosis. It is thought that the CAF-1 counterpart in *Arabidopsis* is necessary for the maintenance of gene expression in the meristem cells, although the exact mechanisms are unknown.

The transcription of the *WUS* gene is directly activated by SPLAYED (SYD), which is similar to the ATPases of the SWI/SNF chromatin remodelling complexes from yeast and metazoans [41]. The *syd* mutant has prematurely terminated shoot meristems and reduced *WUS* expression. SYD has been shown to be involved in the regulation of the stem cell pool maintenance via transcriptional control of *WUS* through direct interaction with its promoter.

In contrast to the recent advances in our understanding of meristematic pluripotent stem cell initiation and maintenance, little is known of the mechanisms that enable a somatic cell to become a totipotent stem cell, which can give rise to a somatic embryo. Embryogenic stem cells are most easily derived from either meristematic or young tissues, in particular those from immature zygotic embryos. However, a functional shoot meristem does not appear to be essential to form embryogenic competence, as suggested by the results with the meristem mutants *wus*, *stm* and *zwille/pinhead* (*zll/pnh*) [42]. Indeed, although they lack a functional embryonic shoot meristem, these mutants can be used to produce embryogenic cells, suggesting that a functional shoot apical meristem is not essential in the establishment of embryogenic cell lines in *Arabidopsis*. Nevertheless, the stem cell regulatory gene *WUS* is involved in embryogenic competence. Using a genetic gain-of-function screening approach, two alleles of the *WUS* transcription factor gene have been found to induce somatic embryo formation when overexpressed in *Arabidopsis* roots, leaf petioles, stems or leaves [43]. This result suggests that the *WUS* gene is not only essential for pluripotent stem cell maintenance non-cell-autonomously, but also that it is involved in the promotion and/or maintenance of totipotent embryogenic stem cells. However, the fact that cells with embryogenic competence were obtained from *wus* mutants suggests that multiple alternative pathways can lead to embryogenic cell formation [42]. Indeed, a number of reports have identified other transcription factor genes involved in the establishment of embryogenic cell capacity. *LEAFY COTYLEDON1* (*LEC1*) encodes a CCAAT box-binding factor HAP3 subunit, which, upon overexpression in vegetative cells, induces the expression of embryo-specific genes and the development of structures with embryonic traits [44,45]. The *lec1* mutants also have a reduced ability to form embryogenic cells and somatic embryos [46]. *LEC1* is thought to be a major embryonic transcriptional regulator that maintains embryogenesis while suppressing vegetative development. Another *LEAFY COTYLEDON* gene, *LEC2*, also induces somatic embryos from vegetative cells when ectopically expressed [47]. *LEC2* encodes a putative B3 domain transcription factor that has functional roles in early through late zygotic embryogenesis. Similar to *lec1*, *lec2* mutants have a reduced ability to form embryogenic cells and somatic embryos [46]. Another transcription factor gene, *BABY BOOM* (*BBM*) of the *AP2/ERF* gene family, was isolated using a screening designed to identify genes that are expressed during the *in vitro* induction of embryo devel-

opment from immature pollen grains (microspore embryogenesis) of *Brassica napus* [48]. *BBM* is preferentially expressed in developing embryos and seeds; however, ectopic expression of *BBM* in *Arabidopsis* and *Brassica* leads to the spontaneous formation of somatic embryos and cotyledon-like structures from seedlings. Finally, when the MADS-domain family transcription factor gene *AGAMOUS-LIKE15* (*AGL15*) was constitutively expressed, it enhanced the formation of secondary embryos from the shoot meristems of cultured zygotic embryos [49].

In addition to transcription factors, the expression of the *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE* (*SERK*) from carrot, which encodes a leucine-rich repeat (LRR) transmembrane receptor-like kinase, has been shown to be associated with individualized embryogenic cells [50]. Overexpression of *AtSERK* in *Arabidopsis* increases the efficiency of the initiation of embryogenic cells in the presence of auxin. Although the mechanism by which SERK functions to promote embryogenic cell competence is unknown, its expression appears to be linked to a cell in the process of becoming totipotent [50].

At this time, the only negative regulatory factor involved in embryogenic cell formation is PKL. Indeed, the *pkl* mutation leads to an increase in the formation of competent cells and the spontaneous formation of somatic embryos from roots [51]. The *PKL* gene encodes a putative CHD3 chromatin remodelling factor that functions to repress embryonic traits during post-embryonic development [52].

The variety of regulatory factors involved in the acquisition of embryogenic competence suggests that numerous independent and/or inter-related pathways are possible. For example, WUS overexpression appears to repress the *LEC1* gene, whereas the overexpression of *AGL15* upregulates *AtSERK*; furthermore, when *LEC2* is ectopically expressed, an increase in *AGL15* is observed [43,49,53]. Moreover, in the roots of the *pkl* mutant, transcripts for both *LEC1* and *LEC2* are derepressed and elevated more than 100-fold [52]. Whether and how these genes work together to promote embryogenic cell formation is not yet known.

What could be the molecular basis for controlling the degree of freedom of pluripotent stem cells versus totipotent embryogenic cells?

In the case of pluripotent stem cells in the shoot meristem, the *WUS–CLV* negative regulatory loop functions to control the size of the stem cell pool. This occurs through spatially limiting the expression domain of *WUS* in the niche that allows a permissive local environment for pluripotent stem cell maintenance (Figure 1). STM activity functions to repress the differentiation of stem cell daughter cells, allowing them to proliferate prior to gaining organ identity. Whether a similar system exists in the root meristem is unclear; however, coordinated niche cell activity also appears to balance stem cell self-renewal, proliferation and differentiation in the root. Both regulatory systems depend on communication between neighbouring cells, which results in a tightly controlled cellular context related to their pluripotent potential. Conversely, for totipotent embryogenic stem cells, no spatial regulation has yet been found for the control of the cell pool. However,

the ectopic expression of a number of transcription factor genes (e.g. *LEC1*, *LEC2*, *BBM*, *WUS* and *AGL15*), or the mutation of the *PKL* gene, which encodes a putative chromatin remodelling factor, results in a somatic-to-embryogenic cell transformation. WUS is the only transcription factor that has been found so far to be involved in both meristematic stem cell and embryogenic stem cell regulation. Interestingly, WUS functions non-cell-autonomously to maintain pluripotent stem cell identity in the meristem niche, whereas it appears to function cell-autonomously when ectopically expressed in a range of somatic cells that become totipotent embryogenic cells. Apparently, the cellular context in which *WUS* is expressed is important to determine the ability of a cell to become pluripotent or totipotent. Which factors form the molecular basis of this conductive cellular context?

In animals, recent studies have highlighted the underlying mechanisms for the plasticity of the stem cell genomes. Chromatin status is directly influenced by the cellular environment, which indirectly controls the degree of tissue differentiation [54]. Epigenetic mechanisms enable the stabilization of a given gene profile that determines self-renewal, differentiation and organ identity. In plants, a similar mechanism could also provide the molecular basis for the control of developmental capacity or degree of freedom in the meristematic stem cells. It has been found that embryonic stem cells in animals, which are equivalent to totipotent cells in plants, have a nuclear


Box 1. Cytological organization: interaction dependent versus physical isolation

Pluripotent plant stem cells located within the root and shoot meristems, are isodiametric, have a high nucleus:cytoplasm ratio, a spherically shaped nucleus containing one or more nucleoli, a dense cytoplasm and a highly fragmented vacuome [8] (Figure I). Meristematic stem cells have strong interactions with and depend on the surrounding cells to create a niche that maintains stem cell identity [6,31,62]. They typically have only a thin primary cell wall and are connected by plasmodesmata essential for intercellular movement of transcription factors that control developmental processes non-cell-autonomously [14–16]. Totipotent embryogenic stem cells can originate from somatic cells from various tissues, or are found in embryogenic calli and have a clearly defined cellular architecture conserved across species [7–9,63]. They have a voluminous, centrally positioned nucleus with a single large nucleolus and a high nucleus:cytoplasm ratio. The cytoplasm is dense and contains localized starch reserves around the nucleus. Their embryogenic cell wall can be modified with a glycoprotein-rich extracellular matrix [64] that contributes to wall thickening. The wall contains few or entirely lacks plasmodesmata and a surrounding callose deposit is frequently found [8,65,66]. Experimentally, such isolation can be achieved by osmotic treatments that result in substantial cell plasmolysis, plasmodesmata rupture and, subsequently, an improved induction of somatic embryogenesis [21,67].

Both pluripotent meristematic stem cells and totipotent embryogenic stem cells share certain cellular characteristics including a high nucleus:cytoplasm ratio, a dense cytoplasm, and a small fragmented vacuome. However, one cytological characteristic that distinguishes embryogenic cells is their nuclear architecture and chromatin structure. The nuclei of pluripotent meristematic stem cells are spherically shaped with several (generally small) nucleoli. Most of the chromatin exists as heterochromatin (electron-dense area when observed with a transmission electronic microscope), uniformly distributed in the nucleus with small regions of euchromatin. The nuclei of totipotent embryogenic stem cells are irregularly shaped with invaginations of the nuclear envelope (leading to several nuclear lobes) and contain one large nucleolus. The nucleus of totipotent embryogenic stem cells contains small regions of heterochromatin (located mainly at the periphery of the nucleus), but most of the chromatin exists as euchromatin (considered as transcriptionally active).

In addition, single somatic cells that become embryogenic are often physically or physiologically isolated from their immediate surroundings owing to wall thickening and the absence or reduced number of plasmodesmata [8,65,66]. Physical isolation can facilitate the reprogramming of their genomic and cellular functions to acquire totipotency and competence for embryogenesis. By contrast, meristematic stem cells have strong interactions with the surrounding cells that dictate their identity and ultimate fate. The stem cell niche in which meristematic stem cells reside appears to be essential in providing them with crucial signals that control their differentiation pathways [5,6]. The intercellular and intracellular characteristics that distinguish these different cell types might provide clues to their inherent developmental capacities.
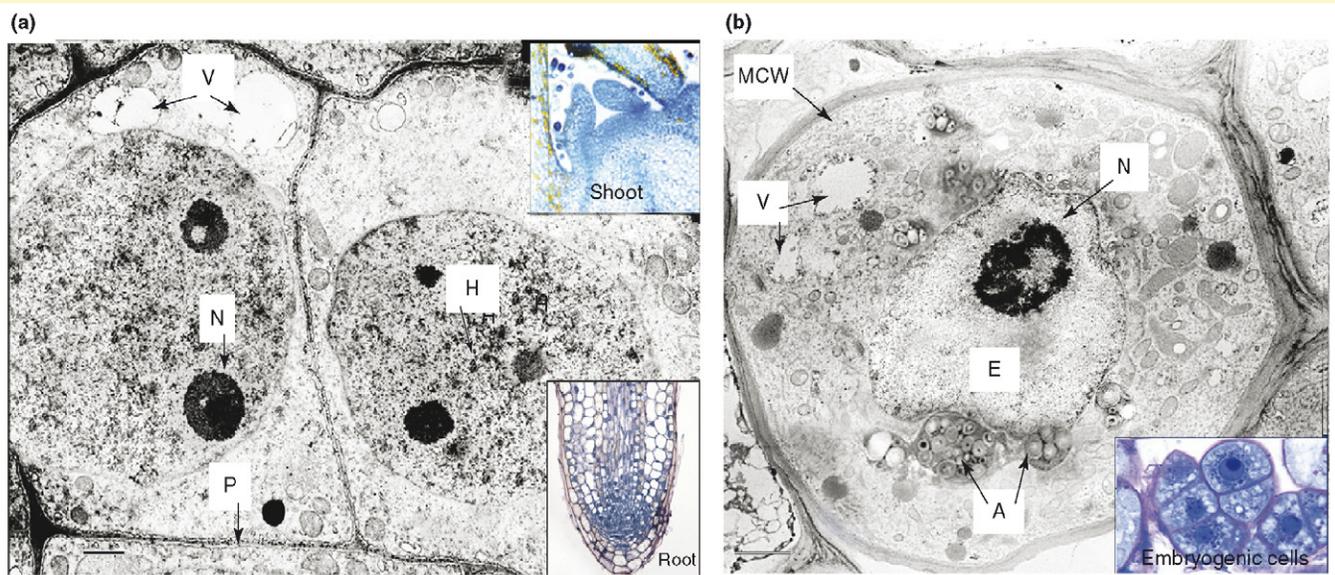
Figure I. Cellular architecture and ultrastructure of **(a)** a pluripotent meristematic stem cell (scale bar = 1 μm) and **(b)** a totipotent embryogenic stem cell (scale bar = 3 μm). Abbreviations: A, amyloplasts adjacent to nucleus; E, euchromatin; H, heterochromatin; MCW, modified cell wall; N, nucleoli, P, plasmodesmata; V, vacuome.

architecture distinct from that of differentiated cells [55,56]. The chromatin of embryogenic cells is richer in the less compact, more transcriptionally active euchromatin, whereas, as differentiation progresses, accumulation of highly condensed, transcriptionally inactive heterochromatin regions appear. Hence, changes in chromatin structure are linked to the developmental capacities of animal cells; condensed chromatin is associated with a differentiated cell identity with specific developmental capacities whereas less compact chromatin allows for pluripotency and higher developmental capacities. The question remains whether similar mechanisms could provide the molecular basis for the totipotency and autonomous orientation of embryonic stem cells of plants. Is chromatin structure the key element in controlling the degree of freedom (unipotency, pluripotency, totipotency) of stem cells?

At the moment, one of the major challenges facing plant developmental biology is to determine interactions between chromatin remodelling factors, transcription factors and genes involved in signalling. In one study [57], the formation of embryogenic cells from carrot epidermal cells was inhibited by treatment with the DNA methylation inhibitor 5-azacytidine. In addition, *LEC1* expression was also reduced, suggesting that methylation of DNA might mediate the regulation of embryogenic gene expression through chromatin remodelling. One common feature of the *LEC1*, *LEC2*, *BBM*, *WUS* and *AGL15* genes and the *pkl* mutant is their ability to promote embryogenic cell competence in the absence of high auxin or stress-related treatments used to establish embryogenic cells. It has recently been shown that *LEC2* overexpression also leads to an increase in the expression of the AUX/IAA transcription factor gene *IAA30,* which is normally expressed in the QC of the root meristem [53,58]. It was suggested that *IAA30* functions to initiate embryogenic cell formation either through changing the plant response to auxin and/or by increasing levels of free IAA [53]. Furthermore, a link between auxin and PKL has recently been established [59]. This report suggests that, through chromatin remodelling, PKL negatively regulates auxin-mediated lateral root formation via stabilization of

the SOLITARY-ROOT/AUX/IAA14 transcription factor-mediated inactivation of the AUXIN RESPONSE FACTOR transcriptional activators ARF7 and ARF19. Thus, the developmental capacity of cells to give rise to lateral roots appears to be regulated by the activity of transcription factors; this activity is dependent on chromatin-modifying factors, which might allow access to specific promoter sites. Whether similar mechanisms function in the initiation of embryogenic cells is unknown, but the common components (e.g. the involvement of the auxin response and PKL) suggest that the regulation of chromatin structure might be an essential factor in the determination of embryogenic capacity.

What future is there for plant stem cells?

Within the past decade, the successful *in vitro* culture of mammalian somatic cells and the potential prospect offered by their use in regenerative medicine have boosted advances in stem cell research. Paradoxically, although the regeneration of whole plants from somatic cells *in vitro* has been known for >40 years, little is known of the molecular and cellular basis of plant stem cell pluripotency and totipotency. In this Opinion article, we propose that the concept of a plant stem cell should be enlarged to include single embryogenic cells as totipotent stem cells outside of a niche (Figure 1). The cellular, physiological and molecular similarities and differences between pluripotent and totipotent plant stem cells are highlighted in Table 1 and Box 1.

Recent studies on animal stem cells indicate that chromatin status is a crucial element in the maintenance of stem cells and in the definition of their development potential [54]. In view of those results, a detailed comparison of how chromatin is organized in pluripotent versus totipotent plant stem cells is essential to understand the differences between these two stem cell types. Studies conducted these past few years in animal systems have revealed that transcription factor networks and epigenetic processes are important for the maintenance of the different stem cell types [54–56,60]. For plant stem cells, these approaches are still in their infancy. Nonetheless, it is now

Table 1. The cellular, physiological, and molecular similarities and differences between pluripotent and totipotent plant stem cells

Pluripotent meristematic stem cells	Totipotent embryogenic stem cells
Cellular	
High nucleus:cytoplasm ratio	High nucleus:cytoplasm ratio
Spherically shaped nucleus	Centralized irregular shaped nucleus
One or multiple nucleoli	Single large nucleolus
No amyloplasts	Amyloplasts adjacent to nucleus
Higher heterochromatin versus euchromatin content	Lower heterochromatin versus euchromatin content
Thin primary cell wall	Modified cell wall with callose deposits
Plasmodesmata	Plasmodesmata rare or absent
Fragmented vacuome	Fragmented vacuome
Physiological	
Stable environment	Stressed and variable environment
Strong interactions with stem cell niche cells	No spatial regulation (no niche)
Molecular	
Negative regulatory CLV–WUS feedback	No regulatory feedback known
WUS functions non-cell-autonomously	WUS functions cell-autonomously
Stem cell pool population regulated in the niche context	No regulation of stem cell pool population
Hierarchical regulation by transcription factor network control stem cell maintenance and fate	Evidence for independent transcription factor networks (LEC1, LEC2, BBM, AGL15, WUS)
Do chromatin-regulated transcriptional profiles determine pluripotent stem cell activity as found in animals?	Do chromatin-related structural changes allow a permissive transcriptional profile for totipotency?

clear that transcriptional and regulatory activities must be viewed in the context of chromatin structure. Because chromatin has been revealed to be highly dynamic, in both plants and animals, it will be necessary to develop more *in vivo* cellular and molecular imagery techniques, such as high resolution real-time fluorescence microscopy [61]. Finally, to understand and compare plant stem cell types with those of animals, a new era of post-functional genomics using 'chromatinomics' approaches that attempt to determine the link between chromatin structure and gene expression must be established [54].

From the application viewpoint, enhancing our understanding of stem cell maintenance and differentiation should lead to useful new molecular tools to control more effectively the vegetative propagation of higher plants. Such tools might be based on constructs with genes encoding transcription factors, such as *WUS*, *BBM* and *LEC1*, under the control of promoters that can be induced precisely at the regeneration phase.

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