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Why Somatic Plant Cells Start to form Embryos?

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Abstract Embryogenesis in plants is not restricted to the fertilized egg cell but can be naturally or artificially induced in many different cell types, including somatic cells. Although genetic components clearly determine the potential of species/genotypes to form somatic embryos, the expression of embryogenic competence at the cellular level is defined by developmental and physiological cues. Competent cells can respond to a variety of conditions by the initiation of embryogenic development. In general, these conditions include alterations in auxin (exogenous and/or endogenous) levels and evoke stress responses. Recent experimental results in the field of developmental and molecular plant biology emphasize the role of chromatin remodelling in the coordination of overall gene expression patterns associated with developmental switches. It can be hypothesized that the initiation of somatic embryogenesis is a general response to a multitude of parallel signals (including auxin and stress factors). This response includes, in addition to cellular and physiological reorganization, the extended remodelling of the chromatin and a release of the embryogenic programme otherwise blocked in vegetative cells by chromatin-mediated gene silencing. In this review I attempt to give a general overview of experimental results supporting the aforementioned hypothesis, leaving the detailed elaboration of special subjects to other chapters.

1 Embryogenesis in Plants—Variations on a Theme

In higher plants, double fertilization generates the embryo and the endosperm simultaneously, the joint development of which leads to a viable seed. Plant zygotic embryogenesis is a process that is deeply hidden in maternal tissues. In addition to the large body of histological data generated in various species, analysis of *Arabidopsis* mutants enlighted the series of events underlying plant embryo development (for a review see Mordhorst et al. 1997). Micromanipulation and in vitro fertilization supplemented by molecular and genomic methods have already revealed additional details and will also contribute to our understanding of plant embryogenesis (Grimanelli et al. 2005; Kranz et al. 1995; Kranz 1999; Sprunck et al. 2005).

However, within higher plants, detours to zygotic embryogenesis became known for a considerable number of species generally referred to as apomixis (more than 400 species belonging to at least 40 different families; Bicknell and Koltunow 2004). During apomixis, the asexual formation of a seed starts from

the maternal tissues of the ovule, avoiding the processes of meiosis and fertilization, leading to embryo development (Bicknell and Koltunow 2004). The widely observed phenomenon of apomixis reveals two important aspects of plant embryogenesis: (1) the fertilization trigger can be substituted by endogenous mechanisms (2) in higher plants other cell types in addition to the fertilized egg cell can maintain or regain the capability for embryogenic development. Although apomictic processes are restricted to the cells of the generative apex or the ovule, there is a large variety of somatic plant cells that can also undergo embryogenic development under appropriate conditions. Natural formation of embryos as vegetative propagules can take place, for example, on leaf margins of *Kalanchoë*, *Bryophyllum* (Yarbrough 1932) or *Malaxis* (Taylor 1967) species. There are many more examples for embryogenesis initiated from in vitro cultured somatic (for a comprehensive overview see Thorpe 1995) or gametic (e.g. microspores; for a review see Reynolds 1997) cells.

In all forms of plant embryogenesis (Fig. 1) certain criteria have to be fulfilled before initiation. The species or genotype has to have the genetic potential to form embryos from somatic cells and one or a few cells of the plant/explant have to be competent to receive a signal (endogenous or exogenous) that triggers the pathway of embryogenic development (commitment) leading to embryo formation even in the absence of further signals. For the in vitro forms of somatic embryogenesis, these conditions (potential, competence, induction, commitment) have to be experimentally optimized.

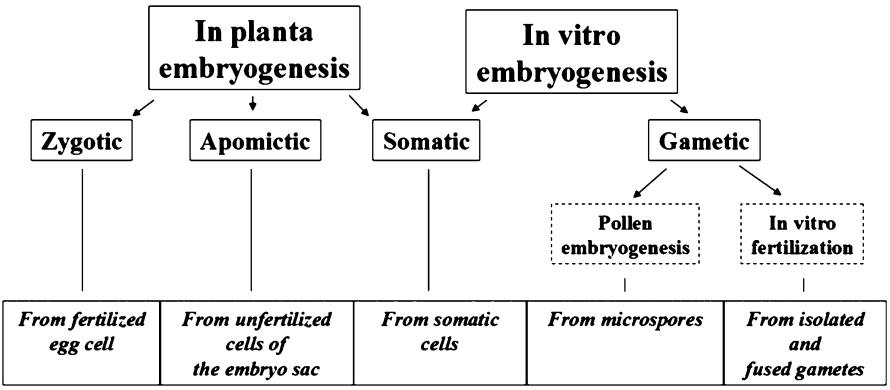

Fig. 1 Various pathways leading to embryo development in higher plants. Embryogenesis in most higher plant species starts with the fertilization of the egg cell that is parallel to the fertilization of the central cell (double fertilization). However, in certain species and in certain conditions, embryogenesis can be initiated in the embryo sac in the absence of fertilization (apomixis). In other species (e.g. in *Kalanchoë* sp.), embryos as vegetative propagules arise on leaf margins (in planta somatic embryogenesis). Embryogenesis can also be artificially induced in somatic or gametic cells in vitro

Although in vitro somatic embryogenesis is practised in many tissue culture laboratories using many species, genotypes and explants, the biological background of the process is still largely unknown. The special conditions required for successful embryo induction are set up experimentally without knowing why a given genotype/explant has embryogenic potential and how and why competence or commitment is achieved or what is the real trigger initiating embryo development.

2 Embryogenic Potential

The potential for somatic embryogenesis is first of all determined at the level of the genotype. It is clearly proved by the successful transfer of the embryogenic capability between embryogenic and recalcitrant genotypes via sexual crossing (Bowley et al. 1993; Kielly and Bowley 1992; Moltrasio et al. 2004). In spite of the continuously increasing group of species where the conditions for somatic embryo induction have been established, there are a number of species that are still recalcitrant to form somatic embryos. Highly embryogenic and recalcitrant genotypes exist even within a given species. It has to be emphasized, however, that in many instances "recalcitrance" could be resolved by optimizing growth conditions of plants or by proper explant selection (Krishna Raj and Vasil 1995). Genetic determinants therefore may only serve to define the conditions when and where embryogenic competence can be expressed (see later). Thus, the embryogenic potential is largely defined by the developmental programme of the plant as well as by environmental cues.

Somatic embryos can develop on all organs of seedlings in certain highly embryogenic genotypes of carrot or alfalfa, indicating a wide expression of embryogenic potential. In most plant species, however, embryogenic competence is restricted to certain tissues of a given genotype. Tissue culture experiences support the view that there exists a kind of gradient in the embryogenic response among the various plant organs. The embryogenic potential is highest in tissues with embryonic origin and decreases towards the hypocotyl, petiole, leaf and root (reviewed by Neumann 2000). But even if embryogenic competence seems to be lost in somatic plant cells, it can potentially be regained. In these "indirect" ways of somatic embryogenesis an intermediate phase of callus formation is required in order to express the embryogenic potential.

Obviously, the embryogenic capability of plant cells continuously decreases during plant ontogenesis, and it is species-dependent. In monocotyledonous plants, including most of the agronomically important cereals, embryogenic competence is mostly restricted to cells with embryogenic or meristematic origin, including immature embryos or seeds, leaf bases (*Gram-*

inae) or tips (*Orchidaceae*), bulb scales (*Liliaceae*), lateral buds, etc. (for a detailed list see Krishna Raj and Vasil 1995). The embryogenic potential of these meristematic cells can be maintained if the explants are cultured in a medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) followed by excessive callus formation. A high frequency of somatic embryogenesis can be achieved after the transfer of these "embryogenic callus" cells to a low-auxin or hormone-free medium.

In contrast to the cells of meristematic tissues, somatic cells of monocotyledonous plants differentiate early and rapidly and this is followed by the loss of their mitotic and morphogenetic capabilities. In this respect it is interesting to note that the regulation of the juvenile-to-adult transition might be different in dicots and monocots (for a review see Chuck and Hake 2005). Although the direct reasons for the early loss of totipotency in monocots are not known, they may be linked to the strict regulation of the synthesis and/or metabolism of endogenous growth regulators such as auxin.

Several attempts have been made to compare embryogenic and closely related recalcitrant genoypes to point out significant differences (for a review see Fehér et al. 2003). In alfalfa (*Medicago sativa* ssp. *varia*), closely related genotypes were selected on the basis of their embryogenic potential (Bögre et al. 1990). Their response to auxin has been compared and characteristic differences could be established. Auxin-responsive genes were induced/repressed at a significantly lower auxin concentration in the embryogenic versus the non-embryogenic genotype (Bögre et al. 1990). Furthermore, auxin inhibited rooting of in vitro grown shoot cuttings also at a much lower concentration (Bögre et al. 1990). Callus growth of the non-embryogenic genotype continued at the same 2,4-D concentration that inhibited cell division in the cells of the embryogenic genotype where this level of 2,4-D triggered somatic embryogenesis. These observations indicated a considerable difference between the auxin sensitivity of the two genotypes. The key role of endogenous hormone metabolism affected by genetic, physiological and environmental cues is well accepted in the induction phase of somatic embryogenesis (Jimenez, this volume).

3
Cellular Competence

Embryogenic competence is expressed finally at the level of single cells. It is very difficult to define, however, what this cellular competence means. According to a widely accepted definition, embryogenic competent cells are those cells which are capable of differentiating into embryos if they receive inducers of differentiation (Halperin 1969). However, embryogenic competence itself needs to be induced in many cases (e.g. during "indirect" somatic embryogenesis, see earlier), and the signals inducing competence and trigger-

ing embryogenic development are not easy to separate. Cellular competence is associated with the dedifferentiation of somatic cells that allows them to respond to new developmental signals.

It is well accepted that embryogenic competent cells can be morphologically recognized as small, rounded cells with rich cytoplasm and small vacuoles. In this respect they are very similar to meristematic cells or zygotes and this similarity is further emphasized by their asymmetric division (Fig. 2). Embryogenic competent cells can also be characterized by the central position of the nucleus and by prominent radiating perinuclear microtubules and actin filaments (Šamaj et al. 2003). Additionally, they exhibit a special cell wall composition that is discussed in detail by Šamaj (this volume).

These types of cells either originate from embryonic/meristematic tissues or can be formed from elongated, vacuolized cells under specific conditions, e.g. after treatment with 2,4-D. However, other hormones (abscisic acid, ABA, cytokinin) or stress treatments (Ikeda-Iwai et al. 2003; Kamada et al. 1993; Nishiwaki et al. 2000; Pasternak et al. 2002) can also induce the formation of the embryogenic competent cell type.

Development of embryogenic competent cells can be best documented in systems where single cells were selected (Nomura and Komamine 1985; Osuga et al. 1999) or video-tracked (Toonen et al. 1994) using carrot suspension cells or *Medicago* leaf protoplasts (Bögre et al. 1990; Dudits et al. 1991; Pasternak et al. 2002; Fehér et al. 2005).

Although video cell tracking of individual carrot cells of a heterogeneous cell suspension culture could not clearly assign a morphological type to the initial cells that could form proembryogenic cell clusters, the highest frequency could be observed in the case of small, spherical, densely cytoplasmic cells (Toonen et al. 1994). The same technology was successfully used to demonstrate that the expression of the somatic embryogenesis receptor kinase (*SERK1*) gene is indeed linked to the embryogenic cell fate (Schmidt

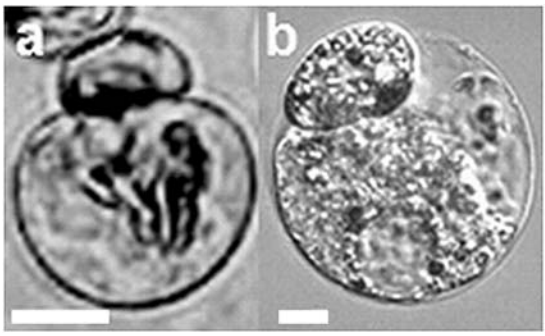

Fig. 2 Morphological similarity of an asymmetrically dividing leaf-protoplast-derived embryogenic alfalfa cell (**a**) and an isolated maize zygote (**b**). The *bar* represents 10 μm

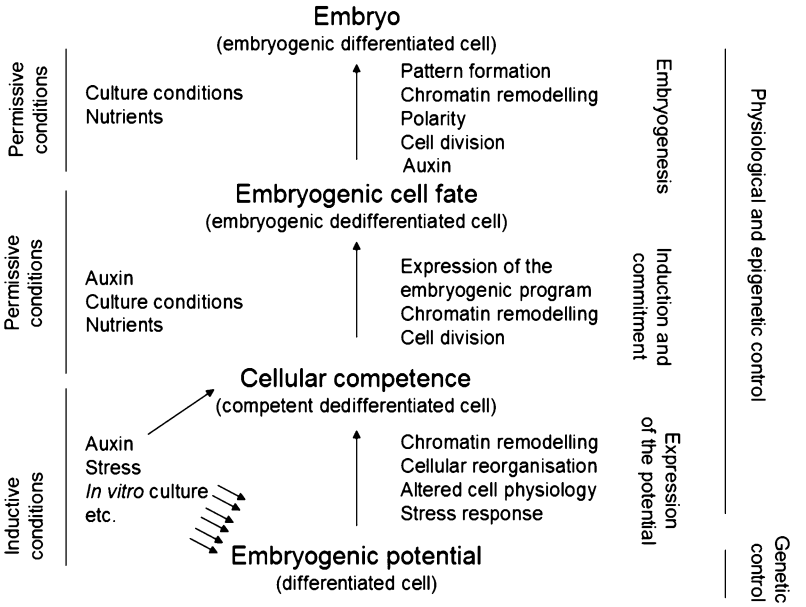

Fig. 3 A hypothetical model of events underlying somatic embryogenesis. A multitude of parallel signals, including auxin (either exogenously supplied or endogenously altered), evoke a wide cellular response including reorganizations at the levels of cell structure, physiology, chromatin and gene expression. As a result, the dedifferentiated cells become competent for embryogenesis. Competent cells will indeed be embryogenic if external and cellular conditions allow the expression of the embryogenic programme that is, in most cases, preceded by or parallel to cell divisions. Further cell divisions together with polarity establishment and pattern formation result in the development of the embryo. The central role of chromatin remodelling can be hypothesised in all phases, including dedifferentiation, embryogenic reprogramming and embryo differentiation. They are all associated with the parallel activation/inactivation of a large number of genes

et al. 1997). Following the division of these small, spherical, dense carrot cells, the JIM8 cell wall epitope was shown to be asymmetrically transferred to the daughter cells from which only those devoid of the epitope remained embryogenic (Toonen et al. 1996).

Another approach was developed by Nomura and Komamine (1985) based on the fractionation of suspension-cultured carrot cells. They could isolate a fraction of small, dense, isodiametric cell type (state 0) that could synchronously develop into somatic embryos under appropriate conditions (Osuga et al. 1999). It was found that the formation of state 1 cells (forming small embryogenic cell clusters) was dependent on auxin, which, however, blocked the further development (Nomura and Komamine, 1985).

Alfalfa leaf protoplasts also represent a rather homogenous and synchronized system that allows detailed investigations both at the single cell and at the cell population level (for a review see Fehér et al. 2005). A fur-

ther advantage of the system is that the development of the cells is dependent on 2,4-D concentration: 1 μM 2,4-D results in the formation of elongated vacuolated cells, while small, cytoplasmicaly rich, embryogenic cells are formed at a tenfold higher concentration (Dudits et al. 1991; Pasternak et al. 2002). Furthermore, the system can be used to compare genotypes with or without embryogenic potential (Bögre et al. 1990; see also earlier). The comparisons made between embryogenic and non-embryogenic cells revealed that the two types exhibit not only characteristic morphological differences but that their physiology is also altered. Among other differences, the embryogenic competent cells have higher cytoplasmic and vacuolar pH values and an altered auxin metabolism (Pasternak et al. 2002). These protoplast-derived cells were activated earlier as was shown by faster medium acidification and earlier BrdU/thymidine incorporation into their genomic DNA as well as by earlier cell divisions (Bögre et al. 1990; Pasternak et al. 2002). The correlation between the plasma membrane pH gradient, the timing of cell activation and embryogenic cell formation was strengthened by several further observations. For example, buffering of the medium by 2-morpholinoethanesulphonic acid slowed down medium acidification, delayed cell division and prevented embryogenic cell formation in the presence of the embryogenic (10 μM) 2,4-D concentration. On the other hand, gradual medium acidification achieved by L-galactolactone accelerated cell division and promoted embryogenic cell formation under non-embryogenic (1 μM 2,4-D) conditions (Pasternak et al. 2002; Fehér et al. 2005). Oxidative stress (iron, copper, menadione, nitric oxide) was also shown to promote both cell division and embryogenic cell formation under non-embryogenic conditions (Pasternak et al. 2002; Ötvös et al. 2005). Some of these changes could be linked to the timing of endogenous auxin (indole acetic acid, IAA) peaks (Pasternak et al. 2002).

The same system seemed to be useful for the identification of genes differentially expressed in vacuolated, non-embryogenic (1 μM 2,4-D) versus dense, embryogenic competent (10 μM 2,4-D) cells. A PCR-based complementary DNA (cDNA) subtraction approach was used to obtain a cDNA population enriched in sequences preferentially expressed in the embryogenic cell type (Fehér et al., unpublished results). The functional classification of 36 differentially expressed genes revealed that most of the proteins indentified are related to cellular reorganization, including stress responses, intracellular membrane transport and secretion, protein synthesis and nuclear functions. The genes had distinct expression patterns during somatic embryogenesis, indicating their participation in various processes underlying the embryo formation from protoplast-derived cells.

Similar molecular approaches resulting in the identification of genes with similarly diverse functions have also been carried out in other embryogenic systems (for a review see Fehér et al. 2003). Further investigations are needed in order to establish the significance of these genes/proteins in somatic em-

bryogenesis, but their diversity indicates the wide range of cellular changes that are associated with embryogenic cell formation. Further details on differential gene expression during somatic embryogenesis are also given by Suprassanna (this volume).

The best-characterized gene that can be associated with embryogenic competence is the gene coding for the somatic embryogenesis receptor kinase (SERK1) identified first by Schmidt et al. (1997) in carrot. Using the SERK promoter fused to the luciferase gene and video cell tracking, it was shown that SERK-expressing single cells could indeed develop into somatic embryos (Schmidt et al. 1997). Furthermore, the ectopic expression of the *AtSERK* gene could facilitate the formation of somatic embryos (Hecht et al. 2001). SERK expression is therefore widely used as a marker of embryogenic competence (Baudino et al. 2001; Nolan et al. 2003; Somleva et al. 2000; Thomas et al. 2004; Ötvös et al. 2005). It was shown that in planta, AtSERK1 expression was first expressed during megasporogenesis and then in the functional megaspore, in all cells of the embryo sac until fertilization and in the embryo up to the heart stage. After this stage, expression was undetectable in any part of the developing seed. Low expression was, however, detected in adult vascular tissues. AtSERK1 gene expression was also observed in the shoot apical meristem and cotyledons of auxin-grown *Arabidopsis* seedlings used to initiate embryogenic callus cultures (Hecht et al. 2001). In other species (Baudino et al. 2001; Nolan et al. 2003; Somleva et al. 2000; Thomas et al. 2004), *SERK* gene homologues were also identified, but they were found to be even more widely expressed, indicating roles for these genes beyond the regulation of embryogenesis. Therefore, it was suggested that the SERK protein is rather a general morphogenetic than strictly an embryogenic marker (Nolan et al. 2003).

4 Induction of the Developmental Switch

Many tissue culture systems use 2,4-D as an efficient inducer of somatic embryogenesis. If we can answer the question why this synthetic auxin is so efficient in this respect, we may get closer to understanding the processes underlying the induction phase of somatic embryogenesis. The first question to be answered is whether 2,4-D is required for the acquisition of competence or for the initiation of the embryogenic cell fate or both. The question is not easy to answer in the case of cultures which are established in the long-term presence of 2,4-D and where embryos are formed only after the removal of 2,4-D (e.g. in the case of carrot). Does the commitment for embryo development happen before or after 2,4-D removal? Now it is well accepted that cell fate determination takes place in the presence of 2,4-D, which blocks the pro-

gression of the development at the same time. That 2,4-D is only a trigger of the cell fate switch is emphasized by experiments with a special *Medicago* cell culture (microcallus suspension culture or MCS) maintained in the presence of another synthetic auxin, namely naphthylacetic acid (NAA) (Dudits et al. 1991; Györgyey et al. 1991,1997). If these cells are transferred to hormone-free medium, they form roots with high frequency. If a large concentration (100 μM) of 2,4-D is applied to the cells for as short a time as a few minutes before the transfer to hormone-free medium, the cells will develop into somatic embryos. However, the first embryos can be observed on the surfaces of the calli only 2–3 weeks following the treatment. On the basis of these experiments, a high efficiency of embryogenesis could also be achieved on carrot hypocotyl surfaces after exposure to 450 μM 2,4-D for 2 h (Kitamiya et al. 2000). Indeed, these observations indicate that 2,4-D is required for the initiation of a programme that can further proceed on its own. Removal of 2,4-D from the induction medium can be important to allow the establishment of cellular polarity, which is one of the first cytological events underlying embryogenic development (Šamaj et al. 2003; for a review see Fehér et al. 2003).

2,4-D is often simply considered as an auxin analogue, but it has distinct and much more diverse effects than natural auxins. For example, 2,4-D has recently been demonstrated to regulate cell elongation and division in a different way from NAA (Campanoni and Nick 2005). That 2,4-D enhances division but simultaneously blocks elongation of cells could also be observed in the case of embryogenic alfalfa leaf protoplasts (Pasternak et al. 2002; Fehér et al. 2005).

As 2,4-D is also used as a herbicide, several attempts have been made to clarify its mode of action. Recent studies have proposed that ethylene is induced in response to auxinic herbicides (Grossmann 2000; Zheng and Hall 2001) and that ethylene in turn triggers ABA biosynthesis (Grossmann and Hansen 2001). The increased expression of the gene coding for 1-aminocyclopropane-1-carboxylic acid synthase which catalyses the rate-limiting step in ethylene biosynthesis as well as the involvement of 9-*cis*-epoxycarotenoid dioxygenase, a key regulator in ABA biosynthesis, has been demonstrated in the action of auxinic herbicides such as 2,4-D (Hansen and Grossmann 2000; Woeste et al. 1999). Further cell damage and death can be attributed to cyanide formation as a co-product of ethylene biosynthesis (Grossmann 1996). A genome-wide analysis of gene expression changes in *Arabidopsis* in response to 1-h treatment with 1 μM 2,4-D (only twice the concentration used to induce somatic embryogenesis in carrot by Kitamiya et al. 2000) has also been reported (Raghavan et al. 2005). In total 148 genes showed increased and 85 genes decreased transcription in response to this treatment. The wide spectrum of 2,4-D action is indicated by the various classes of genes affected, including genes involved in transcription, metabolism, signal transduction, cellular communication, protein turnover, subcellular localization,

cellular transport and interaction with the cellular environment in addition to the 25% of the genes indentified that could not be classified.

These findings are in agreement with many observations made in experimental systems where 2,4-D was used to trigger somatic embryogenesis. Additionally, ABA has been reported to induce somatic embryogenesis in seedlings (Nishiwaki et al. 2000). Application of ABA to immature zygotic sunflower embryos resulted in the induction of somatic embryogenesis under sucrose conditions which otherwise allow only caulogenesis to occur (Charriére et al. 1999). Direct experimental evidence of the contribution of endogenous ABA to the induction phase of somatic embryos was provided by Senger et al. (2001). These authors showed that reduced cellular ABA levels in *Nicotiana plumbaginifolia* resulted in disturbed morphogenesis at the preglobular embryoid formation stage, which could be reversed by exogenous ABA application. ABA is considered to be a "stress hormone" in plants. Indeed, it has been widely reported that application of stress conditions can also induce or promote somatic embryo formation (for a review see Fehér et al. 2003). In alfalfa leaf protoplast-derived cells, various oxidative stress-inducing agents were shown to induce embryogenic cell formation under conditions where normally elongated, vacuolated cells develop (Pasternak et al. 2002). H_2O_2 and nitric oxide have also been shown to promote somatic embryogenesis (Kairong et al. 1999; Ötvös et al. 2005).

That oxidative stress and the stress responses are indeed an inherent part of 2,4-D-induced somatic embryogensis is well demonstrated by a microarray study. As a suitable experimental system, soybean cotyledones were placed with their abaxial side down on a medium containing 40 mg l^{-1} (approximately 200 μM) 2,4-D (Thibaud-Nissen et al. 2003). Embryos appeared only on the adaxial side of explants after 21 days of culture. The gene expression pattern of the separated abaxial and adaxial parts was compared at different time points on a 9280-clone cDNA microarray. Clustering of the microarray data revealed that oxidative burst/detoxification, cell wall modification and cell division related genes significantly increased their expression after 7 days in culture. At 14 days, cell division activity was decreased, but the transcription of stress-responsive genes was enhanced. Proteomic analysis of somatic embryogenesis in *M. truncatula* also resulted in the identification of thioredoxin and 1-Cys-peroxiredoxin among the 16 proteins associated with embryogenic development (Imin et al. 2005).

In addition to induction of ABA and ethylene synthesis, 2,4-D has also been shown to increase endogenous auxin (IAA) levels in plant cells (Michalczuk et al. 1992a, b). The general role of auxin in the initiation of embryogenesis is supported by the findings that an auxin surge has been shown to accompany fertilization in carrot (Ribnicky et al. 2001) and that 2,4-D could induce the development of unfertilized isolated egg cells of wheat in vitro (Kranz et al. 1995). The appropriate endogenous auxin level of explants can be a key requirement for somatic embryogenesis. Even in those systems where

no exogenous auxin is required for somatic embryo induction, the importance of the endogenous auxin level can be recognized. For example, ABA could induce embryogenesis in carrot seedlings only if the shoot tips, regions of auxin synthesis, were present (Nishiwaki et al. 2000). Ikeda-Iwai et al. (2003) reported that various stress treatments also promoted subsequent somatic embryo induction in shoot tip and flower bud explants. In alfalfa leaf protoplasts sodium nitroprusside as a NO donor could promote embryogenic cell formation only in the presence of auxin (Ötvös et al. 2005).

5 Determination of Embryogenic Cell Fate

Obviously, the initiation of embryogenic development in a differentiated cell requires a complete cellular reprogramming. Differentiated functions have to be deregulated and, following a transition phase, a new programme leading to embryo development has to be started. Although this reorganization is accompanied by profound morphological and physiological changes, reprogramming of the overall gene expression pattern is of utmost importance. During recent years it has become well accepted that the precise control of chromatin modifications in response to developmental and environmental cues determines the correct spatial and temporal expression of the genes (Li et al. 2002). The higher order of chromatin stabilizes gene expression patterns determining the regions of the genome that are silent or active in a given cell or at a given developmental phase (Wagner 2003). Experimental evidence has highlighted the importance of regulating chromatin structure in embryogenic transition. For example, chromatin-mediated gene silencing has been shown to play key roles in determining embryo and endosperm development in *Arabidopsis*. Mutations in *Arabidopsis* genes coding for similar proteins ("polycomb" group) that have been shown to have chromatin silencing functions during drosophila development have been identified. These mutations resulted in fertilization-independent endosperm (*fie*) or seed (*fis*) formation (Chaudhury et al. 2001; Grossniklaus et al. 2001; Luo et al. 1999; Ohad et al. 1999). Another mutation, *medea*, is defective in the protein involved in the same regulatory pathway (Grossniklaus et al. 1998; Kiyosue et al. 1999). These observations suggest that the embryogenic programme is repressed by chromatin-based gene silencing and becomes released in response to fertilization.

A further *Arabidopsis* mutant, *pickle (pkl)*, has a phenotype characterized by the postembryonic expression of embryo-specific markers and the spontaneous regeneration of somatic embryos in roots (Ogas et al. 1997, 1999). The product of the *pkl* gene was characterized as a chromatin-remodelling factor that represses embryogenesis-related gene expression and regulates the developmental transition from an embryogenic to a vegetative state (Ogas et al.

1999). In addition to chromatin organization, the direct regulation of genes involves specific transcription factors. Until now, several transcription factors (*leafy cotyledon 1 and 2, wuschell, baby boom*) have been identified to be involved in zygotic embryogenesis and to result in ectopic embryo formation if expressed in vegetative tissues (Boutilier et al. 2002; Lotan et al. 1998; Stone et al. 2001; Zuo et al. 2002; Sauer and Friml, this volume). The link between chromatin remodelling and these transcription factors has been demonstrated by the release of the repression of *lec1* expression in *pickle* mutants that can lead to the development of embryos on roots (Ogas et al. 1999). *Pickle* has been shown to repress embryogenic cell fate in all vegetative tissues (Henderson et al. 2004), but it was also demonstrated that the derepression of embryogenic functions in *pickle* mutants is selective (Dean Rider et al. 2003).

On the basis of the aforementioned evidence, one can hypothesize that during the induction of somatic embryogenesis the remodelling of chromatin results in the release of the embryogenic programme otherwise repressed by chromatin-based silencing mechanisms in vegetative plant cells. Polycomb-like chromo-domain-containing proteins have been shown to be expressed during carrot somatic and zygotic embryogenesis (Kiyosue et al. 1998). Furthermore, the expression of *lec1* during somatic embryogenesis has already been demonstrated in carrot and alfalfa (Yazawa et al. 2004; Fehér et al., unpublished results). It is interesting to note that in carrot *c-lec1* transcripts are already present in embryogenic cultures and the gene is strongly expressed 1 day after the removal of 2,4-D from the medium (Yazawa et al. 2004), but in alfalfa where a 1-h 2,4-D shock was followed by several weeks of culturing in hormone-free conditions, *ms-lec1* expression increased only at the time of the differentiation of embryos (3 weeks after induction; Fehér et al., unpublished results). This observation further supports the hypothesis that in the carrot system embryogenic commitment takes place before the removal of 2,4-D.

If we accept the primary role of chromatin remodelling in the initiation of the embryogenic programme, the main question still remains: what is the main signal and how does that signal result in chromatin remodelling and reprogramming of gene expression during somatic embryogenesis? In this respect it is interesting to note that the ectopic expression of the homeotic transcription factor *wuschel* in the root has been shown to induce shoot stem cell identity and leaf development on its own, floral development together with *leafy*, and embryogenesis together with auxin (Gallois et al. 2004). These results indicate that although auxin is required, it is insufficient to initiate embryogenesis in somatic plant cells on its own. A plausible model of the induction of somatic embryogenesis therefore might be be based on (at least) two factors: auxin, which is responsible for an appropriate cellular environment, and other unknown factor(s), including stress, which trigger the embryogenic programme.

6
Conclusions and Future Prospects

While the inducers of somatic embryogenesis are highly variable, the common cellular response has to be rather general. In vitro somatic embryogenesis is associated with artificial conditions, high levels of exogenous growth regulators and many other stress factors. These extreme and stressful conditions may result in a general stress response in cells showing extended chromatin reorganization. The presence of auxin as a growth regulator might also be important in order to provide the cells with the required developmental flexibility, e.g. promoting dedifferentiation. In this view, the general applicability of 2,4-D for the induction of somatic embryogenesis rests on its ability to evoke stress and auxin-responses at the same time (see earlier).

The extended chromatin reorganization caused by the inducing conditions might result in the "accidental" release of the embryogenic programme normally repressed by chromatin-mediated gene silencing mechanisms. Auxin (exogenous and/or endogenous) is also required for the expression of the embryogenic programme by ensuring cell survival, providing the suitable physiological background, inducing cell division and/or providing further necessary pathways. The large number of cellular events that have to be coordinated during the formation of embryogenic cells define together only a narrow window that indeed permits the initiation and progression of embryogenic development. That is why not all cells of an explant subjected to the same treatment are capable of developing into embryos, and why various explants, genotypes and species need different conditions for successful induction. This hypothesis, which should be validated by further experimental data on both zygotic and somatic embryogenesis, is summarized in Fig. 3.

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