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The initiation phase of somatic embryogenesis: what we know and what we don't

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ABSTRACT Somatic plant cells are not terminally differentiated and can therefore regain totipotency and initiate embryo development under appropriate conditions. Although this phenomenon is well known for more than 50 years, the details are still mysterious as we do not know why certain genotypes, explants or cells are more amenable for somatic embryogenesis than the others. It is also not known why so many different conditions can be used to initiate somatic embryogenesis and what are the key molecular steps being common in all cases. Recent progress in plant molecular and developmental biology now allows us to establish new hypotheses on the way of transition of somatic cells to the embryogenic state. One of the possible hypotheses is presented in this short review.

Acta Biol Szeged 52(1):53-56 (2008)

KEY WORDS

auxin
cell physiology
chromatin
competence
embryogenic potential
gene expression
transcription factors

Plant cells possess remarkable developmental plasticity. One of the most intriguing examples of this plasticity is somatic embryogenesis during which differentiated plant cells regain totipotency and develop into embryos. Despite the fact that somatic embryogenesis is widely used for *in vitro* plant propagation the biological background of this plant-specific phenomenon is hardly known. It is not surprising therefore that a special issue of the *Science* magazine listed among the current 125 most important scientific questions the one: "How does a single somatic cell become a whole plant?" (Kennedy and Norman 2005). Exploration of the physiological and molecular events underlying somatic embryogenesis is of general interest as it may serve to improve practical applications and provide basic knowledge on the acquisition of totipotency (e.g. by stem cells (Grafi and Avivi 2004)) and the regulation of developmental switches (Costa and Shaw 2007). In this respect the induction phase of somatic embryogenesis is of primary interest. Although during the last couple of years there was a great progress in the identification of key molecular players involved in the process (Fehér 2006; Feher et al. 2003) we are still far to understand how dedifferentiation leads to cellular totipotency and why it is expressed in only certain cells of certain genotypes.

A hypothesis of somatic embryo induction based on the current knowledge

Somatic embryogenesis occurs in a wide variety of species and explants but the genetic determination of embryogenic capability is clear from studies where it was transferred from embryogenic to non-embryogenic genotypes (e.g. Moltrasio

et al. 2004). As the number of tissue culture systems achieving somatic embryogenesis in formerly recalcitrant species is increasing, there is a view that embryogenic capability of somatic plant cells is a rather general feature but the appropriate conditions allowing the expression of this trait greatly varies (Fehér 2006). It can be hypothesized that although plant cells in general have the capability for embryogenesis, the expression of this trait (the acquisition of embryogenic competence) is dependent on many circumstances mainly determined by the given physiological state of the cell which is determined by its genetic and developmental conditions and by environmental cues. Briefly: the genetically determined embryogenic potential may allow the expression of embryogenic competence under appropriate conditions which will result in the initiation of embryo development in response to an appropriate developmental signal in those cells only where the physiological conditions are favorable (Fig. 1). This complicated interaction of genetic and physiological factors may explain why only certain genotypes and certain cells can go through the whole process of somatic embryogenesis.

The agents used to induce *in vitro* embryogenesis in somatic plant cells are highly variable ranging from various plant hormones to stress treatments (Feher et al. 2003). The most widely used inducer, however, is auxin, especially 2,4-dichlorophenoxyacetic acid (2,4-D). It can be stated in general, that most if not all treatments reported to induce somatic embryogenesis, including 2,4-D, can affect the auxin balance within the cells. In this respect the source of the explant has also a significant contribution: explants with high levels of endogenous auxin may be more responsive (Jiménez and Thomas 2006) and may not even require exogenous auxin for induction (Ikeda-Iwai et al. 2003). In many systems, following the induction, somatic embryo development proceeds

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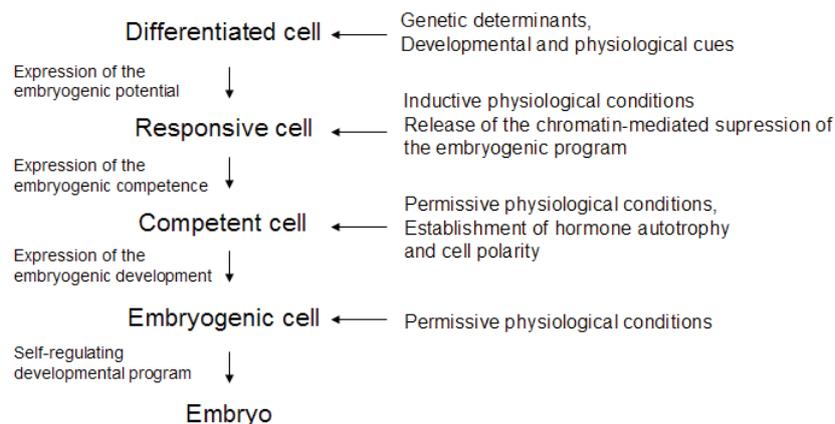


Figure 1. A hypothesis on the induction of somatic embryogenesis in differentiated plant cells. Genetically and developmentally determined cellular differentiation inhibits or allows the expression of embryogenic potential depending on the genotype, developmental state and tissue type. Cells exhibiting this potential are responsive to strong aspecific signals such as auxin imbalance and stress that may result in the release of the chromatin-mediated repression of embryogenic development. The embryogenic program then proceeds on a self-regulated way under permissive physiological conditions ensuring the establishment of auxin autotrophy and polarity.

under auxin free conditions (e.g. Dudits et al. 1991) which indicates that the cells become capable for self-supporting auxin synthesis. Establishment of auxin synthesis and polar auxin transport is a key step in meristem formation underlying embryo development (Nawy et al. 2008).

Several attempts have been made to identify key genes which may govern somatic embryogenesis in response to the induction signal (Thibaud-Nissen et al. 2003; Rose and Nolan 2006; Suprasanna and Bapat 2006; Tang and Newton 2006; Domoki et al. nd; Legrand et al. 2007; Yazawa and Kamada 2007; Zeng et al. 2006, 2007). These attempts resulted in the identification of many genes the expression of which is up- or down regulated during embryogenesis. These gene expression changes clearly indicate that somatic embryo induction evokes a general cellular reorganization characterized by the expression of stress responses, the entry into the cell division cycle, the alteration of cellular metabolism etc (Fehér et al. 2003). Although signaling molecules like the somatic embryogenesis receptor kinase (SERK; (Schmidt et al. 1997; Hecht et al. 2001)) or the MADS-box transcription factor baby boom (BBM; (Boutilier et al. 2002)) could also be identified and their overexpression enhances the regeneration/embryogenic capability of the cells (Srinivasan et al. 2007), a single key gene responsible for the induction of the developmental pathway could not be identified in these approaches.

Important molecular aspects of somatic embryogenesis could be revealed, however, due to the studies of various *Arabidopsis* mutants. The transcription factors Leafy Cotyledon1 and 2 (LEC1 and 2; have been shown to be required for early as well as late steps during zygotic embryo development and their overexpression may induce ectopic embryo

development in leaf cells (Lotan et al. 1998; Stone et al. 2001, 2008); (Kwong et al. 2003)). The Wuschel transcription factor having an important role in shoot meristem organization and maintenance can evoke similar response if overexpressed in transgenic plants (Mayer et al. 1998; Zuo et al. 2002). The expression of these genes could also be linked to somatic embryogenesis (Yazawa et al. 2004; Gaj et al. 2005; Singla et al. 2007); (Braybrook et al. 2006; Domoki et al. nd; Rose and Nolan 2006).

The most remarkable observation, however, is related to the *pickle* mutant (Ogas et al. 1999). In *pickle* mutants in place of some of the root meristems embryos develop. The *pickle* gene codes for a chromatin remodeling ATPase and its activity is required to suppress the embryogenic program (including the expression of embryogenesis related gene expression like of LEC1 and 2) in somatic cells (Ogas et al. 1999; Dean Rider et al. 2003; Henderson et al. 2004; Li et al. 2005; Rider et al. 2004). This observation means that there is a need to change of our thinking on the “induction” of somatic embryogenesis as it is rather a “release from suppression” (Fehér 2006). Now it is well accepted that the overall gene expression pattern of an eukaryotic cells is controlled on the chromatin level via the organization of the chromatin into loose active and compact inactive or silenced regions (Li et al. 2002).

Somatic embryogenesis may therefore occur if the genes responsible for the embryogenic developmental program are released from chromatin-mediated gene silencing in vegetative cells. This may happen in response to strong aspecific signals, such as high auxin dose and/or sublethal stress, which evoke the activation of large chromatin regions (Fehér 2006). This hypothesis may explain why less differentiated cells (e.g. immature embryos) are more amenable for somatic embryo-

genesis and why various aspecific signals can evoke similar embryogenic response.

Open questions and future prospects

Despite the current above reported progress in their identification, many embryogenesis-related genes are not known yet. Especially embryogenesis-related chromatin remodeling factors and their regulation are of special interest in this respect. The identification of specific genomic regions silenced in vegetative but active in embryogenic cells would be of great significance. Although the central role of auxin is well accepted, we do not know how embryogenic cells become hormone autonomous. The physiological conditions allowing the expression of totipotency should also be more clearly defined. The comparison of the molecular events following fertilization and somatic embryo induction is required. Similarly, the comparison of basic information gained with embryogenic competent plant cells and pluripotent animal stem cells can result in more general conclusions. Finally, the acquired knowledge should be converted into practical procedures improving the embryogenic response of plant cells.

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