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Review

Somatic embryogenesis — Stress-induced remodeling of plant cell fate ☆

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ABSTRACT

Plants as sessile organisms have remarkable developmental plasticity ensuring heir continuous adaptation to the environment. An extreme example is somatic embryogenesis, the initiation of autonomous embryo development in somatic cells in response to exogenous and/or endogenous signals. In this review I briefly overview the various pathways that can lead to embryo development in plants in addition to the fertilization of the egg cell and highlight the importance of the interaction of stress- and hormone-regulated pathways during the induction of somatic embryogenesis. Somatic embryogenesis can be initiated *in planta* or *in vitro*, directly or indirectly, and the requirement for dedifferentiation as well as the way to achieve developmental totipotency in the various systems is discussed in light of our present knowledge. The initiation of all forms of the stress/hormone-induced *in vitro* as well as the genetically provoked *in planta* somatic embryogenesis requires extensive and coordinated genetic reprogramming that has to take place at the chromatin level, as the embryogenic program is under strong epigenetic repression in vegetative plant cells. Our present knowledge on chromatin-based mechanisms potentially involved in the somatic-to-embryogenic developmental transition is summarized emphasizing the potential role of the chromatin to integrate stress, hormonal, and developmental pathways leading to the activation of the embryogenic program. The role of stress-related chromatin reorganization in the genetic instability of *in vitro* cultures is also discussed. This article is part of a Special Issue entitled: Stress as a fundamental theme in cell plasticity.

1. Introduction

Plants are sessile organisms and have an intricate relation with their environment as they are fully dependent on the specific resources and general life conditions present in their surroundings. As all of the environmental parameters are dynamic, plants have to ensure their continuous adaptation. The environment has a dramatic influence on plant form, function, and development resulting in extensive phenotypic plasticity [1]. In order to optimize resource exploitation as well as to overgrow harmful conditions, plants maintain the capacity for unlimited growth. This is due to sustained stem cell activity in specific locations of the plant body, the meristems. Meristematic plant stem cells perpetuate themselves by cell division and give rise to derivative cells that are the founders of cell files differentiating into new tissues and organs [2]. In the case of plants, organ formation is largely post-embryonic, continuous, and strongly influenced by the environment.

Plants are frequently exposed to herbivore and pathogen attacks as well as harsh environmental impacts (frost, storm, fire, *etc.*), and therefore exhibit extensive regeneration abilities to ensure their survival. Plants can heal local damages by tissue regeneration, but more remarkably they can replace whole organs by *de novo* organogenesis and, as the more extreme example, can regenerate the whole plant body even from a single cell through somatic embryogenesis [3–5]. These pathways can be readily induced under appropriate *in vitro* conditions in the case of many plant species [6]. Despite the fact that the regeneration ability of plants is widely exploited for the vegetative propagation of cultivated plant varieties, our present knowledge on the molecular background of the above regeneration pathways is rather scarce. However, the application of modern genetic, transcriptomic, epigenetic and cellular imaging approaches during the past few years has resulted in interesting insights into the molecular mechanisms underlying the regeneration ability of plants.

Plant somatic cells are considered to differentiate in a more flexible way as compared to those of animals. It is generally believed that differentiated plant cells under certain circumstances can revert to an earlier developmental state (dedifferentiate) and can regain pluri- or totipotency. Subsequently, the cells change their developmental fate under the influence of hormonal and environmental cues and regenerate new tissues, organs or the whole body. Until recently, this phenomenon was considered responsible for the extensive regeneration ability of plants. Accumulating data indicate, however, that under *in vitro* conditions shoot and root regeneration may also be initiated from adult stem cells present around the veins throughout the plant body [7]. This pathway is rather similar to the organ regeneration observed

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in the case of certain animals [8]. Although this finding raised a debate about the inherent pluri/totipotency of differentiated plant cells [8,9], it cannot explain all types of plant regeneration that most likely can follow various pathways [3,4,10] such as during somatic embryogenesis ([11]; and see Section 3 for details).

The induction of embryo development from differentiated plant cells (somatic embryogenesis) is the most extreme and therefore the most investigated but at the same time, probably the least understood type of plant regeneration. The plant-specific phenomenon of somatic embryogenesis is the strongest argument for the totipotency of differentiated plant cells. It is not really known, however, why and how differentiated plant cells re-acquire totipotency and/or the embryonic cell fate, and why this phenomenon is restricted only to certain genotypes, explants, or cells. More than two decades ago it was already recognized that cellular stress plays an important role in the cell fate switch leading to embryo development from differentiated cells and somatic embryogenesis was proposed being a developmental stress response [12]. This view is now widely accepted but the underlying mechanisms are still hardly known. In this chapter, which is dedicated to Dénes Dudits for his 70th birthday, I try to summarize present knowledge on the initiation phase of somatic embryogenesis highlighting the potential role of chromatin reorganization integrating stress, hormonal, and developmental pathways during this remarkable developmental switch.

2. Plant embryogenesis — variation on a theme

Unlike in animals, the life cycle of plants alternates between multicellular haploid (gametophyte) and diploid (sporophyte) generations. The fusion of haploid gametes generated by the female (embryo sac) and male (pollen) gametophytes, respectively, results in the formation of the zygote that develops into the diploid sporophyte through embryogenesis. However, the initiation of embryo development is not restricted to the fertilized egg cell in plants. In a number of plant species, embryo-containing seeds can develop from un-reduced embryo sac initials (gametophytic apomixis) or from somatic cells of the ovule (sporophytic apomixis) without egg cell fertilization [13]. During these processes, cells of the developing ovule recruit or hijack the molecular machinery of the sexual program so that meiosis and egg cell fertilization are not required for the asexual seed formation. In other plant species reproducing asexually, including *Kalanchoe* species [14,15], embryo-like propagules and plantlets develop at leaf margins. In addition to these natural ways of asexual embryogenesis, embryo development can be initiated *in vitro* from sporophytic [5,16] or gametophytic cells [17,18].

Once embryos are initiated due to any of the aforementioned pathways, their developmental program is rather similar and they go through analogous developmental phases although with some differences [15,19–22]. *In vitro* formed embryos are frequently larger and formed by a higher number of cells and have a less organized surface as compared to their zygotic equivalents [19,23–25]. This is most likely due to the lack of the maternal environment and the endosperm which are known to regulate embryo growth and development within the ovule [26,27]. In the absence of the surrounding seed tissues, the maturation of *in vitro* developing embryos also differs as they do not desiccate nor get dormant [19,20,22].

The initial events of embryogenesis also exhibit variability. Embryo development from the zygote starts with an asymmetric cell division [28]. Plant egg cells have an intrinsic polarity that is transiently lost and re-established following fertilization and becomes fixed due to the differential expression of homeotic transcription factors [29]. In this way, the first division of the zygote establishes the apical–basal axis of the plant: the apical cell develops into the embryo proper while the basal cell forms the suspensor and contributes to the development of the root meristem. Interestingly, while asymmetric division of embryogenic cells is frequently observed in the case of *in vitro* somatic embryogenesis [11,16], symmetric division is characteristic for embryogenic microspore-derived cells which otherwise would divide asymmetrically in order to differentiate into the cell types of the mature pollen [20]. Suspensor-like structures may arise as results of asymmetric division of somatic and the symmetric division of microspore cells, but they are often undeveloped or degenerated. The contribution of these suspensor-like cells to the development of the root pole in the case of *in vitro* grown embryos is unlikely [11]. Nevertheless, somatic or microspore embryos develop functional root meristems. The *Arabidopsis hanaba taranu* mutant exhibits altered auxin distribution in the embryo and develops a normal root meristem in a hypophysial cell-independent manner indicating that it is not the suspensor-derived lineage but a developmental auxin gradient that is important for root meristem establishment [30]. In this context it needs to be mentioned that the embryo-like structures developing at the leaf-margins of *Kalanchoe* species exhibit a defective root meristem and produce only adventitious roots that can be the consequence of their multicellular origin obviously avoiding suspensor development [15].

The various forms of embryogenesis also share key regulator genes/proteins [31,32]. These are multifunctional regulators of plant development including embryogenesis, like the homeotic transcription factor WUSCHEL (WUS), an important regulator of plant stem cell fate in the shoot meristem [33], the LEAFY COTYLEDON 1 and LEAFY COTYLEDON 2 (LEC1 and LEC2) and the AGAMOUS-LIKE 15 (AGL15) transcription factors associated with seed development and maturation [34], the BABY BOOM 1 (BBM1) transcription factor involved in the growth regulation of organ primordia [35], and the SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) associated with brassinosteroid signaling and morphogenesis [36]. Not only the key regulators are shared but the overall gene expression patterns of somatic and zygotic embryos are also comparable. The cotton somatic and zygotic embryo transcriptomes were compared in details and the expression patterns of genes associated with metabolism, cellular processes, and embryo development were found to be highly similar [25]. The main difference highlighted by this investigation was the effect of *in vitro* culture conditions on somatic embryo development characterized by the expression of a high number of stress-associated genes [25].

The above short summary indicates that plant embryogenesis may be initiated from different cell types in various *in planta* or *in vitro* environments and proceeds through generally the same developmental pathway that is affected, however, by the diverse (*in vitro* or maternal) environmental conditions. The main differences among the various embryogenic pathways are in the initiation phase. Somatic embryogenesis itself exhibits a large variation in this respect (Fig. 1). As it is discussed below, accumulating data support the view that somatic embryogenesis can start in several ways and most likely it is not only one molecular mechanism that can trigger embryo development in plants.

3. The many faces of the initiation phase of *in vitro* plant embryogenesis

It is well accepted that the developmental switch resulting in somatic embryogenesis is triggered in cells transiently exposed to strong stress and/or high non-physiological concentration of growth regulators. According to a generally accepted model, the inducing conditions result in the dedifferentiation of somatic plant cells followed or paralleled by the reacquisition of developmental totipotency. At this totipotent stage, the cells are competent to perceive appropriate developmental signal(s) that prompt the commitment toward embryogenesis, which thereafter proceeds autonomously under permissive *in planta* or *in vitro* conditions. Accumulating pieces of evidence indicate that this view is too much generalized and there might exist different pathways leading to somatic embryogenesis. In this respect, especially the differentiation state of the initial explant, and the direct, or indirect,