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Stress induces cell dedifferentiation in plants ☆

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ABSTRACT

Accumulating evidence lends support to the proposal that a major theme in plant responses to stresses is dedifferentiation, whereby mature cells acquire stem cell features (e.g. open chromatin conformation) prior to acquisition of a new cell fate. In this review, we discuss data addressing plant cell plasticity and provide evidence linking stress, dedifferentiation and a switch in cell fate. We emphasize the epigenetic modifications associated with stress-induced global changes in chromatin structure and conclude with the implications for genetic variation and for induced pluripotent stem cells in animals. It appears that stress is perceived as a signal that directs plant cells to undergo reprogramming (dedifferentiation) as a means for adaptation and in preparation for a stimulus-based acquisition of a new cell fate. This article is part of a Special Issue entitled: Stress as a fundamental theme in cell plasticity.

1. Cell dedifferentiation

Dedifferentiation has often been studied, both in plants and animals, with respect to cell cycle activity often leading to the erroneous assumption that cycling cells such as plant calli are essentially dedifferentiating cells [1,2]. The term dedifferentiation was initially coined to describe the reversal of cells from a given differentiated state into a more primordial state ('an indifferent embryonic cell type') as deduced from changes in cell shape and morphology [3,4]. Indeed, early reports on cellular dedifferentiation highlighted changes in cell morphology such as those that occur during limb regeneration or when mature cells are removed from their natural location in the *soma* and placed in a tissue culture environment. These changes were often interpreted as a sign that cells assume a more generalized embryonic state characterized by structural 'simplicity' [5,6]. Yet, in the absence of intrinsic features of dedifferentiating cells, the ultimate proof for dedifferentiation was the capacity of cells to differentiate into cell types other than that of the donor cell(s) [7].

Some of the inherent features of dedifferentiating cells were highlighted via the study of plant protoplasts, which provide a suitable experimental tool for studying dedifferentiation. Fully differentiated cells that are cemented in the framework of a leaf tissue, most of which are engaged in photosynthesis, can be easily separated by treatment with cell wall-degrading enzymes (e.g. cellulase) to yield a large population of protoplasts (plant cells devoid of cell walls). These cells are not yet committed to any specific fate but have acquired pluripotency demonstrated by their capability to differentiate into different cell types depending upon the type of stimulus supplied. Reentry into the cell cycle is induced by application of the phytohormones, auxin and cytokinin that give rise to cell proliferation and the formation of callus from which shoots and roots can be formed to yield fertile plants [8–10]. The study of protoplasts revealed one important feature of dedifferentiation, namely, an open chromatin conformation (also known as euchromatin or transcriptionally active/competent chromatin), which is often associated with the disruption of nucleolar structure and shutdown of rRNA gene transcription [11–14]. This decrease in rRNA gene transcription results in low production of ribosome subunits and a consequent reduction in protein synthesis, which is consistent with the quiescent nature of stem cells. Open chromatin conformation is currently recognized as an inherent feature characterizing dedifferentiation as well as the stem cell state in both plants and animals [2,15,16]. Notably, the open chromatin conformation of pluripotent cells was uncovered in earlier research, though overlooked by others, via electron microscopy examination of erythropoietic cells in animals or meristematic cells in plants [17–20]. The importance of chromatin conformation for the establishment of the stem cell state in plants is demonstrated by the overrepresentation of chromatin modifier genes (CMGs) in *Arabidopsis* shoot apical meristem cells. Accordingly, transcriptome analysis performed by Yadav et al. [21] revealed that cells within the meristem territory including the central zone, the rib meristem and the peripheral zone display unusual expression of CMGs; out of the 445

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CMGs present on the ATH1 array, these three cell types express (>256 expression signal) 297, 283 and 282 CMGs, respectively [16].

2. Switching cell fate in plants

Dedifferentiation underlies cell plasticity, that is, the capacity of mature differentiated cells to switch fate. Switching fate can occur as an integral part of the normal developmental program of plants. One example is the development of secondary meristems such as the interfascicular cambium from parenchyma cells located between vascular bundles and the cork cambium that is generated via switching fate of parenchyma or collenchyma cells located beneath the epidermis [22]. Tissue culturing is perhaps the most studied example of switching fate in plants as mature differentiated cells placed in a tissue culture environment with appropriate phytohormones can be induced to reenter the cell cycle, proliferate and often form callus from which shoots and roots as well as embryos can be formed to generate fertile plants [23–25]. In this respect, stress has long been known to induce somatic embryogenesis in a variety of plant species [26]. Another well-known example, is the formation of regenerative xylem as well as phloem anastomoses – naturally-occurring regenerative sieve tubes – from cortical parenchyma cells following wounding [27,28]. Accordingly, wounding of *Cucurbita* and *Arabidopsis* stems that disrupts vascular bundle continuity results in the formation of regenerative xylem and sieve tubes from interfascicular parenchyma cells that restores bundle functionality. However, switching cell fate is most common in plants following exposure to biotic and abiotic stresses. For instance, various stresses such as drought or an excess/shortage of nutrients as well as phytohormone application are sensed by the plant leading to significant changes in root architecture [29]. Most apparent is an increase in lateral root formation from a subset of root pericycle cells that generate a new meristem as well as an increase in the number and length of root hairs via redifferentiation of existing root epidermal cells to trichoblasts [29–31]. It has been suggested that reprogramming of specific target cells in response to stress (e.g. P depletion) is fundamental for stress adaptation [30].

Cell-fate switch is most noticeable under biotic stress. There are numerous examples demonstrating the dramatic effect of insects, mites and pathogenic organisms or symbionts including bacteria, fungi and viruses on plant biology. The effects of these organisms can manifest as abnormal growths often referred to as nodules, galls, tumors or neoplasms [32,33]. The most studied examples include the crown gall-forming disease in eudicots caused by *Rhizobiaceae* species, that is, *Agrobacterium tumefaciens* (updated name, *Rhizobium radiobacter*), first reported more than a century ago by Smith and Townsend who studied a naturally occurring tumor or gall on cultivated *Argyranthemum frutescens* (Paris daisy) [34]. Another example is *Rhizobium leguminosarum*, the nitrogen-fixing legume symbiont that induces dedifferentiation and cell divisions in root cortical cells leading to formation of root nodules [35,36]. Plant-parasitic cyst nematodes are known to induce the formation of a syncytium (a multinucleated cell) that involves the redifferentiation and fusion of hundreds of root cells to generate novel plant cell types that serve as a unique feeding site [37].

3. Chromatin structure

Switching fate is a dramatic event in the life of a cell and a very complex process, which requires the acquisition of competence to switch fate (i.e. dedifferentiation) followed by signal-dependent execution of the new cell fate. Conceivably, this process requires extensive reorganization of chromatin to bring about repression of genes related to the previous differentiated state and activation of other genes for driving the ensuing fate of the cell. The basic structural unit of chromatin is the nucleosome, which comprises DNA wrapped around a core histone octamer composed of two of each of histones H2A, H2B, H3 and H4. Core histone proteins possess a common structural motif, the histone fold, which is necessary for the interactions between core histone proteins and duplex DNA [38]. The X-ray crystal structure of the nucleosome reveals that the histone amino-terminal tails are unstructured and protruding outside the nucleosomal disk [39] where they can contact neighboring nucleosomes. The N-terminal tails can undergo multiple types of reversible chemical modifications, including acetylation, methylation and phosphorylation, on multiple amino acid residues. These reversible modifications impart chromatin with the capacity to change conformation upon perceiving a signal via recruitment of proteins that dictate a particular chromatin structure that is either transcriptionally active or transcriptionally inactive. Beside histones, the DNA itself can be chemically modified by methylation at position 5 of the pyrimidine ring of cytosine [40]. The dynamics of DNA and histone modifications are driven by the activities of several groups of enzymes that add or remove a specific chemical group (e.g. methyl, acetyl) from the DNA or from the histone proteins. Addition of a methyl group to DNA is catalyzed by various DNA methyltransferases, while removal of the methyl group is generally carried out by a base excision repair pathway induced by the activity of the DME/ROS1 family of DNA glycosylases or by the activity of 5-methylcytosine deaminases that convert 5-methyl-C to thymidine. The activity of the latter enzyme is coupled with G/T mismatch DNA glycosylase activity that corrects the G/T mismatch [41]. Acetyl groups are added to histones by histone acetyltransferases (HATs) and are removed by histone deacetylases (HDACs), while methyl groups are added by a large group of histone methyltransferases (SET domain-containing proteins) and removed by the activities of lysine-specific demethylase 1 (LSD1) and the jumonji class of histone demethylases [42–44].

Histone modifications can affect promoter activity and in general, gene promoters can be found in three basic states that are determined, at least partly, by such histone modifications. These include a restrictive/inactive state established by repressive epigenetic marks (e.g. trimethylated H3K9/K27), a permissive/active state determined by epigenetic marks such as trimethylated H3K4 and acetylated H3K9, and promoters that possess 'bivalent' restrictive and permissive epigenetic marks simultaneously (e.g. trimethylated H3K27 and trimethylated H3K4). This unique state is found in some non-expressed genes or genes expressed at low levels in human embryonic stem cells [45,46] suggesting that in these cells, tissue-specific regulatory genes may be 'primed' for transcription but held in check until cells perceive a specific signal for differentiation that dictates either gene activation or gene silencing [47]. Interestingly, 'bivalent' domains appear to be widespread in the genomes of somatic cells of *Arabidopsis* and rice where a large proportion of genes possessing the repressive marks (e.g. H3K27me1 or H3K27me3) also contain permissive marks such as H3K4me3 and acetylated H3K9 [48–50]. This is particularly relevant for plants whose sessile life style has led to the development of short and long term mechanisms to cope with and survive the challenging environment.

4. Does stress induce dedifferentiation?

The idea that stress might induce plant cells to acquire stem cell properties emerged from the transcriptome analysis of dedifferentiating protoplast cells. Comparison of the protoplast gene expression profile with available databases reveals unexpected similarity with the expression profile of *Arabidopsis* leaves induced to senesce prematurely by exposure to the dark [51,52]. Indeed, exposing tobacco plants to the dark for extended periods results in leaves turning yellow accompanied by chromatin decondensation and the disruption of nucleolar structure—properties of stem cells [52]. Similarly, decondensation of pericentric heterochromatin has been reported during late stages of leaf senescence in *Arabidopsis* [53]. Furthermore, *Arabidopsis* mesophyll cells respond to a variety of stress conditions including dark, heat and wounding by activation of the meristem-specific *ANAC2* promoter along with pericentric chromatin decondensation [14]. Thus, it appears that a general response of plant cells to acute stress that often induces premature