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Historical review of research on plant cell dedifferentiation

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Abstract Plant cell dedifferentiation has long attracted interest as a key process for understanding the plasticity of plant development. In early studies, typical examples of plant cell dedifferentiation were described as physiological and cytological changes associated with wound healing or regenerative development. Subsequently, plant tissue and cell culture techniques, in which exciting progress was achieved after discovery of the hormonal control of cell proliferation and organogenesis in vitro in the 1950s, have been used extensively to study dedifferentiation. The pioneer studies of plant tissue/cell culture led to the hypothesis that many mature plant cells retain totipotency and related dedifferentiation to the initial step of the expression of totipotency. Plant tissue/cell cultures have provided experimental systems not only for physiological analysis, but also for genetic and molecular biological analysis, of dedifferentiation. More recently, proteomic, transcriptomic, and epigenetic analyses have been applied to the study of plant cell dedifferentiation. All of these works have expanded our knowledge of plant cell dedifferentiation, and current research is contributing to unraveling the molecular mechanisms. The present article provides a brief overview of the history of research on plant cell dedifferentiation.

Keywords Dedifferentiation · History · Tissue culture · Totipotency

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Introduction

The individual body of a multicellular organism, either animal or plant, contains multiple types of tissues, each consisting of cells with a specific morphology and function. All of these cells are derived from the unicellular zygote through proliferation and stepwise specialization, i.e., cell differentiation, often mediated by the stem cell stage(s). Mature differentiated cells stably maintain their specialized characteristics but in some cases these attributes are totally or partially lost and the cells return to a more juvenile state by the process termed dedifferentiation. Although dedifferentiation can be induced in both animal and plant cells, it occurs more readily in a wider range of cells in plants than in animals, reflecting the rather unstable and plastic determination of cellular characters in plants (Buvat 1989). Dedifferentiation of plant cells was well recognized by the early twentieth century (Child 1912). Since then, plant cell dedifferentiation has attracted much interest in the context of the plasticity that characterizes plant development. Research on plant cell dedifferentiation greatly expanded with development of tissue and cell culture techniques and the more recent introduction of novel concepts and technologies. Here I present a historical review of the trends of studies on plant cell dedifferentiation, keeping in mind conceptual changes and variation.

Early studies of plant cell dedifferentiation

When plants are injured by physical means such as cutting, in appropriate conditions, cells adjacent to the injury site proliferate to form soft tissue cushion called callus, which covers the cut surface. Callus formation is often accompanied by cork formation or tissue reunion, and is sometimes

followed by organogenesis (Blackman and Matthaei 1901; Buvat 1989; Kostoff 1928). Cellular changes during such wound healing processes were investigated as cell dedifferentiation in early studies (Bloch 1941, 1952).

Histological observations of wound responses including callus formation had been carried out already in the late nineteenth century and were described in several sections of textbooks of plant physiology and anatomy published in the 1870s and 1880s (Sachs 1887; Thomé 1879; Tschirch 1889). In these sections, however, the word "dedifferentiation" (*Entdifferenzierung* in German) or a dedifferentiation-like concept did not appear, implying the absence of the established view relating the wound response of plants to dedifferentiation at this time. In 1912, however, Child stated, with respect to dedifferentiation induced by tissue isolation and involved in regenerative development, "it has long been known that in plants many cells are capable of undergoing dedifferentiation" and "the occurrence of dedifferentiation has been accepted by the botanists as a fact for many years" (Child 1912). It seems therefore that, from the end of the nineteenth century to the beginning of the twentieth century, recognition of wound-induced changes of plant cells as a typical example of dedifferentiation was expanded and became widely accepted.

In the following decades, physiological and cytological processes associated with wound healing in plants were investigated in more detail (Blackman and Matthaei 1901; Bloch 1935; Buvat 1989). Findings obtained from these studies were reviewed by Bloch (1941, 1952), who featured dedifferentiation as an important aspect of the wound reactions of plant cells, and defined dedifferentiation as a "process by which mature or specialized cells lose their differentiated character and rejuvenate". Dedifferentiation-related cellular events described in these articles were cell wall extension, mitochondrial changes, nuclear growth and division, and cell division. The description of nuclear growth largely depended on observations made on regenerating moss and liverwort tissues (Heitz 1925) and on wounded leaves of *Peperomia blanda* (Fischer 1934). The other cytological descriptions were mostly based on the studies by Buvat with various kinds of plant tissues, which were originally published in the 1940s and later summarized together with subsequent works in a book chapter by the author (Buvat 1989).

Bloch's reviews devoted many paragraphs to the problem of what types of plant cells are capable of reacting to wound stimuli to dedifferentiate (Bloch 1941, 1952). From a search of the literature and his own observations, Bloch concluded that most mature plant cells, unless degenerated, could more or less dedifferentiate, although the reactivity varied considerably among cell types. In this respect, particular emphasis was placed on the occurrence of dedifferentiation in large, vacuolate cells and in cells with thickened and even lignified walls (Bloch 1926). It is noteworthy that dedifferentiation was not considered to be an irregular process only induced under externally stressed conditions but was commented not to be infrequent in the normal course of plant development. These discussions led to the view that plasticity is characteristic of plant development.

At this time the compound that mediates wound-induced dedifferentiation was also of major interest to researchers. This "wound hormone" had long been assumed to be produced in injured sites and induce callus formation in the neighboring regions. Haberlandt (1921) tested experimentally the wound hormone hypothesis and obtained supporting evidence, which initiated the quest to identify the wound hormone. A bioassay system with bean pod tissues was particularly effective in research into the wound hormone (Bonner and English 1938), leading to identification of the wound hormones as fatty acid derivatives named traumatic acid and traumatin (English et al. 1939; Zimmerman and Coudron 1979). However, given the lack of evidence for generality of their function in a wide range of plant species, nowadays these substances are not considered to be important factors in the wound induction of plant cell dedifferentiation.

Development of in vitro culture techniques and their application to research on dedifferentiation

The early history of plant tissue culture is well documented (Gautheret 1983; White 1936, 1946). The origin of plant tissue culture was traced to a paper by Haberlandt, which first formulated clearly the idea of culturing isolated vegetative cells of higher plants to cast light on the characteristics and capabilities intrinsic to the plant cell as an autonomous "elementary organism" (Haberlandt 1902). Subsequently, for about 40 years, however, plant tissue cultures fully meeting Harberlandt's concept, in which cells can proliferate unlimitedly not as a part of organs but as cells free from organs, could not be experimentally confirmed despite extensive efforts. During this period, although culture media and aseptic culture methods were much improved, in vitro culture for an extended period was only possible with meristematic materials such as root tips and buds, which can be regarded as organ culture rather than tissue culture (Gautheret 1983; White 1936, 1946).

Successful plant tissue culture was achieved independently by three researchers in the late 1930s. In 1939, White reported unlimited growth of cells derived from proliferating procambial tissue of a hybrid of *Nicotiana glauca* × *N. langsdorffii* in an artificial nutrient condition (White 1939). The material used in this experiment was a type of genetic tumor. At almost the same time, Gautheret

and Nobécourt achieved long-term maintenance of cell proliferation in tissue cultures initiated from normal tissues of carrot by incorporating in the culture medium indole-3-acetic acid (IAA), which had been identified as auxin 5 years previously (Gautheret 1939; Nobécourt 1939).

In the 1950s, identification of an additional phytohormone, cytokinin, dramatically changed plant tissue culture strategies. Using an IAA-supplemented tissue culture of tobacco as a bioassay system, Skoog and coworkers isolated the first-known cytokinin, kinetin, as a cell-division-promoting substance from DNA degradation products (Miller et al. 1955, 1956). Examination of the effects of various concentrations of IAA and kinetin on tobacco pith tissue culture showed that both kinetin and IAA were required for stimulation of cell proliferation. In addition, high and low ratios of kinetin to IAA favored shoot and root formation, respectively, whereas in the presence of high concentrations of both kinetin and IAA, unorganized growth of cell mass was promoted (Skoog and Miller 1957). In subsequent work, kinetin, IAA, and other cytokinins and auxins were tested in a variety of tissue culture systems and plant species, and substantially similar effects were observed in many cases (for relatively early examples, see Bonnett and Torrey 1965; Engvild 1973; Schraudolf and Reinert 1959; Wolter 1968). These studies indicated that the balance of phytohormones, especially cytokinin and auxin, is a major determinant of cell proliferation and morphogenesis in plant tissue culture. While phytohormone applications became widespread in tissue culture, the term "callus", originally used for a cushion of proliferating cells formed during wound healing, was extended to include unorganized masses of dividing cells induced and maintained in the presence of exogenous phytohormones. Phytohormone-induced callus formation in tissue culture as well as wound-induced callus formation have been generally considered to involve cell dedifferentiation.

Temporal requirements for exogenously applied phytohormones during in vitro organogenesis were utilized for physiological dissection of the processes of tissue culture responses. The first systematic experiments were conducted with tissue culture of *Convolvulus arvensis*, in which leaf explants were cultured with various compositions of cytokinin and auxin and transferred onto media with different phytohormone compositions at various times (Christianson and Warnick 1983, 1985). Based on the results obtained, Christianson and Warnick divided the process of organogenesis in vitro into three phases: the first phase is acquisition of competence, in which cells become able to respond to organogenesis induction signals, i.e., competent for organogenesis, under a relatively broad range of phytohormone conditions; the second phase involves organogenesis induction, in which competent cells are canalized and determined by an appropriate phytohormone balance for a specific pathway of organogenesis; and the third phase is morphological differentiation, in which organ development occurs independently of external phytohormones (Christianson and Warnick 1985). The primary phase apparently corresponds to cell dedifferentiation, and thus Christianson and Warnick's physiological dissection approach presented a novel experimental distinction of dedifferentiation from the other phases, and has been successfully applied to tissue cultures of several plant species including the model plant *Arabidopsis thaliana* (Arabidopsis). To establish a procedure for *Agrobacterium tumefaciens*-mediated transformation of Arabidopsis, a two-step tissue culture technique, consisting of pre-culture with a high concentration of auxin and subsequent culture with a high concentration of cytokinin, was developed for efficient induction of shoot regeneration (Akama et al. 1992; Valvekens et al. 1988). In this culture system, as the pre-culture not only activates cell proliferation to initiate callus formation but also enhances regenerative responses to the subsequent culture, cells are considered to dedifferentiate to acquire the competence for organogenesis during the pre-culture period.

Organogenesis in tissue culture relies on de novo formation of the shoot or root apical meristems, which produce the aboveground or belowground organs, respectively, but not the entire plant body. Although both the aboveground and belowground organs can be regenerated from a tissue culture system by sequential induction of shoot organogenesis and root organogenesis, the regenerants lack cotyledons and hypocotyls. Induction of regeneration of the entire plant body became possible after the discovery of somatic embryogenesis, an additional regenerative pathway distinct from organogenesis. Somatic embryogenesis in vitro was first reported in the late 1950s (Reinert 1959; Steward et al. 1958). In these studies embryo-like structures, later called somatic embryos, were formed from cultured carrot cells and developed into complete plantlets. This finding contributed to establishment of the concept that a somatic plant cell retains the potential to differentiate into all cells that constitute a whole plant body, i.e., totipotency. However, it was unclear whether somatic embryos were of unicellular or multicellular origin. Vasil and Hildebrandt (1965) showed that single cells dissociated from pith-derived callus of tobacco proliferated to form cell masses and eventually gave rise to rooted plantlets, but in this case the developmental process from cell mass to plantlet was not determined. The final proof for this question was obtained five more years later in the Reinert laboratory (Backs-Hüsemann and Reinert 1970). Serial observation of morphogenesis starting from a single cell released in a cell-suspension culture of carrot provided direct evidence that a single cell could develop into a somatic embryo and thus demonstrated totipotency of a somatic plant cell.

Since the early observations of Steward, Reinert and their co-workers, somatic embryogenesis has been reported for many plant species, and a variety of protocols for induction of somatic embryos have been described (Thorpe and Stasolla 2001; Williams and Maheswaran 1986). Tissue culture-induction pathways for somatic embryogenesis may be either direct or indirect (Williams and Maheswaran 1986). Direct somatic embryogenesis is induced on the surface of tissue explants without an intervening callus stage. For this induction, treatment with diverse stresses, such as high osmotic pressure, heavy metal ions, NaCl, wounding, or extreme concentrations of auxin, are highly effective (Dudits et al. 1995; Harada et al. 1990). In many systems for indirect somatic embryogenesis, tissue is first cultured on medium that contains a high concentration of auxin to activate cell division and induce generation of embryogenic cells, and then the resultant cell mass is transferred to medium without or with a reduced level of auxin, on which somatic embryo development occurs (Thorpe and Stasolla 2001). In both direct and indirect procedures, the initial phase of somatic embryogenesis involves cell dedifferentiation. In response to an external stimulus, somatic cells dedifferentiate and acquire embryogenic competence prior to the commitment to embryogenesis (Fehér et al. 2003; Namasivayam 2007).

Several plant tissue and cell culture methods were developed not for cell proliferation or plant regeneration but for xylogenesis as model systems for studying cytodifferentiation. In most xylogenic cultures, quiescent cells of parenchymatous tissues are induced to differentiate into tracheary elements by application of auxin and cytokinin (Sugiyama and Komamine 1990). The transdifferentiation process from mesophyll cells to tracheary elements is considered to consist of three stages: dedifferentiation, during which mesophyll cells lose their capability for photosynthesis; canalization of cell fate from procambial initials to tracheary element precursor cells; and differentiation into tracheary elements (Fukuda 1997). Therefore, findings obtained from studies with xylogenic cultures provide insights into the regulation of dedifferentiation as well as tracheay element-specific differentiation events. For example, isolated mesophyll cells of *Zinnia elegans* transdifferentiate into tracheary elements independent of cell-cycle progression (Fukuda and Komamine 1981), which implies that dedifferentiation can be decoupled from the cell cycle.

To end this section, as an in vitro experimental system particularly suitable for studying plant cell dedifferentiation, special note is warranted of cultures of protoplasts, i.e., individual cells devoid of their cell walls. The first landmark for protoplast culture was development of a method for enzymatic isolation of protoplasts by digestion of cell walls with cellulase (Cocking 1960). In 1970, cell wall regeneration and cell division were observed in protoplasts isolated from the mesophyll of tobacco and suspension-cultured cells of soybean (Kao et al. 1970; Nagata and Takebe 1970). In 1971, Takebe and colleagues succeeded in plant regeneration from tobacco mesophyll protoplasts (Nagata and Takebe 1971; Takebe et al. 1971). It can be emphasized that, in this experiment, more than 80 % of the protoplasts gave rise to colonies capable of regeneration into whole plants (Nagata 1985). This work thus provided a further demonstration of the high potential for differentiation of single isolated plant cells. The protoplast culture technique has been used for diverse applications, including production of somatic hybrid plants through protoplast fusion (Melchers et al. 1978). Protoplasts also serve as physiological tools for basic plant scientific research (Galun 1981). Of particular importance, primary cultures of protoplasts isolated from fully differentiated tissues such as leaf mesophylls offer experimental platforms advantageous for analysis of the dedifferentiation process because, in such protoplast cultures, pronounced changes considered to be associated with dedifferentiation are induced rapidly with reasonably good synchrony in a relatively homogenous population of single cells during protoplast isolation and in response to phytohormones before the commencement of cell division (Galun 1981; Jiang et al. 2013).

Modern approaches to elucidating molecular mechanisms of dedifferentiation

Molecular-level mechanistic studies of plant development arose after the inception of model plants suitable for molecular genetic research and establishment of reliable methods of plant transformation in the 1980s, and subsequently have been expanded by adoption of new technologies and novel concepts. Modern molecular approaches have been applied in research on plant cell dedifferentiation, which has yielded many important findings on a variety of aspects of dedifferentiation particularly in the last decade. As these findings are reviewed and discussed in several recent articles (Grafi and Barak 2014; Ikeuchi et al. 2013; Jiang et al. 2013; Neelakandan and Wang 2011; Sugimoto et al. 2011) and other articles in the present issue, I will not discuss this work in detail and instead describe the major trends in current research on plant cell dedifferentiation in the following sections.

Molecular genetic analysis of tissue culture responses

Among closely related species, and even among ecotypes or cultivars of the same species, tissue culture responses are sometimes highly variable, which might be partly attributable to genetic differences in dedifferentiation capability. Genetic aspects of variation in tissue culture responses

have been studied mostly in crops (Bolibok and Rakoczy-Trojanowska 2006; Henry et al. 1994; Sugiyama 2000). For example, in tomato the genetic basis underlying the superior regeneration capacity introduced from *Lycopersicon peruvianum* was investigated and the chromosomal location was determined for the most influential allele *Rg-1* using restriction fragment length polymorphism (RFLP) analysis (Koornneef et al. 1987, 1993). Unlike this example, it is often difficult to account for genetic variation in tissue culture response by simple Mendelian inheritance because the traits are under quantitative control by many genes rather than qualitative control by a few genes (Bolibok and Rakoczy-Trojanowska 2006). Therefore, quantitative trait locus (QTL) mapping has been widely used, usually in combination with RFLP or other DNA-polymorphism analysis, for genetic studies of tissue culture responses. Particularly in rice and barley, QTL analyses have been performed extensively in relation to the capability for callus formation and shoot regeneration by in vitro culture systems. Several major QTLs have been detected in both species (Komatsuda et al. 1993; Mano and Komatsuda 2002; Takeuchi et al. 2000; Mano et al. 1996; Taguchi-Shiobara et al. 1997). Of these QTLs, only one QTL gene of rice has been isolated and shown to encode ferredoxin-nitrite reductase (Nishimura et al. 2005). Natural variation in shoot regeneration capability among Arabidopsis ecotypes also has been subjected to QTL analysis, which recently identified a gene encoding a receptor-like kinase as a previously unknown determinant of shoot regeneration (Motte et al. 2014).

Genetic studies focused more on understanding the basic aspects of tissue culture responses have been carried out with mutants of Arabidopsis (Sugiyama 2000, 2014). Many temperature-sensitive mutants of Arabidopsis impaired in organogenesis in vitro were isolated from a mutagenized population and used for genetic dissection of the process from dedifferentiation to organ regeneration (Konishi and Sugiyama 2003; Ozawa et al. 1998). Through identification of the mutated genes in these mutants and their molecular characterization, several gene functions required for dedifferentiation and organogenesis have been revealed (Sugiyama 2014). This analysis suggests that different levels of RNA processing factors involved in pre-mRNA splicing and pre-rRNA processing reflect different levels of competence for cell division, and that an increase in the expression of those factors constitutes an essential part of dedifferentiation of incompetent cells (Ohbayashi et al. 2011; Ohtani and Sugiyama 2005; Ohtani et al. 2013).

Epigenetic analysis of dedifferentiation

Nuclear enlargement was recognized as an important character of dedifferentiating plant cells already in early studies (Fischer 1934; Heitz 1925). For a long time, however, research on the nuclear changes during dedifferentiation was limited and mostly had a cytological focus (e.g., Feldman and Torrey 1977; Jordan et al. 1987). Only in the last decade has the molecular basis of the dedifferentiation-associated nuclear changes been extensively investigated in plants.

The main sources of our present knowledge on this issue are chromatin and epigenetic analyses with mesophyll protoplasts of tobacco, Arabidopsis, and cucumber (Avivi et al. 2004; Grafi et al. 2007; Ondřej et al. 2009; Tessadori et al. 2007; Williams et al. 2003; Zhao et al. 2001). These studies show that large-scale chromatin reorganization, including drastic decondensation of heterochromatin domains, occurs during preparation of protoplasts before administration of phytohormones. While chromatin reorganization in protoplasts is accompanied by an increase in acetylation at the K9 and K14 residues of histone H3, one of the epigenetic markers of transcriptionally active chromatin (Williams et al. 2003), it is not accompanied by a remarkable decrease in the global levels of DNA methylation and histone H3K9 dimethylation, which are epigenetic markers of heterochromatin (Tessadori et al. 2007). Nevertheless, local alterations were detected in DNA methylation patterns, which might participate in the activation of expression of specific genes in dedifferentiating protoplasts (Avivi et al. 2004).

The importance of nuclear changes at the chromatin level in plant cell dedifferentiation also has been indicated by analysis of mutants impaired in histone modifiers and chromatin remodelers. A well-known example is the Arabidopsis *pickle* (*pkl*) mutant. *PKL* encodes an ATP-dependent chromatin remodeler of the CHD3 subfamily (Ogas et al. 1999). Roots of the loss-of-function mutant of this gene display embryonic characters, and form callus and somatic embryos when excised and cultured on phytohormone-free medium (Ogas et al. 1997). On the basis of these phenotypes, PKL was inferred to repress the embryonic identity and prevent dedifferentiation though regulation of chromatin structure. Recent studies have investigated the roles of PKL, other chromatin remodelers, histone modifications, and gene transcription in cell identity determination and their mutual relationships (Aichinger et al. 2009; Bouyer et al. 2011). The results of such studies are helpful to understand how chromatin controls differentiated and undifferentiated states of plant cells.

Analysis of dedifferentiation from a cell-cycle perspective

Reactivation of cell division, i.e., re-entry into the cell cycle, is not always, but is generally, a characteristic part of plant cell dedifferentiation. Therefore, components of the cell-cycle machinery can be considered to have important roles in dedifferentiation. In a strict sense, dedifferentiation from a cell-cycle perspective is confined to the process of

preparation for the cell-cycle re-entry prior to cell division, and thus changes in the cell-cycle regulators in non-proliferative cells may provide insights into their possible roles in dedifferentiation. From this point of view, notable are CDKA;1, an A-class cyclin-dependent kinase, and D-type cyclins, CycD1 and CycD3, among core regulators of the cell cycle. In Arabidopsis and radish, active expression of *CDKA;1* is detectable not only in dividing cells but also in non-dividing cells of some root tissues such as the pericycle and stelar parenchyma, which are generally regarded to have the capacity to reinitiate cell proliferation readily in response to a mitotic stimulus (Hemerly et al. 1993; Martinez et al. 1992). In addition, expression of *CDKA;1* is rapidly induced in quiescent tissues by wounding, a common trigger of dedifferentiation (Hemerly et al. 1993). These patterns link *CDKA;1* expression with competence for cell proliferation (Hemerly et al. 1993), which is reinforced by genetic dissection of the dedifferentiation process (Ozawa et al. 1998). In cell suspension cultures of Arabidopsis that have been starved of three growth requirements, namely a carbon source, auxin, and cytokinin, expression of *CycD2* and *CycD3* is induced by addition of a carbon source alone and cytokinin alone, respectively (Soni et al. 1995). This result indicates that, similar to *CDKA;1*, the *CycD* genes are expressed in the preparatory stage of cell division and thus are associated with dedifferentiation. Furthermore, overexpression of *CycD3* is capable of replacing cytokinin for induction of callus in Arabidopsis tissue culture (Riou-Khamlichi et al. 1999), suggesting that cytokinin-dependent *CycD3* expression is crucial for re-entry into the cell cycle.

Cyclins and CDKs are modulated by many other factors (Dewitte and Murray 2003). An important dedifferentiation-regulatory role is reported for Kip-related proteins (KRPs), a group of modulators that are characterized by the ability to bind CDKs and negatively affect their functions. Overexpression and gene-silencing analysis of *KRP* genes of Arabidopsis indicate that these KRPs downregulate cell proliferation and prevent dedifferentiation (Anzola et al. 2010). More importantly, it was also demonstrated that auxin-dependent *KRP* expression is controlled through chromatin rearrangement by the chromatin remodeler PROPORZ1 (PRZ1). Following this and other recent findings, chromatin-level regulation of the plant cell cycle is now of great interest (Raynaud et al. 2014).

Proteome and transcriptome analysis of dedifferentiation

Proteins and mRNAs that are specifically expressed in association with a certain phenomenon of interest are usually suitable markers for molecular characterization of the phenomenon. Such molecular markers for plant tissue culture responses were searched for initially by comparison of electrophoretic patterns, subtractive hybridization, or differential screening. Most such studies were focused on the processes of organ regeneration and somatic embryogenesis, and comparatively little attention has been paid to the dedifferentiation phase (De Klerk et al. 1997; Komamine et al. 1992; Zimmerman 1993). Nevertheless, a number of dedifferentiation-related proteins and mRNAs were identified in the 1980s and 1990s from this research (Ramagopal 1989, 1994; Schmidt et al. 1997; Takahashi and Nagata 1992a, 1992b; Takahashi et al. 1989). Of these molecular markers, *par* genes identified in tobacco mesophyll protoplast cultures are characterized by auxin-dependent expression and temporal expression prior to the S phase of the first round of resumed cell division, and therefore are considered to participate in the G_0-to-G_1 or G_1-to-S phase transition linked with auxin-stimulated dedifferentiation (Nagata et al. 1994). In embryogenic cultures of carrot, expression of *Somatic Embryogenesis Receptor-like Kinase* (*SERK*) is correlated with competence for somatic embryogenesis (Schmidt et al. 1997). Transgenic experiments with the *SERK* homolog of Arabidopsis (*AtSERK*) confirm that *SERK* enhances embryogenic competence in cultures and thus is functionally associated with the acquisition of competence during dedifferentiation (Hecht et al. 2001).

More recently, new technologies such as microarray analysis, RNA-seq by next generation sequencing, and mass spectrometric characterization of proteins have been applied to comprehensive profiling of transcriptomes and proteomes in various in vitro plant culture systems. The most extensive studies with a focus on dedifferentiation have been performed with callus-inducing cultures and protoplast cultures of Arabidopsis (Che et al. 2006; Chitteti and Peng 2007; Chitteti et al. 2008; Chupeau et al. 2013; Damri et al. 2009; Liu et al. 2010; Sugimoto et al. 2010; Xu et al. 2012). Comprehensive gene expression data associated with dedifferentiation have been obtained not only from protoplast and callus cultures but also from studies of various reprogramming phenomena involving dedifferentiation. Examples are transcriptome analyses of transdifferentiation of *Zinnia* mesophyll cells into tracheary elements (Demura et al. 2002), root nodule formation in the legume *Medicago truncatula* (Lohar et al. 2006), incision and tissue reunion of the Arabidopsis stem (Asahina et al. 2011), and chloronema regeneration from excised leaf tissues of the moss *Physcomitrella patens* (Nishiyama et al. 2012).

These transcriptome or proteome profiling studies show that global changes occur in gene/protein expression patterns in association with plant cell dedifferentiation. They have also identified many genes and proteins that may be involved in the regulation of dedifferentiation, some of which will be mentioned below. In addition, the omics data sets have enabled researchers to make overall comparisons between seemingly disparate physiological

processes, which have sometimes yielded remarkable results. For instance, transcriptome profiles of dedifferentiating protoplasts and senescing leaf cells of Arabidopsis show striking similarities (Damri et al. 2009). This finding, together with several other lines of evidence, has led to the hypothesis that a common response of plant cells to stresses converges on dedifferentiation (Grafi et al. 2011). Transcriptome analysis indicates that callus induced by phytohormones from various organ segments of Arabidopsis is enriched with root tip-expressed transcripts, and genetic analysis suggests that callus initiation and lateral root formation are under the same genetic control as pericycle cell division (Sugimoto et al. 2010). These findings raise the question of whether plant callus formation necessarily involves the dedifferentiation process (Sugimoto et al. 2011).

Analysis of transcriptional regulation of dedifferentiation

Transcriptional regulation is a major focus of recent research on plant cell dedifferentiation and considerable effort has been applied to identify transcription factors that control dedifferentiation. We currently have a list of more than 20 transcription factors of Arabidopsis for which functional involvement in dedifferentiation is experimentally suggested by their inductive or suppressive effects on callus initiation (Ikeuchi et al. 2013). A portion of these transcription factors are critical regulators of embryonic cell fate or meristematic stem cell identity, such as LEAFY COTYLEDON1 (LEC1) and WUSCHEL (WUS). Ectopic overexpression of the genes for these transcription factors induces somatic embryogenesis and callus formation (e.g., Lotan et al. 1998; Zuo et al. 2002). In *Physcomitrella patens*,

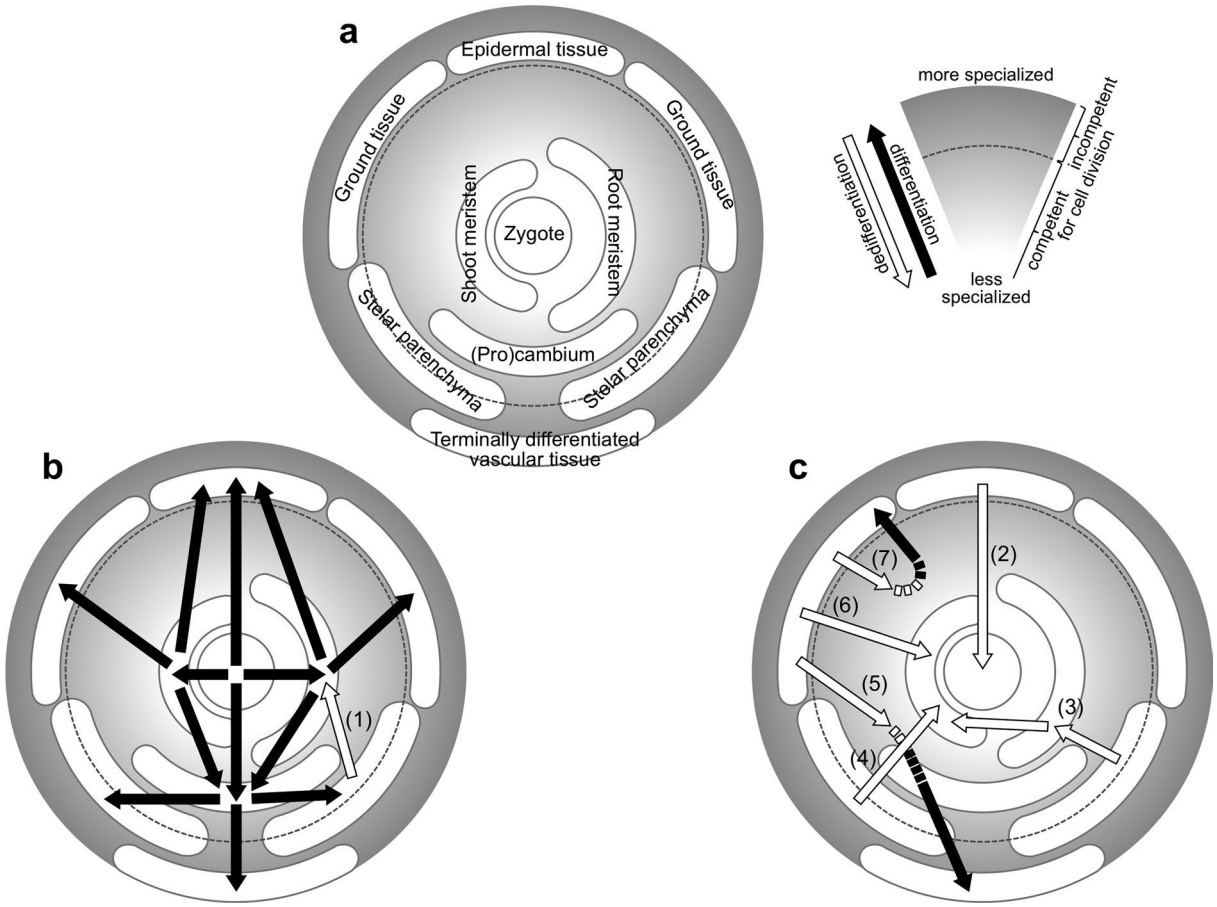

Fig. 1 Schematic illustration of various processes involving differentiation and dedifferentiation of plant cells. **a** Map of plant cells and tissues arranged concentrically by their putative levels of specialization and competence. **b** Processes of differentiation and dedifferentiation in the normal course of plant development. **c** Examples of dedifferentiation and redifferentiation processes induced by external stimuli. *Solid* and *open arrows* indicate differentiation and dedifferentiation processes as changes in the centrifugal direction toward the periphery of the diagram and in the opposite direction, respectively. (*1*) The initial process of lateral root formation from the pericycle shown as a form of dedifferentiation. (*2*) Stress-induced direct embryogenesis from epidermal cells. (*3*) Hormonal induction of organogenic callus from the root pericycle. (*4*) Wound-induced formation of organogenic callus from the stelar parenchyma. (*5*) Transdifferentiation of mesophyll cells into tracheary elements. (*6*) Organogenic callus formation from mesophyll protoplast culture. (*7*) Tissue reunion in the incised stem. Callus growth may correspond to unorganized or disorganized proliferation of cells in a state near the central zone

PpWOX13LA and PpWOX13LB, members of the WUS-related homeobox (WOX) protein family, are required for transformation of leaf cells into chloronema stem cells (Sakakibara et al. 2014). These findings collectively imply a general linkage exists between acquisition of stem cell fate and dedifferentiation in plants.

Several transcription factors have been localized in pathways connecting external stimuli and reactivation of cell division. For example, the AP2/ERF transcription factor WOUND INDUCED DEDIFFERENTIATION 1 (WIND1), which was originally identified as a gene differentially expressed between seedlings and cultured cells by transcriptomic analysis, mediates wound-induced callus formation via cytokinin signaling (Iwase et al. 2011). In tissue culture for hormonal induction of callus formation from pericycle cells, LBD16, 17, 18, and 29, which are auxin-responsive LOB domain transcription factors, direct callus initiation via the lateral root development program (Fan et al. 2012). Analysis of wound healing and tissue reunion in incised Arabidopsis stems revealed that the NAC transcription factor ANAC071 and the AP2/ERF transcription factor RAP2.6L are involved in the reactivation of cell division in the regions above and below the incision, respectively (Asahina et al. 2011).

Current epigenetic studies have provided increasing evidence that chromatin rearrangement is of great significance for gene expression of many transcription factors. As is the case for cell-cycle regulation, transcriptional control of dedifferentiation and regeneration is now meeting epigenetic control (Xu and Huang 2014).

Concluding remarks

Beginning with its description as a wound response, the study of plant cell dedifferentiation has grown together with the development of plant tissue/cell culture techniques and is currently a highly active field of plant science. Accumulation of molecular-level information has enabled comparison of cell dedifferentiation processes between plants and animals, which implies that a substantially similar mechanism might underlie dedifferentiation in evolutionarily distant organisms (Grafi 2004). In addition, plants have been suggested to possess multiple pathways for cell dedifferentiation, which are triggered by different stimuli and mediated by different regulators. There is even a pathway that has long been called dedifferentiation but recently it has been questioned whether it is truly dedifferentiation or instead differentiation (Sugimoto et al. 2011). This somewhat complicated situation is, at least in part, due to the ambiguous and variable definition and conceptual changes of the term "dedifferentiation". At this point, it may be meaningful to recall the view of dedifferentiation outlined by Bloch (1941): dedifferentiation is a process of loss of differentiated characters and rejuvenation, which is not infrequent in normal plant development. Subsequently, acquisition of organogenic or embryogenic competence was added to the notion of dedifferentiation in the context of expression of totipotency. Based on these views, considering "dedifferentiation" in a relatively broad sense, dedifferentiation corresponds to any change in the direction toward a less specialized, more juvenile, and more pluricompetent state, and can embrace almost all phenomena that have been regarded as, or associated with, dedifferentiation in the history of the dedifferentiation research (Fig. 1). Detailed and comparative analysis of each of these dedifferentiation processes would unravel diversity in the regulatory molecular networks and identify a core common mechanism of plant cell dedifferentiation.

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