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How Cells Acquire Pluripotentiality: the Perspective of Chromatin Structure

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The remarkable regenerative capacity displayed by plants and certain vertebrates such as amphibians is largely based on the ability of somatic cells to undergo dedifferentiation. While dedifferentiation is commonly associated with reentry into the cell cycle, its distinguishing feature is the withdrawal from a given differentiated state into a 'stem cell' like state that confers pluripotentiality. This aspect of dedifferentiation has hardly been studied, largely because of lack of a suitable experimental system. In recent years, we are using plant protoplasts (plant cells devoid of cell walls) a valuable experimental tool for studying how cells dedifferentiate and acquire pluripotentiality. The fully differentiated, non-dividing mesophyll cells can be separated from their original tissue by cell-wall-degrading enzymes generating a large population of protoplasts that are not yet committed to any specific fate but have acquired pluripotentiality; their subsequent fate depends on the type of stimulus applied. Only upon application of phytohormones (auxin and cytokinin) can these protoplasts reenter the cell cycle, proliferate and form calli (masses of dividing cells) from which shoots and roots can be regenerated to form the entire fertile plant. Hence, the transition of a differentiated mesophyll cell into the cell cycle is resolved into two distinct phases: acquisition of pluripotentiality (dedifferentiation),

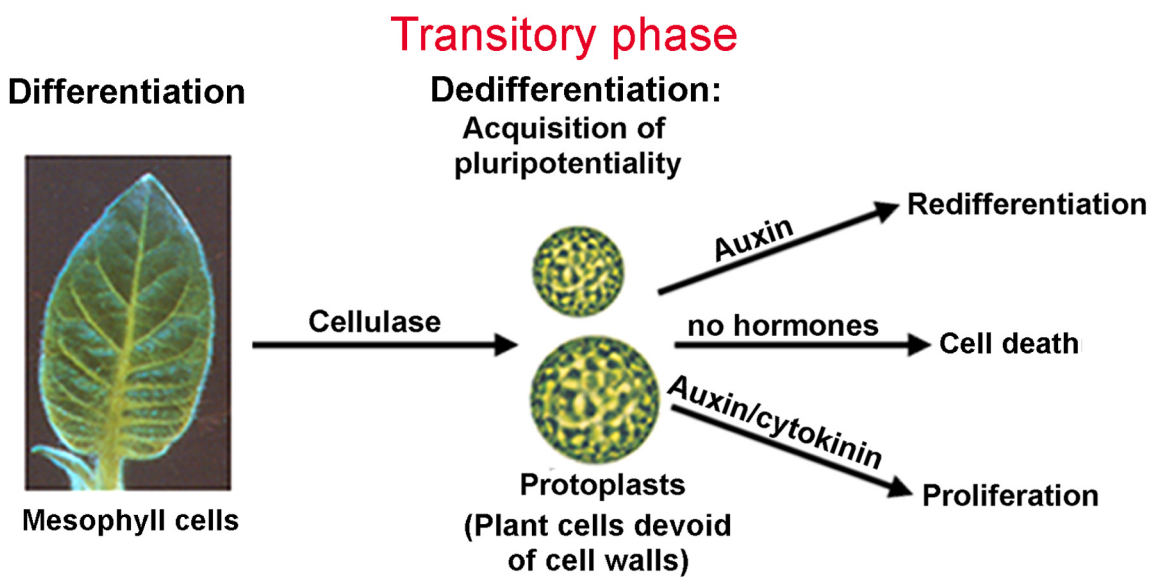

Fig. 1 *Plant protoplasts as a model system to study dedifferentiation. Differentiated mesophyll cells become pluripotent upon removal of cell walls. At this stage, additional signals determine cell fate: auxin and cytokinin induce reentry into S phase, auxin by itself may induce redifferentiation, whereas in the absence of hormonal application, cells die.*

followed by a signal-dependent reentry into S phase. As demonstrated in the protoplast system, dedifferentiation precedes not only reentry into S phase but also re-differentiation or cell death (Fig. 1). By monitoring chromatin structure two distinct phases of chromatin decondensation were evident: an early phase that occurs during acquisition of pluripotentiality followed by a second phase that precedes reentry into S phase.

We are currently characterizing inherent developmental, biochemical and molecular features of pluripotent cells. We found that acquisition of pluripotentiality was accompanied by posttranslational modifications of histone H3, redistribution of heterochromatin protein 1 (HP1), increased telomere length, as well as by disruption of the nucleolar domain, which was accompanied by chromatin condensation of the 18S ribosomal DNA (Fig. 2). Moreover, chromatin decondensation appears to be subdomain-specific leading to activation of silent genes such as *VIP1* and *NO APICAL MERISTEM (NAM)*-like genes. While *VIP1* encodes a VirE2-interacting protein which is required for *Agrobacterium* tumorigenecity, NAM-domain proteins are assumed to be involved in establishing new stem cell lineages in plants. Thus, NAM may confer pluripotentiality in plants, similarly to Oct3/4 in animals. Currently, efforts are directed toward assessing the significance of *NAM* genes for the establishment of pluripotentiality using T-DNA insertion mutants and NAM overexpressing plants. In addition, we study mechanisms underlying chromatin remodeling focusing on methyl-CpG-binding domain (MBD) proteins, histone modifications, as well as the mechanism(s) regulating telomere length during dedifferentiation.

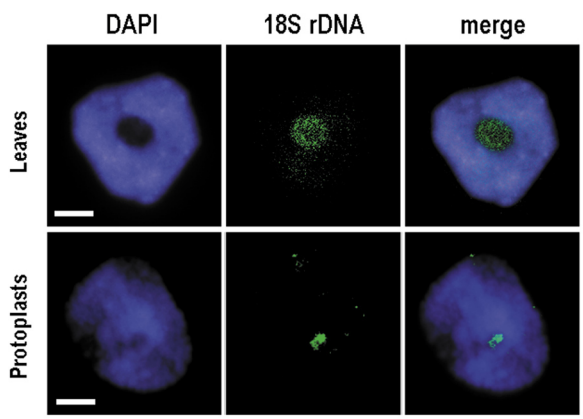

Fig. 2 *Reorganization of the nucleolar domain during acquisition of pluripotentiality. Fluorescence in situ hybridization (FISH) analysis demonstrating the dispersed chromatin configuration of the 18S rDNA gene cluster (green) in differentiated leaf cells versus condensed configuration in pluripotent protoplasts. Bar= 10 μm.*

Selected Publications

Zhao, J., Morozova, N., Williams, L., Libs, L., Avivi, Y., and Grafi, G. (2001) Two phases of chromatin decondensation during cellular dedifferentiation of plant cells: distinction between competence for cell-fate switch and a commitment for S phase. J. Biol. Chem. 276, 22772-22778.

Fass, E., Shahar, S., Zhao, J., Zemach, A., Avivi, Y., and Grafi, G. (2002) Phosphorylation of histone H3 at lysine 10 cannot account directly for the detachment of human heterochromatin protein 1_ from mitotic chromosomes in plant cells. J. Biol. Chem. 277, 30921-30927.

Zemach, A. and Grafi, G. (2003) Characterization of *Arabidopsis thaliana* methyl-CpG-binding domain (MBD) proteins. Plant J. 34, 565-572.

Williams, L., Zhao, J., Morozova, N., Li, Y., Avivi, Y. and Grafi G. (2003) Chromatin reorganization accompanying cellular dedifferentiation is associated with modifications of histone H3, redistribution of HP1, and activation of E2F-target genes. Dev. Dyn. 228, 113-120.

Grafi G. (2004) How cells dedifferentiate: a lesson from plants. Dev. Biol. 268, 1-6.

Avivi Y., Morad V., Ben-Meir H., Zhao J., Kashkush K., Tzfira T., Citovsky V., and Grafi G. (2004) Reorganization of Specific Chromosomal Domains and Activation of Silent Genes in Plant Cells Acquiring Pluripotentiality. Dev. Dyn. 320, 12-22.

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