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Cellular dedifferentiation: The perspective of chromatin structure

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In multicellular organisms, cellular dedifferentiation is the major process underlying totipotency, regeneration and formation of new stem cell lineages; in animals, dedifferentiation is often associated with carcinogenesis. The study of cellular dedifferentiation in animals, particularly early events related to cell fate-switch and determination, is limited by the lack of a suitable, convenient experimental system. In contrast, plant protoplasts (plant cells devoid of cell wall) provide an outstanding experimental tool for the study of the biochemical and molecular basis of cellular dedifferentiation. The fully differentiated, non-dividing mesophyll cells of tobacco leaves can be easily separated from their original tissue by cell wall-degrading enzymes, giving rise to a large population of protoplasts. Following treatment with phytohormones (auxin and cytokinin) protoplasts re-enter the cell cycle and proliferate. Using this system we recently found that dedifferentiation of tobacco mesophyll cells proceeds by two functionally distinct phases of chromatin decondensation: the first is a transitory phase that confers competence for cell fate switch, followed, under appropriate hormonal conditions, by a second phase, representing a commitment for the mitotic cycle (Zhao et al., 2001).

The stepwise manner by which chromatin decondensation occurs during cellular dedifferentiation suggests that heterochromatin is a rather heterogeneous structure displaying different levels of chromatin compaction. We propose that the first phase of chromatin decondensation – probably a key step in cellular plasticity, i.e., the interplay between differentiation, proliferation and cell death - occurs in a less condensed

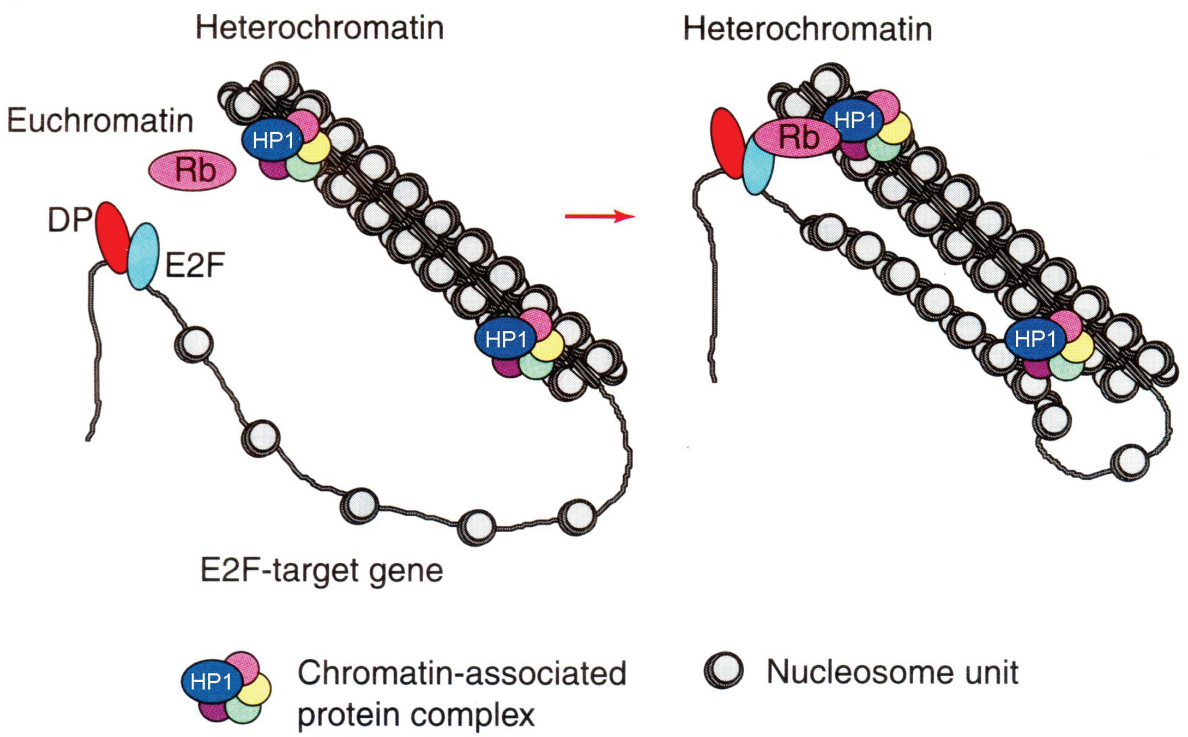

Fig. 1 *The Rb protein - a bridge to heterochromatin. Direct interaction between pRb and HP1 protein leads to relocation of a euchromatic E2F-target gene into the proximity of heterochromatin thus inducing its silencing.*

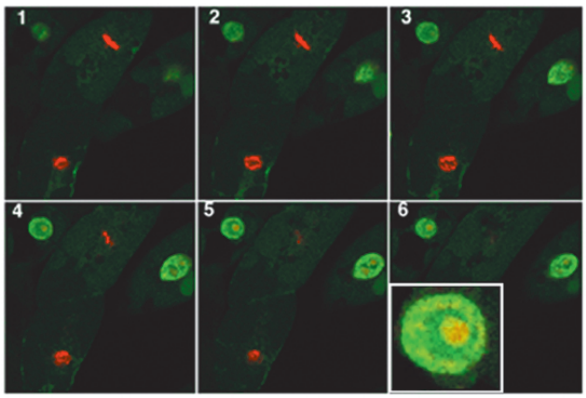

Fig. 2 *Detachment of GFP-HP1g from chromosomes during mitosis of plant cells. Confocal images showing a Z series (slices of 2.2 mm, 1-6) of nuclei at interphase and mitosis.*

heterochromatin which is likely to be located at the boundary region between eu- and heterochromatin (will be referred to as 'border chromatin'). We assume that genes located at the border chromatin have an important biological role in cellular plasticity. We found that the retinoblastoma protein and heterochromatin protein 1 (HP1), both located at border chromatin, can interact with each other (Williams and Grafi, 2000) providing evidence for the molecular machinery involved in pRb-mediated heterochromatin formation and gene silencing.

To further investigate mechanisms contributing to heterochromatin formation and maintenance in plants, we generated transgenic tobacco plants and transgenic BY-2 cycling cells expressing the human HP1γ fused to green fluorescence protein (GFP) and studied the dynamics of GFP-HP1γ association with chromatin. Our results showed that GFP-HP1γ is localized to discrete domains in tobacco interphase nuclei, tending to bind heterochromatic regions. During mitosis, GFP-HP1γ is detached from chromosomes concomitantly with phosphorylation of histone H3 on serine 10, but reassembles onto chromosomes as cells exit mitosis. GST pull-down assays showed that HP1γ is capable of binding histone H3 from tobacco leaves, an interaction that requires the chromo but not the chromo shadow domain. By searching GenBank databases we found that plants possess HP1-like proteins containing both chromo and chromo shadow domains. We characterized an HP1-like protein from tomato, designated LeHP1-L, that binds plant histone H3 in a chromo domain-dependent manner. In vitro studies showed that the human histone methyltransferase SUV39H1 methylated histone H3 prepared from tobacco leaves, enabling it to interact both with huHP1γ and LeHP1-L. Our results suggest that formation of heterochromatic subdomains in the plant nucleus is mechanistically similar to that of animals and is mediated, at least in part, by the association of HP1-like proteins with methylated histone H3.

Selected Publications

Williams, L. and Grafi, G. (2000) The retinoblastoma proetin – A bridge to heterochromatin. Trends Plant Sci. 5, 239-240.

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., Avivi, Y., Zemach, A., Shahar, S., and Grafi G. (2001) Plants possess HP1-like proteins that bind methylated histone H3: implications for heterochromatin formation. Submitted

Acknowledgements

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