Summary of PhD. thesis

Cell division represents one of fundamental attributes of all living creatures. Basic molecular mechanisms operating during cell proliferation seem to be evolutionary conserved among eukaryotes. The cell cycle is divided in four subsequent phases; the most regulatory events are concentrated in G_1/S and G_2/M checkpoints.

The key regulatory proteins, cyclin-dependent kinases (CDKs), govern the progress through the whole cell cycle. Their function is strictly dependent on catalytic cyclin subunits. The corresponding cyclin partner binding CDK determines the time window of the specific CDK/cyclin complex action in individual cell cycle phase. To become fully active, the complex requires further posttranslational modification including activatory phosphorylation and dephosphorylation of the CDK on specific amino acid residues.

Plant cell cycle, besides well-conserved mechanisms common to all eukaryotes, exhibits other specific mechanisms resulting from plant survival strategy. The G_1/S transition is strongly affected by external and internal signals, mainly phytohormones and metabolites, reflecting the elementary conditions suitable for accomplishment of the whole cell cycle. The central molecule responding to these signals at G_1/S is D cyclin, whose expression is regulated by cytokinin and sucrose (Riou-Khamlichi *et al.*, 1999; Riou-Khamlichi *et al.*, 2000). The control of plant G_2/M transition, however, still remains a bit obscure. The cell at G_2/M is primarily monitoring whether the DNA replication has been finished, and in other eukaryotes, it consequently activates CDK phosphatase Cdc25 that is serving as all-or-nothing positive signal for the entry into mitosis (e.g. Moreno *et al.*, 1990; O'Farrell, 2001). Although there has been great effort to identify a homologue to this enzyme in plants, unfortunately the regulatory protein responsible for dephoshorylation of higher plant CDK at G_2/M is so far unknown. Nevertheless, it was documented for several times that this regulation is operating in plants and moreover it was shown to be under the positive control of cytokinins (Zhang *et al.*, 1996; Zhang *et al.*, 2005). Recently, the screening of *Arabidopsis* and rice genomes revealed a small gene coding for Cdc25 CDK phosphatase catalytic domain (Landrieu *et al.*, 2004). This gene is, however, unable to complement yeast *CDC25* mutant strains and moreover its overexpression in plants does not exhibit phenotypic changes (Landrieu *et al.*, 2004; Sorrell *et al.*, 2005). Based on these findings, Boudolf et al. (2006) suggested recently that plants could lack Cdc25 phosphatase and that its role might have been evolutionarily replaced by a B-type CDK-dominated

pathway. However, this hypothesis neglects the fact that CDK undergoes before mitosis activatory dephosphorylation and inhibition of this action (e.g. by application of lovastatin - inhibitor of isoprenoid-type cytokinin synthesis) causes arrest of the cells in G_2 phase (Laureys *et al.*, 1998). Moreover, as described by Sorrell et al. (2002) and Orchard et al. (2005) the G_2/M specific CDKB is most likely phosphoregulated at the same evolutionary conserved amino acid residues as CDKA is. All these arguments together with the fact, that the counteracting CDK kinase Wee1 at G_2/M transition has been repeatedly identified in higher plants (Sun *et al.*, 1999; Sorrell *et al.*, 2002; Gonzalez *et al.*, 2004) strongly support the important role of CDK activatory dephosphorylation in plants. As the search for plant homologue fails until now it is useful to employ the plants carrying foreign *cdc25* gene and thus to study the effect of this regulatory step on plant cell cycle dependent processes. The results concerning plants with introduced fission yeast (model organism of eukaryotic cell cycle regulation) *cdc25* gene (*Spcdc25*) confirmed that the yeast protein is functional in plants fulfilling the same role as in donor organism (Bell *et al.*, 1993; Zhang *et al.*, 1996; Zhang *et al.*, 2005).

Given the Spcdc25 phosphatase overexpression in plants affects the duration of G_2 phase and promotes the entry into mitosis we could expect that the processes dependent on cell division regulation would be influenced. Bell *et al.* (1993) transformed tobacco with fission yeast *Spcdc25* cDNA and showed that there is a dramatic impact of *Spcdc25* overexpression on plant habitus and ontogenesis without any detail analysis. These findings stimulated us to study the effect of *Spcdc25* expression on different plant processes closely related to cell division regulation and supply further pieces to the mosaic of morphological, developmental and biochemical changes induced by overexpression of the mitotic activator thus to propose a model of the interaction with plant growth and development.

In this thesis the results analysing the impact of *Spcdc25* expression on processes differing primarily in organisation levels under *in vitro* conditions, i.e. on *de novo* organ formation (paper 1), on the flowering onset (paper 3) and on cell suspensions characteristics (paper 4,5), were included. A new methodical approach used as a part of the *Spcdc25* detection experiments was also incorporated in the thesis (paper 2).

The main conclusions on the effect of *Spcdc25* expression are as follows:

- The changes in *de novo* organ formation mimic the cytokinin effect as there is promotion of shoot formation and restriction of rhizogenesis in transgenics.
- *Spcdc25* and sucrose act synergistically on flower induction thus confirming the key role of cell division and saccharide signalling in flowering onset.
- The acceleration of the entry into mitosis substantially influenced the orientation of cell division and cells expressing *Spcdc25* become cytokinin-independent at G_2/M transition.
- Besides changes in rate and orientation of cell division, a shift in carbohydrate status was found, indicating the interaction of cell cycle regulation with plant metabolism being a complex one.

➢ **In conclusion, the results further confirm the existence and importance of CDK activatory dephosphorylation at plant G_2/M transition and support the model of cytokinin stimulation of this regulatory step. The results also indicate the possibility, that the decision about mitosis onset generates a signal emanated towards plant metabolism.**