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DISSERTATION SUMMARY

Characterization of the plant anaphase-promoting complex: gene expression and protein-protein interaction studies

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Endoreduplication, the duplication of the genome without division, is a widespread process during the development of various plant organs. It supposes the modification of the cell cycle, G2-M transition needs to be inhibited and DNA-synthetic phase maintained. Investigation of alfalfa nodulation led to the identification of *ccs52*, a cell cycle switch gene encoding a putative regulator of the transition from division to differentiation. Its animal orthologs, members of the Cdh1/fizzy-related family, contribute to the activation of the anaphase-promoting complex (APC).

The anaphase-promoting complex is a cell cycle-regulated ubiquitin-protein ligase whose activity is essential for progression through mitosis. APC is active during mitosis and G1 cell cycle phase; it mediates the ubiquitination of various regulatory proteins, targeting them for degradation by the 26S proteasome. Although APC is a large multiprotein complex (it has at least 12 subunits in *S. cerevisiae*) and its phosphorylation state varies during the cell cycle, its full activation requires the binding of one member of either the fizzy or the fizzy-related family of activator proteins. These activators are thought to confer substrate specificity to the complex by recognizing proteins containing either D- or/and KEN-boxes. Proteins with such motifs (*e.g.* securin, an inhibitor of sister chromatid separation or mitotic cyclins, that have to be degraded for the inactivation of cyclin-dependent kinases) are key regulators of mitotic events.

Despite the recent development in understanding APC function and regulation many questions remain unanswered. This is particularly true for the plant APC which is still unexplored.

The completed Arabidopsis genome allows to identify homologs of almost all of vertebrate APC subunits. In order to give a first characterization of the plant complex and to get an insight into its interaction with Ccs52 proteins, full length cDNAs of certain of the predicted subunits were isolated from an Arabidopsis cDNA library. These were the TPR repeats containing CDC23, CDC16, CDC27.2 as well as the CDC27/HOBBIT; APC2 that contains a C-terminal cullin-homology domain, the Doc domain protein APC10, CDC26 and the APC11 RING-H2 finger protein.

In attempt to define the molecular organization of the plant APC, we tested all possible interactions among the cloned subunits as well as their association with the members of the Arabidopsis Ccs52 family by yeast-two hybrid analysis. Strong interaction was observed between APC2 and APC11 and the yeast two-hybrid result was verified by co-immunoprecipitation of the epitope-tagged proteins.

To study the role of these genes during the cell cycle, Arabidopsis cell suspension was synchronized with aphidicholin. The APC-subunit coding genes have constant expression level during the cell cycle, which suggests the importance of posttranslational modifications in the regulation of the activity of the complex. In contrast, the expression of the three *ccs52* genes shows a strong cell cycle regulation, and this coincides well with the pattern of the corresponding substrates.

Although the structure of the APC may depend on multiple protein-protein interactions, our results demostrate a pairwise interaction between the subunits APC2 and APC11. The gene expression studies support that the transcriptional regulation of APC function is mainly based on cell cycle phase-specific expression of the APC activator genes.

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