



September 13, 2010

**Report established by Jean-Philippe REICHELLED  
on the thesis report of Ondřej SMETANA**

Mr Ondřej SMETANA is submitting to the Charles University in Prague and to the University of Strasbourg a thesis report aiming to obtain a doctoral degree. Mr Ondřej SMETANA has performed his PhD researches both at the IBMP of Strasbourg and at the Charles University of Prague under the joint supervision of Dr. Marie-Edith CHABOUTÉ and Prof. Zdeněk OPATRŇÝ.

The thesis report (155 pages) is entitled «Molecular and cellular aspects of programmed cell death in response to genotoxics in plants». The main goal of the PhD project of Mr Ondřej SMETANA was to characterize the mechanisms occurring in the response to genotoxic stress induced by Double Strain Breaks (DSB) in the tobacco BY-2 cell suspension and in Arabidopsis plants.

The first part of the dissertation is a general introduction (39 pages) dealing with knowledge about cell cycle regulation, DNA damage response and programmed cell death (PCD) mechanisms in plant and other organisms. This part of the manuscript is clearly written and well documented. The objectives of the project, which are briefly exposed at the end of the introduction, are well defined.

The chapter dedicated to the methods (18 pages) gives a good overview of the methods used during the PhD work of Ondřej SMETANA.

The results (66 pages) are composed of three chapters. The first one describes experiments dedicated to setup a PCD inducing system in the tobacco BY-2 cell suspension. Ondřej SMETANA uses the DSB inducing agent Bleomycin (BLM) and characterizes the PCD both at cellular and molecular levels, showing that it involves the ATM pathway. He also shows that PCD is associated with a specific cell cycle arrest at the G2/M transition.

In the second chapter, Ondřej SMETANA analyses the effects of ectopic expression of the NtE2F transcription factor on the DNA damage-induced cell death in BY-2. By combining BY-2 synchronization, flow-cytometry and mitotic index measurements, he shows convincingly that NtE2F overexpression is able to bypass the G2/M checkpoint induced by BLM. Another conclusion of this study is that overexpression of NtE2F has an inhibitory effect on bleomycin-mediated cell death.

The third chapter aims to characterize the genotoxic-induced PCD in the Arabidopsis root apical meristem (RAM). Ondřej SMETANA shows that BLM-treatment has several effects in the root (alteration of the meristem architecture, modification of auxin maxima, induction of endoreduplication, induction of PCD in the RAM). Another further approach aims to find actors of the BLM-induced PCD signalization by isolation and analysis of several knock-out mutants or overexpression lines (caspases, DNA damage signaling proteins, cell cycle regulators). The data established/confirmed that the ATM pathway is involved in the BLM-induced PCD signalization.



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The report is ending in a general discussion summarizing the main data and proposing a hypothetical model of BLM-induced cell death regulation. In this model, the role of Reactive Oxygen Species is not well defined. The perspectives of the work could be more developed.

In conclusion, Mr Ondřej SMETANA presents a very good scientific work in a clearly exposed report containing only few mistakes (typo, missing references,...). It makes no doubt that this work will be finalized by publications in the near future.

Therefore, I consider this thesis fully suitable for the defense.

Perpignan, September 13, 2010

Jean-Philippe Reichheld, Chargé de Recherches CNRS, HDR



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