Proteomic Analysis of Plant Cell Nuclei Purified by Flow Sorting

Beáta Petrovská[1], Hana Jeřábková[1], Ivo Chamrád[2], Jan Vrána[1], René Lenobel[2], Jana Uřinovská[2], Marek Šebela[2], and Jaroslav Doležel[1]

[1] Centre of Plant Structural and Functional Genomics, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany AS CR, Šlechtitelů 31, CZ-783 71 Olomouc, Czech Republic

[2] Department of Protein Biochemistry and Proteomics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic

SUMMARY

Many proteins are involved in maintaining nuclear organization, gene expression and nuclear and cell division. However, except for histones and a few other nuclear proteins, only a fraction of these proteins is known in plants. The **plant nuclear proteome** has not been well explored yet. Biochemical composition of plant sub-cellular components may be altered during their isolation and during subsequent protein purification. The conventional multi-step fractionation procedure is both laborious and liable to contamination. We have developed a single step method based on **flow sorting.** The method allows purification of G1, S and G2 phase nuclei, and minimizes the risk of contamination by non-nuclear proteins. Preliminary results obtained using G1 phase barley root tip cell nuclei indicate that **flow sorting coupled with a protein/peptide separation and mass spectrometry** will permit a comprehensive characterization of the plant nuclear proteome.

AIM OF THE STUDY

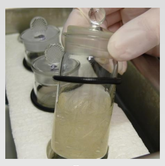
I. Developing a new protocol for purification of plant nuclei:

- More efficient (identification of as yet unknown plant nuclear proteins),
- More sensitive (low abundance proteins),
- Less time consuming (avoiding mechanical homogenization of tissues, filtration, nuclei solubilization, separation on a density gradient),
- Not altering nuclear proteins,
- Avoiding contamination by non-nuclear proteins.

II. Identification of the plant nuclear proteins

1. Sample preparation

1. barley seedlings

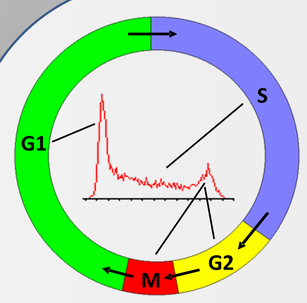


2. mild fixation of roots in formaldehyde

3. dissection of root tips

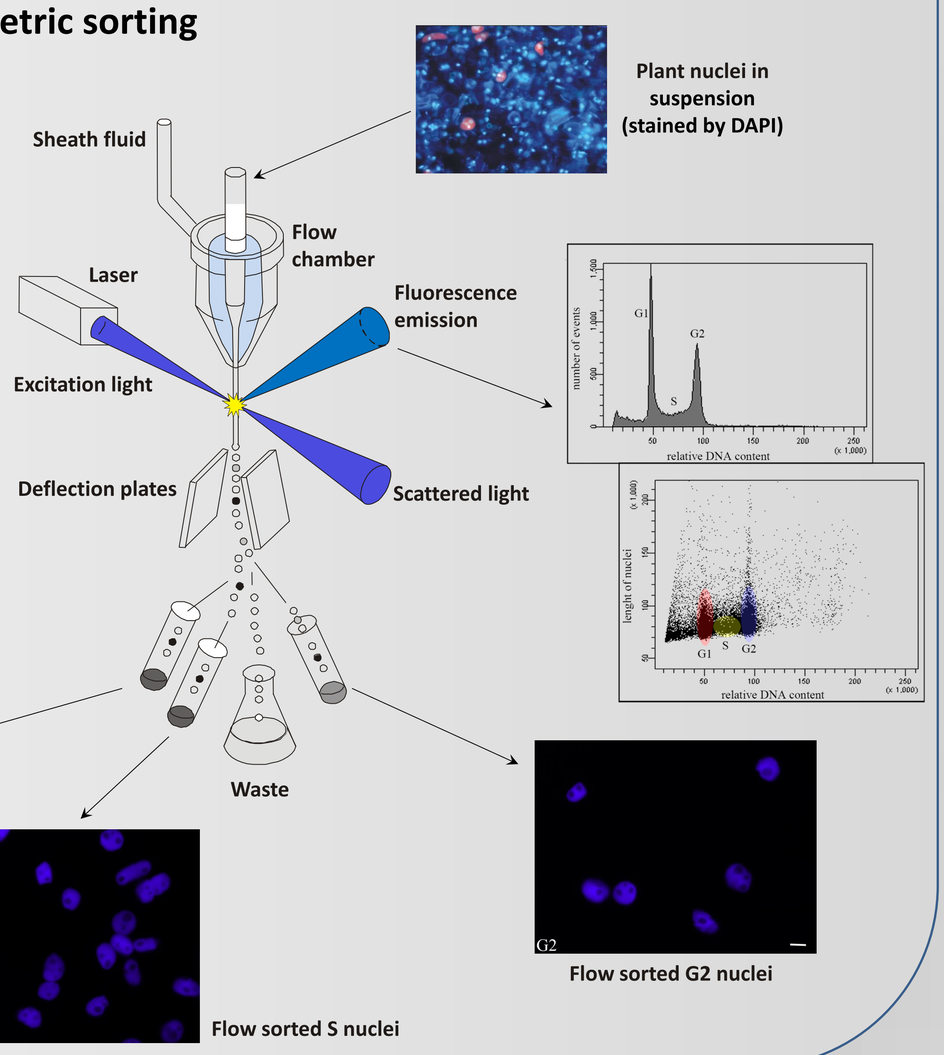
4. release of nuclei to LB01 buffer by mechanical homogenization

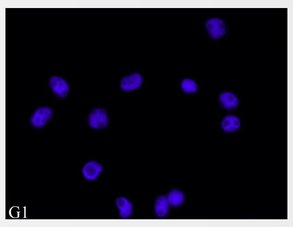
2. Flow cytometric sorting



FACSAria SORP

- Provides the opportunity to study nuclear proteome in different phases of cell cycle
- 5 million of sorted nuclei for protein/peptide separation and MS
- Sorting: 200 nuclei/sec >>> 5 million nuclei sorted from 12 samples in ~180 min



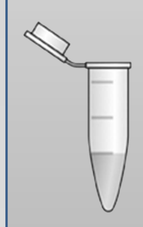


Flow sorted G1 nuclei

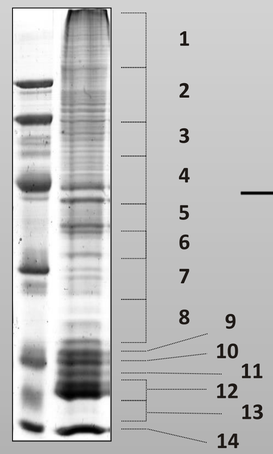
Flow sorted S nuclei

Flow sorted G2 nuclei

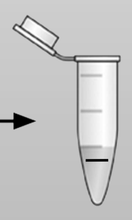
3. Proteomic analyses



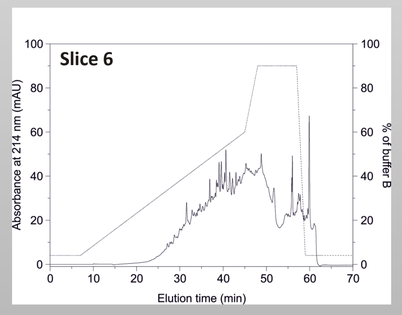
DNase digestion in a lysis buffer



Protein separation



In-gel digestion



Separation by reversed-phase nanoLC

NanoLC-ESI-MS and MS/MS

NanoLC-MALDI-MS and MS/MS

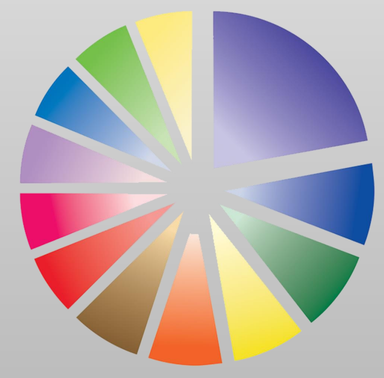
Total number of protein identifications

LC-MALDI-TOF/TOF

LC-ESI-Q/TOF

GO

Biological process



- Translation; *p*-value 1.21E-39
- Ribosome biogenesis; *p*-value 4.01E-25
- Ribonucleoprotein complex biogenesis; *p*-value 1.16E-24
- Cellular macromolecular complex assembly; *p*-value 3.92E-23
- Cellular macromolecular complex subunit organization; *p*-value 6.29E-22
- Chromatin organization; *p*-value 2.72E-20
- Nucleosome assembly; *p*-value 1.07E-29
- Nucleosome organization; *p*-value 1.07E-29
- Chromatin assembly; *p*-value 2.72E-29
- Protein-DNA complex assembly; *p*-value 4.29E-29
- DNA packaging; *p*-value 3.68E-28
- Chromatin assembly or disassembly; *p*-value 1.26E-25

803 distinct proteins identified in G1 phase barley nuclei

GO term enrichment analysis = 99 % nucleus-related !!!

CONCLUSION

- Proteomic analysis is feasible using flow sorted population of plant cell nuclei.
- Coupling FCM, protein/peptide separation and MS provides an elegant and powerful means to determine the composition of nuclear proteome at defined phases of the cell cycle.
- Our approach can be extended further to studying the chromosomal proteome.

This research was supported by grants from the Czech Science Foundation (14-28443S, P501/12/G090), the National Program of Sustainability I (LO1204), the European Social Fund (Operational Program Education for Competitiveness CZ.1.07/2.3.00/20.0165), and Internal Grant Agency of Palacky University, Olomouc (Prf/2013/003).