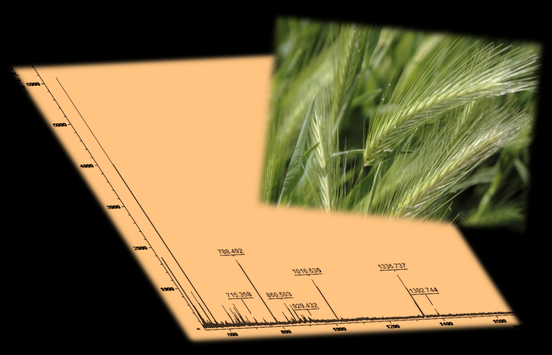


POSTGENOMIC ERA IN PLANT RESEARCH

A. PLANT NUCLEAR PROTEOM

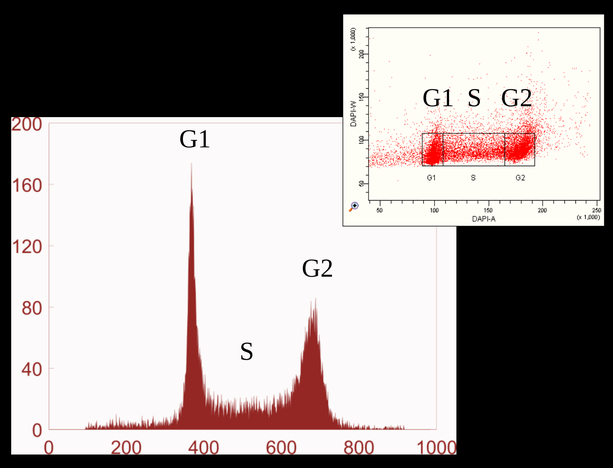
The separation of high-purity nuclei from plants is a difficult task. Usually, a series of fractionation process is needed to obtain purified nuclei.

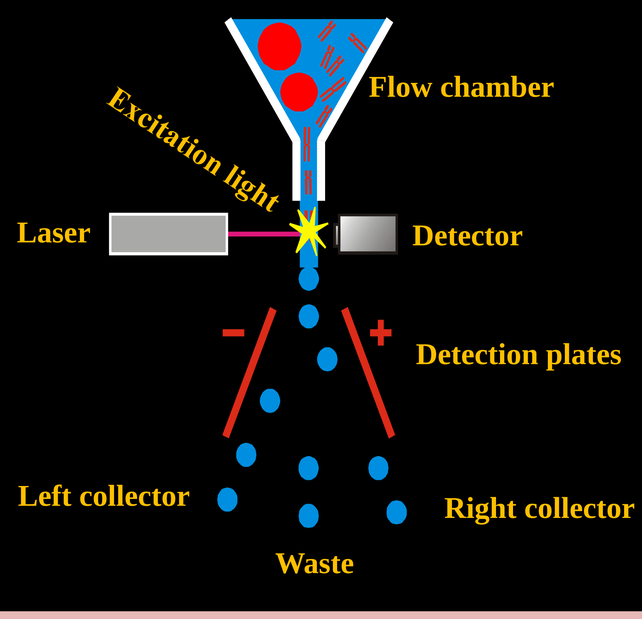
B. PLANT CHROMOSOMAL PROTEOM

Until now – limited amount of data available on composition of plant chromosomal proteins. Pioneering work was performed only with human metaphase chromosomes.

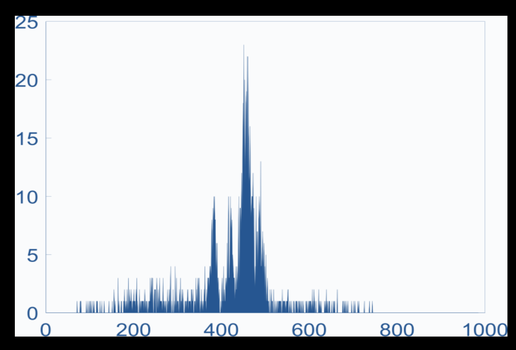
SOLUTION

Nuclei sorting





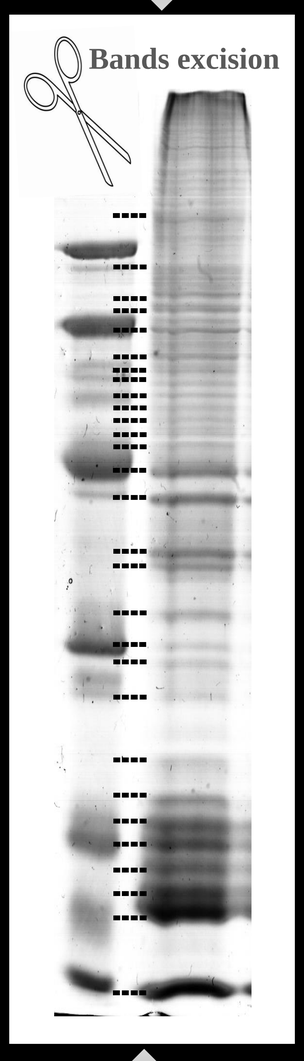
Chromosome sorting



1. Isolation of nuclei or chromosomes and their sorting by flow cytometry

2. Protein extraction

3. 1-DE separation



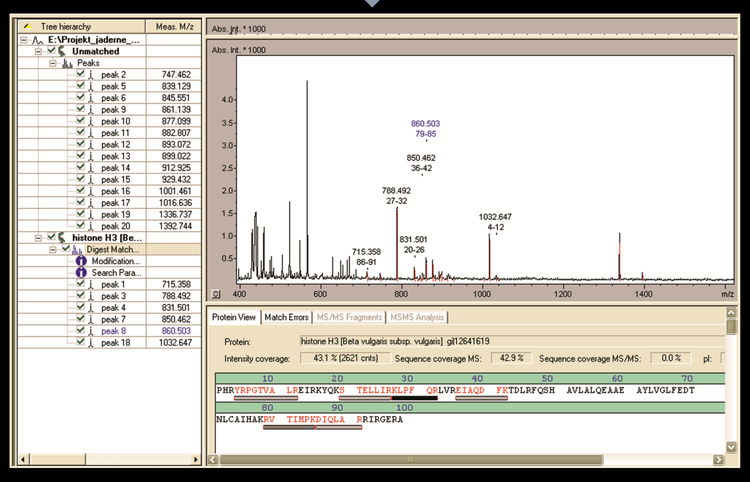
or 2-DE gel

4. Enzymatic digestion

5. Peptide separation by nanoLC coupled to MS (and MS/MS) analyses

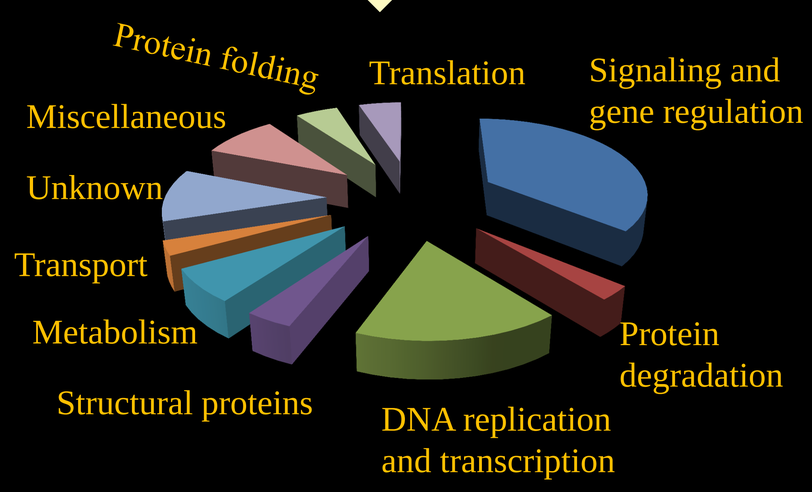
6. Database search

7. Protein identification



MALDI-TOF mass spectrometry of tryptic digests of SDS-PAGE-separated barley nuclear proteins. Right bottom panel - a mass spectrum of histone H3 acquired on a Microflex LRF20 MALDI-TOF instrument operating in the reflectron mode for positive ions; CHCA was used as a matrix. On the left side - a list of peptides, which were assigned in the protein sequence database NCBInr.

8. Functional classification



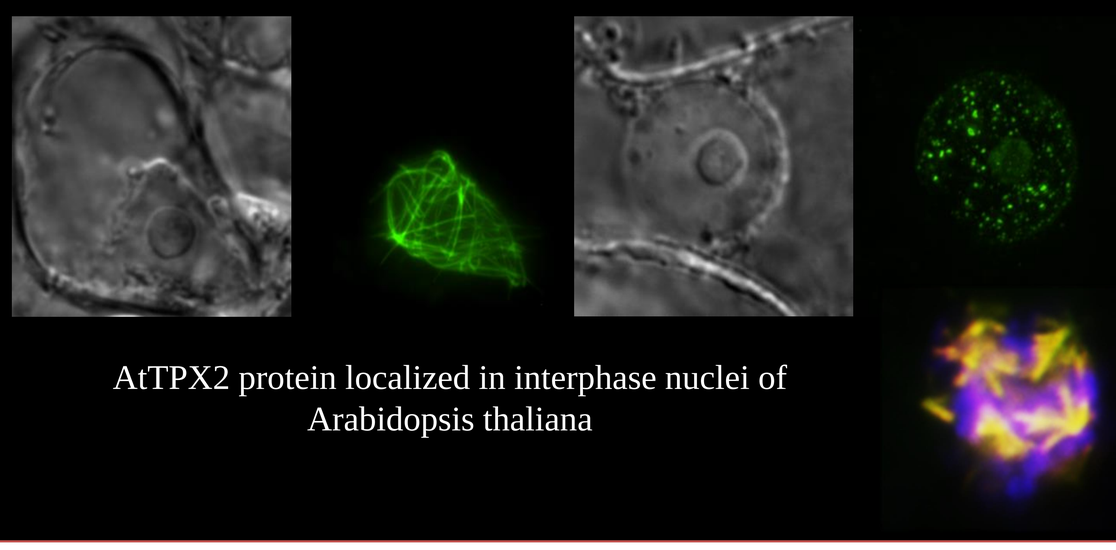
9. Identification of phosphorylation sites

(understanding of protein interaction within the nucleus and its function as a cellular regulator)

10. Histones modification

11. Functional analysis

Immunolocalization, localization of GFP/RFP fusion proteins, reverse genetic approaches (RNAi, amiRNA, T-DNA knockout mutant lines)



AtTPX2 protein localized in interphase nuclei of Arabidopsis thaliana

In collaboration with **Marek Šebela**, Department of Protein Biochemistry and Proteomics, CRH, Olomouc, Czech Republic.

The Centre of Plant Structural and Functional Genomics

Institute of Experimental Botany AS CR, v. v. i.

C. R. HANÁ