Proteomic Analysis of Plant Cell Nuclei
Purified by Flow Sorting

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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
   - 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

3. Proteomic analyses
   - DIA digestion in a lysis buffer
   - In-gel digestion
   - Protein separation
   - Separation by reversed-phase nanoLC
   - NanolC-ESI-MS and MS/MS
   - NanolC-MALDI-MS and MS/MS

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.

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