



ABSTRACT

“A proteomics approach the mapping proteins to organelles”

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Cells are organized spatially and functionally into sub-cellular compartments. Changes in sub-cellular localization are involved in regulation of interactions and stability. Studying global changes in protein localization can provide useful insights into numerous cellular functions.

Many sub-cellular compartments unfortunately cannot be purified to homogeneity making assignment of their genuine residents difficult. Proteins from the same organelle however, co-sediment and hence exhibit similar distributions in a density gradient. Sub-cellular localization can be therefore assigned by comparing distributions of unknown proteins to those of known organelle markers.

LOPIT (localization of organelle proteins by isotope tagging) is a high-throughput proteomics based technique for protein localization to sub-cellular organelles^{a,b,c} which we have developed. In this approach, organelles are partially separated by density gradient centrifugation and proteins within fractions quantified using differential stable isotope labeling.

Over the past few years we have applied LOPIT technique to study sub-cellular location of proteins within Arabidopsis, and have been able to assign many hundreds of proteins to different sub-cellular. More recently we have extended the technology to map changes in location upon a given perturbation. We have also applied the technology to the mapping of organelle proteins in other organisms and demonstrated its use in the mapping of protein complexes to sub-cellular structures^{d,e}.

A review of the LOPIT technique will be given along with a description of how we are extending it to dynamically map protein redistribution.

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