Specific Proteomic Analysis of Mitochondria in Dopamine Neurons

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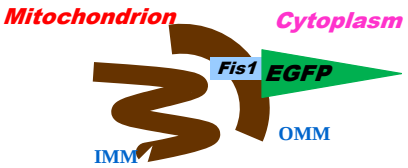
INTRODUCTION

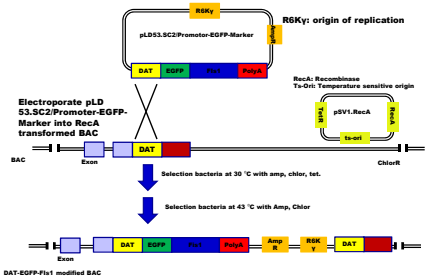
- Altered mitochondrial dynamics in dopamine (DA) neurons have been related to several neurological diseases including Parkinson's disease.
- In addition, heterogeneities of mitochondria within different cell types have been well demonstrated.
- Neuroproteomic studies are beginning to contribute to our understanding of the molecular mechanisms involved in normal neuronal function, as well as in neurological and psychiatric disease.
- The extreme cellular complexity of the nervous system is a major limitation in proteomic analysis, since it is difficult to characterize specific changes in protein expression or modification in specific neuronal sub-types. Here, we have developed methods to enrich for mitochondria specific for DA neurons

METHODS

Labeling mitochondria by expression of EGFP-tagged Fis1



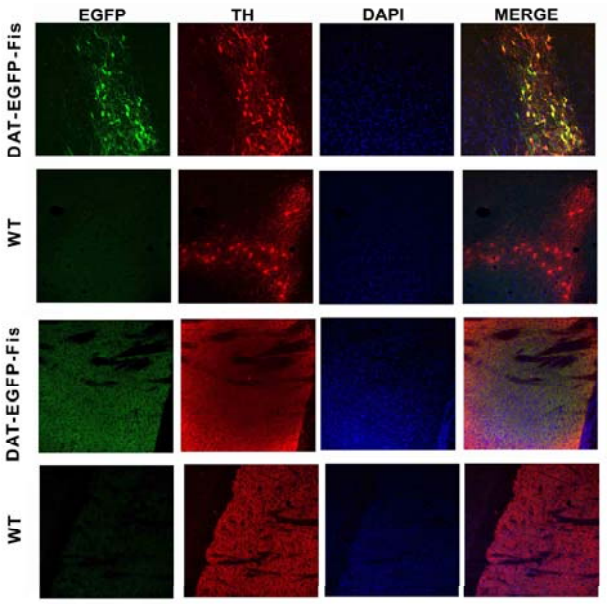
Generating BAC transgenic mice



➢The DAT is expressed in neurons in the SNc and VTA, and cell bodies axons, and axon terminals of DA neurons are strongly labeled in BAC DAT-GFP mice. We therefore utilized the transcriptional unit and its associated regulatory elements of the DAT promoter were used to drive expression of GFP-Fis1 in DA neurons in BAC transgenic mice.

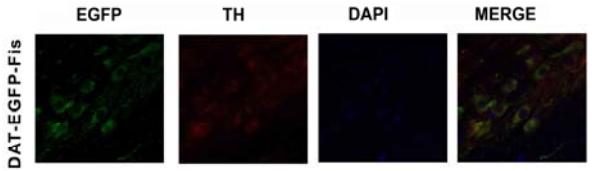
RESULTS

Specific Expression of EGFP in DA neurons



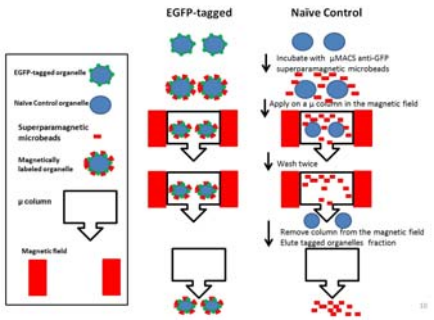
➢**EGFP expression and its co-localization with endogenous TH in DAT-EGFP-Fis1-C modified BAC-transgenic mice in substantia nigra (SNc) (top two panels) and caudate putamen (bottom two panels).** Brain sections of DAT-EGFP-Fis1-C modified BAC transgenic (DAT-EGFP-Fis) or wild-type control mice (WT) were double-immunostained with antibodies to EGFP (labeled by Alexa Fluor 488, green) and to TH (labeled by Alexa Fluor 546, red), as well as stained by DAPI. The green-red-blue *merged* image indicates the co-localization *of* EGFP *and* TH in the transgenic mice group. Images were acquired with an Olympus Fluoview FV*1000* laser scanning confocal microscope using a 20 × 1.4 NA oil immersion objective with 405 nm, 473 nm, and 559 nm lasers.

Expression of EGFP in mitochondria



➢ **Possibility of localization of EGFP to mitochondria.** Images were acquired with an Olympus Fluoview FV*1000* laser scanning confocal microscope using a 100 × 1.4 NA oil immersion objective with 405 nm, 473 nm, and 559 nm lasers

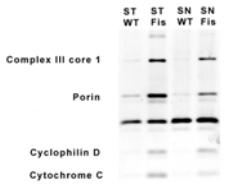
Immunoprecipitated EGFP-tagged mitochondria



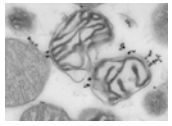
➢Optimized methods to allow for specific, efficient and reproducible isolation of pure EGFP-tagged organelles.
➢We compared and evaluated five different types of magnetic beads to isolate of EGFP-tagged mito, (and nuclei or synaptic vesicles , not shown).
➢μMACS anti-GFP superparamagnetic microbeads showed the lowest nonspecific binding and the highest efficiency.

High Purity of Immunoprecipitated EGFP-tagged Mitochondria

Immunoblotting analysis



EM analysis

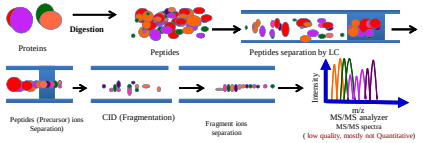


High- abundance proteins identified by LC-MS/MS analysis.

	SN	ST
Total number proteins identified	236	157
Total number of proteins confirmed as mitochondrial proteins	203	142
% Confirmed mitochondrial proteins	86%	90%

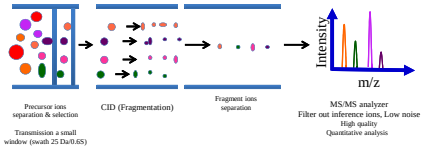
Ongoing Experiments

Traditional LC-MS/MS workflow.



➢ Proteins are initially digested into peptides which are then separated by LC and analyzed by mass spectrometry. The peptides ions are further separated in the first chamber of the mass spectrometer generating MS spectra, and then are fragmented into fragment ions generating MS/MS spectra.
➢ A drawback of this technique is that MS/MS spectra are only used for peptide identification and MS data (rather than more selective MS/MS data) are used for peptide quantification.

Data-independent MS/MS^{All} (SWATH^{TM}) technique



➢ In the SWATH approach, the first quadrupole is stepped at 25 *amu* increments across the mass range of interest so that ions within each 25 amu window are transmitted into the collision cell.
➢ This assay acquires a sequential series of isolation windows (SWATHs) for each given precursor mass range across the LC and thus yields amore quantitative MS/MS data for peptides (proteins) in a complex sample in one single LC-MS/MS run.

CONCLUSIONS

This approach provides us with a tool to further understand mitochondria in DA neurons in normal and pathological conditions, for example in Parkinson's disease mouse models .

ACKNOWLEDGMENTS

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➢ EGFP-Fis1 constructs were kindly provided by Dr. Masayuki Matsushita.