

Separation of transmembrane, membrane-associated and cytosolic proteins

Christel Brou, Alain Israël
Institut Pasteur, Paris, France

INTRODUCTION

This procedure allows separating the true transmembrane proteins(TM) from the membrane-associated proteins (MAP). For example, AIP4/Itch or members of Nedd4 family are MAPs, whereas other E3 ubiquitin ligases (like DTX) appear to be true transmembrane proteins. Non-activated Notch is a TM protein.

MATERIALS

- Stable cell lines expressing the protein of interest
- dounce homogenizer
- ultracentrifuge

METHODS

- MAPs = membrane-associated proteins
 - TM = true transmembrane proteins
 - MB = membrane fraction = TM + MAPs
 - cells: from 50 cm² confluent adherent cells to several plates (depending on cell line)
1. Harvest the cells in 1 ml PBS/plate, in falcon 15 ml tubes
 2. Centrifuge 1500 rpm, 5 min
 3. Resuspend the pellet in 0,1x PBS (containing a cocktail of protease inhibitors and Ca and Mg if necessary). 2 ml at least (should correspond to more than 5 fold the cell pellet volume), leave the cells swelling 10 minutes on ice
 4. Dounce (pestle B) 20 to 50 strokes depending on the cell type. You should control under the microscope that the cells are broken, and the nuclei intact; for example: 20x for 293T, at least 50x for MEF cells
 5. Back into the 15 ml falcon tube, measure the volume and add PBS 10x to have 1X final (about 180µl), take a sample (= TOT XT)
 6. Centrifuge at 4°C, 2500 rpm, 20 min: nuclei are in the pellet
 7. Supernatant = Cyto+mb, take an aliquot
 8. Centrifuge this supernatant in TLA55 tubes (Beckman ref 337448) in TL100 rotor TLA55, 55000 rpm (100 000 g), 1hour at 4°C.
 - Supernatant = cyto S100
 - Pellet = MB
 9. Resuspend the pellet with the pipetman in NaCarbonate 100 mM, pH 11,5 in 1ml (difficult to resuspend well), leave on ice 30 min.

10. Centrifuge again 55000 rpm 1 hour at 4°C
 - supernatant = MAPs
 11. It is better to wash the pellet again with the same buffer, and centrifuge
 12. Pellet = TM, can be resuspended in the buffer of your choice (it is difficult ! maybe laemmli is the best)
 13. Load on gel 10 to 20 μ l of each fraction (depending on the corresponding volume compared to the total extract) : TOT, cyto+MB, nuclei, S100, MB, TM, MAP
- Good controls for WB : EGFR (TM), rab 8 or beta-catenin (MAP)

BIBLIOGRAPHY

- Fujiki Y, Hubbard AL, Fowler S, Lazarow PB. Isolation of intracellular membranes by means of sodium carbonate treatment: application to endoplasmic reticulum. 1982. *J Cell Biol.* 93:97-102
- Mulugeta S, Beers MF. Processing of surfactant protein C requires a type II transmembrane topology directed by juxtamembrane positively charged residues. 2003. *J Biol Chem.* 278:47979-86