Systematic subcellular localization in the Human Protein Atlas project – deciphering the cytoskeletal proteomes.

R Schutten[1], H Ait Blal[1], F Danielsson[1], M Hjelmare[1], D Mahdessian[1], M Skogs[1], C Stadler[1], A Aberg[1], M Wiking[1], M Uhlén[1] and E Lundberg*[1.]

[1]Science for Life Laboratory, Royal Institute of Technology (KTH), Stockholm, Sweden.

* Corresponding Author: emma.lundberg@scilifelab.se

Keywords: Confocal Microscopy, Cytoskeleton, Human Protein Atlas, Subcellular localization.

Compartmentalization of biological processes is a fundamental principle of eukaryotic cells that concentrates molecules needed for a specific function and enables multiple processes to occur in parallel. Despite a great deal of research, basic questions about the spatial organization of many proteins and biological processes remain unanswered. As part of the Swedish Human Protein Atlas project, we are systematically localizing the human proteome on a subcellular level using an antibody-based approach[1-3].

A semi-automated pipeline has been established combining sample preparation, high-resolution confocal microscopy and image analysis to allow investigation of 500 proteins in three cell lines per month. The validation criteria are strict and include systematic gene silencing using siRNA[4], paired antibodies, and fluorescently tagged proteins (FP) as a complementary technique[5]. Also, RNA-sequencing is used to cherry pick cell lines for each protein.

To date, over 10000 human proteins have been localized, of which 800 to cytoskeletal structures. Interestingly, a global analysis indicate that 50% of all proteins localize to multiple compartments, almost 50% show variation between cell lines and 5-10 % show cell cycle dependent expression[2,5]. A major challenge is to harvest all information embedded in the high-resolution images. Currently this is addressed by automated image analysis and refined classification of intricate subcellular patterns. For instance, the microtubule patterns have been subclassified into microtubules, plusends, minusends, centrosome, centriole, primary cilia and cytokinetic bridge.

Here we discuss the importance of spatial proteomics and present the content and results of the Subcellular Protein Atlas as well as the challenges to maximize the biological output with emphasis on cytoskeletal structures. We also demonstrate how super-resolution microscopy can be used to localize proteins in primary cilia on a nm scale as well as present a group of non-cytoskeletal proteins with a cytoplasmic rod&ring like pattern.

1. www.proteinatlas.org/subcellular
2. Fagerberg et al, J Proteome Res, 2011, 10(8):3766-77
3. Uhlen et al, Nat Biotechnol, 2010, 28(12);1248-50
4. Stadler et al, J Proteomics, 2012, 75(7):2236-51
5. Stadler et al, Nat Methods, 2013, 10(4):315-23