ATLAS ANTIBODIES

Immunofluorescence in Cell Lines

The need for subcellular localization studies

One major rationale for investigating the subcellular location of a specific protein is that location is often tightly connected to function. For example, proteins locating to the nucleus are frequently implicated in gene regulation, proteins in mitochondria with energy production and Golgi-related proteins are often associated with protein modification and sorting. In addition to elucidating functional characteristics of proteins, subcellular location information can also facilitate protein interaction studies. For two proteins to interact, it is a prerequisite that they locate to the same defined site. Thus, knowing the subcellular location of a protein is a key step towards understanding function and probable interaction patterns.

The Human Protein Atlas

The Human Protein Atlas project has created a complete map of protein expression in all major organs and tissues in the human body[1,2,3]. To accomplish this, highly specific antibodies directed against all of the different human proteins were generated and subsequent protein profiling was established in a multitude of tissues and cells. The Human Protein Atlas (www.proteinatlas.org) consists of three separate parts, each using a particular approach to study the spatial distribution of human proteins.

The Tissue Atlas shows the distribution of the proteins across all major tissues and organs in the human body.
The Cell Atlas shows the subcellular location of proteins in single cells.
The Pathology Atlas shows the impact of gene expression levels in all major cancer types for survival of patients.

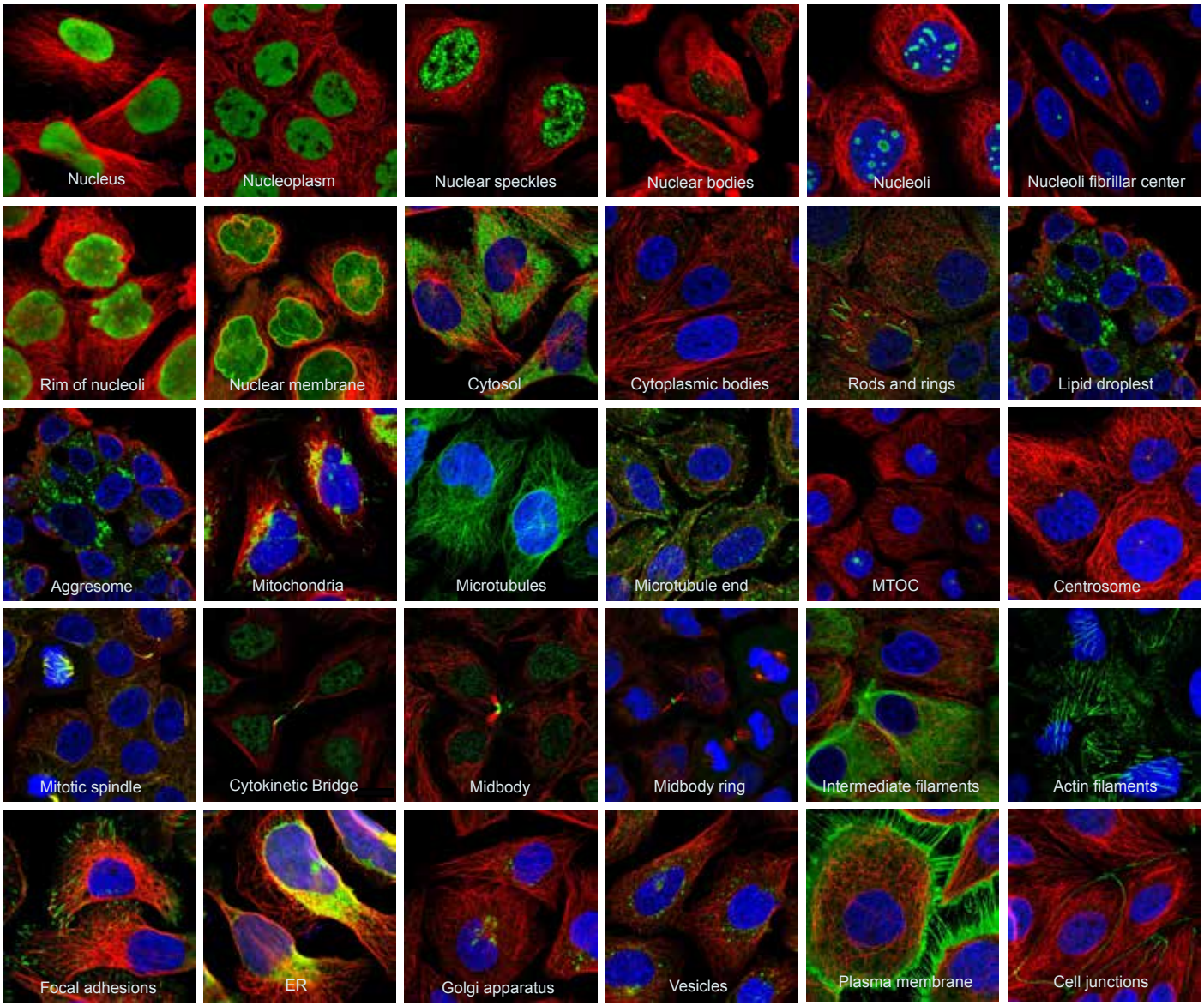

Figure 1.
The protein targets on the Human Protein Atlas portal are assigned to one or several of 30 subcellular locations, here exemplified by; nucleus (HPA036022), nucleoplasm (HPA024344), nuclear speckles (HPA041411), nuclear bodies (HPA001907), nucleoli (HPA026512), nucleoli fibrillar center (HPA061017), rim of nucleoli (HPA011384), nuclear membrane (HPA001209), cytosol (HPA018295), cytoplasmic bodies (HPA066137), rods and rings (HPA001400), lipid droplets (HPA016607), aggresome (HPA056406), mitochondria (HPA024089), microtubules (HPA038786), microtubule end (HPA026678), microtubule organizing center (MTOC) (HPA034964), centrosome (HPA037682), mitotic spindle (HPA046743), cytokinetic bridge (HPA037708), midbody (HPA044257), midbody ring (HPA058265), intermediate filaments (HPA002465), actin filaments (HPA050627), focal adhesions (HPA004835), endoplasmic reticulum (ER) (HPA003230), golgi apparatus (HPA035275), vesicles (HPA041224), plasma membrane (HPA018530) and cell junctions (HPA063559).The HPA product number refers to the antibody used in the presented image. Antibody staining is shown in green, nuclear reference in blue and microtubules reference in red. For further information about subcellular staining patterns, please visit the subcellular dictionary on Human Protein Atlas web portal: https://www.proteinatlas.org/learn/dictionary/cell

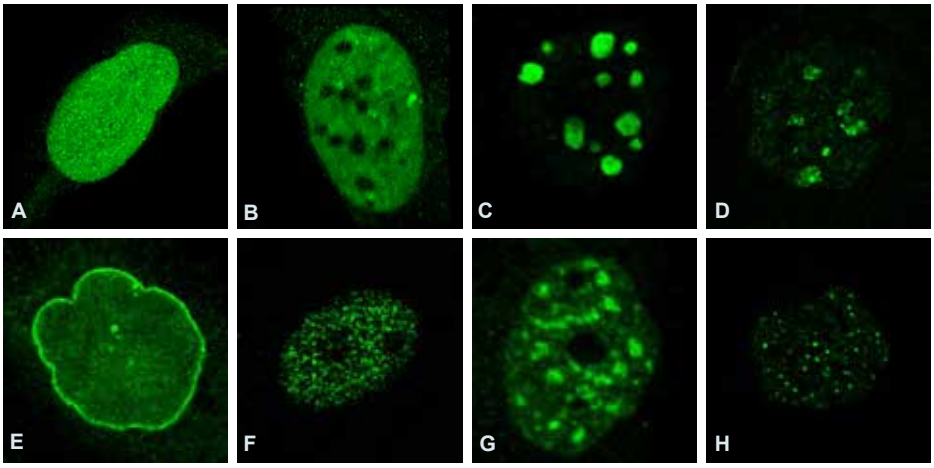

Figure 2.
Diversity of nuclear staining patterns shown by eight different antibodies in cell line U2-OS or A-431. A) Smooth nuclear staining by Anti-MECP2 (HPA000593), B) nuclear staining without nucleoli by Anti-RBM14 (HPA006628), C) nucleoli staining by Anti-ZSCAN1 (HPA007938), D) nucleoli fibrillar center staining by Anti-FOXL2NB (HPA061017), E) nuclear membrane staining by Anti-UNC84B (HPA001209), F) nuclear granular staining by Anti-REPIN1 (HPA036022) G) nuclear speckled staining by Anti-RBM25 (HPA003025), H) nuclear bodies stained by Anti-TERF2 (HPA001907).

ICC-IF on the Human Protein Atlas

To acquire spatial information of proteins on a subcellular level, high resolution fluorescent confocal microscopy was performed[1,4,5].

On the Human Protein Atlas portal, all proteins are localized in the U-2 OS cell line. In addition, two other cell lines are selected based on RNA transcript levels for the protein coding gene of interest. The three cell lines are stained with the same antibody.

Standardized immunofluorescent based procedures are used when staining the different human proteins. In addition to the investigated protein of interest (green channel), a set of reference markers are used in each image; an antibody based marker to α-tubulin to visualize the microtubules (red), an antibody towards KDEL to visualize the endoplasmic reticulum (yellow) as well as the nucleic acid dye DAPI (blue) to visualize the nuclei. These markers serve as controls for sample fixation, permeabilization and immunostaining[6], as well as guidance in the image annotation for assignment of subcellular location.

Antibody dilution and immunostaining procedures are automated and confocal microscopy images are acquired and annotated manually including comparing the protein localization with existing literature.

The proteins are localized to 30 different cellular structures (Figure 1) defining 13 major organelle proteomes. The staining is further analysed by scoring intensity on a four-graded scale and assigned staining characteristics e.g. smooth, granular, speckled or fibrous.

Figure 2 illustrates differences in nuclear substructures. The six different proteins localize to the nucleus, nucleoli or nuclear membrane and show varying staining characteristics.

On the Human Protein Atlas portal, each staining experiment can be viewed in a virtual microscope. Four different channels are clickable for visualization of the three markers in blue, red and yellow and antibody staining in green (Figure 3). A validation score of the observed staining is assigned for each cell line and is classified as either Validated, Supported, Approved or Uncertain. The scores are based on concordance with RNA sequencing data, experimental gene/protein characterization data in the UniProtKB/Swiss-Prot database as well as with other antibodies against the same target.

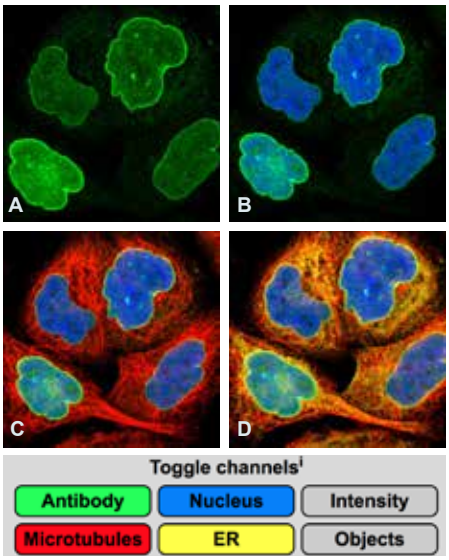

Figure 3.
Anti-SUN2 (HPA001209) shows staining of nuclear membrane in A-431 cells. By clicking on the different channels, the images can be visualized with one or several reference markers. **A**) Antibody staining shown in green, **B**) addition of DAPI nuclear reference in blue, **C**) addition of microtubules reference in red and **D**) addition of endoplasmic reticulum (ER) reference in yellow.

References:
1)Thul PJ et al. A subcellular map of the human proteome. Science 2017 356(6340): eaal3321
2)Uhlén M. et al. Tissue-based map of the human proteome. Science 2015 347(6220):1260419.
3) Uhlén M. et al. Towards a knowledge-based Human Protein Atlas. Nat Biotechnol 2010 28(12):1248-50.
4) Barbe L. et al. Toward a confocal subcellular atlas of the human proteome. Mol Cell Proteomics 2008 7(3):499-508.
5) Stadler C. et al. Immunofluorescence and fluorescent-protein tagging show high correlation for protein localization in mammalian cells. Nat Methods. 2013 Apr;10(4):315-23 2013.
6) Stadler C. et al. A single fixation protocol for proteomewide immunofluorescence localization studies. J Proteomics 2009 73(6):1067-78

Summary

- Subcellular location studies are a key step in elucidating protein function and interaction. ICC-IF images showing the localizations for all human protein targets are accessible through the Human Protein Atlas portal.
- In the Human Protein Atlas, subcellular location information of proteins is obtained by using high resolution fluorescent confocal microscopy in three different human cell lines. Each protein is assigned to one or several of 30 subcellular locations by manual annotation.
- All ICC-IF images shown on the Human Protein Atlas portal can be visualized through the interactive “virtual microscope”, enabling the user to toggle the different clickable multicolor images, which are displaying the staining for the protein of interest as well as the organelle probes.

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