nature reviews molecular cell biology
https://doi.org/10.1038/s41580-026-00947-3

Review article

Check for updates

Subcellular localization as a driver of protein function

Alina Sigaeva[1,2,7], Charlotte Hutchings[3,7], Anthony Cesnik[4], Kathryn S. Lilley[3] & Emma Lundberg[1,4,5,6]

Abstract

Biological functions depend on the spatiotemporal distribution of proteins within cells. Key cellular activities such as signal transduction, metabolism, cell cycle and cell death are driven by the interactions of proteins that are localized in multiple cellular compartments. Such multilocalization can even allow protein with identical sequences to display multifunctionality, a phenomenon known as moonlighting. Despite its biological importance, the relationship between protein localization and function remains underexplored. In this Review, we discuss the known mechanisms of protein localization (including RNA transport, role of proteoforms and molecular interactions) and how subcellular localization controls protein function. Proper regulation of protein localization is crucial for specialized cell and tissue functions, including cell differentiation, polarization and the epithelial–mesenchymal transition. Protein mislocalization can also have important roles in pathological processes, such as in cancer, neurodegeneration and autoimmunity. We end with a discussion of current technological and conceptual challenges in the field of subcellular proteomics and spatial biology. Addressing these challenges will allow us to link the dynamic nature of protein localization and function across biological scales and contexts, with great impact on fundamental cell biology and clinical applications.

Sections

Introduction

Mechanisms of protein localization

Protein localization dynamically fine-tunes protein function

Protein localization is instrumental for cell differentiation and identity

Protein mislocalization in disease

Conclusion and future perspective

[1]Science for Life Laboratory, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, Stockholm, Sweden. [2]Neurosensorik/Animal Navigation, Institute of Biology and Environmental Sciences, Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany. [3]Cambridge Centre for Proteomics, Department of Biochemistry, University of Cambridge, Cambridge, UK. [4]Bioengineering Department, Stanford University, Stanford, CA, USA. [5]Pathology Department, Stanford University, Stanford, CA, USA. [6]Chan Zuckerberg Biohub, San Francisco, CA, USA. [7]These authors contributed equally: Alina Sigaeva, Charlotte Hutchings. e-mail: emmalu@stanford.edu

Introduction

The regulation of cellular functions such as preserving genetic information, producing energy and building blocks, and sending and receiving signals to and from the microenvironment is achieved through the subcellular compartmentalization of molecules, including proteins, which are involved in nearly every biological process. Spatially separated cellular niches combined with temporally regulated expression allow the cell to isolate and concentrate local molecular interactions that facilitate protein function.

Proteins display a remarkable degree of multilocalization across the cell, with over 50% of all annotated human proteins currently reported to be multilocalizing[1,2]. The estimates for other model organisms are somewhat lower, possibly owing to lower subcellular complexity, fewer systematic studies and more reliance on computational predictions rather than experimental evidence. Nevertheless, multilocalization has been reported for at least 30% of proteins in *Saccharomyces cerevisiae* (yeast)[3], 27% in *Mus musculus* (mouse), 14% in *Drosophila melanogaster* (fruitfly), 12% in *Arabidopsis thaliana* (thale cress) and 9% in *Oryza sativa* (rice)[4]. Thus, protein multilocalization appears to be a common phenomenon across the tree of life.

Multilocalization can dramatically expand the interaction landscape and the resulting cellular function(s) of a protein. Whereas the functionality of differential localization is well studied for protein groups such as transcription factors and metabolic enzymes, the functions of most proteins in their non-canonical locations remain unknown. There is a large disparity between the number of proteins annotated as multilocalizing and the number which are considered multifunctional (Supplementary Fig. 1a). The number of described functions tends to be higher for the proteins that have been studied for a longer time (Supplementary Fig. 1b). It is important to note that different experimental and conceptual methodologies for studying protein localization and its relation to protein function have resulted in varying definitions of protein multilocalization and multifunctionality, as discussed in Box 1. Regardless of the definitions used, the prevalence of both protein multilocalization and multifunctionality and the connection between the two are drastically underestimated. Therefore, our understanding of cellular behaviours and functions will benefit from systematic large-scale mechanistic studies of proteins at the subcellular level. The state-of-the-art methods used to identify and control subcellular protein localization are discussed in a complementary Review paper[5].

In this Review, we discuss the functionality and mechanisms of subcellular protein localization. First, we describe what is known about the mechanisms underlying protein localization, ranging from pretranslational localization of mRNAs to the active relocation of proteins. We discuss the importance of localization for protein function and the ways in which localization changes can fine-tune functionality and promote the evolution of multifunctional proteins. We provide examples of the effects of protein localization on establishing distinct cell types and phenotypes and discuss the growing body of literature describing protein mislocalization in disease. Finally, we outline future research directions that will advance our understanding of correct subcellular protein localization and its importance for protein function.

Mechanisms of protein localization

Many cellular processes control protein localization (Fig. 1), usually through tightly regulated molecular interactions that actively guide proteins to locations of specific activities within the cell. Here, we discuss such interactions and the mechanisms that compartmentalize the proteome and contribute to the control of biological functions. We begin by discussing the pretranslational localization of mRNAs by RNA-binding proteins (RBPs) and organelles. We then consider mechanisms which facilitate the cotranslational or post-translational localization of proteins, including but not limited to those that are directed by the presence of specific protein sequence motifs and structures. We highlight the role of PTMs and proteoforms (distinct molecular forms of a protein) in defining and dynamically altering protein localization, and the interplay of various localization mechanisms.

RNA localization and local translation

The control of subcellular localization begins even before protein synthesis. Protein-coding mRNAs contain *cis*-acting RNA elements known as RNA 'zipcodes', which are recognized and bound by a myriad of RBPs and form messenger ribonucleoprotein (mRNP) granules (a type of biomolecular condensate)[6]. Examples of mRNP granules contributing to localized translation include neuronal granules[7] and germ granules[8,9]. RBPs regulate the translation, degradation and localization of mRNAs to spatiotemporally regulate protein synthesis[10–12].

RBPs can limit the availability of ribosome-binding sites in mRNAs to repress their translation and can interact with motor proteins to facilitate active mRNA transport. A well-studied example is ZBP-1 (also known as IGF2BP1), which binds the 3′ untranslated region of *ACTB* mRNA (encoding β-actin) and interacts with the microtubule motor protein KIF11 (ref. 13) to move the resulting mRNP to the leading edge of motile fibroblasts[14–16], axonal growth cones[17–19] and dendritic filopodia[20] (Fig. 1a). Phosphorylation of ZPN1[21,22] results in mRNA release and localized synthesis of β-actin to facilitate directional movement and growth[23,24]. This localization mechanism can be further regulated by RNA modifications and structures, such as N^6-methyladenosine (m^6A) switches[25], and there is increasing evidence that these features regulate mRNA interactions with RBPs to control their recruitment to cellular compartments[25–28]. Nevertheless, the full impact of RNA modifications and structure on mRNA localization, protein localization and protein function remains underexplored. In addition to direct interactions between mRNAs and RBPs, recent studies have demonstrated that organelles can also control mRNA localization and local translation. Both mRNA and ribosomes are transported across the cell by endosomes and lysosomes[29–33]. The FERRY complex preferentially binds both mRNAs of nuclear-encoded mitochondrial proteins and the endosomal GTPase RAB5, thereby recruiting the transcripts to early endosomes in proximity of mitochondria[32,33] (Fig. 1a).

Despite an increased appreciation of the importance of mRNA localization in supporting localized protein synthesis and function, systematic association between mRNA and protein localization for each gene has not yet been established. Rapid ongoing advances in in situ spatial transcriptomics, growing databases of RBP motifs[34] and techniques in development for targeted relocalization of endogenous RNA (for example, CRISPR-TO[35]) are improving our understanding of mRNA localization and interactions in cells. This knowledge, together with new high resolution tools for investigating local translation[36], will probably facilitate a transition from studying the effect of mRNA localization on the location and function of a few proteins at a time to gaining a systematic understanding of this mechanism and its physiological importance.

Sequence-guided and structure-guided localization

Following translation, the primary amino acid sequence and the folded structure of a protein contain localization signals and domains that bind

Box 1 | Defining multilocalizing, multifunctional and moonlighting proteins

Proteome-wide studies of subcellular localization have identified a large proportion of multilocalization[1,2]. Meanwhile, an increasing number of studies have demonstrated the capacity of proteins to carry out multiple cellular functions[93,94]. Here, we reflect on what it truly means to be a multilocalizing or multifunctional protein and define key nomenclature.

Proteins can be annotated as multilocalizing for a number of reasons. Some proteins, including glycolysis enzymes, tricarboxylic acid cycle enzymes and transport proteins occupy multiple locations within a single cell at a given time. Other proteins, such as those involved in the cell cycle, can be found at different locations within the same cell at different points in time. Multilocalization can occur on a larger scale with proteins displaying variable spatial distributions in different cell types or tissues. Here, we consider a protein to be 'multilocalizing' when the same protein has been identified in two or more discrete cellular compartments in any scale of space or time. The notion of discrete cellular compartments, often imposed by the scale and limited resolution of current experimental methods, hampers the classification of protein distribution gradients within compartments. In reality, niches exist even within cellular compartments, and protein gradients across these niches have an important role in cellular function[218].

The question of multilocalization naturally extends to consider how the cell regulates changes in protein localization. Proteins exhibiting variable cellular localization in different conditions can be described as 'differentially localized'. Differential localization can be caused by proteins moving from one location in the cell to another ('relocalization') or by local changes in protein turnover[219]. In both cases, differential localization can occur without any change in total protein abundance.

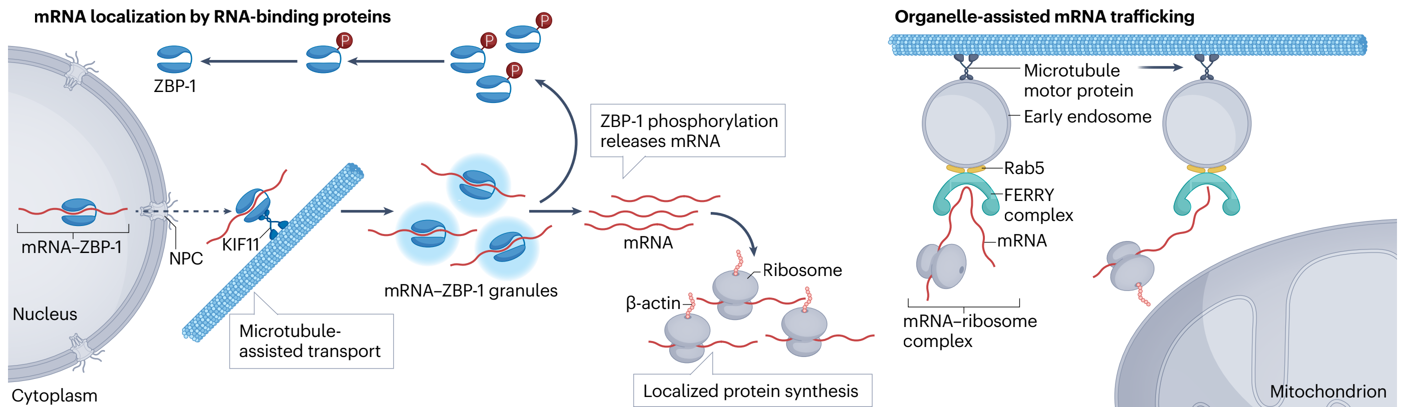
Next, the concept of protein function warrants attention as the definition of a multifunctional protein differs across the literature. We define 'protein function' based on the hierarchy imposed by the Gene Ontology consortium, in which 'molecular function' represents the molecular-level activity of a protein, and 'biological process' is the higher-level biological role achieved by the molecular function[220]. Thus, a 'multifunctional protein' should carry out two or more molecular functions, and proteins that carry out the same molecular function in different biological processes (for example, enzymes using different substrates for the same reaction) would not be considered multifunctional. This definition of multifunctionality does not mean that the protein has to perform different molecular functions at the same time, within the same cell — or even within the same cell type — as demonstrated by the example of crystallins, which are classical multifunctional proteins[221].

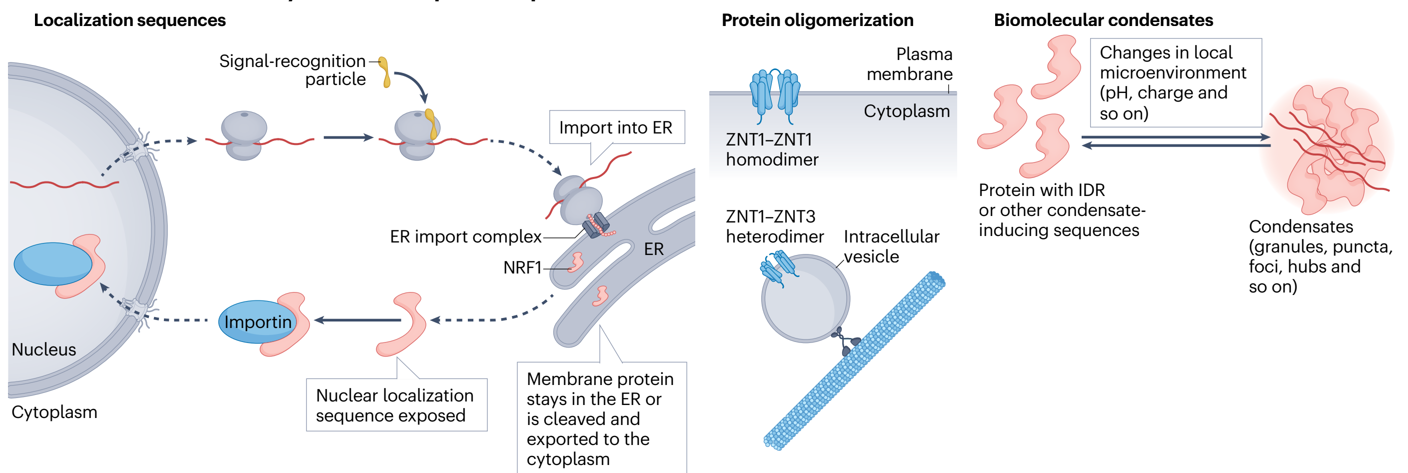
To define a protein as multilocalizing or multifunctional, there must be a clear definition of what we consider to be a single protein entity. Indeed, a single gene can give rise to multiple proteoforms through alternative splicing and post-translational modifications (PTMs), and proteoforms can oligomerize to provide further molecular and functional diversity[55]. Therefore, we broadly define a 'protein' as including all proteoforms resulting from a single gene, that is, a proteoform family[222]. Protein homologues are encoded by separate genes and are therefore considered to be independent proteins; these proteins may have similar sequences and structures if gene duplications were recent or followed by minimal sequence divergence. The molecular diversity of proteins is important to define in the present context, because although multilocalization and multifunctionality are affected by proteoform diversity, the current methods to interrogate these characteristics are often unable to distinguish between proteoforms[5].

Finally, in this Review, we also discuss examples of 'moonlighting proteins'. Such proteins represent an extreme subset of multi-functional proteins, in which multiple functions are reported for identical polypeptide sequences, thus excluding protein homologues and proteoforms generated by alternative splicing, gene fusions or proteolysis[93,125]. Although multilocalization is not a prerequisite for being a moonlighting protein, the two are often discussed together as multilocalization is one of the main ways in which moonlighting functions can evolve[126,128]. It is important to point out that, as we are learning more about the ways in which a protein can be multifunctional, the original definition of moonlighting proteins begins to be challenged by the research community[223]. We might need to refine our nomenclature for multifunctional and moonlighting proteins to better reflect the underlying biological complexity.

to the molecules required to guide them to their final destination(s) in the cell. Classic examples of such sequences include nuclear localization signals (NLSs) and nuclear export signals, which recruit importin and exportin proteins, respectively, to facilitate transport across the nuclear membrane[37–39]. Similarly, mitochondrial localization signals are bound by proteins such as TOM40 for import into mitochondria[40,41], and membrane localization signals lead to binding with signal recognition particle proteins before their import to the endoplasmic reticulum and vesicular export to the cell membrane[42,43] (Fig. 1b). Although localization signals are widely used and commonly conserved across the tree of life (Box 2), they often appear to be functionally redundant and ambiguous (containing overlapping sequences that target multiple compartments). In addition, organelle targeting seems to be permissive, as a large proportion (20–30%) of randomly generated peptides can act as localization signals that drive protein translocation[44].

Discovering and searching for localization signals with experimental and machine-learning methodologies has greatly improved our understanding of protein localization. However, there is increasing evidence that other physicochemical characteristics of the amino acid sequences (for example, isoelectric point, hydrophobicity)[45] also affect subcellular localization of proteins. Moreover, many localization signals need to be recognized by an adaptor protein for translocation, and specific adaptors might not be available in the cell. The cellular context becomes more important for artificial proteins or proteins coming from different species. For example, whereas NLSs are generally conserved across the tree of life, certain yeast NLSs are functional in plants, but not in mammalian cells[46]. Moreover, a protein can contain conflicting localization signals of varying strength that are exposed or shielded in different subcellular environments. Therefore, proteins that contain localization signals are not always present in the designated compartment and those without a known localization signal may localize to a specific compartment, as is the case for the 69% of proteins annotated as nuclear in UniProt, which do not contain a known NLS[45].


a mRNA localization and local protein synthesis
mRNA localization by RNA-binding proteins
ZBP-1
P
ZBP-1 phosphorylation releases mRNA
mRNA–ZBP-1
NPC
KIF11
mRNA–ZBP-1 granules
mRNA
Ribosome
β-actin
Nucleus
Microtubule-assisted transport
Localized protein synthesis
Cytoplasm
Organelle-assisted mRNA trafficking
Microtubule motor protein
Early endosome
Rab5
FERRY complex
mRNA
mRNA–ribosome complex
Mitochondrion


b Protein localization driven by interactions of protein sequences and structures
Localization sequences
Signal-recognition particle
Import into ER
ER import complex
ER
NRF1
Importin
Nucleus
Cytoplasm
Nuclear localization sequence exposed
Membrane protein stays in the ER or is cleaved and exported to the cytoplasm
Protein oligomerization
Plasma membrane
Cytoplasm
ZNT1–ZNT1 homodimer
ZNT1–ZNT3 heterodimer
Intracellular vesicle
Biomolecular condensates
Changes in local microenvironment (pH, charge and so on)
Protein with IDR or other condensate-inducing sequences
Condensates (granules, puncta, foci, hubs and so on)

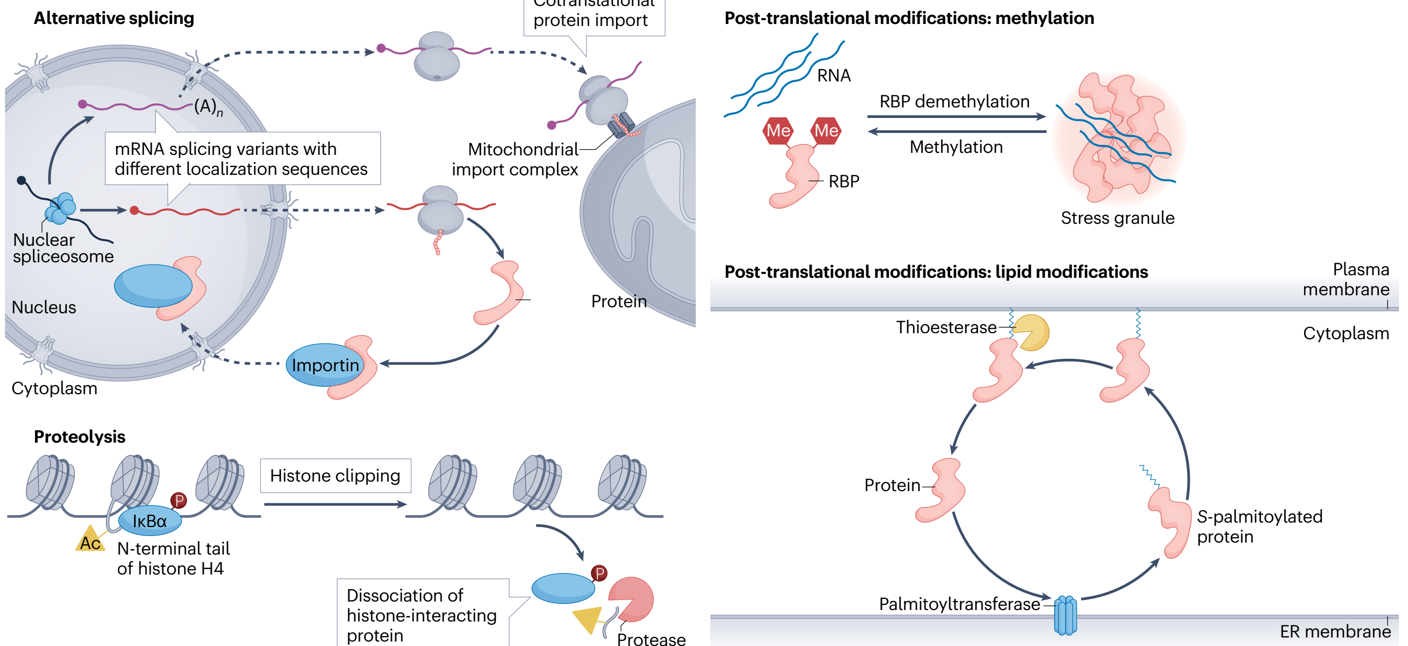

c Proteoform-driven localization
Alternative splicing
Cotranslational protein import
$(A)_n$
mRNA splicing variants with different localization sequences
Mitochondrial import complex
Nuclear spliceosome
Nucleus
Protein
Importin
Cytoplasm
Post-translational modifications: methylation
RNA
RBP demethylation
Me
Methylation
RBP
Stress granule
Post-translational modifications: lipid modifications
Plasma membrane
Thioesterase
Cytoplasm
Protein
S-palmitoylated protein
Palmitoyltransferase
ER membrane
Proteolysis
Histone clipping
P
IκBα
Ac
N-terminal tail of histone H4
Dissociation of histone-interacting protein
Protease

Fig. 1 | Cellular mechanisms of protein localization. a, mRNA localization. mRNAs can specifically interact with RNA-binding proteins (RBPs) that target them to a specific cellular compartment for localized protein synthesis. ZBP-1 binds *ACTB* mRNA and interacts with the microtubule motor protein KIF11 to move the resulting mRNP granule to the motile edge of the cell. mRNAs can also interact with adaptor proteins, such as the RNA/ribosome intermediary (FERRY) complex on the surface of organelles – in this case, endosomes – and are transported together with these organelles. **b**, Localization through protein interactions. A protein can contain multiple localization signals of different strength, which might be shielded or exposed in specific conditions, resulting in interactions with different adaptor proteins. Endoplasmic reticulum (ER) localization sequences found in the nascent polypeptide are recognized by signal recognition particles, which trigger interaction with an ER import complex and subsequently cotranslational import into the ER matrix or insertion into its membrane. Some resident ER proteins, such as the transcription factor NRF1 (ref. 217), can be cleaved and exported back to the cytoplasm, exposing nuclear localization sequences recognized by importins, which facilitate the entry of NRF1 into the nucleus. Other protein–protein interactions, such as oligomerization, can affect protein localization. Homodimerization of ZNT1 results in plasma-membrane localization, whereas ZNT1–ZNT3 heterodimers localize to intracellular vesicles. The assembly of biomolecular condensates is often controlled by interactions between intrinsically disordered regions (IDRs) of proteins in the subcellular microenvironment. **c**, Proteoform localization. One gene can produce multiple proteoforms with differing subcellular localizations. Alternative splicing can result in loss or gain of localization signals. Proteins can undergo various post-translational modifications affecting localization. For example, proteolysis of the acetylated (Ac) N-terminal tail of histone H4 ('histone clipping') reduces the interaction of this histone with phosphorylated (P) IκBα, triggering dissociation of IκBα from the chromatin during intestinal cell differentiation. Arg methylation and demethylation of RBPs control the assembly of stress granules. S-palmitoylation can promote protein localization to the plasma membrane. The dashed arrows represent translocation. NPC, nuclear pore complex.

There is an increasing number of transformer-based protein language models that use multimodal protein information, including protein sequences, to better predict protein folding[47,48] and subcellular localization[45]. So far, none of these models have been entirely successful in predicting protein localization, with performance varying for different organelles and especially for smaller compartments and for multilocalizing proteins. The performance of such language models will improve as they capture sequence information beyond localization signals, including intrinsically disordered regions (IDRs), charge patterns and structural motifs such as interaction domains. For more in-depth discussion of the advances in this field, we refer the reader to a complementary review[5].

Sequence and structure features contribute to localization by influencing the molecular interactions of proteins. Homodimerization of ZNT1 (also known as SLC30A1) results in canonical localization to the plasma membrane, where ZNT1 exports zinc to protect the cell from zinc toxicity, whereas heterodimers of ZNT1 with ZNT3 localize to intracellular vesicles[49] (Fig. 1b). Similar differences in subcellular localization were also shown for other combinations of ZNT homodimers and heterodimers. In some cases, the effects of molecular interactions go beyond relocalization of the protein and lead to the formation of a subcellular compartment. Indeed, interactions between proteins, RNA molecules and other molecules can lead to phase separation and the formation of biomolecular condensates[50,51] (Fig. 1b). The propensity of a protein to contribute to such condensates is heavily influenced by its properties, including specific amino acid composition, PTMs and the presence of IDRs or other polymerization-promoting domains[52]. The functional importance of dynamic condensates is increasingly recognized and it is currently thought that up to 20% of the proteome can be dynamically localized to mesoscale biomolecular condensates[51]. Importantly, the same sequence and structure features that can drive the formation of larger condensates, also regulate the recruitment of proteins to smaller complexes that do not undergo phase separation[53,54], thereby regulating protein localization across a multitude of scales.

Proteoform-level regulation of localization

Protein localization can be regulated by controlling the generation and expression of proteoforms with differential localization. A single gene can give rise to proteoforms[55] owing to PTMs, sequence variations, alternative splicing and proteolysis. The mechanisms discussed above can result in differential localization of these proteoforms. Alternative splicing of mRNAs can produce proteoforms with different localization signals, leading to different subcellular localization (Fig. 1c). A recently discovered example is CDK5RAP2, which has a centrosome localization signal in exon 17 that is missing from its shorter splicing isoform. The proteoform produced from this alternatively spliced transcript has cytoplasmic localization and contributes to microtubule formation in developing neurons[56]. Alternative splicing can generate proteoforms that interact with sets of factors as different as those of proteins encoded by independent genes[57], which also probably contributes to pleiotropy and differential localization. Similarly, the protein-sequence variation introduced by proteolytic cleavages can control proteoform localization by removing localization signals or influencing protein interactions. In some cases, cleavage of one protein can cause a change in localization of another protein. For example, proteolysis of the acetylated N-terminal tail of histone H4, a process referred to as 'histone clipping', reduces the interaction of this histone with phosphorylated NF-κB inhibitor-α (IκBα) during intestinal cell differentiation (Fig. 1c). Consequently, IκBα dissociates from the chromatin and remains in the nucleoplasm, which is required for differentiation[58].

A second layer of proteoform-level regulation of subcellular localization is PTMs, which alter protein conformation and interactions and regulate the accessibility of localization signals. For example, the phosphorylation of STAT transcription factors promotes their nuclear import upon cytokine stimulation[59]. Modifications such as Arg methylation regulate protein recruitment to and assembly of ribonucleoprotein granules and other membraneless organelles[60] (Fig. 1c). Inclusion into membranes can be directed by lipid modifications such as S-palmitoylation[61] (Fig. 1c), whereas the secretion of extracellular proteins often depends on complex glycosylation patterns and crosstalk with other modifications, for example in the case of beta-glucuronidase[62,63]. Hence, the addition or removal of PTMs can act as a regulatory switch linking cell signalling pathways to protein localization and function.

Together, these examples provide motivation to study proteoforms, specifically, the precise roles of proteoform variation in localization and functionality across the proteome and pleiotropy across the genome. The extent of proteoform variation, however, remains largely uncharacterized[64]. Current technology often does not allow robust detection of different proteoforms, measuring their abundance or interrogating their localization. Spatial proteomics methods typically

Box 2 | Protein multilocalization and multifunctionality across the tree of life

The compartmentalized distribution of biomolecules is a fundamental feature of life. Even the simplest prokaryotes contain intracellular domains enriched in specific molecules to perform specific functions. As in eukaryotic cells, these domains include membraneless[224] and membrane-bound compartments[225,226]. Bacterial encapsulin proteins can form shells that isolate certain parts of the cytoplasm but that have pores that permit the exchange of molecules[227]. Similarly, viruses in cells create protein-enclosed compartments (capsids) with controlled molecular import and export[228]. Viral capsid proteins and bacterial encapsulin proteins are possibly evolutionary connected[229].

Both cell compartmentalization and protein multifunctionality pose a challenge for protein targeting, which in various organisms has been solved in multiple non-exclusive ways. mRNA localization has been described from bacteria and yeast to plants and vertebrates[11,12], as well as in over 500 human cell lines[230,231]. On the one hand, mRNA localization has intuitive energetic advantages, as a single localized mRNA transcript can be translated into thousands of copies of protein, eliminating the need to individually transport each of the protein molecules. On the other hand, relocalization of existing proteins owing to changes in localization-signal accessibility permits highly dynamic shifts in protein distribution, possibly enabling better temporal control of the process. A disadvantage of protein localization signals is that they are often hijacked by pathogens, including by viruses[232,233] and fungi[234]. Localization signals are typically not very specific: a wide range of unrelated sequences can promote protein translocation to a specific compartment[44]. This property not only makes localization signals robust to mutations but also allows proteins to explore new subcellular locations and interaction partners, possibly conferring an evolutionary advantage. Translocation of existing proteins between membrane-bound compartments is usually facilitated by protein transport complexes such as the nuclear pore complex, which was already present in the last eukaryotic common ancestor[235] and is similar to transport structures of certain bacteria with membrane-encapsulated DNA[236]. Specific protein import machinery is not necessarily highly conserved; for example, the general structure of the eukaryotic mitochondrial import complex is preserved, but its individual proteins evolved independently and converged in function[237].

Another way to control and change protein distributions in the cell relies on having several similar proteins with distinct subcellular localizations, either encoded by one gene and resulting from alternative splicing[238] or originating from gene duplication and subsequent evolution of the paralogs. Whereas (alternative) splicing is not present in prokaryotes, gene duplication is and can result in long-term gene preservation and independent evolution of similar, yet distinct, proteins. Duplication of a gene encoding a moonlighting protein can either lead to the complete specialization of the paralogs[239] or to the partial preservation of moonlighting activity[240]. One example of gene duplication leading to divergent subcellular localization is malate dehydrogenase (MDH). Most animals express two MDH isoforms: cytosolic MDH, which is encoded by *MDH1*, and mitochondrial MDH, which is encoded by *MDH2* and has a mitochondrial import sequence[241]. Land plants have evolved at least six MDH isoforms that variably localize in the cytosol, mitochondria, peroxisomes and plastids[242]. In both cases, MDH isoforms catalyse the same reaction but contribute to different biological processes.

Subcellular localization controls protein function throughout the tree of life and in viruses. Many mechanisms of protein localization have arisen through both parallel and convergent evolution, highlighting the importance of subcellular localization for protein, cell and organism function.

rely on antibodies designed to be broadly specific to a gene, targeting as many protein isoforms as possible, and shotgun proteomics workflows rely on peptide identifications that neither address the full combinatorial nature of PTMs within a protein nor accurately map splicing variants[64,65]. Although some studies have begun to predict and experimentally map the subcellular distribution of PTMs[66–68], the phenotypic consequences of such distributions, the effects of PTM localization and the crosstalk between different PTMs remain understudied. Furthermore, proteoforms have largely been analysed in bulk samples that pool thousands of individual cells and provide information in averages. Advances in cell fractionation[69], large-scale isolation of cell types[70] and single-cell proteoform detection[71–73] will make identifying the precise subcellular localizations of specific proteoforms a promising area of future research.

Interplay of protein localization mechanisms

The wide range of mechanisms available to the cells to control protein localization raises the question: How do these mechanisms interact with each other? The accessibility of various localization signals to transport proteins can be irreversibly modulated by alternative splicing[56,74] or reversibly modulated by PTMs[75,76]. Moreover, the local availability of interaction partners for translocation (of both proteins and mRNA) or, conversely, molecules that mask localization signals, might change the preferred localization. Subcellular localization of a specific protein molecule is clearly a result of the interplay between various targeting mechanisms. For example, β-actin, discussed above as a protein with mRNA-driven localization, is not exclusively located at the cellular leading edge and similar regions, and has important functions in the nucleus. However, β-actin does not possess an NLS and is cotransported into the nucleus with cofilin[77].

Perhaps the best characterized examples of subcellular localization facilitated by multiple targeting mechanisms are membrane proteins trafficked through the endomembrane system. The endomembrane system comprises the endoplasmic reticulum, Golgi complex, endosomes, lysosomes, peroxisomes and lipid droplets. This orchestra of organelles is responsible for the synthesis, processing and trafficking of membrane proteins, which are estimated to account for approximately one-third of the proteome[78]. The passage of a protein through the endomembrane system involves mRNA targeting, cotranslational and post-translational protein localization. These mechanisms are largely dependent on interactions between the target protein and organelle-specific proteins; a number of excellent reviews have been dedicated to the role of the endoplasmic reticulum[79], Golgi complex[80] and intracellular vesicles[81–83] in protein targeting, sorting and recycling. Apart from being irreplaceable for correct localization of integral-membrane proteins, endomembrane-assisted trafficking

allows cells to distribute proteins faster and on a larger scale compared with the transport of individual molecules, and it contributes to spatial organization of signalling pathways.

Whether all protein targeting mechanisms are used in equal measure for all proteins remains unknown. There may be an evolutionary pressure for using some localization mechanisms over others, depending on the intrinsic properties of the proteins, such as their structure, abundance, turnover rates, size, shape, as well as their function, target location and the timescale on which relocalization needs to occur. Better characterization of the role of different localization mechanisms in subcellular targeting of particular proteins requires simultaneous analysis of the different mechanisms by which protein localization is controlled, from sequence to local molecular interactions. Methods for identifying subcellular protein localization are briefly summarized in Box 3 and are discussed in detail, together with the corresponding resources and databases, in the complementary Review paper[5].

Protein localization dynamically fine-tunes protein function

The evolution of diverse mechanisms that control protein subcellular localization and the conservation of these mechanisms across the tree of life (Box 2), points to the importance of location for protein function. Two main factors explain this strong relationship. Firstly, the subcellular localization of a protein determines its interactions with other proteins and with nucleic acids, lipids and substrates for enzymatic reactions. Secondly, subcellular localization can directly influence the stability, structure and, consequently, behaviour of a protein through changes in pH and salt concentration[84,85], redox state[86,87], molecular crowding[88–90] and solvating properties[91]. This change may be of particular importance for proteins with IDRs as they have the potential to adopt different structures and functions depending on cellular conditions[85,92]. Hence, proteins correctly localized to specific subcellular compartments have access to the necessary physicochemical

Box 3 | Methods to study protein localization

There is a wide range of techniques used to determine the subcellular location of a protein, which differ in resolution and certainty, samples used, speed, throughput and automation. The currently used methodologies can be broadly classified into the following categories:

(1) Computational predictions: the amino acid sequence of a protein can be directly used to assign localization, traditionally by identifying or predicting localization signals for various organelles, including the nucleus[243], nucleolus[244,245], endoplasmic reticulum and secretory pathway[246,247], mitochondria[248,249] and peroxisomes[250]. More complex machine-learning models can deduce subcellular localization information from protein sequences even in the absence of known localization signals[45,251,252].

(2) Proximity labelling: these methods use nonspecific enzymes to covalently label and identify proteins within a small physical radius of known subcellular marker proteins (for example, structural components of mitochondria). The marker proteins ('baits') are genetically fused to a labelling enzyme, typically a peroxidase or biotin ligase[253–258]. Following treatment of the cell with the necessary substrate (hydrogen peroxide or biotin, respectively), proteins near the bait are covalently labelled ('preys'), thus allowing pull down and identification by mass spectrometry. Given the limited labelling distance, prey proteins are classified as localizing to the subcellular compartment represented by the marker bait protein[200]. A protein complex with sterically competing, mutually exclusive components probably has differential localization or performs different functions. Recently, a large-scale integrated map of protein complexes[259] demonstrated such differential composition for hundreds of protein complexes.

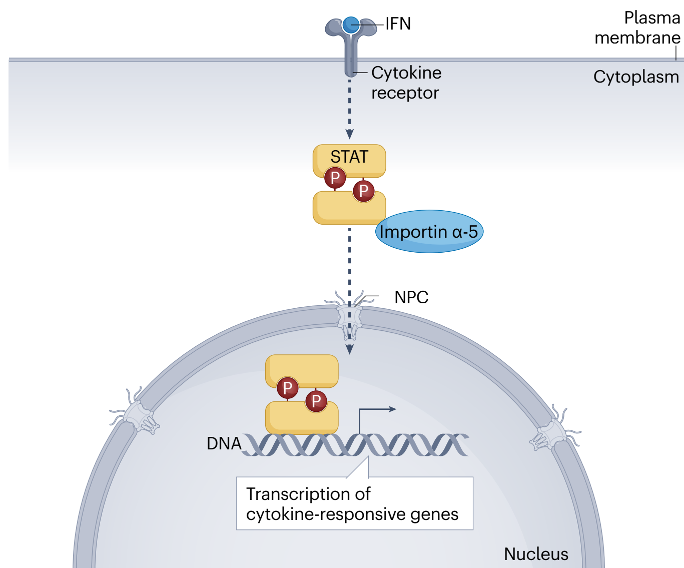
(3) Isolation and enrichment of compartments: subcellular fractionation can be used to physically separate organelles including the nucleus[260], mitochondria[261,262], lysosomes[263] and peroxisomes[264], as well as membraneless compartments (condensates)[51,265]. Similarly, laser capture microdissection can extract from cells specific subcellular regions based on microscopic visualization[266–268]. In both cases, proteins in the different samples can then be detected by mass spectrometry to define the proteome of a given compartment.

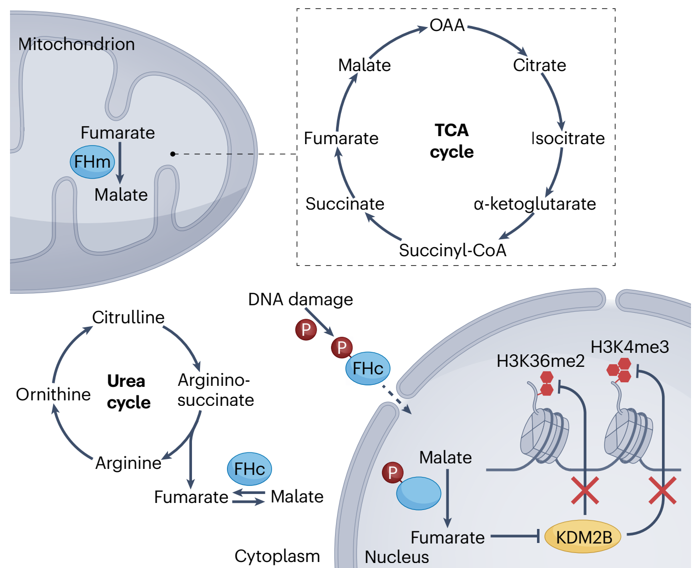
(4) Protein correlation profiling: these methods use differential centrifugation, density centrifugation or sequential detergent treatment of cell lysate to separate proteins into multiple fractions without organelle isolation or purification[2,67,201,205,269,270]. Protein subcellular localization is predicted by comparing the abundance profiles of proteins with unknown localization to those of established marker proteins, thereby generating cell-wide proteome maps.

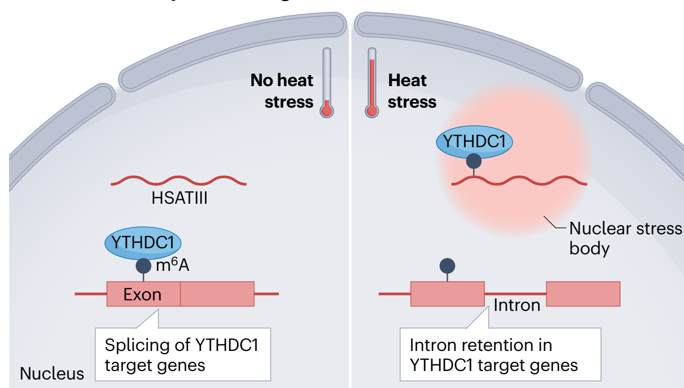
(5) Imaging-based techniques: antibodies or genetic tags are used to label proteins of interest and visualize them in situ. Optical confocal microscopy is most commonly used to detect the labels, but other modalities, such as mass cytometry or electron microscopy, can be suitable for specific applications. Protein subcellular localization is then deducted from the staining patterns of easily recognizable organelles (with the recognized-pattern repertoire drastically expanded by the use of image-to-location deep learning models such as CytoSelf[271], Subcell[208] and DINO[272]) or the overlap between signals from the protein(s) of interest and marker proteins or chemical dyes with known subcellular localization.

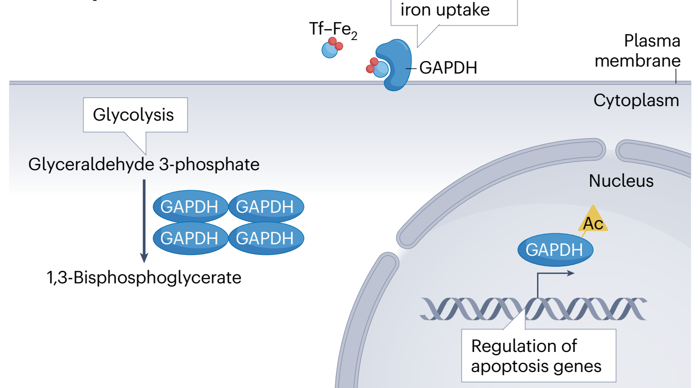
(6) Multiple measurements: multiplexed and multimodal measurements can extract multiple layers of information on molecule localization from a single sample. Multiplexed imaging technologies[273] such as those used for protein localization[274–276], for single-molecule RNA localization[277] or for both[278], allow one to overlay and compare the distributions of multiple molecules of interest. A recently introduced systematic framework allows simultaneous mapping of the subcellular transcriptome and proteome in a single sample[279]: cells are gently lysed and fractionated by density equilibrium centrifugation to separate organelles. The resulting fractions undergo phase separation, with RNA in the aqueous phase further analysed by RNA sequencing and proteins in the organic phase measured by mass spectrometry.

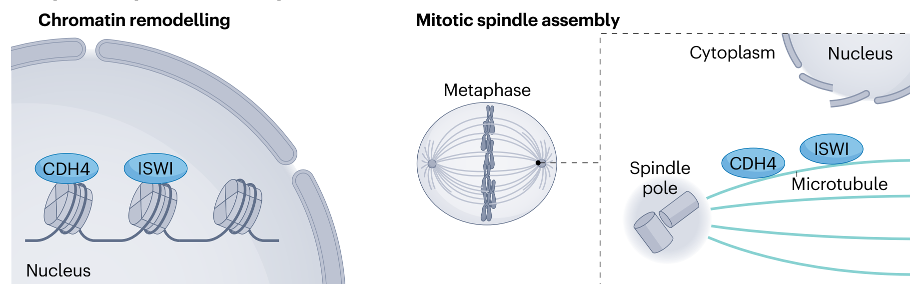
For a detailed discussion and comparison of the existing methods for determining and manipulating subcellular protein localization, we refer the reader to a complementary Review[5].











environment and interactors to carry out their function. Importantly, although an extension of the 'one gene, one protein' hypothesis could cause a misconception that a protein resides in a single location to carry out a single function, many proteins have been shown to be multilocalizing and multifunctional[1,93,94]. Here, we discuss how multilocalization and its temporal regulation expands the functional repertoire of proteins.

Multilocalization regulates protein function

Multilocalization contributes to protein function in several ways (Fig. 2). For some proteins, multilocalization arises as a direct consequence of the translocation required to carry out their function. Classical examples include, but are not limited to transcription factors and transport proteins, which respectively undergo or facilitate nucleo-cytoplasmic shuttling[95–99]. For example, stimulation of the cell

Fig. 2 | Multilocalization is crucial for protein function. **a**, A single protein function can be driven by translocation. For example, activation of STAT transcription factors by kinase receptors of cytokines, such as interferons (IFN) at the cell membrane, involves their phosphorylation and dimerization. STAT homodimers or heterodimers bind to importin α-5 and are imported to the nucleus, where they activate cytokine-responsive genes. **b**, A single protein function may contribute to multiple processes in a cell, for example, interconversion of fumarate and L-malate by fumarate hydratase (FH). Two proteoforms of fumarate hydratase exist that catalyse the reversible conversion of fumarate and l-malate. The canonical mitochondrial isoform (FHm) contributes to the tricarboxylic acid (TCA) cycle in mitochondria, whereas the non-canonical cytoplasmic isoform (FHc) drives regulation of fumarate levels in the cytoplasm following its generation by the urea cycle and amino acid metabolism. Additionally, FHc inhibits the histone demethylase activity of KDM2B in the nucleus following DNA damage. **c**, The level of protein function can be regulated by changes in its subcellular localization, for example, sequestration of the N^6-methyladenosine (m^6A)-reader YTHDC1 from the nucleoplasm into nuclear stress bodies. Sequestration occurs during heat stress, owing to YTHDC1 binding of m^6A at the long non-coding RNA HSATIII instead of at m^6A-dependent splice sites, and results in intron retention in hundreds of mRNAs. **d**, A protein may carry out distinct molecular functions in different subcellular compartments, for example, the moonlighting protein GAPDH. This protein can be controlled by post-translational modification (not shown) and oligomerization to function in glycolysis in the cytoplasm, extracellular vesicle formation (not shown) and iron transport at the cell membrane, and acetylation (Ac)-mediated apoptosis in the nucleus. **e**, Multilocalization contributes to spatiotemporal regulation of protein function, for example, to the multifunctionality of CDH4 and ISWI throughout the eukaryotic cell cycle. During interphase, CDH4 and ISWI localize to the nucleus, where they regulate transcription through their chromatin remodelling activities. During mitosis, disintegration of the nuclear envelope results in the release of CDH4 and ISWI into the cytoplasm, where they contribute to microtubule assembly and stability. The dashed arrows represent translocation. H3K36me2, dimethylated histone H3 Lys36; NPC, nuclear pore complex; Tf-Fe_2, iron-binding transferrin; OAA, oxaloacetic acid.

by interferon or interleukin results in transphosphorylation of STAT family transcription factors by receptor-associated Janus kinases[100–102] (Fig. 2a). Phosphorylated STATs undergo dimerization in the cytosol before their binding to importin α-5, which transports them into the nucleus through the nuclear pore complex[103–105]. Once inside the nucleus, STATs directly bind to specific sites in the promoters of cytokine-responsive genes to trigger an immune response[106,107]. Given the importance of subcellular localization for the function of transcription factors, their intracellular distribution is tightly regulated through multiple mechanisms, including protein synthesis and degradation[108], nuclear import[109] and export[59], and retention inside[110] or outside of the nucleus[111]. The dynamic interplay between these mechanisms can create complex oscillating systems, with transcription factor concentrations changing across different cellular compartments over relatively short time periods[112] to fine-tune gene expression in response to internal or external stimuli[113].

Other proteins reside in multiple subcellular compartments simply because their function is utilized separately in these locations. For example, humans express two isoforms of fumarate hydratase, the enzyme responsible for the reversible interconversion of fumarate and L-malate (Fig. 2b). These proteoforms are generated from a single gene through use of alternative translation initiation sites. The canonical, longer isoform localizes to the mitochondria, where it contributes to the tricarboxylic acid cycle, whereas the shorter isoform, which lacks a mitochondrial localization signal, resides in the cytosol and regulates the levels of fumarate generated by the urea cycle and amino acid catabolism[114–116]. Following DNA damage, the cytosolic fumarate hydratase isoform is phosphorylated and translocated to the nucleus, where it catalyses the production of fumarate to inhibit the activity of histone denethylase KDM2B[117]. Hence, the same molecular function of a protein can be utilized for different biological processes in different locations in the cell.

Even when a protein's function is limited to a single location, changes in subcellular localization can be exploited to alter the protein activity in response to internal or external stimuli. In the case of the m^6A reader YTHDC1, recognition and binding of a heat-stress-induced m^6A modification at the long non-coding RNA HSATIII results in sequestration of the protein from the nucleoplasm to nuclear stress bodies, thereby suppressing m^6A-dependent splicing of hundreds of pre-mRNAs[118,119] (Fig. 2c). Other membraneless organelles including nuclear speckles, paraspeckles, P-bodies, Cajal bodies and stress granules have also been shown to act as sponges that sequester proteins from their action locations and repress their functionality[50]. The dynamic formation and dissolution of these phase separated condensates facilitates many spatiotemporally defined processes[52,120]. For example, a recent study determined that 1,910 proteins in HeLa cells are involved in condensate formation in response to oxidative stress[121]. Mounting evidence suggests that the dynamic and transient formation of such condensates occurs during many core cellular responses, including transcription activation[122] and signal transduction[123]. Indeed, the formation of biomolecular condensates not only physically concentrates or sequesters proteins but also alters the proximal chemical environment and resulting solvation, charge, folding and reactivity of constituent proteins[54,91]. In the case of signal transduction, dynamic signalling complexes often consist of proteins with multiple similar domains connected by IDRs. Interactions between these domains allow the proteins to assemble into dynamic helical filaments, forming biomolecular condensates known as signalosomes[124]. This process creates transient clusters of signalling molecules, thereby increasing their local concentrations and enabling rapid cellular responses to activation signals.

Multilocalization facilitates multifunctionality

An increasing number of proteins are now recognized as having multiple distinct functions when differently localized within the cell[93]. The extreme manifestation of this phenomenon are 'moonlighting proteins' – proteins with identical sequences (that is, not the product of gene fusions or alternative splicing) that carry out diverse cellular functions[93,125–127]. Unsurprisingly, multilocalization is considered one of the main factors driving the evolution of such moonlighting properties, as the protein is exposed to different chemical spaces with alternative interacting partners[126,128]. One well-documented example is glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme that contributes to glycolysis in the cytoplasm, but has additional roles including extracellular vesicle formation[129] and iron transport at the plasma membrane[130,131], and the regulation of apoptosis genes in the nucleus[132,133] (Fig. 2d). These moonlighting functions depend on the protein's PTM and oligomerization status[134,135]. In normal cellular conditions, antioxidant proteins such as glutaredoxins maintain a state of redox balance, in which GAPDH remains in the cytosol[136]. However, in conditions of oxidative stress, the oxidation or S-nitrosylation of GAPDH results in nuclear translocation, which triggers apoptosis and

a switch from glycolysis to the pentose phosphate pathway[136–139]. The pentose phosphate pathway allows rerouting of carbohydrates into metabolic reactions which produce reducing equivalents in the form of NADPH that counteract oxidative stress[137]. Interestingly, the different functions of GAPDH appear to have a biologically meaningful connection. Whether such a functional connection is common for all moonlighting proteins remains an open question.

With the number of recognized moonlighting proteins on the rise and a large proportion of multilocalizing proteins having only one annotated function (Supplementary Fig. 1a), there is a clear need to functionally characterize proteins at their non-canonical subcellular location. Although several databases have been generated to collate moonlighting proteins (MoonProt[93], Multitasking Proteins DataBase[140], PlantMP[141] and MoonDB[142]), there is not yet a systematic method for identifying protein multifunctionality and moonlighting on a subcellular level. Instead, protein functionality is typically studied protein-by-protein and the discovery of moonlighting tends to be serendipitous.

Spatiotemporal dynamics of protein function

Given the strong relationship between protein localization and function, it is important to understand how protein localization is temporally regulated. A key biological process that relies on the spatiotemporal regulation of proteins is the eukaryotic cell cycle, and both multilocalization and multifunctionality have been extensively reported for cell cycle-associated proteins[143] (Fig. 2e). The chromatin remodellers CDH4 and ISWI, for example, localize to the nucleus to regulate transcription during interphase, but are released following the dissipation of the nuclear envelope during mitosis, which allows them to localize to and regulate the assembly and stability of the mitotic spindle[144,145]. Ki-67 localizes to the nucleolus in interphase, but relocates to and facilitates the generation of the chromosome periphery during mitosis owing to the hyperphosphorylation of its disordered repeat domains, which creates alternating charge blocks and increases their tendency to undergo liquid–liquid phase separation[146]. Another temporal process, the circadian rhythm, can also affect protein localization. Proteins that drive circadian rhythms — cryptochrome and Period proteins — as well as proteins under circadian regulation such as CNT2, change their localization based on the time of day, often without changes in mRNA or protein abundance[147,148]. It is important to emphasize that asynchronous cyclical processes in a cell population manifest as subpopulations in different phases. Bulk, single time-point measurements provide only an averaged snapshot of all cell states in a population; they cannot be used to distinguish between temporally regulated variation in protein localization within an asynchronous cell population and differential protein localization between stable subsets of the cell population. It is thus possible that a larger portion than currently described of the reported multilocalizing proteins change their subcellular localization periodically over the course of some cyclical cellular processes.

Overall, protein subcellular localization provides a means to regulate the structure, interactions and subsequent function of a protein and, thus, is a key factor contributing to the 'Goldilocks principle' whereby conditions must be just right for a protein to conform to its necessary structure and function. In turn, protein differential localization allows functionality to be regulated over space and time by altering the activity level of a given function or permitting a change of function. Importantly, protein differential localization can and often does occur in the absence of a change in total protein abundance[149,150], meaning that abundance-focused studies potentially miss a large portion of the changes in protein behaviour. In addition, as mentioned in Box 1, much of the existing literature on protein localization and function does not include proteoform-specific evidence. Therefore, the extent to which alternative splicing, PTMs, oligomerization, conformational changes or other yet unknown mechanisms contribute to the widespread phenomena of protein localization, multilocalization and multifunctionality is largely unknown.

Protein localization is instrumental for cell differentiation and identity

Cell differentiation is achieved through spatiotemporal changes in protein localization and abundance, which are often triggered by the translocation of transcription factors[151]. Some transcription factors are known moonlighting proteins[152], such as β-catenin (also known as catenin beta-1). In normal epithelial cells, β-catenin is part of the adherens junctions, helping maintain the integrity of epithelial layers; free cytoplasmic β-catenin in these cells is ubiquitylated and degraded by the proteasome (Fig. 3a). However, as the cell disassembles its junctions during the epithelial–mesenchymal transition, β-catenin is released into the cytoplasm, where it escapes degradation, and translocates to the nucleus, where it acts as a transcription factor that promotes the transition[153].

Cell type-specific protein localization is not limited to transcription regulators. Despite organelles performing similar core functions in different cells, even closely related cell types can exhibit substantially variable organellar proteomes[154]. Approximately one-fifth of all mitochondrial proteins show differential abundance in astrocytes and neurons[155], and half of centrosome-interacting proteins in neural cells localize to the centrosome only at certain stages of cell differentiation, without changing the total abundance levels[156]. Cell type-specific organellar proteomes are thought to contribute to cell identity by performing cell type-specific functions[154]. For example, the multilocalizing RBP PRPF6 is generally ubiquitous in nuclear spliceosome complexes[157]. In neural stem cells (but not in mature neurons), it additionally localizes to centrosomes, together with other RNA-binding and RNA-processing proteins[156] (Fig. 3b). Mutating the RNA-targeting function of PRPF6 results in neurodevelopmental disorders, suggesting that pre-mRNA splicing is a brain-specific function of PRPF6 and is probably performed at the centrosome. Despite the importance of correct protein localization for specific cell and tissue functions in multicellular organisms, cell type-specific protein localization remains underexplored.

Certain cell types, such as epithelial cells and neurons, rely on establishing specialized domains within a single compartment. Examples include the apical and basal membranes of epithelial cells (parts of the plasma membrane compartment)[158], outer segments of photoreceptors (parts of the cytoplasm, cilia and plasma membrane)[159,160] and presynaptic active zones of neurons (parts of the cytoplasm and plasma membrane)[161]. As we discuss in Box 1, different patterns of protein distribution within compartments are not considered to represent multilocalization but instead are termed protein gradients. These gradients are often overlooked during protein localization studies but can be a fundamental part of the cell identity. For example, epithelial cell polarization depends on the correct localization of so-called polarity factors that control the recruitment and exclusion of domain-specific proteins. The endomembrane system has an important role in this process[158,162,163], as membrane proteins are sorted into the vesicles that are subsequently transported to either apical or basolateral membranes. Once at their destination, the vesicles undergo selective and

controlled fusion with the plasma membrane and deliver polarity factors and their targets to the required location. One of the key apical polarity factors is the atypical protein kinase C (aPKC), a protein that phosphorylates other membrane-associated polarity factors to exclude them from the apical domain of epithelial cells[158] (Fig. 3c). In non-epithelial cells, disturbed epithelial cells or cancer cells, certain aPKC isoforms change their subcellular localization and can be found in other parts of the membrane, cytoplasm, or nucleus[164–167]. These isoforms regulate cell proliferation, cell cycle and glucose metabolism, especially in the context of cancer[166,168], and atypical nuclear expression of aPKC has been suggested as a cancer prognostic marker[164,166,168], yet the exact relationship between subcellular localization and function of aPKC isoforms is not clear. Hence, we need to reassess the granularity of annotations and take into account local minima and maxima when characterizing the spatial distribution of proteins and their functional impact.

Despite strong evidence for the importance of subcellular protein distribution in establishing cell identity, routine cell phenotyping is still based almost exclusively on transcript or protein abundance and does not include protein localization. This bias is an understandable consequence of technical limitations, higher cost and lower throughput of subcellular spatial analysis, and of general lack of well-validated baseline datasets. Nevertheless, a growing body of evidence highlights the clinical importance of specific and correct subcellular protein localization, which we discuss below.

Protein mislocalization in disease

Various diseases with a genetic component have been shown to involve protein mislocalization[169–171]. As illustrated by the example of PRPF6, multilocalizing and moonlighting proteins can perform cell type-specific functions in one of their possible subcellular locations. In some cases, a mutation might affect localization at only one of the possible locations, leading to a loss of only one of the protein functions. If this happens in a cell type-specific manner, such a specific mislocalization might affect only a subset of cells in the organism, leading to the development of a tissue-specific pathology. However, most clinical evidence remains anecdotal and does not provide systematic analysis of the links and the causal relationship between mutations, protein mislocalization and pathological consequences. Nevertheless, hundreds of pathogenic variants associated with diseases such as cancer, immunodeficiencies, metabolic diseases and neurological and neuromuscular disorders, have been directly linked to protein mislocalization[172]. Intriguingly, variants of the same gene known to cause different diseases tended to show differential localization patterns. Subcellular targeting of proteins can be disrupted by a number of different factors, affecting the protein localization mechanisms discussed above. Protein mislocalization, misfolding, incorrect PTMs and incorrect interactions with other molecules can negatively affect protein function, resulting in pathology. These processes are interdependent, and each of them can be either the cause or the consequence of the other. Table 1 summarizes some examples of pathological changes of function caused by protein mislocalization.

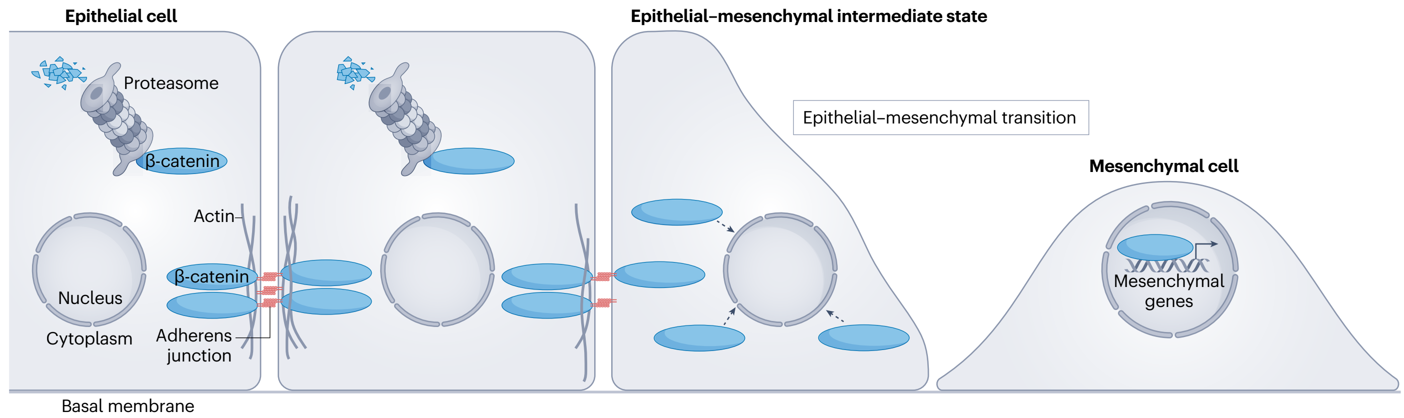
Mislocalized pathogenic protein variants are over-represented in the organelles of the secretory pathway[172], and disrupted endomembrane trafficking is known to be implicated in the development of neurodegenerative conditions[173,174], cancer[162] and metabolic disorders[175]. Furthermore, pathogenic variants that undergo mislocalization most often carry mutations in the domains involved in protein folding or insertion into the membrane[172]. Protein folding relies on controlled chemical conditions and on interactions with chaperones that assist in the process. Proteins can be misfolded owing to mutations, PTMs affecting the interactions between amino acids or unwanted protein interactions. Misfolded proteins often aggregate, although the presence of stable soluble misfolded proteins with altered activity[176] and interactions[177] might be a widespread phenomenon[178]. Aggregation starts a vicious cycle, where one misfolded molecule triggers aggregation of otherwise normal polypeptide chains. Aggregated proteins fail to both correctly localize and to perform their function. Conversely, protein mislocalization may result in misfolding owing to the lack of correct binding partners, failure to be inserted into a membrane or altered folding environment, possibly leading to proteotoxicity, as seen in amyotrophic lateral sclerosis[179]. Protein mislocalization accompanied by misfolding is one of the hallmarks of neurodegenerative conditions such as Parkinson's disease or Alzheimer's disease (Table 1).

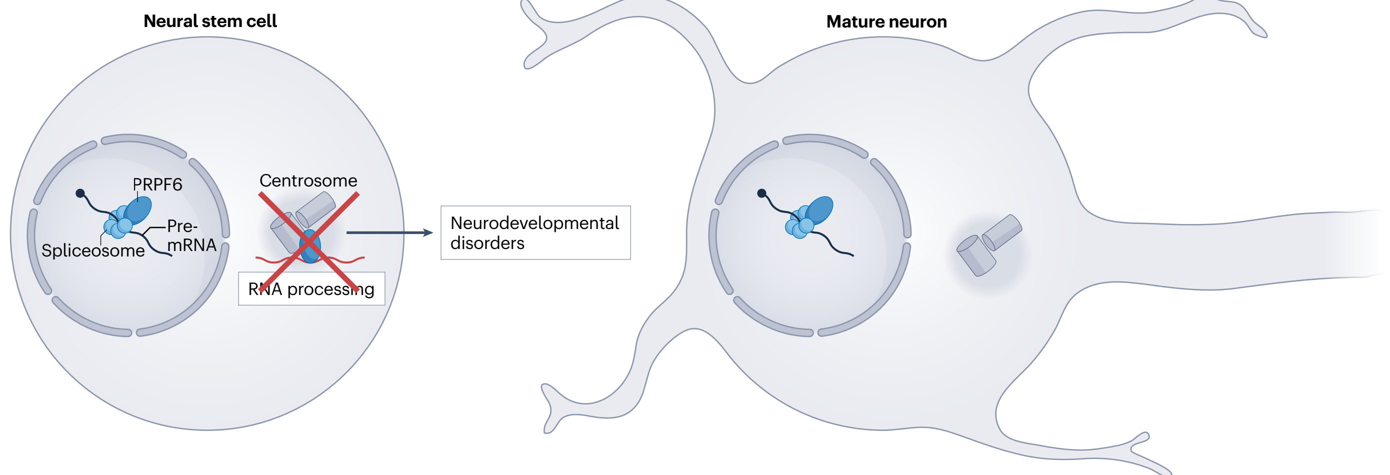
In addition to changes in protein folding and membrane insertion, mutations that change the solubility of a protein may also trigger changes in the ability of proteins to dynamically and reversibly associate with biomolecular condensates, with pathological consequences[45,180,181]. Indeed, 36,777 pathological mutations in 1,745 human proteins were shown to be involved in biomolecular condensate dysregulation in disease[182]. For example, mutations in the tumour suppressor SPOP can interfere with phase separation and prevent the protein from correctly functioning as a substrate adaptor of the cullin–RING E3 ubiquitin ligase in nuclear speckles, thus contributing to the formation of solid tumours[183]. By contrast, deacetylation of HP1γ promotes nuclear condensation, which results in drug resistance in myeloma[184].

Protein mislocalization can also be triggered by environmental stresses. Viruses can protect themselves from nuclear restriction enzymes (such as IFI16) by triggering their relocalization to the cytoplasm of the infected cell and subsequently to the extracellular space[185]. Relocalization exposes IFI16 to different interaction partners, including cytoplasmic viral-RNA sensor RIG-I[186]. These new interactions promote inflammation and, in the case of extracellular IFI16, can contribute to the development of autoimmune disorders, with the mislocalized protein acting as an autoantigen[185]. Similar unwanted interactions, both inside and outside of the cell, have been shown to contribute to the role of moonlighting proteins in cancer progression[187]. Therefore, protein mislocalization can directly cause pathology through both loss and gain of function in the new molecular context (Table 1).

Protein mislocalization can directly lead to the breakdown of correct molecular interactions. In Usher syndrome, usherin (also known as USH2A), a cilium-associated scaffold and transport protein, is truncated and loses its localization signal. Mislocalization of usherin to the cytoplasm of cochlear hair cells[188] and photoreceptors[189] results in a congenital disorder characterized by hearing and vision loss. Moreover, mutant usherin in photoreceptors promotes the mislocalization of its interaction partners[189]. Proteins localized to the wrong compartment can engage in unwanted interactions, as is the case of the chromatin remodeller and tumour suppressor ARID1B. Mutant ARID1B lacking functional NLS is localized to the cytoplasm, where it interacts with the RAF–ERK signalling pathway and promotes oncogenesis, in contrast to its canonical function[190]. Similar switches can explain the contradictory functions that have been described for other proteins.

Mislocalized proteins can also be exposed to new protein-modifying enzymes and PTMs, which often change their conformation, activity and ability to interact with other molecules. Certain PTMs change the protein propensity for aggregation and might influence the development of neurodegenerative disorders[191]. PTMs can directly


a Protein relocalization in cell differentiation
Epithelial cell
Epithelial–mesenchymal intermediate state
Proteasome
β-catenin
Actin
Nucleus
β-catenin
Cytoplasm
Adherens
junction
Epithelial–mesenchymal transition
Mesenchymal cell
Mesenchymal
genes
Basal membrane


b Cell-specific and tissue-specific functions
Neural stem cell
Mature neuron
PRPF6
Centrosome
Pre-
mRNA
Spliceosome
RNA processing
Neurodevelopmental
disorders

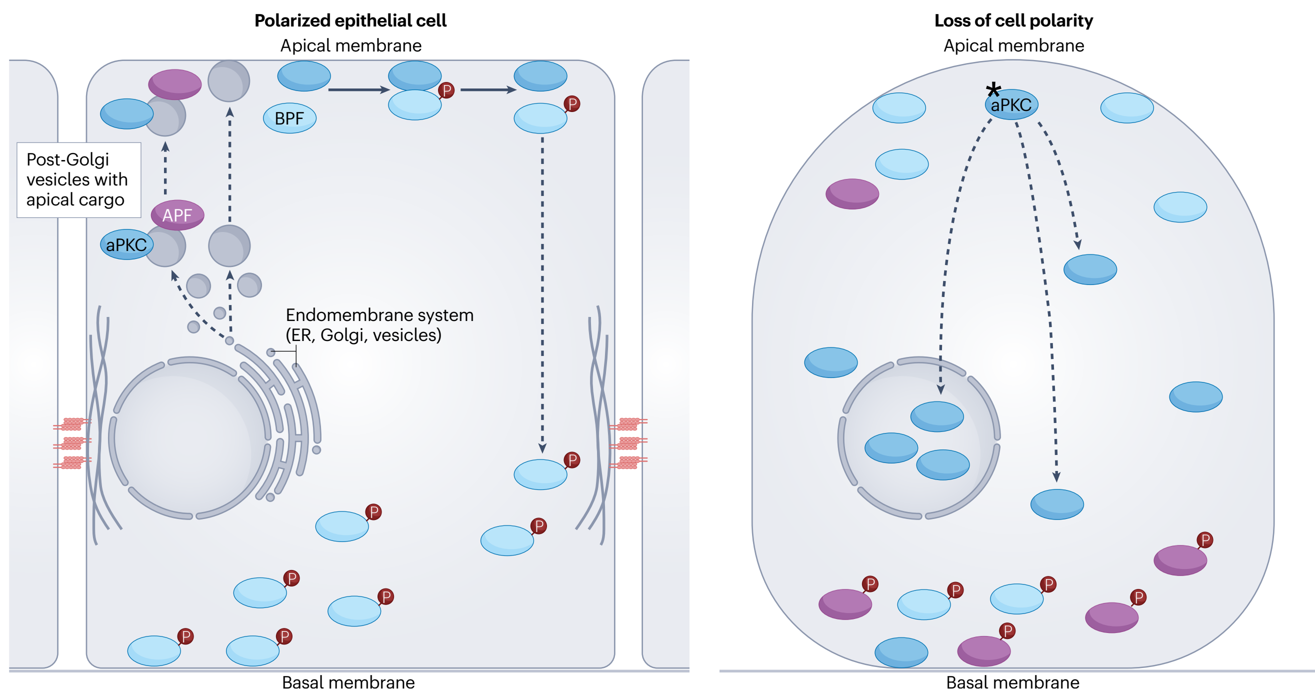

c Protein gradients establish cell polarity
Polarized epithelial cell
Apical membrane
Loss of cell polarity
Apical membrane
BPF
P
Post-Golgi
vesicles with
apical cargo
APF
aPKC
Endomembrane system
(ER, Golgi, vesicles)
aPKC
Basal membrane
Basal membrane

Fig. 3 | Examples of cell type-specific protein relocalization. a, In healthy epithelia, β-catenin is localized at cell adherens junctions, thereby connecting them to the cytoskeleton. In these cells, cytoplasmic β-catenin undergoes proteasomal degradation. During epithelial–mesenchymal transition, β-catenin is removed from the junctions, is no longer marked for degradation and can enter the nucleus, where it acts as a transcription regulator that promotes the transition. **b**, The RNA-binding protein PRPF6 is a ubiquitously expressed component of nuclear spliceosome complexes. However, in neural stem cells it is additionally localized to centrosomes, where it exhibits RNA-processing activity. Mutations disrupting this activity lead to neurodegenerative disorders but have no pronounced effects in other cell types and tissues, highlighting the tissue-specific and location-specific function of centrosomal PRPF6. **c**, Apical–basal polarity in epithelia is maintained by proteins that recruit or prohibit other proteins from accumulating at a respective side of the cell. One of these proteins, atypical protein kinase C (aPKC), phosphorylates (P) basal and lateral polarity factors (BPF; for example, LLGL2 or EPB41L5), thereby preventing them from entering the cellular apical domain; by contrast, apical polarity factors (APF) can accumulate there. Moreover, trafficking of polarity factors is often facilitated by the endomembrane system, with cargo proteins sorted into the vesicles targeting the apical or the basal membrane. These vesicles are transported to the corresponding side of the cell, where they undergo controlled and specific fusion with the plasma membrane, thereby delivering the polarity factors and their targets. Mutations in aPKC (denoted by the asterisk) result in aPKC redistribution to the cytoplasm, nucleus and other membrane domains, where its off-site phosphorylating activity has negative effects on the distribution and function of other proteins. The dashed arrows represent translocation. ER, endoplasmic reticulum.

regulate protein localization, for example, reversible S-palmitoylation, which allows cells to dynamically control protein localization through the enzymes that add and remove palmitate residues to their substrates. S-palmitoylation has a key role in neurons, and unsurprisingly, many neurodegenerative conditions characterized by protein mislocalization are also associated with reduced activity of palmitoylating and de-palmitoylating enzymes[192]. Finally, mislocalization of the protein-modifying enzymes themselves can lead to aberrant patterns of PTMs and atypical activation of downstream signalling pathways, which seems to drive certain forms of Cushing syndrome[193].

Owing to the important role of protein mislocalization in various diseases, new therapeutics that aim to correctly relocalize proteins of interest are emerging[194–197]. The efficiency of other extremely promising therapeutics, such as selective protein degradation using molecular glue degraders or proteolysis-targeting chimeras (PROTACs)[198], is also influenced by the subcellular localization of the target[199]. In some clinical contexts it might be necessary to induce protein degradation only in one specific subcellular location, and out-of-place degrader activity can lead to inadvertent negative effects, especially in the case of multilocalizing and multifunctional proteins. It is, therefore, imperative that we continue to work on mapping subcellular protein distribution patterns, understanding how they are established, regulated and disrupted in various biological contexts, and determine the functional consequences of changing subcellular protein localization.

Conclusion and future perspective

Subcellular localization of proteins has emerged as an important mechanism for the control of protein function. The development of diverse methodologies for the study of protein subcellular localization has led to the generation of high-quality spatial maps and revealed that most proteins display complex spatiotemporal distributions[1,2,67,150,200–205]. The ways in which the cell establishes and regulates these distributions are largely independent of protein abundance[149,150] and extremely relevant for cell function.

Unfortunately, large-scale mechanistic studies that link protein localization to function are still lacking. One reason is inability to achieve location-specific manipulation of protein abundance using established gene-centric methods, such as CRISPR tools. Hence, although CRISPR and similar technologies have been extremely powerful in elucidating gene functions, they fall short from disentangling protein multilocalization, multifunctionality and genetic pleiotropy. Our understanding of the link between protein localization and function would greatly benefit from the development of robust tools for controlling and transiently modifying subcellular protein distributions. Some of the advances in this area are discussed in a complementary Review paper[5].

Several other major challenges remain in our understanding of the relationship between protein subcellular location and function. Nearly all published reports of localization or function refer only to a single protein entity without providing proteoform information of PTMs, oligomerization, alternative splicing or sequence variants. This shortcoming is partly owing to the challenges associated with measuring proteoforms, especially when they are rare, transiently expressed or context specific. As the dynamic range and sensitivity of mass spectrometry-based proteomics continues to increase and validated proteoform-specific antibodies become widely available for imaging-based experiments, our ability to distinguish proteoforms is also expected to improve and reveal an additional layer of regulatory complexity in the relationship between protein localization and function.

Protein localization and function also need to be studied in a wider range of contexts. Even different human cell lines show substantial changes in mRNA expression, protein abundance and phosphorylation[206], protein localization[1] and protein–protein interactions[207]. Cell type-specific and tissue-specific variation in subcellular proteomes is likely to be even more pronounced and underexplored, particularly for proteins localized to specialized cell structures that only exist in certain cell types or at specific times. Similar blind spots probably exist for protein localizations that change in response to internal or external stimuli. Varying penetrance of protein localization phenotypes complicates the efforts to classify proteins as multilocalizing and/or multifunctional. The number of both multilocalizing and multifunctional proteins is likely substantially higher than current estimates. It is possible that proteins present in different locations at the same time in the same cell have a different chance of being multifunctional than proteins present in different locations in different cell types. Substantially more data are needed to answer these intriguing questions with certainty. New machine-learning models[208] could speed up the annotation of imaging data for both cell-specific and tissue-specific localizations. Moreover, large language models should be improved to accurately predict protein localization, as well as the potential for protein multilocalization and multifunctionality, based on the protein sequence and multimodal data representing cellular context (transcriptomes, interactomes).

Finally, the subcellular study of proteins must move away from treating subcellular compartments as discrete, uniform and constant. Such an approach not only limits the discovery of functional heterogeneity between cell types but also prevents the consideration of

Table 1 | Pathological loss or gain of function caused by protein mislocalization

Disease	Protein	Biological trigger	Pathogenic effect of mislocalization	Description
Usher syndrome	Usherin	Mutation resulting in a truncated protein	Mislocalization of interacting proteins	Usherin mislocalization from cilia triggers mislocalization of its interaction partners and loss of function, resulting in combined hearing and vision loss[188,189]
Cancer	ARID1B	Mutations in nuclear localization sequence	New interaction partners	ARID1B is mislocalized to the cytoplasm, where it can activate RAF–ERK signalling, thereby promoting oncogenesis[190]
Autoimmune response	IFI16	Viral infection, interaction with viral proteins	Protein serves as an alarmin (autoantigen)	Egressing virions can trap IFI16, leading to its accumulation in the extracellular space, which can trigger an autoimmune response[185]
Familial amyotrophic lateral sclerosis	FUS	Mutations in the C terminus	Phase separation into stress granules	Mutant FUS is exposed to the wrong cellular environment (cytoplasm), where it accumulates in stress granules, possibly leading to proteotoxicity[179]
Amyotrophic lateral sclerosis	TDP-43	Abnormal PTMs	Misfolding, aggregation	Aberrant PTMs promote changes in conformation, hindering correct protein interactions and localization[191]
Alzheimer's disease	Tau protein	Abnormal phosphorylation	Misfolding, aggregation	Aberrant PTMs promote changes in conformation, hindering correct protein interactions and localization[191]
Huntington's disease, Alzheimer's disease, other neurodegenerative diseases	Multiple proteins	Mutations and dysregulation of palmitoylating and de-palmitoylating enzymes	Aggregation	Disturbed palmitoylation patterns affect protein trafficking in neurons, contributing to the progression of neurodegenerative diseases[192]
Cushing syndrome	PKAc	Mutations in the catalytic subunit	Wrong molecular interactions, failure to assemble in the signalling islands (hubs)	Mutated PKAc is excluded from the signalling islands, which leads to its dysregulation, indiscriminate diffusion within the cells and aberrant phosphorylation patterns[193]

ARID1B, AT-rich interactive domain-containing protein 1B; FUS, fused in sarcoma; IFI16, gamma-interferon-inducible protein 16; PKAc, protein kinase A, catalytic subunit; PTMs, post-translational modifications; TDP-43, TAR DNA-binding protein 43.

variation between and within organelles of the same cell across space and time[209–211]. A clear example is the transient and dynamic formation of membraneless organelles and biomolecular condensates. In addition, various intracellular vesicles often have similar morphology, despite being a heterogeneous population with vastly different molecular composition and functions. This heterogeneity poses a problem for image-based localization classification models, which might not be able to differentiate between biologically meaningful organelle classes and subclasses. Proteins can also localize to organelle contact sites[212,213] and display distinct sub-organellar distributions. Recently, for example, nucleolar proteins were shown to reside in three sub-phases of nucleoli based on the sequence of their IDRs[214]. New computational tools focusing on the integration of different experimental modalities, such as microscopy images and protein–protein interaction data, can capture smaller and more transient subcellular protein assemblies, while still identifying conventional organelles, thereby recapitulating the hierarchical architecture of the cell, and, importantly, providing separate lines of evidence for protein localization and putative function[215]. Exploring and classifying protein localization across multiple scales and in different biological contexts[216] could reveal tighter co-localization patterns and facilitate the annotation of functionally related proteins.

Overall, the landscape of subcellular protein distributions, interactions and functions is incredibly complex, and exploring it fully will require integrated data-driven methodologies. We will need to map subcellular protein and proteoform distributions in a variety of contexts, including perturbations at the level of specific proteins, cells, tissues and entire organisms. It will be necessary to develop new detection techniques with increased sensitivity, selectivity and spatial resolution, and new bioinformatics methods and pipelines to process, combine and analyse the massive datasets. It is crucial that the community continues to make cell and tissue maps publicly available to accelerate the construction of models that would be able to capture the connection between protein location and function. Guidelines on data collection, reporting and accessibility would permit more efficient resource allocation and better integration of the data coming from different sources into more complex, larger-scale models. New insights from this research will help refine our nomenclature for multifunctional and moonlighting proteins to better reflect the underlying biological reality. Fully understanding the relationship between protein localization and function is a monumental task that will undoubtedly require concerted community effort and cooperation, yet can reward us with new avenues for both fundamental and clinical research.

Published online: 18 February 2026

References

1. Thul, P. J. et al. A subcellular map of the human proteome. *Science* **356**, eaal3321 (2017).
2. Geladaki, A. et al. Combining LOPIT with differential ultracentrifugation for high-resolution spatial proteomics. *Nat. Commun.* **10**, 331 (2019).
3. Jiang, J. Q. & Wu, M. Predicting multiplex subcellular localization of proteins using protein-protein interaction network: a comparative study. *BMC Bioinforma.* **13**, S20 (2012).
4. The UniProt Consortium et al. UniProt: the Universal Protein Knowledgebase in 2025. *Nucleic Acids Res.* **53**, D609–D617 (2025).
5. Leineweber, W., Tei, R., Mäkiniemi, Ting, A. & Lundberg, E. Technologies to measure and modulate protein subcellular localization. *Nat. Rev. Mol. Cell Biol.* https://doi.org/10.1038/s41580-026-00957-1 (2026).
6. Oleynikov, Y. & Singer, R. H. RNA localization: different zipcodes, same postman? *Trends Cell Biol.* **8**, 381–383 (1998).
7. Fernandopulle, M. S., Lippincott-Schwartz, J. & Ward, M. E. RNA transport and local translation in neurodevelopmental and neurodegenerative disease. *Nat. Neurosci.* **24**, 622–632 (2021).

8. Brangwynne, C. P. et al. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* **324**, 1729–1732 (2009).
9. Ramat, A., Haidar, A., Garret, C. & Simonelig, M. Spatial organization of translation and translational repression in two phases of germ granules. *Nat. Commun.* **15**, 8020 (2024).
10. Chekulaeva, M. Mechanistic insights into the basis of widespread RNA localization. *Nat. Cell Biol.* **26**, 1037–1046 (2024).
11. Das, S., Vera, M., Gandin, V., Singer, R. H. & Tutucci, E. Intracellular mRNA transport and localized translation. *Nat. Rev. Mol. Cell Biol.* **22**, 483–504 (2021).
12. Engel, K. L., Arora, A., Goering, R., Lo, H.-Y. G. & Taliaferro, J. M. Mechanisms and consequences of subcellular RNA localization across diverse cell types. *Traffic Cph. Den.* **21**, 404–418 (2020).
13. Song, T. et al. Specific interaction of KIF11 with ZBP1 regulates the transport of β-actin mRNA and cell motility. *J. Cell Sci.* **128**, 1001–1010 (2015).
14. Kislauskis, E. H., Zhu, X. & Singer, R. H. Sequences responsible for intracellular localization of beta-actin messenger RNA also affect cell phenotype. *J. Cell Biol.* **127**, 441–451 (1994).
15. Oleynikov, Y. & Singer, R. H. Real-time visualization of ZBP1 association with β-actin mRNA during transcription and localization. *Curr. Biol.* **13**, 199–207 (2003).
16. Tyagi, S. & Alsmadi, O. Imaging native beta-actin mRNA in motile fibroblasts. *Biophys. J.* **87**, 4153–4162 (2004).
17. Welshhans, K. & Bassell, G. J. Netrin-1-induced local β-actin synthesis and growth cone guidance requires zipcode binding protein 1. *J. Neurosci.* **31**, 9800–9813 (2011).
18. Zhang, H. L. et al. Neurotrophin-induced transport of a beta-actin mRNP complex increases beta-actin levels and stimulates growth cone motility. *Neuron* **31**, 261–275 (2001).
19. Leung, K.-M. et al. Cue-polarized transport of β-actin mRNA depends on 3′UTR and microtubules in live growth cones. *Front. Cell. Neurosci.* **12**, 300 (2018).
20. Eom, T., Antar, L. N., Singer, R. H. & Bassell, G. J. Localization of a β-actin messenger ribonucleoprotein complex with zipcode-binding protein modulates the density of dendritic filopodia and filopodial synapses. *J. Neurosci.* **23**, 10433–10444 (2003).
21. Hüttelmaier, S. et al. Spatial regulation of β-actin translation by Src-dependent phosphorylation of ZBP1. *Nature* **438**, 512–515 (2005).
22. Sasaki, Y. et al. Phosphorylation of zipcode binding protein 1 is required for brain-derived neurotrophic factor signaling of local β-actin synthesis and growth cone turning. *J. Neurosci.* **30**, 9349–9358 (2010).
23. Shestakova, E. A., Singer, R. H. & Condeelis, J. The physiological significance of β-actin mRNA localization in determining cell polarity and directional motility. *Proc. Natl Acad. Sci. USA* **98**, 7045–7050 (2001).
24. Katz, Z. B. et al. β-Actin mRNA compartmentalization enhances focal adhesion stability and directs cell migration. *Genes. Dev.* **26**, 1885–1890 (2012).
25. Flamand, M. N. & Meyer, K. D. m^6A and YTHDF proteins contribute to the localization of select neuronal mRNAs. *Nucleic Acids Res.* **50**, 4464–4483 (2022).
26. Kudrin, P., Singh, A., Meierhofer, D., Kuśnierczyk, A. & Ørom, U. A. V. N4-acetylcytidine (ac4C) promotes mRNA localization to stress granules. *EMBO Rep.* **25**, 1814–1834 (2024).
27. Liu, N. et al. N^6-methyladenosine-dependent RNA structural switches regulate RNA–protein interactions. *Nature* **518**, 560–564 (2015).
28. Madugalle, S. U., Meyer, K., Wang, D. O. & Bredy, T. W. RNA N^6-methyladenosine and the regulation of RNA localization and function in the brain. *Trends Neurosci.* **43**, 1011–1023 (2020).
29. Liao, Y.-C. et al. RNA granules hitchhike on lysosomes for long-distance transport, using annexin A11 as a molecular tether. *Cell* **179**, 147–164.e20 (2019).
30. Cioni, J.-M. et al. Late endosomes act as mRNA translation platforms and sustain mitochondria in axons. *Cell* **176**, 56–72.e15 (2019).
31. Higuchi, Y., Ashwin, P., Roger, Y. & Steinberg, G. Early endosome motility spatially organizes polysome distribution. *J. Cell Biol.* **204**, 343–357 (2014).
32. Schuhmacher, J. S. et al. The Rab5 effector FERRY links early endosomes with mRNA localization. *Mol. Cell* **83**, 1839–1855.e13 (2023).
33. Quentin, D. et al. Structural basis of mRNA binding by the human FERRY Rab5 effector complex. *Mol. Cell* **83**, 1856–1871.e9 (2023).
34. Sasse, A. et al. A resource of RNA-binding protein motifs across eukaryotes reveals evolutionary dynamics and gene-regulatory function. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-025-02733-6 (2025).
35. Han, M. et al. Programmable control of spatial transcriptome in live cells and neurons. *Nature* **643**, 241–251 (2025).
36. Tomuro, K. et al. Sequence grammar and dynamics of subcellular translation revealed by APEX-Ribo-Seq. Preprint at *bioRxiv* https://doi.org/10.1101/2025.05.26.656194 (2025).
37. Stewart, M. Function of the nuclear transport machinery in maintaining the distinctive compositions of the nucleus and cytoplasm. *Int. J. Mol. Sci.* **23**, 2578 (2022).
38. Hutten, S. & Kehlenbach, R. H. CRM1-mediated nuclear export: to the pore and beyond. *Trends Cell Biol.* **17**, 193–201 (2007).
39. Lange, A. et al. Classical nuclear localization signals: definition, function, and interaction with importin α. *J. Biol. Chem.* **282**, 5101–5105 (2007).
40. Becker, T., Song, J. & Pfanner, N. Versatility of preprotein transfer from the cytosol to mitochondria. *Trends Cell Biol.* **29**, 534–548 (2019).
41. Melin, J. et al. Presequence recognition by the tom40 channel contributes to precursor translocation into the mitochondrial matrix. *Mol. Cell. Biol.* **34**, 3473–3485 (2014).
42. Hegde, R. S. & Keenan, R. J. The mechanisms of integral membrane protein biogenesis. *Nat. Rev. Mol. Cell Biol.* **23**, 107–124 (2022).
43. Luirink, J. SRP-mediated protein targeting: structure and function revisited. *Biochim. Biophys. Acta Mol. Cell Res.* **1694**, 17–35 (2004).
44. Dunn, C. D. & Paavilainen, V. O. Wherever I may roam: organellar protein targeting and evolvability. *Curr. Opin. Genet. Dev.* 58–59, 9–16 (2019).
45. Kilgore, H. R. et al. Protein codes promote selective subcellular compartmentalization. *Science* **387**, 1095–1101 (2025).
46. Hicks, G. R. *Nuclear Import and Export in Plants and Animals* 61–82 (Springer, 2005).
47. Hayes, T. et al. Simulating 500 million years of evolution with a language model. *Science* **387**, 850–858 (2025).
48. Abramson, J. et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **630**, 493–500 (2024).
49. Golan, Y., Berman, B. & Assaraf, Y. G. Heterodimerization, altered subcellular localization, and function of multiple zinc transporters in viable cells using bimolecular fluorescence complementation. *J. Biol. Chem.* **290**, 9050–9063 (2015).
50. Hirose, T., Ninomiya, K., Nakagawa, S. & Yamazaki, T. A guide to membraneless organelles and their various roles in gene regulation. *Nat. Rev. Mol. Cell Biol.* **24**, 288–304 (2023).
51. Keber, F. C., Nguyen, T., Mariossi, A., Brangwynne, C. P. & Wühr, M. Evidence for widespread cytoplasmic structuring into mesoscale condensates. *Nat. Cell Biol.* **26**, 346–352 (2024).
52. Jeon, S. et al. Emerging regulatory mechanisms and functions of biomolecular condensates: implications for therapeutic targets. *Signal. Transduct. Target. Ther.* **10**, 4 (2025).
53. Mittag, T. & Pappu, R. V. A conceptual framework for understanding phase separation and addressing open questions and challenges. *Mol. Cell* **82**, 2201–2214 (2022).
54. Banani, S. F., Lee, H. O., Hyman, A. A. & Rosen, M. K. Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* **18**, 285–298 (2017).
55. The Consortium for Top Down Proteomics, Smith, L. M. & Kelleher, N. L. Proteoform: a single term describing protein complexity. *Nat. Methods* **10**, 186–187 (2013).
56. Nakamura, A., Ikeda, M., Kusayanagi, S. & Hayashi, K. An alternative splice isoform of mouse CDK5RAP2 induced cytoplasmic microtubule nucleation. *IBRO Neurosci. Rep.* **13**, 264–273 (2022).
57. Yang, X. et al. Widespread expansion of protein interaction capabilities by alternative splicing. *Cell* **164**, 805–817 (2016).
58. Marruecos, L. et al. Dynamic chromatin association of IκBα is regulated by acetylation and cleavage of histone H4. *EMBO Rep.* **22**, e52649 (2021).
59. Reich, N. C. STATs get their move on. *JAK-STAT* **2**, e27080 (2013).
60. Hofweber, M. & Dormann, D. Friend or foe — post-translational modifications as regulators of phase separation and RNP granule dynamics. *J. Biol. Chem.* **294**, 7137–7150 (2019).
61. Chen, B., Sun, Y., Niu, J., Jarugumilli, G. K. & Wu, X. Protein lipidation in cell signaling and diseases: function, regulation, and therapeutic opportunities. *Cell Chem. Biol.* **25**, 817–831 (2018).
62. Colley, K. J. et al. in *Essentials of Glycobiology* (eds Varki, A. et al.) (Cold Spring Harbor Laboratory, 2022).
63. Shipley, J. M., Grubb, J. H. & Sly, W. S. The role of glycosylation and phosphorylation in the expression of active human beta-glucuronidase. *J. Biol. Chem.* **268**, 12193–12198 (1993).
64. Aebersold, R. et al. How many human proteoforms are there? *Nat. Chem. Biol.* **14**, 206–214 (2018).
65. Sinitcyn, P. et al. Global detection of human variants and isoforms by deep proteome sequencing. *Nat. Biotechnol.* **41**, 1776–1786 (2023).
66. Krahmer, N. et al. Organellar proteomics and phospho-proteomics reveal subcellular reorganization in diet-induced hepatic steatosis. *Dev. Cell* **47**, 205–221.e7 (2018).
67. Martinez-Val, A. et al. Spatial-proteomics reveals phospho-signaling dynamics at subcellular resolution. *Nat. Commun.* **12**, 7113 (2021).
68. Duan, J. et al. Stochiometric quantification of the thiol redox proteome of macrophages reveals subcellular compartmentalization and susceptibility to oxidative perturbations. *Redox Biol.* **36**, 101649 (2020).
69. Tran, J. C. et al. Mapping intact protein isoforms in discovery mode using top-down proteomics. *Nature* **480**, 254–258 (2011).
70. Melani, R. D. et al. The Blood Proteoform Atlas: a reference map of proteoforms in human hematopoietic cells. *Science* **375**, 411–418 (2022).
71. Melby, J. A. et al. High sensitivity top–down proteomics captures single muscle cell heterogeneity in large proteoforms. *Proc. Natl Acad. Sci. USA* **120**, e2222081120 (2023).
72. Johnson, K. R., Gao, Y., Greguš, M. & Ivanov, A. R. On-capillary cell lysis enables top-down proteomic analysis of single mammalian cells by CE–MS/MS. *Anal. Chem.* **94**, 14358–14367 (2022).
73. Su, P. et al. Single cell analysis of proteoforms. *J. Proteome Res.* **23**, 1883–1893 (2024).
74. Wu, W. et al. Alternative splicing controls nuclear translocation of the cell cycle-regulated Nek2 kinase. *J. Biol. Chem.* **282**, 26431–26440 (2007).
75. Soutoglou, E., Katrakili, N. & Talianidis, I. Acetylation regulates transcription factor activity at multiple levels. *Mol. Cell* **5**, 745–751 (2000).
76. Maures, T. J., Su, H.-W., Argetsinger, L. S., Grinstein, S. & Carter-Su, C. Phosphorylation controls a dual-function polybasic nuclear localization sequence in the adapter protein SH2B1β to regulate its cellular function and distribution. *J. Cell Sci.* **124**, 1542–1552 (2011).
77. Kelpsch, D. J. & Tootle, T. L. Nuclear actin: from discovery to function. *Anat. Rec.* **301**, 1999–2013 (2018).
78. Wallin, E. & Heijne, G. V. Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms. *Protein Sci.* **7**, 1029–1038 (1998).
79. Sánchez, W. N., Driessen, A. J. M. & Wilson, C. A. M. Protein targeting to the ER membrane: multiple pathways and shared machinery. *Crit. Rev. Biochem. Mol. Biol.* **60**, 33–79 (2025).

80. Brownfield, B. A. & Fromme, J. C. Structural insights into traffic through the Golgi complex. *Curr. Opin. Cell Biol.* **94**, 102505 (2025).
81. Gopaldass, N., Chen, K.-E., Collins, B. & Mayer, A. Assembly and fission of tubular carriers mediating protein sorting in endosomes. *Nat. Rev. Mol. Cell Biol.* **25**, 765–783 (2024).
82. Carosi, J. M., Denton, D., Kumar, S. & Sargeant, T. J. Receptor recycling by retromer. *Mol. Cell. Biol.* **43**, 317–334 (2023).
83. Homma, Y., Hiragi, S. & Fukuda, M. Rab family of small GTPases: an updated view on their regulation and functions. *FEBS J.* **288**, 36–55 (2021).
84. Oliveira, V. M. de et al. Effects of pH and salt concentration on stability of a protein G variant using coarse-grained models. *Biophys. J.* **114**, 65–75 (2018).
85. Bugge, K. et al. Interactions by disorder — a matter of context. *Front. Mol. Biosci.* **7**, 110 (2020).
86. Meng, J. et al. Precision redox: the key for antioxidant pharmacology. *Antioxid. Redox Signal.* **34**, 1069–1082 (2021).
87. Reichmann, D., Voth, W. & Jakob, U. Maintaining a healthy proteome during oxidative stress. *Mol. Cell* **69**, 203–213 (2018).
88. Alfano, C. et al. Molecular crowding: the history and development of a scientific paradigm. *Chem. Rev.* **124**, 3186–3219 (2024).
89. Despa, F., Orgill, D. P. & Lee, R. C. Molecular crowding effects on protein stability. *Ann. N. Y. Acad. Sci.* **1066**, 54–66 (2006).
90. Speer, S. L., Stewart, C. J., Sapir, L., Harries, D. & Pielak, G. J. Macromolecular crowding is more than hard-core repulsions. *Annu. Rev. Biophys.* **51**, 267–300 (2022).
91. Kilgore, H. R. et al. Distinct chemical environments in biomolecular condensates. *Nat. Chem. Biol.* **20**, 291–301 (2024).
92. Aftab, A., Sil, S., Nath, S., Basu, A. & Basu, S. Intrinsic disorder and other malleable arsenals of evolved protein multifunctionality. *J. Mol. Evol.* **92**, 669–684 (2024).
93. Chen, C. et al. MoonProt 3.0: an update of the moonlighting proteins database. *Nucleic Acids Res.* **49**, D368–D372 (2021).
94. Bertolini, E., Babbi, G., Savojardo, C., Martelli, P. L. & Casadio, R. MultifacetedProtDB: a database of human proteins with multiple functions. *Nucleic Acids Res.* **52**, D494–D501 (2024).
95. Jagga, B. et al. Structural basis for nuclear import selectivity of pioneer transcription factor SOX2. *Nat. Commun.* **12**, 28 (2021).
96. Cai, H. et al. Nucleocytoplasmic shuttling of a GATA transcription factor functions as a development timer. *Science* **343**, 1249531 (2014).
97. Meyer, T. & Vinkemeier, U. Nucleocytoplasmic shuttling of STAT transcription factors. *Eur. J. Biochem.* **271**, 4606–4612 (2004).
98. Yang, Y. et al. Nuclear transport proteins: structure, function and disease relevance. *Signal. Transduct. Target. Ther.* **8**, 425 (2023).
99. She, Z.-Y. & Yang, W.-X. Nucleocytoplasmic shuttling of SOX14A and SOX14B transcription factors. *Oncotarget* **8**, 46955–46968 (2017).
100. Schindler, C., Shuai, K., Prezioso, V. R. & Darnell, J. E. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. *Science* **257**, 809–813 (1992).
101. Shuai, K., Stark, G. R., Kerr, L. M. & Darnell, J. E. A single phosphotyrosine residue of stat91 required for gene activation by interferon-γ. *Science* **261**, 1744–1746 (1993).
102. Stark, G. R., Kerr, I. M., Williams, B. R. G., Silverman, R. H. & Schreiber, R. D. How cells respond to interferons. *Annu. Rev. Biochem.* **67**, 227–264 (1998).
103. McBride, K. M. Regulated nuclear import of the STAT1 transcription factor by direct binding of importin-alpha. *EMBO J.* **21**, 1754–1763 (2002).
104. Fagerlund, R., Melén, K., Kinnunen, L. & Julkunen, I. Arginine/lysine-rich nuclear localization signals mediate interactions between dimeric STATs and importin α5. *J. Biol. Chem.* **277**, 30072–30078 (2002).
105. Sekimoto, T. Extracellular signal-dependent nuclear import of Stat1 is mediated by nuclear pore-targeting complex formation with NPI-1, but not Rch1. *EMBO J.* **16**, 7067–7077 (1997).
106. Horvath, C. M., Wen, Z. & Darnell, J. E. A STAT protein domain that determines DNA sequence recognition suggests a novel DNA-binding domain. *Genes. Dev.* **9**, 984–994 (1995).
107. Khan, K. D. et al. Induction of the Ly-6A/E gene by interferon alpha/beta and gamma requires a DNA element to which a tyrosine-phosphorylated 91-kDa protein binds. *Proc. Natl Acad. Sci. USA* **90**, 6806–6810 (1993).
108. Ruvkun, G. & Lehrbach, N. Regulation and Functions of the ER-Associated Nrf1 Transcription Factor. *Cold Spring Harb. Perspect. Biol.* **15**, a041266 (2023).
109. Capece, D. et al. NF-κB: blending metabolism, immunity, and inflammation. *Trends Immunol.* **43**, 757–775 (2022).
110. Guo, X. et al. Mechanistic insights and implications of FOXO–SNAI interplay. *BioEssays* **44**, 2200070 (2022).
111. Huang, D., Wang, Y., Tang, J. & Luo, S. Molecular mechanisms of suppressor of fused in regulating the hedgehog signalling pathway. *Oncol. Lett.* **15**, 6077–6086 (2018).
112. Zambrano, S., De Toma, I., Piffer, A., Bianchi, M. E. & Agresti, A. NF-κB oscillations translate into functionally related patterns of gene expression. *eLife* **5**, e09100 (2016).
113. Aqdas, M. & Sung, M.-H. NF-κB dynamics in the language of immune cells. *Trends Immunol.* **44**, 32–43 (2023).
114. Adam, J. et al. A role for cytosolic fumarate hydratase in urea cycle metabolism and renal neoplasia. *Cell Rep.* **3**, 1440–1448 (2013).
115. Ajalla Aleixo, M. A., Rangel, V. L., Rustiguel, J. K., De Pádua, R. A. P. & Nonato, M. C. Structural, biochemical and biophysical characterization of recombinant human fumarate hydratase. *FEBS J.* **286**, 1925–1940 (2019).
116. Mescam, M., Vinnakota, K. C. & Beard, D. A. Identification of the catalytic mechanism and estimation of kinetic parameters for fumarase. *J. Biol. Chem.* **286**, 21100–21109 (2011).
117. Jiang, Y. et al. Local generation of fumarate promotes DNA repair through inhibition of histone H3 demethylation. *Nat. Cell Biol.* **17**, 1158–1168 (2015).
118. Ninomiya, K. et al. m^6 A modification of HSATIII lncRNAs regulates temperature-dependent splicing. *EMBO J.* **40**, e107976 (2021).
119. Timcheva, K. et al. Chromatin-associated YTHDC1 coordinates heat-induced reprogramming of gene expression. *Cell Rep.* **41**, 111784 (2022).
120. Lyon, A. S., Peeples, W. B. & Rosen, M. K. A framework for understanding the functions of biomolecular condensates across scales. *Nat. Rev. Mol. Cell Biol.* **22**, 215–235 (2021).
121. Matsushima, T. et al. Localizatome: a database for stress-dependent subcellular localization changes in proteins. *Database* **2025**, baaf028 (2025).
122. Sabari, B. R. Biomolecular condensates and gene activation in development and disease. *Dev. Cell* **55**, 84–96 (2020).
123. Su, Q., Mehta, S. & Zhang, J. Liquid–liquid phase separation: orchestrating cell signaling through time and space. *Mol. Cell* **81**, 4137–4146 (2021).
124. Bienz, M. Signalosome assembly by domains undergoing dynamic head-to-tail polymerization. *Trends Biochem. Sci.* **39**, 487–495 (2014).
125. Jeffery, C. J. Moonlighting proteins. *Trends Biochem. Sci.* **24**, 8–11 (1999).
126. Singh, N. & Bhalla, N. Moonlighting proteins. *Annu. Rev. Genet.* **54**, 265–285 (2020).
127. Jeffery, C. J. Protein moonlighting: what is it, and why is it important? *Philos. Trans. R. Soc. B* **373**, 20160523 (2018).
128. Copley, S. D. An evolutionary perspective on protein moonlighting. *Biochem. Soc. Trans.* **42**, 1684–1691 (2014).
129. Dar, G. H. et al. GAPDH controls extracellular vesicle biogenesis and enhances the therapeutic potential of EV mediated siRNA delivery to the brain. *Nat. Commun.* **12**, 6666 (2021).
130. Sheokand, N. et al. Secreted glyceraldehye-3-phosphate dehydrogenase is a multifunctional autocrine transferrin receptor for cellular iron acquisition. *Biochim. Biophys. Acta* **1830**, 3816–3827 (2013).
131. Malhotra, H. et al. Moonlighting protein glyceraldehyde-3-phosphate dehydrogenase: a cellular rapid-response molecule for maintenance of iron homeostasis in hypoxia. *Cell. Physiol. Biochem.* **52**, 517–531 (2019).
132. Chang, C.-H. et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* **153**, 1239–1251 (2013).
133. Hara, M. R. et al. *S*-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nat. Cell Biol.* **7**, 665–674 (2005).
134. Sirover, M. A. The role of posttranslational modification in moonlighting glyceraldehyde-3-phosphate dehydrogenase structure and function. *Amino Acids* **53**, 507–515 (2021).
135. Sirover, M. A. Moonlighting glyceraldehyde-3-phosphate dehydrogenase: posttranslational modification, protein and nucleic acid interactions in normal cells and in human pathology. *Crit. Rev. Biochem. Mol. Biol.* **55**, 354–371 (2020).
136. Inadomi, C. et al. Overexpression of glutaredoxin protects cardiomyocytes against nitric oxide-induced apoptosis with suppressing the S-nitrosylation of proteins and nuclear translocation of GAPDH. *Biochem. Biophys. Res. Commun.* **425**, 656–661 (2012).
137. Fuentes-Lemus, E., Usgame, K., Fierro, A. & López-Alarcón, C. Enzymes of glycolysis and the pentose phosphate pathway as targets of oxidants: Role of redox reactions on the carbohydrate catabolism. *Redox Biochem. Chem.* **11**, 100049 (2025).
138. Talwar, D. et al. The GAPDH redox switch safeguards reductive capacity and enables survival of stressed tumour cells. *Nat. Metab.* **5**, 660–676 (2023).
139. Mustafa Rizvi, S. H. et al. Oxidized GAPDH transfers *S*-glutathionylation to a nuclear protein Sirtuin-1 leading to apoptosis. *Free. Radic. Biol. Med.* **174**, 73–83 (2021).
140. Franco-Serrano, L. et al. MultitaskProtDB-II: an update of a database of multitasking/moonlighting proteins. *Nucleic Acids Res.* **46**, D645–D648 (2018).
141. Su, B., Qian, Z., Li, T., Zhou, Y. & Wong, A. PlantMP: a database for moonlighting plant proteins. *Database J. Biol. Databases Curation* **2019**, baz050 (2019).
142. Ribeiro, D. M., Briere, G., Bely, B., Spinelli, L. & Brun, C. MoonDB 2.0: an updated database of extreme multifunctional and moonlighting proteins. *Nucleic Acids Res.* **47**, D398–D402 (2019).
143. Piano, V. multitasking proteins: exploring noncanonical functions of proteins during mitosis. *Biochemistry* **64**, 2123–2137 (2025).
144. Yokoyama, H., Rybina, S., Santarella-Mellwig, R., Mattaj, I. W. & Karsenti, E. ISWI is a RanGTP-dependent MAP required for chromosome segregation. *J. Cell Biol.* **187**, 813–829 (2009).
145. Yokoyama, H. et al. CHD4 is a RanGTP-dependent MAP that stabilizes microtubules and regulates bipolar spindle formation. *Curr. Biol. CB* **23**, 2443–2451 (2013).
146. Yamazaki, H., Takagi, M., Kosako, H., Hirano, T. & Yoshimura, S. H. Cell cycle-specific phase separation regulated by protein charge blockiness. *Nat. Cell Biol.* **24**, 625–632 (2022).
147. Öllinger, R. et al. Dynamics of the circadian clock protein PERIOD2 in living cells. *J. Cell Sci.* **127**, 4322–4328 (2014).
148. Jaballah, N. et al. The scaffold protein PDZK1 governs diurnal localization of CNT2 on the plasma membrane in mouse intestinal epithelial cells. *J. Biochem.* **174**, 193–201 (2023).
149. Christopher, J. A. et al. Global proteomics indicates subcellular-specific anti-ferroptotic responses to ionizing radiation. *Mol. Cell. Proteom.* **24**, 100888 (2025).
150. Hein, M. Y. et al. Global organelle profiling reveals subcellular localization and remodeling at proteome scale. *Cell* **188**, 1137–1155.e20 (2025).
151. Guo, X., Peng, K., He, Y. & Xue, L. Mechanistic regulation of FOXO transcription factors in the nucleus. *Biochim. Biophys. Acta* **1879**, 189083 (2024).

152. Lamouille, S., Xu, J. & Derynck, R. Molecular mechanisms of epithelial–mesenchymal transition. *Nat. Rev. Mol. Cell Biol.* **15**, 178–196 (2014).
153. Rim, E. Y., Clevers, H. & Nusse, R. The Wnt pathway: from signaling mechanisms to synthetic modulators. *Annu. Rev. Biochem.* **91**, 571–598 (2022).
154. Schieweck, R. & Götz, M. Pan-cellular organelles and suborganelles — from common functions to cellular diversity? *Genes. Dev.* **38**, 98–114 (2024).
155. Russo, G. L. et al. CRISPR-mediated induction of neuron-enriched mitochondrial proteins boosts direct glia-to-neuron conversion. *Cell Stem Cell* **28**, 524–534.e7 (2021).
156. O'Neill, A. C. et al. Spatial centrosome proteome of human neural cells uncovers disease-relevant heterogeneity. *Science* **376**, eabf9088 (2022).
157. Makarov, E. M., Makarova, O. V., Achsel, T. & Lührmann, R. The human homologue of the yeast splicing factor prp6p contains multiple TPR elements and is stably associated with the U5 snRNP via protein-protein interactions. *J. Mol. Biol.* **298**, 567–575 (2000).
158. Buckley, C. E. & St Johnston, D. Apical–basal polarity and the control of epithelial form and function. *Nat. Rev. Mol. Cell Biol.* **23**, 559–577 (2022).
159. Kennedy, B. & Malicki, J. What drives cell morphogenesis: a look inside the vertebrate photoreceptor. *Dev. Dyn.* **238**, 2115–2138 (2009).
160. Imanishi, Y. Protein sorting in healthy and diseased photoreceptors. *Annu. Rev. Vis. Sci.* **5**, 73–98 (2019).
161. Choi, J., Rafiq, N. M. & Park, D. Liquid–liquid phase separation in presynaptic nerve terminals. *Trends Biochem. Sci.* **49**, 888–900 (2024).
162. Baschieri, F. & Farhan, H. Endomembrane control of cell polarity: relevance to cancer. *Small GTPases* **6**, 104–107 (2015).
163. Klinkert, K., Rocancourt, M., Houdusse, A. & Echard, A. Rab35 GTPase couples cell division with initiation of epithelial apico-basal polarity and lumen opening. *Nat. Commun.* **7**, 11166 (2016).
164. Kojima, Y. et al. The overexpression and altered localization of the atypical protein kinase C λ/ι in breast cancer correlates with the pathologic type of these tumors. *Hum. Pathol.* **39**, 824–831 (2008).
165. Tokinaga-Uchiyama, A. et al. Aberrant nuclear localization of aPKCλ/ι is associated with poorer prognosis in uterine cervical cancer. *Int. J. Gynecol. Pathol.* **38**, 301–309 (2019).
166. Ni, S. et al. PKC iota promotes cellular proliferation by accelerated G1/S transition via interaction with CDK7 in esophageal squamous cell carcinoma. *Tumor Biol.* **37**, 13799–13809 (2016).
167. Zhao, H.-Y. et al. Perturbation of mammary epithelial cell apicobasal polarity by RHBDF1-facilitated nuclear translocation of PKCζ. *Biol. Res.* **57**, 90 (2024).
168. Tyagi, K., Roy, A. & Mandal, S. Protein kinase C iota promotes glycolysis via PI3K/AKT/mTOR signalling in high grade serous ovarian cancer. *Mol. Biol. Rep.* **51**, 983 (2024).
169. Suk, T. R. & Rousseaux, M. W. C. The role of TDP-43 mislocalization in amyotrophic lateral sclerosis. *Mol. Neurodegener.* **15**, 45 (2020).
170. Ko, S. B. H. et al. Corticosteroids correct aberrant CFTR localization in the duct and regenerate acinar cells in autoimmune pancreatitis. *Gastroenterology* **138**, 1988–1996.e3 (2010).
171. Assoni, A. F., Foijer, F. & Zatz, M. Amyotrophic lateral sclerosis, FUS and protein synthesis defects. *Stem Cell Rev. Rep.* **19**, 625–638 (2023).
172. Lacoste, J. et al. Pervasive mislocalization of pathogenic coding variants underlying human disorders. *Cell* **187**, 6725–6741.e13 (2024).
173. Dong, J. et al. Endosomal traffic disorders: a driving force behind neurodegenerative diseases. *Transl. Neurodegener.* **13**, 66 (2024).
174. Muraleedharan, A. & Vanderperre, B. The endo-lysosomal system in Parkinson's disease: expanding the horizon. *J. Mol. Biol.* **435**, 168140 (2023).
175. Gilleron, J. & Zeigerer, A. Endosomal trafficking in metabolic homeostasis and diseases. *Nat. Rev. Endocrinol.* **19**, 28–45 (2023).
176. Nissley, D. A. et al. Universal protein misfolding intermediates can bypass the proteostasis network and remain soluble and less functional. *Nat. Commun.* **13**, 3081 (2022).
177. Lan, P. D. et al. Synonymous mutations can alter protein dimerization through localized interface misfolding involving self-entanglements. *J. Mol. Biol.* **436**, 168487 (2024).
178. Halder, R. et al. How soluble misfolded proteins bypass chaperones at the molecular level. *Nat. Commun.* **14**, 3689 (2023).
179. Dormann, D. et al. ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import. *EMBO J.* **29**, 2841–2857 (2010).
180. Mensah, M. A. et al. Aberrant phase separation and nucleolar dysfunction in rare genetic diseases. *Nature* **614**, 564–571 (2023).
181. Das, T. et al. Tunable metastability of condensates reconciles their dual roles in amyloid fibril formation. *Mol. Cell* **85**, 2230–2245.e7 (2025).
182. Banani, S. F. et al. Genetic variation associated with condensate dysregulation in disease. *Dev. Cell* **57**, 1776–1788.e8 (2022).
183. Bouchard, J. J. et al. Cancer mutations of the tumor suppressor SPOP disrupt the formation of active, phase-separated compartments. *Mol. Cell* **72**, 19–36.e8 (2018).
184. Li, X. et al. Deacetylation induced nuclear condensation of HP1γ promotes multiple myeloma drug resistance. *Nat. Commun.* **14**, 1290 (2023).
185. Bawadekar, M., De Andrea, M., Gariglio, M. & Landolfo, S. Mislocalization of the interferon inducible protein IFI16 by environmental insults: Implications in autoimmunity. *Cytokine Growth Factor. Rev.* **26**, 213–219 (2015).
186. Jiang, Z. et al. IFI16 directly senses viral RNA and enhances RIG-I transcription and activation to restrict influenza virus infection. *Nat. Microbiol.* **6**, 932–945 (2021).
187. Adamo, A. et al. Moonlighting proteins are important players in cancer immunology. *Front. Immunol.* **11**, 613069 (2021).
188. Crane, R., Tebbe, L., Mwoyosvi, M. L., Al-Ubaidi, M. R. & Naash, M. I. Expression of the human usherin c.2299delG mutation leads to early-onset auditory loss and stereocilia disorganization. *Commun. Biol.* **6**, 933 (2023).
189. Tebbe, L. et al. The usherin mutation c.2299delG leads to its mislocalization and disrupts interactions with whirlin and VLGR1. *Nat. Commun.* **14**, 972 (2023).
190. Animireddy, S. et al. Aberrant cytoplasmic localization of ARID1B activates ERK signaling and promotes oncogenesis. *J. Cell Sci.* **134**, jcs251637 (2021).
191. Schaffert, L.-N. & Carter, W. G. Do post-translational modifications influence protein aggregation in neurodegenerative diseases: a systematic review. *Brain Sci.* **10**, 232 (2020).
192. Ramzan, F., Abrar, F., Mishra, G. G., Liao, L. M. Q. & Martin, D. D. O. Lost in traffic: consequences of altered palmitoylation in neurodegeneration. *Front. Physiol.* **14**, 1166125 (2023).
193. Omar, M. H. et al. Mislocalization of protein kinase A drives pathology in Cushing's syndrome. *Cell Rep.* **40**, 111073 (2022).
194. Ng, C. S. C., Liu, A., Cui, B. & Banik, S. M. Targeted protein relocalization via protein transport coupling. *Nature* **633**, 941–951 (2024).
195. Till, N. A., Ramanathan, M. & Bertozzi, C. R. Induced proximity at the cell surface. *Nat. Biotechnol.* **43**, 702–711 (2025).
196. Shao, Q., Duong, T. N., Park, I., Orr, L. M. & Nomura, D. K. Targeted protein localization by covalent 14–3–3 recruitment. *J. Am. Chem. Soc.* **146**, 24788–24799 (2024).
197. Gibson, W. J. et al. Bifunctional small molecules that induce nuclear localization and targeted transcriptional regulation. *J. Am. Chem. Soc.* **145**, 26028–26037 (2023).
198. Tsai, J. M., Nowak, R. P., Ebert, B. L. & Fischer, E. S. Targeted protein degradation: from mechanisms to clinic. *Nat. Rev. Mol. Cell Biol.* **25**, 740–757 (2024).
199. Simpson, L. M. et al. Target protein localization and its impact on PROTAC-mediated degradation. *Cell Chem. Biol.* **29**, 1482–1504.e7 (2022).
200. Go, C. D. et al. A proximity-dependent biotinylation map of a human cell. *Nature* **595**, 120–124 (2021).
201. Orre, L. M. et al. SubCellBarCode: proteome-wide mapping of protein localization and relocalization. *Mol. Cell* **73**, 166–182.e7 (2019).
202. Fu, X., Zhang, S. & Liu, P. Co-immunoprecipitation for identifying protein–protein interaction on lipid droplets. *Biophys. Rep.* **10**, 102 (2024).
203. Fasimoye, R. et al. Golgi-IP, a tool for multimodal analysis of Golgi molecular content. *Proc. Natl Acad. Sci. USA* **120**, e2219953120 (2023).
204. Morgenstern, M. et al. Quantitative high-confidence human mitochondrial proteome and its dynamics in cellular context. *Cell Metab.* **33**, 2464–2483.e18 (2021).
205. Schessner, J. P., Albrecht, V., Davies, A. K., Sinitcyn, P. & Borner, G. H. H. Deep and fast label-free dynamic organellar mapping. *Nat. Commun.* **14**, 5252 (2023).
206. Chen, H. et al. Omics analyses uncover host networks defining virus-permissive and -hostile cellular states. *Mol. Cell. Proteom.* **24**, 100966 (2025).
207. Huttlin, E. L. et al. Dual proteome-scale networks reveal cell-specific remodeling of the human interactome. *Cell* **184**, 3022–3040.e28 (2021).
208. Gupta, A. et al. SubCell: vision foundation models for microscopy capture single-cell biology. Preprint at *bioRxiv* https://doi.org/10.1101/2024.12.06.627299 (2025).
209. Zhang, Z. et al. A subcellular map of translational machinery composition and regulation at the single-molecule level. *Science* **387**, eadn2623 (2025).
210. Park, H. et al. Spatial snapshots of amyloid precursor protein intramembrane processing via early endosome proteomics. *Nat. Commun.* **13**, 6112 (2022).
211. Chen, L. et al. Mitochondrial heterogeneity in diseases. *Signal. Transduct. Target. Ther.* **8**, 311 (2023).
212. Gamuyao, R. & Chang, C.-L. Imaging and proteomics toolkits for studying organelle contact sites. *Front. Cell Dev. Biol.* **12**, 1466915 (2024).
213. Calì, T. et al. Key challenges and recommendations for defining organelle membrane contact sites. *Nat. Rev. Mol. Cell Biol.* **26**, 776–796 (2025).
214. King, M. R. et al. Macromolecular condensation organizes nucleolar sub-phases to set up a pH gradient. *Cell* **187**, 1889–1906.e24 (2024).
215. Schaffer, L. V. et al. Multimodal cell maps as a foundation for structural and functional genomics. *Nature* **642**, 222–231 (2025).
216. Cesnik, A. et al. Mapping the multiscale proteomic organization of cellular and disease phenotypes. *Annu. Rev. Biomed. Data Sci.* **7**, 369–389 (2024).
217. Radhakrishnan, S. K., Den Besten, W. & Deshaies, R. J. p97-dependent retrotranslocation and proteolytic processing govern formation of active Nrf1 upon proteasome inhibition. *eLife* **3**, e01856 (2014).
218. Stenström, L. et al. Mapping the nucleolar proteome reveals a spatiotemporal organization related to intrinsic protein disorder. *Mol. Syst. Biol.* **16**, e9469 (2020).
219. Currie, J. et al. Simultaneous proteome localization and turnover analysis reveals spatiotemporal features of protein homeostasis disruptions. *Nat. Commun.* **15**, 2207 (2024).
220. Ashburner, M. et al. Gene Ontology: tool for the unification of biology. *Nat. Genet.* **25**, 25–29 (2000).
221. Piatigorsky, J. et al. Gene sharing by delta-crystallin and argininosuccinate lyase. *Proc. Natl Acad. Sci. USA* **85**, 3479–3483 (1988).
222. Shortreed, M. R. et al. Elucidating proteoform families from proteoform intact-mass and lysine-count measurements. *J. Proteome Res.* **15**, 1213–1221 (2016).
223. Chapple, C. E. et al. Extreme multifunctional proteins identified from a human protein interaction network. *Nat. Commun.* **6**, 7412 (2015).
224. Antifeeva, I. A. et al. Liquid–liquid phase separation as an organizing principle of intracellular space: overview of the evolution of the cell compartmentalization concept. *Cell. Mol. Life Sci.* **79**, 251 (2022).

225. Cornejo, E., Abreu, N. & Komeili, A. Compartmentalization and organelle formation in bacteria. *Curr. Opin. Cell Biol.* **26**, 132–138 (2014).
226. Sagulenko, E. et al. Structural studies of planctomycete gemmata obscuriglobus support cell compartmentalisation in a bacterium. *PLoS ONE* **9**, e91344 (2014).
227. Giessen, T. W. Encapsulins. *Annu. Rev. Biochem.* **91**, 353–380 (2022).
228. Morgan, C. J. et al. An essential and highly selective protein import pathway encoded by nucleus-forming phage. *Proc. Natl Acad. Sci. USA* **121**, e2321190121 (2024).
229. Giessen, T. W. The structural diversity of encapsulin protein shells. *ChemBioChem* **25**, e202400535 (2024).
230. Chouaib, R. et al. A dual protein–mRNA localization screen reveals compartmentalized translation and widespread co-translational RNA targeting. *Dev. Cell* **54**, 773–791.e5 (2020).
231. Fazal, F. M. et al. Atlas of subcellular RNA localization revealed by APEX-Seq. *Cell* **178**, 473–490.e26 (2019).
232. Mattola, S. et al. Nuclear entry and egress of parvoviruses. *Mol. Microbiol.* **118**, 295–308 (2022).
233. Redrejo-Rodríguez, M., Muñoz-Espín, D., Holguera, I., Mencía, M. & Salas, M. Nuclear and nucleoid localization are independently conserved functions in bacteriophage terminal proteins. *Mol. Microbiol.* **90**, 858–868 (2013).
234. He, Q., McLellan, H., Boevink, P. C. & Birch, P. R. J. All roads lead to susceptibility: the many modes of action of fungal and oomycete intracellular effectors. *Plant. Commun.* **1**, 100050 (2020).
235. Neumann, N., Lundin, D. & Poole, A. M. Comparative genomic evidence for a complete nuclear pore complex in the last eukaryotic common ancestor. *PLoS ONE* **5**, e13241 (2010).
236. Sagulenko, E. et al. Nuclear pore-like structures in a compartmentalized bacterium. *PLoS ONE* **12**, e0169432 (2017).
237. Schneider, A. Evolution of mitochondrial protein import — lessons from trypanosomes. *Biol. Chem.* **401**, 663–676 (2020).
238. Link, S., Grund, S. E. & Diederichs, S. Alternative splicing affects the subcellular localization of Drosha. *Nucleic Acids Res.* **44**, 5330–5343 (2016).
239. Li, X., Zelenka, P. S. & Piatigorsky, J. Differential expression of the two δ-crystallin genes in lens and non-lens tissues: Shift favoring δ2 expression from embryonic to adult chickens. *Dev. Dyn.* **196**, 114–123 (1993).
240. Espinosa-Cantú, A., Ascencio, D., Barona-Gómez, F. & DeLuna, A. Gene duplication and the evolution of moonlighting proteins. *Front. Genet.* **6**, 227 (2015).
241. Birktoft, J. J. et al. Comparison of the molecular structures of cytoplasmic and mitochondrial malate dehydrogenase. *Biochem. Soc. Trans.* **17**, 301–304 (1989).
242. Baird, L. M., Berndsen, C. E. & Monroe, J. D. Malate dehydrogenase in plants: evolution, structure, and a myriad of functions. *Essays Biochem.* **68**, 221–233 (2024).
243. Lu, J. et al. Types of nuclear localization signals and mechanisms of protein import into the nucleus. *Cell Commun. Signal. CCS* **19**, 60 (2021).
244. Scott, M. S., Troshin, P. V. & Barton, G. J. NoD: a Nucleolar localization sequence detector for eukaryotic and viral proteins. *BMC Bioinforma.* **12**, 317 (2011).
245. Hatanaka, M. Discovery of the nucleolar targeting signal. *BioEssays N. Rev. Mol. Cell. Dev. Biol.* **12**, 143–148 (1990).
246. Blobel, G. & Sabatini, D. D. Controlled proteolysis of nascent polypeptides in rat liver cell fractions. I. Location of the polypeptides within ribosomes. *J. Cell Biol.* **45**, 130–145 (1970).
247. Sabatini, D. D. & Blobel, G. Controlled proteolysis of nascent polypeptides in rat liver cell fractions. II. *Locat. polypeptides rough. microsomes. J. Cell Biol.* **45**, 146–157 (1970).
248. Dudek, J., Rehling, P. & van der Laan, M. Mitochondrial protein import: common principles and physiological networks. *Biochim. Biophys. Acta* **1833**, 274–285 (2013).
249. Schmidt, O., Pfanner, N. & Meisinger, C. Mitochondrial protein import: from proteomics to functional mechanisms. *Nat. Rev. Mol. Cell Biol.* **11**, 655–667 (2010).
250. Baker, A., Lanyon-Hogg, T. & Warriner, S. L. Peroxisome protein import: a complex journey. *Biochem. Soc. Trans.* **44**, 783–789 (2016).
251. Jiang, Y. et al. MULocDeep: a deep-learning framework for protein subcellular and suborganellar localization prediction with residue-level interpretation. *Comput. Struct. Biotechnol. J.* **19**, 4825–4839 (2021).
252. Thumuluri, V., Almagro Armenteros, J. J., Johansen, A. R., Nielsen, H. & Winther, O. DeepLoc 2.0: multi-label subcellular localization prediction using protein language models. *Nucleic Acids Res.* **50**, W228–W234 (2022).
253. Kubitz, L. et al. Engineering of ultraID, a compact and hyperactive enzyme for proximity-dependent biotinylation in living cells. *Commun. Biol.* **5**, 657 (2022).
254. Branon, T. C. et al. Efficient proximity labeling in living cells and organisms with TurboID. *Nat. Biotechnol.* **36**, 880–887 (2018).
255. Choi-Rhee, E., Schulman, H. & Cronan, J. E. Promiscuous protein biotinylation by Escherichia coli biotin protein ligase. *Protein Sci. Publ. Protein Soc.* **13**, 3043–3050 (2004).
256. Roux, K. J., Kim, D. I., Raida, M. & Burke, B. A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. *J. Cell Biol.* **196**, 801–810 (2012).
257. Lam, S. S. et al. Directed evolution of APEX2 for electron microscopy and proximity labeling. *Nat. Methods* **12**, 51–54 (2015).
258. Lee, A. et al. OrthoID: profiling dynamic proteomes through time and space using mutually orthogonal chemical tools. *Nat. Commun.* **15**, 1851 (2024).
259. Fischer, S.N. et al. hu.MAP3.0: atlas of human protein complexes by integration of >25,000 proteomic experiments. *Mol Syst Biol* **21,** 911–943 (2025).
260. Komatsu, S. Isolation, purity assessment, and proteomic analysis of nuclei. *Methods Mol. Biol. Clifton NJ* **1696**, 81–90 (2018).
261. Afanasyeva, M. A. et al. Isolation of large amounts of highly pure mitochondria for 'Omics' studies. *Biochem. Biokhimiia* **83**, 76–85 (2018).
262. Liao, P.-C., Bergamini, C., Fato, R., Pon, L. A. & Pallotti, F. Isolation of mitochondria from cells and tissues. *Methods Cell Biol.* **155**, 3–31 (2020).
263. Schmidt, H. et al. Enrichment and analysis of secretory lysosomes from lymphocyte populations. *BMC Immunol.* **10**, 41 (2009).
264. Saleem, R. A., Smith, J. J. & Aitchison, J. D. Proteomics of the peroxisome. *Biochim. Biophys. Acta* **1763**, 1541–1551 (2006).
265. Wheeler, J. R., Jain, S., Khong, A. & Parker, R. Isolation of yeast and mammalian stress granule cores. *Methods San. Diego Calif.* **126**, 12–17 (2017).
266. Mund, A. et al. Deep Visual Proteomics defines single-cell identity and heterogeneity. *Nat. Biotechnol.* **40**, 1231–1240 (2022).
267. Ezzoukhry, Z. et al. Combining laser capture microdissection and proteomics reveals an active translation machinery controlling invadosome formation. *Nat. Commun.* **9**, 2031 (2018).
268. Dong, Z. et al. Spatial proteomics of single cells and organelles on tissue slides using filter-aided expansion proteomics. *Nat. Commun.* **15**, 9378 (2024).
269. Mulvey, C. M. et al. Using hyperLOPIT to perform high-resolution mapping of the spatial proteome. *Nat. Protoc.* **12**, 1110–1135 (2017).
270. Itzhak, D. N., Tyanova, S., Cox, J. & Borner, G. H. Global, quantitative and dynamic mapping of protein subcellular localization. *eLife* **5**, e16950 (2016).
271. Kobayashi, H., Cheveralls, K. C., Leonetti, M. D. & Royer, L. A. Self-supervised deep learning encodes high-resolution features of protein subcellular localization. *Nat. Methods* **19**, 995–1003 (2022).
272. Doron, M. et al. Unbiased single-cell morphology with self-supervised vision transformers. Preprint at *bioRxiv* https://doi.org/10.1101/2023.06.16.545359 (2023).
273. Mund, A., Brunner, A.-D. & Mann, M. Unbiased spatial proteomics with single-cell resolution in tissues. *Mol. Cell* **82**, 2335–2349 (2022).
274. Kennedy-Darling, J. et al. Highly multiplexed tissue imaging using repeated oligonucleotide exchange reaction. *Eur. J. Immunol.* **51**, 1262–1277 (2021).
275. Schürch, C. M. et al. Coordinated cellular neighborhoods orchestrate antitumoral immunity at the colorectal cancer invasive front. *Cell* **182**, 1341–1359.e19 (2020).
276. Goltsev, Y. et al. Deep profiling of mouse splenic architecture with CODEX multiplexed imaging. *Cell* **174**, 968–981.e15 (2018).
277. Janesick, A. et al. High resolution mapping of the tumor microenvironment using integrated single-cell, spatial and in situ analysis. *Nat. Commun.* **14**, 8353 (2023).
278. Merritt, C. R. et al. Multiplex digital spatial profiling of proteins and RNA in fixed tissue. *Nat. Biotechnol.* **38**, 586–599 (2020).
279. Villanueva, E. et al. System-wide analysis of RNA and protein subcellular localization dynamics. *Nat. Methods* **21**, 60–71 (2024).

Acknowledgements

We thank L. Magni for early discussions related to moonlighting proteins and W. Leineweber for comments and feedback during writing manuscript. E.L. was supported by the Wallenberg Foundation (grant no. 2021.0346), Erling Persson Foundation, Göran Gustafsson Foundation, Schmidt Futures, the Bridge2AI Program (NIH Common Fund; grant no. OT2 OD032742), PROMINENT team supported by the Cancer Grand Challenges partnership funded by Cancer Research UK (grant no. CGCATF-2021/100010) and the National Cancer Institute (grant no. OT2CA278713), the Cancer Cell Map Initiative (NCI Center for Cancer Systems Biology; grant no. U54 CA274502), Stanford Institute for Human-Centered AI, Chan Zuckerberg Initiative, Phil and Penny Knight Initiative for Brain Resilience at the Wu Tsai Neurosciences Institute, Danaher and Param Hansa Philanthropies. A.S. was supported by a Rubicon grant from the Dutch Research Council (NWO, grant no. 019.231EN.013). C.H. was funded through a BBSRC CASE award with AstraZeneca (grant no. BB/W509929/1).

Author contributions

A.S., C.H. and A.C. researched data for the article; E.L., A.S., C.H. and K.S.L. substantially contributed to discussion of content; E.L., A.S., C.H. and A.C. wrote the article; all authors reviewed and/or edited the manuscript before submission.

Competing Interests

E.L. is an adviser for Element Biosciences, Cartography Biosciences, Genbio.ai and Pixelgen Technologies AB.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41580-026-00947-3.

Peer review information *Nature Reviews Molecular Cell Biology* thanks Jennifer Lippincott-Schwartz and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

© Springer Nature Limited 2026