Protein localization in disease and therapy

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Summary

The eukaryotic cell is organized into membrane-covered compartments that are characterized by specific sets of proteins and biochemically distinct cellular processes. The appropriate subcellular localization of proteins is crucial because it provides the physiological context for their function. In this Commentary, we give a brief overview of the different mechanisms that are involved in protein trafficking and describe how aberrant localization of proteins contributes to the pathogenesis of many human diseases, such as metabolic, cardiovascular and neurodegenerative diseases, as well as cancer. Accordingly, modifying the disease-related subcellular mislocalization of proteins might be an attractive means of therapeutic intervention. In particular, cellular processes that link protein folding and cell signaling, as well as nuclear import and export, to the subcellular localization of proteins have been proposed as targets for therapeutic intervention. We discuss the concepts involved in the therapeutic restoration of disrupted physiological protein localization and therapeutic mislocalization as a strategy to inactivate disease-causing proteins.

Key words: Human disease, Nucleo-cytoplasmic transport, Protein trafficking, Subcellular protein mislocalization, Theraputic mistargeting, Theraputic rescue

Introduction

All eukaryotic cells are surrounded by plasma membranes and contain elaborate organelles and a complex endomembrane system. These organelles provide distinct compartments for different metabolic activities. Protein translation is confined to only one of these compartments, the cytosol. The translocation of proteins is a fundamental requirement for proteins to be able to exert their functions in different organelles. Indeed, approximately half of the proteins generated by a cell have to be transported into or across at least one cellular membrane to reach their functional destination (Chacinska et al., 2009).

The regulation of protein trafficking relies on information that is encoded within the protein sequence and occurs by two major mechanisms, namely co-translational and post-translational translocation (Rapoport, 2007; Schnell and Hebert, 2003; Wickner and Schekman, 2005). Proteins targeted to the mitochondria, peroxisomes or the nucleus are post-transcriptionally translocated (Fig. 1) (Schmidt et al., 2010; Suntharalingam and Wente, 2003; Terry et al., 2007; Wickner and Schekman, 2005; Wolf et al., 2010). Even though protein trafficking into each of these organelles involves distinct mechanisms, there are some common features, such as the presence of signal sequences, which are recognized by specific receptors, and the import through membrane-spanning pores. Whereas the transport systems for the post-translational translocation of proteins into the mitochondria rely on the activity of chaperones to provide unfolded polypeptide chains as translocation substrates (Rapoport, 2007; Schmidt et al., 2010), the nuclear envelope is perforated by nuclear pores that allow the passage of folded proteins either by simple diffusion or mediated by soluble transport receptors (Strambio-De-Castillia et al., 2010; Suntharalingam and Wente, 2003; Terry et al., 2007). By contrast, many other proteins, including those destined for the secretory pathway and integral membrane proteins, are transported into the endoplasmic reticulum (ER) during synthesis, a process known as co-translational translocation (Fig. 1) (Wickner and Schekman, 2005).

Subcellular localization is essential to protein function and has been suggested as a means to achieve functional diversity and, at the same time, economize on protein design and synthesis (Butler and Overall, 2009). Subcellular localization determines the access of proteins to interacting partners and the post-translational modification machinery and enables the integration of proteins into functional biological networks. Aberrantly localized proteins have been linked to human diseases as diverse as Alzheimer's disease, kidney stones and cancer. In this Commentary, we will, therefore, summarize our current knowledge on the mechanisms that regulate subcellular protein localization and those that have been implicated in the pathogenesis of human disease, and we will discuss emerging therapeutic strategies that target protein localization.

Subcellular mislocalization of proteins in human diseases

Protein translocation accomplishes the movement of material and information within the eukaryotic cell and is essential for the normal functioning of the cell. The protein transport machinery of cells ensures that the right amount of protein is present at the right time and place (Fig. 1). Aberrant protein localization that is caused by mutation, altered expression of cargo proteins or transport receptors or by deregulation of components of the trafficking machinery is a prominent feature of many human diseases. Deregulation of protein trafficking can lead to mislocalization of proteins and hence their inactivation (i.e. loss of function), misregulation or a harmful activity at the wrong place (i.e. toxic gain of function). Abnormalities in the subcellular localization of proteins that are important for the signaling, metabolic or structural properties of the cell can cause disorders that involve biogenesis, protein aggregation, cell metabolism or signaling. Table 1 lists some proteins whose localization to the wrong subcellular

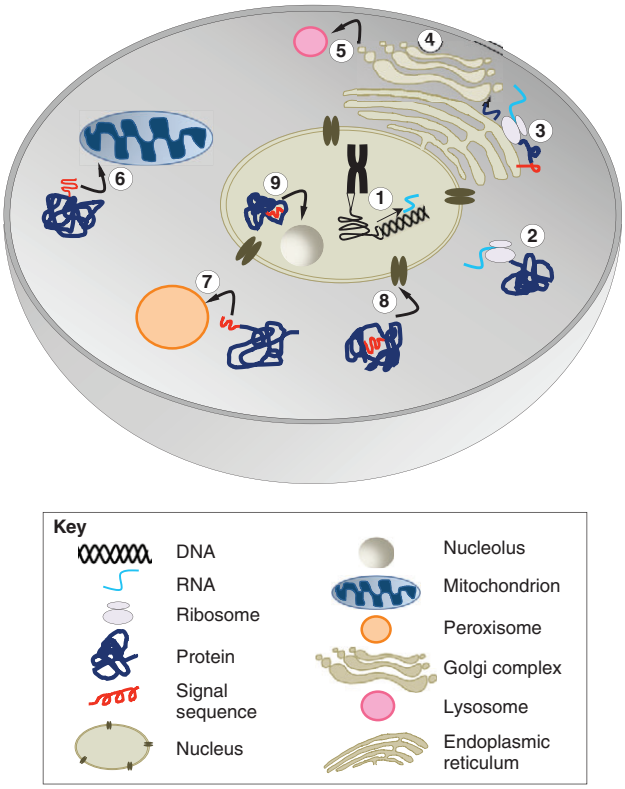


Fig. 1. Schematic overview of intracellular protein trafficking. The major components of the eukaryotic cell are the cytosol, the nucleus, the nucleolus, the endoplasmic reticulum (ER), the Golgi complex, mitochondria and the peroxisome. Whereas gene transcription takes place within the nucleus (1), protein synthesis is confined to the cytosol and takes place either on free RNA ribosomes (2) or on ribosomes associated with the ER (3). Most proteins destined to be secreted from the cell (4), or to reside in the plasma membrane, the lysosomes (5), the Golgi apparatus or the ER, follow the secretory pathway and enter the ER before the end of translation. Proteins targeted to the mitochondria (6), peroxisome (7) and nucleus (8) are translocated after their synthesis is complete. Subnuclear localization signals include nucleolar retention signals (9), nuclear-matrix-targeting signals and signals that target proteins to splicing speckles (Mekhail et al., 2007).

compartment has been associated with human diseases, and in the following sections we will discuss some of the mechanisms that can lead to such changes in protein localization.

Mislocalization through alterations of the protein trafficking machinery

Dysregulation of the protein trafficking machinery can have dramatic effects on general protein transport processes, modifying cell morphology and physiology. Along these lines, changes in the nuclear pore complex (NPC) have been linked to several genetic disorders (Chahine and Pierce, 2009). For example, in patients with familial atrial fibrillation, the homozygous mutation R391H in the nucleoporin NUP155 has been shown to reduce nuclear envelope permeability and affect the export of *Hsp70* mRNA and import of HSP70 protein (Zhang et al., 2008). That study was the first to link a nucleoporin defect to cardiovascular disease. Mutations in other components of the NPC, such as the nucleoporin p62 protein and ALADIN (alacrima achalasia adrenal insufficiency neurologic disorder, officially known as AAAS) are thought to cause the neurodegenerative diseases infantile bilateral striatal necrosis and triple A syndrome, respectively (Basel-Vanagaite et al., 2006; Kiriyama et al., 2008). Mutant ALADIN prevents nuclear entry of the DNA repair proteins aprataxin and DNA ligase I and, therefore, results in increased DNA damage and subsequent cell death caused by oxidative stress (Kiriyama et al., 2008).

In a similar fashion, protein import into other organelles can be affected by mutations in the trafficking machinery. For instance, mutations in the peroxin gene *PEX7*, which encodes a peroxisomal import receptor that is responsible for the transport of several essential peroxisomal enzymes, have been found to cause the peroxisome biogenesis disorder rhizomelic chondrodysplasia punctata type 1 (RCDP1) (Braverman et al., 1997).

Mislocalization through altered protein targeting signals

Targeting signals tend to be conserved between proteins and thus sensitive to alterations (Laurila and Vihinen, 2009). Considerable effort has been devoted to developing reliable methods to predict the effect of mutations on the subcellular localization of disease-related proteins (Emanuelsson et al., 2007; Laurila and Vihinen, 2009; Nair and Rost, 2008). A substantial amount of experimental data has been collected on mislocalization of disease-causing nuclear proteins. For example, loss of the nuclear localization signal (NLS) in the sex-determining region Y protein (SRY) has been shown to be associated with XY sex reversal in Swyer syndrome. Similarly, missense mutations within two NLSs that reduce the nuclear localization of SRY have been characterized in patients with this syndrome (McLane and Corbett, 2009).

A similar alteration has been reported for the non-canonical NLS of the cell-type specific transcriptional activator short stature homeobox (*SHOX*) gene in patients with Léri–Weill dyschondrosteosis and Langer mesomelic dysplasia, two rare genetic disorders that result in dwarfism. The missense mutation R173C within the SHOX NLS abolishes nuclear localization and consequently the downstream transcriptional activation by SHOX (Sabherwal et al., 2004). Interestingly, alterations within one copy of the NLS lead to phenotypes identical to SHOX haploinsufficiency in Léri–Weill dyschondrosteosis, as only half the amount of protein can be imported into the nucleus. If both NLS copies are mutated, all of the SHOX protein remains in the cytoplasm, which leads to the clinically severe, homozygous form of Langer mesomelic dysplasia (Sabherwal et al., 2004).

Additional mutations in several NLSs of disease-relevant proteins have been identified, including trichorhinophalangeal syndrome I (TRPS1) (Kaiser et al., 2004), aristaless related homeobox (ARX) (Shoubridge et al., 2010) and forkhead box P2 (FOXP2) (Mizutani et al., 2007), which are involved in tricho-rhino-phalangeal syndrome, X-linked lissencephaly with ambiguous genitalia and a speech–language disorder, respectively. In contrast with the previously described examples, mutations that disrupt the zinc finger domain of the transcription factor autoimmune regulator (AIRE), which are present in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, have been reported to cause cytoplasmic retention despite an intact nuclear targeting signal (Bjorses et al., 2000). Mutations within the nucleolar localization signal (NoS) of the ribosomal protein S19 (RPS19) have also been reported to be involved in disease development: in patients with Diamond–Blackfan anemia these mutations prevent the correct targeting of RPS19 to the nucleoli (Da Costa et al., 2003a).

Table 1. Mislocalized proteins that have been associated with human diseases

Protein	Disease	Mechanism	Mislocalization	Reference
SRY	Swyer syndrome	Mutation of NLS	Loss of nuclear localization	(McLane and Corbett, 2009)
SHOX	Léri–Weill dyschondrosteosis	Mutation of NLS	Cytoplasmic retention	(Sabherwal et al., 2004)
TRPS1	TRPS	Mutation of NLS	Loss of nuclear localization	(Kaiser et al., 2004)
ARX	XLAG	Mutation of NLS	Loss of nuclear localization	(Shoubridge et al., 2010)
FOXP2	Speech–language disorder	Mutation of NLS	Loss of nuclear localization	(Mizutani et al., 2007)
AIRE	APECED	Mutation of ZFD	Cytoplasmic retention	(Bjorses et al., 2000)
RPS19	Diamond–Blackfan anemia	Mutation of NoS	Loss of nucleolar localization	(Da Costa et al., 2003b)
AGT	Primary hyperoxaluria type 1	Polymorphism and/or mutation	Mitochondrial mislocalization	(Djordjevic et al., 2010)
hsMOK2	Laminopathy	Mutation of lamin A/C	Formation of nuclear aggregates	(Dreuillet et al., 2008)
SHOC2	Noonan-like syndrome	Acquired N-myristoylation	Mislocalization to the plasma membrane	(Cordeddu et al., 2009)
Rhodopsin	Retinitis pigmentosa	Mutations	ER retention	(Mendes et al., 2005)
AVPR2	Nephrogenic diabetes insipidus	Mutations	ER retention	(Robben et al., 2006)
ATP7B	Wilson disease	H1069Q mutation	ER retention	(Payne et al., 1998)
ABCA1	Tangier disease	Mutations	Loss of plasma membrane localization	(Tanaka et al., 2003)
Tau	Neurodegenerative diseases	Hyperphosphorylation	Mislocalization to dendritic spines	(Hoover et al., 2010)
TARDBP	ALS and FTLD	Unknown	Cytoplasmic mislocalization	(Winton et al., 2008)
FUS	FTLD	Mutations	Cytoplasmic mislocalization	(Vance et al., 2009)
FOXO	Various types of cancer	Post-translational modifications	Cytoplasmic mislocalization	(Dansen and Burgering, 2008)
p53	Various types of cancer	Mutations, post-translational modifications	Cytoplasm	(Fabbro and Henderson, 2003)

APECED, autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy; ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; TRPS, trichorhinophalangeal syndrome; XLAG, X-linked lissencephaly with absent corpus callosum and ambiguous genitalia.

Genetic alterations that affect protein targeting signals have also been associated with several metabolic disorders that are characterized by defects in specific organelle functions, such as lysosomal and peroxisomal functions. Mislocalization of the peroxisomal enzyme alanine–glyoxylate aminotransferase (AGT) to the mitochondria in patients with the hereditary kidney-stone disease primary hyperoxaluria type 1 (PH1) has been shown to be caused by the synergistic interaction between the common P11L polymorphism and a disease-specific G170R mutation (Djordjevic et al., 2010). Whereas the polymorphism generates a cryptic mitochondrial targeting sequence (Purdue et al., 1991), the polymorphism and mutation together inhibit AGT dimerization and allow unfolding of the protein, which is a requirement for subsequent mitochondrial import (Danpure, 2006).

In contrast with genetic alterations, inactivation of protein targeting signals by post-transcriptional modifications and protein–protein interactions represents an important regulatory mechanism of reversible protein translocation. A striking example is the phosphorylation-dependent unmasking of the NLS that is present in nuclear factor κB (NF-κB), a transcription factor known to be crucial for inflammatory processes and the development and progression of several malignancies (Karin and Greten, 2005; Luo et al., 2005). In unstimulated cells the NF-κB NLS is masked by the inhibitor protein IκB. Most activating agents employ a common pathway that involves the IκB kinase (IKK)-mediated phosphorylation of IκB, causing the ubiquitylation and subsequent degradation of IκB proteins, allowing the nuclear translocation of NF-κB through unmasking of the NLS (Hayden and Ghosh, 2008). Nuclear localization of NF-κB is associated with tumorigenesis and various inflammatory diseases and hence altering its cellular localization has been considered as an attractive therapeutic target.

Mislocalization through changes in protein interaction or modification

In addition to altering the localization signal of a protein, disease-related mutations can result in mislocalization of a protein through protein sequestration. Mutations within the *LMNA* (lamin A/C) gene, which cause laminopathies, have recently been reported to result in mislocalization of the DNA-binding transcriptional repressor zinc finger protein 239 (ZNF239, also known as MOK2). The pathogenic lamin A/C mutant protein sequesters ZNF239 into nuclear aggregates, and this process is thought to subsequently deregulate ZNF239 target genes (Dreuillet et al., 2008).

Hereditary genetic changes that alter the post-translational modifications that are relevant for correct protein localizations are also associated with human diseases. In a recent study, Cordeddu et al. reported a genetic change in patients with Noonan-like syndrome that introduces an N-myristoylation site in SHOC2, a leucine-rich repeat-containing protein. This acquired fatty acid modification results in aberrant SHOC2 targeting to the plasma membrane and impaired translocation to the nucleus upon growth factor stimulation (Cordeddu et al., 2009).

Mislocalization of misfolded proteins

Aberrant folding of proteins with important cellular roles can cause diseases in which loss of the protein function rather than its mislocalization drives the pathology (Hung et al., 1997; Payne et al., 1998; Skach, 2000; Tanaka et al., 2003). Misfolding can also result in proteins that retain intrinsic function yet become misrouted and, as a consequence of their mislocalization, cease to function normally (Conn et al., 2007). Accordingly, aberrant localization of misfolded proteins can have deleterious gain-of-function or dominant-negative effects in many diseases. Several hereditary genetic disorders have been linked to altered trafficking of misfolded G-protein-coupled receptors (GPCRs); examples of such disorders include retinitis pigmentosa (Conn et al., 2007; Mendes et al., 2005) and nephrogenic diabetes insipidus (Robben et al., 2006). In some disease states, the defect in cell surface membrane expression of GPCRs is due to a dominant-negative effect of the misfolded receptor on its wild-type counterpart owing to their association in the ER and misrouting of the resulting complex (Brothers et al., 2004; Conn et al., 2007; Gehret et al., 2006; Karpa

et al., 2000). Mislocalized misfolded proteins that exert toxic gain-of-function or dominant-negative effects are increasingly recognized as a cardinal feature of many neurodegenerative diseases (Box 1).

Mislocalization of signaling proteins

Signal transduction involves the transmission of a signal in time and space. Because proteins cannot diffuse as quickly as small-molecule second messengers, the subcellular localization of signaling proteins in proximity to their downstream targets is a key element of many signal transduction circuits (Scott and Pawson, 2009). Translocation of signaling proteins provides an efficient means to carry the signal over a substantial distance or between cellular compartments. Deregulation of the spatiotemporal signaling dynamics has been shown to be involved in tumorigenesis, tumor growth and metastasis (Kau et al., 2004; Wang and Hung, 2005). Several important tumor suppressors require the ability to localize to the nucleus to perform their function, and their cytoplasmic localization can serve as an inactivation mechanism that gives rise to uncontrolled cell proliferation and the onset of disease (Fabbro and Henderson, 2003; Salmena and Pandolfi, 2007; Turner and Sullivan, 2008; Yashiroda and Yoshida, 2003). Consequently, mislocalization of nuclear proteins to the cytoplasm has been proposed as a generalized mechanism for the inactivation of tumor suppressors (Kau et al., 2004; Salmena and Pandolfi, 2007).

The transcription factor forkhead box O3a (FOXO3a), a member of the forkhead family of proteins, provides an example of a tumor suppressor whose function is altered by mislocalization. FOXO3a regulates the expression of, for example, the apoptotic proteins FasL (Brunet et al., 1999) and Bim (Gilley et al., 2003), and the cell cycle inhibitor p27 (Dijkers et al., 2000). Cytoplasmic localization of FOXO3a has been shown to correlate with poor survival in breast cancer (Hu et al., 2004), whereas nuclear localization correlates with an increased sensitivity to radiation (Chen et al., 2008). Three oncokinases have been shown to regulate FOXO3a localization: AKT (Brunet et al., 1999), IKK (Hu et al., 2004) and extracellular signal-regulated kinase 1/2 (ERK1/2) (Yang et al., 2008). Whereas all three kinases phosphorylate FOXO3a on different residues (AKT on T32, S253 and S315; IKK on S644; and ERK1/2 on S294, S344 and S425), the outcome is the same, namely export of FOXO3 from the nucleus and subsequent degradation.

The roles of the cell cycle inhibitors p21 and p27 are also regulated by their cellular localization. p21 and p27 are traditionally considered to be tumor suppressors that act in the nucleus and become oncogenic when localized in the cytoplasm. p21 was originally shown to inhibit tumorigenesis and was suggested as a good candidate for gene therapy (Katayose et al., 1995; Yang et al., 1995). However, it subsequently became apparent that p21 is also associated with anti-apoptotic functions when it is localized in the cytosol. It was shown that the binding of p21 to mitogen-activated protein kinase (MAPK) kinase kinase 5 (MAP3K5, also known as ASK1) in the cytoplasm inhibits the MAPK cascade (Asada et al., 1999; Huang et al., 2003). In addition, phosphorylation of p21 on T145 by AKT induces nuclear export of p21, which enhances cell growth (Li et al., 2002; Rossig et al., 2001; Zhou et al., 2001). Furthermore, cytoplasmic p21 was found to have an oncogenic role in mammary tumorigenesis and metastasis in vivo (Cheng et al., 2010). The physiological relevance of p21 in the cytoplasm is emphasized further by the clinical observation that cytoplasmic localization of p21 is a poor prognostic marker for breast cancer (Xia et al., 2004), is involved in mediating resistance to anticancer drugs (Koster et al., 2010; Ruan et al., 1999; Zhang et al., 1995) and has been shown to result in a poor response to tamoxifen (Pérez-Tenorio et al., 2006).

Similar to p21, the cell cycle inhibitor p27 has been found to have oncogenic roles when localized in the cytoplasm. AKT phosphorylates p27 on T157, which blocks the nuclear import of p27 (Liang et al., 2002; Shin et al., 2002; Viglietto et al., 2002). Phosphorylation of p27 on S10 has also been shown to induce cytoplasmic localization of p27 without leading to its degradation (Rodier et al., 2001). As is the case for p21, the cytoplasmic localization of p27 has been found to be a poor prognostic factor in cancers, including breast (Liang et al., 2002), hepatocellular (Nan et al., 2004), colon (Ogino et al., 2009) and ovarian cancer (Rosen et al., 2005), and Barrett's carcinoma (Singh et al., 1998).

Box 1. Aberrant protein localization in neurodegenerative diseases

Despite a wealth of experimental data on neurodegenerative diseases, no consensus has yet emerged on the nature of the neuropathogenic species and how they promote degeneration of neurons in, for example, Alzheimer's, Parkinson's, Huntington's and prion diseases, amyotrophic lateral sclerosis (ALS) or frontotemporal lobar degeneration (FTLD). Considerable evidence has been accumulated in recent years to indicate that the earlier stages of the protein mislocalization process are more directly tied to pathogenesis than the filamentous protein aggregates themselves. The formation of large protein aggregates and inclusion bodies might even represent a beneficial defense mechanism that operates to eliminate irreversibly aggregated proteins. Accordingly, it has been shown in mouse models that neurofibrillary tangles composed of aggregates of a hyperphosphorylated form of the microtubule-associated protein tau (MAPT) exert negligible neurotoxicity compared with that of soluble tau (Oddo et al., 2006; Santacruz et al., 2005). Mislocalization of tau to dendritic spines has recently been reported to mediate a synaptic dysfunction that is associated with impaired brain function at the preclinical disease stages that immediately precede neurodegeneration. The accumulation of hyperphosphorylated tau within intact dendritic spines impairs glutamate receptor trafficking or synaptic anchoring (Hoover et al., 2010). In patients with ALS and FTLD, affected neurons exhibit a striking redistribution of TAR DNA-binding protein (TARDBP, also known as TDP43) or the ALS-associated protein fused in sarcoma (FUS) from the nucleus to the cytoplasm (Neumann et al., 2006). Interestingly, a recent study showed that TDP43-mediated neurotoxicity was associated with an increased cytoplasmic localization of this protein, whereas TDP43 inclusion bodies were not necessary for the toxicity and did not affect the risk of cell death (Barmada et al., 2010; Kwiatkowski et al., 2009; Lagier-Tourenne and Cleveland, 2009; Vance et al., 2009).

Aggregation of amyloidogenic proteins can result in the sequestration and, hence, aberrant localization of numerous proteins, including importin alpha and phosphorylated SMAD3, which suggests that there is a functional impairment of nuclear trafficking in Alzheimer's disease (Chalmers and Love, 2007; Zhu et al., 2002). It has been hypothesized that an altered localization of transcription factors such as NF-κB, activating transcription factor 2 (ATF2), cAMP response element-binding (CREB), p53, E2F transcription factor and NF-E2-related factor 2 (NRF2) might contribute to cell death commitment in several neurodegenerative diseases (Chu et al., 2007).

In addition to tumor suppressors, it has gradually become clear that receptor tyrosine kinases (RTKs), traditionally regarded as cell-membrane-bound proteins, are also able to translocate to and function in the nucleus (Carpenter and Liao, 2009; Gomes et al., 2008; Reilly and Maher, 2001; Sehat et al., 2010; Stachowiak et al., 1997; Wang and Hung, 2009). Recently, a new trafficking mechanism for EGFR from cell surface to the nucleus has been reported. EGFR trafficking involves endocytosis, followed by retrograde trafficking from the Golgi to the ER by COPI-mediated vesicular transport. The receptor is then translocated through the ER to the nucleus, where SEC61B is able to release it from the inner nuclear membrane (Wang et al., 2010a; Wang et al., 2010b). It is worthwhile mentioning that the EGFR remains membrane-bound throughout the entire trafficking pathway from the cell surface to the nucleus. Nuclear localization of EGFR is correlated with many tumor types (Hadzisejdic et al., 2010; Hoshino et al., 2007; Lo et al., 2005a; Psyrri et al., 2008; Xia et al., 2009) and has been shown to be involved in mediating resistance to the anticancer agents cetuximab (Li et al., 2009) and cisplatin (Hsu et al., 2009). Consistently, nuclear EGFR has been associated with cell proliferation, DNA repair, drug resistance and nuclear EGFR and ERBB2 have been shown to bind to promoters and activate transcription (Hanada et al., 2006; Hung et al., 2008; Lin et al., 2001; Lo et al., 2005b; Wang et al., 2004).

Therapeutic manipulation of protein localization in human diseases

Because the deregulation of intracellular protein transport is crucially involved in the pathophysiology of a broad range of medical conditions and diseases, it offers molecular targets at many different levels for the attempt to normalize or to interfere with protein localization with a therapeutic strategy. In particular, cellular processes that link protein folding, cell signaling, and nuclear import and export to subcellular localization of proteins have been proposed as targets for therapeutic intervention, and in some cases agents have been developed to successfully influence subcellular protein distribution in disease states (Table 2).

Stabilizing correct protein folding

Many disease-causing mutations disrupt the three-dimensional conformation but not the active domain of the affected polypeptide, which results in an unstable rather than an inactive protein. In recent years, it has been demonstrated that the subcellular localization and function of some of these unfolded proteins is salvageable through the binding of small molecule compounds that stimulate correct folding of these proteins or that stabilize their native-like conformation. In analogy to protein chaperones, these small molecule agents have been named pharmacological chaperones and have been shown to reverse the intracellular retention of several different misfolded proteins (Morello et al., 2000; Ringe and Petsko, 2009). For instance, there is considerable experimental evidence that the responsiveness of patients with mild phenylketonuria to an established therapy using tetrahydrobiopterin is owing to its function as a pharmacological stabilizer of the mutated enzyme phenylalanine hydroxylase (Pey et al., 2004).

A number of other pharmacological chaperones are in late-stage clinical trials, including drugs for transthyretin-based amyloidosis, the imino sugar isofagomine for Gaucher disease and 1-deoxygalactonojirimycin for Anderson–Fabry disease (Ringe and Petsko, 2009). GPCRs comprise the largest family of drug targets for such pharmacological chaperons, so this approach has enormous potential to promote the functional rescue of those proteins involved in human diseases (Conn et al., 2007).

Targeting signaling pathways that regulate subcellular localization

The subcellular distribution of many disease-relevant proteins can be influenced by binding to other biomolecules and by post-translational modification, including phosphorylation, acetylation, ubiquitylation, farnesylation and proteolytic processing (Fig. 2) (Butler and Overall, 2009). Accordingly, manipulation of the upstream regulatory processes has the potential to restore the correct location and function of aberrantly localized proteins. In addition, from a chemical and pharmacological perspective, the

Table 2. Examples of agents that can interfere with protein trafficking

Agent	Direct target	Localization effect	Potential application	Reference
IN3	Gonadotropin-releasing hormone receptor (GNRHR)	Cell surface expression of GNRHR	Reproductive disorders	(Finch et al., 2010)
Rapamycin	MTOR	Restoration of nuclear TARDBP	Various neurodegenerative diseases	(Caccamo et al., 2009)
CF35Es	Mutant rhodopsin	Proper rhodopsin trafficking	Retinitis pigmentosa	(Ohgane et al., 2010)
SMIP001/004	Unknown	Nuclear p27KIP localization	Prostate cancer	(Rico-Bautista et al., 2010)
ETP-45648	PI3K	Nuclear FOXO localization	Various types of cancer	(Link et al., 2009)
CHS828	IKK	Cytoplasmic NF-κB	Various types of cancer	(Olsen et al., 2004)
PITs	Pleckstrin homology domain	Loss of AKT at the plasma membrane	Various types of cancer	(Miao et al., 2010)
Palmostatin B	APT1	Loss of precise RAS localization	Lung cancer	(Dekker et al., 2010)
Tipifarnib	Farnesylated proteins	Mistargeting of farnesylated proteins	Hematologic malignancies	(Martinelli et al., 2008)
Poloxin	PLK1	PLK1 mislocalization	Various types of cancer	(Reindl et al., 2008)
Resveratrol	SIRT1	Nuclear FOXO1	Various types of cancer	(Frescas et al., 2005)
GSIs	NOTCH1	Loss of ICN1	T cell acute lymphoblastic leukemia	(Real et al., 2009)
Elliticine	Unknown	Increased nuclear p53 localization	Various types of cancer	(Xu et al., 2008)
INCAs	Calcineurin and NFAT	Cytoplasmic NFAT	Inflammatory and autoimmune diseases	(Roehrl et al., 2004)
WGA	N-Acetyl-D-Glucosamine (GlcNac)	Unspecific nuclear exclusion	ND	(Gasiorowski and Dean, 2003)
bimax1/2	Importin-α	Resistance to nuclear cargo release	Viral infection, Atherosclerosis	(Kosugi et al., 2008)
LMB analogues	CRM1	Unspecific nuclear trapping	Various types of cancer	(Mutka et al., 2009)

ND, not determined.

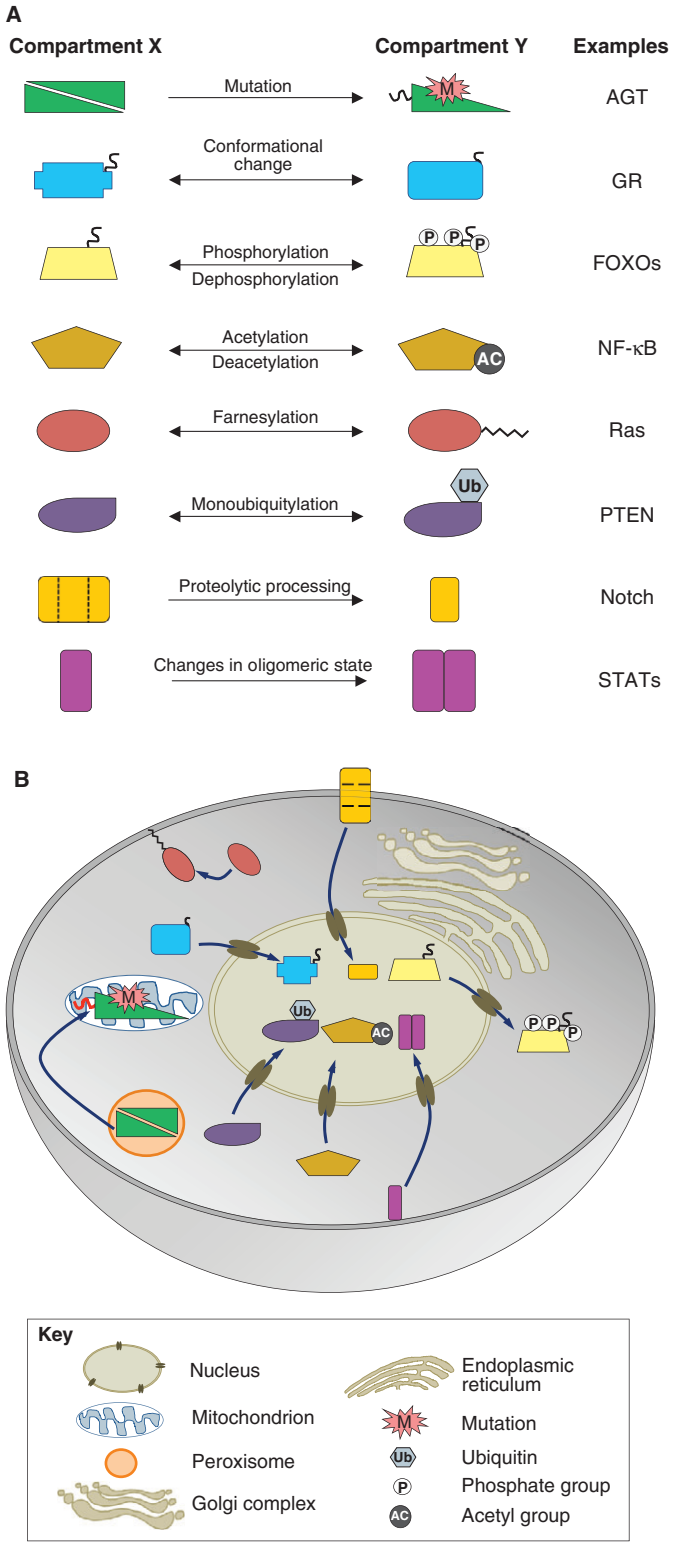


Fig. 2. Examples of mechanisms that regulate protein translocation. (**A**) The subcellular distribution of many disease-relevant proteins has been shown to be influenced by genetic alterations, binding to other biomolecules and by post-translational modifications including mutation, phosphorylation, acetylation, ubiquitylation, farnesylation and proteolytic processing (Butler and Overall, 2009). (**B**) Changes in cellular localization as a result of the mechanisms described for the specific example proteins shown in A. GR, glucocorticoid receptor.

action of many proteins that are involved in these signaling pathways can be modulated by small molecules.

The role of protein kinase inhibitors in intracellular protein trafficking is probably the most studied modification of such a process, and these inhibitors have contributed to many of the concepts of spatiotemporal regulation of protein function. The phosphoinositide 3-kinase (PI3K)–AKT signaling network represents a paradigm for the development of protein kinase inhibitors that influence subcellular translocation of disease-relevant downstream targets (Carnero et al., 2008; Hennessy et al., 2005). PI3K stimulates the production of phosphatidylinositol (3,4,5)-triphosphate [PtdIns(3,4,5)P_3], which results in the translocation of the downstream kinases PDK1 and AKT from the cytoplasm to the inner surface of the plasma membrane. Activated AKT phosphorylates a broad range of substrate proteins and in turn regulates the subcellular trafficking of downstream targets, such as members of the FOXO family of transcription factors (Zanella et al., 2010a), the cell cycle inhibitor p27 (Liang et al., 2002), the tuberous sclerosis protein 2 (tuberin) (Cai et al., 2006), the E3 ubiquitin ligase MDM2 (Mayo and Donner, 2001) and the glucose transporters GLUT1 and GLUT4. Numerous studies using the PI3K inhibitors LY294002 and wortmannin indicate that these translocation events are sensitive to upstream pathway inhibition (Anderson et al., 1998; Brunet et al., 1999; Cai et al., 2006; Kim et al., 2009; Wolf et al., 2010). Several small chemical compounds that specifically inhibit different lipid and protein kinases within the PI3K–AKT pathway, including PI3K, AKT and PDK, have been shown to influence the subcellular localization of downstream targets. For example, a nuclear accumulation of FOXO transcription factors has been demonstrated upon treatment with the selective PI3K inhibitors ETP-45658, PI-301 and PIK-75, the PI3Kδ inhibitor D000, the AKT inhibitors AI, AI-VIII and AI-X, as well as the protein tyrosine kinase inhibitor genistein (Zanella et al., 2008).

In contrast to therapeutic strategies that attempt to restore normal protein folding and localization, other therapies aim to deliberately cause protein mislocalization, and thereby inhibit protein function. Accordingly, therapeutic mislocalization of oncoproteins has emerged as a promising treatment strategy for cancer diseases. For example, perifosine, a lipid-based compound that targets the pleckstrin homology domain of AKT, and therefore prevents its translocation to the plasma membrane (Kondapaka et al., 2003), represents an example for this approach and is currently being tested in Phase II and III clinical trials (Penel et al., 2010). In addition, recent studies report the development of allosteric non-lipid-based small molecules that target the interaction of phosphatidylinositol 3-phosphate (PtdIns3*P*) and the pleckstrin homology domains of different proteins at the plasma membrane (Kim et al., 2010; Miao et al., 2010). Similarly, the Polo-like kinase 1 (PLK1) can be inhibited by small molecules that interfere with its intracellular localization by inhibiting the function of the Polo-box domain, which is responsible for the interaction with the intracellular anchoring sites (Reindl et al., 2008). A recent study reported that the acyl protein thioesterase 1 (APT1) inhibitor palmostatin B, which perturbs depalmitoylation, can interfere with oncogenic Ras signaling, thereby causing loss of precise steady-state localization of palmitoylated Ras (Dekker et al., 2010). Conversely, the antitumor activity of farnesyltransferase inhibitors (FTIs) against non-Ras-dependent cancers suggests that these signal transduction inhibitors can exert their effects by mistargeting other farnesylated proteins (Sousa et al., 2008).

There is a wide variety of non-kinase components of signaling pathways, including proteases, histone deacetylases, phosphatases, ubiquitin ligases, farnesyltransferases and protein chaperones, whose manipulation has been shown to influence the subcellular localization of disease-relevant proteins. Inhibitors of the protease γ-secretase, initially developed for the treatment of Alzheimer's disease, have been shown to prevent the proteolytic generation of the intracellular domain of Notch molecules, and hence their subsequent nuclear translocation and the upregulation of Notch target genes (Real et al., 2009). Resveratrol, a small-molecule activator of NAD-dependent deacetylases (the sirtuins) overrides the phosphorylation-dependent nuclear exclusion of forkhead box O1 (FOXO1) that is caused by growth factors and results in nuclear translocation of FOXO1 in hepatocytes. Selective modulation of the FOXO–sirtuin interactions represents a promising therapeutic modality for metabolic disorders (Frescas et al., 2005). The immunodepressive drugs cyclosporin A and FK506 inhibit the phosphatase activity of calcineurin and, in turn, the dephosphorylation-mediated unmasking of the NLS in the nuclear factor of activated T cells (NFAT) transcription factor, preventing its nuclear import. More specific inhibitors of NFAT–calcineurin association (e.g. INCA) have been developed and shown to inhibit nuclear NFAT localization (Roehrl et al., 2004).

Nuclear import as a therapeutic target

Therapeutic targeting of the import of nuclear proteins provides another strategy for the indirect manipulation of protein localization. However, this has not yet been extensively explored (Davis et al., 2007). In fact, there is currently no small-molecule compound available to interfere with the nuclear import of proteins, but insight into the process of nuclear protein import has provided promising anticancer, antiviral and anti-inflammatory strategies (Faustino et al., 2007). For example, replication of human immunodeficiency virus 1 (HIV1) requires the nuclear translocation of the HIV1 pre-integration complex (PIC). The HIV1 protein integrase has been proposed as the karyophilic agent that recruits the cellular nuclear import machinery to transport HIV1 cDNA through an intact nuclear envelope. These observations thus highlight important potential therapeutic targets for impeding the progression of HIV and AIDS (Chahine and Pierce, 2009).

Several general regulators of the nuclear import process, including the NPC, the transport receptors and the import partners have also been proposed as targets for therapeutic intervention (Chahine and Pierce, 2009; Davis et al., 2007). Monoclonal antibodies directed against the FG-repeats of nucleoporins, which are thought to mediate the sequential binding of nuclear import receptors during the translocation, have been used successfully in rat liver nuclear envelopes to prevent cargo association with the NPC and block the translocation of proteins (Gasiorowski and Dean, 2003; Snow et al., 1987). Nuclear import can also be blocked by the plant lectin wheat germ agglutinin (WGA), but the mechanism underlying this phenomenon is still elusive (Chahine and Pierce, 2009).

Interfering with transport receptors represents an alternative approach to modulate the nuclear import of proteins. This concept has been proved valid with the development of peptide nuclear import inhibitors on the basis of activity profiling of systematically mutated NLS peptide templates. Using this method, the peptides bimax1 and bimax2, which specifically inhibit the classical nuclear import pathway, mediated by importin alpha, were generated (Kosugi et al., 2008). However, the therapeutic use of the currently available agents for the inhibition of the nuclear import is limited, as they block the transport of all nuclear proteins through the pore in a non-specific manner.

Targeting nuclear export

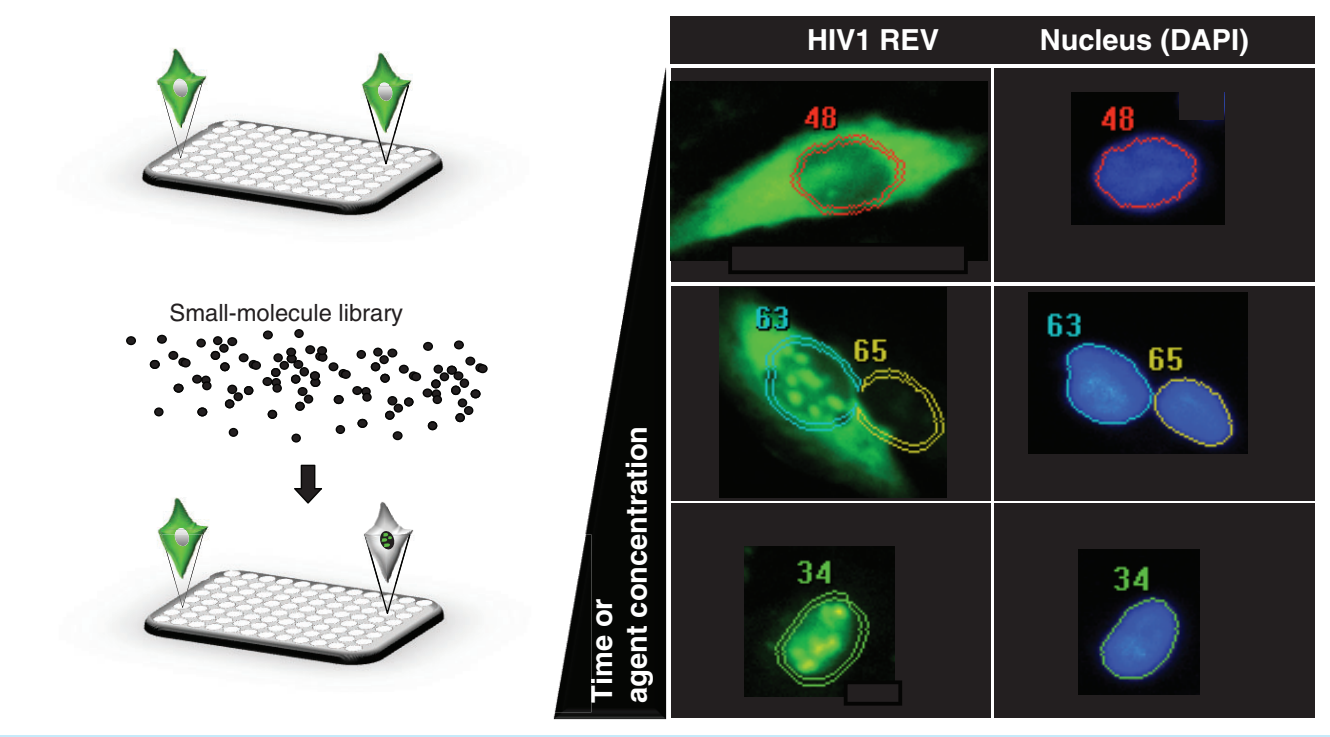
Like the inhibition of the nuclear import through a variety of drugs, targeting the nuclear export machinery is a non-selective strategy to trap proteins in a specific cellular compartment. Recent evidence indicates that nuclear export inhibitors might be of substantial therapeutic use (Mutka et al., 2009). For instance, blocking the nuclear export dependent on CRM1 (chromosome region maintenance 1; also referred to as exportin1 or Xpo1) to restore the functions of nuclear tumor suppressors has been considered as an attractive therapeutic approach for the treatment of cancer (Turner and Sullivan, 2008). The first specific CRM1 inhibitor, leptomycin B (LMB), was originally identified as an antifungal agent isolated from *Streptomyces* bacteria (Hamamoto et al., 1983) and, in the late 1990s, was shown to be an inhibitor of the nucleo-cytoplasmic translocation of HIV mRNA, which is mediated by the RNA-binding protein HIV1 Regulator of virion (Rev) (Wolff et al., 1997). LMB covalently binds to a single cysteine residue and prevents binding of the nuclear export signal (NES) to CRM1 (Kudo et al., 1999). Unfortunately, LMB was found to exhibit severe toxicities in a Phase I clinical trial, which means that it is currently not suitable for therapeutic use (Newlands et al., 1996). In addition to LMB, ratjadones, a group of myxobacterial cytotoxins that are chemically unrelated to LMB, have been shown to block CRM1-dependent nuclear export by an identical mechanism (Kalesse et al., 2001; Meissner et al., 2004).

The recent advent of high-content screening technology (Box 2) has provided a unique tool to conduct large-scale experiments, with the aim of identifying nuclear export inhibitors with image-based monitoring of NES-containing reporter proteins (Zanella et al., 2010b; Zanella et al., 2008; Zanella et al., 2009). Several chemical series that contain compounds that block the nuclear export by known and new pharmacological mechanisms were identified by this method (Kau et al., 2003; Link et al., 2009). A medicinal chemistry approach based on modifying LMB resulted in several semi-synthetic LMB derivatives that maintain the high potency of LMB, but are up to 16-fold more well-tolerated than LMB in vivo and show substantial efficacy in multiple mouse xenograft models (Mutka et al., 2009). These data provide proof of concept that nuclear export can be inhibited with manageable toxicities in vivo and should further fuel efforts to conduct massive image-based screens aimed at the identification of reversible less-toxic inhibitors of the general nuclear export machinery.

Conclusions

Proteins exert their biological functions within the spatiotemporal context of an intact cell. To be at the right place at the right time is of paramount importance for a protein to gain access to appropriate molecular interaction partners. As a consequence, aberrant protein localization is a prominent feature of a broad range of medical conditions and diseases. Protein mislocalization could be caused by alterations such as mutations within signal sequences, changes in post-translational modifications or expression level of the cargo protein itself or by deregulation of the protein trafficking machinery. Deleterious gain-of-function or dominant-negative effects caused by aberrant subcellular localization of misfolded proteins have been implicated in the pathophysiology of several neurodegenerative diseases. Deregulation of the

Box 2. High-content screening to monitor protein localization



High-content screening, a technology that combines the efficiency of high-throughput techniques with the ability of cellular imaging to collect quantitative data from complex biological systems, is ideally suited to systematically observe translocation events in response to chemical or genetic perturbation on a large scale in time and space. The subcellular localization of target proteins that lie downstream of the perturbation provides a visual read-out for measuring the activity of signaling pathways and supports the identification of small molecule compounds or relevant therapeutic targets that might be exploited pharmaceutically to restore or interfere with protein localization and function (Kau et al., 2003; Kau et al., 2004; Zanella et al., 2010c; Zanella et al., 2008). The analysis of the subcellular relocalization of signaling proteins, including NF-κB (Taylor, 2010), FOXO (Kau et al., 2003; Link et al., 2009; Zanella et al., 2010c; Zanella et al., 2008; Zanella et al., 2009), NFAT (Wolff et al., 2006), p27Kip1, p53 (Xu et al., 2008), estrogen receptor alpha (Dull et al., 2010), p38 (Trask et al., 2006; Trask et al., 2009), Ets domain transcriptional repressor (ERF1) (Granas et al., 2006), AKT (Lundholt et al., 2005; Rosado et al., 2008; Wolff et al., 2006), glucokinase (Wolff et al., 2008) and HIV1 regulator of virion (REV) (Zanella et al., 2010b), have shed light on molecular mechanisms that underlie the spatiotemporal regulation of protein translocation and led to the identification of small molecule agents that interfere with the corresponding signaling networks.

The figure illustrates a strategy of using high-content screening to monitor the localization of the fluorescently tagged reporter protein HIV1 REV (green). Nuclear segmentation was performed on the basis of a DAPI nuclear stain (blue). The binary image is processed further to separate the foreground into separate islands, which are then labeled such that each cell has its own identifier (numbers). The difference in the measurements between the nuclear and the nuclear plus cytoplasmic masks corresponds to a ring mask that is assigned to the cytoplasm. On the basis of the segmentation of the nucleus and the cytoplasm, the relative distribution of the fluorescent reporter protein between the two cell compartments can be quantified and the effect of a small-molecule library on the nuclear export of proteins can be assessed.

spatiotemporal dynamics of signaling proteins has been shown to promote tumorigenesis and metastasis. Similarly, aberrant localization of several essential enzymes results in different metabolic diseases.

The substantial progress in our understanding of the mechanisms involved in the pathogenesis of many of these diseases, together with major technological advances that allow the image-based identification of small-molecule compounds or molecular targets, have opened new horizons for therapeutic intervention. Consistent with this development, targeting protein localization has been conceptualized as a promising therapeutic strategy for the treatment of several human diseases. However, the spectrum of agents that specifically target protein localization in clinical use or preclinical development is still limited. From a conceptual point of view, these agents can be divided into two main classes: relocators that restore physiological protein localization and function, and mislocators that aim to deliberately cause protein mislocalization and thereby inhibit the function of disease-causing proteins. Non-selective strategies to trap proteins in specific cellular compartments, including the inhibition of nuclear protein import and export, have been developed and hold promise for developing effective future therapies against viral infections and cancer. In some cases pharmacological chaperones have been developed to successfully stabilize polypeptide folding and thereby restore the correct localization and function of the protein. Small-molecule and biological inhibitors of signaling proteins represent the fastest growing segment of agents that influence protein localization. The development of high-quality antibodies and organelle-specific

fluorescent dyes will facilitate the characterization of pathological protein localization and could be used as predictive and diagnostic biomarkers for many human diseases. As cancer is increasingly perceived as a disease of pathway lesions associated with the reversible mislocalization of essential signaling proteins, this strategy is expected to have its most immediate impact on the development of new anticancer therapies.

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