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Small Peptide Recognition Sequence for Intracellular Sorting

Kailash N. Pandey
Department of Physiology, Tulane University Health Sciences Center, School of Medicine, New Orleans, LA 70112

Abstract

Increasing evidence indicate that complex arrays of short signals and recognition peptide sequence ensure accurate trafficking and distribution of transmembrane receptors and/or proteins and their ligands into intracellular compartments. Internalization and subsequent trafficking of cell-surface receptors into the cell interior is mediated by specific short-sequence peptide signals within the cytoplasmic domains of these receptor proteins. The short signals usually consist of small linear amino acid sequences, which are recognized by adaptor coat proteins along the endocytic and sorting pathways. In recent years, much has been learned about the function and mechanisms of endocytic pathways responsible for the trafficking and molecular sorting of membrane receptors and their ligands into intracellular compartments, however, the significance and scope of the short sequence motifs in these cellular events is not well understood. Here a particular emphasis has been given to the functions of short-sequence signal motifs responsible for the itinerary and destination of membrane receptors and proteins moving into subcellular compartments.

Keywords

Membrane receptors; short sequence signals; endocytosis; intracellular trafficking receptor sorting; receptor recycling; endosomes; lysosomes

2. Introduction

The carboxyl-terminus domains of various membrane receptors and/or membrane proteins play important roles in mediating the adaptive changes, which accelerate their trafficking and sorting from the cell surface into the intracellular compartments [1–4]. The carboxyl-terminal tail of membrane receptors and/or proteins is required for internalization, sorting, down-regulation, and desensitization processes. In the recent years, the short sequence signal motifs mediating the molecular mechanisms of receptor endocytosis and trafficking have received considerable interest. The proper and directed operation of the endocytic pathway of membrane receptors and their bound ligand requires numerous critical sorting decisions along the endocytic trafficking pathway. At the plasma membrane, receptors may either remain at the cell surface or be rapidly internalized into coated pits and vesicles [5]. Subsequently, the receptors can proceed and be delivered to the lysosomes or may recycle

Address for Correspondence: Kailash N. Pandey, Ph.D. Department of Physiology, SL-39 Tulane University Health Sciences Center 1430 Tulane Ave, New Orleans, LA 70112 Tel: (504) 988-1628 Fax: (504) 988-2675 kpandey@tulane.edu.

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back to the plasma membrane. Alternatively, the receptors could be destined to go to endosomes or plasma membrane involving the trans-Golgi networks (TGN) or without TGN.

The complex array of routing and trafficking decisions is directed by a set of sorting-signal sequence motifs in the cytoplasmic domains of the receptor molecules, which recognize the routing pathways and deliver the ligand-receptor complexes to their intended locations in the intracellular compartments. Indeed, more studies are required to delineate the new signal motifs of membrane receptors and/or membrane proteins, which direct internalization, trafficking, and sorting events. This review focuses on the characteristic features and specific functions of short sequence signal motifs involved in trafficking and intracellular routing of membrane receptors or membrane proteins, which are either targeted to endosomes, lysosomes, and/or related subcellular organelles or recycled back to the plasma membrane.

3. Intracellular trafficking of membrane receptors

The targeting and sorting of individual membrane receptor and/or protein is directed by their intrinsic sequence-based signal motifs in the endocytic and secretory pathways [1,6–8]. It is envisioned that the receptor-mediated endocytosis of various ligand-receptor complexes may involve sequential sorting steps through which ligand-receptor complexes and membrane proteins could eventually be degraded, recycled back to the cell surface, or released into the cell exterior [9]. During the sequential processes of endocytosis of ligand-receptor complexes via the intracellular sorting pathway, the ligand-receptor complexes may proceed according to one or more possibilities: i) the majority of ligand-receptor complexes are targeted to the lysosomes, leading to degradation of the ligand-receptor complexes, ii) during the endocytic process of certain ligand-receptor complexes, the acidic pH of endosomes induces dissociation of the ligand from the receptor, the ligand is released intact into the cell exterior and the receptor is re-inserted into the plasma membrane, and iii) in certain instances, the ligand is dissociated from the receptor in endosomes, subsequently a population of receptor molecules may recycle back to the plasma membrane and the ligand remains in the lysosomes. Internalization of macromolecules is usually carried out by two main structural devices formed on the plasma membrane, which include clathrin-coated vesicles and caveolae, and both seem to function in a short peptide sequence-dependent manner.

3.1 Clathrin-mediated intracellular trafficking

It is thought that most ligand-receptor complexes are concentrated in the clathrin-coated pits of the plasma membrane, which are cleaved off to produce clathrin-coated vesicles [10–12]. The vesicles rapidly lose their coats, a change that facilitates their fusion with early endosomes, which are maintained at a slightly acidic pH and function as platforms for many ligand-receptor complexes. The bound ligand-receptor complexes are delivered to the lysosomes, where both ligand and receptor are degraded [9,13–15]. Under certain conditions, however, the ligand is delivered to lysosomes for degradation, while the receptor returns to the plasma membrane to bind new ligand for additional rounds of internalization and this process is referred to as retroendocytosis [9]. Alternatively, some endosomal receptor proteins recycle back to the plasma membrane to maintain a steady-state condition [16].

The clathrin-mediated endocytosis is regarded as an established mechanism for the internalization of cargo as well as a large number of membrane receptors and proteins from the cell surface to the intracellular compartments [17–19]. A clathrin-containing coated pit originates by deepening invagination and dissociation from the plasma membrane and yields

a clathrin-coated vesicle, involving the mechanochemical force generated by dynamin [20]. The coated vesicles usually give rise to endosomes, recycling endosomes, and/or lysosomes. In this dynamic process, the membrane receptors with bound ligand or activated membrane proteins within the vesicles, are rapidly internalized and delivered to the endosomes. However, within the endosomes, the trafficking molecules are either directed to lysosomes, where the ligand-receptor complexes or the membrane protein are degraded, or via recycling endosomes, the receptor can be inserted into the plasma membrane, where it can bind to a new ligand. The proposed schematic pathways indicating the internalization, subsequent trafficking, and subcellular sorting of membrane receptors and/or protein in different subcellular compartments have been presented in (Fig. 1).

3.2 Caveolae-mediated trafficking

On the other hand, caveolae are assembled by the protein caveolin, which is constituted in the endoplasmic reticulum and then travels to the plasma membrane. Caveolae participate in the internalization of various types of macromolecules and also function as docking sites for the assembly of intracellular signaling networks [21–23]. A number of cargo molecules are known to be internalized involving caveolae such as transforming growth factor-beta receptor (TGF-β R), ubiquitinated epidermal growth factor (EGF) receptor, integrins, adenosine receptors, and glutamate transporter [24–26]. Caveolae-dependent endocytic mechanism also requires dynamin in a short peptide sequence-dependent manner; however, there is only limited similarity between clathrin-mediated and caveolae-dependent internalization mechanisms.

4. Characteristic features of different small-sequence peptide motifs and intracellular trafficking

The small functional signal motifs have been instrumental in our understanding of the endocytosis and intracellular trafficking of transmembrane receptor-ligand complexes from the plasma membrane into the cell interior. The signal motifs usually constitute short linear arrays of amino acids, which consist of four to seven amino-acid residues [1,27]. Among them, only two or three amino acid residues are usually important to the functional characteristics of that particular sequence-signal motif. The critical functional residues are most likely the bulky hydrophobic amino acids. However, it has been suggested that the charged amino acid residues are important determinants of specificity and exhibit functional significance in the endocytosis and molecular sorting of membrane receptors and/or membrane proteins.

The hallmark characteristic of endocytic and trafficking signals, which specifies and distinguishes these signals from other sequence motifs is their presence in the cytoplasmic domains of transmembrane receptors. In certain circumstances, the trafficking and sorting determinants seem to be folded structures and the critical amino acid residues are not particularly colinear. A compilation of membrane receptors and/or membrane proteins with known signal sequence motifs is presented in Table 1. However, much work remains to be accomplished to identify new motifs for internalization, trafficking, and intracellular distribution of various membrane receptors and proteins. Morover high performance algorithms for the identification of short sequences should provide an extremely useful tool to search through the genome databases for new signal motifs involved in the internalization and trafficking of membrane receptors and proteins [28].

4.1 Role of dileucine-dependent signal-sequence motifs

Dileucine (LL)-based sequence motifs exhibit a broad range of functions in endocytosis and trafficking of various membrane receptors and/or membrane proteins [1,29–32]. The

dileucine motif is recognized by adaptor proteins (APs), which regulate trafficking of various membrane proteins involving both endocytic and secretory pathways. The dileucine motif is characterized by four to seven amino acid residues, which precede dileucine residues. The LL residues are usually preceded by a polar residue and a negatively charged amino acid residue, which may be aspartic acid, glutamic acid, or phosphoserine. Although, dileucine motifs with acidic amino acid residues are constitutively active, those LL motifs that contain serine residue are activated by phosphorylation [31]. Some membrane receptors contain more than one dileucine motif; one of the leucine residues can be substituted for tyrosine-based signal motifs. In CD3 and mannose-6-phosphate receptors, LL motifs correspond to a distinct class of signal that include [DE] XXXL[LI] and DXXLL motifs. These features of dileucine-type signal motifs suggest that they can be recognized at the plasma membrane as well as in intracellular locations [29].

Dileucine motifs function in the regulation of both endocytosis and the secretory pathway [33,34]. In the cytoplasmic tail of GABA receptor, LL motifs can act at the level of the TGN to control the expression of receptors on the cell surface [34]. The LL motif present in the amino-terminal region of glucose transporter 8 (GLUT 8) has been suggested to regulate intracellular sequestration [35,36]. These findings indicated that amino-terminal dileucine motifs in Glut 8 transporters constitute a docking site that is responsible for endocytic processes. The dileucines have been indicated to serve as endocytic signals for various membrane proteins and/or receptors [35,37]. Both LL- and tyrosine-based signals interact with clathrin-associated APs complexes, which help to recruit membrane proteins into clathrin-coated pits and/or vesicles. During endocytosis, AP-2 plays a central function in the formation of clathrin-coated vesicles. The earlier studies have shown that certain dileucine motifs bind to mu subunits of APs, but some [DE] XXXL[LI] motifs interact with the gamma and sigma 1 subunits of AP-1, as well as with delta and sigma 3 subunits of AP-3 [38,39]. A splice variant of the high-density lipoprotein (HDL) receptor, which is known as scavenger receptor II (SR-BII), is internalized via clathrin-containing endocytic vesicles and contains the dileucine motif in the carboxyl-terminal domain [40]. Dileucine-based signal motifs have been identified in various other vesicular transport membrane proteins such as the vesicular acetylcholine transporter (VAchT), vesicular GLUT 1 (VGLUT1), and tyrosinase [29,41,42].

The dileucine DXXLL signal motifs also participate in the recycling of membrane proteins between the TGN and endosomes [1]. Certain transmembrane proteins contain [D/E]XXXL[L/I] motifs and/or YXXphi signals, both of which have been shown to function in internalization and subcellular trafficking events. The [D/E]XXXL [L/I] signal motifs interact and/or bind to mu adaptins as well as to beta subunits of APs [43–46]. Interestingly a recent crystallographic study has demonstrated that the AP-2 adaptor core binds to the dileucine motif of CD4, and has suggested that the interaction between a dileucine-motif-containing-protein and AP-2 complex is dynamic in nature [47]. The LL-based sorting motifs in mannose-6-phosphate receptors are recognized by ADP-ribosylation-factor (ARF)-dependent clathrin adaptor proteins, referred to as GGAs (Golgi-localizing, gamma-adaptor homology domain, ARF-binding protein). GGAs display a critical function in packaging of mannose-6-phosphate receptors into clathrin-coated vesicles in the TGN, which could be regulated by the phosphorylation state of GGAs [48]. To ascertain the function of dileucine-based signals in relation to [D/E]XXXL [L/I] or DXXXL motifs, it should be emphasized that the former is recognized by heterotetrameric adaptor complexes such as AP-1, AP-2, AP-3, and AP-4, which later bind GGA adaptor proteins [49–51].

4.2 Role of NPXY-type signal-sequence motifs

The low-density lipoprotein (LDL) mutant receptor was shown to have normal ligand binding characteristics; however, its defective receptor internalization pointed to a critical

function of the cytoplasmic domain in overall endocytosis and trafficking [52]. Those initial studies were instrumental in identification of the FXNPXY sequence motif as the first recognized trafficking and sequestration signal of membrane receptors. The substitution of a cysteine residue for a tyrosine residue in NPXY (Asn-Pro-X-Tyr) motif of the cytoplasmic domain of the LDL receptor in a patient with familial hypercholesterolemia rapidly abrogated its internalization [53]. It has been suggested that hypercholesterolemia results from a tyrosine-phenylalanine mutation in the NPXY motif of the LDL receptor [3,52]. In a large part, the NPXY signal motifs mediate rapid internalization of membrane proteins, including members of LDL receptors, beta-1 integrin, megalin, and beta-amyloid precursor protein families, as well as certain receptor tyrosine kinases. These include members of the insulin receptor, epidermal growth factor (EGF) receptor family, and neurotrophin receptors [1,54–56].

The NPXY motifs recruit clathrin and adaptor protein molecules and act as cargo recognition motifs for their delivery to endosomes and lysosomes [3,57]. The NPXY motifs initially recruit AP-2 at the plasma membrane and activate the mu2 subunit of AP-2, after which the beta-2 subunit of AP-2 binds clathrin at the cell surface, leading to clathrin-mediated endocytosis [1,3,58,59]. The NPXY motifs have also been shown to recruit and interact with the phosphotyrosine domain of the adaptor protein desabled-2 (Dab-2), which directly interacts with the NPXY motifs and leads to clathrin-mediated endocytosis by activating clathrin and AP-2 [60,61]. The AP-2 is composed of four subunits (alpha, beta2, mu2, and sigma2), which interacts with NPXY motifs of membrane receptors and/or proteins in the internalization process. The FDNPVY sequence motif binds to components of the clathrin coat and in this context, the NPXY residues adopt a beta-turn structure [62]. Furthermore, chimeric insertion of the FDNPVY sequence into the transferrin receptor leads to rapid internalization, whereas insertion of only NPVY sequence does not enhance endocytosis of this receptor protein [63]. Similarly, endocytosis of the beta-amyloid precursor protein is directed by a longer sequence motif, GYENPTY, in which the first tyrosine residue seems to have a more critical role in the internalization events [64].

Several studies have demonstrated that proteins containing phosphotyrosine-binding (PTB) domains such as Dab-1 and Dab-2 participate in LDL receptor internalization and Dab-1 and Dab-2 directly bind FXNPXY sequence motifs located in various members of the LDL receptor family [65,66]. However, overexpression of the PTB domain of either Dab-1 or Dab-2 impedes internalization of the LDL receptor, leading to the accumulation of receptors on the cell surface. Both Dab-1 and Dab-2 contain signal motifs, which bind to clathrin and AP-2 at the carboxyl-terminus at their PTB domain [57,60,67]. Similarly, Grb-2 has been shown to facilitate the internalization of EGF receptor proteins [68]. The NPXY motifs in the carboxyl-terminal domain of beta-5 integrin act as the molecular switch for distinct biological processes of integrin activation, endocytosis, and sorting [54,69,70] Furthermore, the NPXY motif in the cytosolic tail of beta-1 integrin, acts in the recruitment of APs and clathrin for endocytosis and internalized cargo assembly.

4.3 Role of GDAY-type signal-sequence motifs

The tetrameric sequence Gly^{920}-Asp^{921}-Ala^{922}-Tyr^{923} (GDAY) motif in the carboxyl terminal-domain of guanylyl cyclase/natriuretic peptide receptor-A (GC-A/NPRA) serves as an internalization signal for endocytosis [71]. The residues Gly^{920} and Tyr^{923} constitute the important elements in the GDAY signal motif; however, Asp^{921} provides an acidic environment for efficient signaling of GDAY during the trafficking of NPRA (Fig. 2). The single mutation Asp^{921} to Ala did not exert a major effect on receptor internalization, but significantly attenuated the recycling of internalized receptors to the plasma membrane. On the other hand, mutation of Gly^{920} and Tyr^{923} to Ala inhibited the internalization of NPRA, but had no discernible effect on the recycling process. These findings suggested that the

tyrosine-based GDAY sequence motif modulates the early internalization of NPRA, whereas Asp^{921} seems to mediate recycling and sorting of the receptor. Thus two overlapping motifs within the GDAY sequence exert different but specific effects on endocytosis and subsequent trafficking of NPRA. Interestingly, guanylyl cyclase-B/natriuretic peptide receptor-B (GC-B/NPRB) has also been shown to be internalized and recycled in hippocampus neurons and C6 glioma cells cultures [72]. The trafficking of GC-B/NPRB occurs in a ligand-dependent manner in response to C-type natriuretic peptide (CNP) stimulation and has been suggested to involve a clathrin-dependent mechanism. Our recent work has suggested that the internalization of GC-A/NPRA also involves clathrin-dependent pathways [73]. The process of receptor internalization of GC-A/NPRA is severely diminished by inhibitors of clathrin proteins such as chlorpromazine and monodensyl cadaverine.

The internalization of platelet-activating factor is also regulated by a putative motif, DPXXY, and that of type-2 vasopressin receptor by the NPXXY sequence [74,75]. Similarly, the YXXL motif also functions in endocytosis of LDL receptor-related proteins [76]. A common feature of these internalization signal motifs, including NPXY and GDAY, is the presence of a tyrosine residue at the end of the tetrapeptide sequence [71,74]. Moreover, tyrosine residues in the mannose-6-phosphate receptor and in the influenza virus hemagglutinin are also involved in endocytosis, even though they are not present in the context of NPXY or YXRF consensus sequences. Therefore, if a universal internalization signal exists, it may not be based on a universal amino acid sequence [3,74]. The critical characteristics of all these sequences might be their specification of a particular conformation, such as a tight beta-turn in protein structure [77]. It has also been proposed that Tyr recognition signals form a small surface loop, however, they differ in structure in terms of the positioning of Tyr in the loop structure [1,63,78]. The substitution of Tyr with residues known to be inactive in endocytosis resulted in disruption of the beta-turn conformation. A similar approach was used to obtain evidence that the PPGY sequence of the acid phosphatase in the cytoplasmic tail forms a type 1 beta-turn with the Tyr in the fourth position [79]. All these studies indicated that the presence of Tyr in the fourth position of internalization signals is critical for receptor endocytosis.

4.4 Role of YXXphi-type signal-sequence motifs

The YXXphi signal sequences exhibit dual specificity, such as an endocytotic functional motif and direct trafficking within the endosomal and/or secretory pathways [1,3,27]. The tyrosine-based YXXphi sorting signals direct the targeting of integral membrane proteins by interacting with the mu1, mu2, mu3, and mu4 subunits of adaptor protein complexes AP-1, AP-2, AP-3, and AP-4, respectively [58,80,81]. In the YXXphi signal motifs, Y is a tyrosine residue, X is any amino acid residue, and phi is an amino acid residue with large bulky hydrophobic side chains. The tetrapeptide sequence YXXphi is present in the cytoplasmic domains of several transmembrane receptors such as transferrin and asialoglycoprotein receptors, which provides trafficking and sorting information during the internalization process. In this tetrapeptide sequence motif, the Y residue is critical for the signal and in most conditions, it cannot even be substituted by other aromatic amino acid residues, since the phenolic hydroxyl group of the tyrosine is essential for generating the endocytic and trafficking signal. The two X residues are also considered to contribute to the specificity and potency of the signals for the endocytic and sorting events. The phi position in the tetrapeptide sequence is thought to accommodate a varying number of amino acids containing bulky hydrophobic side chains [82,83]. However, both tyrosine and phi residues are important in internalization and trafficking events. It is also thought that the identity of amino acid residues at the phi position confers the specificities and properties of the trafficking and sorting signals.

In certain instances, the YXXphi signal motifs also direct lysosomal sorting and contain acidic residues at the X position [82]. Furthermore, the tetrameric YXXphi motif may also involve a glycine residue preceding the tyrosine residue (YGXphi). The substitution of alanine in place of glycine interferes with lysosomal targeting, but does not affect endocytosis [84]. The YXXphi signal motifs with internalization specificity are usually located within 10–40 amino acid residues from the transmembrane spanning domain, but not at the carboxyl-terminus of the endocytotic receptors [85]. Furthermore, the tetrameric YXXphi motifs for lysosomal targeting are usually located at six to eight amino acid residues from the transmembrane spanning domain at the carboxyl-terminal of the membrane receptors [1]. The mutations in X residues, which substantially decrease the interaction with AP-2, have little effect on the internalization process [86]. The presence of a glycine residue before the critical tyrosine residue of YXXphi signals helps to recognize lysosomal membrane proteins [49]. The YSGL motif interacts with currently unknown intracellular proteins and governs constitutive internalization of chemokine-CXCR3 receptors [87]. Previous studies have shown that YXXphi signals similar to YKKL motifs within the carboxyl-terminal of the protease-activated receptor-1 (PAR-1), regulates the clathrin- and dynamin -dependent internalization process [58].

Indeed, some additional motifs in addition to those mention above, also play critical roles in the internalization and trafficking of membrane receptors and/or membrane proteins. The YXXGL motifs influence the internalization and sorting of P_2X_4 receptors [88]. Similarly, the VXXSL motif seems to be critical for the surface expression of voltage-gated K^+ channels [89]. Interestingly, the GTALL motif has been suggested to direct the leutinizing hormone (LH) receptor from the degradative to the recycling pathway [90]. It has also been shown that a HLVNK motif plays a dual role either as a TGN sorting signal for the localization of CD44 in the basolateral membrane or as an internalization motif necessary for the transcytosis of CD44 in the apical membrane [91–93].

5. Conclusions

The specific short-sequence motifs located in the intracellular cytoplasmic domain of various membrane receptors and/or membrane proteins regulate the internalization, trafficking, and/or sorting into the subcellular compartments. This review delineates the function of various specific short-sequence signal motifs, which inherently control the endocytosis, trafficking, and sorting processes of many membrane receptors and/or membrane proteins. These processes include the interactions of ligand-receptor complexes, which can govern the rate at which the receptor traverses the intracellular compartments, the intrinsic regulation of receptor or protein, and protein-protein interactions during the intracellular trafficking. The internalization and trafficking signal motifs are recognized by various interacting proteins and direct itinerant routing of protein molecules to facilitate endocytotic and/or biosynthetic products to their intended final destinations. The internalization and sorting-signal motifs include tyrosine-based sequences such as NPXY, GDXY and YXXphi and dileucine-based motifs (LL and LL/I), most often located in the carboxyl-terminal domains of the targeting membrane receptors and/or membrane proteins.

In addition, endosomes and/or lysosomes can be accessed through the biosynthetic pathways, where the internalized receptor and/or proteins can be transported via an intracellular route to endosomes and eventually, destined to reach either, recycling endosomes, lysosomes, and/or the plasma membrane. The cellular regulation and expression of targeted protein molecules involve intracellular trafficking and movement into subcellular compartments in a small peptide-sequence-dependent manner. The short peptide sequence-based signals are of special interest in the molecular trafficking and sorting, which are responsible for decoding of these motifs. It is particularly important that we continue to

define the functions of short-sequence motifs in the endocytosis, trafficking, and sequestration of specific membrane receptors or membrane proteins. It is reasonable to suggest that a short peptide sequence motif might be distinct for an individual receptor trafficking and sorting, yielding a unique signaling cascade output. Precise characterization of short peptide sequence-dependent receptor trafficking and sorting will be crucial for developing the next generation therapeutic targets.

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Abbreviations

AP	adaptor protein
PTB domain	phosphotyrosine-binding domain
PTI domain	phosphotyrosine-interacting domain
GC-A	guanylyl cyclase-A
NPRA	natriuretic peptide receptor-A
ANP	atrial natriuretic peptide
BNP	brain natriuretic peptide, GC-B, guanylyl cyclase-B
NPRB	natriuretic peptide receptor-B
CNP	C-type natriuretic peptide, ARF, ADP (adenosine diphosphate) – ribosylation factor
GGA	Golgi-localizing, gamma-adaptor homology domain, ARF-binding protein, TGN, trans-Golgi network

6. References and annotations

Papers of particular interest, published within the period of review, have been highlighted as:

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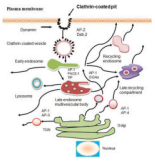

Figure 1. Intracellular pathways of receptor-medicated endocytosis and trafficking

The binding of ligand to specific cell surface receptors leads to a selective recruitment of ligand-receptor complexes into clathrin-coated pits on the plasma membrane. The coated pit invaginates and pinches-off into a vesicle in the cytosol, which triggers the recruitment of adapter proteins and other interacting molecules. The clathrin -dependent routes require dynamin to achieve the fission of the membrane invaginations and vesicle internalization. The ligand-receptor complexes within the cargo entering via the clathrin pathway are usually directed to early endosomes. From the endosomes, the receptors and ligands are sorted to various subcellular locations, where the internalized molecules are either sorted to degradative compartments such as the late endosomes, and/or lysosomes, or recycled to the plasma membrane via recycling endosomes. The recycled molecules can participate in several rounds of endocytosis. Alternatively, the internalized cargo molecules may be sequestered in endosomes for a longer period of time and continue to spark signaling events. Some early and late endosomes also contain membrane structures in the lumen, which are referred to as multi-vesicular bodies (MVBs). The endosomal and lysosomal system can also transmit and receive cargo from the trans-Golgi network (TGN). The critical molecules involved in the trafficking at different locations have been indicated as AP-1, AP-2, AP-3, AP-4, Dab-1, GGA, PACS-1, and TIP.

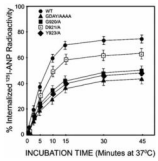

Figure 2. Role of the GDAY motif in the internalization and trafficking of wild-type mutant guanylyl cyclase/natriuretic peptide receptor-A

HEK-293 cells expressing wild-type, G920A, D921A, Y923A or GDAY/AAAA mutant receptors were preincubated for 1 h at 4 °C with labeled ^{125}I-atrial natriuretic peptide (^{125}I-ANP). Surface ^{125}I-ANP binding to wild-type and mutant receptors was performed at 4°C. Subsequently, cells were warmed at 37 °C for the indicated time periods to permit the internalization of ligand–receptor complexes into the cell interior. Internalization of receptor was quantified at different time points. Copyright @2005 The Biochemical Journal.

Table 1

Important short-sequence motifs involved in the internalization and trafficking of membrane receptors and/or proteins

The degenerate short tetra- or hexa-peptide sequence motifs contain tyrosine or phenylalanine residues, followed by hydrophobic or aromatic residues. Certain motifs also contain acidic residues in conjunction with tyrosine. The dileucine-type of signal motifs is also essential for internalization and trafficking of certain membrane receptors and/or proteins into the cell interior.

Signal Motifs	Targeted Pathways	Membrane Receptor/Protein
DXXLL	TGN-to-endosome sorting	VHS domain of GGAs
YXXΦ	Internalization, lysosomal and basolateral targeting	Transferrin receptor, LAMP-1, CD1
YXXGL	Internalization	P_2X receptor
DSLL	TGN to endosomal targeting	Beta-adrenergic receptor
YENPTY	Internalization	Beta-amyloid precursor protein
YKYSKV	Internalization, lysosomal targeting	CD-Mannose-6-phosphate receptor
HLVNK	TGN sorting and basolateral localization	CD44
YSKV	Internalization, lysosomal targeting	CI-Mannose-6-phosphate receptor
GDAY	Internalization	GC-A/natriuretic peptide receptor-A
FQQI	Internalization, lysosomal targeting	GLUT4
GYQTI	Internalization	Igp-A/lamp-1
GYEQF	Internalization	Igp-B/lamp-2
FDNPVY	Internalization	LDL receptor
GTALL	Internalization	LH receptor
GYRHV	Lysosomal sorting	Lysosomal acid phosphatase
FENTLY	Internalization, lysosomal targeting	Mannose phosphate receptor
YSAF	Internalization	Polymeric Ig receptor
YEQGL	Internalization	P_2X receptor (ATP-gated ion channel)
YQRL	TGN to plasma membrane recycling	TGN38
YTRF	Internalization	Transferrin receptor
YQPL	Internalization	T-cell receptor (CD3)
LL	Internalization, secretory pathway	Fc receptor
LI	Internalization, secretory pathway	MHC class II invariant chain

GGAs, Golgi-localizing gamma-adaptor homology domain; GLUT4, glucose transporter 4;GC-A, guanylyl cyclase-A; LDL, low density lipoprotein; LH, leutinizing hormone; TGN, trans-Golgi network; MHC, major histocompatibility complex.