

Functional diversity of protein C-termini: more than zipcoding?

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The carboxylated (C)-terminus of proteins, which includes the single terminal α -carboxyl group and preceding residues, is uniquely positioned to serve as a recognition signature for a variety of cell-biological processes, including protein targeting, subcellular anchoring and the static and dynamic formation of macromolecular complexes. The terminal sequence motifs can be processed by posttranslational modifications, thereby providing a means to increase sequence diversity and to regulate interactions. Several classes of protein domains have been identified that are either designed for or are capable of interacting with protein C-termini – these include PDZ and TPR domains. The interactions between these protein domains and various terminal epitopes play an important role in specifying cell-biological functions. The combination of diversity and the plasticity of the chemistry of C-termini provides mechanisms for spatial and temporal specificity that are exploited by a variety of biological processes, ranging from specifying prokaryotic protein degradation to nucleating mammalian neuronal signaling complexes. Understanding the diverse functions of protein C-termini might also provide an important indexing criterion for functional proteomics.

Proteins are linear amino acid polymers flanked by amino (N-) and carboxyl (C-) termini. The distinct chemistry at the N- and C-termini orients the polarity of a protein, thus contributing to the folding kinetics and providing two positionally and chemically unique recognition signatures essential for a variety of biological processes. A well-known function is their ability to ‘zipcode’ – that is, to serve as a cellular targeting signal.

Most proteins have their terminal regions exposed and accessible for various biochemical reactions. This is presumably due to the sequential nature of amino acid polymerization during protein synthesis and the energy cost of burying termini inside the hydrophobic core found in a typical protein. Increasing evidence suggests that the exposed terminal epitopes are important recognition anchors for protein–protein and protein–lipid interactions, which form the molecular basis for the diverse functions of protein C-termini considered in the following discussion.

The amino- and carboxyl-termini of proteins have rather distinct roles. The functions of the amino-terminal sequence are related to ubiquitination-induced degradation [1] as well as to sorting [2]. By contrast, the C-terminus is where the translation process terminates, and it was initially thought that the last few amino acids might interact with various release factors involved in translational termination [3]. It is now believed that the protein C-termini, acting as unique recognition signatures, are involved in a variety of biological processes through either static or dynamic interactions with other proteins

and/or non-protein biomolecules such as lipids (Fig. 1). This review focuses on the variety of modifications and functional diversity of protein carboxyl-terminal epitopes and the domains that bind to them. We discuss several well-studied C-terminal motifs and the mechanisms by which they regulate diverse biological functions well beyond the conventional cellular targeting – ‘zipcoding’ – role of proteins in the secretory pathway. The wealth of C-terminal sequence diversity revealed from genome sequencing projects and protein databases should herald a plethora of functions to be discovered in the coming years.

Interaction domains for carboxylated termini

Many cellular processes depend on sequential protein–protein interactions, leading to the formation of protein complexes with appropriate compositions and stoichiometries that serve as the basis for the propagation of information through a signaling system. Protein C-termini interact with other proteins that are often signaling scaffold proteins containing protein modules designed to mediate protein–protein interactions [4,5]. Proteins that possess more than one such interaction module with either identical or different binding specificities constitute molecular scaffolds capable of organizing macromolecular protein complexes that facilitate signaling efficacy and provide spatial specificity.

The PDZ domain, a well-known protein–protein interaction module occurring in more than 600 proteins, provides an illustration of protein–protein interactions involving C-termini (Fig. 2a). This domain was originally identified in the postsynaptic density protein 95 kDa (PSD95), the *Drosophila* tumor-suppressor Dlg1 and the tight-junction protein Zo-1. The PDZ domain has a carboxylate-binding groove. Its preferential binding to a short COOH-terminal peptide (3–4 residues in the binding pocket) was revealed by random peptide-selection studies [6] and was evident in the co-crystal of PDZ3 of PSD95 [7]. The PDZ domain might mediate either static or dynamic interactions in a protein complex. For example, a protein termed INAD (inactivation no afterpotential D) comprises five PDZ domains that organize a protein complex that appears to be constitutively bound to TRP channels, phospholipase C and calmodulin; however, the interaction of INAD PDZ domains with other proteins such as rhodopsin might be dynamic [8].

In addition to controlling composition, interactions mediated by PDZ domains have now been shown to

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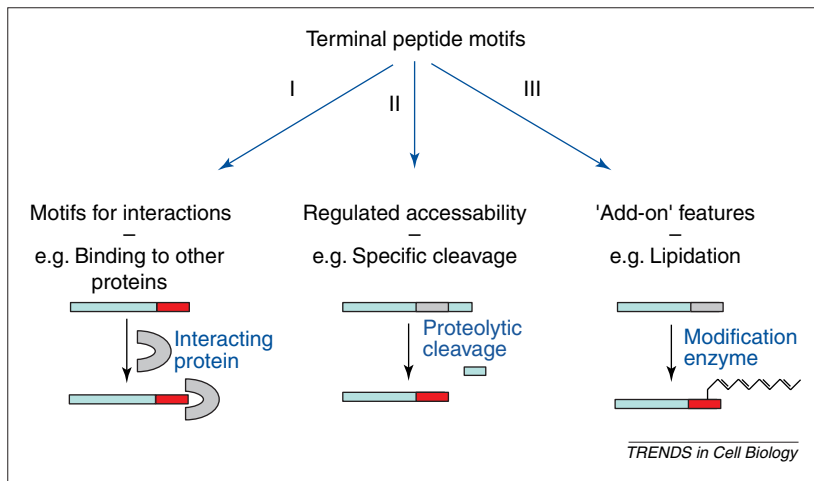


Fig. 1. Regulation by recognition motifs in protein termini. Examples of three pathways are shown in which protein carboxyl-termini might serve as substrates for a variety of biochemical reactions, thereby regulating the physiological roles of the parent protein. Rectangular bars represent polypeptides. The active C-terminus is highlighted in red; the non-active C-terminus is highlighted in gray. (I) The protein terminus serves directly as a binding site; (II) the protein terminus is latent for biochemical reaction before specific cleavage; (III) the protein terminus undergoes a particular posttranslational modification through enzymatic addition of a non-peptide moiety.

directly regulate the function/activity of the PDZ binding targets. One such interaction takes place between the cystic fibrosis transmembrane conductance regulator (CFTR) and CFTR-associated protein of 70 KDa (CAP70). The membrane-bound CFTR protein is a chloride channel containing an ATP-binding cassette (ABC). The multivalent binding of CAP70 PDZ domains to CFTR triggers transient oligomerization of CFTR proteins, thereby inducing the formation of more favorable conductance states that result in potentiation of chloride channel activity [9].

As well as the above, increasing evidence supports the notion that PDZ binding is not restricted to merely being a 'glue' to bind and assemble macromolecular complexes [10]. Interactions involving PDZ domains appear to be a general mechanism for localizing membrane proteins to specific subcellular membrane domains and for coupling them to proteins in signal-transduction pathways. PDZ domains are also involved in other cellular functions such as regulation of gene expression and targeted protein degradation. For example, TAZ ('transcriptional co-activator with PDZ domain') is a transcription factor whose activity is coupled to PDZ domain binding [11].

The PDZ domain, as a C-termini interaction scaffold, is evolutionarily conserved. Similar protein folds that function as interaction scaffolds for C-termini have been found in a variety of organisms, including prokaryotes, such as bacteria, where PDZ-like domains are involved in C-terminal sequence-specific proteolysis [12–15] (also see following discussion).

In addition to PDZ domain proteins [16], there are other proteins or protein domains that can bind to protein C-termini. For example, hydrophilic C-terminal interacting domains such as tetratricopeptide repeat (TPR) domains are found in the

protein PEX5, which is involved in the targeting of peroxisomal proteins (Fig. 2b) [17,18]. In addition, hydrophobic membrane-bound receptors such as ERD2 bind to C-termini. ERD2 is a putative seven-transmembrane receptor for the specific KDEL tetrapeptide C-terminal signal that is found in resident proteins of the endoplasmic reticulum (ER) lumen [19]. Interestingly, recent evidence suggests that the WW domain [20] and the zeta form of the 14-3-3 protein [21] might also interact with the carboxylated terminus of their interaction partners. The activity of the aforementioned domains appears to be restricted to binding ability, and there are also a large number of protein modification enzymes such as proteases that act upon protein C-termini. A list of the domains can be found at: www.molecularinteraction.org.

An unusual case of protein domain-mediated C-terminus interaction occurs in the nNOS PDZ domain. This domain has the capability to interact with either a terminal peptide [6,22] or an internal peptide [23] of PSD95. However, incubation of the purified PDZ domain with a library of peptides revealed that the nNOS PDZ, in the absence of other protein domains or its cofactor, preferentially interacts with carboxylated terminal epitopes [6]. The precise *in vivo* implications of the multiple binding modes of the nNOS PDZ domain are not fully understood.

Modified forms of protein C-termini

The C-termini of many proteins are posttranslationally modified, processed and matured in order to function properly. The diversity of protein C-termini is further increased as a result of protein posttranslational modifications, which have important biological roles such as cell-surface localization, protein targeting and signal transduction. Three major classes of C-terminal modifications and their functional implications are briefly discussed below. It should be noted that these modification mechanisms are not restricted to protein C-termini and that there are other modifications targeted to protein C-termini.

Protein lipidation

Lipidation is one of the maturation processes modifying many proteins found in both prokaryotes and eukaryotes, including nuclear lamins, viral oncogene products and Ras-related GTP-binding proteins [24,25]. C-terminal lipidation includes two major classes: ether linkage (such as Ras and GRK) and amide linkage (such as glypiated proteins and trimeric G-protein γ). Lipidated proteins have an increased affinity for cell membranes. The preferential partition of lipid-anchored proteins provides a number of physiological benefits, including the spatial specificity of biochemical activity and increased local concentration, that might facilitate protein–protein interaction by expediting lateral movement or by providing rapid crosstalk or oligomerization.

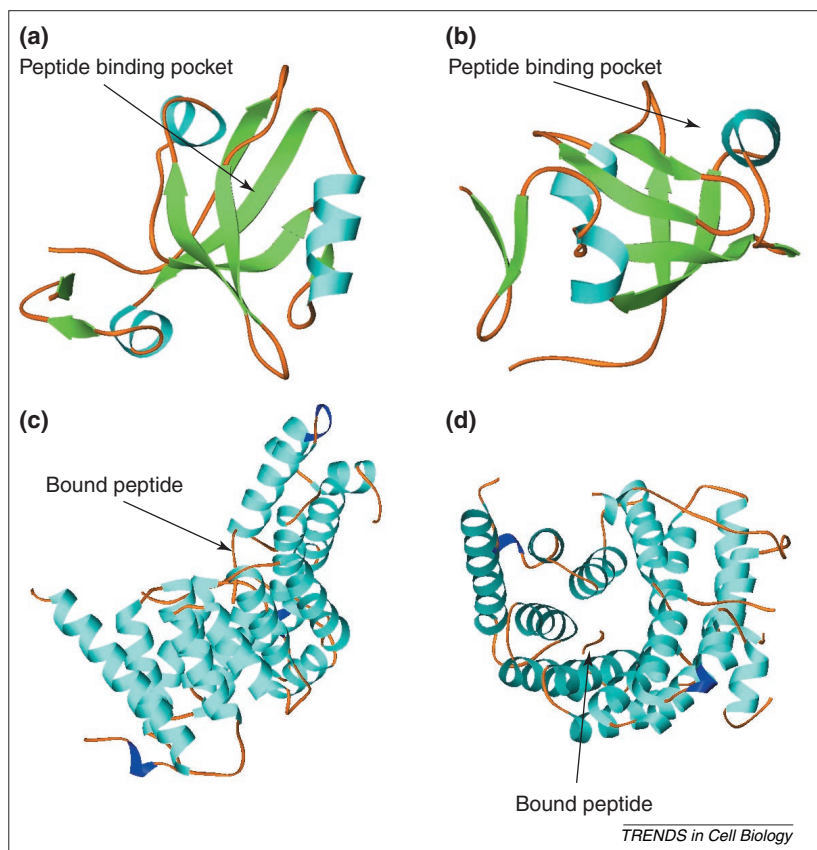


Fig. 2. Structures of two protein domains that interact with protein carboxyl-termini. (a) Structure of the PSD95 PDZ3 domain [7] in two orientations, with the unoccupied peptide-binding pocket facing out (a) or facing up (b). (c, d) Structure of the PEX5 tetratricopeptide repeat (TPR) domain [55] with a bound peptide. The complex is shown in two orientations. The binding pockets (a, b) or the bound peptides (c, d) are marked as indicated.

A number of C-terminal motifs are sites for lipidation, including -CaaX, -CC and -CXC (where x = any amino acid) [26]. For example, proteins terminating in the CaaX-COOH motif (found in proteins such as Ras and yeast α -factor mating pheromone) undergo prenylation at the cysteine residue fourth from the end, trimming of the last three amino acids of the protein and carboxyl-methylation [26]. The catalytic α and β subunits of phosphodiesterase (PDE) also undergo posttranslational C-terminal prenylation [27]. The α subunit is farnesylated on the first cysteine of the C-terminal motif CCVQ, whereas the β subunit is geranylgeranylated on the cysteine of CRIL, which is consistent with the specificity rules for protein prenyl transferases. Limited proteolysis of the C-termini of the PDE catalytic subunits correlates with loss of membrane binding, indicating that the prenyl groups might be responsible for anchoring the enzyme in the membrane [27]. The requirement for the C-terminal lipid to act as an anchor for the proper function of PDE highlights the importance of these groups, not only for regulating the localization of proteins onto the membranes but also for acting as an oriented molecular 'handle' for interacting with other proteins in the proximity of certain membrane domains.

Phosphorylation, glycosylation and acetylation

In addition to lipid modification, other types of posttranslational modification to the protein C-termini might induce altered chemistry in response to certain conditions or stimuli and might be involved in regulating protein activity.

One example is the well-studied tumor-suppressor gene product p53, which is a transcription factor found inactivated in many human tumors. The full-length p53 protein can exist as a non-DNA-binding latent form, which can be activated to bind to DNA by C-terminal modifications such as phosphorylation, acetylation and glycosylation [28–30]. Phosphorylation of C-terminal serine residues such as Ser392, located in the –1 position of the p53 C-terminus, by casein kinase II, or binding of the antibody Pab421 to a C-terminal epitope, can activate the DNA-binding activity of p53 *in vitro* [31]. Similar results have been obtained through acetylation of the C-terminal lysine residues in the transcriptional coactivator termed CREB-binding protein (CBP)/p300 [32]. Interestingly, CBP/p300 has a cofactor that contains a series of TPR motifs [33], protein domains that are involved in binding to carboxylated C-termini (as discussed earlier).

Proteolysis

The ligation or cleavage of a protein peptide bond in order to expose or remove a carboxylated terminus provides a means to regulate biochemical reactions that take place at protein C-termini by altering the accessibility of the carboxylated epitope. In *Escherichia coli*, abnormal proteins can arise as a result of various mechanisms, including premature termination of translation/transcription, denaturing conditions and improper folding. To channel these proteins into a degradation pathway, an 11-residue carboxyl terminal sequence with a non-polar tail, -AANSENALAA-COOH, is added by stable 10Sa RNA encoded by SsrA and released from the ribosome [34]. This SsrA tag serves as the signal for protease recognition in various cellular compartments – periplasm (by the Tsp protease), membranes (HflB) and cytoplasm (ClpXP, C1pAP and Hflb) [35]. The HflB, ClpXP and C1pAP proteases are members of the Clp protease family that possess a PDZ-like protein-interaction domain. It is thought that this domain plays a key role in determining substrate selectivity through C-terminal recognition [36]. Recent evidence suggests that the Lon protease also acts upon the C-terminal 20 residues of Sula, which, together with another potential cofactor, eventually leads to the degradation of Sula by Lon [37,38]. In addition, the PDZ-like domain of the SpoIVB serine peptidase is used for self-cleavage [13]. The existence of other families of prokaryotic serine peptidases with PDZ domains makes it likely that PDZ domain-mediated C-terminal recognition is utilized by the bacterial degradation machinery to recognize substrates. The use of a separate

recognition (binding) and degradation (catalytic) domain might be a general mechanism for achieving selective protein degradation.

The susceptibility of the C-terminus to proteolysis and the likely involvement of PDZ domains in this process have implications for subcellular anchoring mediated by terminal epitopes. PDZ proteins in mammalian neurons interact with the C-termini of ionotropic glutamate receptor 2 (NR2) subunits and play a role in localizing and/or anchoring them to certain subcellular locations. The ionotropic glutamate receptor 2A (NR2A) subunit is susceptible to certain C-terminal truncations in heterologous expression systems [39], suggesting the possibility that the proteolysis might be a physiologically regulated mechanism to rearrange the synaptic NR2A–PDZ interactions, thereby affecting synaptic remodeling.

Another line of evidence for regulated specific cleavage of the C-terminus came from the molecular cloning of rhodanase, a mitochondrial sulfur transferase. The protein sequence determined from native rhodanase lacks the last three C-terminal amino acids encoded by the cDNA [40]. More interestingly, addition of a serine or arginine residue at the C-terminus alters the enzymatic activity significantly. In addition, the C-terminal 16 amino acids of p53, including the carboxylated terminus, are required for p53 degradation [41]. This evidence further supports the notion of activity regulation through proteolytic cleavage at protein C-termini.

Subcellular targeting

Signals and mechanisms for protein sorting and trafficking have been studied extensively and are summarized in several recent reviews [42–46]. The complexity of terminal recognition is interpreted at multiple cell-biological levels. A well-studied phenomenon is protein sorting mediated by various short peptidic epitopes, including the terminal tetrapeptide signals –KDEL and –KKXX of luminal and transmembrane ER proteins [47]. These carboxylated tetrapeptidic epitopes are recognition signatures for receptor complexes; their interactions with the corresponding receptors – ERD2 for the KDEL tetrapeptide signal and, most likely, COPI for the KKXX motif – form the basis for ER localization and retrieval activity.

Because a given protein can have more than one signal that is involved in targeting the protein to different locations, the interaction of KDEL or KKXX localization signals with their receptors does not always lead to a dominant ER localization outcome. In fact, two different ER localization signals appear to have different ‘ER localization potentials’. For example, a cell-surface marker protein, when fused with a KKXX terminus, has a lower ‘leaky’ surface expression than that of a marker with a KDEL terminus, consistent with the notion that KKXX has a stronger ER localization potential [48]. In addition to

terminal epitopes, protein sorting processes involve a number of other short epitopes, including internal ER localization signals such as RXR [49] and ER export signals such as the di-acidic DXE motif of the vesicular stomatitis virus G glycoprotein (VSV-G) [50,51], the FCYENE motif in the potassium channel Kir [52] and the FxxxFxxxF motif in the dopamine D1 receptor [53]. It is not uncommon for more than one epitope with opposite sorting activity (ER localization versus ER export, for example) to be found in any one protein, raising an interesting question concerning the functional interplay of carboxylated terminal signals with internal signals. The combination and differential accessibility of these signals as a result of oligomeric protein assembly might actually contribute to a specific mechanism that serves as a checkpoint for quality control. Through such a mechanism, proteins that are not fully assembled would expose certain subcellular (such as ER) localization signals, thereby abrogating surface expression of the protein. This notion is supported by several lines of emerging evidence, including observations on the assembly and functional expression of potassium channels [49].

The sorting function of the terminal signal peptide is not restricted to proteins that enter the secretory pathway. For example, two types of *cis*-acting peroxisomal targeting signals, PTS1 and PTS2, mediate the import of most peroxisomal matrix proteins. PTS1 is a C-terminal tripeptide: –S/A/C-K/R/M-L(M)-COOH [54]. Pex5p, a member of the TPR family of proteins, is a receptor for the C-terminal peptide PTS1.

Concluding remarks

Protein C-terminal sequences possess sufficient diversity to provide recognition signatures for protein interactions. Because many novel and conserved C-terminal sequences have been observed in known proteomes from organisms such as budding yeast (H. Yang and M. Li, unpublished), it is likely that the current understanding of the functional roles of protein C-termini is very incomplete. In addition, there are a variety of posttranslational modifications that provide a regulatory mechanism allowing for reversible and/or irreversible changes of protein termini by altering accessibility and/or chemical properties. Identification and understanding of the chemical modifications and the physiological implications of them are important in order to fully define the functional roles of protein C-termini.

At the cell-biological level, depending upon whether and how a given machinery for posttranslational modifications is subcellularly positioned and/or temporally regulated, the resultant modified protein C-termini might possess biochemical heterogeneity temporally and/or spatially, leading to multiple distinctive biological functions of one protein in different regions of the same cell and/or during different physiological states.

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