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Ubiquitination of Gap Junction Proteins

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Abstract Gap junctions are plasma membrane domains containing arrays of channels that exchange ions and small molecules between neighboring cells. Gap junctional intercellular communication enables cells to directly cooperate both electrically and metabolically. Several lines of evidence indicate that gap junctions are important in regulating cell growth and differentiation and for maintaining tissue homeostasis. Gap junction channels consist of a family of transmembrane proteins called connexins. Gap junctions are dynamic structures, and connexins have a high turnover rate in most tissues. Connexin43 (Cx43), the best-studied connexin isoform, has a half-life of 1.5–5 h; and its degradation involves both the lysosomal and proteasomal systems. Increasing evidence suggests that ubiquitin is important in the regulation of Cx43 endocytosis. Ubiquitination of Cx43 is thought to occur at the plasma membrane and has been shown to be regulated by protein kinase C and the mitogen-activated protein kinase pathway. Cx43 binds to the E3 ubiquitin ligase Nedd4, in a process modulated by Cx43 phosphorylation. The interaction between Nedd4 and Cx43 is mediated by the WW domains of Nedd4 and involves a proline-rich sequence conforming to a PY (XPPXY) consensus motif in the C terminus of Cx43. In addition to the PY motif, an overlapping tyrosine-based sorting signal conforming to the consensus of an YXXϕ motif is involved in Cx43 endocytosis, indicating that endocytosis of gap junctions involves both ubiquitin-dependent and -independent pathways. Here, we discuss current knowledge on the ubiquitination of connexins.

Keywords Gap junction · Connexin · Ubiquitin · Endocytosis · Degradation

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Introduction

Gap junctions are plasma membrane domains consisting of arrays of intercellular channels that provide for diffusion of ions and small molecules (<1 kDa) between neighboring cells (Saez et al., 2003). In vertebrates, gap junction channels are made of a family of transmembrane proteins called connexins (Willecke et al., 2002). Gap junctional intercellular communication (GJIC) enables cells to directly cooperate both electrically and metabolically. Gap junctions have important roles in tissues containing electrically excitable cells. For instance, electrical coupling via gap junctions is important in synchronizing the contraction of heart muscle (Severs et al., 2004). Furthermore, electrical synapses via gap junctions are a ubiquitous feature of neural circuits in the mammalian brain (Connors & Long, 2004). Gap junctions also have important roles in tissues that do not contain electrically excitable cells. Several lines of evidence indicate that gap junctions are important in regulating cell growth and differentiation and for maintaining tissue homeostasis (Loewenstein, 1979; Yamasaki & Naus, 1996). Dysfunctional intercellular communication via gap junctions has been implicated as a causative factor in heart failure, neuropathologies, deafness, skin disorders and cataracts (Mesnil, 2002; Wei, Xu & Lo, 2004). Gap junctions have a high turnover rate, and many pathological conditions are associated with abberant endocytic trafficking of connexins. Increasing evidence suggests that

ubiquitin plays a key role in gap junction endocytosis. This review will discuss current knowledge on the ubiquitination of connexins.

Formation and Degradation of Gap Junctions

Connexins are expressed in most cell types, and most cells and tissues usually express several different connexin isoforms (Bruzzone, White & Paul, 1996; Sohl, Maxeiner & Willecke, 2005). There are 21 known connexins in the human genome, of which the best studied is connexin43 (Cx43) (Sohl & Willecke, 2003). Connexins span the membrane four times, and both the amino terminus and the carboxy terminus are localized on the cytosolic side of the membrane (Sosinsky & Nicholson, 2005). Connexins are cotranslationally inserted into the endoplasmic reticulum membranes and transported via the Golgi apparatus and the *trans*-Golgi network to the plasma membrane (Segretain & Falk, 2004) (Fig. 1). A subpopulation of the connexins, presumably those with abnormal conformation, undergo endoplasmic reticulum-associated degradation (ERAD) (VanSlyke, Deschenes & Musil, 2000; VanSlyke & Musil, 2002). Along with their trafficking from the endoplasmic reticulum to the plasma membrane, the connexins oligomerize into hexameric structures called connexons (Segretain & Falk, 2004). Connexons can consist of identical or different connexin isotypes (Sosinsky & Nicholson, 2005). At the plasma membrane, the connexons can dock with connexons in the adjacent cell and thereby form intercellular channels (Segretain & Falk, 2004). These channels are assembled in plasma membrane domains called gap junctions. As observed by transmission electron microscopy, the two plasma membrane domains of a gap junction are apparently separated by a gap of 2–3 nm (Goodenough & Revel, 1970; Revel & Karnovsky, 1967). Connexons at

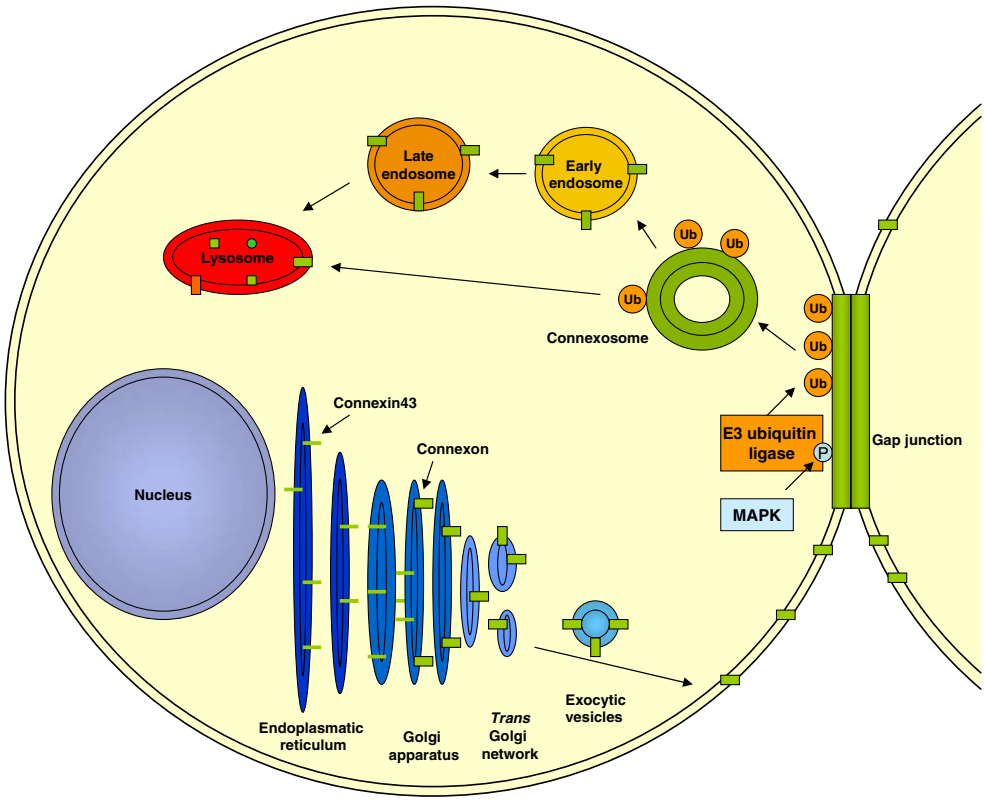

Fig. 1 A working model for ubiquitination of Cx43. Cx43 is synthesized at the endoplasmic reticulum and transported via the Golgi apparatus and the *trans*-Golgi network to the plasma membrane. When Cx43 reaches the plasma membrane, it has oligomerized into connexons. Newly synthesized connexons are added to the peripheries of existing gap junctions and dock with connexons in the adjacent cell. During endocytosis of gap junctions, both membranes of the junction are internalized into one of the adjacent cells and form a connexosome. Degradation of connexosomes occurs in lysosomes, either by direct fusion between the two organelles or by maturation of the connexosome into a multivesicular endosome and trafficking of connexins via early and late endosomes prior to connexin degradation in lysosomes. Degradation of Cx43 gap junctions is also dependent on proteasomal activity. Phosphorylation of Cx43 is thought to act as a signal for recruitment of an E3 ubiquitin ligase, e.g., Nedd4, which ubiquitinates Cx43. Nedd4 can also bind to Cx43 independently of the Cx43 phosphorylation state. In addition to a ubiquitin-dependent pathway for Cx43 endocytosis, a ubiquitin-independent pathway exists, in which the YXXϕ motif in the Cx43 C terminus is important

the plasma membrane that are not part of gap junction plaques, also called hemichannels, may have important functions that do not involve direct intercellular communication (Stout, Goodenough & Paul, 2004).

Gap junctions are dynamic structures. Connexons are continually added to the edges of existing gap junctions, while channels are removed by endocytosis from the center of the gap junction (Gaietta et al., 2002; Lauf et al., 2002). Connexins usually have a high turnover rate, with a half-life of 1.5–5 h (Crow et al., 1990; Fallon & Goodenough, 1981; Laird, Puranam & Revel, 1991; Traub et al., 1989). During endocytosis of gap junctions, both membranes of the junction are internalized into one of the adjacent cells and thereby form a double-membrane vacuole called an annular gap junction or connexosome (Jordan et al., 2001; Laird, 2006; Larsen & Hai, 1978). Endocytosis of gap junctions has been suggested to involve clathrin (Larsen et al., 1979; Leithe & Rivedal, 2004a; Piehl et al., 2007). Internalization of gap junctions requires proteasomal activity (Fernandes, Girao & Pereira, 2004; Laing et al., 1997; Laing & Beyer, 1995; Leithe & Rivedal, 2004a,b Musil et al., 2000; Qin et al., 2003). However, the precise role of the proteasome in gap junction endocytosis is not known.

Following internalization of gap junctions, connexins are degraded in lysosomes (Jordan et al., 2001; Laing et al., 1997; Lan et al., 2005; Larsen & Hai, 1978; Mazet, Wittenberg & Spray, 1985; Naus et al., 1993; Qin et al., 2003; VanSlyke et al., 2000). Some electron microscopic studies suggest that connexosomes are able to fuse directly with lysosomes (Murray et al., 1981; Risinger & Larsen, 1983; Vaughan & Lasater, 1990). Other electron microscopic studies have shown that connexosomes sometimes contain single membranes, presumably representing connexosomes that have started their degradation process (Gregory & Bennett, 1988; Larsen & Hai, 1978; Mazet et al., 1985; Severs et al., 1989). Based on immunoelectron microscopic experiments, we have suggested that internalized Cx43 gap junctions undergo a maturation process from double-membrane vacuoles to multivesicular endosomes with a single limiting membrane (Leithe, Brech & Rivedal, 2006a). This transformation of the connexosome was found to be associated with trafficking of Cx43 to the early and late endosome, prior to degradation of Cx43 in lysosomes (Leithe et al., 2006a).

Most connexin isoforms are phosphoproteins. Phosphorylation of connexins occurs primarily in the C-terminal tail. Protein kinases that are involved in connexin phosphorylation include mitogen-activated protein kinase (MAPK), protein kinase C (PKC), protein kinase A (PKA), cdc2/cyclinB, casein kinase 1, v-src and c-src (Lampe & Lau, 2004). Phosphorylation of connexins regulates multiple steps in the life cycle of gap junctions, including connexin trafficking to the plasma membrane, assembly of connexons into gap junctions and gating of gap junction channels. There is also significant evidence that phosphorylation of connexins is important in regulating the internalization and degradation of gap junctions (Asamoto et al., 1991; Hossain, Ao & Boynton, 1998; Laird, 2005; Leithe & Rivedal, 2004a,b; Oh, Grupen & Murray, 1991). Modulation of the gap junction turnover rate has been suggested to be an important mechanism for regulating the level of GJIC (Berthoud et al., 2004; Laird, 2005; Musil et al., 2000; Thomas et al., 2003). There is increasing evidence that ubiquitin is a key protein in the regulation of gap junction degradation.

Ubiquitin

Ubiquitin is a 76-amino acid globular protein that is highly conserved in eukaryotic cells (Hershko & Ciechanover, 1998). Ubiquitin is able to be covalently conjugated to other proteins in a process called ubiquitination. Covalent conjugation of ubiquitin is essential for the proteolysis of most proteins, during both constitutive degradation and degradation as a result of changes in the cellular environment. Conjugation of ubiquitin to a protein can also regulate its activity or location. Ubiquitin is involved in a variety of cellular processes and is essential in regulating cell growth, proliferation and differentiation. Dysfunction of ubiquitin-mediated processes is causally related to various diseases, including malignant transformation (Glickman & Ciechanover, 2002).

Ubiquitin is conjugated to other proteins by forming a bond between the carboxy-terminal glycine of ubiquitin and the ε-NH_2 group of a lysine residue on the substrate protein. Ubiquitin can also be conjugated to the α-NH_2 group of the N-terminal amino acid of the substrate (Ben Saadon et al., 2004; Ciechanover & Ben Saadon, 2004). Ubiquitination is a multistep process that involves the sequential action of three classes of enzymes (Pickart, 2001). First, a ubiquitin-activating enzyme, called E1, forms a thiol-ester bond with the C terminus of ubiquitin (glycine76) in an adenosine triphosphate-dependent manner. Then, a ubiquitin-conjugating enzyme, known as E2, accepts ubiquitin from E1 by a *trans*-thiolation reaction. Finally, a ubiquitin ligase, known as E3, catalyses the transfer of ubiquitin from the E2 enzyme to the substrate protein.

Proteins destined for proteasomal degradation are usually conjugated to a polyubiquitin chain in which successive ubiquitins are linked by lysine48-glycine76 isopeptide bonds (Chau et al., 1989). Proteins can also be conjugated to a single ubiquitin, multiple monoubiquitins or polyubiquitin chains in which the ubiquitins are linked through

other lysine residues than lysine48, e.g., lysine63. Such protein modifications are involved in various nonproteasomal processes, e.g., DNA repair and endocytosis (Hofmann & Pickart, 1999; Spence et al., 2000).

In most organisms, including humans and yeast, there is one E1 enzyme (McGrath, Jentsch & Varshavsky, 1991; Zacksenhaus & Sheinin, 1990). There are several E2 family members. For instance, *Saccharomyces cerevisiae* encodes 13 E2s, while there are at least 25 E2s in mammalian cells (Weissman, 2001). Database analysis suggests that approximately 1,000 E3s exist in yeast and mammalian cells (Pickart & Eddins, 2004). Thus, the organization of the ubiquitin conjugating cascade is hierarchical (Glickman & Ciechanover, 2002). E3s are crucial in determining the timing and substrate selection in ubiquination reactions. Thus, E3s are the key regulatory determinants in the ubiquitination process. The E3 ubiquitin ligases can be classified into three major families: (1) HECT (homologous to E6-AP carboxy terminal) E3s, (2) RING (really interesting new gene) E3s and (3) the U-box E3s.

The discovery of the HECT E3s was a consequence of the finding that oncogenic human papillomaviruses encode a protein called E6 that specifically induces degradation of p53 (Mantovani & Banks, 2001). It was found that E6 serves as an adaptor between p53 and the cellular protein E6-associated protein (E6-AP). E6-AP was identified as a ubiquitin ligase that induces polyubiquitination and proteasomal degradation of p53 (Scheffner et al., 1990, 1993). Subsequently, a family of proteins that are closely related to E6-AP in an approximately 350-residue region at their carboxyl termini, termed the HECT domain, was identified (Huibregtse et al., 1995). Within the HECT domain is a strictly conserved cysteine residue that acts as a site for thiol ester formation with ubiquitin transferred from an E2. This cysteine residue is essential for substrate ubiquitination. Thus, HECT domain E3s participate directly in catalysis by forming a bond with ubiquitin during the ubiquitination reaction. Another feature sheared by many HECT E3s, but not E6-AP, is the WW domain, a short motif that can bind phosphoserine and phosphothreonine or PY (XPPXY, where P is proline, X is any amino acid and Y is tyrosine) motifs in the target protein and thereby mediate substrate recognition (Nguyen et al., 1998).

Besides E6-AP, the best-characterized HECT E3 is the essential *S. cerevisiae* enzyme Rsp5. Rsp5 ubiquitinates at least 13 plasma membrane channels and receptors (Dupre, Urban-Grimal & Haguenauer-Tsapis, 2004). For these substrates, the consequence of ubiquitination is internalization. Rsp5 also ubiquitinates soluble proteins, of which the best known is Rbp1, the large subunit of RNA polymerase II (Huibregtse, Yang & Beaudenon, 1997). In this case, ubiquitination leads to proteolysis by proteasomes. The properties of Rsp5 are conserved in mammalians. The mammalian orthologue of Rsp5 is Nedd4 (neural precursor cell expressed, developmentally downregulated 4) (Ingham, Gish & Pawson, 2004). The best-known substrate of Nedd4 is ENaC (amiloride-sensitive epithelial sodium channel). Nedd4 binds to a PY motif in ENaC via its WW domain and ubiquitinates lysine residues within the ENaC cytoplasmic region (Schild et al., 1996; Staub et al., 1996, 1997). This results in downregulation of the channel, presumably through internalization and degradation via the lysosomal pathway.

The second family of E3 ligases, the RING E3s, constitute the majority of the E3 ligases. The RING E3s are characterized by a series of histidine and cysteine residues with a spacing that allows for the coordination of two zinc ions in a cross-brace structure called the "RING finger" (Vandemark & Hill, 2002). The RING fingers of E3s can bind E2s. Other domains of E3 bind the substrate. In contrast to the HECT E3s, the RING E3 does not bind ubiquitin but is thought to help transfer ubiquitin directly from E2 to the substrate by forming a tight bond between E2 and substrate.

RING E3s can be classified as either single subunits or multisubunits. The single-subunit E3s consist of just the RING finger E3 protein. For instance, the E3 Mdm2 (mouse double minute 2), a RING E3 responsible for constitutive ubiquitination of p53, contains both a p53-binding domain and an E2-binding RING finger (Capili et al., 2001; Fang et al., 2000). Another single-subunit E3 is Cbl (Casitas B-cell lymphoma). Cbl is critically involved in the ubiquitination and downregulation of receptor tyrosine kinases, including the epidermal growth factor (EGF) and platelet-derived growth factor receptors (Joazeiro et al., 1999; Levkowitz et al., 1999). Deletions that disrupt the Cbl RING abrogate EGF-induced degradation, and these mutant proteins can be oncogenic.

In multisubunit E3s, the RING finger protein is one subunit of a multiprotein complex (Petroski & Deshaies, 2005). These E3s contain a RING finger subunit that functions in E2 recruitment. In addition, they contain a member of the Cullin protein family that binds the RING finger protein and structural adaptors that link the Cullin to substrate recognition elements. The archetypal multisubunit E3s are the SCF (Skp1-Cullin1-F-box protein) ubiquitin ligases (Feldman et al., 1997; Skowyra et al., 1997). SCF E3s have important roles in the regulation of the G_1/S cell cycle transition.

The third family of E3 ligases, the U-box E3s, is a relatively small group (Cyr, Hohfeld & Patterson, 2002). Similar to RING E3s, the U-box E3s function as bridging factors between the E2 and the substrate. Many known substrates of U-box E3s are misfolded proteins, including the fibrosis transmembrane receptor and the glucocorticoid receptor (Connell et al., 2001).

The ubiquitination of proteins is often tightly regulated (Gao & Karin, 2005). Several mechanisms are known to underlie this regulation. For instance, the activity of E3 may be turned on by E3 phosphorylation. Ubiquitination of a substrate can also be regulated by covalent modifications of the substrate that affect the interaction between E3 and the substrate. E3 ligases have substrate-interacting domains that recognize a specific sequence or structural element in the substrate. Modifications of the E3-binding domains of the substrate, e.g., by phosphorylation, glycosylation, acetylation or hydroxylation, are known to modulate the recognition of substrates by their cognate E3s (Fang & Weissman, 2004). This increases the repertoire of substrates that can be targeted to a given ligase and links protein ubiquitination and turnover to numerous signaling pathways.

Ubiquitination is a dynamic and reversible process. Deubiquitinating enzymes cleave ubiquitin from proteins and disassemble ubiquitin chains (Wing, 2003). Since ubiquitin is a long-lived protein and is normally reused several times, deubiquitinating enzymes play crucial roles in many cellular processes.

Ubiquitination of Cx43

Laing & Beyer published in 1995 the first evidence that ubiquitin is involved in Cx43 degradation. The authors used the Chinese hamster ovary cell line CHO-ts20, which expresses a thermolabile E1 ubiquitin-activating enzyme. It was found that the Cx43 protein level was increased under conditions in which the ubiquitin-activating enzyme was defective, indicating the involvement of ubiquitin in Cx43 degradation (Laing & Beyer, 1995). The data also indicated that ubiquitin can be conjugated to Cx43. It was suggested that ubiquitination of Cx43 could play a role both in ERAD of Cx43 and in Cx43 endocytosis (Laing et al., 1997; Laing & Beyer, 1995).

Subsequent experiments in our laboratory indicated that ubiquitination of Cx43 can be regulated by phosphorylation (Leithe & Rivedal, 2004a). As a model system to study endocytosis of Cx43 gap junctions, we used the rat liver epithelial cell line IAR20, which under normal growth conditions expresses relatively high levels of Cx43 endogenously, most of which is organized as gap junctions at the plasma membrane. In agreement with previous studies, EGF-induced phosphorylation of Cx43 was found to be associated with inhibition of gap junction channels. EGF was also found to induce internalization and degradation of Cx43 gap junctions. We hypothesized that EGF-induced phosphorylation is a signal for conjugation of ubiquitin to Cx43. Coimmunoprecipitation studies supported this hypothesis (Leithe & Rivedal, 2004a). The EGF-induced ubiquitination of Cx43 was found to occur concomitantly with Cx43 hyperphosphorylation and internalization of Cx43 gap junctions. The EGF-induced hyperphosphorylation, ubiquitination, internalization and degradation of Cx43 were found to be mediated by the MAPK pathway. Based on these observations, we hypothesized that phosphorylation of Cx43 could act as a binding site for a ubiquitin ligase, which would induce Cx43 ubiquitination (Leithe & Rivedal, 2004a). It is currently unclear whether the ability of EGF to induce ubiquitination and endocytosis of Cx43 gap junctions is specific to rat liver epithelial cells or whether this also occurs in other cell types. However, heparin-binding EGF has been suggested to induce internalization and degradation of Cx43 in cardiomyocytes (Yoshioka et al., 2005).

The tumor-promoting PKC activator 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induces hyperphosphorylation of Cx43 and inhibition of GJIC in several cell types (Berthoud et al., 1993; Enomoto et al., 1984; Leithe et al., 2003; Murray & Fitzgerald, 1979; Yotti, Chang & Trosko, 1979). TPA also induces loss of Cx43 at the plasma membrane and Cx43 degradation (Asamoto et al., 1991; Leithe & Rivedal, 2004b; Rivedal, Yamasaki & Sanner, 1994). The TPA-induced degradation of Cx43 was associated with strongly increased ubiquitination of Cx43 (Leithe & Rivedal, 2004b). The TPA-induced ubiquitination of Cx43 involved both PKC and the MAPK pathway. Coimmunoprecipitation studies indicated that Cx43 is modified by one to four ubiquitins in response to TPA or EGF treatment. Experiments using antibodies that differentiate between mono- and polyubiquitinated proteins suggested that Cx43 under these conditions is modified by multiple monoubiquitins rather than a polyubiquitin chain (Leithe & Rivedal, 2004b).

A recent study by Leykauf et al. (2006) supports the hypothesis that phosphorylation and ubiquitination of Cx43 are tightly linked processes. Using the rat liver epithelial cell line WB-F344, the authors found that Cx43 binds the rat E3 ubiquitin ligase Nedd4 in both cell-free and cellular systems. All three WW domains of rat Nedd4 were observed to bind to rat Cx43. It was suggested that phosphorylation of Cx43 was not indispensable for Nedd4 binding. However, it was found that phosphorylation of Cx43 may modulate the binding of Nedd4. The Cx43 C-terminal sequence contains a proline-rich region corresponding to the consensus of a PY motif (Thomas et al., 2003). Such PY motifs have been shown to act as ligands for WW domain-containing proteins, including the Nedd4/Nedd4-like family of E3 ubiquitin ligases (Ingham et al., 2004). Leykauf et al. (2006) found that WW2 is the only rat Nedd4 WW domain that binds robustly to the PY motif of Cx43.

The PY motif of Cx43 is overlapped by a tyrosine-based sorting signal conforming to the consensus of a YXXϕ

(where Y is tyrosine, X is any amino acid and ϕ is an amino acid with a bulky hydrophobic side chain) motif (Thomas et al., 2003). This motif is found in the cytosolic domains of several plasma membrane proteins (Bonifacino & Traub, 2003). The motif is involved in mediating internalization from the plasma membrane as well as targeting certain proteins to lysosomes (Canfield et al., 1991; Williams & Fukuda, 1990). Similarly to other tyrosine-based sorting signals, the YXXϕ motif mediates endocytosis by interacting with components of coated domains involved in protein sorting, localized at the plasma membrane or on endosomes (Bonifacino & Traub, 2003). For instance, YXXϕ signals have been shown to interact with AP-2, an adaptor protein found in clathrin coats at the plasma membrane (Honing et al., 1996; Ohno et al., 1995). Thomas et al. (2003) have shown that the tyrosine residue at position 286, which is essential for the function of both the PY and the YXXϕ motifs, is a crucial amino acid involved in turnover of Cx43 transfected in the human hepatocellular carcinoma cell line SKHep1. A mutational analysis of the region around tyrosine286 suggested that the YXXϕ motif plays a major role in regulating Cx43 endocytosis. Substitution of the proline residue at position 283 in the PY motif was found to have only a minor effect on Cx43 endocytosis. These results suggest that in SKHep1 cells the tyrosine-based motif is more important than the PY motif in Cx43 turnover under normal growth conditions.

When Cx43 endocytosis is counteracted by hypertonic medium, which blocks clathrin-mediated endocytosis, ubiquitinated forms of Cx43 accumulate (Leithe & Rivedal, 2004a). Based on these observations, we have suggested that Cx43 ubiquitination occurs at the plasma membrane. Leykauf et al. (2006) found that depletion of Nedd4 by RNA interference caused accumulation of Cx43 gap junctions at the plasma membrane. It was also found that Nedd4 colocalized with Cx43 both at the plasma membrane as well as in intracellular vesicles. Taken together, these results suggest that ubiquitin might play a role in the internalization of Cx43 gap junctions. Further studies are required to define the role of ubiquitin in Cx43 gap junction endocytosis.

Internalization of Cx43 gap junctions requires proteasomal activity (Fernandes et al., 2004; Laing et al., 1997; Laing & Beyer, 1995; Leithe & Rivedal, 2004a,b; Musil et al., 2000; Qin et al., 2003). However, the precise role of the proteasome in gap junction endocytosis is not known. Proteasomal inhibitors were found to counteract TPA- and EGF-induced ubiquitination of Cx43 (Leithe & Rivedal, 2004b). This opens the possibility that the proteasome might play an indirect role in endocytosis of Cx43 by affecting the Cx43 ubiquitination level. However, the proteasome might also play additional roles in gap junction endocytosis, and further studies are required to understand the function of the proteasome in Cx43 degradation.

Ubiquitin and Cx26

OCP1 (organ of Corti protein1) is abundantly expressed in the organ of Corti (Chen et al., 1995; Henzl et al., 2001, 2004). Sequence data suggest that OCP1 harbors a consensus F-box motif and is a subunit of a SCF E3 ubiquitin ligase (Henzl et al., 2001, 2004). Interestingly, Henzl et al. (2001, 2004) have shown that OCP1 binds Cx26, one of the major gap junction proteins in the epithelial support complex of the organ of Corti. Further studies are needed to determine the OCP1 binding site on Cx26 and the role of this interaction in Cx26 trafficking.

Conclusions

Several lines of evidence indicate that ubiquitination of Cx43 is important in regulating Cx43 degradation. Ubiquitination of Cx43 appears to occur at the plasma membrane. Thus, ubiquitin might be involved in the internalization process of Cx43 at the plasma membrane but could also be involved in the subsequent intracellular trafficking of Cx43. An important subject for future studies will be to determine what role ubiquitin might play in Cx43 gap junction endocytosis. In this regard, it will also be important to define the ubiquitin conjugating sites on Cx43.

Dysfunctional intercellular communication via gap junctions has been implicated as an important factor in many pathological conditions, including cancer (Mesnil, 2002; Wei et al., 2004). Often, such loss of gap junctional communication can be attributed to aberrant trafficking of connexins (Leithe et al., 2006b). It will in the future be important to investigate whether this dysfunction in connexin trafficking can be due to aberrant connexin ubiquitination.

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