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Review

Degradation of connexins and gap junctions

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ABSTRACT

Connexin proteins are short-lived within the cell, whether present in the secretory pathway or in gap junction plaques. Their levels can be modulated by their rate of degradation. Connexins, at different stages of assembly, are degraded through the proteasomal, endo-/lysosomal, and phago-/lysosomal pathways. In this review, we summarize the current knowledge about connexin and gap junction degradation including the signals and protein–protein interactions that participate in their targeting for degradation.

1. Introduction

Connexins have an unexpected short half-life of only 1–5 h even when their half-lives have been determined in tissues [1–5]. It is still puzzling that connexins, either unassembled or assembled into connexons (hemichannels) and especially when assembled and localized at gap junction plaques have – and apparently need – such a fast turnover. The degradation of connexins, connexons, and gap junctions, and alterations of their turnover accompany various physiological and pathological conditions (e.g., cell migration, mitosis, ischemia, etc.). This was discussed at one of the roundtables (themed "Internalization and degradation pathways of connexins and gap junctions") that were entertained at the 2013 International Gap Junction Conference (Charleston, SC, http://academicdepartments.musc.edu/igjc2013) and chaired by the senior authors of this article. Discussed topics, emerging concepts and hypotheses, and other relevant issues related to the turnover of connexins and gap junctions are summarized here.

Gap junction plaques are internalized from the plasma membrane as vesicle-like double-membrane structures termed annular gap junctions. The first electron microscopy images of these structures were published by Bjorkman from granulosa cells of the ovarian follicle [6]. Numerous other ultrastructural studies have shown the presence of annular gap junctions in several cell types and differentiating tissues [7–12]. A few ultrastructural studies reported annular gap junctions enclosed within double-membrane structures, which would be consistent with autophagic degradation of endocytosed gap junctions [9,13,14]. Live-imaging studies have also shown gap junction internalization [15–18]. Their internalization as double-membrane structures is in agreement with studies reporting that docked hemichannels (or connexons) in gap junction plaques appear inseparable under physiological conditions [19,20]. This internalization occurs through a combined endo/exocytic process [21], because one of the cells acts as an acceptor and the other acts as a donor (Fig. 1).

Studies of wild type and mutant connexins have addressed their subcellular localization, channel function, internalization and/or turnover in transfected cells. The turnover of connexins is determined by the rates of synthesis and degradation, which occurs through the proteasomal and the lysosomal pathways. Several reviews on different aspects of connexin and gap junction degradation have been recently published [22–26].

This review will focus on the roles of these pathways on degradation of connexins within the secretory pathway vs. those in gap junction plaques, and the signals and protein–protein interactions that participate in targeting connexins for degradation through the proteasomal vs. the lysosomal pathways. First, we will briefly describe the different pathways and then describe the role of these pathways in the degradation of connexins and gap junctions localized in different cellular compartments.

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2. Proteasomal and lysosomal degradation pathways

2.1. The 26S proteasome

The 26S proteasome is a cytoplasmic protein complex formed by a barrel-shaped 20S core particle, which contains the proteolytic activity, and two 19S regulatory particles, one on each side of the core particle. The regulatory particle is composed of a ring-shaped base made of six ATPases and a lid [27]. Several proteasome inhibitors have been used to study the function of the proteasome including ALLN (N-Acetyl-L-leucyl-L-leucyl-L-norleucinal), lactacystin, ZL_3VS (carboxybenzyl-leucyl-leucyl-leucine vinyl sulfone), epoxomicin and bortezomib [28,29].

Degradation of cellular proteins by the proteasome can be ubiquitin-dependent or ubiquitin-independent. Modification of proteins by ubiquitin, a three-step process, results from the activity of an ubiquitin-activating enzyme (E1, the rate-limiting step), an ubiquitin-conjugating enzyme (E2) and an ubiquitin-protein ligase (E3). This modification can be attached to the protein as one (mono) or several (multiple mono) single ubiquitin molecules, or as multimers of ubiquitin (poly-ubiquitin chain). Although lysine48-conjugated ubiquitin chains have been associated with targeting proteins for proteasomal degradation, more recent studies suggest that these poly-ubiquitin chains can be formed by linkage of ubiquitin molecules between one of the six other lysines available for conjugation. Although it was initially thought that ubiquitination occurred only on lysine residues of the target protein, ubiquitination has been found in threonines, serines or cysteines and the amino group of the N-terminal residue of the protein targeted for proteasomal degradation (reviewed in [30]). It has been hypothesized that the variety of proteasome-targeting ubiquitin motifs may determine the degradation rate of the target protein.

2.2. Lysosomal degradation pathways

Cells have evolved two degradation pathways that depend on the activities of lysosomal enzymes, endo-/lysosomal and phago-/lysosomal (termed macroautophagy, or simply, autophagy). The endo-/lysosomal pathway is designed for the uptake of extracellular nutrients and factors via the formation and internalization of vesicles from the plasma membrane. These vesicles then fuse with endosomes and lysosomes to degrade the cargo.

Macroautophagy is a lysosomal degradation pathway essential for cell survival that is known to be activated by nutrient depletion. It is responsible for the degradation of structures already located in the cytoplasm such as non-functional organelles, protein aggregates, and invading pathogens [31–34]. During autophagy, materials targeted for degradation are first sequestered by an isolation membrane (phagophore) forming a double-membrane vesicle (autophagosome), which then fuses with a lysosome where the cargo is degraded.

Because lysosomes are involved in both the endo-/lysosomal and auto-/phagosomal degradation pathways, the manipulation of specific components allows these pathways to be distinguished. The identification of autophagy-related genes and experimental manipulation of their levels have allowed evaluation of the contribution of autophagy to connexin/gap junction degradation. The microtubule-associated protein 1 light chain 3 (LC3) is considered the most specific autophagosomal marker [35]. LC3 is proteolytically processed shortly after translation and converted into LC3-I. LC3-I is converted to LC3-II after being recruited to developing phagophores and covalently conjugated to phosphatidyl-ethanolamine of the phagophore membrane. LC3-II remains on autophagosomes for most of their lifetime [35,36]. Other autophagosome-related genes include Atg5, Atg6 (Beclin) and Atg7.

3. Signals involved in degradation of connexins

Several modifications including phosphorylation, ubiquitination, SUMOylation, methylation, acetylation, nitrosylation, and glutamate γ-carboxylation have been found in connexins (reviewed recently in [37,38]). Of these, phosphorylation and ubiquitination are known to be involved in protein degradation. Evidence that phosphorylation and ubiquitination also play a role in the internalization of gap junction plaques and degradation of connexins has been obtained. The role of phosphorylation in internalization and degradation of gap junctions has been reviewed [39].

3.1. Ubiquitination

Cx43 and avian Cx45.6 are polyubiquitinated [40,41]. In NRK cells and TPA-treated rat liver epithelial IAR20 cells, Cx43 is multiply mono-ubiquitinated [42,43]. Several E3 ubiquitin ligases are involved in ubiquitination of connexins. Nedd4, Smurf2 and TRIM21 are among the E3 ubiquitin ligases that interact with Cx43 [44–46]. All of them are associated with gap junction plaques (Fig. 2). Ubiquitination of connexins at gap junction plaques appears to have a role in endocytosis and/or intracellular vesicle trafficking [42,47]. An interaction of E3 ubiquitin ligases with connexins that are either rapidly degraded by endoplasmic reticulum-associated degradation or in their transit to the plasma membrane may not be revealed by immunofluorescence, because the connexins are not concentrated in a particular area (as they are in gap junction plaques).

Nedd4 interacts via its WW domains with the carboxyl-terminus of Cx43 and ubiquitinates Cx43 in gap junctions [42,46]. It has been proposed that the resulting ubiquitinated Cx43 recruits Eps15 (a member of the clathrin-coat-associated sorting proteins [CLASPs] (reviewed in [48]) to gap junction plaques and mediates their internalization [42]. CLASPs encode conserved peptide sequences that contact ubiquitin-moieties across their surface (termed ubiquitin interacting motifs [UIMs]) and thus ensure ubiquitin signal-specificity. Interestingly, alternative CLASPs such as Eps15/Eps15R and Epsin1/2 may replace the canonical CLASP, AP-2, allowing clathrin-mediated endocytosis to occur independent of AP-2.

The Nedd4-interacting protein 2 (NDFIP2) is a short-lived protein that is degraded in the lysosome. siRNA silencing of NDFIP2 decreased the number of gap junction plaques associated with a small increase in Cx43 half-life, whereas overexpression of NDFIP2 increased the number and size of gap junction plaques and decreased the half-life of Cx43 [49]. Thus, it is possible that Nedd4-interacting proteins like NDFIP2 may regulate internalization of Nedd4-ubiquitinated Cx43 (and other connexins) from the plasma membrane.

Smurf2 modulates Cx43 endocytosis and degradation [45]. Treatment of IAR20 cells with the tumor promoter, 12-O-tetradecanoylphorbol 13-acetate (TPA), a protein kinase C activator, promotes Cx43 phosphorylation and ubiquitination, recruitment of Smurf2 to gap junction plaques and increases interaction between these proteins [43,45,50,51]. Treatment with a siRNA against Smurf2 leads to an increase in the size of gap junction plaques and in gap junction intercellular communication, and to prevention of the TPA-induced decrease in Cx43 at gap junctions [45]. The ubiquitin-binding proteins, Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate) and Tsg101 (tumor susceptibility gene 101), components of the endosomal sorting complex required for transport (ESCRT) have been identified as important for targeting of annular gap junction-endosome intermediates for lysosomal fusion and degradation [47] (Fig. 2).

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