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Review

Endocytosis and post-endocytic sorting of connexins ☆

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ABSTRACT

The connexins constitute a family of integral membrane proteins that form intercellular channels, enabling adjacent cells in solid tissues to directly exchange ions and small molecules. These channels assemble into distinct plasma membrane domains known as gap junctions. Gap junction intercellular communication plays critical roles in numerous cellular processes, including control of cell growth and differentiation, maintenance of tissue homeostasis and embryonic development. Gap junctions are dynamic plasma membrane domains, and there is increasing evidence that modulation of endocytosis and post-endocytic trafficking of connexins are important mechanisms for regulating the level of functional gap junctions at the plasma membrane. The emerging picture is that multiple pathways exist for endocytosis and sorting of connexins to lysosomes, and that these pathways are differentially regulated in response to physiological and pathophysiological stimuli. Recent studies suggest that endocytosis and lysosomal degradation of connexins is controlled by a complex interplay between phosphorylation and ubiquitination. This review summarizes recent progress in understanding the molecular mechanisms involved in endocytosis and post-endocytic sorting of connexins, and the relevance of these processes to the regulation of gap junction intercellular communication under normal and pathophysiological conditions. This article is part of a Special Issue entitled: The Communicating junctions, composition, structure and characteristics.

Contents

Abbreviations: CHO, Chinese hamster ovary; CIP75, connexin43-interacting protein of 75-kDa; CIP85, connexin43-interacting protein of 85-kDa; Dab2, disabled-2; EEA1, early endosomal autoantigen 1; EGF, epidermal growth factor; ENaC, amiloride-sensitive epithelial sodium channel; ERAD, endoplasmic reticulum-associated degradation; ESCRT, endosomal sorting complex required for transport; HECT, homologous to E6-AP carboxy terminal; GPCR, G protein-coupled receptor; Hrs, hepatocyte growth factor-regulated tyrosine kinase substrate; MAPK, mitogen-activated protein kinase; Nedd4, neural precursor cell expressed, developmentally downregulated 4; NRK, normal rat kidney; OCP1, Organ of Corti protein 1; PLC β3, phospholipase Cβ3; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; RING, really interesting new gene; SCF, Skp1/Cul1/F-box complex; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; Tsg101, tumor-susceptibility gene product 101; ZO-1, Zonula occludens 1

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1. Introduction

Connexins are tetramembrane spanning proteins that are able to form channels between adjacent cells. These channels assemble into intercellular plasma membrane domains known as gap junctions [1]. The connexin gene family constitutes 20 members in humans [2,3]. Connexins are expressed in nearly all cell types, both during development and in the adult organism [4]. Most tissue types express several connexin isoforms. Gap junctions enable adjacent cells in solid tissues to directly exchange ions, such as K^+ and Ca^{2+}, second messengers, such as inositol triphosphate, ATP, cAMP, and cGMP, and small metabolites, including glucose and glutamate. Gap junctions have a fundamental role in tissues containing electrically excitable cells. For instance, gap junctions function as electrical synapses between neurons and have important roles in the synchronous contraction of the heart muscle by mediating electrical coupling between cardiomyocytes [5,6]. Gap junctions also have critical roles in non-excitable tissues, by regulating cell growth and differentiation, embryonic development and tissue homeostasis [7,8]. Loss of functional gap junction channels has been implicated as a causative factor in multiple diseases, including heart failure, neuropathology, deafness, skin disorders and cataracts [9]. There is also substantial evidence that loss of connexin expression is important in cancer development, and several members of the connexin gene family act as tumor suppressors [10,11].

Intercellular communication via gap junctions is controlled by multiple mechanisms. The most rapid regulation of gap junction channels is achieved by altering the conductance of single channels or their probability of opening [12]. Slower regulation of gap junctions can be achieved by changing the rate of connexin synthesis, trafficking of connexins to the plasma membrane or assembly of connexins into gap junctions [12]. There is also increasing evidence that modulation of connexin endocytosis and sorting to lysosomes may be critically involved in controlling gap junction intercellular communication [13–16]. Here, we review recent progress in understanding the mechanisms involved in the regulation of connexin endocytosis and post-endocytic sorting to lysosomes. We also discuss how modulation of the connexin endocytosis and degradation rates might play important roles in controlling the level of functional gap junctions under normal and pathological conditions.

2. Synthesis and trafficking of connexins to the plasma membrane

Connexin proteins span the membrane four times and have their amino and carboxy termini localized on the cytosolic side of the membrane [4]. The four transmembrane regions, the two extracellular loops and the amino terminus contain several identical residues among the various connexins. In contrast, the length and amino acid compositions of the carboxyl terminus and the intracellular loop vary extensively between connexins. Connexin proteins are most commonly referred to by their predicted molecular weight in kilodaltons. The best-studied connexin isoform is connexin 43 (Cx43).

Connexins are generally considered to be co-translationally inserted into the endoplasmic reticulum, although cell-free studies indicate that Cx26 can also insert into membranes post-translationally or directly into the plasma membrane [17–19]. Connexins with correct conformation are transported via the Golgi apparatus and the *trans*-Golgi network to the plasma membrane [20–23]. Cx26 may also follow an alternative route to the plasma membrane that bypasses the conventional secretory pathway [24–26]. Along their trafficking from the endoplasmic reticulum to the plasma membrane, connexins oligomerize into hexameric structures that form a cylinder with an aqueous pore extending through the channel, called a connexon. Connexons can consist of six identical or different connexin isotypes, termed homomeric and heteromeric connexons, respectively [4]. In contrast to the oligomerization of most other multisubunit integral membrane proteins, Cx43 and Cx46 oligomerize after they have left the endoplasmic reticulum, probably in the *trans*-Golgi network [20,27,28]. On the other hand, Cx32 oligomerizes within the endoplasmic reticulum, suggesting that the site of oligomerization is isoform specific [29,30]. The trafficking of connexons from the *trans*-Golgi network to the plasma membrane is mediated by microtubules [31–34]. A recent study by del Castillo et al. shows that transport of connexons from the trans-Golgi network to the plasma membrane is regulated by the integral membrane protein consortin [35].

At least 40% of newly synthesized wild-type Cx43 and Cx32 and up to 100% of some mutant forms of Cx32 have been estimated to undergo endoplasmic reticulum-associated degradation (ERAD) [36,37]. Although Cx32 and Cx43 are the only connexin isoforms known to undergo ERAD, it is anticipated that ERAD is a general mechanism underlying quality control of connexins. The degradation of Cx32 and Cx43 at the endoplasmic reticulum is strongly affected by various cytosolic stressors [37]. Oxidative or thermal stress inhibits dislocation to the cytosol and subsequent proteasomal degradation of Cx32 and Cx43 [37]. The wild-type connexin protein escaping ERAD in response to cytosolic stress remains in a full-length, membrane-integrated form, and is able to undergo trafficking via the secretory pathway and form functional gap junctions at the plasma membrane [37]. Reducing connexin ERAD has been hypothesized to be a mechanism for upregulating gap junctional communication under normal physiological conditions and under pathological conditions, such as ischemia-reperfusion injury and fever [38]. In line with the notion that controlled ERAD of connexins may have important physiological roles, androgen depletion has been shown to induce degradation of a major fraction of Cx32 and Cx43 at the endoplasmic reticulum in human prostate cancer cells [39]. The enhanced degradation of connexins in response to androgen depletion is associated with reduced gap junctional intercellular communication. The degradation of Cx43 at the endoplasmic reticulum is regulated in part by CIP75 (connexin43-interacting protein of 75-kDa) [40]. CIP75 belongs to the UbL (ubiquitin-like)-UBA (ubiquitin-associated) protein family, which is involved in the translocation of proteins across the endoplasmic reticulum membrane, and in mediating the delivery of proteins to proteasomes during ERAD [41]. Recently, Su et al. found that CIP75 stimulates proteasomal degradation of Cx43 at the endoplasmic reticulum in a process independent of Cx43 ubiquitination [42].

3. Assembly of connexons into gap junctions

Connexons are delivered to the plasma membrane at non-gap junctional sites, and diffuse laterally to the periphery of the gap junction plaques where they dock with connexons in neighboring cells to form intercellular channels [23,33]. Docking between connexons occurs by a “lock and key” mechanism involving six protrusions from each connexon [4,43,44]. By electron microscopy, gap junctions can be identified as intercellular areas in which the two plasma membrane domains are tightly apposed to each other. The two membranes of a gap junction appear to be separated by an extracellular gap of 2–4 nm for which the junction is named [45,46]. The conductance and selectivity of a gap junction channel is strongly affected by its connexin composition. Connexons in one cell can dock with connexons consisting of different connexin isoforms in the adjacent cell to form a so-called heterotypic gap junction channel [1]. The connexin composition of the gap junction channels differs significantly between cell and tissue types. Different combinations of connexin isoforms enable the various cell types to form gap junctions with different conductive and selective properties. Moreover, expressing several connexin isoforms might have a compensating role, enabling cells to express functional gap junctions even if one of the isoforms is mutated or lost. Although the best-known function of connexins is to form gap junction channels, substantial evidence indicates that connexons at the plasma membrane that are not assembled in gap junctions may