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Review

Regulation of connexins by the ubiquitin system: Implications for intercellular communication and cancer

Edward Leithe *

Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital, and Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Oslo, Norway

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ABSTRACT

The connexins constitute a family of integral membrane proteins that form intercellular channels, enabling adjacent cells to directly exchange ions and small molecules. The connexin channels assemble into distinct plasma membrane domains known as gap junctions. Intercellular communication via gap junctions has an important role in regulating cell growth and differentiation, as well as in maintaining tissue homeostasis. Connexin43 (Cx43), the most ubiquitously expressed connexin isoform in human tissues, has been shown to act as a tumor suppressor and is frequently downregulated during cancer development. Cx43 has a short half-life, and modulation of the Cx43 turnover rate represents an important mechanism by which the level of gap junctional intercellular communication is regulated under basal conditions. Moreover, many growth factors, oncogenes, and tumor promoters are potent inducers of Cx43 endocytosis and endolysosomal degradation, resulting in loss of gap junctions. Emerging evidence indicates that the ubiquitin system has a major role in these processes. Recent studies have shown that ubiquitination is also involved in the autophagy-mediated degradation of Cx43 in a process mediated by the proto-oncogenic E3 ubiquitin ligase NEDD4. Moreover, ubiquitination of connexins has been implicated in modulating the level of intercellular communication via gap junctions in response to oxidative stress. This review article provides an overview of our current understanding of the role of the ubiquitin system in the regulation of connexins and discusses how the malfunction of these processes may contribute to the loss of intercellular communication via gap junctions during carcinogenesis.

Contents

Abbreviations: AMSH, associated molecule with the SH3 domain of STAM (signal transducing adaptor molecule); ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; CIP75, Cx43-interacting protein of 75 kD; CIP85, Cx43-interacting protein of 85 kDa; Cx, connexin; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transition; Eps15, epidermal growth factor receptor substrate 15; ERAD, endoplasmic reticulum-associated degradation; ESCRT, endosomal sorting complex required for transport; HECT, homologous to E6-AP carboxy terminal; Hrs, hepatocyte growth factor-regulated tyrosine kinase substrate; IP_3, inositol trisphosphate; IGF-1, insulin-like growth factor-1; LC-MS/MS, liquid chromatography tandem mass spectrometry; MAPK, mitogen-activated protein kinase; NEDD4, neural precursor cell-expressed developmentally downregulated gene 4; OCP1, organ of Corti protein 1; PKC, protein kinase C; PTEN, phosphatase and tensin homolog; RING, really interesting new gene; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SCF, Skp1–Cullin–Fbox; siRNA, small interfering RNA; SMURF2, SMAD ubiquitination regulatory factor-2; SUMO, small ubiquitin-related modifier; TGF-β, transforming growth factor-β; TPA, 12-O-tetradecanoylphorbol-13-acetate; Tsg101, tumor susceptibility gene 101 protein; ZO-1, zonula occludens-1.

* Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital, P.b. 4950 Nydalen, NO-0424 Oslo, Norway.
E-mail address: eleithe@rr-research.no.

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1. Introduction

The connexins constitute a family of transmembrane proteins that form channels between adjacent cells, enabling the direct intercellular exchange of ions and small molecules (< ~1.5 kD) [1]. These channels assemble into distinct plasma membrane domains known as gap junctions [2]. In humans, the connexin protein family constitutes 21 members, of which the most ubiquitously expressed is connexin43 (Cx43) [3]. The molecules transferred between cells via gap junction channels include nucleotides, amino acids, sugars, and signaling mediators such as inositol trisphosphate (IP_3), cyclic adenosine monophosphate (cAMP) and adenosine triphosphate (ATP) [1]. Gap junctions enable neighboring cells to communicate both electrically and metabolically, and they are considered to have essential roles in coordinating the activities of individual cells in tissues [1]. A large body of experimental work suggests that connexins are important in controlling cell growth and differentiation and that dysregulation of connexins and intercellular communication via gap junctions is involved in cancer pathogenesis [4–8]. In line with this notion, several members of the connexin protein family have been shown to act as tumor suppressor proteins, and they are frequently downregulated during cancer development [6].

Despite their highly complex organization, gap junctions are dynamic plasma membrane structures [9]. The connexin pool that constitutes the gap junctions is continuously replaced as newly synthesized connexins are added to the edges of gap junctions while older connexins are removed from the center of the junction by endocytosis [10,11]. Following endocytosis, connexins may undergo degradation in lysosomes or be recycled to the plasma membrane [9,12–14]. Considering that they are transmembrane proteins, connexins have a high turnover rate, exhibiting a half-life of only 1.5 to 5 hours in most tissue types [15–18]. Modulation of the connexin turnover rate has been suggested to be an important mechanism by which cells regulate the level of functional gap junctions under basal conditions [9,12,14,19–23]. Moreover, many growth factors, oncogenes, and tumor promoters are potent inducers of connexin endocytosis and degradation, resulting in the loss of gap junctions at the plasma membrane [5,6]. The accurate regulation of connexin degradation has also been implicated in modulating the level of intercellular communication via gap junctions in response to oxidative stress [14,24]. The emerging picture is that the ubiquitin system has a central role in controlling the level of functional gap junctions at the plasma membrane under basal conditions, in response to exposure to growth factors or tumor promoters, and in response to oxidative stress. This review article provides an overview of our current understanding of the role of the ubiquitin system in the regulation of connexins and discusses how the malfunction of these processes may contribute to an aberrant expression of connexins and the loss of intercellular communication via gap junctions during carcinogenesis.

2. The life cycle of connexins

Connexins span the membrane four times, and the N- and C-termini are localized on the cytosolic side of the membrane [2] (Fig. 1A). Connexins are cotranslationally inserted into the endoplasmic reticulum [25]. A subpool of newly synthesized connexins undergoes endoplasmic reticulum-associated degradation (ERAD), which is mediated by proteasomes [24,26]. Connexins that are spared from ERAD are transported via the Golgi apparatus and the *trans*-Golgi network to the plasma membrane. During their sorting to the plasma membrane, connexins oligomerize into hexameric structures called connexons, which may consist of six identical or different connexin isotypes [2] (Fig. 1B). At the plasma membrane, the connexons are able to dock with connexons in the adjacent cell to form intercellular channels (Fig. 1B).

Following their assembly into gap junctions, connexons migrate toward the center of the gap junction, where they are removed by endocytosis [10,11]. During endocytosis of gap junctions, both membranes of the junction are internalized into one of the adjacent cells, forming a double-membrane vacuole called an annular gap junction or connexosome [27–32] (Fig. 2). Endocytosis of Cx43 gap junctions is mediated by clathrin and dynamin [31–34]. The process also involves CIP85 (Cx43-interacting protein of 85 kDa), a putative Rab GTPase-activating protein [35,36]. After gap junction internalization, connexins may undergo degradation in lysosomes [20,26–28,30–32,37–39] (Fig. 2). Connexins may follow different pathways en route to lysosomes [9,12,40–42]. One pathway involves the fusion between annular gap junctions and lysosomes [22,38,43,44]. A second pathway involves the engulfment of the annular gap junctions by autophagosomes, which subsequently fuse with lysosomes [45–48]. Notably, connexins are not only targets for autophagosomal degradation, but they are also involved in modulating autophagosome biogenesis [49]. In a third degradation pathway, annular gap junctions may undergo a maturation process to form connexin-enriched multivesicular endosomes, followed by the sorting of connexins to lysosomes via the endolysosomal pathway [30,50,51]. In addition to their role in mediating degradation of connexins during ERAD, proteasomes are involved in modulating the sorting of connexins from the plasma membrane to lysosomes [19–22].

Increasing evidence suggests that connexons may undergo recycling to the plasma membrane following internalization [13,14,45]. Mild hyperthermia or oxidative stress has been shown to negatively regulate the postendocytic delivery of connexons to lysosomes and instead promote the recycling of connexons back to the plasma membrane, where they can participate in the formation of functional gap junctions [14]. Thus, the postendocytic sorting of connexons may serve as a control point to modulate gap junction levels. The fraction of the internalized connexin pool that is subjected to recycling may also vary according to the cell-cycle stage. For instance, Cx43 has been shown to undergo recycling to the plasma membrane in late phases of mitosis, a process that may be important for the rapid reassembly of gap junctions after cytokinesis [13]. It was recently reported that entire annular gap junctions are also able to recycle to the plasma membrane to form gap junctions [45].

Gap junction channels are tightly regulated in response to a variety of stimuli, including changes in calcium concentration, pH, and voltage [52]. Intercellular communication via gap junctions is also controlled by post-translational modifications of connexins [53]. Phosphorylation regulates several steps in the life cycle of connexins, including the assembly of connexons into gap junctions, gating of gap junction channels, and gap junction internalization and degradation [54,55]. Among the protein kinases known to regulate connexin phosphorylation are mitogen-activated protein kinase (MAPK), protein kinase C