FEBS Letters xxx (2014) xxx–xxx

FEBS Letters
journal homepage: www.FEBSLetters.org

Review

Roles and regulation of lens epithelial cell connexins

Viviana M. Berthoud *, Peter J. Minogue, Patricia Osmolak, Joseph I. Snabb, Eric C. Beyer

Department of Pediatrics, Pritzker School of Medicine, University of Chicago, Chicago, IL 60637, United States

ARTICLE INFO

Article history:
Received 27 November 2013
Revised 19 December 2013
Accepted 30 December 2013
Available online xxxx

Edited by Wilhelm Just

Keywords:
Connexin43
Connexin50
Cataract
Lens

ABSTRACT

The avascular lens of the eye is covered anteriorly by an epithelium containing nucleated, metabolically active cells. This epithelium contains the first lens cells to encounter noxious external stimuli and cells that can develop compensatory or protective responses. Lens epithelial cells express the gap junction proteins, connexin43 (Cx43) and connexin50 (Cx50). Cx43 and Cx50 form gap junction channels and hemichannels with different properties. Although they may form heteromeric hemichannels, Cx43 and Cx50 probably do not form heterotypic channels in the lens. Cx50 channels make their greatest contribution to intercellular communication during the early postnatal period; subsequently, Cx43 becomes the predominant connexin supporting intercellular communication. Although epithelial Cx43 appears dispensable for lens development, Cx50 is critical for epithelial cell proliferation and differentiation. Cx43 and Cx50 hemichannels and gap junction channels are regulated by multiple different agents and likely contribute to both normal lens physiology and to pathology.

1. Introduction

The lens is a transparent organ whose main function is to transmit light and focus it on the retina. It is suspended between the aqueous humor and the vitreous. The cells of the lens communicate through an extensive network of gap junctions that are critical for cell homeostasis and maintenance of transparency, since the lens has no direct blood supply.

The lens contains two cell types: epithelial cells that constitute a single layer along the anterior surface and fiber cells that form the bulk of the organ. These two cell types originate during embryogenesis from the lens vesicle when cells in the posterior region elongate to form the primary fibers. Afterwards, epithelial cells near the lens equator differentiate into fiber cells. Epithelial-to-fiber cell differentiation involves cell elongation and loss of nuclei and organelles and occurs throughout the lifespan of the organism. Connexin46 (Cx46) and connexin50 (Cx50) are the two most abundant gap junction proteins in lens fiber cells [1,2]. These two connexins co-localize at gap junction plaques and can form mixed hexamers [1,3]. Substantial attention has been paid to Cx46 and Cx50, since cataracts develop in people or animals with mutations of these genes and in "knock-out" mice. These connexins and their roles in the lens have been recently reviewed [4].

This review will focus on the role of connexins for epithelial cell function. These cells are critically important for the lens, since they contain most of its metabolic, synthetic and active transport machinery [5]. Moreover, since fiber cells lose their nuclei, the epithelial cells are the only lens cells capable of proliferation. Thus, the division of these cells directly contributes to lens growth. Proliferation and differentiation of lens epithelial cells are influenced by various growth factors (including FGFs, BMPs, and TGFβ) and signaling cascades (including MAPK/ERK and Wnt/Fz) (reviewed by [6]).

2. Connexins expressed in lens epithelial cells

The gap junctions between epithelial cells are morphologically and physiologically distinct from those between fiber cells. Electron micrographs have shown that epithelial gap junctions contain tightly grouped connexons (with a near crystalline organization similar to junctions between cardiac myocytes or hepatocytes) while those between fiber cells are more randomly dispersed [7,8]. While the basis for the "crystalline" appearance of gap junctions is poorly understood (despite multiple electron microscopy studies), this difference suggested that epithelial and fiber cell gap junctions might have different protein components and might have some different physiological properties [8]. Epithelial cell gap junctions are differentially regulated from those between fiber cells; for instance, they are more sensitive to closure in response to cytoplasmic acidification [8]. Two connexins are extensively expressed by epithelial cells, Cx43 and Cx50. Immunofluorescence studies show that the distributions of Cx43 and Cx50 are substantially overlapping, with some gap junction plaques containing both connexins and others containing only Cx43 or Cx50 (Fig. 1 and [9]).

* Corresponding author. Address: Department of Pediatrics, University of Chicago, 900 East 57th St., KCBD 5150, Chicago, IL 60637, United States. Fax: +1 (773) 834 1329.
E-mail address: vberthou@peds.bsd.uchicago.edu (V.M. Berthoud).

0014-5793/$36.00 © 2014 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
http://dx.doi.org/10.1016/j.febslet.2013.12.024

3. Cx43 and Cx50 channels

3.1. Gap junction channels

The properties of Cx43 and Cx50 channels in epithelial cells can be extrapolated from studies of these connexins performed in exogenous expression systems. Both Cx43 and Cx50 form functional homomeric/homotypic gap junction channels (i.e., two hemichannels composed of the same connexin isoform docked to each other), but these channels differ in some properties including voltage gating, single channel conductance and permeability. Cx50 channels are more sensitive to transjunctional voltage than Cx43 channels. The single channel conductance of the main state of Cx50 channels is about 220 pS [10] whereas that of Cx43 channels is about 100 pS [11,12]. Cx43 channels exhibit similar permeabilities to both cations and anions [13], but Cx50 channels are more permeable to cations than anions [10]. While both Cx43 and Cx50 make gap junction channels that are permeable to glutathione, the permeability of Cx43 channels to glutathione is greater [14,15]. These channels may have different relative size selectivities, since Cx43 channels are more permeable to some larger gap junction tracers (including Lucifer yellow and Alexa594) than ones formed of the chicken Cx50 ortholog [16]. The differences in voltage gating and permeability between gap junction channels formed of Cx43 and Cx50 are influenced by differences in N-terminal amino acids between these connexins [16].

It is not entirely clear whether Cx43 and Cx50 can combine to form functional channels containing both connexins. Gap junction channels containing two different connexins can be heterotypic (formed by the docking of hemichannels composed of different connexins) or heteromeric (formed by the mixing of two different connexins within a hemichannel). Cx43 and Cx50 do not form heterotypic channels in *Xenopus* oocyte pairs [17]. However, they may form heteromeric channels, since *Xenopus* oocytes co-injected with Cx43 and Cx50 cRNAs have lower junctional conductances than ones injected with either cRNA alone [18]. Moreover, some Cx50 mutants (Cx50P88S and Cx50S50P) do not localize to gap junction plaques when expressed by themselves, but they do so when transfected into cells that endogenously express Cx43 or when they are co-expressed with Cx43 [18,19]; this "rescue" of mutant protein trafficking by wild type Cx43 suggests that they may interact and form heteromeric connexons.

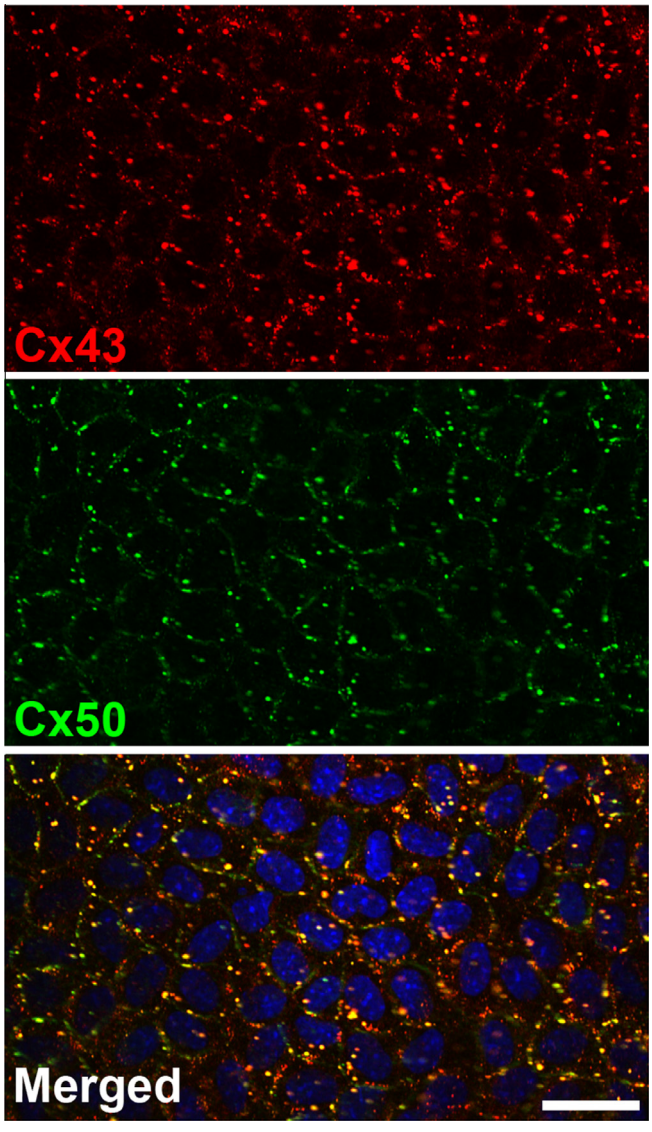

Fig. 1. Immunofluorescence of Cx43 and Cx50 in the epithelium. Confocal images showing the distribution of Cx50 (green) and Cx43 (red) in a flat mount of the epithelium removed from the lens of a 1.9 month old C3H mouse. These images illustrate the variations in relative proportions of Cx43 and Cx50 and their co-localization. In some areas, cells have an increased proportion of Cx43 whereas in other areas, cells show an increased proportion of Cx50 punctate staining. While some of the cells show a high degree of co-localization between the two connexins, others show a more uniform punctate staining with some co-localization between Cx43 and Cx50. Bar, xx μm.

3.2. Cx43 and Cx50 hemichannels

Both Cx43 and Cx50 can form functional hemichannels. They have primarily been studied in non-lens cells or in exogenous expression systems where hemichannel opening is induced by incubation in extracellular solutions containing very low concentrations of divalent cations.

Cx43 hemichannels have unitary conductances of ~220 pS (about twice the conductance of a single Cx43 intercellular channel) [20]. In addition to opening by exposure to low concentrations of extracellular divalent cations, Cx43 hemichannels open in response to metabolic inhibition, some cytokines, and oxidative stress [21]. Opening of Cx43 hemichannels is modulated by intracellular pH concentration and the phosphorylation status of the protein. Cx43 hemichannels are permeable to a variety of common dye tracers (like Lucifer yellow, ethidium, DAPI and propidium) and can allow the release of cytoplasmic small molecules (including ATP, glutamate, NAD^+, glutathione, PGE2, and ascorbate) [22,23].

The electrophysiological properties and regulation of Cx50 hemichannels have been extensively characterized. Cx50 hemichannels open in response to reduction of extracellular calcium and transmembrane depolarization; they are closed by extracellular acidification [24]. When expressed in *Xenopus* oocytes, Cx50 forms inwardly rectifying, high conductance (470 pS) single hemichannels [25]. In HeLa cells, the single channel conductance of the main state of Cx50 channels is 352 pS [26]. Hemichannels formed of Cx50 are also sensitive to extracellular monovalent cations. Replacement of extracellular Na^+ with K^+ (or other monovalent cations) potentiates Cx50 hemichannel current; apparently, K^+ reduces the ability of divalent cations like Ca^{2+} to close Cx50 hemichannels [27].

3.3. Pharmacology

Some of the relatively non-selective gap junction channel "blockers", like octanol, heptanol, flufenamic acid, and glycyrrhetinic acid derivatives inhibit both Cx43 and Cx50 homomeric/homotypic channels. However, Cx43 and Cx50 channels differ in some pharmacological properties. Cx50 gap junction channels are inhibited by quinine (IC50 73 μM), mefloquine (IC50 ~1.1 μM) and several of