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Connexin in Lens Physiology and Cataract Formation

Mauricio A. Retamal[1]*, Carmen G. León-Paravic[1], Christian A. Verdugo[5], Constanza A. Alcaino[1,2], Rodrigo Moraga-Amaro[3,4] and Jimmy Stehberg[3,4]

[1]*Laboratorio de Fisiología, Facultad de Medicina. Clínica Alemana - Universidad del Desarrollo, Santiago, Chile*
[2]*Laboratorio de Neurobiología, Departamento de Fisiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile*
[3]*Laboratorio de Neurobiologia, Departamento de Ciencias Biologicas, Facultad de Ciencias Biologicas & Facultad de Medicina, Universidad Andres Bello, Santiago, Chile*
[4]*Centro de Investigaciones Biomedicas, Facultad de Ciencias Biologicas & Facultad de Medicina, Universidad Andres Bello, Chile*
[5]*Tecnología Médica, Facultad de Medicina, Clínica Alemana - Universidad del Desarrollo, Santiago, Chile*

Abstract

Connexins are a family of proteins that forms hemichannels that communicate the cytoplasm with the extracellular space. When two hemichannels [each one from two neighboring cells] make contact, they form a gap junction channel, which communicates the cytoplasm of adjacent cells. The molecular mechanisms that control the opening and closing of both functional hemichannels and gap junction channels is still matter of intense scrutiny. The lens is a transparent structure located in the anterior segment of the eye, which is critical for normal vision. Its main function is to refract the light, focusing it on the retina. Given this function, the lens requires great transparency and homogeneity which are attained by being avascular to avoid light scattering. To compensate for the lack of blood vessels, lens cells have intercellular connections formed by gap junction channels, which allow passive flux of nutrients and metabolites throughout the entire lens. Cataracts are produced by opacity of the lens, so less light reaches the retina. Recent evidence suggests that dysfunction of gap junction channels and hemichannels may induce cataract formation. Here we review general properties of gap junction channels and hemichannels. Then, we show the role of these channels in lens physiology and cataract formation with emphasis on rodent models lacking particular connexin genes and single point mutations in humans associated to hemichannel dysfunction. Finally, we raise the question of how environmental factors may affect hemichannel and gap junction activity and in turn induce or accelerate cataract formation by discussing the evidence that link molecular modifications [i.e phosphorylation and oxidation] of gap junction channels and hemichannels with cataract formation.

Keywords: Hemichannels; Gap junction channels; Connexons; Lens opacity

Abbreviations: Cxs: Connexins; NO: Nitric Oxide; MW: Molecular Weight; ER: Endoplasmic Reticulum; Ca^{+2}: Calcium Ion; Mg^{+2}: Magnesium Ion; ATP: Adenosine Triphosphate; ADP: Adenosine Diphosphate; cAMP: Cyclic Adenosine Monophosphate; IP3: Inositol 1,4,5-Trisphosphate; PKA: Protein Kinase A; PKC: Protein Kinase C; PKG: Protein Kinase G; MAPK: Mitogen-Activated Protein Kinase; GSNO: S-Nitrosoglutathione; TPA: phorbol 12-myristate 13-acetate; H_2O_2: Hydrogen Peroxide; CO_2: Carbon Dioxide

Introduction

The lens, a transparent biconvex structure in the anterior segment of the eye, is critical for normal vision. The main function of the lens is to refract the light, focusing it on the retina. To maintain an accurate focus, lenses constantly modify their curvature due to changes in the degree of ciliary muscle contraction. This process is called "accommodation". The human lens has three zones: i] capsule, which is a basal membrane produced by lens epithelial cells, ii] cortex, formed by epithelial cells undergoing differentiation to fiber cells, and iii] nucleus, formed by fiber cells. Considering its function in light transmission and accommodation, the lens requires transparency and homogeneity. The lens is an avascular tissue to avoid light scattering. It obtains its nutrients by diffusion from surrounding fluids, including the aqueous humor and the interstitial fluid surrounding the vasculature around the cornea. To compensate for the lack of blood vessels, the lens has a micro-circulatory system that allows circulation of nutrients, electrolytes and signals necessary for cellular function and sustenance, as well as for the maintenance of transparency [1]. Lens microcirculation is based on an intricate network of intercellular connections via gap-junction channels. These channels are formed by transmembrane proteins called connexins [Cxs] and allow passive fluxes of solutes and nutrients throughout the lens [2,3]. Six connexin molecules form a hemichannel that spans the plasma membrane of one cell. Two hemichannels from adjacent cells dock and form a gap-junction channel.

Cataract is a pathological condition in which the lens becomes opaque, thus reducing the amount of light reaching the retina. The causes for cataract formation are diverse, including gene mutations and posttranslational protein modifications. Protein abnormalities associated to cataract formation include α, β and γ- crystallin [3,4], intermediate filament [i.e filensin and phakinin] [3,5] and membrane proteins such as: aquaporin [AQP0] [3] and connexins 46 and 50 [6]. In this review we provide an overview of the link between channels formed by connexins and cataract formation, with special emphasis on mutations and connexin posttranslational modifications that result in altered hemichannel properties.

Connexins, general properties

Connexins [Cxs] are a family of membrane proteins that have four transmembrane domains, two extracellular loops, one intracellular loop and intracellular C and N-termini (Figure 1). In mammals, at least

***Corresponding author:** Dr. Mauricio A. Retamal, Laboratorio de Fisiología. Clínica Alemana – Universidad del Desarrollo, Av Las Condes #12438. Lo Barnechea, Santiago, Chile, Tel: +56-2-3279407; Fax: +56-2-3279306; E-mail: mretamal@udd.cl

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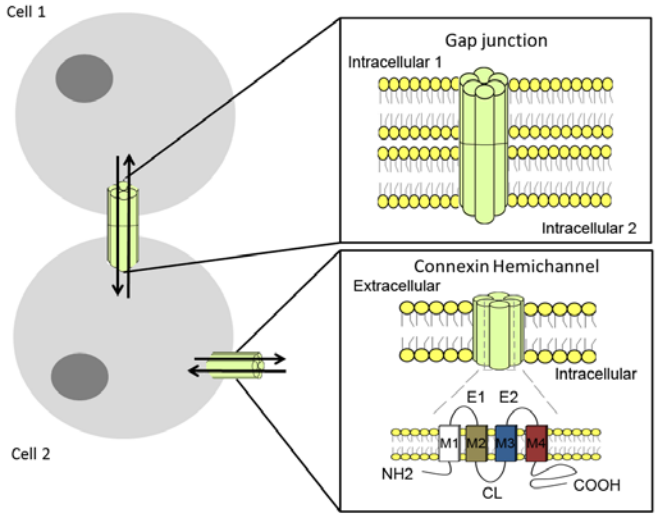

Figure 1: Scheme representing the general topology of Cx channels. Each connexin molecule [lower insert, bottom] has four transmembrane domains [M1-M4], two extracellular loops [E1 and E2], one cytoplasmic loop [CL] and both the N and C-terminus on the cytoplasmic side. Six Cx molecules form a hemichannel and docking of two hemichannels forms a gap junction channel. Thus, hemichannels connect the intracellular and the extracellular space, whereas gap-junction channels connect neighboring cells [cell 1 and cell 2].

20 connexin isoforms have been described [7]. They are named on the basis of their predicted molecular weight [i.e., the molecular weight of Cx46 is expected to be 46 kDa]. Cx isoforms exhibit considerable homology; the most variable region is the C-terminus which varies in length between isoforms and accounts for most of their differences in molecular weight. Cx23 has a very short C-terminus compared to Cx62, which has the longest one. The C-terminus contains a number of regulatory sites, including consensus phosphorylation sites [8-11] and sites for oxidation [12,13], protein-protein interactions [14-16] and cleavage [17,18]. All Cx isoforms [except for Cx23; [19]] have six conserved extracellular cysteines, which have been proposed to form disulfide-bridges essential for hemichannel docking, and thus formation of the gap-junction channel [20].

In mammals, almost all cell types express one or more Cx isoforms. However, there are important differences in levels and patterns of expression of different isoforms. For example, Cx43 is the most ubiquitous [21,22], whereas Cx46 is only present in lens [23] and lung [24]. Three connexin isoforms are expressed in mammalian lens; cortical epithelial cells express Cx43 [21] and Cx50 [25], whereas nuclear fiber cells express Cx46 [23,26] and Cx50 [27,28]. The importance of both Cx46 and Cx50 in maintaining lens transparency is well documented [29-31].

Connexin gap-junction channels

A gap-junction channel is formed by the docking of two hemichannels at the so-called junctional membrane of adjacent cells. The gap junctions allow the passage of large molecules up to 1.2 kDa due to their large pore diameter [about 13 Å at its narrowest point] [32]. Therefore, neighboring cells can share molecules such as ATP, ADP, glucose, glutathione and glutamate, as well as second messengers such as cAMP, IP3 and Ca^{+2} [33-38]. However, gap junctions are somewhat selective on the basis of molecular size, charge and shape [39-44] e.g., gap junctions formed by Cx32 are much less permeable to ATP than those formed by Cx43 [38].

The mechanisms for gap junction opening and closing ["gating"] have been studied in detail. The main factor is the transjunctional voltage [defined as the voltage between the membranes of adjacent cells]. So far, two voltage sensors have been described in each hemichannel: the "fast gate" located at the cytoplasmic part of the channel, and the "slow gate" also called "loop gate" that is located at the extracellular side [45]. Another control mechanism for gap junction gating is through phosphorylation. Cxs show multiple sites for phosphorylation by several kinases, including PKC, PKA, PKG, MAPk p38 and Src [11,46]. Phosphorylation [of serine, threonine and/or tyrosine residues] may elicit changes in gap junction ion conductance [47-53], Cx turnover [11,54-59], gap junction assembly [58,60-63] and large-solute permeability [64-69]. Changes in intracellular pH also regulate gap junction function. Intracellular acidification reduces gap junction permeability. However, the sensitivity to pH is isoform-dependent, Cx46 and Cx50 being the most sensitive isoforms [70]. Interestingly, the human Cx50 C-terminus truncated form is equally sensitive to acidification than wild type Cx50 [18], while ovine Cx50 truncated form is less sensitive to pH [17]. The mechanism of the pH effect is thought to be titration of histidine residues located at the carboxyl tail and/or cytoplasmic loop [70]. Increases in intracellular free Ca^{+2} concentrations can reduce intercellular communication through gap junctions via Ca^{+2}-calmodulin and PKC activation. This process is believed to occur in permeabilized or ATP-depleted cells by an increase in free intracellular Ca^{+2} concentration that binds to calmodulin [71]. Then, Ca^{+2}-calmodulin complex reduces intercellular communication mediated by Cx32 [72,73], Cx43 [74], Cx44 [75] or Cx50 [76]. In vitro measurements, suggest that Cx32 has a *Kd* for Ca^{+2}-calmodulin of about 3.5 μM [77] while Cx43 has a *Kd* close to 1 μM [78]. Both Ca^{+2}-calmodulin and PKC can be activated by Ca^{+2} and decrease gap junction communication in lens epithelial cells in culture [79].

Connexin hemichannels

Hemichannels, composed of six Cx, are assembled in the endoplasmic reticulum, Golgi apparatus or post Golgi vesicles, and transported to the plasma membrane [80,81]. The presence of undocked functional hemichannels acting as channels at the plasma membrane has been demonstrated in several cell types, using morphological [82-84], biochemical [12,60,61,85,86], electrophysiological [13,87-92], optical [81,93] and functional [i.e., dye uptake and ATP and glutamate release] [94-98] assays. The role of functional hemichannels at the plasma membrane in physiological or pathophysiological processes is presently under vigorous scrutiny.

Connexin hemichannels and cell physiology

For several years it was thought that undocked hemichannels had to be in a constant closed state. It was believed that if opened, molecules such as ATP, amino acids and cofactors would leave the cell massively while Ca^{2+} would enter the cells, having deleterious effects. However, recent studies show that in some circumstances hemichannels can open under physiological conditions without affecting cell viability. An important consequence is that hemichannel-mediated release of ATP [95,99,100], cyclic ADP-ribose [cADPR] [101,102], prostaglandin E2 [PGE_2] [86] and glutamate [98] may play physiological roles in cell signaling. Although some of these studies were carried out under non-physiological conditions [i.e., in the absence of Ca^{+2} and Mg^{+2}], it is clear that hemichannels may open in solutions with physiological concentrations of Ca^{+2} and Mg^{+2}, as observed in the generation and spreading of calcium waves in several cell types [95,96,103-105]. In addition, hemichannels appear to be involved in Ca^{+2} permeation across the plasma membrane [106,107], osteoblast viability induced

by biphosphonates [108], cell proliferation [109], cell migration [110], light processing by the retina [111,112] mechanotransduction [113] glucose uptake [114], wound repair [115], blood-brain barrier permeability [116] and water permeable channels in colonocytes [117]. However, signals that induce hemichannel response [opening] under physiological conditions remain unknown. Some putative mechanisms that can induce hemichannel activation under experimental conditions are dephosphorylation [118,119], changes of redox potential [12,13], plasma membrane depolarization [13,87,90,120], decrease in extracellular Ca^{+2} concentration [121,122] and unsaturated fatty acids [123]. (For more details about hemichannel gating mechanisms see [124]). In conclusion, Cx hemichannel gating is controlled by different molecular mechanisms and their opening results in the release of paracrine-autocrine molecules which in turn may modulation different important cell functions.

Connexin hemichannels and cell death

As stated above, hemichannels form large pores at the plasma membrane, permeable to molecules up to 1.2 kDa. Therefore, massive and/or prolonged hemichannel opening has been proposed to induce or accelerate cell death in some pathological conditions. These include Charcot-Marie-Tooth disease [125,126], metabolic alterations such as ischemia [12,94,127-130], oculodentodigital dysplasia [**131**], hidrotic ectodermic dysplasia [132], keratitis-ichthyosis-deafness syndrome [KIDS] [133], inflammatory processes [114,134,135], cadmium-induced oxidative cellular stress [136] deafness [137] and cataract [138,139]. Hemichannel- induced cell death is due mainly to an uncontrolled massive loss of important metabolites such as ATP, amino acids and reduced glutathione [140], loss of ion gradients and massive entry of Ca^{+2} [106,107], which can activate proteases, causing irreversible cell damage.

In summary, an increasing body of evidence supports the idea that controlled hemichannel opening allows physiological autocrine/paracrine cell signaling, but in contrast, massive and/or uncontrolled hemichannel opening may induce or accelerate cell death [124].

Connexins and lens physiology

Lens formation begins with the thickening of surface ectodermal cells to form the lens placode, which then invaginates to form a vesicle of epithelial cells known as the lens vesicle [141]. The posterior originally cuboidal lens vesicle cells [which will give rise to epithelial cells] elongate into long fiber cells, known as primary fibers, that fill the vesicle lumen [142,143]. Secondary fiber cells are produced thoughout life by mitosis of the epithelial progenitor cells located at the germinative zone just above the lens equator. Fiber cells maturate by eliminating organelles to obtain lens transparency [144,145]. The mammalian lens is vascularised during development. The capillary network covering the lens regresses late during embryonic development in humans, and before eye opening in rodents (for a review see [146]).

Gap junctions are believed to have a crucial role in the intercellular transmission of signalling molecules required for differentiation during embryonic development. Diverse gap junctions have been shown to mediate distinct mechanisms to control the formation of lens primary and secondary fiber cells [6]. Cx43 is expressed the earliest, at the lens placode stage [147]. Studies in chicks suggest that the early expression of Cx43 [148] is associated to its high sensitivity to pCO_2 [149]. Cx46 and Cx50 are first synthesized at the lens vesicle stage at the time of primary fiber elongation [148,150,151]. Cx50 is involved in epithelial cell proliferation and delayed fiber cell maturation [150,152]. Sellito and coworkers showed that knock-in of Cx46 instead of Cx50 in Cx50 knockout mice did not restore mitosis, suggesting that Cx50, but not Cx46, is selectively involved in intercellular transmission of signals mediating growth factor effects in lens epithelia [152].

In adult animals under physiological conditions mature fiber cells in the interior of the lens have minimum metabolism due to their lack of intracellular organelles. Two types of proteins are usually highly accumulated within mature fiber cells. First, the soluble and transparent protein crystallin [α, β and γ], which makes up for 80–90% of the soluble proteins in the lens and is involved in giving transparency [153,154] as well as in other functions [155]. The other major protein present in mature fiber is aquaporin 0 [AQP0, also known as major intrinsic protein [MIP]]. Besides functioning as a water channel at pH 6.5 [physiologically normal for the lens] and at low calcium concentrations [156], AQP0 is also reported to form 11–13-nm thin junctions between lens fibers [157] and to act as an adhesion molecule [158] interacting with Cx50 in differentiating lens fibers [159] to enhance the formation of functional gap junctions [160]. Mature fiber cells express Cx46 and Cx50, which are needed for the coupling of both peripheral and interior fiber cells [161-163] and for maintaining lens transparency [164-166], although the role of Cx50 can be compensated by the knock-in of Cx46 [167]. Gap junctions are essential to maintain the intracellular ionic composition necessary for crystallin solubility [2,165,168] and Cx46 gap junctions are essential in the regulation of intracellular calcium levels. Elevated calcium affects transparency by activation of cysteine proteases known as calpains, which are believed to be involved in cleavage and degradation of α- and β-crystallins in the lens [169,170]. Lens also requires a low oxygen level to maintain its transparency [146,171-173]. Whereas oxygen partial pressures below 5% [38 mmHg] are considered hypoxic in most tissues, the oxygen pressure at the lens in humans fluctuates between 1-3% [7–25 mmHg; 174,175]. Cx46 may allow cell survival during hypoxia in lens; this notion seems plausible given that a recent report has shown Cx46 to be involved in cancer cell survival under "hypoxic" conditions [176].

The need to maintain oxygen levels low in the lens is very important [174,175] not only for transparency, but to regulate lens size as oxygen can increase lens growth [177]. Excessive oxidation of proteins in fiber cells at the nucleus of the lens induces nuclear cataracts [178-180]. Fiber cells have minimal antioxidant capabilities and require antioxidant compounds such as glutathione, which are produced by epithelial cells. Such antioxidant molecules diffuse from the epithelial cells at the surface of the lens down to the lens nucleus through gap junctions [181]. The products of their oxidation at the lens nucleus diffuse back to the epithelial cells at the lens surface to be reduced.

In conclusion, channels formed by connexins are important for lens development and for normal cellular function in the adult lens under physiological conditions. They help to maintain ionic composition and metabolite supply necessary to attain transparency. Furthermore, they are also crucial for the transport of antioxidant molecules such as glutathione and to transport back to the lens surface oxidised molecules to be reduced.

Connexins and cataracts

Nutrition and excretion from lens fiber cells occur through gap junctions; the fluxes of nutrients and metabolites are determined by their chemical concentration gradients [2,165]. As mentioned above, Cxs 43, 46 and 50 are the Cxs isoforms expressed in the lens. In animal models [knockout] or human families with single-point mutations of Cx 46 and 50 isoforms there is formation of cataracts (Table 1),

suggesting that Cx function is essential to maintain lens transparency [6,182,183]. In contrast with the abundant studies on cataracts induced by genetic alterations of connexins, post-translational modifications of gap junctions and hemichannels have been much less studied.

Cx43: Connexin 43 plays an important role in eye development [167]. In newborn Cx43 knockout mice, the spaces between lens epithelial cells are larger than those in wild-type lens and there are also intracellular vacuoles associated with early stages of cataract formation

	Human Cx 46 mutation related to cataract.	
AA	***Cataract phenotype***	***Ref***
G2N	Nuclear pulverulent and posterior polar cataracts	260
D3Y	Pulverulent congenital cataracts	219
L 11S	"Ant-egg" cataract	221
T19M	Posterior-polar cataract	261
F32L	Autosomal dominant congenital nuclear pulverulent cataract	262
F32L	Autosomal dominant congenital cataract ADCC	263
R33L	Granular embryonal cataract	264
V28M	Variable cataract	220
E42K	Congenital Nuclear cataract	265
V44M	Nuclear cataract	266
D47N	Congenital nuclear cataract	267
P59L	Autosomal dominant "nuclear punctate" cataracts	268
N63S	Autosomal dominant congenital cataract ADCC	218
R67H	Autosomal dominant congenital cataract with incomplete penetrance	269
R76G	Total cataract	220
T87M	"Pearl box" cataract	270
N188T	Congenital nuclear pulverulent cataract	271
P187L	Homogeneous "zonular pulverulent" cataracts	272
P187S	Conqenital nuclear pulverulent cataracts	273
	Human Cx SO mutations related t o cataract.	
AA	***Cataract phenotype***	***Ref***
L7Q	Pulverulent cataract with smaller eyes	274
G22R	Dense cataract and microphthalmia	275
R23T	Progressive dense nuclear	276
R23T	Nuclear cataract	277
131T	Nuclear cataract	278
T39R	Congenital cataract and microcornea	279
V44E	Cataract and microcornea	280
W45S	Jellyfish-like cataract and microcornea	281
G46R	Congenital cataract and microcornea	279
D47N	Nuclear pulverulent cataract	282
D47Y	Nuclear cataract	283
D47A	Nuclear cataract	284
D47A	Nuclear cataract	285
E48K	Zonular nuclear pulverulent	286
S50P	whole cataracts and microphthalmia	287
V64A	Dominant congenital cataract	288
V64G	Nuclear cataract	289
V79L	Full moon like with Y-sutural opacities	290
P88S	Zonular pulverulent	291
P88Q	"Balloon-like" cataract with Y -sutural opacities	292
P88Q	Lamellar pulverulent cataract	252
P189L	Nuclear cataract and microcornea	293
R198Q	Posterior subcapsular cataract and microcornea	280
R198W	congenital cataract-microcornea syndrome	294
T203N	Autosomal dominant cataract	295
Δ- 248	total cataract and nystagmus	296
Δ -CT	Triangular nuclear cataract	297
S258F	Autosomal dominant nuclear cataract	298
S276F	Pulverulent nuclear cataract	299
L281C	Zonular cataract	300

Table 1: Cx46 and Cx50 single-point mutations that induce cataract formation in humans. The Δ symbol represents truncation of the protein at a given point. AA denotes the amino acid mutated and cataract phenotype is a short commentary about the phenotype and location of the cataract.

[30]. Since Cx43 knockout mice die shortly after birth, it was not possible to follow later cataract development. It will be necessary to use inducible knockout mice to learn about the role of Cx43 in cataract formation in adult animals. On the other hand, most Cx43 single-point mutations in humans are correlated with heart malformations [184], non-syndromic autosomal recessive deafness [185], or oculodentodigital dysplasia [ODDD] [186-191], suggesting that Cx43 dysfunction may not be directly causal of cataract formation. However, only one Cx43 mutation, Y17S [which is associated to ODDD] does form cataract [192]. The Y17S mutant, when expressed in C6 cells, does form gap junctions and functional hemichannels, but their activity measured as scrape-loading and propidium iodide uptake respectively were decreased compared to wild type Cx43 [192]. It is conceivable that Cx43 Y17S induces cataract formation by gap junction and/or functional hemichannel loss of function. Based on the fact that most Cx43 mutations known to date except Y17S do not form cataracts, the role of Cx43 in cataract formation is still under debate. The existence of functional Cx43 hemichannels has been confirmed in several systems under both physiological and pathophysiological conditions [124]. However, there is still no evidence on the presence of functional Cx43 hemichannels in the lens.

Cx43 hemichannels are sensitive to nitric oxide [NO]. NO induces S-nitrosylation [covalent binding of NO to the sulfur of a cysteine residue] of one or more cysteines present in the C-terminus. This has been shown in astrocytes [12] and vascular endothelial [193] cells in culture. This posttranslational modification was associated with an increase in Cx43 hemichannel opening [12,114]. Hemichannel opening is then followed by cell damage or cell death. It is possible that under oxidative stress, such as in diabetes, Cx oxidation may contribute to cataract formation [183]. The result of gap junction oxidation depends on the cell type. Oxidative stress reduces Cx43 expression and gap junction formation in the heart [194], but hydrogen peroxide increases gap junction communication in astrocytes [195]. The effect of oxidative stress on lens Cx43 has not yet been investigated.

UV light [196] and low power density microwaves [197] decrease Cx43 gap junction communication. These changes have been associated with early cataract development. However, it is not known whether Cx46 and Cx50 are also affected. In these studies Cx43 gap junctions and functional hemichannels were not differentiated.

In lens epithelial cells, Cx43 is phosphorylated by PKC [198]. In a rabbit lens epithelail cell line [N/N1003A] it has been reported that Cx43 phosphorylation by PKC-gamma is important to prevent the spread of apoptosis in lenses under oxidative stress [199] due to a disassembly of gap junction plaques [200,201]. A decrease of gap junction communication induced by TPA [a PKC activator] was also observed in bovine [202] and canine [203] lens epithelial cells, where the zonula occludens protein-1 [ZO-1] appears to be essential for gap junction disassembly induced by PKC-gamma [204]. This TPA induced-phosphorylation was associated with resistance to degradation by a proteosome dependent pathway [205], and stimulate the interactions PKC-gamma with Cx43 and Cav-1 [206]. Additionally, increases in intracellular Ca^{+2} concentrations reduce intercellular communication in lens epithelial cells due to Cx43 phosphorylation [207]. As PKC-mediated phosphorylation of Cx43 hemichannels decreases their permeability for large solutes [208], it is possible that PKC-gamma reduces cell death in the lens by decreasing the spread of damaging molecules, as a result of gap junction-mediated transport and by closing hemichannels.

In conclusion, to which extent Cx43 dysfunction is involved in cataract formation is still debated. Nevertheless, based on studies on other tissues, Cx43 gap junction and hemichannel function may be affected by oxidative stress and UV light. In the lens, Cx43 has been shown to be involved in responses to oxidative stress.

Cx46: Cx46 forms functional hemichannels when expressed in heterologous systems [120,209,210]. Expression in *Xenopus* oocytes, induces the appearance of hemichannels with a conductance of about 300 pS [211]. The membrane currents were increased when the extracellular Ca^{+2} concentration was diminished [211], and decreased when the intracellular pH was below 6.5 [212]. In addition to the currents associated with Cx46 hemichannel opening, the oocytes became permeable to fluorescent molecules with molecular weights close to 500 Da [13,22,120,211]. If oocytes expressing Cx46 are put together, allowing plasma membrane contact, they form gap junctions. After a couple of hours it is possible to detect trans-junctional currents and molecular transfer between neighboring cells [38,213,214,215]. In addition to their sensitivity to plasma membrane-voltage changes, Cx46 hemichannels are also mechanosensitive [216].

The importance of Cx46 in lens transparency is well documented. Mice lacking Cx46 develop nuclear cataracts associated with crystallin proteolysis [31]. Cx46 deletion induces an increase in the intracellular Ca^{+2} concentrations that reaches 1 μM in fiber cells [169]. The mechanism of this Ca^{+2} increase is still unknown. In Cx46-knockout mice crystalline aggregation and proteolysis are due to activation of calcium-dependent proteases, m-calpain and Lp82. Thus, Cx46 under physiological conditions is involved in maintaining intracellular calcium homeostasis, but when absent, protease activation and cataractogenesis are induced [217]. Single-point mutations of Cx46 also cause cataract formation (see Table). Mutations D3Y, N63S and frameshift at codon 380 [f380], induce "zonular pulverulent" cataract [218,219], whereas mutations V28M and R76G induce variable and total cataract phenotypes, respectively [220]. Mutation L11S induces congenital "ant-egg" cataract phenotype [221]. Functionally, mutation N63S and f380 diminish the ability of Cx46 to form functional gap junctions and hemichannels when expressed in Xenopus laevis oocytes [222,223].

In rat and bovine lens during late development [two-weeks into gestation], Cx46 is found mainly non-phosphorylated, but a month after birth it becomes mostly phosphorylated in serine residues [224,225]. It has been proposed that serine phosphorylation induces Cx46 co-localization with Cav-1, associated with lipid rafts [226]. A PKC activator [TPA] does not decrease gap junction communication of lentoid ovine cells, suggesting that PKC does not alter gap junction properties [227]. These data suggest that PKC does phosphorylate Cx46, but does not alter gap junction function. However, PKC decreases Cx46 hemichannel currents when expressed in Xenopus laevis oocytes [228]. On the other hand, chicken Cx56 [closely related to rat Cx46] is phosphorylated in S118 and S493 by PKC and PKA *in vitro* and *in vivo* [229]. PKC-gamma phosphorylation of Cx56 has been reported to reduce gap junction communication between lens fibers cells [230].

Rats injected with sodium selenite develop cataracts after about 1-2 weeks of treatment. Cataract formation is correlated with Cx46 dephosphorylation without changes in protein levels [231]. This is in agreement with the fact that PKC-gamma knockout mice lens cells are more susceptible to oxidative damage [232], suggesting that reduced phosphorylation of Cx46 hemichannels and/or gap junction elicits cell damage. Moreover, casein kinase II phosphorylates rat Cx46,

increasing hemichannel activity at the plasma membrane [233]. More experiments are needed to clarify the role of PKC in Cx46 hemichannel and gap junction modulation in lens.

Oxidative stress induces cataract formation [183,234], NO being one of the reactive molecules involved in this process [235-238]. No information is yet available concerning modulation of Cx46 hemichannels/gap junction channels by NO *in vivo*. A NO donor [GSNO] changes both electrical properties [opening/closing kinetics and voltage dependence] and large-molecule permeability of Cx46 hemichannels expressed in Xenopus laevis oocytes [13]. NO-induced Cx46 modification seems to be due to S-nitrosylation of one or more intracellular Cys [13]. These results suggest that NO induces cataract formation by altering Cx46 hemichannel properties. Because it is known that Cx46 hemichannels are mechanosensitive [216], we proposed that osmotic changes may induce ATP release to the extracellular space through hemichannels. The activation of P2X receptors has been correlated with lens epithelial cell volume regulation [239]. Because NO decreases Cx46 hemichannel permeability to large molecules [13], an increase of NO concentration due to disease [i.e diabetes], will decrease ATP release through functional hemichannels, thus changing the lens cellular osmotic response.

Another condition that induces cataracts is a change in concentration of unsaturated fatty acids in the aqueous humor [240-242]. Human lens epithelial cells die after being exposed to micromolar concentrations of unsaturated fatty acids [i.e., arachidonic acid and linoleic acid]. Saturated fatty acids are less effective in inducing cell death [241]. The effect of linoleic acid is not due to lipid peroxidation products [241] or changes in activity of Na^+-K^+-ATPase [243], but rather depends on the albumin concentration in the aqueous humor [244]. Recently, Retamal et al. [123] showed that Cx46 hemichannels are affected by unsaturated fatty acids. In this work low concentration of linoleic acid [0.1 μM] was shown to increase Cx46 hemichannel currents, whereas levels higher than 100 μM decreased currents. The linoleic acid effect was observed in less than 5 min. Linoleic acid did not affect Cx46 gap junction currents in this time span, suggesting that cataracts are due to an effect on hemichannels, not on gap junctions. However, experiments with longer exposure are needed to determine to which extent linoleic acid affects gap junctions. Other unsaturated fatty acids also affect Cx46 hemichannel properties, arachidonic acid being the most potent [123]. The effect of linoleic acid was shown to result from direct interaction with Cx46, rather than modification of the physical properties of the plasma membrane, or involvement of second messengers. Taken together, these data suggest that cataract formation induced by unsaturated fatty acid may be mediated by changes in functional hemichannel properties. However, the possibility that gap junctions are affected by linoleic acid after being exposed for long periods of time cannot be ruled out. There is currently no evidence showing effects of unsaturated fatty acids on Cx43 and Cx50 hemichannels.

Currently, only Cx46 and Cx50 have been determined to be C-terminus truncated by calpain in lens cells under physiological conditions [245]. Truncation of Cx46 C-terminus modifies hemichannel sensitivity to NO [13], pH [246], facilitates voltage-gating [92] and oligomerization [247]. Therefore, truncation of the C-terminus of Cx46 hemichannels in the lens may change their properties.

The presence of Cx46 hemichannels in lens cells has been clearly established by the work of Ebihara and co-workers [248] who have determined the electrophysiological and permeability properties of these hemichannels expressed in fiber cells.

In conclusion, dysfunction of Cx46 can cause cataract associated to crystallin proteolysis. Moreover, cataract formation is associated to reduced Cx46 phosphorylation. In vitro studies suggest that Cx46 hemichannel permeability may be affected by oxidative stress, and by unsaturated fatty acids, both of which may in the long term induce cataract formation.

Cx50: Initially Cx50 was known as lens intrinsic membrane protein MP70 [249]. Like Cxs 43 and 46, Cx50 forms functional hemichannels when expressed in heterologus systems [83,88,250]. In HeLa cells Cx50 forms hemichannels that are voltage dependent with a conductance of about 350 pS [88]. To date, no reports have been published regarding the presence of functional Cx50 hemichannels in lens epithelial cells.

Mice lacking Cx50 develop cataracts [31] and microphthalmia [150]. Several Cx50 single-point mutations producing cataract have been reported (Table 1). Among them, mutations P88S and P88Q prevent Cx50 reaching the plasma membrane to form hemichannels or gap junctions [251,252]. Mutation W45S does form gap junctions and hemichannels but they are not functional [139]. The E48K mutant forms functional hemichannels [253] but it seems that their activity is not sufficient to maintain lens transparency. Furthermore, evidence from the G46V mutant suggests that an enhanced hemichannel activity may be deleterious [138].

Cx50 is sensitive to low extracellular pH which closes functional hemichannels and gap junctions [254]. Cx50 expressed in Xenopus laevis oocytes and exposed to CO_2 is blocked by about 78% when the pH reaches 6.83 [255]. Actually Cx50 is one of the most CO_2 [pH] - sensitive Cx isoforms [256]. Like Cx46, Cx50 is also C-terminus truncated by calpain [245]. Cx50 truncation causes loss of pH sensitivity [17] and may also induce decrease in conductance with no changes in voltage dependence, single-channel gating characteristics or unitary conductance [18]. These results suggest that the Cx50 C-terminus modulates the pH response and macroscopic conductance but does not participate in single-channel conductance, voltage dependence, or gating properties.

Protein kinase A phosphorylates Cx50 in the C-terminus (S395), increasing the activity of both gap junctions [measured as intercellular dye-transfer] and functional hemichannels [measured as dye uptake] [257]. Chicken ortholog Cx45.6 is also truncated by caspase-3 during lens development [258]. Cx50 is also phosphorylated by PKCgamma, at serine and threonine residues [226], reducing Cx50 gap-junction plaque size [228,259] and increasing the number of hemichannels at the non-apposing plasma membrane [259]. It has been observed that H_2O_2 activates PKC in the lens [226], thus suggesting that cell damage induced by oxidative stress may result in part in higher Cx50 hemichannel levels at the plasma membrane.

In conclusion, Cx50 gap junction dysfunction causes cataract. Cx50 is highly sensitive to acidification and evidence from other tissues suggests that cellular damage by oxidative stress might be mediated in part by increases in Cx50 hemichannel levels.

Future Directions

The role of gap junctions, particularly of Cx46 and Cx50 in lens physiology and lens opacity is well documented, basically because gap junctions are the "circulatory system" that preserves the intracellular composition in the lens. With defective gap junction function, metabolite supply and catabolite removal decrease and cells malfunction or simply die. The role of functional hemichannels is not well understood. Although Cx43, Cx46 and Cx50 form functional hemichannels in

several cell types, only recently functional Cx46 hemichannels have been reported in lens fiber cells. The question remains as to whether Cx43 and Cx50 also form functional hemichannels in lens cells. Studies on the properties of Cx gap junctions and functional hemichannels in different cell types different from lens fiber cells suggest that their function may be regulated by several factors including pH, oxygen levels and oxidative stress. A novel research field is being opened by questions pertaining to how environmental factors may affect hemichannel and gap junction activity and in turn induce or accelerate cataract formation. Notwithstanding, present evidence suggests that Cx gap junctions and possibly functional hemichannels may constitute novel pharmacological targets for cataract prevention and treatment.

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