JCB Article

Dominant cataracts result from incongruous mixing of wild-type lens connexins

Francisco J. Martinez-Wittinghan,[1] Caterina Sellitto,[1] Leping Li,[1] Xiaohua Gong,[2] Peter R. Brink,[1] Richard T. Mathias,[1] and Thomas W. White[1]

[1]Department of Physiology and Biophysics, State University of New York, Stony Brook, NY 11794
[2]School of Optometry, University of California, Berkeley, CA 94720

Gap junctions are composed of proteins called connexins (Cx) and facilitate both ionic and biochemical modes of intercellular communication. In the lens, Cx46 and Cx50 provide the gap junctional coupling needed for homeostasis and growth. In mice, deletion of Cx46 produced severe cataracts, whereas knockout of Cx50 resulted in significantly reduced lens growth and milder cataracts. Genetic replacement of Cx50 with Cx46 by knockin rescued clarity but not growth. By mating knockin and knockout mice, we show that heterozygous replacement of Cx50 with Cx46 rescued growth but produced dominant cataracts that resulted from disruption of lens fiber morphology and crystallin precipitation. Impedance measurements revealed normal levels of ionic gap junctional coupling, whereas the passage of fluorescent dyes that mimic biochemical coupling was altered in heterozygous knockin lenses. In addition, double heterozygous knockout lenses retained normal growth and clarity, whereas knockover lenses, where native Cx46 was deleted and homozygously knocked into the Cx50 locus, displayed significantly deficient growth but maintained clarity. Together, these findings suggest that unique biochemical modes of gap junctional communication influence lens clarity and lens growth, and this biochemical coupling is modulated by the connexin composition of the gap junction channels.

Introduction

Gap junctions facilitate the intercellular exchange of small molecules and are composed of proteins called connexins (Cx;* Harris, 2001). Gap junctional communication can be divided simply into two modes, ionic and biochemical. Ionic coupling, i.e., the exchange of major cytoplasmic ions like K^+, allows for essential functions such as the coordinated contraction of cardiac myocytes and the propagation of action potentials through electrical synapses (Bruzzone et al., 1996). Biochemical communication, i.e., the intercellular sharing of larger solutes like cAMP, is required for many homeostatic processes, and its perturbation by mutation of connexin genes leads to a wide variety of human hereditary diseases (White and Paul, 1999; Evans and Martin, 2002). Although all connexins mediate ionic coupling without apparent selectivity, the spectrum of larger biochemical solutes that can be exchanged between coupled cells is highly dependent on connexin type (Goldberg et al., 1999; Valiunas et al., 2002).

There are at least 20 connexin genes in vertebrates, and most organs express a distinct subset of these, suggesting that biochemical coupling may be finely tuned to meet the needs of each tissue (Bruzzone et al., 1996; Willecke et al., 2002). This hypothesis was confirmed by studies of genetically manipulated mice that showed a restricted ability of connexins to substitute for one another in vivo (Plum et al., 2000; White, 2002). An excellent example of this lack of redundancy is provided by the lens, which predominantly expresses two connexins, Cx46 and Cx50. In mice, knockout of Cx50 resulted in significantly reduced lens growth and mild nuclear cataracts (White et al., 1998; Rong et al., 2002). In contrast, deletion of Cx46 produced severe cataracts without altering ocular growth (Gong et al., 1997). Homozygous replacement of the Cx50 coding region with that of Cx46 by genetic knockin corrected the nuclear cataracts but not the growth deficit that resulted from knockout of the Cx50 gene (White, 2002). Together, these studies suggested that a loss of connexin diversity prevented normal lens development and that distinct modes of coupling provided by Cx46 and Cx50 were required.

The lens is composed of only two distinct types of cell, a simple epithelium lining the anterior surface and highly dif-

Address correspondence to Thomas W. White, Dept. of Physiology and Biophysics, BST 5-147, State University of New York, Stony Brook, NY 11794-8661. Tel.: (631) 444-9683. Fax: (631) 444-3432.
E-mail: thomas.white@sunysb.edu

*Abbreviations used in this paper: Cx, connexin; R_s, electrical series resistance.

Key words: knockin; knockover; connexin; lens; intercellular communication

The Journal of Cell Biology, Volume 161, Number 5, June 9, 2003 969–978
http://www.jcb.org/cgi/doi/10.1083/jcb.200303068

The Journal of Cell Biology

ferentiated fibers that constitute the rest of the lenticular mass (White and Bruzzone, 2000b). Epithelial cells undergo mitosis and differentiate to give rise to new lens fibers, which lose intracellular organelles and accumulate high concentrations of crystallins to produce the high refractive index needed for accommodation. Gap junctional communication joins all the cells of the lens into a functional syncytium (Miller and Goodenough, 1986; Rae et al., 1996), and this feature allows the metabolically active epithelium to maintain in fiber cells the precise intracellular conditions necessary to prevent precipitation of the crystallins and cataract formation (Mathias et al., 1997). These processes of epithelial cell division and fiber differentiation continue throughout life at the lens equator, adding new fibers to the lens surface that enshroud older fibers and produce radially symmetric lens growth.

In the mouse lens, Cx50 and Cx46 have distinct but overlapping patterns of gene expression. Cx50 is abundantly transcribed in epithelial cells and continues to be expressed in the differentiating lens fibers (Rong et al., 2002). In contrast, Cx46 is poorly represented in the lens epithelium but undergoes a large up-regulation coincident with fiber differentiation (Gong et al., 1997). It follows that animals heterozygous for the knockin gene would coexpress these two connexins in a novel subset of cells where they could potentially mix together into the same intercellular channels (White and Bruzzone, 1996, 2000a; Beyer et al., 2001). Mixing of connexin subunits in such heteromeric channels would continue to provide ionic communication (Brink et al., 1997) but could dramatically alter the range of biochemical solutes shared between coupled cells (Bevans et al., 1998; Beyer et al., 2001). Cx46 and Cx50 can mix together to form heteromeric channels in vivo, and these channels provide a robust ionic coupling in vitro (Jiang and Goodenough, 1996; Hopperstad et al., 2000).

Here we have used mouse genetics to manipulate the connexin content of the Cx46 and Cx50 loci at will. We show that forced mixing of Cx46 and Cx50 in heterozygous knockin mice did little to diminish ionic coupling, or the biochemical coupling required for normal growth, but dominantly interfered with the biochemical coupling required for lens clarity. We also show that Cx46, when expressed alone from the Cx50 locus in knockover animals, could maintain lens clarity without supporting normal growth. These results imply that lens intercellular communication is highly specialized and that normal growth and differentiation are not regulated by the magnitude of ionic coupling. More precisely, the data suggest distinct modes of biochemical coupling provided by Cx46 and Cx50 are required for homeostasis. In addition, the results obtained with these mice offer a novel mechanistic insight into the pathology of human cataracts that result from dominant mutations in lens connexin genes (Francis et al., 1999; White and Paul, 1999; Graw and Loster, 2003).

Results

Mice used in this study

This manuscript describes the phenotypes of five novel strains of genetically engineered mice where two different autosomal connexin loci, Cx46 and Cx50, have been concurrently manipulated. The Cx46 locus can be either wild-type (+) or knockout (−). For the Cx50 locus, there are three possible states: wild-type (+) or knockout (−), with the additional possibility of being knockin for the Cx46 coding sequence (46). These knockin alleles of the Cx50 locus express the Cx46 protein under the spatial and temporal control of the native Cx50 gene regulatory machinery (White, 2002). For clarity, textual descriptions of the animals throughout the text and figures are accompanied by the complete genotype of both the Cx46 and Cx50 genes written in the preceding notation. For example, the genotype of knockin mice where the native Cx46 locus is wild-type and the Cx50 locus is homozygous for the knockin allele is also written out as [$Cx46^{(+/+)}$ $Cx50^{(46/46)}$]. Thus, homozygous knockin mice express a total of four copies of Cx46, two from the native locus and two from the Cx50 locus. A complete list of the genotypes and phenotypes of animals published previously and described in the present work is provided in Table I.

Cx46 redistributes in knockin lenses

The lens is a sphere of cells composed of an anterior epithelium covering a solid mass of underlying fibers. In wild-type mammalian lenses, Cx50 is expressed in both epithelial cells and fibers, whereas Cx46 is restricted to the fibers (Paul et al., 1991; Gong et al., 1997; Dahm et al., 1999; Rong et al., 2002). To determine if the spatial arrangement of Cx46 was altered by genetic replacement of Cx50 with Cx46 (White,

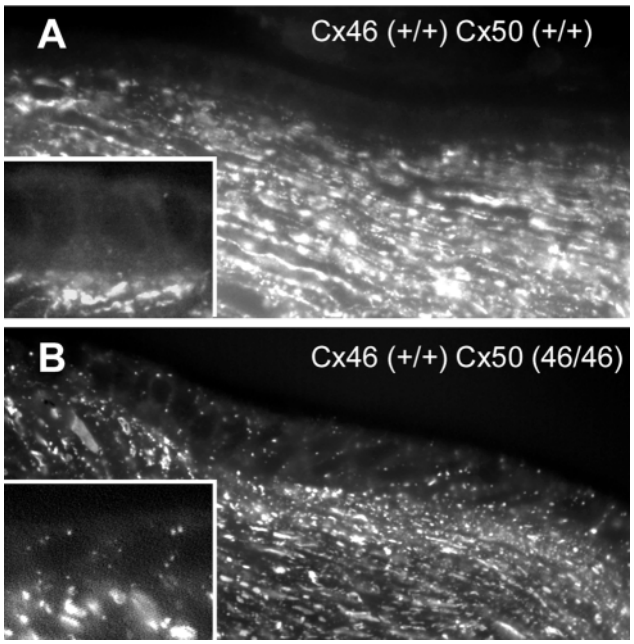

Figure 1. **Altered Cx46 distribution in knockin lenses.** (A) Sections of wild-type lenses stained with an anti-Cx46 antibody produced a strong punctate labeling pattern in fiber cells with no Cx46 expression detected in the epithelial cells (inset). (B) Consistent with the documented differences in expression of Cx46 and Cx50, knockin lenses displayed a similar strong staining in fibers in addition to a novel punctate labeling between epithelial cells (inset). Thus, heterozygous knockin mice would coexpress Cx46 and Cx50 in epithelial cells.

Table I. **Correlation of lens connexin genotypes and phenotypes**

Genotype		Phenotype	
Cx46 gene	**Cx50 gene**	**Lens size**	**Lens clarity**
Previous studies[a]			
(+/−)	(+/+)	Normal	Clear
(−/−)	(+/+)	Normal	Cataract
(+/+)	(+/−)	Normal	Clear
(+/+)	(−/−)	Small	Cataract
(+/+)	(46/46)	Small	Clear
Present work			
(+/−)	(+/−)	Normal	Clear
(+/+)	(+/46)	Normal	Cataract
(+/−)	(+/46)	Normal	Cataract
(−/−)	(+/46)	Normal	Cataract
(−/−)	(46/46)	Small	Clear

[a]Gong et al., 1997; White et al., 1998; Rong et al., 2002; White, 2002.

2002), wild-type and knockin lenses [$Cx46^{(+/+)}$ $Cx50^{(46/46)}$] were sectioned and stained with an antibody to Cx46. Wild-type lenses displayed the expected pattern of Cx46 immunostaining with abundant punctate labeling between fibers and no detectable signal in the epithelium (Fig. 1 A). In contrast, knockin lenses showed a similar pattern of fiber labeling in addition to a novel staining of Cx46 between epithelial cells (Fig. 1 B). These data are consistent with the differences in distribution of Cx46 and Cx50 and suggest that the knockin not only reduced channel diversity but also produced a shift in Cx46 distribution. One important outcome of this shift was that heterozygous knockin animals [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$], [$Cx46^{(+/-)}$ $Cx50^{(+/46)}$], or [$Cx46^{(-/-)}$ $Cx50^{(+/46)}$], coexpressed Cx46 and Cx50 in epithelial cells, a consequence that could potentially alter biochemical coupling patterns in the lens.

Coexpression of Cx46 and Cx50 at the Cx50 locus produces cataract

Genetic dissection of the overlapping influences of connexin identity, location, quantity, and mixing on lens clarity was achieved by interbreeding of Cx46 knockout [$Cx46^{(-/-)}$ $Cx50^{(+/+)}$] (Gong et al., 1997), Cx50 knockout [$Cx46^{(+/+)}$ $Cx50^{(-/-)}$] (White et al., 1998), and Cx50 knockin [$Cx46^{(+/+)}$ $Cx50^{(46/46)}$] (White, 2002) animals. This breeding strategy produced five novel lens connexin genotypes that were then compared with five previously published animal models (Table I). As expected, adult wild-type lenses were clear (Fig. 2 A) when viewed on equatorial edge with transmitted light. Interestingly, the loss of one Cx46 and one Cx50 allele in a double heterozygous knockout lens [$Cx46^{(+/-)}$ $Cx50^{(+/-)}$] (Fig. 2 B) did not disturb lens clarity, suggesting that overall connexin quantity was not important for homeostasis. Consistent with this idea, Cx46 knockover lenses, where native Cx46 was completely deleted and homozygously knocked into the Cx50 locus [$Cx46^{(-/-)}$ $Cx50^{(46/46)}$], displayed normal clarity, although they were notably smaller than wild-type (Fig. 2 C). Since the knockout of Cx50 produced cataract (White et al., 1998; Rong et al., 2002), these results suggested that connexin location and identity were more important than connexin quantity because the most significant difference between the Cx50 knockout and the Cx46 knockover was the locus-dependent pattern of expression of Cx46. Together, the double heterozygous knockout and knockover results demonstrate that lens clarity can be maintained by only

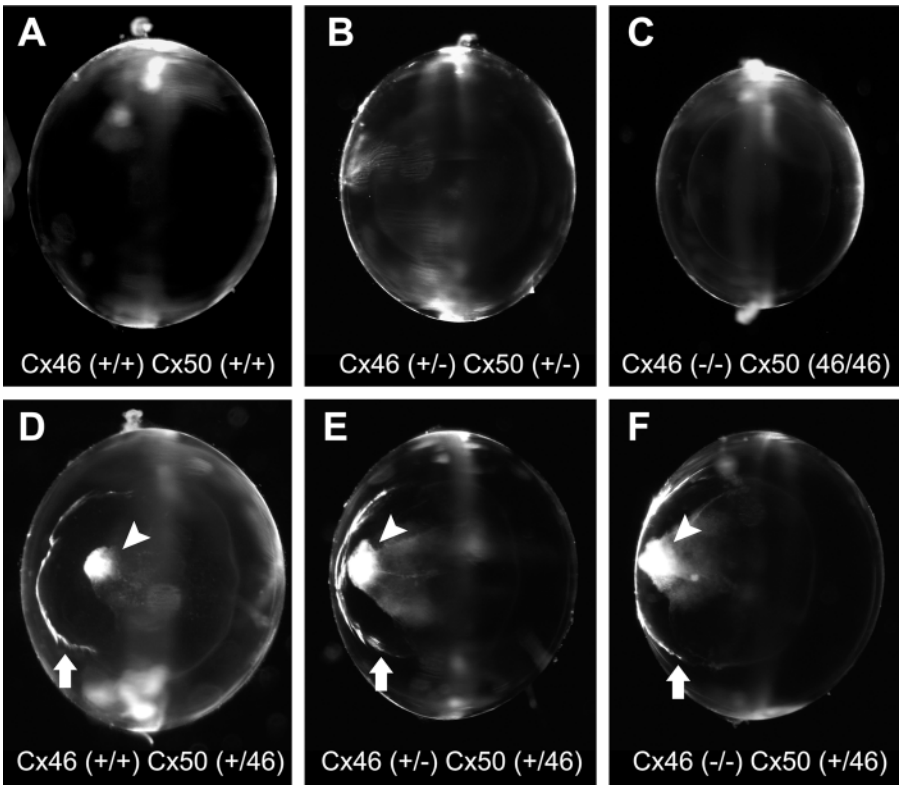

Figure 2. **Cataracts form in heterozygous knockin lenses.** (A) Wild-type lenses were clear when viewed on equatorial edge. (B) Double heterozygous knockout lenses appeared normal, so overall connexin quantity did not influence clarity. (C) Knockover lenses, expressing only knockin Cx46, were smaller than normal but clear. Thus, Cx46 alone could maintain clarity if it was expressed from the Cx50 locus. (D) Heterozygous knockin lenses developed dense cataracts in the central anterior region of the lens (arrowhead) in addition to an irregular diffraction under the epithelium (arrow). (E and F) A similar cataract developed in heterozygous knockin lenses lacking one or two copies of the native Cx46 gene. Therefore, illicit mixing of Cx46 and Cx50 in heterozygous knockins caused dominant cataracts that were independent of overall Cx46/Cx50 stoichiometry.

two of the four connexin alleles normally expressed, provided that one of the alleles is expressed from the Cx50 locus.

In contrast, heterozygous knockin lenses, where the Cx50 locus expresses one copy of wild-type Cx50 and one copy of knockin Cx46, developed dense cataracts (Fig. 2, D–F). The resulting lens opacity was distinct from those resulting from knockout of either Cx46 (Gong et al., 1997) or Cx50 (White et al., 1998) and consisted of a densely opaque core located in the central anterior region of the lens and a larger irregular diffraction more closely underlying the epithelium. This phenotype occurred in all heterozygous knockin mice and was independent of whether the native Cx46 locus was wild-type [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$] (Fig. 2 D), heterozygous [$Cx46^{(+/-)}$ $Cx50^{(+/46)}$] (Fig. 2 E), or null [$Cx46^{(-/-)}$ $Cx50^{(+/46)}$] (Fig. 2 F) for Cx46. Closer comparison of Fig. 2 B and 2 F elegantly illustrates the detrimental consequences of forced coexpression and the analytical power of the genetic manipulation. Both of these lenses express one allele of Cx46 and one allele of Cx50. In Fig. 2 B, they are on their native loci [$Cx46^{(+/-)}$ $Cx50^{(+/-)}$] with no negative effects, whereas in Fig. 2 F their expression is incongruously mixed on the Cx50 locus [$Cx46^{(-/-)}$ $Cx50^{(+/46)}$] and results in prominent cataract. These data demonstrated that improper mixing of Cx46 and Cx50 in heterozygous knockin mice caused a dominant loss of lens clarity that was not dependent on either the overall lens connexin quantity or stoichiometry.

Heterozygous knockin lenses grow normally

The interbreeding of genetically engineered animals also allowed dissection of the potentially overlapping influences of Cx50 quantity and mixing on lens growth. Knockout of Cx50 impaired lens growth in addition to causing cataract (White et al., 1998; Rong et al., 2002), and the impaired growth phenotype was not rescued by homozygous knockin of Cx46 (White, 2002). In both cases, the impaired growth rate was most evident in early postnatal life. To determine if heterozygous knockin lenses grew normally, lens mass was quantified as a function of age. As shown in Fig. 3 A, there were no significant differences in the growth rate of wild-type and heterozygous knockin [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$] lenses between 2 and 12 wk of age, a period in which the Cx50-deficient lenses clearly manifest a growth defect (White et al., 1998; White, 2002).

To determine if the presence of native Cx46 influenced growth in the heterozygous knockin, lens mass was compared between mice of different genotypes at 6 wk of age (Fig. 3 B). As published previously, Cx50 knockout lenses [$Cx46^{(+/+)}$ $Cx50^{(-/-)}$] were significantly smaller than wild-type (45% reduction in mass, $P < 0.001$, Student's t test). Since homozygous knockin of Cx46 failed to rescue normal growth (White, 2002), it was not surprising that the Cx46 knockover lenses [$Cx46^{(-/-)}$ $Cx50^{(46/46)}$], which differ from knockin by the loss of native Cx46, displayed a similar growth deficiency to the Cx50 knockout (41% smaller, $P < 0.001$) despite maintaining normal clarity. These data suggested that Cx50-dependent growth failure and cataracts were independent phenotypes and that normal growth required at least one copy of Cx50. Consistent with this idea, heterozygous knockin lenses were not significantly different in size from wild-type at 6 wk of age ($P = 0.43$). Like

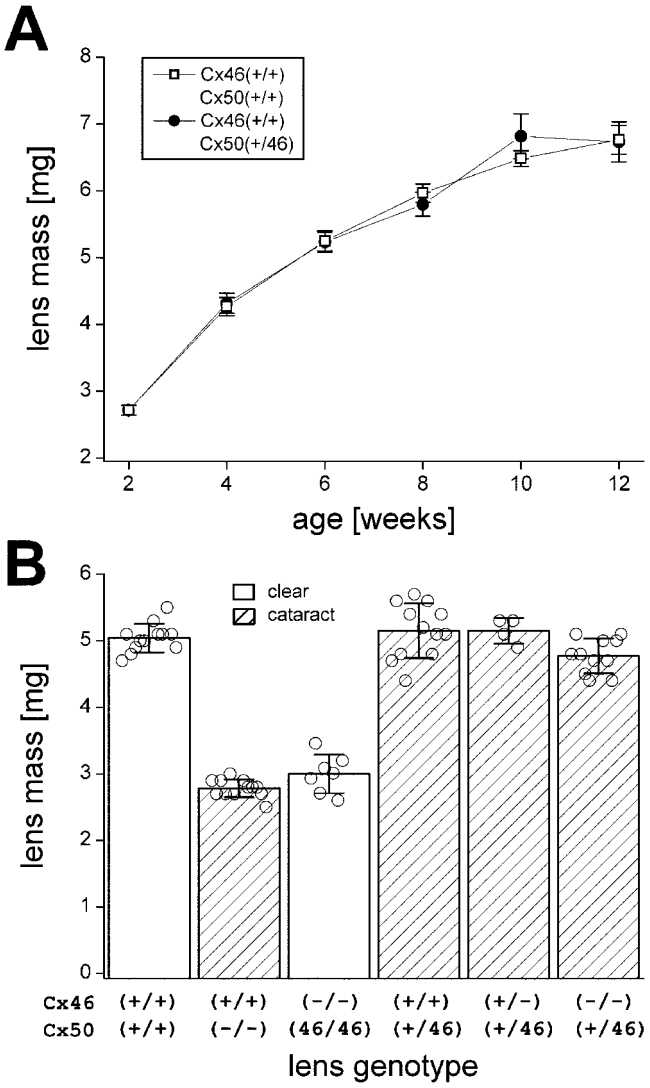

Figure 3. **Lens growth is not affected by knockin heterozygosity.** (A) There were no differences in mass between wild-type and heterozygous knockin lenses throughout early postnatal life ($n = 6$ for each genotype at each age). (B) At 6 wk of age, wild-type lenses had a mass of 5.04 ± 0.22 mg (mean ± SD). As reported previously, Cx50 knockout lenses were 45% smaller than wild-type ($P < 0.001$). Similar to Cx50 knockouts, Cx46 knockover lenses were 41% smaller ($P < 0.001$). Heterozygous knockin lenses were not significantly different in size from wild-type ($P = 0.43$) independent of the state of the Cx46 locus ($n = 4$–12 lenses for each genotype). Thus, knockin heterozygosity did not affect lens growth.

the dominant cataract phenotype described above, normal growth occurred in all heterozygous knockin lenses and was independent of the state of the Cx46 locus [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$], [$Cx46^{(+/-)}$ $Cx50^{(+/46)}$], or [$Cx46^{(-/-)}$ $Cx50^{(+/46)}$]. Thus, the inappropriate mixing of Cx46 and Cx50 in heterozygous knockin mice did not impair Cx50-dependent lens growth, and this normal growth was not influenced by the overall ratio of Cx46 to Cx50.

Histology of heterozygous knockin lenses

Cataractogenesis in the Cx46 and Cx50 knockout lenses initiated in the early postnatal period (Gong et al., 1997; White et al., 1998). To examine this developmental stage of

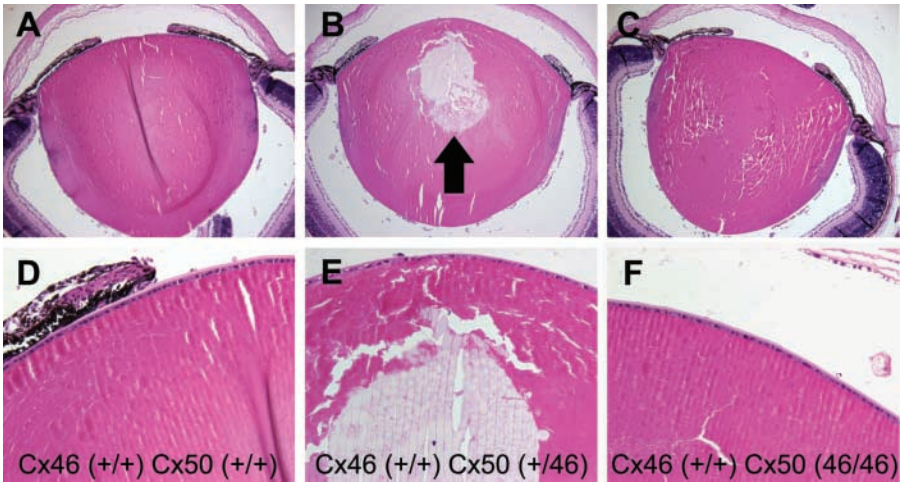

Figure 4. **Heterozygous knockin lenses develop histological abnormalities.** (A) Wild-type lenses were uniformly stained with eosin throughout the section. (B) In contrast, heterozygous knockin lenses displayed a large clear zone in the central anterior region of the lens (arrow). (C) This clear zone was not apparent in homozygous knockin lenses. (D) At higher magnification, wild-type lenses had a normal anterior epithelium and well developed lens fibers. (E) In the heterozygous knockin lenses, the clear zone was composed of opaque fibers exhibiting an irregular pattern of circular eosinophilic staining. (F) In contrast, homozygous knockin lenses had a healthy appearance indistinguishable from the wild-type lenses. Thus, profound pathological changes in heterozygous knockin lenses could be detected by postnatal day 7.

heterozygous knockin lenses for pathological changes, juvenile eyes were fixed, serially sectioned, and stained with hematoxylin and eosin. Examination of the equatorial regions of lenses of all genotypes at low magnification (Fig. 4, A–C) showed normal cell elongation, accumulation of high concentrations of eosinophilic cytoplasmic protein, and loss of fiber cell nuclei with age, all markers of normal fiber differentiation (Wride, 1996). However, prominent differences in lens histology were detected in the more centrally located fiber cells that are highly dependent on junctional communication for nutrient exchange.

At postnatal day 7, wild-type [$Cx46^{(+/+)}$ $Cx50^{(+/+)}$] (Fig. 4 A) or homozygous knockin [$Cx46^{(+/+)}$ $Cx50^{(46/46)}$] (Fig. 4 C) lenses had a normal appearance, with an anterior epithelium and well developed lens fibers. In addition, the fibers were uniformly stained with eosin in all of the serial sections. In contrast, heterozygous knockin lenses [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$] exhibited a large opaque zone in the central anterior region of the lens (Fig. 4 B) that corresponded to the location of the dense cataract seen in intact lenses. At higher magnification (Fig. 4 E), this opaque zone was composed of fibers that had lost the strong eosin staining seen in the cytoplasm of neighboring cells. Furthermore, the opaque fibers exhibited a fine irregular pattern of circular eosinophilic staining, suggestive of fiber vesiculation. These erratic histological features were specific to heterozygosity of the knockin allele and were not seen at higher magnification in wild-type or homozygous knockin lenses (Fig. 4, D and F). Like the cataract itself, these morphological changes occurred in all heterozygous knockin mice regardless of whether the Cx46 locus was wild-type, heterozygous, or null for Cx46 (unpublished data). These results showed that illicit mixing of Cx46 and Cx50 in heterozygous knockin mice dominantly compromised early postnatal lens homeostasis, and these morphological changes were very evident by postnatal day 7.

Cataracts contain precipitated crystallins

During normal differentiation, lens fibers express a highly specialized group of cytoplasmic proteins called crystallins (Wride, 1996). A characteristic feature of many lens opacities is the conversion of soluble crystallins into an insoluble form (Piatigorsky, 1980), which was observed in both the Cx46 and Cx50 knockout mice (Gong et al., 1997; White et al., 1998) and corrected in homozygous knockin animals (White, 2002). Therefore, the effect of knockin heterozygosity on the solubility of crystallin proteins was examined. Wild-type and heterozygous knockin [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$] lenses from adult animals were separated into soluble and insoluble components that were analyzed by Western blotting for αA-, αB-, β-, and γ-crystallins. All four of the crystallin proteins examined were highly abundant in the soluble fractions from either genotype of lens (Fig. 5, lanes 1 and 2), demonstrating that the changes in gene transcription and translation that accompany fiber differentiation occurred normally in the heterozygous knockin animals.

The ability of lens fibers to maintain the differentiated state of transparency requires that these soluble crystallin proteins remain in solution, and gap junctional coupling allows the metabolically active epithelium to maintain within fiber cells the precise cytoplasmic conditions to prevent precipitation (Mathias et al., 1997). Consistent with the pres-

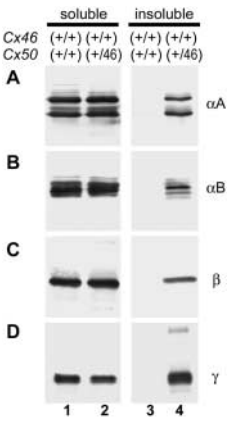

Figure 5. **Opacities in heterozygous knockin lenses result from precipitated crystallins.** (A) Biochemical analysis of crystallin solubility revealed that, as expected, αA-crystallin was present in the soluble fractions of both wild-type (lane 1) and heterozygous knockin lenses (lane 2) but only in the insoluble fraction of the heterozygous knockins (lane 4). (B–D) In a similar fashion, αB- (B), β- (C), and γ-crystallin (D) were also detected in all soluble fractions but only in the insoluble fraction from heterozygous knockin lenses. Thus, knockin heterozygosity caused lens opacities by converting the normally soluble crystallin proteins into insoluble forms.

ence of dense opacities seen in intact lenses, αA-, αB-, β-, and γ-crystallins were detected in the insoluble fraction of heterozygous knockin lenses [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$] but not wild-type lenses (Fig. 5, lanes 3 and 4). These data demonstrate that improper mixing of Cx46 and Cx50 in heterozygous knockin lenses converted crystallin proteins to insoluble forms that contributed to cataractogenesis. These data further show that the defect was not in the initial synthesis or solubility of the crystallins, but it was in the gap junctional coupling dependent maintenance of cytoplasmic homeostasis.

Heterozygous lenses are well coupled ionically

The inappropriate mixing of Cx46 and Cx50 in heterozygous knockin mice perturbed normal crystallin solubility and produced cataracts, an outcome which did not occur in wild-type or homozygous knockin lenses (White, 2002). This defect could have resulted from a perturbation of normal patterns of either ionic or biochemical coupling, or a reduction in the magnitude of both. To determine the extent of gap junctional communication in heterozygous knockin lenses [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$], we directly quantified the intercellular passage of ions using electrical impedance techniques (Mathias et al., 1981). Young adult lenses (4–6 wk old) were impaled at a 45° angle from the anterior posterior axis, and the electrical series resistance (R_s) was recorded at different radial depths. In both wild-type and heterozygous knockin lenses, R_s increased at greater depths consistent with the increasing number of fiber cells coupled by low resistance gap junctions between the recording electrode and the lens surface (Fig. 6 A). Conversion of these R_s data to values of resistivity allowed for averaging the initial 20% of lens fiber depth and showed an ∼24% increase in resistivity in heterozygous knockin animals (Fig. 6 B). This slight increase in resistance was consistent with the fact that the unitary conductance of Cx46 is smaller than Cx50, and functional replacement of one copy of Cx50 with one copy of Cx46 should result in a net reduction of ionic coupling if the total number of open channels remained constant.

Magnitudes of gap junctional communication are normally expressed as conductance, and conversion of normalized resistivity values to coupling conductance (Gong et al., 1998; Baldo et al., 2001) yielded a measure of the ionic gap junctional communication that could be directly compared with earlier studies of knockout lenses. Consistent with the increase in resistance caused by substitution of a smaller channel, coupling conductance was reduced by ∼20% in heterozygous knockin animals compared with wild-type (Fig. 6 C). More importantly, the level of ionic coupling in heterozygous knockin lenses was much higher than the ∼35% reduction observed in either the Cx50 [$Cx46^{(+/+)}$ $Cx50^{(+/-)}$] or Cx46 [$Cx46^{(+/-)}$ $Cx50^{(+/+)}$] heterozygous knockout lenses (Gong et al., 1998; Baldo et al., 2001). Since neither of the heterozygous knockouts develop cataracts (Gong et al., 1997; White et al., 1998; Rong et al., 2002), and the levels of ionic coupling in heterozygous knockins are intermediate between those of wild-type and heterozygous knockouts lenses, a deficiency in the magnitude of ionic coupling cannot be the cause of crystallin precipitation in heterozygous knockin mice. These results

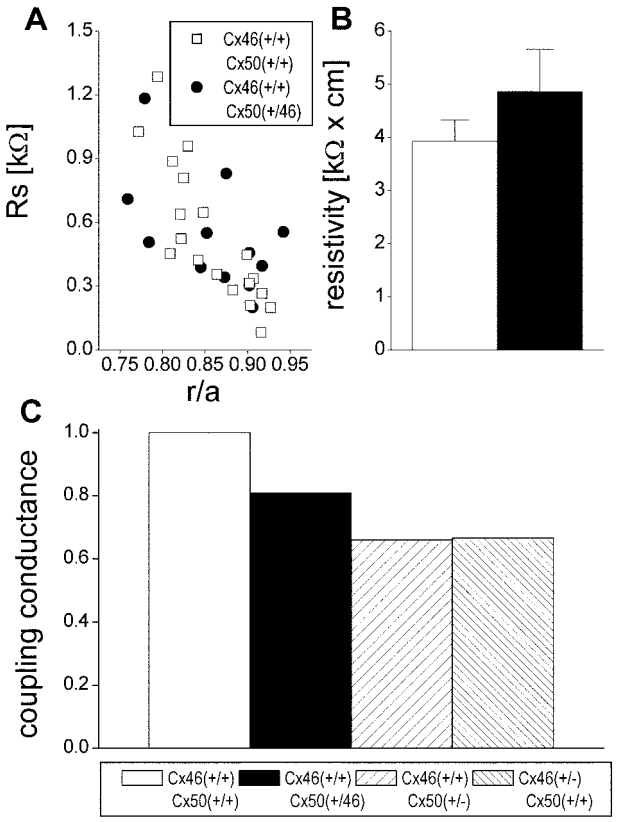

Figure 6. **Heterozygous lenses have high levels of ionic coupling.** (A) A plot of the R_s as a function of fractional radial depth (r/a), where "r" is the radial distance of the recording electrode from the lens center and "a" is the radius of the lens. In both wild-type (□) and heterozygous knockin lenses (●), R_s increased as a function of depth due to the increasing numbers of fiber cells coupled by gap junctions. (B) A plot of R_s data converted to resistivity, averaging the r/a values from 0.95 to 0.75 showed an ∼24% increase in resistivity in heterozygous knockins. (C) Conversion of the resistivity values to coupling conductance allowed comparison to values previously published for the heterozygous lens connexin knockouts (Gong et al., 1998; Baldo et al., 2001). Functional replacement of one copy of Cx50 with one copy of Cx46 restored approximately half of the communication lost after deletion of one Cx50 allele, consistent with the known unitary conductances of Cx46 and Cx50. Although coupling conductance was reduced ∼20% in heterozygous knockin animals, this level of ionic coupling was much higher than that observed in either Cx46 or Cx50 heterozygous knockout lenses, which do not develop cataracts. Thus, a deficiency of ionic coupling cannot be the cause of cataractogenesis in heterozygous knockins.

suggest that incongruous mixing of Cx46 and Cx50 in heterozygous knockin lenses did not significantly reduce ionic coupling but instead altered the spectrum of biochemical coupling sufficiently to produce cataractogenesis.

Heterozygous lenses exhibit restricted dye coupling

Documenting the actual intercellular metabolites responsible for the observed cataractogenesis is technically challenging (Goldberg et al., 1999, 2002), but fluorescent dyes can be qualitatively used to reveal approximate patterns of biochemical coupling within the living lens (Miller and Goodenough, 1986; Rae et al., 1996; White et al., 1998). Our data suggest that heterozygous knockin lenses uniquely coexpress Cx46 and Cx50 in the lens epithelium, and this may have altered

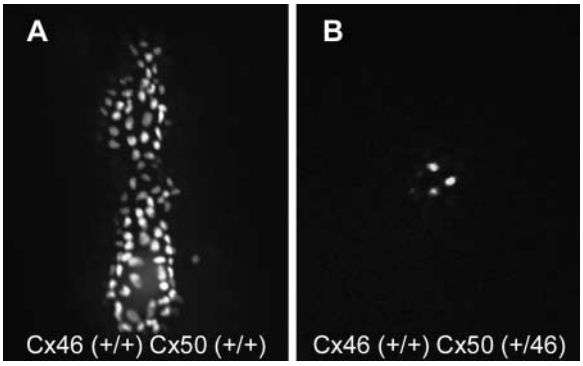

Figure 7. **Heterozygous lenses have reduced patterns of dye coupling.** Microelectrodes delivering DAPI were placed into centrally located anterior epithelial cells. Injection of single epithelial cells in wild-type lenses resulted in the intercellular passage of DAPI to more than 50 neighboring epithelial cells (A). The dye spread pattern in wild-type lenses tended to be more extensive along the axis of the underlying fiber cells, which are also highly coupled to the epithelium. In contrast, DAPI was transferred to many fewer neighboring epithelial cells in heterozygous knockin lenses (B). Thus, there appeared to be a qualitative difference in the extent of DAPI spread, and by analogy biochemical coupling, between wild-type and heterozygous knockin lenses. A and B are representative of four and five epithelial injections, respectively.

biochemical coupling within these cells. To determine if the extent of epithelial dye coupling was altered, we directly visualized the intercellular passage of DAPI. This dye was chosen for two reasons; first DAPI accumulates and concentrates within the nuclei of cells, and second, its intercellular passage has been shown to be highly dependent on which connexin isoforms are providing intercellular coupling (Elfgang et al., 1995; Cao et al., 1998). Since the only nuclei in the central anterior surface of the lens are within the underlying monolayer of epithelial cells (Fig. 4), these two features made DAPI an ideal candidate to probe qualitative changes in biochemical coupling in the intact lens epithelium.

Young adult lenses (4–6 wk old) were impaled through the central anterior capsule, and the resting membrane voltage was monitored throughout the injection. In four out of four trials, injection of a single epithelial cell in wild-type lenses [$Cx46^{(+/+)}$ $Cx50^{(+/+)}$] resulted in the intercellular passage of DAPI to a large number of neighboring cells (Fig. 7 A). The dye spread pattern in wild-type lenses tended to be more extensive along the axis of the underlying fiber cells, which radiate outward from the anterior suture and are highly coupled to the epithelium (Rae et al., 1996; White et al., 1998). In contrast, DAPI was transferred to many fewer neighboring epithelial cells ($\leq$10) in heterozygous knockin lenses [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$] in five independent injections (Fig. 7 B). Thus, DAPI was able to pass through gap junctions in all lenses, but there was also a large qualitative difference in the extent of dye spread. Proper quantitation of dye permeation and selectivity is highly laborious (Valiunas et al., 2002) and well beyond the scope of the present study, but these qualitative differences between wild-type and heterozygous knockin lenses support our hypothesis that biochemical coupling was altered by the forced coexpression of Cx46 and Cx50 in epithelial cells.

Discussion

We have shown that forced mixing of Cx46 and Cx50 produced a dominant cataract phenotype without adversely affecting lens growth. This cataract resulted from disruption of lens fiber morphology and crystallin precipitation, and these defects were not a consequence of reduced levels of ionic gap junctional coupling but instead correlated with alterations in dye coupling. The most logical conclusion is that altered biochemical coupling, resulting from inappropriate mixing of Cx46 and Cx50, perturbed cellular homeostasis but not lens growth. These findings suggest that unique biochemical modes of gap junctional communication influence lens clarity and lens growth, and this biochemical coupling is modulated by the connexin composition of the gap junction channels.

As a solid avascular sphere, the lens is an organ that is uniquely dependent on gap junctional communication for providing nutrients and removing metabolic wastes (Piatigorsky, 1980; Mathias et al., 1981). A microcirculation model that relies on ionic coupling through gap junctions generates the osmotic fluxes and voltage gradients that drive biochemical modes of coupling in the lens (Donaldson et al., 2001). Briefly, an ionic current enters the lens along the extracellular spaces between cells. After crossing fiber cell membranes in the lens interior, the current flows back toward the surface via an intracellular pathway provided by ionic coupling through gap junction channels. The circulating current generates fluid flow and a voltage gradient, forces which in turn help carry larger biochemical solutes into the lens and between the coupled cells (Mathias et al., 1997). In Cx46 knockout mice, this ionic current was completely lost in the inner lens fibers resulting in severe cataracts (Gong et al., 1997, 1998). In the Cx50 knockout, the inner ionic current was reduced 66% compared with wild-type, producing a milder cataract phenotype (White et al., 1998; Baldo et al., 2001). In support of our conclusion that altered biochemical coupling alone can produce cataract, heterozygous knockin lenses [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$] retained high levels of ionic coupling, which presumably reflected a normally operating microcirculation, yet they displayed a severe cataract.

Surprisingly, knockover lenses [$Cx46^{(-/-)}$ $Cx50^{(46/46)}$] lacked the cataract normally resulting from either Cx46 or Cx50 knockout (Gong et al., 1997; White et al., 1998). In the Cx46 knockout, Cx50 expression from its native locus was unable to maintain clarity, whereas Cx46 expression from the Cx50 locus in the knockover prevented cataract. As many prior studies have established that levels of Cx46 and Cx50 protein synthesis show no apparent compensatory upregulation after genetic manipulation (Gong et al., 1998; White et al., 1998, 2001; Baldo et al., 2001; Rong et al., 2002; White, 2002), Cx46 would be expected to provide a lower level of ionic coupling than Cx50 when expressed from the same promoter, due to its smaller unitary conductance (140 pS versus 220 pS). At the very least, knockover lenses would be expected to have less ionic coupling than wild-type due to an overall reduction in channel number. These data suggest that the combination of ionic and biochemical coupling provided by Cx46, when exclusively ex-

pressed from the Cx50 locus, was far more efficient than that provided by Cx50 in maintaining lens clarity.

Lens growth results from the regulated proliferation of epithelial cells that migrate toward the equatorial region where they differentiate into new fiber cells (Wride, 1996). Since homozygous knockin of Cx46 failed to rescue growth (White, 2002), it was not surprising that the knockover lenses displayed the same lens growth defect as the Cx50 knockout. It was less obvious why heterozygous knockin lenses were able to grow normally despite having severe cataracts. The fact that this normal growth was evident regardless of whether the native Cx46 gene was even present suggests that lens growth, like clarity, was not dependent on the overall magnitude of coupling and that the incongruous mixing of Cx46 and Cx50 did not alter the biochemical coupling that participates in the regulation of epithelial proliferation and fiber differentiation. Normal lens growth was also observed in heterozygous Cx50 knockouts (White et al., 1998; Rong et al., 2002), implying that one allele of Cx50 provides sufficient biochemical coupling. Consistent with this, we have seen that lenses lacking both alleles of Cx46 and one copy of Cx50 [$Cx46^{(-/-)}$ $Cx50^{(+/-)}$] still grow normally (unpublished data). Together, these data suggest that normal lens growth only requires one copy of Cx50 and is independent of the remaining connexin composition of the lens.

In wild-type mice, Cx46 and Cx50 are normally coexpressed in fibers but not in the lens epithelium. Additionally, they are known to naturally cooligomerize into heteromeric channels within the fiber cells (Konig and Zampighi, 1995; Jiang and Goodenough, 1996), and heteromeric channels composed of Cx46 and Cx50 provide robust levels of ionic conductance in vitro (Ebihara et al., 1999; Hopperstad et al., 2000). In the knockin mice, our immunocytochemistry data showed that Cx46 was translocated into the epithelium where it could inappropriately mix with Cx50 in heterozygous animals. Heteromeric mixing of connexins has been documented to profoundly alter the biochemical coupling of signaling molecules such as cyclic nucleotides or fluorescent dyes that mimic their size and charge (Bevans et al., 1998; Valiunas et al., 2002). This novel mixing of Cx46 and Cx50 in lens epithelial cells produced a dominant cataract phenotype that was much more severe than the Cx50 knockout phenotype and was also independent of total lens connexin stoichiometry or quantity. From the data presented here, we conclude that incongruous mixing of Cx46 and Cx50 in epithelial cells altered the spatial patterns of biochemical coupling necessary to sustain lens clarity.

Ideally, one would like to directly document the proposed changes in biochemical coupling in the lens responsible for the observed cataractogenesis, but this is technically difficult, especially in a three-dimensional optically active tissue. Instead, we have shown that DAPI permeation was qualitatively different in the epithelial cells of heterozygous knockin lenses compared with wild-type lenses. DAPI has a similar charge and molecular mass as many cytoplasmic signaling molecules, and it is not unreasonable to assume that alterations in this dye's spread may reflect changes in permeation in other types of endogenous metabolites. In any case, we have also shown by impedance techniques that intercellular communication to ions was not disrupted in the heterozygous knockin lenses and that our impedance data were quantitatively reconciled with an approximately one to one replacement of functional channels if one considered differences in the unitary conductance of Cx46 and Cx50 (Srinivas et al., 1999; Hopperstad et al., 2000). If one accepts that the principal mechanism used by connexin channels to convey information between cells is intercellular coupling to ions and small metabolites, then our data showing high levels of ionic coupling and altered dye spread logically infer that altered biochemical coupling must be responsible for cataracts in the heterozygous knockin mice.

This explanation also provides a potential mechanistic insight into the pathology of human congenital cataracts caused by mutations in connexin genes (Francis et al., 1999; White and Paul, 1999; Graw and Loster, 2003). To date, only dominant cataracts caused by heterozygous missense mutations in Cx50 have been identified, and these patients generally exhibit normal eye and lens growth (Shiels et al., 1998; Francis et al., 1999; Polyakov et al., 2001). In contrast, the recessive phenotype of the Cx50 knockout mice includes microphthalmia in addition to cataracts (White et al., 1998; Rong et al., 2002). The heterozygous knockin lenses [$Cx46^{(+/+)}$ $Cx50^{(+/46)}$] described in this article offer a resolution to this discrepancy, since the knockin allele behaves like a dominant mutation. Like the human cataract pedigrees, one normal Cx50 allele is sufficient to ensure normal lens growth, and one altered Cx50 allele is sufficient to disrupt homeostasis and cause cataract. It is highly improbable that all of the missense connexin mutants associated with cataracts will be devoid of functional activity, since a subset of missense mutations associated with other connexin disorders have been shown to retain gap junction channel activity in vitro (Oh et al., 1997; Ressot et al., 1998; Bruzzone et al., 2003). Our results expand the pathological paradigm and now allow for the consideration of functional mutant, or incongruously expressed wild-type, connexins to dominantly alter biochemical communication without disrupting ionic coupling.

A summary of our current and previously published data (Table I) shows an emerging pattern of connexin-specific gap junction functions in the lens. The two predominant connexins of the lens fulfill unique functions; Cx46 is primarily responsible for maintaining lens clarity, whereas Cx50 mainly provides for normally regulated growth. In addition, the contributions of Cx50 to normal growth and the maintenance of clarity are independent of each other, and Cx46 better fulfills the latter role in the knockover animals [$Cx46^{(-/-)}$ $Cx50^{(46/46)}$]. Here, we have shown that connexin-specific differences in biochemical coupling differentially regulate lens growth and clarity and that the spectrum of larger biochemical solutes that need to be exchanged between lens cells is indeed finely tuned to meet the needs of this tissue.

Materials and methods

Animals

Cx46 knockout, Cx50 knockout, and Cx50/Cx46 knockin mice have been described previously (Gong et al., 1997; White et al., 1998; White, 2002). Double heterozygous knockouts were produced by mating Cx46 knockout with Cx50 knockout animals. Knockover animals were produced by mat-

ing homozygous Cx46 knockout mice with homozygous knockin mice and then intercrossing the heterozygous progeny. Genotypes of all animals were confirmed by PCR analysis of genomic DNA from tail biopsies using previously described protocols (Gong et al., 1997; White et al., 1998; White, 2002).

Growth analysis

Lenses from eyes of age-matched animals were dissected, blotted dry on tissue paper, and individually weighed. Differences between lenses from various genotypes were compared by the Student's unpaired *t* test.

Microscopy

Eyes were dissected and immediately transferred to Petri dishes containing prewarmed M199 medium with Hanks Salts (M199) on a 37°C stage. Lenses were dissected and transferred to prewarmed poly-lysine–coated Petri dishes with glass bottoms (World Precision Instruments) filled with M199. Lenses were oriented onto their equatorial edges and photographed on an Olympus SZX9 microscope equipped with an Olympus C3030 zoom digital camera.

Immunofluorescence

Adult eyes were dissected, cut open at the posterior pole, fixed in 1% formaldehyde in PBS overnight, rinsed in PBS, and embedded in Optimal Cutting Temperature compound in liquid N_2. Frozen sections (10–12 μM) were cut on a cryostat, air dried onto Superfrost Plus slides, and blocked with 3% BSA in PBS. Sections were stained with rabbit antisera against Cx46 (Paul et al., 1991), diluted in 3% BSA in PBS, and washed with PBS. Primary antisera were visualized with a Cy3-conjugated goat anti–rabbit antibody (Jackson ImmunoResearch Laboratories) and photographed on an Olympus BX51 microscope using an Optronics MagnaFire digital camera.

Histology

Postnatal day 7 eyes were dissected, cut open at the posterior pole, fixed in 4% formaldehyde in PBS overnight, rinsed in PBS, and dehydrated through an ethanol series. Eyes were cleared in xylene and embedded in paraffin. Paraffin sections (2–3 μM) were cut on a 0.5-cm diamond knife, floated on water, and dried onto Superfrost Plus slides overnight at 37°C. Sections were stained with hematoxylin and eosin and photographed.

Western blotting

Lenses were dissected, and equal wet weights were homogenized in 0.1 M NaCl, 0.1 M Na_2HPO_4, 10 mM ascorbic acid, and protease inhibitors (10 μg/ml each of chymostatin, leupeptin, and pepstatin). After centrifugation at 14,000 *g* for 20 min at room temperature, the soluble fraction was recovered and stored at −80°C. The insoluble fraction was subjected to a second wash with 1 ml of 0.1 M NaCl, 0.1 M Na_2HPO_4 followed by a subsequent wash with 1 ml of 20 mM NaOH. The insoluble fraction was finally washed in 1 ml of 1 mM Na_2CO_3 after which the pellets were resuspended in sample buffer and stored at −80°C. Equal aliquots of samples from each genotype were electrophoresed on 10% SDS–polyacrylamide gels, transferred to nitrocellulose membranes, and incubated with antibodies specific for αA-, αB-, β-, and γ-crystallin proteins. Primary antibodies were detected with alkaline phosphatase–conjugated goat anti–rabbit IgG (Jackson ImmunoResearch Laboratories) using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates (Sigma-Aldrich).

Impedance

Gap junctional ionic coupling was measured as described previously (Gong et al., 1998; Baldo et al., 2001). Briefly, lenses were dissected and placed anterior surface down into a 37°C chamber filled with Tyrode solution (138 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, 5 mM Hepes, pH 7.4). A stochastic current containing a wide range of frequencies was passed through a microelectrode into a fiber cell near the center of the lens, while the induced voltage was recorded with a second electrode placed at different radial depths from the lens surface. Voltage and current data were input into a fast Fourier analyzer to compute the real time frequency domain impedance. Gap junctional ionic coupling was calculated from impedance data using previously described models (Mathias et al., 1981; Baldo et al., 2001).

Dye coupling

Gap junctional coupling to dyes mimicking larger biochemical solutes was measured as described previously (White et al., 1998). Briefly, 4–6-wk-old lenses were dissected and mounted on their posterior surface on poly-L-lysine–coated 35-mm Petri dishes filled with 37°C M199. High resistance microelectrodes were filled with 1 mg/ml DAPI (Molecular Probes) dissolved in 120 mM K-aspartate, 10 mM NaCl, 1 mM $MgCl_2$, 3 mM Mg-ATP, and 5 mM Hepes, pH 7.2. Centrally located epithelial cells were impaled through the lens capsule from the anterior lens surface. DAPI was iontophoretically injected for 5 min, while continuously monitoring the membrane potential of the impaled cell. Injected lenses were photographed on an Olympus BX51 microscope using an Optronics MagnaFire digital camera.

This work was funded by National Institutes of Health grants EY13849 (to X. Gong), EY14604 (to P.R. Brink), EY06391 (to R.T. Mathias), and EY13163 (to T.W. White).

Submitted: 11 March 2003
Revised: 24 April 2003
Accepted: 24 April 2003

References

Baldo, G.J., X. Gong, F.J. Martinez-Wittinghan, N.M. Kubar, N.B. Giluly, and R.T. Mathias. 2001. Gap junctional coupling in lenses from alpha(8) connexin knockout mice. *J. Gen. Physiol.* 118:447–456.

Bevans, C.G., M. Kordel, S.K. Rhee, and A.L. Harris. 1998. Isoform composition of connexin channels determines selectivity among second messengers and uncharged molecules. *J. Biol. Chem.* 273:2808–2816.

Beyer, E.C., J. Gemel, A. Martinez, V.M. Berthoud, V. Valiunas, A.P. Moreno, and P.R. Brink. 2001. Heteromeric mixing of connexins: compatibility of partners and functional consequences. *Cell Commun. Adhes.* 8:199–204.

Brink, P.R., K. Cronin, K. Banach, E. Peterson, E.M. Westphale, K.H. Seul, S.V. Ramanan, and E.C. Beyer. 1997. Evidence for heteromeric gap junction channels formed from rat connexin43 and human connexin37. *Am. J. Physiol.* 273:C1386–C1396.

Bruzzone, R., T.W. White, and D.L. Paul. 1996. Connections with connexins: the molecular basis of direct intercellular signaling. *Eur. J. Biochem.* 238:1–27.

Bruzzone, R., V. Veronesi, D. Gomes, M. Bicego, N. Duval, S. Marlin, C. Petit, P. D'Andrea, and T.W. White. 2003. Loss-of-function and residual channel activity of connexin26 mutations associated with non-syndromic deafness. *FEBS Lett.* 533:79–88.

Cao, F., R. Eckert, C. Elfgang, J.M. Nitsche, S.A. Snyder, D.F. Hulser, K. Willecke, and B.J. Nicholson. 1998. A quantitative analysis of connexin-specific permeability differences of gap junctions expressed in HeLa transfectants and *Xenopus* oocytes. *J. Cell Sci.* 111:31–43.

Dahm, R., J. van Marle, A.R. Prescott, and R.A. Quinlan. 1999. Gap junctions containing alpha8-connexin (MP70) in the adult mammalian lens epithelium suggests a re-evaluation of its role in the lens. *Exp. Eye Res.* 69:45–56.

Donaldson, P., J. Kistler, and R.T. Mathias. 2001. Molecular solutions to mammalian lens transparency. *News Physiol. Sci.* 16:118–123.

Ebihara, L., X. Xu, C. Oberti, E.C. Beyer, and V.M. Berthoud. 1999. Co-expression of lens fiber connexins modifies hemi-gap-junctional channel behavior. *Biophys. J.* 76:198–206.

Elfgang, C., R. Eckert, H. Lichtenberg-Frate, A. Butterweck, O. Traub, R.A. Klein, D.F. Hulser, and K. Willecke. 1995. Specific permeability and selective formation of gap junction channels in connexin-transfected HeLa cells. *J. Cell Biol.* 129:805–817.

Evans, W.H., and P.E. Martin. 2002. Gap junctions: structure and function. *Mol. Membr. Biol.* 19:121–136.

Francis, P.J., V. Berry, A.T. Moore, and S. Bhattacharya. 1999. Lens biology: development and human cataractogenesis. *Trends Genet.* 15:191–196.

Goldberg, G.S., P.D. Lampe, and B.J. Nicholson. 1999. Selective transfer of endogenous metabolites through gap junctions composed of different connexins. *Nat. Cell Biol.* 1:457–459.

Goldberg, G.S., A.P. Moreno, and P.D. Lampe. 2002. Gap junctions between cells expressing connexin 43 or 32 show inverse permselectivity to adenosine and ATP. *J. Biol. Chem.* 277:36725–36730.

Gong, X., E. Li, G. Klier, Q. Huang, Y. Wu, H. Lei, N.M. Kumar, J. Horwitz, and N.B. Gilula. 1997. Disruption of alpha3 connexin gene leads to proteolysis and cataractogenesis in mice. *Cell.* 91:833–843.

Gong, X., G.J. Baldo, N.M. Kumar, N.B. Gilula, and R.T. Mathias. 1998. Gap junctional coupling in lenses lacking alpha3 connexin. *Proc. Natl. Acad. Sci. USA.* 95:15303–15308.

Graw, J., and J. Loster. 2003. Developmental genetics in ophthalmology. *Ophthalmic Genet.* 24:1–33.

Harris, A.L. 2001. Emerging issues of connexin channels: biophysics fills the gap.

Q. Rev. Biophys. 34:325–472.

Hopperstad, M.G., M. Srinivas, and D.C. Spray. 2000. Properties of gap junction channels formed by Cx46 alone and in combination with Cx50. *Biophys. J.* 79:1954–1966.

Jiang, J.X., and D.A. Goodenough. 1996. Heteromeric connexons in lens gap junction channels. *Proc. Natl. Acad. Sci. USA.* 93:1287–1291.

Konig, N., and G.A. Zampighi. 1995. Purification of bovine lens cell-to-cell channels composed of connexin44 and connexin50. *J. Cell Sci.* 108:3091–3098.

Mathias, R.T., J.L. Rae, and R.S. Eisenberg. 1981. The lens as a nonuniform spherical syncytium. *Biophys. J.* 34:61–83.

Mathias, R.T., J.L. Rae, and G.J. Baldo. 1997. Physiological properties of the normal lens. *Physiol. Rev.* 77:21–50.

Miller, T.M., and D.A. Goodenough. 1986. Evidence for two physiologically distinct gap junctions expressed by the chick lens epithelial cell. *J. Cell Biol.* 102:194–199.

Oh, S., Y. Ri, M.V. Bennett, E.B. Trexler, V.K. Verselis, and T.A. Bargiello. 1997. Changes in permeability caused by connexin 32 mutations underlie X-linked Charcot-Marie-Tooth disease. *Neuron.* 19:927–938.

Paul, D.L., L. Ebihara, L.J. Takemoto, K.I. Swenson, and D.A. Goodenough. 1991. Connexin46, a novel lens gap junction protein, induces voltage-gated currents in nonjunctional plasma membrane of *Xenopus* oocytes. *J. Cell Biol.* 115:1077–1089.

Piatigorsky, J. 1980. Intracellular ions, protein metabolism, and cataract formation. *Curr. Top. Eye Res.* 3:1–39.

Plum, A., G. Hallas, T. Magin, F. Dombrowski, A. Hagendorff, B. Schumacher, C. Wolpert, J. Kim, W.H. Lamers, M. Evert, et al. 2000. Unique and shared functions of different connexins in mice. *Curr. Biol.* 10:1083–1091.

Polyakov, A.V., I.A. Shagina, O.V. Khlebnikova, and O.V. Evgrafov. 2001. Mutation in the connexin 50 gene (GJA8) in a Russian family with zonular pulverulent cataract. *Clin. Genet.* 60:476–478.

Rae, J.L., C. Bartling, J. Rae, and R.T. Mathias. 1996. Dye transfer between cells of the lens. *J. Membr. Biol.* 150:89–103.

Ressot, C., D. Gomes, A. Dautigny, D. Pham-Dinh, and R. Bruzzone. 1998. Connexin32 mutations associated with X-linked Charcot-Marie-Tooth disease show two distinct behaviors: loss of function and altered gating properties. *J. Neurosci.* 18:4063–4075.

Rong, P., X. Wang, I. Niesman, Y. Wu, L.E. Benedetti, I. Dunia, E. Levy, and X. Gong. 2002. Disruption of Gja8 (alpha8 connexin) in mice leads to microphthalmia associated with retardation of lens growth and lens fiber maturation. *Development.* 129:167–174.

Shiels, A., D. Mackay, A. Ionides, V. Berry, A. Moore, and S. Bhattacharya. 1998. A missense mutation in the human connexin50 gene (GJA8) underlies autosomal dominant "zonular pulverulent" cataract, on chromosome 1q. *Am. J. Hum. Genet.* 62:526–532.

Srinivas, M., M. Costa, Y. Gao, A. Fort, G.L. Fishman, and D.C. Spray. 1999. Voltage dependence of macroscopic and unitary currents of gap junction channels formed by mouse connexin50 expressed in rat neuroblastoma cells. *J. Physiol. London*. 517:673–689.

Valiunas, V., E.C. Beyer, and P.R. Brink. 2002. Cardiac gap junction channels show quantitative differences in selectivity. *Circ. Res.* 91:104–111.

White, T.W. 2002. Unique and redundant connexin contributions to lens development. *Science.* 295:319–320.

White, T.W., and R. Bruzzone. 1996. Multiple connexin proteins in single intercellular channels: connexin compatibility and functional consequences. *J. Bioenerg. Biomembr.* 28:339–350.

White, T.W., and D.L. Paul. 1999. Genetic diseases and gene knockouts reveal diverse connexin functions. *Annu. Rev. Physiol.* 61:283–310.

White, T.W., and R. Bruzzone. 2000a. Gap junctions: fates worse than death? *Curr. Biol.* 10:R685–R688.

White, T.W., and R. Bruzzone. 2000b. Intercellular communication in the eye: clarifying the need for connexin diversity. *Brain Res. Brain Res. Rev.* 32:130–137.

White, T.W., D.A. Goodenough, and D.L. Paul. 1998. Targeted ablation of Connexin50 in mice results in microphthalmia and zonular pulverulent cataracts. *J. Cell Biol.* 143:815–825.

White, T.W., C. Sellitto, D.L. Paul, and D.A. Goodenough. 2001. Prenatal lens development in connexin43 and connexin50 double knockout mice. *Invest. Ophthalmol. Vis. Sci.* 42:2916–2923.

Willecke, K., J. Eiberger, J. Degen, D. Eckardt, A. Romualdi, M. Guldenagel, U. Deutsch, and G. Sohl. 2002. Structural and functional diversity of connexin genes in the mouse and human genome. *Biol. Chem.* 383:725–737.

Wride, M.A. 1996. Cellular and molecular features of lens differentiation: a review of recent advances. *Differentiation.* 61:77–93.