An Important Regulatory Role for PKCy in Lens Cell Differentiation

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Abstract
Purpose: The purpose of this study was to determine if Protein Kinase Cy (PKCy) is involved in the regulated loss of Cx43 during lens epithelial cell differentiation to fiber cells.

Methods: NIN 1003A rabbit lens epithelial cells were treated with the PKCy activator, phorbol-12-myristate 13 acetate (TPA) at 400nM, for up to 24 hours and Western blotting was performed to quantitate PKCy, Cx43 and Cx50 protein levels. The distribution of Cx43 in the control (b612p-Prkcctm1St1) and PKCy knockout (B6;129p-Prkcctm1St1) mouse lenses was determined from dissected epithelial, cortical, and nuclear regions. Light microscopy was utilized to examine lens structure for control and knockout mouse lenses.

Results: Long-term exposure of lens epithelial cells in culture to TPA resulted in the degradation of Cx43 but not Cx50. Since hyper-phosphorylation of Cx43 has been shown to result in the down-regulation of Cx43, we examined the effects of a PKCy knockout mouse, where phosphorylation of Cx43 by PKCy does not occur. In normal mouse lenses, PKCy was found in the epithelial, cortical, and nuclear regions of the whole lens but was absent from the knockout mice. In the wild type mice Cx43 was found only in the epithelial layers. In contrast, in the PKCy knockout mouse lenses, Cx43 persisted into the outer and cortical regions of the lens. The location of Cx50 and Cx46 were identical in both wild type and knockout mice. Light and electron microscopy demonstrated that in the bow region the characteristic V-shape arrangement of the nuclei was disrupted in the knockout mice. However, fiber cell differentiation appeared otherwise normal despite the abnormal and persistent distribution of Cx43 in the knockout mouse lenses.

Conclusions: PKCy is required for the degradation of Cx43 during lens differentiation. The loss of PKCy does not alter Cx50 or Cx46 levels. The presence of the hyperphosphorylation sites at residues 363 - 375 of Cx43, not found in Cx50, are probably required for the PKCy-catalyzed loss of Cx43. This would not occur in the absence of PKCy as observed in the knockout mice.

Introduction
The mammalian lens is an avascular tissue which contains four distinct regions of differentiation. These include the central epithelium, the equatorial epithelium, the cortical fiber zone and the nuclear fiber zone. The actual transition of a lens epithelial cell to a lens fiber cell occurs in the dynamic equatorial zone located in the bow region of the lens. The exact mechanism of differentiation of lens epithelial cells is not clear yet. But this complex process which starts with the induction of proliferation followed by the cell cycle arrest and differentiation initiation hints at a high degree of cross talk between the signaling pathways that control these events. Some progress has been made in determining the molecular and cellular processes of lens epithelial cell differentiation in recent years. But the specific role for the classic isoform of Protein Kinase Cy (PKCy) in lens cell differentiation has not been elucidated yet. PKCy is present abundantly in the lens and has a very significant role in controlling the gap junction activity and regulating the connexin (Cx) proteins. Part of the lens epithelial to fiber cell differentiation process involves a loss of Cx43 and maintenance and control of Cx50 and Cx46. We suggest that it is the hyper-phosphorylation of Cx43 by PKCy which causes loss of Cx43 during differentiation. Cx50 and Cx46 do not contain the high serine rich tail present in Cx43 and survive into the mature fiber cell.

Degradation of Cx43 and not Cx50 by PKCy activation

Results: Long term exposure of the lens epithelial cells to TPA resulted in the degradation of Cx43, where as it had no effect on Cx50. Long term exposure to TPA also resulted in the degradation of PKCy, but the constitutively active catalytic sub-unit was intact.

Conclusions
• Long term exposure of lens epithelial cells to TPA (activator of PKCy) results in severe degradation of Cx43, but has no effect on Cx50. The presence of the phosphorylation sites at residues 363-375 of Cx43, not found in Cx50, are probably required for the PKCy-catalyzed loss of Cx43.
• The PKCy KO mouse lens has ~200% more total Cx43 than the control lens. Moreover, in the wild type mouse lenses, Cx43 is present only in the outer region in the epithelial cells, whereas in the PKCy knock out mouse lenses Cx43 persisted into the cortical region. The distribution of Cx46 and Cx50 were identical both in the wild type and PKCy knock out mouse lens (not shown).

Distribution of Cx43 in Control and KO lenses

Fig 5: Lenses were collected from wild type and PKCy knock out mice and homogenized or dissected into outer, cortical and nuclear regions. Each sample was then dissolved in cell lysis buffer, sonicated and sample loading buffer was added. Samples were immunoblotted with anti Cx43 NT antisera.

Results: The PKCy knock out mouse lens has ~200% more total Cx43 than the control lens. Moreover, in the wild type mouse lenses, Cx43 was present only in the outer region in the epithelial cells, whereas in the PKCy knock out mouse lenses Cx43 persisted into the cortical region. The distribution of Cx46 and Cx50 were identical both in the wild type and PKCy knock out mouse lens (not shown).

Fig 6: The knock out mouse lens has less nuclear organization. The characteristic V-shape arrangement of the nuclei in the bow region is disrupted in the PKCy knock out mouse lens. However, the general lens structure is maintained and develops somewhat normally.

Conclusions
• Long term exposure of lens epithelial cells to TPA (activator of PKCy) results in severe degradation of Cx43, but has no effect on Cx50. The presence of the phosphorylation sites at residues 363-375 of Cx43, not found in Cx50, are probably required for the PKCy-catalyzed loss of Cx43.
• The PKCy KO mouse lens has ~200% more total Cx43 on average than the control mouse lens.
• Cx43 persists into the cortical region of the mouse lens in PKCy KO mouse, whereas it is found only in the outer epithelial region in WT mouse. Loss of PKCy does not alter CX50 and Cx46 distribution.

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