Gap Junctions in the Lens: Is Location Everything?

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Abstract

The primary function of the ocular lens is as a light-focusing structure. To this end the lens is avascular, with a highly ordered arrangement of cells that acts to reduce light scattering. However, the avascular nature of the lens presents the problem of supplying cells deep within the lens with nutrients and removing waste products of cellular metabolism, i.e. maintaining cellular homeostasis. To solve this problem the lens possesses intercellular channels, called gap junctions, that facilitate cell-to-cell communication and are regulated by changes in the subcellular distribution and age-dependent processing of the gap junction protein subunits, called connexins. The lens expresses three types of connexins (Cx): Cx43 in the anterior surface epithelial cells and connexins 46 and 50 in the fibre cells. Therefore, to fully examine the role of GJs in lens homeostasis, regional differences in GJ structure and function need to be quantified.

In this thesis I present the results of experiments designed to map the differentiation-dependent changes to subcellular distribution and posttranslational processing of Cx46 and Cx50 in mouse and rat lenses. My results quantitatively show for the first time that Cx50 undergoes two discrete cleavage events in a similar manner to that previously shown for Cx46, and that qualitatively Cx46 does the same in the mouse lens. Such posttranslational processing has important effects on the proposed lens circulation model (Mathias *et al.*, 1997) that accounts for lens homeostasis. In addition, I have examined the functional contribution of gap junctions to localised intercellular communication in different regions of wildtype and Cx46-KO mouse lenses, using highly localised uncaging of fluorescein with Two-Photon Excited Flash Photolysis (TPEFP) in half lenses. The results of these experiments have provided the first supporting evidence for a macromolecule-permeable pathway, previously suggested by Shestoplaov and Bassnett (2000, 2003), that acts in conjunction with gap junctions to facilitate intercellular communication in the lens core. My results show that this pathway acts at a localised level to confer cell-to-cell communication that is as effective as that mediated by gap junctions, and therefore a re-evaluation of current models of lens homeostasis is now required.

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Abbreviations

AAH ..... artificial aqueous humour

AICS ..... artificial intracellular solution

Ar^+ ..... argon ion (laser)

BSA ..... bovine serum albumin

^oC ..... degrees celsius

cDNA ..... complementary deoxyribose nucleic acid

Cx ..... connexin

DF ..... differentiating fibre

dH_20 ..... distilled water

EGTA ..... ethylene glycol-bis(2-amino-ethylether)-N,N, N', N' -tetra-acetic acid

FAM ..... 5', 6'-carboxyfluorescein

GFP ..... green fluorescent protein

GJ ..... gap junction

HEPES ..... N-2-Hydroxyethylpiperazine-N'-2-ethansulphonic acid

IDL ..... interactive data language

kDa ..... kiloDalton

KI ..... knockin

KO ..... knockout

MF ..... mature fibre

MP (70) ..... membrane protein (of 70kDa)

mA ..... milliamp

ml ..... millilitre

µl ..... microlitre

mm ..... millimetre

µm ..... micrometer

mOsm ..... milliosmole

mV ..... millivolt

min ..... minute

NA ..... numerical aperture

nm ..... nanometre

OCT ..... optimal cutting temperature

Osm ..... osmole

PBS ..... phosphate buffered saline

PFA ..... paraformaldehyde

R/A ..... normalised radial position = distance from lens centre (R) divided by total lens radius (A)

s ..... second

SDS-PAGE ..... sodium dodecyl sulphate polyacrylamide gel electrophoresis

SEH ..... sucrose EDTA HEPES solution

sRhod ..... sulforhodamine B

TP ..... two-photon

TPEFP ..... two-photon excited flash photolysis

TPEMS ..... two-photon excited microsurgery

UV ..... ultraviolet

WGA ..... wheat germ agglutinin

w/v ..... weight/volume

WT ..... wildtype