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Non-essential role for cilia in coordinating precise alignment of lens fibres

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ABSTRACT

The primary cilium, a microtubule-based organelle found in most cells, is a centre for mechano-sensing fluid movement and cellular signalling, notably through the Hedgehog pathway. We recently found that each lens fibre cell has an apically situated primary cilium that is polarised to the side of the cell facing the anterior pole of the lens. The direction of polarity is similar in neighbouring cells so that in the global view, lens fibres exhibit planar cell polarity (PCP) along the equatorial-anterior polar axis. Ciliogenesis has been associated with the establishment of PCP, although the exact relationship between PCP and the role of cilia is still controversial. To test the hypothesis that the primary cilia have a role in coordinating the precise alignment/orientation of the fibre cells, IFT88, a key component of the intraflagellar transport (IFT) complex, was removed specifically from the lens at different developmental stages using several lens-specific Cre-expressing mouse lines (MLR10- and LR-Cre). Irrespective of which Cre-line was adopted, both demonstrated that in IFT88-depleted cells, the ciliary axoneme was absent or substantially shortened, confirming the disruption of primary cilia formation. However no obvious histological defects were detected even when IFT88 was removed from the lens placode as early as E9.5. Specifically, the lens fibres aligned/oriented towards the poles to form the characteristic Y-shaped sutures as normal. Consistent with this, in primary lens epithelial explants prepared from these conditional knockout mouse lenses, the basal bodies still showed polarised localisation at the apical surface of elongating cells upon FGF-induced fibre differentiation. We further investigated the lens phenotype in knockouts of Bardet–Biedl Syndrome (BBS) proteins 4 and 8, the components of the BBSome complex which modulate ciliary function. In these BBS4 and 8 knockout lenses, again we found the pattern of the anterior sutures formed by the apical tips of elongating/migrating fibres were comparable to the control lenses. Taken together, these results indicate that primary cilia do not play an essential role in the precise cellular alignment/orientation of fibre cells. Thus, it appears that in the lens cilia are not required to establish PCP.

1. Introduction

The primary cilium is a microtubule-based cell membrane projection that emanates from a modified centriole, the basal body. The ciliary membrane contains various receptors or channels so that the cilium can act as a sensor or an antenna to detect diverged signals from extracellular space (Berbari et al., 2009; Satir et al., 2010). Among several signalling pathways that are known to be associated with cilia the most intensively examined is the Hedgehog pathway (Hh); indeed, cilia are the dominant domain for enabling the Hh pathway to initiate its signalling cascade (Briscoe and Therond, 2013). An emerging new pathway that has also been linked to the cilium is the Wnt/Frizzled planar cell polarity (PCP) pathway that provides a mechanism for orienting cells relative to the axis along the plane of the tissue (Jones et al., 2008; Ross et al., 2005). However, in contrast to the consensus on the role of cilia in the Hh pathway, the link between cilia and PCP is still controversial (Wallingford and Mitchell, 2011). Primary cilia are found in almost all vertebrate cells and in several tissues cilia are indispensable for their development and maintenance. Indeed disrupted ciliary function is related to a broad range of diseases including polydactyly, obesity, retinal degeneration and renal disease. These are collectively known as ciliopathies, and many syndromes are linked to genetic mutations in cilia-related genes (Waters and Beales, 2011).

The eye lens is comprised of two types of cells, the highly elongated fibre cells that constitute the bulk of the lens and the epithelial cells that form a thin layer on the anterior surface of the fibres (Fig. 1A). The lens

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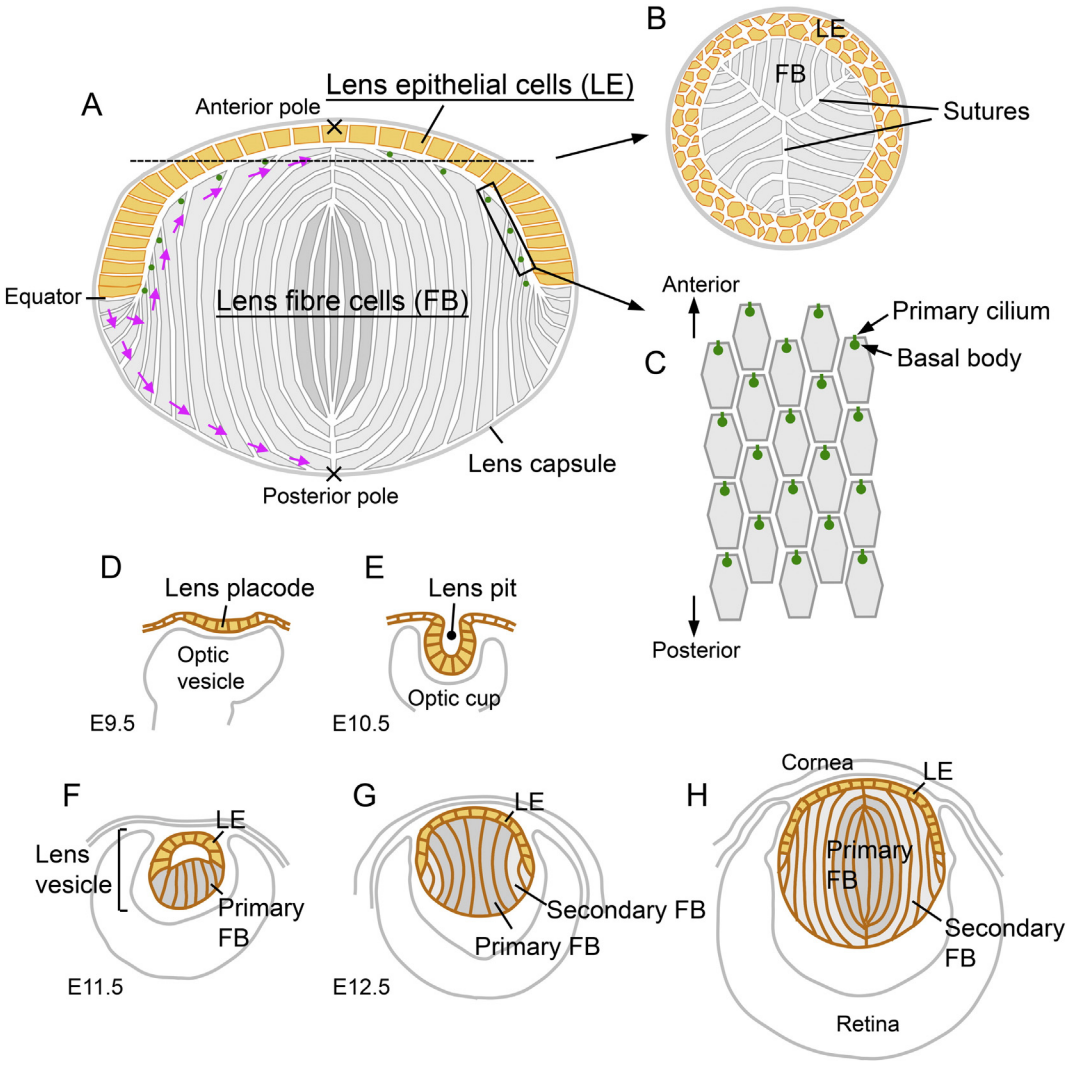

Fig. 1. Lens structure (A–C) and development (D–H). (A) Most of the proliferative activity in the lens epithelium (yellow) is found in the region anterior to the equator. Cells that shift posterior to the equator exit the cell cycle and initiate terminal differentiation into lens fibre cells (grey). The apical and basal tips of the elongating fibres undergo directed migration (purple arrows) to the anterior and the posterior poles, respectively. At the poles, each fibre tip meets the tip from an equivalent fibre from another segment and collectively these aligned junctions form the lens sutures. The epithelial and fibre cells are contained within a thick extracellular matrix, the lens capsule. (B) A cross section around the anterior pole shows Y-shaped sutures formed by the aligned apical tips of the fibre cells. (C) A superficial view shows the hexagonal apical surfaces of the fibres with anteriorly polarised primary cilia/basal bodies. (D–H) Lens morphogenesis begins with the thickening of the surface ectoderm in the head region adjacent to the optic cup to form the lens placode (D). Following invagination to form the lens pit (E), the cells round up and separate from the surface ectoderm to form the lens vesicle (F). The cells in the posterior half of the lens vesicle elongate and undergo terminal differentiation to form the primary fibre cells that fill the vesicle lumen (G). The cells in the anterior half of the vesicle form an epithelial layer and this establishes the characteristic anterior–posterior polarity of the lens (G). Secondary fibre cells differentiate from the lens epithelial cells at the lens equator and progressively accumulate around the primary fibre cells so that the primary fibres are internalised and comprise the lens nucleus (H).

epithelial cells are proliferative and are the progenitors for additional fibre cells that are progressively added to the fibre mass as the lens grows throughout life. The lens fibre cells align and pack regularly as they elongate and undergo terminal differentiation. During this process they also undergo directed migration towards the poles and eventually meet up with equivalent fibres from other segments of the lens. Because this behaviour is highly coordinated, the points of contact at their tips form distinctive Y-shaped suture lines (Fig. 1B, Kuszak et al., 2006). Primary cilia/basal bodies have been detected in both lens epithelial and fibre cells and interestingly, they show asymmetric localisation on the apical surfaces (Sugiyama et al., 2010). This is most pronounced in the fibre cells of the lens cortex; each fibre has a hexagonal apical surface and the cilium/basal body is localised towards the anterior side (the side facing towards the anterior pole, Fig. 1C). Since the direction of this polarity is similar in neighbouring cells, in the global view the lens fibres show planar polarity along the equatorial-anterior polar axis (Sugiyama et al., 2010). Given the role of primary cilia as antenna to detect extracellular signals and also their involvement in PCP formation, we hypothesised that the primary cilia on the fibre cells detect a global guidance signal (presumably coming from the anterior pole) to promote directed fibre cell migration to form lens PCP (Sugiyama et al., 2011).

Hh signalling plays critical roles during eye development. During eye formation Hh is essential to separate the two presumptive eye regions and its failure leads to cyclopia (Gongal et al., 2011). Hh is also required for closure of the choroid fissure during eyecup formation and its defective signalling causes coloboma (Gongal et al., 2011). It is also known that Hh mediates a switch between lens and anterior pituitary placodal cell identity; Hh signal suppresses lens fate and promotes the specification of anterior pituitary cells (Gunhaga, 2011). In accord with this, reduction of Hh activity leads to development of ectopic lenses whereas over-expression blocks lens formation (Gunhaga, 2011). Hh has also been reported to play an essential role during early lens development since conditional knockout (cKO) of Smo, the essential transducer of

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