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A gradient of matrix-bound FGF-2 and perlecan is available to lens epithelial cells

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ABSTRACT

Fibroblast growth factors play a key role in regulating lens epithelial cell proliferation and differentiation via an anteroposterior gradient that exists between the aqueous and vitreous humours. FGF-2 is the most important for lens epithelial cell proliferation and differentiation. It has been proposed that the presentation of FGF-2 to the lens epithelial cells involves the lens capsule as a source of matrix-bound FGF-2. Here we used immunogold labelling to measure the matrix-bound FGF-2 gradient on the inner surface of the lens capsule in flat-mounted preparations to visualize the FGF-2 available to lens epithelial cells. We also correlated FGF-2 levels with levels of its matrix-binding partner perlecan, a heparan sulphate proteoglycan (HSPG) and found the levels of both to be highest at the lens equator. These also coincided with increased levels of phosphorylated extracellular signal-regulated kinase 1 and 2 (pERK1/2) in lens epithelial cells that localised to condensed chromosomes of epithelial cells that were Ki-67 positive. The gradient of matrix-bound FGF-2 (anterior pole: 3.7 ± 1.3 particles/μm^2; equator: 8.2 ± 1.9 particles/μm^2; posterior pole: 4 ± 0.9 particles/μm^2) and perlecan (anterior pole: 2.1 ± 0.4 particles/μm^2; equator: 5 ± 2 particles/μm^2; posterior pole: 1.9 ± 0.7 particles/μm^2) available at the inner lens capsule surface was measured for the bovine lens. These data support the anteroposterior gradient hypothesis and provide the first measurement of the gradient for an important morphogen and its HSPG partner, perlecan, at the epithelial cell-lens capsule interface.

The eye lens comprises a lens capsule that encases a single layer of epithelial cells covering just the anterior hemisphere and the fibre cells that make the bulk of the lens. The lens grows throughout life through continuous epithelial cell proliferation and their differentiation into lens fibre cells at the lens equator. Fibroblast growth factors (FGFs) 1 and 2 regulate both lens cell proliferation and differentiation (McAvoy and Chamberlain, 1989), although of the two, FGF-2 is absolutely required (Garcia et al., 2005; Zhao et al., 2006). It, like FGF-1 is synthesized in the retina and ciliary body (Lovicu et al., 1997) and the FGFs are secreted into the aqueous and vitreous humours (Caruelle et al., 1989; Schulz et al., 1993), diffusing through the lens capsule where they can potentially bind to the heparan sulphate proteoglycan (HSPG) (De Iongh and McAvoy, 1992), perlecan (Iozzo, 1998; Tholozan et al., 2007). FGF-2 can be released from the capsule by matrix metalloproteinases (Tholozan et al., 2007) and bind to FGF receptors to activate the mitogen-activated protein kinase (MAPK) signalling cascade, resulting in the phosphorylation of MAPK1 (ERK1; Upadhya et al., 2013) that is localised in the nuclei of epithelial cells (Lovicu and McAvoy, 2001) effecting cell proliferation and differentiation (Golestaneh et al., 2004; Le and Musil, 2001; Lovicu and McAvoy, 2001) via specific transcription factors (Krishna and Narang, 2008).

Lens epithelial cell proliferation and differentiation are FGF-concentration dependent processes (McAvoy and Chamberlain, 1989). It is proposed that there is an anteroposterior gradient of FGF-1 and FGF-2 (Lovicu and McAvoy, 2005; Lovicu et al., 2011; McAvoy and Chamberlain, 1989), but so far this gradient at the cell-lens capsule interface has not been measured. Previous studies based on cross sections of rat lens capsules (Lovicu and McAvoy,

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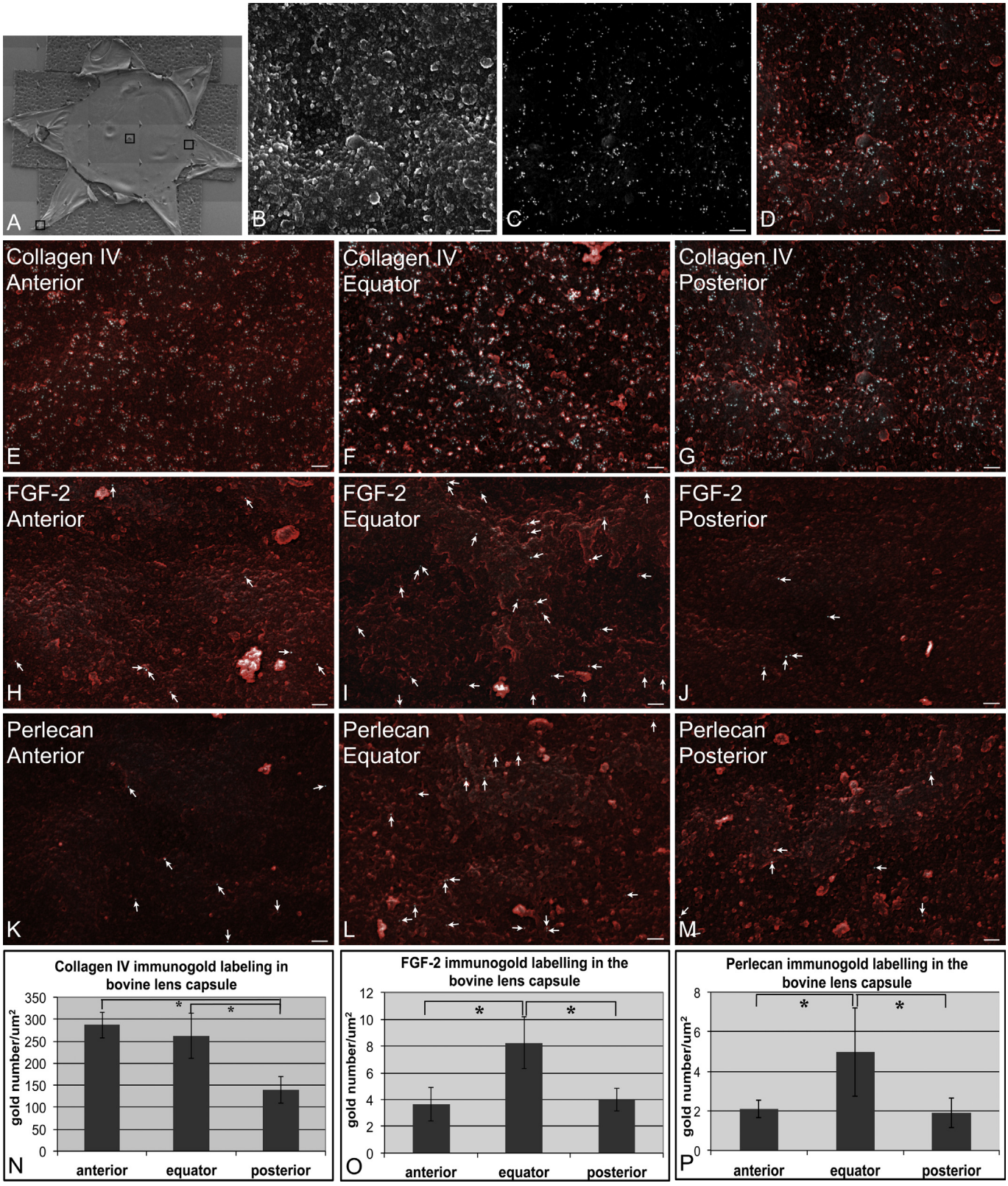

Fig. 1. Measurement of FGF-2, collagen IV and perlecan at the epithelial cell – lens capsule interface by immunoelectron microscopy. Bovine lens capsules were flat-mounted and washed in deionised water for one hour to remove epithelial cells. They were then incubated with either rabbit anti-collagen IV polyclonal antibody (Abcam, UK), or rabbit anti-FGF-2 polyclonal antibody (Calbiochem, USA) or mouse anti-perlecan monoclonal antibody (Chemicon International, USA) for 2 h at room temperature, followed by an hour of incubation with the appropriate secondary antibodies (either goat anti-rabbit or goat anti-mouse IgG both conjugated with 10 nm gold; BBInternational, Cardiff, UK). Negative controls were incubated with PBS instead of primary antibodies. Immunogold labelled capsules were viewed in a Hitachi SU-70 FEG scanning electron microscope (Hitachi Hight-Technologies Europe GmbH, Germany). Each labelling was independently repeated three times. A. An example of a lens capsule prepared for scanning electron microscopy. Three images were then randomly taken at the equator and the anterior and posterior poles (black squares). B. The secondary electron image shows the capsular surface and its compacted meshwork. C. A backscatter electron image of the same area as in panel B shows a large number of white gold particles that indicate collagen IV labelling. D is the combined secondary/backscatter image of panels B and C to show the location of gold particles on the lens capsule. E–M: Representative images show the immunogold labelling of collagen IV (E–G), FGF-2 (H–J) and perlecan (K–M) in different regions of the inner surface of the bovine lens capsule. More gold particles were present after collagen IV labelling than after FGF-2 and perlecan labelling (white arrows). N–P: The quantification of gold particles detected on lens capsules after Collagen IV, FGF-2 and perlecan labelling. Significant differences tested by Student's *t*-Test are shown (*). B–M. Scale bars = 100 nm.