data, along with immunoblotting and transfection analyses (Osler and Bader, 2004) demonstrate the specificity of B846 for Bves.

The retina

The central nervous system (CNS) will give rise to the optic cup and stalk as a lateral outpocketing. The following description of Bves expression concentrates on the developing chicken, and a similar protein distribution was seen in the embryonic mouse eye. Bves protein is detected in CNS cells adjacent to the central canal (Figure 33A) and is sustained in this layer throughout the CNS and in the diencephalon as it evaginates to form the optic vesicle (figure 33B). This staining is confined to the apical aspect of the epithelium adjacent to the lumen of the optical vesicle (figure 33B, inset). Next, strong expression is seen at the apical surfaces of the inner and outer epithelial layers as they merge to form the mature retina (Figure 34A-H). It should be noted that the inner and outer layers of the optic cup are parts of a single, continuous sheet and that the infolding of the optic cup brings the apical surfaces of this epithelium into apposition (see Figure 30). Close inspection (Figure 34B-C) shows that Bves is present in both the inner and outer layers of the optic cup. Thus, Bves staining is observed continuously in an apical position throughout the course of retinal cup formation and differentiation even though this epithelium is continuously reshaped during the early phases of diencephalon outgrowth and subsequent invagination. At this point in development, few if any pigment granules are seen in the outer layer (Figure 34D). As the inner and outer layers appose, a continuous band of Bves staining is observed at their junction (Figure 34E-G; note that this section does not contain the lens), and pigment granules are now abundant in the RPE (figure 34H). When the day 19

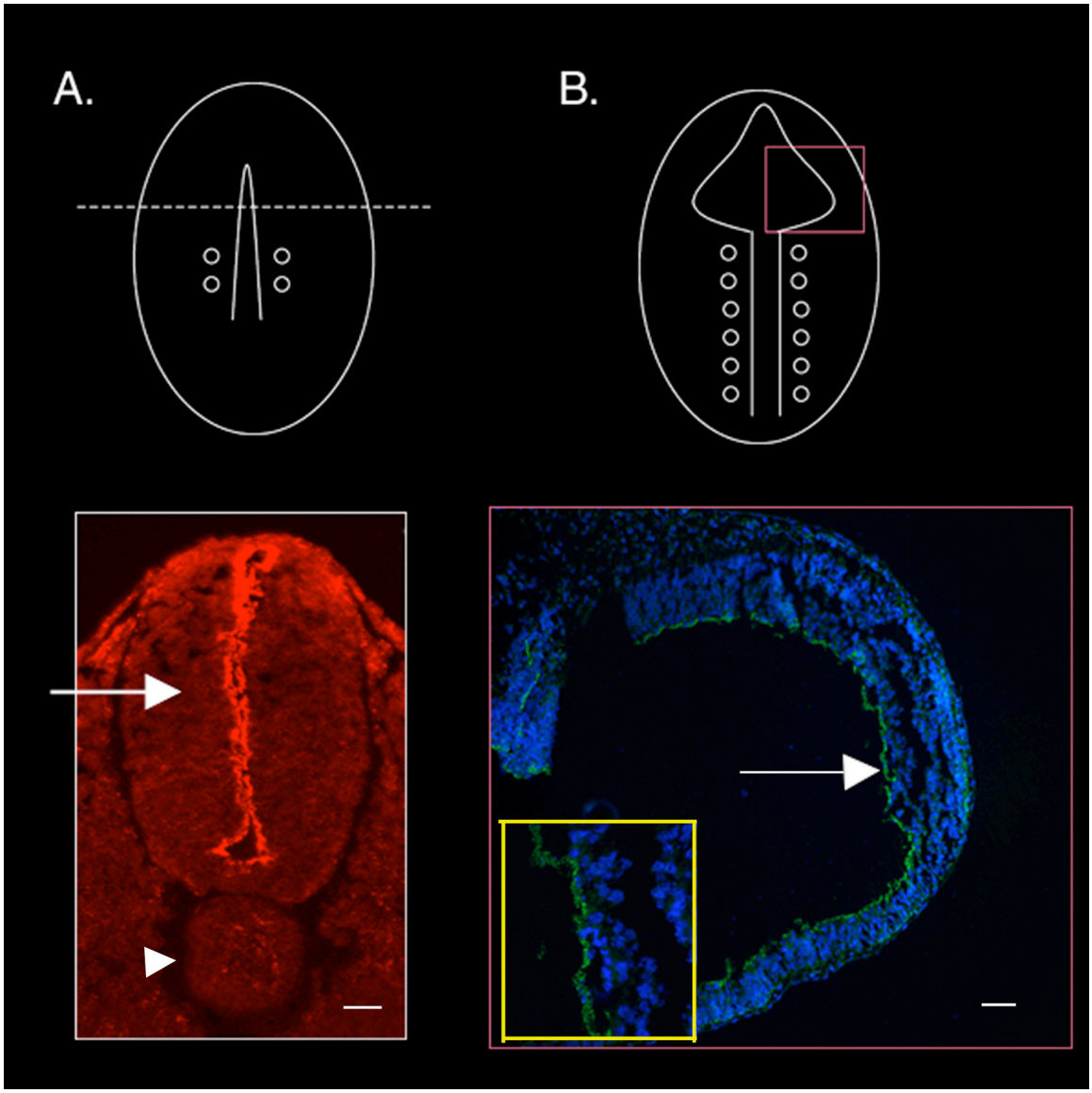

Figure 33. Bves is expressed in the CNS and during optic vesicle formation. (A) The dotted line in the schematic represents the plane of the section shown in the accompanying micrograph (lower left). Immunohistochemical analysis shows Bves expression (red, arrow) in the epithelial lining of the chick neural tube at stage 7 (scale bar = 50 um). The position of the notochord is shown (arrowhead). The diagram in figure 1A represents this stage. (B) The upper diagram depicts a stage 9 chick embryo in the longitudinal plane illustrating the formation of the optic vesicle. The pink box in the schematic represents the area of the section shown. Bves expression (green, arrow, lower right) in the outpocketing of the diencephalon during early optic vesicle formation of a stage 9 chick shows the apical position in this staining in the epithelium (scale bar = 50 um). (Inset) A higher power view (scale bar = 10 um) is given to show the apical position of Bves in the neuroectoderm. DAPI (blue) was used to stain nuclei.

embryonic chick retina was examined, Bves staining was localized to the RPE, photoreceptors, outer nuclear layer, and the ganglion cell layer (figure 34I-L).

Our previous studies have shown that Bves colocalizes with adherens junctions proteins, such as E-cadherin and β-catenin during epithelial sheet formation *in vitro* (Wada et al., 2001). To determine whether this is a common feature in diverse embryonic epithelia, colocalization studies with immunochemical markers of cell adhesion molecules were conducted. We find that Bves has extensive overlap of staining with β-catenin, a marker of the adherens junction (Aberle et al., 1996) in the apical/lateral region of the optic cup epithelia both before (Figure 34M-O) and after epithelial apposition (Figure 34P-R). This overlap is confined to the apical-most portion of the cell as staining of adherent junction proteins extends more basally. It should be noted that additional, non-overlapping staining of β-catenin is also observed in the RPE.

The lens

The lens is derived from surface ectoderm where Bves is broadly expressed prior to any morphological evidence of lens induction (data not shown). As the lens vesicle is formed by invagination of the surface ectoderm, Bves staining is observed on the inner surface of the vesicle, i.e. the apical/lateral surface of this epithelium (diagram in Figure 35A; Figure 35B). Again, extensive but not complete overlap of Bves staining with adhesion junction markers, in this case E-cadherin is observed in the apical position of the lens vesicle epithelium (Figure 35B-D). The next stage of lens development is characterized by the growth or extension of primary fibers that fill the lens cavity (Figure 35A). As cellular extension proceeds, high levels of Bves are seen at the advancing edge of the primary fibers that course anteriorly during their

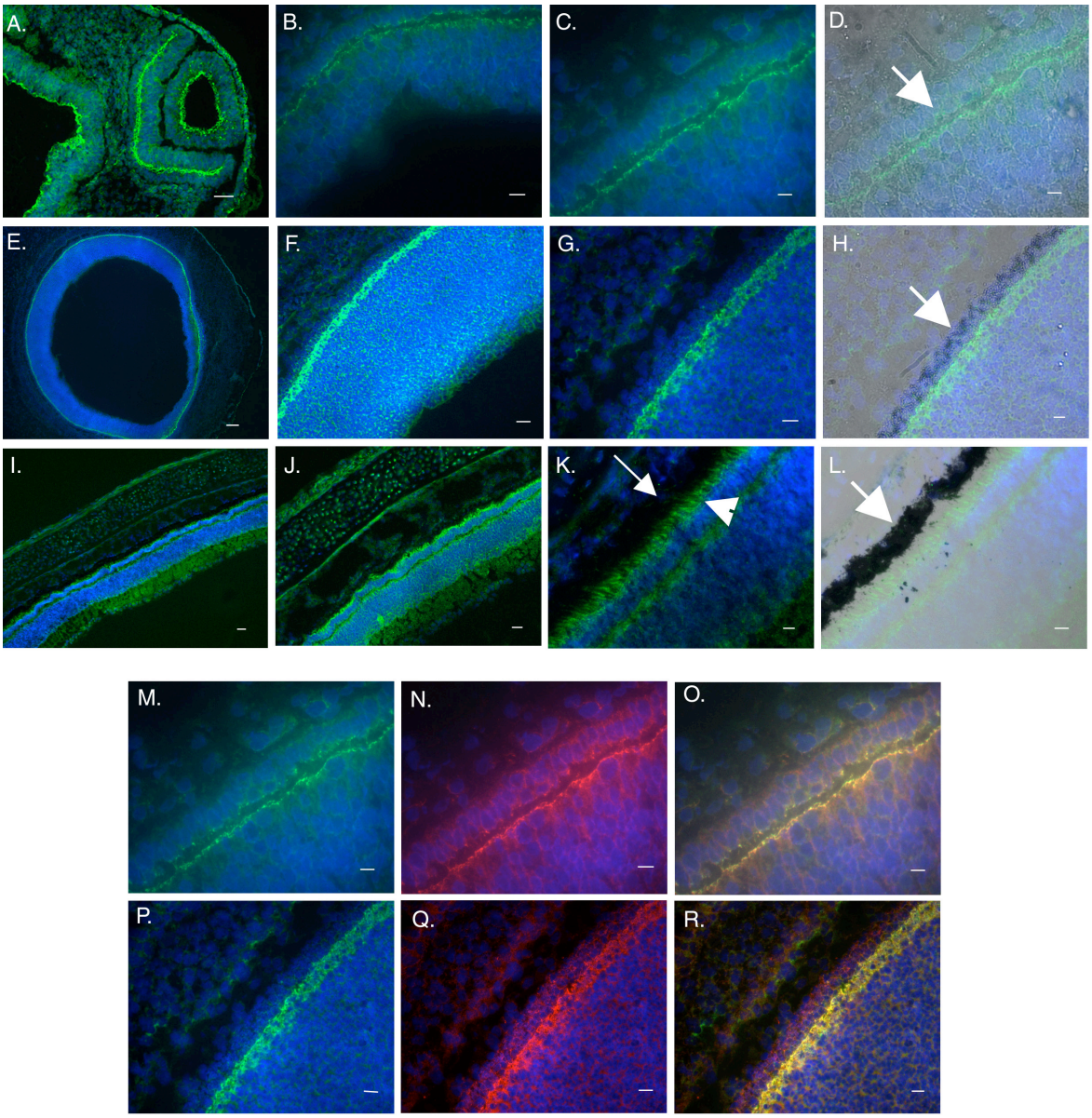

Figure 34. Bves localization during formation and apposition of inner and outer retinal layers. (A-D) Bves expression (green) during inner and outer optic cup apposition in stage 18 chick embryos in (A) (scale bar = 100 um), (B) (scale bar = 25 um), (C,D) (scale bar = 10 um). Phase contrast is overlapped with images (D,H,L) to illustrate pigment localization in the RPE (arrows). Note that pigment granules have not accumulated in D. (E-H) Bves (green) expression after the optic cup layers have fused in the day 8 chick embryo in (E) (scale bar = 100 um), (F) (scale bar = 25 um), (G,H) (scale bar = 10 um). (I-L) Bves (green) remains expressed in the differentiated retinal layers (photoreceptors (arrowhead) and RPE (arrow)) derived from the epithelial inner and outer optic cup layers in the day 19 chick in (I) (scale bar = 100 um), (J) (scale bar = 25 um), (K,L) (scale bar = 10 um). Bves (green) expression overlaps with adherens junction marker, ß-catenin (red), during (M-O, scale bar = 10 um) and after (P-R, scale bar = 10 um) optic cup apposition. Overlap of Bves and ß-catenin expression is represented as yellow in the merge (O,R). Note that -catenin staining is broader than that of Bves. DAPI (blue) was used to stain nuclei.

differentiation to obliterate the lens vesicle cavity (Figure 35E). After the lens vesicle cavity has been obliterated, Bves continues to be expressed in the anterior undifferentiated epithelial cells (Figure 35H). After these cells migrate past the bow region and begin to elongate, the expression of Bves is not seen on the lateral surface of the elongating lens fibers, but may remain localized at the apical regions of these cells even during their pronounced elongation (Figure 35H). While E-cadherin staining is observed in this same region, it is more broadly expressed through the lens fibers (Figure 35E-J). High power magnification of a day 4 embryo shows overlap of staining in the anterior lens epithelium (Figure 35K-M). Bves continues to be expressed in the differentiated layers in the anterior epithelium and apically in the primary fibers.

The cornea

Bves is broadly expressed in surface ectodermal cells prior to corneal induction and differentiation in the chicken (Figures 31, 34A, 35H) and mouse (data not shown). This staining is not confined to regions of the presumptive cornea and is present in much of the ectoderm of the head. When the surface ectoderm becomes stratified as seen in this six week old mouse preparation, Bves staining is seen in the corneal epithelium, but is absent in the primary and secondary corneal stroma, endothelium and the sclera (Figure 36). The same restriction of Bves staining to corneal epithelium is observed in the chick (Figure 36F-G). Bves staining within the corneal epithelium varies from basal to apical strata (Figure 36B-C). In the deep layers, prominent punctate staining around the periphery of cells is observed while rather homogeneous reactivity is observed around the entirety of cells in the intermediate or wing cell layers (Figure 36C). Finally, as cells orient themselves perpendicular to the apical/basal axis in the most superficial region, strong homogeneous staining is seen throughout these cells.