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Early Divergence of Central and Peripheral Neural Retina Precursors During Vertebrate Eye Development

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Abstract

During development of the vertebrate eye, optic tissue is progressively compartmentalized into functionally distinct tissues. From the central to the peripheral optic cup, the original optic neuroepithelial tissue compartmentalizes, forming retina, ciliary body and iris. The retina can be further sub-divided into peripheral and central compartments, where the central domain is specialized for higher visual acuity, having a higher ratio and density of cone photoreceptors in most species. Classically, models depict a segregation of the early optic cup into only two domains, neural and non-neural. Recent studies, however, uncovered discrete precursors for central and peripheral retina in the optic vesicle, indicating that the neural retina cannot be considered as a single unit with homogeneous specification and development. Instead, central and peripheral retina may be subject to distinct developmental pathways that underlie their specialization. This review focuses on lineage relationships in the retina and revisits the historical context for segregation of central and peripheral retina precursors before overt eye morphogenesis.

Keywords

optic cup; optic vesicle; fate map; lineage; avian eye

Introduction

Eye morphogenesis during vertebrate development occurs through a complex series of morphogenetic steps. A single prospective eye-field can be identified in the blastoderm of the early embryo but the first overt indicators of eye development are the optic sulci on

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either side of the neural folds. During development, the continuing evagination of the eye anlagen forms the optic vesicles. Zebrafish, exceptionally, undergo a cavitation process to form the optic vesicles (Schmitt and Dowling, 1994). The basic morphology of mature eye is recognizable when the optic vesicle invaginates, forming a bi-layered cup in which the inner epithelium is 'neural' and the outer is the pigmented epithelium. During this complex developmental process, functional compartmentalization becomes recognizable. Over time, and consistent with a morphogenetic wave of differentiation, the sensory neural retina, ciliary body, and ultimately iris resolve from this simple cup structure, along the central to peripheral axis respectively (Figure 1). When describing the entire eye, this axis is also referred to as the anterior-posterior axis, in keeping with common understanding that the anterior segment of the eye refers to the ciliary body, iris and lens, and the posterior segment refers to the sensory retina.

In the mature eye, the retina, ciliary body, and iris each differentiates directly from the neuroepithelium and has a distinct function. The outer epithelium for all three tissues has a simple pigmented structure and may have regional differences, although this is not yet clear. Within the neural retina a further sub-division can be described between the central and peripheral aspects. In some species, the central retina is structurally different than the peripheral retina, and is organized into an area of high visual acuity (Prince, 1956). There are a wide variety of characteristics that set the central apart from the peripheral retina, and not all are found in the eyes of all vertebrates: Different photoreceptor densities are found centrally and cones often dominate; specialized pigments can be deposited, as in the lutein of the human macula; and in some species a foveal depression is found. Even in mice, where none of these features are noted, a recent report detailed that the retinal ganglion cells (RGCs) were organized differently in the central retina (Della Santina et al., 2013). All of these features lead to enhanced processing at the fundus, the area opposite the pupil. The central retina is small, for example comprising only 3% of the overall area of the human eye. However, potential mechanisms guiding the development of this essential area are currently unknown.

A major focus of studies on eye development is understanding the mechanisms underlying emergence of distinct tissues in the mature eye. The first obvious distinction, between neural and pigmented epithelium, has been described in many studies over decades (Chow and Lang, 2001; Bharti et al., 2006). The further compartmentalization of the eye epithelium into distinct functional units during development is largely modeled on the basis of gene expression and mis-expression (Cho and Cepko, 2006; Dias da Silva et al., 2007; Liu et al., 2007; Davis et al., 2009). These studies have described the general cues for differentiation of the most peripheral tissues (iris and ciliary body) but do not describe how the neural retina is compartmentalized.

The classic model of eye development postulates a single origin for the neural retina (Spemann, 1901) and is largely based upon observation of interplay between overlying surface ectoderm and the optic vesicle during both lens and neural retina development, which leads to placement of a single neural retina precursor at the distal tip of the OV (Figure 2A). Upon invagination to form the optic cup, the retinal precursor is displaced behind the developing lens and inner and outer optic cup epithelia are resolved, separated by

the hinged epithelium forming the peripheral-most optic cup lip (OCL), advertised as the precursor of the ciliary body and iris. Thus, the neural retina is proposed to develop predominately through expansion of this precursor population. It has been demonstrated that the most central portion of the neural retina differentiates early, and the peripheral margins remain proliferative and differentiate last. In fish and frog species, a precursor population is maintained at the margin of the sensory retina, and continually adds tissue to the sensory retina (Raymond et al., 2006; Agathocleous and Harris, 2009). However, this is an adult mode of eye growth, and the equivalent structure in avian and mammalian species, the ora serrata, does not make a significant contribution to the differentiated eye (Fischer and Reh, 2000; Ghai et al., 2008; Lamba et al., 2008; Fischer et al., 2014). Warm-blooded vertebrates have a protracted period of embryonic eye growth that may have different characteristics. Recent direct lineage analyses in the developing chick eye found that peripheral retina is added to the inner optic cup (OC) from a multipotent precursor population in the optic cup lip (Figure 2B) (Venters et al., 2011).

A single neural retina origin model of eye development exposes its entirety to similar environments through early induction stages. If, in contrast, central and peripheral retina diverge early in development, each sub-unit can be independently instructed towards a specific functional assignment. Subdivision of the retina, early in development opens up many questions regarding its distinct specification, which may occur earlier in development than previously thought. Most importantly, it would inform future studies on where to look for potential inductive mechanisms involved in the generation of the unique features of the macula and fovea.

There are several recent reports and detailed reviews on the molecular pathways involved in eye development, and there is a significant understanding of the regional specification of the early optic tissue into neural retina and pigmented epithelium through molecular networks (Heavner and Pevny, 2012; Sinn and Wittbrodt, 2013; Fuhrmann et al., 2014; Zagozewski et al., 2014). Beyond this broader demarcation, there are gene expressions that support that the early retinal tissue is compartmentalized in the newly formed optic cup (Vogel-Hopker et al., 2000; Martinez-Morales et al., 2005; Oron-Karni et al., 2008). However, a straightforward molecular definition of central versus peripheral tissue based on marker expression is complicated by the fact that the same cells types are found in both, albeit in different arrangements. Therefore, the majority of the genetic program may be the same, with differences in onset timing, developmental history and subtle environmental cues.

New findings do hint at additional early compartments within the optic vesicles, and demonstrate that specified neural retina tissue is found at unexpected positions. The entire dorsal optic vesicle microenvironment is the site of molecular cues that direct the dorsal towards pigmented epithelium differentiation and, therefore, the neural retinal domain may be located much more ventrally than previously described (Steinfeld et al., 2013). Recently it was shown that ectopic neural retinal tissue can be induced by overexpression of the transcription factor Lhx1 (Kawaue et al., 2012), similar to well described ectopic inductions from the action of FGFs or Sox2 (Park and Hollenberg, 1989; Hyer et al., 1998; Zhao et al., 2001a; Galy et al., 2002; Ishii et al., 2009). Lhx1 is normally expressed in the caudal OV, and its expression overlaps with the precursor for the central neural retina as identified

through direct cell marking (Venters et al., 2013). Together, these data indicate the central neural retina may already have a distinct molecular identity in the optic vesicle.

Generally speaking, beyond a basic description of histological development or gene expression patterns in the fully differentiated eye, there is very little information on the a priori events in central retina development, whether that be the primate macula, or the avian area centralis (reviewed by Gregory-Evans et al., 2013, Gregory-Evans and Gregory-Evans, 2011; Schulte and Bumsted-O'Brien, 2008). Pax6 mutations and a few other loci have been implicated in human foveal malformation syndromes, but the mechanistic failing has not been modeled (Azuma et al., 2003; Michaelides et al., 2012). Specific signaling environments have also been described, leading to the hypothesis that the developing chick central retina is not influenced by retinoic acid or thyroid hormone signaling (Trimarchi et al., 2008; Billings et al., 2010). It is not clear if this is related to a generalized early differentiation state, or a cone photoreceptor specification event.

This review will, for the most part, focus on studies that have looked at the lineage relationships in the developing eye. Lineage relationships between groups of cells can often reveal functional compartments, especially given that many simple gene expressions do not reveal the actual state of the signaling event that they are associated with. Once the optic cup is formed, differences between central and peripheral neural retina are easiest to observe and define, and this stage is where most studies have based their analyses. Therefore, the review will begin at optic cup stages and subsequently move earlier in development to re-introduce several classic, and detailed, studies that yield intriguing data in support of the dual origin model.

Optic Cup stages: Central and Peripheral Retina Grow Differently

There are some indications that the central retina may be set apart at very early developmental stages. The growth characteristics of the central and peripheral retina highlight clear morphogenetic differences between these two compartments and indicate timing disparity as differentiated cell types emerge. It is well documented that the central neural retina begins to differentiate first in most species. Early born RGCs in the central retinal of the chick begin to exit the cell cycle by the end of E2 (Kahn, 1974; Prada et al., 1991), before optic cup morphogenesis. The successive exit of retinal cell types continues over several days with overlapping exit timing along the central-peripheral eye axis (Belecky-Adams et al., 1996; Rapaport et al., 2004). Similarly, the RPE displays a central-peripheral mitotic gradient (Bodenstein and Sidman, 1987). These proliferation characteristics are underscored by clone behavior in the retina, where clonal expansion is several times greater in peripheral versus central retina (Fekete et al., 1994).

Recent direct lineage analyses, in the chick, identified an ordered, continual anterior-driven expansion of the entire optic cup, including the peripheral neural retina, from a long-lived progenitor pool at the OCL (Figure 3) (Venters et al., 2011). This is in contrast to a model whereby the neural periphery merely remains proliferative resulting in increased peripheral clone expansion.

This conclusion mirrors reports in other animals, using various methodology to demark cell populations in the developing eye. Early studies into growth patterns in the optic cup have been done using chimeras, either mixtures of different species, or animals with different genetic markers that can be followed. In Xenopus, chimeras are made using pigmented and albino cells, and can be targeted directly to the forming eye. Small pieces of the donor optic vesicle were transplanted into the edge of a newly formed optic cup and these produced large sectors of donor RPE tissue in the eye (Cooke and Gaze, 1983; Conway and Hunt, 1987; Hunt et al., 1987a). In general, the pigmented donor cells stayed together as a coherent population, and enlarged along the central to peripheral axis. When examined in vivo over time, it was clear that the pigmented cell clusters increased in size by adding new cells at the anterior-most edge of the group; the posterior-most cells did not markedly expand. Pigmented cells were also found in the adjacent iris, the most anterior portion of the optic cup (Conway and Hunt, 1987; Hunt et al., 1987a). This defines a growth pattern specific to the peripheral retina domain, in which the anterior edge is a germinal center and lays down new tissue over time. When donor cells were not introduced into the peripheral OC domain, they remained as small cohorts of cells near the optic nerve (Hunt et al., 1987b).

The growth characteristics of the mammalian retina are similar to those uncovered in other species. In mouse embryos, chimeras are made by mixing blastocysts at the 8-cell stage; therefore the presence of a central versus peripheral domain can only be inferred. Even with that caveat, a pattern emerged where pigmented cells in the periphery of the eye were grouped into elongated patches that extended through the peripheral RPE into the iris (Schmidt et al., 1986; Bodenstein and Sidman, 1987a; Collinson et al., 2004). In contrast, the area around the optic nerve contained pigmented cells grouped randomly and as single cells. Because of the indirect nature of this labeling technique, it cannot be known if all the pigmented cells in a given patch are clonally related. However, the pattern matches closely to results from direct lineage analyses in the Xenopus and chick eye.

Therefore, in the RPE, the central domain is a site of rapid differentiation and low proliferation, while the remainder of the eye is built through continued proliferation and addition by cells in the peripheral domain. Is this also true for the inner epithelium of the optic cup? This has been harder to analyze in Xenopus since there are no robust markers to label the cells. Rather, chimeric mice and retroviral labels applied to the chick embryo have been used. The majority of reports detail either the radial/column clonal relationships, or the tangential/lateral migration of neuronal subtypes. There are only a handful of studies that describe marked cell populations in the inner epithelium growing along the central-peripheral axis. Again, these studies do not define a strictly clonal relationship, but the pattern is consistent with production from a peripheral germinal center and there is a tendency for cohorts to have a long axis that is aligned along the central-to-peripheral eye axis (Goldowitz et al., 1996; Reese et al., 1999; Rice et al., 1999). The anterior-directed growth of the optic cup has also been described in the fish using chimeras that are easily scored simultaneously in the outer and inner epithelia [pigmentation and GFP label respectively, (Centanin et al., 2011)].

Classically, the Xenopus and fish studies describe an anterior-directed growth from the area of CMZ (Ciliary Margin Zone) just posterior to the ciliary body, at the edge of the sensory

retina. The CMZ is a germinal niche for the continual addition of retinal tissue in the adult fish and frog and in those species the retina expands anteriorly throughout adulthood (Hollyfield, 1968; Johns, 1977; Perron et al., 1998; Raymond et al., 2006; Centanin et al., 2011). It has been proposed that an equivalent germinal center for the outer epithelium lays down RPE to match the growth of the inner epithelium (Centanin et al., 2011). The mouse and adult chick eye are not thought to have an active CMZ and the edge of the sensory retina is only identifiable late in embryogenesis, as the ora serrata (reviewed in Kubota et al., 2002; Fischer et al., 2013). However, the *embryonic* morphogenesis of the eye is similar and may be considered an accelerated stage in fish and frogs, where both species go through a larval stage with a functioning visual system.

A unified model from the variety of studies in several species shows that the peripheral retina grows in a long-term anterior-directed clonal manner (Bodenstein and Sidman, 1987a; Hunt et al., 1987a) while the central retina expands only slightly (Fekete et al., 1994). This is true for both the inner and outer epithelia (Schmidt et al., 1986; Bodenstein and Sidman, 1987; Hunt et al., 1987b; Reese et al., 1999; Rice et al., 1999; West, 1999; Centanin et al., 2011; Venters et al., 2011). Tagged population/clone distribution within a single tissue yields information regarding the growth potential of particular locations. In addition, whether a tagged cell population is restricted to a single tissue fate (unipotential) or populates multiple tissues (multipotential; Figure 4) further defines tissue subunits and helps identify critical points when cells become restricted to a particular lineage.

Optic Cup stages: Central Retina Precursors are Unipotential and Peripheral Retina Precursors are Multipotential

A s*egregated* neural retina precursor tissue in the newly formed optic cup would, regardless of differences in proliferation potential along the eye axis, distribute progeny only to the inner epithelium of the optic cup. In contrast, the ongoing anterior-directed eye growth from a *less restricted* precursor could distribute progeny into all eye domains with the exception of the central retina. The cells at the lip of the newly formed optic cup give rise to progeny that populate both the inner and outer epithelia; that is, both RPE and NR (Figures 2, and 3). This has been shown by direct lineage tracing of a single cell (in Xenopus, (Wetts et al., 1989) and of a small population of cells at the OCL (in the chick, (Venters et al., 2013). In the mouse and fish eye, very few studies have been able to follow progeny into both epithelia, and as most studies were based on chimeric animals, it is not as clear that the cells on both sides of the OCL hinge are clonally related (Rice et al., 1999; Centanin et al., 2011). Both fish and mouse experiments demonstrate spokes of chimeric tissue that extend from the posterior to the anterior in both epithelia. In the mouse eye (Rice et al., 1999) about half of the spokes in the neural retina mirror spokes in the overlying RPE, supportive of a non-restricted progenitor. In contrast, Centanin et al, (2011) have shown that only 2% of their inner epithelium spokes are not aligned with spokes in the outer epithelium, more supportive of unipotent precursors. It might be premature, however to conclude that mice and fish eyes do not also have this in common with frog and chicken. Several studies have demonstrated that the inner and outer epithelia seem to slide relative to each other during development (Holt, 1980; O'Gorman et al., 1987; Goldowitz et al., 1996; Picker et al., 2009; Kwan et al.,

2012). This movement would offset one epithelium from another, although this would be an early phenomenon, and expected to resolve near the end of development.

Problematically, very few studies have analyzed the most anterior tissues, including the ciliary body, iris and hinge area at the lip of the optic cup. Generation of a permanent tracing reagent allowed long term lineage analysis and revealed that there is a bipotentiality, as relates to an inner and outer epithelia fate, maintained at the OCL for the peripheral retina (Venters et al., 2008; Venters et al., 2011). As discussed later, while the peripheral neural retina is derived from an OV population that contributes to both the NR and the RPE, the central neural retina derives from an OV population that contributes only to the neural retina. Thus they are fated, in a broad tissue sense, to be uni and bi-potent respectively (Figure 4). Therefore the dual retina model (Figure 2) postulates that the central retina has a dedicated precursor in the OV and the peripheral retina is added progressively at OC stages from a progenitor pool at the OCL shared with anterior eye tissues.

While we can describe the anatomy and cellular make-up of the central neural retina necessary for its specialized role, we are still lacking information regarding both the morphogenesis of the central retina and the mechanisms through which it develops in relation to the entirety of the eye. Recognition of an early segregation of this functional domain permits study of central retina ontogeny as it relates to function. We are now in the position to address questions regarding the size and scope of both central and peripheral retinal precursor pools, and identify developmental requirements for their coordinated incorporation into a functioning eye.

Optic Vesicle stages: Origins of the Central and Peripheral Retina

The final position of optic tissues in mature eyes is easily extrapolated following morphogenesis into an optic cup. Likewise, growth dynamics and potentialities are more easily scored once the cup has formed. However, the different characteristics of the peripheral and central retina in the early optic cup highlight that acquisition of central or peripheral characteristics may occur earlier in development, in the optic vesicles. The optic vesicles develop as bilateral evaginations of the anterior neuroepithelim. Classic work placed the neural retina precursor at the distal optic vesicle (Spemann, 1901) (Figure 2A). However, in higher vertebrates, there has been little direct fate mapping of OV cells as they transition into an optic cup and no data to substantiate the general model of the distal OV origins of the neural retina. The rostro-caudal axis of the distal OV translates to the naso-temporal axis of the peripheral retina (Dutting and Thanos, 1995; Venters et al., 2013). Recent studies have shown that the distal OV does not contribute to the central retina, situated immediately dorsal to the optic stalk. Rather, central retina derives independently from a caudal OV position (Figure 2, Figure 5Ci, Venters et al., 2013). A more widespread origin of the central retina from both the distal and dorsal OV has also been reported (Figure 5Cii, Dutting and Thanos, 1995; Shin and O'Brien, 2009). Despite differences in the finite details of the outcome and/or interpretation of these direct fate mapping in the OV, it is clear that there are territories in the OV that distribute to either the central or peripheral retina, not both. Technically comparing the various reports, the use of permanent genetic tagging facilitates high spatial resolution analysis, leading to the discovery of two distinct OV

precursors for the neural retina- a dedicated central retina precursor in the caudal OV and a multipotent precursor for the peripheral retina along the distal OV (Venters et al., 2011; Venters et al., 2013).

Segregation of precursors of central versus peripheral retinal domains in the OV is further supported by retrospective, non-targeted OV labeling with replication-incompetent retrovirus (Peters and Cepko, 2002). The analysis of resulting clone size and distribution within the retina showed clones occupying either central or peripheral retina. Demonstration of separate central and peripheral retinal precursor domains in the OV argues against the simple derivation of the retina from a single precursor domain within the OV.

Optic Vesicle stages: Fate Potential in Retinal Precursor Populations

Direct lineage mapping from the OV has generally addressed the distribution of cells to either the inner or outer epithelium of the optic cup. OV mapping uncovered a specific origin for uniquely central retina while peripheral retina derived from a bi-potent precursor (Venters et al., 2013) suggesting a lineage restriction for the central neural retina even at OV stages. Previous studies had placed the central neural retinal precursors at the distal tip of the OV (Shin and O'Brien, 2009). The newer study employed non-diluting, permanent cell tagging techniques and produced a refined pattern. The potentiality of OV tissues has also been studied through assessing the potential of OV fragments and partial OV ablations to generate RPE or neural retina, and compensate for ablated fragment (Figure 5D). A broad dorso-ventral divide in optic vesicle potential can be shown; dorsal OV generating pigmented epithelium and ventral OV generating neural retina suggesting unipotence for each of these OV domains. However, it is unlikely that such a simplified state can be implied, as a dependence on extraocular influences were noted for the potential of partial OVs (Kagiyama et al., 2005; Kobayashi et al., 2009). Variant OV potentialities have been similarly noted through the capacity of various regions of the OV to compensate for ablated OV territories in ovo. If a tissue/location is already committed to a single fate it should have less potential to compensate for the removed tissue than a multipotent precursor tissue. As in explant studies, the dorsal OV is slanted towards an RPE fate while the ventral OV, specifically the anterior-ventral quadrant, was competent for both fates in generating a more normal OC morphology (Hirashima et al., 2008). Patterning defects recorded from ablations as early as 8 somites highlight early demarcation of fate potential in the OV. The discovery that all but the central eye precursors reside in the distal OV puts a multipotent tissue distally, while fate restricted precursors reside in the dorsal and caudal OV (Figure 4). Ablation/explant studies have been largely analyzed on the framework of a single neural retina precursor model. Reassessment with the less rigid framework of fate potential, allowed in the dual retinal origin model, can conclude that the eye recovers so long as the remnant includes either a portion of the multipotent precursor, or the central neural retina precursor (Figure 5D). These ablation studies only assess the recovery of early eye morphology based on having both an inner neural retina and an outer pigmented epithelium; the central to peripheral axis was not examined.

Optic Vesicle stages: Placement of Central and Peripheral Retinal Domains Through Morphogenetic Transition from Vesicle to Cup

Mitotic differences noted between central and peripheral retina in the optic cup are already evident in the optic vesicle. The distribution of peripheral precursors is more extensive than central retina precursors, and quantified clone size in the central retina is approximately half that of clone size in the peripheral retina in a retrospective clonal growth analysis of OV tracing (Peters and Cepko, 2002). As differentiation of the neural retina proceeds in a central to peripheral wave (Kahn, 1974; Prada et al., 1991), central retinal cells exit the cell cycle earliest. This may account for some of the difference observed in clone size but continued addition of new retinal cells from labeled progenitors incorporated into the OCL provides a substantial contribution to the more extensive peripheral distribution (Venters et al., 2011; Venters et al., 2013). Cell cycle withdrawal of the earliest differentiating retinal cell type, RGCs, occurs in the optic vesicle for the central retinal population (Kahn, 1974; Prada et al., 1991). The precursor cells of the central neural retina, already initiating differentiation, therefore may be less responsive to proposed instructive signals patterning the OV than the peripheral precursors (reviewed in Esteve and Bovolenta, 2006; Fuhrmann, 2010; Heavner and Pevny, 2012; Kim and Kim, 2012).

Along with expansive growth, distinct morphological changes are evident as the optic vesicle transitions to a cup (Figure 6). Invagination from the caudo-ventral OV places the presumptive central neural retina behind the lens placode (Romanoff, 1960; Bellairs and Osmond, 2005). This displacement towards the dorsal OV situates the central retinal precursor dorsal to the developing optic stalk, formed from proximal aspects of the dorsal and ventral neuroepithelium (Hilfer et al., 1981). Central-peripheral proliferation differences preferentially expand the peripheral OC, such that it progressively accounts for more of the optic cup epithelium and the central domain is confined to a central position (Gloor et al., 1985). Overt development of the ventral optic cup is delayed in comparison to the dorsal. A proximal position of the caudal OV populates the ventral hinge of the new OC (Holt, 1980) and may provide the population that yields the ventral optic cup (Hunt et al., 1988). The morphogenetic changes between OV and OC stages result in the ventro-caudally derived central retina situated adjacent to the dorsally derived central RPE, dorsal to the optic stalk and behind the lens. With development, the expansion of the peripheral domain from the OCL accounts for the majority eye growth without impacting the position of the central population.

Optic Vesicle Stages: Acquisition of Central and Peripheral Retinal Characteristics

While assays of optic competence identify ages when the potential of OV domains is restricted, they use the ability to produce grossly normal eyes as readout. The competence for OV compartments to generate inner or outer OC epithelia can be ascribed through morphological criteria, whereas subtle retinal patterning defects are necessary to dissect patterning of the central-peripheral retinal axis such as photoreceptor composition and retinotectal projection. The role of specific molecular gradients along naso-temporal, dorso-

ventral, and central-peripheral eye axes has been examined and can be used as a reference for establishment of retinal axes (reviewed in Schulte and Bumsted-O'Brien, 2008; Feldheim and O'Leary, 2010; Reese, 2011). Most information is available for positional specification along the naso-temporal and dorso-ventral axes but a central-peripheral axis is inherent within both divisions.

In this line of inquiry, central retina precursor domains and instructive influences in the OV have been addressed through ablations and subsequent effect on "central" functional characteristics, but these experiments are challenging to analyze. Anatomically, the central foveal retina can be distinguished histologically in the mature eye. Identification in the chick has utilized the cone-rich/rod-free photoreceptor composition of the central retina or retinotectal projection assays to assign central characteristics. Distinct differences are seen in the outcome, depending on the method of analysis. Schulte et al. (Schulte et al., 2005) demonstrated that the rod-free central retina develops normally in the chick eye after removal of the distal OV, the site of the presumed central precursors. In contrast, the rod free zones were perturbed or missing after ablation of the dorsal or rostral OV respectively. If the loss of the central retina in ablated embryos resulted directly from ablation of a distinct central retinal precursor population, these data would be in some accord with the retinotectal mapping results of Dutting and Thanos (Dutting and Thanos, 1995).

However, the perturbed visual pigment pattern could similarly result from loss of extrinsic signals to a specified photoreceptor population (Bradford et al., 2005; Schulte et al., 2005). The rod pattern in perturbed retinas supports a ventral OV compensation in the resultant eye (Hunt et al., 1988). While surgical ablation experiments carry an inherent caveat, especially when comparing work between different groups, partial OV ablations suggest tissue fated for the ventral OC cannot replace the central retina. It appears, at least in the chick eye, that some aspects of photoreceptor development are fixed by OV stages and a central, rod-free compartment cannot be reconstituted from an ectopic OV compartment and requires not only OC morphogenesis but also an already specialized neural retina precursor.

Positional identity within the retina is also reflected in connections between retinal axons and the brain, and retinotectal maps provide a framework for retinal positional information. The central retina differentiates early and sends axons to a specific target and retinotectal projection after ablation of specific OV domains yields insight into specification of retinal positioning. Retinotectal patterning along the naso-temporal retinal axis is initiated by OV stages in the chick eye (Figure 5). The native projection of the central cells is not reassigned during compensatory growth following partial OV ablation, although peripheral cells are able to respond to their new position (Crossland et al., 1974; Matsuno et al., 1992; Dutting and Thanos, 1995; Mueller et al., 1998). Therefore, specificity of retinal axon projection may already be determined for the central retina precursor in the OV.

In contrast to photoreceptor patterning, removal of the rostral/nasal OV had no effect on normal RT projection (Matsuno et al., 1992; Dutting and Thanos, 1995) fitting with positional specificity being fixed in central retina precursors. As the readout for the two assays depend on the behavior of retinal cell types with temporally distinct birthdates, the early-born RGCs are most likely to have determined positional information, while some

plasticity could remain in the later born photoreceptors (Kahn, 1974; Spence and Robson, 1989; Prada et al., 1991; Belecky-Adams et al., 1996; Adler and Raymond, 2008). However, it is as yet unclear if targeted ablation of a specifically central retina precursor, distinct from the peripheral, would result in a loss of a cone-rich central retinal compartment. Lineage and functional assays identify, however, that a central retina precursor can be identified in the OV and that central retinal characteristics are less plastic than those of the peripheral retina following perturbation at OV stages.

Little is known about the central neural retina as distinct from the peripheral retina at early stages of eye development. Direct and indirect lineage analysis identifying distinct precursors for both, and the central characteristics that are fixed at OV stages highlight the early emergence of this compartment and raises questions regarding our understanding of intrinsic embryonic differences between the two.

Early Optic Tissue

Scant information is available regarding the origins of specific eye compartments in earlier stage embryos. The presumptive eye-field can be visualized by expression of relevant genes in the anterior of primitive streak and early head-fold stage embryos (reviewed in Graw, 2010; Heavner and Pevny, 2012; Sinn and Wittbrodt, 2013) but, as yet, no clear demarcation can be made for locations that will distribute to retinal subdomains. Some information, however, is available on the general origin of the eye in the presumptive eye-field of the blastoderm. Direct lineage analyses (Figure 4A) map forebrain and optic precursors to the embryonic midline, anterior to the mature primitive streak and to positions immediately lateral to its rostral end (Schoenwolf et al., 1989a; Schoenwolf et al., 1989b; Schoenwolf and Sheard, 1990; Garcia-Martinez et al., 1993; Hatada and Stern, 1994). Ventral forebrain and ventral optic vesicle derive from medial positions and the more lateral position, in contrast, contributed cells to both the optic vesicles and lateral walls of the brain (Schoenwolf and Alvarez, 1989; Schoenwolf et al., 1989a; Schoenwolf and Sheard, 1990).

With subsequent development, the prospective eye territory is incorporated into the anterior neural folds. The anterior-posterior subdivisions of the anterior neural plate have been reviewed (Rubenstein et al., 1998) and fate mapping, via chick-quail chimeras, positions the presumptive eye with the forebrain (Couly and Le Douarin, 1987; Cobos et al., 2001). By systematic transplantation of tissue using an ordered grid, Cobos et al (Cobos et al., 2001), circumscribe both the extent of the eye primordia at HH8 in the anterior neural fold and domains that preferentially populate either the naso-temporal or central- peripheral retina along the anterior-posterior or medio-lateral axis of the neural ridge respectively (Figure 6A). Although these studies hint at some segregation of cells that incorporate into specific optic vesicle positions, a more comprehensive study is needed to determine differences in central and peripheral retina derivation at such early stages.

Before gene expression was used to marked prospective eye fields, investigators localized an early embryonic eye-field through experimentation to test the eye-potential of regions of the blastoderm. Eye precursor territories were described through examination of the potency of circumscribed regions of the blastoderm to generate eye tissues after transplant to the

chorio-allantoic membranes or embryonic coelomic cavity. Eye forming potential was detected in a blastoderm territory that included the middle third of pre- primitive streak stage embryos (Hoadley, 1926), or in definitive-streak stage chick embryos was restricted to the blastoderm immediately anterior and anterior-lateral to the primitive streak (Hoadley, 1926; Hunt, 1932; Clarke, 1936; Rawles, 1936) (Figure 4A). Like gene expression, such studies do not predict whether all, any, or parts of the identified optic precursor population will normally give rise to the eye, simply identifying tissues that are competent to differentiate as optic tissue when isolated from their normal developmental regime.

Potentiality studies in the eye-forming regions of the blastoderm give some insight that tissue-specific potential are varied in the eye-forming regions of the blastoderm (Figure 4A). RPE differentiation was seen, both with and without neural retina differentiation from the anterior blastoderm (Hoadley, 1926; Hunt, 1932; Clarke, 1936). Refinement of the assay highlighted earlier embryos yielding only RPE and later stages both RPE and neural retina (Hoadley, 1926). Further, they suggest a gradient of optic potential along the medio-lateral axis of the proposed eye-field, lateral tissue differentiating RPE and medial tissues both RPE and neural retina (Clarke, 1936). The unavoidable variability of these types of experiments and the lack of information currently available regarding specification and derivation of optic compartments at early developmental stages does not allow direct superimposition of blastoderm potential on ultimate eye fates but is supportive of early determination of specific eye tissues or compartments.

The alternative model of retinal development proposes the central neural retina is already separately defined by OV stages while the peripheral retina derives from a shared precursor pool that generates both pigmented and non-pigmented tissue. As yet, it is not clear if this difference marks an inherent difference in potentiality or a restriction/gain of potential with maturation of the two precursor populations. Potentiality and lineage studies in the eye-forming regions of the blastoderm give some insight that such differences may arise early in development, but further experimentation with newer molecular tools is needed to resolve when the different progenitors are established.

Summary

The mature eye comprises several tissues that progressively resolve with development. Little information is available regarding lineage relationships between mature optic cup tissues and the mechanisms that couple fate commitment to eye morphogenesis. A combination of molecular and direct lineage analyses highlight differences between how we demark tissue precursor populations of the eye and mechanistic models of normal eye morphogenesis. Refinement of our understanding of eye development and how it underlies domain specific eye diseases and developmental disorders requires amalgamation of knowledge mined with such complimentary tools.

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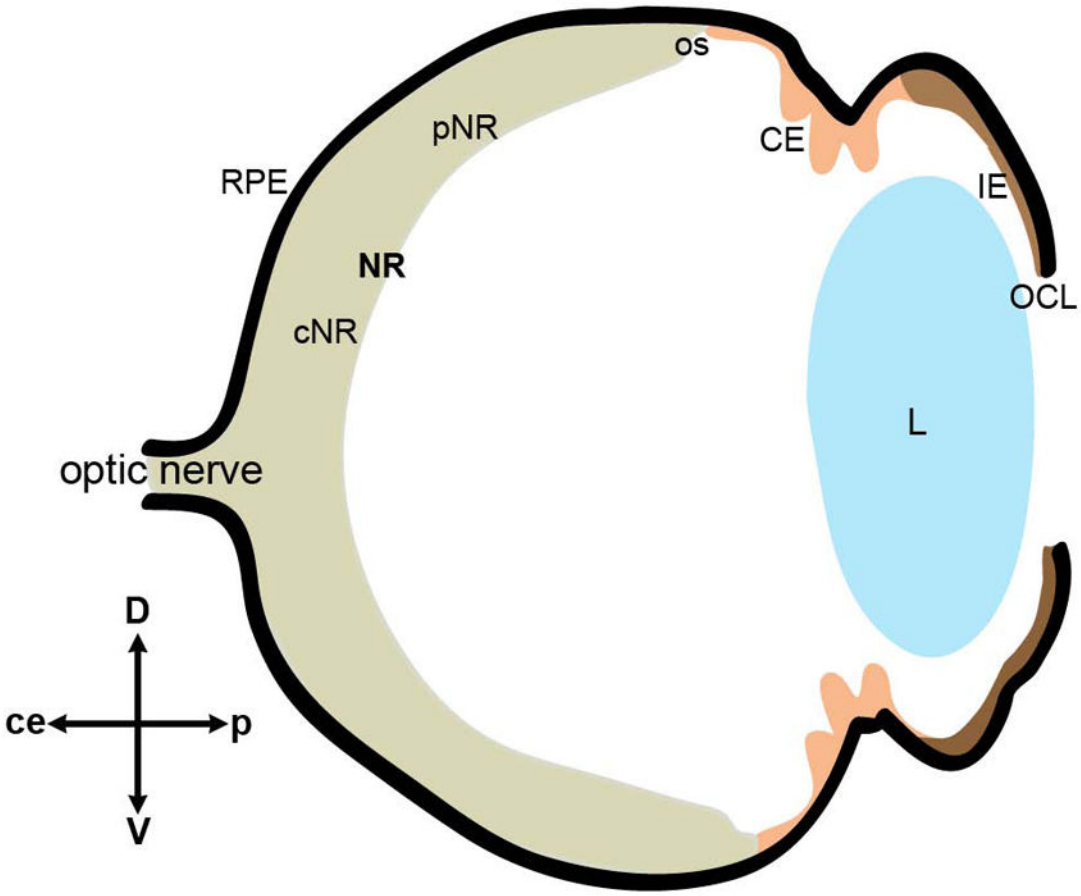

Figure 1. Optic Cup Tissues

Scheme of a sagittal section through the mature eye, depicting the central (posterior) to peripheral (anterior) arrangement of optic tissues. The ciliary body, iris, and lens are collectively called the anterior segment of the eye.

L-lens, C-central, P-peripheral, D-dorsal, V-ventral, OCL-optic up lip, IE-iris epithelia, CE-ciliary epithelia, os-ora serrata, NR-neural retina, pNR-peripheral neural retina, cNR-central neural retina.

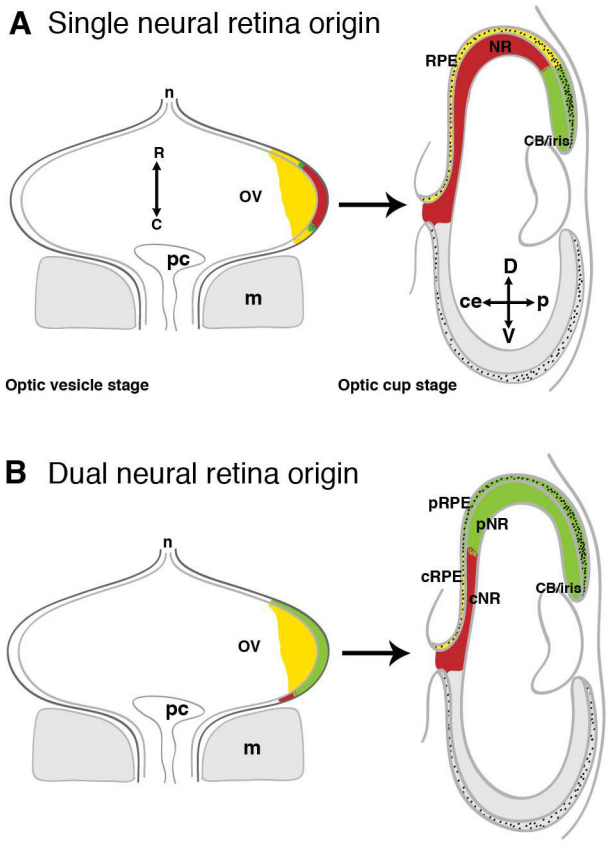

Figure 2. Models of Eye Development

(**A**) **Single retinal origin model of eye development** place a single neural precursor (red) at the distal OV. Anterior eye tissues, ciliary body and iris (green), arise from the boundary between NR and RPE (yellow) precursor zones. The entirety of the NR is derived from a single precursor domain at the distal OV. (**B**) **Dual retinal origin model of eye development.** The retina can be divided into two domains, central (red) and peripheral (green), based on their OV origin. The cNR precursor is positioned at the caudal OV. The peripheral NR shares an OV origin with anterior eye tissues.

OV-optic vesicle, pc-prechordal plate, m-paraxial mesoderm, L-lens; CB-ciliary body, axis points: R-rostral, C-caudal, ce-central, p-peripheral, D-dorsal, V-ventral.

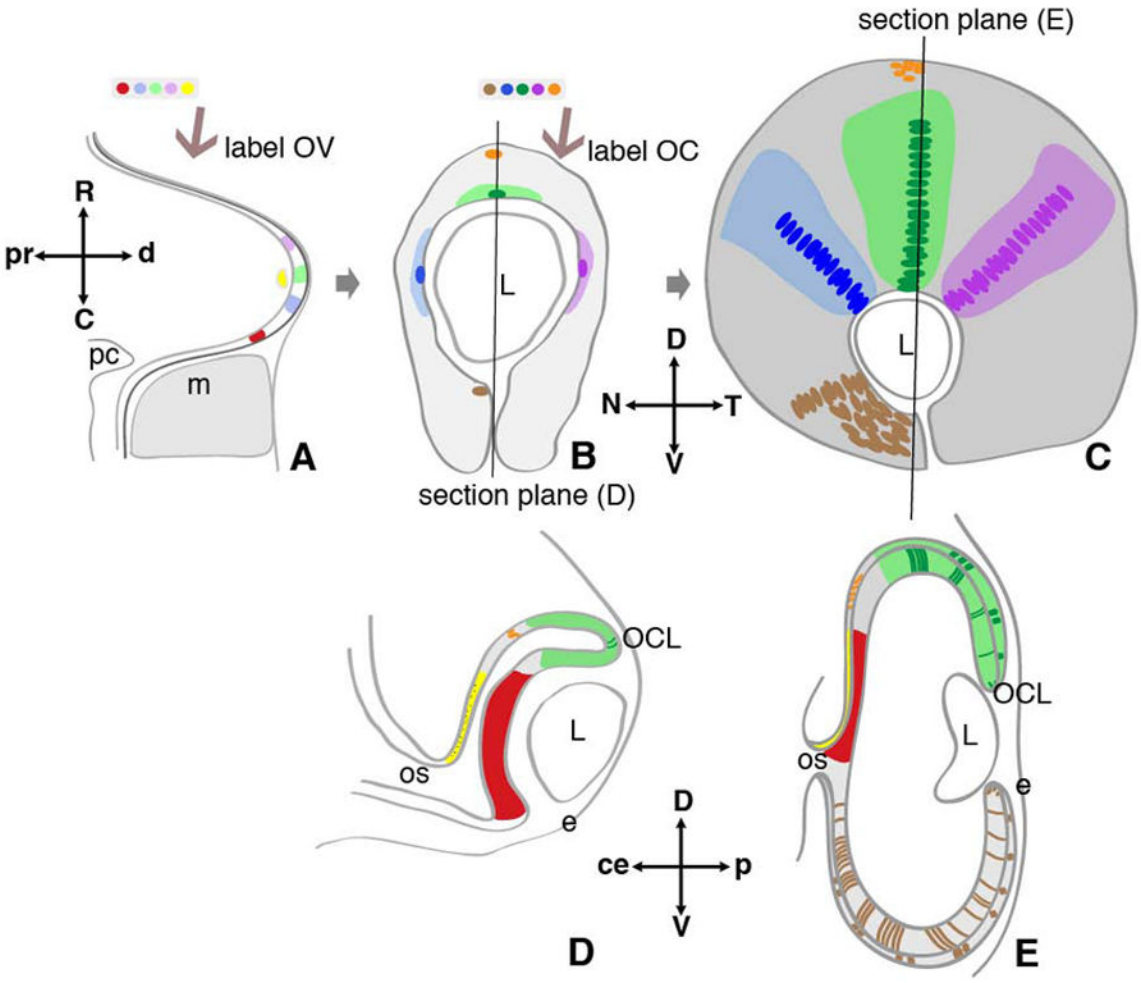

Figure 3. Lineage relationships during eye morphogenesis

(**A–E**) Schematic views of eye at optic vesicle (**A**), early (**B**), and definitive **(C)** optic cup stages in wholemount (**A–C**) and section (**D and E**) view. Cells along the rostrocaudal axis of the distal optic vesicle (**A,** pale blue, green purple dots) distribute along the nasotemporal OCL axis of the early optic cup (pale blue, green, purple sectors in **B**. Labeling the early OCL (darker blue, green, purple dots, **B**) results in radial distribution of marked cells from the peripheral towards the central optic cup (**C and E**). Non-OCL label is displaced to the posterior over time (orange dots, **B, C**). The ventral optic cup is labeled after tagging progenitors at the newly formed ventral OCL (brown dots, **B, C, E**); cells expand circumferentially in the ventral domain as development continues. In section view (**D and E**), OV label at the caudal OV (red, A) and dorsal OV (yellow, A) are restricted to the central neural retina and central retinal pigmented epithelium (red and yellow label, **D and E**). (**E**) Representative distribution from OCL labeling of a subpopulation of the dorsal (dark green) and ventral (brown) OCL following several days of development.

Pc-prechordal plate, m-paraxial mesoderm, L-lens, os-optic stalk, e-surface ectoderm, OCL-optic cup lip, R-rostral, C-caudal, pr-proximal, d-distal, D-dorsal, V-ventral, N-nasal, T-temporal.

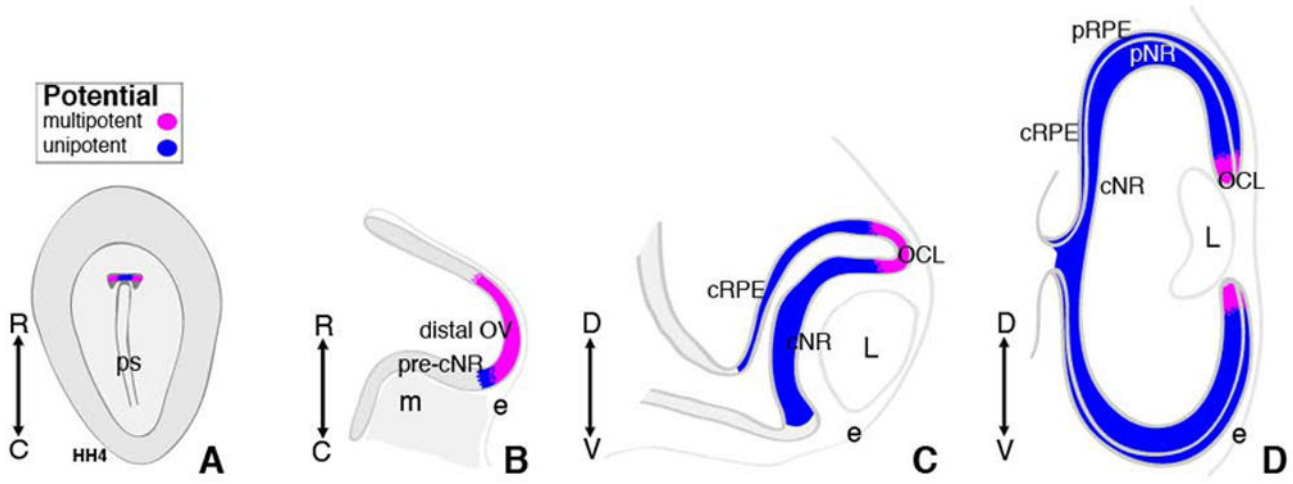

Figure 4. Peripheral eye domain retains multipotency through optic cup morphogenesis

Wholemount (**A**) and sectional (**B–D**) representation of HH Stage 4 embryo (**A**), optic vesicle (**B**), early (**C**) and later (**D**) optic cup stage eyes. (**A**) Eye forming potential was seen rostral to the primitive streak (grey). RPE and neural retina were formed from lateral tissue (pink) and RPE from medial tissue (blue). (**B**) The distal optic vesicle (magenta) is multipotent in respect to its normal contribution to inner and outer optic cup and, subsequently, multiple eye tissues. A caudal optic vesicle domain (blue) is restricted to a single, central neural retinal fate. (**C, D**) With development, multipotency is retained in the OCL in optic cup and fate-restricted tissue throughout the rest of the eye.

ps-primitive streak, OV-optic vesicle, cNR-central neural retina, m-paraxial mesoderm, e-ectoderm, L-lens, cRPE-central retinal pigmented epithelium, OCL-optic cup lip, pNR-peripheral neural retina, pRPE-peripheral retinal pigmented epithelium, R-rostral, C-caudal, D-dorsal, V-ventral.

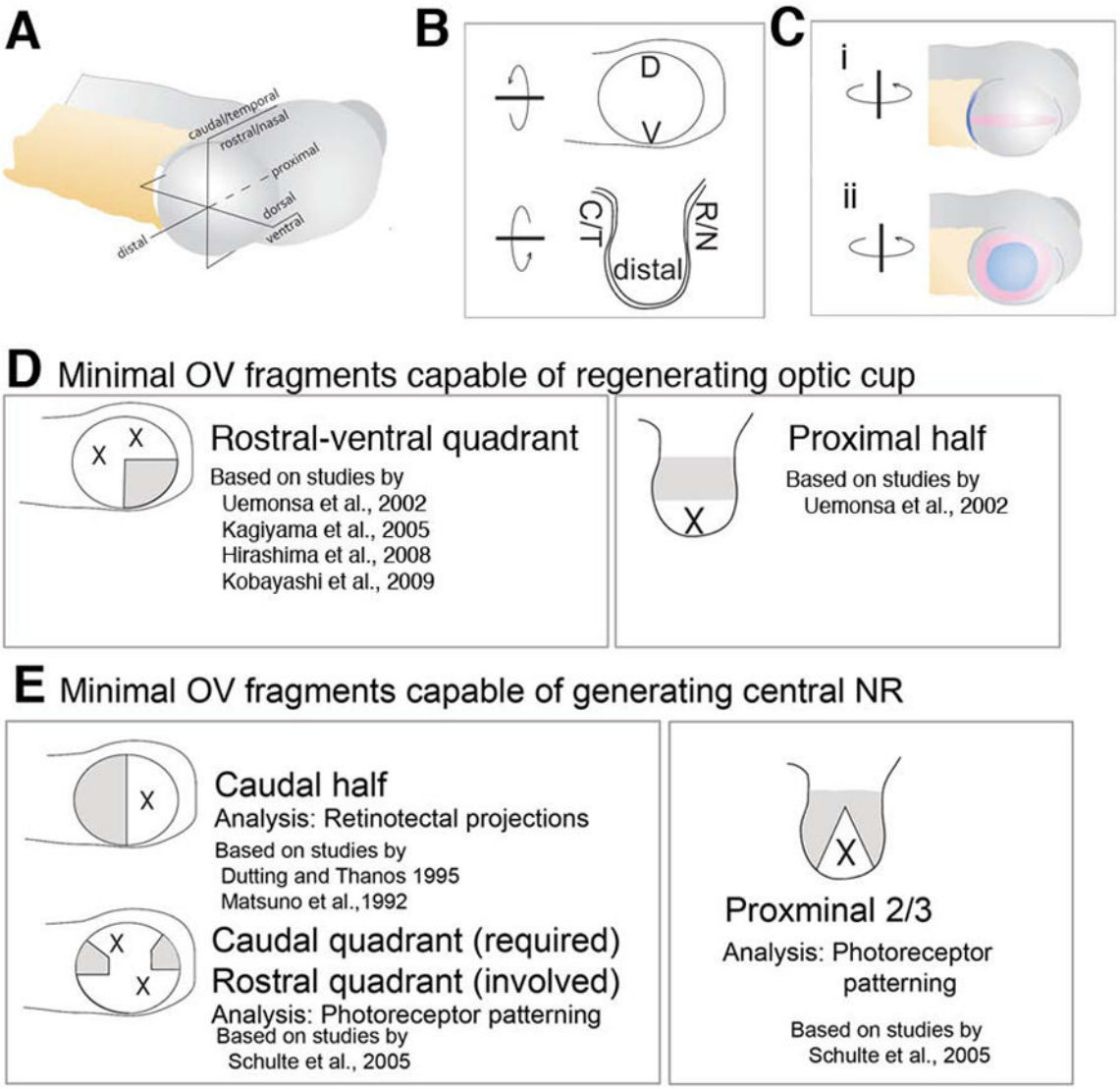

Figure 5. Summary of optic vesicle domains identified through direct lineage analysis and ablation experiments

(**A**) Schematic of optic vesicle to demonstrate major axes and (**B**) Rotated views and simplified graphics to highlight an individual axis. (**C**) Comparison of results of direct lineage analysis: (i) derived from non-diluting permanent retroviral tagging; (ii) from DiI membrane labeling. As in Figure 4, magenta indicates multipotent progenitor domains and blue indicates restricted domains, giving rise to only central neural retinal tissue. (**D**) Summary of several ablation studies, in which an optic cup was formed and analyzed for early morphology, see text for details. (**E**) Summary of ablation studies, in which an optic cup was formed and analyzed for mature features, either photoreceptor complement or retinotectal projection, see text for details.

D-dorsal, V-ventral, N-nasal, T-temporal, R-rostral, C-caudal.

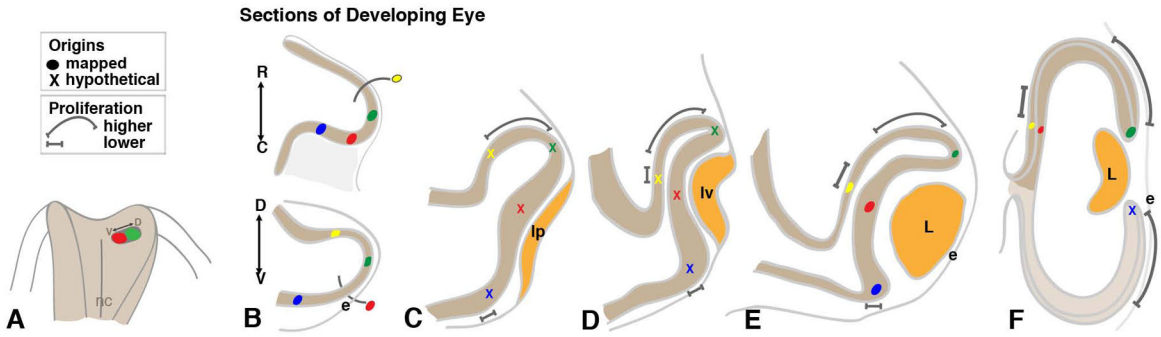

Figure 6. Optic cup morphogenesis

(**A**) Scheme of the head region of a neural fold stage (HH8) embryo highlighting areas that distributed to optic tissue (grey), central (red) and peripheral (green) neural retina in mature optic cup. (**B–F**) Sectional representation of progressive stage of eye development. Fate mapped regions are marked with colour, see key below. Broad proliferation zones are indicated by bars. (**B**) Optic vesicle before lens placode formation in two different views: coronal (top) and transverse (lower) view. Tagged domains not in section plane are indicated by arc. (**C–F**) Schematic sections present dorsal-ventral view of eye. (**C**) Transitional stage of OV to OC morphogenesis with a distinct lens placode. (**D**) Invaginating OV and developing lens vesicle. (**E**) Early OC with lens. The ventral OC has not formed. (**F**) OC and lens. Dorsal and ventral OC domains have formed. KEY: Yellow-dorsal OV, green-distal OV, red-caudal OV, blue-ventral, proximal OV.

OV-optic vesicle, OC-optic cup, L-lens, e-ectoderm