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Gene expression is dynamically regulated in retinal progenitor cells prior to and during overt cellular differentiation

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ABSTRACT

The retina is comprised of one glial and six neuronal populations that are generated from a multipotent pool of retinal progenitor cells (RPCs) during development. To give rise to these different cell types, RPCs undergo temporal identity transitions, displaying distinct gene expression profiles at different stages of differentiation. Little, however, is known about temporal differences in RPC identities prior to the onset of overt cellular differentiation, during the period when a retinal identity is gradually acquired. Here we examined the sequential onset of expression of regional markers (i.e., homeodomain transcription factors) and cell fate determinants (i.e., basic-helix-loop-helix transcription factors and neurogenic genes) in RPCs from the earliest appearance of a morphologically-distinct retina. By performing a comparative analysis of the expression of a panel of 27 homeodomain, basic-helix-loop-helix and Notch pathway genes between embryonic day (E) 8.75 and postnatal day (P) 9, we identified six distinct RPC molecular profiles. At E8.75, the earliest stage assayed, murine RPCs expressed five homeodomain genes and a single neurogenic gene (*Pax6*, *Six3*, *Six6*, *Rx*, *Otx2*, *Hes1*). This early gene expression profile was remarkably similar to that of 'early' RPCs in the amphibian ciliary marginal zone (CMZ), where RPCs are compartmentalised according to developmental stage, and homologs of *Pax6*, *Six3* and *Rx* are expressed in the 'early' stem cell zone. As development proceeds, expression of additional homeodomain, bHLH and neurogenic genes was gradually initiated in murine RPCs, allowing distinct genetic profiles to also be defined at E9.5, E10.5, E12.5, E15.5 and P0. In addition, RPCs in the postnatal ciliary margin, where retinal stem cells are retained throughout life, displayed a unique molecular signature, expressing all of the early-onset genes as well as several late-onset markers, indicative of a 'mixed' temporal identity. Taken together, the identification of temporal differences in gene expression in mammalian RPCs during pre-neurogenic developmental stages leads to new insights into how regional identities are progressively acquired during development, while comparisons at later stages highlight the dynamic nature of gene expression in temporally distinct RPC pools.

The mature retina is a multicellular neural tissue that is responsible for receiving and processing visual information. It is comprised of one glial and six neuronal cell types that are segregated into three distinct retinal layers – the ganglion cell layer (GCL), inner nuclear layer (INL) and outer nuclear layer (ONL). The GCL is populated by retinal ganglion cells (RGCs) and displaced amacrine cells, the INL contains bipolar, amacrine, and horizontal cell interneurons and Müller glial cells, and the ONL contains rod and cone photoreceptors.

In mouse, eye development begins at E8.5, with the optic vesicles forming from a bilateral evagination of the diencephalon (Wawersik et al., 2000). Slightly later at E10, the vesicles invaginate to form a bilayered optic cup, with the inner and outer layers giving rise to the retina and retinal pigmented epithelium (RPE), respectively. Retinal cell differentiation begins at ~E11.5, with the seven retinal cell types generated in a stereotyped, albeit overlapping order (Cepko et al., 1996; Young, 1985). In the first wave of differentiation, which occurs between ~E11.5–E18.5, RGCs, cone photoreceptors, horizontal and amacrine cell interneurons differentiate, while rod photoreceptor, bipolar and Müller glial cell production peaks postnatally. Birthdating and lineage tracing studies in several vertebrate species have revealed that

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all seven retinal cell types are derived from a multipotent pool of RPCs (Carter-Dawson and LaVail, 1979; Prada et al., 1991; Turner and Cepko, 1987; Turner et al., 1990; Wetts and Fraser, 1988; Young, 1985). Notably however, RPC clonal composition is highly variable in these studies, indicating that RPCs are heterogeneous with respect to their developmental potential. Clonal heterogeneity is due in part to the presence of both multipotent RPCs, which give rise to all retinal cell types, as well as committed retinal precursors with fixed lineages (Cayouette et al., 2006; Godinho et al., 2007; Pearson and Doe, 2004). In addition, clonal heterogeneity can be explained in part by the stochastic nature of RPC fate decisions, with differentiative divisions that give rise to the more frequent retinal cell types occurring with higher probabilities (Gomes et al., 2011; He et al., 2012).

While intrinsic and extrinsic determinants are both involved in specifying retinal cell identities, clonal analyses suggest that cell fates are intrinsically determined (Belliveau and Cepko, 1999; Belliveau et al., 2000; Cayouette et al., 2003; Rapaport et al., 2004). Thus, the competence of RPCs to give rise to different cell fates is likely determined by the expression of unique combinations of intrinsic determinants (Holt et al., 1988; Livesey and Cepko, 2001; Turner and Cepko, 1987; Wetts and Fraser, 1988). One would thus predict that the genes that confer a retinal regional identity and those that specify distinct retinal cell fates would be expressed in a temporally dynamic fashion. Indeed, several studies have revealed that RPC gene expression profiles are temporally dynamic (Blackshaw et al., 2004; Cayouette et al., 2003; Gamsiz et al., 2012; Mizeracka et al., 2013; Trimarchi et al., 2008; Zhang et al., 2006). Notably, this heterogeneity is also observed at the single cell level, with individual RPCs displaying unique molecular signatures, consistent with the idea that RPCs at different stages of development/differentiation are intermingled in the central retina (Trimarchi et al., 2008).

In the retina, previous expression profiling analyses have primarily focused on the molecular signatures of RPCs between E12.5 and P21 in mouse, during the period of active cellular differentiation, whereas the molecular signatures of murine RPCs prior to the onset of cellular differentiation have largely been ignored. In general, the acquisition of a cellular identity, including the specification of a neural cell fate, is a progressive event that requires neural progenitors to first acquire a regional identity, which is often conferred by homeodomain transcription factors, followed by subtype specification and differentiation, which is induced by other transcription factors and signaling molecules (Ohsawa and Kageyama, 2008; Zuber et al., 2003). Some insights into the progressive nature of RPC fate specification have come from analyses of the CMZs of fish and amphibians, which are populated by self-renewing RPCs that give rise to new retinal cells throughout the lifetime of the animal (Casarosa et al., 2005; Perron et al., 1998). In the Xenopus CMZ, located at the periphery of the mature retina, RPCs are arranged in a highly organised, spatiotemporal gradient according to their developmental stage and potential (Fig. 1A) (Harris and Perron, 1998; Hitchcock et al., 2004). Specifically, undifferentiated stem cells are located at the peripheral margin of the CMZ, while progressively more developmentally restricted RPCs are found sequentially in more central regions (Dorsky et al., 1995; Perron et al., 1998; Wetts et al., 1989). Five subregions have been identified in the Xenopus CMZ based on the spatiotemporal expression profiles of various homeodomain, basic-helix-loop-helix (bHLH) and Notch pathway genes (Fig. 1A). The first subregion (i.e., zone I) contains retinal stem cells, while zones II–IV contain progressively more committed RPCs, and zone V is comprised of the mature retinal layers (Casarosa et al., 2005). Interestingly, the timing of neurogenesis in the Xenopus CMZ closely follows that observed in the central retina, suggesting that temporal identity transitions, as revealed by distinct patterns of gene expression, may also be conserved in both regions (Amato et al., 2004).

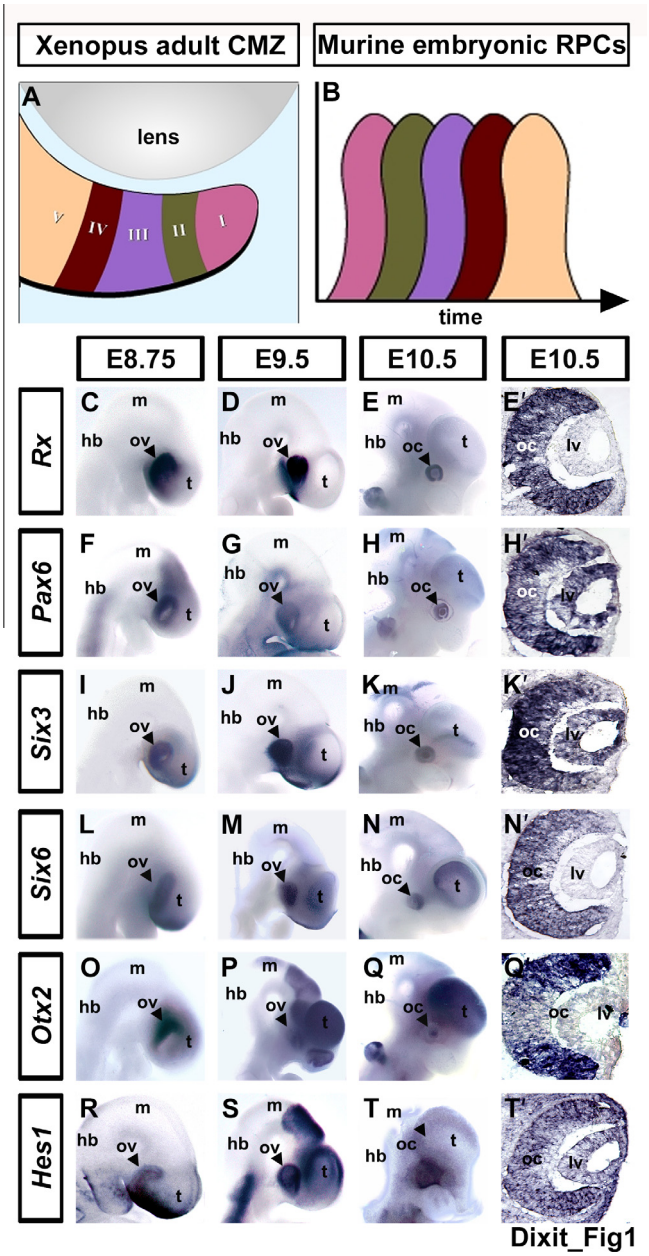

Fig. 1. Expression of early onset genes during early to mid retinogenesis (E8.75–10.5). (A) Schematic representation of Xenopus CMZ compartmentalisation as reported by Perron et al. (1998). Each zone is marked by the combined expression of specific genes (not shown). (B) Schematic representation of the murine progenitor populations described herein. Similar molecular profiles were observed in retinal progenitors at distinct embryonic stages in mouse. (C–T′) RNA *in situ* hybridisation analysis of *Rx* (C–E′), *Pax6* (F–H′), *Six3* (I–K′), *Six6* (L–N′) *Otx2* (O–Q′), and *Hes1* (R–T′) in whole embryos and retinal sections during formation of bilateral optic vesicles and induction of the lens placode (E8.5), invagination of the lens placode and optic vesicles to form the optic pit (E9.5) and initiation of retinal cell differentiation (E10.5). Arrowheads in wholemounts denote detectable expression. hb, hindbrain; m, midbrain; di, diencephalon; t, telencephalon; ov, optic vesicles; oc, optic cup; lv, lens vesicle.

Here we characterised temporal changes in gene expression during the initial acquisition of an RPC identity in mouse, as well as during the period of cellular differentiation. Specifically, we performed a comparative analysis of gene expression from E8.75, when the optic vesicles first form, to P9, when differentiation is largely complete (except in the peripheral-most retina). While