PERSPECTIVE

The role of Islet-1 in cell specification, differentiation, and maintenance of phenotypes in the vertebrate neural retina

Many blinding diseases, such as retinitis pigmentosa, age-related macular degeneration, and glaucoma involve the permanent loss of retinal neurons, especially photoreceptors or the centrally projecting retinal ganglion cells. Stem cells have been proposed as a potential source of cells for neuronal transplantation, due to their capacity for proliferative expansion and the potential to generate different retinal cell types. Understanding the developmental biology of retinal cells will be the key to the success of such a strategy. In the developing retina, a complex series of transcription factors sequentially activates genes involved in development, thus defining the adult cell type. Among these, the LIM-homeodomain transcription factor Islet-1 (*Isl1*) is expressed by developing and mature ganglion, cholinergic amacrine, ON-bipolar, and horizontal cells in the retina of most of the vertebrates that have been studied. Because *Isl1*-null mutant mice die at embryonic day 9.5, before the onset of retinogenesis, the role of Isl1 in retinal neurogenesis remained largely unknown. However, Isl1 conditional knockout has been generated to identify the exact role of Isl1 in retinal development. Thus, the conditional inactivation of this transcription factor during mouse retinogenesis disrupts retinal function and also results in marked reductions in mature bipolar, amacrine, and ganglion cells, and a substantial increase in horizontal cells. Therefore, Isl1 seems to play a highly conserved role in cell specification, differentiation, and maintenance of phenotypes of retinal cell types. An understanding of factors such as Isl1 that are involved in vertebrate retinogenesis might be exploitable to reprogram transplanted retinal stem cells.

The Islet-1 transcription factor and retinal development: The retina is a component of the central nervous system and plays an essential role in the acquisition and processing of visual information. The mature retina contains six major types of neurons which are distributed in three nuclear layers separated by two plexiform layers. The outer nuclear layer is occupied by photoreceptors, the inner nuclear layer by bipolar, horizontal, and amacrine, and the ganglion cell layer by retinal ganglion cells (RGCs) and displaced amacrine cells. Furthermore, three basic types of glial cell are found in most of the vertebrate retinas, Müller cells, astroglia, and microglia. During retinogenesis, seven types of cell are generated from multipotent progenitors in sequences relatively conserved among vertebrate species: RGCs are generated first, followed in overlapping phases by horizontal cells, cones, amacrine cells, rods, bipolar cells, and finally Müller glial cells. This retinal cell generation is governed by the combined action of various intrinsic and extrinsic factors. Among the intrinsic factors, the LIM-homeodomain (LIM-HD) transcription factors form a combinatorial "LIM code" that contributes to the specification of cell types (Bathi et al., 2008). Characterization of the expression of one of these LIM-HD proteins, Islet-1 (Isl1), reveals its presence in differentiating and mature RGCs, cholinergic amacrine cells, ON-bipolar cells, and horizontal cells in the retina of several vertebrates (**Figure 1**) (Bejarano-Escobar et al., 2015). The pattern of Isl1 expression during vertebrate retinal development is consistent with that expected for a transcription factor involved in retinal cell differentiation, with it following the typical gradients of cell maturation described during retinal ontogeny. In early stages of retinal development, Isl1 immunoreactivity is detected in nuclei of differentiating ganglion cells located in the inner surface of the retina (**Figure 1A**, **B**) (Francisco-Morcillo et al., 2006; Bejarano-Escobar et al., 2012; Álvarez-Hernán et al., 2013). As development proceeds, abundant ovoid Isl1-positive nuclei are also found dispersed over the neuroblastic layer (**Figure 1B**). In more mature retinas, the expression of Isl1 in subpopulations of ganglion, amacrine, and bipolar cells is consistent across vertebrate species (**Figure 1C–E**). However, Isl1 expression in horizontal cells has been described in the retina of fish, amphibians, reptiles, and birds (**Figure 1D**), but not in that of the mouse (**Figure 1E**) (Francisco-Morcillo et al., 2006; Elshatory et al., 2007a; Bejarano-Escobar et al., 2012; Álvarez-Hernán et al., 2013). Although Isl1 immunoreactivity has been described in the nuclei of developing rods and cones (Francisco-Morcillo et al., 2006), Isl1 mRNA has never been detected in the outer nuclear layer of different vertebrate species (Elshatory et al., 2007a). The staining in the photoreceptor layer is likely a result of cross-reactivity to Isl2, another LIM-HD transcription factor known to be expressed in the nuclei of photoreceptors. Therefore, Isl1 seems not to be involved in

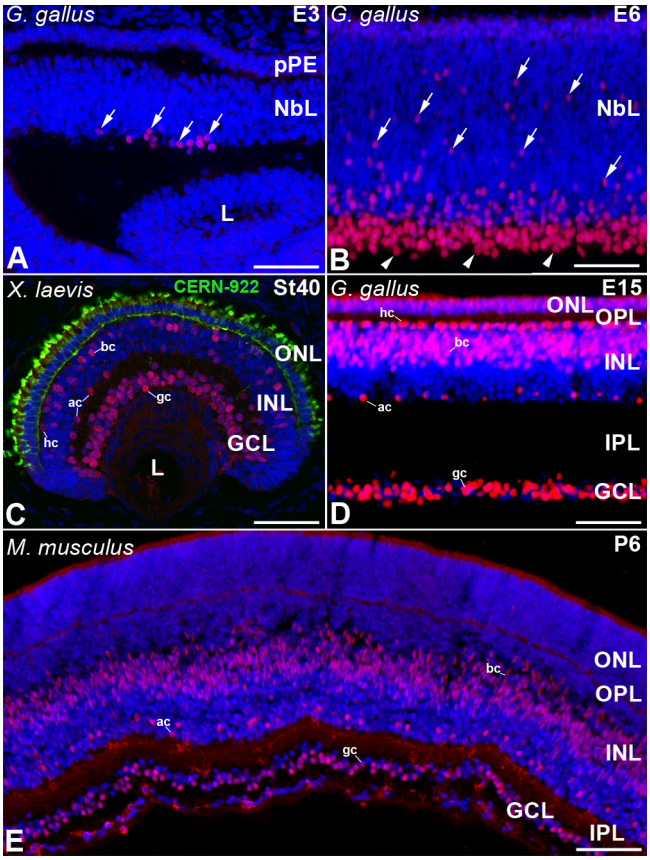

Figure 1 Expression patterns of Isl1 in the developing *Gallus gallus* (A, B, D), *Xenopus laevis* (C), and *Mus musculus* (E) retinas.
Sections were labelled with DAPI, and single-labelled with antibodies against Isl1 (red) (A, B, D, E), or doubly immunostained with anti-Isl1 (red)/CERN-922 (green) (C). At E3, Isl1 is mainly detected in sparse nuclei of differentiating ganglion cells located near the vitreal surface of the chicken retina (arrows in A). In more advanced stages (E6), the nuclei of cells located in the presumptive ganglion cell layer (GCL) appear strongly immunolabeled (arrowheads in B), but also there are nuclei of migratory neuroblasts dispersed throughout the retinal tissue (arrows in B). In the St40 *X. laevis* retina, abundant nuclei were immunoreactive for Isl1 in the GCL, but also in the INL (C). Thus, nuclei located in the amacrine cell layer, bipolar cell layer, and horizontal cell layer were detected with this antibody (C). Isl1 is never detected in the nuclei of CERN-922-immunoreactive photoreceptors (C). Similar staining patterns are found in the E15 chicken retina (D) and in the P6 mouse retina (E). However, immunoreactive horizontal cells are not detected in the mouse retina (E). Developmental stages referred to as: E, day of embryonic development; P, postnatal day; St, *Xenopus laevis* developmental stages (Nieuwkoop and Faber, 1967), Normal table of *Xenopus laevis* (Daudin), North Holland, Amsterdam, The Netherlands, 1967. Isl-1: Islet-1; ac: amacrine cell; bc: bipolar cell; gc: ganglion cell; GCL: ganglion cell layer; hc: horizontal cell; INL: inner nuclear layer; IPL: inner plexiform layer; L: lens; NbL: neuroblastic layer; ONL: outer nuclear layer; OPL: outer plexiform layer; pPE: presumptive pigment epithelium. Scale bars denote 100 μm in A–D, 150 μm in E.

photoreceptor maturation.

Because conventional *Isl1* knockout mice do not survive beyond E9.5, the role of Isl1 in retinogenesis remained largely unknown. Nonetheless, using a conditional gene knockout strategy, various workers have explored the functional mechanisms of Isl1 during specification and differentiation of retinal cell types. Recent studies have revealed an essential role for Isl1 in regulating several genes involved in RGC differentiation (Mu et al., 2008; Pan et al., 2008; Li et al., 2014; Wu et al., 2015). For several years, it has been considered that Isl1 and Pou4f2 (POU domain, class 4, transcription factor 2, also known as Brn3b) function downstream of Math5/Atoh7 to regulate the expression of a common set of RGC-specific genes (Mu et al., 2008; Pan et al., 2008). Thus, Isl1 and Brn3b/Pou4f2 interact physically to form a complex which can bind to DNA motifs of target genes involved in the differentiation of the RGCs (Li et al., 2014), findings which support the hypothesis that Math5/Atoh7 endows the post-mitotic precursors with RGC competence and activates the expression of *Isl1* and *Brn3b/Pou4f2* to initiate the RGC differentiation program. It has recently been shown, however, that ectopic expression of Isl1 and Brn3b/Pou4f2 in *Math5/Atoh7* knockout mice is sufficient to specify RGC fate (Wu et al., 2015). Elshatory et al. (2007b) have demonstrated that the deletion of *Isl1* in the developing mouse retina significantly reduces not only ganglion cells (by 71%) but also amacrine and bipolar cells. Indeed, there were 93% fewer cholinergic amacrines in adult Isl1-null retinas compared with the wild type. Moreover, there is also a marked reduction (76%) in mature ON- and OFF-bipolar cells. The authors concluded that Isl1 has an important role in cholinergic amacrine cell development, and that it is required for engaging bipolar differentiation pathways but not for general bipolar cell specification. Concerning the possible role of Isl1 in horizontal cell differentiation, Suga et al. (2009) found that, in the chicken retina, while the expression of Lim1 transcription factor is restricted to type I horizontal cells, that of Isl1 is restricted to type II/III. The overexpression of Isl1 during the period of horizontal cell differentiation repressed endogenous Lim1 expression, and increased the number of type II horizontal cells at the expense of type I. Both factors are therefore involved in the subtype-specific morphogenesis of post-migratory retinal horizontal cells. Surprisingly, Isl1 is not expressed in developing and mature horizontal cells in the mouse retina (**Figure 1E**) (Elshatory et al., 2007a), although it is directly involved in regulating horizontal cell number in this species (Whitney et al., 2011).

Transcription factors and retinal regeneration: Retinal regeneration has been demonstrated to occur in fish, frogs, and embryonic and postnatal chickens. However, the spontaneous repair and regenerative capacity of the mammalian retina appears limited compared to teleosts and amphibians. Thus, such retinal degenerations as retinitis pigmentosa, age-related macular degeneration, and glaucoma often end with the death of retinal neurons such as photoreceptors or RGCs, and this is generally regarded to be the irreversible cause and end-stage of blindness. The differentiation of cells in the adult retina of cold-blooded vertebrates during growth and regeneration involves a recapitulation of mechanisms that control the sequence of cell production during retinal development. Therefore, understanding the combinatorial expression of the transcription factors involved in retinogenesis might lead to new genetic treatments for retinal degenerations. A recent potential alternative that has emerged is to use stem cell transplantation therapy to replace host cells within the neural retina. Human prenatal retinal tissue was one of the first donor sources to be examined in patients, but the use of human fœtal tissue is problematic due to ethical issues surrounding its procurement and to the limitations in the amount of donor material that can be obtained. Human pluripotent stem cells are another potential donor source for retinal cell transplantation. Embryonic stem cells (ESCs) can be maintained and expanded indefinitely in culture as undifferentiated cells. However, their use has significant limitations including ethical issues and the risk of teratoma formation. In contrast, induced pluripotent stem cells (iPSCs) can be obtained from somatic cells of adult tissues, and therefore constitute a unique, powerful, and patient-specific tool for modelling disease and developing cell-based retinal degenerative disease therapies. The chief problem, however, is to understand the developmental cues that differentiate stem cells into the specific adult cell types required to repair damaged retinal tissue. Therefore, studies that identify the transcription factors and cofactors that regulate the establishment of stem cell multipotency and eventual cell specification and differentiation of various retinal cell types and subtypes may lay the groundwork to improve stem-cell-mediated regeneration, and eventually lead to the development of effective retinal degenerative disease therapies. Indeed, the presence of Isl1 in different retinal neuroblasts could point to a line of investigation into a possible function of Isl1 in retinal repair and regeneration.

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