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Expression of the *insulinoma-associated 1* (*insm1*) gene in *Xenopus laevis* tadpole retina and brain

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Abstract

The *insulinoma-associated 1* (*insm1*) gene is involved in the differentiation of several neuronal and endoderm derived cell types. *insm1* is expressed in the retina and brain of several vertebrates including *Xenopus laevis*. We report the detailed expression pattern of *insm1* in the *X. laevis* tadpole retina and brain. *X. laevis insm1* is expressed in most of the ciliary marginal zone of the mature retina and the optic tectum, dorsal pallium, hypothalamus and preoptic areas of the developing tadpole brain. Overall, *insm1* is expressed in regions of the tadpole brain and retina harboring populations of progenitor cells.

Keywords

Xenopus laevis; insm1; ciliary marginal zone; optic tectum; dorsal pallium; hypothalamus

INTRODUCTION

The *insulinoma-associated 1*(*insm1* or *IA-1*) gene is a conserved gene encoding a DNA-binding zinc finger transcription factor. *insm1* has been studied in several model organisms. Insm1 proteins include seven N-terminal SNAG transcriptional repressor motifs, five C-terminal Cys2-His2 zinc fingers and a putative nuclear localization signal (Parlier et al. 2008). In the mouse nervous system, *insm1* is involved in the differentiation or transition of progenitors into neurons or neurogenic progenitors. In mouse, it is expressed in all proliferating neural cells, including retina, spinal cord, and fore- mid-, and hindbrain and thought to be expressed late in progenitor cell development, during the final cell division as late progenitors give rise to neurons (Duggan et al. 2008). Regulation of mouse *insm1* by

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ascl1 is important in the differentiation of central serotonergic and noradrenergic monoaminergic neurons (Jacob et al. 2009). In *insm1* null mice serotonergic precursors begin to differentiate but fail to produce serotonin due lack of tryptophan hydroxylase 2, which is coordinately regulated by *insm1* and *asc1.* The olfactory epithelium of embryonic *insm1* null mice exhibits a decrease in basal progenitors and an increase in apical progenitors as well as fewer terminally dividing progenitors, suggesting that insm1 is involved in the transition of progenitors to a basal position, which favors neurogenic and terminal division (Rosenbaum et al. 2011).

In the zebrafish retina, *insm1* regulates cell cycle kinetics of the rod progenitor cells, is needed for the differentiation of rod and cone photoreceptors, and, as in mice, is negatively regulated by Notch-Delta signaling (Forbes-Osborne et al. 2013). Zebrafish *insm1* in the retina is genetically upstream of *neuroD* as well as *ath5/atoh7* and photoreceptor specification genes, *crx* and *nr2e3*. *insm1* was also studied in zebrafish retinal regeneration where, in the event of retinal damage, *insm1a* is necessary for müller glia dedifferentiation by suppressing *ascl1a* and itself (Ramachandran et al. 2012). Furthermore, Insm1 defines the zone of Müller glia activity via suppression of *hb-efg*$_a$ expression and stimulation of Müller glia progenitor cell cycle exit. In the mouse retina, *insm1* expression was upregulated upon inhibition of *notch* signaling by application of the inhibitor DAPT (Nelson et al. 2007). An Insm1:LacZ transgene was expressed almost exclusively in non-proliferating cells in the retina. These cells were primarily found at the ventricular surface, although some were found in the ganglion cell layer. The authors concluded that cells expressing the transgene were primarily nascent photoreceptors.

insm1 is normally expressed during development and is regulated by a heterodimer of neuroD and E47, which binds to an E-box found in the promoter (Breslin et al. 2003). However, *insm1* expression can be reactivated in cancer. *insm1* is strongly expressed in tumors of neuroendocrine origin (Goto et al. 1992; Lan et al. 1993; Wang et al. 2009). This, combined with the absence of *insm1* in adult tissues, has lead to the evaluation of the *insm1* promoter in gene therapy against small-cell lung cancer, pediatric medulloblastoma, neuroblastoma, and retinoblastoma tumors (Pedersen et al. 2006; Wang et al. 2009; Christensen et al. 2010; Akerstrom et al. 2013).

Xenopus insm1 has been mainly studied in endoderm-derived cells. It is expressed in all endocrine cells and acts downstream of *ngn3* and upstream of *pax6* and *neuroD* in the transcriptional cascade for differentiation, suggesting that *insm1* gene hierarchy found in the zebrafish retina is conserved and utilized in other species and organ systems (Gierl et al. 2006; Mellitzer et al. 2006; Pearl et al. 2009). *insm1* has also been studied in the *Xenopus* nervous system where it functions downstream of *Xash1 (ascl1)* in the formation of the noradrenergic primary neurons in the developing heart field near the cement gland (Parlier et al. 2008). Although *insm1* has been studied in the *Xenopus* nervous system, there are few reports on *insm1* expression in the *Xenopus* brain and no reports on *insm1* expression in the mature *Xenopus* retina.

We have a long-standing interest in the development of progenitor cells in the mature and regenerating *X. laevis* retina (Bailey et al. 2004; Kelly et al. 2007; Martinez-De Luna et al.

2011) and in gene expression in the developing brain (El-Hodiri et al. 2003; Kelly et al. 2007). Therefore, we sought to characterize *insm1* expression in retinal progenitor cells. Here, we report that *X. laevis insm1* is expressed in a subset of the progenitor cell population in the tadpole retina and brain.

RESULTS AND DISCUSSION

Expression in retinal progenitor cells

Xenopus laevis insm1 is expressed in the developing eye (Parlier et al. 2008). In order to more precisely characterize *insm1* expression in the retina, we performed *in situ* hybridization using retinal sections from *X. laevis* embyros at stages 38 to 45 (Figure 1). *insm1* is expressed in the ciliary marginal zone (CMZ) of the tadpole retina, a region of the peripheral retina containing retinal progenitor and stem cells (RPCs and RSCs, respectively). The CMZ can be divided into 4 zones, based on state of retinal neural development (Perron et al. 1998). The zones contain RPCs of increasing development in a distal – proximal arrangement (Figure 1A). At stage 38, *insm1* is expressed in the retinal progenitor cells (RPCs) but absent from the retinal stem cells (RSCs) at the distal edge (zone 1) of the CMZ. *insm1* expression is also seen in the undifferentiated neuroepithelium adjacent to the ventral CMZ (Figure 1A). Similar to stage 38, retinal *insm1* expression in stage 41 and 45 embryos is restricted to the CMZ (Figure 1B-D). Further examination of stage 41 embryos demonstrates that *insm1* expression begins distally in zone 2 of the CMZ similar to zone 2 marker, *notch1* (compare Figure 1 C and H to F and I). *insm1* expression expands proximally to zones 3 and 4 of the CMZ comparable to *neuroD* expression(compare Figure 1 C and H to G and J), which marks those zones (Perron *et al.,* 1998). This pattern of expression in the CMZ is similar to that of zebrafish *insm1a*. *insm1a* expression is also observed outside the CMZ in the regenerating zebrafish retina (Morris et al. 2011), in the ONL and INL, in rod progenitors and glia-derived progenitors, respectively. The *X. laevis* expression pattern is markedly different from that of the mouse retina (Nelson et al. 2007) in that expression is not observed in the frog ONL. Retinal regeneration in *Xenopus* involves the recruitment of progenitor cells, primarily from dedifferentiated retinal pigmented epithelium (RPE) (Yoshii et al. 2007). It will be interesting to discover if *insm1* is expressed in RPE-derived progenitor cells during *X. laevis* regeneration as it is in glia-derived progenitors of the regenerating zebrafish retina.

Expression in the developing tadpole brain

insm1 is also expressed in the developing tadpole brain (Parlier et al. 2008). To further characterize *insm1* expression in *Xenopus* brain, we performed whole mount *in situ* hybridization on brains isolated from stage 45 tadpoles. *insm1* is expressed in the optic tectum, dorsal pallium, rhombomeres, hypothalamus and preoptic area (Figure 2 D-F). This pattern is similar to that of *foxn4* and *lhx5*, which are also expressed in similar progenitor cell-containing regions of the brain (Figure 2 G – L**)** (Moreno et al. 2004; Moreno et al. 2005; Kelly et al. 2007). These genes differ in expression pattern in the forebrain where *insm1* and *foxn4* are expressed in the dorsal pallium while *lhx5* is expressed in the olfactory bulbs (compare Figure 2 D, G with J). The expression patterns of these genes also differ in the hindbrain: *insm1* is expressed in the rhombomeres while *foxn4* and *lhx5* are expressed in

the reticular formation (compare Figure 2 D with G, J). This is in general agreement with the expression of *insm1* in late progenitor populations in the mouse brain (Duggan et al. 2008). It would be interesting to discover whether *insm1* has a role in regulating proliferation in the brain, as has been demonstrated for the zebrafish retina (Ramachandran et al. 2012).

MATERIALS AND METHODS

In situ hybridizations were performed using digoxigenin-labeled antisense riboprobes generated by *in vitro* transcription of linearized DNA templates. Probes for *insm1* (Parlier et al. 2008), *foxn4* (Kelly et al. 2007), *lhx5* (Bachy et al. 2001), *neurod* (Lee et al. 1995), and *notch1* (Coffman et al. 1990) were prepared as described. Embryos for *in situ* hybridization were generated and staged according to standard criteria (Nieuwkoop 1994; Sive 2000). Wholemount *in situ* hybridizations were performed using *Xenopus laevis* embryos were performed as described previously (Sive 2000). Section *in situ* hybridizations were performed using 8 μM sections of fixed, paraffin-embedded tadpoles (Viczian et al. 2003). Whole mount brain *in situ* hybridizations were performed on brains isolated from fixed stage 45 embryos (Colombo et al. 2004).

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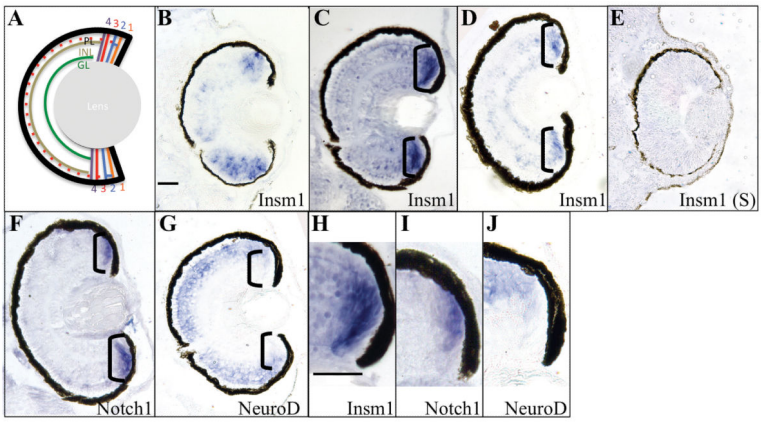

Figure 1. *Insm1* is expressed in retinal progenitor cells (RPCs)

A. Schematic representation of the mature *Xenopus* tadpole retina. B – J. *In situ* hybridization performed using sectioned *Xenopus laevis* embryos. *insm1* is expressed in retinal progenitor cells (RPCs) and undifferentiated neuroepithelium at stage 38. At stages 41 (C, H) and stage 45 (D), *insm1* expression was detected in RPCs located in zones 2 and 3 of the ciliary marginal zone (CMZ) but not in the retinal stem cells of zone 1. Compare the expression of *insm1* at stage 41 (C, H) to expression of *notch1*, a zone 2 marker, at stage 41 (F, I). Both are absent in the most distal zone 1 of the CMZ. Also compare the proximal expression of *insm1* (C, H) to zone 3 and 4 marker, *neuroD* at stage 41 (G, J). The CMZ is indicated by black brackets. Scale bars (1 μ M) for (B-G) and (H-J) are indicated in (A) and (F), respectively. Abbreviations: GL – ganglion cell layer, INL – inner nuclear layer, PL – photoreceptor layer.

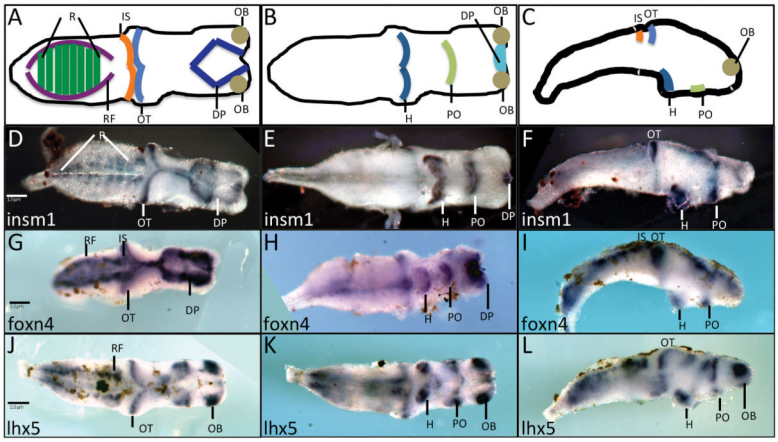

Figure 2. *insm1* expression in the developing brain

A – C. Diagrammatic representation of selected brain regions as viewed in dorsally (A), ventrally (B) or laterally (C). D – L. Whole mount *in situ* hybridizations of stage 45 dissected brains using probes for *insm1* (D – F), *foxn4* (G – I), or *lhx5* (J – L). *insm1* is expressed in the dorsal pallium, optic tectum and rhombomeres as viewed dorsally (D). *insm1* expression was also detected ventrally (E) and laterally (F) in the hypothalamus, preoptic area and isthmus. Scale bars (1 μ M) are indicated in (D) for (D-F), (G) for (G-I) and (J) for (J-L). Abbreviations: DP – dorsal pallium, H – hypothalamus, IS – isthmus, OB – olfactory bulb, OT – optic tectum, PO – preoptic area, RF – reticular formation, R – rhombomeres.