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Transcriptional Activation by Islet1 Isoforms

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Transcriptional Activation by Islet1 Isoforms

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Master of Arts

in

Psychology

by

Amanda Grace Kautzman

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May 2014

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by

Amanda Grace Kautzman

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This thesis is dedicated to my late grandmother, Doris Kautzman, who was and ever will be the driving force behind my desire to keep learning.

ABSTRACT

Transcriptional Activation by Islet1 Isoforms

by

Amanda Grace Kautzman

The present study examined the role of alternative splicing as a mechanism to create neuronal diversity within the developing retina. The *Isl1* gene has two alternatively spliced isoforms, α and β, that are present in the developing retina. The Isl1β isoform lacks a critical portion of a protein-binding domain with which Isl1 binds to Lhx3 in a known transcriptional complex (Isl1:Lhx3:Ldb1). Developmental analysis of *Isl1* expression reveals the *Isl1α* isoform is expressed at higher levels than Isl1β throughout development and persisting into adulthood. *Isl1* isoforms are present in distinct subsets of retinal ganglion cells, with Isl1β-expressing cells being restricted to cells with soma sizes under 175μm^2. A luciferase assay demonstrates the Isl1β isoform is functionally distinct from Isl1α and is not capable of activating the Isl1:Lhx3:Ldb1 complex as Isl1α is. These results suggest that Isl1β containing-complexes may have unique gene targets from Isl1α and implicate the alternative splicing of *Isl1* as a mechanism contributing to neuronal differentiation during retinal development.

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Introduction

The complexity of the central nervous system can be accredited to a combination of cell intrinsic factors as well as environmental cues during development that influence cell genesis, fate determination, migration, arborization, in addition to others. The different influences on these events provide the developing nervous system with the potential for great diversity. Many of these developmental mechanisms have been studied extensively in the visual system, making it an ideal system to study how complexity in the nervous system arises. There are five neuronal cell classes in the retina that contain numerous subtypes, each of which exhibit unique morphologies, connectivity, positions, and gene expression profiles. This paper will explore differentiation within specific neuronal populations of the developing retina by the transcription factor Islet1 (Isl1).

Alternative RNA splicing plays an essential role in diversifying gene regulation and function. RNA splicing is the process by which internal sequences are removed from precursor-mRNA (pre-mRNA) while being processed into mature mRNA, as a complex of proteins called a spliceosome remove the intron and join the flanking exons (Query & Konarska, 2013). This mechanism can generate multiple unique mRNAs through a process known as alternative splicing, leading to the translation of different protein sequences (Nester, Hyman, & Malenka, 2008). This explains how 150,000 different mRNAs are coded for by only about 30,000 genes in the human genome (Elliot & Ladomery, 2011), which could in turn greatly increase the complexity of the nervous system (Wan et al., 2011).

Isl1 is an alternatively spliced LIM-homeodomain (LIM-HD) transcription factor that plays many roles in development. LIM-HD transcription factors represent a highly conserved subset of homeobox-containing proteins (Hobert & Westphal, 2000). They are capable of binding to other LIM-HD proteins as well as other proteins, transcription factor families, and kinases (Bach 2000). The diversity of binding partners allows for participation in a wide range of developmental events. For example, LIM-HD proteins can form complexes with POU-HD transcription factors to repress gene transcription, while forming complexes with RLIM factors leading to transcriptional activation. They also interact with the Lbd (Lim domain-binding protein 1) co-factor family, which greatly increases their ability to affect gene transcription. Lbd proteins intrinsically dimerize and allow LIM-HD proteins to interact with specific regulatory proteins like Otx2 or Isl1 (Hobert & Westphal, 2000). In addition, Ldb proteins can form either homodimers or heterodimers to further diversify the combination of complexes that can assemble.

The LIM-HD transcription factor, Isl1, has been studied in many tissues including cardiac and skeletal muscle, pancreas, kidney, brain, and spinal cord. It has been shown to bind and regulate insulin promoters and has been implicated as a key component of motor neuron specification (Ando, Shioda, Handa, & Kataoka, 2003). Isl1 has been shown to play a role in the differentiation of bipolar cells, cholinergic amacrine cells, and retinal ganglion cells (RGCs) in the developing retina: when Isl1 is conditionally knocked out of the embryonic retina, these cell types are born, but later are not detectable and therefore either died or switched fates (Elshatory et al.,

2007a). This implicates *Isl1* as a gene that could be important for cells to maintain a differentiated state.

Recently, two alternatively spliced isoforms of Isl1, the canonical alpha form (α) and alternative beta (β) form, were described in pancreatic cell lines (Ando et al., 2003). The Isl1β isoform is missing 23 amino acids in the C-terminus that corresponds to the protein-binding domain LBD (Lhx3-binding domain). Figure 1A shows the two isoforms of Isl1 and highlights the missing amino acids in the Isl1β isoform. The two isoforms are of interest because Isl1 forms a well characterized transcriptional ternary complex with two other proteins, Lhx3 and Ldb1 (Isl1:Lhx3:Ldb1) (Figure 1B). Therefore, the absence of this Lhx3-binding domain in Isl1β suggests there may be functional differences between the two isoforms of Isl1, such as their ability to form transcriptional complexes and activate gene targets.

Both isoforms of *Isl1* are expressed in the developing mouse retina and persist into adulthood (Whitney, 2013). Reverse transcriptase PCR with a primer pair capable of amplifying both *Isl1* transcripts showed that *Isl1α* and *Isl1β* are expressed in the mouse retina at E12.5, E14.5, P1 and adulthood (Figure 2A). Quantitative RT-PCR also showed that *Isl1α* is also more abundant than *Isl1β* and this difference becomes more pronounced as development proceeds (Figure 2B; Whitney, 2013). Both isoforms are also present in other central nervous system tissue such as the brain and spinal cord. Western blots using an antibody recognizing both isoforms, Isl1-Pan, confirmed that both of these proteins are expressed in the retina (Figure 3A).

Isl1 isoform immunostaining from adult retinal tissue reveals a population of retinal ganglion cells that only express the Isl1β isoform (Whitney, 2013). An Isl1 antibody was produced and its specificity was confirmed in Western blots of HEK293T cells that were transfected with overexpression plasmids designed to express Isl1α, Isl1β, and Lhx3 proteins (Figure 3B). Since no Isl1β-specific antibody exists, Isl1β cells are recognized as Isl1-Pan-positive and Isl1α-negative cells. Figure 4A shows a subset of RGCs that are Isl1-Pan-positive as well as Isl1α-negative RGCs, indicating they are a population of RGCs that only express Isl1β (blue). These cells were also ChAT-negative and therefore were verified as RGCs and not displaced cholinergic amacrine cells. These Isl1β RGCs make up 25.7% of all Isl1-Pan-positive cells within the GCL (Whitney, 2013).

Isl1β is not equally distributed across all RGC subtypes as they are restricted to certain ranges of soma size, indicating that they might be indicative of particular cell types. At least 22 morphologically distinct RGC cell types have been described (Volgyi et al., 2009) and they range from a soma size of roughly 60μm^2 to 600μm^2. (Coombs et al., 2009) The soma sizes of Isl1-Pan-positive cells within the GCL were found to be in the range from about 50μm^2 to 300μm^2 but Isl1β RGCs, however, are restricted to the range of RGCs under 175μm^2 (Figure 4B). These cells are likely from multiple subsets of RGCs since they were found to be randomly spaced from one another while single populations of RGCs exhibit non-random spacing (Whitney, 2013). This data suggests that the presence of different isoforms of Isl1 may play an important role in the differentiation of specific RGC subtypes.

Given the expression profile of Isl1 isoforms and the previous discovery of an Isl1β-only population of RGCs in the adult retina, we hypothesize that each Isl1 isoform has a specific role in the development or differentiation of these RGCs. In addition, we speculate that Isl1β is not capable of forming the same Isl1:Lhx3:Ldb1 complex as Isl1α due to its missing functional domain. This paper seeks to evaluate the functional role of Isl1 alternative splicing in the developing retina.

Methods

Isl1α, Isl1β, Lhx3 Overexpression Plasmids

Gateway cloning (Invitrogen) was used to make Isl1α and Isl1β overexpressing plasmids. The open reading frame (ORF) of Isl1α was amplified from adult mouse retinal cDNA using primers with attB adapter sequences. This product was gel-purified and inserted into a donor vector (pDONR221, Invitrogen) to create an empty vector using BP Clonase to mediate the BP recombination reaction. One Shot Stbl3 chemically competent E. coli (Invitrogen) were transformed with this reaction, and kanamycin resistant clones were selected for overnight culture in LB broth. A QIAprep Spin Miniprep kit (Qiagen) was used to extract plasmid DNA from the bacteria, and the successful recombination of the Isl1α ORF into the donor vector was confirmed by restriction digest and sequencing. The Isl1α-containing entry vector was then recombined with a destination vector, using LR Clonase, and transformed into E. coli for plasmid amplification and purification, as described above. The destination vector, pLenti CMV/TO GFP-Zeo Dest (Campeau et al.,

2009; Addgene plasmid #17431), will express the gene of interest and GFP using two different constitutive CMV promoters. Once the sequence of the ORF in the destination vector was validated, the plasmid was replicated and purified from E. coli using an EndoFree Plasmid Maxi kit (Qiagen). The Isl1β ORF was generated using deletion PCR, to remove the 69 nucleotides from Isl1α that are not present in Isl1β (Whitney, 2013). Once isolated, the Isl1β ORF was also cloned into the same destination vector described above, and large amounts were purified.

HEK293T Transfection and Luciferase Assay

HEK293T cells were transiently transfected with combinations of experimental plasmids designed to express Isl1α, Isl1β, Lhx3, or GFP (control plasmid with no gene of interest inserted). Cells were also co-transfected with a luciferase reporter that has been previously shown to be specifically activated by the Isl1:Lhx3:Ldb1 complex (Hx:RE) as well as CMV-β-Galactosidase (β-Gal) (Lee et al., 2008). The cells maintained in a media of high glucose (4.5g/L) DMEM plus 10% Fetal Bovine Serum, with penicillin (100 U/mL) and streptomycin (100 µg/mL), in a T 75 tissue culture flask. Media was changed every two days and cells were passaged once per week or when cells reached 75% confluency. Cells were counted and plated in 12 well tissue culture plates at 150,000 cells per well and media was changed 24 hours later. 48 hours post-plating, cells were transiently transfected with the transfection reagent TurboFect (Thermo Scientific). 250ng of Hx:RE reporter plasmid, 20ng of β-Gal, and 400ng of experimental plasmids were transfected into

each well, and the transfectant was removed 16 hours later. Cell lysates were collected 24 hours from the start of transfection.

Cell lysates were assayed for luciferase (Promega) and β-Gal activity on a Perkin-Elmer plate reader, and 3 wells of the same condition were assayed and averaged to serve as internal experimental replicates. Luciferase units were corrected by subtraction of untransfected well units, and then were normalized using β-Gal. Each bar represents mean fold induction with respect to the β-Gal control and error bars represent SEM. Results are representative of three independent experiments. A one-way ANOVA and Tukey's post-hoc test were used to determine significance. Asterisks indicate a significant difference with a p-value <0.05.

Protein Collection

For luciferase assays, HEK293T cells were lysed in 100ul per well of cold 1X Passive Lysis Buffer (Promega). Cells were lifted from plate with cell scraper, vortexed, and centrifuged at 12,000g for two minutes at 4^{o}C. Protein lysates were stored at -80^{o}C.

Results

Transcriptional Activation of Isl1 Isoforms

Since the two isoforms of Isl1 were found to be expressed in different populations of RGCs, functional differences between these two proteins were assessed using a transcriptional activation assay. A luciferase assay was used to test

directly if the two Isl1 isoforms function distinctly in their ability to regulate gene expression. HEK293T cells were transfected with either the Isl1α or Isl1β isoform, as well as Lhx3, to allow the Isl1 complex to form (HEK293T endogenously express Ldb1). In addition, cells were co-transfected with a Hexamer Response Element (Hx:RE) luciferase reporter that has been shown to be activated specifically by the formation of this complex (Lee et al., 2003). The Hx:RE plasmid was previously shown to have a DNA sequence specific to the Isl1:Lhx3:Ldb1 complex that is not recognized by either Isl1 or Lhx3 alone (Lee et al., 2008).

The activation of luciferase by the Isl1α:Lhx3:Ldb1 complex was found to be 5 fold greater than the Isl1β:Lhx3:Ldb1 complex (Figure 5) and this luciferase activation in Lhx3+Isl1α overexpressing cells was significantly different from Lhx3+Isl1β and all other conditions (p-value = 0.035), while activation of Lhx3+Isl1β was indistinguishable from Lhx3 alone. These data demonstrate a functional difference between the two isoforms of Isl1, and furthermore suggest that the β isoform might not be capable of forming a complex with Lhx3 and Ldb1.

Discussion

This paper identified that through alternative splicing, the *Isl1* gene creates two functionally distinct isoforms. This luciferase assay is the first demonstration that Isl1 isoforms have different transcriptional activation abilities and therefore, are likely contributing to the differentiation of RGC subtypes. This finding allows for a deeper understanding of how the retina forms and provides first direct evidence for

the importance of having two isoforms of Isl1, as Isl1α was shown to activate the Isl1:Lhx3:Ldb1 complex significantly more robustly than Isl1β. The data also show that the Isl1β isoform does not further enhance activation binding of Lhx3 and Ldb1. It remains unknown how Isl1β is functioning in specific RGC subtypes. Perhaps it is forming a unique complex with other LIM-HD transcription factors and binds to a unique promoter sequence distinct from the Isl1:Lhx3:Ldb1 complex.

The data presented in this paper enhances what was known about the developmental expression profile and protein localization of Isl1α and Isl1β in the retina (Whitney, 2013). It was previously shown that Isl1β is present in a subset of RGCs, but the mechanism by which Isl1β is contributing to the differentiation of these cells remains unclear (Whitney, 2013). We can now confidently presume that the Isl1 isoforms are contributing to RGC differentiation given their ability to regulate gene transcription through different mechanisms. These results suggest that alternative splicing of Isl1 creates different isoforms which differ in their ability to alter gene transcription, and that this difference may contribute to retinal differentiation.

The current findings allow for expansion of the combinational code of LIM-HD transcription factors. It was previously known that a complex of Isl1, Lhx3, and Ldb1 proteins cause cells become motorneurons, while with Lhx3 and Ldb1 complexes, without Isl1, become V2 interneurons (Bhati et al., 2000). These complexes were shown to bind to different transcriptional promoter sequences and provided the first demonstration that Isl1 plays a role in fate determination in the

periphery. Given that Isl1 isoforms also function distinctly in the developing retina, this further expands the potential number of LIM-HD complexes that Isl1 can complex with, and offers insight into the extent of cell type diversity within the developing nervous system.

Until this study, Isl1 isoforms had only been identified in the earlier described pancreatic cell lines (Bhati et al, 2000). Isl1 alternative splicing is conserved between mice and rat species, but is not conserved between rodents and humans. This is surprising given 100% homology of their protein sequences, but it remains unclear if there are splice variants in humans that exhibit similar functions. In the retina, Isl1 has also been shown to interact with other transcription factors and form complexes independent of Isl1:Lhx3:Ld1b. For example, Isl1 binds to a POU domain transcription factors like Pou4f2 and Brn3b, and they co-regulate target genes in developing RGCs (Pan et al., 2008 & Li et al., 2014). Isl1 also participates in protein-protein interactions in other cellular tissues. For instance, Isl1 promotes insulin-gene transcription by direct binding to the basic helix-loop-helix (bHLH) transcription factor, BETA2 (Peng et al, 2005). In addition, Isl1 binds to the estrogen receptor in the rat arcuate nucleus and implicates Isl1 as a player in controlling brain function via interaction with steroid hormones (Gay et al., 2000). Lastly, Isl1 has been documented to influence gene transcription by binding with multiple factors to form "enhancer complexes" that bind to specific intronic sequences within other genes. Isl1 complexes with two transcription factors, TBX20 and GATA4, and this

binding to the *FGF10* gene causes regulation of downstream targets affecting development of cardiac outflow muscles in humans (Golzio et al., 2012).

The idea of alternative splicing as a mechanism for directly creating neuronal diversity through differentiation is a relatively new idea. The retinoid-related orphan receptor (Rorb) was described to have two isoforms that are expressed at different time points in development and directly affect neuronal differentiation (Liu et al., 2013). Pax6, a critical transcription factor to eye development, has an alternatively spliced isoform that is necessary for proper development of retina architecture (Azuma et al., 2005). Alternatively spliced isoforms can also be expressed during varied developmental windows implicating isoform-specific physiological roles. For example, the two PAC1-R isoforms, Hip and Hop1, have varied expression patterns throughout postnatal retinal development and are likely playing distinct instructive roles (Denes et al., 2014).

Isl1 is not the only LIM-HD transcription factor with known alternative isoforms; Lhx9 and Lhx7 have also been documented, but their effect on retinal development still remains to be explored (Failli et al, 2000). Since LIM-HD proteins are able to interact with one another though binding of their homeodomains, it is unclear if any of these other transcription factor family members may be interacting with Isl1. Given the findings of this paper, it would be prudent to explore the role of Isl1 isoforms in forming complexes with other documented binding partners in the retina.

While a functional difference was shown between the two isoforms of Isl1, further studies are needed to assess the consequence of this *in vivo*. One way this could be accomplished is by creating conditional-knockout mice that are genetically engineered to lack both of the Isl1 isoforms in the retina by utilization of Cre-Lox technology. Cells from these same animals can be electroporated to re-introduce one of the isoforms and explore the functional consequence of just having one specific Isl1 isoform. One caveat to this experiment is that mice lacking Isl1 specifically in the retina have previously been shown to have the birth of all cell types followed by subsequent death or switching off ate (Elshatory et al., 2007b), therefore electroporation experiments would need to occur *in utero* before these events occur. Given that Isl1β is endogenously expressed in much lower quantities than Isl1α, retinas from Isl1β-only rescue retinas may provide striking *in vivo* insight into the function of *Isl1* alternative splicing.

This paper provides the first evidence that Isl1 isoforms have differing activational abilities and therefore suggests a possible role for the alternative splicing of *Isl1* in the differentiation of RGCs, contributing to neuronal diversity. Further studies are needed to elucidate the mechanism by which each isoform is helping to specify the cell fate of their cells they are present in.

References

Ando, K., Shioda, S., Handa, H., & Kataoka, K. (2003). Isolation and characterization of an alternatively spliced variant of transcription factor Islet-1. J. Molec. Endocrin. 31, 419-425.

Azuma, N., Tadokoro, K., Asaka, A., Yamada, m., Yamaguchi, Y., Handa, H., Matsushima, S., Watanabe, T., Kohsaka, S., Kida, Y., Shiraishi, T., Ogura, T., Shimamura, K., & Nakafuku, M. (2006). The *Pax6* isoform bearing an alternative spliced exon promotes the development of the neural retinal structure. Hum. Mol. Genet. 14(6), 735-745.

Bach, I. (2000). The LIM domain: regulation by association. Mech. Dev. 91(1-2), 5-17.

Bhati, M., Lee, C., Nancarrow, A.L., Lee, M., Craig, V.J., Guss, J.M., Mackay, J.P. & Matthews, J.M. (2008). Implementing the LIM code: the structural basis for cell type-specific assembly of LIM-homeodomain complexes. EMBO J. 27(14), 2018-2029.

Campeau, E., Ruhl, V.E., Rodier, F., Smith, C.L., Rahmberg, B.L., Fuss, J.O., Campisi, J., Yaswen, P., Cooper, P.K., & Kaufman, P.D. (2009). A versatile viral system for expression and depletion of proteins in mammalian cells. PLoS One 4(8), e6529.

Coombs, J., Van Der List, D., Wang, G.Y., & Chalupa, L.M. (2009). Morphological properties of mouse retinal ganglion cells. Neuroscience 140, 123-136.

Denes, V., Czotter, N., Lakk, M., Berta, G, & Gabriel, R. (2014). PAC1-expressing

structures of neural retina alter their PAC1 isoform splicing during postnatal development. Cell Tissue Res. 355, 279-288.

Elliot, D., & Ladomery, M. (2011). Molecular Biology of RNA. New York, NY: Oxford University Press.

Elshatory, Y., Everhart, D., Deng, M., Xie, X., Barlow, R.B., & Gan, L. (2007a). Islet-1 controls the differentiation of retinal bipolar and cholinergic amacrine cells. J. Neurosci. 27(46), 12707-12720.

Elshatory, Y., Deng, M., Xie, X., & Gan, L. (2007b). Expression of the LIM-homeodomain protein Isl1 in the developing and mature mouse retina. J. Comp. Neurol. 503(1), 182-197.

Failli, V., Rogard, M., Mattei, M.G., Vernier, P., Retaux, S. (2000). Lhx9 and Lhx9alpha LIM-homeodomain factors: genomic structure, express patterns, chromosomal location, and phylogenetic analysis. Genomics 64(3): 307-317.

Gay, F., Anglade, I., Gong, Z., & Salbert, G. (2000). The LIM/homeodomain protein islet-1 modulates estrogen receptor functions. 14(10), 1627-1648.

Golzio, C., Havis, E., Daubas, P., Nuel, G., Babarit, C., Munnich, A., Vekemans, M., Zaffran, S., Lyonnet, S., & Etchevers, H.C. (2012). ISL1 Directly Regulates *FGF10* Transcription during Human Cardiac Outflow Formation. PLOS One. 7(1), e30677.

Hobart, O. and Westphal, H. (2000). Functions of LIM-homeobox genes. Trends Genet. 16(2), 75-83.

Kay, J.N., Voinescu, P.E., Chu, M.W., & Sanes, J.R. (2011). Neurod6 expression

defines new retinal amacrine cell subtypes and regulates their fate. Nat. Neurosci. 14: 965-972.

Lee, S.K., and Pfaff, S.L. (2003). Synchronization of neurogenesis and motor neuron specification by direct coupling of bHLH and homeodomain transcription factors. Neuron 38, 731-745.

Lee, S., Lee, B., Joshi, K., Pfaff, S.L., Le,, J.W., & Lee, S.K. (2008) A regulatory network to segregate the identity of neuronal subtypes. Developmental Cell 14, 877-889.

Renzhong, L., Ruonala, R., Sapkota, D., Hu, Z., & Mu, X. (2014). Isl1 and Pou4f2 form a complex to regulate target genes in developing retinal ganglion cells. PLOS One. 9(3), e92105.

Liu, H., Kim, S.Y., Fu, Y., Wu, X., Ng, L., Swarooop, A., & Forrest, D. (2013). An isoform of retinoid-related orphan receptor β directs differentiation of retinal amacrine and horizontal interneurons. Nat. Commun. 2(2): 109-118.

Nester, E., Hyman, S., & Malenka, R. (2008). Molecular neuropharmacology: a foundation for clinical neuroscience, second edition. New York, NY: McGraw-Hill Professional Publishing.

Pan, L., Deng, M., Xie, X., & Gan, L. (2008). ISL1 and BRN3B co-regulate the differentiation of murine retinal ganglion cells. Development. 135(11), 1981-1990.

Query, C.C., & Konarska, M.M. (2013). Strutural biology: Spliceosome's core exposed. Nature 493, 615-616.

Volgyi, B., Chheda, S., & Bloomfield, S.A. (2009). Tracer coupling patterns of the ganglion cell subtypes in the mouse retina. J Comp. Neurol. 512(5), 664-687.

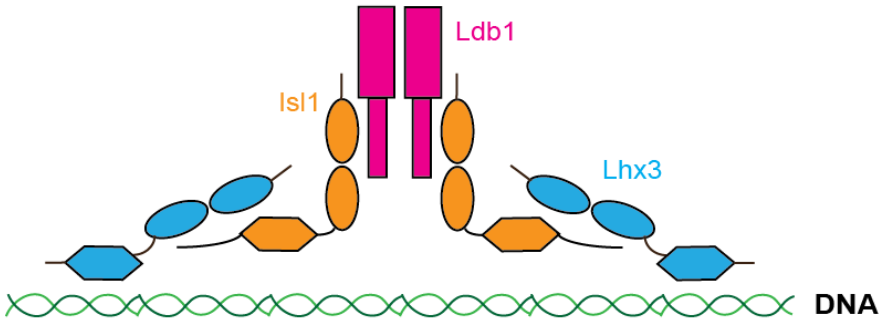
Wan, J., Masuda, T., Hackler Jr., L., Torres, K.M., Merbs, S.L., Zack, D.J., & Qian, J. (2011). Dynamic usage of alternative splicing exons during mouse retina development. Nuc. Acids Res. 39(18), 7920-7930.

Whitney, I.E. (2013). Identifying genetic determinants of distinct cell types in the mouse retina. (Doctoral dissertation). University of California at Santa Barbara, Santa Barbara.

Figure 1

1A

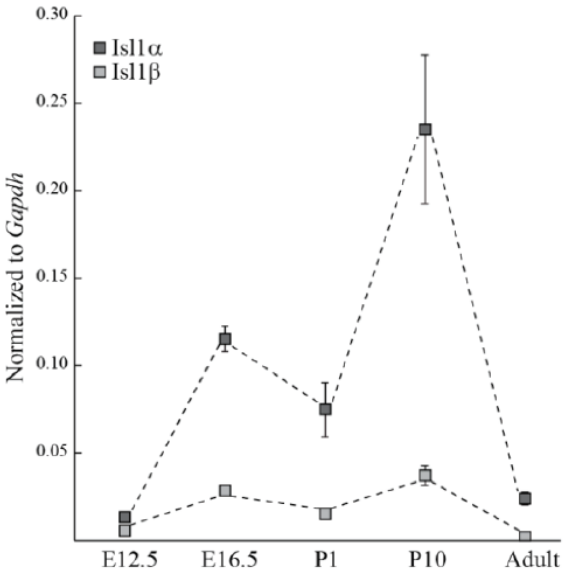
1B



Structure of Isl1 isoforms and Isl1 transcriptional complex. **(A)**. Isl1β is missing 23 amino acids that correspond to one of the Lhx3-binding domains (Ldb1) with which Isl1 binds to Lhx3. Asterisk denotes region of missing amino acids in Isl1β, relative to Isl1α **(B)**. Hexameric transcriptional complex formed with Ldb1 dimer (pink), Isl1 (orange), and Lhx3 (blue). Figure adapted from Matthews et al., 2006.

Figure 2

2A



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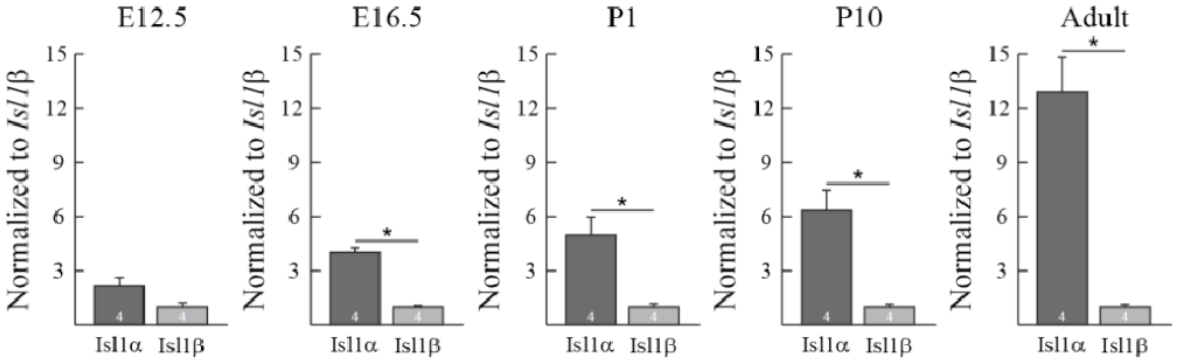
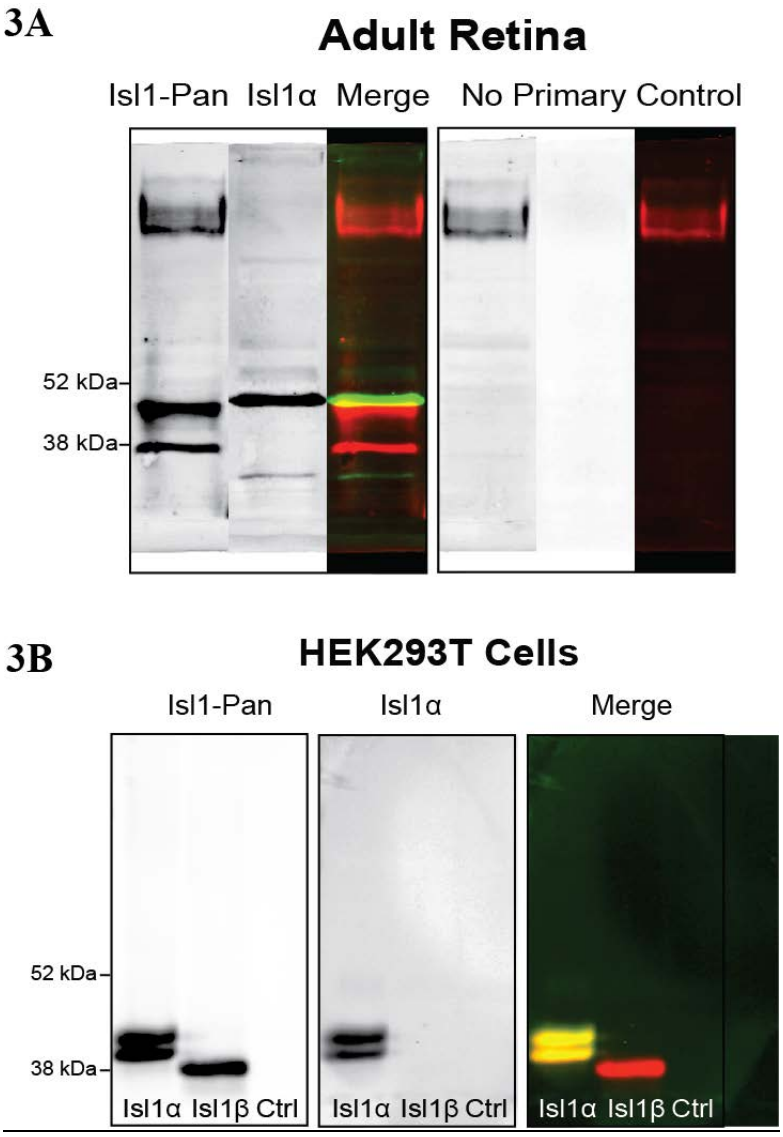


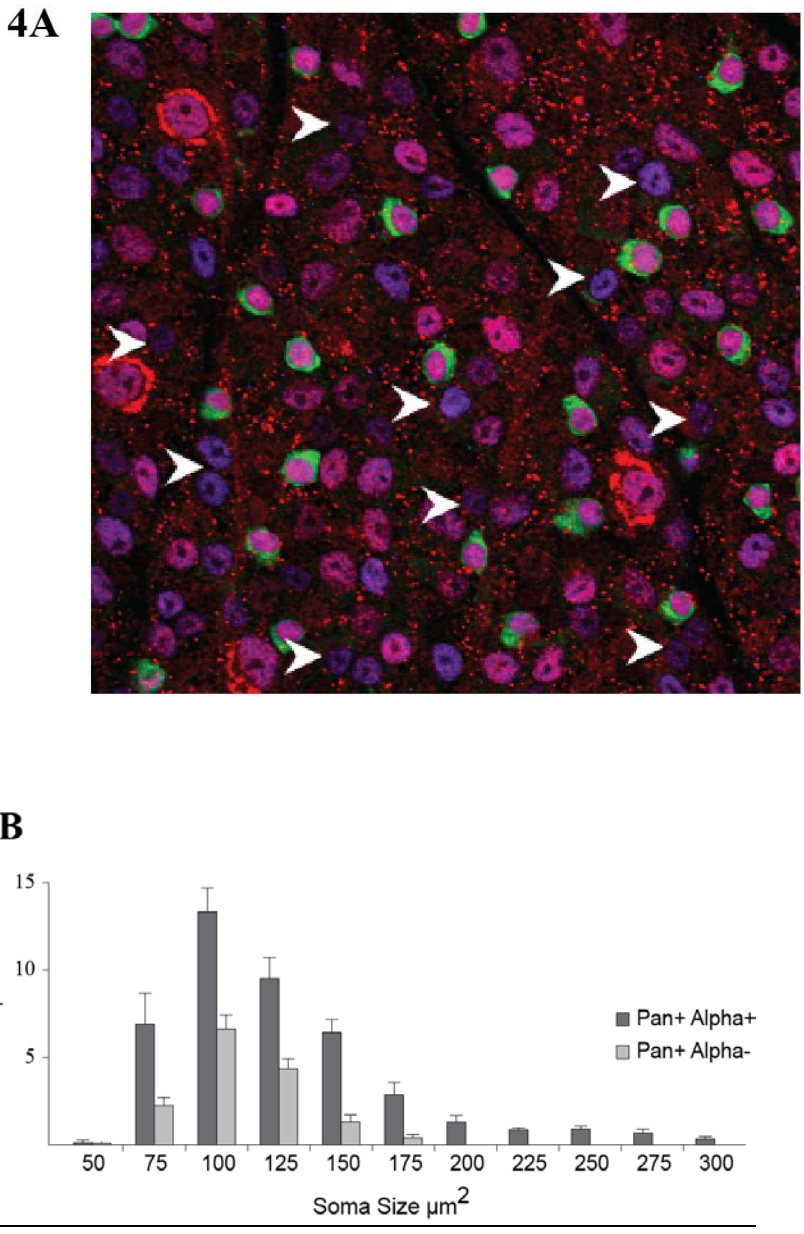
Developmental expression of Isl1 isoforms. **(A)**. RT-PCR with Isl1 isoform-specific primers with values normalized to Gapdh, a housekeeping gene. **(B)**. qPCR (real time RT-PCR) showing fold increase of Isl1α expression over Isl1β expression. A two-way mixed ANOVA and post-hoc t-test show Isl1α expression is significantly higher than Islβ at E16.5, P1, P10, and adult (p-value <0.05). Figure taken from Whitney dissertation, 2013.

Figure 3



Immunoblotting of HEK293T cells and adult retinal extracts for Isl1 with Isl1-Pan and Isl1α antibodies. **(A)**. Immunoblotting adult retinal lysates show overlap, though not complete, of the Isl1α antibody band with the Isl1-Pan alpha antibody band. **(B)**. HEK293T cells were used to specify Isl1-Pan and Isl1α antibodies. Bands for Isl1α (smaller kDa) and Isl1β at 39 kDa and 36 kDa correspond to the predicted molecular weights, respectively. The larger band in the doublet recognized by the Isl1-Pan antibody corresponds to a phosphorylated version of the protein that is not recognized by the Isl1α antibody. Figure taken from Whitney dissertation, 2013.

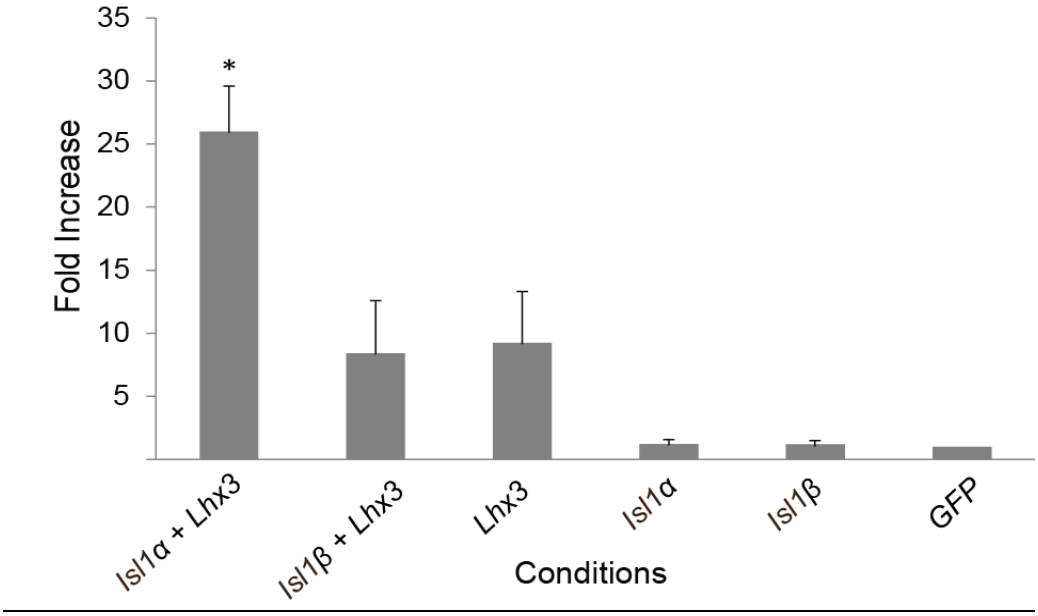
Figure 4



Soma size analysis of Isl1β-positive cells. **(A)**. Representative image showing Islβ-only cells in blue (white arrowheads). Isl1α-positive cells are red, ChAT-positive cells are green, and Isl1-Pan -positive cells are blue. Isl1β-only cells were identified as cells being Isl1-Pan-positive and Isl1-α-negative. **(B)**. Soma size was measured from about 2000 cells in 8 fields in the ganglion cell layer of one retina. Isl1β-only cells soma sizes range from about 50µm^2 to 175µm^2, compared to Isl1-Pan-positive soma sizes ranging from 50µm^2 to 300µm^2. Figure adapted from Whitney dissertation, 2013.

Figure 5

5A



Transcriptional activation of Isl1 isoforms assessed by luciferase assay. **(A)**. Isl1α+Lhx3 has ~20 fold increase over Isl1β+Lhx3 in the ability to turn on transcription of the Hx:RE luciferase reporter. Isl1α+Lhx3 is significantly different from all other conditions (asterisk denotes p-value <0.05). All results normalized to GFP.