Laidlaw Scholars Program: Regulation of Runx3 expression in early proprioceptor development

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Abstract

Diversification of sensory neuron (SN) subtypes enables the distinction among various stimuli. SN subtype specification gives rise to nociceptors, mechanoreceptors, and proprioceptors. In particular, proprioceptors are responsible for limb positioning and essential for motor control. Among other associated molecular players, Runt-related transcription factor 3 (Runx3) is critical for early proprioceptor development. However, the mechanisms by which Runx3 expression is induced and selectively maintained within proprioceptive progenitors are unclear. Following the completion of a literature study on the regulation of Runx3 expression in early proprioceptor development during Summer I of the Laidlaw Scholars Program, we developed a model for the induction of Runx3 expression in the proprioceptive population of dorsal root ganglia (DRG) SNs: retinoic acid (RA) signalling activates the expression of Sox11 transcription factors (TFs), which cooperate with Brn3a TFs to bind to upstream Runx3 elements, thus inducing Runx3 expression in early proprioceptors. We also propose several experiments to test this hypothesis during Summer II of the Laidlaw Scholars Program. Elucidating the mechanisms of early proprioceptor development will not only contribute to the current understanding of sensory neurogenesis but also inform potential ataxia and neuropathy modelling and treatment approaches.

Introduction

Neuronal diversity enables the distinction among various stimuli, including touch, pressure, pain, and others [1]. As such, elucidating the mechanisms of sensory neuron (SN) subtype diversification is essential for understanding how we are able to perceive and interact with our surroundings. Cell bodies of SNs are located in dorsal root ganglia (DRG), which flank the spinal cord. DRG SNs extend their axons into the periphery and through a central collateral, communicate sensory information to the spinal cord and brain [2]. The major DRG SN subtypes consist of nociceptors (pain), mechanoreceptors (touch), and proprioceptors (limb positioning). These neuronal subtypes are characterized by the differential expression of tropomyosin receptor-kinase A (TrkA), TrkB, TrkC, respectively, as well as Runt-related transcription factor 1 (Runx1) and Runx3 [1]. Early proprioceptor development is achieved through an interplay of TrkC, Runx3, and associated signalling molecules [1, 3]. Runx3, in particular, plays a critical role in early proprioceptor development through the maintenance of the proprioceptive fate and repression of alternative lineages [1, 3, 4]. While certain molecular players have been implicated in regulating Runx3 expression, the current understanding of Runx3 regulation in early proprioceptor development is unclear. As such, for Summer I of the Laidlaw Scholars Program, I sought to answer the research question: During the development of DRG SNs, what are the mechanisms that regulate Runx3 expression in the TrkC+ proprioceptive neuronal population?

In this report, I will summarize: (1) the findings of the literature review and describe the model developed during Summer I and (2) discuss the specific aims and experimental approaches for completion during Summer II. With regard to Summer I, I will begin by highlighting the function

and importance of Runx3 in early proprioceptor development. I will then provide an overview of the types of gene regulation and then contextualize these regulatory mechanisms for Runx3 expression in proprioceptor development. I will follow with a discussion of the transcription factors and signalling molecules implicated in Runx3 regulation. Lastly, I will synthesize the discussed information to propose a model for the regulation of Runx3 expression in early proprioceptor development. With regard to Summer II, I will close this summary with the specific aims and proposed experimental approaches to test the model.

Summer I: Background & Literature Review

The role of Runx3 expression in early proprioceptor development

Early proprioceptor development is characterized by an interplay among TrkC, Runx3, and the target-derived signal neurotrophin-3 (NT3), which serves as the ligand for TrkC [1]. Runx3 expression is required for TrkC promotion and maintenance, and TrkC-NT3 signalling is required for proprioceptor survival [3, 4, 5]. During early proprioceptor development, TrkC expression is induced on embryonic day 11.0 (E11.0), and shortly followed by Runx3 expression (Figure 1) [3, 6]. By E12.0, most TrkC+ neurons express Runx3 (Figure 1) [3].

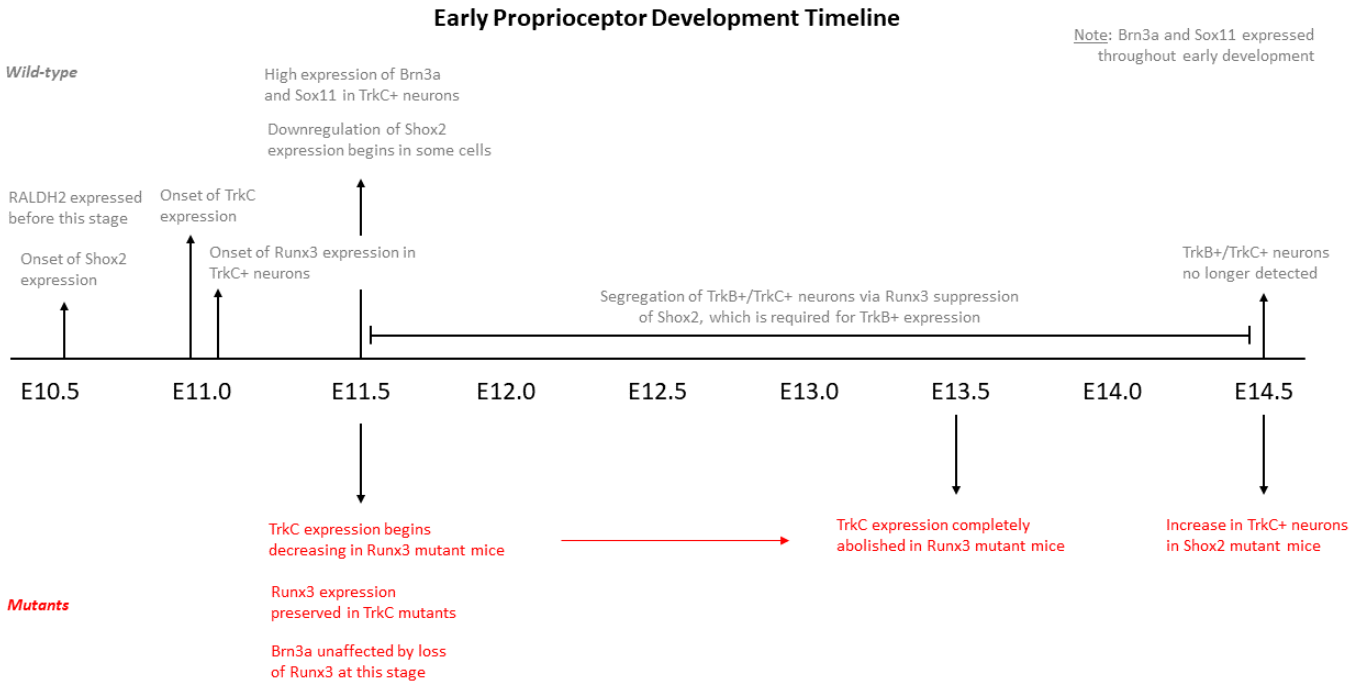
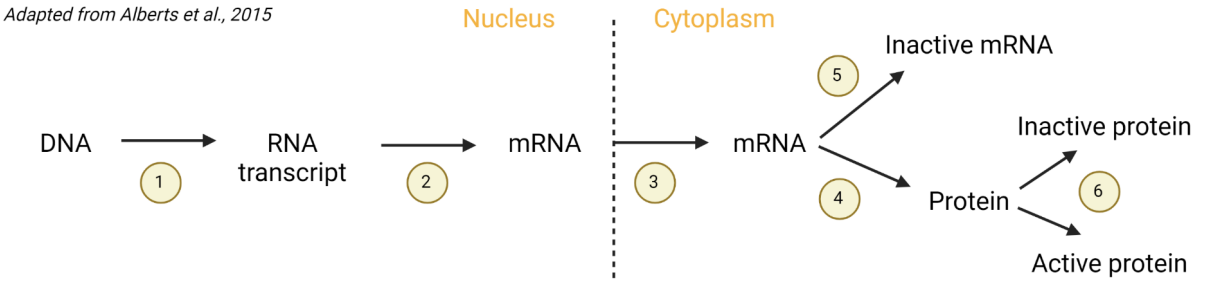


Figure 1. Early proprioceptor development timeline.

During these early developmental stages, Runx3 plays an important dual role. First, Runx3 is required for maintaining TrkC expression as evidenced by the abolishment of TrkC expression in Runx3 mutant mice by E13.5 (Figure 1) [3]. Second, Runx3 functions in segregating the mechanoreceptive/proprioceptive (TrkB+/TrkC+) hybrid neuronal population between E11.5 and

E14.5 (Figure 1). Specifically, Runx3 represses Shox2, which is necessary for maintaining TrkB expression. Therefore, Runx3 helps restrict and maintain TrkC expression in proproceptive progenitors, thus driving the proprioceptive fate [4]. Differential Runx3 expression also appears to determine potential for proprioceptor survival during programmed cell death [7]. In the absence of Runx3, early proprioceptors exhibit a reduction in proprioceptive markers and are also unable to properly project to their targets. Moreover, Runx3 mutant mice display limb ataxia and a decrease in TrkC+ proprioceptors [8, 9]. In essence, Runx3 plays a critical role in early proprioceptor development.

Types of gene regulation



		Specific to Runx3	Specific to Runx3 in proprioceptor development
1	**Transcriptional regulation**	1) P1 and P2 (with CGI) regions 2) TAD, TE1, and TE2 3) R1, R2, and R3	1) P2 region (with CGI) 2) R1, R2, and R3 3) BRNF binding sites 4) SRY binding site
2	**RNA processing control**	Runx3 splice variants: p27, p33, p44, p46	Runx3 expressed as the p44 transcript via P2-mediated transcription
3	**RNA transport & localization**	Nuclear localization signal (NLS)	
4	**Translational regulation**	Different P1 vs. P2 5'UTR translation efficiencies	
5	**mRNA degradation**	Runx3 p44 isoform lacks a classical poly-A tail	
6	**Protein activity control**	1) CBF-beta binding partner 2) RD and other protein domains 3) Post-translational modifications	1) CBF-beta binding partner 2) RD, TAD, and MH2 domains 2) Acetylation and ubiquitination in degradation

Figure 2. Types of gene regulation specific to Runx3 in proprioceptor development.

From transcription to translation, gene expression and activity may be regulated on several levels (Figure 2). During DNA transcription, expression may be regulated via transcriptional activators and repressors. Following production of an RNA transcript, alternative splicing and processing determine the selection and abundance of a particular transcript. After exiting the nucleus and entering the cytosol, expression is then regulated by localization signals that control the

particular destination of transcripts. During translation, some transcripts are degraded in the cytoplasm while others are translated into protein products. Lastly, on the protein activity level, the activation (possibly by binding to co-factors), deactivation, degradation, and localization of proteins also serve as mechanisms of expression regulation [10]. Overall, it is important to understand Runx3 regulation within the framework of these types of gene regulation (Figure 2).

Types of Runx3 regulation in early proprioceptor development

The *Runx* gene family encodes for TFs containing a runt domain (RD). In particular, *Runx3* comprises six exons and is approximately 67 kb in size, the smallest of the *Runx* genes [11]. On the transcriptional level, Runx3 expression is regulated by distal and proximal promoter regions, P1 and P2, respectively [11, 12]. Unlike the P1 region, the P2 promoter contains a CpG island (CGI), which can serve as a site for epigenetic modifications [11]. For instance, separate from its role in proprioceptor development, Runx3 also acts as a gastric tumor suppressor; approximately half of gastric cancers exhibit hypermethylation of the P2 CGI, leading to inactivation of Runx3 (Figure 4C) [13]. In proprioceptor development, Runx3 expression is mediated by the P2 promoter (Figure 4B). As well, during early proprioceptor development, the P2 promoter appears to be regulated by three upstream enhancer elements, R1, R2, and R3 (Figure 2, 3, 4A) [6].

On the levels of RNA processing and translation, alternative splicing produces various transcripts, resulting in four Runx3 variants: p46, p33, p27 and p44 (Figure 2) [11, 12]. The p46, p33, and p27 isoforms are P1-derived, while the p44 isoform is P2-derived [11, 14]. Accordingly, Runx3 is expressed in the p44 isoform in proprioceptor progenitors. Moreover, P1 and P2 correspond to different 5’ UTRs, which lead to varying translation efficiencies [15]. As demonstrated in Runx1 expression, the short 5’ UTR of P1 and the long 5’ UTR of P2 support different translation mechanisms, where translation via the P1-5’UTR is more efficient than that of the 5’ P2-5’UTR [12, 15]. However, the existence and details of a similar mechanism for the regulation of Runx3 expression are not yet known. Following translation, Runx3 proteins are localized to the nucleus via a nuclear localization signal (NLS) contained in the C-terminus [15].

On a post-translational level, Runx3 proteins may be subject to modifications that regulate its activity (Figure 2). For example, Runx3 proteins are ubiquitinated by Smurf proteins and subsequently degraded by the ubiquitin ligase-mediated pathway [16]. This degradation may be prevented through acetylation via p300 proteins, which function as histone acetyltransferases (HATs) [17]. Both acetylation and ubiquitination target Runx3 lysine residues. Thus, increased p300-mediated acetylation competes for target lysine residues in the Runx3 protein, preventing ubiquitination, and decreasing Runx3 degradation [16, 17]. Runx3 proteins also interact with histone deacetylases (HDAC); Runx3 activity decreases with HDAC activity whereby deacetylation via HDACs destabilizes Runx3 proteins, leading to their degradation [17]. As HATs and HDACs control Runx3 stability and expression duration, it is possible that these

enzymes function in regulating Runx3 regulation in early proprioceptor development (Figure 4C).

Moreover, Runx3 protein activity is also regulated through heterodimerization to its binding partner core-binding factor subunit beta (CBFβ), which increases Runx3 TF DNA binding affinity [18]. Specifically, CBFβ binds to the RD of Runx proteins. As such, Runx3 isoforms with truncated RD regions bind unstably to CBFβ, and likewise, different CBFβ isoforms bind to the RD with different stabilities [11, 12, 18]. It is important to note, however, that Runx3 proteins are able to bind to DNA as monomers while CBFβ proteins alone are unable to do so [18]. In fact, it was previously demonstrated that in zebrafish SN development, heterodimerization with CBFβ is not necessary for Runx3 transactivational function. Moreover, high levels of Runx3 proteins were able to compensate for a lack of CBFβ expression. Accordingly, these results suggest a significant but perhaps not critical role for CBFβ expression in SN development [19]. However, it is important to note that results obtained in the zebrafish model system may not necessarily translate to humans and other model systems, leaving open the possibility of Runx3 regulation via cofactor binding. In addition to the RD, other Runx3 domains interact with binding partners to regulate its activity; for example, the Runx3 transcriptional activation domain (TAD) located in the C-terminal interact with the MH1 and MH2 domains of Smad proteins to regulate transcription of target genes [20, 21]. As of yet, it is unclear whether these mechanisms play a role in proprioceptor development.

Based on the previous discussion, it is clear that there are a number of regulatory mechanisms for Runx3 expression. Considering the current gap in literature, we will specifically focus on two mechanisms of Runx3 regulation in early proprioceptor development: (1) regulation via transcription factors and (2) regulation via signalling molecules (Figure 2).

Transcription factors implicated in the regulation of Runx3 expression

Our focus on transcriptional regulation is justified by the recently proposed "broad-to-restricted" model for sensory neuron subtype specification. Under this framework, early sensory neuron progenitors are initially transcriptionally unspecialized before becoming transcriptionally specialized throughout early SN development. Indeed, many TFs, including Runx3, are initially co-expressed with other "broad-to-restricted" TFs before restricting to specific neuronal subtypes. This TF selection process is not intrinsic to the cell but rather controlled by extrinsic cues, including target-derived signals. Notably, the regulation of Runx3 in proprioceptive progenitors entails both the onset of its expression and its restriction and selective maintenance within this particular population [22].

Previous studies have demonstrated a role for transcription factor Brn3a in regulating Runx3 expression in TG [23]. Encoded by *Pou4f1*, Brn3a is a pan-neuronal POU-homeodomain transcription factor that regulates subtype specification in the DRG and TG [23, 24, 25]. Brn3a is

expressed early on and maintained throughout early proprioceptor development (Figure 1) [23, 25]. Brn3a-deficient embryos at E10.5 and E12.5 lack Runx3 expression but not TrkC expression in TG; at E13.5, these embryos lose TrkC expression and continue to lack Runx3 expression. Thus, Runx3 is implicated as a downstream target of Brn3a. Further study with ChIP-sequencing assays of E13.5 embryos revealed a conserved element -94 kb upstream of the Runx3 transcription start site (TSS) that may serve as a potential binding site for Brn3a. Notably, this region was significantly histone H3 acetylated, implying euchromatin formation at the -94 kb site [23].

Building upon the discovery of the Brn3a-binding site located at the -94 kb site in Runx3, transgenic mice studies revealed three regulatory elements—R1, R2, and R3—and their roles in promoting and repressing Runx3 expression (Figure 3). R1, R2, and R3 play slightly varying functions throughout early proprioceptor development. At E11.5, all three REs are active and confer P2-mediated Runx3 expression in TrkC+ neurons, though R1 appears to play a predominating role (Figure 3). At E12.5, R2 no longer confers Runx3 expression, whereas individual R1 and R3 deletions result in dramatic and intermediate decreases in Runx3 expression, respectively (Figure 3). R1 also functions in repressing P2-mediated Runx3 expression in TrkA+ neurons. Thus, cell-type specificity of Runx3 expression is, in part, achieved through a combination of R1-mediated promotion of Runx3 expression in TrkC+ neurons and repression of Runx3 expression in TrkA+ neurons. Consistent with prior studies, the previously identified -94 kb Brn3a binding site (BRNF-R3) colocalizes with R3, implicating a role for Brn3a in R3-mediated Runx3 expression [6, 23].

Importantly, mutation of the BRNF-R1 site exhibited a less severe phenotype than the R1-mutants. However, a major difference in phenotype was not observed when comparing BRNF-R3 mutants with R3 mutants. Therefore, it is likely that R1 activity is regulated by additional TFs. Consistent with this notion, the R1 region also contains a conserved SRY-binding site [6]. Intriguingly, SRY-box containing gene 11 (Sox11) is a transcription factor expressed in developing sensory neurons [26, 27]. During DRG sensory neuron development, Sox11 is expressed by E11.5, coinciding with the onset of Runx3 expression (Figure 1) [3, 6, 27]. Sox11 mutant mice exhibited normal expression of TrkC and other neurotrophin receptors in TG, thus uncoupling Sox11 expression from TrkC expression [27]. Considering Sox11 as a candidate regulatory molecule for Runx3, which maintains TrkC expression, this result may initially appear contradictory. However, it is possible that other SoxC group proteins compensate for Sox11 deficiency [28]. In fact, functional redundancy of Sox4 and Sox11, which are both structurally similar SoxC factors, has been previously demonstrated in retinal ganglion development, implicating a compensatory mechanism for Sox11 absence during development [29]. Additionally, as Runx3 expression and TrkC expression are cross-maintained by one another, Sox11 as a candidate regulatory molecule is consistent with the idea that Runx3 is induced by a

TrkC-independent system [3]. These circumstances suggest that Brn3a-mediated R3 activity and Brn3a-Sox11-mediated R1 activity function together to regulate Runx3 expression [6].

Signalling molecules associated with the regulation of Runx3 expression

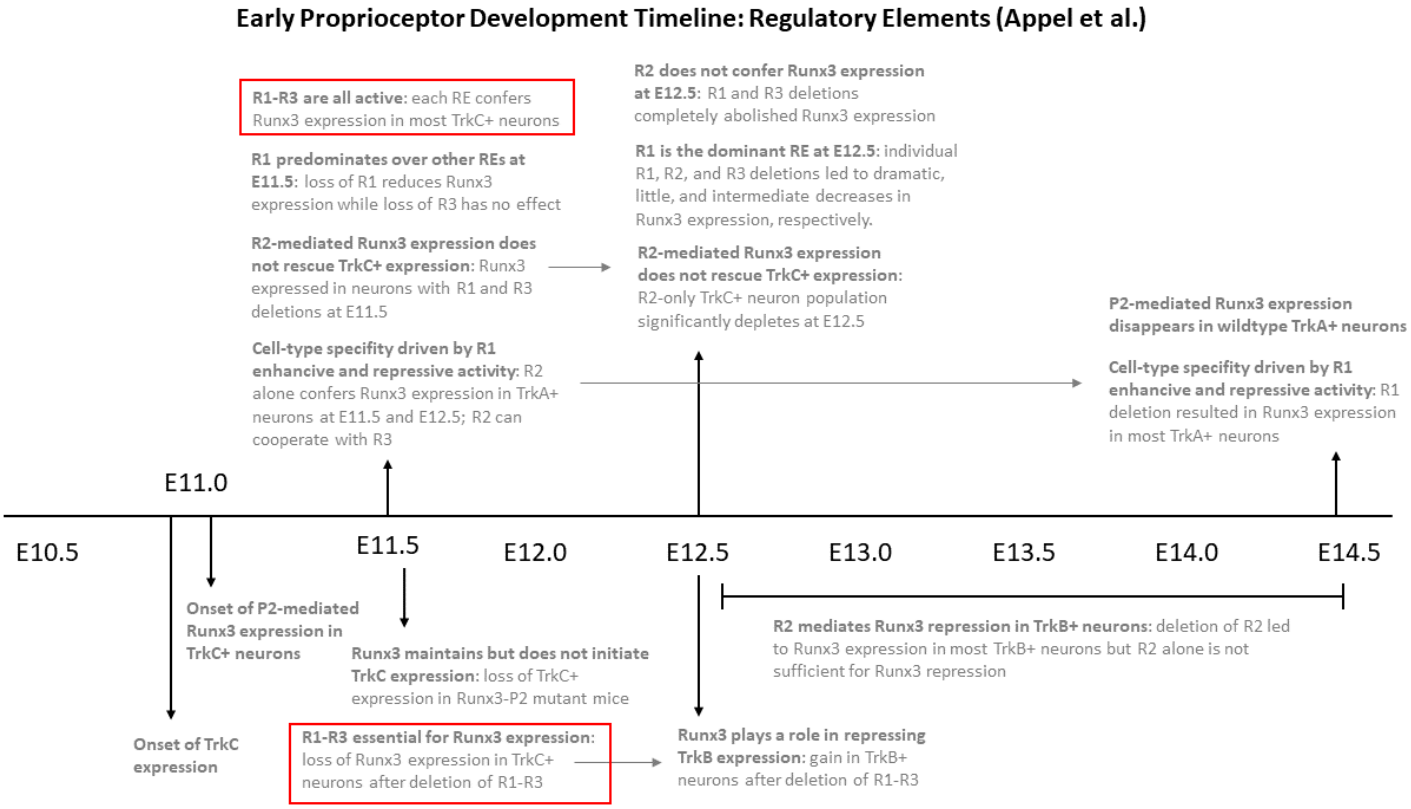


Figure 3. Regulatory elements—R1,R2, and R3—in early proprioceptor development.

Considering the role of signalling molecules in Runx3 regulation, it is important to note that Runx3 expression is independent of target-derived signals such as NT3. Chick embryos with ablated peripheral targets exhibited no change in both Runx3 mRNA and protein expression as compared to control embryos, suggesting a mechanism for Runx3 regulation independent of peripheral targets [30]. Moreover, as previously mentioned, the onset of Runx3 expression occurs around E11.0, shortly after the onset of TrkC expression [3, 6]. However, Runx3 expression is detected in TrkC mutants at E11.5, evidencing a TrkC-independent mechanism for Runx3 induction [3]. Regulation of Runx3 expression is, therefore, uncoupled from both TrkC expression and NT3 activity.

Interestingly, retinoic acid (RA) signalling has been shown to activate the transcription of Brn2, Sox1, and Sox6 [31]. RA signalling involves various molecular players including RA-synthesizing retinaldehyde dehydrogenases (RALDH1, RALDH2, and RALDH3), RA-inactivating CYP26 enzymes, and retinoic acid receptors (RARs) and retinoid X receptors

(RXRs) (Figure 4A). RA exists in the major form all-trans RA (ATRA) and isoforms 9-*cis*-RA, and 13-*cis*-RA, where ATRA is the primary ligand during development [31, 32]. Additionally, RA interacts with a variety of binding partners, including retinol-binding proteins 1 and 4 (RBP1 and RBP4) as well as cellular retinoic-acid-binding proteins 1 and 2 (CRABP1 and CRABP2) (Figure 4A) [31]; though it is important to note that the relevant binding partners in proprioceptor development have not yet to be identified. With the evidence for Brn3a and Sox11 function in SN development, it is possible that RA signalling activates one or more of these TFs to regulate Runx3 expression [23, 24, 26, 27].

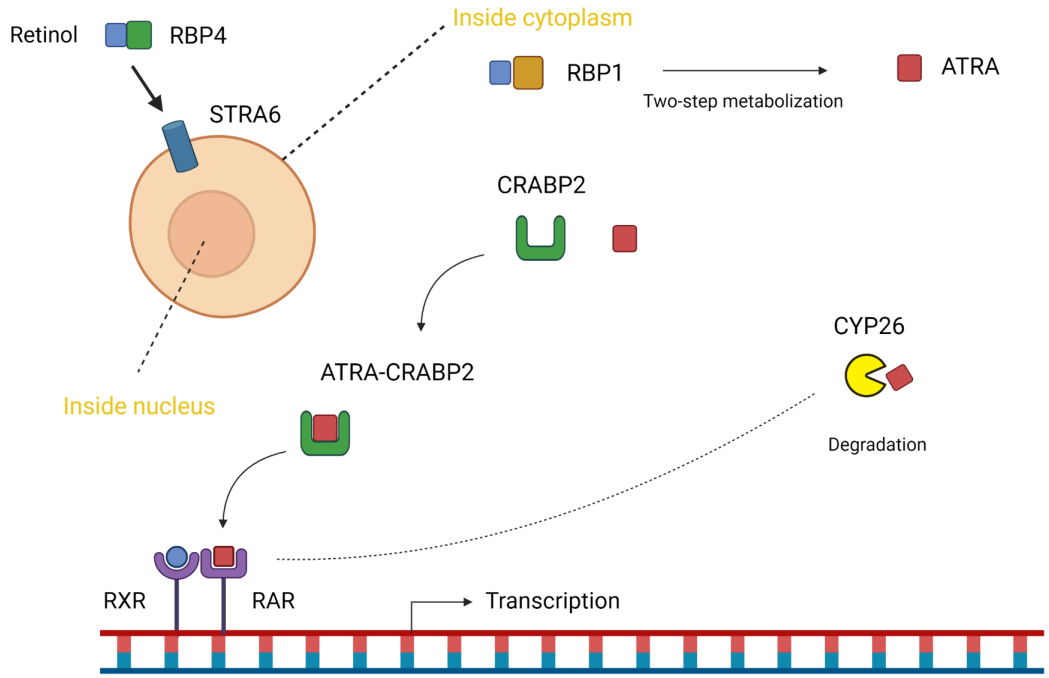


Figure 4A. Mechanisms of RA signalling.

Several lines of evidence support the involvement of RA signalling in inducing Runx3 expression during early proprioceptor development. First, RA signalling has been implicated in regulating Runx3 expression in the hematopoietic developmental system. Experiments using RAR and RXR agonist and antagonist treatment of human myeloid leukemia cell line 60 (HL-60) cells support the notion that Runx3 induction is likely regulated via RAs through RARα signalling [33]. Second, RA signalling activity has been implicated in neural differentiation. It was demonstrated that P19 cells of mice embryonal carcinoma (EC) differentiate into neurons, glia, and fibroblasts when treated with concentrations of RA greater than 5 x 10^{-8} M [34, 35]. Third, RA signalling has been shown to function specifically in proprioceptor development. During E9.5 to E10.0, before the onset of TrkC and Runx3 expression, RA-synthesizing enzyme RALDH2 is expressed (Figure 1). RALDH2 mutant mice and triple RALDH1, RALDH2, and RALDH3 knockout mice were separately shown to be unable to induce Runx3 expression in TrkC+ neurons [7]. Furthermore, consistent with previous studies demonstrating RA

dose-dependency, RA-induced Runx3 expression levels in Islet+ neurons were positively correlated with RA concentration [7, 34, 35].

Finally, RA signalling has been implicated to target Sox11. Neuro-2a (N2a) neuroblastoma cells, which serve as a model for developing neurons, displayed increases in Sox11 mRNA expression when grown in an RA-containing medium. Even more so, only N2a cultures treated simultaneously with RA and Sox11 siRNA exhibited a reduction in Sox11 mRNA expression [26]. As such, it is possible that Sox11 is a downstream target of RA signalling. Additionally, RA signalling has been previously shown to function in a dose-dependent manner whereby Runx3 expression positively correlates with RA concentration [7, 33, 34, 35]. As graded concentrations of RA have been previously shown to function in motor neuron specification in chick embryos, it is possible that RA signalling is able to play a role in sensory neuron specification via dose-dependent function [31]. Taken together, RA signalling appears to induce Runx3 expression during proprioceptor development and other developmental systems. RA signalling also appears to activate Sox11, which may cooperate with Brn3a, during neuronal differentiation.

A model for the regulation of Runx3 expression in early proprioceptor development

Based on the previous discussion, we have constructed a model for the regulation of Runx3 in early proprioceptor development involving RA signalling, Sox11, and Brn3a (Figure 4B). First, retinoids expressed in and around the vicinity of the DRG enter developing sensory neurons, prompting RA signalling (Figure 4A, 4B). Once inside the cell, retinoids are metabolized into ATRA and other RA isoforms. Presumably together with its binding partners, the RA-complex activates RARs and RXRs, which bind to and activate Sox11 transcription (Figure 4A, 4B). Once Sox11 is activated, Sox11 and Brn3a bind to their respective sites in R1 and R3, inducing P2-mediated Runx3 expression in proprioceptive progenitors (Figure 4B). Finally, accessibility to BRNF and SRY binding sites may be regulated via epigenetic modifiers, potentially helping to confer cell-type specificity of Runx3 expression in proprioceptor progenitors (Figure 4C).

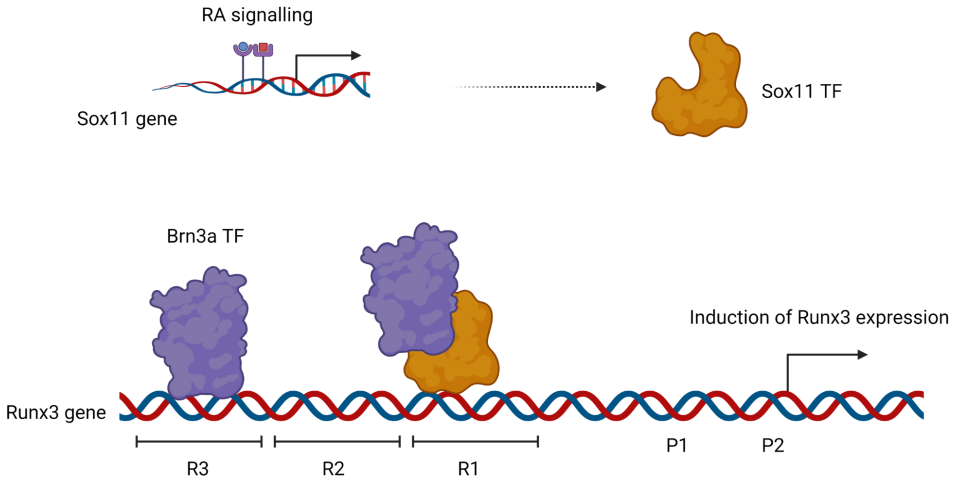


Figure 4B. Proposed model for the regulation of Runx3 expression in early proprioceptor development.

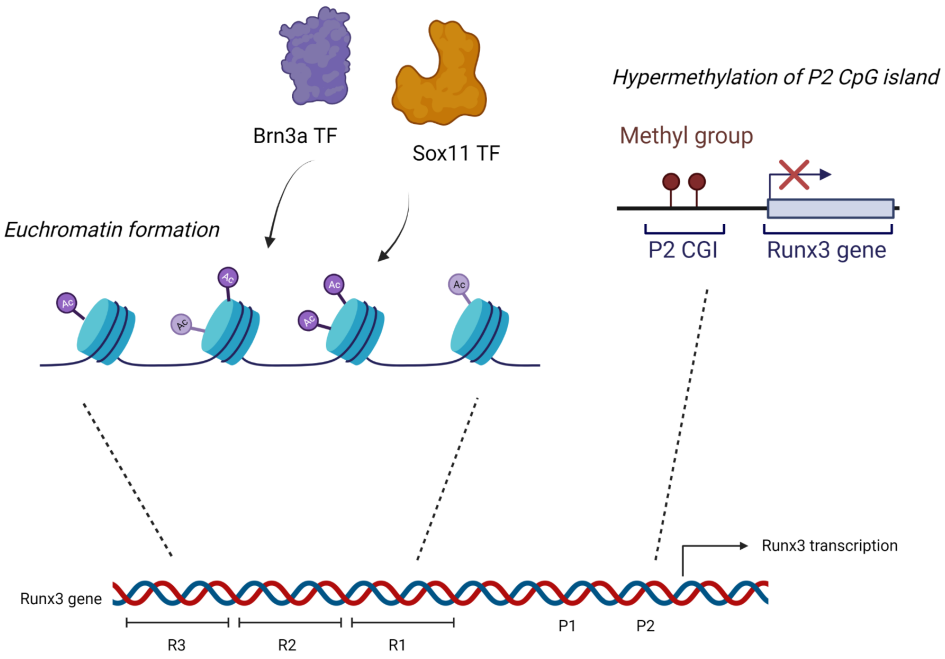


Figure 4C. Epigenetic modifications of the *Runx3* gene.

Summer II: Experimental Validation of the Developed Model

In sum, to further our understanding of Runx3 regulation in early proprioceptor development, we propose a model for Runx3 onset based on the literature study. Specifically, we hypothesize that RA signalling activates Sox11, which cooperates with Brn3a to bind to Runx3 enhancer elements, inducing Runx3 expression in the proprioceptive population. To test this idea, we propose several experiments to conduct during Summer II of the Laidlaw Scholars Program. Experiments are proposed with consideration for feasibility and efficiency, and prioritization on the potential for productive conclusions within the six-to-eight week timeline. Accordingly, two model systems were chosen: (1) embryonic mice at E10.5 and/or E11.5 for *in vivo* analysis and (2) a Runx3 GFP embryonic stem cell (ESC) reporter line for *in vitro* analysis. Embryonic mice will confirm *in vivo* expression of the molecular players of interest at the relevant developmental stages. A mouse Runx3GFP ESC reporter line serves as an *in vitro* model system for developing proprioceptors *in vivo*. Runx3GFP cultures fluoresce green in cells that express Runx3, providing a useful and quantifiable readout to help track Runx3 expression under varying conditions. Fluorescence can be viewed using a fluorescence microscope available at Columbia University Irving Medical Center.

Aim 1: Confirm in vivo expression of the relevant molecules during Runx3 induction in embryonic mice

Experimental Approach: To test the presence of Sox family TFs and RA signalling molecules at the relevant developmental stage with respect to Runx3 expression, the spatiotemporal mRNA expression of Sox family genes, RALDH genes, RAR/RXR genes, and potential binding partners will be analyzed. DRG-containing tissue from embryonic mice at E10.5, E11.0, and E11.5 will be collected and isolated. Digoxigenin-labeled probes complementary to the selected target genes will be developed. Through RNA *in situ* hybridization (ISH), target transcripts in the collected tissue samples will be probed for and visualized. After color development, a purple-blue color will indicate mRNA expression and can be observed with a light microscope. Tissue samples probed for TrkC will serve as a control group. Under the hypothesis, expression of Sox transcripts and RA signalling-related transcripts in and around the vicinity of the DRG is expected, indicating the expression of those transcripts in embryonic mice DRG at the relevant developmental stage. Experiment 1 will narrow down the relevant candidate regulatory molecules of interest; expressed molecules will be prioritized in the subsequent experiments.

Aim 2: Examine the role of RA signalling in Runx3 induction during early proprioceptor development

Experimental Approach: To test the idea that RA signalling leads to downstream induction of Runx3 expression, specifically in the context of proprioceptor development, the effects of RA signalling activation and inhibition on Runx3 expression will be analyzed. Runx3GFP ES cells will be differentiated at the relevant time just before Runx3 induction during proprioceptor development. These ES cell cultures will then be treated with various RA signalling agonists and inhibitors—such as RAR and RXR agonists and inhibitors, CYP26 enzymes, RALDH inhibitors, among others. Agonists and inhibitors will be selected based on their expression at the relevant developmental stage in embryonic mice as demonstrated by the results from Experiment 1. Runx3 expression will be observed and quantified via GFP fluorescence using a fluorescence microscope. Runx3GFP ESCs that were not treated with RA signalling agonists and inhibitors and differentiated at the same developmental stage will serve as a control group. Under the hypothesis, we expect to generally see increased GFP expression in cultures treated with RA-signalling agonists and decreased to no GFP expression in cultures treated with RA-signalling inhibitors.

Aim 3: Examine the role of Sox11 in Runx3 induction during early proprioceptor development

Experimental Approach: To test the role of Sox11 and other Sox family proteins in regulating Runx3 expression in developing proprioceptors, Runx3 expression in response to Sox gene silencing will be analyzed. Runx3GFP ES cells will be differentiated at the relevant time just before Runx3 induction during proprioceptor development. These ES cell cultures will be transfected with Sox small interfering RNAs (siRNAs) in order to silence expression of Sox11 and other Sox factors. Other Sox factors of interest will be selected based on expression at the relevant developmental stage in embryonic mice as demonstrated by the results from Experiment 1. Runx3 expression at several developmental stages before and during its onset will be observed

and quantified using GFP fluorescence. Mock-transfected ES cell cultures (ESCs transfected with the delivery agent only) will serve as a control group. Under the hypothesis, little to no Runx3 expression in the transfected cultures and normal levels of Runx3 expression in the non-transfected cultures are expected.

Aim 4: Elucidate changes in the epigenetic status of the Runx3 locus throughout early proprioceptor development

The final experiment focuses on better understanding the potential epigenetic modifications influencing the regulation of Runx3 expression. This aspect is particularly intriguing for three reasons: (1) the BRNF-R3 binding region was found to be significantly histone H3 acetylated at E13.5 [23] and (2) hypermethylation of the P2 CpG island leads to inactivation of Runx3 in gastric tumors [13] (3) in addition, HATs and HDACs are also known to interact with Runx3 to control protein stability and expression duration; as such, HATs and HDACs expression during early proprioceptor development may be potentially important for post-translational Runx3 regulation [20, 21]. Concurrent with the literature study, I have learned the basic analysis pipeline and skills necessary for studying scRNA-seq data in the bioinformatics program R Seurat; this includes obtaining publicly accessible datasets from the Gene Expression Omnibus (GEO), executing the relevant scripts and commands, defining the dimension and resolution parameters for a given scRNA-seq dataset, and data filtering through cluster filtering. I have also learned the basic principle underlying ATAC-seq and am familiarized with ATAC-seq data. As such, with this final experiment, I will apply knowledge gained during Summer I to experimentation during Summer II.

Experimental Approach: To understand the role of epigenetic modifiers and chromatin accessibility in Runx3 induction, we will use RNA-sequencing datasets and Assay for Transposase-Accessible Chromatin with Sequencing (ATAC-seq) datasets newly acquired by the De Nooij Laboratory as well as other publicly available datasets found on the GEO. First, in order to understand epigenetic modifier function in Runx3 induction, we will use RNA-seq datasets to analyze the expression of histone deacetylases (HDACs), histone acetyltransferases (HATs), and DNA methyltransferases (DNMTs) in proprioceptors at E11.0 to E13.5. Second, in order to define Runx3 chromatin accessibility throughout early proprioceptor development, we will use ATAC-seq datasets to analyze the changes in chromatin accessibility of Sox11 and Brn3a binding sites in proprioceptors at E11.0 to E13.5. Under the hypothesis, we expect to see an increase in HAT expression, as well as a potential reduction of HDAC and DNMT expression, in proprioceptive neurons during and after Runx3 induction. We also expect to see an increase in the accessibility of BRNF and SRY binding regions around Runx3 induction at E11.0.

Summary

Overall, I conducted a literature review to ultimately develop a model for Runx3 regulation in early proprioceptor development that summarizes several lines of evidence studied during

Summer I of the Laidlaw Scholars Program. I learned how to analyze scRNA-seq data as well as proposed several specific aims and experiments for application during Summer II of the program. Upon completion of this project, I presented the literature findings and proposed model from Summer I and the experimental approaches for Summer II to the De Nooij Laboratory. With this two-part project, I aim to further the current understanding of early proprioceptor development. Through the elucidation of the regulatory mechanisms of Runx3 expression in early proprioceptor development, I hope to contribute to the development of sensory neuron stem cell derivation protocols. In turn, this may have potential clinical applications for approaching ataxia and peripheral neuropathy disease modelling and treatment.

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