UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of ***Doctor of Philosophy*** *of*

IAN BELL

on Thursday, January 11th, 2024 at 1:30p.m. (SSC 2315)

Thesis Title: Loss of Nkd1 is dominant to loss of Axin2 in regulating Wnt signaling.

Examination Committee:

Dr. Jaideep Mathur, Molecular and Cellular Biology (Exam Chair)
Dr. Nina Jones, Dept. of Molecular and Cellular Biology
Dr. Terry Van Raay, Dept. of Molecular and Cellular Biology
Dr. Joseph Yankulov, Dept. of Molecular and Cellular Biology
Dr. Greg Kelly, Dept. of Physiology and Pharmacology, University of Western Ontario (External Examiner)

Advisory Committee:
Dr. Terry Van Raay (Adv)
Dr. Nina Jones
Dr. Andrew Bendall

Abstract: Canonical Wnt signaling is a crucial regulatory pathway in early embryonic development and adult stem cell maintenance and its misregulation leads to numerous diseases. Thus, understanding the regulation of this pathway becomes vitally important. The canonical Wnt pathway signals through the stabilization of β-catenin in the cytoplasm allowing for β-catenin to relocate to the nucleus to activate Wnt target genes. Axin2 and Nkd1 are widely utilized negative feedback regulators in Wnt signaling where Axin2 functions to destabilize cytoplasmic β-catenin, and Nkd1 functions to inhibit the nuclear localization of β-catenin. Here, we set out to further understand how Axin2 and Nkd1 regulate Wnt signaling by creating *axin2*$^{-/-}$ and *nkd1*$^{-/-}$ single mutants and *axin2*$^{-/-}$;*nkd1*$^{-/-}$ double mutant zebrafish using sgRNA/Cas9. All three Wnt regulator mutants were viable and had impaired heart looping, neuromast migration defects, and behavioral abnormalities in common, but there were no signs of synergy in the *axin2*$^{-/-}$;*nkd1*$^{-/-}$ double mutants. Further, Wnt target gene expression by qRT-PCR and RNA-seq analysis, and protein expression by mass spectrometry demonstrated that the *axin2*$^{-/-}$;*nkd1*$^{-/-}$ double mutant resembled the *nkd1*$^{-/-}$ phenotype, suggesting that loss of Nkd1 is dominant over the loss of Axin2. In support of this, the data further demonstrates that Axin2 uniquely alters the properties of β-catenin-dependent transcription, having novel readouts of Wnt activity compared to *nkd1*$^{-/-}$ or the *axin2*$^{-/-}$;*nkd1*$^{-/-}$ double mutant. We also tested the sensitivity of the Wnt regulator mutants to exacerbated Wnt signaling, where the single mutants displayed characteristic heightened Wnt sensitivity, resulting in an eyeless phenotype. Surprisingly, this phenotype was rescued in the double mutant, where we speculate that cross-talk between Wnt/β-catenin and Wnt/Planar cell polarity pathways could lead to altered Wnt signaling in

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some scenarios. Collectively, the data emphasizes both the commonality and the complexity in the feedback regulation of Wnt signaling.

Curriculum Vitae: Ian obtained his Bachelor of Science in Molecular Biology and Genetics at the University of Guelph in 2019. In the fall of 2019, he entered directly into the Ph.D. program under the supervision of Dr. Van Raay.

Publications: Bell I, Khan H, Stutt N, Horn M, Hydzik H, Lum W, Rea V, Clapham E, Hoeg L, and Van Raay T. Loss of Nkd1 is dominant over loss of Axin2 in regulating Wnt signaling. Manuscript submitted for peer review.

Bell I, Horn H, Van Raay T, Bridging the gap between non-canonical and canonical Wnt signaling through Vangl2. 2022. Semin Cell Dev Biol.

Rea V, Bell I, Ball T, Van Raay T. Gut-derived metabolites influence neurodevelopmental gene expression and Wnt signaling events in a germ-free zebrafish model. 2022. Microbiome.

Johnson R, Zalm J, Chen A, Bell I, Van Raay T, Al-Abdul-Wahid S, Manderville R. Unraveling the Chemosensing Mechanism by the 7-(Diethylamino)coumarin-hemicyanine Hybrid: A Ratiometric Fluorescent Probe for Hydrogen Peroxide. 2022. Anal Chem.