Name: Ryan Trevena

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Major Professor: Dr. Francisco Pelegri

Degree Objective: Ph.D. Endocrinology and Reproductive Physiology

Background: B.S. Genetics, Cell Biology and Development; University of Minnesota - TC

Current Research Project:

An oocyte has a remarkable capacity to restore the totipotency of a mammalian cell by nuclear reprogramming. Since the first instance of a mammalian clone, Dolly the sheep, there have been many efforts to harness nuclear reprogramming for reproductive and therapeutic cloning. Subsequent studies have largely focused on livestock production, conservation of endangered animals and generation of embryonic stem cells. The availability of high-quality oocytes is a central limitation to the feasibility of using somatic cell nuclear transfer (SCNT) in these efforts. Thus, an alternative method, interspecies somatic cell nuclear transfer (iSCNT), or using oocyte and somatic nuclei from two relative species to generate cytoplasmic hybrid (cybrid) embryos was established. Several examples of iSCNT have been described in the literature, but these efforts have been met with major limitations regarding proper and complete nuclear remodeling given interspecies nuclear-cytoplasmic incompatibilities. In these reports, some common cellular and molecular mechanisms implicated by the failure to produce progeny or derive functional embryonic stem cells include: activation of the zygotic genome, mito-nuclear interactions, and silencing of somatic genes. Evolutionary genetic divergence has been shown to inversely correlate with the interspecies cybrid embryo's capacity to facilitate proper nuclear reprogramming for all three of the aforementioned embryonic processes. Given this paradigm, understanding the genetic and developmental parameters that permit compatible nucleocytoplasmic interactions is essential to establish higher efficiencies in iSCNT. My current research project uses the *Danionin* family that encompasses the developmental model, zebrafish (*Danio rerio*), to study two parameters that we hypothesize contribute to nucleocytoplasmic incompatibilities in embryogenesis of interspecies cybrids: i) improper regulation of the first wave zygotic genes by maternal transcription factors to completely activate the zygotic genome and ii) loss of robustness of gene regulatory networks (GRNs) responsible for nuclear reprogramming.

Specifically, previous reports of iSCNT in mammalian species have implicated improper degradation of maternal transcripts, incomplete activation of transcription factors, and subsequent irregular gene expression as likely causes for defective development of interspecies cybrids. Despite these findings, there remains little known regarding the basis of atypical regulation of the developmental programs and subsequent zygotic gene expression. Our studies will begin to characterize the underpinning regulatory mechanisms that discern whether two species are compatible for iSCNT. Within a given phylogenetic tree, the most closely related species are expected to be compatible for successful iSCNT, but not the less related, which is the point in the phylogeny that reflects a compatibility boundary. Thus, by using zebrafish and its relative species of various phylogenetic relatedness, we can systematically generate interspecies cybrids of varying relation for this characterization. Using developmental profiling, I will define a compatibility boundary by assessing developmental competence indicative of successful or failed iSCNT. Concurrently, we will assess the transcriptomic profiles of these cybrids to identify differential gene expression of the first wave zygotic genes in incompatible cybrids that exhibit developmental anomalies, such as gastrulation defects that have been outlined in previous studies of distant taxonomical relation (i.e. bovine-pig cybrids, camel-rabbit, tiger-pig). Transcripts with changes in gene expression will be analyzed for enriched motifs to identify genes normally activated at MBT that are improperly regulated in the cybrid embryos. These motif enrichments will be used to analyze candidate maternal transcription factors whose targets are enriched in the set of misregulated genes. Taken together, this characterization will provide needed context as to the genetic and molecular basis for improper facilitation of a key developmental event, the zygotic genome activation. Furthermore, this study will expand knowledge of the role that loss of gene regulatory network robustness plays in cybrid nucleocytoplasmic incompatibilities. Using single-embryo RNAseq, we will assess how variations in transcriptional programs, due to loss in GRN robustness, leads to developmental defects in interspecies combinations beyond a compatibility boundary. This analysis, with the ZGA gene expression assessment, will provide a comprehensive understanding of the key transcriptional elements responsible for improper or incomplete nuclear reprogramming of interspecies cybrid embryos generated by iSCNT.

Publications:

Wegner, K. A., Cadena, M. T., Trevena, R., Turco, A. E., Gottschalk, A., Halberg, R. B., … Vezina, C. M. (2017). An immunohistochemical identification key for cell types in adult mouse prostatic and urethral tissue sections. *PLoS ONE*, *12*(11), e0188413.

Nair, S., Welch, E.L., Moravec, C.E., Trevena, R.L., Pelegri, F.J. (2021). Maternal *too much information/prc1-like* is required for microtubule reorganization during both cytokinesis and axis induction. Development. [In Review]

National Presentations:

Trevena RL, Pelegri FJ (2021) A Contrasting Characterization of Interspecies Hybrid Viability and Sterility among Diverged *Danionin* Species. International Zebrafish Conference. Virtual.

*Trevena RL, Pelegri FJ (2020) Assessing the Mid-blastula Transition within an Interspecies Model System across *Danionin* Species. Society of Developmental Biology. Virtual. Abstract #505

*Trevena RL, Chamberlain TC, Pelegri FJ (2019) Nuclear Reprogramming of Interspecies Somatic Cell Nuclear Transfer (isSCNT) in a *Danionin* Genus Model System. ASCB|EMBO Meeting. Washington, DC. Abstract 4488

Trevena RL, Chamberlain TC, Selvaraj M, Pelegri FJ (2018) Systematic Investigation of Interspecies Somatic Nuclear Transfer (isSCNT) Boundaries in the *Danio* Genus. International Zebrafish Conference. Madison, WI. Abstract 428

Other Presentations:

Trevena RL, Chamberlain TC, Selvaraj M, Pelegri FJ (2019) Systematic Investigation of Interspecies Somatic Nuclear Transfer (isSCNT) Boundaries in the *Danio* Genus. Endocrinology and Reproductive Physiology Spring Symposium. Madison, WI.

*Trevena RL, Chamberlain TC, Pelegri FJ (2020) Nuclear Reprogramming of Interspecies Somatic Cell Nuclear Transfer in a *Danionin* Model. Endocrinology and Reproductive Physiology Spring Symposium. Madison, WI.

Trevena RL, Nair S, Welch EL, Moravec CE, Pelegri FJ (2021) Maternal *too much information/prc1-like* (*tmi/prc1l*) is Required for Microtubule Reorganization during Axis Induction. Endocrinology and Reproductive Physiology Spring Symposium. Madison, WI.

*Denotes an Oral Presentation

Teaching and Mentorship:

Teaching Assistant:

Genetics 133 Genetics in the News, Spring 2019
OB/GYN 956 Advanced Responsible Conduct of Research, Spring 2020

Guest Lecturer:

Genetics in the News [Genetics 133]
Spring 2019
- Bacterial Genetics and the Microbiome

Developmental Genetics for Conservation [Genetics 527]
Fall 2018, Fall 2019, Fall 2020, Summer 2021
- Cloning and Synthetic Biology

Modern Research Skills in Genome Editing and Analysis [Genetics 677]
- Bioinformatic Analysis of Single Generation Cripsr-cas9 Edits in the Early Zebrafish Embryo
    - Lecture and laboratory instruction

Mentorship:

S. Isaac, Biological SIGNALS Fellow, Mentorship in gynogenetic embryo manipulation by early pressure, *In* vitro Fertilization, zebrafish husbandry, CRISPR, *In situ* hybridization. [Summer 2018]

Julia Oelke, Androgenesis of *Danionin* species cytoplasmic hybrids, Bioinformatic analyses, [Fall 2019 – Spring 2020]

Benton Veire, Danionin hybrid sperm/fertility assessments, *In* vitro Fertilization, zebrafish husbandry, Immunohistochemistry, confocal microscopy; Bumblebee/honeybee cloning [Summer 2019 – Spring 2021]

Olivia Thompson, Midboby formation and axis induction defect analyses, [Summer 2021 – Current]

ERP Service:

Recruitment 2018

Recruitment 2019

Recruitment 2020

Recruitment 2021

ERP Student Committee 2018-2021