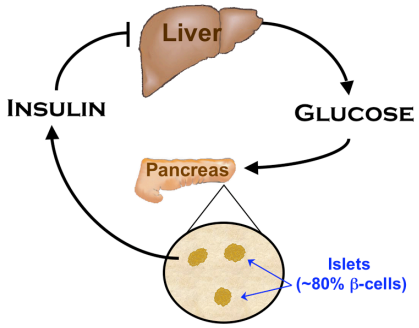
Research Plan: Transcriptional circuitry and the pancreatic beta-cell

My research goal is to understand the systems-level transcriptional mechanisms underlying mammalian cell specification using the pancreatic beta-cell as a model. To accomplish this, I will combine genome-wide transcription factor binding, gene expression perturbation using genetic manipulations, comparative genomics, and physiological approaches. Other endodermally-derived cells like hepatocytes employ many of the same master regulators, and comparison of the complex genetic architectures of these related yet distinct cell-types may help further elucidate the basic principles of their functional organization.

Background

Vertebrates are organized collections of hundreds of different cell types that maintain phenotypic identity by a combination of epigenetic programming and dynamic transcriptional regulation. Tissue-specific transcription factors are thought to contribute significantly to cellular phenotype determination in vertebrates by forming and maintaining stable regulatory networks, but the exact regulatory connections employed to define a mammalian tissue remain unknown. Establishing the contribution of master transcriptional regulators, whose disruption impairs proper cellular function, to the mechanisms of tissue specification would help answer how a multicellular organism differentially exploits one genome to create functionally diverse tissues.

One of the most highly specialized and evolutionarily conserved tissues found among mammals are pancreatic islets. Islets are largely composed (>80%) of pancreatic beta-cells, which uniquely regulate insulin release upon glucose stimulation. Pancreatic beta-cells are an excellent model for understanding how master regulators control cellular phenotype because of their exquisite sensitivity to functional disruption and their direct association with diabetes. Beta-cells are largely eliminated in Type I diabetes, and beta-cell malfunction contributes to a large fraction of Type II diabetes. A number of master regulators of beta-cell function, many originally identified because their haploinsufficiency causes beta-cell failure and diabetes, were recently discovered to function in a highly interconnected regulatory network. Underscoring the complexity of cellular genetic networks, three of these factors, HNF1α, HNF1β, and HNF4α, are central to the regulatory circuitry of the principal liver cell type, the hepatocyte—yet liver function remains unaffected by their haploinsufficiency.



My postdoctoral research found that key beta-cell master regulators can regulate and integrate multiple, diverse tissue-specific processes, such as glucose metabolism, signal transduction, insulin secretion, and miRNA transcription through promoter binding. Building on these discoveries, I have selected a set of transcription factors for investigation based on their well-known roles in beta-cell function (Table). Many of these factors are also required for proper development of the endocrine pancreas, and also regulate transcription in hepatocytes. Hepatocytes are ideal for comparative studies, as they perform highly conserved physiological roles that differ from islets, yet employ many of the same master regulators.

Table. Transcriptional regulators of β-cell function

Regulator	*Protein class*	*KO phenotype*
Ipf1	POU-Homeodomain	No pancreas, diabetes
Hnf4α	Nuclear receptor	Embryonic lethal
Hnf1α	POU-homeodomain	Impaired beta-cell function, insulin secretion
Hnf1β	POU-homeodomain	Impaired beta-cell function, insulin secretion
NeuroD1	Helix-loop-helix	Decreased islet cells, diabetes
Foxa2	Winged helix	Embryonic lethal
CREB	Leucine zipper	Poor islet proliferation
Nkx2.2	NK-homeodomain	Impaired beta-cell development, diabetes

Mapping potential regulatory circuitry

An unbiased, steady-state map of potential regulatory pathways will be constructed by mapping the promoters bound in vivo by transcriptional regulators known to be key to beta-cell function in both mouse and human tissues. To map these bound regions, I am employing chromatin immunoprecipitation of the regulators listed above with microarrays representing the transcriptionally active portion of the human genome to identify a comprehensive collection of promoters occupied in primary human islets. These bound promoters are a combination of active, direct regulatory interactions and potential regulatory events (e.g. regions activated under alternate environmental stimuli). The subsequent map of the beta-cell circuitry can reveal unexpected regulatory linkages. For instance, I recently found that HNF regulators co-bind a disproportionate number of other transcriptional regulators, most of which had not previously been suggested to be downstream of the HNF factors (Figure 1). Similar DNA binding results for IPF1 and NeuroD1 in human pancreatic islets have recently expanded the previously mapped regulatory networks of the HNF factors, and revealed a number of novel transcriptional programs potentially controlled by specific combinations of master regulators.

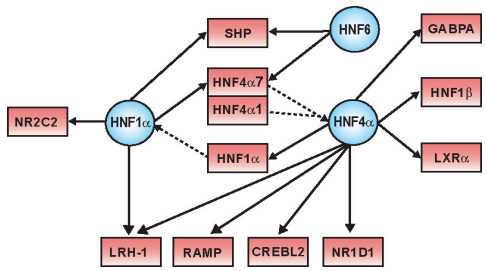


Figure 1. Portion of the potential transcriptional regulatory network in human pancreatic islets (Odom *et al.* 2004). Blue circles represent the transcription factor proteins, red rectangles represent promoter regions.

In addition, similar approaches can be used to identify the miRNAs bound by and thus potentially downstream of beta-cell master regulators. miRNAs are a recently discovered class of endogenous small RNAs that repress partially complementary mRNAs; mRNAs targeted by miRNAs often code for transcriptional regulators. The regulatory relationship between beta-cell transcription factors and miRNAs remains an unexplored level of genome-wide control. We have shown recently that miRNA loci are regulated in a tissue-specific manner by master regulators similarly to genes, and that ablation of master regulators that occupy a particular miRNA locus has functional consequences for the downstream miRNA.

Perturbing regulatory circuitry—gene expression effects

The dynamic response of the beta-cell regulatory circuitry mapped above will be determined by chronic and acute perturbation of the beta-cell regulatory network. Mice with either germline or conditional knockouts of many of the factors above are available (e.g. Foxa2, Hnf1α, Hnf4α, Nkx2.2). Profiling the gene expression changes that exist between wild-type mice and mice chronically lacking a key beta-cell factor reveals the portions of a genetic program which cannot be established in the absence of that factor. A complementary technique is to use lentivirus-drive RNAi to ablate a transcriptional regulator in MIN6 insulinoma cells or isolated islets. The resultant gene expression changes reveal the acute, short-term effects of removal of a transcription factor. Comparison of these expression sets can allow the regulatory and developmental redundancies to be established. For instance, genes that alter their expression regardless of how the regulator is removed are directly dependent on the factor, and comparison with the binding data will allow direct targets to be determined. In contrast, those genes uniquely changing upon RNAi-driven ablation may be direct targets where long-term compensation exists from other, functionally redundant transcription factors. Gene expression changes which occur over longer developmental time, but not in acute RNAi ablation, could reflect accumulation of more subtle network perturbations. Thus, analysis of a single factor's effect on gene expression in a chronic versus acute knock-down can annotate the potential regulatory connections determined in the differentiated tissue.

Because many beta-cell transcription factors operate in highly redundant networks, and removal of single factors can on occasion have limited functional effect, my laboratory will determine the

overlapping roles played by individual members using a fractional factorial design to remove transcriptional regulators systematically. These combinatorial deletion experiments are readily performed in the genetically tractable mouse MIN6 insulinoma cell line using lentivirus-driven RNAi. Recent results have suggested that separate lentiviruses against up to three regulators can be transfected into MIN6 cells. Simultaneous removal of redundant factors will have disproportionately strong effects on genes that are co-regulated; thus, this process could identify combinatorial requirements for tissue-specific gene expression on a global basis. The gene expression dependencies determined from the MIN6 insulinoma line will be confirmed using pancreatic islets.

Perturbing regulatory circuitry—physiological effects

The physiological effects of loss of transcriptional regulators on beta-cell function will be investigated using two approaches. First, in combination with MIN6 or pancreatic islets which have been perturbed using lentivirus approaches above, I will employ standard protocols to monitor insulin biosynthesis and secretion in response to glucose and other secretagogues, as well as rates of proliferation in the case of MIN6 cells. These studies, complementary to gene expression studies, will reveal how changes in regulatory architecture affect relevant physiological features of the beta-cell.

To determine how combinations of regulators stably interact in tissue-specific gene expression programs, I will compare the network information from the pancreatic beta-cell to identical data from hepatocytes. Hepatocytes use many of the same transcriptional regulators as the beta-cell in somewhat different combinations to execute distinct biological functions. Thus, comparison of these two network architectures will allow the comparison of two steady state equilibria, as opposed to genetic or biochemical perturbation-driven effects. Through this combination of complementary experimental approaches, my laboratory will elucidate how a highly conserved vertebrate cell type uses control of complex, redundant transcriptional networks to execute a single function—the biosynthesis and secretion of insulin.

Teaching Proposal

One of my principal goals in teaching is to convey scientific concepts in a manner that is exciting and provocative as well as intellectually rigorous. In addition, I am interested in reaching out to scientifically curious non-scientists. As we have seen from the recent media coverage of the stem cell debates over the last four years, even otherwise well-educated citizens frequently have a poor understanding (occasionally bordering on dangerous) of the concepts, promise, and limitations of biology and biotechnology. Here, I outline in course-catalog format two courses I would like to develop as a faculty member:

Classical and Genomics Approaches to Transcription

In this advanced seminar series, we will discuss technologies employed today to investigate how eukaryotes orchestrate transcription by discussion of primary literature to illustrate methodology. The initial classes will demonstrate how classical techniques ranging from gel mobility assays to transient transfections can be used to investigate transcription. Sequencing the genome of many different higher vertebrates has provided unprecedented data to begin using comparative genomics to identify non-coding sites that may represent regulatory regions. Bioinformatics techniques that infer regulatory importance based on these conserved genomic sequences will be explored. Whole genome transcript analysis and chromatin immunoprecipitation on genomic arrays offer a powerful complementary set of tools that permit the actual outputs and inputs of these programs to be determined. Finally, recent computational approaches and algorithms used to fuse all these datasources into dynamic and predictive models will be discussed and criticized. This course is intended for graduate students with moderate to significant research experience.

A Lasting Revolution: The Advent, Promise, and Limitations of Modern Biology

In this overview course, the extraordinary revolutions that have occurred in the human understanding of living systems in the last sixty years will be covered. Much of the focus will be on understanding the impact that the technical and conceptual developments in biology have had on society. All subjects in this class will be treated with scientific rigor; famous and occasionally notorious stories in biological research will be presented to vividly demonstrate the nature of scientific inquiry. Subjects to be covered will focus on those with high impact on public policy, and include: antibiotics, imaging technologies, genetics and genomics, invention and exploitation of crop monoculturing, stem cells, nucleus transfer technology (cloning), and emerging diseases and bioterrorism. Class participation and presentations are expected of all participants.