Determination of regulation by the transcription factor PU.1 in differentiation of hematopoietic stem cells

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Background: The differentiation of hematopoietic stem cells into specific cell lineages is determined by the binding patterns of transcription factors whose expression is not limited to a single cell lineage[1]. The comparison of binding patterns for transcription factors has yielded the observations that within the same cell type transcription factors tend to colocalize on a genome-wide scale, and that in different cell types, or at different developmental stages, the same factor can express different genome-binding patterns[2]. The transcription factor PU.1 is required for the regulation of differentiation of hematopoietic stem cells into specific cell lineages – the level and pattern of expression of PU.1 specifies fate and developmental phases[1]. Although PU.1 is naturally high in earliest T-cell precursors, PU.1 can repress T-cell specific genes in these cells and T-cell precursors then repress PU.1 during the commitment phase of T-cell specification[3,4,5]. The basis of the regulatory roles of PU.1 have not yet been fully defined – PU.1 has been thought to have a primarily positive regulatory role, but it is evident PU.1 has a negative regulatory function in the specification of hematopoietic stem cells into T-cells.

Specific Aims: (1) Determine the basis of the negative regulatory roles of PU.1. **(2)**Identify and confirm genes involved in T-cell development negatively regulated by PU.1.

Motivation: I became interested in the role PU.1 plays in regulation of the development of T-cells during my rotation in Dr. Rothenberg's lab at California Institute of Technology. The Rothenberg lab has been a major contributor in the study of the specification of hematopoietic stem cells into T-cells and in defining the regulatory roles of PU.1 and has a large ChIP-Seq data set for PU.1 binding sites submitted for publication. My rotation project was to work with Ameya Champhekar in identifying the *cis*-regulatory modules of PU.1 binding genomic regions in early T-cells. I did this by cloning genes of interest with conserved PU.1 binding sites and then testing whether PU.1 regulates the gene in B-cell, T-cell, and Myeloid cell lines using a luciferase assay to detect activity. If PU.1 regulated the genes, I mutated the PU.1 binding site in the constructs to determine whether binding directly to the gene is required for regulation. I became particularly interested in the negative regulatory role of PU.1 when examining the ChIP-Seq and RNA-Seq data for the genes I was cloning – I began to wonder how PU.1, as well as other transcription factors that are present at multiple decision points in the cell lineage specification pathway, may play multiple roles to create a robust cell lineage pathway. The fact that PU.1 represses T-cell genes early during differentiation of hematopoietic stem cells and then the T-cell genes repress PU.1 during commitment to the T-cell lineage suggests that PU.1 plays an important negative regulatory role during T-cell development. It has been shown that PU.1 has a positive regulatory role, so I wanted to look into the negative regulatory role that would increase the impact of PU.1 in the determination of the cell fate of hematopoietic stem cells. The Rothenberg Lab has the experience and means to complete the experiments and analysis necessary for this project.

Proposal: To determine negative regulatory role of PU.1, I would use bioinformatics to compile a list of potential genes negatively regulated by PU.1 and molecular biology to confirm whether the genes are negatively regulated. The methods I would use to determine if PU.1 has a negative regulatory role have been used in my previous bioinformatics research projects involving RNA-Seq and ChIP-Seq data and in my rotation project in the Rothenberg lab. I would begin by examining ChIP-Seq and RNA-Seq data to identify PU.1 binding sites where histone methylation marks occur. Histone methylation marks for genes positively regulated by PU.1 in

the fate determination of hematopoietic stem cells have been identified, and so I would be looking for genes where different histone methylation marks or recruitment of repression complexes occur. After creating a list of genes with potential negatively regulated PU.1 binding sites, I would look for any conserved regions of the PU.1 binding sites that may act as the negative *cis*-regulatory modules for PU.1.

Once I had a list of target genes, I would then proceed by cloning the genes of interest into P2C2 cells that express PU.1. To determine if PU.1 acts as a repressor, I would insert my cloned target genes into two sets of P2C2 cells – one set that expresses PU.1 as an obligate repressor, or direct dominant negative, construct – a PU.1 fusion with the Drosophila Engrailed repression domain, and a set that expresses wildtype PU.1. A subset of target genes with PU.1 binding sites that are more likely to be enriched for potential negative regulatory elements could then be identified by which genes have the same response to wildtype PU.1 and the obligate repressor construct of PU.1. The subset of genes with negative regulatory elements could also be identified using a dominant activator, such as a PU.1 fusion with the VP16 domain of a viral transactivator, or a PU.1 fusion to p300 as an obligate enhancer element activator. By using both the dominant activator technique and obligate repressor technique, I can more thoroughly identify the subset of target genes that are the most likely to contain negative regulatory elements because they would have the same response to the wildtype and obligate repressor, but different responses to dominant activator versions of PU.1.

To further establish that the genes of interest are in fact negatively regulated, I would show that removal of wildtype PU.1 increases the genes' expression. For each target gene, I would transfect the gene's PU.1 target regulatory sequence into a cell type that is not PU.1-dependent for survival. Then, I would measure the gene's expression level to determine if it increases in the absence of PU.1; if this is the case then the gene would be shown to be negatively regulated by PU.1. Over the course of the experiment I would expect to see the list of target genes dramatically decrease – there are a large number of PU.1 binding sites for genes that are expressed during the differentiation of hematopoietic stem cells and a large number of those genes are potentially positively regulated by PU.1; however by refining the list of potentially negatively regulated genes we can also confirm the list of positively regulated genes. A histone mark for repression by PU.1 could then potentially be identified when comparing the ChIP-Seq data for the list of genes that are shown to have negative regulation by PU.1.

Broader Impacts: The proposed project would change how students are taught about how transcription factors' regulatory roles affect cell specification and development by defining the regulatory role of PU.1 and increasing the knowledge of the regulatory network for the differentiation pathway of hematopoietic stem cells. The integration of bioinformatics and molecular biology techniques in the project would modernize how researchers identify and confirm regulatory basises of transcription factors and would produce data sets for use by other experimental scientists. The project would also present the opportunity to have high school students and undergraduates participate in research. Upon completion of this project I would publish and present my results to the scientific community at conferences in order to share the knowledge gained.

References: **(1)** Iwasaki H et al., *Immunity*, 2007, 26:726-740. **(2)** Heinz S et al., *Molecular Cell*, 2010, 38:576-589. **(3)** Franco CB et al., *PNAS*, 2006, 103(32):11993-11998. **(4)** Anderson MK et al., *Immunity*, 2002, 16:285-296. **(5)**Yui MA et al., *The Journal of Immunology*, 2010, 185:284-293.