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"Identifying Genes that Regulate Organogenesis: A Tol2-Based Gal4VP16 Gene-Trap Screen in Zebrafish"

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Background: Organogenesis is the tightly orchestrated process by which embryonic primordia develop into fully-differentiated and functional organs. In the context of the adult organ, genes which play a role in organogenesis and embryo patterning are utilized for such vital homeostatic processes as tissue maintenance, renewal and regeneration after injury; their function is altered in many human disease states ranging from genetic syndromes to cancer. The zebrafish (*Danio rerio*) is an ideal model vertebrate organism in which to study organogenesis: the developing zebrafish is transparent, allowing one to visualize developing organs; many zebrafish organs exhibit striking histologic similarity to human tissues; and zebrafish are small enough to be used in forward genetic screens. A gene-trap screen is a method for identifying genes which are expressed in a tissue restricted and a temporally restricted manner during development. We have designed a novel Gal4VP16-based gene-trap screen in zebrafish to accomplish the following aims: (1) identify genes which are expressed in developing organs; (2) to study the function of those genes when expression is reduced or lost; and (3) to enable the expression of transgenes in those organs under the regulation of Gal4VP16.

Design: The Tol2 transposon has been engineered to carry a Gal4-based gene-trap construct (figure 1). Gal4VP16 is an engineered transcription factor which binds to specific DNA sequences (termed UAS) and activates the expression of downstream genes. This gene-trap plasmid construct is injected with Tol2 transposase mRNA into single-cell zebrafish embryos. The transposase enzyme catalyzes the transposition of the vector from the donor plasmid to random sites throughout the genome. When the construct lands in an expressed region of the genome, Gal4VP16 is expressed under the control of the trapped gene's regulatory elements as a fusion protein with the expressed sequences. The Gal4VP16 fusion protein binds to the UAS element present within the vector and activates GFP expression. The gene trap vector is potentially mutagenic because it disrupts exon splicing and open reading frames. Because GFP is driven by Gal4VP16 expression, lines derived in this manner can be used to express any transgene in the GFP positive domains.

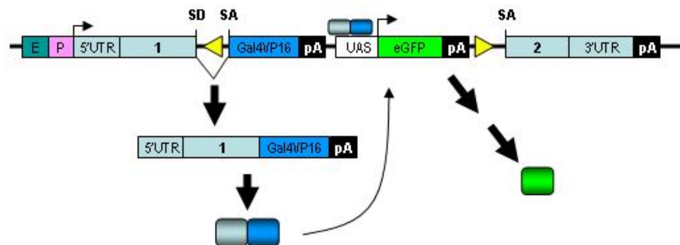

Figure 1. The gene trap vector is randomly distributed throughout the genome. If the vector lands in a coding sequence, transcription of the "trapped" gene results in splicing to the vector's splice acceptor site and the production of a Gal4VP16 fusion protein (if the reading frame is the same). Gal4VP16 mediates expression of GFP, and identifies the trapped gene's expression pattern.

Methods: Wild-type single cell-stage embryos were injected with the Gal4VP16;UAS:GFP construct and transposase mRNA and reaised to sexual maturity. Germline mosaic injected fish were incrossed to obtain F1 embryos which are screened for GFP expression. Stable lines were generated by outcrossing F1 adults showing

distinct, tissue restricted expression patterns to wild-type adults and raising the GFP+ F2 embryos. To detect the ability of Gal4VP16 to transactivate expression of additional transgenes, plasmid constructs encoding UAS:mCherry were injected into F2 single-cell stage embryos. Genomic DNA sequences flanking the gene-trap vector were amplified by linker-mediated PCR. PCR products from GFP+ embryos were compared to PCR products obtained from GFP- clutchmates by agarose gel electropheresis to identify PCR products likely to be associated with the GFP expressing allele. The PCR products were cloned and sequenced. Flanking sequences were compared to genomic sequence data obtained through the Danio rerio Sequencing Project at the Sanger Center using various genomic search tools.

Results: Twenty-seven distinct GFP expression patterns have been recovered as stable F2 lines from the initially injected F0 founders. GFP expression is observed in the CNS, spinal cord, muscle, pancreas, heart, hematopoietic cells, and other developing tissues. Gal4VP16 activity has been demonstrated in the GFP+ tissues for several lines by injecting UAS:mCherry transgene into single cell stage embryos. mCherry fluorescence is observed in the same tissues in which GFP fluorescence is observed. The insertion sites of two lines have been mapped thus far.

Conclusions: Our gene-trap approach has been utilized to identify regions of the genome harboring genes which are expressed during organogenesis. Trapped genes drive expression of Gal4VP16 in tissue restricted patterns resulting in GFP fluorescence. Gal4VP16 expressed in those tissues transactivates expression of UAS:mCherry from an injected plasmid specifically in the GFP+ tissues. A putative gene-trap has been cloned in one line expressing GFP in the pineal and lens. Several lines containing UAS-regulated transgenes (constitutively active beta-catenin, Notch intracellular domain, mCherry, nitroreductase) are being raised and crossed with the Gal4VP16 lines to investigate the function of transgene expression in those tissues.