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Large-scale Mutagenesis in Zebrafish (Retrovirus, Transposon, and ENU)

Cheol-Hee Kim

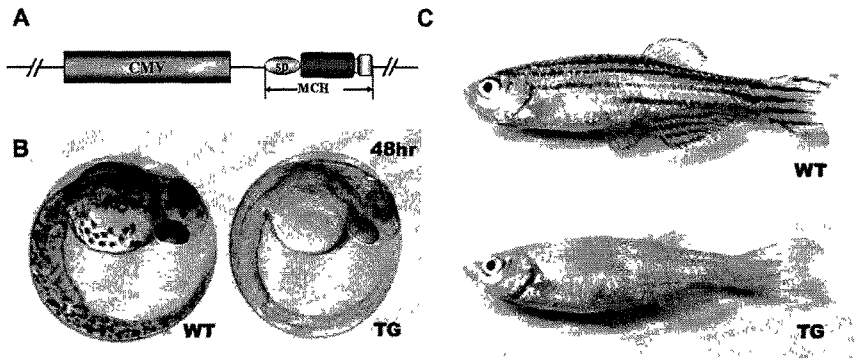
School of Bioscience and Biotechnology, Chungnam National University

Due to its small size, short generation time, and the external development and optical clarity of its embryos, the zebrafish (*Danio rerio*) has been the vertebrate most amenable to forward genetics. Large scale screens using ethylnitrosourea (ENU) as a chemical mutagen have yielded mutations in hundreds of genes, affecting every aspect of development. However, the cloning of chemically mutated genes is quite laborious. Insertional mutagenesis provides an alternative whereby the identification of the mutated gene is greatly facilitated by the presence of a molecular tag at the site of the mutagenic lesion. Specifically, by using the retroviral vector, the sequencing of up to a few kilobases of DNA flanking the insertion has been sufficient to identify the mutated gene. Moloney murine leukemia virus vectors are able to infect zebrafish cells due to the substitution of the vesicular stomatitis virus glycoprotein (VSV-G) for the envelope protein. VSV-G is thought to bind to a common membrane phospholipid, explaining the broad host range of VSV. Retroviral vectors are ideal mutagens as they are thought to integrate roughly randomly throughout the genome and integrate cleanly. Over 500 insertional mutations have been recovered in a large-scale screen in the Nancy Hopkins lab, and the mutated genes have been identified for over 280 loci (1).

Members of the Tc1/mariner superfamily of transposable elements isolated from vertebrate species are inactive due to the accumulation of mutations. A representative of a subfamily of fish elements estimated to be last active > 10 million years ago has been reconstructed, and named *Sleeping Beauty* (SB). Multiple transposase binding sites within the terminal inverted repeats, a transpositional enhancer sequence, unequal affinity of the transposase to the binding sites and the activity of the cellular HMGB1 protein all contribute to a highly regulated assembly of SB synaptic complexes, which is likely a requirement for the subsequent catalytic steps. SB catalyzes efficient cut-and-paste transposition in a wide range of vertebrate cells in tissue culture, and in somatic tissues as well as the germline of the zebrafish in vivo, indicating its usefulness as a vector for transgenesis and insertional mutagenesis (2). The *Tol2* element is a member of the hAT (hobo/Activator/ Tam3) transposable element family. About 20 copies are present in the medaka fish genome and, unlike many other hAT

family elements, virtually all the copies are autonomous or potentially autonomous, containing an intact transposase gene. In more than half of excision events, breakage and rejoining of DNA molecules occur within the 8-bp target site duplication region, removing the entire Tol2 sequence and retaining parts of the target site duplications. In the reminder of the excision events, either the left or the right terminal region is left and the other end is lost together with its flanking region. Insertion of Tol2 occurs without detectable preference for target sequences and creates a target site duplication of exactly 8 bp. A gene transfer vector using Tol2 has already been established in zebrafish. Foreign DNA fragements inserted in Tol2 can be efficiently delivered to the chromosomes by transposition (3).

Currently, in my lab we are trying to establish an insertional mutagenesis system equip with a new transgene reporter, MCH. Melanin-concentrating hormone (MCH) is a cyclic neuropeptide synthesized as a preprohormone in the hypothalamus of all vertebrates. This neuropeptide binds to G-protein-coupled seven transmembrane receptor(s) to mediate its function. MCH was named after its function in fish, in which it causes aggregation or concentration of melanin granules in melanophores, thus regulating body color. The function of central MCH that has attracted most attention is its involvement in regulating food intake and energy homeostasis in mammals (4). The following figure shows an example of screening transgenic zebrafish based on body color change.



Finally, I will talk about the positional cloning of two ENU mutations; *headless* and *mind bomb*. The vertebrate organizer can induce a complete body axis when transplanted to the ventral side of a host embryo by virtue of its distinct head and trunk inducing properties. Wingless/Wnt antagonists secreted by the organizer have been identified as head inducers. Their ectopic expression can promote head formation, whereas ectopic activation of Wnt signalling during early gastrulation blocks head formation. These observations suggest that the ability of head inducers to inhibit Wnt signalling during formation of anterior structures is what distinguishes them from trunk inducers that permit the operation of posteriorizing Wnt signals. I will describe the zebrafish *headless* (*hdl*) mutant and show that its severe head defects are due to a mutation in T-cell factor-3 (Tcf3), a member of the Tcf/Lef

family. Loss of Tcf3 function in the *hdl* mutant reveals that *hdl* represses Wnt target genes. I provide genetic evidence that a component of the Wnt signalling pathway is essential in vertebrate head formation and patterning (5). Lateral inhibition, mediated by Notch signaling, leads to the selection of cells that are permitted to become neurons within domains defined by proneural gene expression. Reduced lateral inhibition in zebrafish *mind bomb* (*mib*) mutant embryos permits too many neural progenitors to differentiate as neurons. Positional cloning of *mib* revealed that it is a gene in the Notch pathway that encodes a RING ubiquitin ligase. Mib interacts with the intracellular domain of Delta to promote its ubiquitylation and internalization. Cell transplantation studies suggest that *mib* function is essential in the signaling cell for efficient activation of Notch in neighboring cells. These observations support a model for Notch activation where the Delta-Notch interaction is followed by endocytosis of Delta and transendocytosis of the Notch extracellular domain by the signaling cell. This facilitates intramembranous cleavage of the remaining Notch receptor, release of the Notch intracellular fragment, and activation of target genes in neighboring cells (6).

References

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