UNIVERSITY of HOUSTON
BIOLOGY AND BIOCHEMISTRY

Characterization of Conserved Non-Coding Elements essential for *wnt1* expression in zebrafish

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Why should we care about Wnt1 regulation?

- Wnt1 is critical for brain development and bone physiology.
- Its expression pattern is conserved in vertebrates, suggesting an ancient and important regulatory mechanism.
- Wnt1 is expressed in a part of the early brain called mes/r1, then localizes to the midbrain and midbrain-hindbrain boundary (MHB)
- Mutations in pathways that regulate Wnt1 could result in developmental abnormalities in humans, and knowing if this is true requires a complete understanding of Wnt1 regulation.

Background

- Wnt1 CNE (conserved noncoding element) regions were amplified from zebrafish and spotted gar genomes and ligated with the *cfos* promoter and EGFP reporter. Tol2 sites allow for transposable insertion into zebrafish genome.
- Zebrafish CNE20 and CNE27 revealed different levels and patterns of expression, yet localized in patterns similar to *wnt1* (midbrain and midbrain-hindbrain boundary)
- Similarly, spotted gar CNE20 and CNE27 maintained expression in midbrain/MHB regions but at varying levels when compared to its zebrafish orthologs

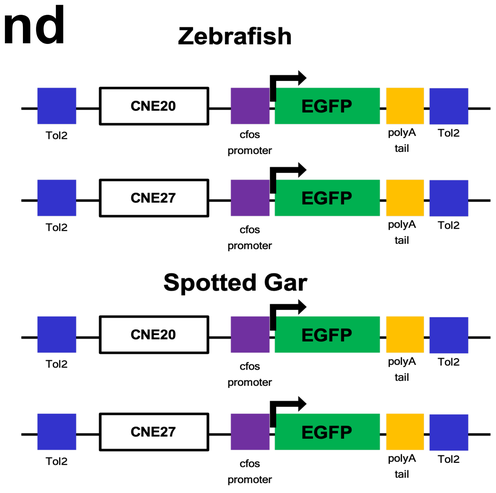

Figure 2. Genetic insert of the different transgenic lines used to characterize the EGFP transcripts. Note all inserts are analyzed in zebrafish.

Goals

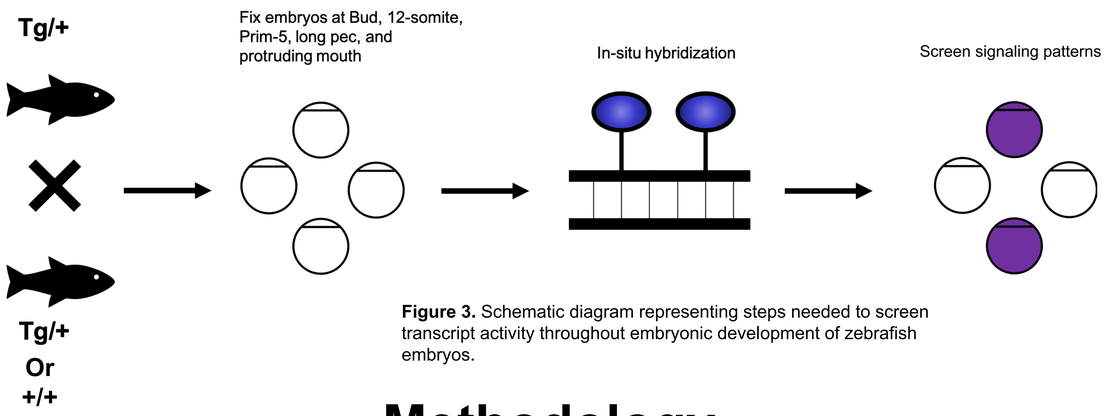

Figure 3. Schematic diagram representing steps needed to screen transcript activity throughout embryonic development of zebrafish embryos.

Methodology

In-situ Hybridization

1. Probe embryos with labeled complementary RNA probe
2. Introduce antibody that binds to probe label
3. React with stain solution to yield a signal
4. Observe and image staining pattern

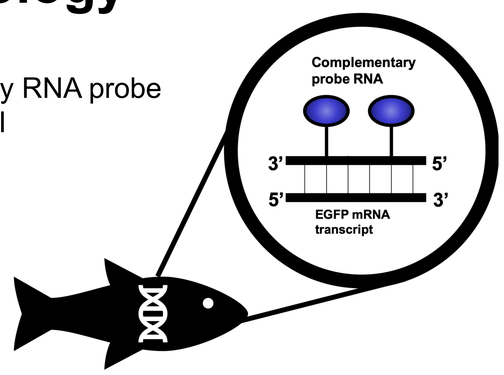

Figure 4. In-situ hybridization visualization. Probe enters cell membrane and binds to complementary EGPF mRNA transcripts. Introduced antibody then binds to DIG antigenic sites. Reaction with NB stain buffer creates a signal with DIG antibody-mRNA complex.

Results

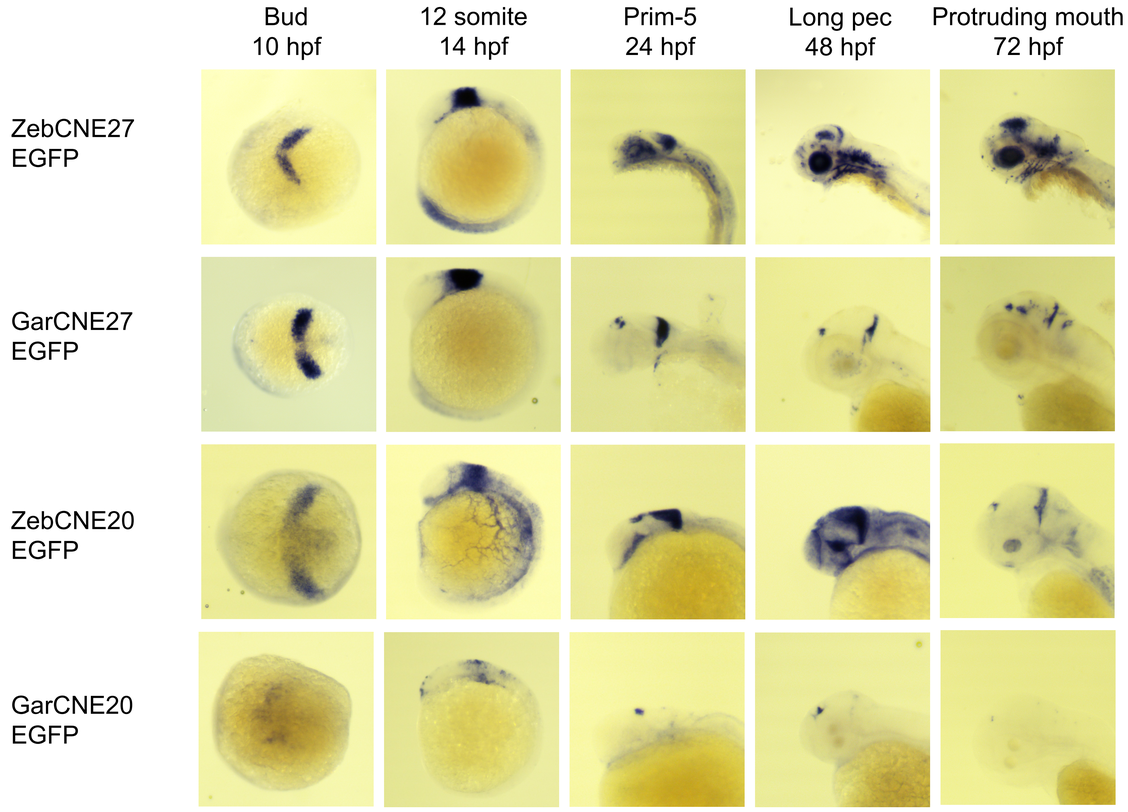

Figure 5. EGFP reporter expression driven by zebrafish or spotted gar CNE elements in transgenic zebrafish. Stage is indicated above each column. Blue staining identifies cells transcribing the reporter gene.

Conclusions

- GarCNE27 activity is more similar to ZebCNE20 than to ZebCNE27. This could indicate similar functions even though they are not respective orthologs
- GarCNE20 ortholog does not appear as prominent as its ortholog in zebrafish
- Expression patterns remain consistent with known wnt1 and wnt10b expression patterns and at the same degree trough their developmental cycle
- Tissue development depends on a network of enhancers that regulate transcription for maturation of segmented brain regions

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References

1. Lekven, A. C., G. R. Buckles, N. Kostakis and R. T. Moon, 2003 wnt1 and wnt10b function redundantly at the zebrafish midbrain-hindbrain boundary. Developmental Biology 254: 172-187.
2. Lekven, A. C., C. J. Lilie, H. C. Gibbs, D. G. Green, A. Singh *et al.*, 2019 Analysis of the wnt1 regulatory chromosomal landscape. Development Genes and Evolution 229: 43-52