Exploring Transcriptional Silencing of Transposons in Zebrafish

Molly Broache
PURAD Fall 2009 Project
Transposons

• Parasitic DNA elements capable of invading host genome

• It is known that transposons are present in the zebrafish genome but it is unknown what role methylation plays in their silencing
DNA methylation

• Epigenetic modification
• Can block the binding of transcription factors or recruit transcriptional repressors

• In plants and mammals, DNA methylation is essential for silencing of transposons
Does DNA methylation repress transcription of transposons in zebrafish?
Dnmt1 is responsible for maintenance of DNA methylation

* s904 (dnmt1 mutant allele) causes a premature stop mutation

Anderson et al, 2009
Dnmt1 mutants have many developmental abnormalities by 6 dpf
Visual assay to identify hypomethylated larvae
\textit{Dnmt1} zebrafish have hypomethylated genomes
In situ data using gypsy as a transposon probe

In situ for ngaro, gypsy, line1-1, line1-3 were developed. These transposons are known to be controlled by the piRNA amplification loop in zebrafish (Houwing, 2008).
Examining Transposon Methylation and Expression in Zebrafish

- Hundreds of transposon families in zebrafish genome
- Approximately 100-200 copies of Dirs and Bhikhari
- Dirs and Bhikari both have ORFS, suggesting they are active
- Used reverse transcription and PCR to amplify expressed sequences of Dirs and Bhik
Bhikhari

Gerster et al, 1999

Bhikhari ORF
Bhikhari LTR
Actin

3dpf WT
RT+
RT-

3dpf mut
RT+
RT-

6dpf WT
RT+
RT-

6dpf mut
RT+
RT-
Dirs1a

- Subfamily of Dirs1_DR retrotransposons
- 11% divergence between the DIRS1a_DR and DIRS1_DR consensus sequences.
- DIRS1a_DR has the presence of stop codons in ORF1-ORF3, while DIRS1_DR does not, suggesting thatDirs1 is non-autonomous
Detection of methylated cytosines with bisulfite conversion

Allele 1 (methylated)  
\[
\begin{align*}
TCTCCACGTCCATCGCT \\
AGAGGAGCAGGTAGCGA \\
\end{align*}
\]

Allele 1 (unmethylated)  
\[
\begin{align*}
TCTCCACGTCCATCGCT \\
AGAGGAGCAGGTAGCGA \\
\end{align*}
\]

Bisulfite treatment  
Alkylation  
Spontaneous denaturation

TUTUUAUGTUUATCGUT  
AGAGGAGUAGGTAGCGA

TUTUUAUGTUUATUGUT  
AGAGGAGUAGGTAGUGA

PCR

TTTTATGTGTATCGGT  
AAAAATACAAATAGCAA

TTTTATGTGTATTTGTT  
AAAAATACAAATAGCAA
Bisulfite conversion with Dirs LTR

Methylation of transposons is variable in WT genomes

52% methylated
Preliminary results suggest that transposons are not regulated by DNA methylation in zebrafish larvae.
Future Directions

• Examine expression of additional transposons in the zebrafish genome
• Investigate the levels of methylation in both WT and $dnmt1$ mutant fish at transposon promoters
• Examine the role of de novo methyltransferases (Dnmt3’s) in transposon control