Characterization of the morphological, phenotypic, and molecular effects of 17α-ethynylestradiol exposure during early development in *Xenopus laevis*

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Introduction

• Estrogenic chemicals in the environment
  – Exposure hypothesized to cause adverse effects
    • Feminization/demasculinization of males
  – Wide variety of species are affected by exposure

• 17α-ethynylestradiol (EE2)
  – Potent estrogen of environmental concern
  – Present in oral contraceptives
    • Not fully removed by conventional sewage treatment
    • Detectable in surface water
Introduction

• *Xenopus laevis*
  – Common laboratory amphibian
  – Exquisitely sensitive to estrogenic exposures during sexual differentiation
    • Male-to-female phenotypic sex reversal
    • Recently discovered sex-linked gene

• EE2 and *X. laevis* used as model systems
  – Morphological and phenotypic effects of EE2 exposure
  – Molecular effects underlying sex reversal
Experimental design

• Dosing Regime*
  – FETAX control and 0.0025% ethanol solvent control
  – 0.1, 1, and 10 µg/L EE2

• Tadpole samples
  – Near sexual differentiation

• Experiment terminated at 96 d
  – Morphometrics and phenotyping
  – Molecular samples
  – Histological samples

*Estrogen equivalent concentrations in surface water normally range from 3-30 ng/L
Days to Metamorphosis

Survival analysis followed by ANOVA, post-hoc Tukey's test; significant differences (p<0.05) denoted by different letters.
Phenotyping: Gross Morphology

Fisher’s Exact Tests; significant differences denoted by different letters
DM-W Based Genotypic Sexing

- *X. laevis* has ZW chromosomal sex determination
  - ZW female; ZZ male
  - *DM-W* resides on the W chromosome

- Multiplex *DM-W/DMRT1* PCR genotyping
  - Genomic DNA
  - PCR products visualized on a gel
Genotypic Sex Ratios

*Initial data from a subsample of EE2 treated animals.
Initial Comparison of Genotyping and Phenotyping

Control Phenotype  | Control Genotype  | SC Phenotype  | SC Genotype  | EE2 Treated Phenotype  | EE2 Treated Genotype

% Ambiguous  | % Female  | % Male
Gross Phenotypic Morphology

1. Genetic female
2. Sex-reversed genetic male
3. Genetic male
Transcriptome Analysis

• Nieukwoop-Faber Stage 53 Tadpoles
  – Undergoing sexual differentiation
  – Control and 100 µg/L EE2 treated animals
    • Male genotype

• **Illumina Sequencing**
  – RNA Seq
  – Single-end read
  – 75 bp read length
Initial Transcriptome Analysis

• CLC Genomics Workbench
  – Reads filtered and trimmed
  – Mapped to *X. laevis* published mRNAs
  – Expression analysis

• General Statistics
  – 70% of reads mapped to an mRNA transcript
  – 95% of transcripts were detected at least once
Transcriptome Analysis

- Overview of changes

- 73% of genes are upregulated at least 2-fold
- 12% of genes are downregulated at least 2-fold
- 15% of genes remain unchanged

22 genes upregulated at least 15-fold
66 genes downregulated at least 15-fold
Types of Genes Impacted

• Up-regulated
  – Estrogen/steroid hormone metabolism
  – Cardiac/skeletal muscle contraction and growth
  – DNA repair

• Down-regulated
  – Redox metabolic activity
  – Axonogenesis and synaptogenesis
  – Metabolism of neurotransmitters
<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen sulfotransferase (sult1e1)</td>
<td>+19</td>
</tr>
<tr>
<td>Frizzled-related protein (frzb-1)</td>
<td>+24</td>
</tr>
<tr>
<td>Troponin T Type 3 (tnnt3)</td>
<td>+37</td>
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<tr>
<td>Cu-Zn superoxide dismutase (sod)</td>
<td>-23</td>
</tr>
<tr>
<td>Synaptosomal associated protein 25 (snap-25)</td>
<td>-85</td>
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<tr>
<td>Sulfotransferase 4a1 (sult4a1)</td>
<td>-23</td>
</tr>
</tbody>
</table>
Biological Relevance of EE2 Exposure

• Male-to-female sex reversal

• May impact individual fitness
  – Delayed metamorphosis and smaller size

• Changes in the male transcriptome at sexual differentiation
  – Estrogen/hormone metabolism
  – Other processes
Additional Ongoing Analysis

• Histology of gonads
  – Gross morphology of small animals unclear

• Parallel wood frog experiment
  – Native, non-model species
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