Glutathione redox dynamics and expression of glutathione-related genes in the developing embryo

Alicia R. Timme-Laragy, Jared V. Goldstone, Barry R. Imhoff, John J. Stegeman,

Mark E. Hahn, Jason M. Hansen

Supplemental File 5.

Supplemental methods- Sampling, RNA extraction, and cDNA synthesis

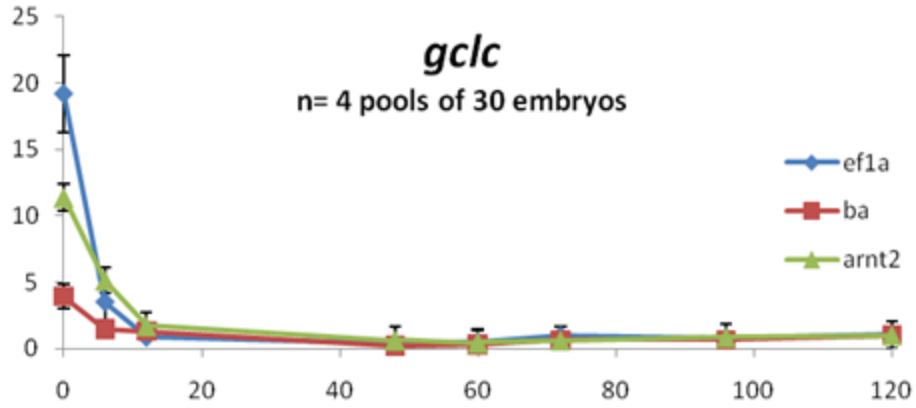
As described previously [1], pools of carefully staged embryos kept at low density at 28.5°C were fixed in liquid nitrogen at 6, 12, 24, 48, 60, 72, 96, and 120 hours post fertilization. Embryos that hatched without stimulation at 48 and 60 hpf timepoints were treated as separate samples. Eggs for the 0 hpf timepoint were manually stripped from 3 females and combined.

RNA was isolated using RNA STAT-60 (Tel-Test B, Inc.,Friendswood, TX) according to the manufacturer's instructions. cDNA was synthesized from 2 µg total RNA using random hexamers and the Omniscript cDNA Synthesis Kit (Qiagen, Valencia, CA).

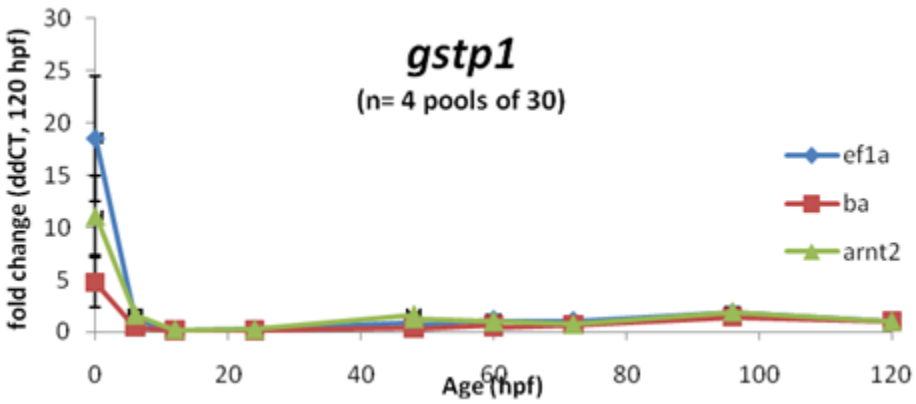
Supplemental methods- measurement of Gene Expression by Quantitative real-time RT-PCR (QPCR)

QPCR was performed using the iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) in a MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad). Each reaction was run in duplicate wells containing cDNA from 5 ng of RNA. Primers and extension temperatures are provided in supplemental Table S1. The PCR conditions used were 95°C for 3.5 minutes followed by 35-40 cycles of 95°C for 15 seconds and 25 seconds at the gene specific temperature (see Table S1). Each run included melt curve analysis to ensure that only a single product was amplified, as well as a no-template control. All primers were tested for amplification efficacy (100 % ± 10 %). Housekeeping genes were selected to be most appropriate for both embryonic development (*β-actin),* and verified with a second housekeeping gene (*ef1a*) [2], as well as *arnt2*, which was one of the most stably expressed genes identified on the microarray between 3-48 hpf. Genes were analyzed using the comparative delta delta C_T method [3].

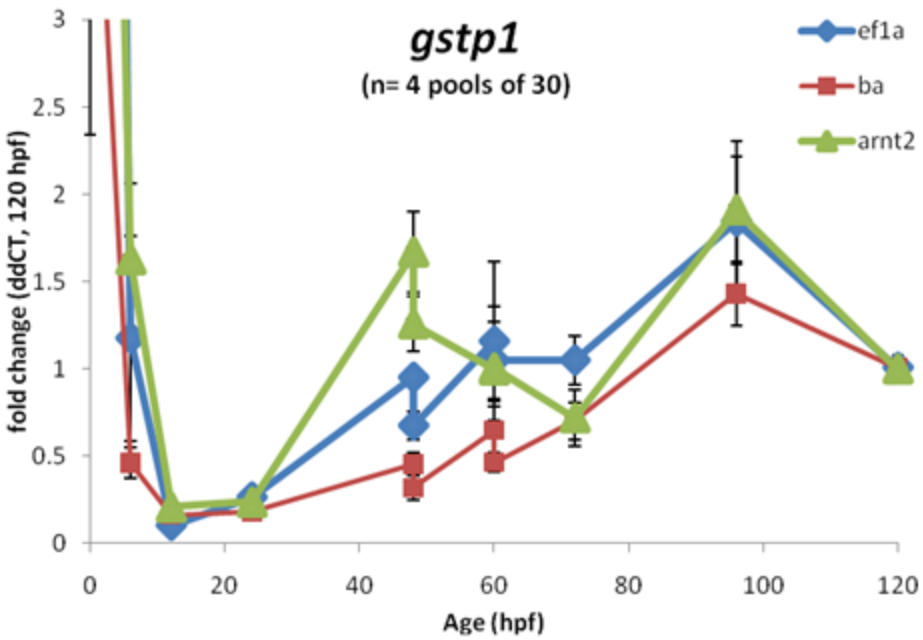
A



B



C



Supplemental file 3. Measurement of gene expression by QPCR. To expand the analysis of developmental expression of two of the most critical glutathione-related genes, *gclc* and *gstp1*

expression was measured by QPCR using cDNA made from RNA isolated from pools of unfertilized eggs and embryos at the indicated times. Detailed methods are available elsewhere [1]. The fold change in expression is normalized to levels found at 120 hpf, and normalized to 3 different housekeeping genes (*ef1a*, *b-actin*, and *arnt2*). A) Expression of *gclc* ; B) expression of *gstp1*, C) expression of *gstp1* graph scaled to show the differences found in hatched vs. unhatched embryos at 48, 60, and 72 hpf. Hatched embryos had slightly lower expression of *gstp1* than unhatched embryos. Data are presented as mean and SEM, $N = 4$ pools of 30 embryos.

References cited

1. Timme-Laragy AR, Karchner SI, Franks DG, Jenny MJ, Harbeitner RC, Goldstone JV, McArthur AG, Hahn ME: **Nrf2b, novel zebrafish paralog of oxidant-responsive transcription factor NF-E2-related factor 2 (NRF2)**. *The Journal of biological chemistry* 2012, **287**(7):4609-4627.
2. McCurley AT, Callard GV: **Characterization of housekeeping genes in zebrafish: male-female differences and effects of tissue type, developmental stage and chemical treatment**. *BMC molecular biology* 2008, **9**:102.
3. Livak KJ, Schmittgen TD: **Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method**. *Methods* 2001, **25**(4):402-408.