

Regenerative Growth Is Impacted by TCDD: Gene Expression Analysis Reveals Extracellular Matrix Modulation

Eric A. Andreasen, Lijoy K. Mathew, and Robert L. Tanguay¹

Department of Environmental and Molecular Toxicology, Environmental Health Sciences Center, Marine and Freshwater Biomedical Sciences Center, Oregon State University, Corvallis, Oregon 97331

Received November 28, 2005; accepted January 20, 2006

Adult zebrafish can completely regenerate their caudal fin following amputation. This complex process is initiated by the formation of an epithelial wound cap over the amputation site by 12 h post amputation (hpa). Once the cap is formed, mesenchymal cells proliferate and migrate from sites distal to the wound plane and accumulate under the epithelial cap forming the blastema structure within 48 hpa. Blastema cells proliferate and differentiate, replacing the amputated tissues, which are populated with angiogenic vessels and innervating nerves during the regenerative outgrowth phase which is completed around 14 days post amputation (dpa). Regenerative outgrowth does not occur in TCDD-exposed zebrafish. To identify the molecular pathways that are perturbed by TCDD exposure, male zebrafish were ip injected with 50 ng/g TCDD or vehicle and caudal fins were amputated. Regenerating fin tissue was collected at 1, 3, and 5 dpa for mRNA abundance analysis. Microarray analysis and quantitative real time PCR revealed that wound healing and regeneration alone altered the expression of nearly 900 genes by at least two-fold between 1 and 5 dpa. TCDD altered the abundance of 370 genes at least two-fold. Among these, several known aryl hydrocarbon responsive genes were identified in addition to several genes involved in extracellular matrix composition and metabolism. The profile of misexpressed genes is suggestive of impaired cellular differentiation and extracellular matrix composition potentially regulated by Sox9b.

Key Words: AHR; TCDD; regeneration; zebrafish; extracellular matrix.

Adult zebrafish have the capacity to regenerate their caudal fin upon amputation, a process that is inhibited by exposure to the ubiquitous environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Zodrow and Tanguay, 2003). Since fin regeneration is a complex *in vivo* process involving wound healing, tissue remodeling, growth, angiogenesis, and neuronal innervation, this model is ideally suited

to reveal common mechanisms of TCDD toxicity observed across species. Identifying mechanisms of TCDD toxicity has been confounded by the wide array of toxicities induced in mammals including acute responses such as epithelial hyperplasia and metaplasia, lymphoid involution, porphyria, wasting syndrome, tumor promotion, and death (reviewed in Birnbaum and Tuomisto, 2000; Gu *et al.*, 2000) in addition to reproductive and developmental toxicities (reviewed in Birnbaum and Tuomisto, 2000). Despite this wide array of toxicities, much of the toxicity of TCDD requires the aryl hydrocarbon receptor (AHR) (Fernandez-Salguero *et al.*, 1996; Mimura *et al.*, 1997). What remains elusive are the downstream effects of AHR that are necessary to produce toxicity. Since toxicity is initiated by activation of the AHR transcription factor by ligands such as TCDD, assessment of gene expression changes will help to clarify the mechanism(s) of TCDD toxicity.

The AHR is a ligand activated, basic helix-loop-helix transcription factor and is a member of the PAS domain family of genes (reviewed in Gu *et al.*, 2000). The AHR signal transduction pathway in fish is similar to that in mammals, with the exception that fish have two or more AHR genes while mammals have only one (reviewed in Hahn *et al.*, 1997; Tanguay *et al.*, 2003). Similar to mammals, two dimerization partners for AHR, AHR nuclear translocator proteins 1 and 2 (ARNT1, ARNT2), have been cloned and characterized in zebrafish. AHR2 and ARNT1 are the members of the AHR pathway in zebrafish that mediate TCDD toxicity including TCDD-impaired larval fin regeneration (Andreasen *et al.*, 2002; Mathew *et al.*, 2005; Prash *et al.*, 2003, 2004, in press). Therefore, despite the added complexity of the fish AHR phylogeny, the functionality of the fish and mammalian AHR pathways are similar.

Amputation and regeneration of the adult zebrafish caudal fin has been used as a system to study epimorphic tissue regeneration (reviewed in Poss *et al.*, 2003). Epimorphic regeneration involves the reprogramming and migration of cells that differentiate and restore a tissue to its original form. The caudal fin consists of a series of boney rays attached to the skeleton by ligaments. Each ray, referred to as a lepidotrichia is

¹ To whom correspondence should be addressed at Oregon State University, Department of Environmental and Molecular Toxicology, 1007 ALS, Corvallis, Oregon 97331-7301. Fax: (541) 737-7966. E-mail: robert.tanguay@oregonstate.edu.

comprised of two hemirays. The hemirays consist of repeating segments joined by ligaments. The area between the hemirays of a lepidotrichia contains blood vessels, nerves, and mesenchymal cells. The fin grows by successive addition of hemiray segments to the most distal (end of fin) segment. After partial amputation of the caudal fin, an apical epithelial wound cap (AEC) forms over the clot at the amputation site within 12 h post amputation (hpa). The AEC cells are derived from non-proliferating local epithelial cells that migrate laterally over the wound. Once the cap is formed, mesenchymal cells proliferate and migrate from sites distal to the wound plane and accumulate under the epithelial cap forming a structure called the blastema within 48 hpa. The cells forming the blastema are believed to be de-differentiated cells that are later reprogrammed to form the regenerating tissue. Scleroblast (bone forming cells giving rise to the hemiray) and blastemal cells proliferate and differentiate, replacing the amputated tissues, which are infused with nerves and blood vessels in a process called regenerative outgrowth. Outgrowth begins at 2 days post amputation (dpa) until the regenerative event is complete at about 14 dpa.

Zebrafish caudal fin regeneration is impaired by TCDD when exposure occurs at any time during the first four days of regeneration (Zodrow and Tanguay, 2003). An epithelial wound cap and blastema forms in the TCDD exposed regenerate, but outgrowth does not occur. The AHR pathway is active in the regenerating fin since both zfAHR2 and TCDD induced CYP1A mRNAs (a marker of an active AHR pathway) are expressed in the regenerating caudal fin (Andreasen *et al.*, 2002; Zodrow and Tanguay, 2003). Several studies in zebrafish have proposed potential mechanisms that initiate AEC, blastema formation, and outgrowth (reviewed in Poss *et al.*, 2003). The effect of TCDD on the expression of these and other genes and pathways involved in fin regeneration was the focus of this work. Microarray analysis revealed that 370 known genes were differentially regulated by TCDD during the first five days of regeneration. The major categories of responsive genes were detoxification enzymes, extracellular matrix, development, retinoic acid signaling, and metabolism. These studies have laid the foundation for the dissection of the pathways required for fin regeneration and those that are modified by TCDD.

MATERIALS AND METHODS

TCDD dosing and fin amputation. Adult male zebrafish (*Danio rerio*) (AB strain, Eugene, OR) were anesthetized with tricaine methanesulfonate (MS-222) and their caudal fins were amputated at the bifurcation of the fin rays. Fish were then injected with phosphatidylcholine liposomes (vehicle control) alone or liposomes incorporated with TCDD (>99% pure, Chemsyn, Lenexa, KS) prepared as previously described (Andreasen *et al.*, 2002), resulting in a final dose of 50 ng TCDD/g fish. At the indicated dpa, fish were euthanized with an over dose of MS-222. Regenerated fin tissue was then isolated by amputation within two ray segments anterior to the original amputation plane. Amputated tissue was then stored in RNAlater (Qiagen, Valencia, CA) and

frozen at -80°C until RNA was isolated from the tissue. Tissues from 10 fish were combined to comprise a single experimental group. Three groups of tissue were obtained from vehicle control or TCDD exposed fish at 1, 3, and 5 dpa. For qRT-PCR studies, fish were similarly dosed with vehicle or TCDD and regenerating tissues were isolated in triplicate (6 fins/group). Additionally, mRNA abundance was determined in non-regenerating (0 dpa) fins.

Total RNA isolation. Fins of vehicle control or TCDD exposed fish were isolated as described above. RNAlater was removed from the samples and total RNA was purified with TRI reagent (Molecular Research Laboratories, Cincinnati, OH) according to the manufacturer's instructions. Total RNA was DNase treated with RQ1 DNase (Promega, Madison, WI) according to the manufacturer's protocol and RNA quantity and quality was determined by UV absorbance. Ribosomal RNA abundance and degree of degradation was determined in electropherogram patterns using the 2100 Bioanalyzer and RNA 6000 Nano chips (Agilent Technologies, Palo Alto, CA).

Affymetrix microarray processing. Probe synthesis, hybridization, and scanning were conducted by the Center for Gene Research at Oregon State University, Corvallis OR. 2.5 μg of total RNA was used to generate biotinylated complementary RNA (cRNA) for each treatment group using the One-Cycle Target Labeling kit (Affymetrix, Santa Clara, CA). Briefly, RNA was reverse transcribed using a T7-(dT)₂₄ primer and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA) and double stranded cDNA was synthesized and purified. Biotinylated cRNA was synthesized from the double stranded cDNA using T7 RNA polymerase and a biotin-conjugated pseudouridine containing nucleotide mixture provided in the IVT Labeling Kit (Affymetrix, Santa Clara, CA). Prior to hybridization, the cRNA was purified, fragmented and 10 μg from each experimental sample was hybridized to zebrafish genome arrays (Zebrafish430_2) according to the Affymetrix GeneChip Expression Analysis Technical Manual (701021 Rev. 5). Affymetrix fluidics station 400 was used to wash the arrays. Arrays were scanned with an Affymetrix scanner 3000. The Affymetrix eukaryotic hybridization control kit and Poly-A RNA control kit were used to ensure efficiency of hybridization and cDNA amplification. All cRNA was synthesized at the same time. Hybridizations were conducted with one replicate of all times and treatments concurrently. Each array image was visualized to discount artifactual signals, scratches or debris. Experiments were MIAME certified. The raw data is listed at the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/projects/geo/>); series record #GSE3667).

The Affymetrix zebrafish genome array was designed to detect over 14,900 transcripts accounting for approximately 33% of the predicted genome. Probe sets comprising 16 different oligonucleotides were designed to detect an individual transcript. Since the zebrafish genome has not been completely sequenced, the microarray may have different probe sets that detect the same transcript. Additionally, annotation of the microarray is incomplete. Sequence used to design the probe sets were found in dbEST (July 2003), GenBank (release 136.0, June 2003), RefSeq (July 2003), and UniGene (Build 54, June 2003). Sequence similarity to known mammalian proteins was determined by identifying full length mRNA sequence for each zebrafish probe set by conducting a BLAST search of each Affymetrix probe set against Genbank (<http://www.ncbi.nlm.nih.gov/BLAST/>), TIGR (<http://tigrblast.tigr.org/tgi/>), and Sanger (http://www.sanger.ac.uk/Projects/D_rerio/) databases. The predicted amino acid sequences from these genes were then compared to mammalian orthologs using pblast at NCBI. The deduced full-length protein predicted from each transcript's probe set was confirmed to be at least 45% identical to its mammalian ortholog.

qRT-PCR validation of mRNA abundance. Total RNA was isolated in triplicate from the regenerating fins of vehicle control or TCDD exposed fish ($n = 6/\text{group}$). cDNA was prepared from 2 mg of total RNA per group using Superscript II (Life Technologies, Gaithersburg, MD) and oligo(dT) primers in a 20 ml volume. Quantitative PCR using gene specific primers was conducted using the Opticon 2 real-time PCR detection system (MJ Research, Waltham, MA). Specifically, 1 ml of each cDNA pool was used for each

PCR reaction in the presence of SYBR Green, using DyNAmo SYBR green qPCR kit according to the manufacturer's instructions (Finnzymes, Espoo Finland). All experimental samples were analyzed concurrently with standard curves generated from known quantities of DNA template covering seven orders of magnitude for each gene in duplicate. Negative controls for each experiment consisted of RNA without reverse transcriptase and another without template. Agarose gel electrophoresis and thermal denaturation (melt curve analysis) were conducted to ensure formation of specific products.

Oligonucleotides. All oligonucleotide primers were synthesized by MWG-Biotech (High Point, NC). Forward primers are prefixed with an F corresponding to sense strands and antisense reverse primers are designated with an R. Primers were designed to amplify sequence within the Affymetrix probe set sequence. Sequence for each primer can be found in Table 1.

Statistical analysis. Affymetrix cell files were imported into GeneSpring 7.1 software (Agilent Technologies, Palo Alto, CA) and GC-RMA processed to correct for background signal and to normalize across arrays. Subsequently, each transcript was normalized to the median signal for each transcript across all arrays to allow for between array comparisons on a relative scale for each gene. All statistical analysis of microarray data was conducted using GeneSpring 7.1 software.

The effects of time on transcript level of vehicle control exposed animals was determined by one-way ANOVA assuming equal variance employing Benjamini and Hochberg multiple testing corrections ($p < 0.05$). Only genes at least two-fold differentially expressed from 1 dpa vehicle control transcript levels were considered for this analysis. Hierarchical clustering using a smooth correlation and default parameters was employed to characterize expression patterns during regeneration in vehicle exposed animals.

In order to determine the effect of TCDD on gene expression, the data set was filtered for significance by first generating a list of genes that were at least two-fold differentially expressed in comparison to 1 dpa control fins in at least one time point independent of TCDD exposure. Effects of time and treatment

were first analyzed by two-way ANOVA analysis assuming equal variance and employing Benjamini and Hochberg multiple testing corrections ($p < 0.05$). The effects of TCDD dosing was determined by filtering control and TCDD data sets for an individual time point for genes that were at least two fold differentially expressed between control and TCDD dosed fish. Statistical significance of TCDD exposure was determined by one-way ANOVA comparing time matched controls to TCDD dosed transcript levels assuming equal variance ($p < 0.05$).

Significant differences of mRNA abundance determined with qRT-PCR were assessed by two-way ANOVA on \log_{10} transformed data. Differences between groups were analyzed by the Tukey method ($p < 0.05$). qRT-PCR analyses were performed using SigmaStat software (Chicago, IL).

RESULTS

Differential Gene Expression during Fin Regeneration

Changes in gene expression were monitored at 1, 3, and 5 dpa in regenerating tissue from adult male zebrafish exposed at the time of amputation to vehicle control or 50 ng/g TCDD using Affymetrix zebrafish genome arrays. These time points were chosen because TCDD impairs regenerative fin outgrowth, a process that begins at 2 dpa. The oligonucleotide array was designed to detect approximately 14,900 transcripts accounting for approximately 33% of the estimated genome. Although each oligonucleotide (probe) on the array was designed to detect a unique transcript, the degree of redundancy is not completely known. The dose of TCDD chosen is approximately the EC_{90} for impairing fin regeneration.

TABLE 1
Oligonucleotides Used for qRT-PCR

Target gene	Sequence 5' to 3'	Affymetrix probe set number
F CYP1A	TGCCGATTTTCATCCCTTCC	Dr.9478.1.S1_at
F CYP1A	AGAGCCGTGCTGATAGTGTC	Dr.9478.1.S1_at
F CYP1B1	TCATCCTGCTACTTGTCCAGG	Dr.Affx.2.29.S1_at
R CYP1B1	TGGATGTGTCTTTGGTCGTG	Dr.Affx.2.29.S1_at
F UDPGT	CACTGAACTACCTATAACGG	Dr.5410.1.a1_at
R UDPGT	TGTTGGAAATGTGTCCGGCC	Dr.5410.1.a1_at
F BAMBI	AGCAAAGACATTTCAAGGAC	Dr.7919.1.S1_at
R BAMBI	TCAGTGTCAATCCAGGATCC	Dr.7919.1.S1_at
F Frizzled7a	TCGAGTACGCATTAGGATCCG	Dr.4823.1.S1_at
R Frizzled7a	ACTGTACAGATACAAAGGTC	Dr.4823.1.S1_at
F cc Chemokine 1	TTGAAGAAAATGCTGAAGCG	Dr.8113.1.S1
R cc Chemokine 1	AACACACACAGTATATCGCC	Dr.8113.1.S1
F Proline-4-hydroxylase Alpha 1	GAGTGTCTGAGCAAGGAGCG	Dr.3932.1.s1_at
R Proline-4-hydroxylase Alpha 1	GATAAGCCGGGAAATACAAGC	Dr.3932.1.s1_at
F Procollagen C-Endopeptidase Enhancer 2	TTCCAGAGGAGGTCGAGGAC	Dr.15092.1.a1_at
R Procollagen C-Endopeptidase Enhancer 2	TTGTCTATCTTTGCTATGGG	Dr.15092.1.a1_at
F Lysyl oxidase 2	AAGACAGAACAGAATCC	Dr.23096.1.a1_at
R Lysyl oxidase 2	CTCACTTGAGAGTTATCCAC	Dr.23096.1.a1_at
F Sox9b	TGACGAGTTGTTCTCCAGAG	Dr.11850.1.s2; Dr.11850.1.S1
R Sox9b	AGGCCACACGTCTATAACCC	Dr.11850.1.s2; Dr.11850.1.S1
F Cadherin-19	AGGATCACCATCTGTGCGG	Dr.14830.1.S1_at
R Cadherin-19	TGCTAACCATCTAGTTTAAAGGGC	Dr.14830.1.s1_at
F B-actin	AAGCAGGAGTACAGATGAGTC	Dr.1109.1.S1_at
R B-actin	TGGAGTCTCAGATGCATTG	Dr.1109.1.S1_at

Importantly, this dose does not lead to overt toxicity such as fin necrosis, hemorrhaging wasting, or alterations in swimming behavior and feeding. Tissues isolated from 10 fish at each time and treatment, were pooled to generate an experimental group. Three independent biological replicates of each time and treatment were analyzed. A total of 18 microarrays were analyzed.

Approximately 11,700 transcripts were detected after filtering out low abundant genes. Among these, 1383 genes were differentially affected at least two fold by time and or TCDD exposure in at least one time point (Fig. 1). Wound healing and regeneration alone altered the expression of 932 genes by at least two-fold between 1 and 5 dpa (Fig. 1). Expression of 99 genes was affected by TCDD alone (Fig. 1). Time and TCDD exposure affected the abundance of 352 genes (Fig. 1). Further analysis of the change of expression of genes over time using one way ANOVA analysis of vehicle exposed animals identified 898 genes that were at least two-fold differentially regulated from 1 to 5 dpa (Fig. 2). Hierarchical clustering analysis was employed to characterize the expression patterns of these vehicle exposed animals. Five major clusters were identified and labeled A–E. The ratio of genes with enhanced to repressed expression was 3 to 2. Since the Affymetrix array is not fully annotated, and the effect of TCDD was the focus of these studies, the identity of these non-annotated genes remains to be determined.

Altered Gene Expression in Response to TCDD Exposure

Gene expression altered by TCDD exposure was determined by first filtering for genes that were at least 2 fold differentially regulated from their time matched control. This gene list was

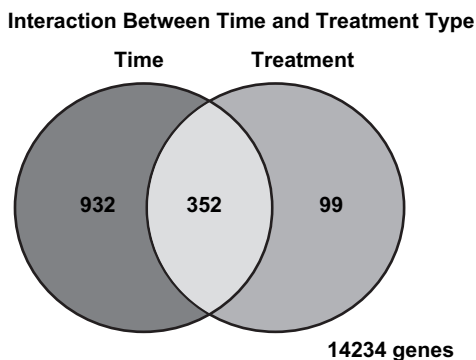


FIG. 1. Venn diagram depicting 1383 transcripts that were at least two-fold differentially regulated from 1 dpa control fins. Fins from vehicle control or TCDD exposed animals were isolated at 1, 3 and 5 dpa. Three groups of 10 pooled fins were isolated for each time and treatment. Two-way ANOVA analysis assuming equal variance and employing Benjamini and Hochberg multiple testing corrections ($n = 3$, $p < 0.05$) identified 932 transcripts that were differentially regulated over time. Ninety-nine transcripts were found to be differentially expressed due to TCDD exposure alone. 352 transcripts were differentially expressed at different time points between vehicle or TCDD exposed animals.

then analyzed for statistical significance employing one-way ANOVA analysis comparing time matched vehicle and TCDD exposed gene profiles. The expression of 370 genes was differentially regulated by TCDD exposure (Fig. 3). The temporal response increased over time with 50, 196, and 265 being differentially expressed at 1, 3, and 5 dpa. This increasing diversity of gene expression parallels the timeline of the pathological signs of toxicity. TCDD exposure resulted in enhancing the abundance of 127 genes of which 67 were identified with mammalian orthologs (Table 2). Genes were categorized into functional groups based upon sequence homology and known function in the literature. Expression of eight genes involved in xenobiotic metabolism including genes known to be inducible by AHR activation such as cytochrome P4501A

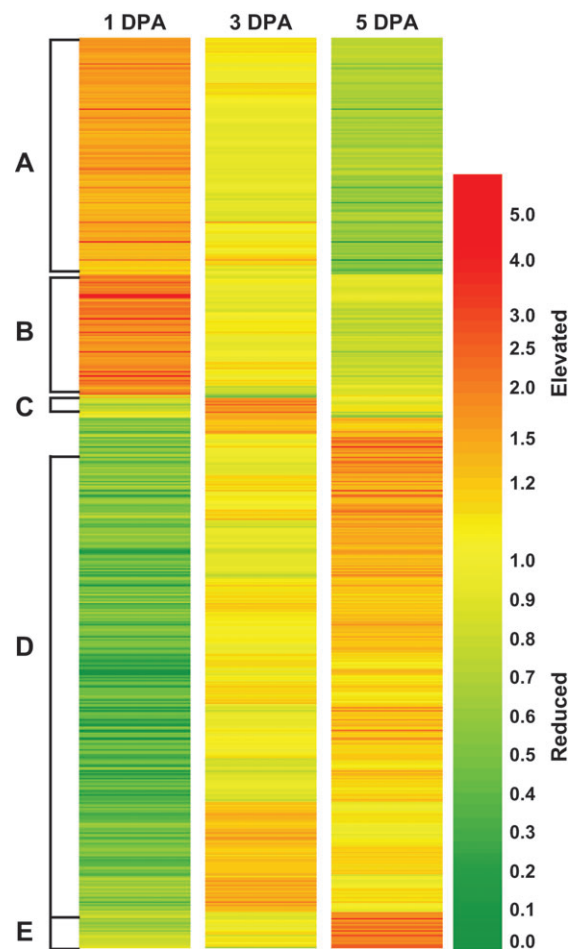


FIG. 2. The effect of time alone on global gene expression is depicted in a heat map. Only genes differentially expressed at least two-fold at any time in vehicle exposed fins were included in analysis. Significance was assessed by one-way ANOVA assuming equal variance employing Benjamini and Hochberg multiple testing corrections ($p < 0.05$). Hierarchical clustering analysis was employed to characterize expression patterns during regeneration in vehicle exposed animals. Five major clusters were identified and labeled A–E. Color bar indicates the relative abundance of each transcript with red being the most abundant.

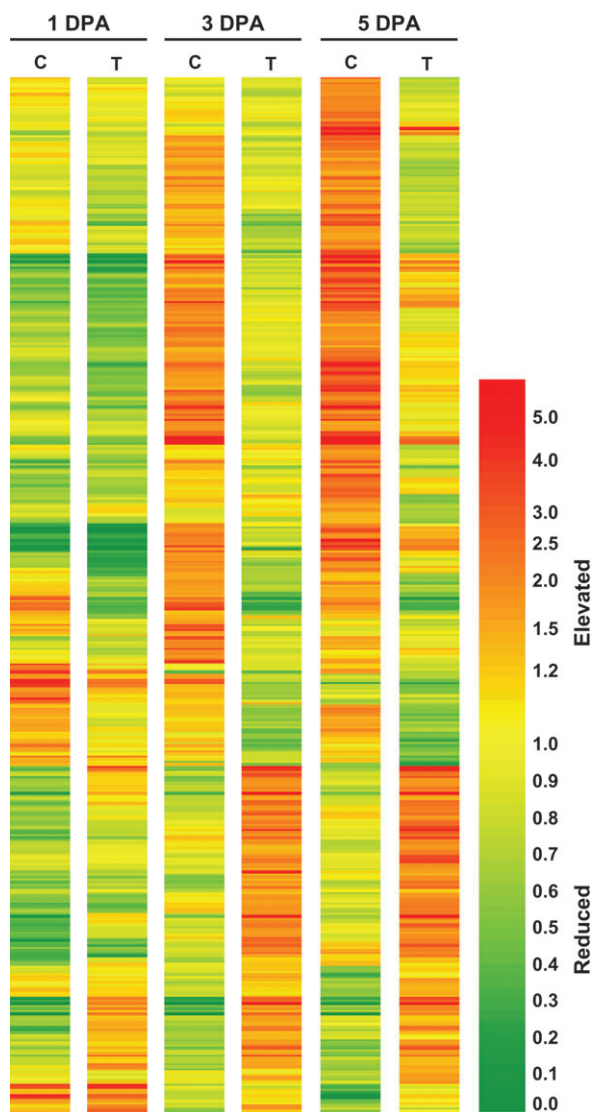


FIG. 3. Heat map depicting relative expression levels of genes at least two fold differentially expressed between vehicle or TCDD exposed fish at the same time in regeneration. One way ANOVA analysis was conducted to determine the significance of TCDD exposure on time matched samples assuming equal variance ($p < 0.05$). Color bar indicates the relative abundance of each transcript with red being the most abundant.

were affected by TCDD confirming that the tissues were exposed to TCDD and had an active AHR pathway as early as 1 dpa. Other major functional groups with enhanced gene expression include extracellular matrix (ECM) and metabolism, development, FGF signaling, and metabolism. TCDD exposure resulted in reduced expression of 243 genes of which 132 were identified and categorized in Table 3. Two major functional groups were identified. Thirty-six genes with reduced expression are components in ECM or metabolism of this matrix. The expression of 21 genes involved in development was negatively affected by TCDD. Other major groups of genes that were negatively affected by TCDD in-

clude retinoic acid metabolism and signaling, wound response, metabolism, and amino acid metabolism. Lack of sufficient sequence information inhibited the identification of 60 induced and 113 repressed genes.

Validation of Gene Expression Profiles

Separate experiments using quantitative real time PCR were conducted to measure the abundance of several of the transcripts listed in Tables 2 and 3, representing high and low responders in distinct functional categories. Separate biological samples from those used in the microarray experiments were collected to ensure that significant responses to TCDD were not experimental artifacts. Animals were dosed with vehicle or TCDD similarly to the microarray experiment and three replicates (six fins per replicate) were each isolated at 1, 3, and 5 dpa. Abundance of seven transcripts that were enhanced by TCDD exposure and four that were repressed by TCDD exposure were analyzed.

Transcripts of the AHR inducible xenobiotic detoxifying enzymes CYP1A, CYP1B1, UDPGT in vehicle exposed fish were initially decreased at 1 dpa in comparison to non-regenerating fins (Figs. 4A, 4B, 4C). However their abundance became elevated by 3 dpa and they were all significantly enhanced by TCDD dosing from 1 to 5 dpa. The low abundance of CYP1A in vehicle control animals lead to an artificially high fold induction (low denominator) and therefore was not included in Figure 4, but is listed in Table 4. Representative genes grouped into the ECM metabolism, immune and development categories also responded to TCDD. Frizzled 7a and Cadherin 19 had elevated expression in response to TCDD as early as 1 dpa and remained elevated to 5 dpa (Figs. 4D and 4E). BAMBI and CC-Chemokine 1 had transient response to TCDD with BAMBI displaying TCDD inducible expression at 3 and 5 dpa and CC-Chemokine 1 not responding to TCDD exposure until 5 dpa (Figs. 4F and 4G). Frizzled 7a, BAMBI and CC-Chemokine 1 message level in vehicle exposed fish was not repressed by regeneration alone unlike the xenobiotic metabolizing enzymes.

Four genes identified as reduced by TCDD exposure on the microarrays were quantified to determine the congruence between gene array and qRT-PCR. All four of these genes had enhanced expression during regeneration in comparison to the non-regenerating fin (Fig. 5). Sox9b was chosen because it was highly responsive to TCDD by microarray analysis. Sox9b expression was reduced by TCDD at all time points between 3 and 22 fold (Fig. 5A). Three genes that were marginally, but significantly repressed by TCDD were quantified by qRT-PCR to assess how well microarrays discern small changes in gene expression. Two genes involved in collagen maturation, lysyl oxidase 2 and prolyl 4-hydroxylase $\alpha 1$, each increased expression during the first five days of regeneration (Figs. 5B and 5C). Additionally both were repressed by TCDD with lysyl oxidase 2 being repressed at all time points and

TABLE 2
Transcripts Enhanced at Least Two-Fold by TCDD Exposure in Comparison with Their Time Matched Vehicle Control

Gene	Affymetrix	Genbank	Fold induction (<i>p</i> value)		
			1 DPA	3 DPA	5 DPA
Xenobiotic metabolism					
CYP1A	Dr.9478.1.S1_at	AB078927.1	100.5 (0.009)	95.0 (0.001)	40.1 (0.011)
CYP1C1	Dr.12329.1.A1_at	BG728978	52.4 (0.002)	69.2 (0.003)	50.6 (0.006)
UDPGT	Dr.5410.1.A1_at	A1588396	8.1 (0.002)	9.4 (<0.001)	8.2 (0.005)
Cytochrome b5	Dr.5040.1.S1_at	BC053263.1	6.7 (0.002)	7.7 (0.006)	6.6 (0.008)
Sulfotransferase	Dr.3583.1.S1_at	AY180110.1	3.2 (0.003)	2.5 (0.037)	2.1 (0.002)
Copper amine oxidase	Dr.7419.1.A1_at	AW343893	2.5 (0.032)	2.5 (0.004)	2.8 (0.001)
CYP1B	Dr.Affx.2.29.S1_at	AF235139.1		4.4 (0.025)	2.8 (0.029)
Glutathione peroxidase	Dr.8000.1.S1_at	AW232570		2.5 (0.007)	3.7 (<0.001)
SQRDL	Dr.9492.1.A1_at	B1882244		2.1 (0.005)	2.0 (<0.001)
Matrix metabolism/componets					
MMP13	Dr.10314.1.S1_a_at	BC049472.1	5.6 (0.043)	9.3 (<0.001)	5.6 (<0.001)
Cadherin-19	Dr.14830.1.S1_at	BM072040	3.2 (0.004)	3.4 (0.003)	3.2 (0.001)
Exostoses 1c	Dr.1169.1.A1_at	BQ131658		2.0 (0.004)	
TIMP2 Like	Dr.15281.1.A1_at	BQ131818			2.8 (0.05)
Thioredoxin	Dr.8723.1.S1_at	BI864002			2.0 (0.02)
Development					
Frizzled 7a	Dr.4823.1.S1_at	AF336123.1	5.4 (0.008)	3.8 (0.001)	3.1 (0.007)
Dlx4b	Dr.153.1.S1_at	NM_131318.1	2.5 (0.012)	2.1 (0.002)	2.1 (0.029)
BAMBI	Dr.7919.1.S1_at	NM_131784.1	2.2 (0.018)	2.1 (0.03)	3.5 (0.009)
Zn Finger	Dr.16562.2.A1_at	BI709799	2.2 (0.026)		2.1 (0.006)
Sox31	Dr.25405.1.A1_at	BM402113		6.8 (0.01)	8.0 (0.01)
Hes5	Dr.1899.3.A1_at	AL729023		2.5 (0.021)	4.0 (0.01)
Mxsd	Dr.5789.1.S1_at	NM_131276.1			3.9 (0.003)
Emx3	Dr.534.1.S1_at	NM_131279.1			3.2 (0.007)
Sprouty4	Dr.12410.1.S1_at	NM_131826.1		2.1 (0.027)	
BMP2a	Dr.1463.1.S1_at	NM_131359.1		2.1 (0.03)	
Egr1	Dr.10183.1.S2_at	AW595366		2.0 (0.007)	
Sox19	Dr.20910.1.S1_at	NM_130908.1		2.0 (0.03)	
MsxA	Dr.5754.1.S1_at	NM_131274.1			2.7 (0.022)
Ddnf	Dr.8195.1.A1_at	NM_131595.1			2.2 (0.026)
Insm1	Dr.19235.1.S1_at	BC053119.1			2.2 (0.001)
Jagged1a	Dr.12590.1.S1_at	NM_131861.1			2.2 (0.027)
Glycogen pathway					
Pp1r3a	Dr.6604.2.A1_a_at	BM530940	3.0 (0.002)	2.7 (0.004)	2.1 (0.032)
ARK5	Dr.4918.1.A1_at	AI544549		2.7 (0.004)	
Endothelial cell sprouting/migration					
ESM-1	Dr.19483.1.A1_at	AL909732	2.2 (0.004)	2.8 (0.017)	2.7 (0.003)
REM1	Dr.15089.1.S1_at	BC045943.1		3.2 (0.003)	2.5 (0.018)
Hey1	Dr.19915.1.S1_at	AJ510221.1			2.5 (0.018)
Fgf signaling					
Sef	Dr.12379.1.S1_at	NM_153660.1		2.4 (0.01)	2.9 (0.005)
Sp8	Dr.9764.1.A1_at	AI793487			2.6 (0.005)
PEA3	Dr.618.1.S1_at	NM_131425.1			2.2 (0.012)
Fgf10	Dr.8853.1.S1_at	AF544025.1			2.2 (0.018)
Reduction/oxidation metabolism and signaling					
Nrf2	Dr.12684.1.S1_at	BC045852.1		2.1 (0.01)	
Dhrs1	Dr.23482.1.A1_at	AI330348			2.0 (0.019)
Chromatin/DNA/RNA synthesis processing					
NT5CP	Dr.3959.1.A1_at	BI430015	6.7 (0.011)	9.0 (0.002)	7.6 (<0.001)
MID1 interacting protein	Dr.428.1.S1_at	BC042326.1	2.4 (0.014)	2.4 (0.008)	

TABLE 2—Continued

Gene	Affymetrix	Genbank	Fold induction (<i>p</i> value)		
			1 DPA	3 DPA	5 DPA
BTB (POZ) domain	Dr.11749.1.A1_at	AI658316			3.1 (0.007)
Histone H2A	Dr.21443.1.A1_at	AI626801			2.9 (0.011)
Immune response					
CC chemokine CCL1	Dr.8113.1.S1_at	NM_131062.1	2.3 (0.015)	3.4 (0.001)	4.5 (0.006)
Scyba	Dr.4047.1.S1_at	NM_131627.1		2.3 (<0.001)	
SOCS-3	Dr.9617.1.A1_at	CD283300			2.2 (0.04)
Clotting					
Coagulation factor V	Dr.18429.1.A1_at	AF515275.1			4.3 (0.003)
Nervous/endocrine system					
Somatostatin-1	Dr.7560.1.S1_at	AF435965.1		5.1 (0.021)	
NPTX1	Dr.8012.1.A1_at	AW280057		3.4 (0.011)	4.5 (0.01)
P2X4a	Dr.10741.1.S1_at	NM_153653.1			2.0 (0.03)
Metabolism					
GCHFR	Dr.12454.1.S1_at	BI981470		3.6 (0.001)	2.0 (0.03)
Transcobalamin II	Dr.6550.1.A1_at	BM859107		2.9 (0.039)	
ANKH	Dr.22947.1.A1_at	AW305395		2.6 (0.042)	3.0 (0.004)
ADP-ribosylation factor-like 4a	Dr.13970.1.S1_at	BC050164.1		2.2 (0.001)	
Carbonic anhydrase II	Dr.20080.1.S1_at	CA975638			3.9 (0.029)
CARP 10	Dr.13585.2.S1_at	AL915399			3.0 (0.048)
ras family GTPase	Dr.13114.1.A1_at	BQ093552			2.8 (0.011)
Troponin I	Dr.17891.1.S1_at	BQ076093			2.7 (0.003)
Argininosuccinate Synthase	Dr.4095.1.A1_at	AI957843			2.4 (0.001)
RA signaling					
Retinol dehydrogenase 14	Dr.16687.2.A1_at	BI980393		2.0 (.005)	
Other					
Zinc finger DHHC domain protein	Dr.9870.1.A1_at	BG305771	3.5 (0.007)	6.8 (<0.001)	4.2 (0.001)
Lrrc4	Dr.14757.1.A1_at	BI981263		3.8 (0.014)	2.2 (0.047)
Prostasin precursor	Dr.23312.1.A1_at	BE017586		3.3 (<0.001)	
Calpain-2 catalytic subunit	Dr.10119.1.A1_at	BE201806			3.0 (0.037)
Cytokeratin 18	Dr.9236.1.A1_at	BG727389			2.6 (0.026)

prolyl 4-hydroxylase α 1 at 3 and 5 dpa. Procollagen c-endopeptidase enhancer 2 (PCOLCE2), another collagen modifying gene was significantly reduced by TCDD only at 1 dpa and the basal expression like the two other collagen metabolizing enzymes increased over time (Fig. 5D).

Comparison of mRNA abundance determined by microarray and qRT-PCR revealed similar results especially when the response to TCDD was robust (Table 4). In order to make comparisons between the two techniques, data is displayed as fold change relative to vehicle control tissues at the same time in regeneration. The expression of all genes responded to TCDD in a similar manner whether analyzed with qRT-PCR or microarray. However there were some discrepancies in response time with some genes that expressed less than a three-fold change in response to TCDD. For instance, both methods revealed significant enhancement of CC-Chemokine and BAMBI gene expression in response to TCDD exposure using microarray analysis, however they were not responsive when using qRT-PCR at 1 dpa (Table 4). Similarly, procollagen

endopeptidase enhancer 2 (PCOLCE2) expression was repressed by TCDD exposure at all times investigated using microarray analysis. This gene product was only repressed 2.2–2.8 fold at 3 and 5 dpa, respectively, when analyzed using microarray analysis and was not found to be repressed by qRT-PCR at these time points (Table 5). Discrepancies in abundance of a transcript in Table 4 may be accounted for by differences in method, but may also be partially due to biological variability between the different biological pools of RNA that were used to conduct the different techniques.

DISCUSSION

Activation of the AHR Pathway

Fin regeneration is a complex process requiring precise regulation of several processes including wound healing, ECM production, revascularization, innervation, and bone formation. Due to the complex morphological changes during

TABLE 3
Transcripts Repressed at Least Two-Fold by TCDD Exposure in Comparison with Their Time Matched Vehicle Control

Gene	Affymetrix	Genbank	Fold reduced (<i>p</i> value)		
			1 DPA	3 DPA	5 DPA
Matrix componets/metabolism					
<i>Chondrogenesis regulation</i>					
Sox9b	Dr.11850.1.S2_at	AY029578.1	9.4 (.015)	14.4 (.002)	10.8 (.001)
RGS-2	Dr.6136.2.S1_at	BE200885			5.9 (.049)
<i>Collagen modification and catabolism</i>					
PCOLCE2	Dr.15092.1.A1_at	BM181793	3.2 (.031)	2.8 (<0.001)	2.2 (.032)
Lysyl oxidase 2 precursor	Dr.23096.1.A1_at	AW510294	2.4 (.008)		2.2 (.046)
Proline 4-hydroxylase alpha 1	Dr.3932.1.S1_at	BC045890.1	2.0 (.023)	4.7 (<0.001)	2.3 (.036)
Lysyl oxidase precursor	Dr.11427.1.S1_at	BG302899		6.1 (.013)	9.6 (.002)
Heat shock protein 47	Dr.106.1.S1_at	NM_131204.1		3.8 (.003)	
FK506 binding protein 14	Dr.6003.1.S1_at	AI974136		2.8 (<0.001)	2.5 (.032)
Lysyl hydroxylase	Dr.7688.1.A1_at	BI896329		2.7 (.009)	2.5 (.025)
PCOLCE	Dr.15554.1.A1_at	CD014267		2.6 (.009)	2.6 (.017)
Proline 4-hydroxylase alpha 2	Dr.19144.1.A1_at	AW059030		2.2 (<0.001)	
FK506 binding protein 9	Dr.23890.1.S1_at	AI943251		2.0 (.006)	
<i>Extracellular matrix componets</i>					
Cartelage Link Protein	Dr.24236.1.S1_at	AL717548	6.6 (.011)	12.9 (.02)	7.6 (.01)
Bone/cartilage proteoglycan 3	Dr.21007.1.S1_at	BQ418916	4.0 (.042)		4.4 (.038)
Dermacan	Dr.4338.1.A1_at	AI588307	3.2 (.034)	3.6 (.003)	3.8 (.02)
Col11a1 like	Dr.3536.1.A1_at	AL724939	2.7 (.017)	2.5 (.02)	
Protogalactin Gal1-L2	Dr.13015.1.S1_at	BQ131778	2.4 (.009)	2.2 (.041)	
Bone/cartilage proteoglycan I	Dr.16232.1.A1_at	BG884528		2.5 (.021)	3.1 (.001)
Col1a1	DrAffx.2.1.S1_at	AY380819			2.5 (.004)
<i>Cell-cell or cell-matrix interaction</i>					
Talin 2	Dr.15009.1.S1_at	BI979725	2.6 (.047)		
Plectin 1	DrAffx.2.25.A1_at	AY289607.1	2.6 (.001)		
Thrombospondin 3	Dr.1089.1.S1_at	NM_173225.1		2.7 (.022)	2.0 (.039)
Cyr61	DrAffx.2.91.S1_at	BI430409			4.6 (.03)
Coagulation factor XIII A chain	Dr.16206.1.A1_at	BQ285074			3.5 (.001)
Spp1 secreted phosphoprotein1	Dr.17754.1.S1_at	BQ092246			2.3 (.018)
Cadherin-11	Dr.251.1.S1_at	BQ285646			2.2 (.009)
Spondin 2b	Dr.563.1.S1_at	NM_131008.1			2.2 (.026)
Gamma-sarcoglycan	Dr.9726.1.A1_at	BE693202			2.2 (.006)
<i>Bone formation</i>					
TrATPase	Dr.1508.1.S1_at	AI882824	5.0 (.024)	2.7 (.016)	
Connexin 43	Dr.582.1.S1_a_at	NM_131038.1		4.7 (<0.001)	3.2 (.004)
BMPER	Dr.12376.1.A1_at	BG985627		2.3 (.042)	3.2 (.021)
Gadd45beta	Dr.1378.2.S1_a_at	BG739102			2.7 (.036)
Gremlin-2	Dr.19674.1.A1_at	AL920950			2.1 (.021)
<i>Non-collagen extracellular matrix metabolism and synthesis</i>					
Hyaluronan synthase 2	Dr.10465.1.S1_at	NM_153650.1		4.0 (.003)	
Sulfatase FP2b	Dr.12108.1.A1_at	BM102082		2.2 (.012)	
Spermine oxidase	Dr.11707.1.S1_at	BC044478.1			2.7 (.026)
RA signaling					
Cyp26b1	DrAffx.2.62.S1_at	AI721901		2.4 (.01)	3.4 (.007)
Cyp26a1	Dr.5757.1.S1_at	NM_131146.1			3.8 (.002)
Retinol binding protein 4	Dr.5479.1.S1_at	AW232202			3.2 (.023)
DHRS3	Dr.2644.1.A1_at	AI626374			2.4 (.013)
Retinoid×receptor alpha	Dr.348.1.S1_at	NM_131217.1			2.1 (.013)
Immune/wound response					
CIQG	Dr.14041.1.S1_at	BI429699	2.5 (.004)	2.2 (.016)	
Granulin	Dr.4748.1.S1_at	AF273481.1	2.3 (.015)		
Chemokine (C-X-C) ligand 12a	Dr.822.1.S2_at	BM184127		3.0 (.016)	2.5 (.001)

TABLE 3—Continued

Gene	Affymetrix	Genbank	Fold reduced (<i>p</i> value)		
			1 DPA	3 DPA	5 DPA
Carboxypeptidase N1	Dr.1128.1.S1_at	BI704530		2.0 (.018)	3.0 (.005)
Complement C1q TNF 5	Dr.965.1.S1_at	AI331382		2.0 (.002)	
Complement factor D precursor	Dr.1999.1.S1_at	AW232266			5.2 (.039)
Serum amyloid A-5 protein	Dr.13131.1.S1_at	BI883568			4.7 (.025)
CD99 antigen-like 2	Dr.25120.1.S1_at	AY078168.1			2.2 (.021)
Development					
CGI-38	Dr.23293.1.A1_at	BE016153	3.2 (.004)		
FABP7a	Dr.20850.1.S1_at	NM_131605.2		7.5 (.001)	7.5 (.029)
Draculin	Dr.8064.1.S1_at	NM_130977.1		4.0 (.008)	
Dapper homolog 2	Dr.6948.1.A1_at	AW117004		2.9 (.002)	
Tubulin beta 2	Dr.21550.1.S1_at	AI721474		2.7 (.001)	
Cysteine-rich protein 2	Dr.5660.1.S1_at	BC044391.1		2.7 (.001)	3.1 (.02)
DIX domain containing 1	Dr.11043.1.A1_at	AF502143.1		2.3 (.001)	
Angiopoietin-related protein 7	Dr.10141.1.A1_at	BE202096		2.3 (.016)	
Midkine-related growth factor	Dr.24241.1.S1_at	NM_131716.1		2.2 (.03)	
Zinc finger protein SLUG	Dr.20.1.S1_at	AI584287		2.1 (<0.001)	
ZIC3	Dr.25653.1.A1_at	AL719376		2.1 (.013)	
Netrin-G1 ligand precursor	Dr.5427.1.A1_at	AW170838		2.0 (.001)	
Growth/differentiation factor 8	Dr.5778.1.S1_at	AY258034.1			3.6 (.002)
Forkhead box C1b	Dr.19254.1.A1_at	BC046028.1			3.5 (.003)
Wnt11r	Dr.8297.1.S1_at	NM_131076.1			3.2 (.004)
Slit2	Dr.10489.1.S1_at	NM_131735.1			2.9 (.002)
Twist1	Dr.8121.1.S1_at	NM_130984.1			2.9 (.011)
Distal-less homeobox gene 1a	Dr.128.1.S1_at	NM_131305.1			2.9 (.008)
Olfactomedin 2	Dr.10562.1.A1_at	BC044164.1			2.5 (.029)
Lrrtm1	Dr.12811.1.A1_at	BI879769			2.5 (.002)
Tgfbr2	Dr.6407.1.A1_at	AW203007			2.4 (<0.001)
Signaling					
Heat-shock protein beta-8	Dr.5342.1.A1_at	AI878489		3.0 (.001)	
Kctd4	Dr.14133.1.A1_at	BQ092295		2.6 (<0.001)	
LMO-4	Dr.18443.1.S1_at	BC049054.1		2.1 (.04)	
Myotubularin-related protein 1	Dr.7782.1.A1_at	AW232596		2.0 (<0.001)	
cMyc	Dr.1.1.S1_at	BC053281.1		2.0 (.002)	
Rev-Erb Beta 1	Dr.6920.1.S1_at	BF937474			5.6 (.016)
FK506 binding protein 5	Dr.2675.1.A1_at	BG308728			3.9 (.014)
Sgk1	Dr.9896.1.A1_at	BI670851			3.3 (.007)
MYC I protein	Dr.16048.1.S1_at	BC045424.1			2.8 (.028)
Hsp70	Dr.20198.1.S1_a_at	NM_131397.2			2.8 (.041)
cJun	Dr.7608.2.S1_at	CD282850			2.0 (.03)
Metabolism					
HSD11b2	Dr.12359.1.A1_at	BG799163	4.6 (.03)		
Ferritin H-1	Dr.12425.5.S1_at	AW232676	2.1 (.008)		
Lactate dehydrogenase A4	Dr.4212.1.S1_at	NM_131246.1		2.1 (.002)	
Insulin-like growth factor 2	Dr.9288.1.S1_at	AF250289.1		2.1 (.017)	
Glucose transporter type 10	Dr.17311.1.A1_at	BM777825		2.1 (.01)	
Glucose transporter type 9	Dr.14747.1.A1_at	BM082885			3.8 (.003)
Insulin-like growth factor 2b	Dr.8145.1.S1_at	NM_131433.1			3.3 (.001)
GPD1	Dr.3275.1.A1_at	BG306810			2.5 (.03)
Amino acid metabolism					
Tryptophan 5-hydroxylase	Dr.17776.1.A1_at	BQ092469		6.7 (.004)	
HGD	Dr.3529.1.S1_at	NM_152966.1		4.3 (.009)	7.8 (.001)
Tryptophan hydroxylase 1	Dr.15819.1.A1_at	BM533772		4.2 (.008)	
BHMT	Dr.2596.1.S1_a_at	BM342901		3.4 (.014)	4.6 (.002)
SLC1a4	Dr.25217.1.S1_at	AI943367		2.8 (<0.001)	2.1 (.042)
Cysteine dioxygenase type I	Dr.8833.1.S1_at	AI957754		2.6 (.021)	3.2 (.034)
Phenylalanine hydroxylase	Dr.4171.1.A1_at	AW421213		2.6 (.008)	3.6 (.002)

TABLE 3—Continued

Gene	Affymetrix	Genbank	Fold reduced (<i>p</i> value)		
			1 DPA	3 DPA	5 DPA
CAT-3	Dr.3740.1.A1_at	AI721361		2.0 (.005)	2.3 (.004)
MAT1A	Dr.3498.1.S1_at	BC045343.1			2.7 (.05)
Glutamine synthetase	Dr.4147.1.S1_at	NM_181559.1			2.3 (.013)
PCBD	Dr.14639.1.S1_at	AF473825.1			2.1 (.008)
Nucleotide metabolism					
GTP cyclohydrolase 1	Dr.14668.1.S1_at	AL717907		3.0 (.015)	5.4 (.002)
Guanine deaminase	Dr.23036.1.A1_at	CD014969			2.7 (.002)
DNA-damage-inducible beta	Dr.1378.1.S1_at	BE016131			2.0 (.04)
RNA Syn/Processin					
Napor	Dr.Affx.1.42.S1_s_at	AF321194.1			2.3 (.002)
Poly A binding protein 1a	Dr.11392.1.A1_at	BQ480987			2.2 (.016)
Calcium and G protein signaling					
Reticulocalbin 1	Dr.1232.1.A1_at	AI793417		3.0 (.008)	
Synaptotagmin IV	Dr.13868.1.S1_at	BI842004		2.7 (.017)	
Copine-2	Dr.5965.1.A1_at	BI982036		2.2 (.022)	2.1 (.003)
Adenylate kinase isoenzyme 4	Dr.1707.1.S1_at	AI667106		2.1 (.018)	2.4 (.005)
GNG3	Dr.10136.1.S1_at	NM_131841.1			2.4 (.004)
INPP5F	Dr.21368.1.A1_at	AI601721			2.0 (.032)
Other					
17-beta-HSD 8	Dr.19643.1.A1_at	AL918850	2.2 (<0.001)	5.6 (.008)	
Calbindin 2	Dr.7048.1.A1_at	AW826677		4.6 (.001)	
CDKN1C	Dr.3502.1.S1_at	AI444436		3.0 (.001)	2.8 (.026)
Carboxypeptidase-like protein X2	Dr.1484.1.A1_at	BM081849		2.8 (.043)	2.8 (.002)
Stromal cell-derived factor 2 like 1	Dr.25140.6.S1_at	BI865716		2.9 (.001)	
Myosin I alpha;	Dr.4817.1.A1_at	AI641073		2.0 (.019)	
Cytoglobin	Dr.4925.1.S1_x_at	NM_152952.1		2.4 (.002)	
Vacuole membrane protein 1	Dr.982.1.S1_at	BI877516		2.3 (.002)	
Dermal papilla derived protein 7	Dr.4293.1.S1_at	BI709309		2.3 (.004)	
ERGIC-53 protein	Dr.6822.1.A1_at	BQ284855		2.1 (.001)	
Cyp8B1	Dr.20589.2.A1_at	BE605469			4.3 (.007)
Vimentin	Dr.8242.1.A1_at	NM_131872.1			3.5 (.002)
ERBB2 1b	Dr.10199.1.S1_at	AI477430			2.7 (.023)
Type I cytokeratin	Dr.5531.1.S1_a_at	BE200701			2.6 (.036)
Scavenger receptor class B1	Dr.6652.1.S1_at	BI983877			2.5 (.047)
Annexin A6	Dr.7658.1.S1_at	AW116978			2.4 (.001)
LMBR1L	Dr.19947.1.S1_at	BC044535.1			2.2 (.038)
Deiodinase, iodothyronine, type II	Dr.12949.1.S1_at	CA975553			2.2 (.033)
SCARB1	Dr.6652.1.S2_at	BC044516.1			2.1 (.037)
Angiotensin-related protein 5	Dr.7681.1.A1_at	AW117142			2.1 (.002)
MPDU1	Dr.1439.1.S1_at	AI588415			2.0 (.042)

regeneration, it is not surprising that enormous transcriptional changes are required to complete this process. Since TCDD inhibits regeneration, the identification of the genes altered by TCDD at the site of toxicity over time is crucial to increase our understanding of the mechanism of toxicity. We know that this regenerative toxicity in the fin is dependent upon AHR2 and ARNT1 signaling, since toxicity is averted if either of these genes are repressed in larval zebrafish during fin regeneration (Mathew *et al.*, 2005). The AHR pathway is active in the regenerating fin prior to outgrowth since cytochrome P4501A (CYP1A), the most well documented AHR regulated gene, is induced by TCDD as early as 1 day following amputation. Induction of CYP1A is not thought to be essential for TCDD

toxicity in zebrafish since translational repression of CYP1A in zebrafish embryos did not alter their response to TCDD (Carney *et al.*, 2004). In addition to CYP1A, we have identified several known AHR regulated xenobiotic metabolizing genes that were induced by TCDD as early as 1 dpa (early responders). Analysis of the promoters of these early responders that remained elevated through 5 dpa revealed ten genes (CYP1A1, CYP1C1, Sulfotransferase, Sulfide:quinone oxidoreductase, MMP13, Cadherin-19, Protein phosphatase 1 regulatory subunit 3A, Endothelial cell-specific molecule 1, Cytochrome b5, Zinc finger DHHC domain protein) that had at least one XRE within -4000 bp of the transcription start site. This suggests that they could be directly regulated by AHR;

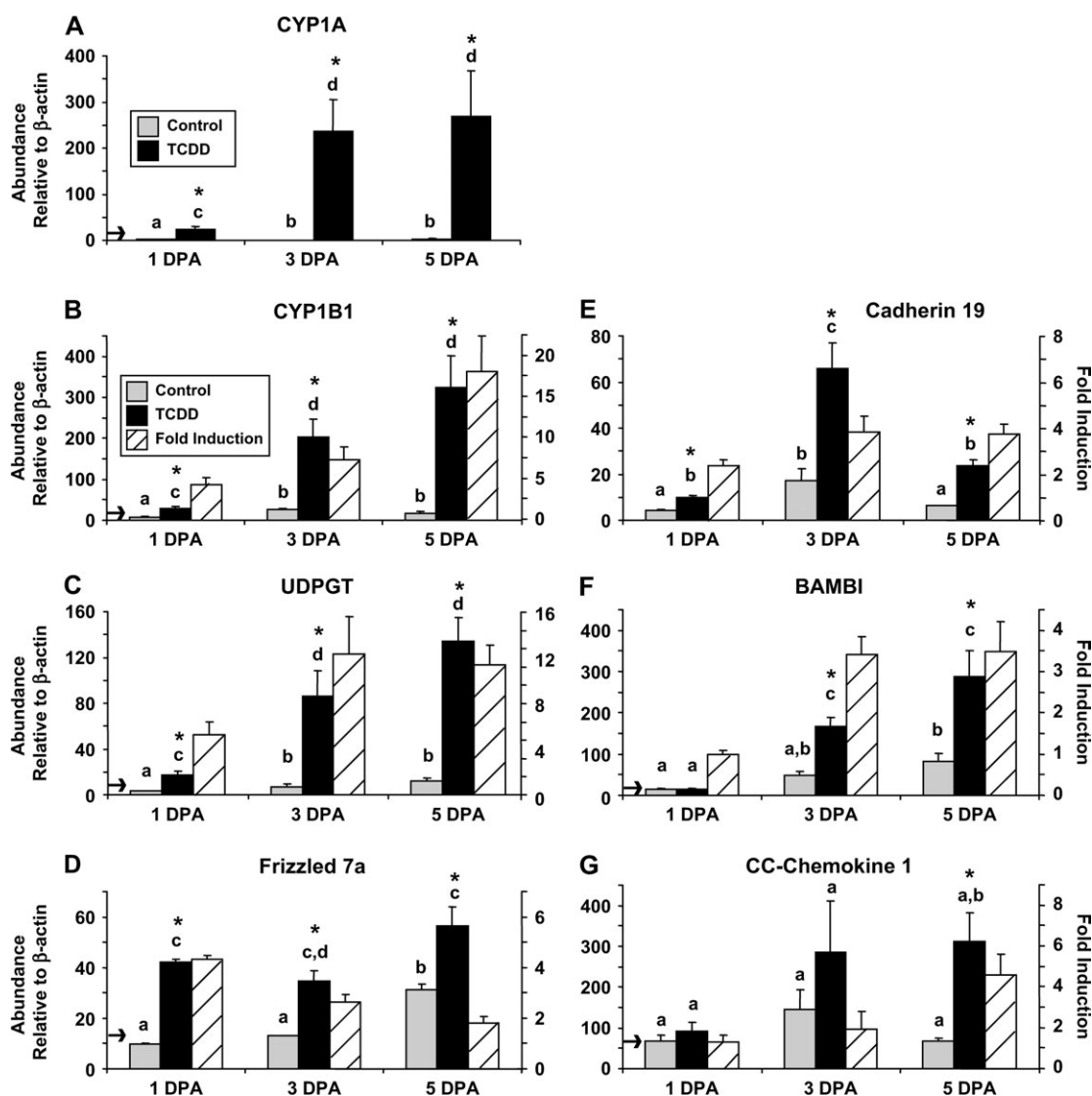


FIG. 4. qRT-PCR detection of TCDD enhanced mRNAs in vehicle or TCDD dosed fish. Gene specific primers were used to quantify mRNAs using real-time PCR. Abundance of (A) CYP1A (B) CYP1B1 (C) UDPGT (D) Frizzled 7a (E) Cadherin 19 (F) BAMBI and (G) CC-Chemokine 1 mRNAs are illustrated as raw data normalized to β -actin mRNA levels and as fold induction in TCDD exposed samples. The low abundance of CYP1A in vehicle control animals lead to an artificially high fold induction (low denominator) and therefore is not depicted. The arrow head on the y-axis indicates the level of expression in non-regenerating fins. Cadherin 19 was not determined in non-regenerating fins. Samples were run concurrently with standard curves dilutions derived from plasmid cDNA or gel purified gene specific PCR products for each primer set. Each bar represents the mean \pm SEM ($n = 3$). Two-way ANOVA was conducted to determine differences in time and treatment expression and was followed by Tukeys test. Asterisk (*) indicates significant difference at the same time in regeneration between vehicle (control) and TCDD exposed fish ($p < 0.05$).

however, this must be experimentally confirmed. Genes differentially regulated by TCDD in embryonic zebrafish, and adult brain, liver and testes of adult Medaka were not identified as being similarly regulated in the regenerating fin with the exception of xenobiotic metabolizing enzymes (Handley-Goldstone *et al.*, 2005; Volz *et al.*, 2005). This is most likely due to the difference in tissue types. However, global gene expression studies in mammalian systems and the regenerating zebrafish fin have revealed misregulation of common pathways suggestive of similar genomic responses, and mechanisms of TCDD toxicity across species. It is noteworthy that genes related

to the ECM and the retinoid pathway are misregulated in mammals and in the regenerating fin (Boverhof *et al.*, 2005; Fletcher *et al.*, 2005b; Frueh *et al.*, 2001; Guo *et al.*, 2004; Hanlon *et al.*, 2005; Mizutani *et al.*, 2004; Thackaberry *et al.*, 2005).

Extracellular Matrix Related Transcripts

The largest category of genes misregulated by TCDD are those related to ECM composition and metabolism. TCDD exposure resulted in repression of 34 of the 41 genes in this

TABLE 4
Comparative Summary of mRNAs Elevated by TCDD Using Microarray and qRT-PCR

	Fold induction					
	Array			qRT-PCR		
	Days post amputation					
	1	3	5	1	3	5
CYP1A1	<i>100</i>	<i>95</i>	<i>40</i>	<i>15</i>	<i>1410</i>	<i>142</i>
CYP1B1	1.3	4.4	2.8	4.3	7.4	18.2
UDPGT	8.1	9.4	8.2	5.2	12.2	11.3
Frizzled 7a	5.4	3.8	3.1	4.3	2.6	1.8
Cadherin 19	3.2	3.4	3.2	2.4	3.8	3.7
BAMBI	2.2	2.1	3.5	1.0	3.4	3.3
CC-Chemokine	2.3	3.4	4.5	1.3	2.0	4.6

Note. Italicized cells represent significant effect of TCDD compared to time matched control as described in the previous tables and figures.

category. This suggests that TCDD impairs the maturation of the ECM. This could occur by directly altering the expression of ECM genes or by impacting genes responsible for controlling cell differentiation and matrix maturation. Structural differences in ECM can occur due to impaired synthesis or catabolism of ECM components including collagen. This is noteworthy as pharmacological inhibition of matrix metalloproteinases (MMPs), proteins involved in metabolism of ECM constituents including collagen, inhibit blastemal proliferation and fin regeneration (Bai *et al.*, 2005). Moreover,

proper regulation of the plasminogen and MMP pathway is known to regulate ECM composition and wound healing (reviewed in Lund *et al.*, 1999). Additionally, numerous drugs known to affect metabolism of collagen, one of the principal constituents of ECM, have been shown to impair collagen organization and fin regeneration (Bechara *et al.*, 2000). Evidence here suggests that both post-translational modifications and metabolism of collagen is impaired by TCDD.

Collagens function to provide tensile strength to tissues and are also involved in cell adhesion, chemotaxis, migration,

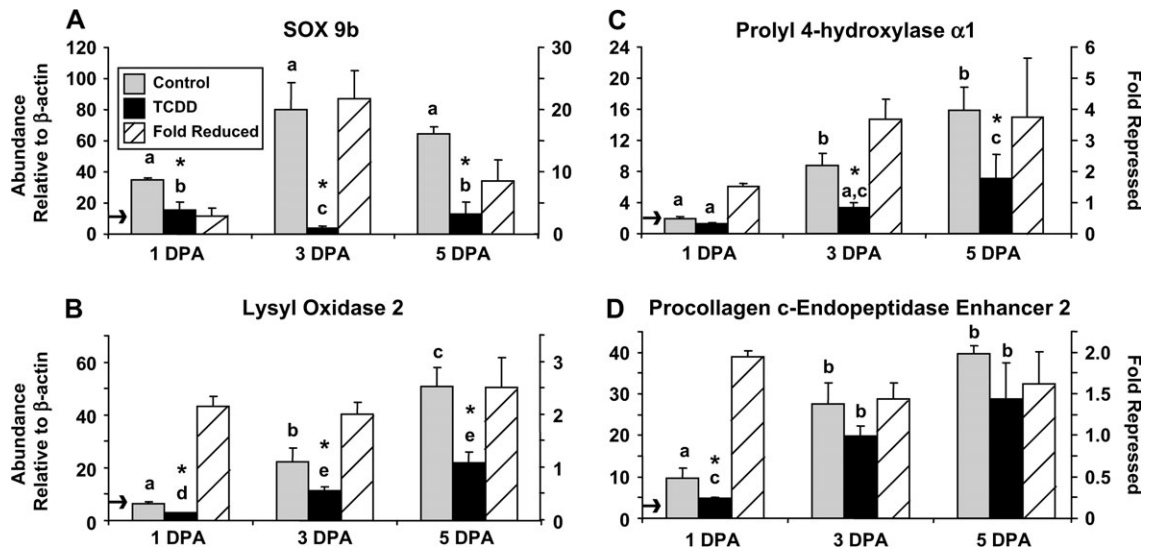


TABLE 5
Comparative Summary of mRNAs Reduced by TCDD Using Microarray and qRT-PCR

	Fold repressed							
	Array			qRT-PCR				
	Days post amputation							
	1	3	5	1	3	5	5	
Sox 9b	<i>9.4</i>	<i>14.4</i>	<i>10.8</i>	3.0	21.7	8.5		
Lysyl Oxidase 2	<i>2.4</i>	<i>1.8</i>	<i>2.2</i>	2.1	2.0	2.5		
Prolyl 4-hydroxylase α 1	<i>2.0</i>	<i>4.7</i>	<i>2.3</i>	1.5	3.7	3.7		
PCOLCE2	<i>3.2</i>	<i>2.8</i>	<i>2.2</i>	2.0	1.4	1.6		

Note. Italicized cells represent significant effect of TCDD compared to time matched control as described in the previous tables and figures.

differentiation, and wound healing. In addition to catabolism by MMPs, proper collagen structure can be compromised by altering synthesis, trafficking, post-translational modification, and fibril assembly reviewed below (reviewed in Canty and Kadler, 2005). In addition to the repression of procollagen 1a1 and 11a1 message by TCDD, the abundance of 11 transcripts involved in collagen synthesis and catabolism were impacted. Abundance of 9 transcripts involved in post translational modification of collagen were repressed by TCDD in the fin. Prolyl-4-hydroxylase α 1 and α 2 (P4H) were both repressed beginning at 1 and 3 dpa respectively. P4H functions to hydroxylate proline residues of collagen in the ER thereby stabilizing the triple helical structure and increasing the denaturing temperature. The observed 3 dpa reduction in lysyl hydroxylase would be expected to impair collagen processing in the ER since it serves to generate sites of carbohydrate attachment and helps to stabilize collagen crosslinks. Impaired hydroxylation of procollagen can lead to its retention in the ER. TCDD reduced the mRNA of three genes, HSP47 and the FK506 binding proteins 9 and 14, involved in proper folding of procollagen chains in the ER, beginning at 3 dpa. FK506 binding proteins 9 and 14 contain peptidylproline *cis-trans* isomerase activity which converts proline to the *trans* conformation allowing proper protein folding. HSP47, repressed by TCDD at 3 dpa, acts as a chaperone involved in folding procollagen in the ER. Fibrillar collagens (types I–III, V, and XI) contain N and C-terminal propeptides that are removed by procollagen peptidases allowing the mature collagen to form fibrils in the ECM. Procollagen C-endopeptidase enhancers 2 and 1 (PCOLCE 2 and 1), repressed by 1 and 3 dpa respectively bind to the C-terminal propeptide enhancing the cleavage of the propeptide by proteases. Collagen fibril assembly may be misregulated since lysyl oxidases (2 and 1), repressed at 1 and 3 dpa, catalyze the first step in cross linking of collagen fibrils in the ECM.

The potential for alteration in ECM turnover by TCDD also exists since MMP13 (collagenase-3) message was greatly enhanced by TCDD exposure throughout the five days of

regeneration. This is not unique to zebrafish since TCDD exposure results in elevated collagenase (MMP1 or 13) in cell culture and in the mouse heart (Partridge *et al.*, 2000; Thackaberry *et al.*, 2005; Villano *et al.*, 2006; Vogel *et al.*, 2004). TIMP2 (tissue inhibitor of metalloproteinase 2, like) was elevated at 5 dpa. TIMP2 inhibits MMP2 activation. In addition to serving as structural architecture, the ECM also serves as a source and store of biologically active molecules which can be released and or activated by MMPs affecting cell growth, cell migration, cell-cell communication and apoptosis, angiogenesis (reviewed in Mott and Werb, 2004). By this mechanism, alterations in collagen abundance and structure could also affect availability of extracellular signaling molecules such as bFGF, TGF- β , and VEGF.

Misexpression of a regulator of ECM production or cell differentiation fate could explain the TCDD-dependent changes in ECM transcript abundance. The HMG-box DNA-binding transcription factor SRY-box 9 (Sox9) may play this role in the regenerating fin. A functional role of Sox9 was first discovered in humans with heterozygous mutations in and around the Sox9 gene resulting in skeletal malformations known as campomelic dysplasia (CD), that is often associated with XY sex reversal and other malformations of internal organs (reviewed in Schafer *et al.*, 1996). Sox9 is considered essential for commitment of mesenchymal cells to chondrocyte differentiation and cartilage formation (Bi *et al.*, 1999; Mori-Akiyama *et al.*, 2003). ECM constituents enhanced by Sox9 during chondrogenesis include procol2a1, procol9a1, procol11a2, CD Rap, aggrecan, cartilage link protein (reviewed in de Crombrugge *et al.*, 2000). In zebrafish there are two Sox9 orthologs, Sox9a and Sox9b, which have distinct and overlapping expression patterns during development which additively are thought to duplicate the mammalian Sox9 function (Chiang *et al.*, 2001; Yan *et al.*, 2005). Both Sox9a and 9b bind specific DNA response elements and have similar transactivation properties (Chiang *et al.*, 2001). Studies using Sox9 mutant variant zebrafish and antisense translational repression determined that both Sox9 genes play an essential

role in chondrogenesis with Sox9b involved in attaining proper numbers of chondrocytes (Yan *et al.*, 2005). This is striking because during development, TCDD exposure impairs chondrogenesis in the head in an AHR2 dependent manner (Prasch *et al.*, 2003; Teraoka *et al.*, 2002). The TCDD dependent morphology remarkably resembles Sox9b morphants (antisense repressed) suggesting that Sox9b could be impaired by TCDD during development (Yan *et al.*, 2005). It may be that Sox9b is required to lay down a provisional matrix in the regenerating fin giving it form, while the tissue is regenerating. Sox9b message was indeed enhanced by amputation 4–8 fold during the first five days of regeneration in comparison to non-regenerating fin tissue. However Sox9b was the most strongly reduced transcript by TCDD during the first five days of regeneration, being repressed to levels similar to non-regenerating fins. Whether Sox9b is directly regulated by AHR2 remains to be determined.

Evidence of functional repression of Sox9 can be illustrated by reduced expression of Sox9 dependent transcripts and repressed chondrogenic markers. This was observed in the fin as cartilage link protein (CRTL) the well-characterized Sox9 dependent gene (Kou and Ikegawa, 2004) was the second strongest repressed gene by TCDD in the regenerates. CRTL plays a crucial role in chondrogenesis by stabilizing proteoglycan aggregates, giving cartilage its tensile strength, elasticity, and organization of chondrocytes. Targeted disruption of CRTL1 is lethal in mice and results in severe chondrodysplasia (Czipri *et al.*, 2003). Of additional importance, once CRTL is proteolytically cleaved, the N-terminus can stimulate proteoglycan and type II collagen synthesis contributing to the ECM (Liu *et al.*, 2000). Another indicator of impaired chondrogenesis is the reduced expression of the regulator of G-protein signaling 2 (RGS-2) at 5 dpa. RGS-2 was recently found to promote late stage chondrogenesis (James *et al.*, 2005).

Transcriptional regulation of Sox9 is not well understood. A potential modifier of Sox9b expression in response to TCDD is the retinoic acid pathway. Sox9 expression responds to retinoic acid in a cell or tissue specific manner, either with enhanced or repressed expression (Afonja *et al.*, 2002; Sekiya *et al.*, 2000, 2001). TCDD is known to alter retinoid storage and metabolism of retinoic acid in liver, kidney, and plasma (Fletcher *et al.*, 2005a). Transcripts of several retinoic acid responsive genes including *cyp26a1*, *cyp26b1*, and short-chain dehydrogenase/reductase 3, each involved in retinoid metabolism, were repressed by TCDD, although only at 3 and 5 dpa. If a repressed retinoid pathway leads to reduced Sox9b expression as early as 1 dpa, then other RA responsive genes should be similarly down regulated. This remains to be determined. However the potential for RA regulation of Sox9b exists since the Sox9b promoter has 10 RAR elements within –4000 bp of the translation start site. Additionally Sox9a has no RAR response elements and only one RXR element suggesting that Sox9b would be preferentially regulated by the RA signaling.

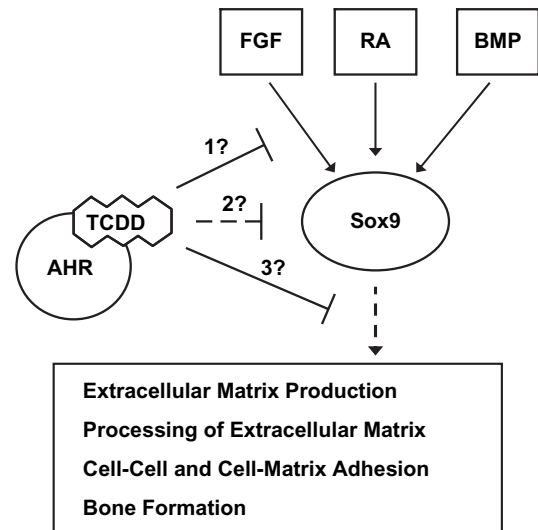


FIG. 6. Potential mechanisms and consequences of Sox9 repression by TCDD. (1) AHR may alter signaling of the FGF, retinoid, and BMP pathways which are all involved in Sox9 expression and fin regeneration. (2) AHR may directly interfere with Sox9 expression or message stability or it could act indirectly through another factor such as AHRR. (3) AHR may act through Sox9 or directly alter ECM environment.

Potential rationale for and consequences of reduced Sox9b expression is illustrated in Figure 6. AHR could alter Sox9 signaling by impairing its expression directly through RA signaling or other pathways such as FGF and BMP signaling known to regulate fin regeneration and Sox9 expression. AHR may interfere with Sox9b expression at its enhancer-promoter directly or through other negative regulators such as AHRR or alter Sox9b message stability. Ultimately the observed alteration in ECM constituents could be directed through AHR via Sox9b or through other AHR mediated mechanisms. The functional result of repressed Sox9b would be impaired EMC production and processing, cell adhesion, and bone formation.

Taken together, these results demonstrate that the regenerating fin is a dynamic tissue characterized by enormous alterations in transcript abundance within five days of amputation. The response to TCDD is drastic with complex changes in the transcriptome over time. The most striking functional category of altered transcripts are those involved in ECM composition or its regulation. It will be essential to determine the role of these responsive genes using the power of the zebrafish model, and to determine if similar TCDD responses occur in other regenerative and developmental models.

ACKNOWLEDGMENTS

We would like to thank Anne-Marie Girard from the Center for Gene Research and Biotechnology at Oregon State University for her valuable assistance. We would also like to thank Eric Johnson and Greg Gonnerman for their technical assistance. This work was supported in part by NIEHS grants ES10820, ES00210, and ES03850.

REFERENCES

- Afonja, O., Raaka, B. M., Huang, A., Das, S., Zhao, X., Helmer, E., Juste, D., and Samuels, H. H. (2002). RAR agonists stimulate SOX9 gene expression in breast cancer cell lines: Evidence for a role in retinoid-mediated growth inhibition. *Oncogene* **21**, 7850–7860.
- Andreasen, E. A., Hahn, M. E., Heideman, W., Peterson, R. E., and Tanguay, R. L. (2002). The zebrafish (*Danio rerio*) aryl hydrocarbon receptor type 1 (zfAHR1) is a novel vertebrate receptor. *Mol. Pharmacol.* **62**, 234–249.
- Bai, S., Thummel, R., Godwin, A. R., Nagase, H., Itoh, Y., Li, L., Evans, R., McDermott, J., Seiki, M., and Sarra, M. P., Jr. (2005). Matrix metalloproteinase expression and function during fin regeneration in zebrafish: analysis of MT1-MMP, MMP2 and TIMP2. *Matrix Biol.* **24**, 247–260.
- Bechara, I. J., Joazeiro, P. P., Mari-Beffa, M., Becerra, J., and Montes, G. S. (2000). Collagen-affecting drugs impair regeneration of teleost tail fins. *J. Submicrosc. Cytol. Pathol.* **32**, 273–280.
- Bi, W., Deng, J. M., Zhang, Z., Behringer, R. R., and de Crombrugge, B. (1999). Sox9 is required for cartilage formation. *Nat. Genet.* **22**, 85–89.
- Birnbaum, L. S., and Tuomisto, J. (2000). Non-carcinogenic effects of TCDD in animals. *Food Addit. Contam.* **17**, 275–288.
- Boverhof, D. R., Burgoon, L. D., Tashiro, C., Chittim, B., Harkema, J. R., Jump, D. B., and Zacharewski, T. R. (2005). Temporal and dose-dependent hepatic gene expression patterns in mice provide new insights into TCDD-mediated hepatotoxicity. *Toxicol. Sci.* **85**, 1048–1063.
- Canty, E. G., and Kadler, K. E. (2005). Procollagen trafficking, processing and fibrillogenesis. *J. Cell Sci.* **118**, 1341–1353.
- Carney, S. A., Peterson, R. E., and Heideman, W. (2004). 2,3,7,8-Tetrachlorodibenzo-p-dioxin activation of the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator pathway causes developmental toxicity through a CYP1A-independent mechanism in zebrafish. *Mol. Pharmacol.* **66**, 512–521.
- Chiang, E. F., Pai, C. I., Wyatt, M., Yan, Y. L., Postlethwait, J., and Chung, B. (2001). Two sox9 genes on duplicated zebrafish chromosomes: Expression of similar transcription activators in distinct sites. *Dev. Biol.* **231**, 149–163.
- Czipri, M., Otto, J. M., Cs-Szabo, G., Kamath, R. V., Vermes, C., Firneisz, G., Kolman, K. J., Watanabe, H., Li, Y., Roughley, P. J., et al. (2003). Genetic rescue of chondrodysplasia and the perinatal lethal effect of cartilage link protein deficiency. *J. Biol. Chem.* **278**, 39214–39223.
- de Crombrugge, B., Lefebvre, V., Behringer, R. R., Bi, W., Murakami, S., and Huang, W. (2000). Transcriptional mechanisms of chondrocyte differentiation. *Matrix Biol.* **19**, 389–394.
- Fernandez-Salguero, P. M., Hilbert, D. M., Rudikoff, S., Ward, J. M., and Gonzalez, F. J. (1996). Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity. *Toxicol. Appl. Pharmacol.* **140**, 173–179.
- Fletcher, N., Giese, N., Schmidt, C., Stern, N., Lind, P. M., Viluksela, M., Tuomisto, J. T., Tuomisto, J., Nau, H., and Hakansson, H. (2005a). Altered retinoid metabolism in female Long-Evans and Han/Wistar rats following long-term 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treatment. *Toxicol. Sci.* **86**, 264–272.
- Fletcher, N., Wahlstrom, D., Lundberg, R., Nilsson, C. B., Nilsson, K. C., Stockling, K., Hellmold, H., and Hakansson, H. (2005b). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) alters the mRNA expression of critical genes associated with cholesterol metabolism, bile acid biosynthesis, and bile transport in rat liver: a microarray study. *Toxicol. Appl. Pharmacol.* **207**, 1–24.
- Frueh, F. W., Hayashibara, K. C., Brown, P. O., and Whitlock, J. P., Jr. (2001). Use of cDNA microarrays to analyze dioxin-induced changes in human liver gene expression. *Toxicol. Lett.* **122**, 189–203.
- Gu, Y. Z., Hogenesch, J. B., and Bradfield, C. A. (2000). The PAS superfamily: Sensors of environmental and developmental signals. *Annu. Rev. Pharmacol. Toxicol.* **40**, 519–561.
- Guo, J., Sartor, M., Karyala, S., Medvedovic, M., Kann, S., Puga, A., Ryan, P., and Tomlinson, C. R. (2004). Expression of genes in the TGF-beta signaling pathway is significantly deregulated in smooth muscle cells from aorta of aryl hydrocarbon receptor knockout mice. *Toxicol. Appl. Pharmacol.* **194**, 79–89.
- Hahn, M. E., Karchner, S. I., Shapiro, M. A., and Perera, S. A. (1997). Molecular evolution of two vertebrate aryl hydrocarbon (dioxin) receptors (AHR1 and AHR2) and the PAS family. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13743–13748.
- Handley-Goldstone, H. M., Grow, M. W., and Stegeman, J. J. (2005). Cardiovascular gene expression profiles of dioxin exposure in zebrafish embryos. *Toxicol. Sci.* **85**, 683–693.
- Hanlon, P. R., Zheng, W., Ko, A. Y., and Jefcoate, C. R. (2005). Identification of novel TCDD-regulated genes by microarray analysis. *Toxicol. Appl. Pharmacol.* **202**, 215–228.
- James, C. G., Appleton, C. T., Ulici, V., Underhill, T. M., and Beier, F. (2005). Microarray analyses of gene expression during chondrocyte differentiation identifies novel regulators of hypertrophy. *Mol. Biol. Cell.* **16**, 5316–5333.
- Kou, I., and Ikegawa, S. (2004). SOX9-dependent and -independent transcriptional regulation of human cartilage link protein. *J. Biol. Chem.* **279**, 50942–50948.
- Liu, H., McKenna, L. A., and Dean, M. F. (2000). An N-terminal peptide from link protein can stimulate biosynthesis of collagen by human articular cartilage. *Arch. Biochem. Biophys.* **378**, 116–122.
- Lund, L. R., Romer, J., Bugge, T. H., Nielsen, B. S., Frandsen, T. L., Degen, J. L., Stephens, R. W., and Dano, K. (1999). Functional overlap between two classes of matrix-degrading proteases in wound healing. *Embo. J.* **18**, 4645–4656.
- Mathew, L. K., Andreasen, E. A., and Tanguay, R. L. (2005). Ahr activation inhibits regenerative growth. *Mol. Pharmacol.* **69**, 257–265.
- Mimura, J., Yamashita, K., Nakamura, K., Morita, M., Takagi, T. N., Nakao, K., Ema, M., Sogawa, K., Yasuda, M., Katsuki, M., et al. (1997). Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. *Genes Cells* **2**, 645–654.
- Mizutani, T., Yoshino, M., Satake, T., Nakagawa, M., Ishimura, R., Tohyama, C., Kokame, K., Kangawa, K., and Miyamoto, K. (2004). Identification of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-inducible and -suppressive genes in the rat placenta: Induction of interferon-regulated genes with possible inhibitory roles for angiogenesis in the placenta. *Endocr. J.* **51**, 569–577.
- Mori-Akiyama, Y., Akiyama, H., Rowitch, D. H., and de Crombrugge, B. (2003). Sox9 is required for determination of the chondrogenic cell lineage in the cranial neural crest. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 9360–9365.
- Mott, J. D., and Werb, Z. (2004). Regulation of matrix biology by matrix metalloproteinases. *Curr. Opin. Cell. Biol.* **16**, 558–564.
- Partridge, N. C., Fiacco, G. J., Walling, H. W., Barmina, O. Y., Jeffrey, J. J., and Ruh, M. F. (2000). Effects of dioxin and estrogen on collagenase-3 in UMR 106–01 osteosarcoma cells. *Arch. Biochem. Biophys.* **382**, 182–188.
- Poss, K. D., Keating, M. T., and Nechiporuk, A. (2003). Tales of regeneration in zebrafish. *Dev. Dyn.* **226**, 202–210.
- Prasch, A. L., Heideman, W., and Peterson, R. E. (2004). ARNT2 is not required for TCDD developmental toxicity in zebrafish. *Toxicol. Sci.* **82**, 250–258.
- Prasch, A. L., Tanguay, R. L., Mehta, V., Heideman, W., and Peterson, R. E. (2006). Identification of zebrafish ARNT1 homologs: TCDD toxicity in the developing zebrafish requires ARNT1. *Mol. Pharmacol.* [Epub ahead of print] PMID: 16306231.
- Prasch, A. L., Teraoka, H., Carney, S. A., Dong, W., Hiraga, T., Stegeman, J. J., Heideman, W., and Peterson, R. E. (2003). Aryl hydrocarbon receptor 2 mediates 2,3,7,8-tetrachlorodibenzo-p-dioxin developmental toxicity in zebrafish. *Toxicol. Sci.* **76**, 138–150.
- Schafer, A. J., Foster, J. W., Kwok, C., Weller, P. A., Guioli, S., and Goodfellow, P. N. (1996). Campomelic dysplasia with XY sex reversal: Diverse

- phenotypes resulting from mutations in a single gene. *Ann. N.Y. Acad. Sci.* **785**, 137–149.
- Sekiya, I., Koopman, P., Tsuji, K., Mertin, S., Harley, V., Yamada, Y., Shinomiya, K., Niguji, A., and Noda, M. (2001). Transcriptional suppression of Sox9 expression in chondrocytes by retinoic acid. *J. Cell. Biochem.* **81**, 71–78.
- Sekiya, I., Tsuji, K., Koopman, P., Watanabe, H., Yamada, Y., Shinomiya, K., Nifuji, A., and Noda, M. (2000). SOX9 enhances aggrecan gene promoter/enhancer activity and is up-regulated by retinoic acid in a cartilage-derived cell line, TC6. *J. Biol. Chem.* **275**, 10738–10744.
- Tanguay, R. L., Andreasen, E. A., Walker, M. K., and Peterson, R. E. (2003). Dioxin toxicity and aryl hydrocarbon receptor signaling in fish. In *Dioxins and Health* (A. Schechter, Ed.), pp. 603–628. Plenum Press, New York.
- Teraoka, H., Dong, W., Ogawa, S., Tsukiyama, S., Okuhara, Y., Niiyama, M., Ueno, N., Peterson, R. E., and Hiraga, T. (2002). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin toxicity in the zebrafish embryo: Altered regional blood flow and impaired lower jaw development. *Toxicol. Sci.* **65**, 192–199.
- Thackaberry, E. A., Jiang, Z., Johnson, C. D., Ramos, K. S., and Walker, M. K. (2005). Toxicogenomic profile of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the murine fetal heart: Modulation of cell cycle and extracellular matrix genes. *Toxicol. Sci.* **88**, 231–241.
- Villano, C. M., Murphy, K. A., Akintobi, A., and White, L. A. (2006). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces matrix metalloproteinase (MMP) expression and invasion in A2058 melanoma cells. *Toxicol. Appl. Pharmacol.* **210**, 212–224.
- Vogel, C. F., Sciallo, E., and Matsumura, F. (2004). Activation of inflammatory mediators and potential role of ah-receptor ligands in foam cell formation. *Cardiovasc. Toxicol.* **4**, 363–373.
- Volz, D. C., Bencic, D. C., Hinton, D. E., Law, J. M., and Kullman, S. W. (2005). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) induces organ-specific differential gene expression in male Japanese medaka (*Oryzias latipes*). *Toxicol. Sci.* **85**, 572–584.
- Yan, Y. L., Willoughby, J., Liu, D., Crump, J. G., Wilson, C., Miller, C. T., Singer, A., Kimmel, C., Westerfield, M., and Postlethwait, J. H. (2005). A pair of Sox: Distinct and overlapping functions of zebrafish sox9 co-orthologs in craniofacial and pectoral fin development. *Development* **132**, 1069–1083.
- Zodrow, J. M., and Tanguay, R. L. (2003). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin inhibits zebrafish caudal fin regeneration. *Toxicol. Sci.* **76**, 151–161.