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Research paper

A novel stress hormone response gene in tadpoles of *Xenopus tropicalis*

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ABSTRACT

Previous work identified a transcribed locus, Str. 34945, induced by the frog stress hormone corticosterone (CORT) in *Xenopus tropicalis* tails. Because thyroid hormone had no influence on its expression, Str. 34945 was dubbed the first "CORT-only" gene known from tadpoles. Here, we examine the genomic annotation for this transcript, hormone specificity, time course of induction, tissue distribution, and developmental expression profile. The location of Str. 34945 on the *X. tropicalis* genome lies between the genes *ush1g* (Usher syndrome 1G) and *fads6* (fatty acid desaturase 6). A blast search showed that it maps to the same region on the *X. laevis* genome, but no hits were found in the human genome. Using RNA-seq data and conventional reverse transcriptase PCR and sequencing, we show that Str. 34945 is part of the 3′ untranslated region of *ush1g*. We find that CORT but not aldosterone or thyroid hormone treatment induces Str. 34945 in tadpole tails and that expression of Str. 34945 achieves maximal expression within 12–24 h of CORT treatment. Among tissues, Str. 34945 is induced to the highest degree in tail, with lesser induction in lungs, liver, and heart, and no induction in the brain or kidney. During natural metamorphosis, Str. 34945 expression in tails peaks at metamorphic climax. The role of *ush1g* in metamorphosis is not understood, but the specificity of its hormone response and its expression in tail make *ush1g* valuable as a marker of CORT-response gene induction independent of thyroid hormone.

1. Introduction

Stress hormones induce a wide range of structural and functional changes in tissues and processes during development that are essential for survival, such as organ maturation and timing of birth (Fowden et al., 1998; Liggins, 1994). Stress hormones also mediate the effects of environmental stressors (Denver, 2009; Hu, et al., 2008; Maher, et al., 2013). When the levels of stress hormones deviate from normal during early-life development, lifelong negative consequences may occur (Crespi and Warne, 2013; Harris and Seckl, 2011). In addition, stress hormones often do not act alone but in concert with other hormones, especially thyroid hormone (Bonett et al., 2010). Despite these critical roles of stress hormones and interactions with thyroid hormone in numerous tissues during development, the underlying tissue-specific molecular developmental pathways and stress hormone-regulated genes are not well characterized.

Amphibian metamorphosis is a valuable model to study the role of hormones and gene regulation due to its exquisite sensitivity and dramatic dependence on thyroid and stress hormones and ease of endocrine manipulation (Buchholz, 2015). The effects of stress hormone on development and subsequent altered stress hormone levels are conserved in tadpoles and humans (Buchholz, 2015). To begin to elucidate the genes involved in stress hormone-regulated development, we previously carried out a microarray study using *Xenopus tropicalis*, the African clawed frog (Kulkarni and Buchholz, 2012). We analyzed global gene expression from tadpole tails treated with corticosterone (CORT, the frog stress hormone), thyroid hormone, CORT + thyroid hormone, or vehicle control for 18 h. The expression of 5,432 genes was altered in response to either one or both hormones. There were 16 patterns of gene regulation due to up- or down-regulation by thyroid hormone and/or CORT. The transcribed locus Str. 34945 was among a small and interesting group of genes because these genes were upregulated in the presence of CORT, and thyroid hormone had no influence on their expression. We designated these genes as "CORT-only" based on this specificity of hormone induction.

No such CORT-only gene had been previously identified or characterized in tadpoles, and thus in the current study we chose to characterize one of the most highly induced CORT-only genes, Str. 34945. The Str. 34945 sequence is unannotated, in that it

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was not associated with a known gene. We updated the genomic annotation of Str. 34945. We then confirmed and further examined the hormonal regulation of Str. 34945, its tissue distribution, and developmental expression profile through metamorphosis in the tadpole tail. Knowledge of when and where this gene is expressed will greatly facilitate mechanistic studies to elucidate the role of CORT on development by virtue of having a convenient method to assay CORT action in tadpoles independent of thyroid hormone.

2. Materials and methods

2.1. Animal husbandry

Breeding was induced by priming male and female wild type *Xenopus tropicalis* with 20 U of ovine luteinizing hormone (Los Angeles Biomedical Research Institute, National Hormone and Peptide Program) in the evening and boosting with 200 U the next morning. The resulting tadpoles were reared at 26C and fed Sera Micron food twice daily with daily water changes. Tadpole tissues were harvested at developmental stages determined by Nieuwkoop and Faber (NF) (Nieuwkoop and Faber, 1994). The use of animals in experiments was in accordance with the guidelines of the University of Cincinnati Institutional Animal Care and Use Committee (IACUC protocol # 06-10-03-01).

2.2. Genomic characterization of Str. 34945

2.2.1. RNA-seq analysis

The datasets used for genome-wide profiling of gene expression were from Buisine et al. (Buisine et al., 2015) and followed the same workflow. Briefly, saturating amounts (>35–40 M reads) of high quality SOLiD strand specific reads were mapped on the *Xenopus tropicalis* genome (v4.1) with the Bioscope pipeline, run with stringent parameters. Only reads mapping at a single genomic location were kept. Read density profiles were normalized over the lowest sequencing depth and displayed on a genome browser. A total of five libraries were processed, corresponding to a collection of larval (tailfin, limb buds) and adult (intestine, kidney, brain) and tissues. Although a more recent version of the genome assembly is available, our use of version 4.1 has no impact on the results while we benefit from the high quality annotation described in (Buisine et al., 2015). Importantly, this annotation is based on the RNA-PET technology, which is designed to specifically capture the 5′ end (and 3′ end, to some extent) of transcripts, thus providing a higher resolution map of transcript boundaries.

2.2.2. Conventional reverse transcriptase PCR analysis

RNA and cDNA from NF54 tails of tadpoles treated with and without 100 nM CORT or vehicle for 24 h. were used in PCR (Takara Bio Inc., Mountain View, CA). Primer sequences for Str. 34945 and coding regions of *ush1g* and *fads6* are listed in Table 1. All PCR products were run on 1% agarose gels and visualized with ethidium bromide. The results were independently obtained 3 times. Controls with no reverse transcriptase produced no bands (data not shown). The PCR product obtained from the fourth exon of *ush1g* to Str. 34945 was spin column purified and sequenced. Genome analyses were conducted using Xenbase (http://www.xenbase.org/, RRID:SCR_003280) (Karimi, et al., 2017).

2.3. Hormone treatments and tissue harvest

To determine hormone induction specificity, premetamorphic tadpoles at stage NF 54 were treated with 100 nM CORT, 100 nM aldosterone (ALDO), and 10 nM triiodothyronine (T3), or EtOH vehicle control by addition to aquarium water for 24 h, as done in previous studies (Kulkarni and Buchholz, 2012; Shewade et al., 2017). A 10 nM T3 daily dose is considered to be the maximum physiologically relevant dose of thyroid hormone, while 100 nM CORT and 100 nM ALDO are physiologically relevant doses for stress hormones (Glennemeier and Denver, 2002; Leloup and Buscaglia, 1977). Tails (n = 10 per treatment) were harvested from Tricaine-anathesized animals, snap frozen, and stored at −80 until RNA isolation. For the time course, premetamorphic tadpoles at stage NF 54 were treated with 100 nM CORT for 0, 2, 4, 12, and 24 h. A parallel experiment was done for 0, 1, 2, 3 and 5 days. Water changes and chemical replacements were preformed every day. Tails (n = 5 per time point) were harvested and stored as above. To determine tissue distribution, premetamorphic tadpoles at stage NF 54 were treated for 24 h with 100 nM of CORT or EtOH vehicle control. The tail, lungs, liver, heart, brain, and kidney were harvested (Patmann et al., 2017) and stored as above. The sample size was n = 6, but for smaller organs two to three individuals were pooled together to obtain enough RNA. For the developmental series, tails (n = 6 per stage) from tadpoles at various developmental stages throughout metamorphosis were harvested and stored as above.

2.4. RNA extraction, cDNA synthesis, and qPCR

Total RNA was extracted by using TRI Reagent RT (Molecular Research Center, Inc.) using manufacturers protocol. One ug of RNA was used in making cDNA using the manufacturer's protocol (Biotools B&M Labs, S.A.). One uL of cDNA was used in 20 uL reactions for quantitative PCR with Taqman Universal master mix (Life Technologies) using FAM labeled primer-probe sets and run on a 7300 Real Time PCR system. The TaqMan primer probe sequences for Str. 34945 were forward: 5′ CTGTAGGACACGTATTTCATGATTAAGC, reverse: 5′ CAACATTAACAGGGTATGATAAAATCAATATATCTTTATTACAAAAT, and probe: 5′ CCTGACGCATTTTGTG. The TaqMan primer probe sequences for the reference gene ribosomal protein L8 (rpL8) were forward: 5′ AGAAGGTCATCTCATCTGCAAACAG, reverse: 5′ CTTCAGGATGGGTTTGTCAATACGA, and probe: 5′ CAACCCCAACAATAGCT. The reaction conditions for were 50 °C 2 min, 95 °C 10 min, then 40 cycles of 95 °C 10 s, 60 °C 1 min. Relative quantification was carried out using the delta delta Ct method (Livak and Schmittgen, 2001).

2.5. Statistical analysis

Data was tested for normalcy using the Shapiro-Wilk test and Bartlett test, but none of the data were normally distributed. Thus,

Table 1
Primer table for conventional PCR.

Gene	Exon span	Primer Name	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Product (bp)
rpl8	E3-6	DRB21b/22b	CGTGGTGCTCCTCTTGCCAAG	GACGACCAGTACGACGAGCAG	577
ush1g	E1-E2	DRB325/326	AGAGACCTGAACTCGCCAGA	GGTGGGAAACATTGTGGCAC	525
ush1g	E3-E4	DRB333/334	CTGGGCGAGGAAGTGAAGTT	CGGTAGTCTTGGCAGCAGAA	528
ush1g/Str. 34945	E4-Str. 34945	KSForward/KSReverse	TTCTGCTGCCAAGACTACCG	CATGTCCCCTTTAGATCCCCC	1404
Str. 34945	—	SSK34/33	ATCGGGTCAAGTCGGTATTATCC	AGGGCAGTGGAAACAGTAACTCAC	274
fads6	E1-E4	DRB327/328	AAAAAGAGCAGCTGGTGGGA	GAGGAACACGCACACAATGG	561

the non-parametric Kruskal-Wallis one-way analysis of variance was performed to identify significant differences in Str. 34945 expression among developmental stages and hormone treatments using JMP statistical analysis software (JMP Pro 12) and R. Pairwise comparisons were done using the Dunn's multiple comparisons test or Welch's *t* test, and a *p*-value <.05 was considered to be statistically significant. All error bars are reported as standard error of the mean.

3. Results

3.1. Genomic analysis of Str. 34945

To determine the identity of Str. 34945, we began by examining RNA-seq data from larval and adult tissues. Read counts achieved peaks in the coding exons of *ush1g*, and read counts at the 3′ untranslated region extended beyond the annotated portion of

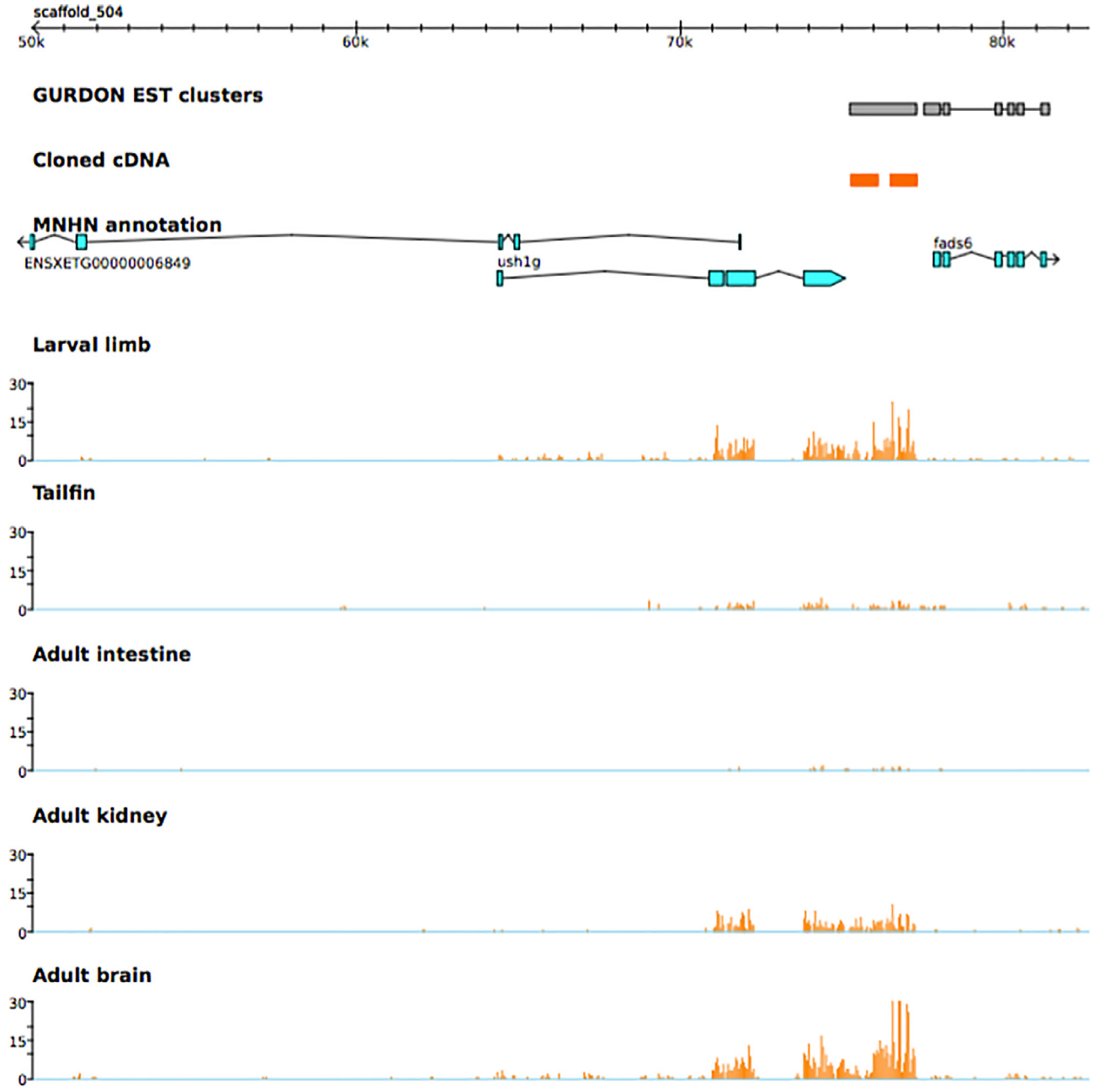

Fig. 1. RNA-seq expression data for Str. 34945 in larval and adult organs. The data for RNA-seq, as well as Gurdon EST clusters, cloned cDNA, and MNHN annotation (Muséum national d'Histoire naturelle), are shown mapped onto Scaffold 504 of the *Xenopus tropicalis* genome v4.1 in the region of *ush1g* and *fads6*. Exon-intron structures for *ush1g* (4 exons) and *fads6* (6 exons) are shown with blue boxes on the MNHN annotation track. The location of Str. 34945 is shown with a large grey box in the Gurdon EST cluster track (the smaller grey boxes are Gurdon ESTs for *fads6*) and with orange boxes on the cloned cDNA track. Read count peaks indicated by the orange histograms are seen predominantly underneath the exons of *ush1g* and not *fads6*. The read count peaks in *ush1g* extend beyond the annotated region of exon 4 to the 3′ end of Str. 34945, suggesting a single continuous transcript including *ush1g* and Str. 34945. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

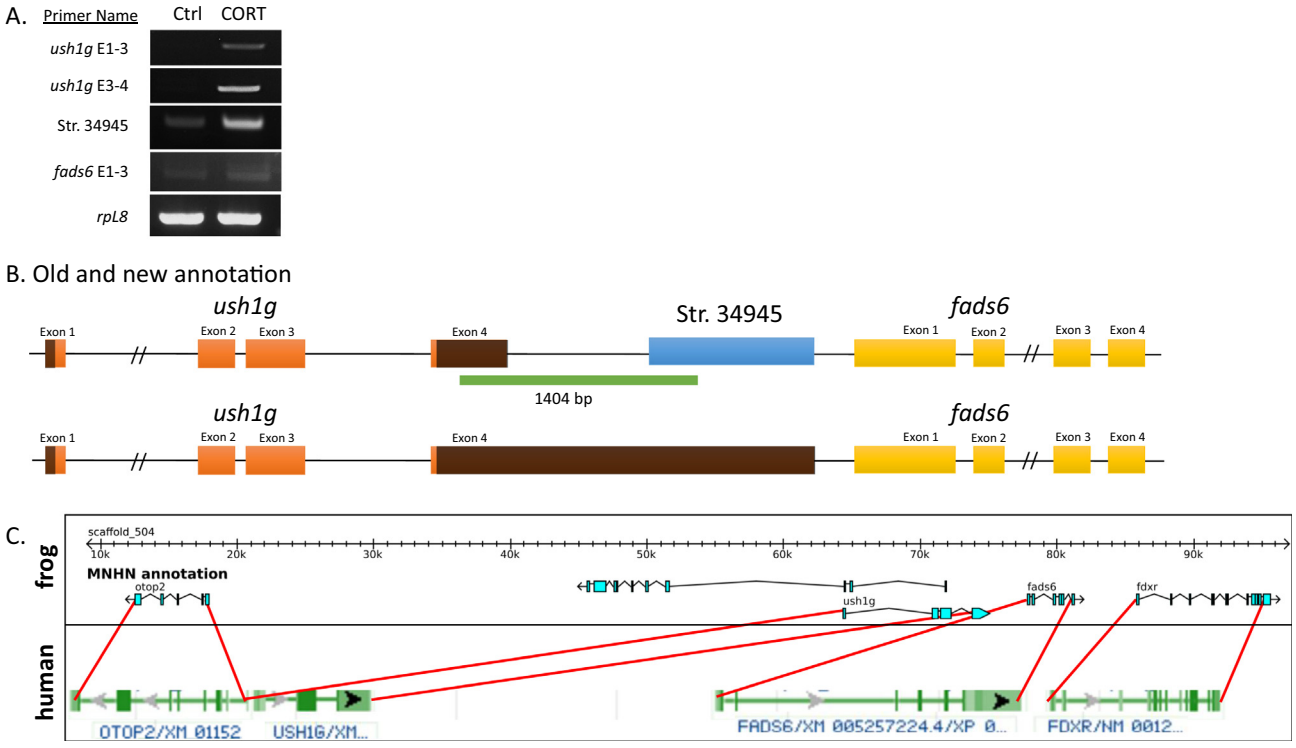

Fig. 2. Str. 34945 is part of the 3′ untranslated region of *ush1g*. **A**. Premetamorphic tadpoles were treated with vehicle control and 100 nM CORT (corticosterone) for 24 h., followed by tail RNA extraction and conventional PCR for the indicated regions of *ush1g*, Str. 34945, *fads6* and *rpL8*. E = exon. CORT induction was observed in *ush1g* and Str. 34945 and not *fads6*. *RpL8* was used as a quality control. B. Current annotation of Str. 34945 from Xenbase is shown above the annotation established by this study. The 5′ and 3′ untranslated regions of *ush1g* are dark, and the *ush1g* exons are orange. The green bar indicates the PCR product, from tail cDNA, whose sequence matched the genomic sequence. C. Synteny analysis of the frog and human genomes indicate that the frog and human *ush1g* are homologs and that the genomic location of *ush1g* is conserved in frogs and humans. The four genes are *otop2* (*otopterin 2*), *ush1g*, *fads6*, and *fdxr* (*ferredoxin reductase*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ush1g and seemed to represent a continuous transcript through a length encompassing all of Str. 34945 (Fig. 1). Essentially no expression of *fads6* was detected in the tissues analyzed.

To support the RNA-seq data that Str. 34945 is a portion of the 3′ untranslated region of *ush1g*, we measured tail expression levels of *ush1g* and *fads6* after 100 nM CORT treatment (Fig. 2A). We found that each exon of *ush1g* was upregulated by CORT, as is Str. 34945. *Fads6* was not well expressed in tail nor was it upregulated by CORT. As a positive control for the *fads6* PCR primers, we observed expression of *fads6* in tadpole brain, which was not regulated CORT (data not shown).

To confirm that Str. 34945 is part of the 3′ untranslated region of *ush1g*, we designed PCR primers to amplify the region between the 3′ end of the current genomic annotation of *ush1g* and the 5′ end of Str. 34945 using conventional reverse transcriptase quantitative PCR. A PCR product of the expected length was obtained and sequenced (Fig. 2B). The sequence matched the *X. tropicalis* genome version 4.1 exactly (Hellsten et al., 2010), confirming that Str. 34945 is a part of the *ush1g* transcript.

3.2. Hormone specificity

To confirm the specificity of hormone regulation for Str. 34945, as suggested by our previous microarray (Kulkarni and Buchholz, 2012), we measured Str. 34945 expression in the tail of *X. tropicalis* after CORT (100 nM), aldosterone (100 nM), and triiodothyronine (T3, 10 nM) treatments. Expression of Str. 34945 mRNA was induced exclusively by CORT and not aldosterone or T3 (Fig. 3). Such verification was important because analysis of another putative "CORT-only" gene from the microarray (*pck1*) revealed some induction by thyroid hormone (Kulkarni and Buchholz, 2012).

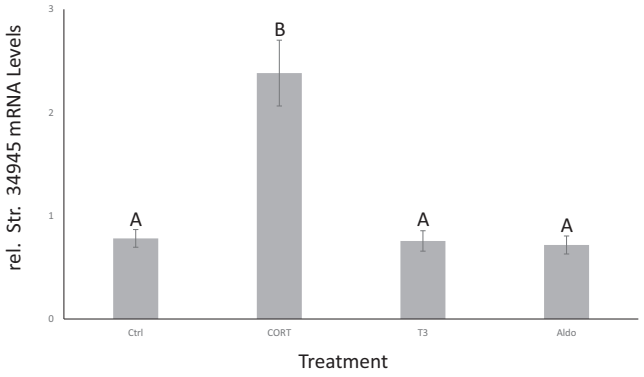

Fig. 3. Hormone induction specificity of Str. 34945 in tadpole tails. Premetamorphic tadpoles were treated with vehicle control, 100 nM CORT (corticosterone), 10 nM T3 (triiodothyronine), and 100 nM ALDO (aldosterone) for 24 h, followed by tail RNA extraction and RT-qPCR for Str. 34945 and the housekeeping gene *rpL8*. Str. 34945 was induced only by CORT and not by T3 or ALDO. Bars show mean mRNA levels relative to the housekeeping gene *rpL8*. Error bars indicate SEM. The letters above the error bars indicate significance groups among treatments based on Dunn's post hoc test ($p < .05$, n = 10 tails per treatment group).

3.3. Time course of Str. 34945 induction by CORT in tails

To determine the optimal treatment time of CORT induction of Str. 34945 expression, we measured Str. 34945 expression after continuous exposure to 0, 2, 4, 12, and 24 h and 0, 1, 2, 3, and 5 days of 100 nM CORT treatment in tadpole tails. Str. 34945 mRNA levels remained low at 0, 2, and 4 h and then maintained a 6- to 8-fold induction from each time point between 12 h to 5 days (Fig. 4).

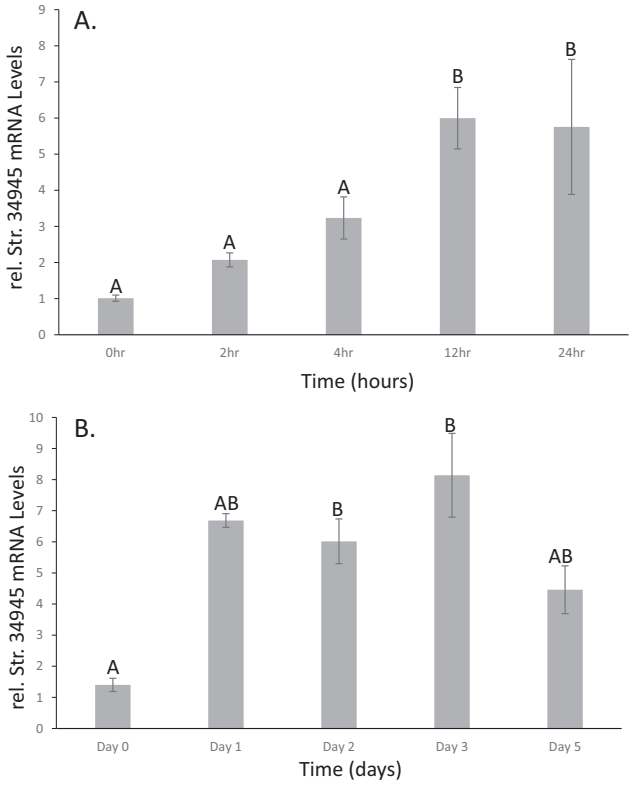

Fig. 4. Time course of Str. 34945 induction by CORT in tadpole tails. Premetamorphic tadpoles were treated with vehicle control and 100 nM CORT (corticosterone) for (**A**) 0, 2, 4, 12, 24 h and (**B**) 0, 1, 2, 3, 5 days, followed by tail RNA extraction and RT-qPCR for Str. 34945 and the housekeeping gene *rpL8*. Maximal expression of Str. 34945 was achieved by 12 h. and was maintained for 5 days. Bars show mean mRNA levels relative to the housekeeping gene *rpL8*. Error bars indicate SEM. The letters above the error bars indicate significance groups among treatments based on Dunn's post hoc test ($p < .05$, $n = 5$ tails per treatment group).

Based on the results obtained, we chose to conduct all further hormone treatments for 24 h.

3.4. Tissue distribution

We next examined which organs other than tail exhibited induction of Str. 34945 expression after exogenous CORT treatment. We examined Str. 34945 mRNA levels in the tail, lungs, liver, heart, brain, and kidney. Str. 34945 expression significantly increased in the tail by 7-fold, lungs by 3-fold, and liver and heart by 2-fold (Fig. 3). Exogenous CORT did not significantly increase Str. 34945 mRNA levels in the brain and kidney (Fig. 5).

3.5. Developmental profile of Str. 34945

Because Str. 34945 expression is regulated by CORT, we expected that Str. 34945 expression levels would follow the developmental profile of CORT level in plasma, which peaks at the climax of metamorphosis at 571 ng/dl in *X. laevis* (Hatey and Jaudet, 1984), which is expected to be similar to *X. tropicalis* because no known endocrine differences have been identified between these two closely related species (Fu et al., 2017; Wang, et al., 2008). We measured Str. 34945 in the tails of tadpoles throughout tadpole development and found that Str. 34945 mRNA began to rise at prometamorphosis (NF 58) and peaked at metamorphic climax (NF 62) (Fig. 6).

4. Discussion

Corticosteroid-dependent developmental processes are complex, and there is still much to learn about the signaling mechanisms and effects of stressors on development (Crespi and Warne, 2013; Kulkarni and Buchholz, 2014). To add to our current understanding, we characterized the expression and genomic annotation of a transcribed locus upregulated by CORT in tadpoles, Str. 39345, which was originally identified in a microarray analysis of hormone regulated genes in tadpole tails (Kulkarni and Buchholz, 2012).

Our interest in this gene derived from the novel pattern of hormone induction. The only previously known CORT-response gene in tadpoles was the transcription factor *klf9*, but *klf9* is also regulated independently by thyroid hormone and synergistically with thyroid hormone and CORT (Bagamasbad et al., 2012; Bonett et al., 2009). CORT is also known to alter deiodinase activity affecting thyroid hormone signaling in a tissue-, stage-, and condition-specific manner, but such altered activity may not be due to CORT action on deiodinase genes at the transcriptional level (Bonett et al., 2010; Martinez-deMena, et al., 2016; Van der Geyten and Darras, 2005). Here, we confirmed the microarray study that CORT induced Str. 34945 expression with no influence from thyroid hormone. Str. 34945 is also not induced by 100 nM ALDO, indicating it is a bone fide "CORT-only" gene. The induction kinetics of this gene leaves open the possibility that it may be a direct response gene as its induction starts to increase between 4 and 12 h, though we have not confirmed this with co-treatment of CORT with cycloheximide or identified a functional glucocorticoid hormone response element. In addition, as expected for a CORT-response gene, expression of Str. 34945 peaked at metamorphic climax (NF 62 in *Xenopus*) when endogenous CORT also reaches a peak in all anurans examined (Kulkarni and Buchholz, 2014; Glennemeier and Denver, 2002).

Interestingly, even though most if not all tissues express glucocorticoid receptors including brain, tail, and lung (Shewade et al., 2017), only four of the six tissues tested induced Str. 34945 in response to CORT, namely tail, lung, liver, heart. The tail gave the greatest degree of induction at about 7-fold, and the fact that thyroid hormone does not affect its expression makes this transcript unique for studies in CORT action and glucocorticoid endocrine disruption. Indeed, a recent study examined the expression of several genes originally identified in our previous microarray study for use in an *ex vivo* tail tip assay for screening glucocorticoid disrupting chemicals, though they did not examine Str. 34945 and did not independently verify that the genes they chose were not inducible by thyroid hormone (Chen et al., 2017).

A further goal for this research was to determine the identity of Str. 34945 beyond a "transcribed locus" as indicated in the Unigene database. The Str. 34945 sequence is derived from six expressed sequence tags (ESTs) from testis and brain of adult frogs and maps to an unannotated genomic region between *ush1g* and *fads6* in the *X. tropicalis* genome. The largest open reading frame of the 2053 bp nucleic acid sequence of Str. 34945 is 90 amino acids and gave no BLAST hits to the human and mouse protein databases. Thus, Str. 34945 does not appear to code for a protein. However, Str. 34945 does have a polyA tail suggesting it may be part of a protein coding transcript.

We next determined if Str. 34945 could be part of the untranslated region of an adjacent gene, i.e., *ush1g* or *fads6*, or could be its own transcript. RNA-seq data suggested that Str. 34945 could be part of a large 3′ untranslated region of *ush1g*, giving no evidence that it could be part of the 5′ untranslated region of *fads6*. If Str. 34945 were part of the *ush1g* transcript, then *ush1g* should be CORT-inducible, which is indeed what we found. However, it was

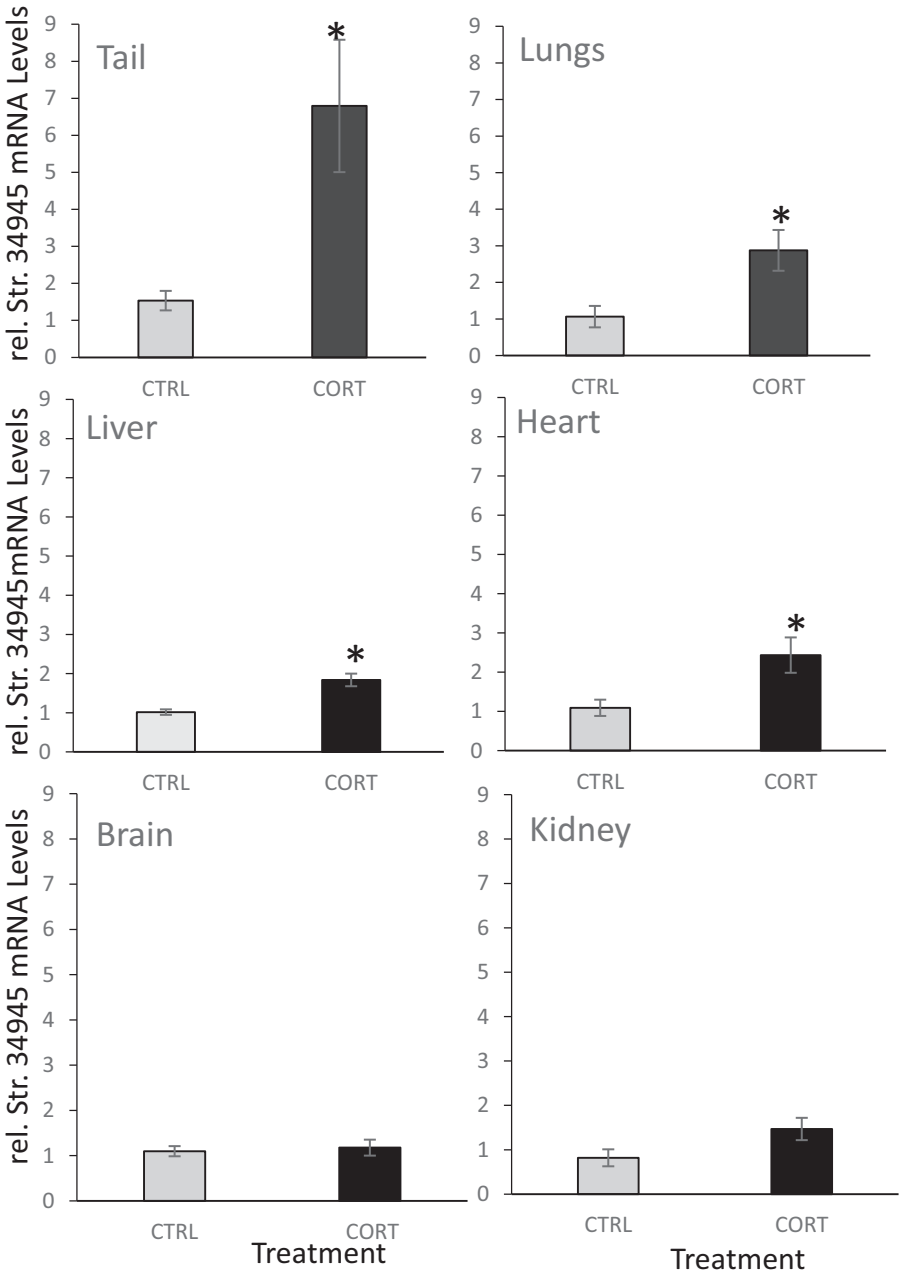

Fig. 5. Tissue expression profile of Str. 34945 in tadpoles. Premetamorphic tadpoles were treated with vehicle control and 100 nM CORT (corticosterone) for 24 h., followed by RNA extraction from harvested tails, lungs, livers, hearts, brains, and kidneys and then by RT-qPCR for Str. 34945 and the housekeeping gene *rpL8*. Str. 34945 was most highly induced in tails, moderately induced in lungs, liver, and heart, and not induced in brain and kidney. Bars show the mean mRNA levels relative to the housekeeping gene *rpL8*. Error bars represent SEM. The asterisks above the error bars indicate significance groups among treatments based on Welch's *t*-test ($p < .05$, n = 6 for tails and brains, n = 5 for livers and kidneys, n = 4 for lungs and hearts per treatment group).

possible that *ush1g* and Str. 34945 were two different CORT-induced transcripts. We reasoned that if the unannotated region between *ush1g* and Str. 34945 were both part of a single transcript, we should be able to amplify it using reverse transcriptase PCR. Indeed, we obtained a PCR product of the expected length, and the sequence matched the genomic region between *ush1g* and Str. 34945 enabling us to conclude that Str. 34945 corresponds to a portion of the 3′ untranslated region of *ush1g*.

Our PCR sequence matched the *X. tropicalis* genome sequence v4.1 exactly with minor differences compared to *X. tropicalis* genome v7.1. Interestingly, the sequence of one of the ESTs comprising Str. 34945, i.e., CX896217, matches genome v7.1 exactly, suggesting potential strain differences in this region of the *X. tropicalis* genome. Using BLAST, the non-repetitive portions of Str. 34945 hit in the unannotated regions of the *X. laevis* genome just 3′ of *ush1g* in chromosome9_10L/S (Session et al., 2016). Also, RepeatMasker (Smit et al. 2013) found a DNA transposon (hobo-Activator) in ~280 bases of the Str. 34945 sequence and there was a DNA transposon at the homologous site in *X. laevis*. No ESTs are known from the coding region of *ush1g* in *X. tropicalis*, but a Unigene from *X. laevis* is known, Xl.81120, which is comprised of three sequences from two cDNA libraries from gastrula and NF53 tadpole whole body.

Using BLAST to compare the non-repetitive region of Str. 34945 against the human and mouse genomes gave no hits. However, synteny including at least four genes with *ush1g* was observed

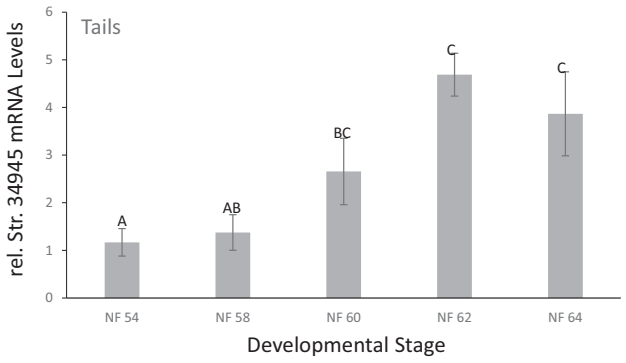

Fig. 6. Developmental expression profile of Str. 34945 in tadpole tails throughout the larval period. Tails from tadpoles at the indicated Nieuwkoop and Faber (NF) stages (Nieukoop and Faber, 1994) were harvested, followed by tail RNA extraction and RT-qPCR for Str. 34945 and the housekeeping gene *rpL8*. The expression of Str. 34945 increased significantly at metamorphic climax (NF 62-64). Bars show the mean mRNA levels relative to the housekeeping gene *rpL8*. Error bars represent SEM. The letters above the error bars indicate significance groups among stages based on Dunn's post hoc test ($p < .05$, $n = 6$ for tails).

between the human and frog genomes (Fig. 2C). Human and frog share the intron exon structure for *ush1g*, and exon 4 in both species begins with the sequence "ATAA", which encodes the STOP codon, followed by long 3′ untranslated regions. The 3′ untranslated region of human *ush1g* is about 2000 bp compared to 3309 bp of *Xenopus* encompassing Str. 34945, but *Xenopus* transcripts tend to have long 3′ untranslated regions (Buisine et al., 2015). Human expression from EST data for *ush1g* is in testis, ovary, endometrium, skin, esophagus, adrenal (Uhlen et al., 2015).

The potential role of *ush1g* in metamorphosis is not known, as nothing is known about this gene in frogs other than its representation in the Unigene expression database and expression in *Xenopus* larval photoreceptor cells (Maerker et al., 2008; Sahly et al., 2012). *Ush1g* is a protein coding gene that is essential in the development and maintenance of the visual and auditory systems in mammals (Reiners, et al., 2006; Sorusch et al., 2017). Also known as SANS (scaffold protein containing ankyrin repeats and SAM domain), *ush1g* is a structural protein that associates with an intracellular protein complex near the base of cilia in photoreceptors and cochlear hair cells (Adato et al., 2005). Mutations in this gene are associated with Usher's syndrome type 1G, which is an autosomal recessive disease that results in congenital sensorineural deafness and retinitis pigmentosa (Weil et al., 2003). Given this previous knowledge of the function and expression of *ush1g*, insight into potential roles in tail resorption and other events of metamorphosis remain obscure.

In summary, we have characterized the first CORT-only response gene, *ush1g*, in tadpoles with respect to genomic annotation, time course of induction, tissue and developmental expression profiles. This gene will be useful in future studies of CORT action during development and metamorphosis and additional studies will be required to understand its developmental roles.

Acknowledgments

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