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

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4 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 hormone specificity, time course of induction, tissue distribution, developmental expression profile, and genomic annotation for this transcript. 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 hours 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 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*. 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.

Key Words: frog metamorphosis, corticosterone, Usher's syndrome type 1G, gene expression

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, Li, & Forhead, 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, life-long negative consequences may occur (Crespi & Warne, 2013; Harris & Seckl, 2011). In addition, stress hormones often do not act alone but in concert with other hormones, especially thyroid hormone (Bonett, Hoopfer, & Denver, 2010; Terrien & Pruinet 2013). 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 & 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 hours. 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. We chose to characterize one of
these genes, Str. 34945, because it was among the most highly induced CORT-only genes. The
Str. 34945 sequence is unannotated, in that it was not associated with a known gene. We
examined Str. 34945 expression in key CORT target tissues and conducted a developmental
expression profile through metamorphosis in the tadpole tail. We also defined the genomic
annotation of Str. 34945. 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 measure CORT action in tadpoles independent of thyroid hormone.

Materials and Methods

1. Animal Husbandry

Breeding was induced by priming male and female wild type *Xenopus tropicalis* with 20U of
ovine luteinizing hormone (Los Angeles Biomedical Research Institute, National Hormone and
Peptide Program) in the evening and boosting with 200U 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 & Faber, 1956). 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. 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 hours, as done in previous studies (Kulkarni & Buchholz, 2012; Shewade, et. al., 2017). Tails (n=10 per treatment) were harvested from Tricaine-anesthetized animals, snap frozen, and stored at -80 until RNA isolation. For the time course, premetamorphic tadpoles at stage NF 54 were treated with 100nM CORT for 0, 2, 4, 12, and 24 hours. A parallel experiment was done for 0, 1, 2, 3 and 5 days. 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 hours with 100nM 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.

3. 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 mL of cDNA was used in 20 mL 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' CTGTAGGACACGTATTTTCATGATTAAGC, 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' CTTTCAGGATGGGTTTGTCAATACGA, and probe: 5' CAACCCCAACAATAGCT. The reaction conditions for were 50°C 2 min, 95°C 10 min, then 40 cycles of 95°C 10 sec, 60°C 1 min. Relative quantification was carried out using the delta delta Ct method (Livak & Schmittgen, 2001).

4. Genomic characterization of Str.34945

RNA-seq analysis:

The datasets used for genome-wide profiling of gene expression were from (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. (Buisine, et. al., 2015). Importantly, this annotation is based on the RNA-PET technology, which is designed to specifically capture the 5' (and 3' end, to some extent) end of transcripts, thus providing a higher resolution map of transcript boundaries.

Conventional reverse transcriptase PCR analysis:

RNA and cDNA from NF54 tails of tadpoles treated with and without 100 nM CORT or vehicle for 24 hrs. were used in PCR (Takara Bio Inc., MountainView, 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).

5. Statistical analysis

Analysis of variance (ANOVA) was performed to identify significant differences in Str. 34945 expression among developmental stages and hormone treatments using JMP statistical analysis software (JMP Pro 12). Pair-wise comparisons were done using the Tukey-Kramer *post-hoc* test, and a *p*-value less than 0.05 was considered to be statistically significant. All error bars are reported as standard error of the mean.

Results

1. Hormone Specificity

To confirm the specificity of hormone regulation for Str. 34945, as suggested by our previous microarray (Kulkarni & Buchholz, 2012), we measured Str. 34945 expression in the tail of *X. tropicalis* after CORT (100nM), aldosterone (100nM), and triiodothyronine (T3, 10nM) treatments. Expression of Str. 34945 mRNA was induced exclusively by CORT and not aldosterone or T3 (Figure 1). Such verification was important because analysis of another

putative "CORT-only" gene from the microarray (*pck1*) revealed some induction by thyroid hormone (Kulkarni & Buchholz, 2012).

2. 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 0, 2, 4, 12, and 24 hours and 0, 1, 2, 3, and 5 days of 100nM CORT treatment in tadpole tails. Str. 34945 mRNA levels remained low at 0, 2, and 4 hours and then maintained a 6- to 8-fold induction from 12 hours to 5 days (Figure 2). Based on the results obtained, we chose to conduct all further hormone treatments for 24 hours.

3. 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 (Figure 3). Exogenous CORT did not significantly increase Str. 34945 mRNA levels in the brain and kidney (Figure 3).

4. 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 (Hatey & Jaudet, 1984). 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) (Figure 4).

5. 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 *ush1g* and seemed to represent a continuous transcript through a length encompassing all of Str. 34945 (Figure 5). 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 (Figure 6A). 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. 6B). 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.

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 & Warne,

2013; Kulkarni & Buchholz, 2014). To add to our current understanding, we characterized the endogenous 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 & 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 & 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 100uM ALDO, indicating it is a bone fide "CORT-only" gene. This gene may be a direct response gene as its induction starts to increase between 4 and 12 hours, though we have not confirmed this with co-treatment of CORT with cycloheximide or identified a glucocorticoid hormone response element. In addition, as expected for a CORT-response gene, expression of Str.34945 peaked at metamorphic climax when endogenous CORT also reaches its peak (Glennemeier, Denver, 2002; Leloup & Buscaglia 1977)

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

229 makes this transcript unique for studies in CORT action and glucocorticoid endocrine disruption.
230 Indeed, a recent study examined the expression of several genes originally identified in our
231 previous microarray study for use in an *ex vivo* tail tip assay for screening glucocorticoid
232 disrupting chemicals, though they did not examine Str. 34945 and did not independently verify
233 that the genes they chose were not inducible by thyroid hormone (Chen, et. al., 2017).

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

242 We next determined if Str. 34945 could be part of the untranslated region of an adjacent
243 gene, i.e., *ush1g* or *fads6*, or could be its own transcript. RNA-seq data suggested that Str. 34945
244 could be part of a large 3' untranslated region of *ush1g*, giving no evidence that it could be part
245 of the 5' untranslated region of *fads6*. If Str. 34945 were part of the *ush1g* transcript, then *ush1g*
246 should be CORT-inducible, which is indeed what we found. However, it was possible that *ush1g*
247 and Str. 34945 were two different CORT-induced transcripts. We reasoned that if the
248 unannotated region between *ush1g* and Str. 34945 were both part of a single transcript, we
249 should be able to amplify it using reverse transcriptase PCR. Indeed, we obtained a PCR product
250 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 5' 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 between the human and frog genomes (Fig. 6C). 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 2000bp compared to 3309bp 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; Soroush 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 time course of induction, tissue and developmental expression profiles, and genomic annotation. 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.

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References Cited

- Adato, A., Michel, V., Kikkawa, Y., Reiners, J., Alagramam, K. N., Weil, D., ... Petit, C. (2005). Interactions in the network of Usher syndrome type 1 proteins, 14(3), 347–356. <http://doi.org/10.1093/hmg/ddi031>
- Bagamasbad, P., Ziera, T., Borden, S. A., Bonett, R. M., Rozeboom, A. M., Seasholtz, A., & Denver, R. J. (2012). Molecular Basis for Glucocorticoid Induction of the Krüppel-Like Factor 9 Gene in Hippocampal Neurons, 153(November), 5334–5345. <http://doi.org/10.1210/en.2012-1303>
- Bonett, R. M., Hoopfer, E. D., & Denver, R. J. (2010). Molecular mechanisms of corticosteroid synergy with thyroid hormone during tadpole metamorphosis. *General and Comparative Endocrinology*, 168(2), 209–219. <http://doi.org/10.1016/j.ygcen.2010.03.014>
- Bonett, R. M., Hu, F., Bagamasbad, P., & Denver, R. J. (2009). Stressor and glucocorticoid-dependent induction of the immediate early gene krüppel-like factor 9: implications for neural development and plasticity. *Endocrinology*, 150(4), 1757–1765. <http://doi.org/10.1210/en.2008-1441>
- Buchholz, D. R. (2015). More similar than you think : Frog metamorphosis as a model of human perinatal endocrinology. *Developmental Biology*, 1–8. <http://doi.org/10.1016/j.ydbio.2015.02.018>
- Buisine, N., Ruan, X., Bilesimo, P., Grimaldi, A., & Alfama, G. (2015). Xenopus tropicalis Genome Re-Scaffolding and Re-Annotation Reach the Resolution Required for In Vivo ChIA-PET Analysis, 1–27. <http://doi.org/10.1371/journal.pone.0137526>
- Chen, X., Li, Y., Zhu, M., & Qin, Z. (2017). ScienceDirect An ex vivo assay for screening

319 glucocorticoid signaling disruption based on glucocorticoid-response gene transcription in
 320 *Xenopus* tails. *Journal of Environmental Sciences*, 1–9.
 321 <http://doi.org/10.1016/j.jes.2017.05.017>

322 Crespi, E. J., & Warne, R. W. (2013). Integrative and Comparative Biology Environmental
 323 Conditions Experienced During the Tadpole Stage Alter Post-metamorphic Glucocorticoid
 324 Response to Stress in an Amphibian, 1–13. <http://doi.org/10.1093/icb/ict087>

325 Denver, R. J. (2009). Stress hormones mediate environment-genotype interactions during
 326 amphibian development. *General and Comparative Endocrinology*, 164(1), 20–31.
 327 <http://doi.org/10.1016/j.ygcen.2009.04.016>

328 Fowden, A. L., Li, J., & Forhead, A. J. (1998). Glucocorticoids and the preparation for life after
 329 birth: are there long-term consequences of the life insurance?, 44, 113–122.

330 Harris, A., & Seckl, J. (2011). Hormones and Behavior Glucocorticoids , prenatal stress and the
 331 programming of disease. *Hormones and Behavior*, 59(3), 279–289.
 332 <http://doi.org/10.1016/j.yhbeh.2010.06.007>

333 Hatey, L., & Jaudet, J. (1984). Variations in Aldosterone and Corticosterone Plasma Levels
 334 during Metamorphosis in *Xenopus laevis* Tadpoles, 65, 59–65.

335 Hellsten, U., Harland, R. M., Gilchrist, M. J., Hendrix, D., Jurka, J., Blitz, I. L., ... Amaya, E. (2010).
 336 charge recombination reaction, it was proposed that structural changes occur- ring in
 337 response to electron transfer decrease the free energy gap between P + and Q, 328(April),
 338 633–637.

339 Hu, F., Crespi, E. J., & Denver, R. J. (2008). Programming Neuroendocrine Stress Axis Activity by
 340 Exposure to Glucocorticoids during Postembryonic Development of the Frog , *Xenopus*

341 laevis, 149(11), 5470–5481. <http://doi.org/10.1210/en.2008-0767>
 342 Kulkarni, S. S., & Buchholz, D. R. (2012). Beyond synergy: Corticosterone and thyroid hormone
 343 have numerous interaction effects on gene regulation in *Xenopus tropicalis* tadpoles.
 344 *Endocrinology*, 153(11), 5309–5324. <http://doi.org/10.1210/en.2012-1432>
 345 Kulkarni, S. S., & Buchholz, D. R. (2014). Corticosteroid signaling in frog metamorphosis, (March
 346 2016). <http://doi.org/10.1016/j.ygcen.2014.03.036>
 347 Liggins, G. C. (1994). The Role of Cortisol in Preparing the Fetus for Birth *.
 348 Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-
 349 Time Quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method, 408, 402–408.
 350 <http://doi.org/10.1006/meth.2001.1262>
 351 Maerker, T., Wijk, E. Van, Overlack, N., Kersten, F. F. J., McGee, J., Goldmann, T., ... Wolfrum, U.
 352 (2008). A novel Usher protein network at the periciliary reloading point between molecular
 353 transport machineries in vertebrate photoreceptor cells, 17(1), 71–86.
 354 <http://doi.org/10.1093/hmg/ddm285>
 355 Maher, J. M., Werner, E. E., Denver, R. J., & Maher, J. M. (2013). Stress hormones mediate
 356 predator- induced phenotypic plasticity in amphibian tadpoles.
 357 <http://doi.org/10.5061/dryad.1kf76>
 358 Martinez-deMena, R., Calvo, R. M., Garcia, L., & Obregon, M. J. (2016). Effect of glucocorticoids
 359 on the activity, expression and proximal promoter of type II deiodinase in rat brown
 360 adipocytes. *Molecular and Cellular Endocrinology*, 428, 58–67.
 361 <http://doi.org/10.1016/j.mce.2016.03.021>
 362 Reiners, J., Nagel-wolfrum, K., Ju, K., Wolfrum, U., & Ma, T. (2006). Molecular basis of human

363 Usher syndrome : Deciphering the meshes of the Usher protein network provides insights
 364 into the pathomechanisms of the Usher disease, 83.
 365 <http://doi.org/10.1016/j.exer.2005.11.010>

366 Sahly, I., Dufour, E., Schietroma, C., Michel, V., Bahloul, A., Perfettini, I., ... Petit, C. (2012).
 367 Localization of Usher 1 proteins to the photoreceptor calyceal processes, which are absent
 368 from mice, 199(2), 381–399. <http://doi.org/10.1083/jcb.201202012>

369 Session, A. M., Uno, Y., Kwon, T., Chapman, J. A., Toyoda, A., Takahashi, S., ... Matsuda, Y.
 370 (2016). Genome evolution in the allotetraploid frog *Xenopus laevis*. *Nature*, 538(7625),
 371 336–343. <http://doi.org/10.1038/nature19840>

372 Shewade, L. H., Schneider, K. A., Brown, A. C., & Buchholz, D. R. (2017). General and
 373 Comparative Endocrinology In-vivo regulation of Krüppel-like factor 9 by corticosteroids
 374 and their receptors across tissues in tadpoles of *Xenopus tropicalis*. *General and*
 375 *Comparative Endocrinology*, 248, 79–86. <http://doi.org/10.1016/j.ygcen.2017.02.007>

376 Sorusch, N., Bauß, K., Plutniok, J., Samanta, A., Knapp, B., Nagel-wolfrum, K., & Wolfrum, U.
 377 (2017). Characterization of the ternary Usher syndrome SANS / ush2a / whirlin protein
 378 complex, 26(6), 1157–1172. <http://doi.org/10.1093/hmg/ddx027>

379 Uhlen, M., Fagerberg, L., Hallstrom, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., ... Ponten,
 380 F. (2015). Tissue-based map of the human proteome. *Science*, 347(6220), 1260419–
 381 1260419. <http://doi.org/10.1126/science.1260419>

382 Van der Geyten, S., & Darras, V. M. (2005). Developmentally defined regulation of thyroid
 383 hormone metabolism by glucocorticoids in the rat. *Journal of Endocrinology*, 185(2), 327–
 384 336. <http://doi.org/10.1677/joe.1.05974>

Weil, D., El-Amraoui, A., Masmoudi, S., Mustapha, M., Kikkawa, Y., Lainé, S., ... Petit, C. (2003).

Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin. *Human Molecular Genetics*, 12(5), 463–471. <http://doi.org/10.1093/hmg/ddg051>

Table 1. Primer table for conventional PCR.

Gene	Exon span	Primer Name	Forward Primer (5'-3')	Reverse Primer (5'-3')	Product (bp)
<i>rpl8</i>	E3-6	DRB21b/22b	CGTGGTGCTCCTCTTG CCAAG	GACGACCAGTACGAC GAGCAG	577
<i>ush1g</i>	E1-E2	DRB325/326	AGAGACCTGAAC TCGCCAGA	GGTGGGAAACAT TGTGGCAC	525
<i>ush1g</i>	E3-E4	DRB333/334	CTGGGCGAGGAA GTGAAGTT	CGGTAGTCTTGGC AGCAGAA	528
<i>ush1g</i> / Str. 34945	E4 - Str.34945	KSForward/ KSReverse	TTCTGCTGCCAAG ACTACCG	CATGTCCCCTTTA GATCCCCC	1404
Str. 34945	—	SSK34/33	ATCGGGTCAAGT CGGTATTATCC	AGGGCAGTGGAA ACAGTAACTCAC	274
<i>fads6</i>	E1-E4	DRB327/328	AAAAAGAGCAGC TGGTGGGA	GAGGAACACGCA CACAATGG	561

Figure legends

Figure 1. 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 hrs, 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 Tukey's honest significant difference test ($p < 0.05$, $n = 10$ tails per treatment group).

Figure 2. 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 hours 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 hrs. 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 Tukey's honest significant difference test ($p < 0.05$, $n = 5$ tails per treatment group).

Figure 3. Tissue expression profile of Str. 34945 in tadpoles. Premetamorphic tadpoles were treated with vehicle control and 100 nM CORT (corticosterone) for 24 hrs., 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 letters above the error bars indicate significance groups among treatments based on Tukey's honest significant difference test ($p < 0.05$, $n = 6$ for tails and brains, $n = 5$ for livers and kidneys, $n = 4$ for lungs and hearts per treatment group).

Figure 4. Developmental expression profile of Str. 34945 in tadpole tails throughout the larval period. Tails from tadpoles at the indicated Nieuwkoop and Faber (NF) stages (Nieuwkoop & Faber, 1956) 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 Tukey's honest significant difference test ($p < 0.05$, $n = 6$ for tails).

Figure 5. 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.

Figure 6. 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 hrs., 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*).

GURDON EST clusters

Cloned cDNA

MNHN annotation

ENSXETG00000006849

Larval limb

Tailfin

Adult intestine

Adult kidney

Adult brain

B. Old and new annotation