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

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23 **Abstract**

24 Previous work identified a transcribed locus, Str. 34945, induced by the frog stress hormone  
25 corticosterone (CORT) in *Xenopus tropicalis* tails. Because thyroid hormone had no influence on  
26 its expression, Str. 34945 was dubbed the first "CORT-only" gene known from tadpoles. Here,  
27 we examine the hormone specificity, time course of induction, tissue distribution, developmental  
28 expression profile, and genomic annotation for this transcript. We find that CORT but not  
29 aldosterone or thyroid hormone treatment induces Str. 34945 in tadpole tails and that expression  
30 of Str. 34945 achieves maximal expression within 12-24 hours of CORT treatment. Among  
31 tissues, Str. 34945 is induced to the highest degree in tail, with lesser induction in lungs, liver,  
32 and heart, and no induction in the brain or kidney. During natural metamorphosis, Str. 34945  
33 expression in tails peaks at metamorphic climax. The location of Str. 34945 on the *X. tropicalis*  
34 genome lies between the genes *ush1g* (Usher syndrome 1G) and *fads6* (fatty acid desaturase 6).  
35 A blast search showed that it maps to the same region on the *X. laevis* genome, but no hits were  
36 found in the human genome. Using RNA-seq data and conventional reverse transcriptase PCR  
37 and sequencing, we show that Str. 34945 is part of the 3' untranslated region of *ush1g*. The role  
38 of *ush1g* in metamorphosis is not understood, but the specificity of its hormone response and its  
39 expression in tail make *Ush1g* valuable as a marker of CORT-response gene induction  
40 independent of thyroid hormone.

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

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## 46 **Introduction**

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

58           Amphibian metamorphosis is a valuable model to study the role of hormones and gene  
59 regulation due to its exquisite sensitivity and dramatic dependence on thyroid and stress  
60 hormones and ease of endocrine manipulation (Buchholz, 2015). The effects of stress hormone  
61 on development and subsequent altered stress hormone levels are conserved in tadpoles and  
62 humans (Buchholz, 2015). To begin to elucidate the genes involved in stress hormone-regulated  
63 development, we previously carried out a microarray study using *Xenopus tropicalis*, the African  
64 clawed frog (Kulkarni & Buchholz, 2012). We analyzed global gene expression from tadpole  
65 tails treated with corticosterone (CORT, the frog stress hormone), thyroid hormone, CORT +  
66 thyroid hormone, or vehicle control for 18 hours. The expression of 5,432 genes was altered in  
67 response to either one or both hormones. There were 16 patterns of gene regulation due to up- or  
68 down-regulation by thyroid hormone and/or CORT. The transcribed locus Str. 34945 was among

69 a small and interesting group of genes because these genes were upregulated in the presence of  
70 CORT, and thyroid hormone had no influence on their expression. We designated these genes as  
71 "CORT-only" based on this specificity of hormone induction. We chose to characterize one of  
72 these genes, Str. 34945, because it was among the most highly induced CORT-only genes. The  
73 Str. 34945 sequence is unannotated, in that it was not associated with a known gene. We  
74 examined Str. 34945 expression in key CORT target tissues and conducted a developmental  
75 expression profile through metamorphosis in the tadpole tail. We also defined the genomic  
76 annotation of Str. 34945. Knowledge of when and where this gene is expressed will greatly  
77 facilitate mechanistic studies to elucidate the role of CORT on development by virtue of having a  
78 convenient method to measure CORT action in tadpoles independent of thyroid hormone.

79

## 80 **Materials and Methods**

### 81 1. Animal Husbandry

82 Breeding was induced by priming male and female wild type *Xenopus tropicalis* with 20U of  
83 ovine luteinizing hormone (Los Angeles Biomedical Research Institute, National Hormone and  
84 Peptide Program) in the evening and boosting with 200U the next morning. The resulting  
85 tadpoles were reared at 26C and fed Sera Micron food twice daily with daily water changes.  
86 Tadpole tissues were harvested at developmental stages determined by Nieuwkoop and Faber  
87 (NF) (Nieuwkoop & Faber, 1956). The use of animals in experiments was in accordance with the  
88 guidelines of the University of Cincinnati Institutional Animal Care and Use Committee (IACUC  
89 protocol # 06-10-03-01).

### 90 2. Hormone Treatments and Tissue Harvest

91 To determine hormone induction specificity, premetamorphic tadpoles at stage NF 54 were  
92 treated with 100 nM CORT, 100 nM aldosterone (ALDO), and 10 nM triiodothyronine (T3), or  
93 EtOH vehicle control by addition to aquarium water for 24 hours, as done in previous studies  
94 (Kulkarni & Buchholz, 2012; Shewade, et. al., 2017). Tails (n=10 per treatment) were harvested  
95 from Tricaine-anesthetized animals, snap frozen, and stored at -80 until RNA isolation. For the  
96 time course, premetamorphic tadpoles at stage NF 54 were treated with 100nM CORT for 0, 2, 4,  
97 12, and 24 hours. A parallel experiment was done for 0, 1, 2, 3 and 5 days. Tails (n = 5 per time  
98 point) were harvested and stored as above. To determine tissue distribution, premetamorphic  
99 tadpoles at stage NF 54 were treated for 24 hours with 100nM of CORT or EtOH vehicle control.  
100 The tail, lungs, liver, heart, brain, and kidney were harvested (Patmann et al, 2017) and stored as  
101 above. The sample size was n = 6, but for smaller organs two to three individuals were pooled  
102 together to obtain enough RNA. For the developmental series, tails (n = 6 per stage) from  
103 tadpoles at various developmental stages throughout metamorphosis were harvested and stored  
104 as above.

105

### 106 3. RNA extraction, cDNA synthesis, and qPCR

107 Total RNA was extracted by using TRI Reagent RT (Molecular Research Center, Inc.) using  
108 manufacturer's protocol. One ug of RNA was used in making cDNA using the manufacturer's  
109 protocol (Biotools B&M Labs, S.A.). One mL of cDNA was used in 20 mL reactions for  
110 quantitative PCR with Taqman Universal master mix (Life Technologies) using FAM labeled  
111 primer-probe sets and run on a 7300 Real Time PCR system. The TaqMan primer probe  
112 sequences for Str. 34945 were forward: 5' CTGTAGGACACGTATTTTCATGATTAAGC,  
113 reverse: 5' CAACATTAACAGGGTATGATAAAATCAATATATCTTTATTACAAAAT, and

114 probe: 5' CCTGACGCATTTTGTG. The TaqMan primer probe sequences for the reference gene  
115 ribosomal protein L8 (rpL8) were forward: 5' AGAAGGTCATCTCATCTGCAAACAG,  
116 reverse: 5' CTTCAGGATGGGTTTGTCAATACGA, and probe: 5'  
117 CAACCCCAACAATAGCT. The reaction conditions for were 50°C 2 min, 95°C 10 min, then  
118 40 cycles of 95°C 10 sec, 60°C 1 min. Relative quantification was carried out using the delta  
119 delta Ct method (Livak & Schmittgen, 2001).

120

#### 121 4. Genomic characterization of Str.34945

##### 122 *RNA-seq analysis:*

123 The datasets used for genome-wide profiling of gene expression were from (Buisine, et. al.,  
124 2015) and followed the same workflow. Briefly, saturating amounts (> 35-40 M reads) of high  
125 quality SOLiD strand specific reads were mapped on the *Xenopus tropicalis* genome (v4.1) with  
126 the Bioscope pipeline, run with stringent parameters. Only reads mapping at a single genomic  
127 location were kept. Read density profiles were normalized over the lowest sequencing depth and  
128 displayed on a genome browser. A total of five libraries were processed, corresponding to a  
129 collection of larval (tailfin, limb buds) and adult (intestine, kidney, brain) and tissues. Although a  
130 more recent version of the genome assembly is available, our use of version 4.1 has no impact on  
131 the results while we benefit from the high quality annotation described in Buisine et al. (Buisine,  
132 et. al., 2015). Importantly, this annotation is based on the RNA-PET technology, which is  
133 designed to specifically capture the 5' (and 3' end, to some extent) end of transcripts, thus  
134 providing a higher resolution map of transcript boundaries.

135

##### 136 *Conventional reverse transcriptase PCR analysis:*

137 RNA and cDNA from NF54 tails of tadpoles treated with and without 100 nM CORT or vehicle  
138 for 24 hrs. were used in PCR (Takara Bio Inc., MountainView, CA). Primer sequences for Str.  
139 34945 and coding regions of *ush1g* and *fads6* are listed in Table 1. All PCR products were run  
140 on 1% agarose gels and visualized with ethidium bromide. The results were independently  
141 obtained 3 times. Controls with no reverse transcriptase produced no bands (data not shown).  
142 The PCR product obtained from the fourth exon of *ush1g* to Str. 34945 was spin column purified  
143 and sequenced. Genome analyses were conducted using Xenbase (<http://www.xenbase.org/>,  
144 RRID:SCR\_003280).

145

## 146 5. Statistical analysis

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

152

## 153 **Results**

### 154 1. Hormone Specificity

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



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

162

## 163 2. Time course of Str. 34945 induction by CORT in tails

164 To determine the optimal treatment time of CORT induction of Str. 34945 expression, we  
165 measured Str. 34945 expression after 0, 2, 4, 12, and 24 hours and 0, 1, 2, 3, and 5 days of  
166 100nM CORT treatment in tadpole tails. Str. 34945 mRNA levels remained low at 0, 2, and 4  
167 hours and then maintained a 6- to 8-fold induction from 12 hours to 5 days (Figure 2). Based on  
168 the results obtained, we chose to conduct all further hormone treatments for 24 hours.

169

## 170 3. Tissue Distribution

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

176

## 177 4. Developmental Profile of Str. 34945

178 Because Str. 34945 expression is regulated by CORT, we expected that Str. 34945 expression  
179 levels would follow the developmental profile of CORT level in plasma, which peaks at the  
180 climax of metamorphosis (Hatey & Jaudet, 1984). We measured Str. 34945 in the tails of  
181 tadpoles throughout tadpole development and found that Str. 34945 mRNA began to rise at  
182 prometamorphosis (NF 58) and peaked at metamorphic climax (NF 62) (Figure 4).

183

## 184 5. Genomic analysis of Str. 34945

185 To determine the identity of Str. 34945, we began by examining RNA-seq data from larval and  
186 adult tissues. Read counts achieved peaks in the coding exons of *ush1g*, and read counts at the 3'  
187 untranslated region extended beyond the annotated portion of *ush1g* and seemed to represent a  
188 continuous transcript through a length encompassing all of Str. 34945 (Figure 5). Essentially no  
189 expression of *fads6* was detected in the tissues analyzed.

190 To support the RNA-seq data that Str.34945 is a portion of the 3' untranslated region of  
191 *ush1g*, we measured tail expression levels of *ush1g* and *fads6* after 100 nM CORT treatment  
192 (Figure 6A). We found that each exon of *ush1g* was upregulated by CORT, as is Str. 34945.  
193 *Fads6* was not well expressed in tail nor was it upregulated by CORT. As a positive control for  
194 the *fads6* PCR primers, we observed expression of *fads6* in tadpole brain, which was not  
195 regulated CORT (data not shown).

196 To confirm that Str. 34945 is part of the 3' untranslated region of *ush1g*, we designed  
197 PCR primers to amplify the region between the 3' end of the current genomic annotation of  
198 *ush1g* and the 5' end of Str.34945 using conventional reverse transcriptase quantitative PCR. A  
199 PCR product of the expected length was obtained and sequenced (Fig. 6B). The sequence  
200 matched the *X. tropicalis* genome version 4.1 exactly (Hellsten et al., 2010), confirming that Str.  
201 34945 is a part of the *ush1g* transcript.

202

## 203 **Discussion**

204 Corticosteroid-dependent developmental processes are complex, and there is still much to  
205 learn about the signaling mechanisms and effects of stressors on development (Crespi & Warne,

206 2013; Kulkarni & Buchholz, 2014). To add to our current understanding, we characterized the  
207 endogenous expression and genomic annotation of a transcribed locus upregulated by CORT in  
208 tadpoles, Str. 39345, which was originally identified in a microarray analysis of hormone  
209 regulated genes in tadpole tails (Kulkarni & Buchholz, 2012).

210 Our interest in this gene derived from the novel pattern of hormone induction. The only  
211 previously known CORT-response gene in tadpoles was the transcription factor *klf9*, but *klf9* is  
212 also regulated independently by thyroid hormone and synergistically with thyroid hormone and  
213 CORT (Bagamasbad et al., 2012; Bonett et al., 2009). CORT is also known to alter deiodinase  
214 activity affecting thyroid hormone signaling in a tissue-, stage-, and condition-specific manner,  
215 but such altered activity may not be due to CORT action on deiodinase genes at the  
216 transcriptional level (Bonett et al., 2010; Martinez-deMena, et al., 2016; Van der Geyten &  
217 Darras, 2005). Here, we confirmed the microarray study that CORT induced Str. 34945  
218 expression with no influence from thyroid hormone. Str.34945 is also not induced by 100uM  
219 ALDO, indicating it is a bone fide "CORT-only" gene. This gene may be a direct response gene  
220 as its induction starts to increase between 4 and 12 hours, though we have not confirmed this  
221 with co-treatment of CORT with cycloheximide or identified a glucocorticoid hormone response  
222 element. In addition, as expected for a CORT-response gene, expression of Str.34945 peaked at  
223 metamorphic climax when endogenous CORT also reaches its peak (Glennemeier, Denver,  
224 2002; Leloup & Buscaglia 1977)

225 Interestingly, even though most if not all tissues express glucocorticoid receptors  
226 including brain, tail, and lung (Shewade et al., 2017), only four of the six tissues tested induced  
227 Str.34945 in response to CORT, namely tail, lung, liver, heart. The tail gave the greatest degree  
228 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.

251 34945 enabling us to conclude that Str. 34945 corresponds to a portion of the 3' untranslated  
252 region of *ush1g*.

253 Our PCR sequence matched the *X. tropicalis* genome sequence v4.1 exactly with minor  
254 differences compared to *X. tropicalis* genome v7.1. Interestingly, the sequence of one of the  
255 ESTs comprising Str. 34945, i.e., CX896217, matches genome v7.1 exactly, suggesting potential  
256 strain differences in this region of the *X. tropicalis* genome. Using BLAST, the non-repetitive  
257 portions of Str. 34945 hit in the unannotated regions of the *X. laevis* genome just 5' of *ush1g* in  
258 chromosome9\_10L/S (Session et al., 2016). Also, RepeatMasker (Smit et al. 2013) found a DNA  
259 transposon (hobo-Activator) in ~280 bases of the Str. 34945 sequence and there was a DNA  
260 transposon at the homologous site in *X. laevis*. No ESTs are known from the coding region of  
261 *ush1g* in *X. tropicalis*, but a Unigene from *X. laevis* is known, Xl.81120, which is comprised of  
262 three sequences from two cDNA libraries from gastrula and NF53 tadpole whole body.  
263 Using BLAST to compare the non-repetitive region of Str. 34945 against the human and mouse  
264 genomes gave no hits. However, synteny including at least four genes with *ush1g* was observed  
265 between the human and frog genomes (Fig. 6C). Human and frog share the intron exon structure  
266 for *ush1g*, and exon 4 in both species begins with the sequence "ATAA", which encodes the  
267 STOP codon, followed by long 3' untranslated regions. The 3' untranslated region of human  
268 *ush1g* is about 2000bp compared to 3309bp of *Xenopus* encompassing Str. 34945, but *Xenopus*  
269 transcripts tend to have long 3' untranslated regions (Buisine, et al., 2015). Human expression  
270 from EST data for *ush1g* is in testis, ovary, endometrium, skin, esophagus, adrenal (Uhlen et al.,  
271 2015).

272 The potential role of *ush1g* in metamorphosis is not known, as nothing is known about  
273 this gene in frogs other than its representation in the Unigene expression database and expression

274 in *Xenopus* larval photoreceptor cells (Maerker et al., 2008; Sahly et al., 2012). *Ush1g* is a  
275 protein coding gene that is essential in the development and maintenance of the visual and  
276 auditory systems in mammals (Reiners, et al., 2006; Sorousch et al., 2017). Also known as SANS  
277 (scaffold protein containing ankyrin repeats and SAM domain), *ush1g* is a structural protein that  
278 associates with an intracellular protein complex near the base of cilia in photoreceptors and  
279 cochlear hair cells (Adato et al., 2005). Mutations in this gene are associated with Usher's  
280 syndrome type 1G, which is an autosomal recessive disease that results in congenital  
281 sensorineural deafness and retinitis pigmentosa (Weil et al., 2003). Given this previous  
282 knowledge of the function and expression of *ush1g*, insight into potential roles in tail resorption  
283 and other events of metamorphosis remain obscure.

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

289

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296

297 **References Cited**

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

385 Weil, D., El-Amraoui, A., Masmoudi, S., Mustapha, M., Kikkawa, Y., Lainé, S., ... Petit, C. (2003).  
 386 Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a  
 387 protein that associates with the USH1C protein, harmonin. *Human Molecular Genetics*,  
 388 12(5), 463–471. <http://doi.org/10.1093/hmg/ddg051>

389

390 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

391

392

393

#### 394 **Figure legends**

395 Figure 1. Hormone induction specificity of Str. 34945 in tadpole tails. Premetamorphic tadpoles  
 396 were treated with vehicle control, 100 nM CORT (corticosterone), 10 nM T3 (triiodothyronine),  
 397 and 100 nM ALDO (aldosterone) for 24 hrs, followed by tail RNA extraction and RT-qPCR for  
 398 Str. 34945 and the housekeeping gene *rpl8*. Str. 34945 was induced only by CORT and not by  
 399 T3 or ALDO. Bars show mean mRNA levels relative to the housekeeping gene *rpl8*. Error bars

400 indicate SEM. The letters above the error bars indicate significance groups among treatments  
401 based on Tukey's honest significant difference test ( $p < 0.05$ ,  $n = 10$  tails per treatment group).

402

403 Figure 2. Time course of Str. 34945 induction by CORT in tadpole tails. Premetamorphic  
404 tadpoles were treated with vehicle control and 100 nM CORT (corticosterone) for (A) 0, 2, 4, 12,  
405 24 hours and (B) 0, 1, 2, 3, 5 days, followed by tail RNA extraction and RT-qPCR for Str. 34945  
406 and the housekeeping gene *rpL8*. Maximal expression of Str. 34945 was achieved by 12 hrs. and  
407 was maintained for 5 days. Bars show mean mRNA levels relative to the housekeeping gene  
408 *rpL8*. Error bars indicate SEM. The letters above the error bars indicate significance groups  
409 among treatments based on Tukey's honest significant difference test ( $p < 0.05$ ,  $n = 5$  tails per  
410 treatment group).

411

412 Figure 3. Tissue expression profile of Str. 34945 in tadpoles. Premetamorphic tadpoles were  
413 treated with vehicle control and 100 nM CORT (corticosterone) for 24 hrs., followed by RNA  
414 extraction from harvested tails, lungs, livers, hearts, brains, and kidneys and then by RT-qPCR  
415 for Str. 34945 and the housekeeping gene *rpL8*. Str. 34945 was most highly induced in tails,  
416 moderately induced in lungs, liver, and heart, and not induced in brain and kidney. Bars show the  
417 mean mRNA levels relative to the housekeeping gene *rpL8*. Error bars represent SEM. The  
418 letters above the error bars indicate significance groups among treatments based on Tukey's  
419 honest significant difference test ( $p < 0.05$ ,  $n = 6$  for tails and brains,  $n = 5$  for livers and kidneys,  
420  $n = 4$  for lungs and hearts per treatment group).

421

422 Figure 4. Developmental expression profile of Str. 34945 in tadpole tails throughout the larval  
423 period. Tails from tadpoles at the indicated Nieuwkoop and Faber (NF) stages (Nieukoop &  
424 Faber, 1956) were harvested, followed by tail RNA extraction and RT-qPCR for Str. 34945 and  
425 the housekeeping gene *rpL8*. The expression of Str. 34945 increased significantly at  
426 metamorphic climax (NF 62-64). Bars show the mean mRNA levels relative to the housekeeping  
427 gene *rpL8*. Error bars represent SEM. The letters above the error bars indicate significance  
428 groups among stages based on Tukey's honest significant difference test ( $p < 0.05$ ,  $n = 6$  for  
429 tails).

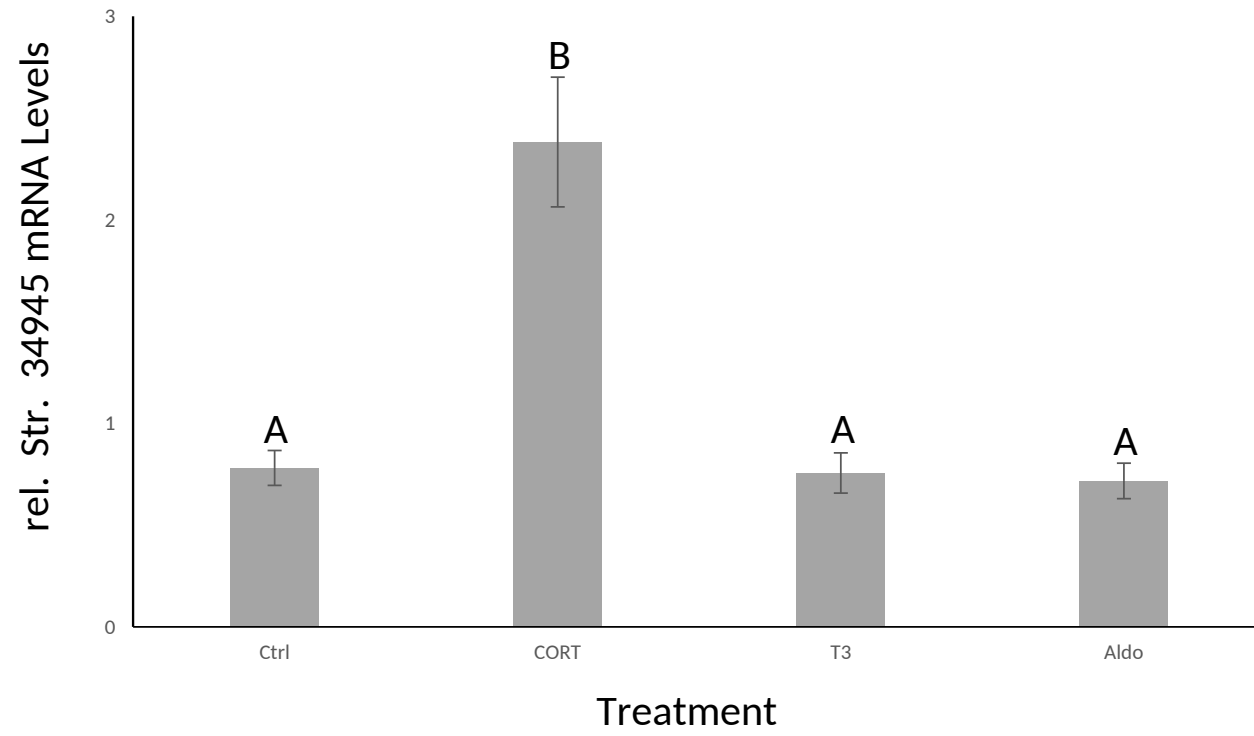
430

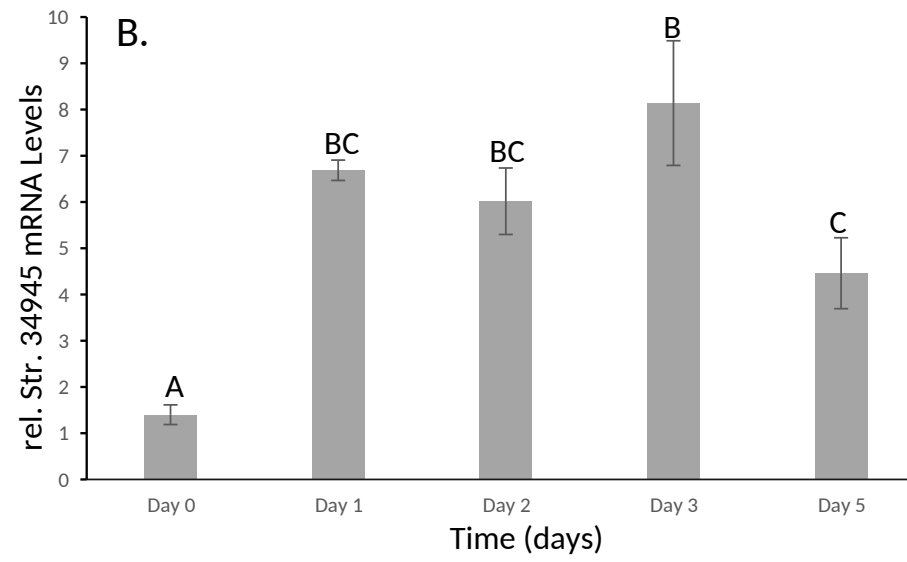
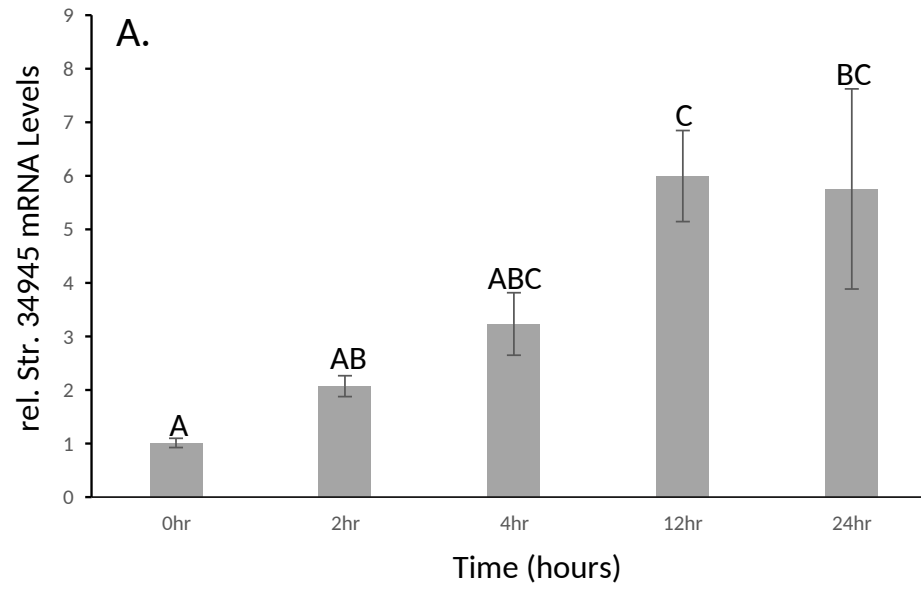
431 Figure 5. RNA-seq expression data for Str. 34945 in larval and adult organs. The data for RNA-  
432 seq, as well as Gurdon EST clusters, cloned cDNA, and MNHN annotation (Muséum national  
433 d'Histoire naturelle), are shown mapped onto Scaffold 504 of the *Xenopus tropicalis* genome  
434 v4.1 in the region of *ush1g* and *fads6*. Exon-intron structures for *ush1g* (4 exons) and *fads6* (6  
435 exons) are shown with blue boxes on the MNHN annotation track. The location of Str. 34945 is  
436 shown with a large grey box in the Gurdon EST cluster track (the smaller grey boxes are Gurdon  
437 ESTs for *fads6*) and with orange boxes on the cloned cDNA track. Read count peaks indicated  
438 by the orange histograms are seen predominantly underneath the exons of *ush1g* and not *fads6*.  
439 The read count peaks in *ush1g* extend beyond the annotated region of exon 4 to the 3' end of Str.  
440 34945, suggesting a single continuous transcript including *ush1g* and Str. 34945.

441

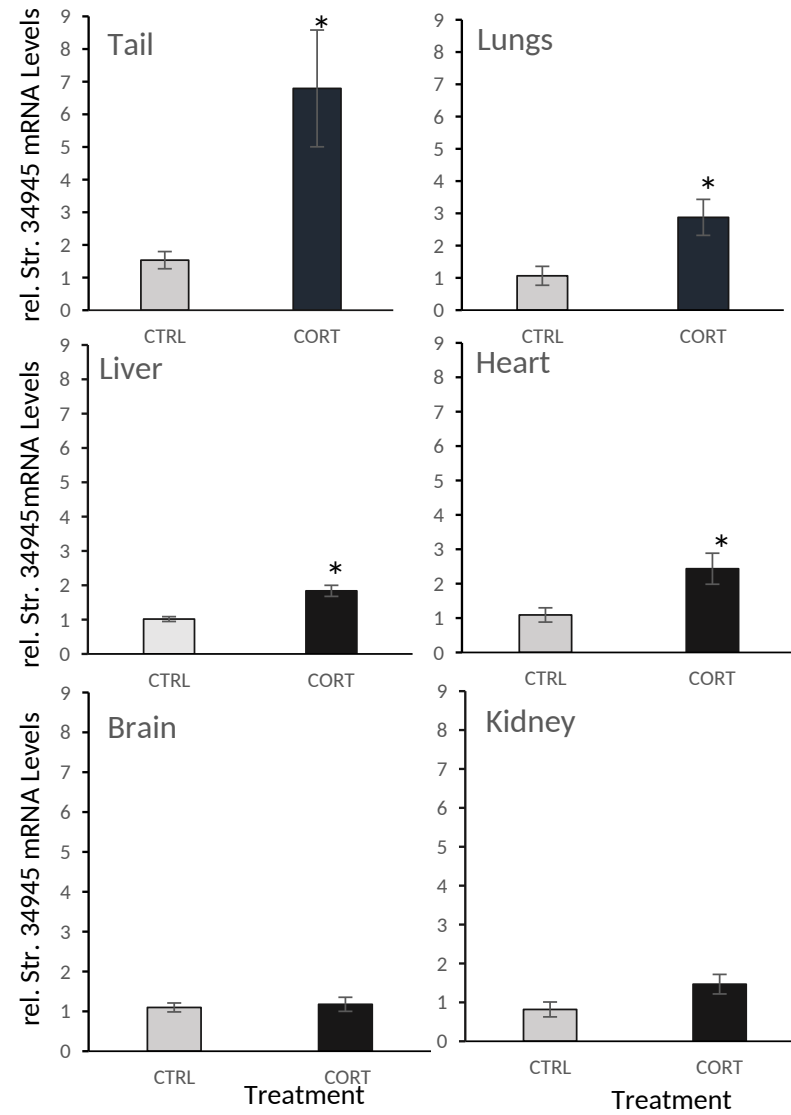
442 Figure 6. Str. 34945 is part of the 3' untranslated region of *ush1g*. A. Premetamorphic tadpoles  
443 were treated with vehicle control and 100 nM CORT (corticosterone) for 24 hrs., followed by tail  
444 RNA extraction and conventional PCR for the indicated regions of *ush1g*, Str. 34945, *fads6* and

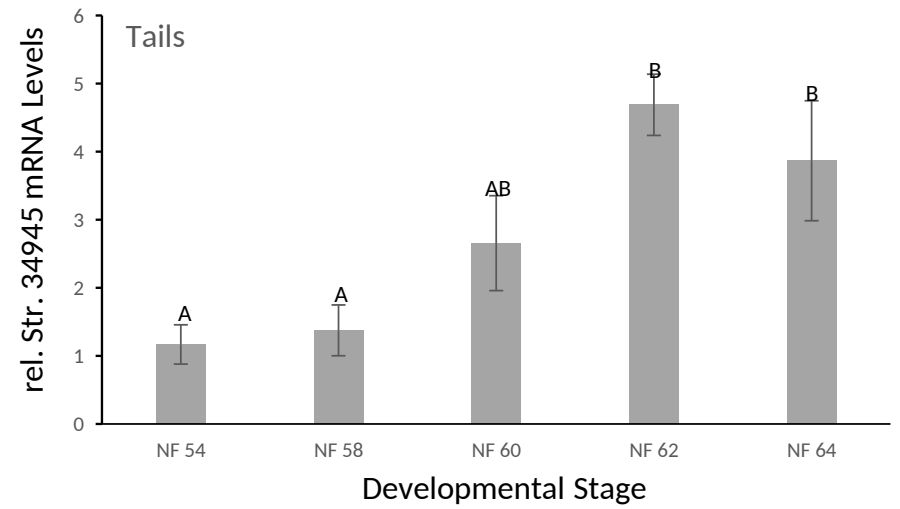
445 *rpL8*. E = exon. CORT induction was observed in *ush1g* and Str. 34945 and not *fads6*. *RpL8* was  
446 used as a quality control. B. Current annotation of Str. 34945 from Xenbase is shown above the  
447 annotation established by this study. The 5' and 3' untranslated regions of *ush1g* are dark, and the  
448 *ush1g* exons are orange. The green bar indicates the PCR product, from tail cDNA, whose  
449 sequence matched the genomic sequence. C. Synteny analysis of the frog and human genomes  
450 indicate that the frog and human *ush1g* are homologs and that the genomic location of *ush1g* is  
451 conserved in frogs and humans. The four genes are *otop2* (*otopterin 2*), *ush1g*, *fads6*, and *fdx*  
452 (*ferredoxin reductase*).  
453  
454

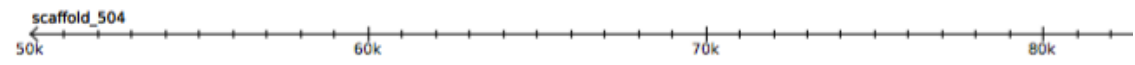












**GURDON EST clusters**

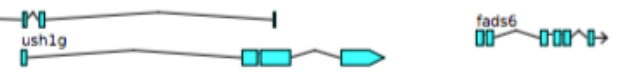


**Cloned cDNA**



**MNHN annotation**

ENSXETG00000006849



**Larval limb**



**Tailfin**



**Adult intestine**

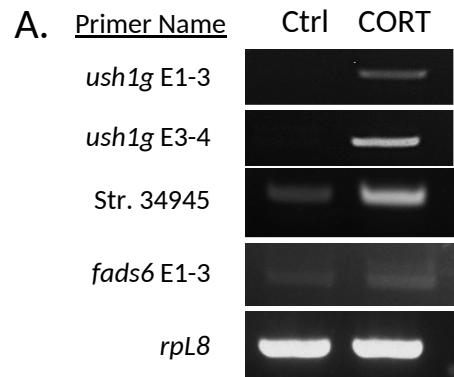


**Adult kidney**



**Adult brain**





**B. Old and new annotation**

