

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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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 genome lies between the genes ush1g (Usher syndrome 1G) and fads6 (fatty acid desaturase 6). 34 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 processes during development that are essential for survival, such as organ maturation and timing 48 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 & Pruinet 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 response to either one or both hormones. There were 16 patterns of gene regulation due to up- or 67 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 Peptide Program) in the evening and boosting with 200U the next morning. The resulting 84 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-anathesized 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

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' CTGTAGGACACGTATTTCATGATTAAGC,
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

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

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)

- 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).

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

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

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. 34945 is a part of the *ush1g* transcript. 201 202

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

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).

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 ushlg, 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 ushlg 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*.

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, XI.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).

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 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; Sorusch 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

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.

289

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

390 Table 1. Primer table for conventional PCR.

391

392

393

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

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

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 annotation established by this study. The 5' and 3' untranslated regions of ush1g are dark, and the 447 448 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 fdxr 452 (ferredoxin reductase). 453

454













B. Old and new annotation

OTOP2/XM 01152.

USH1G/XM...



FDXR/NM 0012..

FRDS6/XM 005257224.4/XP 0.