ELSEVIER

Gene Expression Patterns 7 (2007) 258–265

GEP

www.elsevier.com/locate/modgep

Zebrafish *Angiotensin II Receptor-like 1a* (*agtrl1a*) is expressed in migrating hypoblast, vasculature, and in multiple embryonic epithelia

B. Tucker [a], C. Hepperle [a], D. Kortschak [a], B. Rainbird [a], S. Wells [a], A.C. Oates [b,*], M. Lardelli [a]

[a] *Centre for the Molecular Genetics of Development and Discipline of Genetics, School of Molecular and Biomedical Science, The University of Adelaide, 5005, SA, Australia*

[b] *Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr 108, 01307 Dresden, Germany*

Received 25 July 2006; received in revised form 14 September 2006; accepted 15 September 2006
Available online 23 September 2006

Abstract

The human gene *AGTRL1* is an angiotensin II receptor-like gene expressed in vasculature, which acts as the receptor for the small peptide APELIN, and a co-receptor for Human Immunodeficiency Virus. Mammalian AGTRL1 has been shown to modulate cardiac contractility, venous and arterial dilation, and endothelial cell migration *in vitro*, but no role in the development of the vasculature, or other tissues, has been described. We report the identification and expression of the zebrafish ortholog of the human gene *AGTRL1*. Zebrafish *agtrl1a* is first expressed before epiboly in dorsal precursors. During epiboly it is expressed in the enveloping layer, yolk syncytial layer and migrating mesendoderm. During segmentation stages, expression is observed in epithelial structures such as adaxial cells, border cells of the newly formed somites, developing lens, otic vesicles and venous vasculature.

Keywords: Zebrafish; Epiboly; Cell migration; G protein-coupled receptor; Somitogenesis; Delta/Notch; Gene expression; Veins; Vasculature; Embryogenesis; Angiotensin receptor

1. Results and discussion

G protein-coupled receptor proteins (GPCRs) are multiple-pass transmembrane domain proteins involved in the signal transduction of many major developmental pathways (Strosberg, 1996; Malbon, 2005). Angiotensin receptor proteins are GPCRs that bind short polypeptide ligands (Angiotensins) and have been intensively studied due to their role in regulation of blood pressure (Thomas and Mendelsohn, 2003). Angiotensin receptors define a vertebrate subfamily of the GPCRs that includes Angiotensin II receptor-like 1 (AGTRL1), also known as APJ or Msr (Devic et al., 1996; Devic et al., 1999; O'Dowd et al., 1993), and Angiotensin II type 1 and 2 receptors (AGTR1, AGTR2). Control of cell migration is a feature of many angiotensin receptor subfamily members, for example endothelial cells (ECs, Benndorf et al., 2003), vascular smooth muscle cells (VSMCs, Chassagne et al., 2002) and neurons (Cote et al., 1999). In addition, the zebrafish chemokine SDF-1 and its angiotensin subfamily receptor *odysseus*/*Cxcr4b* are involved in the directional migration of primodial germ cells (Doitsidou et al., 2002; Knaut et al., 2003) and angiotensin II acts through AGTR1 to stimulate migration of rat VSMCs (Jing et al., 2002). Angiotensin receptors are also involved in vascular smooth muscle cell proliferation during development (Sayeski and Ali, 2003).

AGTRL1 binds the short polypeptide ligand Apelin (Tatemoto et al., 1998) and acts as a co-receptor for human and simian HIV strains (Choe et al., 1998; Edinger et al., 1998). Recently, a role for Apelin/AGTRL1 has emerged in control of cardiovascular function (Chen et al., 2003). Apelin is known to be a potent stimulator of cardiac contractil-

* Corresponding author. Tel.: +49 351 210 2845; fax: +49 351 210 2020.
E-mail address: oates@mpi-cbg.de (A.C. Oates).

1567-133X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.modgep.2006.09.006

ity (Szokodi et al., 2002), an arterial and venous dilator (Cheng et al., 2003) and can stimulate gastric cell differentiation *in vitro* (Wang et al., 2004). In mouse, *Agtrl1* has been shown to be an early marker of vascular development (Devic et al., 1999; Saint-Geniez et al., 2003; Saint-Geniez et al., 2002). However, targeted mutation of *Agtrl1* in mice had no observable effect on embryonic development or histology of various tissues that were examined (Ishida et al., 2004), and so potential embryonic functions for *AGTRL1* remain unclear. In this paper we report the discovery and developmental expression of the zebrafish ortholog of human *AGTRL1* in a screen for genes expressed during somitogenesis.

1.1. Isolation of the zebrafish ortholog of human AGTRL1/APJ

In a whole mount *in situ* transcript hybridization screen for genes involved in somitogenesis and neurogenesis (Tamme et al., 2001), we discovered a cDNA clone (BR131) of a gene with sequence similarity to the Angiotensin Receptor-like subfamily of the GPCRs, expressed at high levels in the epithelia separating newly formed somites and at lower levels in other epithelial structures (Figs. 2, 3). We isolated a cDNA clone containing the entire open reading frame from a 9 to 16 h post fertilisation (hpf) library and compared its putative peptide sequence with those of other Angiotensin Receptor-like genes (Fig. 1A). Phylogenetic analysis revealed that the zebrafish gene is orthologous to the human gene *AGTRL1* (Fig. 1B; see Section 2). Recent data base searches have revealed another zebrafish *AGTRL1* ortholog, therefore we have named the gene studied in this manuscript zebrafish *agtrl1a* (encoding the putative protein Agtrl1a).

1.2. agtrl1a expression during blastula and gastrula stages

To define the tissues in which *agtrl1a* is expressed during embryonic development we performed whole mount *in situ* transcript hybridisation on embryos throughout the first 24 h post fertilisation (hpf). No maternal *agtrl1a* expression was detected prior to MBT. Zygotic *agtrl1a* expression was first detected at the oblong stage (3.7 hpf) in a radially asymmetric domain in the deep cells (Fig. 2A,A′), persisting to dome stage (4.3 hpf, Figs. 2E,E′); this expression domain was absent in Maternal-Zygotic *one-eyed pinhead* (*MZoep*) embryos, indicating a dependence on Nodal signalling (Gritsman et al., 1999); (data not shown). *agtrl1a* expression is located dorsally, as shown by double staining with probes for transcripts of *agtrl1a* and for the dorsal marker *chordin* (*chd*, Schulte-Merker et al., 1997); (Figs. 2C and D). At dome stage (4.3 hpf), *agtrl1a* was also expressed in a superficial layer of cells, likely the enveloping layer (EVL) (Fig. 2E,E′). Starting at germ-ring stage (5.7 hpf), *agtrl1a* transcripts accumulated in the margin (arrowheads), and could be observed in flattened ring shapes adjacent to the yolk over the animal pole (arrows; Fig. 2F,F′). To help distinguish these cell types, thin sections were cut and examined, and were consistent with expression in the EVL, yolk syncytial layer (YSL) and the marginal hypoblast (Fig. 2G1-3).

During epibolic gastrulation (6–10 hpf), *agtrl1a* was expressed in a dispersed hypoblastic cell population, the majority of which appeared to migrate animally and dorsally away from the margin (Figs. 2H–K′). These cells were absent in *MZoep* embryos, indicating a mesendodermal identity, but the larger flattened, predominantly animal pole staining pattern remained, confirming this as YSL expression (Fig. 2I,I′). Some of the *agtrl1a*-positive hypoblastic cells accumulated at the dorsal midline (arrow), and others appeared to form adaxial precursors (arrowheads) or contribute to the prechordal plate (asterisk; Fig. 2J′ and K′). The arrangement and number of these cells was not dramatically perturbed in either *spadetail* (*spt*) or *casanova* (*cas*) mutant embryos (data not shown), indicating that they are not entirely of anterior trunk paraxial mesoderm, or endodermal fate (Dickmeis et al., 2001; Griffin et al., 1998; Kikuchi et al., 2001). Expression of *agtrl1a* at the 2 somite stage was consistent with cephalic mesoendodermal identity (Fig. 2L). Thus, migratory cells are located in regions that normally contribute to heart, head mesenchyme, pharyngal endoderm, vasculature and myeloid blood lineages. We conclude that expression of *agtrl1a* in migrating cells is a feature conserved with other angiotensin receptor subfamily members.

1.3. agtrl1a expression during segmentation and pharyngula stages

In segmentation and pharyngula stage embryos, *agtrl1a* was expressed in a range of epithelial tissues (Fig. 3). At 14 hpf, *agtrl1a* expression was evident in the otic vesicle (Figs. 3A and C) and in the epithelium covering the retina, being maintained as these cells invaginated to form the lens (Figs. 3A, B, and D). We observed *agtrl1a* expression in the tailfin primordium before 24 hpf (Fig. 3C). *agtrl1a* was also expressed in vascular primordia and then in forming vessels such as the middle cerebral vein (Fig. 3E) and primary caudal vein (Figs. 3F and G). We did not observe expression in the dorsal aorta, suggesting that *agtrl1a* expression may be restricted to venous vasculature. Thus, vascular expression is evolutionarily conserved for vertebrate *AGTRL1* genes. In addition to cranial vasculature, clusters of *agtrl1a*-positive cells were observed in the pharyngeal region at 24 hpf (Fig. 3H). To test whether these cells might be the endoderm of the pharyngal pouches, we examined expression in *oep* and in *cas*, where pharyngeal expression of *nkx2.5* is absent due to failure of endodermal differentiation (Alexander et al., 1999; Schier et al., 1997). We find that *agtrl1a* expression was absent in both *oep* and *cas* mutants at 24 hpf, specifically in the pharyngeal region and presumptive mouth (Figs. 3I,J, asterisks).

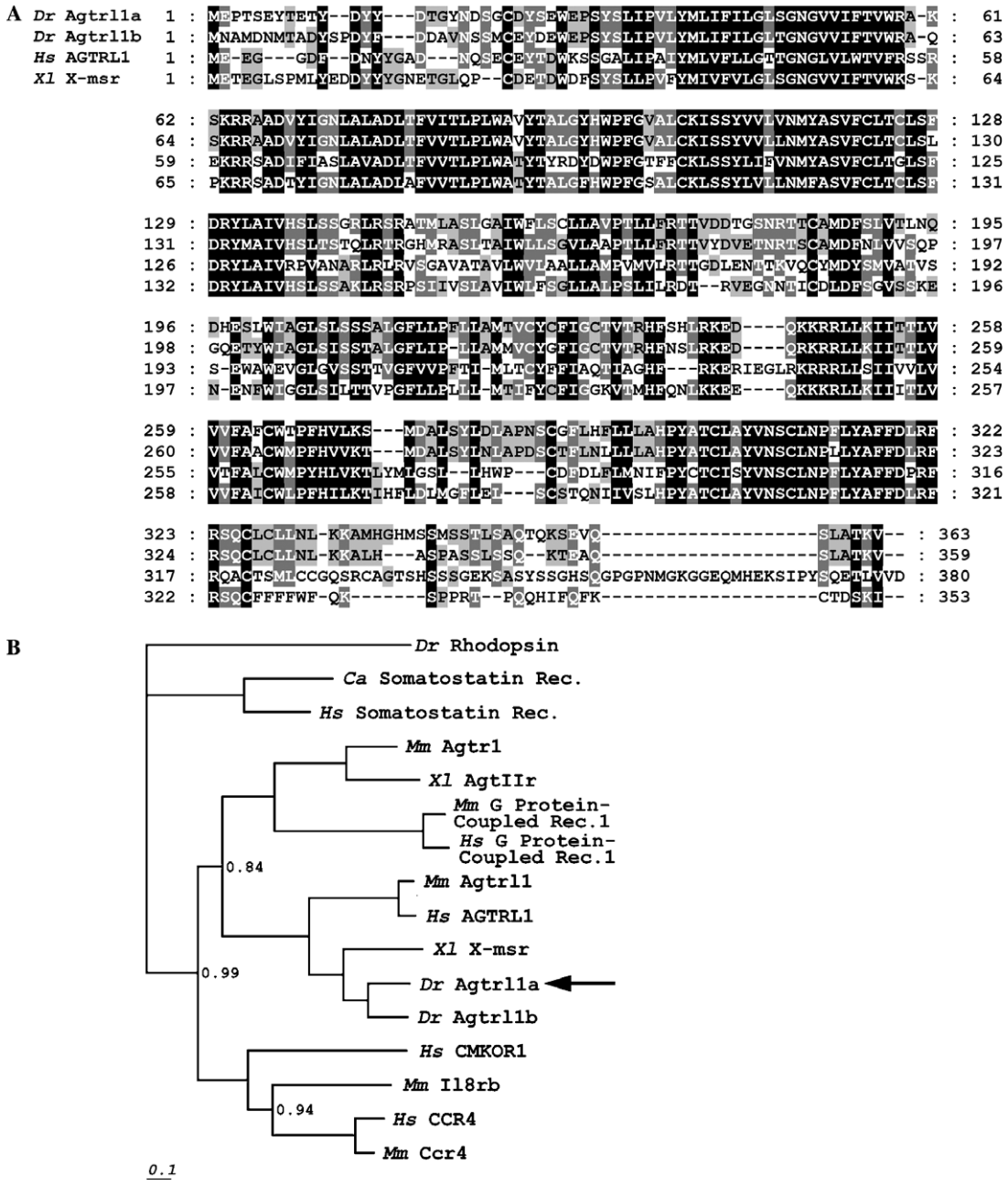

Fig. 1. Sequence and phylogenetic analysis of zebrafish *agtrl1a*. (A) ClustalW alignment of a putative translation of zebrafish *agtrl1a* (*Dr* Agtrl1a) against a duplicate ortholog *agtrl1b* (*Dr* Agtrl1b) and their human (top) and *Xenopus* (bottom) orthologs. Residues conserved in all four sequences are boxed in black shading while those conserved in three or two of the four proteins are boxed in grey. (B) Phylogenetic analysis (MRBAYES) of DNA sequences of zebrafish *agtrl1a* (*Dr* Agtrl1a, see arrow) and closely and distantly related G protein-coupled receptors. Zebrafish rhodopsin was used as outgroup. Values for node posterior probabilities are indicated where these were less than 1. See Section 2 for sequence accession numbers. The correspondence between branch length and nucleotide substitutions per site is indicated below the tree.

1.4. agtrl1a expression and regulation during somitogenesis

During somitogenesis, *agtrl1a* was expressed in the posterior presomitic mesoderm (PSM) and lateral tail bud, adaxial cells, and in stripes in 3–5 of the most recently formed somitic epithelia (Figs. 3A and C, Fig. 4). In addition, *agtrl1a* shows variability in the anterior PSM where new somites are forming, with either one or two strong stripes of expression (Figs. 4A and B). To establish the part of a somite in which *agtrl1a* is expressed we stained embryos simultaneously for *agtrl1a* and *myod* (Weinberg et al., 1996) or *dld* (Dornseifer et al., 1997) expression. The stripes of *agtrl1a* are complementary to those of *myod* (Fig. 4A and B) and overlap those of *dld* (Fig. 4C). Note, however, that *agtrl1a* expression overlaps that of *myod* in adaxial cells (Figs. 4A and B). Thus, *agtrl1a* is expressed in the anterior half of newly formed or forming somites. To test whether the variable

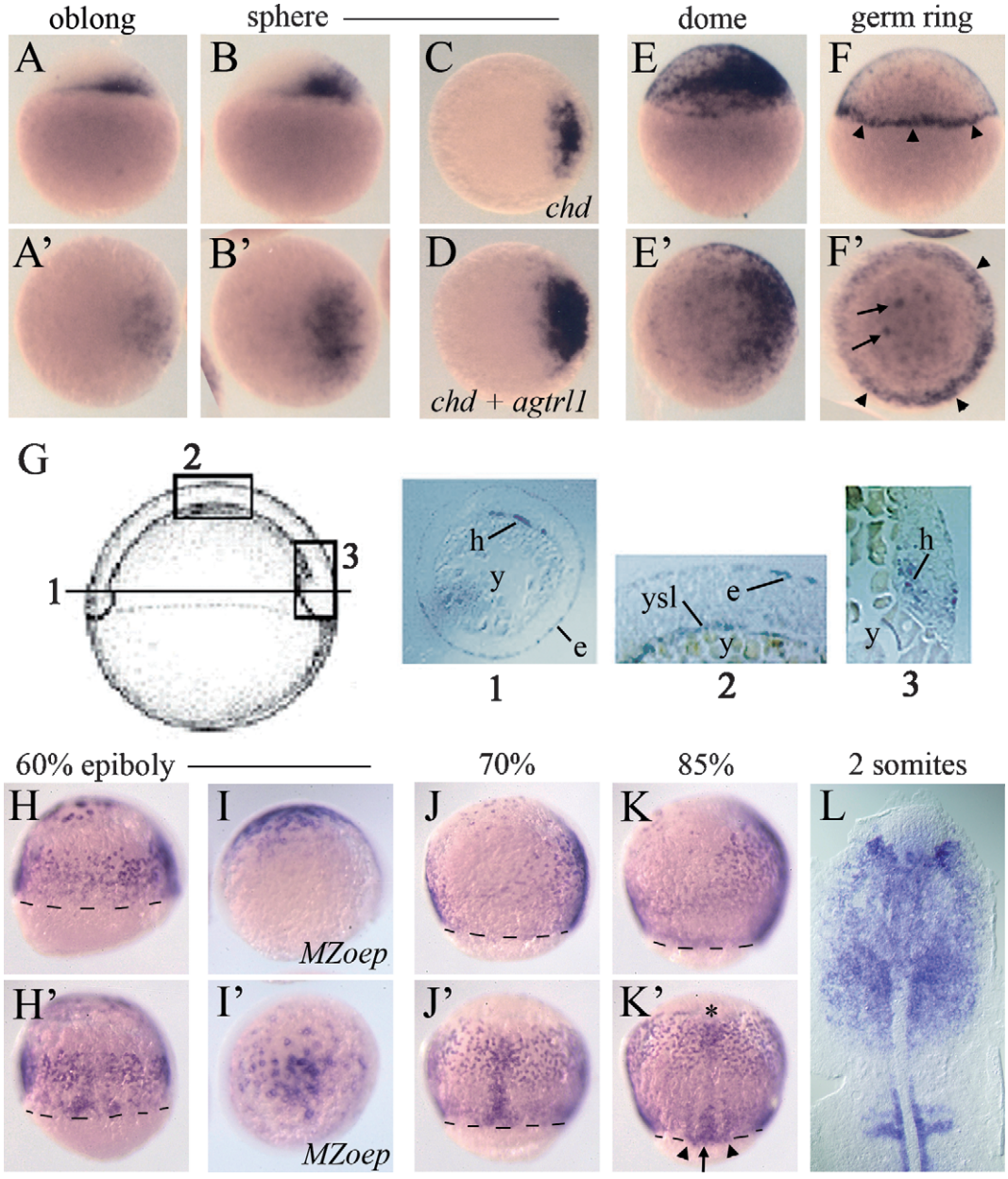

Fig. 2. Expression of *agtrl1a* during blastula and gastrula stages *In situ* transcript hybridisations on embryos up to 11 hpf. (A–F) Expression pattern of *agtrl1a* through blastula stages. Lateral views A, B, E, and F (upper panels) and animal pole views A′, B′, E′, F′ (lower panels), all with dorsal to right. C shows dorsal expression domain of *chd*, D shows embryo co-hybridized with *chd* and *agtrl1a* riboprobes. In F and F′ arrowheads marks the germ-ring and arrows indicate the YSL nuclei. (G) Sections through 6 hpf embryos after *in situ* transcript hybridisation against *agtrl1a*. The diagram shows the positions of the sections. (1) Horizontal section through mesendodermal part of the embryo (shield to top), (2) vertical section through the embryo, (3) vertical section through the shield; y, yolk; e, enveloping layer, h, hypoblast, ysl, yolk syncitial layer. (H–K) Expression of *agtrl1a* during gastrula stages. Lateral views with dorsal to right H–K, dorsal views H′, J′, K′ and animal view I′. Dashed lines mark the gastrula margin; in K′ the asterisk, arrow and the arrowheads mark the prechordal plate, the axial mesoderm, and the adaxial cells, respectively. I, I′ shows *agtrl1a* expression in *MZoep* embryos. (L) Flat mounted embryo with anterior up, showing *agtrl1a* expression in the head.

PSM expression observed reflects dynamic changes like *her1* cyclic gene transcription (Holley et al., 2000; Sawada et al., 2000), we stained embryos simultaneously for *agtrl1a* and *her1*. *agtrl1a* expression domains in the PSM were always static in comparison to the wavefronts of *her1* expression, indicating that *agtrl1a* is not expressed cyclically (Fig. 3D). Combined, these data indicate that cells that will form the anterior epithelial border of a somite begin to express *agrtl1a* approximately an hour before the morphological appearance of the furrow.

A number of mutations are known to affect the formation of somites (van Eeden et al., 1996). Mutations affecting Notch signalling (e.g. *beamter bea/deltac*) (Julich et al., 2005), *deadly seven* (*des/notch1a*) (Holley et al., 2002), *mind bomb* (*mib*) (Itoh et al., 2003), and *after eight* (*aei/deltad*) (Holley et al., 2000) lead to a loss of coordination

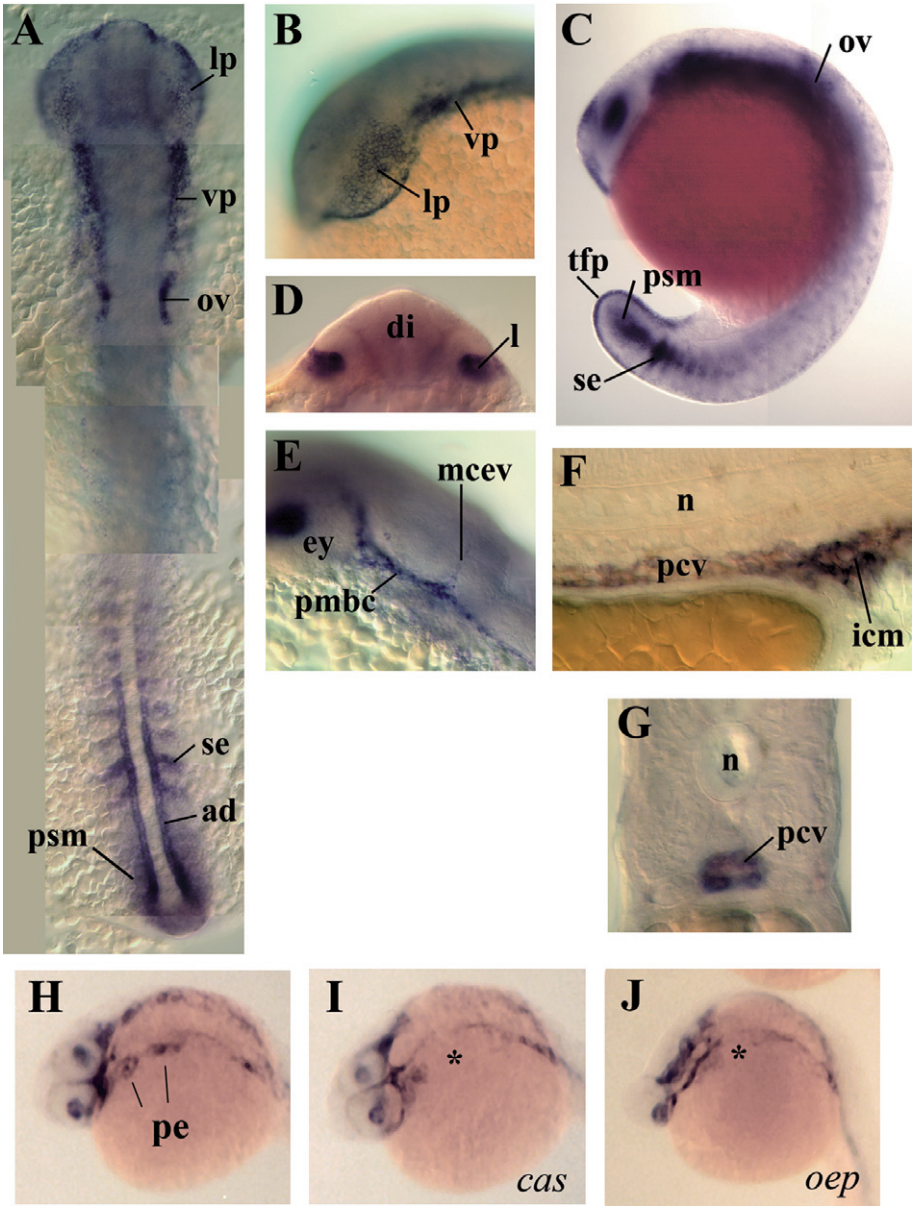

Fig. 3. Expression of *agtrl1a* during segmentation and pharyngula stages *in situ* transcript hybridisations showing *agtrl1a* expression in embryos from 14 to 24 hpf. (A) Dorsal axial view of 14 hpf embryo. Expression is observed in the epithelium covering the retina (lens primordium, lp), putative vascular precursors (vp) lateral to mid- and hindbrain, in otic vesicles (ov), in somitic epithelia (se), presomitic mesoderm (psm) and adaxial cells (ad). (B) Lateral view of head of embryo in A. (C) Lateral view of an embryo at 18.5 hpf showing *agtrl1a* expression in otic vesicles (ov), tail fin primordium (tfp), presomitic mesoderm (psm) and the most recently formed somite epithelium (se). (D) Transverse section at the level of the diencephalon (di) at 24 hpf showing expression in the developing lens (l). Dorsal is up. (E) Lateral view of the developing head of an embryo at 24 hpf showing *agtrl1a* expression in developing vasculature. The primordial midbrain channel (pmbc), middle cerebral vein (mcev) and developing eye (ey) are indicated. (F) Lateral view of the yolk extension and cloaca region of an embryo at 24 hpf showing expression in the primary caudal vein (pcv) and intermediate cell mass (icm). (G) Transverse section at the level of the yolk extension at 24 hpf showing expression in the primary caudal vein. (H–J) *agtrl1a* expression in the pharyngeal endoderm (pe) in wt (H), is absent in *cas* (I) and *oep* (J) embryos (asterisk) at 24 hpf seen in lateral oblique view, anterior to left.

of cyclic gene expression in the cells of the PSM (Jiang et al., 2000; Oates and Ho, 2002), while mutation of the *fused somites* (*fss*/*tbx24*) gene results in loss of any division of paraxial mesoderm into somites (Nikaido et al., 2002). To understand the regulation of *agtrl1a* in the paraxial mesoderm, we compared *agtrl1a* expression in mutant backgrounds to wild-type (Figs. 4E–I). Reduced "salt and pepper" expression of *agtrl1a* was observed in the somitic regions of mutant embryos with defective Notch signalling (Figs. 4F–H) while no expression could be seen in this region in the *fss*/*tbx24* mutant (Fig. 4I). *agtrl1a* expression in adaxial cells was not affected in any of the above mutant backgrounds. Thus, *agtrl1a* expression is downstream of mechanisms controlling the patterning of paraxial mesoderm into segments.

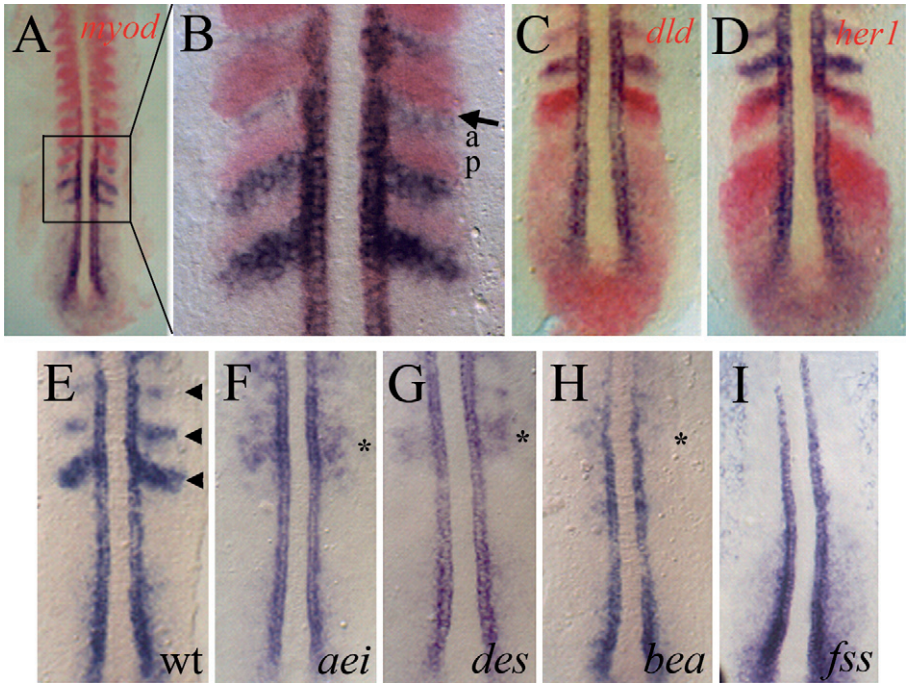

Fig. 4. Expression of *agtrl1a* in somites and in mutants affecting somitogenesis (A–D) *In situ* transcript hybridisations of 15–16 hpf (12–14 somite) wild-type embryos, dorsal views over the posterior notochord (centre), anterior to top. (A and B) *agtrl1a* and *myod*; (C) *agtrl1a* and *dld*; (D) *agtrl1a* and *her1*: *agtrl1a* expression is stained blue while *myod*, *her1* and *dld* expression is red. B is a magnification of the region marked with the box in A; a, anterior somite half, p, posterior somite half, arrow indicates the most recently formed somite furrow. (E–I) *In situ* transcript hybridisation to detect *agtrl1a* expression at 16 hpf in mutant zebrafish embryos. (E) Wild-type embryo, normal somites are indicated with arrowheads. Embryos homozygous for the mutations (F) *after eight* (*aei*^{tr233}), (G) *deadly seven* (*des*^{tp37}), (H) *beamter* (*bea*^{tm98}) with region of disrupted *agtrl1a* expression indicated with an asterisk, and (I) *fused somites/tbx24* (*fss/tbx24*^{te314a}).

2. Experimental procedures

2.1. Cloning of agtrl1a cDNA

Clone BR131 was isolated in a whole mount *in situ* transcript hybridisation screen for genes involved in somitogenesis and neurogenesis (Tamme et al., 2001). Primers BaRa1 (5′-ACTACAGTAGACG ACAC TGGG-3′ and 5′-TCTTCAGCACATGAAAAGGCG-3′) were designed from BR131 sequence and used to screen λ-bacteriophage sub-libraries (Lardelli, 2002) generated from a 9 to 16 hpf library kindly donated by D. Grunwald (University of Utah, Salt Lake City). A cDNA clone containing the entire open reading frame of *agtrl1a* was subsequently isolated and sequence submitted to GenBank with the Accession No. DQ983235.

2.2. Phylogenetic analysis

AGTRL-related DNA sequences (accession numbers below) were aligned using ClustalW. Bayesian analysis was conducted using the MRBAYES v3.1.2 program with *Danio rerio* rhodopsin as an outgroup (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Markov Chain Monte Carlo convergence was conducted essentially as described (Larget and Simon, 1999) using a General Time-Reversible model with invariable sites and gamma distribution values included (GTR + I + G, Tamura and Nei, 1993; Yang, 1993). We ran four simultaneous MCMC chains for 40000 generations five times to generate five distinct data sets, each with distinct random seeds. Trees were sampled every 40 generations, and a total of 751 trees were saved for each data set. The data sets were summarized and statistically analysed independently and in conjunction to confirm consistency between runs. The combined dataset summary was used to determine the most probable tree topology, branch lengths, and to calculate final Bayesian posterior probabilities. All MCMC analysis was performed using MRBAYES v3.1.2. For our phylogenetic analysis we have used the *agtrl1a* sequence previously deposited by others (see accession number BC056308) but note that we believe this to contain the entire open reading frame (see Fig. 1A), *agtrl1b* (BC097125), *C. auratus*-somatostatin-r (AF252879), *H. sapiens*-somatostatin-r2 (BC019610), *M. musculus*-G-protein-coupled-r1 (BC032934), *H. sapiens*-G-protein-coupled-r1 (BC067833), *X. laevis*-mesenchyme-associated-serpentine-r (XLXMSRGEN), *M. musculus*-angiotensin-rlike1 (BC039224), *M. musculus*-angiotensin-r1 (BC036175), *X. laevis*-angiotensin-II-r (S73274), *H. sapiens*-b-chemokine-r-CCR4 (AB023889), *M. musculus*-chemokine-r (MMU15208), *M. musculus*-il8-r-beta (BC051677), *H. sapiens*-chemokine-orphan-r1 (BC036661), *D. rerio*-rhodopsin (NM_131084), *H. sapiens*-AGTRL1 (NM_005161).

2.3. In situ transcript hybridization on whole-mount zebrafish embryos

Embryos were raised at 28.5°C and staged as previously described (Kimmel et al., 1995). *In situ* transcript hybridisation was performed as described (Tamme et al., 2001) using single-stranded RNA probes labelled with digoxigenin-UTP or FITC-UTP (Roche Ltd, Basel, Switzerland). Riboprobes were synthesized directly from cDNA clones in the Bluescript SK vector (Stratagene) or were synthesised used T7 RNA polymerase after PCR amplification of the template with M13 and M13R primers.

2.4. Sectioning of embryos

6 hpf embryos were prepared routinely for paraffin embedding after *in situ* hybridisation against *agtrl1a*. 5 μm thick sections were cut with a rotary microtome.

Acknowledgments

This work was supported by funds from Australian Research Council distributed through The Centre for the Molecular Genetics of Development and by infrastructure funding from The School of Molecular and Biomedical Sciences at The University of Adelaide. ACO was supported by a Ludwig Institute for Cancer Research Travelling Fellowship, and the Max Planck Society. The authors thank Robert Ho (University of Chicago), in whose lab part of this work was carried out, Mario Caccamo (Sanger Centre, Cambridge) for help with gene duplications, and Yohanna Arboleda, Laurel Rohde and Carl-Philipp Heisenberg (MPI-CBG, Dresden) for their expert input. Experimentation involving animals was carried out under the auspices of the Animal Ethics Committee of The University of Adelaide, Princeton University, and the University of Chicago.

References

Alexander, J., Rothenberg, M., Henry, G.L., Stainier, D.Y., 1999. casanova plays an early and essential role in endoderm formation in zebrafish. Dev. Biol. 215, 343–357.

Benndorf, R., Boger, R.H., Ergun, S., Steenpass, A., Wieland, T., 2003. Angiotensin II type 2 receptor inhibits vascular endothelial growth factor-induced migration and *in vitro* tube formation of human endothelial cells. Circ. Res. 93, 438–447.

Chassagne, C., Adamy, C., Ratajczak, P., Gingras, B., Teiger, E., Planus, E., Oliviero, P., Rappaport, L., Samuel, J.L., Meloche, S., 2002. Angiotensin II AT(2) receptor inhibits smooth muscle cell migration via fibronectin cell production and binding. Am. J. Physiol. Cell Physiol. 282, C654–C664.

Chen, M.M., Ashley, E.A., Deng, D.X., Tsalenko, A., Deng, A., Tabibiazar, R., Ben-Dor, A., Fenster, B., Yang, E., King, J.Y., Fowler, M., Robbins, R., Johnson, F.L., Bruhn, L., McDonagh, T., Dargie, H., Yakhini, Z., Tsao, P.S., Quertermous, T., 2003. Novel role for the potent endogenous inotrope apelin in human cardiac dysfunction. Circulation 108, 1432–1439.

Cheng, X., Cheng, X.S., Pang, C.C., 2003. Venous dilator effect of apelin, an endogenous peptide ligand for the orphan APJ receptor, in conscious rats. Eur. J. Pharmacol. 470, 171–175.

Choe, H., Farzan, M., Konkel, M., Martin, K., Sun, Y., Marcon, L., Cayabyab, M., Berman, M., Dorf, M.E., Gerard, N., Gerard, C., Sodroski, J., 1998. The orphan seven-transmembrane receptor apj supports the entry of primary T-cell-line-tropic and dualtropic human immunodeficiency virus type 1. J. Virol. 72, 6113–6118.

Cote, F., Do, T.H., Laflamme, L., Gallo, J.M., Gallo-Payet, N., 1999. Activation of the AT(2) receptor of angiotensin II induces neurite outgrowth and cell migration in microexplant cultures of the cerebellum. J. Biol. Chem. 274, 31686–31692.

Devic, E., Paquereau, L., Vernier, P., Knibiehler, B., Audigier, Y., 1996. Expression of a new G protein-coupled receptor X-msr is associated with an endothelial lineage in *Xenopus laevis*. Mech. Dev. 59, 129–140.

Devic, E., Rizzoti, K., Bodin, S., Knibiehler, B., Audigier, Y., 1999. Amino acid sequence and embryonic expression of msr/apj, the mouse homolog of *Xenopus* X-msr and human APJ. Mech. Dev. 84, 199–203.

Dickmeis, T., Mourrain, P., Saint-Etienne, L., Fischer, N., Aanstad, P., Clark, M., Strahle, U., Rosa, F., 2001. A crucial component of the endoderm formation pathway, Casanova, is encoded by a novel sox-related gene. Genes Dev. 15, 1487–1492.

Doitsidou, M., Reichman-Fried, M., Stebler, J., Koprunner, M., Dorries, J., Meyer, D., Esguerra, C.V., Leung, T., Raz, E., 2002. Guidance of primordial germ cell migration by the chemokine SDF-1. Cell 111, 647–659.

Dornseifer, P., Takke, C., Campos-Ortega, J.A., 1997. Overexpression of a zebrafish homologue of the Drosophila neurogenic gene Delta perturbs differentiation of primary neurons and somite development. Mech Dev 63, 159–171.

Edinger, A.L., Hoffman, T.L., Sharron, M., Lee, B., Yi, Y., Choe, W., Kolson, D.L., Mitrovic, B., Zhou, Y., Faulds, D., Collman, R.G., Hesselgesser, J., Horuk, R., Doms, R.W., 1998. An orphan seven-transmembrane domain receptor expressed widely in the brain functions as a coreceptor for human immunodeficiency virus type 1 and simian immunodeficiency virus. J. Virol. 72, 7934–7940.

Griffin, K.J., Amacher, S.L., Kimmel, C.B., Kimelman, D., 1998. Molecular identification of spadetail: regulation of zebrafish trunk and tail mesoderm formation by T-box genes. Development 125, 3379–3388.

Gritsman, K., Zhang, J., Cheng, S., Heckscher, E., Talbot, W.S., Schier, A.F., 1999. The EGF-CFC protein one-eyed pinhead is essential for nodal signaling. Cell 97, 121–132.

Holley, S.A., Geisler, R., Nusslein-Volhard, C., 2000. Control of her1 expression during zebrafish somitogenesis by a delta-dependent oscillator and an independent wave-front activity. Genes Dev. 14, 1678–1690.

Holley, S.A., Julich, D., Rauch, G.J., Geisler, R., Nusslein-Volhard, C., 2002. her1 and the notch pathway function within the oscillator mechanism that regulates zebrafish somitogenesis. Development 129, 1175–1183.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Ishida, J., Hashimoto, T., Hashimoto, Y., Nishiwaki, S., Iguchi, T., Harada, S., Sugaya, T., Matsuzaki, H., Yamamoto, R., Shiota, N., Okunishi, H., Kihara, M., Umemura, S., Sugiyama, F., Yagami, K., Kasuya, Y., Mochizuki, N., Fukamizu, A., 2004. Regulatory roles for APJ, a seven-transmembrane receptor related to angiotensin-type 1 receptor in blood pressure *in vivo*. J. Biol. Chem. 279, 26274–26279.

Itoh, M., Kim, C.H., Palardy, G., Oda, T., Jiang, Y.J., Maust, D., Yeo, S.Y., Lorick, K., Wright, G.J., Ariza-McNaughton, L., Weissman, A.M., Lewis, J., Chandrasekharappa, S.C., Chitnis, A.B., 2003. Mind bomb is a ubiquitin ligase that is essential for efficient activation of Notch signaling by Delta. Dev. Cell 4, 67–82.

Jiang, Y.J., Aerne, B.L., Smithers, L., Haddon, C., Ish-Horowicz, D., Lewis, J., 2000. Notch signalling and the synchronization of the somite segmentation clock. Nature 408, 475–479.

Jing, T., He, G., Liu, J., Wang, G., Wu, H., Wang, H., 2002. Role of angiotensin II and angiotensin II receptors in vascular smooth muscle cell migration *in vitro*. Chin. Med. J. (Engl) 115, 649–653.

Julich, D., Hwee Lim, C., Round, J., Nicolaije, C., Schroeder, J., Davies, A., Geisler, R., Lewis, J., Jiang, Y.J., Holley, S.A., 2005. beamter/delta C and the role of Notch ligands in the zebrafish somite segmentation, hindbrain neurogenesis and hypochord differentiation. Dev. Biol. 286, 391–404.

Kikuchi, Y., Agathon, A., Alexander, J., Thisse, C., Waldron, S., Yelon, D., Thisse, B., Stainier, D.Y., 2001. casanova encodes a novel Sox-related protein necessary and sufficient for early endoderm formation in zebrafish. Genes Dev. 15, 1493–1505.

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F., 1995. Stages of embryonic development of the zebrafish. Dev. Dyn. 203, 253–310.

Knaut, H., Werz, C., Geisler, R., Nusslein-Volhard, C., 2003. A zebrafish homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor. Nature 421, 279–282.

Lardelli, M., 2002. Generation and PCR screening of bacteriophage lambda sublibraries enriched for rare clones (the sublibrary method). Methods Mol. Biol. 192, 391–399.

Larget, B., Simon, D., 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16, 750–759.

Malbon, C.C., 2005. G proteins in development. Nat. Rev. Mol. Cell Biol. 6, 689–701.

Nikaido, M., Kawakami, A., Sawada, A., Furutani-Seiki, M., Takeda, H., Araki, K., 2002. Tbx24, encoding a T-box protein, is mutated in the zebrafish somite-segmentation mutant fused somites. Nat. Genet 31, 195–199.

Oates, A.C., Ho, R.K., 2002. Hairy/E(spl)-related (Her) genes are central components of the segmentation oscillator and display redundancy with the Delta/Notch signaling pathway in the formation of anterior segmental boundaries in the zebrafish. Development 129, 2929–2946.

O'Dowd, B.F., Heiber, M., Chan, A., Heng, H.H., Tsui, L.C., Kennedy, J.L., Shi, X., Petronis, A., George, S.R., Nguyen, T., 1993. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. Gene 136, 355–360.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Saint-Geniez, M., Masri, B., Malecaze, F., Knibiehler, B., Audigier, Y., 2002. Expression of the murine msr/apj receptor and its ligand apelin is upregulated during formation of the retinal vessels. Mech. Dev. 110, 183–186.

Saint-Geniez, M., Argence, C.B., Knibiehler, B., Audigier, Y., 2003. The msr/apj gene encoding the apelin receptor is an early and specific marker of the venous phenotype in the retinal vasculature. Gene Expr. Patterns 3, 467–472.

Sawada, A., Fritz, A., Jiang, Y.J., Yamamoto, A., Yamasu, K., Kuroiwa, A., Saga, Y., Takeda, H., 2000. Zebrafish Mesp family genes, mesp-a and mesp-b are segmentally expressed in the presomitic mesoderm, and Mesp-b confers the anterior identity to the developing somites. Development 127, 1691–1702.

Sayeski, P.P., Ali, M.S., 2003. The critical role of c-Src and the Shc/Grb2/ERK2 signaling pathway in angiotensin II-dependent VSMC proliferation. Exp. Cell Res. 287, 339–349.

Schier, A.F., Neuhauss, S.C., Helde, K.A., Talbot, W.S., Driever, W., 1997. The one-eyed pinhead gene functions in mesoderm and endoderm formation in zebrafish and interacts with no tail. Development 124, 327–342.

Schulte-Merker, S., Lee, K.J., McMahon, A.P., Hammerschmidt, M., 1997. The zebrafish organizer requires chordino. Nature 387, 862–863.

Strosberg, A.D., 1996. G protein coupled R7G receptors. Cancer Surv. 27, 65–83.

Szokodi, I., Tavi, P., Foldes, G., Voutilainen-Myllyla, S., Ilves, M., Tokola, H., Pikkarainen, S., Piuhola, J., Rysa, J., Toth, M., Ruskoaho, H., 2002. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. Circ. Res. 91, 434–440.

Tamme, R., Mills, K., Rainbird, B., Nornes, S., Lardelli, M., 2001. Simple, directional cDNA cloning for *in situ* transcript hybridization screens. Biotechniques 31, 938–942. 944, 946.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.

Tatemoto, K., Hosoya, M., Habata, Y., Fujii, R., Kakegawa, T., Zou, M.X., Kawamata, Y., Fukusumi, S., Hinuma, S., Kitada, C., Kurokawa, T., Onda, H., Fujino, M., 1998. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem. Biophys. Res. Commun. 251, 471–476.

Thomas, W.G., Mendelsohn, F.A., 2003. Angiotensin receptors: form and function and distribution. Int. J. Biochem. Cell Biol. 35, 774–779.

van Eeden, F.J., Granato, M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.P., Jiang, Y.J., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., Warga, R.M., Allende, M.L., Weinberg, E.S., Nusslein-Volhard, C., 1996. Mutations affecting somite formation and patterning in the zebrafish, Danio rerio. Development 123, 153–164.

Wang, G., Anini, Y., Wei, W., Qi, X., AM, O.C., Mochizuki, T., Wang, H.Q., Hellmich, M.R., Englander, E.W., Greeley Jr., G.H., 2004. Apelin, a new enteric peptide: localization in the gastrointestinal tract, ontogeny, and stimulation of gastric cell proliferation and of cholecystokinin secretion. Endocrinology 145, 1342–1348.

Weinberg, E.S., Allende, M.L., Kelly, C.S., Abdelhamid, A., Murakami, T., Andermann, P., Doerre, O.G., Grunwald, D.J., Riggleman, B., 1996. Developmental regulation of zebrafish MyoD in wild-type, no tail and spadetail embryos. Development 122, 271–280.

Yang, Z., 1993. Maximum likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. Mol. Bio. Evol. 10, 1396–1401.