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RESEARCH ARTICLE

Molecular structure and developmental expression of zebrafish *atp2a* genes

Yen-Yu Lai · Chiung-Wen Pai · I-Ting Tsai · Chi-Yuan Chou · Chia-Ti Tsai · Yau-Hung Chen

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Abstract

We isolated two *atp2a* genes, *atp2a1* and *atp2a2a*, from embryonic zebrafish. Amino acid sequences deduced from zebrafish *atp2a* genes are aligned with orthologue proteins from other species, the results showed that they share high percentage of identities (82%-94%) and acidic pIs (5.03-5.33). Whole mount *in situ* hybridization experiments showed that *atp2a1* and *atp2a2a* are maternal inherited genes which can be detected at 1-cell stage embryos and express in the entire animal pole from 6 hours post-fertilization (hpf) to 12 hpf. At the later stages (48-96 hpf), expression of *atp2a1* was restricted in head and trunk muscles as well as in some neurons. In contrast to the strongly expression of *atp2a1* in head muscle, expression of *atp2a2a* was detected in head muscle in a fainter manner. In addition, transcripts of *atp2a2a* were observed in the developing heart during early cardiogenesis. The present studies not only help us to comparatively analyze *atp2a* genes across species, but also provide useful information about expressions during early embryogenesis that will help in further investigations of functional studies of Atp2a in the future.

Keywords Calcium; Cardiogenesis; Muscle; SERCA; Zebrafish

Y.-Y. Lai · I-T. Tsai · Y.-H. Chen(✉)
Department of Chemistry, Tamkang University, Tamsui, Taiwan
e-mail: yauhung@mail.tku.edu.tw

Y.-Y. Lai
Graduate Institute of Life Sciences, Tamkang University, Tamsui, Taiwan

C.-W. Pai · C.-Y. Chou
Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan

C.-T. Tsai(✉)
Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan
e-mail: fang31@ms39.hinet.net

Introduction

Calcium (Ca^{2+}) plays many important physiological roles, such as neuron transmission, blood coagulation, heart beat, and muscle contraction (Berridge et al., 1998; MacLennan, 2000; Varga-Szabo et al., 2009). Cytosolic Ca^{2+} concentrations are delicately regulated by many membrane receptors and/or Ca^{2+} pump. For example, in muscle cells, intracellular Ca^{2+} concentration is regulated by (i) activation of ryanodine receptors (RyRs) and inositol triphosphate receptors (IP3) in the sarcoplasmic reticulum (SR) membrane to release Ca^{2+} from the SR to the cytosol; and (ii) activation of SR (or ER) Ca^{2+}-ATPase (SERCA/ATP2A), a Ca^{2+} pump, to retrieve cytosolic Ca^{2+} from the cytosol to the SR lumen (Berridge et al., 1998; Fill and Copello, 2002; Mikoshiba, 2007; Berridge, 2009). Increased cytosolic Ca^{2+} activates troponin C that initiates actin/myosin sliding, thus contracting the muscles; instead, reduced cytosolic Ca^{2+} relaxes the muscles (McLennan et al., 1997; Gordon et al., 2000).

ATP2A family genes encode all kinds of the SERCA Ca^{2+}-ATPases, including SERCA1(ATP2A1), SERCA2(ATP2A2), and SERCA3(ATP2A3). Those enzymes catalyze the hydrolysis of ATP coupled with the translocation of Ca^{2+} from the cytosol to the SR lumen, and played many physiological roles, especially muscular excitation and contraction. The molecular structure of *ATP2A* family genes has been determined extensively in fly (Magyar et al., 1995), sea urchin (Gunaratne and Vacquier, 2006), chicken (Machuca et al., 1999) and mammals (Brandl et al., 1986; Lytton and MacLennan, 1988; Wu and Lytton, 1993; Schleef et al., 1996). In zebrafish, there are at least four *atp2a* genes, such as *atp2a1*, *atp2a2a*, *atp2a2b*, and *atp2a3* were reported (Lo et al., 2003; Hirata et al., 2004; Ebert et al., 2005), but their expression information during early embryogenesis are still limited.

As it might be expected, any defect in the above Ca^{2+} signaling pathways induces motor disorder or other pathological defects. For example, Brody disease, a rare inherited disorder of skeletal muscle function in human, is due to *SERCA1* muta-

tion (Odermatt et al., 1996). The clinical signs and symptoms of Brody disease include exercise-induced impairment of muscle relaxation, stiffening and cramps (Brody, 1969; MacLennan, 2000). In mice, inactivation of SERCA leads to neonatal lethality because their diaphragm functions are impaired (Pan et al., 2003). In zebrafish, mutation in *atp2a1* leads to muscle relaxation defect which is similar to human *SERCA1*-deficient phenotypes (Hirata et al., 2004; Olson et al., 2010). These observations indicate that biological functions of SERCA1 are conserved between mammals and zebrafish.

On the other hand, mutations in *SERCA2* (*ATP2A2*) causes Darier disease, an autosomal-dominant skin disorder in human (Sakuntabhai et al., 1999). The symptoms of Darier disease are loss of adhesion between epidermal cells (acantholysis) and abnormal keratinization (Sakuntabhai et al., 1999). In mice, the ablation of *ATP2A2* was lethal, but heterozygous *SERCA2*$^{-/-}$ mice manifested impaired cardiac contractility and delayed cardiomyocyte relaxation (Periasamy et al., 1999). These observations suggest that the biological functions of SERCA2 between human and mice might be diverse. To elucidate the physiological functions of *atp2a* genes during early embryogenesis, it is worthy to analyze comparatively *atp2a* genes across species. Here, we report the spadiotemporal expressions of two zebrafish *atp2a* genes. *atp2a1* was characterized as fast-muscle-specific, whereas *atp2a2a* was found to be expressed in fast-muscle and heart during early embryogenesis. This information may provide more insight into the molecular structure and expression patterns of the lower vertebrate *atp2a* genes.

Materials and Methods

Database searches and phylogenetic analysis

Database searches were carried out using the Blast program at the National Center for Biotechnology Information (Altschul et al., 1997). cDNA clones encode Ata2a1 and Atp2a2a were purchased from Open Biosystem (USA). The presumptive Ata2a1 and Atp2a2a amino acid sequences were determined with the Wisconsin Sequence Analysis Package v.10.0 (GCG). The Gap program of that package was used for pair comparisons, and the Pileup and Prettybox programs used for multiple comparisons. ExPASy ProtParam tool (http://expasy.org/tools/protparam.html) was used to predict the pI and MW of Ata2a1 and Atp2a2a. The Clustalw molecular evolution genetic program was used for our phylogenic tree analysis (http://www.ebi.ac.uk/clustalw/).

Fish embryos staging

Mature zebrafish (AB strain) were raised at the zebrafish facility of the Life Sciences Development Center, Tamkang University. The fish were maintained at 28°C with a photoperiod of 14 hr light and 10 hr dark, in an aquarium supplied with freshwater and aeration (Chen et al., 2009a; Wang et al., 2009a). Embryos were produced using standard procedures (Westerfield, 1995) and were staged according to standard criteria: hours postfertilization, hpf; or days postfertilization (dpf; Kimmel et al., 1995).

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

We corrected 100 embryos per stage to extract their total RNA. RNA isolation and first-strand cDNA synthesis procedures were according to the previous report with minor modification (Chen et al., 2000; 2001; Wang et al., 2009b). Primer sets (ATP2A1-F: 5'-ACCACCAACCAGATGTGTGTCACA-3', ATP-2A1-R: 5'-AACTGGACCAGTCAGGGGCA-3'; ATP2A2A-F: 5'-TGCCACCGCTCTAGGCTTCAAC-3', ATP2A2A-R: 5'-TG-AGCAGCTCGTCCAAGAGGATG-3'; and β-actin-F: 5'-GT-CCCGTACGCCTCTGGTCG-3', β-actin-R: 5'-GCCGGAC-TCATCGTACTCCTG-3') were designed based on the sequences encoding of putative zebrafish *atp2a1*, *atp2a2a*, and β-actin.

Whole mount *in situ* hybridization, cryosection and images

The procedures for whole mount *in situ* hybridization, and cryosection have been described previously (Chen et al., 2009b; Pai and Chen, 2010; Peng et al., 2010), except that *atp2a1* and *ata2a2a* (this study) were used as probes. They were digoxigenin-labeled, after we cloned their partial DNA fragments. All embryos were observed under a microscope (DM 2500, Leica, Germany) equipped with Nomarski differential interference contrast optics (Kramer Scientific) and a digital camera (Cannon, Japan).

Results and Discussion

Comparison of deduced amino acid sequences

By searching GenBank, we found two putative zebrafish *atp2a* sequences (*atp2a1*: NM_001007029, and *atp2a2a*: NM_200965). The deduced amino acid sequence of zebrafish Atp2a1 revealed a 994-amino acid polypeptide (Fig. 1). The zebrafish Atp2a1 polypeptide shares sequence identities of 82-91% of the reported Atp2a1 of human, dog, rat, mouse, chicken and *Xenopus*. On the other hand, the deduced amino acid sequence of zebrafish Atp2a2a revealed a 996-amino acid polypeptide (Fig. 2). The zebrafish Atp2a2a polypeptide shares sequence identities of 85-94% of the reported Atp2a2 of human, dog, rat, mouse, chicken and *Xenopus*. In addition, we used the Clustalw program to determine the phylogenic similarities between zebrafish Atp2a1/Atp2a2a and that of other known species. The phylogenic tree generated by the program showed that zebrafish Atp2a1/Atp2a2a (76%) was more closely related to *Xenopus* Atp2a1/Atp2a2a than those from higher vertebrates and microorganisms (data not shown). Moreover, *atp2a1*/

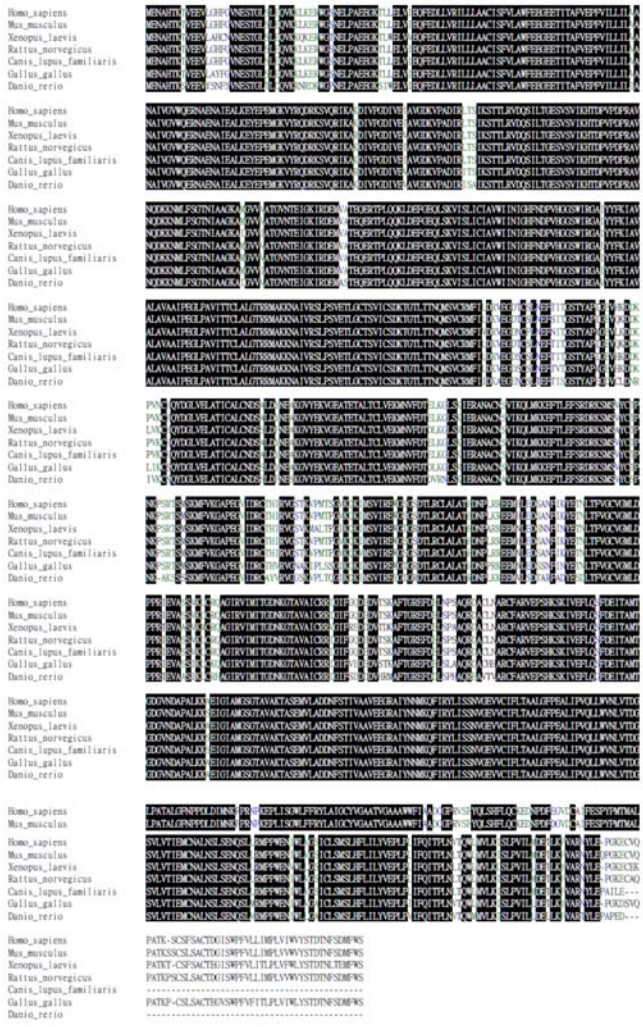

Figure 1. Comparison of the deduced amino acid sequence of zebrafish Atp2a1 with those of other known species. The information was obtained from the GenBank nucleotide sequence database with the following accession numbers: human (NM_004320), dog (XM_855027), rat (NM_058213), mouse (NM_007504), chicken (NM_205519) and *Xenopus* (NM_001092974) Atp2a1. Amino acid residues similar to those of the zebrafish Atp2a1 are presented in black.

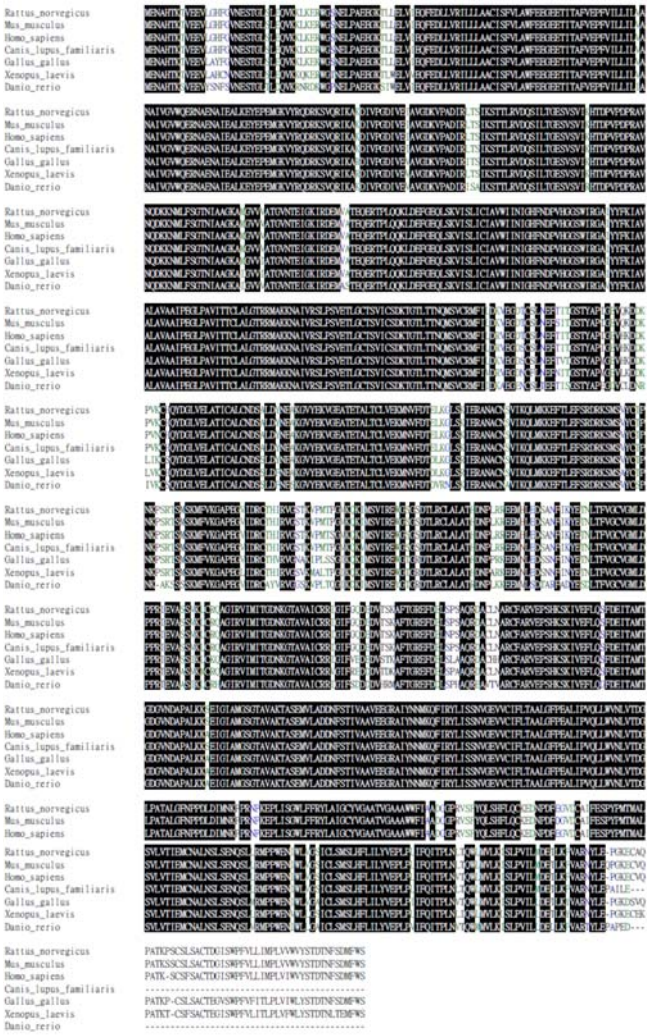

Figure 2. Comparison of the deduced amino acid sequence of zebrafish Atp2a2a with those of other known species. The information was obtained from the GenBank nucleotide sequence database with the following accession numbers: human (NM_170665), dog (NM_ 001003214), rat (NM_001110139), mouse (NM_001110140), chicken (XM_415130) and *Xenopus* (NM_001086935) Atp2a2. Amino acid residues similar to those of the zebrafish Atp2a2 are presented in black.

atp2a2 gene transcripts from selected vertebrates and their molecular features are summarized in Table 1. These data reveal that Atp2a1 and Atp2a2 proteins have acidic pIs (Atp2a1: 5.07-5.25; Atp2a: 5.03-5.33) and are highly negative charged at pH 7.0.

Developmental expression of zebrafish *atp2a1* and *atp2a2a*

Developmental expression of zebrafish *atp2a1* and *atp2a2a* transcripts were determined by RT-PCR, which revealed that endogenous *atp2a1* expressed from 18 hpf to 7 dpf, and *atp2a2a* expressed from 1-cell (0 hpf) to 7 dpf (Fig. 3). Although RT-PCR products of *atp2a1* were undetectable from 1-cell (0 hpf) to 6 hpf, weak signals were detected by nested RT-PCR analysis (data not shown). These observations indicated that zebrafish *atp2a1* and *atp2a2a* are maternal inherited genes.

Spatiotemporal expression of zebrafish *atp2a1* transcripts during early development

To determine the spatiotemporal expression patterns of *atp2a1* during early development, we performed whole mount in situ hybridization using a *atp2a1* antisense DIG-labeled riboprobe. Zebrafish *atp2a1* transcripts were first detected from 1-cell stage to cleavage period (Fig. 4A, B) in a very weak manner (arrow head indicates), and extended their expression from the gastrula period to the early segmentation stages (Fig. 4C, D). At 18-, 24-, and 31-hpf, the zebrafish *atp2a1* transcripts were restricted to trunk somites (Fig. 4E, E', F, G, H). Cryosections

Table 1. Summary of atp2a1/atp2a2 genes from selected vertebrates.

Gene names (species)	Coding region (aa)	Mw (kDa)	pI	GenBank accession number	References
atp2a1					
Human	994	109.3	5.08	NM_004320	Brandl et al., 1986
Dog	993	109.2	5.07	XM_855027	NCBI
Rat	994	109.4	5.13	NM_058213	Wu and Lytton, 1993
Mouse	994	109.4	5.13	NM_007504	Schleef et al., 1996
Chicken	994	109.0	5.25	NM_205519	Karin et al., 1989
Xenopus	1042	115.4	5.14	NM_001092974	Klein et al., 2002
Zebrafish	994	108.8	5.10	NM_001007029	Hirata et al., 2004
atp2a2					
Human	1042	114.8	5.23	NM_170665	Lytton and MacLennan, 1988
Dog	997	109.7	5.26	NM_001003214	Autry and Jones, 1997
Rat	997	109.7	5.27	NM_001110139	Gunteski-Hamblin et al., 1988
Mouse	1044	114.9	5.23	NM_001110140	Hsu et al., 1993
Chicken	1042	114.8	5.23	XM_415130	NCBI
Xenopus	996	109.5	5.03	NM_001086935	Klein et al., 2002
Zebrafish *atp2a2a*	996	109.5	5.08	NM_200965	Ebert et al., 2005
Zebrafish *atp2a2b*	1035	113.6	5.33	NM_001030277	Lo et al., 2003

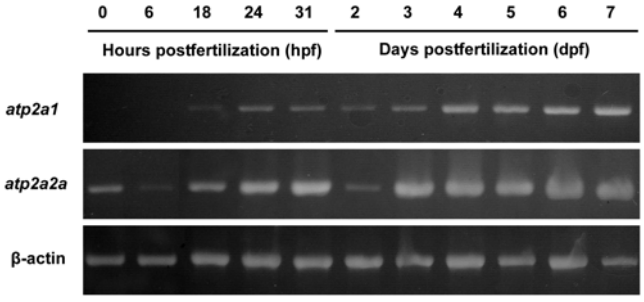

Figure 3. RT-PCR analysis of *atp2a1* and *atp2a2a* gene transcripts, using total RNA extracted from the embryos of different developmental stages. Top panel: *atp2a1*; middle panel: *atp2a2a*; and bottom panel: loading control (β-*actin*). Stage of each sample is indicated on the top.

of 24-hpf embryos revealed that *atp2a1* signals distributed in the entire somite, including the presumptive epaxial and hypaxial muscle regions (Fig. 4G-a, -b, -c). By 48- and 72-hpf, zebrafish *atp2a1* expressed strongly in trunk somites, fin buds, pharyngeal arches as well as in the head muscle progenitors (Fig. 4I, I', I'', J, J', J''). Cryosections revealed *atp2a1* signals were undetected at heat regions (Fig. 4I-a, -b, -c, 4J-a, -b, -c). Expression of zebrafish *atp2a1* continued from 4-dpf to 6-dpf in the all head muscles as well as in the trunk somites (Fig. 4K, K', K'', L, M). By 7-dpf, *atp2a1* signals were detected in head muscles, pharyngeal arches and trunk somites in a fainter manner (Fig. 4N). On the other hand, *atp2a1* signals were undetectable in heart, notochord, and central neuron system. On the basis of these observations, we conclude that zebrafish *atp2a1* is a maternally inherited gene, restricting its expression in head and trunk muscles.

Atp2a1 plays important roles on skeletal muscle contraction of vertebrates

We have shown that *atp2a1* transcripts were detected strongly in the presumptive epaxial and hypaxial muscle regions of 18-48-hpf zebrafish embryos. In mice, canonical *ATP2A1* transcripts were measured in the adults' skeletal muscles but a neonatal isoform, *SERCA1b*, is exclusively expressed in myoblasts and myotubes during embryogenesis (Zádor et al., 2007). In human, *ATP2A1* transcripts were found in the adults' skeletal muscles (Zhang et al., 1995; Loukianov et al., 1998). These observations indicate that *atp2a1* genes are not ubiquitously expressed, but are restricted in muscles among vertebrates. In zebrafish, previous study has shown that SERCA1 is only expressed in muscle but not in spinal cord or notochord at 15 and 19 hpf (Hirata et al., 2004). Furthermore, inactivation of *atp2a1* leads to muscle contraction defects in zebrafish as well as in mice and human (Zhang et al., 1995; Odermatt et al., 1996; Pan et al., 2003; Hirata et al., 2004). Maves et al. (2007) used microarray analysis and found that *atp2a1* involved during zebrafish fast muscle differentiation. In particular, SERCA1 null mutant mice exhibit abnormal diaphragm functions; the insufficient function and development of the diaphragm may be due to the lack of SERCA1b (Pan et al., 2003). Taken together, we suggest that Atp2a1 plays important roles on skeletal muscle contraction and might play a role during vertebrates' muscle differentiation, especially in fast muscle.

Spatiotemporal expression of zebrafish *atp2a2a* transcripts during early development

Again, the spatiotemporal expression patterns of *atp2a2a* during early development were examined by whole mount in situ

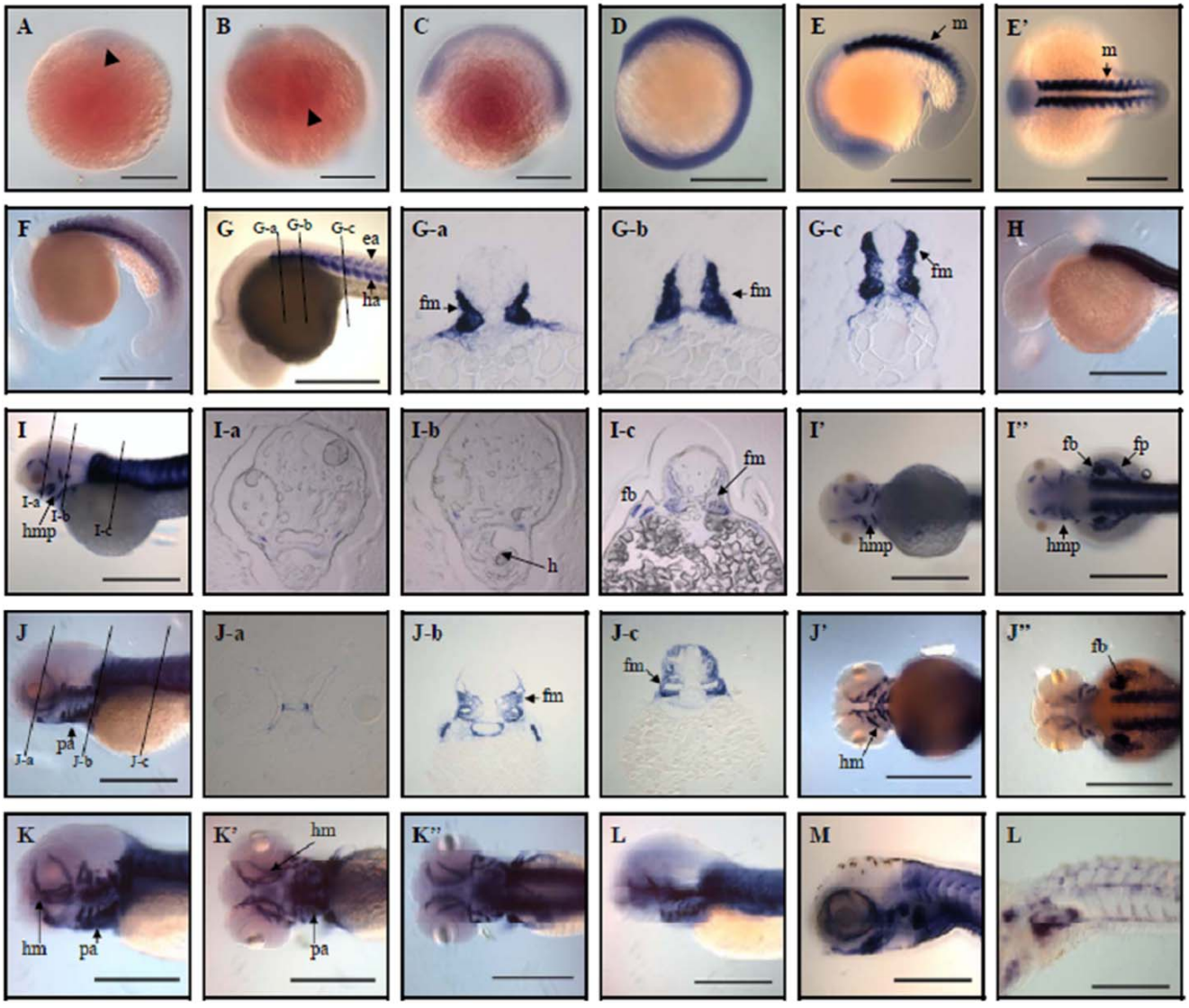

Figure 4. *atp2a1* expression during early embryonic stages. (A) One-cell and (B) 4-cell stages, lateral view. (C) At 6-hpf and (D) 12-hpf, lateral view. (E) At 18-hpf, lateral view, and (E') dorsal view. (F) At 20-hpf and (G) 24-hpf, lateral view. Cross-sections along the plane indicated by lines were shown in (G-a, G-b, G-c). (H) At 31-hpf and (I) 48-hpf, lateral view. Cross-sections along the plane indicated by lines were shown in (I-a, I-b, I-c). (I') At 48-hpf, ventral view, and (I'') dorsal view. (J) At 72-hpf, lateral view. Cross-sections along the plane indicated by lines were shown in (J-a, J-b, J-c). (J') At 72-hpf, ventral view, and (J'') dorsal view. (K) At 96-hpf, lateral, (K') ventral, and (K'') dorsal views. (L, M, N) At 5-7-dpf, lateral view. Scale bars, 250 μm. (ea, epaxial muscle; fb, fin bud; fbp, fin bud precursor from somite number 4; fm, fast muscle; h, heart; ha, hypaxial muscle; hm, head muscle; hmp, head muscle progenitors; m, muscle; pa, pharyngeal arches)

hybridization. Results showed that zebrafish *atp2a2a* transcripts were first observed from 1-cell stage to cleavage period (Fig. 5A, B), and their expression extended from the gastrula period to the early segmentation stages (Fig. 5C, D). By 18-hpf, the zebrafish *atp2a2a* transcripts were detected in lateral plate mesoderm, and in the bilateral heart field (Fig. 5E, E'), and that cardiac cells expressing *atp2a2a* were fused in a single heart tube and elongated by convergent extension by 24- and 31-hpf (Fig. 5F', G). Cryosections of 24-hpf embryos revealed *atp2a2a* signals distributed in the entire somite, including the presumptive epaxial and hypaxial muscle regions (Fig. 5F, F-a, -b, -c,). By 48- and 72-hpf, zebrafish *atp2a2a* expressed strongly in trunk somites, fin buds, pharyngeal arches as well as in the heart (Fig. 5H, H', H'', I, I'). Again, cryosections revealed *atp2a2a* signals were detected in head muscle precursor, pharyngeal arches, heat regions as well as the presumptive epaxial and hypaxial muscles (Fig. 5H-a, -b, -c,). Expression of zebrafish *atp2a2a* continued at 4-dpf in the all head muscles (Fig. 5J, J'). In contrast, the expression of *atp2a2a* was in a fainter manner in head and trunk region

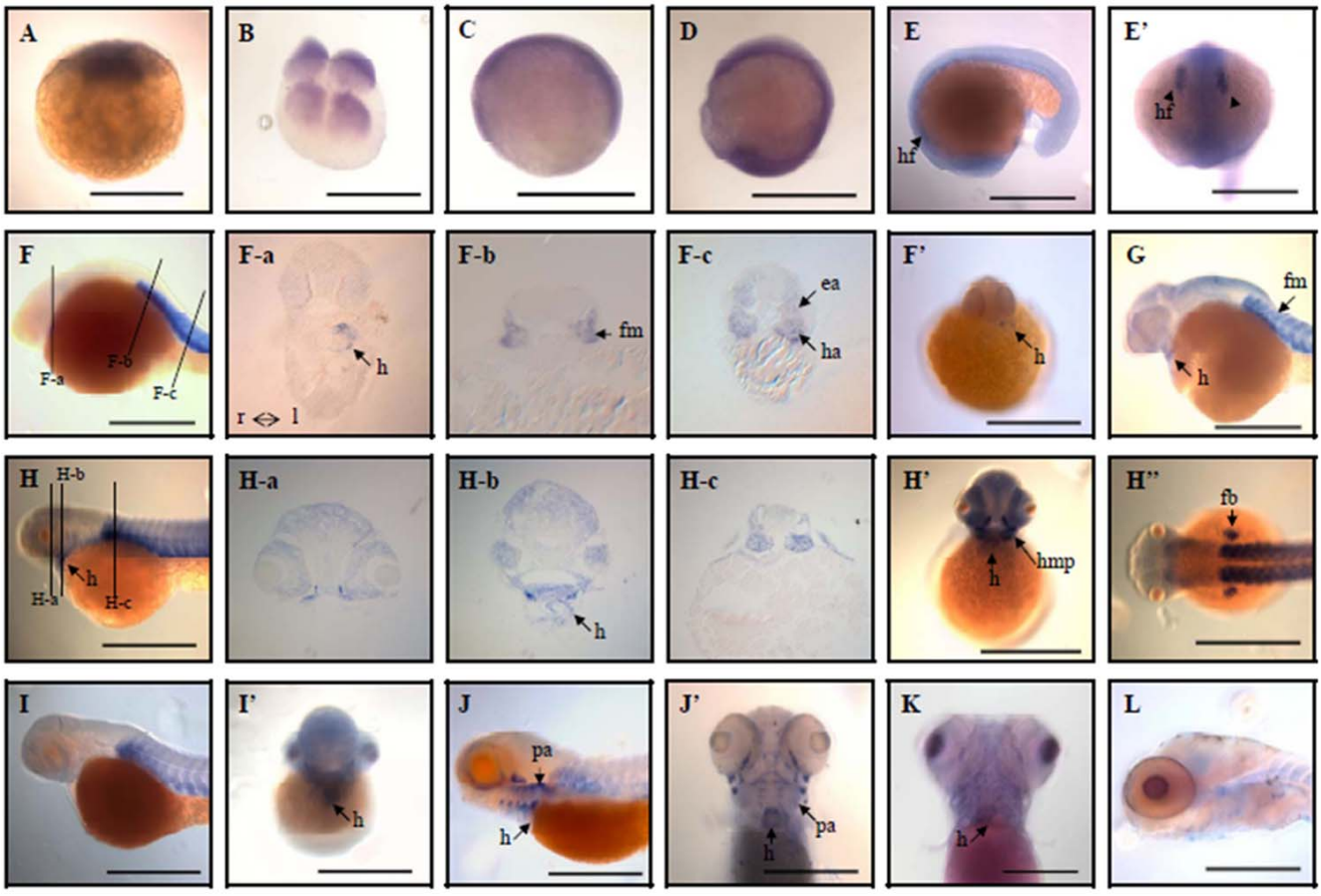

Figure 5. *atp2a2a* expression during early embryonic stages. (A) One-celled and (B) 4-celled stages, lateral view. (C) At 6-hpf and (D) 12-hpf, lateral view. (E) At 18-hpf, lateral view, and (E') dorsal view. (F) At 24-hpf, lateral view. Cross-sections along the plane indicated by lines were shown in (F-a, F-b, F-c). (F') At 24-hpf, ventral view. (G) At 31-hpf and (H) 48-hpf, lateral view. Cross-sections along the plane indicated by lines were shown in (H-a, H-b, H-c). (H') At 48-hpf, ventral view, and (H'') dorsal view. (I) At 72-hpf, lateral view, and (I') ventral view. (J) At 96-hpf, lateral view, and (J') ventral view. (K) At 5-dpf, ventral view. (L) At 6-dpf, lateral view. Scale bars, 250 μm. (ea, epaxial muscle; fb, fin bud; fm, fast muscle; h, heart; hmp, ha, hypaxial muscle; head muscle progenitors; hf, heart field; l, left direction; pa, pharyngeal arches; r, right direction.)

at 5-6 dpf (Figs. 5K, L). Specially, we found that the expression of *atp2a2a* was not significantly reduced in heart region (Fig. 5J' vs. 5K, arrows). On the basis of these observations, we conclude that zebrafish *atp2a2a* is a maternally inherited gene, restricting its expression in trunk muscles as well as in heart.

Atp2a2a might play important roles during heart development of vertebrates

In adult heart, *SERCA2* expression levels are highly associated with cardiac functions, therefore, *SERCA2* could be used as a marker for detection of cardiomyopathy (Castilho et al., 2007; Andersson et al., 2009; Dai and Rabinovitch, 2009; Kontaraki et al., 2010; Stokke et al., 2010; Summerfield et al., 2010). We have shown that *atp2a2a* transcripts were detected strongly in the developing heart of 18-96 hpf zebrafish embryos (Fig. 4). These observations conform to the previous study (Langenbacher et al., 2005) which described a *SERCA2*-null zebrafish mutant, *tremblor* (*tre*), having heart defects such as contraction and fibrillation disorder. Furthermore, *ATP2A2*-null mutant mice were lethal, but heterozygous *SERCA2*$^{-/-}$ mice manifested impaired cardiac contractility and delayed cardiomyocyte relaxation (Periasamy et al., 1999). In human, it always appears that SERCA2 is down-regulated in patients with heart failure, but for cardiomyopathy, it is more controversial. Taken together, we suggest that Atp2a2a might play important roles during heart development of vertebrates.

In conclusion, this study highlights the distinct expression pattern of two structurally related zebrafish *atp2a* genes, *atp2a1* and *atp2a2a*. They are both maternally inherited and muscle-restricted genes. Expression of *atp2a1* is mainly restricted in fast muscles whereas *atp2a2a* is restricted in fast muscle and heart. This information may provide more insight into the molecular structure and expression patterns of the lower vertebrate *atp2a* genes.

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References

Altschul SF, Madden TL, Scha¨ffer AA, Zhang J, Zhang Z, Miller W, and Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389-3402.

Andersson KB, Birkeland JA, Finsen AV, Louch WE, Sjaastad I, Wang Y, Chen J, Molkentin JD, Chien KR, Sejersted OM, and Christensen G (2009) Moderate heart dysfunction in mice with inducible cardiomyocyte-specific excision of the Serca2 gene. J. Mol. Cell Cardiol. 47: 180-187.

Autry JM and Jones LR (1997) Functional co-expression of the canine cardiac Ca2+ pump and phospholamban in Spodoptera frugiperda (Sf21) cells reveals new insights on ATPase regulation. J. Biol. Chem. 272: 15872-15880.

Berridge MJ (2009) Inositol trisphosphate and calcium signalling mechanisms. Biochim. Biophys. Acta. 1793: 933-940.

Berridge MJ, Bootman MD, and Lipp P (1998) Calcium-a life and death signal. Nature 395: 645-648.

Brandl CJ, Green NM, Korczak B, and MacLennan DH (1986) Two Ca2+ ATPase genes: homologies and mechanistic implications of deduced amino acid sequences. Cell 44: 597-607.

Brody IA (1969) Muscle contracture induced by exercise. New Eng. J. Med. 281: 187-192.

Castilho PC, Landeira-Fernandez AM, Morrissette J, and Block BA (2007) Elevated Ca2+ ATPase (SERCA2) activity in tuna hearts: comparative aspects of temperature dependence. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 148: 124-132.

Chen YH, Lee WC, Cheng CH, and Tsai HJ (2000) Muscle regulatory factor gene: zebrafish (*Danio rerio*) *myogenin* cDNA. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 127: 97-103.

Chen YH, Lee WC, Liu CF, and Tsai HJ (2001) Molecular structure, dynamic expression and promoter analysis of zebrafish (Danio rerio) myf-5 gene. Genesis 29: 22-35.

Chen YH, Wang YH, Yu TH, Wu HJ, and Pai CW (2009a) Transgenic zebrafish line with over-expression of Hedgehog on the skin: a useful tool to screen Hedgehog-inhibiting compounds. Transgenic Res. 18: 855-864.

Chen YH, Lin YT, and Lee GH (2009b) Novel and unexpected functions of zebrafish CCAAT box binding transcription factor (NF-Y) B subunit during cartilages development. Bone 44: 777-784.

Dai DF and Rabinovitch PS (2009) Cardiac aging in mice and humans: the role of mitochondrial oxidative stress. Trends Cardiovasc. Med. 19: 213-220.

Ebert AM, Hume GL, Warren KS, Cook NP, Burns CG, Mohideen MA, Siegal G, Yelon D, Fishman MC and Garrity DM (2005) Calcium extrusion is critical for cardiac morphogenesis and rhythm in embryonic zebrafish hearts. Proc. Natl. Acad. Sci. USA 102: 17705-17710.

Fill M and Copello JA (2002) Ryanodine receptor calcium release channels. Physiol. Rev. 82: 893-922.

Gordon AM, Homsher E, and Regnier M (2000) Regulation of contraction in striated muscle. Physiol. Rev. 80: 853-924.

Gunaratne HJand Vacquier VD (2006) Cloning of a sea urchin sarco/endoplasmic reticulum Ca^{2+} ATPase. Biochem. Biophys. Res. Commun. 339: 443-449.

Gunteski-Hamblin AM, Greeb J and Shull GE (1988) A novel Ca2+ pump expressed in brain, kidney, and stomach is encoded by an alternative transcript of the slow-twitch muscle sarcoplasmic reticulum Ca-ATPase gene. J. Biol. Chem. 263: 15032-15040.

Hirata H, Saint-Amant L, Waterbury J, Cui W, Zhou W, Li Q, Goldman D, Granato and Kuwada JY (2004) *accordion*, a zebrafish behavioral mutant, has a muscle relaxation defect due to a mutation in the ATPase Ca^{2+} pump SERCA1. Development 131: 5457-5468.

Hsu DK, Donohue PJ, Alberts GF and Winkles JA (1993) Fibroblast growth factor-1 induces phosphofructokinase, fatty acid synthase and Ca(2+)-ATPase mRNA expression in NIH 3T3 cells. Biochem. Biophys. Res. Commun. 197: 1483-1491.

Karin NJ, Kaprielian Z and Fambrough DM (1989) Expression of avian Ca2+-ATPase in cultured mouse myogenic cells. Mol. Cell. Biol. 9: 1978-1986.

Kimmel C, Ballard WW, Kimmel SR, Ullmann B and Schilling TF (1995) Stages of embryonic development in the zebrafish. Dev. Dyn. 203: 253-310.

Klein SL, Strausberg RL, Wagner L, Pontius J, Clifton SW and Richardson P (2002) Genetic and genomic tools for *Xenopus* research: The NIH *Xenopus* initiative. Dev. Dyn. 225: 384-391.

Kontaraki JE, Parthenakis FI, Nyktari EG, Patrianakos AP and Vardas PE (2010) Myocardial gene expression alterations in peripheral blood mononuclear cells of patients with idiopathic dilated cardiomyopathy. Eur. J. Heart Fail. 12: 541-548.

Langenbacher AD, Dong Y, Shu X, Choi J, Nicoll DA, Goldhaber JI, Philipson KD and Chen JN (2005) Mutation in sodium-calcium exchanger 1 (NCX1) causes cardiac fibrillation in zebrafish. Proc Natl Acad Sci USA. 102: 17699-17704.

Lo J, Lee S, Xu M, Liu F, Ruan H, Eun A, He Y, Ma W, Wang W, Wen Z and Peng J (2003) 15000 unique zebrafish EST clusters and their future use in microarray for profiling gene expression patterns during embryogenesis. Genome Res. 13: 455-466.

Loukianov E, Ji Y, Baker DL, Reed T, Babu J, Loukianova T, Greene A, Shull G and Periasamy M (1998) Sarco(endo)plasmic reticulum Ca2+ ATPase isoforms and their role in muscle physiology and pathology. Ann. NY Acad. Sci. 853: 251-259.

Lytton J and MacLennan DH (1988) Molecular cloning of cDNAs from human kidney coding for two alternatively spliced products of the cardiac Ca^{2+}-ATPase gene. J. Biol. Chem. 263: 15024-15031.

Machuca I, Domenget C and Jurdic P (1999) Identification of avian sarcoplasmic reticulum Ca(2+)-ATPase (SERCA3) as a novel 1,25(OH)(2)D(3) target gene in the monocytic lineage. Exp. Cell Res. 250: 364-375.

MacLennan DH (2000) Ca^{2+} signaling and muscle disease. Eur. J. Biochem. 267: 5291-5297.

MacLennan DH, Rice WJ and Green NM (1997) The mechanism of Ca^{2+} transport by sarco(endo)plasmic reticulum Ca^{2+}-ATPase. J. Biol. Chem. 272: 28815-28818.

Magyar A, Bakos E and Váradi A (1995) Structure and tissue-specific expression of the Drosophila melanogaster organellar-type Ca(2+)-ATPase gene. Biochem. J. 310: 757-763.

Maves L, Waskiewicz AJ, Paul B, Cao Y, Tyler A, Moens CB and Tapscott SJ (2007) Pbx homeodomain proteins direct Myod activity to promote fast-muscle differentiation. Development. 134:

3371-3382.

Mikoshiba K (2007) IP3 receptor/Ca^{2+} channel: from discovery to new signaling concepts. J. Neurochem. 102: 1426-1446.

Odermatt A, Taschner PEM, Khanna VK, Busch HFM, Karpati G, Jablecki CK, Breuning MH and MacLennan DH (1996) Mutations in the gene-encoding SERCA1, the fast-twitch skeletal muscle sarcoplasmic reticulum Ca^{2+} ATPase, are associated with Brody disease. Nat. Genet. 14: 191-194.

Olson BD, Sgourdou P and Downes GB (2010) Analysis of a zebrafish behavioral mutant reveals a dominant mutation in atp2a1/SERCA1. Genesis 48: 354-361.

Pai CW and Chen YH (2010) Transgenic expression of prothymosin alpha on zebrafish epidermal cells promotes proliferation and attenuates the UVB-induced apoptosis. Transgenic Res. 19: 655-665.

Pan Y, Zvaritch E, Tupling AR, Rice WJ, de Leon S, Rudnicki M, McKerlie C, Banwell BL and MacLennan DH (2003) Targeted disruption of the *ATP2A1* gene encoding the sarco(endo)plasmic reticulum Ca^{2+}ATPase isoform 1 (SERCA1) impairs diaphragm function and is lethal in neonatal mice. J. Biol. Chem. 278: 13367-13375.

Peng HC, Wang YH, Wen CC, Wang WH, Cheng CC and Chen YH (2010) Nephrotoxicity assessments of acetaminophen during zebrafish embryogenesis. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 151: 480-486.

Periasamy M, Reed TD, Liu LH, Ji Y, Loukianov E, Paul RJ, Nieman ML, Riddle T, Duffy JJ, Doetschman T, Lorenz JN and Shull GE (1999) Impaired cardiac performance in heterozygous mice with a null mutation in the sarco(endo)plasmic reticulum Ca^{2+}-ATPase isoform 2 (SERCA2) gene. J. Biol. Chem. 274: 2556-2562.

Sakuntabhai A, Ruiz-Perez V, Carter S, Jacobsen N, Burge S, Monk S, Smith M, Munro CS, O'Donovan M et al. (1999) Mutations in *ATP2A2*, encoding a Ca^{2+} pump, cause Darier disease. Nat. Genet. 21: 271-277.

Schleef M, Simon-Chazottes D, Lengeling A, Klocke R, Jockusch H, YardenY and Guenet J (1996) The gene encoding sarcoplasmic reticulum calcium ATPase-1 (Atp2a1) maps to distal mouse chromosome 7. Mamm. Genome 7: 788.

Stokke MK, Hougen K, Sjaastad I, Louch WE, Briston SJ, Enger UH, Andersson KB, Christensen G, Eisner DA, Sejersted OM et al. (2010) Reduced SERCA2 abundance decreases the propensity for Ca^{2}+ wave development in ventricular myocytes. Cardiovasc. Res. 86: 63-71.

Summerfield N, Peters ME, Hercock CA, Mobasheri A and Young IS (2010) Immunohistochemical evidence for expression of fast-twitch type sarco(endo)plasmic reticulum Ca2+ ATPase (SERCA1) in German shepherd dogs with dilated cardiomyopathy myocardium. J. Vet. Cardiol. 12: 17-23.

Varga-Szabo D, Braun A and Nieswandt B (2009) Calcium signaling in platelets. J. Thromb. Haemost. 7: 1057-1066.

Wang YH, Cheng CC, Lee WJ, Chiou ML, Pai CW, Wen CC, Chen WL, Chen YH (2009a) A novel phenotype-based approach for systematically screening antiproliferation metallodrugs. Chem-Biol. Interact. 182: 84-91.

Wang YH, Wen CC, Yang ZS, Cheng CC, Tsai JN, Ku CC, Wu HJ and Chen YH (2009b) Development of a whole-organism model to screen new compounds for sun protection. Mar. Biotechnol. 11: 419-429.

Westerfield M (1995) The zebrafish book. Third edition. University of Oregon Press.

Wu KD, Lytton J 1993 Molecular cloning and quantification of sarcoplasmic reticulum Ca(2+)-ATPase isoforms in rat muscles. Am. J. Physiol. 264: C333-C341.

Zádor E, Vangheluwe P and Wuytack F (2007) The expression of the neonatal sarcoplasmic reticulum Ca^{2+} pump (SERCA1b) hints to a role in muscle growth and development. Cell Calcium. 41: 379-388.

Zhang Y, Fujii J, Phillips MS, Chen HS, Karpati G, Yee WC, Schrank B, Cornblath DR, Boylan KB and MacLennan DH (1995) Characterization of cDNA and genomic DNA encoding SERCA1, the Ca(2+)-ATPase of human fast-twitch skeletal muscle sarcoplasmic reticulum, and its elimination as a candidate gene for Brody disease. Genomics. 30: 415-424.