Research article

Dev Reprod 2024;28(4):121-128
https://doi.org/10.12717/DR.2024.28.4.121

Development & Reproduction

ISSN 2465-9525 (Print)
ISSN 2465-9541 (Online)

PACRG is Expressed on the Left Side of the Brain Vesicle in the Ascidian *Halocynthia* Larva

†Gil Jung Kim

Department of Marine Bioscience, Gangneung-Wonju National University, Gangneung 25457, Korea

Received: September 5, 2024
Revised: October 20, 2024
Accepted: November 12, 2024

†Corresponding author
Gil Jung Kim
Department of Marine Bioscience, Gangneung-Wonju National University, Gangneung 25457, Korea.
Tel: +82-33-640-2415
E-mail: gjkim@gwnu.ac.kr

ORCID
Gil Jung Kim
https://orcid.org/0000-0001-6441-2694

Conflict of interests
The author declares no potential conflict of interest.

Acknowledgements
This study has been worked with the support of a research grant of Gangneung-Wonju National University in 2023.

Authors' contributions
The article is prepared by a single author.

Ethics approval
This article does not require IRB/IACUC approval because there are no human and animal participants.

Abstract

The ascidian larvae, which display a chordate ground body plan, are left-right asymmetric in several structures, including the brain vesicle. In ascidian larvae, the ocellus and otolith pigment cells, which are thought to detect light and gravity respectively, are located on the right side of the brain vesicle, while the coronet cells, which are presumed to be dopaminergic, are located on the left side. To study how left-right asymmetry of the brain vesicle in the ascidian *Halocynthia roretzi* larva is determined, I attempted to isolate a gene that is expressed in the brain vesicle. As a result, an ascidian Parkin co-regulated gene (*PACRG*) orthologue was cloned. Expression of *PACRG* begins weakly in the head region of the late tailbud embryos, and it thereafter is observed on the left side of the brain vesicle of the larvae just before hatching. The location of *PACRG* expression is estimated to overlap with the area stained by the coronet cell-specific antibody. Thus, it is suggested that *PACRG* might be involved in the formation of the left-side structures of the brain vesicle, including coronet cells, during ascidian embryogenesis.

Keywords: Ascidian, *PACRG*, Coronet cells, Left-right asymmetry, Brain vesicle

INTRODUCTION

The adult body of most bilaterians shows stereotypic left-right asymmetry. In vertebrates, the internal organs are not symmetric, as the placement of the heart and liver is determined along the left-right axis, and there are different structures in the left and right regions of the brain (Hamada et al., 2002; Levin, 2005). In the early stages of vertebrate embryonic development, the morphology is bilaterally symmetrical, but later the symmetry becomes broken along the left-right axis. The decisive event in left-right axis formation is the expression of *Nodal* gene on the left side of the embryos (Boorman & Shimeld, 2002; Hirokawa et al., 2006; Schweickert et al., 2017). The important thing among them is that the *Nodal* gene appears to be expressed in the lateral plate mesoderm. The mechanisms for expressing the *Nodal* gene only on the left side of the embryo differs among vertebrates. It was suggested that the liquid flow produced by motile cilia in the node may be responsible for left-right axis formation (Okada & Hirokawa, 2009; Vandenberg & Levin, 2013; Blum et al., 2014). One of the essential genes activated by Nodal appears to encode the transcription factor Pitx2, which normally is expressed only on the left side of vertebrate embryos. It is likely that Nodal signaling establishes left-right polarity by activating Pitx2 on the left side is conserved throughout all vertebrates (Blum et al., 2014; Little & Norris, 2021).

Ascidians are phylogenetically the closest relatives of vertebrates. The ascidian larvae, which display a chordate ground body plan, are left-right asymmetric in several structures, including the brain vesicle (Hirano & Nishida, 2000; Boorman & Shimeld, 2002; Ryan et al., 2016). The only two melanized pigment cells of ascidian larvae exist in the brain vesicle and they belong to two different sensory organs, ocellus and otolith. The ocellus has a cup-shaped pigment cell containing numerous small melanin granules, while the otolith contains a single large melanized pigment cell attached to the ventral surface of the brain vesicle (Nishida & Satoh, 1989; Nicol & Meinertzhagen, 1991). The ocellus and otolith are part of the organ involved in sensing light and gravity, respectively. The ocellus is located on the right side of the brain vesicle. However, the otolith is present near the center of the brain vesicle but exists slightly to the right. The coronet cells, which have been speculated to be components of a sensory system, are asymmetrically located on the left side of the brain vesicle (Nicol & Meinertzhagen, 1991; Taniguchi & Nishida, 2004). The coronet cells are dopaminergic, but their function is not yet known (Moret et al., 2005). The coronet cells were received their name from the coronet cells of the saccus vasculosus, an organ that serves as a photoperiodic and seasonal sensor in teleosts (Nakane et al., 2013; Kourakis et al., 2021).

Parkin co-regulated gene (*PACRG*) was first reported as a gene closely related to the Parkinson's disease associated gene *Parkin* (*PARK2*) in humans (West et al., 2003). *PACRG* is oriented in a head-to-head array with the *Parkin* gene on human chromosome 6. Expression of the *PACRG* and *Parkin* genes appears to be regulated by a shared bi-directional promoter (Imai et al., 2003; West et al., 2003; Stephenson et al., 2018). It is likely that *Parkin* and *PACRG* encoded proteins interact and function in the same biological pathways. *Parkin* encodes an RBR E3 ubiquitin ligase involved in tagging defective proteins for degradation by proteasome (Shimura et al., 2001; Spratt et al., 2014). The exact function of the PACRG is not well understood, but it is implicated in protecting cells against protein aggregation, potentially by a mechanism related to that of the Parkin. *PACRG* is an evolutionarily very highly conserved gene, which is present from green algae to mammals (West et al., 2003; Dawe et al., 2005; Ikeda et al., 2007). During early embryogenesis, *PACRG* expression is specifically localized to epithelia where leftward flow arises, that is, Kupffer's vesicle in zebrafish, the gastrocoel roof plate in *Xenopus* and the posterior notochord in mammals (Thumberger et al., 2012). The expression of *PACRG* is observed in various tissues of adult mice, and it is particularly prominent in regional brain area of newborn mice such as the ependymal cells and cilia lining the ventricles (Brody et al., 2008; Wilson et al., 2009). In human, PACRG is expressed in astrocytes throughout the brain and in pigmented noradrenergic neurons of the locus coeruleus (Taylor et al., 2007). In *Xenopus*, PACRG-specific signals are detected in multiciliated choroid plexus cells, thalamic nuclei and in the ventral midline of tadpole larvae (Thumberger et al., 2012). The *PACRG*-MO (morpholino oligonucleotide) injected *Xenopus* embryos result dose dependently in left-right asymmetry, neural tube closure and gastrulation defects. These results suggest that PACRG may play an important role in motile cilia formation, and function in development and left-right axis formation of the brain in vertebrates.

In this study, I report an ascidian *PACRG* orthologue that is specifically expressed on the left side of the brain vesicle. The brain region expressing *PACRG* appears to include coronet cells known as dopaminergic cells.

MATERIALS AND METHODS

1. Animals and embryos

Adults of ascidian *Halocynthia roretzi* were purchased about a month before the spawning season from fishermen in the vicinity of Institute of Ocean Science Education, Gangneung-Wonju National University. Ascidians were reared in seawater at 9℃ under 24-hour lighting. Eggs were

https://doi.org/10.12717/DR.2024.28.4.121

spawned under temperature and light control, and they were fertilized with a suspension of non-self sperm. Fertilized eggs were raised at 13℃. Embryos were collected at appropriate stages and fixed for whole-mount *in situ* hybridization and immunofluorescence staining. Tadpole larvae hatched at about 35 hours after fertilization.

2. Isolation of Parkin co-regulated gene

The information (Gene ID: Harore.g00012573) on the *PACRG* of *H. roretzi* was obtained from the Aniseed: Ascidian Network for In Situ Expression and Embryological Data (https://www.aniseed.fr; Brozovic et al., 2018). To obtain a full-length cDNA sequence, 5' RACE and 3' RACE were performed with the SMART RACE cDNA Amplification Kit (Ambion, Austin, TX, USA). The *Halocynthia PACRG* cDNA was obtained, consisting of 1,385 nucleotides and encoding predicted protein of 226 amino acids.

3. *In situ* hybridization and immunohistochemistry

The *PACRG* probe for *in situ* hybridization were prepared with a digoxigenin RNA labeling kit (Roche, Basel, Switzerland). The probe was prepared from the full-length *PACRG* cDNA. *In situ* hybridization was performed as described by Miya et al. (1997), except that the probes was not hydrolyzed by alkaline treatment.

The Hpr-1 monoclonal antibody specifically stains the coronet cells in *Halocynthia* late-tailbud embryos and larvae (Darras & Nishida, 2001; Taniguchi & Nishida, 2004). Coronet cells were previously thought to be hydrostatic pressure organ cells (Eakin & Kuda., 1971; Moret et al., 2005). Immunohistochemical staining for coronet cells was carried out by standard methods using a TSA fluorescein system (PerkinElmer Life Sciences, Waltham, MA, USA) according to the manufacturer's protocol.

RESULTS

1. Isolation of Parkin co-regulated gene in *Halocynthia*

To study how left-right asymmetry of the brain vesicle in *Halocynthia* larva is determined, I attempted to isolate a gene that is expressed in the brain vesicle. As a result, an ascidian *PACRG* orthologue cDNA, consisting of 1,385 nucleotides and encoding predicted protein of 226 amino acids, was cloned (Fig. 1). The overall degree of amino acid identity between the *Halocynthia* PACRG and the vertebrate PACRGs is approximately 80%, except for the N-terminus (data not shown; Thumberger et al., 2012). The N-terminus of the Human PACRG protein is about 30 amino acids longer than that of the *Halocynthia* PACRG protein (data not shown).

2. Expression of Parkin co-regulated gene and position of coronet cells in *Halocynthia* larva

I examined the expression pattern of *PACRG* mRNA at various developmental stages during *Halocynthia* embryogenesis using whole-mount *in situ* hybridization. Maternal expression of *PACRG* did not observe (Fig. 2). Zygotic expression of *PACRG* was first detected in the head region at the late tailbud stage (yellow arrow in Fig. 2M). Expression of *PACRG* appeared to occur only on the left side of the brain vesicle at the just before hatching stage (yellow arrows in Fig. 2N and P). Since the colorimetric reaction took more than 48 hours, it is estimated that the transcripts of *PACRG* are expressed at very low levels.

To confirm the location of *PACRG* expression in the brain vesicle, I compared the staining of coronet cells with the expression of *PACRG* in the larvae just before or just after hatching (Fig. 3). In ascidians, *Ciona* and *Halocynthia*, each coronet cell projects a small globular body into the

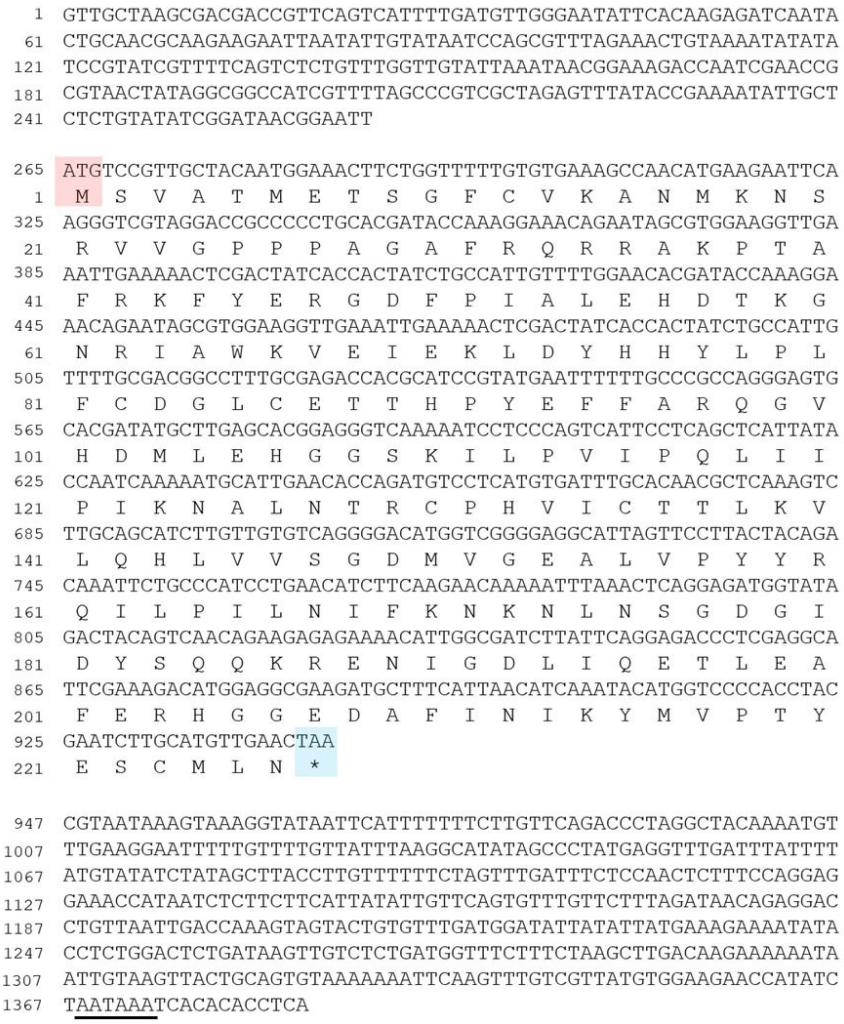

Fig. 1. Nucleotide and deduced amino acid sequence of a cDNA clone for the Parkin co-regulated gene (*PACRG*) in the ascidian *Halocynthia roretzi*. The 1,385-bp insert includes a single open reading frame that encodes a polypeptide of 226 amino acids. An asterisk and an underline indicate the termination codon and the potential signal sequence for polyadenylation, respectively.

lumen of the brain vesicle (Eakin & Kuda., 1971; Katz, 1983; Kim et al., 2006). The globular bodies of coronet cells were specifically recognized by the Hpr-1 antibody in *Halocynthia* larvae (yellow arrowheads in Fig. 3A and C). Coronet cells stained by the Hpr-1 antibody were located on the left side of the brain vesicle (Fig. 3C). This location is thought to be the same as the site of *PACRG* expression (yellow arrow in Fig. 3D). Therefore, *PACRG* is presumed to be expressed on the left side of the brain vesicle, including coronet cells, in *Halocynthia* larva.

DISCUSSION

*PACRG*s are the evolutionarily very highly conserved genes, which are present in all metazoans, as well as flagellated protozoans (Dawe et al., 2005; Ikeda et al., 2007). PACRG does not have any conserved protein domains that might provide evidence of protein function. *PACRG* encodes a small size protein that is mostly in an alpha-helical structure. It is thought that the PACRG in *Halocynthia* would exhibit similar characteristics. PACRGs perform various functions. In

https://doi.org/10.12717/DR.2024.28.4.121

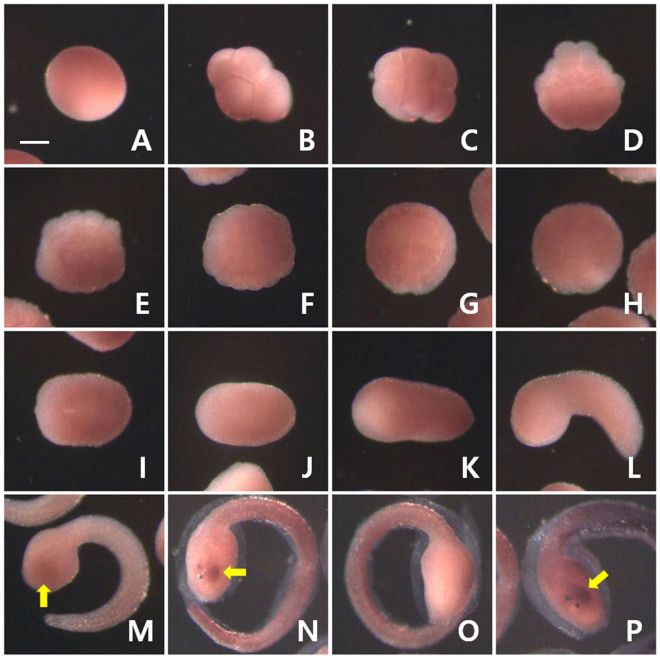

Fig. 2. Expression pattern of *PACRG* during *Halocynthia* embryogenesis. (A) A fertilized egg. (B, C) 8-cell; (D) 32-cell; (E, F) 64-cell; (G, H) 110-cell; (I) Neural plate; (J) Neurula; (K) Early tailbud; (L) Mid tailbud; (M) Late tailbud embryos. (N–P) Larvae just before hatching. (B, L, M, N) Lateral views. (C, F, H) Animal pole views. (D, E, G) Vegetal pole views. (I, J, K, P) Dorsal views. (O) Ventral view. Expression of *PACRG* (yellow arrows) first begins to appear in the head region at the late tailbud stage, and is detected only on the left side of the brain vesicle at the just before hatching stage. The tunic in the tail region of larvae was stained nonspecifically. Scale bar, 100 μm. *PACRG*, Parkin co-regulated gene.

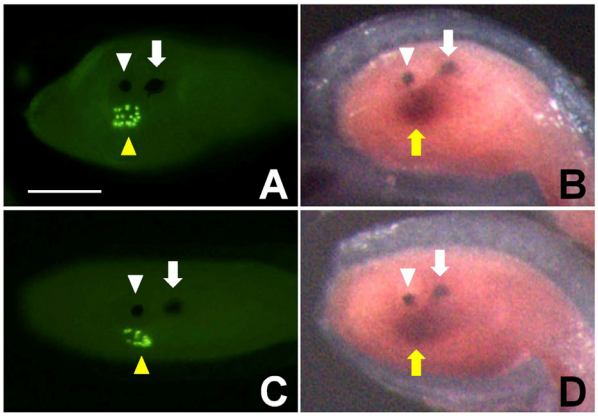

Fig. 3. *PACRG* appears to be expressed in the same area where coronet cells are located on the left side of the brain vesicle in *Halocynthia* larvae. Anterior is to the left. (A, B) Lateral views. (C, D) Dorsal views. White arrowheads indicate the otolith pigment cell, while white arrows point the ocellus pigment cell. Yellow arrowheads indicate cells that express the Hpr-1 antigen in A and C. Yellow arrows represent the expression of *PACRG* in B and D. Scale bar, 100 μm. *PACRG*, Parkin co-regulated gene.

Chlamydomonas, PACRG protein plays a role in regulating dynein-driven microtubule sliding in motile cilia (Dymek et al., 2019). PACRG and FAP20 (flagellar-associated protein 20) form the inner junction of axonemal doublet microtubules and regulate ciliary motility. PACRG also localizes to a subset of non-motile cilia in sensory neurons from the nematode *Caenorhabditis elegans*, where it regulates signaling processes linked to gustatory plasticity (Loucks et al., 2016). It has been

reported that direct interactions between MEIG1 (the meiosis-expressed gene 1) and PACRG are essential for the formation of mature sperm cells in mammals (Lehti & Sironen, 2016; Hasse et al., 2023). In elongating spermatids, MEIG1 binds to PACRG to form a temporary complex in the manchette structure that assists with the elongation process by transporting cargo proteins for sperm formation. It is thus necessary to investigate the function of PACRG in ascidians.

PACRG mRNA is expressed in various brain area of vertebrates. The expression of *PACRG* transcripts is found in specific area of the brain where dopaminergic neurons are abundant, such as the substantia nigra in various vertebrates (Brody et al., 2008; Wilson et al., 2009; Thumberger et al., 2012; Stephenson et al., 2018). In the ascidian *Halocynthia* larva, *PACRG* is expressed on the left side of the brain vesicle, which includes coronet cells known as dopaminergic cells. Therefore, it is possible that the PACRG is involved in the development of coronet cells and in the formation of left-right asymmetry of the brain vesicle in *Halocynthia* larva.

The *Nodal* and *Pitx2* genes are also involved in the formation of left-right asymmetry in ascidians. It has been reported that *Nodal* and *Pitx2* are expressed on the left side of the ascidian embryos at the neurula stage, although the expression is restricted to the epidermis and is not evident in the mesoderm, unlike in vertebrates (Morokuma et al., 2002; Shimeld & Levin, 2006). There is no structure within ascidian embryos in which liquid flow is made by ciliary movements. Moreover, although cilia are present prior to molecular asymmetries, they are not motile in the ascidian *Ciona* embryos (Thompson et al., 2012). Ascidian embryos rotate along the anterior-posterior axis at the neurula stage, known as neurula rotation (Nishide et al., 2012; Yamada et al., 2019). After the rotation, contact between the left-side epidermal cells and the inner vitelline (chorionic) membrane induces *Nodal* expression in the left-side epidermis. It appears that an unknown chemical signal(s) originated from the vitelline membrane are involved in the *Nodal* expression. The ascidian larval brain vesicle had lost left-right asymmetry in the absence of Nodal signaling (Nishide et al., 2012; Kourakis et al., 2021). These results suggest that the expression of *PACRG* on the left side of the brain vesicle in *Halocynthia* is regulated by Nodal signaling during the neural and tailbud stages.

REFERENCES

Blum M, Feistel K, Thumberger T, Schweickert A (2014) The evolution and conservation of left-right patterning mechanisms. Development 141:1603-1613.

Boorman CJ, Shimeld SM (2002) Pitx homeobox genes in *Ciona* and amphioxus show left-right asymmetry is a conserved chordate character and define the ascidian adenohypophysis. Evol Dev 4:354-365.

Brody KM, Taylor JM, Wilson GR, Delatycki MB, Lockhart PJ (2008) Regional and cellular localisation of Parkin co-regulated gene in developing and adult mouse brain. Brain Res 1201:177-186.

Brozovic M, Dantec C, Dardaillon J, Dauga D, Faure E, Gineste M, Louis A, Naville M, Nitta KR, Piette J, Reeves W, Scornavacca C, Simion P, Vincentelli R, Bellec M, Aicha SB, Fagotto M, Guéroult-Bellone M, Haeussler M, Jacox E, Lowe EK, Mendez M, Roberge A, Stolfi A, Yokomori R, Brown CT, Cambillau C, Christiaen L, Delsuc F, Douzery E, Dumollard R, Kusakabe T, Nakai K, Nishida H, Satou Y, Swalla B, Veeman M, Volff JN, Lemaire P (2018) ANISEED 2017: Extending the integrated ascidian database to the exploration and evolutionary comparison of genome-scale datasets. Nucleic Acids Res 46:D718-D725.

Darras S, Nishida H (2001) The BMP/CHORDIN antagonism controls sensory pigment cell specification and differentiation in the ascidian embryo. Dev Biol 236:271-288.

Dawe HR, Farr H, Portman N, Shaw MK, Gull K (2005) The Parkin co-regulated gene product,

https://doi.org/10.12717/DR.2024.28.4.121

PACRG, is an evolutionarily conserved axonemal protein that functions in outer-doublet microtubule morphogenesis. J Cell Sci 118:5421-5430.

Dymek EE, Lin J, Fu G, Porter ME, Nicastro D, Smith EF (2019) PACRG and FAP20 form the inner junction of axonemal doublet microtubules and regulate ciliary motility. Mol Biol Cell 30:1805-1816.

Eakin RM, Kuda A (1971) Ultrastructure of sensory receptors in Ascidian tadpoles. Z Zellforsch Mikrosk Anat 112:287-312.

Hamada H, Meno C, Watanabe D, Saijoh Y (2002) Establishment of vertebrate left-right asymmetry. Nat Rev Genet 3:103-113.

Hasse T, Zhang Z, Huang YM (2023) Molecular dynamics study reveals key disruptors of MEIG1-PACRG interaction. Proteins 91:555-566.

Hirano T, Nishida H (2000) Developmental fates of larval tissues after metamorphosis in the ascidian, *Halocynthia roretzi*. II. Origin of endodermal tissues of the juvenile. Dev Genes Evol 210:55-63.

Hirokawa N, Tanaka Y, Okada Y, Takeda S (2006) Nodal flow and the generation of left-right asymmetry. Cell 125:33-45.

Ikeda K, Ikeda T, Morikawa K, Kamiya R (2007) Axonemal localization of *Chlamydomonas* PACRG, a homologue of the human Parkin-coregulated gene product. Cell Motil Cytoskeleton 64:814-821.

Imai Y, Soda M, Murakami T, Shoji M, Abe K, Takahashi R (2003) A product of the human gene adjacent to parkin is a component of Lewy bodies and suppresses Pael receptor-induced cell death. J Biol Chem 278:51901-51910.

Katz MJ (1983) Comparative anatomy of the tunicate tadpole, *Ciona intestinalis*. Biol. Bull 164:1-27.

Kim JE, Seo HJ, Kim GJ (2006) Brain vesicle structure and formation of the hydrostatic pressure receptors in larvae of the ascidian (*Halocynthia roretzi*). Korean J Fish Soc 39:94-99.

Kourakis MJ, Bostwick M, Zabriskie A, Smith WC (2021) Disruption of left-right axis specification in *Ciona* induces molecular, cellular, and functional defects in asymmetric brain structures. BMC Biol 19:141.

Lehti MS, Sironen A (2016) Formation and function of the manchette and flagellum during spermatogenesis. Reproduction 151:R43-R54.

Levin M (2005) Left-right asymmetry in embryonic development: A comprehensive review. Mech Dev 122:3-25.

Little RB, Norris DP (2021) Right, left and cilia: How asymmetry is established. Semin Cell Dev Biol 110:11-18.

Loucks CM, Bialas NJ, Dekkers MP, Walker DS, Grundy LJ, Li C, Inglis PN, Kida K, Schafer WR, Blacque OE, Jansen G, Leroux MR (2016) PACRG, a protein linked to ciliary motility, mediates cellular signaling. Mol Biol Cell 27:2133-2144.

Miya T, Morita K, Suzuki A, Ueno N, Satoh N (1997) Functional analysis of an ascidian homologue of vertebrate Bmp-2/Bmp-4 suggests its role in the inhibition of neural fate specification. Development 124:5149-5159.

Moret F, Christiaen L, Deyts C, Blin M, Vernier P, Joly JS (2005) Regulatory gene expressions in the ascidian ventral sensory vesicle: Evolutionary relationships with the vertebrate hypothalamus. Dev Biol 277:567-579.

Morokuma J, Ueno M, Kawanishi H, Saiga H, Nishida H (2002) *HrNodal*, the ascidian nodal-related gene, is expressed in the left side of the epidermis, and lies upstream of *HrPitx*. Dev Genes Evol 212:439-446.

Nakane Y, Ikegami K, Iigo M, Ono H, Takeda K, Takahashi D, Uesaka M, Kimijima M,

Hashimoto R, Arai N, Suga T, Kosuge K, Abe T, Maeda R, Senga T, Amiya N, Azuma T, Amano M, Abe H, Yamamoto N, Yoshimura T (2013) The saccus vasculosus of fish is a sensor of seasonal changes in day length. Nat Commun 4:2108.

Nicol D, Meinertzhagen IA (1991) Cell counts and maps in the larval central nervous system of the ascidian *Ciona intestinalis* (L.). J Comp Neurol 309:415-429.

Nishida H, Satoh N (1989) Determination and regulation in the pigment cell lineage of the ascidian embryo. Dev Biol 132:355-367.

Nishide K, Mugitani M, Kumano G, Nishida H (2012) Neurula rotation determines left-right asymmetry in ascidian tadpole larvae. Development 139:1467-1475.

Okada Y, Hirokawa N (2009) Observation of nodal cilia movement and measurement of nodal flow. Methods Cell Biol 91:265-285.

Ryan K, Lu Z, Meinertzhagen IA (2016) The CNS connectome of a tadpole larva of *Ciona intestinalis* (L.) highlights sidedness in the brain of a chordate sibling. eLife 5:e16962.

Schweickert A, Ott T, Kurz S, Tingler M, Maerker M, Fuhl F, Blum M (2017) Vertebrate left-right asymmetry: What can nodal cascade gene expression patterns tell us? J Cardiovasc Dev Dis 5:1.

Shimeld SM, Levin M (2006) Evidence for the regulation of left-right asymmetry in *Ciona intestinalis* by ion flux. Dev Dyn 235:1543-1553.

Shimura H, Schlossmacher MG, Hattori N, Frosch MP, Trockenbacher A, Schneider R, Mizuno Y, Kosik KS, Selkoe DJ (2001) Ubiquitination of a new form of alpha-synuclein by parkin from human brain: Implications for Parkinson's disease. Science 293:263-269.

Spratt DE, Walden H, Shaw GS (2014) RBR E3 ubiquitin ligases: New structures, new insights, new questions. Biochem J 458:421-437.

Stephenson SEM, Aumann TD, Taylor JM, Riseley JR, Li R, Mann JR, Tomas D, Lockhart PJ (2018). Generation and characterisation of a *parkin-PACRG* knockout mouse line and a *PACRG* knockout mouse line. Sci Rep 8:7528.

Taniguchi K, Nishida H (2004) Tracing cell fate in brain formation during embryogenesis of the ascidian *Halocynthia roretzi*. Dev Growth Differ 46:163-180.

Taylor JM, Song YJ, Huang Y, Farrer MJ, Delatycki MB, Halliday GM, Lockhart PJ (2007). Parkin Co-Regulated Gene (*PACRG*) is regulated by the ubiquitin-proteasomal system and is present in the pathological features of Parkinsonian diseases. Neurobiol Dis 27:238-247.

Thompson H, Shaw MK, Dawe HR, Shimeld SM (2012) The formation and positioning of cilia in *Ciona intestinalis* embryos in relation to the generation and evolution of chordate left-right asymmetry. Dev Biol 364:214-223.

Thumberger T, Hagenlocher C, Tisler M, Beyer T, Tietze N, Schweickert A, Feistel K, Blum M (2012) Ciliary and non-ciliary expression and function of *PACRG* during vertebrate development. Cilia 1:13.

Vandenberg LN, Levin M (2013) A unified model for left-right asymmetry? Comparison and synthesis of molecular models of embryonic laterality. Dev Biol 379:1-15.

West AB, Lockhart PJ, O'Farell C, Farrer MJ (2003) Identification of a novel gene linked to parkin via a bi-directional promoter. J Mol Biol 326:11-19.

Wilson GR, Tan JT, Brody KM, Taylor JM, Delatycki MB, Lockhart PJ (2009). Expression and localization of the Parkin co-regulated gene in mouse CNS suggests a role in ependymal cilia function. Neurosci Lett 460:97-101.

Yamada S, Tanaka Y, Imai KS, Saigou M, Onuma TA, Nishida H (2019) Wavy movements of epidermis monocilia drive the neurula rotation that determines left-right asymmetry in ascidian embryos. Dev Biol 448:173-182.

https://doi.org/10.12717/DR.2024.28.4.121