EvoDevo Research
on the Acquisition of Larval Skeleton in Echinoderms

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Abstract

How the divergent morphologies emerged is a key issue for evolutionary biology. Recent prosperity of EvoDevo contributed to solve such a question. But it is still unclear how the concepts in EvoDevo are integrated into the Modern Synthesis. This is because EvoDevo researches did not fully evaluate the effect of developmental changes on actual organisms, especially about novel structures of multicellular organisms. In this study, I dealt with the larval skeleton of echinoderms as an example for evolution of a novel structure. Larval skeleton is the derived feature that is seen in larvae of some echinoderm classes: sea urchins, brittle stars and sea cucumbers. Recent studies suggested that larval skeleton of echinoderms arose by heterochronic shift of the adult skeletogenic machinery that all echinoderms possess. But the molecular basis of this evolution has not been studied. Here, I surveyed what kind of molecular evolution was involved in the evolution and found that heterochronic shift of *alx1* into larval stage may be an important change. To validate the importance of this change for the actual morphological evolution, I artificially expressed *alx1* gene in starfish embryos that have no skeleton and thus possess ancestral state. Although the attempt did not induce any morphological change, the comprehensive genetic analysis using Next generation sequencer revealed that slight changes occurred in terms of gene expression. Some of them seemed

actually involved in skeletogenesis of starfish. These results indicate that the larval expression of alx1 was highly likely to be involved in the evolution of larval skeleton but insufficient for induce ectopic mineralization. The results also suggested that some changes in developmental toolkit could be neutral or potentially adaptive in a certain situation. From such findings, I discussed that the complex trait that requires multiple genetic changes could evolve in the natural population through neutral genetic changes.

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References

Abitua PB, Wagner E, Navarrete IA, Levine M. 2012. Identification of a rudimentary neural crest in a non-vertebrate chordate. Nature 492: 104-107.

Albagli O, Klaes A, Ferreira E, Leprince D, Klämbt C. 1996. Function of ets genes is conserved between vertebrates and Drosophila. Mech Dev 59: 29-40.

Aldana M, Balleza E, Kauffman S, Resendiz O. 2007. Robustness and evolvability in genetic regulatory networks. J Theor Biol 245: 433-448.

Amore G, Yavrouian RG, Peterson KJ, Ransick A, McClay DR, Davidson EH. 2003. Spdeadringer, a sea urchin embryo gene required separately in skeletogenic and oral ectoderm gene regulatory networks. Dev Biol 261: 55-81.

Benson S, Sucov H, Stephens L, Davidson E, Wilt F. 1987. A lineage-specific gene encoding a major matrix protein of the sea urchin embryo spicule: I. Authentication of the cloned gene and its developmental expression. Dev Biol 120: 499-506.

Bidula S, Kenawy H, Ali YM, Sexton D, Schwaeble WJ, Schelenz S. 2013. Role of

ficolin-A and lectin complement pathway in the innate defense against pathogenic aspergillus species. Infect Immun 81: 1730-1740.

Cameron CB, Garey JR, Swalla BJ. 2000. Evolution of the chordate body plan: New insights from phylogenetic analyses of deuterostome phyla. Proc Natl Acad Sci USA 97: 4469-4474.

Carroll SB, Grenier JK, Weatherbee SD. 2010. From DNA to diversity : molecular genetics and the evolution of animal design. Malden: Blackwell.

Cheers MS, Ettensohn CA. 2005. P16 is an essential regulator of skeletogenesis in the sea urchin embryo. Dev Biol 283: 384-396.

Chow G, Benson SC. 1979. Carbonic anhydrase activity in developing sea urchin embryos. Exp Cell Res 124: 451-453.

Crow JF, Kimura M. 1970. An introduction to population genetics theory. New York: Harper and Row.

Darwin C. 1859. On the Origin of Species by Means of Natural Selection : or the Preservation of Favored Races in the Struggle for Life. London: John Murray.

Davidson EH. 2006. The regulatory genome: gene regulatory networks in

development and evolution. San Diego, CA: Academic Press.

Davidson EH, Cameron RA, Ransick A. 1998. Specification of cell fate in the sea urchin embryo: summary and some proposed mechanisms. Development 125: 3269-3290.

Davidson EH, Erwin DH. 2006. Gene regulatory networks and the evolution of animal body plans. Science 311: 796-800.

Davidson EH, Erwin DH. 2010. Evolutionary innovation and stability in animal gene networks. J Exp Zool B Mol Dev Evol 314: 182-186.

Dehal P, Satou Y, Campbell RK, Chapman J, Degnan B, De Tomaso A, Davidson B, Di Gregorio A, Gelpke M, Goodstein DM, Harafuji N, Hastings KEM, Ho I, Hotta K, Huang W, Kawashima T, Lemaire P, Martinez D, Meinertzhagen IA, Necula S, Nonaka M, Putnam N, Rash S, Saiga H, Satake M, Terry A, Yamada L, Wang H-G, Awazu S, Azumi K, Boore J, Branno M, Chin-bow S, DeSantis R, Doyle S, Francino P, Keys DN, Haga S, Hayashi H, Hino K, Imai KS, Inaba K, Kano S, Kobayashi K, Kobayashi M, Lee B-I, Makabe KW, Manohar C, Matassi G, Medina M, Mochizuki Y, Mount S, Morishita T, Miura S, Nakayama A, Nishizaka S, Nomoto H, Ohta F, Oishi K, Rigoutsos

I, Sano M, Sasaki A, Sasakura Y, Shoguchi E, Shin-i T, Spagnuolo A, Stainier D, Suzuki MM, Tassy O, Takatori N, Tokuoka M, Yagi K, Yoshizaki F, Wada S, Zhang C, Hyatt PD, Larimer F, Detter C, Doggett N, Glavina T, Hawkins T, Richardson P, Lucas S, Kohara Y, Levine M, Satoh N, Rokhsar DS. 2002. The draft genome of *Ciona intestinalis*: Insights into chordate and vertebrate origins. Science 298: 2157-2167.

Delsuc F, Brinkmann H, Chourrout D, Philippe H. 2006. Tunicates and not cephalochordates are the closest living relatives of vertebrates. Nature 439: 965-968.

Denes AS, Jekely G, Steinmetz PR, Raible F, Snyman H, Prud'homme B, Ferrier DE, Balavoine G, Arendt D. 2007. Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria. Cell 129: 277-288.

Duloquin L, Lhomond G, Gache C. 2007. Localized VEGF signaling from ectoderm to mesenchyme cells controls morphogenesis of the sea urchin embryo skeleton. Development 134: 2293-2302.

Dunn CW, Hejnol A, Matus DQ, Pang K, Browne WE, Smith SA, Seaver E, Rouse

GW, Obst M, Edgecombe GD, Sorensen MV, Haddock SH, Schmidt-Rhaesa A, Okusu A, Kristensen RM, Wheeler WC, Martindale MQ, Giribet G. 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. Nature 452: 745-749.

Erwin DH, Davidson EH. 2009. The evolution of hierarchical gene regulatory networks. Nat Rev Genet 10: 141-148.

Ettensohn CA. 2009. Lessons from a gene regulatory network: echinoderm skeletogenesis provides insights into evolution, plasticity and morphogenesis. Development 136: 11-21.

Ettensohn CA, Illies MR, Oliveri P, Jong DLD. 2003. Alx1, a member of the Cart1/Alx3/Alx4 subfamily of Paired-class homeodomain proteins, is an essential component of the gene network controlling skeletogenic fate specification in the sea urchin embryo. Development 130: 2917-2928.

Ettensohn CA, Kitazawa C, Cheers MS, Leonard JD, Sharma T. 2007. Gene regulatory networks and developmental plasticity in the early sea urchin embryo: alternative deployment of the skeletogenic gene regulatory network. Development 134: 3077-3087.

Frederiksen PD, Thiel S, Larsen CB, Jensenius JC. 2005. M-ficolin, an innate immune defence molecule, binds patterns of acetyl groups and activates complement. Scand J Immunol 62: 462-473.

Freitas R, Gómez-Marín C, Wilson Jonathan M, Casares F, Gómez-Skarmeta José L. 2012. Hoxd13 Contribution to the Evolution of Vertebrate Appendages. Dev Cell 23: 1219-1229.

Galant R, Carroll SB. 2002. Evolution of a transcriptional repression domain in an insect Hox protein. Nature 415: 910-913.

Gao F, Davidson EH. 2008. Transfer of a large gene regulatory apparatus to a new developmental address in echinoid evolution. Proc Natl Acad Sci USA 105: 6091-6096.

Garfield D, Haygood R, Nielsen WJ, Wray GA. 2012. Population genetics of cis-regulatory sequences that operate during embryonic development in the sea urchin *Strongylocentrotus purpuratus*. Evol Dev 14: 152-167.

Garfield DA, Runcie DE, Babbitt CC, Haygood R, Nielsen WJ, Wray GA. 2013. The impact of gene expression variation on the robustness and evolvability of a developmental gene regulatory network. PLoS Biol 11: e1001696.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 29: 644-652.

Hinman VF, Davidson EH. 2007. Evolutionary plasticity of developmental gene regulatory network architecture. Proc Natl Acad Sci USA 104: 19404-19409.

Holland PW, Booth HA, Bruford EA. 2007. Classification and nomenclature of all human homeobox genes. BMC Biol 5: 47.

Illies MR, Peeler MT, Dechtiaruk AM, Ettensohn CA. 2002. Identification and developmental expression of new biomineralization proteins in the sea urchin *Strongylocentrotus purpuratus*. Dev Genes Evol 212: 419-431.

Janies D. 2001. Phylogenetic relationships of extant echinoderm classes. Can J Zool 79: 1232-1250.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform 9: 286-298.

Katoh-Fukui Y, Noce T, Ueda T, Fujiwara Y, Hashimoto N, Tanaka S,

Higashinakagawa T. 1992. Isolation and characterization of cDNA encoding a spicule matrix protein in Hemicentrotus pulcherrimus micromeres. Int J Dev Biol 36: 353-353.

Kitajima T, Urakami H. 2000. Differential distribution of spicule matrix proteins in the sea urchin embryo skeleton. Dev Growth Differ 42: 295-306.

Kitano H. 2004. Biological robustness. Nat Rev Gen 5: 826-837.

Kurokawa D, Kitajima T, Mitsunaga-Nakatsubo K, Amemiya S, Shimada H, Akasaka K. 1999. HpEts, an ets-related transcription factor implicated in primary mesenchyme cell differentiation in the sea urchin embryo. Mech Dev 80: 41-52.

Lemaire P, Garrett N, Gurdon JB. 1995. Expression cloning of Siamois, a Xenopus homeobox gene expressed in dorsal-vegetal cells of blastulae and able to induce a complete secondary axis. Cell 81: 85-94.

Li B, Dewey CN. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12: 323.

Lindskog S. 1997. Structure and mechanism of carbonic anhydrase. Pharmacol Ther 74: 1-20.

Littlewood DTJ, Smith AB, Clough KA, Emson RH. 1997. The interrelationships of the echinoderm classes: morphologicalandmolecular evidence. Biol J Linn Soc 61: 409-438.

Livingston BT, Killian CE, Wilt F, Cameron A, Landrum MJ, Ermolaeva O, Sapojnikov V, Maglott DR, Buchanan AM, Ettensohn CA. 2006. A genome-wide analysis of biomineralization-related proteins in the sea urchin *Strongylocentrotus purpuratus*. Dev Biol 300: 335-348.

Lynch VJ, Tanzer A, Wang Y, Leung FC, Gellersen B, Emera D, Wagner GP. 2008. Adaptive changes in the transcription factor HoxA-11 are essential for the evolution of pregnancy in mammals. Proc Natl Acad Sci USA 105: 14928-14933.

Mann K, Poustka AJ, Mann M. 2008a. In-depth, high-accuracy proteomics of sea urchin tooth organic matrix. Proteome Sci 6: 33.

Mann K, Poustka AJ, Mann M. 2008b. The sea urchin (*Strongylocentrotus purpuratus*) test and spine proteomes. Proteome Sci 6: 22.

Mann K, Wilt FH, Poustka AJ. 2010. Proteomic analysis of sea urchin (*Strongylocentrotus purpuratus*) spicule matrix. Proteome Sci 8: 33.

Matsubara M. 2006. Diversity and evolution of larval morphology in Echinoderm. In: Graduate School of Science: Kyoto University.

Matsushita M, Fujita T. 2002. The role of ficolins in innate immunity. Immunobiology 205: 490-497.

McCauley BS, Weideman EP, Hinman VF. 2010. A conserved gene regulatory network subcircuit drives different developmental fates in the vegetal pole of highly divergent echinoderm embryos. Dev Biol 340: 200-208.

McCauley BS, Wright EP, Exner C, Kitazawa C, Hinman VF. 2012. Development of an embryonic skeletogenic mesenchyme lineage in a sea cucumber reveals the trajectory of change for the evolution of novel structures in echinoderms. EvoDevo 3: 1-11.

McGonnell IM, Graham A, Richardson J, Fish JL, Depew MJ, Dee CT, Holland PW, Takahashi T. 2011. Evolution of the Alx homeobox gene family: parallel retention and independent loss of the vertebrate Alx3 gene. Evol Dev 13: 343-351.

Mitsunaga K, Akasaka K, Shimada H, Fujino Y, Yasumasu I, Numanoi H. 1986. Carbonic anhydrase activity in developing sea urchin embryos with special

reference to calcification of spicules. Cell Differ 18: 257-262.

Morino Y, Koga H, Tachibana K, Shoguchi E, Kiyomoto M, Wada H. 2012. Heterochronic activation of VEGF signaling and the evolution of the skeleton in echinoderm pluteus larvae. Evol Dev 14: 428-436.

Nakano H, Hibino T, Oji T, Hara Y, Amemiya S. 2003. Larval stages of a living sea lily (stalked crinoid echinoderm). Nature 421: 158-160.

Ohno S. 1970. Evolution by gene duplication: London: George Alien & Unwin Ltd. Berlin, Heidelberg and New York: Springer-Verlag.

Oliveri P, Tu Q, Davidson EH. 2008. Global regulatory logic for specification of an embryonic cell lineage. Proc Natl Acad Sci USA 105: 5955-5962.

Palumbi SR, Wilson AC. 1990. Mitochondrial DNA diversity in the sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis*. Evolution: 403-415.

Pancer Z, Rast JP, Davidson EH. 1999. Origins of immunity: transcription factors and homologues of effector genes of the vertebrate immune system expressed in sea urchin coelomocytes. Immunogenetics 49: 773-786.

Panganiban G, Irvine SM, Lowe C, Roehl H, Corley LS, Sherbon B, Grenier JK, Fallon JF, Kimble J, Walker M, Wray GA, Swalla BJ, Martindale MQ,

Carroll SB. 1997. The origin and evolution of animal appendages. Proc Natl Acad Sci USA 94: 5162-5166.

Patel RK, Jain M. 2012. NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. PLoS One 7: e30619.

Paul CRC, Smith AB. 1984. The early radiation and phylogeny of echinoderms. Biol Rev 59: 443-481.

Pespeni MH, Barney BT, Palumbi SR. 2013a. Differences in the regulation of growth and biomineralization genes revealed through long-term common-garden acclimation and experimental genomics in the purple sea urchin. Evolution 67: 1901-1914.

Pespeni MH, Chan F, Menge BA, Palumbi SR. 2013b. Signs of adaptation to local pH conditions across an environmental mosaic in the california current ecosystem. Integr Comp Biol 53: 857-870.

Pespeni MH, Garfield DA, Manier MK, Palumbi SR. 2012. Genome-wide polymorphisms show unexpected targets of natural selection. Proc Biol Sci 279: 1412-1420.

Pisani D, Feuda R, Peterson KJ, Smith AB. 2012. Resolving phylogenetic signal

from noise when divergence is rapid: a new look at the old problem of echinoderm class relationships. Mol Phylogenet Evol 62: 27-34.

Putnam NH, Butts T, Ferrier DEK, Furlong RF, Hellsten U, Kawashima T, Robinson-Rechavi M, Shoguchi E, Terry A, Yu J-K, Benito-Gutierrez E, Dubchak I, Garcia-Fernandez J, Gibson-Brown JJ, Grigoriev IV, Horton AC, de Jong PJ, Jurka J, Kapitonov VV, Kohara Y, Kuroki Y, Lindquist E, Lucas S, Osoegawa K, Pennacchio LA, Salamov AA, Satou Y, Sauka-Spengler T, Schmutz J, Shin-I T, Toyoda A, Bronner-Fraser M, Fujiyama A, Holland LZ, Holland PWH, Satoh N, Rokhsar DS. 2008. The amphioxus genome and the evolution of the chordate karyotype. Nature 453: 1064-1071.

Rafiq K, Cheers MS, Ettensohn CA. 2012. The genomic regulatory control of skeletal morphogenesis in the sea urchin. Development 139: 579-590.

Rast JP, Smith LC, Loza-Coll M, Hibino T, Litman GW. 2006. Genomic insights into the immune system of the sea urchin. Science 314: 952-956.

Revilla-i-Domingo R, Oliveri P, Davidson EH. 2007. A missing link in the sea urchin embryo gene regulatory network: hesC and the double-negative specification of micromeres. Proc Natl Acad Sci USA 104: 12383-12388.

Rizzo F, Fernandez-Serra M, Squarzoni P, Archimandritis A, Arnone MI. 2006. Identification and developmental expression of the ets gene family in the sea urchin (*Strongylocentrotus purpuratus*). Dev Biol 300: 35-48.

Robinson M, Oshlack A. 2010. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol 11: R25.

Ronshaugen M, McGinnis N, McGinnis W. 2002. Hox protein mutation and macroevolution of the insect body plan. Nature 415: 914-917.

Röttinger E, Besnardeau L, Lepage T. 2004. A Raf/MEK/ERK signaling pathway is required for development of the sea urchin embryo micromere lineage through phosphorylation of the transcription factor Ets. Development 131: 1075-1087.

Ryan JF, Burton PM, Mazza ME, Kwong GK, Mullikin JC, Finnerty JR. 2006. The cnidarian-bilaterian ancestor possessed at least 56 homeoboxes: evidence from the starlet sea anemone, Nematostella vectensis. Genome Biol 7: R64.

Sea_Urchin_Genome_Sequencing_Consortium. 2006. The Genome of the sea urchin *Strongylocentrotus purpuratus*. Science 314: 941-952.

Sharma T, Ettensohn CA. 2010. Activation of the skeletogenic gene regulatory

network in the early sea urchin embryo. Development 137: 1149-1157.

Shimizu-Nishikawa K, Katow H, Matsuda R. 1990. Micromere differentiation in the sea urchin embryo: immunochemical characterization of primary mesenchyme cell-specific antigen and Its biological roles. Dev Growth Differ 32: 629-636.

Shoguchi E, Satoh N, Maruyama YK. 2000. A starfish homolog of mouse T-brain-1 is expressed in the archenteron of Asterina pectinifera embryos: Possible involvement of two T-box genes in starfish gastrulation. Dev Growth Differ 42: 61-68.

Smith J, Davidson EH. 2008. A new method, using cis-regulatory control, for blocking embryonic gene expression. Dev Biol 318: 360-365.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688-2690.

Sucov HM, Benson S, Robinson JJ, Britten RJ, Wilt F, Davidson EH. 1987. A lineage-specific gene encoding a major matrix protein of the sea urchin embryo spicule: II. Structure of the gene and derived sequence of the protein.

Dev Biol 120: 507-519.

Sun J, Nishiyama T, Shimizu K, Kadota K. 2013. TCC: an R package for comparing tag count data with robust normalization strategies. BMC Bioinformatics 14: 219.

True JR, Carroll SB. 2002. Gene co-option in physiological and morphological evolution. Annu Rev Cell Dev Biol 18: 53-80.

Vaughn R, Garnhart N, Garey J, Thomas W, Livingston B. 2012. Sequencing and analysis of the gastrula transcriptome of the brittle star Ophiocoma wendtii. EvoDevo 3: 19.

Wada H, Satoh N. 1994. Phylogenetic relationships among extant classes of echinoderms, as inferred from sequences of 18s rdna, coincide with relationships deduced from the fossil record. J Mol Evol 38: 41-49.

Waddington CH. 1942. Canalization of development and the inheritance of acquired characters. Nature 150: 563-565.

Wei Z, Angerer RC, Angerer LM. 2006. A database of mRNA expression patterns for the sea urchin embryo. Dev Biol 300: 476-484.

Yamashita M. 1985. Embryonic development of the brittle-star *Amphipholis*

kochii in laboratory culture. Biol Bull 169: 131-142.

Yamazaki A, Furuzawa Y, Yamaguchi M. 2010. Conserved early expression patterns of micromere specification genes in two echinoid species belonging to the orders clypeasteroida and echinoida. Dev Dyn 239: 3391-3403.

Yamazaki A, Kidachi Y, Minokawa T. 2012. "Micromere" formation and expression of endomesoderm regulatory genes during embryogenesis of the primitive echinoid Prionocidaris baculosa. Dev Growth Differ 54: 566-578.

Tables

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Figures

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