

Supplementary information

Gene regulation of adult skeletogenesis in starfish and modifications during gene network co-option

Authors: Atsuko Yamazaki^{1*}, Shumpei Yamakawa^{2*}, Yoshiaki Morino¹, Yasunori Sasakura³, Hiroshi Wada¹

*Equal contribution

Affiliations:

¹Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

²Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8572, Japan

³Shimoda Marine Research Center, University of Tsukuba, Shimoda, Shizuoka, 415-0025, Japan

Corresponding authors:

Atsuko Yamazaki, Faculty of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan. Email: yama0205@gmail.com

Hiroshi Wada, Faculty of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan. Email: hwada@biol.tsukuba.ac.jp

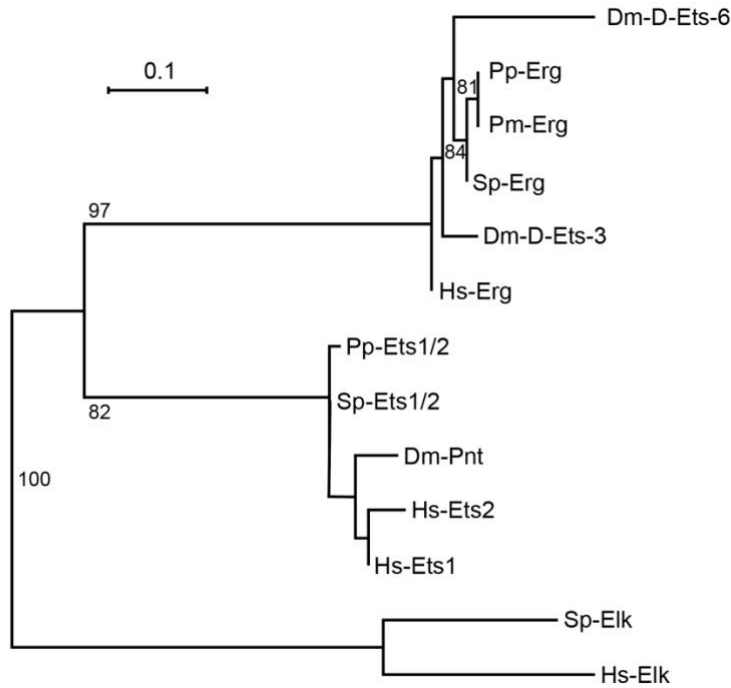
Supplementary information

Figure S1. Target sequences from larvae injected with transcription activator-like effector nuclease (TALEN) mRNAs.

Target	Left arm	Right arm	
	TCCTTGGGCAGGGGCATT	CGGCAGGGTAGTCAAAGCAGCTGCCTTTGGCA	
Control (n=5)	TCCTTGGGCAGGGAGCATT	CGGCAGGGTAGTCAAAGCAGCTGCCTTTGGCA	0 bp (5/5)
	TCCTTGGGCAGGGGCATT	CGGCAGGGTAGTCAAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CGGCAGGGTAGTCAAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CGGCAGGGTAGTCAAAGCAGCTGCCTTTGGCA	
TALEN (n=18)	TCCTTGGGCAGGGAGCATT	CGGCAG-----TCAAAGCAGCTGCCTTTGGCA	-5 bp (10/18)
	TCCTTGGGCAGGGAGCATT	CGGCAG-----TCAAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CGGCAG-----TCAAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CGGCAG-----TCAAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CGGCAG-----TCAAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CGGCAG-----TCAAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CGGCAG-----TCAAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CGGCAG-----TCAAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CGGCAG-----TCAAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CG-----GCAGCTGCCTTTGGCA	-14 bp (3/18)
	TCCTTGGGCAGGGAGCATT	CG-----GCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CG-----GCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CG-----AAAGCAGCTGCCTTTGGCA	-12 bp (3/18)
	TCCTTGGGCAGGGAGCATT	CG-----AAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCATT	CG-----AAAGCAGCTGCCTTTGGCA	
	TCCTTGGGCAGGGAGCAT	-----GCCTTTGGCA	-23 bp
	TCCTTGGGCAGGGAGCA	-----GTCAAAGCAGCTGCCTTTGGCA	-12 bp

Each sequence shows the target sequence of our designed TALEN and is from the control or TALEN mRNA-injected embryos. Target sequences included an 18-bp left-arm binding site, a 16-bp spacer region and a 17-bp right-arm binding site (the left and right binding sites are highlighted in orange). No insertions or deletions were found in genomes from control embryos (0 bp, 5/5), while various deletions were detected in genomes from TALEN mRNA-injected embryos (-5 bp, 10/18; -14 bp, 3/18; -12 bp, 4/18; and -23 bp, 1/18). Such deletions were detected in left/right arm binding sites and spacer regions. Single base mismatch was found at the left binding site of the clone from the control embryos (indicated by the orange letter "A" in the top sequence). This substitution was considered an endogenous polymorphism of the starfish.

Figure S2. Molecular phylogenetic tree of Ets-family genes for identification of *erg* gene.



The numbers at the nodes are the bootstrap values (only those $\geq 50\%$ are shown), obtained by 1000 replicates. Tree was constructed based on the amino acid sequences of the Ets domain using the maximum likelihood (ML) method. The amino acid substitution model was LG + G + F. Accession numbers are as follows: Dm-D-Ets-6, NP_001285547.1; Pm-erg, ADA79646.1; Sp-erg, NP_999833.1; Dm-D-Ets-3, NP_729142.1; Hs-Erg, NP_001317954.1; Pp-Ets1/2, BAJ33504.1; Sp-Ets1/2, NP_999698.1; Dm-Pnt, NP_524461.2; Hs-Ets2, NP_001243224.1; Hs-Ets1, NP_001155894.1; Sp-Elk, NP_999792.1; Hs-Elk, AAH48296.1. Abbreviations: Hs, *Homo sapiens*; Dm, *Drosophila melanogaster*; Sp, *Strongylocentrotus purpuratus*; Pp, *Patiria pectinifera*; Pm, *Patiria miniata*.

Supplementary data

The Sequences for *Pp-erg* and *Pp-clect*

Underlines indicate for sequences that correspond to primers. The sequences of *Pp-erg* and *Pp-clect* were obtained from PCR amplification using cDNA and transcriptomic data, respectively.

>*Pp-erg*

AGATCATCAGGATGAAGCAGGAGCCGGACCACGGAAGTCACGGCCACGAGGCCGGTAGCCTCGGCG
GGCGGGTGGTGGAAAGCCGAGTCCCCGTTGGACTGCAGCGTGACCAAGAGGCAACCCACGGGGCTAT
GACGGAGGCCACCCAGGCGGCACCCCCTTACCCGTGCCCGCCTTACCCCGGATCGGAATCGGACCGTG
CTCGCTACCCCCGCCAATGTCACAACCAACGAGAAACGGGTCAATTGTGCCTGCTGACCCCAACATGT
GGACGATCGACCACGTGCAGCAGTGGGTACAGTGGGCGGTGAGGGAGTACTCCCTGCAGGACGTGCT
GGTCAGTCGGTTCGGCATTGACGGCAAACAGCTCTGCAAGATGACCCGCGAGGACTTCACGCGACTGA
CCAGCTCTACAACGCCGACGTGCTCCTCTCCCATCTCAACTTCTCAAGCAAGCCCCACTGCCAAACCT
GACATCAGACGACGTAGACAAAGCTCTCAGCCTTCCCCAGGGCTCCGCCTAGCTCACAAGCAACGAA
CATTGGAACGGCTCAGCCCATCACCGAGAAGAAATACACGTGCAATGCTACAAATTACTACCCGGATCA
GATGCAAAAAGCAAGTGCCAGTGC GTTCCCGTACCCAGTCAGCTCCCACGTGGACACAAATTCCCGGA
TGCAACGAACAGAGCCTTCGTGCGCTGAGTCGCTGGTGC GCGGCAGGCAAACAGCTGGGCCTCGTCT
GTGCCTGTGCTCCTCAAAGGATTTACACAGACGACGACGTCAATTCCCAAGGCTGGCATCGAATCC
TCATCCCAGATCAGACCAGATCCGTATCAAGTCTTCGGTCCCACCAGCAGAACGCTTGCAAATCCAGAA
AACCCAGCAAGCAGCTTAGGAGGACATGTCTGGAACCGGAGTGGTCAGATCCAGCTGTGGCAGTTCCT
ACTGGAGCTCCTGTCCGACAGCTCCAACGCCAACTGCATCACGTGGGAGGGCACCAACGGGGAGTTCA
AGATGACCGACCCGGACGAGGTGGCCCGCCGCTGGGGCGAGCGCAAGAGCAAACCCAACATGAACTA
CGACAAGCTCAGCCGGGCCCTGCGTACTACTACGACAAGAACATTATGACCAAGGTCCACGGCAAGC
GCTACGCCTACAAGTTCGACTTCGCGGGGCTGGCCAGGCCATGCAGCCGGTGCAGGCCGACCCACAGC
GTCTACCGTTACCAGTCGGACTTGAGCTACCTGGCCAGGGCTATCACCACCCAGCAAGATCAACTTT
GTGGGCACGCCATCCCCTCCACCAACGCCGGCCTCTCAGCTCGCACGGATCCTATTGGTCGTCGCCC
AGCGCCGCAATATCTACCCGAGCAGTCACGTGACCCATCCGCACAGTCACGTGTCACCGCACATAGGC
ACGTATTACGGGTAGACTGTGACGATGCCGTTGTTGTTTTGACTGATAAAAGTGCCATGGTTTAAGAA
AAACTCGAATACCGATGGTTAAAAAAAACGTAACGAACCTACTCCAGCCTGTTTTAAACGGCATC
TTCAACAACCAGGATTAAGGTAGCCGCCGAGGTACCTTGTGGTTATTTTAATCGTGAACTGA

>*Pp-clect*

CTCTCCGATCTCAAACAGTAAGGTCACCGTGTGGTAACTAGGACAGTATGTTGGCTTGACTTGTTA
GTTGGTCAGTCAGTGGTTGGTTGTTGGTAGGAGTAAGTCAGTCTTGTAGGCCAAGTAAATAAGCAG
TTCACTTGTTTTGTTGGTCGGTCAGTAATTCGTTTGGTTCAGTTGGTTGACCTGTAAGTTGGTTGGTT

AGTAAACCGCTGAGGGCGCATCAGACGGTTTGCAGTCGGTCATTTTATAGTTGGCCAGTATGCGGTGT
TCAGTAGATAGCCTCAAAGCATTTTTGTTGTTGGTTAGTTGCTTGGCTGTAGGTTGATATGTTTGCCAT
TGCAATACCAACCACCCAACAACCATCCAACCTGAATAGCTGAACTATACAGCGAATACCAAAAATAAGG
AGGCCGTCTCCCTCTAGAAAAGTGAACATTTTGAGAAAAAATAATTTGGCTAAAATTACATTGTCATA
TTGACTAAAATATCCTGAAGGACAATTAACAACAGCTGATGTGGCAGCCTGAGTGTCTATGTAAACAG
GAGGTATCTCTTTATACGGGCCTCAATTATTTTCTTAACTTCAAATTAGTTCTTATCCTATGAGTCCCCG
TTTTCTGTTGTGAAGCTGGACAAGGGACTTGGCTCCGAACAACTGCATAATGCATGATTGACTCATC
CCTGTTGCATATGCAAATAAATGGGCGGGTCGAAAAGTTTACCGCGCATGG GCACACGAGTTCGCGA
TGCTGTAGACTAGGTTTGATCATTTTTGTTTGGCTTTTATAGTCGACCTGCATTCGAGGTGCAGCTCGCC
TGAGACACAGATCGACACCCAGGTTGAGGAACAGACCCCCAGCCGTTCAAATGCAGTTTTTCTCCCT
TGCTTGTGGTTCTACTGGTCGCCGGCCTGGCCACGGCCCAACGGCTCAACAACCTGCCCCGACGATGTGG
TCCGGCGCCGGCGAATACTGCTACCAGTCTTCAAGCAGCCTCTGCCCTGGCAGGAAGCGAGCAACTC
TGCTCCAAGTTCTCCTCCTGCGCCACGGGAGCGGGCCAGCCTGCTGAAGCTCGACTCACCCCAGGCC
GACACGCGGCTGGAGAAATCCTGAGTCCAAAAGGCAACCCCAGCCCTCCAACACGTGGATCGGCTT
GGGCGATCCCATGAACACGGGTCAGCTCACCTGGCCTGACGGTACCACCACACAGAGCGTAGGCTACA
ACAACCTCGGGCAGGGAGGCCGGAACGGAACTGCGCTCTGAAGGACACCCGCACGAACCAGTGGAC
CTTCGCGCCCTGCACGCAAGCGAACTCCTTCGTCTGTCAGATGCAGCGGAGCGCCCCTCTGCAGACCGG
CGGCCCAGGCACCAATTTCCCTTCACGACCTGTCTAAAAGTCGGCTCGGACGAACAATTTACGAACAA
TTTGTGCAGCGAACAAGAGGAATTCGTACAAAACTGAACAACCCAATCGGGATCCAAGATGGCCGTA
CGAGAAGTAGCACCAAGTATAGTCCCTGCCCCAGTCTACCAACATCCAGCGTGTTCATCCAGTATA
GGTCGAGTACCGGCGATGACCATCAAAAAGTATTTGCTCCACTCTGTAAAACCTTCTTCTGGTACAAACA
TTCATCGCTATCTTGTGAAGCTTTCTTCAGATGTGAGCAATAATTGAACTACAGAAAAAATGACAGCA
TTCACTGAACAGTCGGACACGAATTTATCGAATAGTAACGCATTTTATCGAATATGTTATCTCATTTTGT
GTTAGTTTGTGTTAGCACTTCTTTGACAAT AATGTTGGCAACGGGATTTTCAAGAAACGAATAAAGGAG
AATCAGTGGTCCATGAAATAAAAAAAGATCGGAAGAGCAC