

The IL2'S Problem, IL4 Evidence in Asterias Rubens Sea Star

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Abstract

IL2-like activity was described in sea star, in the past but first sea star genome studies did not confirm the presence of IL2 in A. Rubens. A new genomic survey of Interleukins: IL2 and IL4 were done, in this invertebrate

Keywords: Immune receptors; Interleukin; Transcription

Introduction

Echinoderms occupy a critical and largely unexplored phylogenetic vantage point from which to infer both the early evolution of bilaterian immunity and the underpinnings of the invertebrate innate and its adaptative immunity. Here, we present a survey of the Asterias Rubens sea star genome for genes associated with immunity. In a preliminary work [1] IL1 and IL1R were detected but not IL2, IL4. Our purpose was to research potential interleukins: IL2, IL4 immune receptors, regulators and effectors, particularly, including unprecedented expansions of innate pathogen recognition genes.

Materials and Methods

Immunized and non-immunized A. Rubens sea stars to HRP (Horse-radish peroxydase) were used. Their axial organs were removed. RNA was extracted using Trizol (Invitrogen) according to manufacturer instructions. Following steps were already described [1], the cDNA was sequenced on the illumina GSII platform sequencing 1. 100 bp from one side of the approximately 200 bp fragments. Sequences were assembled using Velvet [2].

Results

Immunized sea star genome shows a first result in the Table 1

- 1) tr|B2RBI8|B2RBI8_HUMAN cDNA, FLJ95535, highly similar to Homo sapiens transcription factor 8 (represses interleukin 2 expression) (TCF8), mRNA, OS = Homo sapiens PE = 2, SV = 1.
- 2) tr|A2RRH1|A2RRH1_HUMAN Interleukin 4 induced 1 OS = Homo sapiens GN = IL4I1, PE = 2, SV = 1

Table 1: Sea star similarities to human *tcf8* (1), to *IL4 induced 1* (2).

One contig (Contig 12015|m.10216) could be annotated via BLASTX to Homo sapiens "cDNA, FLJ95535, highly similar to Homo sapiens transcription factor 8 (represses interleukin 2 expression) (TCF8), mRNA" from the TREMBL database, with an e-value of 3e-40. On an aligned region of 96 amino acids, 79 positive and 71 identical amino acids were found.

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5'GGAGGATTCACTGGGTGAACTCTCCCAAACCGCGACCGCAGGAGACGAGATCGTCCTCT
ACGTACCTCTGCTGGACTCTACTCATGCACCCAGTGCCCAAGACATTCCAGAAGCACAG
CTCCCTGCTCAGACACGTGTACGAGCACTCAGGTGAGCCAAAAAGTAAGCGTCCACACGA
GTGTAAGGAGTGGCGCAAGGCCTTTAAGCACAAGCACCACTCATGGAGCACGCGCTCT
TCATAGTGGTGAGAAGCCATACCAGTGTGACAAGTGCCTTAAGAAGTTCTCTCACTCCGG
CTCCTACAGCCAGCACATGAACCATCGCTACAGCTACTGCCGTAAGAGGACAATCTCAA
CAGGGGATGAGCAAAGGTGGTATGGATGAAGATGAGGAGATGATGGAAGATGGAGAAT
TAAAAGATGTAAGCAGAGTGGG3'

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Figure 1: *tcf 8* sequence (5'-3').

Another contig allows us to report the IL4 obtained sequence in immunized sea stars to HRP

One contig (Contig10756|m.9341) could be annotated via BLASTX to Homo sapiens "Interleukin 4 induced 1" from the TREMBL database, with an e-value of 2.28e-16. On an aligned region of 453 amino acids, 187 positive and 105 identical amino acids were found.

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5'TCTGGTTGTTGAGGCTCGAGATCGTCTCGGAGGTGGAACCCACACTCTGGTGGACCCCTC
CATTGGTTATGTGGATTGGGCGGAGCTTACGTCGGCCCCACCCAGAATCGTCTTCTCCG
TATGGCCAAAGAACTGGGCGTGAAAACCTACAAGATCAACGAAGCTGAGAGGCTTGTTTG
GGTTGATAATCAACGTGGTTACCCTTTCAAGGGATCATGGCCCAAGTTCTGGAACCCCTC
CGTCAATCTGGATTTGAATAACGCTATCCGAGAGATCGACAGACTGGAGATATGATTTT
AGTGGATGCACCTTGGGACGCACCAAATGCCGAAGAGCTGGATAACATGACGATGAAAGA
ATGGATCGACAAGACATGTGGACAGAAAGCATCCGCCGTCTTTTATTACAACCTGTGTA
GGTTGGCGTTTGCATGGAACCCCAAGAGCTATCGGTCCTCTTCTTCGCGTGGTATCTGAA
ACAGACAGGTGGATACGTTTCGATTTATATCGAGTGATAATGGCGGCCAGGAGCGGAAGTT
TGTTGGTGGATCTCAGCAGATAAGCATTAAGATGGGAGAGAAGATCGGCAAAGAGAACAT
CAAGATGGAGAGTCCAGTAGCTCAGATTAATCAGAGTGAGGAGTCTGTTTCTGTGCACAC
CATCAACGGAGATGTCTATCAGGCTAAGTACGTGATCAGCGCAATGGCCCCACCTTTGAC
CCAGAAGATTCACTATGACCCGCCCTGCCTCCTAGGAGAAAACAGCTGATATCCAGAGC
CCCTGTAGGCAGTGTCAAGTGCATGGTGTACTACGACAAGACATGGTGGAAAAGATAG
AGATTTTGTGGCTCCACCTTCAACGTTGACCCAGCAAGTCCGATGGTGTATTCACTTGA
TGACACAAAACCTGATGGCAGTCAACCCAGCCCTTATCTGTTTGTGGCTGCTAACAAGGC
CAGAGAGTTCTGCGGGATCAGCAAAGAGGAAAAGACGAGACAGGCTGTGCAGATGTTACAG
TGCTGCCTTCGGTGGAGCAGAGGAAGCCTTACACCCTATCAACTACAAGGAGAAGAACTG
GATGAATGAGCAGTACTCCGGTGGCTGCTACACCTTACCTATGGCCCTGGTGTCTGTGAC
TCAACTTGGAAAAGAGCTCCGTAAACCATTTGGCAGATTGTATTTTGTGGGACTGAAAAC
TGCGACACACTGGGCTGGCTACATGGAAGGAGCTATACAGGCTGGAGAGAGAGCTGCTAG
GGAGATTCAGCACAGAATGGGAAAAGATTGACGAGAATGAAATCTGGCAGGATGAACCAGA
ATCAAAGGAGGTGCCAGCCCTCCGATACCGACCACCTTCTACGAACGCAACGCTCCATC
TGTAACCTGGCTTGTAAATTCATTGGTGTACTGTTGTCTGTCTGCTGTTATCGGAGT
CTTCGGATCTGCCCTGAAGAAAAATAAACCACATCGATCGACGAGCCGAGACAAAAC
CAACTCCTGAAGATATTCCTGAGTGAGCCAAACTCCATTTCATAGAAAAATTTATCAATTT
AATGCGATTCATTGAGAGATTTATTTCAAGATATTGTCTTTGAGAAAAGAAAAAATACTG
TCAATATAAAACAAATGTGGAGGCGCTTCAAACAAGCAGAGTTTGTCAAGAAAACGTCTTA
CAAAAAACCGAAAACAAAACCACAAGTTTACAAAGGTAATAAAAAATAAACAATAATTTGA
TTATATTGCATGACTTTAATCATGTGATGTGGTTGAATGCCGGCAGAGTTACTATTTTAT
TTTATTAATTTGGTGTAGGTTTCTATTAGTTTTTGGTTTAGTAGCATTGTTTCTTTA

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CAATGAACTAACCCTAGGGCTCAGTTTCATGGAAGTGCCTAAGCATAAAAAAGTTGCTAA
GCACAGACAAATTATGCTAACCCGATTAACGTTACCAGCTAAAATACAGCTATACATGTAC
ACAATGTAATGGTATCCTGCTTAAATGTGCTTAGCGGGAAAATTTCTGCTTGCCACTT
GTGTGATAACTAGTGATATATCACAAAAGTGTATCTTGTGTTTATTGAATAGCTCGCCTTG
TACATGTAGGACCTTTTCTTTTTTCTTGGCTGGACGTATGAAAGTCAATTAATCCTTAAA
AAAGGAACTGAAGTAATGGAAAGAAAGTCAAGATTTGTGTGAAGTTGCAGTGGAAAGCTG
TCAAAGTTTCCTCTGAAGAATGATCTGTTTGAATAAGATGGTCACTTTGTGGATGACT
GTATGAGGCGCCATAAATATGCCTTTTTTAAATAATAATAAATAATTATTTTTGTAGGTTT
TATTATTATCACATTAGTTTGAATGGTGACTTTGAAGTACACTGAA3'

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Figure 2: Sequence of IL4 induced 1.

Discussion

We have learnt that sea star T lymphocytes can release soluble lymphokine mediators, after stimulation with P.W.M (Pokeweed mitogen) sepharose 6 MB beads with mitogenic properties [3]. IL2-like was discovered in Legac., et al. [4], using flow cytometric discrimination, but first genomic studies [1] didn't corroborate this cytometric result. So, recently, we took back genomic works. We mainly found TCF8, which represses interleukin 2 expressions in man, but what did it do in sea star? Is it an indirect evidence of IL2 in Asterias Rubens? It remains enigmatic. We also found IL4 in immunized sea stars only. IL4 induced 1: an interleukin induced by B lymphocytes and why not by sea star B lymphocytes? [1]. The possible absence of true IL2 may reflect the difficulties of identifying these rapidly evolving genes, which have slow protein domain complexity and complex intron - exon structure. Nevertheless, members of most cytokine and chemokine families and their receptors were detected in the sea star genome.

Conclusion

In conclusion, our data are consistent with the hypothesis that a true cytokine network exists in Asterias Rubens genome.

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