ASTERIAS Rubens: Evidence of Immune Genes

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Abstract
In recent papers, we have found that the Asterias Rubens genome shown Ig Kappa genes. It is discovered that the Ig Kappa chain V-IV region S107 B precursor gene was present in non-immunized sea stars. On the other hand, the NF-kappa B gene, into 2 subunits, was shown in these invertebrates.

Keywords: ASTERIAS Rubens; Immune Genes; NF-Kappa; BLASTX

Introduction
In recent papers, we have found that the Asterias rubens sea star genome, shown Igkappa genes [1,2] We try, in the present study, to determine the existence of Igkappa gene in non-immunized Asterias rubens. On the other hand, we attempted to study the NF-Kappa B gene, in the immunized and non-immunized sea star genome. The NF-kappa-B factor was implicated in the immune response in mammals [3]. We discovered the interleukin17 receptor B in sea star genome [4] and we recall that interleukin 17 receptor B mediated the activation of NF-Kappa B in mammals [3]. So it seemed really interesting to study the NF-Kappa B genes in an Invertebrate.

Material and Methods
Immunized and non-immunized sea stars to HRP (Horse-radish peroxydase) were used [1]. The sea star axial organs were removed. RNA was extracted using Trizol (Invitrogen) according to manufacturer instructions. CDNA was normalized using double strand specific nuclease essentially as described by Zhulidov., et al. [5]. cDNA was fragmented using DNA fragmentase (New England Biolabs), according to the manufacturer’s instructions. After ligation of adapters for Illumina’s GSII sequencing system, 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: Zerbino., et al. [6]. Assembled nodes were used for further assembled including Beta vulgaris EST-Data from NCBI in MIRA.

Results
Results are summarized in two parts:
A. Controls
B. HRP

Controls
The first contig shows the presence, in non-immunized Asterias rubens of the gene: Igkappa chain V-IV region S107 B precursor which has been recently isolated [2] from immunized sea stars:

One contig (Contig3053|m.6472) could be annotated via BLASTX to mouse “Ig kappa chain V-IV region S107B” from the SWISSPROT database, with an e-value of 0.004. On an aligned region of 50 amino acids, 23 positive and 16 identical amino acids were found.

The second contig, with its sequence, indicates the presence of « Nuclear factor NF-kappa-B p100 in « Controls »:

One contig (Contig12117|m.10680) could be annotated via BLASTX to mouse "Nuclear factor NF-kappa-B p100 subunit" from the SWISSPROT database, with an e-value of 7.00E-071. On an aligned region of 242 amino acids, 170 positive and 138 identical amino acids were found.

The third contig with its sequence reveals the presence of another subunit of NF Kappa-B (p105):

One contig (Contig675|m.5295) could be annotated via BLASTX to mouse “Nuclear factor NF-kappa-B p105 subunit” from the SWISS-PROT database, with an e-value of 4.00E-071. On an aligned region of 518 amino acids, 263 positive and 192 identical amino acids were found.
HRP

2 contigs (a and b) show the presence also of two subunits of Nuclear factor in immunized Asterias Rubens

a) One contig (Contig11285|m.9708) could be annotated via BLASTX to mouse “Nuclear factor NF-kappa-B p100 subunit” from the

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SWISSPROT database, with an e-value of 1.00E-031. On an aligned region of 203 amino acids, 107 positive and 78 identical amino acids were found.

5': GACA AATT CGAC ACTTA CAAAAGC ATCTCA ACCCG AGTAGGAAAGGA ATCTC TTAT 3'

GCAGTAAATTTTAATTTGATAATTACGATATTTTGTGCT CCCCTTTGATCAGTTTTG
TCACACAAACCGTGGAAAGACTCTAGATCTGATATTTCGCCA ACACGCAACCTGTAAC
AGTCTTGTAGATCTCAACACTTCACTGATTTGGACTCCCAGAAC
AACA CTTTGTTGACTTGGCAGTACACTGTTTCAAGCCAATTTTGAAGGAATCTCAGCCGCTG
CTTCGATA AAATGAGAAAGACTCTCACAACACTTCAAACTGAGATACCTGCTCATCTCATA
AAACAGA AACAGA AAGCCGAGAGACTGAAACAGATGAGACGAGATATATCTTCT
ATGAGTGATGACTATGTCTGAAATCTCAGA AACTCTGACAAATTTGGAAGAATCTGAGAGCCGACT
GTTTACCTCGATTTGCACTGCTGTGCTGGCTGTGCTGACAGGGCTGTAGGAGGTGAGGACT
AGATTTAACCAGAGCGTGGTGAACAGACCTTAAACCTGAGGTTCCACAGAGTCAATTCATC
ATGCAACAAAAGGCGTCCAGAGAGGTTACCTCTCAGACTTCCAGGGGTCAACAATGC
GTTAATCTGGAATGATTGCGTGGATGTTTGGAGGTGAGTTGGGACTCTTAAATGTCATTATCCTC
AGAATCTTGATGGCTTCACTGCAGCACACATTTCAGTTCAGATCTCTGATTTGAAGAAC

b) One contig (Contig2602|m.5791) could be annotated via BLASTX to mouse “Nuclear factor NF-kappa-B p105 subunit” from the SWISSPROT database, with an e-value of 2.00E-058. On an aligned region of 398 amino acids, 209 positive and 153 identical amino acids were found.

5': GCTACCCATGCAAATGCTACGAGAAGAATGCTCACAATGCCAACCCAGGCTTACAG
GTTA ATCCGAAATCAGACACGACCTAACATTTGCTAGA CACGCAGACGACGACGACGACG
ATACGACGGCGGCGCTTCCAGAGCCGTGGTATAGCTTTGAAAGGATCAAGACTTGA
AGATTTAACCAGAGCGTGGTGAACAGACCTTAAACCTGAGGTTCCACAGAGTCAATTCATC
ATGCAACAAAAGGCGTCCAGAGAGGTTACCTCTCAGACTTCCAGGGGTCAACAATGC
GTTAATCTGGAATGATTGCGTGGATGTTTGGAGGTGAGTTGGGACTCTTAAATGTCATTATCCTC
AGAATCTTGATGGCTTCACTGCAGCACACATTTCAGTTCAGATCTCTGATTTGAAGAAC

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Discussion and Conclusion

These results have proven to be of particular importance

First, it is shown that the isolated Igkappa gene [2] with 2 Ig sites exists also in non-immunized animals. Second, it appears that NF Kappa-B factor is present in sea star immune system, under 2 subunits p100 (daltons) and p105 (daltons): undoubtedly it plays a role in the immune function of Asterias rubens, when compared to mammal’s one [7]. The sea star immune complex appears more and more sophisticated.

Bibliography


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