

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 Iggkappa genes [1,2] We try, in the present study, to determine the existence of Iggkappa 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: Iggkappa 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.

5' :GTTGATCATAAGATCAGAGAATATAAAGTGGCTCAGTCCTGAAAACAGTTTGTACAAAA
 CTATTTTTCTTCATACAAAATTTATTAGTGTGCTCCTGCAAAACGGCTGATAACTAAAACC
 AGTATGGCTCTCTTTCTCCGGTTACGGTGATGGTCTGGCTGTGATTGGGCTGACCAGG
 GCTGGATGTCACCGCTATGGACTGAATATCGTAACAACCTGCTACCGTTAATGGGTCAT
 GAAAAGTCGTGGACCGATGCCGAAAACCACTGCCGGCAGTTCCTTACCATCAGCGGACAG
 GGTCACTTTGCATCCGTGCACAGCTCTGACGAAGCGGATTTCTGCTCCAGTATTCGACA
 TCTTCATTGACCTCTGCCTTGGACAAAACAAGTTTGGCTTGGGATGTCCGACCAATCAAAT
 GAAGGCACCTTTCAGCTGGAGTGACGGCACAGCGTTTCGACTACGAGGAATGGCTTCCTGGA
 CAACCGGATAATGCAGGCTCAAATGAAGATTGCGGTGAAATTAAGTTTCTTGGGGTGCC
 CATCGAATGGGCTGGAATGATGGCCGCTGCTCCGATCACC AATCACACGTCTGCAAGATG
 CCAATGCCAACTGTTCTTTGAATACAAAACCGATGAAAAGCAAACATGCAGCCTCCATAACA
 TTTTCATCTAGAGAAAACAAAATCACTTATTCGCCGGGACATGACTGAATTCAGAGTTG
 TACAAAGTTGATTTTCAGCTCTACCAGAATGGTTATTGAATCGTCCACAAAATTC AATGA
 GCCGAAGCATTGATCAACTCAACAAAAGTAACTCATCCACAACAGTAACTCAATAGAGTCA
 CTCAAAACAGTTAATCGTCAGTGTCAACCCACAACAGTTTCTCGCCAGAGTCACTCACAAC
 AGTCACTCGCCAGAGTCACTTACAACAGTTACTCACCAGAGTCACTTGGCAGCCACAACA
 GTTACTCGTCAGAGTCACTCACAACAATTACTCATCGGAGTCACTCACAACAGTTACTCG
 CCAGACTTAATCATAACAGTTACTCGCCAGAGTCACTCACAACAGTTACTCGCCAGACTT
 AATCACAACAGTTTCTCACCAGAGTCACTCAATTCATTACAAAAGAATTTTTTCCGATTT
 TGAATAATTATGGCTAATATTA TACTCAGAGCACTATGCATTA AAAACATAGAGGCTAAAAT
 GTCGATTATTTGGACAACAAAATGCTTGCAATTTAATCATTATTTTGCTTTTTAGTTT
 TTAGCTGAGCCAACCATTTGTTTTCGAAGAGTTAGACTAGTTTAAATCTTTAACTTTGAAT
 AATTTTGTATATAAAAGCACACCATACAATTTACATGCAAACGACAATTTAATGCTTAGT
 CATCGAATTCGGAAGGTA ACTATTTAGAGAAAATTTTACCATTAACAGAAAATTTAGTTTT
 GAGGGGATTTTATCAAATCGACATTTGATCAATCGATTGTTAAGGCAAAGTTTGGGTTTAT
 GGAACCATATTTCCATCGTTTAATTTAATAATGTAATTA AATACAATACCACTAAATTTAT
 GTCCCATTTGCCACCTTTTATGCCAGATGACTTTTCTATACAATTA AAGCCATTGGACAC
 TTTTCGGTAAAACAGTATTGTCCAAAGGCCAAACTTTGTGTATCACA ACTTATATATACAA
 TAACAACCTGTGAAAATTAGGCTCAATCAGTCATTGGAGTCAAGGAGAAAATAACGGGAA
 AACCCACTCCTGTTTTTCGCGCTTTTCGC 3'

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.

5' :AGAAGATTGAAAACCGGAAGAATTGTGACACTGCAACTTGTCACACTTGAAGCACC AATT
 TAAAAGATGTCAGACGAGGAGAGGTCTGATTCCTGCAGTCAGTTGAGATCCCAAATGAC
 ATGTTACAGCAAGCATTTCTTAAACAATAACGGAGCATCAAACCAATCCTCGGAAATGAAT
 TTATCTGTTCTGCCAACTGACCTTCACTCAACTTGAGGTCATGACAGGAATGAGTCAACAG
 ACGCTAAATGACCTCTCTGCTCCCAAACCTCCGCATCATAGAGCAACCCAAAACAGCGTGGC
 TTTTCGGTTCCGTTACGACTGCGAAGGGCCATCACATGGTGGTCTCCCGGTCAACATAGC
 CAGAGAGGAAAGAAGACCTTCCCATCAGTTGAGATACTTAACTATACTGGACCAGCAAGA
 ATGTTGTTTCTTTGGTAACCAGTGAAGATCTACCTAAACCCATGCACATAGCATCGTT
 GGCAAACACTGCACTGATGGATCATGCACTGTGCAGGTGGACCTACAGACATGACTGCA

TCCTCCCTTCAATCCATGCAGATTACAGGAGATTCTGGTTTTGCCCGTGGATGTATGACG
AATCAGTTCAACCAGTCGGATGCCAGGAATATTGAAGTTAGCTATTTGGTGTAAAGAA
ATAAATTTGATGCAAAAATAGGTGGTGT'TTGT'TTCCCAAAAAGACTGTATAGTTGTACA
TAGTGCTTCGTAAATGCCTTGTGAAGTTATTAAGTGAGATATTTACTTTGTATATC
GGTTAAATCTTAAGGTATTATGTAGTTGAAGAACTTGAAACGTCTAATTTCTAACTAG
GTGTTAAGATAACTGTTTTTGTATCAGAAAATAACTGTGGGAAACATAGCAAAACGCT
GGAAGATTAGAATGATTTTTGGGAATATTTTCCTTGTGTAATAGGTTTTTTTTCTTTTC
TGAAGATACATTTTTTACAGAATTTTGTCTGCAACATATATCATACAAATTTTCGGCTTA
ACTGTCGTCAGGACATTGTCTGTATGTTTGTAAATGTAAATGTTTGTGCTTTATTGTC
ATTGCATCAGTGAACAAAAGGGAGCACTACTTTGTTAGTGCAGGGATCTTTTTATTGTA
CCAGCTTTGTAAAGCCTTGTAGCCATAAAGAAATGTTTCTTAAATACTGTTTTTCAAT
CTATTTGATAGTCGTCAGAAGGATTTAAACAGAACATATTTTAGGAAGTCTGTTTT
AATTTTGTGGACAGTTCACTTAAAGCAGAAACATGGTGATAGTTAGCTCATTGTAAAGT
AAAGGAGATGGCTGTGATGGGAATTGCATTGGTGAAAGCATAATAGGCCCAATGCTGTTT
ATCAACCTGTTTGT'TTATGAACAATGTGTAGCCTCCAAACACACTCTAGTTTAAATCAT
ATTAGTTTGGATTTGTGTACATCACTACCTATGAAAGAGTCTTGGACAAGTTGTGCAAAA
TGCTGCATCTTTGT'TTAAAGAACATCATTATTGCTTTGTATCACATTACATAGGAGGTGA
TTGGTCTCTTGTGTCTCTAGATTGCCAATGTAATGGTGAGATTGACAATGTAATGTGCGCA
TGAGTTCCAATTTAGCATCCATTGTGGTTCGAATAGATTTTTTTACATTTAAAGGCAGTG
GACACTATTGGTAATTGTCAAAGACTAGCATTACAGTTGGTGTATCTCAACATTTGCAT
AAAATAACAACCTCAGTAATAATTTCAAATTAAGATTTATTAATTTGTATTCAAGTTTG
CAAAATGTTACAGACTATTTCTTTTACAATAGAAGATTTAAAAGCAATGTTGTGTAATACT
ACTGCATTTACCTTCAAATAAAGAATTTATAATTTAAATTTGCAACCCTCCTACTGTTG
GTACAATTATCTAAAAAGTTGATGCACACTATCGTGGCTTCAAATACTAACCAGCTCCCC
CCCCGCCAGTTTTATAGAGCTCCTTTAGCAGTAAATATTGATATATATTTGGTTTGCA
GTAACACCATGTGTGTATCTACTTGGCAGGTAGAGTTTGTTCCTAGAGAAGTGTCTTGCT
TTATTCAAATACCGCGGAGAAGATGTTCCGGGGATCGGGAGACTTCTCAGTTCAGAAAAG
AATTGCTCTGTTTTTCAACTATTACCTGGGCGGATTTGATAGAACATATGCTGGGACTA
CTACTTAAGCTTATAGGATCGTAATTAAGT
CAAAGTATAGTACACTACAAATCCAAGATGCACAATTTAGTGACAACGGCAATTACGTCT
GCCAAGTAGGCAATAACAGAGCCTATGGTGATCTTACAGTAACGTATGATGGAATCGTGC
TAATTAAGGCAATCGAGTCATTGAAATGGAGCAAGATATGAACTTCACATGCTTTATTG
ATGGCGACTCATCTGTTGAGGTCACATGAAAAAGGAAAATACAATGCTACCATCTCCTG
AGCTGCCTAACATTGAAGTAAAGGGAAGCTCTCTGCTTATAAAGAAGGCATCGACTGCCA
ATGGTGGTAAATACACCTGCAGTGGATTGTACAACAAAAACAATTCAGTTGAACAAAATA
TTTATGTAGGAGGGGAAATGCCAATCAAGTCCCCAATTCAGTGAAGTTTACAGAAGGAG
AGCGTGCTCGCTTTGAGTGTGTCATTCTCCAGCCAGAAGGCGACCCCAAACCTACTTTCC
AATGGATAGTGAGCACTGCAAACTACACCTTTGAGCTGACTGAGACGATTGAGGGAAAGT
GCTACTCCATTGAGCCGTCCAAGCACAAGACTGGATCCATCTTGCACATGCTGGATGTAG
TCAAGGAAGACAGAGGAAACTATACTTGCATTGCAACCAGTAACGT 3'

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

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.

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5' :GACAAATTCGACACTTACAAAAAGCATCTCAACCCGAGTAGGAAGGAATCTCTTTTAGTT
GCAGTAAATTTTGAATTTGTATAATTCAGTATTTTGTGCTCCCTTTGGTATCAGTTTAGA
TCCACACAACCTGTGAAAAACTTCAGTACTTACTAGATTTTCGCCAACGCAACGGTAAACG
AGTCATTTGATTTTGACCATCATCAACTGAAGCAACGCACGTAATACACACAACAAACGG
AACATTTTGTGTGTAGTTTCCAGCGATTTCGAGAAGCAAATCAAAGACAAGATGTCTTTAC
CCAGTGATGTTGAAACAGACTCCGTCATGGATAGTCCAGCAGAGATTCATATGAACATGA
ATAAGCTACAATCTAAACTTCCCAGCGTTACTCAAGACGAGAGATTTGACTCCGGAATTG
ACTCGTTACGTTCTGTTGATTCGGCGTACTGCTTGAGCTTCGAAAGGGAATCGAGCCTGG
CTTCGATAAATGAGAAGCGTCTCTCACATCACACCTGCAACAGCTCCATCTTTCACATG
AAACAAGAACAGAAACCGAGAAGACTGAAACGACAGTAGAAGACATCGATGAAGCTTATC
ATGATGAGTGACTATGTCTGAAACACTCGACAATTTGGAAGAAAAGTGAAGAATTGTGG
AATATCTGAACAAAGATGCACGGGACGTCTTACAGATGATGCCTTCGACCAAGACCAAG
AGGGAGATACGCCCTTCATCTTGCTATTTATTCATAAGGAAGTGGACTTCGCAGAAAAAT
TCATCATCTTTGTTGCAGATCCTGAGTTACTGAACATCAGCAATGATCTTATGCAGACTC
CTTTACACCTTAGCGTATTAACAAGGCAACAAGATATCTGTGCTGTTCTCGTCTTGGGCA
ATGCCCAAATCGACTGCACCGACCGAAACGGCGACACTCCTCTTCATATTGCATGCAGAC
TGAGAGATGAGGGCTGTATCAGAGCTCTGACTGAAGGAATATCTCCACTCGAGCGTAAGA
GAGGGATGTTCCACAGAATAGAGCAAGTGGGGTACAACAGCTTCCACAGAATCTTGAAC
TCAGAAACTTTGAAGGCTACACATGCATCCATATTGCAGGATTCGCTTGATAGCGTCGATC
AGTTGGAGTACCTTGTGCAGCTAGGCGGCGACATAAATGCCCGGATGGAAAAGAGCGGAA
GGACCATTCTCCACTACGCTGTAGAGGCGGGTACTTTTCTCTTTGTCAGTACCTCATTG
CGAACTTGGGTGCCAATGTTAATGCGTTGACCTTTGACCAGTGCACACCC 3'
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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.

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5' :GCTACCCTATGCAGCAAATGTACCAGCAAAGTCCACAATGCCAACACCCAGGCTCTACAG
GTTATCCGAACCAACAGCAGTTAACATTGCAGCAGCAGCAGCAGCAGCAACAGCAAA
ATCACCAGGGTGCGGGCTTCCAGAGCCGTGGTATAAGCTTTGAAAAGGATCAAGACTTGA
AGATTACCCGCGAGACTGAAGTGGTGCAGGACACACAACCAGCGGTTCTACCTACAGTCA
CTCTTATGGGGAGAGAGAGATTGATAGCGATCTTGAAGTAGATTCTCAAATACTTGAGG
ATGCAACAAAAGGCCCTGTCAGGCGATTTGAATCCTGTGTGCAGAGTGGAGTTGGAGTTA
GCACTTTTGAAGTACAAAAGTGCCAGAGATATTTCTGTGCAGACTGATGATATCCAT
TGGATTATGAATCCCTCGCTTGGCGTCTTGCTGAGCGTACATCTCGTGCATTGCGTGACT
TTGCAATCACTGCTGATATGAAGATGCTGTTAGCGGTCCAGAGGCACCTGACTGCTGTGG
AGGATGAGAATGGTGACACACCTTTCATCTGGCAATTATCCACAAGAAATACGACGTTG
CTTTGGGACTCTTAAATGTCATTTATCAGCATTCCTCATCAGAAGATCATTAAATCATCACA
ACAAACTTCATCAGACGCCCCGTGCATGTTGCTGTAATCACAGAACAGCCTGACATTGTAG
AGTTACTTCTCCGTTGTGGAGCTGATCCAAACATCATGGATCATAACGGGAGTATGCCTA
TCCACCTAGCTGCTCATAATCGTCTTAGTGATATAACAGAGGCTTAGTCCAAGGGGTGG
AACCCTTCTGAAAAGTCCAACGCTTCTATTGATCCAATCCCTACTGAAATCAACCTCA
AGAATCTTGATGGCTTCACTGCAGCACACATTTTCAGTTCAGATCTCTGATTTGAAGAACC
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TCAAAGTTTTGGTGAAAAACGGAGCTGATATTAATGTTTCAGGATGGTAAAAGTGGTCGCA
 CCACCCTCCATTACGCCGTGGAGCAGGCAAACCTCTCACTGCTTGGATACCTGATTGCTG
 ATGCAGATGCAGATATCCACATTCAGACGTTTGCTGGTGATACCCCACTTCATCTTGCTT
 GCAGCCTTGATTACGTTGCTGTTGCTGCAGTTCTGGTTAGTGCTGGAGCAGAACCAAGCA
 TTGAGAACCTTGACACCTCTTCCAACGAGATGGCTGGGGAGACTGGTGTAAATATCTGATG
 AAGGACATGTGGAGATATTTGAAGACGAGTACAATAATGAGATACTTAGTGGCAAGACAT
 CAGTGGATCTGGCATGCTCTGAGAGGATGTTGAAAATTTGAATGGTGAGGACTACATAT
 CCAGTTCAGTGTGAGAATCCACACCTAGATTTGAAGAGAGTTTGGTATCAGCTGTTTCTG
 GTTCTACGCCTCTAAGACCAATATGTCTGATTCTTACACCTCTACTGCAAATAAATCAA
 GCACTAGCCTCCGCTTCACTCCACAGGAGATTTGAATCGCCTGGATTCTTAACTCGTC
 AGAAATTATCGTCGCTCCTGGATGAGATAAAAACCCACAGGATTGGTTGTCTCTT 3'

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.

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