



Asterias rubens: Evidence of NF-kappa B genes



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ABSTRACT

In the present paper we show a survey of the *Asterias rubens* sea star genome for genes associated with NF-kappa-B proteins implied in the immune response. The NF-kappa B gene, into 2 subunits, was found in this invertebrate.

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In recent papers, we have found that the *A. rubens* sea star genome has shown Ig kappa genes (Leclerc et al., 2011; Vincent et al., 2014). We discovered also the interleukin 17 receptor B (Leclerc et al., 2013) which mediated the activation of NF-kappa B in mammals (Hayden et al., 2006). So it seemed really interesting to study the NF-kappa B genes in such an invertebrate.

1. Material and methods

Immunized and non-immunized sea stars to HRP (Horse-radish peroxidase) were used (Leclerc et al., 2011). The sea star axial organs were removed. RNA was extracted using Trizol (Invitrogen) according to manufacturer's instructions. cDNA was normalized using double strand specific nuclease essentially as described by Zhulidov et al. (2004). 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. (2007). Assembled nodes were used for further assembled including *Beta vulgaris* EST-Data from NCBI in MIRA.

2. Results

Results are summarized as following:

A) Controls

The first contig shows the presence, in non-immunized *A. rubens* of the gene: NF-kappa-B 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': AGAAGATTGAAAACCGAAGAATTGTGACACTGCAACTTGTACACTTGAAGCACCAATT
TAAAAGATGTCAGACGAGGAGAGGTCTGATTCCCTGCAGTCAGTTGAGAT
CCCAAATGAC
ATGTTACAGCAAGCATTCTTAAACAATAACGGAGCATCAAACCAATCCTC
GGAAATGAAT
TTATCTGTTCTGCCAACTGACCTTCATCAACTTGAGGTCATGACAGGAATGA
GTCAACAG
ACGCTAAATGACCTCTCTGCTCCCAAACCTCCGCATCATAGAGCAACCCAAAC
AGCGTGCC
TTTCGGTTCGGTACGACTGCGAAGGGCCATCATGTTGGTCTCCCGG
TCAACATAGC
CAGAGAGGAAAGAAGACCTTCCCATCAGTTGAGATACTTAACTATACTGG
ACCAGCAAGA
ATTGTTGTTTCCCTTGTAACCAGTGAAGATCTACCTAAACCCCATGCACATA
GCATCGTT
GGCAAACACTGCACTGATGGATCATGCACTGTGCAGGTCGGACCTACAGA
CATGACTGCA
AGCTTTCCAAACCTTGGCATCCTTCATGTAACCTCGCAAGAATGTTGAGAG
GGTCTTGAAA
GATCGTCTTCGTGAACAGCAGAAGCTTTATAGGACTTTTCTTGGTGATAA
GGGATTCGTA
CTGGAGCACAGCATCTGCTTGAGACTGAGCCAGCCCTTGCCAAGAAAGC
CAAGGAAATG
GCCAAGGAGATGGATCTGTCTAAGGTGTGCTTGTGTTTTCAAGCCTACCT
TCCAGACAGT
CAGGGACATTACACGGCTCCTCTGGCTCCTGTACATCACTACCCATCTTTG
ACAGCAAG
GCCCCTGGTGCAACAACCTTAAAAATCTGCCGTATGGACAAGAGCTCAGG
ATGTGTGACA
GGGAATGACAGGATATCTGCTTTGTGATAAAGTTCAAAGGACGACAT
TTATGTGAGG
TTTTTTGAGATGAACCAAGCGGAAGTGGGCC 3'.

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ATGGCGACTCATCTGTTGAGGTCACATGGAAAAAGGAAAATACAATGCTA
 CCATCTCCTG
 AGCTGCCTAACATTGAAGTAAAGGGAAGCTCTCTGCTTATAAAGAAGGCA
 TCGACTGCCA
 ATGGTGGTAAATACACCTGCAGTGGATTGTACAACAAAACAATTCCAGTT
 GAACAAACTA
 TTTATGTAGGAGGGGAAATGCGAATCAAGTCCCCAATTCAGTGAAGTTT
 ACAGAAGGAG
 AGCGTGCTCGCTTTGAGTGTGTCATTCTCCAGCCAGAAGGGCAGCCCAAA
 CCTACTTTCC
 AATGGATAGTGAGCACTGCAAACACTACACCTTTGAGCTGACTGAGACGATT
 GAGGGAAGTC
 GCTACTCCATTGAGCCGTCCAAGCACAAAGACTGGATCCATCTTGACATG
 CTGGATGTAG
 TCAAGGAAGACAGAGGAAACTATACTTGATTGCAACCAGTAACGT 3'

B) HRP.

2 contigs (a and b) show the presence also of two subunits of nuclear factor in immunized *A. 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.
- b) Another 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.

3. Discussion and conclusion

Echinoderms occupy a critical and largely unexplored phylogenetic situation from the point of view of the evolution. According to the present study, it appears that NF kappa-B factor is present in sea star immune genome, under 2 subunits p100(daltons) and p105(daltons). It seems fundamental when compared to another invertebrate the arthropod in its specificity. Undoubtedly the NF-kappa B plays an important role in the immune function of *A. rubens*. Nevertheless this role remains enigmatic, when compared to mammal 's one (Scheinman et al., 1993). In conclusion, the sea star immune system, in its discovery appears more and more sophisticated.

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