

A NEW PRESUMED IGTKAPPA GENE IMPLIED IN THE SEA STAR IMMUNE RESPONSE

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ABSTRACT

The existence of another candidate IgKappa gene of 697 nucleotides, was recently, demonstrated, in the sea star *Asterias rubens* genome. This last gene was cloned and shown, with its sequence, in the present study. Its potential role in the sea star immune response was accentuated.

Keywords: Sea Star, Igkappa Gene, Axial Organ

1. INTRODUCTION

We had discovered another candidate IgKappa gene of 697 nucleotides (Leclerc *et al.*, 2011) in 2014.

2. MATERIALS AND METHODS

Like in 2011, we used sea stars, after immunizations to Horse-Radish Peroxydase (HRP), to study their genomes. Experimental methods were the same than those chosen, at that time (Leclerc *et al.*, 2013; Leclerc and Otten, 2013).

As for the cloning of the gene, we used the experimental protocol concerning the SMART kit PCR cDNA Synthesis (Clontech).

This SMART was specifically designed for the recovery of the target gene.

Poly A fraction was purified, first stalk of DNA was synthesized with an oligodT.

A non-specific amplification was performed in: 5'-CAGATTCAGAAACACATGTATTTCC-3' and followed by a specific one in: 5'-TTTAGCATGGCATGTAAAGACACC-3.

The PCR products showed in agarose gel 4 bands for the negative control and one band for the specific PCR. This last was purified and sequenced on Illumina 's GSII platform sequencing.

3. RESULTS

In **Fig. 1**, we recall the obtained transcriptome of assimilated Ig kappa chain V-V region K2, which is composed of 697 nucleotides.

After cloning we obtained an assimilated Ig Kappa chain V-V region K2 as shown in **Fig. 2**.

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Locus_7375_Transcript_1_1_Confidence_1.000_Length_697
5'TTCAGATTCAGAAACACATGTATTTCCATCTCTATGAAAGAAGTAC
GACAAGTACATCTTTGCTAAGTT
CATTAAATCTGACTATAACTTAAAACTTTTTGTCGTTCCGATGACGTCA
CATCGTTGGATGAAGCAAGTG
GTCCACGAGTTCAACACTCTTTTGAAGTCAACAAAACCTTCAACAAAAG
ACTTTAAAACCCCTTTTACGAGTT
TAACACAGCATCAAGTGAAGATGTTCTTAATAGCTTTTGCGGTGTTTT
CTCTGTTGTGTTACGGAGGGAG
AGCGTGTGATCTCAGCGGCCAGCCAATGGATGTCGTTGGCTGAGGTAG
GAGCGGAGGCAATACTTACCTGT
CCAACGACTTAGAGGAGTGCACGGCCAATTGTAAGGTTTCATGGTA
TCTTCTGAAGAATAAGACATATA
CACCGATTAGTTCATGTAACAAGTTTATGAGAATTCGAAAACAGGT
TTTCAATATCTGGTGGTCCCAA
CTGGACTCTTACTCTCAGCGGAGTTGAGCCGAATGATGCTCGCAAAT
ACAGGTGCAAGGTAAAGAGTAAT
GAAACAAAACCAGCAACTTCAGAAAGCATGACACTCATTATACCATC
AACCATCAACATTACGTTGAGTG
CAGCATCTTCTCAAATAACTGGTAGGGGAAGTGGTGTCTTTACATGCC
ATGCTAAAAACACGAAAG
>sp|P01635.1|KV5A3_MOUSE RecName: Full=Ig kappa chain V-V region K2;
Flags: Precursor
Length=115
Score = 35.4 bits (80), Expect = 0.005
Identities = 27/101 (27%), Positives = 45/101 (45%), Gaps = 8/101 (8%)
Frame = +3
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Fig. 1. DNA sequence of "second" candidate IgKappa gene

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5'CAGATTTCAGAAACACATGTATTTCATACTTCTATGAAGAAGTACGA
CAAGTACATCTTT
GCTAAGTTCATTTAATCTGACTATTAACCTTAAAACTTTTGTGCGTTCGA
TGACGTCACAT
CGTTGGATGAAGCAAGTGGTCCACGAGTTC AACACTCTTTTGAAGTC
AACAAAACTTCAA
CAAAGACTTTAAAACCCCTTTTACGAGTTT AACACAGCATCAAGTGAA
GATGTTCTAATA
GCTTTTGGGTGTTTTCTCTGTGTGTTACGGAGGGAGAGCGTGTGAT
CTCACGGGCCAG
CCAATGGATGTCGGCTGAGGTAGGAACGGAGGCAACTTACCTG
TCCAATAACTTA
GAGGAGTGCACGGCCAATTGTAAGGTTTCATGGTATCTTCTGAAGAA
AAGACATATACA
CCGATTAGTTCATGTATACAAGTTTATGAGAATTTCGAAAACAGGTTT
TCAATATCTGGT
GGTCCCAACTGGACTCTTACTCTCAGCGGAGTTGAGCCGAATGATGC
TCGCAAATACAGG
TGCAAGGTAAAGAGTAATGAAACAAAACCGCAACTTCAGAAAAGCA
TGACACTCATTATA
CCATCAACCATCAACATTACGTTGAGTGCAGCATCTTCTCAAATAACT
GGTAGGGGAAGT
GGTGTCTTTACATGCCATGCTAA
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Fig. 2. DNA Sequence of the "assimilated" IgKappa chain V-V region K2

4. CONCLUSION

At the beginning of the year 2013, we had found a first candidate IgK gene (Leclerc and Otten, 2013) which possessed an IgSF domain.

We supposed that this second cloned gene (**Fig. 2**), was implicated in the response to the antigen HRP as the first one (Leclerc and Otten, 2013).

In fact we don't know, at the moment, if there are, 2 candidate genes or more in response to HRP.

But, if we draw up a conclusion.

Currently, two IgKappa genes, in sea star genome have been cloned and our data contributes greatly to knowledge the molecular and genetic bases of non-self recognition by Invertebrates.

5. REFERENCES

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