

## True New Igkappa Genes in Echinodermata

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### ABSTRACT

An Igkappa transcriptome was discovered in the genome of the Ophiurid: *Ophiocomina nigra*. So, with the Asterid: *Asterias rubens*, and with the Crinoïd : *Antedon bifida* 3 classes of Echinodermata, out of 5, possess each, an Igkappa gene. It remains enigmatic, when we have a look on the 2 other classes without this gene : the Echinids and the Holothurids. Our work is of great novelty: it speaks of Adaptative immunity in Invertebrates.

**Keywords:** *Invertebrates ; Crinoïd ; Ophiurid ; Asterid ; Igkappa gene; Adaptative Immunity.*

**Abbreviations:** *HRP: horse-radish peroxydase*

### INTRODUCTION

Recently, investigations performed in our laboratory, have provided evidence, that the Ophiurid : *Ophiocomina nigra* (Echinodermata), presented antibody reactions (1) with cellular and humoral reactions to Horse-radish peroxydase antigen (HRP)

These reactions are similar to those observed in the sea star *Asterias rubens*, another Echinodermata (2) and we know that the *Asterias rubens* genome contains the sea star Igkappa gene with Ig sites(3), a Fab gene, a Fc receptor gene (4)

The aim of this work consists to explore Igkappa genes in the genome of the fifth classes of Echinodermata, mainly in Asterids(*Asterias rubens*) in Ophiurids (*Ophiocomina nigra*) and Crinoïds (*Antedon bifida*).

### MATERIALS AND METHODS

2) a *Ophiocomina nigra*, *Asterias rubens*, *Antedon bifida* were collected to the Biologic station of Roscoff (France). Digestive coeca were excised and treated with Uptizol (Interchim) to obtain m RNA *Ophiocomina nigra*, mRNA *Asterias rubens*, mRNA *Antedon bifida*.

2) b Preparation of library(RNA), by the use of the Kappa mRNA hyper Prep kit.

2) c Sequencing : the sequencing was done with a NextSeq 500 Illumina (2.75 bases)

Transcriptome was assembled from RNA-Seq fastq files using Trinity v2.1.1 (5) with default parameters. A BLAST database was created with the assembled transcripts using makeblastdb application from ncbi-blast+ (v2.2.31+). The sequences of transcripts of interest were then blasted against this database using blastn application from ncbi-blast+ (6)) with parameter word size 7.

### RESULTS

#### A) *Ophiocomina Nigra*

The sequencing in 5'-3' offers a candidate gene called Igkappa gene and the ophiurid transcriptome shows this gene by the mean of a blast against homo sapiens : this was very highly significant (E-value of 2,00E-12)

Undoubtly it brings the evidence of the existence of an Ophiurid-IGKappa gene, when presented in this brief table :

QueryID	QueryName	SubjectID	Identity	Length	Mismatch	Gapopen	E-value
BC030813.1	Igk	TRINITY_DN64572_c0_g1_i1	89.47	57	6	0	2,00E-12

Now, when we have a look on the transcriptome sequence, it may be observed Ig sites in translated amino-acids sequence :

## True New Igkappa Genes in Echinodermata

>BC030813.1 Homo sapiens immunoglobulin kappa locus, mRNA (cDNA clone MGC:22645 IMAGE:4700961), complete cds

```
5'GAGGAAGTCTCAGTTAGGACCCAGAC
GGAACCATGGAAGCCCCAGCGCAGCTTC
TCTTCTCTCTGCTAC
TCTGGCTCCCAGATAACCACTGGAGAAATA
GTGATGACGCAGTCTCCAGCCACCCTGT
CTGTGTCTCCAGG
GGAAAGAGCCACCCTCTCCTGCAGGGCC
AGTCAGAGTGTTACCAGCAACTTAGCCT
GGTACCAGCAGACA
CCTGGGCAGTCTCCCAGGCTCGTCATCTA
TGGTGCATCCAGCAGGGCCAGTGGTGTC
CCAGCCAGGTTCA
GTGGCAGTGGGTCTGGGACAGAGTTCAC
TCTACCATCAGCAGCCTGCAGTCTGAA
GATTTTGCAGTTTA
TTACTGTCAGCAGTATAATAAGTGGCCGC
ACACTTTTGGCCAGGGGACCAAGCTGGA
CATCAAACGAACT
GTGGCTGCACCATCTGTCTTCATCTTCCC
GCCATCTGATGAGCAGTTGAAATCTGGA
ACTGCCTCTGTTG
TGTGCCTGCTGAATAAATCTATCCCAGG
GAGGCCAAAGTACAGTGGAAAGGTGGATA
ACGCCCTCCAATC
GGGTAACCTCCAGGAGAGTGTACAGAG
CAGGACAGCAAGGACAGCACCTACAGCC
TCAGCAGCACCTG
ACGCTGAGCAAAGCAGACTACGAGAAAC
ACAAAGTCTACGCCTGCGAAGTCACCCA
TCAGGGCCTGAGCT
CGCCCGTACAAAGAGCTTCAACAGGGG
AGAGTGTTAGAGGGAGAAGTGCCCCAC
CTGCTCCTCAGTTC
CAGCCTGACCCCTCCCATCCTTTGGCCT
CTGACCCTTTTCCACAGGGGACCTACCC
CTATTGCGGTCC
TCCAGCTCATCTTTCACCTACCCCCCTC
CTCTCTTTGGCTTTAATTATGCTAATGTT
GGAGGAGAATG
AATAAATAAAGTGAATCTTTGCAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAA 3'
```

### B) *Asterias Rubens*

As precedently described, we can present, that time, the complete sequence of the sea star IGKappa gene (3) in 5'-3' :

```
GGA TCC GGA GGA ATG CGTG GCAA
CATGGCGTCTCTATGGATGTTCTTCTT
TGTCGTGGGGATAACTTTACAACGGAGT
TTGGCGATTTACACGTTTCGCG
```

```
AGCAACCGTCGGACACTAGCGCGTTGCA
GGGGAGCACAGTGGTGCTTCAC
TGCTCCGTTGAGCAGTACATAAACACCA
CGGCCATCGTTTTGGTGGAGCCG
TGACTCGGTTCATCAGCCACAACAAAGAC
CTGAAACTGTCCAGTCTAAACA
CCGACCAGCTCCAAAGGTACTCGATTTT
AGGCGACGCATCTCGGGGGGAA
TTCAACCTTAAAATAGTGAAC TTTACCG
CCACAGACGCCGCCAGTTACCG
CTGTCAGATG TAA GAA TTC
```

### C) *Antedon Bifida*

*Antedon bifida* IGKappa gene (7) was discovered more recently when compared to the 2 other Echinodermata.

The sequence of the transcriptome, in 5'-3' shows :

>TRINITY\_DN9178\_c0\_g1\_i2 (Igk):

```
5'AGCGAATGAAAAAGAAGAACCGGCCA
AAAAAAGTACTTTCTACCAAAGAAGCGAA
TGAAAA
AGAAGAACCGGCCAAAAAAGTACTTTCT
ACCAAAGAAGAACTGAAATAGAAGAA
CTAAC
CGAAACAAGTATTTCTACAAAATCAGTTT
CTGCCAGTGATATATTCTTGGTACAAC TTT
CACACTGGAGATGGGATTCTGCGTAGGA
CCTGAACACAAACCGTTTACAGGAGATT
TCGA
CGGTGACGGTAATGAAGATCTTCTGTTTC
ACAATTCAAAGACAGGCTCGAAAAAGAT
ATA
CTATGCAAGTTGTGACGGCTCTTTTAATG
GTGATAGGTTCGTGGAGAAGAGAGATGAA
TTT
TTGCTACGTAAGTGGATATGATCTATACAT
TGGTGATTTCAACGGCGATGGTCGATCCGA
TATGCTGTGTCATCGTCCTCAGTATGGTC
AGATTTGGGTTGTGTTGGCGCAACCTGG
GGG
TGTATTCACTGCTAACCCGTGGTCGTATA
GTCCCAATTGGTGCAAGGCCACCACTGAT
AA
AGTATATATTGGAGACTTCAACGCAGACG
GTCGGGATGATATTCTTTGCCACACACAA
AG
TTCGGGTTACATTGCAATATATTATGCATT
ATACACTGGTTATTTTTCTACCTCTACAAC
ATATCGCTTTACACGAAGTATGAGTTGGT
GCAGAGGTACATATCAAAGAGTGTATACT
GG
AGATTTCAAACGGAGACCGAAGGGTTGAT
```

## True New Igkappa Genes in Echinodermata

ATGCTCTGCCACGACTACTCATCTGGCTA  
CAT  
ATATGTAGCAGTAGCCACAGCGACTGGTG  
GATTCACCTCTGCCACATGGAGCAGAAGT  
AT  
GGGCTGGTGCAAGCATTTCGAACTCTAAG  
CTCAGCATTGGAGATTTCAATAAAGATAA  
CCG  
CGACGACATCATGTGCAGCGACACAAAT  
GGTCCTTACTGGATAGCATTCTCTCTGTA  
CAA  
CGGTTTCGTTTTTCATCTAAAAGCTGGACCC  
GTAAACAAAAGTGGTGTACATCTGGCAAT  
GA  
TGTGTTAGTTTCGGATGTGAATGGAGATG  
GTGGGGATGATTTGATGTGCCATAATGAA  
GC  
CGACGGCATCAAGTACATATCGATCAACC  
ATAAGGCCTAAAGCAAGTTCCTCTCAATA  
TT  
ACAGAAAATTCACCACAAATGATTCA  
TTTTGTACTGAACCTCAATTCAAATTCAA  
TT  
AAAATTTACATAAACGTTAACGGAAGGAT  
ACAATCAACTAAAATAATGTTTCATTATTAT  
TTTTTCGTCGATAACCTAAACAAAATCAG  
ATAAGAAATTATACAATAATATACTGTAAAC  
GTATTATACAAAATAATTAATGTATATTA  
AGCTACTGTACTTAGAAATGTACTTGTACG  
CTTATTAATATTAATAAGCCTAATGCCCGG  
GTTGATAATAATAAATACATTTTTGCAAG  
TTCAAAAAAAAAAAAAAAAAAAAAAAAAAAA  
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAC  
TCAAAA  
GTCCCAGGCCCCACCCGACCTACTGAA  
CCAGAAAG3'

## CONCLUSION DISCUSSION

Fortunally, we find again in this last Echinodermata (A.bifida), the IgKappa gene, the Fc receptor gene, we discovered in the Asterid: Asterias rubens and in the Ophurid: Ophiocomina nigra.

Fab gene was found also in A.bifida, but the e-value was not significant.

A considerable role may be assigned to crinoïd IgKappa gene and Fc receptor gene (7)in Crinoïd' Immunology.

For the third time, we find an invertebrate primitive antibody in an Echinodermata: it's a great novelty. Echinodermata and Adaptive Immunity become fundamental

In Invertebrate immunological studies and we can speak of 3 true IKGkappa genes, always in Invertebrates.

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