

Evidence of Irf4 Gene, Irf2, Irf8 Genes in an Invertebrate: The Sea Star *Asterias rubens*

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Abstract

IRF4 gene, IRF2 and IRF8 genes have been studied in sea star which is the only invertebrate, to possess these factors, playing a rôle in the immune adaptative immunity of *A. rubens*.

Keywords: IRF4 gene; Adaptative immune system; Immune cells

Introduction

IRF regulatory factors are ancient molecules conserved throughout the evolution of metazoans; they play a fundamental roles in innate and adaptative immune system [1]. To date 11 IRF family have been described in vertebrates and invertebrates [2].

Previous studies showed that in vertebrates, IRF4 was expressed in most types of immune cells, and had critical functions in B cell differentiation and immunoglobulin production [3].

Other studies on chicken found that IRF4 was mainly expressed in the bursa, bursal lymphocytes [4] and thymus [5]. Con A induced the expression of IRF4 in spleen cells [4].

As for IRF8 in mouse, it specifically bound to the expression of type I IFR and IFR-inducible MHC Class I genes. IRF2 had the same rôle and played a regulatory one in cells of the immune system. The aim of this work is the research of such factors in an invertebrate : the sea star *Asterias rubens*.

Materials and Methods

Sea stars were obtained from the Biology Institute (Gothenburgh University). Immunizations to HRP, genomic studies were already described [6]. 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 [7].

Results

First results (a) concern significative IRF4 gene, found in immunized sea stars to HRP, when compared to mouse genome :

a) One Contig (Contig8452|m.8105) could be annotated via BLASTX to *Mus musculus* "Interferon regulatory factor 4" (IRF4), with an e-value of 3.2e-12. On an aligned region of 132 amino acids, 62 positive and 42 identical amino acids were found.

```
5'GCCAGGTGTGCATGTACCTGAGCCAGCCGTATGGAAGA  
CGAGACTGCGTACTGCTCTCAATAAACTCCCTGACATCG  
ATGAGGTCCACGAGAAGACGCAACTAGACATCCCCTGAAC  
CCTATAGAGTCTACAGACTGCTGCCCAAAAAACCATCCA  
ACAGCGCAATGAAAAACGGCTTCGACCAAGCCCAACGT  
ATGCCAGCAGGACTCTACCACAGGAGTGGACATTCGA
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TGAGTAGTTACTCCGAGCGGTACAGTCCATATGACGTGC  
GACCTGGAACCTTATTACACTGATGCATACGACCGGCAAA  
ATCATTTCGAGTTACCAACAACCGAGTCATCATTGGTATG  
ATCCGAGCCGTCCCGGTGTTGATTATGGCTACCAGCAAG  
CATACACCAACTGTTACCCAAACAGCACAGCAGCCGACC  
TTGACATGATCAATGACGTCATCACACGTGACATCGATG  
GCAACCACGGCCACCATCCCCAAGCCACGCCACTACC  
CCCCAGGGAGCG3'
```

Second results (b) concern IRF2 gene in immunized and non-immunized sea stars to HRP, when compared to mouse genome.

b) One Contig (Contig13667) could be annotated via BLASTX to *Mus musculus* "Interferon regulatory factor 2" (IRF2_MOUSE), with an e-value of 1.3e-39. On an aligned region of 144 amino acids, 101 positive and 75 identical amino acids were found.

```
5'TATGTAATCCATCAGCCCCCTCCGTACGTAGAGGGGTG  
AATAAGCAGCGCCTCTGTTGCATGGATATGGGGGTGGAG  
GGCTTATAACTGATGCGGTTGGTGAGAGCGGGGATGGAA  
GGCTGGAAAAGATTGCTAACTCTTGACGAGGTTGGGGAAG  
AGTACGGAGGAAGCTCGTGTGCGATTGGGGAACCTCTGAT  
TGCTGAATGTAACACTCGAATGCCAAACATCTGTATTCA  
TCTGTGATTGGCTGACTTCCGTTATGCTCTTTCCAAAGCTC  
AGGTGAGAAATTGTTGAGAAGTCGGATGATGCGTGGACGT  
AATACTGTCTCGAGTCTGGAGAATTCTTGATTGCACTTTG  
AATAAACTGGTCTTTGGCGATCTTCTGATAGTCCTACTG
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```
GTCTTCTTGTGTATCGTACTGCCATCACCCACAGGTTTGC
TTTTTCTCGCCTTCTTCGGAATCAACTTGTAATCTTGTA
AGCATCGTTCCCTTTACTCTTCCCCTGATCCTTGACCTCA
AGAATGTCCGGAAGTGAATTGATTGCACACCGGAAGTTC
GCTTCCAGATCTTGGGATTCGCCTTCGGGCAATTGGAT
ACCGGGTCGTATTTTTTTGTATGGATGGCCCAAGCACGA
AATAGCGATGCATCTTTGTCACAAGTCCAGCCGTGTCTT
GCGGCATGTTTCCAGGGTATCTTAACCAACTGTTCCTTTT
CATCCAACCACTCCAAGCCGGGTATGGTTTTCTCAACGA
TGTTACGGATGAGCCATGGTCGCAGACGCATCCTCTCGA
CCGGGCGAACCGACG 3'
```

Third results (c) concern IRF8 gene in immunized and non-immunized sea stars to HRP, when compared to mouse genome.

c) One Contig (c35396_g1_i1) could be annotated via BLASTX to *Mus musculus* "Interferon regulatory factor 8" from the Swissprot database (IRF8_MOUSE), with an e-value of 1e-12. On an aligned region of 53 amino acids, 42 positive and 33 identical amino acids were found

```
5'GGTAAATTCAAGCCAGGTGTGCATGTACCTGAGCCAGC
CGTATGGAAGACGAGACTGCGTACTGCTCTCAATAAACT
CCCTGACATCGATGAGGTCCACGAGAAGACGCAACTAGA
CATCCCTGAACCCTATAGAGTCTACAGACTGCTGCCCAA
AAAACCATCCAACAGCGCAATGAAAAACCGGCTTCCGAC
CAGCCCAACGT 3'
```

Discussion and Conclusion

Echinoderms occupy a critical and largely unexplored phylogenetic situation from the point of view of the evolution and of studies of the sea star immune system. Scepticism occurred as for the existence of the primitive sea star antibody [8]. To day it seems obvious that the discovery of IRF4 in immunized sea stars, of IRF2 and IRF8 in both immunized and non-immunized sea stars to HRP corroborate the presence of such an invertebrate primitive antibody in *Asterias rubens*: an Igkappa primitive antibody. In conclusion, these IRF genes control the regulation of this last one.

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