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Complement System in *Asterias rubens* Genome: Comparisons with Rainbow Trout Complement. Notions of Innate and Adaptative Immunity

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ABSTRACT

Seven complement components have been discovered in 2013 in *Asterias rubens* genome when compared to mouse one. Another component (C6) which is present in mouse was found: in sea star, when, also, compared to rainbow trout genome: "*Oncorhynchus mykiss*". Innate and Adaptative Immunity in Sea Star Immune system are evoked, in the present paper, through the sea star IGKappa gene and IPA (Invertebrate Primitive Antibody)

INTODUCTION

We have recently described the "Sea star complement Evidence" [1]. We remarked that C6 and C7 components were missing in sea star transcriptome when compared to mouse one.

An extensive study allowed us to research these components in less evolved animals (phylogenetically speaking) than mouse. Genomic features of the rainbow trout: *Oncorhynchus mykiss* have helped us, in this study.

At this point, we were attempting to determine how many similar complement components might be present in *Asterias rubens* (Invertebrate) and in *Oncorhynchus mykiss* (Vertebrate).

On the other hand, we recall it was considered that just Innate Immunity occured in this Invertebrate.

We confirm, in the present report, Adaptative immunity exists also in it.

MATERIALS AND METHODS

Sea stars *Asterias rubens* were used.

Immunizations to HRP (Horse-radish Peroxydase) and genomic studies were already described [1]. RNA sea star was obtained by using Trizol (Invitrogene) then cDNA was obtained.

After ligation of adapters for Illumina's GSII sequencing system, the cDNA was sequenced on the Illumina GSII platform sequencing.

1100 bp from one side of the approximately 200 bp fragments. Sequences

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were assembled using Velvet (Zerbino and Birney (2008) [2].

C9 from C1 complement genes and Sea star IGKappa gene were studied.

RESULTS

We recall that three complement components: C1r, C4, C1 inhibitor of the classical activation pathway have been fully sequenced in rainbow trout [3] and the well-known C6 was discovered in trout in 2006 [4].

Sea star C1q subunits A, B, C, were sequenced in *A. rubens* [1].

C2, C4B, and C3 which is central in mammals to both the classical and alternative pathways, C9, C5, C8 were also sequenced in *Asterias rubens* [1].

As for C6, it was shown as following, when compared to *Oncorhynchus mykiss* genome:

One contig (Contig11285|m.9708) could be annotated via BLASTX to *Oncorhynchus mykiss* "Complement component C6" from the Trembl database, with an e-value of 3.75e-13. On an aligned region of 113 amino acids, 37 positive and 56 identical amino acids were found.

5'GACAAATTCGACACTTACAAAAAGCATCTCAACCCGAGTAGGAAGGAATCTCTTTTAGTT
GCAGTAAATTTTGAATTTGTATAATTCAGTATTTTGTGCTCCCTTTGGTATCAGTTTAGA
TCCACACAACCTGTGAAAAACTTCAGTACTTACTAGATTTCGCCAACGCAACGGTAAACG
AGTCATTTGATTTTGACCATCATCAACTGAAGCAACGCACGTAATACACACAACAAACGG
AACATTTTGTGTGTAGTTTCCAGCGATTCGAGAAGCAAATCAAAGACAAGATGTCTTTAC
CCAGTGATGTTGAAACAGACTCCGTCATGGATAGTCCAGCAGAGATTCATATGAACATGA
ATAAGCTACAATCTAAACTTCCCAGCGTTACTCAAGACGAGAGATTTGACTCCGGAATTG
ACTCGTTACGTTCTGTTGATTCGGCGTACTGCTTGAGCTTCGAAAGGGAATCGAGCCTGG
CTTCGATAAATGAGAAGACGTCTCTCACATCACACCTGCAACAGCTCCATCTTTCACATG
AAACAAGAACAGAAACCGAGAAGACTGAAACGACAGTAGAAGACATCGATGAAGCTTATC
ATGATGAGTGTACTATGTCTGAAACACTCGACAATTTGGAAGAAACTGCAAGAATTGTGG
AATATCCTGAACAAAGATGCACGGGACGTCTTACAGATGATGCCTTCGACCAAGACCAAG
AGGGAGATACGCCCCTTCATCTTGCTATTATTCATAAGGAAGTGGACTTCGCAGAAAAAT
TCATCATCTTTGTTGCAGATCCTGAGTTACTGAACATCAGCAATGATCTTATGCAGACTC
CTTTACACCTTAGCGTATTAACAAGGCAACAAGATATCTGTCGTGTTCTCGTCTTGGGCA
ATGCCCAAATCGACTGCACCGACCGAAACGGCGACACTCCTCTTCATATTGCATGCAGAC
TGAGAGATGAGGGCTGTATCAGAGCTCTGACTGAAGGAATATCTCCACTCGAGCGTAAGA
GAGGGATGGTTCCACAGAATAGAGCAAGTGGGGTACAACAGCTTCCACAGAATCTTGAAC
TCAGAAACTTTGAAGGCTACACATGCATCCATATTGCAGGATTCGCTTGTAGCGTCGATC
AGTTGGAGTACCTTGTGCAGCTAGGCGGCGACATAAATGCCCCGGATGGAAAGAGCGGAA
GGACCATTCTCCACTACGCTGTAGAGGCGGGTGACTTTTCTCTTTGTCAGTACCTCATTG
CGAACTTGGGTGCCAATGTTAATGCGTTGACCTTTGACCAGTGCACACCC3'

C7 was not found in sea star genome.

DISCUSSION AND CONCLUSION

Asterias rubens, although considered to be more primitive than lower vertebrates (as trout) seems to have evolved much more sophisticated immune innate defense mechanisms. We find much more complement components in the sea star than in trout: 8 out of 9, when compared to mouse genome

Phylogenetically (From a point of view) the sea star could be situated in "an evolutive cul de sac".

It might evolved more quickly than rainbow trout, in term of innate immunity.

As for adaptative immunity, rainbow trout is more evolved [5] than *Asterias rubens* which presents an "invertebrate primitive antibody" in response to antigenic injury [6]. The "invertebrate primitive antibody" is correlated to the sea star IGKappa gene.

The sea star Igkappa gene is clearly the oldest IgKappa gene of the immune system of animals.

It shows already two Ig sites! The forms of Igkappa genes are all found in vertebrates, they share many details with the sea star, including the presence of Ig sites.

The preservation of the Igkappa gene in immunized and non-immunized sea stars is an excellent opportunity for further experiments. It is important to notice that the Igkappa chain V-III region HAH of *Tupaia chinensis* is situated (in the assumptions behind the theory of evolution) between the Igkappa chain precursor V-II region (RPMI/133) and Igkappa chain precursor V-IV region/121.

The preservation of the IgKappa gene for so extended a period of evolution in organisms as distinctively different as sea star, fish, rodent, mammal, indicates that it plays an essential role in the survival of the organisms, role in the regulation of the immune response.

Additionally, the existence of members of the IgKappa gene family with conserved functional characters, indicate that the sea star IgKappa gene has evolved prior to the evolutionary divergence between Invertebrate and Vertebrates: It must be claimed.

The main point to conclude is the following: the sea star *Asterias rubens* has evolved the ability to develop innate and adaptative immunity with its IPA (Invertebrate primitive antibody) in which Fab gene Fc receptor gene MHC genes were found [7-11] like in two other Echinodermata [12,13].

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