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Organizers: **A Giglio[1], P Brandmayr[1], F Talarico[1], A Mazzei[1], S Marsico[2], A Naccarato[3], F Cavaliere[1], ML Vommaro[1], MC Granieri[1]**

[1]*Department of Biology, Ecology and Life Sciences, University of Calabria, Rende, Italy*
[2]*Department of Pharmacy, Heath and Nutritional Sciences*
[3]*CNR-Institute of Atmospheric Pollution Research, Rende*

Characterization of the complement system in a colonial protochordate: C3 complement receptors and opsonic role of C3

A Peronato, N Franchi, L Schiavon, L Ballarin
Department of Biology, University of Padua, Padua, Italy

The complement system is one of the most ancient immune modulator mechanism of bilaterian metazoans, able to influence ancient cells and factors of both innate and adaptive immunity. Three complement-activation pathways are known in vertebrates: the classical, the alternative and the lectin pathways: all of them converge on the cleavage of C3.

The compound ascidian *Botryllus schlosseri* is a reliable model organism for the study of immunobiology. As an invertebrate, *B. schlosseri* relies only on innate immunity for its defense and immunocytes. Recently, in the same species, we demonstrated the presence of homologues of mammalian C3, Bf, MBL and MASP1, referred to as BsC3, BsBf, BsMBL and BsMASP, respectively. All the complement components identified so far, are expressed by morula cells, the most abundant circulating hemocytes.

In mammals, once the complement system is activated, a cascade of reactions that involves proteolysis and polymerization occurs resulting in the cleavage of the third complement component (C3) to C3a and C3b, the former exerting a chemokine–like activity, the latter acting as opsonin and, ultimately, activating the lytic pathway. The best-known receptor for C3a in mammals is C3aR, whereas CR1 is the receptor able to recognize and bind C3b on the microbial surfaces.

In the present work, we described, in *B. schlosseri*, two new genes showing homology with vertebrate C3aR and CR1, respectively, and studied their transcription in the course of the colonial blastogenetic cycle. Results indicate that their mRNAs are located in different immunocyte types suggesting the presence of an important cross-talk between phagocytes and morula cells. In addition, we continued our analysis of the role of C3 in *Botryllus* immunity by studying the modulation of BsC3 transcription during the colonial blastogenetic cycle and the effect of *bsc3* knockdown on immune responses.

Only morula cells, and no other immunocytes type, were labelled by the antisense probe for BsC3aR, whereas phagocytes and young, undifferentiated cells known as hemoblasts were the cells stained by the probe for BsCR1.

Both the *bsc3ar* and *bscr1* genes are constitutively transcribed as almost all morula cells and phagocytes, respectively, resulted labelled by the antisense probe in the ISH assay, independently of their previous challenge with zymosan, a known activator of *B. schlosseri* hemocytes. However, a modulation in the extent of transcription occurs during the colonial blastogenetic cycle as the amount of BsC3aR mRNA abruptly decreased at TO, whereas no differences were observed when EC and MC were compared. This is probably related to the renewing of circulating cells at TO, when 20-30% of hemocytes undergo cell death by apoptosis and are replaced by new, differentiating cells entering the circulation in the same period.