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Elvira De Matthaeis, Andrea Di Giulio, Marzio Zapparoli

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ANNA PERONATO, NICOLA FRANCHI, LAURA DRAGO, LORIANO BALLARIN

Dipartimento di Biologia, Università di Padova

CHARACTERISATION OF COMPLEMENT SYSTEM OF A COLONIAL PROTOCHORDATE: STUDY OF THE EXPRESSION OF C3, CR1, C3AR AND THEIR ROLE OF C3 IN NONSELF RECOGNITION.

The complement system is one of the most ancient immune modulation mechanism of bilaterian metazoans. 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. It relies only on innate immunity for its defense and immunocytes. Recently, in the same species, we demonstrated of the lectin and alternative pathways. 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, C3 is cleaved 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 phagocyte surfaces. In the present work, we describe, in *B. schlosseri*, one genes showing similarity with vertebrate C3aR and three genes with similarity to CR1 (two soluble forms and one transmembrane), and studied their transcription in the course of the colonial blastogenetic cycle. Results indicate that their mRNAs are located in different immunocytes suggesting the presence of a 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 and the soluble CR1s, whereas phagocytes and young, undifferentiated cells known as hemoblasts were the cells stained by the probe for the membrane-linked BsCR1. Both the *bsc3ar* and *bscr1* genes are constitutively transcribed; however, a modulation of transcription occurs during the colonial blastogenetic cycle as the amount of BsC3aR mRNA abruptly decreased at take-over, whereas no differences were observed when early-cycle and mid-cycle 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.