Immune repertoire in the transcriptome of *Littorina littorea* reveals new trends in lophotrochozoan proto-complement evolution

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Immune repertoire in the transcriptome of *Littorina littorea* reveals new trends in lophotrochozoan proto-complement evolution.

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Summary

The evolution of complement system in invertebrates is poorly investigated. While the repertoire of complement genes in several Ecdysozoa lineages is found substantially different from that of Deuterostomia, the composition and function of the complement in the second protostome lineage, Lophotrochozoa, remains unclear. Here we report the general description of new transcriptomic data on the common periwinkle, *Littorina littorea*, and trace the evolutionary trajectories of the ancestral proto-complement repertoire. The repertoire is defined as immune cascade providing the minimum set of C3-associated molecules required for C3b amplification, opsonization of the targets and their phagocytosis: thioester protein (TEP) C3, serine protease C2/factor B (Bf) and complement receptors (CR). The reference transcriptome of *L. littorea* was built from the dual-species RNA-seq experiment with the periwinkle and its tissue digenean parasite *Himasthla elongata*. Five TEPs, including the ortholog of the C3, are found expressed in the in the mollusk's inflamed tissues. The homolog of the complement receptors CR1/CR2 is also expressed, however the ortholog of Bf is not. The extensive phylogenetic analysis showed that the C3 ortholog and the complement receptors are retained in all key lophotrochozoan taxa: Mollusca, Annelida and Brachiopoda. However, the Bf ortholog was lost at least three times independently in different lineages: i) Cephalopoda, ii) a common ancestor of all Gastropoda and iii) one of the Annelida lineage, Clitellata. Both C3 and Bf molecules were retained in bivalve species, brachiopods and annelid worms from the Polychaeta lineage. Hypothetically, the function of the lost Bf in these animals can be compensated by Factor L (Lf) – the serine protease first found in *L. littorea* and homologous to both, the Bf and the arthropod factor C (Cf). The contrast differences in proto-complement repertoire between the sister mollusk' taxa, Bivalvia and Gastropoda (the conserved and modified sets, respectively), can underlie differences in their susceptibility to digenean infection.

Keywords: innate immunity evolution, transcriptomics, host-parasite immune conflict, Mollusca, Trematoda.

Abbreviations: SSW – sterile seawater, C3 –complement component C3, *Ll*C3 – *L. littorea* ortholog of complement component C3, Bf – complement factor B and component C2, Cf – *Limulus* clotting factor C, Lf – *Littorina littorea* serine protease factor L, MASP – mannan-binding protein-associated serine protease, MAC – membrane attack complex, TEP – thioester protein family, α2M – alpha-2-macroglobulin (TEP), *Ll*α2M – *L. littorea* ortholog of α2M, CD109 – CD109 antigen (TEP), *Ll*CD109 – *L. littorea* ortholog of CD109, CR – complement receptor, *Ll*CR – *L. littorea* homolog of CR1/CR2 genes.