Haematopoietic and proteomic responses to wounding stress in the common sea star *Asterias rubens*

Akademisk avhandling

för filosofie doktorsexamen i zoofysiologi som enligt naturvetenskapliga fakultetens beslut kommer att försvaras offentligt fredagen den 17 oktober 2008, kl.10.00 i föreläsningssalen, Sven Lovén centrum för marina vetenskaper, Kristineberg, Fiskebäckskil

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Dissertation Abstract

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Many species of echinoderms have a remarkable ability to regenerate lost tissues, including the sea star *A. rubens*. The initial step in regeneration is wound healing, necessary to prevent disruption of body fluid balance, and to limit the invasion of pathogens. Injury initiates an immune response, where the circulating cells are activated. In general, invertebrates have a well developed innate immune system that is mediated by circulating blood cells. In the sea star these kinds of cells, the coelomocytes, respond with a rapid and massive accumulation at the wound site.

The aim of this thesis was to identify and localize the haematopoietic tissues, the source of stem cells for renewal of the coelomocytes, as well as to increase knowledge of response to wounding, with focus on the coelomocytes and their protein expression, in the common sea star *A. rubens*. Synergistic effects of hypoxia were also investigated.

It could be concluded that cells in the coelomic epithelium respond with proliferation when triggered with mitogenic factors and show a protein expression pattern very similar to the pattern of circulating coelomocytes. The shape and behaviour of cells migrating out of the coelomic epithelium show high similarities with the behaviour of coelomocytes, in terms of phagocytosis and network formation. Tiedemann body and axial organ are also proposed as haematopoietic tissues, since a significant increase in proliferation was seen also in these tissues after triggering with mitogens as well as a pattern of protein expression similar to coelomocytes.

Total coelomocyte count (TCC), as well as the expression of the heat shock protein (HSP) 70, is known to increase with severe stress in sea stars. The stresses investigated here, wounding together with hypoxia, induced a significant increase in TCC even after 1 hour while 6 hours after wounding TCC had increased approximately two-fold. Western blot analysis revealed highly elevated coelomocyte cytoplasmic HSP70-expression 3 hours after wounding. Non-wounded sea stars exposed to hypoxia and wounded animals kept in normoxia, showed enhanced HSP70 expression only after 24 hours. This synergistic stress response of wounding together with hypoxia may suggest ecological consequences, since the hypoxic areas in the ocean are growing.

Protein fractions separated by size from the coelomic fluid of wounded *A. rubens*, had slightly different effects on coelomocytes/haemocytes from three groups of invertebrates, the mollusc *Mytilus edulis*, the tunicate *Ciona intestinalis* and the echinoderm, *A. rubens* itself, where also effects on explants of coelomic epithelium were examined. The fraction containing proteins of the size 15-70 kDa showed a significant cytotoxic effect on the sea star coelomocytes and tissue samples, but seemed not to be cytotoxic for mussel or tunicate cells. This fraction, with smaller proteins, may contain cytokine-like molecules such as interleukin (IL)-1 and IL-6, but also the invertebrate form of lysozyme, molecules previously described in the sea star.

The two-dimensional gel electrophoresis method was developed for analyzing the protein content of coelomocytes. The methodology was optimized in terms of sample preparation, pI interval, gel gradient and staining procedure. The analysis of protein spots using MALDI-TOF/TOF mass spectrometry resulted in 9 identified protein homologues out of 18, of which 6 were found significantly up- or down-regulated. Sample preparation and methodological choices can and should be developed depending on the purpose of a study. The protocol developed here will be useful in future proteomic studies, maybe also for other marine organisms. The databases searched included the sea urchin *Strongylocentrotus purpuratus*, but the search result show no closer relationship between *S. purpuratus* and *A. rubens*, than to other invertebrates or even vertebrates within the database. Future proteomic studies of *A. rubens* may give valuable information about the wound healing and regeneration processes in sea stars as well as in other animals including humans.

Keywords: echinoderm, sea star, *Asterias rubens*, coelomocyte, TCC, haematopoietic tissue, HSP70, 2-DE, mass spectrometry