

REPORT OF MEETING

XIVth scientific meeting of the Italian Association of Developmental and Comparative Immunobiology (IADC), 14 - 16 February 2013, Department of Biological Chemical Pharmaceutical Science and Technology, University of Palermo, Palermo, Italy

Organizers: **N Parrinello, V Arizza, M Cammarata, MG Parisi, M Vazzana, A Vizzini**

Department of Biological Chemical Pharmaceutical Science and Technology, University of Palermo, Palermo, Italy

Plenary lecture

From signal transduction to gene expression in innate immunity: learning from the mussel model

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Antimicrobial peptides (AMP) are certainly one of the major effectors of the anti-infectious innate immunity. They are present in all the living creatures, including bacteria and plants. They are of multiple amino acid sequences and characteristics. In addition, several AMP families are simultaneously present in the same animal, not only in immune circulating cells, but also in various epithelia. Complete molecular cascades of signal transduction from pathogen recognition to AMP activity are known in several vertebrates, but only in *Drosophila* (Ecdysozoa), although still in debate. The present lecture will present the few we know on the mussel *Mytilus* (Lophotrochozoa).

First, biochemical analysis revealed the existence of particular AMPs, grouped in several families, including several isoforms, with different antibacterial capabilities. Synthetic fragments of peptides have been developed for applied biotechnology against bacteria and virus infections.

Second, were molecular biology approaches, which extended the diversity of AMP mRNAs, including within the same mussel, suggesting a complex system of molecular effectors. At that time, we realized how naïve was the idea of a primitive simple innate immune defense.

Third, expression regulations and specificities have been investigated following challenges with bacteria, fungi, and physical stresses. No strict correlation has been yet established between structure and function of AMPs in mussel.

Last, focus was on recognition-signal transduction steps by analogy with *Drosophila* in which the Toll-NF- κ B pathway controlled the synthesis of AMPs. Blast of sequences was less successful than looking for conserved domains of partners within EST databases. Thus, Illumina read

assemblies have completed identified sequences and revealed new ones. Regulation of gene expression following challenges gave some light on the existence of the cascade in Lophotrochozoa.

In conclusion, mussels possess some of the NF- κ B pathway intermediates. But most of them remain to be identified and the functionality of such complex system in the course of real infection is still pending.

Present lecture resulted from data obtained in collaboration with colleagues and students from the Italian Universities of Padua, Trieste, Palermo, and the CSIC-Vigo from Spain.

Session 1. Chairmen: N Parrinello, University of Palermo, Palermo, Italy; E Ottaviani, University of Modena and Reggio Emilia, Modena, Italy; L Abelli, University of Ferrara, Ferrara, Italy; L Ballarin, University of Padua, Padua, Italy
Immunity evolution. Responses and mechanisms

Evolution of the intracellular transport mechanisms in eukaryotes: ciliates and mammals use the same translocation and nuclear localization signals

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In the ciliate *E. raikovi*, self/non-self recognition phenomena are controlled by cell type-specific, water-borne signal proteins (pheromones) by their binding to target cell-surface receptors. The downstream signal transduction pathway activated by the pheromone-receptor interactions of self type (that promote the vegetative, mitogenic cell growth) involves the phosphorylation of a nuclear protein kinase, designated Er-MAPK1, which is structurally similar to the "intestinal-cell kinase" and "male germ cell-associated kinase" described in mammals. To identify the sequence segments responsible for Er-MAPK1 nuclear localization, mouse fibroblasts were

In the 3'-UTR region of Ci-GPxb cDNA we have found a typical SECIS-element, confirming that this protein may be a selenium GPx. Phylogenetic reconstruction, performed with Bayesian methods using metazoan GPxs, indicate that Ci-GPxb emerges in the tree with the tetrameric GPxs, confirming as previously hypothesized. Preliminary data, obtained by homology modeling, confirmed the tetrameric structure and show that the GPxb is similar to GPx3 from *Homo sapiens*, a selenium protein. Thus, we propose to name this gene *ci-gpx3*. As expected, Ci-GPxb clustered with other GPxb7s. The transcription of both these genes, measured by RT-sqPCR, resulted inducible by Cd, Cu and Zn, which have different effects. The peroxidase activity decreases in the cell-free extract from specimens treated with each considered metals, probably in relation to metal-induction of GSH biosynthesis, as indicated by the presence of positive correlation by the time-dependent Cd accumulation an increase of ROS and GSH production. The data presented here improved our knowledge about the evolution of the antioxidant system in metazoans and emphasize the importance of a complex regulation for the antioxidant system, including the transcription of *ci-gpx7* and *ci-gpx3* genes, which can create an efficient detoxification pathway allowing *C. intestinalis* to survive in the presence of metals in the environment.

Session 2. Chairman: G Scapigliati, University of Tuscia, Viterbo, Italy
Environmental stress responses

Amyloidogenesis as stress response: winner or loser process?

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Contacts with foreign molecules from bacteria (LPS), fungi (PMA), parasites, and chemicals can provoke serious stress stimuli able in turn to induce protective responses. The defense answers, due to non-self recognition, belong to acquired immunity, or to innate immunity. In the last case any stress event sets off a mix of responses from immune and neuroimmune systems. Animals generally show fundamental biological principles revealing conserved regulation of the involved processes. This is also true for cellular stress responses, a complex and dynamic process of restoring cellular homeostasis characterized by the same specific stages. We have previously shown that cellular stress conditions promote in different animal models (invertebrates and vertebrates) the same massive morphological and physiological modifications (Fowler *et al.*, 2006; Grimaldi *et al.*, 2012). Any kind of insult mimicking a stress condition (sundry chemical, immune, neuroendocrine and inflammatory) provokes, always and in any type of

cell/tissue, detectable series of events that start with ROS over expression, ACTH axis activation, and cytokine such as IL-18 production. These general overexpressions sustain a massive amyloid fibril synthesis that provides a resistant scaffold in turn driving melanin deposition. (Falabella *et al.*, 2011; Grimaldi *et al.*, 2012). Now we show that the amyloid production is not only linked to precursor melanin activation and synthesis but it is fundamentally important to guaranty a state of cellular redox equilibrium due to regulation of ROS presence, *i.e.*, amyloid fibrils production could be considered as a basic cellular compensatory response endeavouring to attenuate oxidative stress in different cell types. Moreover, the relationship between amyloidogenesis and stressors allows to surmise a new background of information on the effects of stress.

Molecular and physiological characterization of *in vivo* Sulfamethoxazole response in *Procambarus clarkii*

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Sulfamethoxazole (SMZ) is one of the most widely sulfonamides employed to treat human urinary infections, in veterinary practice and aquaculture. SMZ, acting as broad spectrum antibiotic microbial drug, blocks the folic acid metabolism. Because of SMZ widespread use considerable amount is indeed expected to be introduced into the environment. The SMZ derived cytotoxicity is mediated by an arylamine bioactivation to the arylhydroxylamine metabolites (S-NOH) of SMZ and it is associated to the generation of reactive oxygen species (ROS). There has been very limited information relating to the toxic potential of SMZ at cellular and molecular level particularly in aquatic or non-target organisms. In the present study, the red swamp crayfish (*Procambarus clarkii*), because of its tolerance to extreme environmental conditions and resistance to diseases, was used as a model organism to profile the molecular and physiological response to SMZ.

Haemato-immunological parameters such as glucose serum levels and total haemocyte count (THC) were altered as compared to controls; moreover a significative increase in Hsp70 serum level was detected for the first time.

Variation at transcriptional levels of proinflammatory genes (*COX 1* and *COX 2*), antioxidant enzymes (*GST* and *MnSOD*), stress response and Fenton reaction inhibitor genes (*HSP70*, *MT* and *FT*) were evaluated and alteration in the canonical gene expression pattern emerged. Considering the above exposed results, specific mechanisms involved in maintaining physiological homeostasis and adaptation in response to perturbation