

SD6

Developmental abnormalities induced by Gadolinium causes a time-dependent miss-expression of regulative and structural genes in *P. lividus* sea urchin embryos

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Gadolinium (Gd) is a metal of the lanthanide series of the elements whose chelates are commonly used as contrast agents for magnetic resonance imaging. Its release into the aquatic milieu has posed serious concerns regarding its noxious effects, and therefore Gd is now considered an emerging environmental pollutant. The sea urchin embryo is an excellent model used in both toxicological and developmental research. We analysed the consequences of embryo exposure to sublethal concentrations of Gd on embryo development, focusing on skeletogenesis and developmental symmetry. We observed a strong inhibition of skeleton growth, frequently displayed by an asymmetrical pattern. Continuous exposure to Gd of sea urchin embryos caused autophagy, but not apoptosis. Results showed an increase of the LC3 protein at 24 and 48h, confirmed by the increased number of autophagosomes and autophagolysosomes observed by confocal microscopy. RT-PCR gene expression analysis showed the misregulation of several genes acting at different functional and hierarchical levels of both the skeletogenic and the left-right axis specification networks. These included: transcription factors (Alx-1, Nodal), signaling molecules (univin, VEGF, VEGF-R, FGF) and skeletal matrix proteins (p16, p19 and msp130). Embryos were exposed to the same Gd concentration and harvested at 6, 24 and 48 hrs post fertilization (hpf). After 24 hpf, Alx-1 and Nodal showed respectively 40% and 60% reduction of their relative transcriptional levels, while only Alx-1 was reduced by 60% at 48 hpf. A 50% reduction of univin, msp130 and p16 was found at 48 hpf, while FGF was reduced by 60%. Taken together, the results pose serious questions on the hazard of Gd in the marine environment and indicate that Gd is able to affect three different levels of the stress response in sea urchin embryos: morphogenesis, survival strategies such as autophagy, and gene expression.

SD7

Identification of a putative GAGA factor in *P. lividus* embryos

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Drosophila GAGA factor, (GAF), encoded by the gene *Trithorax-like* (*Trf*), initially identified as a regulator of developmental genes (Hox gene), has been subsequently shown to be involved in different processes: chromatin remodelling, "Polycomb responsive element" function and insulator/boundary functions (1,2). Multifactorial gene regulation by GAF has been attributed to its ability to recognize and specifically bind to GAGAG consensus DNA motif by its C2H2 zinc finger domain and to interact with other regulatory factors by its BTB/POZ domain. The roles it plays seem to be conserved along evolution but its vertebrate orthologue, c-KROX, has been only recently identified and characterized (3). We have now evidence of the presence of a putative GAGA factor in sea urchin and we are investigating its role in the regulation of the expression of the *P. lividus* E- histone genes cluster. GAGA sites have been shown to be necessary for the correct temporal expression, during embryogenesis, of early histone genes and for the function of the *sns5* chromatin insulator present in the cluster (4). We have identified, by in silico analysis, a factor which shares with *Drosophila* and vertebrate GAGA factor the presence of both N-terminal BTP/POZ and C2H2 Zinc finger domains. By RT-PCR we have isolated a 2.5kb cDNA corresponding to the entire coding region. One step RT-PCR, performed with RNA from developing embryos, has revealed that this factor is always expressed until larval stage. An antibody has been raised against the putative GAGA factor and its specificity

assessed by western blot. Finally, we have performed ChIP experiments whose results strongly support the hypothesis that this new factor can bind to the *sns5* insulator.

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- [2] Matharu NK et al. 2010 JMB 400 (3): 434-447
- [3] Fuda NJ et al. 2015. PLoS Genet 11(3): 100-108
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SD8

The nucleic acid-binding protein *PcCNBP* is transcriptionally regulated during the immune response in red swamp crayfish *Procambarus clarkii*

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Cellular nucleic acid binding proteins (CNBPs) represent a highly conserved protein family among vertebrates; they harbour seven tandem zinc finger repeats CCHC type and have been described as transcriptional and translational regulator. To date, there is little characterisation of CNBP in invertebrates since its structure and function have been analyzed solely in *Drosophila melanogaster*. However no CNBP has been investigated in other arthropod systems. In an effort to isolate immune-related genes in *Procambarus clarkii*, a partial mRNA coding a zinc finger containing protein was found to be up-regulated during the response to white spot syndrome virus (WSSV) infection. The red swamp crayfish *P. clarkii*, represents an attractive animal model because of its tolerance to extreme environmental conditions and resistance to diseases. Thus it has become an important crustacean model organism for virological studies. In this study, a CNBP homolog from the red swamp crayfish *Procambarus clarkii* was characterised. The full-length cDNA of *PcCNBP* was of 1257 bp with a 5'-untranslated region (UTR) of 63 bp and a 3'-UTR of 331 bp with a poly (A) tail, and an open reading frame (ORF) of 864 bp encoding a polypeptide of 287 amino acids with the predicted molecular weight of about 33 kDa. The predicted protein possesses 7 tandem repeats of 14 amino acids containing the CCHC zinc finger consensus sequence, two RGG-rich single-stranded RNA-binding domain and a Nuclear localization signal, strongly suggesting that *PcCNBP* was a homolog of vertebrate CNBP. Analyses of transcriptional expression profile showed that *PcCNBP* was constitutively expressed among different tissues from of the adult crayfish, under normal physiological conditions. Moreover, qRT-PCR assays indicate that the transcriptional expression of *PcCNBP* responds to bacterial and viral stimulations.

SD9

The analysis of the HSA20/21 Syntenic Association in Cercopithecini allows a Discussion on Neocentromeres Scattering in Primate Genomes

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In spite of many expectations there is not any room for the concept that mammalian genomes have a default chromosome rearrangement rate, and that sister taxa have an intelligible and predictable chromosome organization. A part from the initial distress, these evidences resulted very stimulating for researchers. *In situ* hybridization studies conveyed to a series of proposed "Ancestral Karyotypes", and to the consequent discussion about, for example, the "conservativeness" of Carnivora, the rapid chromosomal evolution in Perissodactyla or in Rodentia and Primates (Supraprimates/Euarchontoglires). Chromosome painting and BACs FISH identified a series of apomorphic syntenic association in primates. Cercopithecini Tribe (Anthropoidea, Cercopithecoidea) is characterized by an apomorphic HSA20/21 syntenic association. This association demonstrates a high rate of polymorphism. We analyzed several species in the wide distributed African tribe of tree-dwellers, often identifying the 20/21 association as an heteromorphic pair in the