A newly identified Galectin from *sea urchin embryos*

Karakostis K. [a,b], Costa C. [a], Zito F. [a], Schröder H.C. [b], Müller W.E.G. [b] , Matranga V. [a]

[a] Consiglio Nazionale delle Ricerche Istituto di Biomedicina e Immunologia Molecolare “Alberto Monroy”, Palermo, Italy

[b] Institut für Physiologische Chemie Angewandte Molekularbiologie Universitätsmedizin der Johannes Gutenberg-Universität, Mainz, Germany

This work was supported by the BIOMINTEC Project (European Commission PITN-GA -2008-215507 grant).

Introduction

Galectins are carbohydrate binding proteins that specifically bind beta-galactoside derivatives (J. Hirabayashi et al, 2002; K. Pfeifer et al, 1993; Liu et al, 2011). The members of the galectin super-family, interacting with cell-surface glyco-conjugates, regulate diverse cellular events including signaling pathways, apoptosis, innate immune and inflammatory responses (Liu and Rabinovich 2005; Liu, 2005). Some galectins involved in biomineralization are found in mammalian osteoblasts and osteocytes (Tanikawa et al. 2010). Recently, a Galectin sequence has been identified in mineralized parts of adult sea urchin (*Strongylocentrotous purpuratus*) (K. Mann et al, 2010). This study was undertaken to isolate and characterize galectin from the *Paracentrotus lividus* sea urchin embryo.

Results

A. Identification of the coding sequence

By RT-PCR and 3’ RACE we amplified a putative galectin family member. The 1309 nt clone includes a 933 nt coding sequence, encoding a 34.7 kDa protein (p*I* = 9.4), containing two tandem carbohydrate-recognition domains (Fig1). The sequence homology, obtained by Blast analysis, suggested *Pl-galectin* as a novel member of the *Galectin-8* family. Thus, we named the protein *Pl-Galectin-8*. *In silico* analysis indicates a non secreted, non glycosylated protein.

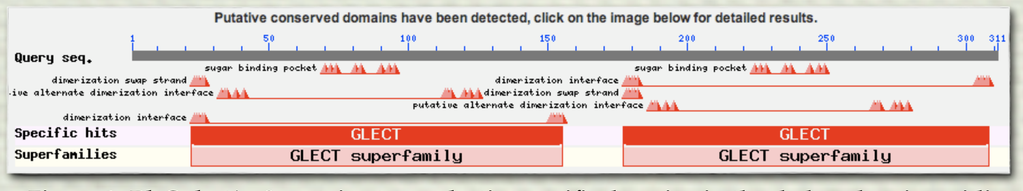

Figure 1. *Pl-Galectin-8* contains two galectin-specific domains in the deduced aminoacidic sequence.

B. Spatio-temporal expression profiling

The expression levels of the *Pl-galectin-8* mRNAs were monitored during the development of the *P. lividus* embryo by whole mount *in situ* hybridization of antisense *Pl-Galectin-8* RNA probe (full CDS) and comparative qPCR. (Russo et al., 2010). *Pl-Galectin-8* is expressed in the foregut of the gastrula and pluteus embryo and expression gradually increases starting from the blastula stage (Fig.2).

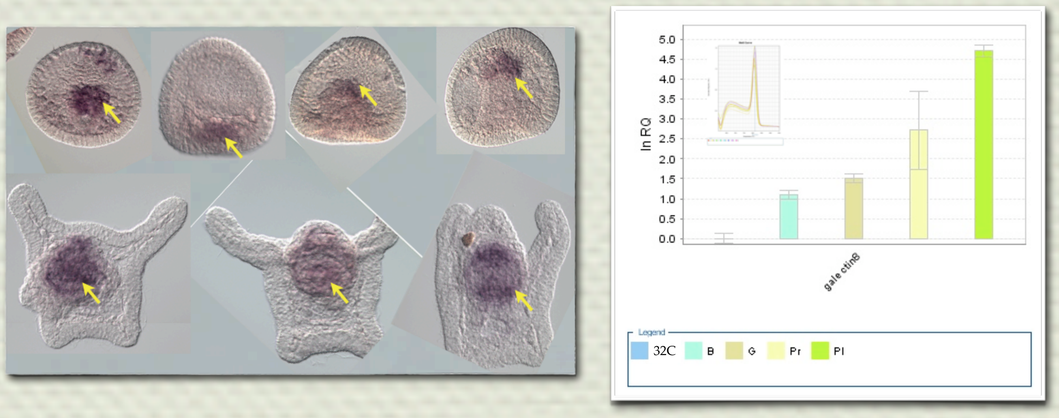

Figure 2. Spatiotemporal expression profile of *Pl-Galectin-8* in embryo development. Left: WMISH in Blastula, Early Gastrula, Middle Gastrula and Late Gastrula (first row) and Pluteus (second row) Right: Temporal expression profile by Comparative qPCR (32C: 32 cells cleavage, B: Blastula, G: Gastrula, Pr: Prism, Pl: Pluteus).

C. Modeling of the putative protein

In silico analysis of the deduced amino-acidic sequence of *Pl-Galectin-8*, indicated high homology (65%) with the Human Galectin-8 (>gi|187609173|pdb|2YXS|A Chain A, Crystal Sturcture Of N-Terminal Domain Of Human Galectin-8 With D-Lactose). We used the Modeller software (http://salilab.org/modeller/9v4/release.html) to create a model structure of the N-terminal *Pl-Galectin-8* based on the Human Galectin-8 (pdb structure: 2YXS). The potential calcium binding aminoacids were identified (Fig.3).

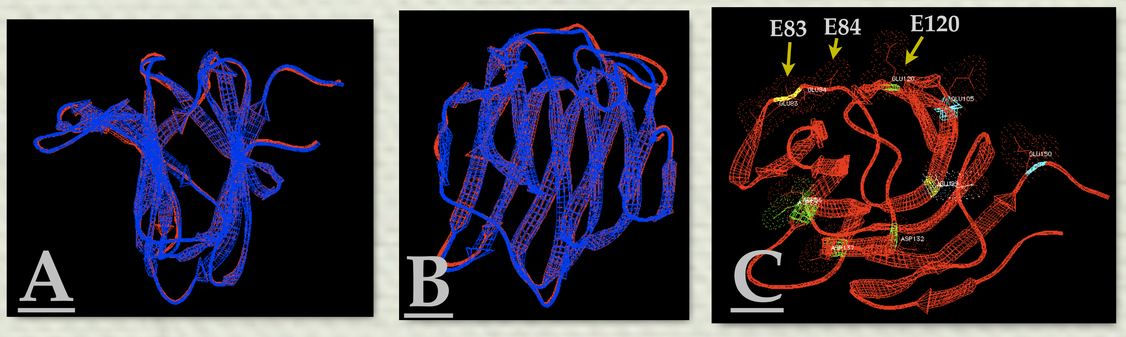

Figure 3. Modeling of Pl-Galectin (in red) based on the crystallized structure of Human Galectin8, 2YXS (in blue). A, B: Superimposition of the two proteins. C: Annotation of the potential calcium binding aminoacids (aa) (E83, E84, E120) In color, more potential calcium binding aa.

D. Preparation of recombinant *Pl-Galectin-8* in E. *coli* st. BL21.AI.

In order to perform functional assays of *Pl-Galectin*, in view of future biomedical applications we cloned the coding sequence (CDS) in the pCOLD-TF expression vector (Takara). The vector fuses a trigger factor protein which facilitates the native expression of the target protein, as well as a 6-Histidines tag in the N-terminal of the fused protein. The protein was expressed in E. *coli (BL21.A1)* following standard procedures and it was purified from the water soluble cell extracts by affinity chromatography (Fig.4).

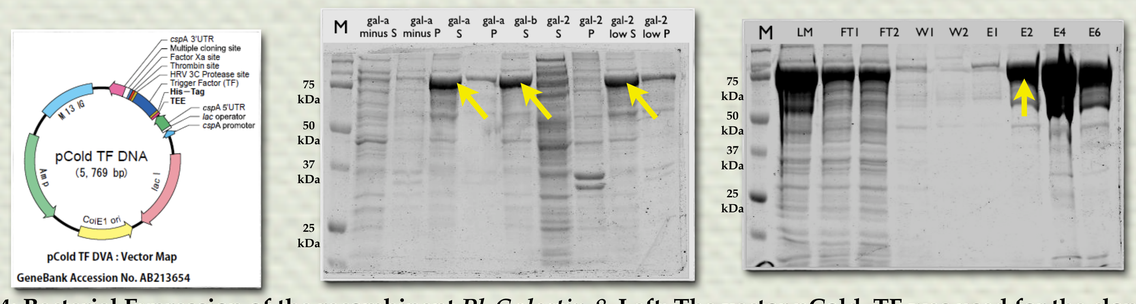

Figure 4: Bacterial Expression of the recombinant *Pl-Galectin-8*. Left: The vector pCold_TF was used for the cloning. Middle: SDS gel electrophoresis of the expression process. Fusion protein is expressed after induction and is found in the native form (75kDa) (minus: un-induced expression, S: water-soluble fraction, P: water insoluble fraction). Right: SDS gel electrophoresis of the purification process. Protein is eluted in the native form, pure from bacterial proteins. LM: Loading Material, FT: flow-through, W: wash, E1-6: elution fractions

E. Functional assays

Galectin polymerization, which is involved in biomineral formation, in the presence of calcium has been previously recorded in a galectin from the sponge *S. Domuncula* (H.C. Schröder et al, 2006). Here, we have initiated biochemical tests to identify similar activity in *P.lividus*. The calcium binding activity of the recombinant *Pl-Galectin-8* protein was assayed by a Calcium-specific electrode; which measures the free calcium cations in a solution. No measurable calcium binding activity was found at protein concentrations varying from 10ng/ml to 1μg/ml (Fig.5). The aggregation of galectin in the presence of $CaCl_2$, is under study.

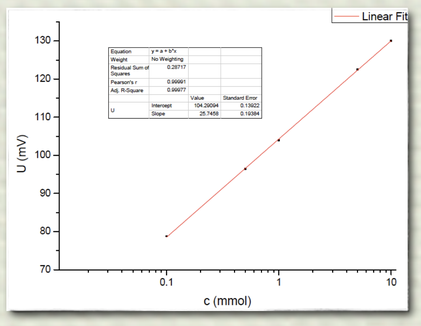

Figure 5: Standard curve of free calcium in solution. Y axis: mV measured by the electrode, X-axis: mmol of $CaCl_2$. Sample measurements showed no deviation from the standard curve.

Conclusions

- A Galectin was identified for the first time in the sea urchin (*P. lividus*). The obtained mRNA sequence was deposited in the EMBL genebank database by the accession number FR716470.
- *Pl-Galectin-8* mRNA expression initiates at the Blastula stage during embryo development and gradually increases until the Pluteus stage. Expression is localized in the foregut of the embryo.
- Modeling of N-terminal domain of *Pl-Galectin-8*, demonstrates a structure similar to the N-terminal human Galectin-8. The model revealed three main potential calcium binding sites.
- A recombinant *Pl-Galectin-8*, was prepared for biochemical studies and antibodies production (on-going now).
- The aggregation of galectin in the presence of calcium, serving for diverse biological events, has been studied. So far, calcium binding activity was assayed without measurable result.
- ☆ This study provides with insight on a newly identified mRNA isolated from *P. lividus* embryos, showing high similarity with *galectin-8*. A molecular tool-set has been prepared, including the cloning of the CDS in various vectors for different purposes (WMISH probe preparation or protein expression) and a recombinant *Pl-Galectin-8* from E. *coli*. Specific polyclonal antibody raised in mice will permit the protein localization in the sea urchin embryo or adult. The functional role of *Pl-Galectin-8* in embryo and adult development is under investigation. Biochemical assays on the recombinant protein aim to reveal the aggregation activity of this protein and it’s role in the sea urchin development.

References

1. Carbohydrate-recognition domains of galectin-9 are involved in intermolecular interaction with galectin-9 itself and other members of the galectin family, Miyanishi et al., 2007, Glycobiology
2. Galectins as modulators of tumour progression. Liu, F.T., G.A. Rabinovich. 2005, Nat. Rev. Cancer. 5:29–41.
3. S-type lectins occur also in invertebrates: high conservation of the carbohydrate recognition domain in the lectin genes from the marine sponge Geodia cydonium. Pfeifer, K., Haasemann, M., Gamulin, V., Bretting, H., Fahrenholz, F. and Müller, W. E. G.,1993, Glycobiol. 3, 179-184.
4. Transcriptional increase and misexpression of 14-3-3 epsilon in sea urchin embryos exposed to UV-B, R. Russo et al.,(2010) Cell Stress and Chaperones 15:993–1001
5 Proteomic analysis of sea urchin (Strongylocentrotus purpuratus) spicule matrix, K. Mann, 2010 , Proteome Science, 8:33
6.Co-expression and Functional Interaction of Silicatein with Galectin, H.C.Shroeder et al, 2006, THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 17, pp. 12001–12009