european journal of histochemistry
a journal of functional cytology

ISSN 1121-760X
volume 60/supplement 1
2016

# PROCEEDINGS OF THE 62nd CONGRESS OF THE ITALIAN EMBRYOLOGICAL GROUP (GEI)

*Naples, June 20-23, 2016*
*Centro Congressi Federico II*
*Complesso dei Santi Marcellino e Festo*
*Largo S. Marcellino 10, Napoli*

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under the auspices of
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Calcein fluorescence suggesting that they succeed in crossing the oocyte plasma membrane. Co NPs, instead, did not cause a reduction of fluorescence suggesting that were unable to pass through the plasma membrane. This different behavior of cobalt and cobalt oxide NPs could be ascribed to different chemical and physical characteristics of their surfaces.
We are obtaining similar results with iron oxide and this might be of use for food and feed supplementation.

## *ANKRD1* OVEREXPRESSION IN THE DEVELOPING MYOCARDIUM CAUSES ANOMALOUS VENOUS RETURN AND MORPHOGENETIC DEFECTS BY IMPARING CARDIAC REMODELING

M. Campione,[1] G.S. Ganzetti,[2] I. Badi,[3] V. Cora,[1] S. Tiso,[1] R. Cinquetti,[3] S. Manzini,[2] G. Chiesa,[2] L. Monti,[3] F. Caicci,[4]
S.B. Bleyl,[5] L. Manni,[4] C. Parolini,[2] F. Acquati[3]

*[1]CNR-Neuroscience Institute, Department of Biomedical Sciences, University of Padua, Italy; [2]Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy; [3]Department of Theoretical and Applied Sciences and Biotechnology and Life Sciences, University of Insubria, Varese, Italy; [4]Department of Biology, University of Padua, Italy [5]Department of Pediatrics, University of Utah, Salt Lake City, UT, USA*

*E-mail: campione@bio.unipd.it*

The acquisition of cardiac anatomical organization is the result of tightly regulated developmental step and even minor mistakes in this chain of events results in congenital heart disease (CHD).[1] Total anomalous pulmonary venous return (TAPVR) is a severe CHD characterized by failure of the pulmonary veins to connect exclusively to the left atrium.[2] We previously identified increased levels of the mechanosensory gene *ANKRD1* in TAPVR patients;[3] however, the link between its increased expression and TAPVR pathogenesis remains unexplored. Here we show that *Ankrd1* defines novel morphogenetic subdomains in the developing myocardium, where it modulates cardiomyocyte structural properties. *Ankrd1* is expressed in discrete subcompartments in the developing mouse heart and its myocardial overexpression in mice strongly impairs cardiac remodeling, including alignment of the developing venous system with the myocardium. Mid-fetal transgenic hearts present complex morphogenetic defects and abnormal pulmonary venous connections accompanied by strong cellular disorganization. Our results define *ANKRD1* as a crucial modulator of heart development, whose regionalized expression is required to refine shape and relative position of cardiac compartments. These findings uncover novel levels of complexity in genetic regulation of cardiac development. We propose that increased *ANKRD1* levels leads to TAPVR as a consequence of impaired remodeling of early venous pole myocardium.

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## FIRST DESCRIPTION OF A HISTAMINE RECEPTOR OF CLASS 2 (HRH2) IN A PROTOCHORDATE: EXPRESSION DURING BLASTOGENESIS AND ROLE IN REGULATION OF CILIARY BEAT FREQUENCY

F. Cima

*Department of Biology, University of Padua, Italy*

*E-mail: francesca.cima@unipd.it*

Histaminergic receptors belong to the family of seven-transmembrane $\alpha$-helix domain receptors classified in mammals into four distinct classes. Despite being widely studied in vertebrates, few data are available on the invertebrate receptors, with only predicted H1 and H2 sequences for non-chordate deuterostomes. We report the first transcript evidence of an H2 receptor for histamine in the colonial ascidian *Botryllus schlosseri* showing a high degree of conservation with HRH2 mammalian and other vertebrate orthologous proteins. The transcript and protein localisation during blastogenic development through *in situ* hybridisation and immunohistochemistry has been described. The mRNA expression appears first in the ciliary tissues of the alimentary system in filter-feeding adults and the buds, with a particular intensity in the pharynx. Transcription is activated very early, beginning from the inner layer of the disc of the secondary bud. From one generation to the next, the transcript signals become more and more intense at the level of the emergence of primordia of the branchial and peribranchial chambers and, finally, in the cells bordering the stigmata, dorsal lamina, and non-glandular ciliated zones of the endostyle. The translated H2 receptor appears as soon as the primordia of branchial and peribranchial chambers form in the secondary bud, and, in the primary buds, is found mainly in the protostigmata before the two layers of branchial and peribranchial epithelial tissue perforate to form the stigmata. In the adult zooid, the H2 receptor is expressed by ciliated mucous cells involved in food progression throughout the whole length of the alimentary canal. The observation of the effects of histamine and histamine-receptor antagonist (ranitidine) and agonist (dimaprit) drugs on explanted branchial tissue has provided confirmation concerning the receptor class and its role in regulating the ciliary beat frequency. The involvement in the local regulation of ciliary activity is of particular concern for evolutionary considerations because HRH2 seems to have been conserved in the pharynx and its developmental derivatives (*e.g.*, upper respiratory tract and middle ear of mammals) during the evolution of chordates.